TM TAMAYO

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-145190- -00000-0000

Fecha: 2013-06-18 16:02:15 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 9

Industria y Comercio
SUPERINTENDENCIA

DELEGATURA PROPIEDAD INDUSTRIAL
División de Nuevas Creaciones

SOLICITUD

PATENTE DE INVENCION PCT/FASE NACIONAL

21. EXPEDIENTE No. _____

54. TITULO ANÁLOGOS DE GLUCAGÓN QUE PRESENTAN ACTIVIDAD DE RECEPTOR DE GIP

51. CLASIFICACIÓN INTERNACIONAL _____

71. SOLICITANTE INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION

DOMICILIO: Indianapolis, IN. 46202 USA.

74. **APODERADO** **CARLOS ORLANDO MAYA RODRIGUEZ**

22. BOGOTA, D.C. **18 de junio de 2013**

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 9

DIRECCIÓN DE NUEVAS CREACIONES
SOLICITUD FASE NACIONAL -PCT

1	**TIPO DE SOLICITUD**	☒ Patente de invención ☒ Capítulo I	☐ Patente de Modelo de Utilidad ☐ Capitulo II

2 DATOS SOLICITUD INTERNACIONAL PCT (86)

Solicitud Internacional No.	PCT/US2011/066164	**Fecha**	2011/12/20
Publicación Internacional No.	WO 2012/088116 A2	**Fecha**	2012/06/28

3 TÍTULO DE LA INVENCIÓN (200 caracteres o espacios máximos)

ANÁLOGOS DE GLUCAGÓN QUE PRESENTAN ACTIVIDAD DE RECEPTOR DE GIP

4 CLASIFICACIÓN INTERNACIONAL (CIP)

5 SOLICITANTE (S) ☐ Esta persona también es inventor. Para datos adicionales utilizar hoja de información complementaria

APELLIDOS O RAZÓN SOCIAL	NOMBRE	IDENTIFICACIÓN	TIPO
1. INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION		EXTRANJERA	

6 DATOS DEL SOLICITANTE

DIRECCIÓN	351 West 10th Street Indianapolis, IN 46202	No. TELÉFONO	
CIUDAD	INDIANAPOLIS	CORREO ELECTRÓNICO	
DEPARTAMENTO/ESTADO		NACIONALIDAD O LUGAR DE CONSTITUCIÓN	
PAÍS DE RESIDENCIA	USA		

7 INVENTOR (ES) Para datos adicionales utilizar hoja de información complementaria

APELLIDOS	NOMBRES	NACIONALIDAD
1.DIMARCHI	Richard, D.	US
2.WARD	Brian	US
3.		
4.		
5.		

DIRECCIÓN DE CORREO ELECTRÓNICO:

8 DATOS INVENTOR (ES)

PAÍS RESIDENCIA	DEPARTAMENTO/ESTADO	CIUDAD	DIRECCIÓN
1.US	Indiana	Carmel	10890 Wilmington Drive, Carmel, IN 46033, US
2.US	Indiana	Indianapolis	351 West 10th Street, Indianapolis, IN 46202, US
3.			
4.			
5.			

OTRO(S) SOLICITANTE(S) Y/O (OTRO(S)) INVENTOR(ES)

☐ Los demás solicitantes y/o (demás) inventores se indican en una hoja a continuación.

9 ☐ **REPRESENTANTE LEGAL** ☒ **APODERADO**

APELLIDOS	NOMBRES	IDENTIFICACIÓN
MAYA RODRIGUEZ	Carlos Orlando	C.C .17117818 T.P .17.402

DIRECCIÓN	Cra. 13 A No. 28-38 Ofc. 230 MZ 2 PARQUE BAVARIA	No. TELÉFONO	3 419841
CIUDAD	BOGOTÁ D.C.	CORREO ELECTRÓNICO	consultas@tmtamayo.net
PAÍS	COLOMBIA	No. RADICACIÓN DE PROTOCOLO DE PODER GENERAL	

10	**DECLARACIONES DE PRIORIDAD** ☒ SI ☐ NO			
	(33) PAÍS DE ORIGEN	CÓDIGO PAÍS	(31) NÚMERO	(32) FECHA (AAAA/MM/DD)
	1.USA	US	61/514,609	2011/08/03
	2.USA	US	61/426,285	2010/12/22
	3.			

11 DECLARACIÓN SOBRE USO DE RECURSOS GENÉTICOS O BIOLÓGICOS

Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de recursos genéticos o biológicos de los que cualquiera de los países miembros de la Comunidad Andina es país de origen.

☐ SI ☒ NO

Nota: En caso afirmativo deberá anexar copia del contrato de acceso de recursos genéticos o productos derivados, o certificado o número de registro, expedido por la Autoridad competente.

12 DECLARACIÓN SOBRE USO DE CONOCIMIENTOS TRADICIONALES

Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de conocimientos tradicionales de comunidades indígenas, afroamericanas o locales de países miembros de la Comunidad Andina.

☐ SI ☒ NO

Nota: En caso afirmativo deberá anexar la licencia o autorización de uso de conocimiento tradicional, o certificado, o número de registro expedido por la Autoridad competente.

13 REDUCCIÓN DE TASAS

Declaro que carezco de medios económicos para presentar la solicitud de patente.

☐ SI ☒ NO

Nota: En caso de ser persona natural y carecer de medios económicos, y por lo tanto, aplique la reducción de tasas a que se refiere la resolución vigente en tarifas, debe firmar la presente solicitud bajo la gravedad de juramento.

Micro, pequeñas y medianas empresas ☐
Universidades públicas o privadas ☐
Entidades sin ánimo de lucro ☐

Debe aportar los documentos que se indican en el numeral 17 de anexos

14 AUTORIZACIÓN DE NOTIFICACIÓN EN LÍNEA ☐ SI ☒ NO

Manifiesto que he leído y entendido perfectamente los términos y condiciones de uso de medios electrónicos para las notificaciones en línea a través de Internet de los actos administrativos proferidos por la Superintendencia de Industria y Comercio que deben ser notificados personalmente y, en consecuencia, autorizo el servicio de notificación a través de internet.

15	**COMPROBANTE DE PAGO O PAGO ELECTRÓNICO**	**N°** 13-0058147	**Fecha** 14-06-2013
	COMPROBANTE POR REIVINDICACIÓN ADICIONAL A 10	**No 13-0058148**	**Fecha: 14-06-2013**

16 FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL

Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)

CARLOS ORLANDO MAYA RODRIGUEZ (Apoderado)

17 ANEXOS

Documentación Técnica
Solicitud Internacional en castellano

1. ☒ Descripción — N° de folios: 388
2. ☒ Reivindicaciones — N° Reivindicaciones: 136
3. ☒ Dibujos y/o figuras — N° folios: 20
4. ☒ Resumen.
5. ☐ Certificado de depósito de material biológico si fuera el caso.
6. ☒ Listado de secuencias de nucleótidos y/o aminoácidos en forma digital si fuera el caso.
7. ☒ Arte final 12 x 12.
8. ☒ Anexo formato digital.

Documentación Jurídica

9. ☒ Poderes, si fuera el caso.
10. ☐ Documento que legalmente pruebe la cesión del inventor al solicitante o a su causante.
11. ☐ Copia del contrato de acceso de recursos genéticos o productos derivados, certificado o número de registro, si fuera el caso.
12. ☐ Copia de la licencia o autorización de Conocimientos Tradicionales, certificado o número de registro, si fuera el caso.
13. **Reducción de tasas**

Micro, pequeñas o medianas empresas

☐ Copia simple de la declaración de renta del año inmediatamente anterior, o en su defecto prueba documental idónea.

☐ Documento de constancia de cumplimiento con lo establecido en la ley 905 de 2004.

Universidades públicas o privadas

☐ Copia acto de reconocimiento institucional emitido por el Ministerio de Educación

Entidades sin ánimo de lucro

☐ Copia de registro vigente en Cámara de comercio.
☐ Hoja de información complementaria.
☐ Otros, especificar

14. ☒ Comprobante de pago de la tasa de presentación de la solicitud.
15. ☐ Comprobante de pago por reivindicación de prioridad.
16. ☐ Comprobante de pago de la tasa por concepto de excedente de palabras en la publicación.
17. ☒ Comprobante de pago por reivindicación adicional a 10.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 0058147

Bogotá D.C., Junio 14 de 2013 - 10:20:55

RECIBIDO DE : TM TAMAYO Y ASOCIADOS NI 830.091.650

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	558992652	14/06/2013	4.310.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE INVENCION	500.000.00	500.000.00
				$500.000.00

SON: **QUINIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____
*****

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-145190- -00000-0000

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 0058148

Bogotá D.C., Junio 14 de 2013 - 10:20:55

RECIBIDO DE : TM TAMAYO Y ASOCIADOS NI 830.091.650

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	558992652	14/06/2013	4.310.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
127	50005-01-01 SOLICITUDES	1607 REIVINDICACION PATENTE UNITARIA ADICIONAL A LAS 10 INIC	30.000.00	3.810.000.00
				$3.810.000.00

SON: **TRES MILLONES OCHOCIENTOSDIEZ MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-******-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-145190- -00000-0000

Fecha: 2013-06-18 16:02:15 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 9

COLONIA

CECBA - LEY404 GCBA
LEGALIZACION
090728 329985
$35.00
12:47:55
28/07/2009

COLEGIO DE ESCRIBANOS · CIUDAD DE BUENOS AIRES · CAPITAL FEDERAL - REPUBLICA ARGENTINA

ACTUACION NOTARIAL
LEY 404

N 011082583

FOLIO 1.318.- PRIMERA COPIA.- ESCRITURA NUMERO SEISCIENTOS CUARENTA Y NUEVE.- En la Ciudad de Buenos Aires, Capital de la República Argentina, a veintidos de julio de dos mil nueve, ante mi, Escribano Autorizante, COMPARECE don **Daniel GOYTIA**, argentino, mayor de edad, casado, Libreta de Enrolamiento número 4.526.965, domiciliado en Bartolomé Mitre 226, 4º piso, de esta Ciudad.- El compareciente es persona de mi conocimiento, doy fe, así como que DICE: **I.-** Que por escritura de fecha 9 de abril de 2008, pasada ante mí al folio 932 del Protocolo de este Registro, al que me remito, doy fe, se protocolizó un **Poder** otorgado por **INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION**, con domicilio en 351 West 10th Street, Indianapolis, IN 46202, US, a su favor, el qué transcripto en sus partes pertinentes literalmente DICE: "... **Poder** - El abajo firmante - (1) - **INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION** - Domiciliado en - (2) - 351 West 10th Street, Indianapolis, IN 46202, US, por el presente otorga a **DANIEL GOYTIA** domiciliado en Buenos Aires, Argentina, poder amplio y suficiente para recabar de las autoridades nacionales que corresponda, sean estas administrativas y/o judiciales, la obtención de **"Patentes, Marcas, Modelos, Diseños y sus renovaciones"** y realizar todos los trámites conducentes para proteger los derechos de la mandante, a cuyo efecto, los faculta para efectuar ante dichas autoridades nacionales, administrativas y/o judiciales todos los trámites necesarios a los objetos indicados; presentar solicitudes, alegatos, oposiciones, protestas, apelaciones y demandas; desistir, arreglar y transigir; aceptar cesiones y transferencias; solicitar testimonios; recibir documentos y otros valores; justificar explotaciones; cobrar y percibir; firmar instrumentos públicos, privados y escrituras públicas; para renunciar o no a las gestiones judiciales; y hacer cuanto fuere necesario ante las autoridades nacionales, judiciales y/o administrativas, sea

N 011082583

como demandante o como demandado, para proteger los intereses del mandante, con facultad de substituir este instrumento y revocar tal substitución. Se deja constancia que las facultades enumeradas en este mandato, son meramente ejemplificativas y no limitativas de otras facultades implícitas, por cuyo motivo, los mandatarios quedan autorizados para ejercer sin restricción alguna todos los derechos que acuerde la ley que se relacionen y/o emerjan y/o que sean consecuencia del objeto del presente poder y ya sea ello en razón de la legislación vigente y/o de la legislación que en el futuro se promulgue. Este documento es efectivo a partir del 31 de enero de 2008 ...".- LO TRANSCRIPTO ES COPIA FIEL, doy fe, de las partes pertinentes del mandato relacionado.- **II.**- Continúa diciendo el compareciente que viene por la presente a **SUSTITUIR** el citado mandato, reservándose para si las facultades, a favor de **"CARLOS TAMAYO & ASOCIADOS" y/o Carlos Orlando Maya Rodriguez y/o Gustavo Calderon Rosero y/o Carmen Graciela Florez y/o Clara Cortes Charry y/o Mayerling Rocio Castelblanco S.**, con domicilio en Carrera 13A Nº 28-38, Buffette 245, Manzana 02, Parque Central Bavaria, Bogotá, **COLOMBIA**, para actuar en dicho país con todas y cada una de las facultades enumeradas precedentemente.- LEIDA y ratificada, así lo expresó, otorgó, aceptó y firmó, por ante mi, doy fe.- "SIGUE UNA FIRMA".- Esta mi sello.- Ante mi: ALEJANDRO M. LIPORACE.- CONCUERDA con su matriz que pasó ante mi, al folio 1.318 del Protocolo del Registro 1331 a mi cargo.- PARA LOS APODERADOS expido esta **PRIMERA COPIA** en UNA FOJA de Actuación Notarial número N 011082583 que sello y firmo en el mismo lugar y fecha de su otorgamiento, doy fe.-

ALEJANDRO M. LIPORACE
Matrícula 3837
ESCRIBANO PUBLICO

7

COLEGIO DE ESCRIBANOS
CIUDAD DE BUENOS AIRES
CAPITAL FEDERAL - REPUBLICA ARGENTINA

LEGALIZACION
LEY 404

L 008968001

EL COLEGIO DE ESCRIBANOS de la Ciudad de Buenos Aires, Capital Federal de la República Argentina, en virtud de las facultades que le confiere la ley orgánica vigente, LEGALIZA la firma y sello del escribano ~~ALEJANDRO MARIA LIPORACE~~ **obrantes en el documento anexo, presentado en el día de la fecha bajo el Nº** ~~090728329985/4~~ **La presente legalización no juzga sobre el contenido y forma del documento.**

Buenos Aires, ~~Martes 28 de Julio de 2009~~

COLEGIO DE ESCRIBANOS
LEGALIZACION
CIUDAD DE BUENOS AIRES
CAPITAL FEDERAL - REPUBLICA ARGENTINA

ESC. RAQUEL COLOMER
COLEGIO DE ESCRIBANOS
CONSEJERA

8

SERIE A 2597588

REPÚBLICA ARGENTINA

MINISTERIO *de*
RELACIONES EXTERIORES
COMERCIO INTERNACIONAL Y CULTO

CANCILLERIA ARGENTINA

APOSTILLE
(Convention de la Haye du 5 octobre 1961)

1. País ARGENTINA
   El presente documento público
2. Ha sido firmado por RAQUEL COLOMER
3. Quien actúa en calidad de FUNCIONARIO HABILITANTE
4. Lleva el sello/timbre de COLEGIO DE ESCRIBANOS DE CAPITAL FEDERAL
5. En BUENOS AIRES 6. El día 29/07/2009
7. Por UNIDAD DE COORDINACION LEGALIZACIONES MINISTERIO DE RELACIONES EXTERIORES, COMERCIO INTERNACIONAL Y CULTO
8. Bajo el Número: 149647/2009
9. Sello/Timbre: 39

LA PRESENTE LEGALIZACION TIENE COMO UNICO EFECTO AUTENTICAR LA FIRMA Y CARACTER DEL OTORGANTE. SIN JUZGAR EL CONTENIDO DEL DOCUMENTO

10. Firma
Dn GUSTAVO EZEQUIEL SANTA ANA
Unidad de Coordinación Legalizaciones
Ministerio de Relaciones Exteriores
Comercio Internacional y Culto

Tipo de Documento: ACTUACION NOTARIAL
Titular del Documento: DANIEL GOYTIA

2009149647

1939917F6B9031883ED305C87367DFC8

Industria y Comercio
SUPERINTENDENCIA

PLANILLA DE RADICACIÓN - DIGITALIZACIÓN DIARIA
RELACIÓN DE ANEXOS

Dependencia:	Fecha: 2013/ 06/ 18/ (AAAA MM DD)	Recorrido:
2020.		

Tipo de Radicación: Entrada: X Salida: ☐ Traslado: ☐ Convenio: ☐

Radicador: | Planilla:

ID	*No. Radicación*	*Consecutivo*	*Item*
1	13-145190	0	
2			
3			
4			
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11			
12			
13			
14			
15			
16			
17			

Item	*Unidad de Conservación*
1	Bolsa
2	Caja
3	Libro
4	Sobre
5	Tomo
6	**Otro**
Item	***Soporte Documental***
7	CD X
8	Disquete
9	DVD
10	Folleto
11	Foto
12	Periódico
13	Plano
14	Publicación Periódica
15	USB
16	**Otro**

Observaciones:
FOLIO 9 CD.

219935
Susan

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

To:
Breen, John P.
BARNES & THORNBURG LLP
11 South Meridian Street
Indianapolis IN 46204
ETATS-UNIS D'AMERIQUE

IP FILES
AUG 0 6 2012
BARNES & THORNBURG LLP

DOCKETED
FOR
BY
DATE
CHE'D BY (PCT Rule 44.1)
DATE

Applicant's or agent's file reference 32993-219935	Date of mailing *(day/month/year)* 26 July 2012 (26-07-2012) **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2011/066164	International filing date *(day/month/year)* 20 December 2011 (20-12-2011)
Applicant INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY...	

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 - 9.011.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Authorized officer BUSSHOFF, Johannes Tel: +49 (0)89 2399-2897

Form PCT/ISA/220 (July 2010)

8/13/12

PATENT COOPERATION TREATY

PCT

IP FILES
AUG 0 6 2012
BARNES & THORNBURG LLP

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
32993-219935		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2011/066164	20/12/2011	22/12/2010
Applicant		
INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY...		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 10 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [X] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. 8
      [X] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [ ] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2011/066164

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2011/066164

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-13, 52-61, 92, 94(completely); 104-135(partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/066164

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/26 C07K14/605
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/155257 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; SMILEY DAV) 23 December 2009 (2009-12-23) Invention 1 page 6, line 10 - page 9, line 5 page 113; table 8 claims 1-96 ----- -/--	1-6, 14-51, 104-136

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2012	26/07/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Niebuhr-Ebel, K

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAN CLARK Q ET AL: "Design of a long acting peptide functioning as both a glucagon-like peptide-1 receptor agonist and a glucagon receptor antagonist", JOURNAL OF BIOLOGICAL CHEMISTRY, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US, vol. 281, no. 18, 1 May 2006 (2006-05-01), pages 12506-12515, XP002529523, ISSN: 0021-9258, DOI: 10.1074/JBC.M600127200 [retrieved on 2006-02-27] abstract page 12506, paragraph 3 - page 12507, paragraph 3 page 12508; table 1 page 12511; table 3 page 12513, paragraph 2 - paragraph 3 -----	1-6, 14-51, 104-136
X	WO 2010/071807 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; KOU BINBIN) 24 June 2010 (2010-06-24) Invention 2 + 3 page 49, line 27 - page 51, line 6 page 59, line 8 - page 60, line 22 page 66, line 15 - page 67, line 5 -----	7-61, 104-136
X	PENGYUN LI ET AL: "Design, synthesis and crystallization of a novel glucagon analog as a therapeutic agent", ACTA CRYSTALLOGRAPHICA SECTION F STRUCTURAL BIOLOGY AND CRYSTALLIZATION COMMUNICATIONS, vol. 63, no. 7, 1 July 2007 (2007-07-01), pages 599-601, XP55010072, ISSN: 1744-3091, DOI: 10.1107/S1744309107028655 Invention 3 abstract page 600; figure 1 -----	52-61, 104-136
A	WO 2008/101017 A2 (INDIANA UNVERSITY RES AND TECH [US]; DAY JONATHAN [US]; PATTERSON JAME) 21 August 2008 (2008-08-21) Invention 15 + 17 claim 65 sequence 495 -/--	92,94, 104-135

4

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	-& DATABASE Geneseq [Online] 27 November 2008 (2008-11-27), "Enhanced potency exhibiting modified human GLP-1 peptide SEQ ID:495.", XP002679991, retrieved from EBI accession no. GSP:ATD14223 Database accession no. ATD14223 sequence -----	92,94, 104-136
A	CHABENNE JOSEPH R ET AL: "Optimization of the native glucagon sequence for medicinal purposes.", JOURNAL OF DIABETES SCIENCE AND TECHNOLOGY NOV 2010 LNKD- PUBMED:21129326, vol. 4, no. 6, November 2010 (2010-11), pages 1322-1331, XP007920819, ISSN: 1932-2968 abstract page 1327, right-hand column, paragraph 2 - page 1328, right-hand column, paragraph 1 figure 5 page 1330, right-hand column, paragraph 2 table 1 -----	92,94, 104-136

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/066164

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009155257 A1	23-12-2009	AU 2009260301 A1	23-12-2009
		CA 2727161 A1	23-12-2009
		CN 102088989 A	08-06-2011
		EP 2307037 A1	13-04-2011
		JP 2011524418 A	01-09-2011
		KR 20110039230 A	15-04-2011
		TW 201010730 A	16-03-2010
		US 2011190200 A1	04-08-2011
		WO 2009155257 A1	23-12-2009
WO 2010071807 A1	24-06-2010	AR 074811 A1	16-02-2011
		AU 2009327418 A1	24-06-2010
		CA 2747499 A1	24-06-2010
		CN 102325539 A	18-01-2012
		EP 2376097 A1	19-10-2011
		KR 20110110174 A	06-10-2011
		PE 03322012 A1	14-04-2012
		SG 172291 A1	28-07-2011
		TW 201028162 A	01-08-2010
		US 2011288003 A1	24-11-2011
		WO 2010071807 A1	24-06-2010
WO 2008101017 A2	21-08-2008	AR 065349 A1	03-06-2009
		AR 080904 A2	16-05-2012
		AU 2008216265 A1	21-08-2008
		BR PI0807728 A2	17-04-2012
		CA 2677932 A1	21-08-2008
		CL 4792008 A1	22-08-2008
		CN 101790538 A	28-07-2010
		CR 11028 A	29-10-2009
		DO P2009000194 A	30-06-2010
		EA 200901108 A1	26-02-2010
		EC SP099622 A	30-10-2009
		EP 2111414 A2	28-10-2009
		JP 2011511753 A	14-04-2011
		KR 20090119876 A	20-11-2009
		MA 31242 B1	01-03-2010
		NZ 578948 A	30-03-2012
		PA 8769301 A1	18-12-2008
		PE 01082009 A1	15-02-2009
		TW 200848423 A	16-12-2008
		US 2010190701 A1	29-07-2010
		WO 2008101017 A2	21-08-2008

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6(completely); 14-51, 104-136(partially)

An analog of glucagon (SEQ ID NO: 1), comprising: a. an amino acid comprising an imidazole side chain at position 1; b. an DPP-IV protective amino acid at position 2, optionally, AIB; c. an acylated amino acid or alkylated amino acid at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the acyl or alkyl group is linked to the amino acid via a spacer; d. an alpha, alpha disubstituted amino acid at position 20; and e. up to ten additional amino acid modifications relative to SEQ ID NO: 1; wherein, when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits a GIP percentage potency of at least 0.1%, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

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2. claims: 7-13(completely); 14-51, 104-136(partially)

as subject 1, except d = an alpha helix stabilizing amino acid at one or more of positions 16-21, optionally, position 16, wherein the analog does not comprise an alpha helix stabilizing amino acid at position 20

---

3. claims: 52-61(completely); 104-136(partially)

A peptide comprising the sequence of HX2X3GTFFSDX10SKYLDX16RX18AX20X21FVQWLX27X28X29GPSSGX35PPPS (SEQ ID NO: 184), wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

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4. claims: 62, 63(completely); 104-136(partially)

A peptide comprising the sequence of SEQ ID NO: 185, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

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5. claims: 64-78(completely); 104-136(partially)

A peptide comprising the sequence of SEQ ID NO: 186, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

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6. claims: 79, 80(completely); 104-136(partially)

A peptide comprising the sequence of SEQ ID NO: 187, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

7-14. claims: 81-88(completely); 89-91, 104-136(partially)

A peptide comprising one of the sequences of SEQ ID NO: 188-195, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

15-19. claims: 92-96(completely); 104-135(partially)

A peptide comprising one of the sequences of SEQ ID NO: 28, 37, 89, 171, 180

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20. claims: 97-102(completely); 104-135(partially)

An analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, comprising: (a) an amino acid comprising an imidazole side chain at position 1, (b) at position 16, an amino acid of Formula IV, wherein n is 1 to 7, wherein each of R and R2 is independently selected from the group consisting of H, C1-C18 alkyl, (C1-C18 alkyl)OH, (C1-C18 alkyl)NH2, (C1-C18 alkyl)SH, (CO-C4 alkyl)(C3-C6)cycloalkyl, (CO-C4 alkyl)(C2-C5 heterocyclic), (CO-C4 alkyl)(C6-C10 aryl)R7, and (C1-C4 alkyl)(C3-C9 heteroaryl), wherein R7 is H or OH, wherein optionally the side chain of the amino acid of Formula IV comprises a free amino group, (c) an a,a-disubstituted amino acid at position 20, (d) up to ten additional amino acid modifications relative to SEQ ID NO: 1,
wherein, when the analog lacks a hydrophilic moiety, the glucagon analog exhibits at least 0.1 % activity of native GIP at the GIP receptor, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

21-109. claims: 103(completely); 104-136(partially)

An analog comprising a parent sequence with a total of up to 3 amino acid modifications relative to the parent sequence, wherein the parent sequence is any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, and 133, wherein the amino acid modifications are selected from the group consisting of several modifications.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

PATENT COOPERATION TREATY

DOCKETED
FOR
BY
DATE
CHE'D BY
DATE

IP FILES
AUG 0 6 2012
PCT
BARNES & THORNBURG LLP

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2011/066164	20.12.2011	22.12.2010

International Patent Classification (IPC) or both national classification and IPC
INV. A61K38/26 C07K14/605

Applicant
INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY...

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Niebuhr-Ebel, K Telephone No. +49 89 2399-0

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☒ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 62-91, 93, 95-103, 136(completely); 104-135(partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 62-91, 93, 95-103, 136(completely); 104-135(partially)

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☒ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☒ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-13, 52-61, 92, 94(completely); 104-135(partially)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	92, 94
	No:	Claims	1-61(completely); 104-135(partially)
Inventive step (IS)	Yes:	Claims	92, 94
	No:	Claims	1-61(completely); 104-135(partially)
Industrial applicability (IA)	Yes:	Claims	1-61, 92, 94(completely); 104-135(partially)
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1 Claims 130-133 and 135 relate to subject-matter considered by this Authority to be covered by the provisions of **Rule 39.1(iv) / 67.1(iv) PCT**.

For the assessment of said claims on the question whether the subject-matter is patentable, no unified criteria exist in the PCT Contracting States. The patentability can be dependent upon the formulation of the claims. Although claims 130-133 and 135 are directed to a method of treatment of the human/ animal body or to the use of a compound in medical treatment, the search and examination have been carried out and based on the alleged effects of the compound/composition.

Re Item IV

Lack of unity of invention

2 The present application does not comply with the requirements of unity of invention.

So far, 109 separate inventions have been identified. Each of them is characterised by an individual "special technical feature"; there is no technical interrelation between these inventions (see below). The applicants are therefore asked to pay additional search fees.

3 In view of the disclosure of the present application, the technical problem to be solved is the provision of novel glucagon analogs.

The available prior art discloses this common concept and solutions thereto. The linking concept is therefore neither novel nor inventive and cannot provide unity of invention. The prior art shows the following technical features:

- D1 (WO2009/155257, by the applicant) discloses numerous glucagon analogs that retain glucagon receptor activity and exhibit improved solubility relative to the native glucagon peptide. The document includes a sequence listing of 115 analogs and the additional modifications include acylation / alkylation, addition of imidazole or [alpha], [alpha]-disubstituted amino acids and modifications of amino acids.

- D2 (Pan et al., 2006) discloses a variety of hybrid peptides made up of glucagon and either GLP-1 or exendin-4 that function as both a glucagon-like peptide-1 receptor agonist and a glucagon receptor antagonist.

The solution in its general form as claimed to the problem posed in the present application is therefore not novel.

4 It follows that there is no common special technical feature for the whole scope of the present application that would define an appreciable contribution (e.g. novel and/or non-trivial) over the prior art. In view of prior art, each claimed variant is considered a separate invention.

5 Concerning inventions 1, 2 and 3, the respective independent claims (1, 7 and 52) each embrace countless glucagon analogs. In case of further prosecution of said inventions the Applicant is requested to point out the supposed unifying concept of the inventions in order to avoid a non-unity objection at a later stage.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The present application relates to glucagon analogs. Following a non-unity objection, four additional search fees were paid. A search was carried out for Inventions 1-3, 15 and 17, which are the subject-matter of the present written opinion.

Invention 1 (claims 1-6 (completely) and 14-51 and 104-136 (partially):

An analog of glucagon, comprising an amino acid comprising an imidazole side chain at position 1, an DPP-IV protective amino acid at position 2, an acylated amino acid or alkylated amino acid at any of positions 9, 10, 12, 16, 20, or 37-43, an alpha, alpha disubstituted amino acid at position 20; and up to ten additional amino acid modifications relative to unmodified glucagon.

6 Reference is made to the following documents:

D1 WO 2009/155257 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; SMILEY DAV) 23 December 2009 (2009-12-23)

D2 PAN CLARK Q ET AL: "Design of a long acting peptide functioning as both a glucagon-like peptide-1 receptor agonist and a glucagon receptor antagonist", JOURNAL OF BIOLOGICAL CHEMISTRY, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US, vol. 281, no. 18, 1 May 2006 (2006-05-01), pages 12506-12515, XP002529523, ISSN: 0021-9258, DOI: 10.1074/JBC.M600127200 [retrieved on 2006-02-27]

7 Novelty (Art. 33 (2) PCT)

7.1 **D1** (WO2009/155257) discloses glucagon analogs that retain glucagon receptor activity and exhibit improved solubility relative to the native glucagon peptide. The glucagon modifications include acylation / alkylation, addition of imidazole or [alpha], [alpha]-disubstituted amino acids and modifications of amino acids.

D2 (Pan et al., 2006) discloses hybrid peptides made up of glucagon and either GLP-1 or exendin-4 that function as both a glucagon-like peptide-1 receptor agonist and a glucagon receptor antagonist.

7.2 The modifications that characterize the glucagon analog of claim 1 are already disclosed in D1 (p. 5, line 14 - p. 9, line 5). Therefore the subject-matter of independent claim 1 and dependent claims 2-6 (completely) and 14-51 and 104-136 (partially) are not considered novel in the sense of **Art. 33 (2) PCT**.

8 Inventive Step (Art. 33 (3) PCT)

8.1 Even if novelty could be established for the claims, they would not be considered inventive for the following reason:

In view of the disclosure of the present application, the technical problem to be solved is the provision of novel glucagon analogs. Claim 1 embraces countless glucagon analogs, but from the examples it is not obvious that any of said peptides has any unexpected or advantageous properties. Furthermore, it is questionable whether all peptides falling under the scope of said claim solve the posed problem, i.e. have glucagon activity. Consequently,

no inventive step can be acknowledged and independent claim 1 and dependent claims 2-6 (completely) and 14-51 and 104-136 (partially) are not considered inventive in the sense of **Art. 33 (3) PCT**.

Invention 2 (claims 7-13 (completely) and 14-51 and 104-136 (partially):

as Invention 1, except that it contains an alpha helix stabilizing amino acid at one or more of positions 16-21, optionally, position 16, wherein the analog does not comprise an alpha helix stabilizing amino acid at position 20

9 Reference is made to the following document:

D3 WO 2010/071807 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; KOU BINBIN) 24 June 2010 (2010-06-24)

10 **Novelty (Art. 33 (2) PCT)**

10.1 **D3** (WO2010/071807) discloses numerous glucagon analogs that have extended half lives . The glucagon modifications include acylation / alkylation, addition of imidazole or [alpha], [alpha]-disubstituted amino acids, alpha helix stabilizing amino acids and modifications of amino acids.

10.2 The modifications that characterize the glucagon analogs of claim 7 are already disclosed in D3. Therefore the subject- matter of claims 7-51 and 104-136 are not considered novel in the sense of **Art. 33 (2) PCT**.

11 **Inventive Step (Art. 33 (3) PCT)**

11.1 Even if novelty could be established for the claims, they would not be considered inventive for the following reason:

In view of the disclosure of the present application, the technical problem to be solved is the provision of novel glucagon analogs. Claim 7 embraces countless glucagon analogs, but from the examples it is not obvious that any

of said peptides has any unexpected or advantageous properties. Furthermore, it is questionable whether all peptides falling under the scope of said claim solve the posed problem, i.e. have glucagon activity. Consequently, no inventive step can be acknowledged and claims 7-51 and 104-136 are not considered inventive in the sense of **Art. 33 (3) PCT**.

Invention 3 (claims 52-61 (completely) and 104-136 (partially):

A peptide comprising the sequence of

HXXGTFTSDX SKYLDXRXAX XFVQWLXXXG PSSGXPPPS

(SEQ ID NO: 184), wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

12 Reference is made to the following documents:

D3 WO 2010/071807 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; KOU BINBIN) 24 June 2010 (2010-06-24)

D4 Pengyun Li ET AL: "Design, synthesis and crystallization of a novel glucagon analog as a therapeutic agent", Acta Crystallographica Section F Structural Biology and Crystallization Communications, vol. 63, no. 7, 1 July 2007 (2007-07-01), pages 599-601, XP55010072, ISSN: 1744-3091, DOI: 10.1107/S1744309107028655

13 **Novelty (Art. 33 (2) PCT)**

13.1 The peptide represented by SEQ ID NO: 184 contains eleven variable positions (X), namely positions 2, 3, 10, 16, 18, 20, 21, 27, 28, 29 and 35, whereby the wild type residue is one of the options at all positions except 2, 28 and 29 . Therefore, the claimed peptides differ from wild type glucagon at least in the following respects: it contains alpha-aminoisobutyric acid (AIB) at position 2, A or G at position 28 and A, R or K at position 29 and an additional C-terminal extension GPSSGAPPPS.

13.2 **D4** describes the exendin- based C- terminal extension of glucagon (GPSSGAPPPS), which "exhibits greatly improved biophysical solubility and stability" (p. 600, left column, first paragraph).

D3 describes that "modifications at position 1 or 2 can increase the peptide's resistance to dipeptidyl peptidase IV (DPP IV) cleavage. For example, the amino acid at position 2 may be substituted with ... amino isobutyric acid" (p. 85 l. 1-4). Starting on p. 86, l. 5 (Passage: "Further modifications and combinations") countless possible modifications are listed: "For example, conservative or non-conservative substitutions, additions or deletions may be carried out at any of positions 2, 5, 7, 10, 11, 12, 13, 14, 17, 18, 19, 20, 21, 24, 27, 28 or 29". On p. 88 of D3, a substitution at position 28 with Glu and a substitution of Thr at position 29 with a charged amino acid selected from the group comprising of Lys and Arg is mentioned. Therefore, the variants disclosed in D3 are considered to fall under the scope of claims 52-61 and 104-136 and are therefore not considered novel in the sense of **Art. 33 (2) PCT**.

14 **Inventive Step (Art. 33 (3) PCT)**

14.1 Even if novelty could be established for the claims, they would not be considered inventive for the following reason:

In view of the disclosure of the present application, the technical problem to be solved is the provision of novel glucagon analogs. Claim 7 embraces countless glucagon analogs, but from the examples it is not obvious that any of said peptides has any unexpected or advantageous properties. Furthermore, it is questionable whether all peptides falling under the scope of said claim solve the posed problem, i.e. have glucagon activity. Consequently, no inventive step can be acknowledged and claims 7-51 and 104-136 are not considered inventive in the sense of **Art. 33 (3) PCT**.

Invention 15 (claim 92 (completely) and 104-136 (partially):

A peptide comprising SEQ ID NO: 28:

HXQGTFTSDK SKYLDERAAQ DFVQWLLDGG PSSGAPPPS

wherein X at position 2 is alpha-aminoisobutyric acid (AIB)

15 Reference is made to the following documents:

D5 WO 2008/101017 A2 (INDIANA UNVERSITY RES AND TECH [US]; DAY JONATHAN [US]; PATTERSON JAME) 21 August 2008 (2008-08-21);

-& DATABASE Geneseq [Online], 27 November 2008 (2008-11-27), "Enhanced potency exhibiting modified human GLP-1 peptide SEQ ID:495.", retrieved from EBI accession no. GSP:ATD14223 , Database accession no. ATD14223

D6 CHABENNE JOSEPH R ET AL: "Optimization of the native glucagon sequence for medicinal purposes.", JOURNAL OF DIABETES SCIENCE AND TECHNOLOGY NOV 2010 LNKD-PUBMED:21129326, vol. 4, no. 6, November 2010 (2010-11), pages 1322-1331, XP007920819, ISSN: 1932-2968

16 **Novelty (Art. 33 (2) PCT)**

16.1 The peptide represented by SEQ ID NO: 28 differs from wild type glucagon in the following respects: it contains AIB at position 2, the substitutions Y10K, S16E, R18A, M27L, N28D, T29G and the C- terminal extension GPSSGAPPPS.

16.2 **D5** (WO2008/101017) discloses glucagon analogs that function as glucagon/ GLP-1 receptor co-antagonists. **D5** discloses numerous peptide modifications such as AIB at position 2 and the C-terminal extension GPSSGAPPPS, but SEQ ID NO: 28 differs from the peptide with the highest homology, i.e. #495 of **D5**, in four residues, namely at positions 10, 20, 27 and 28 and a C-terminal cyteine.

16.3 **D6** discloses the "optimization" of the native glucagon sequence by introducing N28D or the C-terminal extension GPSSGAPPPS.

16.4 The exact sequence of SEQ ID NO: 28 is not disclosed in the prior art, and therefore the subject- matter of claim 92 and its dependent claims are considered novel in the sense of **Art. 33 (2) PCT**.

17 **Inventive Step (Art. 33 (3) PCT)**

17.1 In view of the prior art, the problem to be solved can be formulated as the provision of alternative glucagon analogs. The proposed solution is the peptide represented by SEQ ID NO: 28.

17.2 The Examples of the application show that this peptide has advantageous properties when administered to mice, such as lowering of the body weight (Fig. 1, 4, 5E and 10) and of the blood glucose levels (Fig. 5F). Therefore, an inventive step in the sense of **Art. 33 (3) PCT** is acknowledged.

Invention 17 (claim 94 (completely) and 104-136 (partially):

A peptide comprising SEQ ID NO: 89:

HXQGTFTSDY SKYLDERAAQ DFVQWLLDGG PSSGAPPPSK G

wherein X at position 2 is alpha-aminoisobutyric acid (AIB)

18 **Novelty (Art. 33 (2) PCT)**

18.1 The peptide represented by SEQ ID NO: 89 differs from wild type glucagon in the following respects: it contains AIB at position 2, the substitutions S16E, R18A, M27L, N28D, T29G and the C-terminal extension GPSSGAPPPSKG.

18.2 **D3** (WO2008/101017) discloses glucagon analogs that function as glucagon/ GLP-1 receptor co-antagonists. **D3** discloses numerous peptide modifications such as AIB at position 2 and the C-terminal extension GPSSGAPPPS, but SEQ ID NO: 89 differs from the peptide with the highest homology, i.e. #495 of **D3**, in four residues, namely at positions 20, 27 and 28 and a C-terminal KG instead of a cysteine.

18.3 The exact sequence of SEQ ID NO: 89 is not disclosed in the prior art, and therefore the subject- matter of claim 94 and its dependent claims are considered novel in the sense of **Art. 33 (2) PCT**.

19 **Inventive Step (Art. 33 (3) PCT)**

19.1 In view of the prior art, the problem to be solved can be formulated as the provision of alternative glucagon analogs. The proposed solution is the peptide represented by SEQ ID NO: 89.

19.2 The Examples of the application show that this peptide has advantageous properties when administered to mice, such as lowering of the body weight (Fig. 4, 5E, 8 and 10) and of the blood glucose levels (Fig. 5F). Therefore, an inventive step in the sense of **Art. 33 (3) PCT** is acknowledged.

Re Item VIII

Certain observations on the international application

20 Independent claims 1, 7 and 52 do not meet the requirements of **Article 6 PCT**. The features "wherein, when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits a GIP percentage potency of at least 0.1%, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor" or "wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor" are, in fact, a mere recitation of the result to be achieved. As a general rule, claims which attempt to define the invention by the result to be achieved are not allowed.

21 Moreover, apart from vague statements and unfounded declarations, the present application does not show any activity related to the peptides claimed in independent claims 1, 7 and 52. Therefore, the claims are not supported by the description (**Art. 6 PCT**). In this context, the Applicant should be aware that the scope of a claim should reflect the **actual technical contribution** of the application. Vague statements or assertions having no technical content provide no basis (**Art. 6 PCT**, see also **PCT Guidelines 5.44**).

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

[Continued on next page]

(54) Title: GLUCAGON ANALOGS EXHIBITING GIP RECEPTOR ACTIVITY

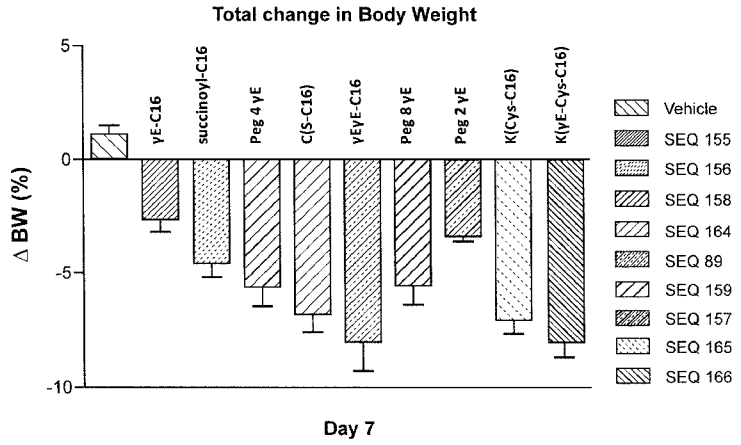

FIGURE 8

(57) Abstract: Provided herein are glucagon analogs which exhibit potent activity at the GIP receptor, and, as such are contemplated for use in treating diabetes and obesity. In exemplary embodiments, the glucagon analog of the present disclosures exhibit an EC50 at the GIP receptor which is within the nanomolar or picomolar range.

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— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

GLUCAGON ANALOGS EXHIBITING GIP RECEPTOR ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/426,285, filed on December 22, 2010, and U.S. Provisional Patent Application No. 61/514,609, filed on August 3, 2011, both applications of which are incorporated by reference in their entirety into the present application.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 190 kilobytes ACII (Text) file named "DOCS-1756766-v1-45700A_SeqListing.txt," created on December 16, 2011.

BACKGROUND

[0003] According to the most recent data from the National Diabetes Fact Sheet of the American Diabetes Association, 23.6 million children and adults in the United States are afflicted with diabetes. Each year, 1.6 million new cases of diabetes are diagnosed in people aged 20 years or older. According to a study recently published in the *Journal of the American Medical Association*, over two-thirds of adults in the United States are either overweight or obese (Flegal et al., *JAMA* 303(3): 235-241 (2010)) and over one third of this population is obese.

[0004] The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic peptide (GIP), are naturally-occurring peptide hormones. Both GLP-1 and GIP stimulate insulin synthesis and secretion in a glucose-dependent manner and do not produce hypoglycemia (*see*, e.g., Nauck et al., J. Clin. Endocrinol. Metab. 76:912-917 (1993) and Irwin et al., Regul. Pept. 153:70-76 (2009)).

[0005] GLP-1 has been shown to be effective as adjunctive therapy for diabetes and is associated with weight loss. What remains unclear about GIP-targeted therapy, however, is whether successful treatment of diabetes and obesity will be achieved through antagonizing

the effects of this hormone (e.g., via GIP receptor antagonism) or through mimicking or enhancing the effects of GIP.

SUMMARY

[0006] Provided herein are peptides that are GIP agonist peptides contemplated for use in treating subjects in need thereof, e.g. with diabetes and obesity.

[0007] Native glucagon does not activate the GIP receptor, and normally has about 1% of the activity of native GLP-1 at the GLP-1 receptor. In some embodiments, the peptides are glucagon analogs comprising a structure based on the amino acid sequence of native human glucagon (SEQ ID NO: 1) but differing at one or more positions as compared to SEQ ID NO: 1, wherein the differences, or modifications, enhance the agonist activity of the analog at the GIP receptor. Such glucagon analogs will have agonist activity at the GIP receptor greater than that of native glucagon and, in some aspects, greater than that of native GIP. In some or any embodiments, the GIP agonist has a GIP percentage potency of at least 0.1%. In some or any aspects of the present disclosures, the glucagon analog additionally exhibits agonist activity at one or both of the glucagon receptor and the GLP-1 receptor. Accordingly, GIP agonists, GIP-GLP-1 co-agonists, GIP-glucagon co-agonists, and GIP-GLP-1-glucagon triagonists are provided herein.

[0008] In exemplary embodiments, the selectivity of the GIP agonist peptide of the present disclosures for the human GLP-1 receptor versus the GIP receptor is less than 100-fold. In some or any embodiments, the GIP agonist peptide has GIP percentage potency within 20-fold or 10-fold different (higher or lower) of the glucagon percentage potency and/or GLP-1 percentage potency.

[0009] In some embodiments of the present disclosures, the glucagon analogs comprise (i) an amino acid comprising an imidazole side chain at position 1, (ii) a DPP-IV protective amino acid at position 2, (iii) an amino acid comprising a non-native acyl or alkyl group, optionally at any of positions 9, 10, 12, 16, 20, or 37-43, and optionally wherein the non-native acyl or alkyl group is linked to such amino acid via a spacer; (iv) an alpha helix stabilizing amino acid at one or more of positions 16, 17, 18, 19, 20 or 21, and (v) up to ten (e.g., up to 1, 2, 3, 4, 5, 6, 7, 8 or 9) additional amino acid modifications relative to SEQ ID NO: 1. In exemplary embodiments, the glucagon analog comprising (i) an amino acid

comprising an imidazole side chain at position 1, (ii) a DPP-IV protective amino acid at position 2, optionally, aminoisobutyric acid, (iii) an amino acid comprising a non-native acyl or alkyl group, optionally at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the non-native acyl or alkyl group is linked to such amino acid via a spacer; (iv) an alpha, alpha disubstituted amino acid at position 20, and (v) up to ten (e.g., up to 1, 2, 3, 4, 5, 6, 7, 8 or 9) additional amino acid modifications relative to SEQ ID NO: 1. In alternative exemplary embodiments, the glucagon analog comprising (i) an amino acid comprising an imidazole side chain at position 1, (ii) a DPP-IV protective amino acid at position 2, optionally, aminoisobutyric acid, (iii) an amino acid comprising a non-native acyl or alkyl group, optionally at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the non-native acyl or alkyl group is linked to such amino acid via a spacer; (iv) an alpha helix stability amino acid at one or more of positions 16-21, optionally, position 16, wherein the analog does not comprise an alpha helix stabilizing amino acid at position 20, and (v) up to ten (e.g., up to 1, 2, 3, 4, 5, 6, 7, 8 or 9) additional amino acid modifications relative to SEQ ID NO: 1. In exemplary embodiments, when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits a GIP percentage potency of at least 0.1% (e.g., at least 1%, at least 10%, at least 20%). In exemplary embodiments , the glucagon analog has less than 100-fold (e.g., less than 50-fold, less than 25-fold, less than 10-fold) selectivity for the human GLP-1 receptor versus the GIP receptor. In exemplary embodiments, the glucagon analog exhibits an EC50 at the GLP-1 receptor which is within 100-fold (e.g., within 50-fold, within 25-fold, within 10-fold) of its EC50 at the GIP receptor.

[0010] Throughout the application, all references to a particular amino acid position by number (e.g.,position 28) refer to the amino acid at that position in native glucagon (SEQ ID NO: 1) or the corresponding amino acid position in any analog thereof. For example, a reference herein to "position 28" would mean the corresponding position 27 for a glucagon analog in which the first amino acid of SEQ ID NO: 1 has been deleted. Similarly, a reference herein to "position 28" would mean the corresponding position 29 for a glucagon analog in which one amino acid has been added before the N-terminus of SEQ ID NO: 1.

[0011] In some embodiments, the GIP agonist peptides comprise an amino acid sequence of any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, and 90, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73,

75, 81, 82, 88, and 92. In some embodiments, the GIP agonist peptides comprise a structure based on a parent sequence comprising any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, and 90, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, and 92, but differs from the parent sequence at one or more positions, as further described herein.

[0012] The invention accordingly provides a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 28. Also provided is a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 37. The invention further provides a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 89. The invention furthermore provides a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 180. The invention moreover provides a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 31.

[0013] The invention provides a peptide comprising the sequence of SEQ ID NO: 184,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEQ ID NO: 184)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog;

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala or Gly;

X_{35} is Ala or a basic amino acid (optionally, Arg or Lys);

wherein, when X_{28} is an acidic amino acid, X_{35} is a basic amino acid;

wherein, when X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41, and

wherein the C-terminal amino acid of the peptide is amidated.

[0014] The invention also provides a peptide comprising the sequence of SEQ ID NO: 184 with up to 3 amino acid modifications relative to SEQ ID NO: 184, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

[0015] The invention additionally provides a peptide comprising the sequence of SEQ ID NO: 185,

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGAPPPS$
(SEQ ID NO: 185)

wherein:

X_2 is AIB;

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{16} is Glu, an alpha, alpha disubstituted amino acid, Lys or

X_{18} is Arg or Ala;

X_{20} is AIB or Gln;

X_{21} is Asp or Glu;

X_{27} is Leu, Nle, or Met;

X_{28} is Ala, Asp or Glu;

X_{29} is Gly of Thr;

and

wherein, when X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41, and

wherein the C-terminal amino acid of the peptide is amidated.

[0016] The invention further provides a peptide comprising the sequence of SEQ ID NO: 185 with up to 3 amino acid modifications relative to SEQ ID NO: 185, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

[0017] Furthermore provided is a peptide comprising the sequence of SEQ ID NO: 186:

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SEQ ID NO: 186)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog;

X_{10} is Tyr or an amino acid covalently attached to a C10 to C26 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is a negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala, Gly or Thr; and

wherein the peptide comprises an amino acid covalently attached to a C10 to C26 acyl or alkyl group, optionally, at position 10, and the C-terminal amino acid of the peptide is amidated.

[0018] Moreover provided is a peptide comprising the sequence ofSEQ ID NO: 186 with up to 3 amino acid modifications relative to SEQ ID NO: 186, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

[0019] The invention provides a peptide comprising the sequence of SEQ ID NO: 187:

$$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$$

(SEQ ID NO: 187)

wherein:

X_2 is AIB;

X_{10} is Tyr or an amino acid covalently attached to a C10 to C26 acyl or alkyl group;

X_{16} is Glu, alpha, alpha-disubstituted amino acid, or Lys;

X_{18} is Arg or Ala;

X_{20} is a negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala, Asp or Glu;

X_{29} is Gly or Thr; and

wherein the peptide comprises an amino acid covalently attached to a C12 to C18 acyl or alkyl group, optionally, at position10, and the C-terminal amino acid of the peptide is amidated.

[0020] The invention also provides SEQ ID NO: 187 with up to 3 amino acid modifications relative to SEQ ID NO: 187, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

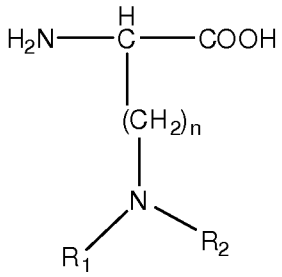
[0021] The invention further provides an analog of any one of SEQ ID NOs: 184, 185, 186, and 187, as described herein, but X_3 or the amino acid at position 3 is Gln or Gln analog or an amino acid which reduces glucagon activity, including, those described herein. In

exemplary embodiments, the amino acid which reduces glucagon activity is an acidic, basic, or hydrophobic amino acid (e.g., Glu, Orn, or Nle). Optionally, the amino acid at position 3 is Glu.

[0022] Furthermore provided herein is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, comprising:

(a) an amino acid comprising an imidazole side chain at position 1,

(b) at position 16, an amino acid of Formula IV:



[Formula IV],

wherein n is 1 to 7, wherein each of R1 and R2 is independently selected from the group consisting of H, C1-C18 alkyl, (C1-C18 alkyl)OH, (C1-C18 alkyl)NH2, (C1-C18 alkyl)SH, (C0-C4 alkyl)(C3-C6)cycloalkyl, (C0-C4 alkyl)(C2-C5 heterocyclic), (C0-C4 alkyl)(C6-C10 aryl)R7, and (C1-C4 alkyl)(C3-C9 heteroaryl), wherein R7 is H or OH, wherein optionally the side chain of the amino acid of Formula IV comprises a free amino group,

(c) an α,α-disubstituted amino acid at position 20,

(d) up to ten additional amino acid modifications relative to SEQ ID NO: 1,

wherein, when the analog lacks a hydrophilic moiety, the glucagon analog exhibits at least 0.1% activity of native GIP at the GIP receptor, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

[0023] Also provided herein are dimers and multimers comprising two or more GIP agonist peptides of the present disclosures. Conjugates comprising a GIP agonist peptide of the present disclosures and a conjugate moiety are additionally provided herein. In some aspects, the conjugate is a fusion polypeptide comprising the GIP agonist peptide of the present disclosures fused to a heterologous peptide. The present disclosures also provides kits comprising the GIP agonist peptides, dimers, multimers, or conjugates of the present disclosures (or a combination thereof).

[0024] Pharmaceutical compositions comprising any of the GIP agonist peptides, dimers, multimers, or conjugates of the present disclosures (or a combination thereof) and a pharmaceutically acceptable carrier, diluent, or excipient are further provided by the present disclosures. The pharmaceutical compositions are preferably sterile and suitable for parenteral administration. The pharmaceutical compositions are contemplated for use in methods of treating or preventing diabetes or obesity, or medical conditions associated with diabetes or obesity . Accordingly, in exemplary embodiments the present disclosure provides a method of reducing weight gain or inducing weight loss, a method of treating or preventing diabetes or obesity, and a method of inducing temporary paralysis of the intestinal tract. Further applications of the peptide analogs and pharmaceutical compositions comprising the same are provided in the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 represents a graph of the body weight (grams) of mice injected with 5 nmol/kg of: a peptide of SEQ ID NO: 28 (○), a peptide of SEQ ID NO: 37 (□), a peptide of SEQ ID NO: 38 ▽or a peptide of SEQ ID NO: 39 (◊), or injected with a vehicle control (△), as a function of time after first injection.

[0026] Figure 2 depicts a graph of the total change in body weight (%), as measured on Day 7) of mice injected with a vehicle control, or with one of the following peptides: a peptide of SEQ ID NO: 138 which peptide is directly attached to a C16 fatty acyl group on Lys at position 40, a peptide of SEQ ID NO: 143 which peptide is acylated via a gamma-Glu-gamma-Glu dipeptide spacer on an 4-amino-Phe residue at position 10, a peptide of SEQ ID NO: 144 which peptide is C16-succinoylated on a 4-aminoPhe at position 10, a peptide of SEQ ID NO: 139 which peptide is directlyattached to a C16 fatty acyl group on Lys at

position 40 (which is followed by a Gly at position 41), a peptide of SEQ ID NO: 140 which peptide is C16-succinoylated on a Lys at position 40 (which is followed by a Gly at position 41), a peptide of SEQ ID NO: 141 which peptide is C16-succinoylated on a Lys at position 40 via a beta-Ala spacer (which Lys is followed by a Gly at position 41), or a peptide of SEQ ID NO: 142 which peptide is directly attached to a C18 fatty acyl group on a Lys at position 40 (which is followed by a Gly at position 41).

[0027] Figures 3A-3C relate to acylated peptides comprising a succinoyl group. Figure 3A depicts the chemical structures of different acylated amino acid residues. From left to right, (i) a Lys residue directly acylated with a C16 fatty acid, (ii) a Lys residue acylated with a C16 fatty acid via a gamma-glutamic acid spacer, (iii) a Lys residue acylated with a C16 fatty acid via a (gamma-glutamic acid-gamma-glutamic acid) dipeptide spacer, (iv) a Lys residue acylated with a C16 succinoyl. Figure 3B represents structures of three exemplary succinic anhydrides. Figure 3C represents a synthesis scheme of SEQ ID NO: 156.

[0028] Figure 4 is a graph of the change in body weight (%) of mice injected with vehicle control (▽), or with a peptide of SEQ ID NO: 152 which peptide comprises a Lys residue at position 16 covalently attached to a C16 fatty acyl group via a gammGlu acid spacer (△), a peptide of SEQ ID NO: 28 which peptide comprises a Lys residue at position 10 covalently attached to a C16 fatty acyl group via a gammaGlu (open hexagon), a peptide of SEQ ID NO: 89 which peptide comprises a Lys at position 40 covalently attached to a C16 fatty acyl group via a gammaGlu-gammaGlu dipeptide spacer (□), a peptide of SEQ ID NO: 145 which peptide comprises a 4-aminoPhe at position 40 covalently attached to C16 fatty acyl group via a gammaGlu-gammaGlu dipeptide spacer (○), or a peptide of SEQ ID NO: 148 which peptide comprises an 4-aminoPhe at position 10 covalently attached to a C16 fatty acyl group via a gammaGlu spacer (◇).

[0029] Figures 5A-5F relate to dual acylated peptides. Figure 5A depicts three types of double acylated compounds: (1) one site comprising two acyl groups in a branched formation, (2) one site comprising two acyl groups in a linear formation, and (3) two sites each connected to a fatty acyl group via a gammaGlu spacer. Figure 5B represents a synthesis scheme of a peptide comprising two acyl groups of different sizes in a branched formation. Figure 5C represents a synthesis scheme of a peptide comprising two acyl groups

of same size in a branched formation. Figure 5D represents a synthesis scheme of a peptide comprising two acyl groups in a linear formation. Figure 5E represents a graph of the change in body weight (%) as measured on Day 7 of the study of mice that received a peptide injection or a vehicle control injection. Figure 5F represents a graph of the change in blood glucose levels (mg/dL) as measured on Day 7 of the study of mice that received a peptide injection or a vehicle control injection.

[0030] Figure 6 depicts S-palmityl alkylation of a Cys residue which is part of a peptide backbone.

[0031] Figure 7 depicts two types of S-palmityl alkylation of a Cys residue, wherein the Cys residue is part of an acylation spacer. In one type (left structure inside box), a Cys is attached to a peptide backbone Lys residue and the Cys is S-palmityl alkylated. In another type (right structure inside box), a Cys is part of a dipeptide spacer (gammaGlu-Cys) of which the gamma Glu is attached to a peptide backbone Lys and the Cys is S-palmityl alkylated.

[0032] Figure 8 represents a graph of change in body weight (%) as measured on Day 7 of mice injected with vehicle control or with one of nine different acylated peptides: SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 164, SEQ ID NO: 89, SEQ ID NO: 159, SEQ ID NO: 157, SEQ ID NO: 165, SEQ ID NO: 166.

[0033] Figures 9A and 9B relate to dimers comprising two peptides wherein at least one comprises an acyl group. Figure 9A depicts a structure of a homodimer, wherein each peptide comprises a Lys residue at the 40^{th} position. Each Lys residue is covalently attached via the epsilon NH2 group to a Cys residue. Each Cys residue is peptide bonded to a gammaGlu residue, which, in turn, is attached to a C16 fatty acyl group. The sulfur atoms of each Cys residue forms a disulfide bridge. Figure 9B depicts a structure of a homodimer that is linked via a thioether bond. Each peptide comprises a Lys residue at the 40^{th} position. The Lys of the top peptide is attached to a Cys residue, which is peptide bonded to a gammaGlu residue, which, in turn, is attached to a C16 fatty acyl group. The sulfur of the Cys residue is linked via a thioether bond to a chemical moiety which, in turn, is attached to the Lys residue of the bottom peptide.

[0034] Figure 10 represents a graph of the change in body weight (%) of mice injected with a vehicle control or with a peptide of SEQ ID NO: 28 (at 1 or 3 nmol/kg), a peptide of SEQ ID NO: 89 (at 1 or 3 nmol/kg), a peptide of SEQ ID NO: 138 (at 3 nmol/kg), or a peptide of SEQ ID NO: 171 (at 3 nmol/kg), as a function of time post-injection.

[0035] Figure 11 represents a graph of the insulin levels, as measured on Day 21, of mice injected with a vehicle control or with a peptide of SEQ ID NO: 28 (at 1 or 3 nmol/kg), a peptide of SEQ ID NO: 89 (at 1 or 3 nmol/kg), a peptide of SEQ ID NO: 138 (at 3 nmol/kg), or a peptide of SEQ ID NO: 171 (at 3 nmol/kg).

[0036] Figures 12A-12C represent a collection of schematics of acylated peptides each comprising an acylated amino acid covalently attached to an acyl group via a "miniPEG" spacer. Figure 12A represents the acylated peptide of SEQ ID NO: 157, Figure 12B represents the acylated peptide of SEQ ID NO: 158, and Figure 12C represents the acylated peptide of SEQ ID NO: 159.

DETAILED DESCRIPTION

[0037] The present disclosures provide GIP agonist peptides (e.g., analogs of native human glucagon (SEQ ID NO: 1) (also referred to as "glucagon analogs"), analogs of any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, and 90, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, and 92, analogs of any of SEQ ID NOs: 184-199) which exhibit agonist activity at the GIP receptor. As used herein, the term "peptide" encompasses a sequence of 2 or more amino acids and typically less than 100, or less than 50 amino acids. The amino acids can be naturally occurring or coded or non-naturally occurring or non-coded. Non-naturally occurring amino acids refer to amino acids that do not naturally occur in vivo but which, nevertheless, can be incorporated into the peptide structures described herein. "Non-coded" as used herein refer to an amino acid that is not an L-isomer of any of the following 20 amino acids: Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr. The term "GIP agonist peptide" refers to a compound that binds to and activates downstream signaling of the GIP receptor. The GIP agonist peptide may have any of the levels of activity at the GIP receptor (e.g., absolute activity level or relative activity level), selectivity for the GIP receptor, or GIP percentage potency, described herein. See, for example, the section

entitled *GIP Receptor Activity*. However, this term should not be construed as limiting the compound to having activity at only the GIP receptor. Rather, the GIP agonist peptides of the present disclosures may exhibit additional activities at other receptors, as further discussed herein. GIP agonist peptides, for example, may exhibit agonist activity at the GLP-1 receptor and/or glucagon receptor.

ACTIVITY OF THE PRESENTLY DISCLOSED PEPTIDES

[0038] *GIP Receptor Activity*

[0039] In some or any embodiments, the peptides of the present disclosures exhibit an EC50 for GIP receptor activation which is in the nanomolar range. In exemplary embodiments, the EC50 of the peptide at the GIP receptor is less than 1000 nM, less than 900 nM, less than 800 nM, less than 700 nM, less than 600 nM, less than 500 nM, less than 400 nM, less than 300 nM, less than 200 nM. In some embodiments, the EC50 of the peptide at the GIP receptor is about 100 nM or less, e.g., about 75 nM or less, about 50 nM or less, about 25 nM or less, about 10 nM or less, about 5 nM or less, or about 1 nM or less. In some or any embodiments, the peptide of the present disclosures exhibits an EC50 for GIP receptor activation which is in the picomolar range. In exemplary embodiments, the EC50 of the GIP agonist peptide at the GIP receptor is less than 1000 pM, less than 900 pM, less than 800 pM, less than 700 pM, less than 600 pM, less than 500 pM, less than 400 pM, less than 300 pM, less than 200 pM. In some embodiments, the EC50 of the peptide at the GIP receptor is about 100 pM or less, e.g., about 75 pM or less, about 50 pM or less, about 25 pM or less, about 10 pM or less, about 5 pM or less, or about 1 pM or less. The term "about" as used herein means greater or lesser than the value or range of values stated by 10 percent, but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

[0040] Suitable methods of determining the EC50 of a peptide for activation of a receptor, e.g., the GIP receptor, are known in the art. One exemplary *in vitro* assay, in which cAMP induction as represented by luciferase activity is measured in HEK293 cells over-expressing the GIP receptor, is described herein at Example 2.

[0041] In some or any embodiments, the peptides (e.g., glucagon analogs, analogs of any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, and 90, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, and 92) of the present disclosures exhibit enhanced activity at the GIP receptor, as compared to native human glucagon. Native glucagon (SEQ ID NO: 1) does not activate the GIP receptor; native glucagon exhibits essentially 0% (e.g., less than 0.001%, less than 0.0001%) activity of native GIP at the GIP receptor. A peptide's relative activity at the GIP receptor relative to native glucagon is calculated as the inverse ratio of (EC50 of the peptide of the present disclosures/EC50 of native glucagon) x 100%.

[0042] A peptide's relative activity at the GIP receptor compared to native GIP is calculated as the inverse ratio of (EC50 of the peptide of the present disclosures/EC50 of native GIP) x 100% (a value also referred to herein as "GIP percentage potency").

[0043] In some or any embodiments of the present disclosures, the peptides of the present disclosures exhibit GIP percentage potency that is at least or about 0.1%. In exemplary embodiments, the peptides exhibit at least or about 0.5%, at least or about 1%, at least or about 5%, at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, or at least or about 100% of the activity of native GIP at the GIP receptor.

[0044] In some embodiments of the present disclosures, the peptides of the present disclosures exhibit activity at the GIP receptor which is greater than that of native GIP. In exemplary embodiments, the GIP agonist peptide exhibits a GIP percentage potency of at least or about 125%, at least or about 150%, at least or about 175% at least or about 200%, at least or about 300%, at least or about 400%, at least or about 500%, at least or about 600%, at least or about 700%, at least or about 800%, at least or about 900%, or at least or about 1000%. In some embodiments, the GIP agonist peptides described herein exhibit a GIP percentage potency of no more than 1000% or no more than 10,000%.

[0045] In some aspects, the peptides of the present disclosures exhibit a GIP percentage potency within the range of about 0.001 to about 10,000 percent, or about 0.01 to about 10,000 percent, or about 0.1 to about 10,000 percent, or about 1 to about 10,000 percent, or

about 0.001 to about 5000 percent, or about 0.01 to about 5000 percent, or about 0.1 to about 5000 percent, or about 0.1 to about 1000 percent.

[0046] *Co-Agonists*

[0047] In some or any embodiments, the peptide of the present disclosures is a co-agonist peptide insofar as it activates the GIP receptor and a second receptor different from the GIP receptor.

[0048] *GIP/GLP-1 Co-Agonists*

[0049] By way of example, the peptide of the present disclosures in some aspects exhibits activity at both the GIP receptor and the GLP-1 receptor ("GLP-1/GIP receptor co-agonists"). In some aspects, the peptides exhibit activity at the GLP-1 and GIP receptors, but the glucagon activity has been significantly reduced or destroyed, e.g., by an amino acid modification at position 3. For example, substitution at this position with an acidic, basic, or a hydrophobic amino acid (glutamic acid, ornithine, norleucine) reduces glucagon activity. In some or any embodiments, the GIP agonist peptide is a peptide which exhibits about 10% or less (e.g., about 5% or less, or about 1% or less, or about 0.1% or less) of the activity of native glucagon at the glucagon receptor.

[0050] In some or any embodiments, the EC50 of the peptide of the present disclosures at the GIP receptor is within about 50-fold or less, about 40-fold or less, about 30-fold or less, about 20-fold or less, or about 10-fold or less, or about 5-fold or less different (higher or lower) from its EC50 at the GLP-1 receptor. For example, the EC50 at the GIP receptor can be 10-fold higher or 10-fold lower than the EC50 at the GLP-1 receptor. In some or any embodiments, the GIP percentage potency of the peptide of the present disclosures is less than or about 50-, 40-, 30-, 20-, 10-, or 5-fold different (higher or lower) from its GLP-1 percentage potency.

[0051] Accordingly, the peptide of the present disclosures has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor. As used herein, the term "selectivity" of a molecule for a first receptor relative to a second receptor refers to the following ratio: EC50 of the molecule at the second receptor divided by the EC50 of the molecule at the first receptor. For example, a molecule that has an EC50 of 1 nM at a first receptor and an EC50 of 100 nM at a second receptor has 100-fold selectivity for the first receptor relative to the

second receptor. In exemplary embodiments, the selectivity of the peptide of the present disclosures for the human GLP-1 receptor versus the GIP receptor is less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 10-fold, less than or about 5-fold).

[0052] In some or any embodiments, the peptides of the present disclosures exhibit enhanced activity at the GLP-1 receptor, as compared to native human glucagon. Native glucagon has about 1% of the activity of native GLP-1 at the GLP-1 receptor. A peptide's relative activity at the GLP-1 receptor relative to native glucagon is calculated as the inverse ratio of (EC50 of the peptide of the present disclosures/EC50 of native glucagon) x 100%.

[0053] A peptide's relative activity at the GLP-1 receptor compared to native GLP-1 is calculated as the inverse ratio of (EC50 of the peptide of the present disclosures/EC50 of native GLP-1) x 100% (a value referred to herein as "GLP-1 percentage potency").

[0054] In some or any embodiments of the present disclosures, the peptides of the present disclosures exhibit a GLP-1 percentage potency of at least or about 0.1%. In exemplary embodiments, the peptides exhibit a GLP-1 percentage potency of at least or about 0.5%, at least or about 1%, at least or about 5%, at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, or at least or about 100%.

[0055] In some embodiments of the present disclosures, the peptides of the present disclosures exhibit activity at the GLP-1 receptor which is greater than that of native GLP-1. In exemplary embodiments, the peptide of the present disclosures exhibits a GLP-1 percentage potency of at least or about 125%, at least or about 150%, at least or about 175% at least or about 200%, at least or about 300%, at least or about 400%, at least or about 500%, at least or about 600%, at least or about 700%, at least or about 800%, at least or about 900%, or at least or about 1000%. In some embodiments, the peptides of the present disclosures exhibit a GLP-1 percentage potency of no more than 1000% or no more than 10,000%.

[0056] *GIP/Glucagon Co-Agonists*

[0057] By way of another example, the peptide of the present disclosures in some aspects exhibits activity at both the GIP receptor and the glucagon receptor ("glucagon/GIP receptor

co-agonists"). In some embodiments, GLP-1 activity has been significantly reduced or destroyed, e.g., by an amino acid modification at position 7, e.g., substitution with Ile, a deletion of the amino acid(s) C-terminal to the amino acid at position 27 or 28, yielding a 27- or 28-amino acid peptide, or a combination thereof. In some embodiments, the peptide of the present disclosures is a peptide which exhibits about 10% or less (e.g., about 5% or less, or about 1% or less, or about 0.1% or less) of the activity of native GLP-1 at the GLP-1 receptor.

[0058] In some or any embodiments, the EC50 of the peptide of the present disclosures at the GIP receptor is within about 50-fold or less, about 40-fold or less, about 30-fold or less, about 20-fold or less, or about 10-fold or less, or about 5-fold or less different (higher or lower) from its EC50 at the glucagon receptor. In some or any embodiments, the GIP percentage potency of the peptide of the present disclosures is less than or about 50-, 40-, 30-, 20-, 10-, or 5-fold different (higher or lower) from its glucagon percentage potency.

[0059] In some embodiments, the peptides of the present disclosures exhibit enhanced activity at the glucagon receptor, as compared to native human glucagon. A peptide's relative activity at the glucagon receptor compared to native glucagon is calculated as the inverse ratio of (EC50 of the peptide of the present disclosures/EC50 of native glucagon) x 100% (a value referred to herein as "glucagon percentage potency").

[0060] In some embodiments of the present disclosures, the peptides of the present disclosures exhibit a glucagon percentage potency of at least or about 0.1%. In exemplary embodiments, the peptides exhibit a glucagon percentage potency of at least or about 0.5%, at least or about 1%, at least or about 5%, at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, or at least or about 100%.

[0061] In some embodiments of the present disclosures, the peptides of the present disclosures exhibit activity at the glucagon receptor which is greater than that of native glucagon. In exemplary embodiments, the peptide of the present disclosures exhibits a glucagon percentage potency of at least or about 125%, at least or about 150%, at least or about 175% at least or about 200%, at least or about 300%, at least or about 400%, at least or about 500%, at least or about 600%, at least or about 700%, at least or about 800%, at least or

about 900%, or at least or about 1000%. In some embodiments, the peptides of the present disclosures exhibit a glucagon percentage potency of no more than 1000% or no more than 10,000%.

[0062] *Triagonists*

[0063] In some embodiments, the peptides of the present disclosures exhibit activity at two or more receptors, other than the GIP receptor. Accordingly, the present disclosures provide in some aspects GIP triagonist peptides. In some aspects, the peptides of the present disclosures exhibit activity at each of the glucagon, GIP and GLP-1 receptors ("glucagon/GIP/GLP-1 tri-agonists").

[0064] In some embodiments, the EC50 of the peptide of the present disclosures at the GIP receptor is within 50-fold or less, 20-fold or less, or 10-fold or less different (higher or lower) than the EC50 of the peptide of the present disclosures at (a) the GLP-1 receptor, (b) the glucagon receptor, or both. In some embodiments, the EC50 of the peptide of the present disclosures at the GIP receptor is within about 40-fold, about 30-fold, about 20-fold different (higher or lower) from its EC50 at the GLP-1 receptor, and optionally within about 50-fold different from its EC50 at the glucagon receptor. In some embodiments, the GIP percentage potency of the peptide of the present disclosures is less than or about 50-fold, 20-fold or 10-fold different (higher or lower) from (a) its GLP-1 percentage potency, (b) its glucagon percentage potency, or both. In some embodiments, the GIP percentage potency of the peptide of the present disclosures is within about 40-fold, about 30-fold, about 20-fold different (higher or lower) from its GLP-1 percentage potency, and optionally within about 50-fold different from its its glucagon percentage potency. In some embodiments, the peptide of the present disclosures does not have at least 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor. In exemplary embodiments, the selectivity of the peptide of the present disclosures for the human GLP-1 receptor versus the GIP receptor is less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 10-fold, less than or about 5-fold).

[0065] *GIP Agonism in the Absence of GLP-1 agonism and glucagon agonism*

[0066] In some embodiments, the peptide of the present disclosures exhibits activity at only the GIP receptor, and not at any other receptor, e.g., GLP-1 receptor, glucagon receptor. In exemplary embodiments, the peptide of the present disclosures exhibits activity at the GIP receptor, and the glucagon and GLP-1 activity have been significantly reduced or destroyed, e.g., by amino acid modifications at positions 3 and 7. In some embodiments, the peptide of the present disclosures is a peptide which exhibits a GLP-1 percentage potency of about 10% or less (e.g., about 5% or less, or about 1% or less, or about 0.1% or less). In some embodiments, the peptide of the present disclosures is a peptide which exhibits a glucagon percentage potency of about 10% or less (e.g., about 5% or less, or about 1% or less, or about 0.1% or less).

[0067] *Activity of Conjugates*

[0068] In some or any embodiments, when the peptide of the present disclosures is conjugated to a heterologous moiety (e.g., a hydrophilic moiety), as further described herein, the peptide of the present disclosures exhibits a decreased activity (e.g., a lower percentage potency or higher EC50) than when the peptide of the present disclosures is in a free or unconjugated form. Thus, it is contemplated that when any of the foregoing absolute activity levels (e.g. GIP percentage potency, GLP-1 percentage potency or glucagon percentage potency, but not relative ratios) is applied to a peptide in conjugated form, e.g. pegylated, such absolute activity levels are reduced by about 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, or 100-fold, and that such fold reduced activity levels are contemplated within the scope of the disclosure. Conversely, when unconjugated, the peptide of the present disclosures exhibits a GIP percentage potency that is about 10-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 100-fold or more higher than the potency of the peptide of the present disclosures when conjugated to a heterologous moiety.

STRUCTURE OF THE PRESENTLY DISCLOSED PEPTIDES

[0069] *Glucagon Analogs*

[0070] In some embodiments, the peptides of the present disclosures are structurally similar to native human glucagon (SEQ ID NO: 1), e.g., is an analog of native human glucagon (also referred to herein as "glucagon analog" or "peptide analog of glucagon"). As

used herein, the terms "glucagon analog" and "peptide analog of glucagon," and the like, refer to peptides that are structurally similar to native human glucagon and these terms do not necessarily imply that the peptides activate the glucagon receptor.

[0071] In some or any embodiments, the peptide of the present disclosures is an analog of native human glucagon (SEQ ID NO: 1) comprising an amino acid sequence based on the amino acid sequence of SEQ ID NO: 1 but differs from SEQ ID NO: 1 inasmuch as the amino acid sequence of the glucagon analog comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and in some instances, 16 or more (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, etc.), specified or optional amino acid modifications. In some or any embodiments, the peptide of the present disclosures comprises a total of 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 additional amino acid modifications (e.g., in addition to the specified amino acid modifications), relative to the native human glucagon sequence (SEQ ID NO: 1). For example, with regard to an analog of glucagon (SEQ ID NO: 1) comprising (a) an amino acid comprising an imidazole side chain at position 1, (b) an DPP-IV protective amino acid at position 2, (c) an acylated amino acid or alkylated amino acid at any of positions 9, 10, 12, 16, 20, or 37-43, (d) an alpha helix stabilizing amino acid at one or more of positions 16, 17, 18, 19, 20, and 21, and (e) up to ten additional amino acid modifications relative to SEQ ID NO: 1, the present disclosures provides an analog of glucagon comprising (a)-(d) with up to 10 additional amino acid modifications in addition to the amino acid modifications specified in (a)-(d). In some or any embodiments, the modifications are any of those described herein, e.g., acylation, alkylation, pegylation, truncation at C-terminus, substitution of the amino acid at one or more of positions 1, 2, 3, 7, 10, 12, 15, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, and 29.

[0072] As used herein an "amino acid modification" refers to (i) a substitution of an amino acid of SEQ ID NO: 1 with a different amino acid (naturally-occurring or coded or non-coded or non-naturally-occurring amino acid), (ii) an addition of an amino acid (naturally-occurring or coded or non-coded or non-naturally-occurring amino acid), to SEQ ID NO: 1 or (iii) a deletion of one or more amino acids of SEQ ID NO: 1.

[0073] In some or any embodiments, the amino acid substitution is a conservative amino acid substitution, e.g., a conservative substitution of the amino acid at one or more of positions 2, 5, 7, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 27, 28 or 29. As used herein,

the term "conservative amino acid substitution" is defined herein as the substitution of one amino acid with another amino acid having similar properties, e.g., size, charge, hydrophobicity, hydrophilicity, and/or aromaticity, and includes exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues:

Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides and esters:

Asp, Asn, Glu, Gln, cysteic acid and homocysteic acid;

III. Polar, positively charged residues:

His, Arg, Lys; Ornithine (Orn)

IV. Large, aliphatic, nonpolar residues:

Met, Leu, Ile, Val, Cys, Norleucine (Nle), homocysteine

V. Large, aromatic residues:

Phe, Tyr, Trp, acetyl phenylalanine

[0074] In alternative embodiments, the amino acid substitution is not a conservative amino acid substitution, e.g., is a non-conservative amino acid substitution.

[0075] As used herein the term "charged amino acid" refers to an amino acid that comprises a side chain that is negative-charged (i.e., de-protonated) or positive-charged (i.e., protonated) in aqueous solution at physiological pH. For example negative-charged amino acids include aspartic acid, glutamic acid, cysteic acid, homocysteic acid, and homoglutamic acid, whereas positive-charged amino acids include arginine, lysine and histidine. Charged amino acids include the charged amino acids among the 20 coded amino acids, as well as atypical or non-naturally occurring or non-coded amino acids. As used herein the term "acidic amino acid" refers to an amino acid that comprises a second acidic moiety (other than the alpha carboxylic acid of the amino acid), including for example, a side chain carboxylic acid or sulfonic acid group.

[0076] In some embodiments, the peptide of the present disclosures comprises an amino acid sequence which has at least 25% sequence identity to the amino acid sequence of native human glucagon (SEQ ID NO: 1). In some embodiments, the peptide of the present

disclosures comprises an amino acid sequence which is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90% or has greater than 90% sequence identity to SEQ ID NO: 1. In some embodiments, the amino acid sequence of the presently disclosed peptide which has the above-referenced % sequence identity is the full-length amino acid sequence of the presently disclosed peptide. In some embodiments, the amino acid sequence of the peptide of the present disclosures which has the above-referenced % sequence identity is only a portion of the amino acid sequence of the presently disclosed peptide. In some embodiments, the presently disclosed peptide comprises an amino acid sequence which has about A% or greater sequence identity to a reference amino acid sequence of at least 5 contiguous amino acids (e.g., at least 6, at least 7, at least 8, at least 9, at least 10 amino acids) of SEQ ID NO: 1, wherein the reference amino acid sequence begins with the amino acid at position C of SEQ ID NO: 1 and ends with the amino acid at position D of SEQ ID NO: 1, wherein A is 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99; C is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 and D is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29. Any and all possible combinations of the foregoing parameters are envisioned, including but not limited to, e.g., wherein A is 90% and C and D are 1 and 27, or 6 and 27, or 8 and 27, or 10 and 27, or 12 and 27, or 16 and 27.

[0077] The analogs of native human glucagon (SEQ ID NO: 1) described herein may comprise a peptide backbone of any number of amino acids, i.e., can be of any peptide length. In some embodiments, the peptides described herein are the same length as SEQ ID NO: 1, i.e., are 29 amino acids in length. In some embodiments, the presently disclosed peptide is longer than 29 amino acids in length, e.g., the presently disclosed peptide comprises a C-terminal extension of 1-21 amino acids, as further described herein. Accordingly, the peptide of the present disclosures in some embodiments, is 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length. In some embodiments, the presently disclosed peptide is up to 50 amino acids in length. In some embodiments, the presently disclosed peptide is longer than 29 amino acids in length (e.g., greater than 50 amino acids, (e.g., at least or about 60, at least or about 70, at least or about 80, at least or about 90, at least or about 100, at least or about 150, at least or about 200, at least or about 250, at least or about 300, at least or about 350, at least or about 400, at

least or about 450, at least or about 500 amino acids in length) due to fusion with another peptide. In other embodiments, the presently disclosed peptide is less than 29 amino acids in length, e.g., 28, 27, 26, 25, 24, 23, amino acids.

[0078] In accordance with the foregoing, in some aspects, the peptide of the present disclosures is an analog of native human glucagon (SEQ ID NO: 1) comprising an amino acid sequence based on SEQ ID NO: 1, which sequence comprises one or more amino acid modifications which affect GIP activity, glucagon activity, and/or GLP-1 activity, enhance stability, e.g., by reducing degradation of the peptide (e.g., by improving resistance to DPP-IV proteases), enhance solubility, increase half-life, delay the onset of action, extend the duration of action at the GIP, glucagon, or GLP-1 receptor, or a combination of any of the foregoing. Such amino acid modifications, in addition to other modifications, are further described below, and any of these modifications can be applied individually or in combination.

AMINO ACIDS COMPRISING A NON-NATIVE ACYL GROUP

[0079] In accordance with some or any embodiments, the GIP agonist peptides which are analogs of glucagon (SEQ ID NO: 1) comprise an amino acid comprising a non-native acyl group (referred to herein as an "acylated amino acid", regardless of how it is prepared, e.g., by incorporation of a previously-acylated amino acid into the peptide, or acylation of the peptide after synthesis). In some or any aspects, the acylated amino acid is located at any of positions 9, 10, 12, 13, 14, 16, 17, 20, 37, 38, 39, 40, 41, 42, or 43 of the glucagon analog. In exemplary aspects, the acylated amino acid is located at any of positions 9, 10, 12, 16, 20, or 40 of the glucagon analog or at any of positions 10, 13, 14, 16, 17, or 40 of the glucagon analog. In exemplary aspects, the acylated amino acid is located at any one or more of positions 10, 14, and 40. In exemplary aspects, the acylated amino acid is located at any of positions 10, 12, or 16 of the peptide analog.

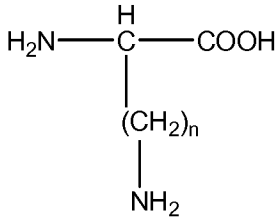
[0080] The acylated amino acid in some embodiments causes the GIP agonist peptide to have one or more of (i) a prolonged half-life in circulation, (ii) a delayed onset of action, (iii) an extended duration of action, (iv) an improved resistance to proteases, such as DPP-IV, and (v) increased potency at any one or more of the GIP receptor, GLP-1 receptor, and glucagon receptor.

[0081] *Direct Acylation*

[0082] In some embodiments, the acyl group is directly linked to an amino acid of the GIP agonist peptide. In accordance with one embodiment, the GIP agonist peptide comprises an acyl group which is attached to the peptide via an ester, thioester, or amide linkage.

[0083] In specific aspects, the GIP agonist peptide comprises an acyl group upon direct acylation of an amine, hydroxyl, or thiol of a side chain of an amino acid of the GIP agonist peptide. In some embodiments, acylation is at position 9, 10, 12, 13, 14, 16, 17, 20, or 40 (e.g., at any one of positions 10, 14, and 40) of the GIP agonist peptide. In this regard, the GIP agonist peptide comprises the amino acid sequence of SEQ ID NO: 1, or a modified amino acid sequence thereof comprising one or more of the amino acid modifications described herein, wherein at least one of the amino acids at positions 9, 10, 12, 13, 14, 16, 17, 20, and 40 (e.g., at any one of positions 10, 14, and 40) of the GIP agonist peptide is an amino acid comprising a side chain amine, hydroxyl, or thiol.

[0084] In some embodiments, the amino acid comprising a side chain amine is an amino acid of Formula I:

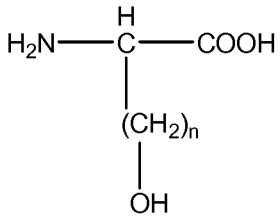


wherein n = 1 to 4

[Formula I]

In some embodiments, the amino acid of Formula I, is the amino acid wherein n is 4 (Lys) or n is 3 (Orn). In some embodiments, the amino acid comprising a side chain amine is an aromatic amino acid comprising a side chain amine. In exemplary aspects, the aromatic amino acid comprising a side chain amine is 4-amino-phenylalanine (4-aminoPhe), p-amino phenylglycine, p-amino homophenylalanine, or 3-amino tyrosine. In exemplary aspects, the aromatic amino acid comprising a side chain amine is 4-amino-Phe.

[0085] In other embodiments, the amino acid comprising a side chain hydroxyl is an amino acid of Formula II:

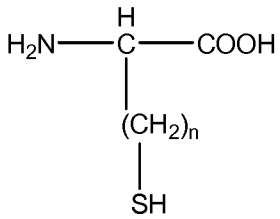


wherein n = 1 to 4

[Formula II]

[0086] In some exemplary embodiments, the amino acid of Formula II is the amino acid wherein n is 1 (Ser). In exemplary aspects, the amino acid of Formula II is the amino acid wherein n is 2 (homoserine). In similar exemplary embodiments, the amino acid comprising a side chain hydroxyl is a Thr or homothreonine. In similar exemplary embodiments, the amino acid comprising a side chain hydroxyl is an aromatic amino acid comprising a side chain hydroxyl. In exemplary aspects, the aromatic amino acid comprising a side chain hydroxyl is tyrosine, homotyrosine, methyl-tyrosine, or 3-amino tyrosine.

[0087] In yet other embodiments, the amino acid comprising a side chain thiol is an amino acid of Formula III:



wherein n = 1 to 4

[Formula III]

In some exemplary embodiments, the amino acid of Formula III is the amino acid wherein n is 1 (Cys).

[0088] In yet other embodiments, the amino acid comprising a side chain amine, hydroxyl, or thiol is a disubstituted amino acid comprising the same structure of Formula I, Formula II, or Formula III, except that the hydrogen bonded to the alpha carbon of the amino acid of Formula I, Formula II, or Formula III is replaced with a second side chain.

[0089] *Acylation Spacers*

[0090] In alternative embodiments, the acyl group is linked via a spacer to an amino acid of the GIP agonist peptide, wherein the spacer is positioned between the amino acid of the GIP agonist peptide and the acyl group. In some embodiments, the GIP agonist peptide

comprises a spacer between the peptide and the acyl group. In some embodiments, the GIP agonist peptide is covalently bound to the spacer, which is covalently bound to the acyl group.

[0091] In some embodiments, the spacer is an amino acid comprising a side chain amine, hydroxyl, or thiol, or a dipeptide or tripeptide comprising an amino acid comprising a side chain amine, hydroxyl, or thiol. The amino acid to which the spacer is attached can be any amino acid (e.g., a singly or doubly α-substituted amino acid) comprising a moiety which permits linkage to the spacer. For example, an amino acid comprising a side chain NH_2, –OH, or –COOH (e.g., Lys, Orn, Ser, Asp, or Glu) is suitable. In this respect, the GIP agonist peptide in some aspects comprises the amino acid sequence of SEQ ID NO: 1, or a modified amino acid sequence thereof comprising one or more of the amino acid modifications described herein, wherein at least one of the amino acids at positions 9, 10, 12, 13, 14, 16, 17, 20, and 37-43 (e.g., position 10, 14, or 40) is an amino acid comprising a side chain amine, hydroxyl, or carboxylate.

[0092] In some embodiments, the spacer is an amino acid comprising a side chain amine, hydroxyl, or thiol, or a dipeptide or tripeptide comprising an amino acid comprising a side chain amine, hydroxyl, or thiol.

[0093] When the acyl group is attached through an amine group of a spacer, the acyl group in some aspects is attached through the alpha amine or through a side chain amine of the spacer amino acid. In the instance in which the acyl group is attached via an alpha amine, the amino acid of the spacer can be any amino acid. For example, the amino acid of the spacer can be a hydrophobic amino acid, e.g., Gly, Ala, Val, Leu, Ile, Trp, Met, Phe, Tyr, 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid. Alternatively, in some aspects, the amino acid of the spacer is an acidic residue, e.g., Asp, Glu, homoglutamic acid, homocysteic acid, cysteic acid, gamma-glutamic acid.

[0094] In the instance in which the acyl group is attached through a side chain amine of the amino acid spacer, the spacer is an amino acid comprising a side chain amine, e.g., an amino acid of Formula I (e.g., Lys or Orn). In this instance, it is possible for both the alpha amine and the side chain amine of the amino acid of the spacer to be attached to an acyl group, such that the GIP agonist peptide is diacylated. Embodiments of the invention include such

diacylated molecules. In some embodiments, the acyl group is attached to a 4-amino-Phe, p-amino phenylglycine, p-amino homophenylalanine, or 3-amino tyrosine.

[0095] When the acyl group is attached through a hydroxyl group of a spacer, the amino acid or one of the amino acids of the dipeptide or tripeptide can be an amino acid of Formula II. In a specific exemplary embodiment, the amino acid is Ser. In similar exemplary embodiments, the acyl group is attached to a Thr or homothreonine. In similar exemplary embodiments, the acyl group is attached via the hydroxyl of an aromatic amino acid comprising a side chain hydroxyl, e.g., tyrosine, homotyrosine, methyl-tyrosine, or 3-amino tyrosine.

[0096] When the acyl group is attached through a thiol group of a spacer, the amino acid or one of the amino acids of the dipeptide or tripeptide can be an amino acid of Formula III. In a specific exemplary embodiment, the amino acid is Cys.

[0097] In some embodiments, the spacer is a hydrophilic bifunctional spacer. In exemplary embodiments, the hydrophilic bifunctional spacer comprises two or more reactive groups, e.g., an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In exemplary embodiments, the hydrophilic bifunctional spacer comprises a hydroxyl group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises an amine group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises a thiol group and a carboxylate. In a specific embodiment, the spacer comprises an amino poly(alkyloxy)carboxylate. In this regard, the spacer can comprise, for example, $NH_2(CH_2CH_2O)_n(CH_2)_mCOOH$, wherein m is any integer from 1 to 6 and n is any integer from 2 to 12, such as, e.g., 8-amino-3,6-dioxaoctanoic acid, which is commercially available from Peptides International, Inc. (Louisville, KY).

[0098] In exemplary embodiments, the spacer comprises a small polyethylene glycol moiety (PEG) comprising a structure $[-O-CH_2-CH_2-]_n$, wherein n is an integer between 2 and 16, (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Such small PEGs are referred to herein as a "miniPEG." In exemplary aspects, the miniPEG is a functionalized miniPEG comprising one or more functional groups. Suitable functional groups include, but are not limited to, an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In exemplary aspects, the miniPEG is a miniPEG acid comprising a structure $\{[-O-CH_2-CH_2-]_n-COO-\}$, wherein n is defined as above. In exemplary aspects, the miniPEG is an amido

miniPEG comprising a structure {-N- CH_2- CH_2-[-O-CH_2-CH_2-]$_n$}, wherein n is defined as above. In exemplary aspects, the miniPEG is an amido miniPEG acid comprising a structure {-N- CH_2- CH_2-[-O-CH_2-CH_2-]$_n$-COO-}, wherein n is defined as above. Suitable reagents for use in acylating an amino acid with a miniPEG are commercially available from vendors, such as Peptides International (Louisville, KY). Also, suitable techniques for acylating an amino acid with a miniPEG are described herein (see Example 1).

[0099] In some embodiments, the spacer is a hydrophobic bifunctional spacer. Hydrophobic bifunctional spacers are known in the art. See, e.g., *Bioconjugate Techniques*, G. T. Hermanson (Academic Press, San Diego, CA, 1996), which is incorporated by reference in its entirety. In exemplary embodiments, the hydrophobic bifunctional spacer comprises two or more reactive groups, e.g., an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In exemplary embodiments, the hydrophobic bifunctional spacer comprises a hydroxyl group and a carboxylate. In other embodiments, the hydrophobic bifunctional spacer comprises an amine group and a carboxylate. In other embodiments, the hydrophobic bifunctional spacer comprises a thiol group and a carboxylate. Suitable hydrophobic bifunctional spacers comprising a carboxylate and a hydroxyl group or a thiol group are known in the art and include, for example, 8-hydroxyoctanoic acid and 8-mercaptooctanoic acid.

[00100] In some embodiments, the bifunctional spacer is not a dicarboxylic acid comprising an unbranched, methylene of 1-7 carbon atoms between the carboxylate groups. In some embodiments, the bifunctional spacer is a dicarboxylic acid comprising an unbranched, methylene of 1-7 carbon atoms between the carboxylate groups.

[00101] The spacer (e.g., amino acid, dipeptide, tripeptide, hydrophilic bifunctional spacer, or hydrophobic bifunctional spacer) in specific embodiments is 3 to 10 atoms (e.g., 6 to 10 atoms, (e.g., 6, 7, 8, 9, or 10 atoms) in length. In more specific embodiments, the spacer is about 3 to 10 atoms (e.g., 6 to 10 atoms) in length and the acyl group is a C12 to C18 fatty acyl group, e.g., C14 fatty acyl group, C16 fatty acyl group, such that the total length of the spacer and acyl group is 14 to 28 atoms, e.g., about 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 atoms. In some embodiments, the length of the spacer and acyl group is 17 to 28 (e.g., 19 to 26, 19 to 21) atoms.

[00102] In accordance with some or any of the foregoing embodiments, the bifunctional spacer can be a synthetic or naturally occurring amino acid (including, but not limited to, any of those described herein) comprising an amino acid backbone that is 3 to 10 atoms in length (e.g., 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid). Alternatively, the spacer can be a dipeptide or tripeptide spacer having a peptide backbone that is 3 to 10 atoms (e.g., 6 to 10 atoms) in length. Each amino acid of the dipeptide or tripeptide spacer can be the same as or different from the other amino acid(s) of the dipeptide or tripeptide and can be independently selected from the group consisting of: naturally-occurring or coded and/or non-coded or non-naturally occurring amino acids, including, for example, any of the D or L isomers of the naturally-occurring amino acids (Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, Tyr), or any D or L isomers of the non-naturally occurring or non-coded amino acids selected from the group consisting of: β-alanine (β-Ala), N-α-methyl-alanine (Me-Ala), aminobutyric acid (Abu), γ-aminobutyric acid (γ-Abu), aminohexanoic acid (ε-Ahx), aminoisobutyric acid (Aib), aminomethylpyrrole carboxylic acid, aminopiperidinecarboxylic acid, aminoserine (Ams), aminotetrahydropyran-4-carboxylic acid, arginine N-methoxy-N-methyl amide, β-aspartic acid (β-Asp), azetidine carboxylic acid, 3-(2-benzothiazolyl)alanine, α-*tert*-butylglycine, 2-amino-5-ureido-n-valeric acid (citrulline, Cit), β-Cyclohexylalanine (Cha), acetamidomethyl-cysteine, diaminobutanoic acid (Dab), diaminopropionic acid (Dpr), dihydroxyphenylalanine (DOPA), dimethylthiazolidine (DMTA), γ-Glutamic acid (γ-Glu), homoserine (Hse), hydroxyproline (Hyp), isoleucine N-methoxy-N-methyl amide, methyl-isoleucine (MeIle), isonipecotic acid (Isn), methyl-leucine (MeLeu), methyl-lysine, dimethyl-lysine, trimethyl-lysine, methanoproline, methionine-sulfoxide (Met(O)), methionine-sulfone ($Met(O_2)$), norleucine (Nle), methyl-norleucine (Me-Nle), norvaline (Nva), ornithine (Orn), para-aminobenzoic acid (PABA), penicillamine (Pen), methylphenylalanine (MePhe), 4-Chlorophenylalanine (Phe(4-Cl)), 4-fluorophenylalanine (Phe(4-F)), 4-nitrophenylalanine ($Phe(4\text{-}NO_2)$), 4-cyanophenylalanine ((Phe(4-CN)), phenylglycine (Phg), piperidinylalanine, piperidinylglycine, 3,4-dehydroproline, pyrrolidinylalanine, sarcosine (Sar), selenocysteine (Sec), O-Benzyl-phosphoserine, 4-amino-3-hydroxy-6-methylheptanoic acid (Sta), 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), 4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA), 1,2,3,4,-tetrahydro-isoquinoline-3-carboxylic acid (Tic),

tetrahydropyranglycine, thienylalanine (Thi) , O-benzyl-phosphotyrosine, O-Phosphotyrosine, methoxytyrosine, ethoxytyrosine, O-(bis-dimethylamino-phosphono)-tyrosine, tyrosine sulfate tetrabutylamine, methyl-valine (MeVal), and alkylated 3-mercaptopropionic acid. In exemplary aspects, the spacer is a Cys residue or a Lys residue.

[00103] In some embodiments, the spacer comprises an overall negative charge, e.g., comprises one or two negative- charged amino acids. In some embodiments, the dipeptide is not any of the dipeptides of general structure A-B, wherein A is selected from the group consisting of Gly, Gln, Ala, Arg, Asp, Asn, Ile, Leu, Val, Phe, and Pro, wherein B is selected from the group consisting of Lys, His, Trp. In some embodiments, the amino acids of the dipeptide spacer are selected from the group consisting of: Ala, β-Ala, Leu, Pro, γ-aminobutyric acid, Glu and γ-Glu.

[00104] In some exemplary embodiments, the GIP agonist peptide comprises an acyl group upon acylation of an amine, hydroxyl, or thiol of a spacer, which spacer is attached to a side chain of an amino acid at position 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40), or at the C-terminal amino acid of the GIP agonist peptide.

[00105] In yet more specific embodiments, the acyl group is attached to the amino acid at any of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40) of the peptide analog and the length of the spacer and acyl group is 14 to 28 atoms. The amino acid at any of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40), in some aspects, is an amino acid of Formula I, e.g., Lys, or a disubstituted amino acid related to Formula I. In more specific embodiments, the peptide analog lacks an intramolecular bridge, e.g., a covalent intramolecular bridge. The glucagon analog, for example, can be a glucagon analog comprising one or more alpha, alpha-disubstituted amino acids, e.g., AIB, for stabilizing the alpha helix of the analog.

[00106] *Acyl Groups*

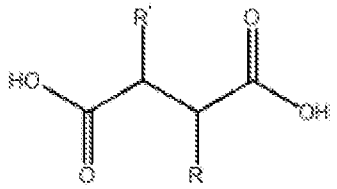
[00107] The acyl group of the acylated amino acid can be of any size, e.g., any length carbon chain, and can be linear or branched. In some specific embodiments, the acyl group is a C4 to C30 fatty acid. For example, the acyl group can be any of a C4 fatty acid, C6 fatty acid, C8 fatty acid, C10 fatty acid, C12 fatty acid, C14 fatty acid, C16 fatty acid, C18 fatty acid, C20 fatty acid, C22 fatty acid, C24 fatty acid, C26 fatty acid, C28 fatty acid, or a C30

fatty acid. In some embodiments, the acyl group is a C8 to C20 fatty acid, e.g., a C14 fatty acid or a C16 fatty acid.

[00108] In an alternative embodiment, the acyl group is a bile acid. The bile acid can be any suitable bile acid, including, but not limited to, cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, taurocholic acid, glycocholic acid, and cholesterol acid.

[00109] In exemplary embodiments, the acyl group is a succinic acid or a succinic acid derivative. By "succinic acid derivative" as used herein is meant a compound comprising a substituted succinic acid or a substituted cyclic succinic acid (i.e., succinic anhydride) or a substituted expanded ring succinic anhydride, (i.e. a 6-8 membered ring comprising the –C(O)-O-C(O)- moiety and 3 to 5 additional carbons), wherein the substituted succinic acid, substituted cyclic succinic acid (i.e., succinic anhydride), or substituted expanded ring succinic anhydride is substituted with one or more alkyl chains or one or more functionalized carbon chains.

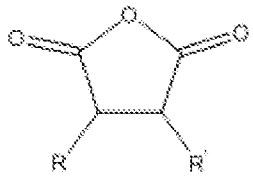
[00110] In exemplary aspects, the succinic acid derivative comprises a structure of Formula V:



[Formula V]

wherein each of R and R' is independently H, a linear or branched C4-C30 carbon chain, or a linear or branched C4-C30 functionalized carbon chain. In exemplary embodiments, R and/or R' is a carbon chain comprising a C4 to C30 alkyl chain. For example, the alkyl group can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl. In exemplary aspects, the functionalized carbon chain comprises a functional group, including, but not limited, carboxyl, sulfhydryl, amine, ketyl, sulfoxyl or amido.

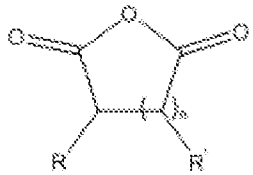
[00111] In exemplary aspects, the succinic acid derivative comprises a succinic anhydride comprising a structure of Formula VI:



[Formula VI]

wherein each of R and R' is independently H, a linear or branched C4-C30 carbon chain, or a linear or branched C4-C30 functionalized carbon chain. In exemplary embodiments, R and/or R' is a carbon chain comprising a C4 to C30 alkyl chain. For example, the alkyl group can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl. In exemplary aspects, the functionalized carbon chain comprises a functional group, including, but not limited, carboxyl, sulfhydryl, amine, ketyl, sulfoxyl or amido.

[00112] In exemplary aspects, the succinic acid derivative is a succinic anhydride derivative, including those of Formula VII:



[Formula VII]

wherein n is 1-4, and

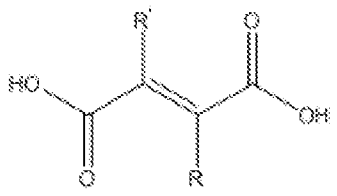
wherein each of R and R' is independently H, a linear or branched C4-C30 carbon chain, or a linear or branched C4-C30 functionalized carbon chain. In exemplary embodiments, R and/or R' is a carbon chain comprising a C4 to C30 alkyl chain. For example, the alkyl group can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl. In exemplary aspects, the functionalized carbon chain comprises a functional group, including, but not limited, carboxyl, sulfhydryl, amine, ketyl, sulfoxyl or amido.

[00113] When only one of R and R' of Formulae V-VI is H, the acylated amino acid is referred to as "Cx Succinoyl." As used herein, the term "Cx Succinoyl," wherein x is an integer, refers to a structure wherein R is an alkyl chain of y carbons and y = x-1, and y does

not include the carbons of succinoyl moiety. For example a structure of Formula VI wherein R is a C15 alkyl group and R' is a H is referred to as C16 Succinoyl. For example, Figure 3A depicts a C16 Succinoyl Lys, wherein a Lys has been succinoylated at the amine. When neither R nor R' of Formulae V-VI is H, then the acylated amino acid is referred to as "Cx,Cx' Succinoyl." As used herein, the term "Cx,Cx' Succinoyl," wherein x and x' are integers, refers to a structure wherein R is an alkyl chain of y carbons and R' is an alkyl chain of y' carbons, and y' = x'-1. For example, a structure of Formula VI wherein R is a C15 alkyl group and R' is a C13 alkyl group is referred to as C16,C14 succinoyl. When the succinic acid derivative is a substituted expanded ring succinic anhydride and neither R nor R' of Formula VII is H, then the acylated amino acid is referred to as "Cx,Cx'-n-Succinoyl." As used herein, the term "Cx,Cx'-n-Succinoyl," wherein x, x', and n are integers, refers to a structure wherein R is an alkyl chain of y carbons, R' is an alkyl chain of y' carbons, and the succinic anhydride ring is extend by n carbons. For example, a structure of Formula VII wherein R and R' are C15 alkyl groups and n = 2 is referred to as C16,C16-2-Succinoyl.

[00114] In exemplary embodiments, the acyl group is a maleic acid or a maleic acid derivative. By "maleic acid derivative" as used herein is meant a compound comprising a substituted maleic acid or a substituted cyclic maleic acid (i.e., maleic anhydride) or a substituted expanded ring maleic anhydride, (i.e. a 6-8 membered ring comprising the –C(O)-O-C(O)- moiety and 3 to 5 additional carbons), wherein the substituted maleic acid, substituted cyclic maleic acid (i.e., maleic anhydride), or substituted expanded ring maleic anhydride is substituted with one or more alkyl chains or one or more functionalized carbon chains.

[00115] In exemplary aspects, the maleic acid derivative comprises a structure of Formula VIII:

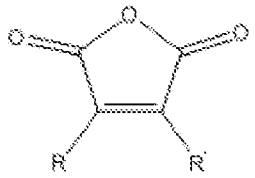


[Formula VIII]

wherein each of R and R' is independently H, a linear or branched C4-C30 carbon chain, or a linear or branched C4-C30 functionalized carbon chain. In exemplary embodiments, R and/or R' is a carbon chain comprising a C4 to C30 alkyl chain. For example, the alkyl group

can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl. In exemplary aspects, the functionalized carbon chain comprises a functional group, including, but not limited, carboxyl, sulfhydryl, amine, ketyl, sulfoxyl or amido.

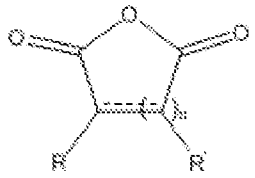
[00116] In exemplary aspects, the maleic acid derivative comprises a maleic anhydride comprising a structure of Formula IX:



[Formula IX]

wherein each of R and R' is independently H, a linear or branched C4-C30 carbon chain, or a linear or branched C4-C30 functionalized carbon chain. In exemplary embodiments, R and/or R' is a carbon chain comprising a C4 to C30 alkyl chain. For example, the alkyl group can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl. In exemplary aspects, the functionalized carbon chain comprises a functional group, including, but not limited, carboxyl, sulfhydryl, amine, ketyl, sulfoxyl or amido.

[00117] In exemplary aspects, the maleic acid derivative is a maleic anhydride derivative, including those of Formula X:



[Formula X]

wherein n is 1-4, there is at least one C=C double bond between two non-carbonyl carbons, and

wherein each of R and R' is independently H, a linear or branched C4-C30 carbon chain, or a linear or branched C4-C30 functionalized carbon chain. In exemplary embodiments, R and/or R' is a carbon chain comprising a C4 to C30 alkyl chain. For example, the alkyl group

can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl. In exemplary aspects, the functionalized carbon chain comprises a functional group, including, but not limited, carboxyl, sulfhydryl, amine, ketyl, sulfoxyl or amido.

[00118] When only one of R and R' of Formulae VIII-IX is H, the acylated amino acid is referred to as "Cx Maleoyl." As used herein, the term "Cx Maleoyl," wherein x is an integer, refers to a structure wherein R is an alkyl chain of y carbons and y = x-1, and y does not include the carbons of maleoyl moiety. For example a structure of Formula IX wherein R is a C15 alkyl group and R' is a H is referred to as C16 Maleoyl. When neither R nor R' of Formulae VIII-IX is H, then the acylated amino acid is referred to as "Cx,Cx' Maleoyl." As used herein, the term "Cx,Cx' Maleoyl," wherein x and x' are integers, refers to a structure wherein R is an alkyl chain of y carbons and R' is an alkyl chain of y' carbons and y' = x'-1. For example, a structure of Formula IX wherein R is a C15 alkyl group and R' is a C13 alkyl group is referred to as C16,C14 maleoyl. When the maleic acid derivative is a substituted expanded ring maleic anhydride and neither R nor R' of Formula X is H, then the acylated amino acid is referred to as "Cx,Cx'-n-Maleoyl." As used herein, the term "Cx,Cx'-n-Maleoyl," wherein x, x', and n are integers, refers to a structure wherein R is an alkyl chain of y carbons, R' is an alkyl chain of y' carbons, and the maleic anhydride ring is extend by n carbons. For example, a structure of Formula X wherein R and R' are C15 alkyl groups and n = 2 is referred to as C16,C16-2-Maleoyl.

[00119] *Methods of Attaching an acyl group*

[00120] Suitable methods of attaching acyl groups to peptides via amines, hydroxyls, and thiols of the peptides are known in the art. See, for example, Example 1 (for methods of acylating through an amine), Miller, *Biochem Biophys Res Commun* 218: 377-382 (1996); Shimohigashi and Stammer, *Int J Pept Protein Res* 19: 54-62 (1982); and Previero et al., *Biochim Biophys Acta* 263: 7-13 (1972) (for methods of acylating through a hydroxyl); and San and Silvius, J Pept Res 66: 169-180 (2005) (for methods of acylating through a thiol); Bioconjugate Chem. "Chemical Modifications of Proteins: History and Applications" pages 1, 2-12 (1990); Hashimoto et al., Pharmacuetical Res. "Synthesis of Palmitoyl Derivatives of Insulin and their Biological Activity" Vol. 6, No: 2 pp.171-176 (1989).

[00121] In some embodiments, the GIP agonist peptide comprises an acylated amino acid by acylation of a long chain alkane by the GIP agonist peptide. In specific aspects, the long chain alkane comprises an amine, hydroxyl, or thiol group (e.g.,octadecylamine, tetradecanol, and hexadecanethiol) which reacts with a carboxyl group, or activated form thereof, of the GIP agonist peptide. The carboxyl group, or activated form thereof, of the GIP agonist peptide can be part of a side chain of an amino acid (e.g., glutamic acid, aspartic acid) of the GIP agonist peptide or can be part of the peptide backbone.

[00122] In exemplary embodiments, the GIP agonist peptide comprises an acyl group by acylation of the long chain alkane by a spacer which is attached to the GIP agonist peptide. In specific aspects, the long chain alkane comprises an amine, hydroxyl, or thiol group which reacts with a carboxyl group, or activated form thereof, of the spacer. Suitable spacers comprising a carboxyl group, or activated form thereof, are described herein and include, for example, bifunctional spacers, e.g., amino acids, dipeptides, tripeptides, hydrophilic bifunctional spacers and hydrophobic bifunctional spacers.

[00123] As used herein, the term "activated form of a carboxyl group" refers to a carboxyl group with the general formula R(C=O)X, wherein X is a leaving group and R is the glucagon analog or the spacer. For example, activated forms of a carboxyl groups may include, but are not limited to, acyl chlorides, anhydrides, and esters. In some embodiments, the activated carboxyl group is an ester with a N-hydroxysuccinimide ester (NHS) leaving group.

[00124] With regard to these aspects, in which a long chain alkane is acylated by the glucagon analog or the spacer, the long chain alkane may be of any size and can comprise any length of carbon chain. The long chain alkane can be linear or branched. In exemplary aspects, the long chain alkane is a C4 to C30 alkane. For example, the long chain alkane can be any of a C4 alkane, C6 alkane, C8 alkane, C10 alkane, C12 alkane, C14 alkane, C16 alkane, C18 alkane, C20 alkane, C22 alkane, C24 alkane, C26 alkane, C28 alkane, or a C30 alkane. In some embodiments, the long chain alkane comprises a C8 to C20 alkane, e.g., a C14 alkane, C16 alkane, or a C18 alkane.

[00125] Also, in some embodiments, an amine, hydroxyl, or thiol group of the GIP agonist peptide is acylated with a cholesterol acid. In a specific embodiment, the GIP agonist peptide

is linked to the cholesterol acid through an alkylated des-amino Cys spacer, i.e., an alkylated 3-mercaptopropionic acid spacer.

[00126] When the acyl group is a succinic acid, succinic acid derivative, maleic acid, or maleic acid derivative the peptide is succinoylated/maleoylated by the reaction of an amine, hydroxyl, or thiol group of the GIP agonist peptide, or spacer, with a succinic acid, succinic acid derivative, maleic acid, or maleic acid derivative of Formula V, Formula VI, Formula VII, Formula VIII, Formula IX or formula X. Methods of succinoylation are described herein.

[00127] *Additional Acyl Groups*

[00128] The peptide in some or any embodiments comprises an acylated amino acid at a position other than positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40). The location of the acylated amino acid may be any position within the GIP agonist peptide, including any of positions 1-29, a position C-terminal to the 29th amino acid (e.g., the amino acid at position 30, 31, 32, 33, 34, 35, 36, 44, 45, 46, 47, etc., at a position within a C-terminal extension or at the C-terminus), optionally together with a second acylated amino acid at any of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40), provided that the GIP agonist activity of the peptide analog is retained, if not enhanced. Nonlimiting examples include positions 5, 7, 11, 13, 14, 17, 18, 19, 21, 24, 27, 28, or 29.

[00129] Consistent with the foregoing, the glucagon analog, in exemplary aspects, comprises two (or more) acylated amino acids, and may be considered a dual acylated peptide or a diacylated peptide, when there are two acyl groups. In exemplary aspects, all of the acylated amino acids are located at two positions of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40). In exemplary aspects, the acylations occur at two of positions 10, 14, and 40. In exemplary aspects, the peptide comprising a first acylated amino acid at position 10 and a second acylated amino acid at position 40.

[00130] In yet additional exemplary embodiments, the glucagon analog comprises two (or more) acyl groups attached to a single amino acid of the peptide backbone. The peptide may be considered as a dual acylated peptide or a diacylated peptide, when there are two acyl groups. The two (or more) acyl groups may be the same acyl group or different acyl groups,

arranged in a branched or linear formation. For example, to achieve a branched formation, the glucagon analog may comprise one acylated amino acid (which is part of the peptide backbone) attached to a spacer comprising at least three functional groups - at least two of which are each covalently attached to an acyl group and one of which is attached to the acylated amino acid of the peptide backbone. In exemplary aspects, a branched formation may be achieved through , e.g., a Lys residue comprising two amine groups (a side chain amine and an alpha amine) for direct attachment to a fatty acyl group or indirect attachment to a fatty acyl group via a spacer. In exemplary aspects, an additional spacer may be placed between the amino acid of the peptide backbone and the spacer comprising at least three functional groups. For example, the amino acid of the peptide backbone may be attached (e.g., via its side chain) to a first spacer, which, in turn, is attached to a second spacer, wherein the second spacer comprises at least three functional groups - at least two of which are each covalently attached to an alkyl group and one of which is attached to the first spacer.

[00131] In exemplary aspects, wherein the alkyl groups are arranged in a linear formation, the glucagon analog comprises one acylated amino acid (which is part of the peptide backbone) directly attached to a first acyl group, which, in turn, is attached to a spacer, which, in turn, is attached to a second acyl group.

[00132] Exemplary structures of dual acylated compounds are depicted in Figure 5A.

[00133] *Hydrophilic moieties and Acyl Groups*

[00134] The GIP agonist peptides comprising an acylated amino acid optionally further comprises a hydrophilic moiety. In some or any embodiments the hydrophilic moiety comprises a polyethylene glycol (PEG) chain. The incorporation of a hydrophilic moiety is accomplished through any suitable means, such as any of the methods described herein. In this regard, the GIP agonist peptide can comprise SEQ ID NO: 1, including any of the modifications described herein, in which at least one of the amino acids at any of positions 9, 10, 12, 13, 14, 16, 17 20, and 37-43 (e.g., at one or more of positions 10, 14, and 40) is an acylated amino acid and at least one of the amino acids at position 16, 17, 21, 24, or 29, a position within a C-terminal extension, or the C-terminal amino acid is a Cys, Lys, Orn, homo-Cys, or Ac-Phe, of which the side chain is covalently bonded to a hydrophilic moiety (e.g., PEG). In some embodiments, the amino acid at any of positions 9, 10, 12, 13, 14, 16, 17, 20, and 37-43 (e.g., at one or more of positions 10, 14, and 40)of the GIP agonist peptide

is attached (optionally via a spacer) to the acyl group and that amino acid is further attached to a hydrophilic moiety or is further attached to a Cys, Lys, Orn, homo-Cys, or Ac-Phe, which is attached to the hydrophilic moiety.

[00135] Alternatively, the acylated glucagon analog can comprise a spacer, wherein the spacer is both acylated and modified to comprise the hydrophilic moiety. Nonlimiting examples of suitable spacers include a spacer comprising one or more amino acids selected from the group consisting of Cys, Lys, Orn, homo-Cys, and Ac-Phe.

[00136] In some aspects, the GIP agonist peptide in some embodiments are acylated at the same amino acid position where a hydrophilic moiety is linked, or at a different amino acid position. Nonlimiting examples include acylation at position 9, 10, 12, 13, 14, 16, 17, 20, or 40 (e.g., at one or more of positions 10, 14, and 40) and pegylation at one or more positions in the C-terminal portion of the glucagon analog, e.g., position 24, 28 or 29, within a C-terminal extension (e.g., 37-43), or at the C-terminus (e.g., through adding a C-terminal Cys).

[00137] In some specific embodiments, the GIP agonist peptide comprising an acylated amino acid lacks an intramolecular bridge, e.g., a covalent intramolecular bridge (e.g., a lactam bridge).

AMINO ACIDS COMPRISING A NON-NATIVE ALKYL GROUP

[00138] In accordance with some or any embodiments, the GIP agonist peptides which are analogs of glucagon (SEQ ID NO: 1) comprise an amino acid comprising a non-native alkyl group (referred to herein as an "alkylated amino acid," regardless of how it is prepared, e.g., by incorporation of a previously-alkylated amino acid into the peptide, or alkylation of the peptide after synthesis). In some or any aspects, the alkylated amino acid is located at any of positions 9, 10, 12, 13, 14, 16, 17, 20, 37, 38, 39, 40, 41, 42, or 43 (e.g., at one or more of positions 10, 14, and 40). In exemplary aspects, the alkylated amino acid is located at any of positions 9, 10, 12, 16, 20, or 40 or at any of positions 10, 13, 14, 16, 17, or 40. In exemplary aspects, the alkylated amino acid is located at any of positions 10, 12, 16, or 40 or at any of positions 10, 12, or 16. In exemplary aspects, the alkylated amino acid is located at any one or more of positions 10, 14, and 40.

[00139] The alkylated amino acid in some embodiments causes the GIP agonist peptide to have one or more of (i) a prolonged half-life in circulation, (ii) a delayed onset of action, (iii) an extended duration of action, (iv) an improved resistance to proteases, such as DPP-IV, and

(v) increased potency at any one or more of the GIP receptor, GLP-1 receptor, and glucagon receptor.

[00140] *Direct Alkylation*

[00141] In some embodiments, the alkyl group is directly linked to an amino acid of the GIP agonist peptide. In accordance with one embodiment, the GIP agonist peptide comprises an alkyl group which is attached to the peptide via an ether, thioether, or amine linkage.

[00142] In specific aspects, the GIP agonist peptide comprises an alkyl group upon direct alkylation of an amine, hydroxyl, or thiol of a side chain of an amino acid of the GIP agonist peptide. In some or any embodiments, the alkyl group is linked to the amino acid of the GIP agonist peptide by reacting the amine, hydroxyl, or thiol with an activated alkyl group. Alkyl groups in some aspects are activated with a leaving group, for example, a halogen, sulfonate ester, pyridylthiol, ammonium salt, or phenoxyl.

[00143] In some or any embodiments, the alkylated amino acid is located at one of positions 9, 10, 12, 13, 14, 16, 17, 20, or 40 (e.g., at any one of positions 10, 14, and 40) or at one of positions 10, 12, or 16. In this regard, the GIP agonist peptide comprises the amino acid sequence of SEQ ID NO: 1, or a modified amino acid sequence thereof, comprising one or more of the amino acid modifications described herein, wherein at least one of the amino acids at positions 9, 10, 12, 13, 14, 16, 17, 20, and 40 (e.g., at any one of positions 10, 14, and 40) is an amino acid comprising a side chain amine, hydroxyl, or thiol.

[00144] In some embodiments, the amino acid comprising a side chain amine is an amino acid of Formula I. In some embodiments, the amino acid of Formula I, is the amino acid wherein n is 4 (Lys) or n is 3 (Orn). In some embodiments, the amino acid comprising a side chain amine is an aromatic amino acid comprising a side chain amine. In exemplary aspects, the aromatic amino acid comprising a side chain amine is 4-amino-phenylalanine (4-aminoPhe), p-amino phenylglycine, p-amino homophenylalanine, or 3-amino tyrosine. In exemplary aspects, the aromatic amino acid comprising a side chain amine is 4-amino-Phe.

[00145] In other embodiments, the amino acid comprising a side chain hydroxyl is an amino acid of Formula II. In some exemplary embodiments, the amino acid of Formula II is the amino acid wherein n is 1 (Ser). In exemplary aspects, the amino acid of Formula II is the amino acid wherein n is 2 (homoserine). In similar exemplary embodiments, the amino acid comprising a side chain hydroxyl is a Thr or homothreonine. In similar exemplary

embodiments, the amino acid comprising a side chain hydroxyl is an aromatic amino acid comprising a side chain hydroxyl. In exemplary aspects, the aromatic amino acid comprising a side chain hydroxyl is tyrosine, homotyrosine, methyl-tyrosine, or 3-amino tyrosine.

[00146] In yet other embodiments, the amino acid comprising a side chain thiol is an amino acid of Formula III. In some exemplary embodiments, the amino acid of Formula III is the amino acid wherein n is 1 (Cys). In some or any exemplary embodiments, the amino acid of Formula III is covalently attached to an alkyl group, e.g., a non-functionalized or functionalized carbon chain. In exemplary aspects, the amino acid is S-palmityl-alkylated (i.e. S-palmitate-alkylated) in which the sulfur atom of a Cys residue is covelantly bound to the β carbon of palmitate. In other embodiments, the amino acid of Formula III is covalently bound to the β carbon of a Cn acetate through the sulfur atom of a Cys residue, wherein n is an integer from 4 to 30. Examples of different ways to S-palmityl alkylate are shown in Figures 6 and 7.

[00147] In yet other embodiments, the amino acid comprising a side chain amine, hydroxyl, or thiol is a disubstituted amino acid comprising the same structure of Formula I, Formula II, or Formula III, except that the hydrogen bonded to the alpha carbon of the amino acid of Formula I, Formula II, or Formula III is replaced with a second side chain.

[00148] *Alkylation Spacers*

[00149] In alternative embodiments, the non-native alkyl group is linked via a spacer to an amino acid of the GIP agonist peptide, wherein the spacer is positioned between the amino acid of the GIP agonist peptide and the non-native alkyl group. In some embodiments, the GIP agonist peptide is covalently bound to the spacer, which is covalently bound to the non-native alkyl group.

[00150] In exemplary embodiments, the glucagon analog is modified to comprise a non-native alkyl group by alkylation of an amine, hydroxyl, or thiol of a spacer, which spacer is attached to a side chain of an amino acid at one of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one of positions 10, 14, and 40). In exemplary embodiments, the alkyl group is attached to the spacer by reacting the amine, hydroxyl, or thiol of the spacer with an alkyl group that has a leaving group (e.g., halogen, sulfonate ester, pyridylthiol, ammonium salt, phenoxyl).

[00151] The amino acid to which the spacer is attached can be any amino acid comprising a moiety which permits linkage to the spacer. For example, an amino acid comprising a side chain NH_2, –OH, or –COOH (e.g., Lys, Orn, Ser, Asp, or Glu) is suitable. In this respect, the alkylated glucagon analog can comprise a modified amino acid sequence of SEQ ID NO: 1, comprising one or more of the amino acid modifications described herein, with at least one of the amino acids at positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40).modified to any amino acid comprising a side chain amine, hydroxyl, or carboxylate.

[00152] In some embodiments, the spacer is an amino acid comprising a side chain amine, hydroxyl, or thiol, or a dipeptide or tripeptide comprising an amino acid comprising a side chain amine, hydroxyl, or thiol. The amino acid to which the spacer is attached can be any amino acid (e.g., a singly or doubly α-substituted amino acid) comprising a moiety which permits linkage to the spacer. For example, an amino acid comprising a side chain NH_2, –OH, or –COOH (e.g., Lys, Orn, Ser, Asp, or Glu) is suitable. In this respect, the GIP agonist peptide in some aspects comprises the amino acid sequence of SEQ ID NO: 1, or a modified amino acid sequence thereof comprising one or more of the amino acid modifications described herein, wherein at least one of the amino acids at positions 9, 10, 12, 13, 14, 16, 17, 20, and 37-43 (e.g., at any one of positions 10, 14, and 40) is an amino acid comprising a side chain amine, hydroxyl, or carboxylate.

[00153] In some embodiments, the spacer is an amino acid comprising a side chain amine, hydroxyl, or thiol, or a dipeptide or tripeptide comprising an amino acid comprising a side chain amine, hydroxyl, or thiol.

[00154] When the alkyl group is attached through an amine group of a spacer, the alkyl group in some aspects is attached through the alpha amine or through a side chain amine of the spacer amino acid. In the instance in which the alkyl group is attached via an alpha amine, the amino acid of the spacer can be any amino acid. For example, the amino acid of the spacer can be a hydrophobic amino acid, e.g., Gly, Ala, Val, Leu, Ile, Trp, Met, Phe, Tyr, 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid. Alternatively, in some aspects, the amino acid of the spacer is an acidic residue, e.g., Asp, Glu, homoglutamic acid, homocysteic acid, cysteic acid, gamma-glutamic acid.

[00155] In the instance in which the alkyl group is attached through a side chain amine of the amino acid spacer, the spacer is an amino acid comprising a side chain amine, e.g., an amino acid of Formula I (e.g., Lys or Orn). In this instance, it is possible for both the alpha amine and the side chain amine of the amino acid of the spacer to be attached to an alkyl group, such that the GIP agonist peptide is dialkylated. Embodiments of the invention include such dialkylated molecules. In some embodiments, the alkyl group is attached to a 4-amino-Phe, p-amino phenylglycine, p-amino homophenylalanine, or 3-amino tyrosine.

[00156] When the alkyl group is attached through a hydroxyl group of a spacer, the amino acid or one of the amino acids of the dipeptide or tripeptide can be an amino acid of Formula II. In a specific exemplary embodiment, the amino acid is Ser. In similar exemplary embodiments, the alkyl group is attached to a Thr or homothreonine. In similar exemplary embodiments, the alkyl group is attached via the hydroxyl of an aromatic amino acid comprising a side chain hydroxyl, e.g., tyrosine, homotyrosine, methyl-tyrosine, or 3-amino tyrosine.

[00157] When the alkyl group is attached through a thiol group of a spacer, the amino acid or one of the amino acids of the dipeptide or tripeptide can be an amino acid of Formula III. In a specific exemplary embodiment, the amino acid is Cys. When the alkyl group is attached through a thiol group of a spacer, the amino acid or one of the amino acids of the dipeptide or tripeptide can be an amino acid of Formula III. In a specific exemplary embodiment, the amino acid is Cys. In exemplary embodiments, the spacer is a Cys residue, which is covalently attached to an alkyl group, e.g., a non-functionalized or functionalized carbon chain. In exemplary aspects, the Cys residue is S-palmityl alkylated (i.e., S-palmitate alkylated), optionally, wherein the Cys residue is attached to a Lys residue which is part of the peptide backbone. In alternative embodiments, the spacer is a dipeptide comprising a Cys residue, which is covalently attached to an alkyl group. In exemplary aspects, the Cys is S-palmityl alkylated, and the Cys is attached to another amino acid of the spacer, which, in turn, is attached to, e.g., a Lys residue which is part of the peptide backbone. Further exemplification of S-palmityl alkylation is provided herein in Example 20.

[00158] In other exemplary embodiments, the spacer is a bifunctional spacer comprising (i) a first end comprising a leaving group that reacts with an alkyl group that comprises an amine, hydroxyl , or thiol and (ii) a second end comprising a functional group that reacts with

the side chain of the amino acid of the glucagon analog. In exemplary aspects, the amino acid of the glucagon analog is an amino acid of Formula I (e.g., Lys) or Formula II (e.g., Ser) and the amino acid is functionalized with a carboxylic acid or carboxylic acid derivative. In alternative exemplary aspects, the amino acid of the glucagon analog is an amino acid of Formula III and the amino acid is functionalized with a haloacetabmide, maleimido, or disulfide. In some embodiments, the amino acid of the glucagon analog is an amino acid comprising a side chain carboxylate, e.g., Glu, Asp, functionalized with an amine, hydroxyl, or thiol.

[00159] In some or any embodiments, the spacer is a hydrophilic bifunctional spacer. In exemplary embodiments, the hydrophilic bifunctional spacer comprises two or more reactive groups, e.g., an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In exemplary embodiments, the hydrophilic bifunctional spacer comprises a hydroxyl group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises an amine group and a carboxylate. In other embodiments, the hydrophilic bifunctional spacer comprises a thiol group and a carboxylate. In a specific embodiment, the spacer comprises an amino poly(alkyloxy)carboxylate. In this regard, the spacer can comprise, for example, $NH_2(CH_2CH_2O)_n(CH_2)_mCOOH$, wherein m is any integer from 1 to 6 and n is any integer from 2 to 12, such as, e.g., 8-amino-3,6-dioxaoctanoic acid, which is commercially available from Peptides International, Inc. (Louisville, KY).

[00160] In exemplary embodiments, the spacer comprises a small polyethylene glycol moiety (PEG) comprising a structure $[-O-CH_2-CH_2-]_n$, wherein n is an integer between 2 and 16, (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Such small PEGs are referred to herein as a "miniPEG." In exemplary aspects, the miniPEG is a functionalized miniPEG comprising one or more functional groups. Suitable functional groups include, but are not limited to, an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In exemplary aspects, the miniPEG is a miniPEG acid comprising a structure $\{[-O-CH_2-CH_2-]_n-COO-\}$, wherein n is defined as above. In exemplary aspects, the miniPEG is an amido miniPEG comprising a structure $\{-N- CH_2- CH_2-[-O-CH_2-CH_2-]_n\}$, wherein n is defined as above. In exemplary aspects, the miniPEG is an amido miniPEG acid comprising a structure $\{-N- CH_2- CH_2-[-O-CH_2-CH_2-]_n-COO-\}$, wherein n is defined as above. Suitable reagents for

use in alkylating an amino acid with a miniPEG are commercially available from vendors, such as Peptides International (Louisville, KY).

[00161] In some embodiments, the spacer is a hydrophobic bifunctional spacer. Hydrophobic bifunctional spacers are known in the art. See, e.g., *Bioconjugate Techniques*, G. T. Hermanson (Academic Press, San Diego, CA, 1996), which is incorporated by reference in its entirety. In exemplary embodiments, the hydrophobic bifunctional spacer comprises two or more reactive groups, e.g., an amine, a hydroxyl, a thiol, and a carboxyl group or any combinations thereof. In exemplary embodiments, the hydrophobic bifunctional spacer comprises a hydroxyl group and a carboxylate. In other embodiments, the hydrophobic bifunctional spacer comprises an amine group and a carboxylate. In other embodiments, the hydrophobic bifunctional spacer comprises a thiol group and a carboxylate. Suitable hydrophobic bifunctional spacers comprising a carboxylate and a hydroxyl group or a thiol group are known in the art and include, for example, 8-hydroxyoctanoic acid and 8-mercaptooctanoic acid.

[00162] In some embodiments, the bifunctional spacer is not a dicarboxylic acid comprising an unbranched, methylene of 1-7 carbon atoms between the carboxylate groups. In some embodiments, the bifunctional spacer is a dicarboxylic acid comprising an unbranched, methylene of 1-7 carbon atoms between the carboxylate groups.

[00163] The spacer (e.g., amino acid, dipeptide, tripeptide, hydrophilic bifunctional spacer, or hydrophobic bifunctional spacer) in specific embodiments is 3 to 10 atoms (e.g., 6 to 10 atoms, (e.g., 6, 7, 8, 9, or 10 atoms) in length. In more specific embodiments, the spacer is about 3 to 10 atoms (e.g., 6 to 10 atoms) in length and the non-native alkyl group is a C12 to C18 alkyl, e.g., C14 alkyl, C16 alkyl, such that the total length of the spacer and alkyl group is 14 to 28 atoms, e.g., about 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 atoms. In some embodiments, the length of the spacer and alkyl group is 17 to 28 (e.g., 19 to 26, 19 to 21) atoms.

[00164] In accordance with some or any of the foregoing embodiments, the bifunctional spacer can be a synthetic or naturally occurring amino acid (including, but not limited to, any of those described herein) comprising an amino acid backbone that is 3 to 10 atoms in length (e.g., 6-amino hexanoic acid, 5-aminovaleric acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid). Alternatively, the spacer can be a dipeptide or tripeptide spacer having

a peptide backbone that is 3 to 10 atoms (e.g., 6 to 10 atoms) in length. Each amino acid of the dipeptide or tripeptide spacer can be the same as or different from the other amino acid(s) of the dipeptide or tripeptide and can be independently selected from the group consisting of: naturally-occurring or coded and/or non-coded or non-naturally occurring amino acids, including, for example, any of the D or L isomers of the naturally-occurring amino acids (Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, Tyr), or any D or L isomers of the non-naturally occurring or non-coded amino acids selected from the group consisting of: β-alanine (β-Ala), N-α-methyl-alanine (Me-Ala), aminobutyric acid (Abu), γ-aminobutyric acid (γ-Abu), aminohexanoic acid (ε-Ahx), aminoisobutyric acid (Aib), aminomethylpyrrole carboxylic acid, aminopiperidinecarboxylic acid, aminoserine (Ams), aminotetrahydropyran-4-carboxylic acid, arginine N-methoxy-N-methyl amide, β-aspartic acid (β-Asp), azetidine carboxylic acid, 3-(2-benzothiazolyl)alanine, α-*tert*-butylglycine, 2-amino-5-ureido-n-valeric acid (citrulline, Cit), β-Cyclohexylalanine (Cha), acetamidomethyl-cysteine, diaminobutanoic acid (Dab), diaminopropionic acid (Dpr), dihydroxyphenylalanine (DOPA), dimethylthiazolidine (DMTA), γ-Glutamic acid (γ-Glu), homoserine (Hse), hydroxyproline (Hyp), isoleucine N-methoxy-N-methyl amide, methyl-isoleucine (MeIle), isonipecotic acid (Isn), methyl-leucine (MeLeu), methyl-lysine, dimethyl-lysine, trimethyl-lysine, methanoproline, methionine-sulfoxide (Met(O)), methionine-sulfone (Met(O_2)), norleucine (Nle), methyl-norleucine (Me-Nle), norvaline (Nva), ornithine (Orn), para-aminobenzoic acid (PABA), penicillamine (Pen), methylphenylalanine (MePhe), 4-Chlorophenylalanine (Phe(4-Cl)), 4-fluorophenylalanine (Phe(4-F)), 4-nitrophenylalanine (Phe(4-NO_2)), 4-cyanophenylalanine ((Phe(4-CN)), phenylglycine (Phg), piperidinylalanine, piperidinylglycine, 3,4-dehydroproline, pyrrolidinylalanine, sarcosine (Sar), selenocysteine (Sec), O-Benzyl-phosphoserine, 4-amino-3-hydroxy-6-methylheptanoic acid (Sta), 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), 4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA), 1,2,3,4,-tetrahydro-isoquinoline-3-carboxylic acid (Tic), tetrahydropyranglycine, thienylalanine (Thi) , O-benzyl-phosphotyrosine, O-Phosphotyrosine, methoxytyrosine, ethoxytyrosine, O-(bis-dimethylamino-phosphono)-tyrosine, tyrosine sulfate tetrabutylamine, methyl-valine (MeVal), and alkylated 3-mercaptopropionic acid. In exemplary aspects, the spacer is a Cys residue.

[00165] In some embodiments, the spacer comprises an overall negative charge, e.g., comprises one or two negative- charged amino acids. In some embodiments, the dipeptide is not any of the dipeptides of general structure A-B, wherein A is selected from the group consisting of Gly, Gln, Ala, Arg, Asp, Asn, Ile, Leu, Val, Phe, and Pro, wherein B is selected from the group consisting of Lys, His, Trp. In some embodiments, the amino acids of the dipeptide spacer are selected from the group consisting of: Ala, β-Ala, Leu, Pro, γ-aminobutyric acid, Glu, and γ-Glu.

[00166] In some exemplary embodiments, the GIP agonist peptide comprises an alkyl group upon alkylation of an amine, hydroxyl, or thiol of a spacer, which spacer is attached to a side chain of an amino acid at position 9, 10, 12, 13, 14, 16, 17, 20, or 37-43, (e.g., at any one or more of positions 10, 14, and 40), or at the C-terminal amino acid of the GIP agonist peptide.

[00167] In yet more specific embodiments, the alkyl group is attached to the amino acid at any of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40), of the peptide analog and the length of the spacer and alkyl group is 14 to 28 atoms. The amino acid at any of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43, (e.g., at any one or more of positions 10, 14, and 40), in some aspects, is an amino acid of Formula I, e.g., Lys, or a disubstituted amino acid related to Formula I. In more specific embodiments, the peptide analog lacks an intramolecular bridge, e.g., a covalent intramolecular bridge. The glucagon analog, for example, can be a glucagon analog comprising one or more alpha, alpha-disubstituted amino acids, e.g., AIB, for stabilizing the alpha helix of the analog.

[00168] *Alkyl Groups*

[00169] The non-native alkyl group of the alkylated amino acid can be of any size, e.g., any length carbon chain, and can be linear or branched. In some specific embodiments, the alkyl group is a C4 to C30 alkyl. For example, the alkyl group can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl.

[00170] In exemplary embodiments, the non-native alkyl group of the alkylated amino acid is a functionalized linear or branched carbon chain of any length. In some specific embodiments, the carbon chain is a C4 to C30 alkyl. For example, the alkyl group can be any

of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl. In exemplary aspects, the functionalized carbon chain comprises a functional group, including, but not limited, carboxy, sulfhydryl, amine, ketyl, sulfoxyl or amido.

[00171] In exemplary embodiments, the non-native alkyl group is a carboxy-functionalized carbon chain of structure –Cx-COOH, wherein x is an integer, optionally an integer between 4 – 30 (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30), wherein the carboxy carbon is the alpha carbon and each of the carbons of Cx are designated beta, gamma, delta, epsilon, etc., wherein the beta carbon is attached to the alpha carbon. For example, wherein, when x is 4, the non-native alkyl group would be designated as follows: C_{ε}-C_{δ}-C -C_{β}-C_{α}OOH. In exemplary embodiments, the carboxy-functionalized carbon chain is attached via a carbon other than the carboxy carbon, i.e., one of the carbons of Cx. In exemplary aspects, the carboxy-functionalized carbon chain is attached via the beta, gamma, delta, or epsilon carbon of the carboxy-functionalized carbon chain to the side chain of the alkylated amino acid. In alternative embodiments, the carboxy-functionalized carbon chain is attached via the beta, gamma, delta, or epsilon carbon of the carboxy-functionalized carbon chain to the side chain of a spacer which spacer is attached to the alkylated amino acid. In exemplary aspects, the carboxy-functionalized carbon chain is attached via the beta carbon of the carboxy-functionalized carbon chain to the side chain of the alkylated amino acid. In alternative embodiments, the carboxy-functionalized carbon chain is attached via the beta carbon of the carboxy-functionalized carbon chain to the side chain of a spacer which spacer is attached to the alkylated amino acid.

[00172] *Methods of Attaching an Alkyl group*

[00173] Methods of attaching an alkyl group to an amino acid are known in the art. For example, an alkyl groups activated with a leaving group may be reacted with an amino acid comprising a nucleophilic side chain, e.g., a side chain comprising an amine, hydroxyl, or thiol. The leaving group in exemplary aspects is a halogen, sulfonate ester, pyridylthiol, ammonium salt, or phenoxyl.

[00174] In exemplary embodiments, the amino acid to be attached to an alkyl group is a Cys residue and the sulfur atom is alkylated, e.g., "S-alkylated." In exemplary embodiments,

the sulfur of the Cys is reacted with the leaving group of an alkyl group comprising a carboxy-functionalized carbon chain of structure –Cx-COOH, wherein x is an integer, optionally an integer between 4 – 30 (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30), wherein the carboxy carbon is the alpha carbon and each of the carbons of Cx are designated beta, gamma, delta, epsilon, etc., wherein the beta carbon is attached to the alpha carbon. For example, wherein, when x is 4, the non-native alkyl group would be designated as follows: C_{ε}-C_{δ}-C -C_{β}-C_{α}OOH. In exemplary embodiments, the carboxy-functionalized carbon chain is attached via a carbon other than the carboxy carbon, i.e., one of the carbons of Cx. In exemplary aspects, the carboxy-functionalized carbon chain is attached via the beta, gamma, delta, or epsilon carbon of the carboxy-functionalized carbon chain to the side chain of the alkylated amino acid. In alternative embodiments, the carboxy-functionalized carbon chain is attached via the beta, gamma, delta, or epsilon carbon of the carboxy-functionalized carbon chain to the side chain of a spacer which spacer is attached to the alkylated amino acid. In exemplary aspects, the carboxy-functionalized carbon chain is attached via the beta carbon of the carboxy-functionalized carbon chain to the side chain of the alkylated amino acid. In alternative embodiments, the carboxy-functionalized carbon chain is attached via the beta carbon of the carboxy-functionalized carbon chain to the side chain of a spacer which spacer is attached to the alkylated amino acid.

[00175] In exemplary aspects, the leaving group is a halogen, such as iodine, bromine, chlorine, or fluorine, sulfonate esters such as tosylate, triflates, or fluorosulfonates, pyridylthiol, ammonium salt, diazonium salts, nitrates, phosphates or phenoxyl.

[00176] In specific aspects, the alkyl group comprises an iodine leaving group and a carboxy-functionalized carbon chain comprising a total of 16 carbons (including the carbon of the carboxylate). Alkylation with such an iodo-carboxylic acid may be referred to as "S-palmityl alkylation" which is synonymous with "S-palmitate alkylation." Further exemplification of S-palmityl alkylation is provided herein in Examples 1 and 20.

[00177] *Additional Alkyl Groups*

[00178] The peptide in some aspects comprises an alkylated amino acid at a position other than positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40). The location of the alkylated amino acid may be any position within the GIP

agonist peptide, including any of positions 1-29, a position C-terminal to the 29th amino acid (e.g., the amino acid at position 30, 31, 32, 33, 34, 35, 36, 44, 45, 46, 47, etc., at a position within a C-terminal extension or at the C-terminus), optionally together with a second alkylated amino acid at any of positions positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40)., provided that the GIP agonist activity of the peptide analog is retained, if not enhanced. Nonlimiting examples include positions 5, 7, 11, 13, 14, 17, 18, 19, 21, 24, 27, 28, or 29.

[00179] Consistent with the foregoing, the glucagon analog, in exemplary aspects, comprises two (or more) alkylated amino acids. In exemplary aspects, all of the alkylated amino acids are located at two positions of positions 9, 10, 12, 13, 14, 16, 17, 20, or 37-43 (e.g., at any one or more of positions 10, 14, and 40). In exemplary aspects, the peptide comprising a first alkylated amino acid at position 10 and a second alkylated amino acid at position 40.

[00180] In yet additional exemplary embodiments, the glucagon analog comprises additional alkyl groups attached to one amino acid of the peptide backbone. The two (or more) alkyl groups may be the same alkyl group or different alkyl groups, arranged in a branched or linear formation. For example, to achieve a branched formation, the glucagon analog may comprise one alkylated amino acid (which is part of the peptide backbone) attached to a spacer comprising at least three functional groups - at least two of which are each covalently attached to an alkyl group and one of which is attached to the alkylated amino acid of the peptide backbone. In exemplary aspects, a branched formation may be achieved through , e.g., a Lys residue comprising two amine groups (a side chain amine and an alpha amine) for direct attachment to a fatty alkyl group or indirect attachment to a fatty alkyl group via a spacer. In exemplary aspects, an additional spacer may be placed between the amino acid of the peptide backbone and the spacer comprising at least three functional groups. For example, the amino acid of the peptide backbone may be attached (e.g., via its side chain) to a first spacer, which, in turn, is attached to a second spacer, wherein the second spacer comprises at least three functional groups - at least two of which are each covalently attached to an alkyl group and one of which is attached to the first spacer.

[00181] In exemplary aspects, wherein the alkyl groups are arranged in a linear formation, the glucagon analog comprises one alkylated amino acid (which is part of the peptide

backbone) directly attached to a first alkyl group, which, in turn, is attached to a spacer, which, in turn, is attached to a second alkyl group.

[00182] Exemplary structures of dual alkylated compounds are derivable from the dual acylated compounds depicted in Figure 5A.

[00183] *Hydrophilic moieties and Alkyl Groups*

[00184] The GIP agonist peptides comprising an alkylated amino acid optionally further comprises a hydrophilic moiety. In some specific embodiments the hydrophilic moiety comprises a polyethylene glycol (PEG) chain. The incorporation of a hydrophilic moiety in some aspects is accomplished through any suitable means, such as any of the methods described herein. In this regard, the GIP agonist peptide can comprise SEQ ID NO: 1, including any of the modifications described herein, in which at least one of the amino acids at any of positions 9, 10, 12, 13, 14, 16, 17, 20, and 37-43 (e.g., at any one or more of positions 10, 14, and 40) of the GIP agonist peptide comprises an alkyl group and at least one of the amino acids at position 16, 17, 21, 24, or 29, a position within a C-terminal extension, or the C-terminal amino acid is a Cys, Lys, Orn, homo-Cys, or Ac-Phe, of which the side chain is covalently bonded to a hydrophilic moiety (e.g., PEG). In some embodiments, the amino acid at any of positions 9, 10, 12, 13, 14, 16, 17, 20, and 37-43 (e.g., at any one or more of positions 10, 14, and 40) of the GIP agonist peptide is attached (optionally via a spacer) to the alkyl group and that amino acid is further attached to a hydrophilic moiety or is further attached to a Cys, Lys, Orn, homo-Cys, or Ac-Phe, which is attached to the hydrophilic moiety.

[00185] Alternatively, the alkylated glucagon analog can comprise a spacer, wherein the spacer is both alkylated and modified to comprise the hydrophilic moiety. Nonlimiting examples of suitable spacers include a spacer comprising one or more amino acids selected from the group consisting of Cys, Lys, Orn, homo-Cys, and Ac-Phe.

[00186] In some aspects, the GIP agonist peptide in some embodiments are alkylated at the same amino acid position where a hydrophilic moiety is linked, or at a different amino acid position. Nonlimiting examples include alkylation at position 9, 10, 12, 13, 14, 16, 17, 20, or 40 (e.g., at any one or more of positions 10, 14, and 40) and pegylation at one or more positions in the C-terminal portion of the glucagon analog, e.g., position 24, 28 or 29, within

a C-terminal extension (e.g., 37-43), or at the C-terminus (e.g., through adding a C-terminal Cys).

[00187] *Additional Alkyl Group embodiment*

[00188] In specific embodiments, the GIP agonist peptide comprising an alkylated amino acid lacks an intramolecular bridge, e.g., a covalent intramolecular bridge (e.g., a lactam bridge).

STABILIZATION OF THE ALPHA HELIX AND ALPHA HELIX STABILIZING AMINO ACIDS

[00189] Without being bound to any particular theory, the GIP agonist peptides which are glucagon analogs comprise a helical structure, e.g., an alpha helix. In some or any embodiments, the GIP agonist peptide comprises amino acids which stabilize the alpha helical structure. Accordingly, in some aspects, the GIP agonist peptide comprises one or more alpha helix stabilizing amino acids. As used herein, the term "alpha helix promoting amino acid" is used interchangeably with the term "alpha helix stabilizing amino acid" and refers to an amino acid which provides increased stability to an alpha helix of the GIP agonist peptides of which it is a part. Alpha helix promoting amino acids are known in the art. See, for example, Lyu et al., *Proc Natl Acad Sci U.S.A.* 88: 5317-5320 (1991); Branden & Tooze, *Introduction to Protein Structure*, Garland Publishing, New York, NY, 1991; Fasman, *Prediction of Protein Structure and the Principles of Protein Conformation*, ed. Fasman, Plenum, NY, 1989). Suitable alpha helix promoting amino acids for purposes herein include, but are not limited to: alanine, norvaline, norleucine, alpha aminobutyric acid, alpha-aminoisobutyric acid (AIB), leucine, isoleucine, valine, and the like. In some embodiments, the alpha helix promoting amino acid is any amino acid which is part of an alpha helix found in a naturally-occurring protein, e.g., Leu, Phe, Ala, Met, Gly, Ile, Ser, Asn, Glu, Asp, Lys, Arg.

[00190] In exemplary embodiments, the alpha helix promoting amino acid provides more stability to the alpha helix as compared to glycine or alanine. In exemplary embodiments, the alpha helix promoting amino acid is an alpha, alpha di-substituted amino acid, e.g., AIB.

Alpha helix: Position of alpha helix promoting amino acids

[00191] In some or any embodiments of the present disclosures, the GIP agonist peptide comprises an amino acid sequence which is similar to native glucagon (SEQ ID NO: 1) and the GIP agonist peptide comprises at least one alpha helix promoting amino acid at one or more of positions 16-21 of the peptide analog (e.g., one or more of positions 16, 17, 18, 19, 20, 21). In some or any embodiments, the peptide analog comprises an alpha helix promoting amino acid at one, two, three, or all of positions 16, 17, 20, and 21.

Alpha helix: Alpha, Alpha Di-Substituted Amino Acids

[00192] In some or any embodiments, the alpha helix promoting amino acid is an alpha,alpha di-substituted amino acid. In specific embodiments, the alpha, alpha di-substituted amino acid comprises R^1 and R^2, each of which is bonded to the alpha carbon, wherein each of R^1 and R^2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R^1 and R^2 together with the alpha carbon to which they are attached form a ring (e.g., a C3-C8 ring). In exemplary embodiments, each of R^1 and R^2 is selected from the group consisting of: methyl, ethyl, propyl, and n-butyl, or R^1 and R^2 together form a cyclooctane or cycloheptane (e.g., 1-aminocyclooctane-1-carboxylic acid). In exemplary embodiments, R^1 and R^2 are the same. In other embodiments, R^1 is different from R^2. In exemplary aspects, each of R^1 and R^2 is a C1-C4 alkyl. In some aspects, each of R^1 and R^2 is a C1 or C2 alkyl. In exemplary embodiments, each of R^1 and R^2 is methyl, such that the alpha, alpha disubstituted amino acid is alpha-aminoisobutyric acid (AIB). In other exemplary embodiments, the alpha, alpha disubstituted amino acid is ACPC.

[00193] In some aspects, the GIP agonist peptide described herein comprises one or more alpha, alpha di-substituted amino acids and the GIP agonist peptide specifically lacks a covalent intramolecular bridge (e.g., a lactam), since the alpha, alpha disubstituted amino acid is capable of stabilizing the alpha helix in the absence of a covalent bridge. In some aspects, the GIP agonist peptide comprises one or more alpha, alpha di-substituted amino acids at the C-terminus (around positions 12-29). In some or any embodiments, one, two, three, four or more of positions 16, 17, 18, 19, 20, 21, 24 or 29 or, one, two, three, four, five, or all of positions 16, 17, 18, 19, 20, or 21 of the GIP agonist peptide is substituted with an α,

α-disubstituted amino acid, e.g., amino iso-butyric acid (AIB), an amino acid disubstituted with the same or a different group selected from methyl, ethyl, propyl, and n-butyl, or with a cyclooctane or cycloheptane (e.g., 1-aminocyclooctane-1-carboxylic acid). For example, substitution of position 20 with AIB enhances GIP activity, in the absence of an intramolecular bridge, e.g., a non-covalent intramolecular bridge (e.g., a salt bridge) or a covalent intramolecular bridge (e.g., a lactam). In some or any embodiments, one, two, three or more of positions 16, 20, 21 or 24 are substituted with AIB. In exemplary embodiments, one, two or all of the amino acids corresponding to positions 2, 16, and 20 of native human glucagon (SEQ ID NO: 1) are substituted with an alpha, alpha disubstituted amino acid such as AIB. In exemplary aspects, the glucagon analog comprises an AIB at positions 2 and 16 or at positions 2 and 20. In other exemplary aspects, the glucagon analog comprises a D-Ser at position 2 and an AIB at position 16 or position 20.

Alpha helix: Intramolecular Bridges

[00194] In some exemplary embodiments, the alpha helix promoting amino acid is an amino acid which is linked to another amino acid of the GIP agonist peptide via an intramolecular bridge. In such embodiments, each of these two amino acids linked via an intramolecular bridge is considered an alpha helix promoting amino acid. In exemplary embodiments, the GIP agonist peptide comprises one or two intramolecular bridges. In exemplary embodiments, the GIP agonist peptide comprises one intramolecular bridge in combination with at least one other alpha helix promoting amino acid, e.g., an alpha, alpha-disubstituted amino acid.

[00195] In some embodiments, the intramolecular bridge is a bridge which connects two parts of the GIP agonist peptide via noncovalent bonds, including, for example, van der Waals interactions, hydrogen bonds, ionic bonds, hydrophobic interactions, dipole-dipole interactions, and the like. In this regard, the glucagon analog comprises a non-covalent intramolecular bridge. In some embodiments, the non-covalent intramolecular bridge is a salt bridge.

[00196] In some embodiments, the intramolecular bridge is a bridge which connects two parts of the GIP agonist peptide via covalent bonds. In this regard, the GIP agonist peptide comprises a covalent intramolecular bridge.

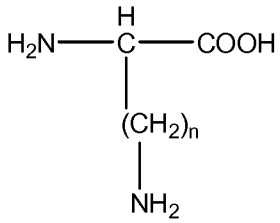
[00197] In some embodiments, the intramolecular bridge (e.g., non-covalent intramolecular bridge, covalent intramolecular bridge) is formed between two amino acids that are 3 amino acids apart, e.g., amino acids at positions i and i+4, wherein i is any integer between 12 and 25 (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25). More particularly, the side chains of the amino acid pairs 12 and 16, 16 and 20 , 20 and 24 or 24 and 28 (amino acid pairs in which i = 12, 16, 20, or 24) are linked to one another and thus stabilize the alpha helix. Alternatively, i can be 17. In some specific embodiments, the GIP agonist peptide comprises an intramolecular bridge between amino acids 17 and 21. In some specific embodiments, the GIP agonist peptide comprises an intramolecular bridge between the amino acids at positions 16 and 20 or 12 and 16 and a second intramolecular bridge between the amino acids at positions 17 and 21. GIP agonist peptides comprising one or more intramolecular bridges are contemplated herein. In specific embodiments, wherein the amino acids at positions i and i+4 are joined by an intramolecular bridge, the size of the linker is about 8 atoms, or about 7-9 atoms.

[00198] In other embodiments, the intramolecular bridge is formed between two amino acids that are two amino acids apart, e.g., amino acids at positions j and j+3, wherein j is any integer between 12 and 26 (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26). In some specific embodiments, j is 17. In specific embodiments, wherein amino acids at positions j and j+3 are joined by an intramolecular bridge, the size of the linker is about 6 atoms, or about 5 to 7 atoms.

[00199] In yet other embodiments, the intramolecular bridge is formed between two amino acids that are 6 amino acids apart, e.g., amino acids at positions k and k+7, wherein k is any integer between 12 and 22 (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, and 22). In some specific embodiments, k is 12, 13, or 17. In an exemplary embodiment, k is 17.

Alpha helix: Amino acids involved in intramolecular bridges

[00200] Examples of amino acid pairings that are capable of bonding (covalently or non-covalently) to form a six-atom linking bridge include Orn and Asp, Glu and an amino acid of Formula I, wherein n is 2, and homoglutamic acid and an amino acid of Formula I, wherein n is 1, wherein Formula I is:



wherein n = 1 to 4

[Formula I]

[00201] Examples of amino acid pairings that are capable of bonding to form a seven-atom linking bridge include Orn-Glu (lactam ring); Lys-Asp (lactam); or Homoser-Homoglu (lactone). Examples of amino acid pairings that may form an eight-atom linker include Lys-Glu (lactam); Homolys-Asp (lactam); Orn-Homoglu (lactam); 4-aminoPhe-Asp (lactam); or Tyr-Asp (lactone). Examples of amino acid pairings that may form a nine-atom linker include Homolys-Glu (lactam); Lys-Homoglu (lactam); 4-aminoPhe-Glu (lactam); or Tyr-Glu (lactone). Any of the side chains on these amino acids may additionally be substituted with additional chemical groups, so long as the three-dimensional structure of the alpha-helix is not disrupted. One of ordinary skill in the art can envision alternative pairings or alternative amino acid analogs, including chemically modified derivatives, that would create a stabilizing structure of similar size and desired effect. For example, a homocysteine-homocysteine disulfide bridge is 6 atoms in length and may be further modified to provide the desired effect.

[00202] Even without covalent linkage, the amino acid pairings described above (or similar pairings that one of ordinary skill in the art can envision) may also provide added stability to the alpha-helix through non-covalent bonds, for example, through formation of salt bridges or hydrogen-bonding interactions. Accordingly, salt bridges may be formed between: Orn and Glu; Lys and Asp; Homo-serine and Homo-glutamate; Lys and Glu; Asp and Arg; Homo-Lys and Asp; Orn and Homo-Glutamate; 4-aminoPhe and Asp; Tyr and Asp; Homo-Lys and Glu; Lys and Homo-Glu; 4-aminoPhe and Glu; or Tyr and Glu. In some embodiments, the analog comprises a salt bridge between any of the following pairs of amino acids: Orn and Glu; Lys and Asp; Lys and Glu; Asp and Arg; Homo-Lys and Asp; Orn and Homo-Glutamate; Homo-Lys and Glu; and Lys and Homo-Glu. Salt bridges may be formed between other pairs of oppositely charged side chains. See, e.g., Kallenbach et al., *Role of the Peptide Bond in Protein Structure and Folding*, in The Amide Linkage: Structural

Significance in Chemistry, Biochemistry, and Materials Science, John Wiley & Sons, Inc. (2000).

[00203] In some embodiments, the non-covalent intramolecular bridge is a hydrophobic bridge. In accordance with one embodiment, the alpha helix of the analog is stabilized through the incorporation of hydrophobic amino acids at positions j and j+3 or i and i+4. For instance, i can be Tyr and i+4 can be either Val or Leu; i can be Phe and i+4 can be Met; or i can be Phe and i+4 can be Ile. It should be understood that, for purposes herein, the above amino acid pairings can be reversed, such that the indicated amino acid at position i could alternatively be located at i+4, while the i+4 amino acid can be located at the i position. It should also be understood that suitable amino acid pairings can be formed for j and j+3.

Alpha helix: Covalent Intramolecular Bridge

[00204] In some embodiments, the covalent intramolecular bridge is a lactam ring or lactam bridge. The size of the lactam ring can vary depending on the length of the amino acid side chains, and in one embodiment the lactam is formed by linking the side chains of an ornithine to a aspartic acid side chain. Lactam bridges and methods of making the same are known in the art. See, for example, Houston, Jr., et al., *J Peptide Sci* 1: 274-282 (2004), and Example 1 herein. In some embodiments, the analog comprises a modified sequence of SEQ ID NO: 1 and a lactam bridge between i and i+4, wherein i is as defined herein above. In some embodiments, the GIP agonist peptide comprises two lactam bridges: one between the amino acids at positions 16 and 20 and another between the amino acids at positions 17 and 21. In some embodiments, the GIP agonist peptide comprises one lactam bridge and one salt bridge. Further exemplary embodiments, are described herein in the section entitled "EXAMPLES." Further exemplary embodiments include the following pairings, optionally with a lactam bridge: Glu at position 12 with Lys at position 16; native Lys at position 12 with Glu at position 16; Glu at position 16 with Lys at position 20; Lys at position 16 with Glu at position 20; Glu at position 20 with Lys at position 24; Lys at position 20 with Glu at position 24; Glu at position 24 with Lys at position 28; Lys at position 24 with Glu at position 28.

[00205] In some embodiments, the covalent intramolecular bridge is a lactone. Suitable methods of making a lactone bridge are known in the art. See, for example, Sheehan et al., *J Am Chem Soc* 95: 875-879 (1973).

[00206] In some aspects, olefin metathesis is used to cross-link one or two turns of the alpha helix of the analog using an all-hydrocarbon cross-linking system. The GIP agonist peptide in this instance comprises α-methylated amino acids bearing olefinic side chains of varying length and configured with either R or S stereochemistry at the j and j+3 or i and i+4 positions. In some embodiments, the olefinic side comprises $(CH_2)n$, wherein n is any integer between 1 to 6. In some embodiments, n is 3 for a cross-link length of 8 atoms. In some embodiments, n is 2 for a cross-link length of 6 atoms. An exemplary GIP agonist peptide comprising an olefinic cross-link is described herein as SEQ ID NO: 17. Suitable methods of forming such intramolecular bridges are described in the art. See, for example, Schafmeister et al., *J. Am. Chem. Soc.* 122: 5891-5892 (2000) and Walensky et al., *Science* 305: 1466-1470 (2004). In alternative embodiments, the analog comprises *O*-allyl Ser residues located on adjacent helical turns, which are bridged together via ruthenium-catalyzed ring closing metathesis. Such procedures of cross-linking are described in, for example, Blackwell et al., *Angew, Chem., Int. Ed.* 37: 3281-3284 (1998).

[00207] In specific aspects, use of the unnatural thio-dialanine amino acid, lanthionine, which has been widely adopted as a peptidomimetic of cystine, is used to cross-link one turn of the alpha helix. Suitable methods of lanthionine-based cyclization are known in the art. See, for instance, Matteucci et al., *Tetrahedron* Letters 45: 1399-1401 (2004); Mayer et al., *J. Peptide Res.* 51: 432-436 (1998); Polinsky et al., *J. Med. Chem.* 35: 4185-4194 (1992); Osapay et al., *J. Med. Chem.* 40: 2241-2251 (1997); Fukase et al., *Bull. Chem. Soc. Jpn.* 65: 2227-2240 (1992); Harpp et al., *J. Org. Chem.* 36: 73-80 (1971); Goodman and Shao, *Pure Appl. Chem.* 68: 1303-1308 (1996); and Osapay and Goodman, *J. Chem. Soc. Chem. Commun.* 1599-1600 (1993).

[00208] In some embodiments, α, ω-diaminoalkane tethers, e.g., 1,4-diaminopropane and 1,5-diaminopentane) between two Glu residues at positions i and i+7 are used to stabilize the alpha helix of the analog. Such tethers lead to the formation of a bridge 9-atoms or more in length, depending on the length of the diaminoalkane tether. Suitable methods of producing

peptides cross-linked with such tethers are described in the art. See, for example, Phelan et al., J. Am. Chem. Soc. 119: 455-460 (1997).

[00209] In yet other embodiments, a disulfide bridge is used to cross-link one or two turns of the alpha helix of the analog. Alternatively, a modified disulfide bridge in which one or both sulfur atoms are replaced by a methylene group resulting in an isosteric macrocyclization is used to stabilize the alpha helix of the analog. Suitable methods of modifying peptides with disulfide bridges or sulfur-based cyclization are described in, for example, Jackson et al., *J. Am. Chem. Soc.* 113: 9391-9392 (1991) and Rudinger and Jost, *Experientia* 20: 570-571 (1964).

[00210] In yet other embodiments, the alpha helix of the analog is stabilized via the binding of metal atom by two His residues or a His and Cys pair positioned at j and j+3, or i and i+4. The metal atom can be, for example, Ru(III), Cu(II), Zn(II), or Cd(II). Such methods of metal binding-based alpha helix stabilization are known in the art. See, for example, Andrews and Tabor, *Tetrahedron* 55: 11711-11743 (1999); Ghadiri et al., *J. Am. Chem. Soc.* 112: 1630-1632 (1990); and Ghadiri et al., *J. Am. Chem. Soc.* 119: 9063-9064 (1997).

[00211] The alpha helix of the GIP agonist peptide can alternatively be stabilized through other means of peptide cyclizing, which means are reviewed in Davies, J. Peptide. Sci. 9: 471-501 (2003). The alpha helix can be stabilized via the formation of an amide bridge, thioether bridge, thioester bridge, urea bridge, carbamate bridge, sulfonamide bridge, and the like. For example, a thioester bridge can be formed between the C-terminus and the side chain of a Cys residue. Alternatively, a thioester can be formed via side chains of amino acids having a thiol (Cys) and a carboxylic acid (e.g., Asp, Glu). In another method, a cross-linking agent, such as a dicarboxylic acid, e.g.,suberic acid (octanedioic acid), etc. can introduce a link between two functional groups of an amino acid side chain, such as a free amino, hydroxyl, thiol group, and combinations thereof.

ADDITIONAL DESCRIPTIONS

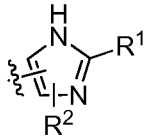
[00212] Provided below are additional descriptions of the glucagon analogs of the present disclosures. As discussed herein, the position of the amino acid in the descriptions below is referenced with regard to the amino acid numbering of SEQ ID NO: 1. Also, while the

descriptions below are discussed in reference to native human glucagon (SEQ ID NO: 1), e.g., modifications of SEQ ID NO: 1, these descriptions do not necessarily imply (i) that such modifications are present in all of the presently disclosed peptides and (ii) that the only method of making the presently disclosed peptides is to start with native human glucagon and modify that sequence. Rather, the descriptions are provided to describe some embodiments of the glucagon analogs of the present disclosures, and the peptides of the present disclosures may be made *de novo* without utilizing native human glucagon as a starting material, as further described in the section entitled "*METHODS OF MAKING PEPTIDES.*"

[00213] *Position 1*

[00214] In some embodiments, the glucagon analog comprises an amino acid modification at position 1, relative to SEQ ID NO: 1, e.g., the glucagon analog comprises an amino acid which is not His at position 1. In exemplary aspects, the glucagon analog comprises a large, aromatic amino acid at position 1. In exemplary embodiments, the large, aromatic amino acid is Tyr, Phe, Trp, amino-Phe (e.g., 4-amino-Phe), chloro-Phe, sulfo-Phe, 4-pyridyl-Ala, methyl-Tyr, or 3-amino Tyr.

[00215] In other exemplary embodiments, the glucagon analog comprises an amino acid comprising an imidazole side chain at position 1. In exemplary aspects, the amino acid at position 1 comprises a structure of Formula A



[Formula A],

wherein each of R1 and R2 independently is selected from the group consisting of H, (C1-6)alkyl, O(C1-6)alkyl, (C1-6)alkyl-OH, F, and (C1-C6)alkyl of which at least one H is replaced by F.

[00216] In exemplary aspects, the amino acid at position 1 is the native residue of glucagon (SEQ ID NO: 1) L-histidine (His), or is a derivative of His (His derivative), e.g., a derivative of His in which the alpha atoms are modified. The His derivative in exemplary aspects is D-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine, homo-

histidine, N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, or alpha, alpha-dimethyl imidiazole acetic acid (DMIA).

[00217] In yet other aspects, the amino acid at position 1 is a DPP-IV protective amino acid, as described herein. In some aspects, the DPP-IV protective amino acid is a derivative of His.

[00218] *Position 2*

[00219] In some embodiments, the presently disclosed peptides comprise a DPP-IV protective amino acid at position 2. As used herein, the term "DPP-IV protective amino acid" refers to an amino acid which achieves substantial resistance of the presently disclosed peptide against dipeptidyl peptidase IV (DPP IV) cleavage. In some aspects, the DPP-IV protective amino acid is one of D-serine, D-alanine, valine, glycine, N-methyl serine, N-methyl alanine, or alpha, aminoisobutyric acid (AIB). In some aspects, the DPP-IV protective amino acid is an α,α-disubstituted amino acid. In some aspects, the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring. In some aspects, the α,α-disubstituted amino acid is AIB or 1-aminocyclopropane-1-carboxylate (ACPC).

[00220] The term "C_1-C_n alkyl" wherein n can be from 1 through 6, as used herein, represents a branched or linear alkyl group having from one to the specified number of carbon atoms. Typical C_1-C_6 alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, hexyl and the like.

[00221] In exemplary embodiments, the DPP-IV protective amino acid is the D-isomer of Ser (D-Ser), or a conservative amino acid substitution thereof. For example, the DPP-IV protective amino acid can comprise a side chain structure of –(C1-C4 alkyl)OH. Optionally, when the side chain structure comprises -(C3 alkyl)OH or –(C4 alkyl)OH, the carbon chain may be straight chained or branched.

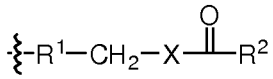
[00222] In exemplary aspects, when the DPP-IV protective amino acid is D-Ser and the amino acid at position 1 is His, the GIP agonist peptide is not conjugated to a heterologous

moiety, e.g., a hydrophilic moiety (e.g., PEG). In other aspects of the present disclosures, the DPP-IV protective amino acid is not D-serine.

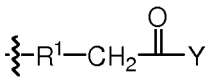
[00223] *Position 3*

[00224] In some embodiments, the glucagon analog comprises at position 3 an acidic, basic, or hydrophobic amino acid residue. Without being bound to any particular theory, such glucagon analogs exhibit a reduced glucagon receptor activity. In some embodiments, the acidic, basic, or hydrophobic amino acid is glutamic acid, ornithine, norleucine. The glucagon analogs that are substituted with, for example, glutamic acid, ornithine, or norleucine in some aspects have about 10% or less of the activity of native glucagon at the glucagon receptor, e.g.,about 1-10%, or about 0.1-10%, or greater than about 0.1% but less than about 10%. In some embodiments, the glucagon analogs exhibit about 0.5%, about 1% or about 7% of the activity of native glucagon.

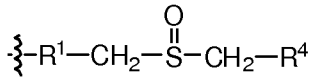
[00225] In some embodiments, the glucagon analog comprises the native amino acid of SEQ ID NO: 1 at position 3, e.g., glutamine, or comprises a glutamine analog. Without being bound to a particular theory, such glucagon analogs comprising a glutamine analog do not exhibit a substantial loss of activity at the glucagon receptor, and in some cases, the glucagon analog comprising the glutamine analog enhances glucagon receptor activity. In some embodiments, the glutamine analog comprises at position 3 an amino acid comprising a side chain of Structure I, II or III:



Structure I



Structure II



Structure III

wherein R^1 is C_{0-3} alkyl or C_{0-3} heteroalkyl; R^2 is NHR^4 or C_{1-3} alkyl; R^3 is C_{1-3} alkyl; R^4 is H or C_{1-3} alkyl; X is NH, O, or S; and Y is NHR^4, SR^3, or OR^3. In some embodiments,

X is NH or Y is NHR^4. In some embodiments, R^1 is C_{0-2} alkyl or C_1 heteroalkyl. In some embodiments, R^2 is NHR^4 or C_1 alkyl. In some embodiments, R^4 is H or C^1 alkyl. In exemplary embodiments, an amino acid comprising a side chain of Structure I is provided where, R^1 is CH_2-S, X is NH, and R^2 is CH_3 (acetamidomethyl-cysteine, C(Acm)); R^1 is CH_2, X is NH, and R^2 is CH_3 (acetyldiaminobutanoic acid, Dab(Ac)); R^1 is C_0 alkyl, X is NH, R^2 is NHR^4, and R^4 is H (carbamoyldiaminopropanoic acid, Dap(urea)); or R^1 is CH_2-CH_2, X is NH, and R^2 is CH_3 (acetylornithine, Orn(Ac)). In exemplary embodiments, an amino acid comprising a side chain of Structure II is provide where, R^1 is CH_2, Y is NHR^4, and R^4 is CH_3 (methylglutamine, Q(Me)); In exemplary embodiments, an amino acid comprising a side chain of Structure III is provided where, R^1 is CH_2 and R^4 is H (methionine-sulfoxide, M(O)); In specific embodiments, the amino acid at position 3 is substituted with Dab(Ac)

[00226] *Position 7*

[00227] In some embodiments, the glucagon analog comprises an amino acid modification at position 7, relative to SEQ ID NO: 1, e.g., the glucagon analog comprises an amino acid other than Thr at position 7. In some aspects, the amino acid at position 7 is a large, aliphatic amino acid, e.g., Ile, Leu, Ala, and the like. Without being bound to a particular theory, glucagon analogs comprising such an amino acid at position 7 are believed to exhibit drastically reduced activity at the GLP-1 receptor.

[00228] *Position 9*

[00229] In some embodiments, the glucagon analog comprises an amino acid modification at position 9, relative to SEQ ID NO: 1, e.g., the glucagon analog comprises an amino acid other than Asp at position 9. In some embodiments, the glucagon analog comprises a negative charged amino acid other than Asp, e.g., Glu, homoglutamic acid, cysteic acid, homocysteic acid. In some aspects, the amino acid at position 9 is an acylated amino acid or an alkylated amino acid, as discussed herein.

[00230] *Position 10*

[00231] In some embodiments, the glucagon analog comprises an amino acid modification at position 10, relative to SEQ ID NO: 1, e.g., the glucagon analog comprises an amino acid other than Tyr at position 10. In some aspects, the amino acid at position 10 is Trp. Without being bound to a particular theory, glucagon analogs comprising such an amino acid at position 10 are believed to exhibit activity at the GIP receptor, GLP-1 receptor, and/or the

glucagon receptor which is not reduced, as compared to the corresponding peptide with a Tyr at position 10.

[00232] In some embodiments, the glucagon analog comprises an an acylated amino acid or an alkylated amino acid, as discussed herein.

[00233] *Position 12*

[00234] In some embodiments, the glucagon analog comprises an amino acid modification at position 12, relative to SEQ ID NO: 1. e.g., the glucagon analog comprises an amino acid other than Lys at position 10. In some aspects, the amino acid at position 12 is a large, aliphatic, nonpolar amino acid, optionally, isoleucine. In some aspects, the amino acid at position 12 is Arg. Without being bound to a particular theory, glucagon analogs comprising a large, aliphatic, nonpolar amino acid, e.g., Ile, exhibit enhanced activity at the GIP receptor. In some embodiments, the glucagon analog comprises an an acylated amino acid or an alkylated amino acid, as discussed herein.

[00235] *Position 15*

[00236] In some embodiments, the glucagon analogs comprise an amino acid modification at position 15, relative to SEQ ID NO: 1, e.g., the glucagon analog comprises an amino acid other than Asp at position 15. In some aspects, the amino acid at position 15 is deleted or is glutamic acid, homoglutamic acid, cysteic acid or homocysteic acid. Without being bound to a particular theory, such glucagon analogs exhibit improved stability, e.g., by way of reducing degradation or cleavage of the analog over time, especially in acidic or alkaline buffers, e.g., buffers at a pH within the range of 5.5 to 8. In some embodiments, the glucagon analogs comprising this modification retains at least 75%, 80%, 90%, 95%, 96%, 97%, 98% or 99% of the original analog after 24 hours at 25°C.

[00237] *Position 16*

[00238] In some embodiments, the glucagon analogs comprise an amino acid modification at position 16, relative to SEQ ID NO: 1, e.g., the glucagon analog comprises an amino acid other than Ser at position 16. In some aspects, the amino acid at position 16 is glutamic acid or with another negative-charged amino acid having a side chain with a length of 4 atoms, or alternatively with any one of glutamine, homoglutamic acid, or homocysteic acid, or a charged amino acid having a side chain containing at least one heteroatom, (e.g.,N, O, S, P) and with a side chain length of about 4 (or 3-5) atoms. In some embodiments, the glucagon

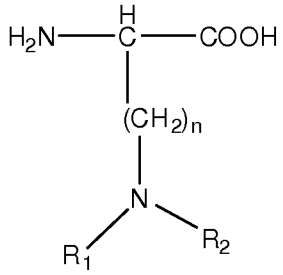
analog comprises at position 16 an amino acid selected from the group consisting of glutamic acid, glutamine, homoglutamic acid, homocysteic acid, threonine or glycine or is an amino acid selected from the group consisting of glutamic acid, glutamine, homoglutamic acid and homocysteic acid.

[00239] Without being bound to a particular theory, such glucagon analogs exhibit enhanced stability, e.g., by way of reducing degradation of the peptide over time, especially in acidic or alkaline buffers, e.g., buffers at a pH within the range of 5.5 to 8. Such glucagon analogs are less susceptible to cleavage of the Asp15-Ser16 peptide bond.

[00240] In some embodiments, the amino acid at position 16 is a negatively charged amino acid, optionally in combination with an alpha helix promoting amino acid (e.g., an alpha, alpha disubstituted amino acid, or AIB) at position 20. Such glucagon analogs exhibit GIP activity.

[00241] In alternative embodiments, the glucagon analog comprises at position 16 a Thr or an alpha helix promoting amino acid, as described above. In some embodiments, the alpha helix promoting amino acid is AIB or Glu.

[00242] In some aspects, the amino acid at position 16 is a positive charged amino acid, e.g., Formula IV:



[Formula IV],

[00243] wherein n is 1 to 16, or 1 to 10, or 1 to 7, or 1 to 6, or 2 to 6, or 2 or 3 or 4 or 5, each of R_1 and R_2 is independently selected from the group consisting of H, C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)NH_2, (C_1-C_{18} alkyl)SH, (C_0-C_4 alkyl)(C_3-C_6)cycloalkyl, (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and (C_1-C_4 alkyl)(C_3-C_9 heteroaryl), wherein R_7 is H or OH, Lys.

[00244] In some embodiments, the glucagon analog comprises an an acylated amino acid or an alkylated amino acid, as discussed herein.

[00245] In yet additional embodiments, the amino acid at position 16 is an amino acid comprising a side chain which is conjugated to a heterologous moiety, as described herein under the section entitled "CONJUGATES."

[00246] *Positions 17 and 18*

[00247] In some embodiments, the glucagon analog comprises an amino acid modification at either or both of positions 17 and 18, relative to SEQ ID NO: 1, such that the dibasic Arg-Arg site at positions 17 and 18 is eliminated. In some embodiments, the glucagon analog comprises an amino acid other than Arg at one or both of positions 17 and 18. Without being bound to any particular theory, it is believed that elimination of the dibasic site improves the in vivo efficacy of the glucagon analog. In some aspects, the amino acid at position 17 is not a basic amino acid. In some aspects, the amino acid at position 17 is an aliphatic amino acid. In some embodiments, the amino acid at position 17 is substituted with another amino acid as described herein, e.g., an amino acid comprising a hydrophilic moiety, an alpha helix promoting amino acid. In some embodiments, the alpha helix promoting amino acid forms a non-covalent intramolecular bridge with an amino acid at j+3 or i+4. In some aspects, the amino acid at position 17 is Gln.

[00248] In some aspects, the amino acid at position 18 is not a basic amino acid. In some aspects, the amino acid at position 18 is an aliphatic amino acid. In some embodiments, the amino acid at position 18 is a small aliphatic amino acid, e.g., Ala.

[00249] In some specific aspects, the amino acid at position 18 is a small aliphatic amino acid, e.g., Ala, and the amino acid at position 17 is Arg. In other aspects, the amino acid at position 18 is a small aliphatic amino acid, e.g., Ala, and amino acid at position 17 is Gln.

[00250] In some aspects, the amino acid at position 17 is an amino acid comprising a side chain which is conjugated to a heterologous moiety, as described herein under the section entitled "CONJUGATES.

[00251] *Position 20*

[00252] In some embodiments, the glucagon analog comprises an amino acid modification at position 20, relative to SEQ ID NO: 1, e.g., the amino acid at postion 20 is an amino acid other than Gln. In some aspects, the amino acid at position 20 is an alpha helix promoting amino acid, e.g. as described above. In some aspects, the amino acid at position 20 is an alpha, alpha disubstituted amino acid, e.g., AIB, ACPC. In some embodiments, the alpha

helix promoting amino acid forms a non-covalent intramolecular bridge with an amino acid at j-3 or i-4.

[00253] In some specific embodiments the amino acid is a hydrophilic amino acid having a side chain that is either charged or has an ability to hydrogen-bond, and is at least about 5 (or about 4-6) atoms in length, for example, lysine, citrulline, arginine, or ornithine. In other aspects, the amino acid at position 20 is Ser, Thr, Ala or AIB.

[00254] In some aspects, the amino acid at position 20 is a an acylated amino acid or alkylated amino acid, as discussed herein.

[00255] In some aspects, the amino acid at position 20 is an amino acid comprising a side chain which is conjugated to a heterologous moiety, as described herein under the section entitled "CONJUGATES.

[00256] Without being bound to a particular theory, such glucagon analogs exhibit enhanced activity at the GLP-1 receptor and/or GIP receptor or exhibit reduced degradation that occurs through deamidation of Gln and/or exhibit increased stability.

[00257] *Position 21*

[00258] In some embodiments, the glucagon analog comprises an amino acid modification at position 21, relative to SEQ ID NO: 1, e.g., the amino acid at postion 21 is an amino acid other than Asp. In exemplary aspects, the amino acid at position 21 is Ser, Thr, Ala or AIB. In other aspects, the amino acid at position 21 is Lys, Arg, Orn, or Citrulline. In some aspects, the amino acid at position 21 is Glu, homoglutamic acid or homocysteic acid. In some aspects, the amino acid at position 21 is an amino acid comprising a side chain which is conjugated to a heterologous moiety, as described herein under the section entitled "CONJUGATES.

[00259] In some embodiments, the amino acid at position 21 is an alpha helix promoting amino acid. In some embodiments, the alpha helix promoting amino acid forms a non-covalent intramolecular bridge with an amino acid at j-3 or i-4.

[00260] Without being bound to a particular theory, such glucagon analogs exhibit reduced degradation that occurs through degradation through dehydration of Asp to form a cyclic succinimide intermediate followed by isomerization to iso-aspartate and/or exhibit increased stability.

[00261] *Position 23*

[00262] In some aspects, the glucagon analog comprises an amino acid modification at position 23, relative to SEQ ID NO: 1. In some aspects, the amino acid at position 23 is an amino acid other than Val, including but not limited to Ile.

[00263] *Position 24*

[00264] In some aspects, the glucagon analog comprises an amino acid modification at position 24, relative to SEQ ID NO: 1. In some aspects, the amino acid at position 24 is an amino acid other than Gln, e.g., Ala, Asn, Cys. In some aspects, the amino acid at position 24 is an amino acid comprising a side chain which is conjugated to a heterologous moiety, as described herein under the section entitled "CONJUGATES.

[00265] *Position 27*

[00266] In some aspects, the glucagon analog comprises an amino acid modification at position 27, relative to SEQ ID NO: 1. In some aspects, the amino acid at position 27 is an amino acid other than Met. In some embodiments, the glucagon analog comprises at position 27 an amino acid which prevents oxidative degradation of the peptide. In some aspects, the amino acid at position 27 is methionine sulfoxide, leucine, isoleucine or norleucine. In some specific embodiments, the amino acid at position 27 is leucine or norleucine.

[00267] In other aspects, the amino acid at position 27 is an aliphatic amino acid (e.g., Gly, Ala, Val, Leu, Ile) or an amino acid of Formula IV, as described herein, e.g., Lys. In exemplary embodiments, the amino acid at position 27 is Val or Lys. Without being bound to any particular theory, such an amino acid modification reduces glucagon activity.

[00268] *Position 28*

[00269] In some aspects, the glucagon analog comprises an amino acid modification at position 28, relative to SEQ ID NO: 1. In some aspects, the amino acid at position 28 is an amino acid other than Asn. In some aspects, the amino acid at position 28 is Ala, Ser, Thr, or AIB. In some aspects, the amino acid at position 28 is a charged amino acid, e.g., a negative-charged amino acid, as further described herein. See section entitled "*Charged C-terminus*." In some aspects, the amino acid at position 28 is Asp.

[00270] In exemplary aspects, the amino acid at position 28 is an amino acid of Formula IV as described herein. The amino acid in exemplary embodiments is Lys. Without being bound to any particular theory, such an amino acid modification reduces glucagon activity.

[00271] *Position 29*

[00272] In some aspects, the glucagon analog comprises an amino acid modification at position 29, relative to SEQ ID NO: 1. In some aspects, the amino acid at position 29 is an amino acid other than Thr. In some aspects, the amino acid at position 29 is Gly. In some aspects, the amino acid at position 29 is Ala.

[00273] In some aspects, the amino acid at position 29 is an amino acid comprising a side chain which is conjugated to a heterologous moiety, as described herein under the section entitled "CONJUGATES.

[00274] *Charged C-terminus*

[00275] In some embodiments, the glucagon analog comprises one or more amino acid substitutions and/or additions that introduce a charged amino acid into the C-terminal portion of the analog, relative to SEQ ID NO: 1. In some embodiments, such modifications enhance stability and solubility. As used herein the term "charged amino acid" or "charged residue" refers to an amino acid that comprises a side chain that is positive-charged or negative-charged (i.e., de-protonated) or positive-charged (i.e., protonated) in aqueous solution at physiological pH. In some aspects, these amino acid substitutions and/or additions that introduce a charged amino acid modifications are at a position C-terminal to position 27 of SEQ ID NO: 1. In some embodiments, one, two or three (and in some instances, more than three) charged amino acids are introduced within the C-terminal portion (e.g., position(s) C-terminal to position 27). In accordance with some embodiments, the native amino acid(s) at positions 28 and/or 29 are substituted with a charged amino acids, and/or in a further embodiment one to three charged amino acids are also added to the C-terminus of the analog. In exemplary embodiments, one, two or all of the charged amino acids are negative-charged. The negative-charged amino acid in some embodiments is aspartic acid, glutamic acid, cysteic acid, homocysteic acid, or homoglutamic acid. In some aspects, these modifications increase solubility or stability. In some embodiments, position 30 is not a charged amino acid. Without being bound to a particular theory, a charged amino acid, e.g., a negative charged amino acid, e.g., Glu, reduced GIP activity.

[00276] *C-terminal truncation*

[00277] In accordance with some embodiments, the glucagon analogs disclosed herein are modified by truncation of the C-terminus by one or two amino acid residues. Such modified glucagon peptides, retain similar activity and potency at the glucagon receptor and GLP-1 receptor. In this regard, the glucagon peptides can comprise amino acids 1-27 or 1-28 of the native glucagon analog (SEQ ID NO: 1), optionally with any of the additional modifications described herein.

[00278] *Charge-neutral C-terminus*

[00279] In some embodiments, the glucagon analog comprises a charge-neutral group, such as an amide or ester, at the C-terminus in place of the alpha carboxylate, relative to SEQ ID NO: 1. Without being bound to any particular theory, such modifications in exemplary aspects increases activity of the glucagon analog at the GLP-1 receptor. Accordingly, in some embodiments, the glucagon analog is an amidated peptide, such that the C-terminal residue comprises an amide in place of the alpha carboxylate of an amino acid. As used herein a general reference to a peptide or analog is intended to encompass peptides that have a modified amino terminus, carboxy terminus, or both amino and carboxy termini. For example, an amino acid chain composing an amide group in place of the terminal carboxylic acid is intended to be encompassed by an amino acid sequence designating the standard amino acids.

[00280] *C-terminal Extensions*

[00281] In some embodiments of the present disclosures, the glucagon analogs comprise a C-terminal extension of 1-21 amino acids fused to the amino acid at position 29. The C-terminal extension may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 amino acids. In some aspects, the C-terminal extension is any of the heterologous peptides described below in the section "CONJUGATES." For example, in some aspects, the extension comprises an amino acid sequence which forms a Trp cage structure, e.g., the extension comprises the amino acid sequence of GPSSGAPPPS (SEQ ID NO: 5), or a conservatively substituted sequence thereof. In alternative aspects, the extension of 1 to 21 amino acids comprises at least one charged amino acid. In exemplary aspects, the extension comprises an amino acid sequence of: X1-X2, wherein X1 is a charged amino acid and X2 is

a small aliphatic amino acid. In some aspects, X1 is a positive charged amino acid, e.g., Arg. In some aspects, extension comprises Arg-Gly.

[00282] In some embodiments, the extension comprises an amino acid sequence of SEQ ID NO: 5 (GPSSGAPPPS), SEQ ID NO: 6 (GGPSSGAPPPS), SEQ ID NO: 7 (KRNRNNIA), or SEQ ID NO: 8 (KRNR). In specific aspects, the amino acid sequence is attached through the C-terminal amino acid of the glucagon analog, e.g., amino acid at position 29. In some embodiments, the amino acid sequence of any of SEQ ID NOs: 5-8 is bound to amino acid 29 of the glucagon analog through a peptide bond. In some specific embodiments, the amino acid at position 29 of the glucagon analog is a Gly and the Gly is fused to one of the amino acid sequences of any of SEQ ID NOs: 5-8.

[00283] In exemplary aspects, the glucagon analog comprises an extension which forms a forms a structure known in the art as a Trp cage (see, e.g., Paschek et al., *Proc Natl Acad Sci USA* 105 (46): 17754-17759 (2008). In some aspects, the extension comprises the amino acid sequence GPSSGAPPPS (SEQ ID NO: 5) or GGPSSGAPPPS (SEQ ID NO: 6) or GPSSGRPPPS (SEQ ID NO: 183) or a sequence of one of the foregoing with 1, 2, or 3 conservative amino acid substitutions. In exemplary aspects, when the extension comprises the amino acid sequence of SEQ ID NO: 183, the amino acid at position 28 is a negative charged amino acid, e.g., Asp or Glu.

[00284] *Other modifications*

[00285] Descriptions of yet other modifications, relative to SEQ ID NO: 1, of the glucagon analogs of the present disclosures are found throughout this application. The above listing is not exhaustive, but merely exemplary.

[00286] In some embodiments, the glucagon analogs described herein are glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into a salt (e.g., an acid addition salt, a basic addition salt), and/or optionally dimerized, multimerized, or polymerized, or conjugated.

EXCLUDED PEPTIDES

[00287] The glucagon analogs of the present disclosures are structurally distinct from the glucagon analogs which exhibit GIP receptor agonist activity described in International Patent Application No. PCT US2009/47447 (filed on June 16, 2009), U.S. Application No. 61/073,274 (filed June 17, 2008); U.S. Application No. 61/078,171 (filed July 3, 2008); U.S.

Application No. 61/090,448 (filed August 20, 2008), U.S. Application No. 61/151,349 (filed February 10, 2009), U.S. Application No. 61/187,578 (filed June 16, 2009), International Patent Application No. PCT/US2010-038825 (filed June 16, 2010); the contents of which are incorporated by reference in their entirety. Accordingly, in any or all embodiments, the glucagon analog of the present disclosures is not any of the glucagon analogs or peptides described in International Patent Application No. PCT/US2009/47447 (filed on June 16, 2009, and published as WO 2010/011439), U.S. Application No. 61/073,274 (filed June 17, 2008); U.S. Application No. 61/078,171 (filed July 3, 2008); U.S. Application No. 61/090,448 (filed August 20, 2008), U.S. Application No. 61/151,349 (filed February 10, 2009), U.S. Application No. 61/187,578 (filed June 16, 2009), International Patent Application No. PCT/US2010/038825 (filed June 16, 2010, and published as WO 2010/148089), U.S. Application No. 61/298,812 (filed January 27, 2010), or International Patent Application No. PCT/US2011/022608 (filed January 26, 2011, and published as WO 2011/094337). In exemplary embodiments, the peptides, glucagon peptides, or glucagon analogs of the present disclosures is not (i.e., excludes) any one or all of the peptides of SEQ ID NOs: 1-262 of WO 2010/011439; SEQ ID NOs: 1-680 of WO 2010/148089, or SEQ ID NOs: 1-1318 of PCT/US2011/022608.).

[00288] In exemplary embodiments, the peptides, glucagon peptides, or glucagon analogs of the present disclosures is not (i.e., excludes) any one or all of the peptides of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246,

247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, and/or 262 of International Patent Application Publication No. WO 2010/011439.

[00289] In exemplary embodiments, the peptides, glucagon peptides, or glucagon analogs of the present disclosures is not (i.e., excludes) any one or all of the peptides of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262 , 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516,

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[00290] In exemplary embodiments, the peptides, glucagon peptides, or glucagon analogs of the present disclosures is not (i.e., excludes) any one or all of the peptides of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262 , 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282,

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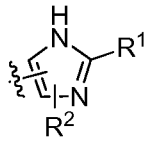
EXEMPLARY EMBODIMENTS

[00291] In exemplary embodiments, the peptide of the present disclosures is an analog of glucagon (SEQ ID NO: 1) comprising (i) an amino acid comprising an imidazole side chain at position 1, (ii) a DPP-IV protective amino acid at position 2, (iii) an acylated amino acid or alkylated amino acid, optionally at any of positions 9, 10, 12, 16, 20, or 37-43, wherein optionally the acyl or alkyl group is linked to the amino acid via a spacer; (iv) an alpha helix stabilizing amino acid at one or more of positions 16-21, and (v) up to ten additional amino acid modifications relative to SEQ ID NO: 1, wherein when the glucagon analog is not conjugated to a heterologous moiety, e.g., a hydrophilic moiety (e.g., PEG), the glucagon analog exhibits at least or about 0.1% (e.g., at least or about 1%, at least or about 10%, at least or about 50%, at least about 80%, at least or about 100%, at least or about 500%) activity of native GIP at the GIP receptor.

[00292] The glucagon analogs described here may comprise any activity profile described herein. See, e.g., the section entitled "*ACTIVITY OF THE PRESENTLY DISCLOSED PEPTIDES*." In exemplary aspects, the glucagon analog exhibits a GIP percentage potency of at least or about 1%, at least or about 10%, at least or about 50%, at least or about 90%, at least or about 100%, at least or about 300%, or at least or about 500%. In some aspects, the glucagon analog also exhibits a GLP-1 percentage potency of at least or about 1%, at least or about 10%, at least or about 50%, at least or about 90%, at least or about 100%, at least or about 300%, or at least or about 500%. In alternative or additional aspects, the glucagon analog exhibits a glucagon percentage potency of at least or about 1%, at least or about 10%, at least or about 50%, at least or about 90%, or at least or about 100%. Accordingly, while the glucagon analogs may be considered as GIP agonist peptides, in some aspects, the glucagon analogs additionally may be considered as a GIP-GLP-1 co-agonist, a GIP-glucagon co-agonist, or a GIP-GLP-1-glucgaon triagonist. For example, the peptide may exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor, wherein the peptide exhibits an EC50 at the GIP receptor which is within 100-fold (e.g., 50-fold, 40-fold, 30-fold, 20-fold, 15-fold, 10-fold, or less) of its EC50 at the GLP-1 receptor and is within 100-fold (e.g., 50-fold, 40-fold, 30-fold, 20-fold, 15-fold, 10-fold, or less) of its EC50 at the glucagon receptor.

[00293] In exemplary embodiments, the glucagon analog comprises an amino acid comprising an imidazole side chain at position 1. In exemplary aspects, the amino acid at position 1 comprises a structure of Formula A



[Formula A],

wherein each of R1 and R2 independently is selected from the group consisting of H, (C1-6)alkyl, O(C1-6)alkyl, (C1-6)alkyl-OH, F, and (C1-C6)alkyl of which at least one H is replaced by F.

[00294] In exemplary aspects, the amino acid at position 1 is the native residue of glucagon (SEQ ID NO: 1) L-histidine (His), or is a derivative of His (His derivative), e.g., a derivative of His in which the alpha atoms are modified. As used herein, the term "His derivative" refers to a chemical moiety comprising an imidazole, e.g., comprising a structure of Formula A, or a substituted imidazole, attached to at least one carbon atom. In exemplary embodiments, the His derivative comprises a structure similar to the structure of histidine, except that the alpha amine, alpha carbon, or alpha carboxylate is replaced with another chemical moiety. In exemplary embodiments, the His derivative is an alpha substituted histidine of which the hydrogen atom linked to the alpha carbon is substituted with another chemical moiety, e.g., methyl, ethyl, propyl, isopropyl, hydroxyl, methoxy, ethoxy, and the like. The His derivative in some aspects is D-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine, homo-histidine, N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, or alpha, alpha-dimethyl imidiazole acetic acid (DMIA).

[00295] In some aspects, the DPP-IV protective amino acid at position 2 at position 2 is one of D-serine, D-alanine, valine, glycine, N-methyl serine, N-methyl alanine, or alpha, aminoisobutyric acid (AIB). In some aspects, the DPP-IV protective amino acid is D-Ser, or a conservative amino acid substitution thereof, or an α,α-disubstituted amino acid. In some aspects, the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group

consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring. In some aspects, the α,α-disubstituted amino acid is AIB. In exemplary aspects, when the DPP-IV protective amino acid is D-Ser, the GIP agonist peptide is not conjugated to a heterologous moiety, e.g., a hydrophilic moiety (e.g., PEG). In other aspects of the present disclosures, the DPP-IV protective amino acid is not D-serine.

[00296] In some aspects, the glucagon analog comprises an alpha helix stabilizing amino acid at any of positions 16, 17, 18, 19, 20, or 21. In some aspects, the glucagon analog comprises an alpha helix stabilizing amino acid at one, two, three, four, five, or all of positions 16, 17, 18, 19, 20, or 21. In exemplary aspects, the glucagon analog comprises an alpha helix stabilizing amino acid at positions 16, 17, 20, and 21. In some aspects, the glucagon analog comprises an alpha helix stabilizing amino acid at positions 16 and 20. In alternative or additional aspects, the glucagon analog comprises an alpha helix stabilizing amino acid at positions 17 and 21.

[00297] In some aspects, when the glucagon analog comprises an alpha helix stabilizing amino acid at position 20, the amino acid at position 20 is an alpha, alpha disubstituted amino acid. In exemplary aspects, the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring. In some embodiemtns, the α,α-disubstituted amino acid is 1-aminocyclopropane-1-carboxylate (ACPC). In some aspects, the α,α-disubstituted at position 20 is AIB. Optionally, in some embodiments, when the amino acid at position 20 is an alpha, alpha disubstituted amino acid, the amino acid at position 16 is an alpha helix stabilizing amino acid other than AIB. In exemplary embodiments, the amino acid at position 16 is a charged amino acid, e.g., a positive-charged amino acid, negative charged amino acid. In some aspects, when the amino acid at position 20 is an alpha, alpha disubstituted amino acid, the amino acid at position 16 is a positive-charged amino acid of Formula IV, e.g., Lys, or is a negative-charged amino acid, e.g., Glu. In some aspects, when the amino acid at position 20 is an alpha, alpha disubstituted amino acid, the amino acid at position 16 is a charge neutral amino acid, e.g., Ser, Ala, Gly.

[00298] Accordingly, in exemplary embodiments, the glucagon analog comprises (i) an amino acid comprising an imidazole side chain at position 1, (ii) a DPP-IV protective amino acid at position 2, optionally, aminoisobutyric acid, (iii) an amino acid comprising a non-native acyl or alkyl group, optionally at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the non-native acyl or alkyl group is linked to such amino acid via a spacer; (iv) an alpha, alpha disubstituted amino acid at position 20, and (v) up to ten (e.g., up to 1, 2, 3, 4, 5, 6, 7, 8 or 9) additional amino acid modifications relative to SEQ ID NO: 1.

[00299] In exemplary embodiments, when the glucagon analog comprises an alpha, alpha disubstituted amino acid at position 20, and when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits a GIP percentage potency of at least 0.1% (e.g., at least 1%, at least 10%, at least 20%). In exemplary embodiments , the glucagon analog has less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold) selectivity for the human GLP-1 receptor versus the GIP receptor. In exemplary aspects, the peptide has an EC50 at the GIP receptor which is less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold) different than its EC50 at the GLP-1 receptor, which, optionally, is less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold), different from its EC50 at the glucagon receptor.

[00300] In exemplary embodiments, when the glucagon analog comprises an alpha, alpha disubstituted amino acid at position 20, the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring. In exemplary embodiments, the the α,α-disubstituted amino acid at position 20

is AIB. Also, in exemplary embodiments, when the glucagon analog comprises an alpha, alpha disubstituted amino acid at position 20, the amino acid at position 16 is an alpha helix stabilizing amino acid other than AIB. In exemplary aspects, the amino acid at position 16 is a charged amino acid, optionally, a negative charged amino acid (e.g., Glu or Asp) or a positive charged amino acid (e.g., Lys or Orn).

[00301] In alternative embodiments, the glucagon analog does not comprise an alpha helix stabilizing amino acid at position 20, and one or more of positions 16, 17, 18, 19, or 21 is an alpha helix stabilizing amino acid. In some aspects, the alpha helix stabilizing amino acid is located at position 16. In some embodiments, the alpha helix stabilizing amino acid is a negative charged amino acid (e.g., Glu), a positive-charged amino acid, (e.g., comprising a structure of Formula IV (e.g., Lys)), or an alpha, alpha disubstituted amino acid. In some aspects, the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring. In specific aspects, the α,α-disubstituted amino acid at position 16 is AIB.

[00302] In additional embodiments, when the glucagon analog does not comprise an alpha helix stabilizing amino acid at position 20, and when one or more of positions 16, 17, 18, 19, or 21 is an alpha helix stabilizing amino acid, the glucagon analog comprises (i) an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 of the peptide analog or (ii) the acylated amino acid or alkylated amino acid is located at position 10, 12, or 16. In some aspects, the glucagon analog comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 of the peptide analog, and optionally the amino acid at position 29 is Gly. The extension of 1 to 21 amino acids in some aspects is any of those described herein – see, e.g., the section entitled "*C-terminal Extensions*." In some aspects, the extension comprises an amino acid sequence which forms a Trp cage structure, e.g., the extension comprises the amino acid sequence of GPSSGAPPPS (SEQ ID NO: 5), or a conservatively substituted sequence thereof. In alternative aspects, the extension of 1 to 21 amino acids comprises at least one charged amino acid. In exemplary aspects, the extension comprises an amino acid sequence of: X1-X2, wherein X1 is a charged amino acid and X2 is a small

aliphatic amino acid. In some aspects, X1 is a positive charged amino acid, e.g., Arg. In some aspects, extension comprises Arg-Gly.

[00303] Accordingly, in alternative exemplary embodiments, the glucagon analog comprises (i) an amino acid comprising an imidazole side chain at position 1, (ii) a DPP-IV protective amino acid at position 2, optionally, aminoisobutyric acid, (iii) an amino acid comprising a non-native acyl or alkyl group, optionally at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the non-native acyl or alkyl group is linked to such amino acid via a spacer; (iv) an alpha helix stability amino acid at one or more of positions 16-21, optionally, position 16, wherein the analog does not comprise an alpha helix stabilizing amino acid at position 20, and (v) up to ten (e.g., up to 1, 2, 3, 4, 5, 6, 7, 8 or 9) additional amino acid modifications relative to SEQ ID NO: 1. In exemplary aspects, the analog does not comprise at position 20 an alpha, alpha di-substituted amino acid, optionally, AIB, or an alpha helix stabilizing amino acid selected from the group consisting of: Leu, Phe, Ala, Met, Gly, Ile, Ser, Asn, Glu, Asp, Lys, and Arg. In exemplary aspects, the glucagon analog is not modified at position 20 compared to SEQ ID NO: 1, and, therefore has Gln residue which is the native amino acid of glucagon at this position.

[00304] In exemplary embodiments, when the glucagon analog comprises an alpha helix stability amino acid at one or more of positions 16-21, optionally, position 16, and the analog does not comprise an alpha helix stabilizing amino acid at position 20, and when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits a GIP percentage potency of at least 0.1% (e.g., at least 1%, at least 10%, at least 20%). In exemplary embodiments , the glucagon analog has less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold) selectivity for the human GLP-1 receptor versus the GIP receptor. In exemplary aspects, the peptide has an EC50 at the GIP receptor which is less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold) different than its EC50 at the GLP-1 receptor, which, optionally, is less than 100-fold (e.g.,

less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold), different from its EC50 at the glucagon receptor.

[00305] In exemplary aspects, when the glucagon analog comprises an alpha helix stability amino acid at one or more of positions 16-21 and the analog does not comprise an alpha helix stabilizing amino acid at position 20, the glucagon analog comprises an alpha helix stabilizing amino acid at position 16, optionally, wherein the alpha helix stabilizing amino acid is a negative charged amino acid (e.g., Glu or Asp) or an alpha, alpha disubstituted amino acid. The α,α-disubstituted amino acid at position 16 may comprise R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring. In exemplary aspects, the amino acid at position 16 is AIB. In exemplary aspects, the glucagon analog comprises at position 18 a small aliphatic amino acid, optionally, Ala, and an Arg at position 17. In exemplary aspects, the glucagon analog comprises the sequence ERAAQ (SEQ ID NO: 200) as positions 16 through 20 or ERAAQD (SEQ ID NO: 201) as positions 16 through 21.

[00306] In exemplary aspects, the glucagon analog comprises an amino acid comprising a non-native acyl or alkyl group at any one of positions 9, 10, 12, 16, 20. In exemplary aspects, the glucagon analog comprises an amino acid covalently attached to a C12 to C18 acyl group or alkyl group at any one or more of positions 9, 10, 12, 13, 14, 16, 17, and 20. In exemplary aspects, the glucagon analog comprises an amino acid covalently attached to a 12 to C18 acyl group or alkyl group at any one or more of positions 10, 14 In some aspects, the glucagon analog comprises an acylated amino acid or alkylated amino acid at position 10, 12, or 16. In exemplary aspects, the acylated amino acid or alkylated amino acid is at position 14. In some aspects, the glucagon analog comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 and comprises an amino acid comprising a non-native acyl or alkyl group at any of positions 37-43 (e.g., 37, 38, 39, 40, 41, 42, 43). In some aspects, the amino acid comprising a non-native acyl or alkyl group is at position 40.

[00307] In exemplary embodiments, the acyl

[00308] In exemplary aspects, the glucagon analog comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 and, in some aspects, the extension forms a structure known in the art as a Trp cage. In some aspects, the extension comprises the amino acid sequence GPSSGAPPPS (SEQ ID NO: 5) or GGPSSGAPPPS (SEQ ID NO: 6) or GPSSGRPPPS (SEQ ID NO: 183) or a sequence of one of the foregoing with 1, 2, or 3 conservative amino acid substitutions. In alternative aspects, the extension comprises at least one charged amino acid, e.g., the extension comprises an amino acid sequence of: X1-X2, wherein X1 is a charged amino acid (e.g., a positive charged amino acid (e.g., Arg)) and X2 is a small aliphatic amino acid. In some aspects, the extension comprises Arg-Gly.

[00309] In exemplary aspects, the acylated amino acid or alkylated amino acid comprises a structure of Formula I(optionally, Lys), Formula II, (optionally, Cys), or Formula III, (optionally, Ser). Optionally, in some aspects, the acylated amino acid or alkylated amino acid comprises a structure of Formula I, e.g., Lys.

[00310] In some embodiments, the acylated or alkylated amino acid is an aromatic amino acid comprising a side chain amine. In exemplary aspects, the aromatic amino acid comprising a side chain amine is 4-amino-phenylalanine (4-aminoPhe), p-amino phenylglycine, p-amino homophenylalanine, or 3-amino tyrosine. In exemplary aspects, the aromatic amino acid comprising a side chain amine is 4-amino-Phe. In exemplary aspects, the acylated or alkylated amino acid is an amino acid of Formula II, n is 2 (homoserine). In exemplary aspects, the acylated or alkylated amino acid is Thr or homothreonine. In exemplary embodiments, the acylated or alkylated amino acid is an aromatic amino acid comprising a side chain hydroxyl, including but not limited to tyrosine, homotyrosine, methyl-tyrosine, or 3-amino tyrosine.

[00311] In exemplary aspects, the glucagon analog comprises an amino acid covalently attached to a Cx-succinoyl, wherein x is an integer between 10 and 26, optionally, between 12 and 18. In exemplary aspects, the Cx-succinoyl is attached to the peptide or glucagon analog via a spacer. The spacer may be any one of those described herein.

[00312] In some aspects, the acylated amino acid or alkylated amino acid is linked to the acyl group or alkyl group via a spacer. In some aspects, the spacer is 3 to 10 atoms in length.

In some aspects, the spacer is an amino acid or dipeptide, and, in some aspects, the spacer comprises one or two acidic amino acid residues, e.g., Glu. In some aspects, the acyl or alkyl group is linked to the amino acid via wherein the total length of the spacer and the acyl group is about 14 to about 28 atoms in length. In some aspects, the spacer comprises a Cys. In some aspects, the spacer comprises one or two gamma-Glu. In some aspects, the spacer comprises a Lys. In some aspects, the spacer comprises a combination of two of Cys, gamma-Glu, and Lys, or two gamma-Glu residues.

[00313] In particular aspects, the spacer is a Cys residue, which is covalently attached to an alkyl group, e.g., a non-functionalized or functionalized carbon chain. In exemplary aspects, the Cys residue is S-palmityl alkylated (i.e., S-palmitate alkylated), optionally, wherein the Cys residue is attached to a Lys residue which is part of the peptide backbone. In alternative embodiments, the spacer is a dipeptide comprising a Cys residue, which is covalently attached to an alkyl group. In exemplary aspects, the Cys is S-palmityl alkylated, and the Cys is attached to another amino acid of the spacer, which, in turn, is attached to, e.g., a Lys residue which is part of the peptide backbone.

[00314] In exemplary aspects, the spacer comprises a small polyethylene glycol moiety (PEG) comprising a structure $[-O-CH_2-CH_2-]_n$, wherein n is an integer between 2 and 16, (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16).

[00315] With regard to the acylated amino acid, the acyl group in some aspects is a C12 to C18 (e.g., C12, C13, C14, C15, C16, C17, C18) fatty acyl group. In some aspects, the acyl group is a C14 or C16 fatty acyl group. In alternative aspects, the acyl group is a succinic acid or a succinic acid derivative (e.g., a succinic acid derivative of Formula V, VI, or VII). In alternative aspects, the acyl group is a maleic acid or a maleic acid derivative (e.g., a maleic acid derivative of Formula VIII, IX, or X).

[00316] With regard to the alkylated amino acid, the non-native alkyl group in some aspects is a carboxy-functionalized carbon chain of structure –Cx-COOH, wherein x is an integer, optionally an integer between 4 – 30 (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30).

[00317] In exemplary aspect, the peptide or glucagon analog comprises two or more acyl or alkyl groups. In this regard, the peptide or glucagon analog may be a diacylated or dual

acylated peptide. The two or more acyl or alkyl groups may be arranged in a linear formation, optionally with intervening spacers. The two or more acyl or alkyl groups may be arranged in a branched formation, as described herein. In exemplary aspects, the two acyl or alkyl groups are attached to a Lys spacer residue.

[00318] In some aspects of the present disclosures, the glucagon analog comprises at least one charged amino acid C-terminal to the amino acid at position 27. For example, in some aspects, the glucagon analog comprises a charged amino acid (e.g., a negative charged amino acid) at position 28. The negative charged amino acid in some aspects is Asp. In alternative aspects, the amino acid at position 28 is a positive charged amino acid, e.g., a positive charged amino acid is an amino acid of Formula I, e.g., Lys.

[00319] In alternative or additional aspects, the glucagon analog comprises an amino acid modification at position 27, at position 29, or at both positions 27 and 29. For example, the amino acid at position 27 is in some aspects is Leu, Nle, Val, or Lys and/or the amino acid at position 29 is in some aspects Gly or Thr.

[00320] The glucagon analogs described herein may comprise additional amino acid modifications, e.g., up to ten (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) additional amino acid modifications, relative to SEQ ID NO: 1, as further discussed herein. In exemplary aspects, the glucagon analog comprises one or more of:

a) a DPP-IV protective amino acid at position 1 of the peptide analog;
b) an acidic amino acid, optionally, Glu, at position 3;
c) an Ile at position 7;
d) an Ile or Arg at position 12;
e) an acidic amino acid, optionally, Glu, at position 15;
f) an aliphatic amino acid, optionally, Ala, at position 18;
g) an acidic amino acid, optionally, Glu, at position 21;
h) an Asn, Ala, or AIB at position 24;
i) an aliphatic amino acid, optionally, Ala, or Leu, or Nle, at position 27;

j) an acidic amino acid, optionally, Glu, or an aliphatic amino acid, optionally, Ala, at position 28;

k) an aliphatic amino acid, optionally, Ala, at position 29;

l) amidation at the C-terminus.

[00321] In accordance with the foregoing, the glucagon analog in exemplary aspects comprises the amino acid sequence of any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, and 90 or any of SEQ ID NOs: 94-100, 102-112, 120-124, 127-131. In exemplary aspects, the glucagon analog comprises or consists of any of SEQ ID NOs: 94-100, 102-112, 120-124, 127-131. In exemplary aspects, the glucagon analog comprises or consists of any of SEQ ID NOs: 28, 29, 31, 37-41, 43-46, 76-80, 83-87, 89, and 90. In exemplary aspects, the glucagon analog comprises of consists of any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 27. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 30. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 32. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 33. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 35. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 36. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 28. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 37. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 89. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 31. In exemplary aspects, the glucagon analog comprises or consists of SEQ ID NO: 180.

[00322] The invention further provides a peptide comprising the sequence of SEQ ID NO: 28. In exemplary aspects, the peptide consists of SEQ ID NO: 28.

[00323] A peptide comprising the sequence of SEQ ID NO: 31 is also provided by the invention. In exemplary aspects, the peptide consists of SEQ ID NO: 31.

[00324] The invention furthermore provides a peptide comprising the sequence of SEQ ID NO: 37. In exemplary aspects, the peptide consists of SEQ ID NO: 37.

[00325] The invention moreover provides peptide comprising the sequence of SEQ ID NO: 89. In exemplary aspects, the peptide consists of SEQ ID NO: 89.

[00326] The invention moreover provides peptide comprising the sequence of SEQ ID NO: 95. In exemplary aspects, the peptide consists of SEQ ID NO: 95.

[00327] The invention moreover provides peptide comprising the sequence of SEQ ID NO: 130. In exemplary aspects, the peptide consists of SEQ ID NO: 130.

[00328] Additionally, a peptide comprising the sequence of SEQ ID NO: 31 is provided herein. In exemplary aspects, the peptide consists of SEQ ID NO: 171.

[00329] A peptide comprising the seuqnce of SEQ ID NO: 180 is also provided by the invention. In exemplary aspects, the peptide consists of SEQ ID NO: 180.

[00330] The invention provides a peptide comprising the sequence of SEQ ID NO: 184,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEQ ID NO: 184)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog;

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala or Gly;

X_{35} is Ala or a basic amino acid (optionally, Arg or Lys);

wherein, when X_{28} is an acidic amino acid, X_{35} is a basic amino acid;

wherein, when X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41, and

wherein the C-terminal amino acid of the peptide is amidated.

[00331] In exemplary aspects, X10 of SEQ ID NO: 184 is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and the peptide optionally comprises Gly at position 41. In exemplary aspects, X10 of SEQ ID NO: 184 is an amino acid covalently attached to a C12-C18 acyl or alkyl group.

[00332] In exemplary aspects, X20 of SEQ ID NO: 184 is Gln, and optionally, the amino acid at position 16 is a negative charged amino acid (e.g., Glu). In exemplary aspects, X18 of SEQ ID NO: 184 is Ala and the peptide comprises E16, R17, A18, A19, and Q20.

[00333] In alternative exemplary aspects, X20 of SEQ ID NO: 184 is AIB. Optionally, X_{16} of SEQ ID NO: 184 is any amino acid other than AIB.

[00334] Also, in exemplary aspects, (i) X_{28} of SEQ ID NO: 184 is an acidic amino acid, optionally Asp or Glu, and X_{35} of SEQ ID NO: 184 is a basic amino acid, optionally Arg or Lys, (ii) only one of X_{27}, X_{28} and X_{29} of SEQ ID NO: 184 is an Ala, (iii) the peptide comprise an amidated Gly at the C-terminus.

[00335] The invention also provides a peptide comprising the sequence of SEQ ID NO: 184 with up to 3 amino acid modifications (e.g., conservative substitutions) relative to SEQ ID NO: 184, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor. In exemplary embodiments, the activity (e.g., the EC50) at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor of the glucagon analog is within 100-fold (e.g., within 50-fold, within 25-fold, within 10-fold) of one another.

[00336] The invention additionally provides a peptide comprising the sequence of SEQ ID NO: 185,

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGAPPPS$

(SEQ ID NO: 185)

wherein:

X_2 is AIB;

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{16} is Glu, an alpha, alpha disubstituted amino acid, Lys or

X_{18} is Arg or Ala;

X_{20} is AIB or Gln;

X_{21} is Asp or Glu;

X_{27} is Leu, Nle, or Met;

X_{28} is Ala, Asp or Glu;

X_{29} is Gly of Thr;

and

wherein, when X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41, and

wherein the C-terminal amino acid of the peptide is amidated.

[00337] The invention also provides a peptide comprising the sequence of SEQ ID NO: 185 with up to 3 amino acid modifications (e.g., conservative substitutions) relative to SEQ ID NO: 185, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor. In exemplary embodiments, the activity (e.g., the EC50) at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor of the glucagon analog is within 100-fold (e.g., within 50-fold, within 25-fold, within 10-fold) of one another.

[00338] The invention also provides a peptide comprising the sequence of SEQ ID NO: 196,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEQ ID NO: 196)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog or an amino acid that reduces glucagon activity (optionally Glu);

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala or Gly;

X_{35} is Ala or a basic amino acid (optionally, Arg or Lys);

wherein, when X_{28} is an acidic amino acid, X_{35} is a basic amino acid;

wherein, when X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41, and

wherein the C-terminal amino acid of the peptide is amidated.

[00339] Additional amino acids that reduce glucagon activity are described herein. See section entitled "Position 3." In exemplary aspects, X_3 is an acidic, basic, or hydrophobic amino acid (e.g., glutamic acid, ornithine, norleucine). In exemplary aspects, X_3 is Glu.

[00340] Furthermore provided is a peptide comprising the sequence of SEQ ID NO: 186:

$$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$$

(SEQ ID NO: 186)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog;

X_{10} is Tyr or an amino acid covalently attached to a C10 to C26 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is a negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala, Gly or Thr; and

wherein the peptide comprises an amino acid covalently attached to a C10 to C26 acyl or alkyl group, optionally, at position 10, and the C-terminal amino acid of the peptide is amidated.

[00341] In exemplary aspects, X_{20} of SEQ ID NO: 186 is AIB. In exemplary aspects, X_{29} is Thr and the peptide does not comprise GPSSGAPPPS (SEQ ID NO: 5). In some aspects, X_{20} of SEQ ID NO: 186 is AIB and X_{16} is an amino acid other than AIB.

[00342] In exemplary aspects, X_{20} is Gln. In some aspects, X_{16} is a negative charged amino acid, optionally, Glu. In exemplary aspects, X_{18} is Ala, and optionally, the peptide comprises E16, R17, A18, A19, and Q20.

[00343] In some aspects, the peptide of SEQ ID NO: 186 comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, and optionally the amino acid at position 29 is Gly. In exemplary aspects, the extension comprises the amino acid sequence GPSSGAPPPS (SEQ ID NO: 5), or a conservatively substituted sequence thereof, or wherein the extension comprises the sequence X1-X2, wherein X1 is a charged amino acid and X2 is a small aliphatic amino acid, optionally, wherein X1 is a positive charged amino acid. In some aspects, the positive charged amino acid is Arg and optionally the peptide comprises or consists of Arg-Gly. In certain aspects, the extension comprises the amino acid sequence GPSSGAPPPS (SEQ ID NO: 5) followed by Lys or Lys-Gly, wherein the Lys is covalently attached to an C10 to C26 acyl group.

[00344] In exemplary aspects, the peptide comprises SEQ ID NO: 186, wherein X_2 is AIB, X_3 is Gln, X_{10} is an amino acid covalently attached to a C10 to C26 acyl or alkyl group, X_{18} is Arg or Ala, X_{20} is AIB or Gln, X_{21} is Asp or Glu, X_{29} is Gly, and the C-terminal amino acid is amidated, wherein Gly at position 29 is fused to GPSSGAPPPS followed by Lys or Lys-Gly, wherein the Lys is covalently attached to a C10-C26 acyl group.

[00345] The invention also provides a peptide comprising the sequence of SEQ ID NO: 186 with up to 3 amino acid modifications (e.g., conservative substitutions) relative to SEQ ID NO: 186, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor. In exemplary embodiments, the activity (e.g., the EC50) at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor of the glucagon analog is within 100-fold (e.g., within 50-fold, within 25-fold, within 10-fold) of one another.

[00346] The invention provides a peptide comprising the sequence of SEQ ID NO: 187:

$$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$$

(SEQ ID NO: 187)

wherein:

X_2 is AIB;

X_{10} is Tyr or an amino acid covalently attached to a C10 to C26 acyl or alkyl group;

X_{16} is Glu, alpha, alpha-disubstituted amino acid, or Lys;

X_{18} is Arg or Ala;

X_{20} is a negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala, Asp or Glu;

X_{29} is Gly or Thr; and

wherein the peptide comprises an amino acid covalently attached to a C12 to C18 acyl or alkyl group, optionally, at position10, and the C-terminal amino acid of the peptide is amidated.

[00347] The invention also provides a peptide comprising the sequence of SEQ ID NO: 187 with up to 3 amino acid modifications (e.g., conservative substitutions) relative to SEQ ID NO: 187, wherein the analog exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor. In exemplary embodiments, the activity (e.g., the EC50) at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor of the glucagon analog is within 100-fold (e.g., within 50-fold, within 25-fold, within 10-fold) of one another.

[00348] The invention provides a peptide comprising the sequence of SEQ ID NO: 198:

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SEQ ID NO: 198)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog or an amino acid that reduces glucagon activity (e.g., Glu);

X_{10} is Tyr or an amino acid covalently attached to a C10 to C26 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is a negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala, Gly or Thr; and

wherein the peptide comprises an amino acid covalently attached to a C10 to C26 acyl or alkyl group, optionally, at position 10, and the C-terminal amino acid of the peptide is amidated.

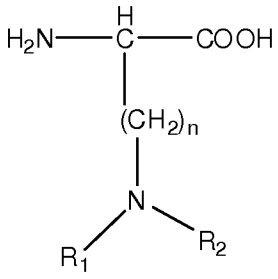
[00349] Additional amino acids that reduce glucagon activity are described herein. See section entitled "Position 3." In exemplary aspects, X_3 is an acidic, basic, or hydrophobic amino acid (e.g., glutamic acid, ornithine, norleucine). In exemplary aspects, X_3 is Glu.

[00350] The invention provides a peptide comprising SEQ ID NO: 184. The invention provides a peptide comprising SEQ ID NO: 185. The invention provides a peptide comprising SEQ ID NO: 196. The invention provides a peptide comprising SEQ ID NO: 186. The invention provides a peptide comprising SEQ ID NO: 187. The invention provides a peptide comprising SEQ ID NO: 198.

[00351] Furthermore provided herein is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, comprising:

(a) an amino acid comprising an imidazole side chain at position 1,

(b) at position 16, an amino acid of Formula IV:



[Formula IV],

wherein n is 1 to 7, wherein each of R1 and R2 is independently selected from the group consisting of H, C1-C18 alkyl, (C1-C18 alkyl)OH, (C1-C18 alkyl)NH2, (C1-C18 alkyl)SH, (C0-C4 alkyl)(C3-C6)cycloalkyl, (C0-C4 alkyl)(C2-C5 heterocyclic), (C0-C4 alkyl)(C6-C10 aryl)R7, and (C1-C4 alkyl)(C3-C9 heteroaryl), wherein R7 is H or

OH, wherein optionally the side chain of the amino acid of Formula IV comprises a free amino group,

(c) an α,α-disubstituted amino acid at position 20,

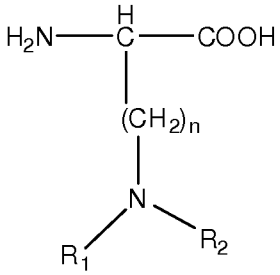
(d) up to ten additional amino acid modifications relative to SEQ ID NO: 1,

wherein, when the analog lacks a hydrophilic moiety, the glucagon analog exhibits at least 0.1% activity of native GIP at the GIP receptor, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

[00352] In exemplary embodiments, the analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, comprises one or more of:

(a) an amino acid comprising an imidazole side chain at position 1,

(b) at position 16, an amino acid of Formula IV:



[Formula IV],

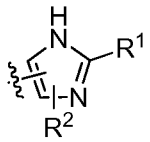
wherein n is 1 to 7, wherein each of R1 and R2 is independently selected from the group consisting of H, C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)NH_2, (C_1-C_{18} alkyl)SH, (C_0-C_4 alkyl)(C_3-C_6)cycloalkyl, (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and (C_1-C_4 alkyl)(C_3-C_9 heteroaryl), wherein R_7 is H or OH, optionally, wherein the side chain of the amino acid of Formula IV comprises a free amino group,

(c) an α,α-disubstituted amino acid at position 20,

(d) up to ten additional amino acid modifications relative to SEQ ID NO: 1,

wherein, when the analog lacks a heterologous moiety, e.g., a hydrophilic moiety (e.g., PEG), the glucagon analog exhibits at least or about 0.1% (e.g., at least or about 1%, at least or about 10%, at least or about 50%, at least or about 80%, at least or about 100%, at least or about 500%) activity of native GIP at the GIP receptor. In exemplary aspects, the peptide has an EC50 at the GIP receptor which is less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold), different than its EC50 at the GLP-1 receptor, which, optionally, is less than 100-fold (e.g., less than or about 90-fold, less than or about 80-fold, less than or about 70-fold, less than or about 60-fold, less than or about 50-fold, less than or about 40-fold, less than or about 30-fold, less than or about 20-fold, less than or about 15-fold, less than or about 10-fold, less than or about 5-fold), different from its EC50 at the glucagon receptor.

[00353] In exemplary embodiments, the glucagon analog comprises at position 1 an amino acid comprising a structure of Formula A



[Formula A],

wherein each of R1 and R2 independently is selected from the group consisting of H, (C1-6)alkyl, O(C1-6)alkyl, (C1-6)alkyl-OH, F, and (C1-C6)alkyl of which at least one H is replaced by F.

[00354] In exemplary aspects, the amino acid at position 1 is the native residue of glucagon (SEQ ID NO: 1) L-histidine (His), or is a derivative of His (His derivative), e.g., D-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine, homo-histidine, N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, or alpha, alpha-dimethyl imidiazole acetic acid (DMIA).

[00355] In exemplary aspects, the glucagon analog comprises the amino acid of Formula IV at position 16 in (b) is homoLys, Lys, Orn, or 2,4-diaminobutyric acid (Dab).

[00356] In exemplary aspects, the amino acid at position 20, e.g., the α,α-disubstituted amino acid, comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring. In exemplary aspects, the α,α-disubstituted at position 20 is AIB. In other exemplary aspects, the α,α-disubstituted at position 20 is ACPC.

[00357] In exemplary aspects, the glucagon analog comprises up to ten additional modifications, relative to SEQ ID NO: 1. In exemplary aspects, the glucagon analog comprises an amino acid substitution, relative to SEQ ID NO: 1, at one or more of positions 2, 12, 17, 18, 21, 24, 27, 28, and 29. In exemplary aspects, the glucagon analog comprises one or more of:

i. a DPP-IV protective amino acid at position 2; optionally AIB or D-Ser;

ii. a large, aliphatic, nonpolar amino acid at position 12, optionally Ile;

iii. an amino acid orther than Arg at position 17, optionally Gln;

iv. a small aliphatic amino acid at position 18, optionally Ala;

v. an amino acid other than Asp at position 21, optionally Glu;

vi. an amino acid other than Gln at position 24, optionally Asn or Ala;

vii. an amino acid other than Met at position 27, optionally Leu;

viii. an amino acid other than Asn at position 28, optionally Ala;

ix. an amino acid other than Thr at position 29, optionally Gly; and

x. an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29.

[00358] In exemplary aspects, the glucagon analog comprises an extension of GPSSGAPPPS or GPSSGAPPPSC.

[00359] In exemplary aspects, the glucagon analog comprising a His at position 1, Lys at position 16 and AIB at position 20 does not comprise Gln-Ala at positions 17-18.

[00360] In other exemplary embodiments, the glucagon analog comprises an amino acid sequence of any of SEQ ID NOs: 48, 52, 53, and 74. Such glucagon analogs are similar in structure to those of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, and 90, except that the former glucagon analogs (SEQ ID NOs: 48, 52, 53, and 74) do not comprise an acylated amino acid or alkylated amino acid.

[00361] In yet other exemplary embodiments, the glucagon analog comprises an amino acid sequence of any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, and 92 or any of SEQ ID NOs: 114-119, 125, 126, and 133, or any of SEQ ID NOs: 139-144, 150-153, 208, 210, and 211. Such glucagon analogs comprise a large, aromatic amino acid at position 1, e.g., Tyr.

[00362] In some embodiments, the GIP agonist peptides comprise an amino acid sequence of any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, and 133. In some embodiments, the GIP agonist peptide comprises an amino acid sequence of any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230.

[00363] In some embodiments, the GIP agonist peptides comprise a structure based on a parent sequence comprising any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, and 133, or any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230, but differs from the parent sequence at one or more positions.

[00364] In some or any embodiments, the peptide of the present disclosures is an analog of a parent sequence comprising any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125,

126, and 133, or any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230 comprising an amino acid sequence based on the amino acid sequence of the parent sequence but differs from the parent sequence inasmuch as the amino acid sequence of the analog comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and in some instances, 16 or more (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, etc.), specified or optional amino acid modifications. In some or any embodiments, the peptide of the present disclosures comprises a total of 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 additional amino acid modifications relative to the parent sequence comprising any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, and 133, or any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230. In some or any embodiments, the modifications are any of those described herein with regard to glucagon analogs, e.g., acylation, alkylation, pegylation, truncation at C-terminus, substitution of the amino acid at one or more of positions 1, 2, 3, 7, 10, 12, 15, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, and 29.

[00365] In some or any embodiments, the modification is an amino acid substitution or replacement, e.g., a conservative amino acid substitution. In some aspects, the conservative substitution is a replacement of the amino acid at one or more of positions 2, 5, 7, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 27, 28 or 29. In alternative embodiments, the amino acid substitution is not a conservative amino acid substitution, e.g., is a non-conservative amino acid substitution.

[00366] In some embodiments, the peptide of the present disclosures comprises an amino acid sequence which has at least 25% sequence identity to the amino acid sequence of the parent sequence, which comprises any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, and 133, or any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230. In some embodiments, the peptide of the present disclosures comprises an amino acid sequence which is at least 30%, at

least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90% or has greater than 90% sequence identity to the parent sequence. In some embodiments, the amino acid sequence of the presently disclosed peptide which has the above-referenced % sequence identity is the full-length amino acid sequence of the presently disclosed peptide. In some embodiments, the amino acid sequence of the peptide of the present disclosures which has the above-referenced % sequence identity is only a portion of the amino acid sequence of the presently disclosed peptide. In some embodiments, the presently disclosed peptide comprises an amino acid sequence which has about A% or greater sequence identity to a reference amino acid sequence of at least 5 contiguous amino acids (e.g., at least 6, at least 7, at least 8, at least 9, at least 10 amino acids) of the parent sequence, wherein the reference amino acid sequence begins with the amino acid at position C of SEQ ID NO: 1 and ends with the amino acid at position D of SEQ ID NO: 1, wherein A is 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99; C is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 and D is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29. Any and all possible combinations of the foregoing parameters are envisioned, including but not limited to, e.g., wherein A is 90% and C and D are 1 and 27, or 6 and 27, or 8 and 27, or 10 and 27, or 12 and 27, or 16 and 27.

[00367] The analogs of the parent sequence comprising any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, and 90, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, and 92, or any of comprising any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230 described herein may comprise a peptide backbone of any number of amino acids, i.e., can be of any peptide length. In some embodiments, the peptides described herein are the same length as SEQ ID NO: 1, i.e., are 29 amino acids in length. In some embodiments, the presently disclosed peptide is longer than 29 amino acids in length, e.g., the presently disclosed peptide comprises a C-terminal extension of 1-21 amino acids, as further described herein. Accordingly, the peptide of the present disclosures in some embodiments, is 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length. In some embodiments, the presently disclosed peptide is up to 50 amino acids in length. In some embodiments, the presently disclosed peptide is longer

than 29 amino acids in length (e.g., greater than 50 amino acids, (e.g., at least or about 60, at least or about 70, at least or about 80, at least or about 90, at least or about 100, at least or about 150, at least or about 200, at least or about 250, at least or about 300, at least or about 350, at least or about 400, at least or about 450, at least or about 500 amino acids in length) due to fusion with another peptide. In other embodiments, the presently disclosed peptide is less than 29 amino acids in length, e.g., 28, 27, 26, 25, 24, 23, amino acids.

[00368] In accordance with the foregoing, in some aspects, the peptide of the present disclosures is an analog of a parent sequence comprising any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, and 133, or any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230 which sequence of the analog comprises one or more amino acid modifications which affect GIP activity, glucagon activity, and/or GLP-1 activity, enhance stability, e.g., by reducing degradation of the peptide (e.g., by improving resistance to DPP-IV proteases), enhance solubility, increase half-life, delay the onset of action, extend the duration of action at the GIP, glucagon, or GLP-1 receptor, or a combination of any of the foregoing. Such amino acid modifications, in addition to other modifications, are further described herein with regard to glucagon analogs, and any of these modifications can be applied individually or in combination.

METHODS OF MAKING PEPTIDES

[00369] The glucagon analogs of the disclosure can be obtained by methods known in the art. Suitable methods of de novo synthesizing peptides are described in, for example, Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2005; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2000; and U.S. Patent No. 5,449,752. Additional exemplary methods of making the peptides of the present disclosures are set forth in Example 1.

[00370] In some embodiments, the peptides described herein are commercially synthesized by companies, such as Synpep (Dublin, CA), Peptide Technologies Corp. (Gaithersburg, MD), and Multiple Peptide Systems (San Diego, CA). In this respect, the peptides can be synthetic, recombinant, isolated, and/or purified.

[00371] Also, in the instances in which the analogs of the disclosure do not comprise any non-coded or non-natural amino acids, the glucagon analog can be recombinantly produced using a nucleic acid encoding the amino acid sequence of the analog using standard recombinant methods. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual. 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994.

[00372] In some embodiments, the glucagon analogs of the disclosure are isolated. The term "isolated" as used herein means having been removed from its natural environment. In exemplary embodiments, the analog is made through recombinant methods and the analog is isolated from the host cell.

[00373] In some embodiments, the glucagon analogs of the disclosure are purified. The term "purified," as used herein relates to the isolation of a molecule or compound in a form that is substantially free of contaminants which in some aspects are normally associated with the molecule or compound in a native or natural environment and means having been increased in purity as a result of being separated from other components of the original composition. The purified peptide or compound include, for example, peptides substantially free of nucleic acid molecules, lipids, and carbohydrates, or other starting materials or intermediates which are used or formed during chemical synthesis of the peptides. It is recognized that "purity" is a relative term, and not to be necessarily construed as absolute purity or absolute enrichment or absolute selection. In some aspects, the purity is at least or about 50%, is at least or about 60%, at least or about 70%, at least or about 80%, or at least or about 90% (e.g., at least or about 91%, at least or about 92%, at least or about 93%, at least or about 94%, at least or about 95%, at least or about 96%, at least or about 97%, at least or about 98%, at least or about 99% or is approximately 100%.

CONJUGATES

[00374] The invention further provides conjugates comprising one or more of the glucagon analogs described herein conjugated to a heterologous moiety. As used herein, the term "heterologous moiety" is synonymous with the term "conjugate moiety" and refers to any molecule (chemical or biochemical, naturally-occurring or non-coded) which is different from the glucagon analogs described herein. Exemplary conjugate moieties that can be

linked to any of the analogs described herein include but are not limited to a heterologous peptide or polypeptide (including for example, a plasma protein), a targeting agent, an immunoglobulin or portion thereof (e.g.,variable region, CDR, or Fc region), a diagnostic label such as a radioisotope, fluorophore or enzymatic label, a polymer including water soluble polymers, or other therapeutic or diagnostic agents. In some embodiments a conjugate is provided comprising an analog of the present invention and a plasma protein, wherein the plasma protein is selected from the group consisting of albumin, transferin, fibrinogen and globulins. In some embodiments the plasma protein moiety of the conjugate is albumin or transferin. The conjugate in some embodiments comprises one or more of the glucagon analogs described herein and one or more of: a peptide (which is distinct from the glucagon and/or GLP-1 receptor active glucagon analogs described herein), a polypeptide, a nucleic acid molecule, an antibody or fragment thereof, a polymer, a quantum dot, a small molecule, a toxin, a diagnostic agent, a carbohydrate, an amino acid.

[00375] In some embodiments, the heterologous moiety is a peptide which is distinct from the glucagon analogs described herein and the conjugate is a fusion peptide or a chimeric peptide. In some embodiments, the heterologous moiety is a peptide extension of 1-21 amino acids. In specific embodiments, the extension is attached to the C-terminus of the glucagon analog, e.g., to amino acid at position 29.

[00376] In some specific aspects, the extension is a single amino acid or dipeptide. In specific embodiments, the extension comprises an amino acid selected from the group consisting of: a charged amino acid (e.g., a negative-charged amino acid (e.g., Glu), a positive-charged amino acid), an amino acid comprising a hydrophilic moiety. In some aspects, the extension is Gly, Glu, Cys, Gly-Gly, Gly-Glu.

[00377] In some embodiments, the extension comprises an amino acid sequence of SEQ ID NO: 5 (GPSSGAPPPS), SEQ ID NO: 6 (GGPSSGAPPPS), SEQ ID NO: 7 (KRNRNNIA), or SEQ ID NO: 8 (KRNR). In specific aspects, the amino acid sequence is attached through the C-terminal amino acid of the glucagon analog, e.g., amino acid at position 29. In some embodiments, the amino acid sequence of any of SEQ ID NOs: 5-8 is bound to amino acid 29 of the glucagon analog through a peptide bond. In some specific embodiments, the amino acid at position 29 of the glucagon analog is a Gly and the Gly is fused to one of the amino acid sequences of any of SEQ ID NOs: 5-8.

[00378] In some embodiments, the heterologous moiety is a polymer. In some embodiments, the polymer is selected from the group consisting of: polyamides, polycarbonates, polyalkylenes and derivatives thereof including, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polymers of acrylic and methacrylic esters, including poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate), polyvinyl polymers including polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, poly(vinyl acetate), and polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses including alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, and cellulose sulphate sodium salt, polypropylene, polyethylenes including poly(ethylene glycol), poly(ethylene oxide), and poly(ethylene terephthalate), and polystyrene.

[00379] In some aspects, the polymer is a biodegradable polymer, including a synthetic biodegradable polymer (e.g., polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone)), and a natural biodegradable polymer (e.g., alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins (e.g., zein and other prolamines and hydrophobic proteins)), as well as any copolymer or mixture thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion.

[00380] In some aspects, the polymer is a bioadhesive polymer, such as a bioerodible hydrogel described by H. S. Sawhney, C. P. Pathak and J. A. Hubbell in Macromolecules, 1993, 26, 581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl

methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

[00381] In some embodiments, the polymer is a water-soluble polymer or a hydrophilic polymer. Hydrophilic polymers are further described herein under "Hydrophilic Moieties." Suitable water-soluble polymers are known in the art and include, for example, polyvinylpyrrolidone, hydroxypropyl cellulose (HPC; Klucel), hydroxypropyl methylcellulose (HPMC; Methocel), nitrocellulose, hydroxypropyl ethylcellulose, hydroxypropyl butylcellulose, hydroxypropyl pentylcellulose, methyl cellulose, ethylcellulose (Ethocel), hydroxyethyl cellulose, various alkyl celluloses and hydroxyalkyl celluloses, various cellulose ethers, cellulose acetate, carboxymethyl cellulose, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, vinyl acetate/crotonic acid copolymers, poly-hydroxyalkyl methacrylate, hydroxymethyl methacrylate, methacrylic acid copolymers, polymethacrylic acid, polymethylmethacrylate, maleic anhydride/methyl vinyl ether copolymers, poly vinyl alcohol, sodium and calcium polyacrylic acid, polyacrylic acid, acidic carboxy polymers, carboxypolymethylene, carboxyvinyl polymers, polyoxyethylene polyoxypropylene copolymer, polymethylvinylether co-maleic anhydride, carboxymethylamide, potassium methacrylate divinylbenzene co-polymer, polyoxyethyleneglycols, polyethylene oxide, and derivatives, salts, and combinations thereof.

[00382] In specific embodiments, the polymer is a polyalkylene glycol, including, for example, polyethylene glycol (PEG).

[00383] In some embodiments, the heterologous moiety is a carbohydrate. In some embodiments, the carbohydrate is a monosaccharide (e.g., glucose, galactose, fructose), a disaccharide (e.g., sucrose, lactose, maltose), an oligosaccharide (e.g., raffinose, stachyose), or a polysaccharide (e.g., starch, amylase, amylopectin, cellulose, chitin, callose, laminarin, xylan, mannan, fucoidan, or galactomannan).

[00384] In some embodiments, the heterologous moiety is a lipid. The lipid, in some embodiments, is a fatty acid, eicosanoid, prostaglandin, leukotriene, thromboxane, N-acyl ethanolamine), glycerolipid (e.g., mono-, di-, tri-substituted glycerols), glycerophospholipid (e.g., phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine,

phosphatidylserine), sphingolipid (e.g., sphingosine, ceramide), sterol lipid (e.g., steroid, cholesterol), prenol lipid, saccharolipid, or a polyketide, oil, wax, cholesterol, sterol, fat-soluble vitamin, monoglyceride, diglyceride, triglyceride, a phospholipid.

[00385] In some embodiments, the heterologous moiety is attached via non-covalent or covalent bonding to the analog of the present disclosure. In exemplary aspects, the heterologous moiety is attached to the analog of the present disclosure via a linker. Linkage can be accomplished by covalent chemical bonds, physical forces such electrostatic, hydrogen, ionic, van der Waals, or hydrophobic or hydrophilic interactions. A variety of non-covalent coupling systems may be used, including biotin-avidin, ligand/receptor, enzyme/substrate, nucleic acid/nucleic acid binding protein, lipid/lipid binding protein, cellular adhesion molecule partners; or any binding partners or fragments thereof which have affinity for each other.

[00386] The glucagon analog in some embodiments is linked to conjugate moieties via direct covalent linkage by reacting targeted amino acid residues of the analog with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of these targeted amino acids. Reactive groups on the analog or conjugate moiety include, e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group. Derivatizing agents include, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride or other agents known in the art. Alternatively, the conjugate moieties can be linked to the analog indirectly through intermediate carriers, such as polysaccharide or polypeptide carriers. Examples of polysaccharide carriers include aminodextran. Examples of suitable polypeptide carriers include polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, e.g., serines, to confer desirable solubility properties on the resultant loaded carrier.

[00387] Cysteinyl residues are most commonly reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid, chloroacetamide to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, alpha-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-

chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

[00388] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

[00389] Lysinyl and amino-terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate.

[00390] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[00391] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively.

[00392] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R-N=C=N-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[00393] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), deamidation of

asparagine or glutamine, acetylation of the N-terminal amine, and/or amidation or esterification of the C-terminal carboxylic acid group.

[00394] Another type of covalent modification involves chemically or enzymatically coupling glycosides to the analog. Sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO87/05330 published 11 Sep. 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

[00395] In some embodiments, the glucagon analog is conjugated to a heterologous moiety via covalent linkage between a side chain of an amino acid of the glucagon analog and the heterologous moiety. In some embodiments, the glucagon analog is conjugated to a heterologous moiety via the side chain of an amino acid at position 16, 17, 21, 24, or 29, a position within a C-terminal extension, or the C-terminal amino acid, or a combination of these positions. In some aspects, the amino acid covalently linked to a heterologous moiety (e.g., the amino acid comprising a heterologous moiety) is a Cys, Lys, Orn, homo-Cys, or Ac-Phe, and the side chain of the amino acid is covalently bonded to a heterologous moiety.

[00396] In some embodiments, the conjugate comprises a linker that joins the glucagon analog to the heterologous moiety. In some aspects, the linker comprises a chain of atoms from 1 to about 60, or 1 to 30 atoms or longer, 2 to 5 atoms, 2 to 10 atoms, 5 to 10 atoms, or 10 to 20 atoms long. In some embodiments, the chain atoms are all carbon atoms. In some embodiments, the chain atoms in the backbone of the linker are selected from the group consisting of C, O, N, and S. Chain atoms and linkers may be selected according to their expected solubility (hydrophilicity) so as to provide a more soluble conjugate. In some embodiments, the linker provides a functional group that is subject to cleavage by an enzyme or other catalyst or hydrolytic conditions found in the target tissue or organ or cell. In some embodiments, the length of the linker is long enough to reduce the potential for steric hindrance. If the linker is a covalent bond or a peptidyl bond and the conjugate is a polypeptide, the entire conjugate can be a fusion protein. Such peptidyl linkers may be any length. Exemplary linkers are from about 1 to 50 amino acids in length, 5 to 50, 3 to 5, 5 to 10, 5 to 15, or 10 to 30 amino acids in length. Such fusion proteins may alternatively be

produced by recombinant genetic engineering methods known to one of ordinary skill in the art.

Conjugates: Fc fusions

[00397] As noted above, in some embodiments, the analogs are conjugated, e.g., fused to an immunoglobulin or portion thereof (e.g.,variable region, CDR, or Fc region). Known types of immunoglobulins (Ig) include IgG, IgA, IgE, IgD or IgM. The Fc region is a C-terminal region of an Ig heavy chain, which is responsible for binding to Fc receptors that carry out activities such as recycling (which results in prolonged half-life), antibody dependent cell-mediated cytotoxicity (ADCC), and complement dependent cytotoxicity (CDC).

[00398] For example, according to some definitions the human IgG heavy chain Fc region stretches from Cys226 to the C-terminus of the heavy chain. The "hinge region" generally extends from Glu216 to Pro230 of human IgG1 (hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by aligning the cysteines involved in cysteine bonding). The Fc region of an IgG includes two constant domains, CH2 and CH3. The CH2 domain of a human IgG Fc region usually extends from amino acids 231 to amino acid 341. The CH3 domain of a human IgG Fc region usually extends from amino acids 342 to 447. References made to amino acid numbering of immunoglobulins or immunoglobulin fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U.S. Department of Public Health, Bethesda, Md. In a related embodiments, the Fc region may comprise one or more native or modified constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE.

[00399] Suitable conjugate moieties include portions of immunoglobulin sequence that include the FcRn binding site. FcRn, a salvage receptor, is responsible for recycling immunoglobulins and returning them to circulation in blood. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. The major contact sites include amino acid residues 248, 250-257, 272, 285,

288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain.

[00400] Some conjugate moieties may or may not include FcγR binding site(s). FcγR are responsible for ADCC and CDC. Examples of positions within the Fc region that make a direct contact with FcγR are amino acids 234-239 (lower hinge region), amino acids 265-269 (B/C loop), amino acids 297-299 (C'/E loop), and amino acids 327-332 (F/G) loop (Sondermann et al., Nature 406: 267-273, 2000). The lower hinge region of IgE has also been implicated in the FcRI binding (Henry, et al., Biochemistry 36, 15568-15578, 1997). Residues involved in IgA receptor binding are described in Lewis et al., (J Immunol. 175:6694-701, 2005). Amino acid residues involved in IgE receptor binding are described in Sayers et al. (J Biol Chem. 279(34):35320-5, 2004).

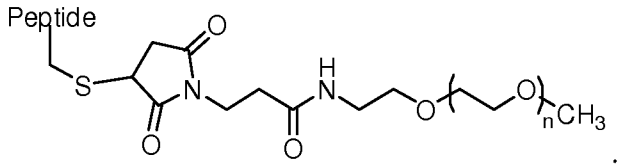
[00401] Amino acid modifications may be made to the Fc region of an immunoglobulin. Such variant Fc regions comprise at least one amino acid modification in the CH3 domain of the Fc region (residues 342-447) and/or at least one amino acid modification in the CH2 domain of the Fc region (residues 231-341). Mutations believed to impart an increased affinity for FcRn include T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591). Other mutations may reduce binding of the Fc region to FcγRI, FcγRIIA, FcγRIIB, and/or FcγRIIIA without significantly reducing affinity for FcRn. For example, substitution of the Asn at position 297 of the Fc region with Ala or another amino acid removes a highly conserved N-glycosylation site and may result in reduced immunogenicity with concomitant prolonged half-life of the Fc region, as well as reduced binding to FcγRs (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et al. 1995, J. Biol. Chem. 276:6591). Amino acid modifications at positions 233-236 of IgG1 have been made that reduce binding to FcγRs (Ward and Ghetie 1995, Therapeutic Immunology 2:77 and Armour et al. 1999, Eur. J. Immunol. 29:2613). Some exemplary amino acid substitutions are described in US Patents 7,355,008 and 7,381,408, each incorporated by reference herein in its entirety.

Conjugates: Hydrophilic moieties

[00402] The glucagon analogs described herein can be further modified to improve its solubility and stability in aqueous solutions at physiological pH, while retaining the high biological activity relative to native glucagon. Hydrophilic moieties such as PEG groups can

be attached to the analogs under any suitable conditions used to react a protein with an activated polymer molecule. Any means known in the art can be used, including via acylation, reductive alkylation, Michael addition, thiol alkylation or other chemoselective conjugation/ligation methods through a reactive group on the PEG moiety (e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group) to a reactive group on the target compound (e.g., an aldehyde, amino, ester, thiol, α-haloacetyl, maleimido or hydrazino group). Activating groups which can be used to link the water soluble polymer to one or more proteins include without limitation sulfone, maleimide, sulfhydryl, thiol, triflate, tresylate, azidirine, oxirane, 5-pyridyl, and alpha-halogenated acyl group (e.g., alpha-iodo acetic acid, alpha-bromoacetic acid, alpha-chloroacetic acid). If attached to the analog by reductive alkylation, the polymer selected should have a single reactive aldehyde so that the degree of polymerization is controlled. See, for example, Kinstler et al., *Adv. Drug. Delivery Rev.* 54: 477-485 (2002); Roberts et al., *Adv. Drug Delivery Rev.* 54: 459-476 (2002); and Zalipsky et al., *Adv. Drug Delivery Rev.* 16: 157-182 (1995).

[00403] In specific aspects, an amino acid residue of the analog having a thiol is modified with a hydrophilic moiety such as PEG. In some embodiments, the thiol is modified with maleimide-activated PEG in a Michael addition reaction to result in a PEGylated analog comprising the thioether linkage shown below:



[00404] In some embodiments, the thiol is modified with a haloacetyl-activated PEG in a nucleophilic substitution reaction to result in a PEGylated analog comprising a thioether linkage.

[00405] Suitable hydrophilic moieties include polyethylene glycol (PEG), polypropylene glycol, polyoxyethylated polyols (e.g., POG), polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), polyoxyalkylenes, polyethylene glycol propionaldehyde, copolymers of ethylene glycol/propylene glycol, monomethoxy-polyethylene glycol, mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol, carboxymethylcellulose, polyacetals, polyvinyl alcohol (PVA), polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, poly (β-amino acids)

(either homopolymers or random copolymers), poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers (PPG) and other polyakylene oxides, polypropylene oxide/ethylene oxide copolymers, colonic acids or other polysaccharide polymers, Ficoll or dextran and mixtures thereof. Dextrans are polysaccharide polymers of glucose subunits, predominantly linked by α1-6 linkages. Dextran is available in many molecular weight ranges, e.g., about 1 kD to about 100 kD, or from about 5, 10, 15 or 20 kD to about 20, 30, 40, 50, 60, 70, 80 or 90 kD. Linear or branched polymers are contemplated. Resulting preparations of conjugates may be essentially monodisperse or polydisperse, and may have about 0.5, 0.7, 1, 1.2, 1.5 or 2 polymer moieties per analog.

[00406] In some or any embodiments, the glucagon analog is conjugated to a hydrophilic moiety via covalent linkage between a side chain of an amino acid of the glucagon analog and the hydrophilic moiety. In some or any embodiments, the glucagon analog is conjugated to a hydrophilic moiety via the side chain of an amino acid at position 16, 17, 21, 24, or 29, a position within a C-terminal extension, or the C-terminal amino acid, or a combination of these positions. In some aspects, the amino acid covalently linked to a hydrophilic moiety (e.g., the amino acid comprising a hydrophilic moiety) is a Cys, Lys, Orn, homo-Cys, or Ac-Phe, and the side chain of the amino acid is covalently bonded to a hydrophilic moiety (e.g., PEG).

Conjugates: rPEG

[00407] In some or any embodiments, the conjugate of the present disclosure comprises the glucagon analog having GIP receptor agonist activity fused to an accessory analog which is capable of forming an extended conformation similar to chemical PEG (e.g., a recombinant PEG (rPEG) molecule), such as those described in International Patent Application Publication No. WO2009/023270 and U.S. Patent Application Publication No. US20080286808. The rPEG molecule in some aspects is a polypeptide comprising one or more of glycine, serine, glutamic acid, aspartic acid, alanine, or proline. In some aspects, the rPEG is a homopolymer, e.g., poly-glycine, poly-serine, poly-glutamic acid, poly-aspartic acid, poly-alanine, or poly-proline. In other embodiments, the rPEG comprises two types of amino acids repeated, e.g., poly(Gly-Ser), poly(Gly-Glu), poly(Gly-Ala), poly(Gly-Asp), poly(Gly-Pro), poly(Ser-Glu), etc. In some aspects, the rPEG comprises three different types of amino acids, e.g., poly(Gly-Ser-Glu). In specific aspects, the rPEG increases the half-life

of the Glucagon and/or GLP-1 agonist analog. In some aspects, the rPEG comprises a net positive or net negative charge. The rPEG in some aspects lacks secondary structure. In some embodiments, the rPEG is greater than or equal to 10 amino acids in length and in some embodiments is about 40 to about 50 amino acids in length. The accessory peptide in some aspects is fused to the N- or C- terminus of the analog of the present disclosure through a peptide bond or a proteinase cleavage site, or is inserted into the loops of the analog of the present disclosure. The rPEG in some aspects comprises an affinity tag or is linked to a PEG that is greater than 5 kDa. In some embodiments, the rPEG confers the analog of the present disclosure with an increased hydrodynamic radius, serum half-life, protease resistance, or solubility and in some aspects confers the analog with decreased immunogenicity.

Conjugates: Multimers

[00408] The invention further provides multimers or dimers of the analogs disclosed herein, including homo- or hetero- multimers or homo- or hetero- dimers. Two or more of the analogs can be linked together using standard linking agents and procedures known to those skilled in the art. For example, dimers can be formed between two peptides through the use of bifunctional thiol crosslinkers and bi-functional amine crosslinkers, particularly for the analogs that have been substituted with cysteine, lysine ornithine, homocysteine or acetyl phenylalanine residues. The dimer can be a homodimer or alternatively can be a heterodimer. In exemplary embodiments, the linker connecting the two (or more) analogs is PEG, e.g., a 5 kDa PEG, 20 kDa PEG. In some embodiments, the linker is a disulfide bond. For example, each monomer of the dimer may comprise a Cys residue (e.g., a terminal or internally positioned Cys) and the sulfur atom of each Cys residue participates in the formation of the disulfide bond. In exemplary aspects, each monomer of the dimer is linked via a thioether bond. In exemplary aspects, an epsilon amine of a Lys residue of one monomer is bonded to a Cys residue, which, in turn, is connected via a chemical moiety to the epsilon amine of a Lys residue of the other monomer. Methods of making such thioether bonded dimers are further described herein. In some aspects, the monomers are connected via terminal amino acids (e.g., N-terminal or C-terminal), via internal amino acids, or via a terminal amino acid of at least one monomer and an internal amino acid of at least one other monomer. In specific aspects, the monomers are not connected via an N-terminal amino acid. In some

aspects, the monomers of the multimer are attached together in a "tail-to-tail" orientation in which the C-terminal amino acids of each monomer are attached together.

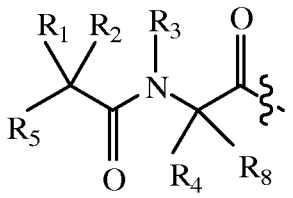
PRODRUGS

[00409] Further provided by the invention are prodrugs of the peptides and analogs described herein. As used herein, the term "prodrug" is defined as any compound that undergoes chemical modification before exhibiting its full pharmacological effects.

[00410] In exemplary embodiments, the prodrug is an amide-based peptide prodrug, similar to those described in International Patent Application Publication No. WO/2010/071807, which published on June 24, 2010. Such prodrugs are intended to delay onset of action and extend the half life of the drug. The delayed onset of action is advantageous in that it allows systemic distribution of the prodrug prior to its activation. Accordingly, the administration of prodrugs eliminates complications caused by peak activities upon administration and increases the therapeutic index of the parent drug.

[00411] In exemplary aspects, the prodrug comprises the structure: A-B-Q; wherein Q is a peptide or analog described herein; A is an amino acid or a hydroxy acid; B is an N-alkylated amino acid linked to Q through an amide bond between A-B and an amine of Q; wherein A, B, or the amino acid of Q to which A-B is linked is a non-coded amino acid, further wherein chemical cleavage half- life (t1/2) of A-B from Q is at least about 1 hour to about 1 week in PBS under physiological conditions. As used herein the term "hydroxy acid" refers to an amino acid that has been modified to replace the alpha carbon amino group with a hydroxyl group.

[00412] In some embodiments the dipeptide prodrug element has the general structure of Formula I:



wherein

R_1, R_2, R_4 and R_8 are independently selected from the group consisting of H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)SH, (C_2-C_3 alkyl)SCH_3, (C_1-C_4

alkyl)$CONH_2$, (C_1-C_4 alkyl)COOH, (C_1-C_4 alkyl)NH_2, (C_1-C_4 alkyl)$NHC(NH_2^+)NH_2$, (C_0-C_4 alkyl)(C_3-C_6 cycloalkyl), (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, (C_1-C_4 alkyl)(C_3-C_9 heteroaryl), and C_1-C_{12} alkyl(A)(W_1)C_1-C_{12} alkyl, wherein W_1 is a heteroatom selected from the group consisting of N, S and O, or R_1 and R_2 together with the atoms to which they are attached form a C_3-C_{12} cycloalkyl or aryl; or R_4 and R_8 together with the atoms to which they are attached form a C_3-C_6 cycloalkyl;

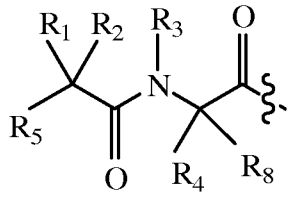
R_3 is selected from the group consisting of C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)NH_2, (C_1-C_{18} alkyl)SH, (C_0-C_4 alkyl)(C_3-C_6)cycloalkyl, (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and (C_1-C_4 alkyl)(C_3-C_9 heteroaryl) or R_4 and R_3 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R_5 is NHR_6 or OH;

R_6 is H, C_1-C_8 alkyl or R_6 and R_2 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of H and OH.

[00413] In other embodiments the dipeptide prodrug element has the general structure of Formula I:



wherein

R_1, R_2, R_4 and R_8 are independently selected from the group consisting of H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)SH, (C_2-C_3 alkyl)SCH_3, (C_1-C_4 alkyl)$CONH_2$, (C_1-C_4 alkyl)COOH, (C_1-C_4 alkyl)NH_2, (C_1-C_4 alkyl)$NHC(NH_2^+)NH_2$, (C_0-C_4 alkyl)(C_3-C_6 cycloalkyl), (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, (C_1-C_4 alkyl)(C_3-C_9 heteroaryl), and C_1-C_{12} alkyl(W_1)C_1-C_{12} alkyl, wherein W_1 is a heteroatom selected from the group consisting of N, S and O, or R_1 and R_2 together with the atoms to which they are attached form a C_3-C_{12} cycloalkyl; or R_4 and R_8 together with the atoms to which they are attached form a C_3-C_6 cycloalkyl;

R_3 is selected from the group consisting of C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)NH_2, (C_1-C_{18} alkyl)SH, (C_0-C_4 alkyl)(C_3-C_6)cycloalkyl, (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and (C_1-C_4 alkyl)(C_3-C_9 heteroaryl) or R_4 and R_3 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R_5 is NHR_6 or OH;

R_6 is H, C_1-C_8 alkyl or R_6 and R_1 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo.

[00414] In some embodiments R_8 is H and R_5 is NHR_6.

[00415] In some embodiments the dipeptide prodrug element has the structure of Formula I, wherein

R_1 and R_8 are independently H or C_1-C_8 alkyl;

R_2 and R_4 are independently selected from the group consisting of H, C_1-C_8 alkyl, C_2-C_8 alkenyl, (C_1-C_4 alkyl)OH, (C_1-C_4 alkyl)SH, (C_2-C_3 alkyl)SCH_3, (C_1-C_4 alkyl)$CONH_2$, (C_1-C_4 alkyl)COOH, (C_1-C_4 alkyl)NH_2, (C_1-C_4 alkyl)$NHC(NH_2+)$ NH_2, (C_0-C_4 alkyl)(C_3-C_6 cycloalkyl), (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and CH_2(C_3-C_9 heteroaryl), or R_1 and R_2 together with the atoms to which they are attached form a C_3-C_{12} cycloalkyl or aryl;

R_5 is NHR_6; and

R_6 is H or C_1-C_8 alkyl.

In other embodiments the dipeptide prodrug element has the structure of Formula I, wherein

R_1 and R_8 are independently H or C_1-C_8 alkyl;

R_2 and R_4 are independently selected from the group consisting of H, C_1-C_8 alkyl, C_2-C_8 alkenyl, (C_1-C_4 alkyl)OH, (C_1-C_4 alkyl)SH, (C_2-C_3 alkyl)SCH_3, (C_1-C_4 alkyl)$CONH_2$, (C_1-C_4 alkyl)COOH, (C_1-C_4 alkyl)NH_2, (C_1-C_4 alkyl)$NHC(NH_2+)$ NH_2, (C_0-C_4 alkyl)(C_3-C_6 cycloalkyl), (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and CH_2(C_3-C_9

heteroaryl), or R_1 and R_2 together with the atoms to which they are attached form a C_3-C_{12} cycloalkyl;

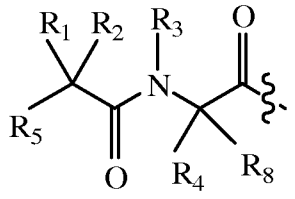
R_3 is C_1-C_{18} alkyl;

R_5 is NHR_6;

R_6 is H or C_1-C_8 alkyl; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo.

[00416] The half life of the prodrug formed in accordance with the present disclosure is determined by the substituents of the dipeptide prodrug element, its location, and the amino acid to which it is attached. For example, the prodrug may comprise a peptide or analog described herein, wherein the dipeptide prodrug element is linked through the alpha amino group of the N-terminal amino acid of the peptide or analog described herein. In this embodiment prodrugs having a $t_{1/2}$ of, e.g., about 1 hour comprise a dipeptide prodrug element with the structure:



wherein

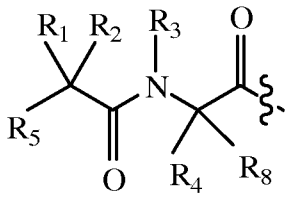
R_1 and R_2 are independently C_1-C_{18} alkyl or aryl; or R_1 and R_2 are linked through -$(CH_2)_p$, wherein p is 2-9;

R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen; and

R_5 is an amine.

[00417] In other embodiments, prodrugs having a $t_{1/2}$ of, e.g., about 1 hour comprise a dipeptide prodrug element with the structure:



wherein

R_1 and R_2 are independently C_1-C_{18} alkyl or (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7; or R_1 and R_2 are linked through -$(CH_2)_p$, wherein p is 2-9;

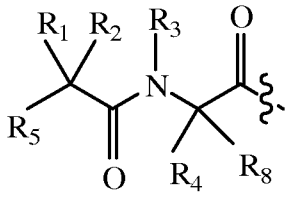
R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen;

R_5 is NH_2; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo.

[00418] Furthermore, prodrugs having the dipeptide prodrug element linked to the N-terminal alpha amino acid of the peptide or analog described herein and having a $t_{1/2}$, e.g., between about 6 to about 24 hours, comprise a dipeptide prodrug element with the structure:



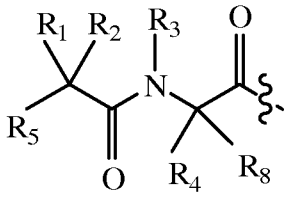
wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_{18} alkyl and aryl, or R_1 and R_2 are linked through $(CH_2)_p$, wherein p is 2-9;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-12 heterocyclic ring;

R_4 and R_8 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl and aryl; and R_5 is an amine;

with the proviso that both R_1 and R_2 are not hydrogen and provided that one of R_4 or R_8 is hydrogen.

[00419] In some embodiments, prodrugs having the dipeptide prodrug element linked to the N-terminal alpha amino acid of the peptide or analog described herein and having a $t_{1/2}$, e.g., between about 12 to about 72 hours, or in some embodiments between about 12 to about 48 hours, comprise a dipeptide prodrug element with the structure:



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wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_4 alkyl)NH_2, and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, or R_1 and R_2 are linked through $(CH_2)_p$, wherein p is 2-9;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-12 heterocyclic ring;

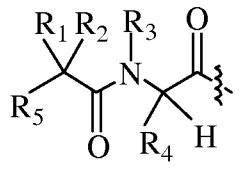
R_4 and R_8 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

R_5 is NH_2; and

R_7 is selected from the group consisting of H, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo;

with the proviso that both R_1 and R_2 are not hydrogen and provided that at least one of R_4 or R_8 is hydrogen.

[00420] In some embodiments, prodrugs having the dipeptide prodrug element linked to the N-terminal amino acid of the peptide or analog described herein and having a $t_{1/2}$, e.g., between about 12 to about 72 hours, or in some embodiments between about 12 to about 48 hours, comprise a dipeptide prodrug element with the structure:



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wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl and (C_1-C_4 alkyl)NH_2, or R_1 and R_2 are linked through $(CH_2)_p$, wherein p is 2-9;

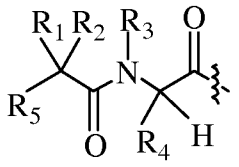
R_3 is C_1-C_8 alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-6 heterocyclic ring;

R_4 is selected from the group consisting of hydrogen and C_1-C_8 alkyl; and

R_5 is NH_2;

with the proviso that both R_1 and R_2 are not hydrogen.

[00421] In other embodiments, prodrugs having the dipeptide prodrug element linked to the N-terminal amino acid of the peptide or analog described here and having a $t_{1/2}$, e.g., between about 12 to about 72 hours, or in some embodiments between about 12 to about 48 hours, comprise a dipeptide prodrug element with the structure:



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wherein

R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl and (C_1-C_4 alkyl)NH_2;

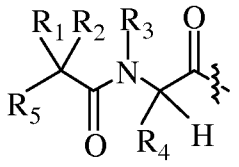
R_3 is C_1-C_6 alkyl;

R_4 is hydrogen; and

R_5 is NH_2;

with the proviso that both R_1 and R_2 are not hydrogen.

[00422] In some embodiments, prodrugs having the dipeptide prodrug element linked to the N-terminal amino acid of the peptide or analog described herein and having a $t_{1/2}$, e.g., between about 12 to about 72 hours, or in some embodiments between about 12 to about 48 hours, comprise a dipeptide prodrug element with the structure:



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wherein

R_1 and R_2 are independently selected from the group consisting of hydrogen and C_1-C_8 alkyl, (C_1-C_4 alkyl)NH_2, or R_1 and R_2 are linked through $(CH_2)_p$, wherein p is 2-9;

R_3 is C_1-C_8 alkyl;

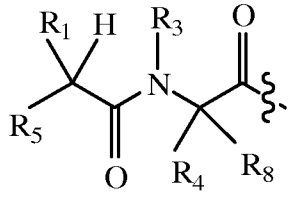
R_4 is (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

R_5 is NH_2; and

R_7 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and (C_0-C_4 alkyl)OH;

with the proviso that both R_1 and R_2 are not hydrogen.

[00423] In addition a prodrug having the dipeptide prodrug element linked to the N-terminal alpha amino acid of the peptide and analog described herein and having a $t_{1/2}$, e.g., of about 72 to about 168 hours is provided wherein the dipeptide prodrug element has the structure:



wherein R_1 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and aryl;

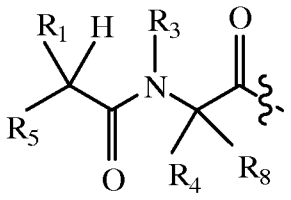
R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen; and

R_5 is an amine or N-substituted amine or a hydroxyl;

with the proviso that, if R_1 is alkyl or aryl, then R_1 and R_5 together with the atoms to which they are attached form a 4-11 heterocyclic ring.

[00424] In some embodiments, the dipeptide prodrug element has the structure:



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wherein R_1 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen;

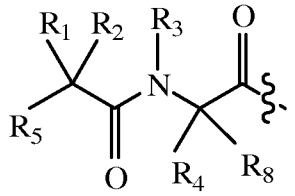
R_5 is NHR_6 or OH;

R_6 is H, C_1-C_8 alkyl, or R_6 and R_1 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo;

with the proviso that, if R_1 is alkyl or (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, then R_1 and R_5 together with the atoms to which they are attached form a 4-11 heterocyclic ring.

[00425] In some embodiments the dipeptide prodrug element is linked to a side chain amine of an internal amino acid of the peptide or analog described herein. In this embodiment prodrugs having a $t_{1/2}$, e.g., of about 1 hour have the structure:



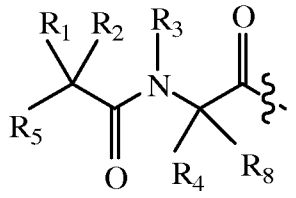
wherein

R_1 and R_2 are independently C_1-C_8 alkyl or aryl; or R_1 and R_2 are linked through $(CH_2)_p$, wherein p is 2-9;

R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen; and R_5 is an amine.

[00426] In some embodiments, prodrugs having a $t_{1/2}$, e.g., of about 1 hour have the structure:



wherein

R_1 and R_2 are independently C_1-C_8 alkyl or (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7; or R_1 and R_2 are linked through -$(CH_2)_p$-, wherein p is 2-9;

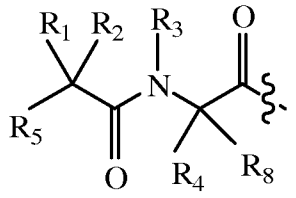
R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen;

R_5 is NH_2; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo.

[00427] Furthermore, prodrugs having a $t_{1/2}$, e.g., between about 6 to about 24 hours and having the dipeptide prodrug element linked to a internal amino acid side chain comprise a dipeptide prodrug element with the structure:



wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl and aryl, or R_1 and R_2 are linked through -$(CH_2)_p$, wherein p is 2-9;

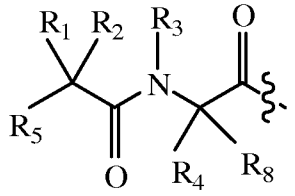
R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-12 heterocyclic ring;

R_4 and R_8 are independently C_1-C_{18} alkyl or aryl; and

R_5 is an amine or N-substituted amine;

with the proviso that both R_1 and R_2 are not hydrogen and provided that one of R_4 or R_8 is hydrogen.

[00428] In some embodiments, prodrugs having a $t_{1/2}$, e.g., between about 12 to about 72 hours, or in some embodiments between about 12 to about 48 hours, and having the dipeptide prodrug element linked to a internal amino acid side chain comprise a dipeptide prodrug element with the structure:



wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl, and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, or R_1 and R_2 are linked through -$(CH_2)_p$-, wherein p is 2-9;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-12 heterocyclic ring;

R_4 and R_8 are independently hydrogen, C_1-C_{18} alkyl or (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

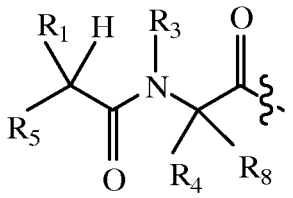
R_5 is NHR_6;

R_6 is H or C_1-C_8 alkyl, or R_6 and R_2 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo;

with the proviso that both R_1 and R_2 are not hydrogen and provided that at least one of R_4 or R_8 is hydrogen.

[00429] In addition a prodrug having a $t_{1/2}$, e.g., of about 72 to about 168 hours and having the dipeptide prodrug element linked to a internal amino acid side chain of the peptide or analog described herein is provided wherein the dipeptide prodrug element has the structure:



wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_{18} alkyl and aryl;

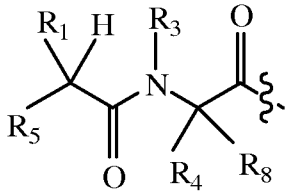
R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen; and

R_5 is an amine or N-substituted amine or a hydroxyl;

with the proviso that, if R_1 and R_2 are both independently an alkyl or aryl, either R_1 or R_2 is linked through $(CH2)_p$ to R_5, wherein p is 2-9.

[00430] In some embodiments, a prodrug having a $t_{1/2}$, e.g., of about 72 to about 168 hours and having the dipeptide prodrug element linked to a internal amino acid side chain of the peptide or analog described herein is provided wherein the dipeptide prodrug element has the structure:



wherein R_1 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

R_3 is C_1-C_{18} alkyl;

R_4 and R_8 are each hydrogen;

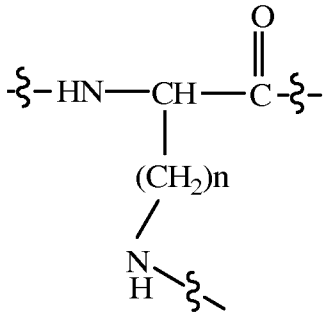
R_5 is NHR_6 or OH;

R_6 is H or C_1-C_8 alkyl, or R_6 and R_1 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo;

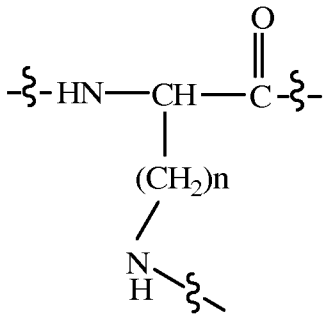
with the proviso that, if R_1 and R_2 are both independently an alkyl or (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, either R_1 or R_2 is linked through $(CH_2)_p$ to R_5, wherein p is 2-9.

[00431] In some embodiments the dipeptide prodrug element is linked to a side chain amine of an internal amino acid of the peptide or analog described herein wherein the internal amino acid comprises the structure of Formula II:



wherein

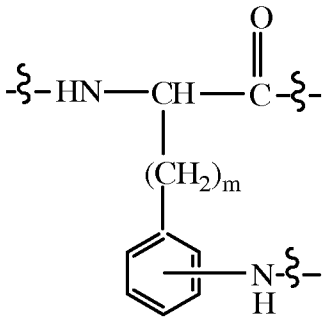
n is an integer selected from 1 to 4. In some embodiments n is 3 or 4 and in some embodiments the internal amino acid is lysine. In some embodiments the dipeptide prodrug element is linked to a primary amine on a side chain of an amino acid located at position 12, 16, 17, 18, 20, 28, or 29 of the peptide or analog described herein. In some embodiments the amino acid at 12, 16, 17, 18, 20, 28, or 29 comprises the structure of Formula II:



wherein n is an integer selected from 1 to 4 and the dipeptide prodrug element is linked to the amino acid side chain via an amide bond. In some embodiments n is 4 and the amino acid is located at position 20.

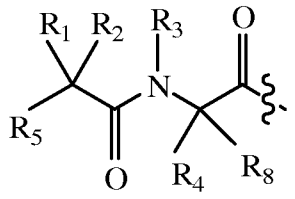
[00432] In a further embodiment the dipeptide prodrug element is linked to the peptide or analog thereof via an amine present on an aryl group of an aromatic amino acid. In some embodiments the aromatic amino acid is an internal amino acid of the peptide or analog

described herein, however the aromatic amino acid can also be the N-terminal amino acid. In some embodiments the aromatic amino acid is selected from the group consisting of amino-Phe, amino-napthyl alanine, amino tryptophan, amino-phenyl-glycine, amino-homo-Phe, and amino tyrosine. In some embodiments the primary amine that forms an amide bond with the dipeptide prodrug element is in the para-position on the aryl group. In some embodiments the aromatic amine comprises the structure of Formula III:



wherein m is an integer from 1 to 3.

[00433] For those embodiments wherein the dipeptide prodrug element is linked to the peptide or analog described herein via an amine present on an aryl group of an aromatic amino acid, prodrugs having a $t_{1/2}$, e.g., of about 1 hour have a dipeptide structure of:

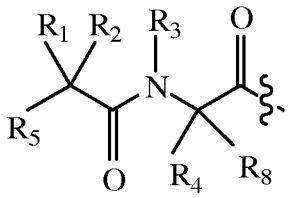


wherein R_1 and R_2 are independently C_1-C_{18} alkyl or aryl;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-12 heterocyclic ring;

R_4 and R_8 are independently selected from the group consisting of hydrogen, C_1-C_{18} alkyl and aryl; and R_5 is an amine or a hydroxyl.

[00434] In some embodiments, the dipeptide prodrug element is linked to the peptide or analog described herein via an amine present on an aryl group of an aromatic amino acid, prodrugs having a $t_{1/2}$, e.g., of about 1 hour have a dipeptide structure of:



wherein R_1 and R_2 are independently C_1-C_{18} alkyl or (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

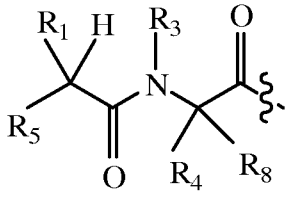
R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-12 heterocyclic ring;

R_4 and R_8 are independently selected from the group consisting of hydrogen, C_1-C_{18} alkyl and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

R_5 is NH_2 or OH; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo.

[00435] Furthermore, prodrugs having the dipeptide prodrug element is linked via an aromatic amino acid and having a $t_{1/2}$, e.g., of about 6 to about 24 hours are provided wherein the dipeptide comprises a structure of:



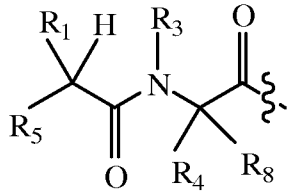
wherein

R_1 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl and aryl, or R_1 and R_2 are linked through -$(CH_2)_p$, wherein p is 2-9;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-6 heterocyclic ring;

R_4 and R_8 are independently selected from the group consisting of hydrogen, C_1-C_{18} alkyl and aryl; and R_5 is an amine or N-substituted amine.

[00436] In some embodiments, prodrugs having the dipeptide prodrug element linked via an aromatic amino acid and having a $t_{1/2}$, e.g., of about 6 to about 24 hours are provided wherein the dipeptide comprises a structure of:



wherein

R_1 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_4 alkyl)NH_2, and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-6 heterocyclic ring;

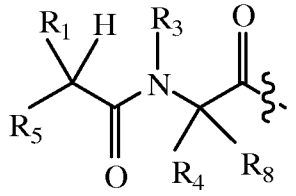
R_4 and R_8 are independently selected from the group consisting of hydrogen, C_1-C_{18} alkyl and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7;

R_5 is NHR_6;

R_6 is H, C_1-C_8 alkyl, or R_6 and R_1 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo.

[00437] In addition, prodrugs having the dipeptide prodrug element is linked via an aromatic amino acid and having a $t_{1/2}$, e.g., of about 72 to about 168 hours are provided wherein the dipeptide comprises a structure of:



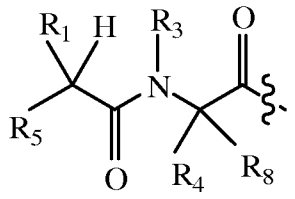
wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl and aryl;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-6 heterocyclic ring;

R_4 and R_8 are each hydrogen; and

R_5 is selected from the group consisting of amine, N-substituted amine and hydroxyl.

[00438] In some embodiments, prodrugs having the dipeptide prodrug element linked via an aromatic amino acid and having a $t_{1/2}$, e.g., of about 72 to about 168 hours are provided wherein the dipeptide comprises a structure of:



wherein R_1 and R_2 are independently selected from the group consisting of hydrogen, C_1-C_8 alkyl, (C_1-C_4 alkyl)COOH, and (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, or R_1 and R_5 together with the atoms to which they are attached form a 4-11 heterocyclic ring;

R_3 is C_1-C_{18} alkyl or R_3 and R_4 together with the atoms to which they are attached form a 4-6 heterocyclic ring;

R_4 is hydrogen or forms a 4-6 heterocyclic ring with R_3;

R_8 is hydrogen;

R_5 is NHR_6 or OH;

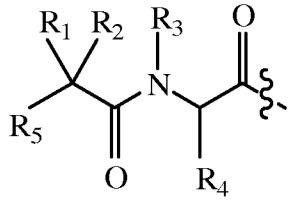
R_6 is H or C_1-C_8 alkyl, or R_6 and R_1 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo.

[00439] In some embodiments the dipeptide prodrug element is linked to an aromatic amino acid via a primary amine present as an aryl substituent of the aromatic amino acid, wherein the aromatic amino acid is located at position 10, 13, 22, or 25 of the peptide or analog described herein (based on the numbering for native glucagon, see e.g., SEQ ID NO:

1). In some embodiments the dipeptide prodrug element linked aromatic amino acid amino acid is located at position 22 of the peptide or analog described herein.

[00440] In accordance with some embodiments the dipeptide prodrug element is linked at the N-terminal amine of the peptide or analog described herein, including for example a glucagon related peptide, or osteocalcin, as well as analogs, derivatives and conjugates of the foregoing, wherein the dipeptide prodrug element comprises the structure:



wherein R_1 is selected from the group consisting of H and C_1-C_8 alkyl;

R_2 and R_4 are independently selected from the group consisting of H, C_1-C_8 alkyl, C_2-C_8 alkenyl, (C_1-C_4 alkyl)OH, (C_1-C_4 alkyl)SH, (C_2-C_3 alkyl)SCH_3, (C_1-C_4 alkyl)$CONH_2$, (C_1-C_4 alkyl)COOH, (C_1-C_4 alkyl)NH_2, (C_1-C_4 alkyl)$NHC(NH_2^+)$ NH_2, (C_0-C_4 alkyl)(C_3-C_6 cycloalkyl), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, CH_2(C_5-C_9 heteroaryl), or R_1 and R_2 together with the atoms to which they are attached form a C_3-C_6 cycloalkyl;

R_3 is selected from the group consisting of C_1-C_8 alkyl, (C_3-C_6)cycloalkyl or R_4 and R_3 together with the atoms to which they are attached form a 5 or 6 member heterocyclic ring;

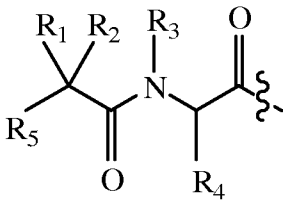
R_5 is NHR_6 or OH;

R_6 is H, or R_6 and R_2 together with the atoms to which they are attached form a 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of H and OH. In some embodiments R_1 is H or C_1-C_8 alkyl, R_2 is selected from the group consisting of H, C_1-C_6 alkyl, CH_2OH, (C_1-C_4 alkyl)NH_2, (C_3-C_6 cycloalkyl) and CH_2(C_6 aryl)R_7 or R_6 and R_2 together with the atoms to which they are attached form a 5 member heterocyclic ring, R_3 is C_1-C_6 alkyl, and R_4 is selected from the group consisting of H, C_1-C_4 alkyl, (C_3-C_6)cycloalkyl, (C_1-C_4 alkyl)OH, (C_1-C_4 alkyl)SH and (C_0-C_4 alkyl)(C_6 aryl)R_7, or R_3 and R_4 together with the atoms to which they are attached form a 5 member heterocyclic ring. In a further embodiment R_3 is CH_3, R_5

is NHR_6, and in an alternative further embodiment R_3 and R_4 together with the atoms to which they are attached form a 5 member heterocyclic ring and R_5 is NHR_6.

[00441] In accordance with another embodiment the dipeptide prodrug element is linked at the N-terminal amine of the peptide or analog described herein, wherein the dipeptide prodrug element comprises the structure:



wherein R_1 is selected from the group consisting of H and C_1-C_8 alkyl;

R_2 and R_4 are independently selected from the group consisting of H, C_1-C_8 alkyl, C_2-C_8 alkenyl, (C_1-C_4 alkyl)OH, (C_1-C_4 alkyl)SH, (C_2-C_3 alkyl)SCH_3, (C_1-C_4 alkyl)$CONH_2$, (C_1-C_4 alkyl)COOH, (C_1-C_4 alkyl)NH_2, (C_1-C_4 alkyl)$NHC(NH_2^+)$ NH_2, (C_0-C_4 alkyl)(C_3-C_6 cycloalkyl), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, CH_2(C_5-C_9 heteroaryl), or R_1 and R_2 together with the atoms to which they are attached form a C_3-C_6 cycloalkyl;

R_3 is selected from the group consisting of C_1-C_8 alkyl, (C_3-C_6)cycloalkyl or R_4 and R_3 together with the atoms to which they are attached form a 5 or 6 member heterocyclic ring;

R_5 is NHR_6 or OH;

R_6 is H, or R_6 and R_2 together with the atoms to which they are attached form a 5 or 6 member heterocyclic ring; and

R_7 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, (C_0-C_4 alkyl)$CONH_2$, (C_0-C_4 alkyl)COOH, (C_0-C_4 alkyl)NH_2, (C_0-C_4 alkyl)OH, and halo. In some embodiments R_1 is H or C_1-C_8 alkyl, R_2 is selected from the group consisting of H, C_1-C_6 alkyl, CH_2OH, (C_1-C_4 alkyl)NH_2, (C_3-C_6 cycloalkyl) and CH_2(C_6 aryl)R_7 or R_6 and R_2 together with the atoms to which they are attached form a 5 member heterocyclic ring, R_3 is C_1-C_6 alkyl, and R_4 is selected from the group consisting of H, C_1-C_4 alkyl, (C_3-C_6)cycloalkyl, (C_1-C_4 alkyl)OH, (C_1-C_4 alkyl)SH and (C_0-C_4 alkyl)(C_6 aryl)R_7, or R_3 and R_4 together with the atoms to which they are attached form a 5 member heterocyclic ring. In a

further embodiment R_3 is CH_3, R_5 is NHR_6, and in an alternative further embodiment R_3 and R_4 together with the atoms to which they are attached form a 5 member heterocyclic ring and R_5 is NHR_6.

PHARMACEUTICAL COMPOSITIONS, USES AND KITS

Salts

[00442] In some embodiments, the glucagon analog is in the form of a salt, e.g., a pharmaceutically acceptable salt. As used herein the term "pharmaceutically acceptable salt" refers to salts of compounds that retain the biological activity of the parent compound, and which are not biologically or otherwise undesirable. Such salts can be prepared *in situ* during the final isolation and purification of the analog, or separately prepared by reacting a free base function with a suitable acid. Many of the compounds disclosed herein are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

[00443] Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Representative acid addition salts include, but are not limited to acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphor sulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isothionate), lactate, maleate, methane sulfonate, nicotinate, 2-naphthalene sulfonate, oxalate, palmitoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, phosphate, glutamate, bicarbonate, p-toluenesulfonate, and undecanoate. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like. Examples of acids which can be employed to form pharmaceutically acceptable acid addition salts include, for example, an inorganic acid, e.g., hydrochloric acid, hydrobromic acid, sulphuric acid, and phosphoric acid, and an organic acid, e.g., oxalic acid, maleic acid, succinic acid, and citric acid.

[00444] Basic addition salts also can be prepared *in situ* during the final isolation and purification of the source of salicylic acid, or by reacting a carboxylic acid-containing moiety with a suitable base such as the hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation or with ammonia or an organic primary, secondary, or tertiary amine. Pharmaceutically acceptable salts include, but are not limited to, cations based on alkali metals or alkaline earth metals such as lithium, sodium, potassium, calcium, magnesium, and aluminum salts, and the like, and nontoxic quaternary ammonia and amine cations including ammonium, tetramethylammonium, tetraethylammonium, methylammonium, dimethylammonium, trimethylammonium, triethylammonium, diethylammonium, and ethylammonium, amongst others. Other representative organic amines useful for the formation of base addition salts include, for example, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines.

[00445] Further, basic nitrogen-containing groups can be quaternized with the analog of the present disclosure as lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; long chain halides such as decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides; arylalkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

Formulations

[00446] In accordance with some embodiments, a pharmaceutical composition is provided wherein the composition comprises a glucagon analog of the present disclosure, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

[00447] The pharmaceutical composition can comprise any pharmaceutically acceptable ingredient, including, for example, acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents,

chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases, pastille bases, pigments, plasticizers, polishing agents, preservatives, sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or wetting agents.

[00448] In some embodiments, the pharmaceutical composition comprises any one or a combination of the following components: acacia, acesulfame potassium, acetyltributyl citrate, acetyltriethyl citrate, agar, albumin, alcohol, dehydrated alcohol, denatured alcohol, dilute alcohol, aleuritic acid, alginic acid, aliphatic polyesters, alumina, aluminum hydroxide, aluminum stearate, amylopectin, α-amylose, ascorbic acid, ascorbyl palmitate, aspartame, bacteriostatic water for injection, bentonite, bentonite magma, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, benzyl benzoate, bronopol, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butylparaben sodium, calcium alginate, calcium ascorbate, calcium carbonate, calcium cyclamate, dibasic anhydrous calcium phosphate, dibasic dehydrate calcium phosphate, tribasic calcium phosphate, calcium propionate, calcium silicate, calcium sorbate, calcium stearate, calcium sulfate, calcium sulfate hemihydrate, canola oil, carbomer, carbon dioxide, carboxymethyl cellulose calcium, carboxymethyl cellulose sodium, β-carotene, carrageenan, castor oil, hydrogenated castor oil, cationic emulsifying wax, cellulose acetate, cellulose acetate phthalate, ethyl cellulose, microcrystalline cellulose, powdered cellulose, silicified microcrystalline cellulose, sodium carboxymethyl cellulose, cetostearyl alcohol, cetrimide, cetyl alcohol, chlorhexidine, chlorobutanol, chlorocresol, cholesterol, chlorhexidine acetate, chlorhexidine gluconate, chlorhexidine hydrochloride, chlorodifluoroethane (HCFC), chlorodifluoromethane, chlorofluorocarbons (CFC)chlorophenoxyethanol, chloroxylenol, corn syrup solids, anhydrous citric acid, citric acid monohydrate, cocoa butter, coloring agents, corn oil, cottonseed oil, cresol, m-cresol, o-cresol, p-cresol, croscarmellose sodium, crospovidone,

cyclamic acid, cyclodextrins, dextrates, dextrin, dextrose, dextrose anhydrous, diazolidinyl urea, dibutyl phthalate, dibutyl sebacate, diethanolamine, diethyl phthalate, difluoroethane (HFC), dimethyl-β-cyclodextrin, cyclodextrin-type compounds such as Captisol®, dimethyl ether, dimethyl phthalate, dipotassium edentate, disodium edentate, disodium hydrogen phosphate, docusate calcium, docusate potassium, docusate sodium, dodecyl gallate, dodecyltrimethylammonium bromide, edentate calcium disodium, edtic acid, eglumine, ethyl alcohol, ethylcellulose, ethyl gallate, ethyl laurate, ethyl maltol, ethyl oleate, ethylparaben, ethylparaben potassium, ethylparaben sodium, ethyl vanillin, fructose, fructose liquid, fructose milled, fructose pyrogen-free, powdered fructose, fumaric acid, gelatin, glucose, liquid glucose, glyceride mixtures of saturated vegetable fatty acids, glycerin, glyceryl behenate, glyceryl monooleate, glyceryl monostearate, self-emulsifying glyceryl monostearate, glyceryl palmitostearate, glycine, glycols, glycofurol, guar gum, heptafluoropropane (HFC), hexadecyltrimethylammonium bromide, high fructose syrup, human serum albumin, hydrocarbons (HC), dilute hydrochloric acid, hydrogenated vegetable oil type II, hydroxyethyl cellulose, 2-hydroxyethyl-β-cyclodextrin, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, 2-hydroxypropyl-β-cyclodextrin, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, imidurea, indigo carmine, ion exchangers, iron oxides, isopropyl alcohol, isopropyl myristate, isopropyl palmitate, isotonic saline, kaolin, lactic acid, lactitol, lactose, lanolin, lanolin alcohols, anhydrous lanolin, lecithin, magnesium aluminum silicate, magnesium carbonate, normal magnesium carbonate, magnesium carbonate anhydrous, magnesium carbonate hydroxide, magnesium hydroxide, magnesium lauryl sulfate, magnesium oxide, magnesium silicate, magnesium stearate, magnesium trisilicate, magnesium trisilicate anhydrous, malic acid, malt, maltitol, maltitol solution, maltodextrin, maltol, maltose, mannitol, medium chain triglycerides, meglumine, menthol, methylcellulose, methyl methacrylate, methyl oleate, methylparaben, methylparaben potassium, methylparaben sodium, microcrystalline cellulose and carboxymethylcellulose sodium, mineral oil, light mineral oil, mineral oil and lanolin alcohols, oil, olive oil, monoethanolamine, montmorillonite, octyl gallate, oleic acid, palmitic acid, paraffin, peanut oil, petrolatum, petrolatum and lanolin alcohols, pharmaceutical glaze, phenol, liquified phenol, phenoxyethanol, phenoxypropanol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, polacrilin, polacrilin potassium, poloxamer,

polydextrose, polyethylene glycol, polyethylene oxide, polyacrylates, polyethylene-polyoxypropylene-block polymers, polymethacrylates, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitol fatty acid esters, polyoxyethylene stearates, polyvinyl alcohol, polyvinyl pyrrolidone, potassium alginate, potassium benzoate, potassium bicarbonate, potassium bisulfite, potassium chloride, postassium citrate, potassium citrate anhydrous, potassium hydrogen phosphate, potassium metabisulfite, monobasic potassium phosphate, potassium propionate, potassium sorbate, povidone, propanol, propionic acid, propylene carbonate, propylene glycol, propylene glycol alginate, propyl gallate, propylparaben, propylparaben potassium, propylparaben sodium, protamine sulfate, rapeseed oil, Ringer's solution, saccharin, saccharin ammonium, saccharin calcium, saccharin sodium, safflower oil, saponite, serum proteins, sesame oil, colloidal silica, colloidal silicon dioxide, sodium alginate, sodium ascorbate, sodium benzoate, sodium bicarbonate, sodium bisulfite, sodium chloride, anhydrous sodium citrate, sodium citrate dehydrate, sodium chloride, sodium cyclamate, sodium edentate, sodium dodecyl sulfate, sodium lauryl sulfate, sodium metabisulfite, sodium phosphate, dibasic, sodium phosphate, monobasic, sodium phosphate, tribasic, anhydrous sodium propionate, sodium propionate, sodium sorbate, sodium starch glycolate, sodium stearyl fumarate, sodium sulfite, sorbic acid, sorbitan esters (sorbitan fatty esters), sorbitol, sorbitol solution 70%, soybean oil, spermaceti wax, starch, corn starch, potato starch, pregelatinized starch, sterilizable maize starch, stearic acid, purified stearic acid, stearyl alcohol, sucrose, sugars, compressible sugar, confectioner's sugar, sugar spheres, invert sugar, *Sugartab*, Sunset Yellow FCF, synthetic paraffin, talc, tartaric acid, tartrazine, tetrafluoroethane (HFC), theobroma oil, thimerosal, titanium dioxide, alpha tocopherol, tocopheryl acetate, alpha tocopheryl acid succinate, beta-tocopherol, delta-tocopherol, gamma-tocopherol, tragacanth, triacetin, tributyl citrate, triethanolamine, triethyl citrate, trimethyl-β-cyclodextrin, trimethyltetradecylammonium bromide, tris buffer, trisodium edentate, vanillin, type I hydrogenated vegetable oil, water, soft water, hard water, carbon dioxide-free water, pyrogen-free water, water for injection, sterile water for inhalation, sterile water for injection, sterile water for irrigation, waxes, anionic emulsifying wax, carnauba wax, cationic emulsifying wax, cetyl ester wax, microcrystalline wax, nonionic emulsifying wax, suppository wax, white wax, yellow wax, white petrolatum, wool fat, xanthan gum, xylitol, zein, zinc propionate, zinc salts, zinc stearate, or any excipient in

the *Handbook of Pharmaceutical Excipients*, Third Edition, A. H. Kibbe (Pharmaceutical Press, London, UK, 2000), which is incorporated by reference in its entirety. *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is incorporated by reference in its entirety, discloses various components used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional agent is incompatible with the pharmaceutical compositions, its use in pharmaceutical compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[00449] In some embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration, such as, for example, at least A, wherein A is 0.0001% w/v, 0.001% w/v, 0.01% w/v, 0.1% w/v, 1% w/v, 2% w/v, 5% w/v, 10% w/v, 20% w/v, 30% w/v, 40% w/v, 50% w/v, 60% w/v, 70% w/v, 80% w/v, or 90% w/v. In some embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration, such as, for example, at most B, wherein B is 90% w/v, 80% w/v, 70% w/v, 60% w/v, 50% w/v, 40% w/v, 30% w/v, 20% w/v, 10% w/v, 5% w/v, 2% w/v, 1% w/v, 0.1% w/v, 0.001% w/v, or 0.0001%. In other embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration range, such as, for example from about A to about B. In some embodiments, A is 0.0001% and B is 90%.

[00450] In some embodiments, the pharmaceutically acceptable ingredient is selected from the group consisting of a sugar (e.g., glucose, sucrose, trehalose, lactose, fructose, maltose, dextran, glycerin, dextran, mellibiose, melezitose, raffinose, mannotriose, stachyose, maltose, lactulose, maltulose, or iso-maltulose, or combinations of these sugars), a sugar alcohol (e.g., glycol, glycerol, erythritol, threitol, arabitol, xylitol, ribitol, mannitol, sorbitol, dulcitol, iditol, isomalt, maltitol, lactitol, or glucitol, or combinations of these sugar alcohols), a salt (e.g., sodium chloride), an emulsifier or surfactant (e.g., polysorbates, such as polyoxyethylene 20 sorbitan monooleate, or other block copolymers of ethylene oxide and propylene oxide), lyoprotectants, and mixtures thereof. For example, excipients such as sugars or sugar alcohols are present, e.g., in a concentration of about 20 mg/mL to about 40 mg/mL, or 25 to 45 mg/mL, such as 35 mg/mL.

[00451] The pharmaceutical compositions may be formulated to achieve a physiologically compatible pH. In some embodiments, the pH of the pharmaceutical composition may be at

least 5, at least 5.5, at least 6, at least 6.5, at least 7, at least 7.5, at least 8, at least 8.5, at least 9, at least 9.5, at least 10, or at least 10.5 up to and including pH 11, depending on the formulation and route of administration, for example between 4 and 7, or 4.5 and 5.5. In exemplary embodiments, the pharmaceutical compositions may comprise buffering agents to achieve a physiological compatible pH. The buffering agents may include any compounds capable of buffering at the desired pH such as, for example, phosphate buffers (e.g.,PBS), triethanolamine, Tris, bicine, TAPS, tricine, HEPES, TES, MOPS, PIPES, cacodylate, MES, acetate, citrate, succinate, histidine or other pharmaceutically acceptable buffers. In exemplary embodiments, the strength of the buffer is at least 0.5 mM, at least 1 mM, at least 5 mM, at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, at least 90 mM, at least 100 mM, at least 120 mM, at least 150 mM, or at least 200 mM. In some embodiments, the strength of the buffer is no more than 300 mM (e.g.,at most 200 mM, at most 100 mM, at most 90 mM, at most 80 mM, at most 70 mM, at most 60 mM, at most 50 mM, at most 40 mM, at most 30 mM, at most 20 mM, at most 10 mM, at most 5 mM, at most 1 mM). For example, the buffer concentration can be about 2 mM to about 100 mM, or about 10 mM to about 50 mM.

[00452] *Routes of Administration*

[00453] The following discussion on routes of administration is merely provided to illustrate exemplary embodiments and should not be construed as limiting the scope in any way.

[00454] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the analog of the present disclosure dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline

cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can comprise the analog of the present disclosure in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the analog of the present disclosure in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

[00455] The analogs of the disclosure, alone or in combination with other suitable components, can be delivered via pulmonary administration and can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa. In some embodiments, the analog is formulated into a powder blend or into microparticles or nanoparticles. Suitable pulmonary formulations are known in the art. See, e.g., Qian et al., Int J Pharm 366: 218-220 (2009); Adjei and Garren, Pharmaceutical Research, 7(6): 565-569 (1990); Kawashima et al., J Controlled Release 62(1-2): 279-287 (1999); Liu et al., Pharm Res 10(2): 228-232 (1993); International Patent Application Publication Nos. WO 2007/133747 and WO 2007/141411.

[00456] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous. The analog of the present disclosure can be administered with a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2- dimethyl-1,3-

dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[00457] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[00458] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl -imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[00459] The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the analog of the present disclosure in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[00460] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

[00461] Additionally, the analog of the present disclosures can be made into suppositories for rectal administration by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[00462] It will be appreciated by one of skill in the art that, in addition to the above-described pharmaceutical compositions, the analog of the disclosure can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

Dose

[00463] The analogs of the disclosure are believed to be useful in methods of treating a disease or medical condition in which GIP receptor agonism, GIP/GLP-1 recptor co-agonism, GIP/glucagon receptor co-agonism, or GIP/GLP-1/glucagon receptor triagonism plays a role. For purposes of the disclosure, the amount or dose of the analog of the present disclosure administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the analog of the present disclosure should be sufficient to stimulate cAMP secretion from cells as described herein or sufficient to decrease blood glucose levels, fat levels, food intake levels, or body weight of a mammal, in a period of from about 1 to 4 minutes, 1 to 4 hours or 1 to 4 weeks or longer, e.g., 5 to 20 or more weeks, from the time of administration. In exemplary embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular analog of the present disclosure and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

[00464] Many assays for determining an administered dose are known in the art. For purposes herein, an assay, which comprises comparing the extent to which blood glucose

levels are lowered upon administration of a given dose of the analog of the present disclosure to a mammal among a set of mammals of which is each given a different dose of the analog, could be used to determine a starting dose to be administered to a mammal. The extent to which blood glucose levels are lowered upon administration of a certain dose can be assayed by methods known in the art, including, for instance, the methods described herein as Example 11.

[00465] The dose of the analog of the present disclosure also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular analog of the present disclosure. Typically, the attending physician will decide the dosage of the analog of the present disclosure with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, analog of the present disclosure to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the invention, the dose of the analog of the present disclosure can be about 0.0001 to about 1 g/kg body weight of the subject being treated/day, from about 0.0001 to about 0.001 g/kg body weight/day, or about 0.01 mg to about 1 g/kg body weight/day. In exemplary embodiments, the dose can be a total weekly dose of about 1 mg to about 40 mg, or about 4 mg to about 30 mg, or about 4 to about 20 mg, or about 10 to about 20 mg, or about 12 mg to about 30 mg.

[00466] In some embodiments, the pharmaceutical composition comprises any of the analogs disclosed herein at a purity level suitable for administration to a patient. In some embodiments, the analog has a purity level of at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99%, and a pharmaceutically acceptable diluent, carrier or excipient. The pharmaceutical composition in some aspects comprise the analog of the present disclosure at a concentration of at least A, wherein A is about about 0.001 mg/ml, about 0.01 mg/ml, 0 about 1 mg/ml, about 0.5 mg/ml, about 1 mg/ml, about 2 mg/ml, about 3 mg/ml, about 4 mg/ml, about 5 mg/ml, about 6 mg/ml, about 7 mg/ml, about 8 mg/ml, about 9 mg/ml, about 10 mg/ml, about 11 mg/ml, about 12 mg/ml, about 13 mg/ml, about 14 mg/ml, about 15 mg/ml, about 16 mg/ml, about 17 mg/ml, about 18 mg/ml, about 19 mg/ml, about 20 mg/ml, about 21 mg/ml, about 22 mg/ml, about 23 mg/ml, about 24 mg/ml, about 25 mg/ml or higher. In some embodiments,

the pharmaceutical composition comprises the analog at a concentration of at most B, wherein B is about 30 mg/ml, about 25 mg/ml, about 24 mg/ml, about 23, mg/ml, about 22 mg/ml, about 21 mg/ml, about 20 mg/ml, about 19 mg/ml, about 18 mg/ml, about 17 mg/ml, about 16 mg/ml, about 15 mg/ml, about 14 mg/ml, about 13 mg/ml, about 12 mg/ml, about 11 mg/ml, about 10 mg/ml, about 9 mg/ml, about 8 mg/ml, about 7 mg/ml, about 6 mg/ml, about 5 mg/ml, about 4 mg/ml, about 3 mg/ml, about 2 mg/ml, about 1 mg/ml, or about 0.1 mg/ml. In some embodiments, the compositions may contain an analog at a concentration range of A to B mg/ml, for example, about 0.001 to about 30.0 mg/ml.

Targeted Forms

[00467] One of ordinary skill in the art will readily appreciate that the analogs of the disclosure can be modified in any number of ways, such that the therapeutic or prophylactic efficacy of the analog of the present disclosures is increased through the modification. For instance, the analog of the present disclosure can be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds, e.g., glucagon analogs described herein, to targeting moieties is known in the art. See, for instance, Wadhwa et al., *J Drug Targeting*, 3, 111-127 (1995) and U.S. Patent No. 5,087,616. The term "targeting moiety" as used herein, refers to any molecule or agent that specifically recognizes and binds to a cell-surface receptor, such that the targeting moiety directs the delivery of the analog of the present disclosures to a population of cells on which surface the receptor (the glucagon receptor, the GLP-1 receptor) is expressed. Targeting moieties include, but are not limited to, antibodies, or fragments thereof, peptides, hormones, growth factors, cytokines, and any other natural or non-natural ligands, which bind to cell surface receptors (e.g., Epithelial Growth Factor Receptor (EGFR), T-cell receptor (TCR), B-cell receptor (BCR), CD28, Platelet-derived Growth Factor Receptor (PDGF), nicotinic acetylcholine receptor (nAChR), etc.). As used herein a "linker" is a bond, molecule or group of molecules that binds two separate entities to one another. Linkers may provide for optimal spacing of the two entities or may further supply a labile linkage that allows the two entities to be separated from each other. Labile linkages include photocleavable groups, acid-labile moieties, base-labile moieties and enzyme-cleavable groups. The term "linker" in some embodiments refers to any agent or molecule that bridges the analog of the present disclosures to the targeting moiety. One of ordinary skill in the art recognizes that sites on

the analog of the present disclosures, which are not necessary for the function of the analog of the present disclosures, are ideal sites for attaching a linker and/or a targeting moiety, provided that the linker and/or targeting moiety, once attached to the analog of the present disclosures, do(es) not interfere with the function of the analog of the present disclosures, i.e., the ability to stimulate cAMP secretion from cells, to treat diabetes or obesity.

Controlled Release Formulations

[00468] Alternatively, the glucagon analogs described herein can be modified into a depot form, such that the manner in which the analog of the present disclosures is released into the body to which it is administered is controlled with respect to time and location within the body (see, for example, U.S. Patent No. 4,450,150). Depot forms of analog of the present disclosures can be, for example, an implantable composition comprising the analog of the present disclosures and a porous or non-porous material, such as a polymer, wherein the analog of the present disclosures is encapsulated by or diffused throughout the material and/or degradation of the non-porous material. The depot is then implanted into the desired location within the body and the analog of the present disclosures are released from the implant at a predetermined rate.

[00469] The pharmaceutical composition in exemplary aspects is modified to have any type of *in vivo* release profile. In some aspects, the pharmaceutical composition is an immediate release, controlled release, sustained release, extended release, delayed release, or bi-phasic release formulation. Methods of formulating peptides for controlled release are known in the art. See, for example, Qian et al., *J Pharm* 374: 46-52 (2009) and International Patent Application Publication Nos. WO 2008/130158, WO2004/033036; WO2000/032218; and WO 1999/040942.

[00470] The instant compositions may further comprise, for example, micelles or liposomes, or some other encapsulated form, or may be administered in an extended release form to provide a prolonged storage and/or delivery effect. The disclosed pharmaceutical formulations may be administered according to any regime including, for example, daily (1 time per day, 2 times per day, 3 times per day, 4 times per day, 5 times per day, 6 times per day), three times a week, twice a week, every two days, every three days, every four days, every five days, every six days, weekly, bi-weekly, every three weeks, monthly, or bi-monthly.

Combinations

[00471] The glucagon analogs described herein may be administered alone or in combination with other therapeutic agents which aim to treat or prevent any of the diseases or medical conditions described herein. For example, the glucagon analogs described herein may be co-administered with (simultaneously or sequentially) an anti-diabetic or anti-obesity agent. Anti-diabetic agents known in the art or under investigation include insulin, leptin, Peptide YY (PYY), Pancreatic Peptide (PP), fibroblast growth factor 21 (FGF21), Y2Y4 receptor agonists, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide (Diamicron); meglitinides, such as repaglinide (Prandin) or nateglinide (Starlix); biguanides such as metformin (Glucophage) or phenformin; thiazolidinediones such as rosiglitazone (Avandia), pioglitazone (Actos), or troglitazone (Rezulin), or other PPARγ inhibitors; alpha glucosidase inhibitors that inhibit carbohydrate digestion, such as miglitol (Glyset), acarbose (Precose/Glucobay); exenatide (Byetta) or pramlintide; Dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin or sitagliptin; SGLT (sodium-dependent glucose transporter 1) inhibitors; glucokinase activators (GKA); glucagon receptor antagonists (GRA); or FBPase (fructose 1,6-bisphosphatase) inhibitors.

[00472] Anti-obesity agents known in the art or under investigation include appetite suppressants, including phenethylamine type stimulants, phentermine (optionally with fenfluramine or dexfenfluramine), diethylpropion (Tenuate®), phendimetrazine (Prelu-2®, Bontril®), benzphetamine (Didrex®), sibutramine (Meridia®, Reductil®); rimonabant (Acomplia®), other cannabinoid receptor antagonists; oxyntomodulin; fluoxetine hydrochloride (Prozac); Qnexa (topiramate and phentermine), Excalia (bupropion and zonisamide) or Contrave (bupropion and naltrexone); or lipase inhibitors, similar to XENICAL (Orlistat) or Cetilistat (also known as ATL-962), or GT 389-255.

[00473] The peptides described herein in some embodiments are co-administered with an agent for treatment of non-alcoholic fatty liver disease or NASH. Agents used to treat non-alcoholic fatty liver disease include ursodeoxycholic acid (a.k.a., Actigall, URSO, and Ursodiol), Metformin (Glucophage), rosiglitazone (Avandia), Clofibrate, Gemfibrozil, Polymixin B, and Betaine.

[00474] The peptides described herein in some embodiments are co-administered with an agent for treatment of a neurodegenerative disease, e.g., Parkinson's Disease. Anti-Parkinson's Disease agents are furthermore known in the art and include, but not limited to, levodopa, carbidopa, anticholinergics, bromocriptine, pramipexole, and ropinirole, amantadine, and rasagiline.

[00475] In view of the foregoing, the invention further provides pharmaceutical compositions and kits additionally comprising one of these other therapeutic agents. The additional therapeutic agent may be administered simultaneously or sequentially with the analog of the present disclosure. In some aspects, the analog is administered before the additional therapeutic agent, while in other aspects, the analog is administered after the additional therapeutic agent.

[00476] *Uses*

[00477] Based on the information provided for the first time herein, it is contemplated that the compositions (e.g., related pharmaceutical compositions) of the present disclosures are useful for treatment of a disease or medical condition, in which e.g., the lack of activity at the GIP receptor, the GLP-1 receptor, or at both receptors, is a factor in the onset and/or progression of the disease or medical condition. Accordingly, the present disclosures provides a method of treating or preventing a disease or medical condition in a patient, wherein the disease or medical condition is a disease of medical condition in which a lack of GIP receptor activation and/or GLP-1 receptor activation is associated with the onset and/or progression of the disease of medical condition. The method comprises providing to the patient a composition or conjugate in accordance with any of those described herein in an amount effective to treat or prevent the disease or medical condition.

[00478] In some embodiments, the disease or medical condition is metabolic syndrome. Metabolic Syndrome, also known as metabolic syndrome X, insulin resistance syndrome or Reaven's syndrome, is a disorder that affects over 50 million Americans. Metabolic Syndrome is typically characterized by a clustering of at least three or more of the following risk factors: (1) abdominal obesity (excessive fat tissue in and around the abdomen), (2) atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and high LDL cholesterol that enhance the accumulation of plaque in the artery walls), (3) elevated blood pressure, (4) insulin resistance or glucose intolerance, (5)

prothrombotic state (e.g.,high fibrinogen or plasminogen activator inhibitor-1 in blood), and (6) pro-inflammatory state (e.g.,elevated C-reactive protein in blood). Other risk factors may include aging, hormonal imbalance and genetic predisposition.

[00479] Metabolic Syndrome is associated with an increased the risk of coronary heart disease and other disorders related to the accumulation of vascular plaque, such as stroke and peripheral vascular disease, referred to as atherosclerotic cardiovascular disease (ASCVD). Patients with Metabolic Syndrome may progress from an insulin resistant state in its early stages to full blown type II diabetes with further increasing risk of ASCVD. Without intending to be bound by any particular theory, the relationship between insulin resistance, Metabolic Syndrome and vascular disease may involve one or more concurrent pathogenic mechanisms including impaired insulin-stimulated vasodilation, insulin resistance-associated reduction in NO availability due to enhanced oxidative stress, and abnormalities in adipocyte-derived hormones such as adiponectin (Lteif and Mather, Can. J. Cardiol. 20 (suppl. B):66B-76B (2004)).

[00480] According to the 2001 National Cholesterol Education Program Adult Treatment Panel (ATP III), any three of the following traits in the same individual meet the criteria for Metabolic Syndrome: (a) abdominal obesity (a waist circumference over 102 cm in men and over 88 cm in women); (b) serum triglycerides (150 mg/dl or above); (c) HDL cholesterol (40 mg/dl or lower in men and 50 mg/dl or lower in women); (d) blood pressure (130/85 or more); and (e) fasting blood glucose (110 mg/dl or above). According to the World Health Organization (WHO), an individual having high insulin levels (an elevated fasting blood glucose or an elevated post meal glucose alone) with at least two of the following criteria meets the criteria for Metabolic Syndrome: (a) abdominal obesity (waist to hip ratio of greater than 0.9, a body mass index of at least 30 kg/m2, or a waist measurement over 37 inches); (b) cholesterol panel showing a triglyceride level of at least 150 mg/dl or an HDL cholesterol lower than 35 mg/dl; (c) blood pressure of 140/90 or more, or on treatment for high blood pressure). (Mathur, Ruchi, "Metabolic Syndrome," ed. Shiel, Jr., William C., MedicineNet.com, May 11, 2009).

[00481] For purposes herein, if an individual meets the criteria of either or both of the criteria set forth by the 2001 National Cholesterol Education Program Adult Treatment Panel or the WHO, that individual is considered as afflicted with Metabolic Syndrome.

[00482] Without being bound to any particular theory, compositions and conjugates described herein are useful for treating Metabolic Syndrome. Accordingly, the invention provides a method of preventing or treating Metabolic Syndrome, or reducing one, two, three or more risk factors thereof, in a subject, comprising providing to the subject a composition described herein in an amount effective to prevent or treat Metabolic Syndrome, or the risk factor thereof.

[00483] In some embodiments, the method treats a hyperglycemic medical condition. In exemplary aspects, the hyperglycemic medical condition is diabetes, diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes, either insulin-dependent or non-insulin-dependent. In some aspects, the method treats the hyperglycemic medical condition by reducing one or more complications of diabetes including nephropathy, retinopathy and vascular disease.

[00484] In some aspects, the disease or medical condition is obesity. In some aspects, the obesity is drug-induced obesity. In some aspects, the method treats obesity by preventing or reducing weight gain or increasing weight loss in the patient. In some aspects, the method treats obesity by reducing appetite, decreasing food intake, lowering the levels of fat in the patient, or decreasing the rate of movement of food through the gastrointestinal system.

[00485] Because obesity is associated with the onset or progression of other diseases, the methods of treating obesity are further useful in methods of reducing complications associated with obesity including vascular disease (coronary artery disease, stroke, peripheral vascular disease, ischemia reperfusion, etc.), hypertension, onset of diabetes type II, hyperlipidemia and musculoskeletal diseases. The present disclosures accordingly provides methods of treating or preventing these obesity-associated complications.

[00486] In some embodiments, the disease or medical condition is Nonalcoholic fatty liver disease (NAFLD). NAFLD refers to a wide spectrum of liver disease ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), to cirrhosis (irreversible, advanced scarring of the liver). All of the stages of NAFLD have in common the accumulation of fat (fatty infiltration) in the liver cells (hepatocytes). Simple fatty liver is the abnormal accumulation of a certain type of fat, triglyceride, in the liver cells with no inflammation or scarring. In NASH, the fat accumulation is associated with varying degrees of inflammation (hepatitis) and scarring (fibrosis) of the liver. The inflammatory cells can

destroy the liver cells (hepatocellular necrosis). In the terms "steatohepatitis" and "steatonecrosis", steato refers to fatty infiltration, hepatitis refers to inflammation in the liver, and necrosis refers to destroyed liver cells. NASH can ultimately lead to scarring of the liver (fibrosis) and then irreversible, advanced scarring (cirrhosis). Cirrhosis that is caused by NASH is the last and most severe stage in the NAFLD spectrum. (Mendler, Michel, "Fatty Liver: Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)," ed. Schoenfield, Leslie J., MedicineNet.com, August 29, 2005).

[00487] Alcoholic Liver Disease, or Alcohol-Induced Liver Disease, encompasses three pathologically distinct liver diseases related to or caused by the excessive consumption of alcohol: fatty liver (steatosis), chronic or acute hepatitis, and cirrhosis. Alcoholic hepatitis can range from a mild hepatitis, with abnormal laboratory tests being the only indication of disease, to severe liver dysfunction with complications such as jaundice (yellow skin caused by bilirubin retention), hepatic encephalopathy (neurological dysfunction caused by liver failure), ascites (fluid accumulation in the abdomen), bleeding esophageal varices (varicose veins in the esophagus), abnormal blood clotting and coma. Histologically, alcoholic hepatitis has a characteristic appearance with ballooning degeneration of hepatocytes, inflammation with neutrophils and sometimes Mallory bodies (abnormal aggregations of cellular intermediate filament proteins). Cirrhosis is characterized anatomically by widespread nodules in the liver combined with fibrosis. (Worman, Howard J., "Alcoholic Liver Disease", Columbia University Medical Center website).

[00488] Without being bound to any particular theory, the compositions and conjugates described herein are useful for the treatment of Alcoholic Liver Disease, NAFLD, or any stage thereof, including, for example, steatosis, steatohepatitis, hepatitis, hepatic inflammation, NASH, cirrhosis, or complications thereof. Accordingly, the present disclosures provides a method of preventing or treating Alcoholic Liver Disease, NAFLD, or any stage thereof, in a subject comprising providing to a subject a composition described herein in an amount effective to prevent or treat Alcoholic Liver Disease, NAFLD, or the stage thereof. Such treatment methods include reduction in one, two, three or more of the following: liver fat content, incidence or progression of cirrhosis, incidence of hepatocellular carcinoma, signs of inflammation, e.g.,abnormal hepatic enzyme levels (e.g., aspartate aminotransferase AST and/or alanine aminotransferase ALT, or LDH), elevated serum

ferritin, elevated serum bilirubin, and/or signs of fibrosis, e.g.,elevated TGF-beta levels. In exemplary embodiments, the compositions are used treat patients who have progressed beyond simple fatty liver (steatosis) and exhibit signs of inflammation or hepatitis. Such methods may result, for example, in reduction of AST and/or ALT levels.

[00489] GLP-1 and exendin-4 have been shown to have some neuroprotective effect. The present disclosures also provides uses of the compositions described herein in treating neurodegenerative diseases, including but not limited to Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, Amylotrophic Lateral Sclerosis, other demyelination related disorders, senile dementia, subcortical dementia, arteriosclerotic dementia, AIDS-associated dementia, or other dementias, a central nervous system cancer, traumatic brain injury, spinal cord injury, stroke or cerebral ischemia, cerebral vasculitis, epilepsy, Huntington's disease, Tourette's syndrome, Guillain Barre syndrome, Wilson disease, Pick's disease, neuroinflammatory disorders, encephalitis, encephalomyelitis or meningitis of viral, fungal or bacterial origin, or other central nervous system infections, prion diseases, cerebellar ataxias, cerebellar degeneration, spinocerebellar degeneration syndromes, Friedreichs ataxia, ataxia telangiectasia, spinal dysmyotrophy, progressive supranuclear palsy, dystonia, muscle spasticity, tremor, retinitis pigmentosa, striatonigral degeneration, mitochondrial encephalo-myopathies, neuronal ceroid lipofuscinosis, hepatic encephalopathies, renal encephalopathies, metabolic encephalopathies, toxin-induced encephalopathies, and radiation-induced brain damage.

[00490] In some embodiments, the compositions are used in conjunction with parenteral administration of nutrients to non-diabetic patients in a hospital setting, e.g., to patients receiving parenteral nutrition or total parenteral nutrition. Nonlimiting examples include surgery patients, patients in comas, patients with digestive tract illness, or a nonfunctional gastrointestinal tract (e.g. due to surgical removal, blockage or impaired absorptive capacity, Crohn's disease, ulcerative colitis, gastrointestinal tract obstruction, gastrointestinal tract fistula, acute pancreatitis, ischemic bowel, major gastrointestinal surgery, certain congenital gastrointestinal tract anomalies, prolonged diarrhea, or short bowel syndrome due to surgery, patients in shock, and patients undergoing healing processes often receive parenteral administration of carbohydrates along with various combinations of lipids, electrolytes, minerals, vitamins and amino acids. The compositions comprising the GIP agonist peptide

and glucagon antagonist peptide, as described herein, and the parenteral nutrition composition can be administered at the same time, at different times, before, or after each other, provided that the composition is exerting the desired biological effect at the time that the parenteral nutrition composition is being digested. For example, the parenteral nutrition may be administered, 1, 2 or 3 times per day, while the composition is administered once every other day, three times a week, two times a week, once a week, once every 2 weeks, once every 3 weeks, or once a month.

[00491] As used herein, the term "treating" includes prophylaxis of the specific disorder or condition, or alleviation of the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms. For example, as used herein the term "treating diabetes" will refer in general to altering glucose blood levels in the direction of normal levels and may include increasing or decreasing blood glucose levels depending on a given situation.

[00492] As used herein an "effective" amount or a "therapeutically effective amount" of a glucagon peptide refers to a nontoxic but sufficient amount of the peptide to provide the desired effect. For example one desired effect would be the prevention or treatment of hypoglycemia, as measured, for example, by an increase in blood glucose level. An alternative desired effect for the glucagon peptides of the present disclosure would include treating hyperglycemia, e.g., as measured by a change in blood glucose level closer to normal, or inducing weight loss/preventing weight gain, e.g., as measured by reduction in body weight, or preventing or reducing an increase in body weight, or normalizing body fat distribution. The amount that is "effective" will vary from subject to subject, depending on the age and general condition of the individual, mode of administration, and the like. Thus, it is not always possible to specify an exact "effective amount." However, an appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

[00493] *SUBJECTS*

[00494] With regard to the above methods of treatment, the patient is any host. In some embodiments, the host is a mammal. As used herein, the term “mammal” refers to any vertebrate animal of the mammalia class, including, but not limited to, any of the monotreme,

marsupial, and placental taxas. In some embodiments, the mammal is one of the mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. In exemplary embodiments, the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). In exemplary embodiments, the mammals are from the order Artiodactyla, including Bovines (cows) and S wines (pigs) or of the order Perssodactyla, including Equines (horses). In some instances, the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). In particular embodiments, the mammal is a human.

KITS

[00495] The glucagon analogs of the present disclosure can be provided in accordance with one embodiment as part of a kit. Accordingly, in some embodiments, a kit for administering a glucagon analog to a patient in need thereof is provided wherein the kit comprises a glucagon analog as described herein.

[00496] In one embodiment the kit is provided with a device for administering the glucagon analog to a subject. The device in some aspects is a syringe needle, pen device, jet injector or other needle-free injector. The kit may alternatively or in addition include one or more containers, *e.g.*, vials, tubes, bottles, single or multi-chambered pre-filled syringes, cartridges, infusion pumps (external or implantable), jet injectors, pre-filled pen devices and the like, optionally containing the glucagon analog in a lyophilized form or in an aqueous solution. The kits in some embodiments comprise instructions for use. In accordance with one embodiment the device of the kit is an aerosol dispensing device, wherein the composition is prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in one embodiment the sterile glucagon composition is prepackaged within the syringe.

[00497] The kits in some embodiments comprise instructions for use. The instructions in some aspects include instructions for use in accordance with any of the methods described herein. The instructions may additionally include instructions for maintaining a healthy diet and/or a physical exercise program. The instructions may be in the form of a paper pamphlet, or in electronic form, e.g., a computer readable storage device comprising the instructions.

[00498] The following examples are given merely to illustrate the present invention and not in any way to limit its scope.

EXAMPLES

EXAMPLE 1

[00499] The following provides exemplary methods of synthesizing the peptide analogs of the present disclosures.

[00500] *Synthesis of peptide fragments of glucagon*

[00501] *Materials:*

[00502] All peptides described herein in the EXAMPLES were amidated unless specified otherwise.

[00503] MBHA resin (4-methylbenzhydrylamine polystyrene resin was used during peptide synthesis. MBHA resin, 100-180 mesh, 1% DVB cross-linked polystyrene; loading of 0.7-1.0 mmol/g), Boc-protected and Fmoc protected amino acids were purchased from Midwest Biotech. The solid phase peptide syntheses using Boc-protected amino acids were performed on an Applied Biosystem 430A Peptide Synthesizer. Fmoc protected amino acid synthesis was performed using the Applied Biosystems Model 433 Peptide Synthesizer.

[00504] *Peptide synthesis (Boc amino acids/ HF cleavage):*

[00505] Synthesis of these analogs was performed on the Applied Biosystem Model 430A Peptide Synthesizer. Synthetic peptides were constructed by sequential addition of amino acids to a cartridge containing 2 mmol of Boc protected amino acid. Specifically, the synthesis was carried out using Boc DEPBT-activated single couplings. At the end of the coupling step, the peptidyl-resin was treated with TFA to remove the N-terminal Boc protecting group. It was washed repeatedly with dimethylformamide (DMF) and this repetitive cycle was repeated for the desired number of coupling steps. After the assembly, the sidechain protection, Fmoc, was removed by 20% piperidine treatment and acylation was conducted using DIC. The peptidyl-resin at the end of the entire synthesis was dried by using dichloromethane (DCM), and the peptide was cleaved from the resin with anhydrous HF.

[00506] For the lactamization, orthogonal protecting groups were selected for Glu and Lys (e.g.,Glu(Fm), Lys(Fmoc)). After removal of the protecting groups and before HF cleavage, cyclization was performed as described previously (see, e.g., International Patent Application Publication No. WO2008/101017).

[00507] *HF treatment of the peptidyl-resin*

[00508] The peptidyl-resin was treated with anhydrous hydrogen fluoride (HF), and this typically yielded approximately 350 mg (~50% yield) of a crude deprotected-peptide. Specifically, the peptidyl-resin (30mg to 200mg) was placed in the HF reaction vessel for cleavage. 500 μL of p-cresol was added to the vessel as a carbonium ion scavenger. The vessel was attached to the HF system and submerged in the methanol/dry ice mixture. The vessel was evacuated with a vacuum pump and 10 ml of HF was distilled to the reaction vessel. This reaction mixture of the peptidyl-resin and the HF was stirred for one hour at 0° C, after which a vacuum was established and the HF was quickly evacuated (10-15 min). The vessel was removed carefully and filled with approximately 35 ml of ether to precipitate the peptide and to extract the p-cresol and small molecule organic protecting groups resulting from HF treatment. This mixture was filtered utilizing a teflon filter and repeated twice to remove all excess cresol. This filtrate was discarded. The precipitated peptide dissolves in approximately 20 ml of 10% acetic acid (aq). This filtrate, which contained the desired peptide, was collected and lyophilized.

[00509] An analytical HPLC analysis of the crude solubilized peptide was conducted under the following conditions [4.6 X 30 mm Xterra C8, 1.50 mL/min, 220 nm, A buffer 0.1% trifluoroacetic acid (TFA)/10% acrylonitrile (CAN), B buffer 0.1% TFA/100% ACN, gradient 5-95%B over 15 minutes]. The extract was diluted twofold with water and loaded onto a 2.2 X 25 cm Vydac C4 preparative reverse phase column and eluted using an acetonitrile gradient on a Waters HPLC system (A buffer of 0.1% TFA/10% ACN, B buffer of 0.1% TFA/10% ACN and a gradient of 0-100% B over 120 minutes at a flow of 15.00 ml/min. HPLC analysis of the purified peptide demonstrated greater than 95% purity and electrospray ionization mass spectral analysis was used to confirm the identity of the peptide.

[00510] *Peptide Acylation*

[00511] Acylated peptides were prepared as follows. Peptides were synthesized on a solid support resin using either a CS Bio 4886 Peptide Synthesizer or Applied Biosystems 430A Peptide Synthesizer. In situ neutralization chemistry was used as described by Schnolzer et al., Int. J. Peptide Protein Res. 40: 180-193 (1992). For acylated peptides, the target amino acid residue to be acylated (e.g., position ten, relative to the amino acid position numbering of SEQ ID NO: 3) was substituted with an N ε -FMOC lysine residue. Treatment of the

completed N-terminally BOC protected peptide with 20% piperidine in DMF for 30 minutes removed FMOC/formyl groups. Coupling to the free ε-amino Lys residue was achieved by coupling a ten-fold molar excess of either an FMOC-protected spacer amino acid (ex. FMOC-Glu-OtBu) or acyl chain (ex. $CH_3(CH_2)_{14}$-COOH)and PyBOP or DEPBT coupling reagent in DMF/*N,N*-diisopropylethylamine (DIEA). Subsequent removal of the spacer amino acid's FMOC group is followed by repetition of coupling with an acyl chain. Final treatment with 100% TFA resulted in removal of any side chain protecting groups and the N-terminal BOC group. Peptide resins were neutralized with 5% DIEA/DMF, dried, and then cleaved from the support using HF/p-cresol, 95:5, at 0°C for one hour. Following ether extraction, a 5% acetic acid (HOAc) solution was used to solvate the crude peptide. A sample of the solution was then verified to contain the correct molecular weight peptide by ESI-MS. Correct peptides were purified by RP-HPLC using a linear gradient of 10% acetonitrile (CH3CN)/0.1% TFA to 0.1% TFA in 100% CH3CN. A Vydac C18 22 mm x 250 mm protein column was used for the purification. Acylated peptide analogs generally completed elution by a buffer ratio of 20:80. Portions were pooled together and checked for purity on an analytical RP-HPLC. Pure fractions were lyophilized yielding white, solid peptides.

[00512] If a peptide comprised a lactam bridge and target residues to be acylated, acylation is carried out as described above upon addition of that amino acid to the peptide backbone.

[00513] Dual acylations or di-acylations are prepared as follows. Peptides are synthesized on a solid support resin using either a CS Bio 4886 Peptide Synthesizer or Applied Biosystems 430A Peptide Synthesizer. In situ neutralization chemistry is used as described by Schnolzer et al., Int. J. Peptide Protein Res. 40: 180-193 (1992). For two site double acylated peptides, the target amino acid residues to be acylated (e.g., position ten and 40, relative to the amino acid position numbering of SEQ ID NO: 3) are substituted with an N ε - FMOC lysine residue. Treatment of the completed N-terminally BOC protected peptide with 20% piperidine in DMF for 30 minutes removes FMOC/formyl groups. Coupling to the free ε-amino Lys residue is achieved by coupling a ten-fold molar excess of either an FMOC-protected spacer amino acid (ex. FMOC-Glu-OtBu) or acyl chain (ex. $CH_3(CH_2)_{14}$-COOH) and PyBOP or DEPBT coupling reagent in DMF/DIEA. Subsequent removal of the spacer amino acid's FMOC group is followed by repetition of coupling with an acyl chain. Final

treatment with 100% TFA results in removal of any side chain protecting groups and the N-terminal BOC group. Peptide resins are neutralized with 5% DIEA/DMF, dried, and then are cleaved from the support using HF/p-cresol, 95:5, at 0°C for one hour. Following ether extraction, a 5% HOAc solution is used to solvate the crude peptide. A sample of the solution is then verified to contain the correct molecular weight peptide by ESI-MS. Correct peptides are purified by RP-HPLC using a linear gradient of 10% CH3CN/0.1% TFA to 0.1% TFA in 100% CH3CN. A Vydac C18 22 mm x 250 mm protein column was used for the purification. Acylated peptide analogs generally complete elution by a buffer ratio of 20:80. Portions are pooled together and checked for purity on an analytical RP-HPLC. Pure fractions are lyophilized yielding white, solid peptides.

[00514] One site branch double acylations are prepared as follows. Peptides are synthesized on a solid support resin using either a CS Bio 4886 Peptide Synthesizer or Applied Biosystems 430A Peptide Synthesizer. In situ neutralization chemistry is used as described by Schnolzer et al., Int. J. Peptide Protein Res. 40: 180-193 (1992). For acylated peptides, the target amino acid residue to be acylated (e.g., position ten relative to the amino acid position numbering of SEQ ID NO: 3) is substituted with an N ε -FMOC lysine residue. Treatment of the completed N-terminally BOC protected peptide with 20% piperidine in DMF for 30 minutes removes FMOC/formyl groups. Coupling to a Lys residue through the C-terminus is achieved by coupling a ten-fold molar excess of an ε-amino and α-amino FMOC-protected spacer amino acid (ex. FMOC-Lys(FMOC)-OH) and PyBOP or DEPBT coupling reagent in DMF/DIEA. Subsequent removal of the spacer amino acid's FMOC groups is followed by coupling each of the ε-amino and α-amino groups with an acyl chain. Final treatment with 100% TFA results in removal of any side chain protecting groups and the N-terminal BOC group. Peptide resins are neutralized with 5% DIEA/DMF, dried, and then are cleaved from the support using HF/p-cresol, 95:5, at 0°C for one hour. Following ether extraction, a 5% HOAc solution is used to solvate the crude peptide. A sample of the solution is then verified to contain the correct molecular weight peptide by ESI-MS. Correct peptides are purified by RP-HPLC using a linear gradient of 10% CH3CN/0.1% TFA to 0.1% TFA in 100% CH3CN. A Vydac C18 22 mm x 250 mm protein column was used for the purification. Acylated peptide analogs generally complete elution by a buffer ratio of 20:80.

Portions are pooled together and checked for purity on an analytical RP-HPLC. Pure fractions were lyophilized yielding white, solid peptides.

[00515] One site linear double acylations are prepared as follows. Peptides are synthesized on a solid support resin using either a CS Bio 4886 Peptide Synthesizer or Applied Biosystems 430A Peptide Synthesizer. In situ neutralization chemistry is used as described by Schnolzer et al., Int. J. Peptide Protein Res. 40: 180-193 (1992). For acylated peptides, the target amino acid residue to be acylated (e.g., position ten, relative to the amino acid position numbering of SEQ ID NO: 3) is substituted with an N ε -FMOC lysine residue. Treatment of the completed N-terminally BOC protected peptide with 20% piperidine in DMF for 30 minutes removes FMOC/formyl groups. Coupling to the free ε-amino Lys residue is achieved by coupling a ten-fold molar excess of either an FMOC-protected spacer amino acid (ex. FMOC-Glu-OtBu) or acyl chain (ex. $CH_3(CH_2)_{14}$-COOH)and PyBOP or DEPBT coupling reagent in DMF/DIEA. Subsequent removal of the spacer amino acid's FMOC group is followed by coupling with an acyl chain functionalized at the end of the fatty acid tail with a protected amino acid sucha as FmocNH-$(CH_2)_{11}$-COOH. The resulting single acylated amino acid is treated with 20% piperidine in DMF to remove the FMOC protecting group, followed by coupling with an acyl chain. Final treatment with 100% TFA results in the removal of any side chain protecting groups and the N-terminal BOC group. Peptide resins are neutralized with 5% DIEA/DMF, dried, and then cleaved from the support using HF/p-cresol, 95:5, at at 0°C for one hour. Following ether extraction, a 5% HOAc solution is used to solvate the crude peptide. A sample of the solution is then verified to contain the correct molecular weight peptide by ESI-MS. Correct peptides are purified by RP-HPLC using a linear gradient of 10% CH3CN/0.1% TFA to 0.1% TFA in 100% CH3CN. A Vydac C18 22 mm x 250 mm protein column is used for the purification. Acylated peptide analogs generally complete elution by a buffer ratio of 20:80. Portions are pooled together and checked for purity on an analytical RP-HPLC. Pure fractions are lyophilized yielding white, solid peptides.

[00516] In the instances of two site or single site double acylations, the two acyl chains to be coupled to the peptide can be the same or different. In the case of two different acyl chains, the target amino acid(s) to be acylated are substituted with two different protecting groups. For example an N ε -FMOC lysine residue and an N ε –ivDde lysine residue (two

site double acylation) or an FMOC Lys (ivDde)-OH (single site double acylation, 852082 Novabiochem). Treatment of the completed N-terminally BOC protected peptide with 20% piperidine in DMF for 30 minutes removed FMOC/formyl groups. The free ε-amino Lys residue is coupled with either an acyl group or spacer amino acid followed by coupling to an acyl group. The resulting single acylated peptide is treated with 2% hydrazine/DMF to remove the(ivDde) protecting group. The free amino Lys residue is coupled with either an acyl group or a spacer amino acid followed by coupling to an acy group. The double acylated peptide is isolated as described above.

[00517] An example synthesis of a dual acylated peptide (Glucagon Aib2 E16 A18 L27 D28 Cex K40(C16γE-K-γEC12)G41-amide, having the amino acid sequence of SEQ ID NO: 211) is shown in Figure 5B and is further described below:

[00518] 0.28gm (0.2mmole) mbha-resin (Midwest Biotech) was placed in a reaction vessel and the following sequence was assembled on a CSBio336 synthesizer using DEPBT/DIEA activated single couplings of Boc amino acids.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha resin

[00519] The Boc protected peptide resin was transferred to a manual reaction vessel and was treated with 20% piperidine/DMF at room temp for 10min. The resin was filtered, washed with DMF 3 times, and an activated solution of Fmoc Lys(ivDde)-OH (EMD-Novabiochem) was added (previously prepared by dissolving 2mmole Fmoc Lys(ivDde) in 4.0ml 0.5M DEPBT/DMF and 0.35ml (2mmole) DIEA wad added.

[00520] The peptide resin was mixed at room temp for 16hrs, then filtered, washed with DMF and was treated with 20% piperidine/DMF for 10min. The resin was filtered, washed with DMF several times, and an activated solution of Fmoc Glu-OBzl was added (previously prepared by dissolving 2mmole Fmoc Glu-OBzl in 4.0ml 0.5M DEPBT/DMF and 0.35ml DIEA was added). The reaction was mixed 1hr at room temp then filtered and the resin washed with DMF. After another treatment with 20% pip/DMF, the resin was washed with DMF several times, and an activated solution of palmitic acid was added to complete the acylation of the α-amine of the side chain Lys at position 40 (previously prepared by dissolving 2mmole palmitic acid in 4.0ml 0.5M DEPBT/DMF and adding 0.35ml DIEA). The reaction was mixed for 1hr.

[00521] The peptide resin was filtered, washed with DMF and treated with 2% hydrazine/DMF at room temp for 15min, the filtered and was washed with DMF several times. An activated solution of Fmoc Glu-OBzl was added (same as above) and the reaction mixed for 1hr at rt. The resin was divided into three portions for final acylation at the ε-lysine amine. In this case, to one portion an activated solution of dodecanoic acid was added. Previously prepared by dissolving 1mmole dodecanoic acid (Aldrich) in 2.0ml 0.5M DEPBT/DMF and 0.175ml DIEA was added. The reaction was mixed 1hr, the filtered, washed with DMF, fthen with dichloromethane. The N-terminal Boc group was removed on treatment with 50%TFA/DCM, and after neutralization with 5%DIEA/DCM, an HF cleavage was run in an ice bath for 1hrs using p-cresol as a scavenger. After evaporation of the HF, the residue was suspended in ethyl ether and the peptide/resin mixture was filtered and washed with ether. The peptide was extracted into aqueous acetic acid and analyzed on HPLC. (4.6x50mm Zorbax SB-C8, 1ml/min, 45°C, 214nm (0.5A) A=0.1%TFA, B=0.1%TFA/90%ACN. The remaining cleavage extract was loaded onto a 21.2x250mm Amberchrom XT20 column and a 0.1%TFA/acetonitrile gradient was run on a Pharmacia FPLC for purification.

[00522] Fractions 83-86 were combined, frozen and lyophilized to give 22.3mg of the peptide of SEQ ID NO: 211 with a purity of 90%+. Theoretical mol.wt.= 5202.9, ESI observed mass = 5202.0

[00523] *Peptide Acylation via Succinoylation*

[00524] Succinoylated peptides are prepared as follows. Peptides are synthesized on a solid support resin using either a CS Bio 4886 Peptide Synthesizer or Applied Biosystems 430A Peptide Synthesizer. In situ neutralization chemistry is used as described by Schnolzer et al., Int. J. Peptide Protein Res. 40: 180-193 (1992). For succincoylated peptides, the target amino acid residue to be acylated (e.g., position ten, relative to the amino acid position numbering of SEQ ID NO: 3) is substituted with an N ε -FMOC lysine residue. Treatment of the completed N-terminally BOC protected peptide with 20% piperidine in DMF for 10 minutes removed FMOC/formyl groups. The resin is filtered, washed with DMF/DCM and re-suspended in DCM. Coupling to the free ε-amino Lys residue is achieved by coupling a ten-fold molar excess of n-hexadecylsuccinic anhydride (TCl) along with 4-dimethylaminopyridine. The resin is mixed overnight, filtered, washed with DCM and

treated with 50% TFA/DCM to remove any side chain protecting groups and the N-terminal BOC. Peptide resins are neutralized with 5% DIEA/DMF, dried, and then are cleaved from the support using HF/p-cresol, 95:5, at 0°C for one hour. Following ether extraction, a 5% HOAc solution is used to solvate the crude peptide. A sample of the solution is then verified by HPLC analysis. The remaining acetic acid solution is loaded onto a 10x250mm Amberchrom XT20 column for purification. An aqueous TFA/ACN gradient is run while collecting fraction and monitoring the UV at 214nm. Pure fractions are lyophilized, yielding solid peptides.

[00525] An example synthesis of a succinoylated peptide (Glucagon Aib2 E16 A18 L27 D28 Cex K40(C16 succinoyl)G41-amide having the amino acid sequence of SEQ ID NO: 156) is shown in Figure Figure 3C and is further described below:

[00526] 0.28gm (0.2mmole) mbha-resin (Midwest Biotech) was placed in a reaction vessel and the following sequence was assembled on a CSBio336 synthesizer using DEPBT/DIEA activated single couplings.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha resin

[00527] Approximately, one third of the Boc protected peptide resin was transferred to a manual reaction vessel and was treated with 20% piperidine/DMF at room temp for 10min. The resin was filtered, washed with DMF 3 times, with dichloromethane twice and re-suspended in 10ml DCM. 324mg (1mmole) n-hexadecylsuccinic anhydride (TCI) was added along with 2-3mg of 4-dimethylaminopyridine (Aldrich).

[00528] The resin was mixed overnight at room temp before filtering, washing with DCM twice, and treating with 50%TFA/DCM for 1-2min. The resin was filtered , washed several times with DCM, neutralized by washing with 5%DIEA/DCM and transferred to a HF reaction vessel. An HF cleavage was run using 5ml liquid hydrogen fluoride and 0.5ml p-cresol scavenger. After stirring 1hr in an ice bath, the HF was removed in vacuo and the residue suspended in ethyl ether. The suspension was filtered using a sintered glass funnel, the solids washed with ether, and the peptide extracted into 15ml 50% aqueous acetic acid. After analysis on HPLC (4.6x50mm Zorbax SB-C8, 1ml/min, 45°C,214nm, A=0.1%TFA, B=0.1%TFA/90%ACN, gradient = 30%B to 90%B over 10min), the cleavage extract was loaded onto a 21.2x250mm Amberchrom XT20 column for purification. An aq

TFA/acvetonitrile gradient was run while collecting fractions and monitoring the UV absorbance. Fractions 55-56 were identified as being single component and were frozen and lyophilized. 32.5mg was recovered with a purity of 90%+ (DLS-027-68B). Theoretical mol. wt.= 4720.3, ESI observed mass = 4717.0

[00529] *Peptide Alkylation (e.g., S-Alkylation)*

[00530] An example synthesis of an alkylated (e.g., S-alkylated) peptide (Glucagon Aib2 E16 A18 L27 D29 Cex Cys40(S-2 palmityl)amide having an amino acid sequence of SEQ ID NO: 164) is shown in Figure 6 and is further described below:

[00531] 0.28 gm 0.2mmole mbha-resin (Midwest Biotech) was placed in a CSBio reaction vessel and the following sequence was assembled on a CSBio336 synthesizer using Boc amino acids and DEPBT/DIEA activated single couplings.

HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSC-amide

[00532] The peptide resin was transferred to an HF reaction vessel and an HF cleavage was run using 10 ml liquid hydrogen fluoride and 1ml p-cresol scavenger in an ice bath for 1hr. After evaporating the HF, the residue was suspended in ethyl ether and the peptide and resin were filtered into a sintered glass funnel. After washing with ethyl ether and quickly air drying, the peptide was extracted into 50% aqueous acetic acid. After analysis via HPLC (4.6x50mm Zorbax SB-C8, 1ml/min, 45°C, 214nm, A=0.1%TFA, B=0.1%TFA/90%ACN, 30% to90%B over 10min), the cleavage extract was diluted 3-4 with water and was loaded onto a 22.2x250mm Amberchrom XT20 column for purification using aq TFA/CAN gradient. An initial pool was re-purified over the same column to give 79mg of 95% purity. Theoretical mol.wt.= 4313.7, ESI observed mass= 4312.0

[00533] 16 mg (3.7μmole) of the above free thiol peptide was suspended in 1 ml methanol and with stirring under a stream of nitrogen, 1.5ul of tetramethylguandine in 1ml methanol (MeOH) was added followed by 3mg (7.8μmole) 2-iodohexadecanoic acid (2-iodopalmitic acid) in 0.5mg tetrahydrofuran (THF). 2-iodohexadecanoic acid was previously prepared by treating 2-bromohexadecanoic acid with potassium iodide in acetone (DLS-027-52). The alkylation reaction was warmed in a bath for 10min during which most of the solvent evaporated. The residue was dissolved in aqueous acetic acid, analyzed by HPLC and was found to be more hydrophobic than the starting peptide. The remaining acetic acid solution

was loaded onto a 10x250mm Amberchrom XT20 column for purification. An aq.TFA/ACN gradient was run while collecting fractions and monitoring the UV at 214nm. Fractions 64-68 were combined, frozen and lyophilized to give 6.1mg of material (the peptide of SEQ ID NO: 164) with a purity of 90%+. Theoretical mol.wt= 4568.1, MALDI observed mass= 4568.79

[00534] When the peptide comprises a Lys residue instead of the Cys residue at the C-terminus, the peptide may be made with the backbone Lys first. The target amino acid residue to be N-alkylated is substituted with an N ε -FMOC lysine residue. Treating of the completed N-terminally BOC protected peptide with 20% piperidine in DMF for 30 minutes removes FMOC/formyl groups from the Lys residue. A Cys residue is coupled to the free ε-amino Lys residue. The Cys residue is then alkylated by reaction with 2-iodopalmitic acid as described above.

[00535] *Peptide Acylation with "miniPEG" spacers*

[00536] An example synthesis of an acylated peptide via a mini-PEG spacer (the peptide having an amino acid sequence of SEQ ID NO: 89) is described below:

[00537] 0.28gm (0.2mmole) 4-methylbenzhydrylamine (mbha) resin (Midwest Biotech, Inc., Fishers, IN) was placed in a reaction vessel and the following sequence was assembled on a CSBio336 synthesizer using Boc amino acids and 3-(diethoxyphosphoryloxy)-3H-benzo[d][1,2,3] triazine-4-one /N,N-Diisopropylethylamine (DEPBT/DIEA) activated single couplings.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha

[00538] Approximately, one third of the Boc protected peptide resin was transferred to a manual reaction vessel and was treated with 20% piperidine/dimethylformamide at room temperature for 10 min. The resin was filtered, washed with dimethylformamide (DMF) several times and an activated solution of Fmoc amidoPEG4 was added (previously prepared by dissolving 487.5mg 1.0mmole N-Fmoc Amido dPEG4 Acid (Peptides International, Louisville, KY) in 2.0ml 0.5M DEPBT/DMF and adding 0.175ml 1mmole diisopropylethylamine). The reaction was mixed at room temp for aprox. 1hr.

[00539] The peptide resin was filtered washed with DMF, and again treated with 20% piperidine/DMF for 10min. The resin was filtered, washed with DMF several times, and an activated solution of Fmoc Glu-OBzl was added (previously prepared by dissolving 470mg 1mmole of Fmoc Glu-γ-OBzl (Aapptec, Louisville, KY) in 2.0ml 0.5M DEPBT/DMF and adding by 0.175ml 1mmole DIEA). The reaction was mixed at room temp for 1hr.

[00540] The peptide resin was again filtered, washed with DMF, and treated with 20% piperidine/DMF at room temp for 10 min. The resin was filtered, washed with DMF several times, and an activated solution of palmitic acid was added (previously prepared by dissolving 256mg 1mmole palmitic acid (Sigma-Aldrich, St. Louis, MO) in 2.0 ml 0.5M DEPBT/DMF and adding 0.175ml DIEA. The reaction was mixed at room temp for 1hr.

[00541] Finally, the peptide resin was filtered, washed with DMF followed by dichloromethane and was treated with 50%TFA/DCM to remove the N-terminal Boc group. After 1- 2 min, the resin was filtered, washed with DCM several times followed by a solution of 5%DIEA/DCM. The air dried peptide resin was transfered to an HF reaction vessel and an HF cleavage was conducted using 5ml liquid hydrogen fluoride and 0.5ml p-cresol scavenger. After mixing 1hr in an ice bath, the HF was removed in vacuo and the residue was suspended in ethyl ether. The peptide/resin mixture was filtered in a sintered glass funnel, washed with ethyl ether, and quickly air dried. The peptide was extracted into 10ml 50% aqueous acetic acid which was analyzed by HPLC (4.6x50mm Zorbax SB-C8, 1ml/min, 214nm, A=0.1%TFA, B=0.1%TFA/90%ACN, gradient = 30%B to 90%B over 10min). The remaining crude extract was diluted 3X with water and was loaded onto a 22.2x250mm Amberchrom XT20 column for purification using an aqueous TFA/ACN gradient. An initial purification pool was re-purified over the same column to give 19mg of product with a purity of 90%+. Theoretical mass =5010.6 , ESI observed mass was 5008.0 .

[00542] The same procedure was repeated using different N-Fmoc Amido dPEG Acids. In one instance, N-Fmoc Amido dPEG8 Acid (Peptides International, Louisville, KY) was used and in another instance, N-Fmoc Amido dPEG2 Acid (Peptides International, Louisville, KY) was used. The structures of the acylated peptides are shown in Figures 12A – 12C.

[00543] *Peptide Dimerization – Disulfide dimerization*

[00544] An example synthesis of a disulfide dimer peptide is described below:

[00545] 0.28gm (0.2mmole) mbha-resin (Midwest Biotech) was placed in a CSBio reaction vessel and the following sequence was assembled on a CSBio336 synthesizer using Boc amino acids and DEPBT/DIEA activated single couplings.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-amide

[00546] The Boc protected peptide resin was transferred to a manual reaction vessel and was treated with 20% piperidine/DMF at room temp for 10min. The resin was filtered, thoroughly washed with DMF, then acylated with an activated solution of Fmoc Cys(Trt) (previously prepared by dissolving 1.17gm 2mmole Fmoc Cys(Trt)-OH (Aapptec) in 4.0ml 0.5M DEPBT/DMF and adding 0.35ml 2mmole diisopropylethylamine). The reaction was mixed at room temp for 2hrs, then filtered washed with DMF and the same procedure was used to add Fmoc Glu-OBzl followed by palmitic acid.

[00547] Finally, the resin was filtered, washed with DMF, with dichlorormethane, and was treated with 50% TFA/DCM for 1-2min. After neutralization with 5%DIEA/DCM, the peptide resin was transferred to and HF reaction vessel and an HF cleavage was run using 10ml liquid hydrogen fluoride and 1ml p-cresol scavenger in an ice bath for 1hr. After evaporating the HF, the residue was suspended in ethyl ether and the peptide and resin were filtered into a sintered glass funnel. After washing with ethyl ether and quickly air drying, the peptide was extracted into 50%aqueous acetic acid. After analysis via HPLC (4.6x50mm Zorbax SB-C8, 1ml/min, 45°C, 214nm, A=0.1%TFA, B=0.1%TFA/90%ACN, 30% to90%B over 10min), the cleavage extract was diluted 3-4 with water and was loaded onto a 21.2x250mm Amberchrom XT20 column for purification using aq TFA/acetonitrile gradient. Purification fractions 64-67 were combined, frozen, and lyophilized to give 58.7mg of material with a HPLC purity of 90%+.

[00548] DLS-027-97A Theoretical mol.wt.= 4866.48, ESI observed mass= 4864.0

HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Cys(SH) γE-C16)G-amide

[00549] 18.6mg (3.8μmole) of the above peptide was dissolved in 2.0ml 3M guanidine/ 0.05M tris (pH8.5) and 1ml dimethylsulfoxide was added. The reaction was stirred at room temp exposed to the air. After 6hrs, an analytical HPLC compared to the starting peptide showed the presence of a more hydrophobic peak.

[00550] The reaction mixture was diluted with 20ml 0.1%TFA and was loaded onto a 10x250mm Amberchrom XT20 column. A purification was run using a 0.1%TFA/acetonitrile gradient while monitoring the UV absorbance at 220nm. Fractions 68-72 were combined and lyophilized to give 5.5mg of purified dimer.

[00551] HPLC purity was 90%+. Theoretical mol. wt. =9743.99, MALDI observed mass= 9745.1. DLS-027-98B. The structure of the resulting dimer is shown in Figure 9A.

[00552] *Peptide Dimerization - Thioether dimerization*

[00553] An example synthesis of a thioether dimer is described below:

[00554] 0.28gm (0.2mmole) mbha-resin (Midwest Biotech) was placed in a reaction vessel and the following sequence was assembled on a CSBio336 synthesizer using DEPBT/DIEA activated single couplings.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha

[00555] One third of the Boc protected peptide resin was transferred to a manual reaction vessel and was treated with 20% piperidine/DMF at room temp for 10min. After washing several time with DMF, an activated solution of Boc Dap(Fmoc) was added (previously prepared by dissolving 426mg 1mmole Boc Dap(Fmoc)-OH (Chem-Impex) in 2.0ml 0.5M DEPBT/DMF and 0.175ml 1mmole DIEA was added). The reaction was mixed at room temp for 2hrs, the filtered, washed with DMF and re-treated with 20% piperidine/DMF as above. After washing with DMF, the resin was acylated with an activated solution of bromoacetic acid (previously prepared by dissolving 139mg 1mmole bromoacetic acid (Aldrich) in 2.0ml 0.5M DEPBT/DMF and adding 0.175ml DIEA). The reaction was mixed at room temp for 1-2hrs, then filtered and the resin washed with DMF followed by DCM. The peptide resin was treaterd with 50%TFA/DCM at room temp for 2min, then filtered, washed with DCM and neutralized with 5% DIEA/DCM. The completed peptide resin was transferred to an HF reaction vessel and an HF cleavage was conducted using 5ml liquid hydrogen fluoride/0.5ml p-cresol. After stirring 1hr in an ice bath, the HF was evaporated and the residue suspended in ethyl ether. The resin/peptide mixture was filtered into a sintered glass funnel and washed with ether. The peptide was extracted into 50% aqueous acetic acid and the crude product was analyzed via HPLC: 4.6x50mm Zorbax SB-C8, 1ml/min, 45°C,214nm, A=0.1%TFA, B=0.1%TFA/90%ACN, gradient= 30%B to 90%B over 10min. The cleavage extract was

loaded onto a 21.2x250mm Amberchrom XT20 column and a purification was run using an aqueous TFA/acetonitrile gradient while monitoring the UV absorbance at 220nm. Fractions 48-53 were combine frozen, and lyophilized to give 18mg of the peptide of SEQ ID NO: 89 with K40(Dap-BrAcetyl), purity =90%+. Theoretical mol. wt.= 4602.8, ESI observed mass= 4616.0

HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Dap-BrAcetyl)G-amide

[00556] 15mg (3.2μmole) of the above K40(Dap-BrAcetyl) peptide and 15mg of the peptide of SEQ ID NO: 89 K40(Cys γE-C16) were dissolved in 3.0ml 7M urea/0.05M tris (pH8.6) and was mixed at room temp while monitoring the HPLC of the reaction progress. After 30min, most of the starting materials were reduced in peak height while a new peak was the major component. The reaction mixture was diluted with 25ml 0.1%TFA and was loaded onto a 10x250mm Amberchrom XT20 column for purification. An aqueous TFA/acetonitrile gradient was run while monitoring the UV absorbance at 220nm. Fractions 57-61 were combined, frozen, and lyophilized to give 7.1mg of thioether dimer. HPLC purity= 90%+, Theoretical mol.wt.=9401.4, MALDI observed mass= 9402.8. The structure of the dimer is shown in Figure 9B.

[00557] *Peptide PEGylation*

[00558] For peptide PEGylation, 40 kDa methoxy poly(ethylene glycol) idoacetamide (NOF) was reacted with a molar equivalent of peptide in 7M Urea, 50mM Tris-HCl buffer using the minimal amount of solvent needed to dissolve both peptide and PEG into a clear solution (generally less than 2 mL for a reaction using 2-3 mg peptide). Vigorous stirring at room temperature commenced for 4-6 hours and the reaction analyzed by analytical RP-HPLC. PEGylated products appeared distinctly from the starting material with decreased retention times. Purification was performed on a Vydac C4 column with conditions similar to those used for the initial peptide purification. Elution occurred around buffer ratios of 50:50. Fractions of pure PEGylated peptide were found and lyophilized. Yields were above 50%, varying per reaction.

[00559] *Analysis using mass spectrometry*

[00560] The mass spectra were obtained using a Sciex API-III electrospray quadrapole mass spectrometer with a standard ESI ion source. Ionization conditions that were used are as follows: ESI in the positive-ion mode; ion spray voltage, 3.9 kV; orifice potential, 60 V.

The nebulizing and curtain gas used was nitrogen flow rate of .9 L/min. Mass spectra were recorded from 600-1800 Thompsons at 0.5 Th per step and 2 msec dwell time. The sample (about 1mg/mL) was dissolved in 50% aqueous acetonitrile with 1% acetic acid and introduced by an external syringe pump at the rate of 5 μL/min.

[00561] When the peptides were analyzed in PBS solution by ESI MS, they were first desalted using a ZipTip solid phase extraction tip containing 0.6 μL C4 resin, according to instructions provided by the manufacturer (Millipore Corporation, Billerica, MA, see the Millipore website of the world wide web at millipore.com/catalogue.nsf/docs/C5737).

[00562] *High Performance Liquid Chromatography (HPLC) analysis:*

[00563] Preliminary analyses were performed with these crude peptides to get an approximation of their relative conversion rates in Phosphate Buffered Saline (PBS) buffer (pH, 7.2) using high performance liquid chromatography (HPLC) and MALDI analysis. The crude peptide samples were dissolved in the PBS buffer at a concentration of 1 mg/ml. 1 ml of the resulting solution was stored in a 1.5 ml HPLC vial which was then sealed and incubated at 37 °C. Aliquots of 100μl were drawn out at various time intervals, cooled to room temperature and analyzed by HPLC.

[00564] The HPLC analyses were performed using a Beckman System Gold Chromatography system using a UV detector at 214 nm. HPLC analyses were performed on a 150 mm x 4.6 mm C18 Vydac column. The flow rate was 1 ml/min. Solvent A contained 0.1% TFA in distilled water, and solvent B contained 0.1% TFA in 90% CH3CN. A linear gradient was employed (40% to 70%B in 15 minutes). The data were collected and analyzed using Peak Simple Chromatography software.

[00565] The initial rates of hydrolysis were used to measure the rate constant for the dissociation of the respective prodrugs. The concentrations of the prodrug and the drug were estimated from their peak areas respectively. The first order dissociation rate constants of the prodrugs were determined by plotting the logarithm of the concentration of the prodrug at various time intervals. The slope of this plot gives the rate constant 'k'. The half lives of the degradation of the various prodrugs were then calculated by using the formula t1/2 = .693/k.

EXAMPLE 2

[00566] This example describes an exemplary method of testing the biological activity of the peptides of the present disclosures, which method involves assaying cAMP synthesis.

[00567] The ability of glucagon analogs to induce cAMP was measured in a firefly luciferase-based reporter assay. HEK293 cells co-transfected with a receptor (glucagon receptor, GLP-1 receptor or GIP receptor) and luciferase gene linked to cAMP responsive element were serum deprived by culturing 16h in DMEM (Invitrogen, Carlsbad, CA) supplemented with 0.25% Bovine Growth Serum (HyClone, Logan, UT) and then incubated with serial dilutions of either glucagon, GLP-1, GIP or novel glucagon analogs for 5 h at 37°C, 5% CO_2 in 96 well poly-D-Lysine-coated "Biocoat" plates (BD Biosciences, San Jose, CA). At the end of the incubation 100 microliters of LucLite luminescence substrate reagent (Perkin-Elmer, Wellesley, MA) were added to each well. The plate was shaken briefly, incubated 10 min in the dark and light output was measured on MicroBeta-1450 liquid scintillation counter (Perkin-Elmer, Wellesley, MA). Effective 50% concentrations were calculated by using Origin software (OriginLab, Northampton, MA.

EXAMPLE 3

[00568] This example describes an exemplary method of assaying the stability of peptides of the present disclosures.

[00569] Each glucagon analog is dissolved in water or PBS and an initial HPLC analysis is conducted. After adjusting the pH (4, 5, 6, 7), the samples are incubated over a specified time period at 37°C and are re-analyzed by HPLC to determine the integrity of the peptide. The concentration of the specific peptide of interest is determined and the percent remaining intact is calculated relative to the initial analysis.

EXAMPLE 4

[00570] This example describes an exemplary method of assaying solubility of peptides.

[00571] A solution (1mg/ml or 3mg/ml) of glucagon (or an analog) is prepared in 0.01N HCl. 100ul of stock solution is diluted to 1ml with 0.01N HCl and the UV absorbance (276nm) is determined. The pH of the remaining stock solution is adjusted to pH7 using 200-250ul 0.1M Na_2HPO_4 (pH9.2). The solution is allowed to stand overnight at 4°C then centrifuged. 100ul of supernatant is then diluted to 1ml with 0.01N HCl, and the UV absorbance is determined (in duplicate).

[00572] The initial absorbance reading is compensated for the increase in volume and the following calculation is used to establish percent solubility:

$$\frac{\text{Final Absorbance}}{\text{Initial Absorbance}} \times 100 = \text{percent soluble}$$

EXAMPLE 5

[00573] This example describes an exemplary method of assaying peptides for binding to a receptor.

[00574] The affinity of peptides to the glucagon receptor is measured in a competition binding assay utilizing scintillation proximity assay technology. Serial 3-fold dilutions of the peptides made in scintillation proximity assay buffer (0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.1% w/v bovine serum albumin) are mixed in 96 well white/clear bottom plate (Corning Inc., Acton, MA) with 0.05 nM (3-[^{125}I]-iodotyrosyl) Tyr10 glucagon (Amersham Biosciences, Piscataway, NJ), 1-6 micrograms per well, plasma membrane fragments prepared from cells over-expressing human glucagon receptor, and 1 mg/well polyethyleneimine-treated wheat germ agglutinin type A scintillation proximity assay beads (Amersham Biosciences, Piscataway, NJ). Upon 5 min shaking at 800 rpm on a rotary shaker, the plate is incubated 12h at room temperature and then is read on MicroBeta1450 liquid scintillation counter (Perkin-Elmer, Wellesley, MA). Non-specifically bound (NSB) radioactivity is measured in the wells with 4 times greater concentration of "cold" native ligand than the highest concentration in test samples and total bound radioactivity is detected in the wells with no competitor. Percent specific binding is calculated as following: % Specific Binding = ((Bound-NSB)/(Total bound- NSB)) X 100. IC_{50} values were determined by using Origin software (OriginLab, Northampton, MA).

EXAMPLE 6

[00575] The following peptides comprising at least Tyr at position 1, AIB at position 2 (for DPP-IV resistance), Lys at position 16, AIB at position 20, and Leu, Ala and Gly at positions 27-29 were made as essentially described in Example 1 and tested in vitro for agonist activity at each of the GLP-1 receptor, glucagon receptor, and GIP receptor as essentially described in Example 2:

The EC50s at the GLP-1 receptor (GLP-1R), the glucagon receptor (GR), and the GIP receptor (GIPR) are provided in Table 1.

TABLE 1

Peptide	SEQ ID NO:	GLP-1 Receptor		Glucagon Receptor		GIP Receptor	
		EC_{50},nM [Std Dev]	relative activity	EC_{50},nM [Std Dev]	relative activity	EC_{50},nM [Std Dev]	relative activity
mt-263	10	0.0100 [0.0154]	154.20%	4.0450 [0.0762]	1.88%	0.0054 [0.0166]	305.91%
mt-402	11	0.0070 [0.0154]	220.60%	0.0298 [0.0762]	256.00%	0.0185 [0.0166]	89.46%
mt-403	12	0.0027 [0.154]	581.89%	0.0077 [0.0762]	987.18%	0.0061 [0.0166]	273.10%
mt-404	13	0.0060 [0.0154]	258.29%	0.1076 [0.0762]	70.80%	0.0327 [0.0166]	50.57%
mt-405	14	0.0022 [0.0154]	717.21%	0.0096 [0.0762]	797.18%	0.0039 [0.0166]	425.45%

Relative activity is activity of peptide relative to the activity of the native hormone

EXAMPLE 7

[00576] Additional peptides mt-395 (SEQ ID NO: 15), mt-396 (SEQ ID NO: 16), mt-397 (SEQ ID NO: 17), and mt-398 (SEQ ID NO: 18) which were based on the structure of mt-263 were made as essentially described in Example 1 and tested in vitro as essentially described in Example 2. The EC50s at each of the GLP-1R, GR, and GIPR are shown in Table 2.

TABLE 2

Peptide	SEQ ID NO:	GLP-1 Receptor		Glucagon Receptor		GIP Receptor	
		EC_{50},nM [Std. Dev]	relative activity	EC_{50},nM [Std. Dev]	relative activity	EC_{50},nM [Std. Dev]	relative activity
mt-263	10	0.0081 [0.0245]	300.61%	3.1371 [0.0298]	0.95%	0.0033 [0.0135]	403.89%
mt-395	15	0.0076 [0.0245]	321.55%	3.4095 [0.0298]	0.87%	0.0025 [0.0135]	537.45%
mt-396	16	0.0093 [0.0245]	262.27%	2.9033 [0.0298]	1.03%	0.0034 [0.0135]	402.69%
mt-397	17	0.0085 [0.0245]	287.88%	5.3528 [0.0298]	0.56%	0.0029 [0.0135]	470.03%
mt-398	18	0.0078 [0.0245]	314.93%	3.7352 [0.0298]	0.80%	0.0031 [0.0135]	433.76%

Relative activity is activity of peptide relative to the activity of the native hormone

EXAMPLE 8

[00577] Additional peptides comprising a Tyr at position 1, AIB at position 2 (for DPP-IV resistance), Glu at position 16 and Lys at position 20 (bridged by a lactam between positions 16 and 20), Leu-Ala-Gly at positions 27-29, and GPSSGAPPPS at positions 30-39 were made as essentially described in Example 1 and tested for agonist activity at each of the GLP-1R, GR, and GIPR as essentially described in Example 2. Other peptides lacking a lactam were made and tested. The results of the activity assays are shown in Table 3.

TABLE 3

Code	SEQ ID NO:	GLP-1R			GR			GIPR		
		EC_{50}, nM	Std Dev	relative activity	EC_{50}, nM	Std Dev	relative activity	EC_{50}, nM	Std Dev	relative activity
mt-217	19	0.222	0.025	11.26%	13.886	0.114	0.82%	9.574	0.019	0.20%
mt-218	20	0.338	0.025	7.40%	16.298	0.114	0.70%	14.283	0.019	0.13%
mt-219	21	0.151	0.025	16.56%	17.628	0.114	0.65%	6.165	0.019	.31%
mt-220	22	0.180	0.025	13.89%	9.670	0.114	1.18%	10.268	0.019	0.19%
mt-225	23	0.098	0.029	29.59%	2.712	0.054	1.99%	1.899	0.017	0.90%
mt-226	24	0.097	0.029	29.90%	3.462	0.054	1.56%	1.467	0.017	1.16%
mt-227	25	0.080	0.029	36.25%	4.244	0.054	1.27%	1.320	0.017	1.29%
mt-228	26	0.146	0.029	19.86%	5.364	0.054	1.01%	2.266	0.017	0.75%

Relative activity is activity of peptide relative to the activity of the native hormone

EXAMPLE 9

[00578] Exemplary peptide analogs of the present disclosures were made as essentially described in Example 1. Each of the peptide analogs comprised an amino acid sequence based on native glucagon (SEQ ID NO: 1) with the native His at position 1, a DPP-IV protective amino acid at position 2, an acylated amino acid at position 10, one or more alpha helix stabilizing amino acids within positions 16-21 of the peptide analog, and other modifications. All peptides were amidated at the C-terminus. The peptides were then tested

for activity at each of the glucagon, GLP-1, and GIP receptors, as described in Example 2. A composite of the results from multiple different activity assays are shown in Table 4.

TABLE 4

SEQ ID NO:	Analog No.	Shorthand Notation of amino acid changes	EC50 (nM) at Glucagon Receptor	EC50 (nM) at GLP-1 Receptor	EC50 (nM) at GIP Receptor
1		Native glucagon	0.025	ND	ND
		Native GLP-1	ND	0.025	ND
2		Native GIP	ND	ND	0.01
27	29	dSer2, K10acyl, E16, A18, L27, D28, G29 +CEX	0.003	0.002	0.085
28	30	Aib2, K10acyl, E16, A18, L27, D28, G29+CEX	0.002	0.002	0.003
29	34	Aib2, K10acyl, E16, A18, L27, D28, GRG29-31	0.007	0.003	0.064
30	36	dSer2, K10acyl, E16, Aib20, E21, L27, D28	0.008	0.0031	0.25
31	37	Aib2, K10acyl, E16, Aib20, E21, L27, D28	0.008	0.0044	0.008
32	44	DMIA1, dSer2, K10acyl, E16, A18, L27, D28	0.004	0.003	0.103
33	49	Aib2, K10acyl, Aib16, A10, D28, G29+CEX	0.005	0.004	0.248
35	53	Aib2, K10acyl, Aib16, A18, V27, K28, G29+CEX	0.011	0.005	0.073
36	54	Aib2, K10acyl, Aib16, A18, K27, D28, G29+CEX	0.008	0.004	0.171
37	61	Aib2, K10acyl, E16, Aib20, E21, L27, D28, G29 +CEX	0.004	0.006	0.002
38	62	Aib2, E3, K10acyl, E16, Aib20, E21, L27, D28, G29 +CEX	0.127	0.008	0.012
39	63	Aib2, E3, K10acyl, E16, L27, D28, G29 +CEX	1.276	0.008	0.026
40	64	Aib2, E3, K10acyl, E16, Aib20, E21, L27, D28	0.042	0.006	0.024
41	65	Aib2, E3, K10acyl, E16, Aib20, L27, D28, G29+CEX	0.221	0.006	0.013

CEX = GPSSGAPPPS (SEQ ID NO: 5)
acyl = C16 acyl

[00579] Each of the peptide analogs of Table 4 demonstrated potent activity at each of the glucagon, GLP-1, and GIP receptors. Notably, each of the peptide analogs exhibited an EC50 at the GIP receptor of <0.5 nM, and most of the peptide analogs exhibited an EC50 at

the GIP receptor of < 0.1 nM. These peptide analogs also exhibited potent activity at the GLP-1 receptor, each peptide analog exhibiting an EC50 ≤0.008 nM. The peptide analogs lacking a Glu at position 3 demonstrated potent activity at the glucagon receptor, each peptide analog of which exhibited an EC50 < 0.03 nM.

[00580] In general, peptide analogs comprising an AIB at position 20 or a combination of Glu at position 16 and a C-terminal extension exhibited highly potent activity at the GIP receptor. Also, peptide analogs comprising AIB at position 2 generally exhibited greater activity at the GIP receptor, as compared to a corresponding peptide analog comprising d-Ser at position 2 (e.g., compare EC50s at GIP receptor between SEQ ID NOs: 2 and 3 or SEQ ID NOs: 5 and 6)

EXAMPLE 10

[00581] Exemplary peptide analogs of the present disclosures were made as essentially described in Example 1. The peptide analogs were similar in structure, comprising an amino acid sequence based on native glucagon (SEQ ID NO: 1) with the native His at position 1, a DPP-IV protective amino acid at position 2, and an alpha helix stabilizing amino acid at position 16, except that the position at which an acylated amino acid occurred varied among this set of peptide analogs. All peptides were amidated at the C-terminus. The peptides were then tested for activity at each of the glucagon, GLP-1, and GIP receptors, as described in Example 2. A composite of the results from multiple different activity assays are shown in Table 5 for some of the peptides made and tested.

TABLE 5

SEQ ID NO:	**Shorthand Notation of amino acid changes**	**EC50 (nM) at Glucagon Receptor**	**EC50 (nM) at GLP-1 Receptor**	**EC50 (nM) at GIP Receptor**
1	Native Glucagon	0.10048	ND	ND
	Native GLP-1	ND	0.01593	ND
2	Native GIP	ND	ND	0.05665
42	Glucagon Aib2, Aib16, amide	0.30892	0.43695	103.93715

43	Glucagon Aib2,K9(rErEC16),Aib16, amide	1.15223	0.23172	15.14376
45	Glucagon Aib2, K12(rErEC16),Aib16, amide	0.04254	0.02049	0.65062

[00582] As shown in Table 5, peptide analogs comprising an acylated amino acid at any of positions 9 and 12, demonstrated an improvement in GIP activity, as compared to the unacylated peptide analog of SEQ ID NO: 42. Not all of the acylated peptide analogs that were made and tested demonstrated an improved GIP activity, however. One of the peptide analogs which was acylated at a position other than position 9 and 12 demonstrated a reduced activity at the GIP receptor.

EXAMPLE 11

[00583] This example describes the *in vivo* activities of exemplary gluagon analogs of the present disclosures.

[00584] The *in vivo* activities of GIP receptor-active glucagon analogs having an amino acid sequence of one of SEQ ID NOs: 28, 37-39 and 134 were tested in diet-induced obese (DIO) mice and compared to the *in vivo* activities of mice that were administered a glucagon agonist analog or a vehicle control. Each test group of mice was made of nineteen mice and each mouse was subcutaneously injected with a 10 nmol/kg dose of peptide or a vehicle control. Body weight and food intake were measured on days 0, 1, 3, 5, and 7 post administration, while fasting blood glucose levels were measured on days 0 and 7. The mice were fasted for 6 hours prior to the measurement of blood glucose on days 0 and 7. Ad lib blood glucose levels were additionally measured on day 5 post-administration.

[00585] All of the mice injected with a GIP receptor-active glucagon analog demonstrated a significant reduction (a reduction between about 11% and about 27%) in body weight seven days following administration, as compared to the mice administered a vehicle control. Additionally all of the mice injected with a GIP receptor-active glucagon analog exhibited a substantial reduction (a reduction between about 43% and about 65%) in blood glucose levels seven days post-administration.

[00586] Another study was carried out in male db/db mice to analyze the *in vivo* activities of GIP receptor–active glucagon analogs. In this study, a 20 nmol/kg dose of a GIP receptor-active glucagon analog having an amino acid sequence of one of SEQ ID NOs: 28, 31, 37, 135 and 136 was administered to the mice via subcutaneous injection. A vehicle control was administered to a control group of mice. Blood glucose levels were assayed prior to administration and 1, 2, 4, 8, 24, 48, and 72 hours after dosing. Body weight was measured before dosing and 72 hours after dosing. Consistent with the results observed in the DIO mice study, all mice that were administered a GIP receptor-active glucagon analog demonstrated a substantial reduction in body weight (a reduction between about 2% and about 5%), as compared to the vehicle control group. Also, all mice that received a GIP receptor-active glucagon analog exhibited a reduction in blood glucose levels.

EXAMPLE 12

[00587] This example describes the structures of additional exemplary peptides.

[00588] Glucagon analogs are made as essentially described in Example 1 and Table 6 describes the structure of these analogs. The glucagon analogs are tested for activity at each of the glucagon receptor, GLP-1 receptor, and the GIP receptor as essentially described in Example 2.

TABLE 6

SEQ ID NO:	Structure	EC50 (nM) at Glucagon Receptor	EC50 (nM) at GLP-1 Receptor	EC50 (nM) at GIP Receptor
48	H(D-Ser)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(-SH)-amide	0.026	0.01	0.433
50	Y(D-ser)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC-amide	0.041	0.108	0.072
51	Y(D-ser)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-TE)-amide	0.255	0.572	0.433
52	H(aib)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC-amide	0.2	0.005	0.006
53	H(aib)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-TE)-amide	3.511	0.027	0.232

54	Y(aib)QGTFTSDYSIYLDKQAA(aib)EFV(aib)WLLAGGPSSGAPPPSC–amide	0.566	0.091	0.467
56	Y(aib)QGTFTSDYSIYLDEQAAKEFV(aib)WLLAGGPSSGAPPPSC–amide, (underlined residues bridged via lactam)	1.266	0.096	1.981
58	Y(aib)QGTFTSDYSIYLDKEAA(aib)KFVNWLLAGGPSSGAPPPSC–amide, (underlined residues bridged via lactam)	0.063	0.014	0.005
59	Y(aib)QGTFTSDYSIYLDKEAA(aib)KFVNWLLAGGPSSGAPPPSC(40K-TE)-amide, (underlined residues bridged via lactam)	0.432	0.063	0.122
60	Y(aib)QGTFTSDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC–amide	0.047	0.0028	0.025
68	Y(aib)QGTFTSD(K(γE-C14-acyl))SIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-ME)-amide	50.1	0.612	0.862
69	Y(aib)QGTFTSD(K(γE-C16-acyl))SIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-ME)-amide	0.055	0.02	0.015
70	Y(aib)QGTFTSDYSIYLDKQAA(aib)EFVC(40K-ME)WLLAGGPSSGAPPPS(K(C16-acyl))-amide	0.018	0.007	0.014
72	Y(aib)EGTFISDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	1.676	2.686	0.035
73	Y(aib)EGTFTSDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	~10	0.91	1.56
74	H(aib)QGTFISDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	2.519	6.42	2.541
75	Y(aib)QGTFISDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	3.116	17.7	2.438
79	H(aib)QGTFTSDK(γE-C16)SKYLDERRA(aib)EFVQWLLDGGPSSGAPPPS-NH_2	0.004	0.006	0.003
80	H(aib)EGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-NH2	1.276	0.008	0.026
81	Y(aib)QGTFTSDK(γE-γE-C16)SIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPS-NH2	0.073	0.008	0.005
175	Y(aib)EGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC-NH_2, wherein the C at the C-terminus is covalently attached to 40 kDa PEG	0.62	0.066	0.100
172	YaibQGTFTSDYSIYLDKQAAaibEFVC(40K-TE PEG)WLLAGGPSSGAPPPSK(C8)-amide	3.06	0.108	0.487

173	YaibQGTFTSDYSIYLDKQAAaibEFVC(40K-TE PEG)WLLAGGPSSGAPPPSK(C12)-amide	0.190	0.011	0.089
174	YaibQGTFTSDYSIYLDKQAAaibEFVC(40K-TE PEG)WLLAGGPSSGAPPPSK(C14)-amide	0.025	0.010	0.019

EXAMPLE 13

[00589] Glucagon analogs were made as essentially described in Example 1 and Table 7 describes the structure of these analogs. The glucagon analogs were tested for activity at each of the glucagon receptor, GLP-1 receptor, and the GIP receptor as essentially described in Example 2.

TABLE 7

SEQ ID NO	Amino Acid Sequence	EC50 (nM) at Glucagon Receptor	EC50 (nM) at GLP-1 Receptor	EC50 (nM) at GIP Receptor
28	HAibQGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amide	0.002	0.002	0.003
89	HAibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSKG(γEγE-C16)-amide	0.0041	0.0021	0.024
90	H Aib QGTFTSDKSK(γEγE-C16)YLDERAAQDFVQWLLDGGPSSGAPPPS-amide	0.0056	0.0027	0.017
91	YAibQGTFTSDK(γEγE-C16)SIYLDKQAAAibEFVNWLLDGGPSSGAPPPS-amide	0.073	0.008	0.005
92	YAibQGTFTSDK(γEγE-C16)SIYLDKQAAAibEFVNWLLDT-amide	0.0105	0.0031	0.021
31	HAibQGTFTSDK(γE-C16)SKYLDERRAAibEFVQWLLDT-amide	0.008	0.0044	0.008

EXAMPLE 14

[00590] Glucagon analogs were made as essentially described in Example 1 and Table 8 describes the structure of these analogs. Full descriptions of these analogs are provided in the sequence listing and the SEQ ID NO: for each is provided in Table 8. The glucagon analogs were tested for activity at each of the glucagon receptor, GLP-1 receptor, and the GIP receptor as essentially described in Example 2.

TABLE 8				
		Glu (0.025)	GLP (0.025)	GIP (0.01)
SEQ ID NO: 28	GIP/GLP-1/glucagon triagonist peptide: HaibQGTFTSDK(γE-C16)SKYLDERAA**Q**DFVQWLLDG**GPSSGAPPPS**-NH_2	0.002	0.002	0.003
SEQ ID NO: 39	GIP/GLP-1 co-agonist peptide: HaibEGTFTSDK(γE-C16)SKYLDERAA**Q**DFVQWLLDG**GPSSGAPPPS**-NH_2	1.276	0.008	0.026
SEQ ID NO: 37	GIP/GLP-1/glucagon triagonist peptide: HaibQGTFTSDK(γE-C16)SKYLDER**R**A**aib**EFVQWLLDG**GPSSGAPPPS**-NH_2	0.004	0.006	0.003
SEQ ID NO: 262	GIP/GLP-1 co-agonist peptide: Haib**E**GTFTSDK(γE-C16)SKYLDER**R**A**aib**EFVQWLLDG**GPSSGAPPPS**-NH_2	0.127	0.008	0.012

[00591] To test *in vivo* activities of these peptides, the peptides of Table 8 (excluding native peptides) were subcutaneously injected into DIO mice (C57B66 mice) at either 5 or 10 nmol/kg every Monday, Wednesday, and Friday, for 4 weeks. The mice were fed a high fat, diabetogenic diet. As shown in Figure 1, the body weight of the mice which received injections of one of these peptides was lowered, as compared, to vehicle control.

EXAMPLE 15

[00592] Acylated peptides comprising the amino acid sequence of SEQ ID NO: 1 (native glucagon) with a Tyr at position 1, AIB at position 2, Glu at position 3, Ile at position 12, Lys at position 16, Gln at position 17, Ala at position 18, AIB at position 20, Glu at position 21, Asn at position 24, Leu at position 27, Ala at position 28, Gly at position 29, followed by the amino acid sequence GPSSGSPPPS (SEQ ID NO: 5), and a C-terminal amidation were made as essentially described in Example 1. The peptides differed by the type of acylation, type of acylation spacer, and/or position of acylated amino acid. The different acylated residues are depicted in Figure 3. The peptides were then tested for *in vitro* activity at each of the glucagon receptor, GLP-1 receptor, and the GIP receptor as essentially described in Example

2. Table 9 summarizes the structure and activities of each peptide.

TABLE 9

Acylated amino acid (position thereof)	**Acyl type**	**Acyl Spacer**	**Amino Acid at 41st position**	**Structure (SEQ ID NO:)**	**EC50 (nM) at Glucagon Receptor**	**EC50 (nM) at GLP-1 Receptor**	**EC50 (nM) at GIP Receptor**
Lys(40)	C16	None	n/a	Y aib **E** GTFTSDYSIYLDKQAA aib EFVNWLL AGGPSSGAPPPS **K(-C16)-**NH_2 (SEQ ID NO: 138)	0.085	0.002	0.001
Lys(40)	C16	None	Gly	Y aib **E** GTFTSDYSIYLDKQAA aib EFVNWLL AGGPSSGAPPPS **K(-C16)G** -NH_2 (SEQ ID NO: 139)	0.203	0.005	0.001
Lys(40)	SuccinoylC16	None	Gly	Y aib **E** GTFTSDYSIYLDKQAA aib EFVNWLL AGGPSSGAPPPS **K(-SuccinoylC16) G** -NH_2 (SEQ ID NO: 140)	0.077	0.001	0.001
Lys(40)	Succinoyl C16	β-Ala	Gly	Y aib **E** GTFTSDYSIYLDKQAA aib EFVNWLL AGGPSSGAPPPS **K(-βAlaSuccinoylC16) G** -NH_2 (SEQ ID NO: 141)	0.058	0.001	0.002
Lys(40)	SuccinoylC18	None	Gly	Y aib **E** GTFTSDYSIYLDKQAA aib EFVNWLL AGGPSSGAPPPS **K(-SuccinoylC18) G** -NH_2 (SEQ ID NO: 142)	0.121	0.004	0.001
4-aminoPhe(10)	C16	γE γE	n/a	Yaib **E** GTFTSD**F(4-aminoγEγEC16)**SIYLDKQAA **aib** EFVNWLLAGGPSSGAPPPS- NH_2 (SEQ ID NO: 143)	0.165	0.002	0.003
4-aminoPhe(10)	Succinoyl C16	None	n/a	Yaib **E** GTFTSD**F(4amino-succinoylC16)**SIYLDKQAA **aib** EFVNWLLAGGPSSGAPPPS- NH_2 (SEQ ID NO: 144)	>0.500	0.004	0.013

[00593] The *in vivo* activities of the peptides listed in Table 9 were tested by injecting into DIO mice (each having an average body weight of 49.0 g) at a dose of 10 nmol/kg on Monday, Wednesday, and Friday for 1 week. The body weight and food intake of the mice were measured on Days 0, 2, 4, 6, and 7, while blood glucose levels were measured on Days 0 and 7.

[00594] As shown in Figure 2, the body weight of mice that received injections of the acylated peptides demonstrated at least a 5% decrease in total body weight by Day 7.

EXAMPLE 16

Acylated peptides comprising the amino acid sequence of native glucagon (SEQ ID NO: 1) with a AIB at position 2, Glu at position 16 (except when position 16 is acylated), Ala at position 18, Leu at position 27, Asp at position 28, Gly at position 29, followed by the amino acid sequence GPSSGSPPPS (SEQ ID NO: 5), and a C-terminal amidation were made as essentially described in Example 1. The peptides differed by the type of acylation, type of acylation spacer, and/or position of acylated amino acid. The peptides were then tested for in vitro activity at each of the glucagon receptor, GLP-1 receptor, and the GIP receptor as essentially described in Example 2. Table 10 summarizes the structure and activities of each peptide.

TABLE 10

Structure					**Glucagon Receptor**		**GLP-1 Receptor**		**GIP-1 Receptor**	
Acylated amino acid (position thereof)	Acyl type	Acyl Spacer	Amino Acid at 41st position	**Amino acid sequence (SEQ ID NO:)**	EC_{50} ST Dev	n *	EC_{50} ST Dev	n *	EC_{50} ST Dev	n *
Lys(10)	C16	γE	n/a	HaibQGTFTSD**K(γE-C16)**SKYLDERAAQDFVQWLL DGGPSSGAPPPS-amide (SEQ ID NO: 28)	0.006 (0.003)	1	0.007 (0.0020)	1	0.003 (0.001)	1
4-aminoPhe (40)	C16	γEγE	Gly	HaibQGTFTSDYSKYLDERAA QDFVQWLLDGGPSSGAPPPS **K(C16)G**-amide (SEQ ID NO: 177)	0.010 (0.001)		0.014 (0.002)		0.021 (0.002)	

4-aminoPhe (40)	succinoylC16	n/a	Gly	HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS**K(SuccinoylC14)G**-amide (SEQ ID NO: 176)	0.012 (0.002)		0.014 (0.011)		0.101 (0.002)	
4-aminoPhe (6)	C16	γE	n/a	HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS**aF(γEγE-C16)**G-amide (SEQ ID NO: 145)	0.002 (0.001)	2	0.002 (0.0011)	2	0.017 (0.0057)	2
4-aminoPhe (10)	C16	γE	n/a	HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS**aF(C16succinoyl)G**-amide (SEQ ID NO: 146)	0.007 0.001)	2	0.007 (0.003)	2	0.052 (0.0205)	2
4-aminoPhe (13)	C16	γE	n/a	HaibQGT**aF(γE-C16)**TSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 147)	0.246 (0.079)	2	0.004 (0.0013)	2	3.131 (N/A)	1
4-aminoPhe (10)	succinoylC16	n/a	n/a	HaibQGTFTSD**aF(γE-C16)**SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 148)	0.008 0.008)	2	0.009 (0.0036)	2	0.009 (0.0020)	2
Lys(13)	C16	γE	n/a	HaibQGTFTSDYSK**aF(γE-C16)**LDERAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 149)	0.011 (0.004)	2	0.007 (0.0036)	2	0.395 (0.2630)	2
Lys(14)	C16	γE	n/a	HaibQGTFTSD**aF(C16succinoyl)**SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 150)	0.006 (0.002)	2	0.004 (0.0018)	2	0.141 (0.046)	2
Lys(16)	C16	γE	n/a	HaibQGTFTSDYSK**K(γE-C16)**LDERAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 151)	0.008 0.001)	2	0.004 (0.0013)	2	0.309 (0.0884)	2
Lys(17)	C16	γE	n/a	HaibQGTFTSDYSKY**K(γE-C16)**DERAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 152)	0.004 (0.001)	2	0.004 (0.0013)	2	0.011	2
Lys(10)	C16	γE	n/a	HaibQGTFTSDYSKYLD**K(γE-C16)**RAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 153)	0.012 (0.006)	2	0.007 (0.0006)	2	0.276	2

4-aminoPhe (40)	C16	γEγE	Gly	HaibQGTFTSDYSKYLDE**K(γE-C16)**AAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 154)	0.008 (0.001)	2	0.004 (0.0019)	2	0.175	2

* number of experiments; different fractions of SEQ ID NOs: 147 and 149 were used in the experiments. Data may be from different experiments.

EXAMPLE 17

[00595] Select peptides from Tables 10 (peptides of SEQ ID NOs: 145, 148, and 152) and peptides of SEQ ID NOs: 28 and 89 were tested for *in vivo* activities in DIO mice (having an original average body weight of 58.0 g) on a high fat, diabetogenic diet. The peptides at a dose of 10 nmol/kg (of the original average body weight) were subcutaneously injected on Days 0 and 3. The body weight and food intake of the mice were measured on Days 0, 1, 3, 5, and 7, while blood glucose levels were measured on Days 0 and 7.

[00596] As shown in Figure 4, the mice that received an injection of one of these peptides demonstrated a decreased body weight over the course of 7 days, as compared to vehicle controls. The mice that received injections of the peptides demonstrated at least a 5% decrease in total body weight by Day 7 of which two demonstrated at least a 10% decrease in total body weight by Day 7.

EXAMPLE 18

[00597] Acylated peptides comprising a "mini-PEG" spacer were made as essentially described in Example 1. Figure 12 represents a schematic of the structures of these acylated peptides. The peptides comprising a mini-PEG spacer were tested for in vitro activity at each of the glucagon receptor, GLP-1 receptor, and the GIP receptor as essentially described in Example 2, and compared to the activities of acylated peptides comprising no spacer or a E spacer. All peptides of this experiment comprised the amino acid sequence of native glucagon (SEQ ID NO: 1) with AIB at position 2, Glu at position 16, Ala at position 18, Leu at position 28, Asp at position 28, Gly at position 29, followed by the amino acid sequence GPSSGSPPPSK (SEQ ID NO: 9), wherein K was an acylated amino acid, and a C-terminal amidation. Table 11 summarizes the structure and activities of each peptide.

TABLE 11

Structure					**Glucagon Receptor**		**GLP-1 Receptor**		**GIP Receptor**	
Acylated amino acid (position thereof)	Acyl type	Acyl Spacer	Amino Acid at 41st position	Amino Acid Sequence	EC_{50} (ST Dev)	n *	EC_{50} (ST Dev)	n *	EC_{50} (ST Dev)	n *
Lys(40)	C16	E	Gly	HaibQGTFTSDYSKYLDE RAAQDFVQWLLDGGPS SGSPPPS**K(γE-C16)**G-amide (SEQ ID NO: 155)	0.004 (0.0004)	2	0.003 (0.0003)	2	0.008 (0.001)	1
Lys(40)	Succinoyl C16	None	Gly	HaibQGTFTSDYSKYLDE RAAQDFVQWLLDGGPS SGAPPPS**K(C16succinoyl)**G-amide (SEQ ID NO: 156)	0.003 (0.0003)	2	0.003 (0.0008)	2	0.006 (0.0009)	1
Lys(40)	C16	PEG2- E	Gly	HaibQGTFTSDYSKYLDE RAAQDFVQWLLDGGA SSGAPPPS**K(Peg2-γE-C16)**G-amide (SEQ ID NO: 157)	0.011 (0.001)	1	0.009 (0.0005)	1	0.021 (0.004)	1
Lys(40)	C16	PEG4- E	Gly	HaibQGTFTSDYSKYLDE RAAQDFVQWLLDGGPS SGAPPPS**K(Peg4-γE-C16)**G-amide (SEQ ID NO: 158)	0.004 (0.0005)	2	0.003 (0.0005)	2	0.005 0.0005)	2
Lys(40)	C16	PEG8- E	Gly	HaibQGTFTSDYSKYLDE RAAQDFVQWLLDGGPS SGAPPPS**K(Peg8-γE-C16)**G-amide (SEQ ID NO: 159)	0.006 (0.0009)	1	0.005 (0.001)	1	0.002 (0.0004)	2

EXAMPLE 19

[00598] Dual acylated peptides were made. Two peptides comprised a single acylated amino acid residue carrying two acylations in a branched formation: one peptide had the branched acylation at position 10, and a 2nd peptide had the branced acylation at position 40. Figure 5 (at the top) depicts the structure of the single acylated Lys residue carrying two acylations in a branched formation. In another instance, a peptide comprised one acylated amino acid residue carrying two acylations in a linear formation. Figure 5A (middle) depicts the structure of the single acylated Lys residue carrying a C12 acylation attached to a C16 acylation via a E spacer in a linear formation. In yet another instance, a peptide comprised comprised two acylated amino acid residues: one at position 10 and another at position 40. Figure 5 (at the bottom) depicts the structure of each acylated Lys residue carrying a C16 acylation via a E spacer. The parentheses denote that the Lys residues are connected via the backbone amino acids at positions 11-39.

[00599] The peptides carrying two acylations were tested tested for *in vitro* activity at each of the glucagon receptor, GLP-1 receptor, and the GIP receptor as essentially described in Example 2 and the results are shown in Table 12.

TABLE 12

Dual Acylated Peptides	EC_{50} (cAMP, nmole)*		
	GCGR	GLPR	GIPR
HaibQGTFTSD **K*(rEC16)** SKYLDERAAQDFVQ WLLDGGPSSGAPPPS-amide (SEQ ID NO: 28)	0.004	0.003	0.007
HaibQGTFTSD **K*K(rEC16)$_2$** SKYLDERAAQDFVQ WLLDGGPSSGAPPPS-amide (SEQ ID NO: 160)	0.024	0.007	0.015
HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS **K*K(rEC16)$_2$**-amide (SEQ ID NO: 161)	0.011	0.007	0.010

HaibQGTFTSD **K*(C12-rEC16)** SKYLDERAAQDFVQWLLDGGPSSGAPPPS -amide (SEQ ID NO: 162)	0.008	0.003	0.033
HaibQGTFTSD **K*(rEC16)** YSKYLDERAAQDFVQWLLDGGPSSGAPPPS **K*(rEC16)**-amide (SEQ ID NO: 163)	0.018	0.011	0.024

[00600] On Day 0, the peptides of Table 12, as well as two dimers (described in Example 22 and Figure 9) and two additional peptides (a peptide of SEQ ID NO: 28 and a peptide of SEQ ID NO: 89) were subcutaneously injected once into DIO mice (at a dose of 10 nmol/kg). The DIO mice had been given a diabetogenic diet prior to injection and the average body weight of the mice was 60 g. The body weight and food intake of the mice were measured on Days 0, 1, 3, 5, and 7, while fasted blood glucose levels were measured on Days 0 and 7.

[00601] As shown in Figure 5E, mice that received a peptide injection exhibited decreased body weight on Day 7 of this study, as compared to mice that were injected with a vehicle control.

[00602] As shown in Figure 5F, blood glucose levels decreased in mice that received a peptide injection, as compared to mice that were injected with a vehicle control.

EXAMPLE 20

[00603] Peptides were S-alkylated in three different ways. In a first way, an S-palmityl akylated Cys residue was part of the peptide backbone. The akylated Cys residue was located at position 40. The structure is shown in Figure 6 (bottom) and is listed as SEQ ID NO: 164 in the sequence listing. In a second way, an S-palmityl akylated Cys residue was attached to a Lys residue, which Lys residue was located at position 40 of the peptide. The resulting structure is shown in Figure 7 (Cys-S—Palmitic) and is listed as SEQ ID NO: 165 in the sequence listing. In a third way, an S-palmityl akylated Cys residue was attached to a spacer residue (gamma-glutamic acid) which was in turn attached to a Lys residue located at position 40 of the peptide. The resulting structure is shown in Figure 7 (γE-Cys-S-palmitic) and is listed as SEQ ID NO: 166 in the sequence listing. The S-alkylated peptides were made as described in Example 1.

[00604] The peptides were tested for *in vitro* activity at each of the glucagon receptor, GLP-1 receptor, and GIP receptor, as essentially described in Example 2. The EC50s at each receptor for each peptide are listed below in Table 13.

TABLE 13

Structure	Glucagon Receptor			GLP-1 Receptor			GIP Receptor		
HaibQGTFTSDYSKYLDERAAQDFVQWLLDG GPSSGAPPPS**C(S-palmityl)**G-amide (SEQ ID NO: 163)	0.006	0.0008	2	0.003	0.0003	2	0.051	0.007	1
HaibQGTFTSDYSKYLDERAAQDFVQWLLDG GPSSGAPPPS**K(Cys S-palmityl)G**-amide (SEQ ID NO: 164)	0.006	0.0013	1	0.003	0.0003	1	0.028	0.001	1
HaibQGTFTSDYSKYKLDERAAQDFVQWLLD GGPSSGAPPPS**K(γE-Cys S-palmityl)G**amide (SEQ ID NO: 165)	0.009	0.0013	1	0.003	0.0003	1	0.043	0.001	1

EXAMPLE 21

[00605] The *in vivo* activities of selected peptides from the previous Examples were tested by subcutaneously injecting 10 nmol/kg of body weight into DIO mice. The average body weight of the mice was 60 g and there were 8 mice per group.

[00606] Among the peptides tested were an acylated peptide with a gamma-glutamic acid spacer, an acylated peptide with a dipeptide spacer of two gamma-glutamic acids, a C16-succinoylated peptide, an acylated peptide with a miniPEG spacer comprising the structure $(-O-CH_2-CH_2-)_n$, wherein n is 2, an acylated peptide with a miniPEG spacer comprising the structure $(-O-CH_2-CH_2-)_n$, wherein n is 4, an acylated peptide with a miniPEG spacer comprising the structure $(-O-CH_2-CH_2-)_n$, wherein n is 8, an S-palmityl akylated peptide, wherein Lys is the backbone residue and Cys is a spacer between the Lys and the acyl group, and an S-palmityl alkylated peptide, wherein Lys is the backbone residue and gamma-glutamic acid-Cys is a dipeptide spacer between the Lys and the acyl group. Body weight and food intake were measured on Days 0, 1, 3, 5, and 7, while blood glucose measurements were taken on Days 0 and 7.

[00607] As shown in Figure 8, many of the tested peptides demonstrated at least a 5% decrease in total change in body weight as measured on Day 7.

EXAMPLE 22

[00608] Three homodimers were made, wherein each homodimer comprised two peptides of SEQ ID NO: 167. The C-terminal Lys residue (at position 40) of each peptide of SEQ ID NO: 167 was amidated (instead of containing an alpha carboxylate) and the epsilon NH2 group of this Lys residue was peptide bonded to a Cys reside, which in turn was bound to a gamma-glutamic acid residue. The gamma-glutamic acid residue was bound to a C16 acyl group. Each half of the dimer was either attached to the other half via a disulfide linkage or a thioether linkage. Example 1 details the synthesis of the homodimers.

[00609] The structures of the resulting products are shown in Figures 9A and 9B.

[00610] The homodimers were tested for in vitro activity at each of the glucagon receptor, GLP-1 receptor, and GIP receptor, as essentially described in Example 2. The EC50s at each receptor for each peptide are listed below in Table 14.

TABLE 14

Structure of each monomer	Glucagon Receptor			GLP-1 Receptor			GIP Receptor		
	EC_{50}	STDev	n*	EC_{50}	STDev	n*	EC_{50}	STDev	n*
Disulfide dimer ***(disulfide with two C16 acylations)*** HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(CysrE-C16)G-amide (SEQ ID NO: 168)	0.004	0.0007	2	0.002	0.0003	2	0.004	0.0002	2
S-acetylDap K40 dimer ***(thioether with one C16 acylation)*** HaibQGTFTSDYSKYLDERAAQDFVQWLLDFGGPSSGAPPPSK(CysrE-C16)G-amide (SEQ ID NO: 169)	0.003	0.0003	2	0.002	0.0003	2	0.009	0.0005	2
Cys-K40 disulfide dimer ***(disulfide with one C16 acylation)*** HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(CysrEC16)G-amide (SEQ ID NO: 170)	0.003	0.0003	1	0.001	0.0003	1	0.014	0.001	1

EXAMPLE 23

[00611] DIO mice (8 animals per group, average body weight of 54 g) were subcutaneously injected with one of 4 peptides described in the table below at either 1 nmol/kg/day or 3 nmol/kg/day, or was given a vehicle control every day. The structures and their in vitro activities at each of the glucagon receptor, GLP-1 receptor, and GIP receptor are provided in Table 15.

TABLE 15

Structure	EC50 (nM) at glucagon receptor [STDev]	EC50 (nM) at GLP-1 receptor [STDev]	EC50 (nM) at GIP receptor [STDev]
HaibQGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amide (SEQ ID NO: 28)	0.002 [0.001]	0.002 [0.001]	0.002 [0.001]
HaibQGTFTSDYSKYLDERAAQDFQVWLLDGGPSSGAPPPSK(γEγE-C16)G-amide (SEQ ID NO: 89)	0.004 [0.001]	0.004 [0.001]	0.020 [0.006]
YaibEGTFTSDYSIYLDKQAAaibEFVNWLLAGGPSSGAPPPSK(C16)-amide (SEQ ID NO: 138)	0.053 [0.015]	0.003 [0.001]	0.002 [0.001]
HaibEGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(γEγE-C16)G-amide (SEQ ID NO: 171)	0.455 [0.137]	0.005 [0.001]	0.038 [0.005]

[00612] Body weight and food intake was measured on every other day, beginning with Day 0 (the day of first administration). Body composition was measured on Days 0 and 19, while ad lib blood glucose levels were measured on Days 0, 7, 14, and 20. ipGTT (1 g/kg) was measured on Day 20 at 0, 15, 20, 60, 120 minutes post-glucose injection). Necropsy (liver and pancreas) and a final bleed was measured on day 22 (after an overnight fast).

[00613] As shown in Figure 10, body weight decreased in all animals given a peptide of Table 15. Food intake as measured on Day 19 was decreased in all animals that were given a peptide of Table 15, as compared to mice given a vehicle control. As shown in Figure 11, the insulin levels (as measured on Day 21) also decreased in animals that were given a peptide,

as compared to a vehicle. Notably, the mice that had demonstrated the greatest amount of weight loss were also the mice that demonstrated the lowest insulin levels

EXAMPLE 24

[00614] Peptides demonstrating body weight lowering capability as assessed in Example 21 were selected for additional mutation studies in which amino acids within the C-terminal half of the peptide were modified in one of several ways. In a first way, the peptide having the sequence of SEQ ID NO: 89 was altered such that the amino acid at position 27, 28, or 29 was substituted with an alanine residue. In another way, the peptide having the sequence of SEQ ID NO: 28 was modified to have a Glu at position 28 and an Arg at position 35. It was theorized that a salt bridge between these two amino acids would form a salt bridge to stabilize the Trp cage structure in the C-terminal portion of the peptide. Lastly, the peptide having the sequence of SEQ ID NO: 28 was modified to include a Gly as the C-terminal amino acid. The peptides, as well as other peptides that look similar to the peptide of SEQ ID NO: 89 or the peptide of SEQ ID NO: 28, were tested for in vitro activities at each of the glucagon receptor, GLP-1 receptor, and GIP receptor as essentially described in Example 2 and the results are provided in Table 16.

TABLE 16

Structure	EC50 (nM) at glucagon receptor	EC50 (nM) at GLP-1 receptor	EC50 (nM) at GIP receptor
HaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPS**K(γEC16)G**- NH_2 (SEQ ID NO: 89)	0.006	0.005	0.028
HaibQGTFTSDK(**γE-C16ac**)SKYLDERAAQDFVQWL**LDGGPSSGAPPPS**-amide (SEQ ID NO: 28)	0.003	0.004	0.004
HaibQGTFTSDYSKYLDERAAQDFVQWL **LA**GGPSSGAPPPS**K(γEγE-C16)G**-NH_2 (SEQ ID NO: 203)	0.003	0.001	0.007

HaibQGTFTSDYSKYLDERAAQDFVQWL LDA GPSSGAPPPS**K(γEγE-C16)G**-NH_2 (SEQ ID NO: 204)	0.003	0.001	0.069
HaibQGTFTSD**K(γE-C16)**SKYLDERAAQDFVQWLLDGGPSSGAPPPS**G**-NH_2 (SEQ ID NO: 205)	0.005	0.002	0.004
YaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPS**K(K-bisγEC16)G**-NH_2 (SEQ ID NO: 208)	0.031	0.025	0.079
YaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPS**K(K-γEC16,γEC8)G**-NH_2 (SEQ ID NO: 210)	0.010	0.005	0.359
YaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPS**K(K-γEC16,γEC12)G**-NH_2 (SEQ ID NO: 211)	0.010	0.006	0.038
HaibQGTFTSDYSKYLDERAAQDFVQWLLDG GPSSGAPPPS**Y(O-2palmitic acid)G-NH_2** (SEQ ID NO: 212)	0.007	0.004	0.339
HaibQGTFTSD**K(γEγE-C16)**SKYLDERAAQDFVQWLLDG GPSSGAPPPS**G**-NH_2 (SEQ ID NO: 207)	0.005	0.002	0.015

EXAMPLE 25

[00615] Peptides were made as essentially described in Example 1 and tested for in vitro activity at each of the glucagon, GLP-1, and GIP receptors as essentially described herein. The structures and EC50 values (nM) of each peptide are provided below in Table 17.

TABLE 17

Peptide	Glucagon EC50 (nM) [STDev]	GLP-1 EC50 (nM) [STDev]	GIP EC50 (nM) [STDev]
Glucagon	0.025 [0.003]		
GLP-1		0.025 [0.003]	

GIP			0.0065 [0.0001]
H**aib**QGTFTSDYSIYLDEKRAKEFVCWL LAGGPSSGAPPPSK-**amide** (SEQ ID NO: 230)	1.644 [0.302]	0.222 [0.034]	0.164 [0.010]

[00616] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[00617] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted.

[00618] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

[00619] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[00620] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as

appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

WHAT IS CLAIMED:

1. An analog of glucagon (SEQ ID NO: 1), comprising:

   a. an amino acid comprising an imidazole side chain at position 1;

   b. an DPP-IV protective amino acid at position 2, optionally, AIB;

   c. an acylated amino acid or alkylated amino acid at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the acyl or alkyl group is linked to the amino acid via a spacer;

   d. an alpha, alpha disubstituted amino acid at position 20; and

   e. up to ten additional amino acid modifications relative to SEQ ID NO: 1;

wherein, when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits a GIP percentage potency of at least 0.1%, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

2. The glucagon analog of claim 1, wherein the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring.

3. The glucagon analog of claim 2, wherein the α,α-disubstituted at position 20 is AIB.

4. The glucagon analog of any one of claims 1 to 3, wherein the amino acid at position 16 is an alpha helix stabilizing amino acid other than AIB.

5. The glucagon analog of claim 4, wherein the amino acid at position 16 is a charged amino acid, optionally, a negative charged amino acid or a positive charged amino acid.

6. The glucagon analog of claim 5, wherein the charged amino acid is Glu or Lys.

7. An analog of glucagon (SEQ ID NO: 1), comprising:

a. an amino acid comprising an imidazole side chain at position 1;

b. an DPP-IV protective amino acid at position 2, optionally, AIB;

c. an acylated amino acid or alkylated amino acid at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the acyl or alkyl group is linked to the amino acid via a spacer;

d. an alpha helix stabilizing amino acid at one or more of positions 16-21, optionally, position 16, wherein the analog does not comprise an alpha helix stabilizing amino acid at position 20; and

e. up to ten additional amino acid modifications relative to SEQ ID NO: 1;

wherein, when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits a GIP percentage potency of at least 0.1%, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

8. The glucagon analog of claim 7, comprising an alpha helix stabilizing amino acid at position 16, optionally, wherein the alpha helix stabilizing amino acid is a negative charged amino acid or an alpha, alpha disubstituted amino acid.

9. The glucagon analog of claim 8, wherein the negative charged amino acid is Glu.

10. The glucagon analog of claim 8, wherein the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally

substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring.

11. The glucagon analog of claim10, wherein the α,α-disubstituted amino acid is AIB.

12. The glucagon analog of any one of claims 7 to 11, comprising at position 18 a small aliphatic amino acid, optionally, Ala, and an Arg at position 17.

13. The glucagon analog of any one of claims 7 to 12, wherein the analog is not modified at position 20 compared to SEQ ID NO: 1.

14. The glucagon analog of any of claims 1 to 13, comprising (i) an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 or (ii) the acylated amino acid or alkylated amino acid is located at position 10, 12, or 16.

15. The glucagon analog of any one of claims 1 to 14, wherein the amino acid at position 1 is His or a His derivative.

16. The glucagon analog of any one of claims 1 to 15, wherein the amino acid at position 2 is an alpha, alpha disubstituted amino acid.

17. The glucagon analog of claim 16, wherein the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring.

18. The glucagon analog of claim 17, wherein the α,α-disubstituted amino acid at position 2 is AIB.

19. The glucagon analog of any of claims 1 to 18, comprising an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29.

20. The glucagon analog of claim 19, wherein the amino acid at position 29 is Gly.

21. The glucagon analog of claim 19 or 20, wherein the extension comprises an amino acid sequence which forms a Trp cage structure.

22. The glucagon analog of claim 21, wherein the extension comprises the amino acid sequence of GPSSGAPPPS (SEQ ID NO: 5), or a conservatively substituted sequence thereof.

23. The glucagon analog of claim 19 or 20, wherein the extension comprises at least one charged amino acid.

24. The glucagon analog of claim 23, wherein the extension comprises an amino acid sequence of: X1-X2, wherein X1 is a charged amino acid and X2 is a small aliphatic amino acid.

25. The glucagon analog of claim 24, wherein X1 is a positive charged amino acid.

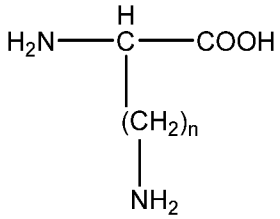
26. The glucagon analog of claim 25, wherein X1 is Arg.

27. The glucagon analog of claim 26, wherein the extension comprises Arg-Gly.

28. The glucagon analog of any of claims 1 to 27, except for claim 14, wherein (i) the amino acid comprising a non-native acyl or alkyl group is located at position 10 or (ii) the glucagon analog comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 and an acylated amino acid or alkylated amino acid at any of positions 37-43, optionally 40.

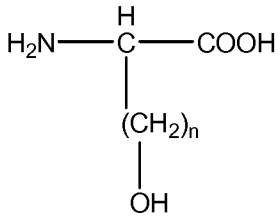
29. The glucagon analog of claim 1 to 27, wherein the acylated amino acid or alkylated amino acid is at position 14.

30. The glucagon analog of any of claims 1 to 29, wherein the acyl group or alkyl group is attached to an amino acid comprising a structure of Formula I (optionally, Lys), Formula II (optionally, Cys), or Formula III (optionally, Ser), wherein each of Formulae I, II, and III, is:



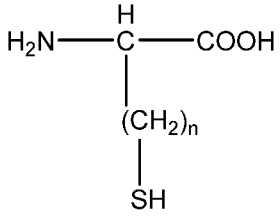
wherein n = 1 to 4

[Formula I];



wherein n = 1 to 4

[Formula II]; and



wherein n = 1 to 4

[Formula III].

31. The glucagon analog of claim 1 to 29, wherein the amino acid of the acyl group or alkyl group is attached to amino-Phe or Tyr.

32. The glucagon analog of any of claims 1 to 31, wherein the acyl group or alkyl group is linked to an amino acid via a spacer 3 to 10 atoms in length.

33. The glucagon analog of any of claims 1 to 32, wherein the spacer is an amino acid or dipeptide.

34. The glucagon analog of claim 33, wherein the spacer comprises one or two acidic amino acid residues.

35. The glucagon analog of any of claims 1 to 34, wherein the acylated amino acid or alkylated amino acid is linked to the acyl or alkyl group via a spacer wherein the total length of the spacer and the acyl group is about 14 to about 28 atoms in length.

36. The glucagon analog of any of claims 1 to 35, wherein the acylated amino acid or alkylated amino acid is attached to a C12 to C18 fatty acyl group.

37. The glucagon analog of claim 36 wherein the acyl group is a C14 or C16 fatty acyl group.

38. The glucagon analog of any of claims 1 to 37, comprising at least one charged amino acid C-terminal to the amino acid at position 27.

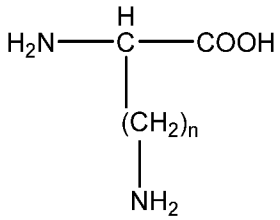
39. The glucagon analog of claim 38, comprising a charged amino acid at position 28.

40. The glucagon analog of claim 38 or 39, wherein the charged amino acid is a negative charged amino acid.

41. The glucagon analog of claim 40, wherein the negative charged amino acid is Asp.

42. The glucagon analog of claim 38 or 39, wherein the charged amino acid is a positive charged amino acid.

43. The glucagon analog of claim 42, wherein the positive charged amino acid is an amino acid of Formula I:



wherein n = 1 to 4

[Formula I].

44. The glucagon analog of claim 43, wherein the amino acid of Formula I at position 28 is Lys.

45. The glucagon analog of any of claims 1 to 44, comprising an amino acid modification at position 27, at position 29, or at both positions 27 and 29.

46. The glucagon analog of claim 45, wherein the amino acid at position 27 is Leu, Nle, Val, or Lys.

47. The glucagon analog of claim 45 or 46, wherein the amino acid at position 29 is Gly or Thr.

48. The glucagon analog of any of claims 1 to 47, comprising one or more of:

i. a DPP-IV protective amino acid at position 1;

ii. an acidic amino acid, optionally, Glu, at position 3;

iii. an Ile at position 7;

iv. an Ile or Arg at position 12;

v. an acidic amino acid, optionally, Glu, at position 15;

vi. an aliphatic amino acid, optionally, Ala at position 18;

vii. an acidic amino acid, optionally, Glu, at position 21;

viii. an Asn, Ala, or AIB at position 24;

ix. an aliphatic amino acid, optionally, Ala, or Leu, or Nle, at position 27;

x. an acidic amino acid, optionally, Glu, or an aliphatic amino acid, optionally, Ala, at position 28;

xi. an aliphatic amino acid, optionally, Ala, at position 29;

xii. amidation at the C-terminus.

49. The glucagon analog of any one of claims 1 to 48, comprising the amino acid sequence of any of SEQ ID NOs: 184-199.

50. The glucagon analog of any one of claims 1 to 48, comprising any of SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, and 230.

51. The glucagon analog of claim 50, comprising the amino acid sequence of any of SEQ ID NOs: 28, 37, 89, 95, 130, 171, and 180.

52. A peptide comprising the sequence of

(a) SEQ ID NO: 184,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEQ ID NO: 184)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog;

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala or Gly;

X_{35} is Ala or a basic amino acid (optionally, Arg or Lys);

wherein, when X_{28} is an acidic amino acid, X_{35} is a basic amino acid;

wherein, when X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41,

wherein the C-terminal amino acid of the peptide is amidated;

or

(b) SEQ ID NO: 184 with up to 3 amino acid modifications relative to SEQ ID NO: 184, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

53. The peptide of claim 52, wherein X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and the peptide optionally comprises Gly at position 41.

54. The peptide of claim 52 or 53, wherein X_{10} is an amino acid covalently attached to a C12-C18 acyl or alkyl group.

55. The peptide of any one of claims 52 to 54, wherein X_{20} is Gln.

56. The peptide of claim 55, wherein the amino acid at position 16 is a negative charged amino acid, optionally, Glu.

57. The peptide of claim 56, wherein X_{18} is Ala.

58. The peptide of claim any one of claims 52 to 54, wherein X_{20} is AIB.

59. The peptide of claim 58, wherein X_{16} is any amino acid other than AIB.

60. The peptide of any one of claims 52 to 59, wherein (i) X_{28} is an acidic amino acid, optionally Asp or Glu, and X_{35} is a basic amino acid, optionally Arg or Lys, (ii) only one of X_{27}, X_{28} and X_{29} is an Ala, (iii) the peptide comprise an amidated Gly at the C-terminus.

61. The peptide of any one of claims 52 to 60, wherein the up to 3 amino acid modifications are conservative substitutions.

62. A peptide comprising the sequence of

(a) SEQ ID NO: 185,

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGAPPPS$
(SEQ ID NO: 185)

wherein:

X_2 is AIB;

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{16} is Glu, an alpha, alpha disubstituted amino acid, Lys or

X_{18} is Arg or Ala;

X_{20} is AIB or Gln;

X_{21} is Asp or Glu;

X_{27} is Leu, Nle, or Met;

X_{28} is Ala, Asp or Glu;

X_{29} is Gly of Thr;

and

wherein, when X_{10} is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41,

wherein the C-terminal amino acid of the peptide is amidated;

or

(b) SEQ ID NO: 185 with up to 3 amino acid modifications relative to SEQ ID NO: 185, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

63. The peptide of claim 62, wherein the up to 3 amino acid modifications are conservative substitutions.

64. A peptide comprising

(a) the sequence of SEQ ID NO: 186:

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SEQ ID NO: 186)

wherein:

X_2 is AIB;

X_3 is Gln or Gln analog;

X_{10} is Tyr or an amino acid covalently attached to a C10 to C26 acyl or alkyl group;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser;

X_{18} is Arg or Ala;

X_{20} is a negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is Ala, Gly or Thr; and

wherein the peptide comprises an amino acid covalently attached to a C10 to C26 acyl or alkyl group, optionally, at position 10, and the C-terminal amino acid of the peptide is amidated;

or

(b) SEQ ID NO: 186 with up to 3 amino acid modifications relative to SEQ ID NO: 186, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

65. The peptide of claim 64, wherein X_{20} is AIB.

66. The peptide of claim 65 wherein X_{29} is Thr and the peptide does not comprise GPSSGAPPPS (SEQ ID NO: 5).

67. The peptide of claim 65 or 66, wherein X_{16} is an amino acid other than AIB.

68. The peptide of claim 64, wherein X_{20} is Gln.

69. The peptide of claim 68, wherein the amino acid at position 16 is a negative charged amino acid, optionally, Glu.

70. The peptide of claim 69, wherein X_{18} is Ala.

71. The peptide of any one of claims 64 to 70, excluding claim 66, comprising an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29.

72. The peptide of claim 71, wherein the amino acid at position 29 is Gly.

73. The peptide of claim 71 or 72, wherein the extension comprises the amino acid sequence GPSSGAPPPS (SEQ ID NO: 5), or a conservatively substituted sequence thereof, or wherein the extension comprises the sequence X1-X2, wherein X1 is a charged amino acid and X2 is a small aliphatic amino acid, optionally, wherein X1 is a positive charged amino acid.

74. The peptide of claim 73, wherein the positive charged amino acid is Arg.

75. The peptide of claim 74, wherein the extension comprises or consists of Arg-Gly.

76. The peptide of claim 73, wherein the extension comprises the amino acid sequence GPSSGAPPPS (SEQ ID NO: 5) followed by Lys or Lys-Gly, wherein the Lys is covalently attached to an C10 to C26 acyl group.

77. The peptide of claim 64, comprising SEQ ID NO: 186, wherein X_2 is AIB, X_3 is Gln, X_{10} is an amino acid covalently attached to a C10 to C26 acyl or alkyl group, X_{18} is Arg or Ala, X_{20} is AIB or Gln, X_{21} is Asp or Glu, X_{29} is Gly, and the C-terminal amino acid is amidated, wherein Gly at position 29 is fused to GPSSGAPPPS followed by Lys or Lys-Gly, wherein the Lys is covalently attached to a C10-C26 acyl group.

78. The peptide of any one of claims 64 to 77, wherein the up to 3 amino acid modifications are conservative substitutions.

79. A peptide comprising

(a) the sequence of SEQ ID NO: 187:

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$
(SEQ ID NO: 187)

wherein:

X_2 is AIB;

X_{10} is Tyr or an amino acid covalently attached to a C10 to C26 acyl or alkyl group;

X_{16} is Glu, alpha, alpha-disubstituted amino acid, or Lys;

X_{18} is Arg or Ala;

X_{20} is a negative charged amino acid or a charge-neutral amino acid, optionally, AIB or Gln;

X_{21} is Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala, Asp or Glu;

X_{29} is Gly or Thr; and

wherein the peptide comprises an amino acid covalently attached to a C12 to C18 acyl or alkyl group, optionally, at position10, and the C-terminal amino acid of the peptide is amidated;

or

(b) SEQ ID NO: 187 with up to 3 amino acid modifications relative to SEQ ID NO: 187, wherein the peptide exhibits agonist activity at each of the human GIP receptor, the human GLP-1 receptor and the human glucagon receptor.

80. The peptide of claim 80, wherein the up to 3 amino acid modifications are conservative substitutions.

81. A peptide comprising the sequence of SEQ ID NO: 188, or a sequence of SEQ ID NO: 188 with up to 3 amino acid modifications relative to SEQ ID NO: 188, wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor.

82. A peptide comprising the sequence of SEQ ID NO: 189, or a sequence of SEQ ID NO: 189 with up to 3 amino acid modifications relative to SEQ ID NO: 189, wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor.

83. A peptide comprising the sequence of SEQ ID NO: 190, or a sequence of SEQ ID NO: 190 with up to 3 amino acid modifications relative to SEQ ID NO: 190, wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor.

84. A peptide comprising the sequence of SEQ ID NO: 191, or a sequence of SEQ ID NO: 191 with up to 3 amino acid modifications relative to SEQ ID NO: 191,

wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor.

85. A peptide comprising the sequence of SEQ ID NO: 192, or a sequence of SEQ ID NO: 192 with up to 3 amino acid modifications relative to SEQ ID NO: 192, wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor, and optionally, wherein the peptide exhibits agonist activity at the human glucagon receptor.

86. A peptide comprising the sequence of SEQ ID NO: 193, or a sequence of SEQ ID NO: 193 with up to 3 amino acid modifications relative to SEQ ID NO: 193, wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor, optionally, wherein the peptide exhibits agonist activity at the human glucagon receptor.

87. A peptide comprising the sequence of SEQ ID NO: 194, or a sequence of SEQ ID NO: 194 with up to 3 amino acid modifications relative to SEQ ID NO: 194, wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor, optionally, wherein the peptide exhibits agonist activity at the human glucagon receptor.

88. A peptide comprising the sequence of SEQ ID NO: 195, or a sequence of SEQ ID NO: 195 with up to 3 amino acid modifications relative to SEQ ID NO: 195, wherein the peptide exhibits agonist activity at each of the human GIP receptor and the human GLP-1 receptor, optionally, wherein the peptide exhibits agonist activity at the human glucagon receptor.

89. The peptide of any one of claims 52 to 88 wherein the C12-C18 fatty acyl or alkyl group is a C14 to C16 fatty acyl or alkyl group, optionally attached via a spacer.

90. The peptide of claim any one claims 52 to 89, wherein the amino acid covalently attached to a C12 to C18 acyl or alkyl group is a Lys or amino-Phe or Tyr or Cys covalently attached to a C12 to C18 acyl or alkyl group.

91. There peptide of any one of claims 52 to 90, wherein the spacer is an amino acid or dipeptide, optionally, wherein the spacer comprises one or two acidic amino acid residues.

92. A peptide comprising the sequence of SEQ ID NO: 28.

93. A peptide comprising the sequence of SEQ ID NO: 37.

94. A peptide comprising the sequence of SEQ ID NO: 89.

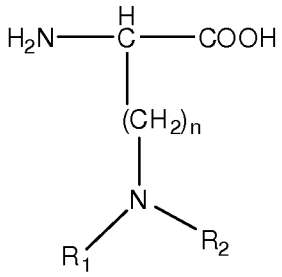
95. A peptide comprising the sequence of SEQ ID NO: 171.

96. A peptide comprising the sequence of SEQ ID NO: 180.

97. An analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, comprising:

(a) an amino acid comprising an imidazole side chain at position 1,

(b) at position 16, an amino acid of Formula IV:



[Formula IV],

wherein n is 1 to 7, wherein each of R1 and R2 is independently selected from the group consisting of H, C1-C18 alkyl, (C1-C18 alkyl)OH, (C1-C18 alkyl)NH2, (C1-C18 alkyl)SH, (C0-C4 alkyl)(C3-C6)cycloalkyl, (C0-C4 alkyl)(C2-C5 heterocyclic), (C0-C4 alkyl)(C6-C10 aryl)R7, and (C1-C4 alkyl)(C3-C9 heteroaryl), wherein R7 is H or OH, wherein optionally the side chain of the amino acid of Formula IV comprises a free amino group,

(c) an α,α-disubstituted amino acid at position 20,

(d) up to ten additional amino acid modifications relative to SEQ ID NO: 1,

wherein, when the analog lacks a hydrophilic moiety, the glucagon analog exhibits at least 0.1% activity of native GIP at the GIP receptor, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

98. The glucagon analog of claim 97, wherein the amino acid at position 1 is His or a His derivative.

99. The glucagon analog of claim 97 or 98, wherein the amino acid of Formula IV in (b) is homoLys, Lys, Orn, or 2,4-diaminobutyric acid (Dab).

100. The glucagon analog of any of claims 97 to 99, wherein the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring.

101. The glucagon analog of claim 100, wherein the α,α-disubstituted at position 20 is AIB.

102. The glucagon analog of any of claims 97 to 101 comprising one or more of:

a. a DPP-IV protective amino acid at position 2; optionally AIB or D-Ser;

b. a large, aliphatic, nonpolar amino acid at position 12, optionally Ile;

c. an amino acid orther than Arg at position 17, optionally Gln;

d. a small aliphatic amino acid at position 18, optionally Ala;

e. an amino acid other than Asp at position 21, optionally Glu;

f. an amino acid other than Gln at position 24, optionally Asn or Ala;

g. an amino acid other than Met at position 27, optionally Leu;

h. an amino acid other than Asn at position 28, optionally Ala;

i. an amino acid other than Thr at position 29, optionally Gly;

j. an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29; and

k. the amino acid sequence set forth as any of SEQ ID NOs: 48, 52, 53, and 74.

103. An analog comprising a parent sequence with a total of up to 3 amino acid modifications relative to the parent sequence, wherein the parent sequence is any of SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, and 127-131, or any of SEQ ID NOs: 48, 52, 53, and 74, or any of SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, and 133, wherein the amino acid modifications are selected from the group consisting of:

a DPP-IV protective amino acid at position 2; optionally AIB or D-Ser;

b. a large, aliphatic, nonpolar amino acid at position 12, optionally Ile;

c. an amino acid orther than Arg at position 17, optionally Gln;

d. a small aliphatic amino acid at position 18, optionally Ala;

e. an amino acid other than Asp at position 21, optionally Glu;

f. an amino acid other than Gln at position 24, optionally Asn or Ala;

g. an amino acid other than Met at position 27, optionally Leu;

h. an amino acid other than Asn at position 28, optionally Ala;

i. an amino acid other than Thr at position 29, optionally Gly;

j. an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29; and

k. the amino acid sequence set forth as any of SEQ ID NOs: 48, 52, 53, and 74.

104. The glucagon analog or peptide of any one of the preceding claims, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

105. The glucagon analog or peptide of any one of the preceding claims, wherein the EC50 of the glucagon analog at the GIP receptor is within about 50-fold from its EC50 at the GLP-1 receptor.

106. The glucagon analog or peptide of any one of the preceding claims, wherein the EC50 of the glucagon analog at the GIP receptor is within about 40-fold from its EC50 at the GLP-1 receptor.

107. The glucagon analog or peptide of any one of the preceding claims, wherein the EC50 of the glucagon analog at the GIP receptor is within about 30-fold from its EC50 at the GLP-1 receptor.

108. The glucagon analog or peptide of any one of the preceding claims, wherein the EC50 of the glucagon analog at the GIP receptor is within about 20-fold from its EC50 at the GLP-1 receptor.

109. The glucagon analog or peptide of any one of the preceding claims, wherein the EC50 of the glucagon analog at the GIP receptor is within about 10-fold from its EC50 at the GLP-1 receptor.

110. The glucagon analog or peptide of any one of the preceding claims, wherein the EC50 of the glucagon analog at the GIP receptor is within about 5-fold from its EC50 at the GLP-1 receptor.

111. The glucagon analog or peptide of any one of the preceding claims, wherein the glucagon analog exhibits a GIP percentage potency of at least 1%.

112. The glucagon analog or peptide of claim 111, wherein the GIP percentage potency is at least 10%.

113. The glucagon analog or peptide of claim 112, wherein the GIP percentage potency is more than 15% or more than 20%.

114. The glucagon analog or peptide of claim 113, wherein the GIP percentage potency is at least 50%.

115. The glucagon analog or peptide of claim 114, wherein the GIP percentage potency is more than or about 100%.

116. The glucagon analog or peptide of any one of the preceding claims, wherein the glucagon analog exhibits a GLP-1 percentage potency of at least 1%.

117. The glucagon analog or peptide of claim 116, wherein the GLP-1 percentage potency is at least 10%.

118. The glucagon analog or peptide of claim 117, wherein the GLP-1 percentage potency is at least 50%.

119. The glucagon analog or peptide of claim 118, wherein the GLP-1 percentage potency is more than or about 100%.

120. The glucagon analog or peptide of any one of the preceding claims, wherein the GIP percentage potency is within 10-fold higher or lower than the GLP-1 percentage potency.

121. The glucagon analog or peptide of any one of the preceding claims, wherein the glucagon percentage potency is at least 1%.

122. The glucagon analog or peptide of claim 121, wherein the glucagon percentage potency is at least 10%.

123. The glucagon analog or peptide of claim 122, wherein the glucagon percentage potency is at least 50%.

124. The glucagon analog or peptide of claim 123, wherein the glucagon percentage potency is more than or about 100%.

125. The glucagon analog or peptide of any one of the preceding claims, wherein the GIP percentage potency is within 10-fold higher or lower than the glucagon percentage potency.

126. A dimer or multimer comprising two or more glucagon analogs or peptides of any one of the preceding claims.

127. A conjugate comprising the glucagon analog or peptide of any one of the preceding claims and a conjugate moiety.

128. The conjugate of claim 127, wherein the glucagon analog is fused to a heterologous peptide analog.

129. A pharmaceutical composition comprising a glucagon analog or peptide of any one of the preceding claims, a dimer or multimer of claim 126, a conjugate of claim 127 or 128, or a combination thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

130. A method of reducing weight gain or inducing weight loss in a subject in need thereof, comprising administering to a patient in need thereof a pharmaceutical composition of claim 129 in an amount effective to reduce weight gain or induce weight loss.

131. The method of claim 130, wherein the amount effective to treat obesity in a subject in need thereof.

132. A method of treating diabetes, comprising administering to a patient in need thereof a pharmaceutical composition of claim 129 in an amount effective to lower blood glucose levels.

133. A method of inducing temporary paralysis of the intestinal tract in a subject in need thereof, comprising administering to a patient in need thereof a pharmaceutical composition of claim 129 in an amount effective to induce temporary paralysis of the intestinal tract.

134. Use of a glucagon analog or peptide of any one of the preceding claims, a dimer or multimer of claim 126, a conjugate of claim 127 or 128, or a combination thereof, in the manufacture of a medicament to treat diabetes, reduce weight gain or induce weight loss, or induce temporary paralysis of the intestinal tract.

135. Use of a glucagon analog or peptide of any one of the preceding claims, a dimer or multimer of claim 126, a conjugate of claim 127 or 128, or a combination thereof, for treating diabetes or obesity, reducing weight gain or inducing weight loss, or inducing temporary paralysis of the intestinal tract.

136. A peptide, optionally a glucagon analog, as described herein.

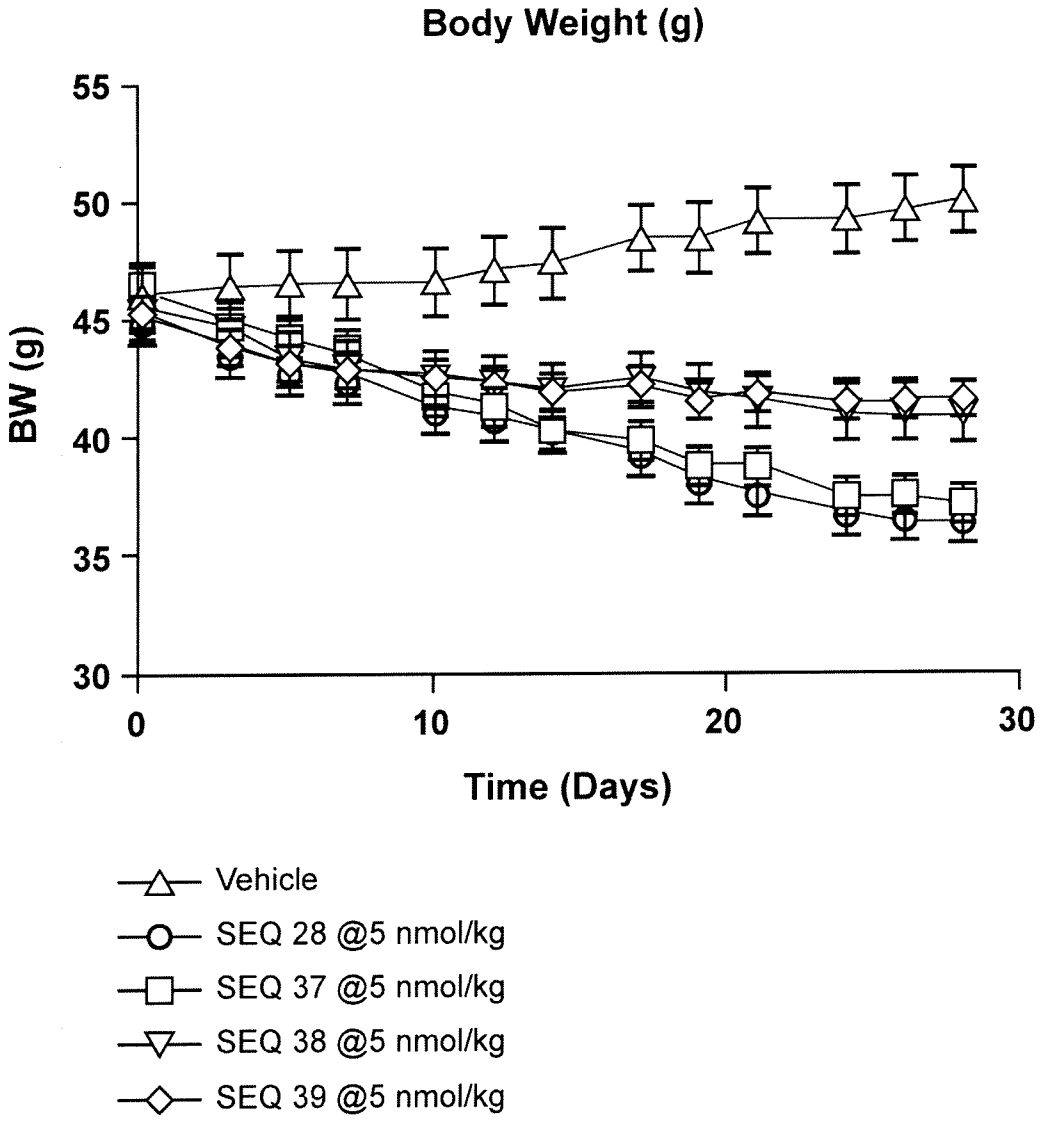

Body Weight (g)
BW (g)
55
50
45
40
35
30
0
10
20
30
Time (Days)
Vehicle
SEQ 28 @5 nmol/kg
SEQ 37 @5 nmol/kg
SEQ 38 @5 nmol/kg
SEQ 39 @5 nmol/kg

FIGURE 1

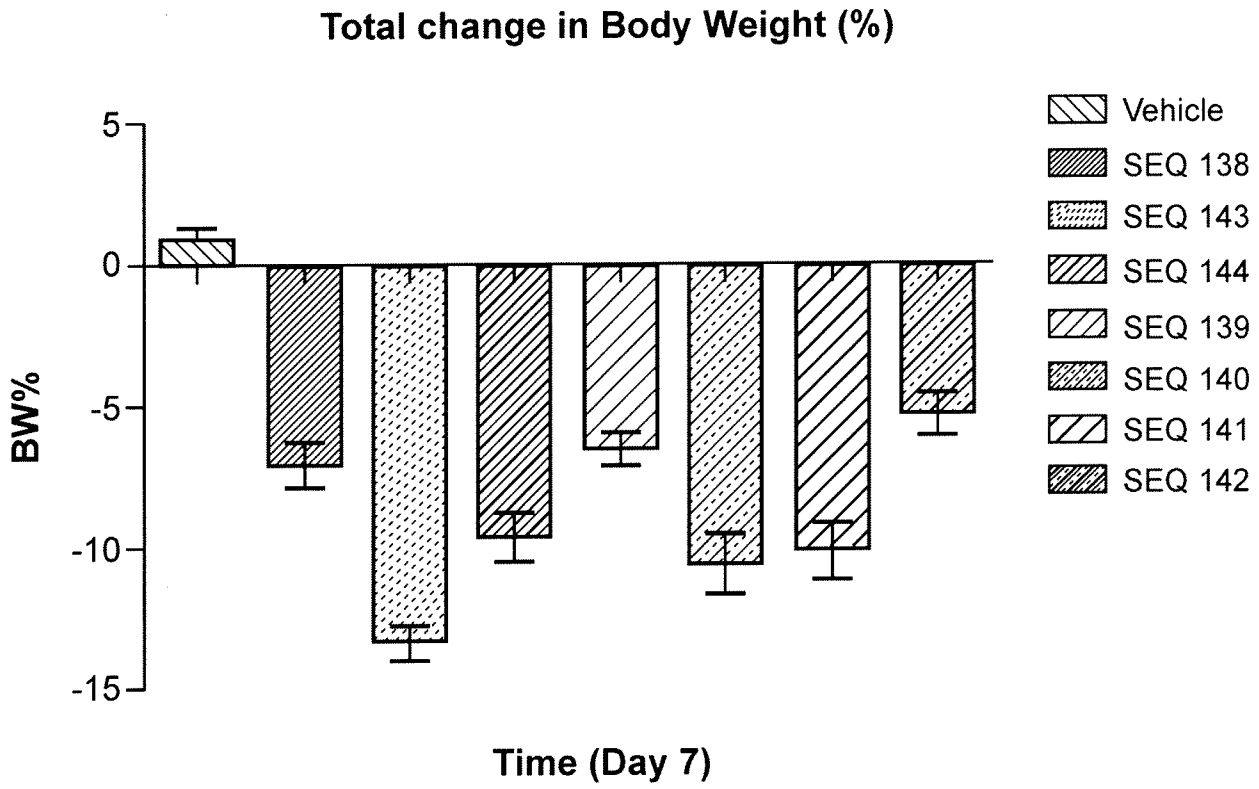

Total change in Body Weight (%)
BW%
5
0
-5
-10
-15
Time (Day 7)
Vehicle
SEQ 138
SEQ 143
SEQ 144
SEQ 139
SEQ 140
SEQ 141
SEQ 142

FIGURE 2

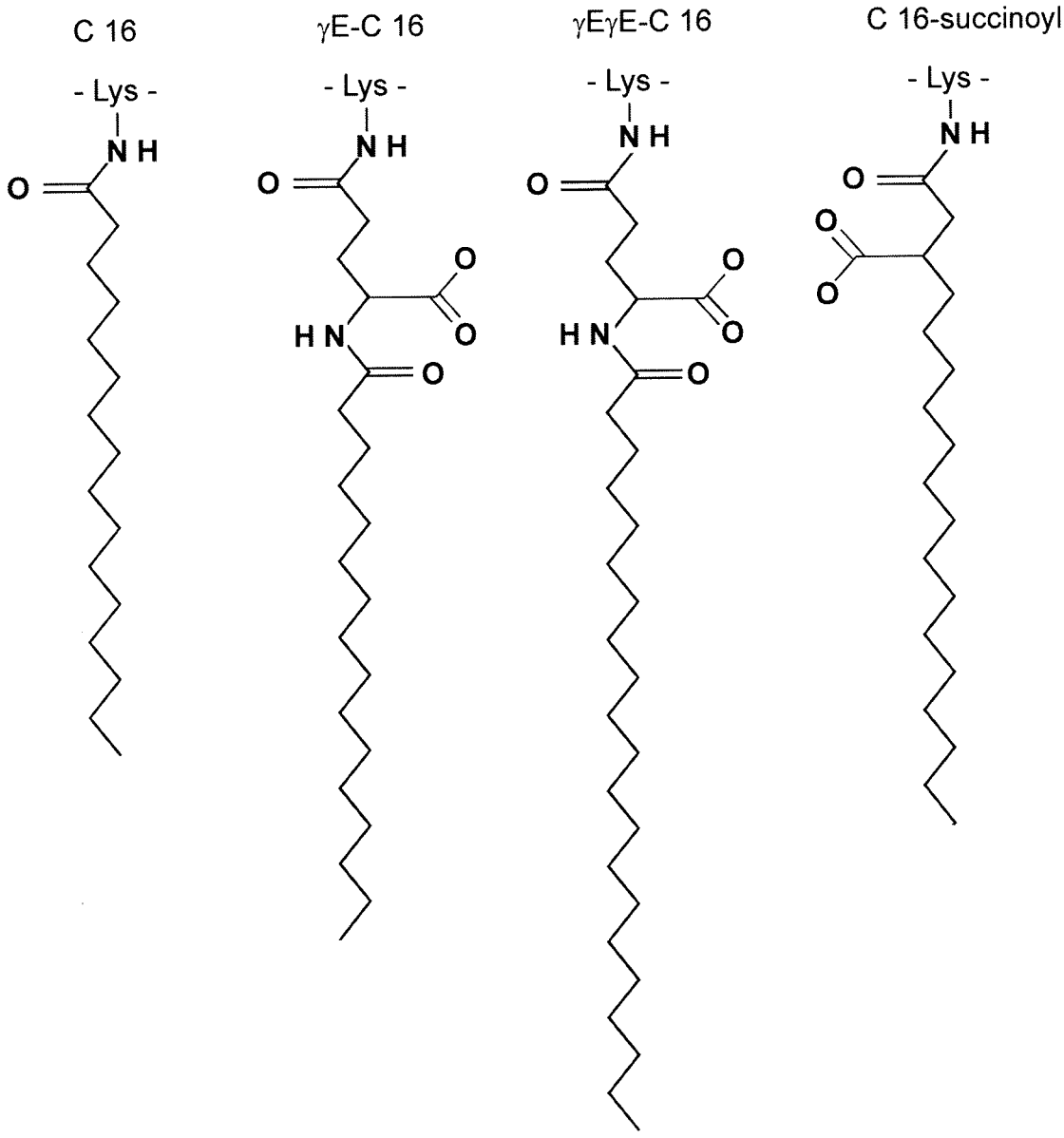
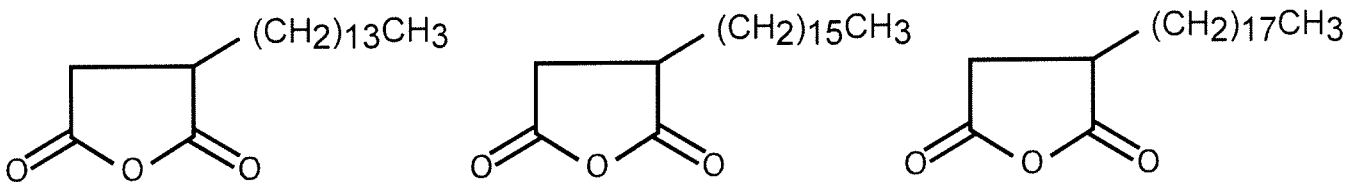

C 16
- Lys -
NH
O
γE-C 16
- Lys -
NH
O
O
O
HN
O
γEγE-C 16
- Lys -
NH
O
O
O
HN
O
C 16-succinoyl
- Lys -
NH
O
O
O

FIGURE 3A



tetradecyl hexadecyl octadecyl

FIGURE 3B

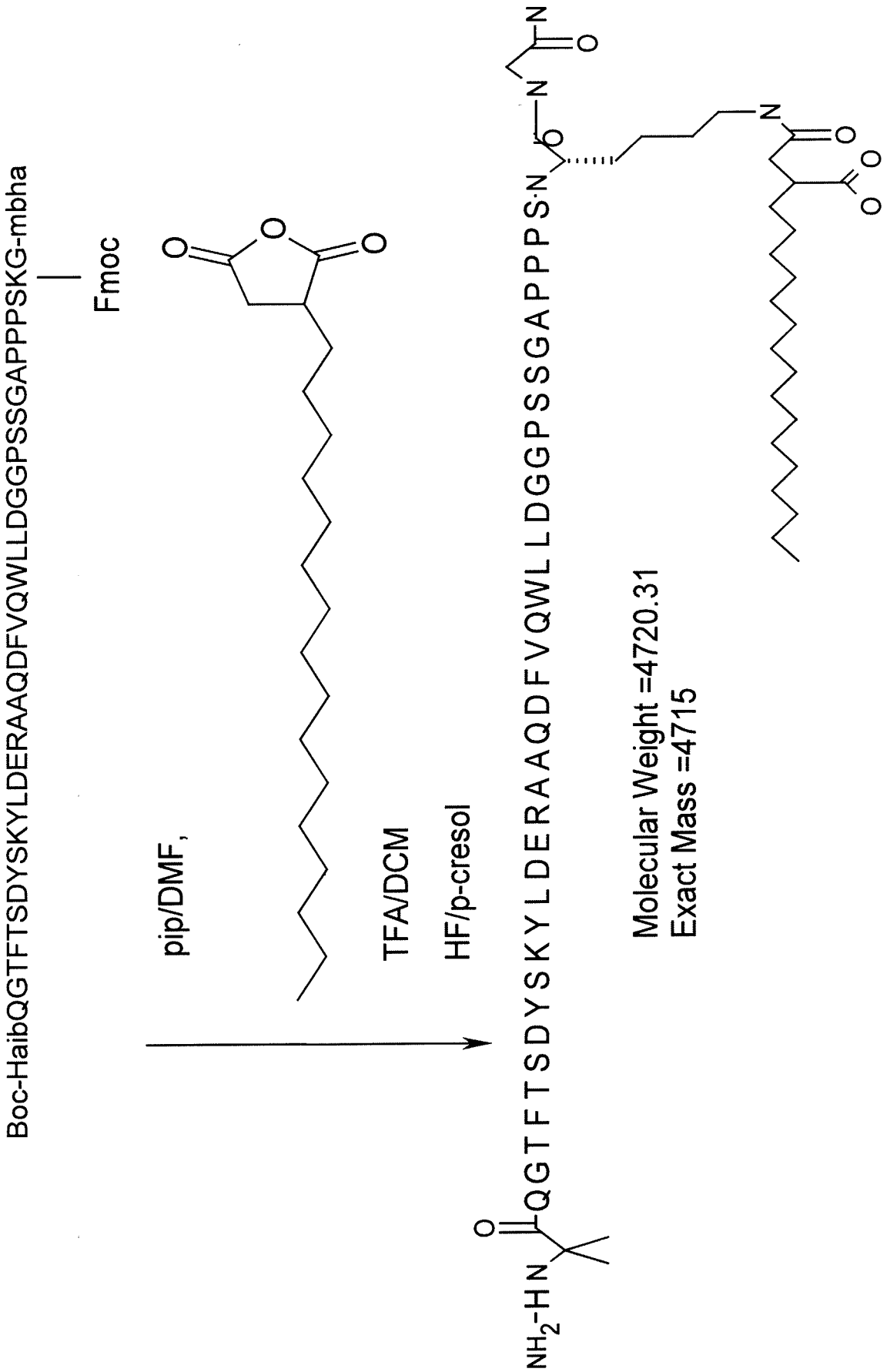

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSKG-mbha
Fmoc
pip/DMF,
TFA/DCM
HF/p-cresol
NH2-HN
QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS
Molecular Weight =4720.31
Exact Mass =4715

FIGURE 3C

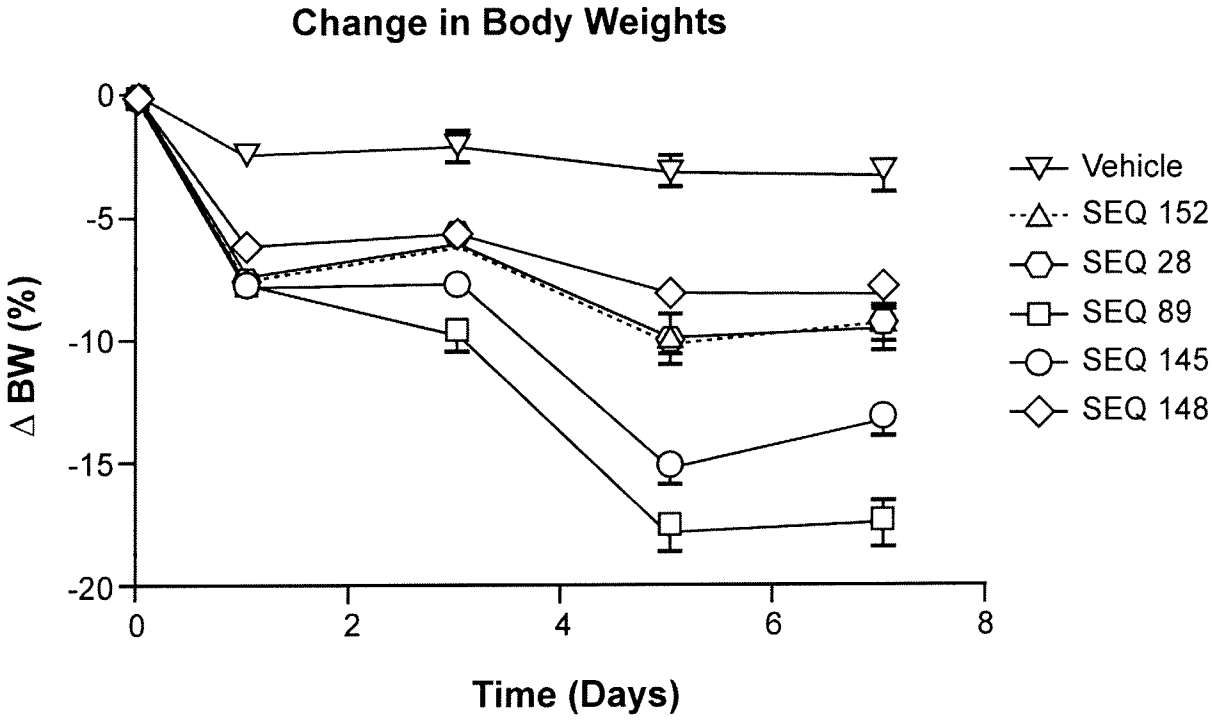

Change in Body Weights
0
-5
-10
-15
-20
Δ BW (%)
0
2
4
6
8
Time (Days)
Vehicle
SEQ 152
SEQ 28
SEQ 89
SEQ 145
SEQ 148

FIGURE 4

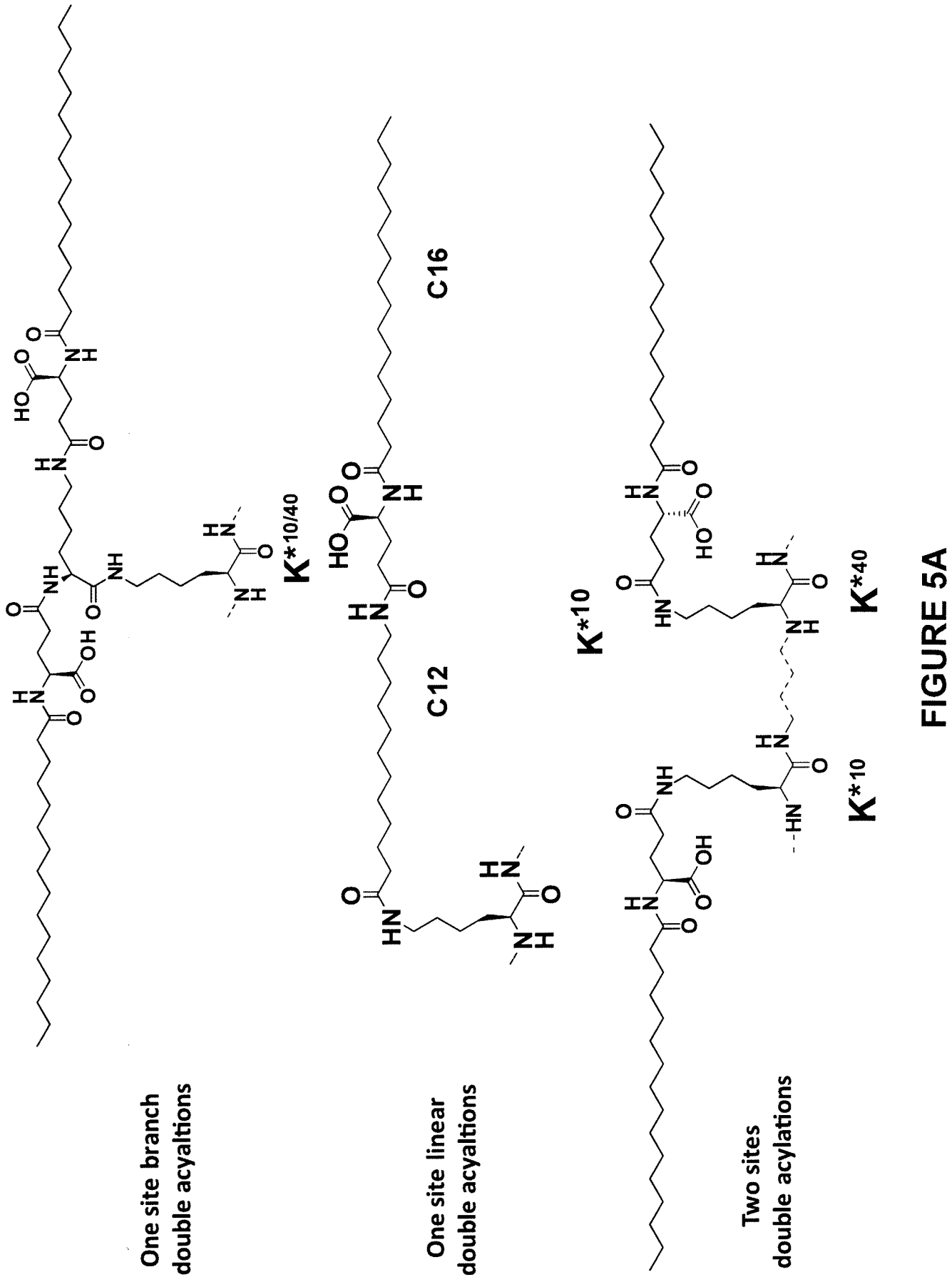

One site branch
double acyaltions
K*10/40
One site linear
double acyaltions
C16
C12
K*10
Two sites
double acylations
K*40
K*10

FIGURE 5A

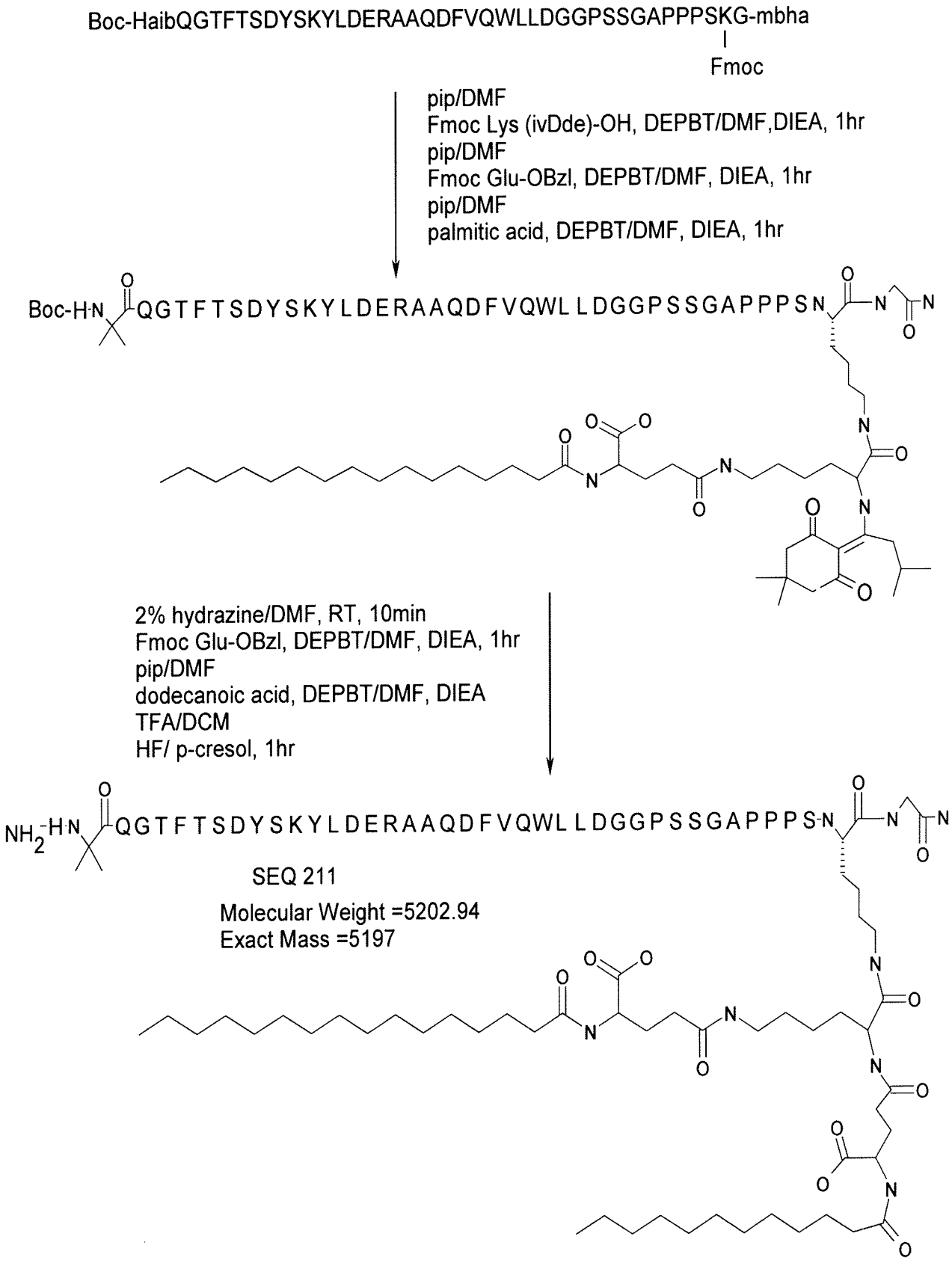

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSKG-mbha
Fmoc
pip/DMF
Fmoc Lys (ivDde)-OH, DEPBT/DMF,DIEA, 1hr
pip/DMF
Fmoc Glu-OBzl, DEPBT/DMF, DIEA, 1hr
pip/DMF
palmitic acid, DEPBT/DMF, DIEA, 1hr
Boc-HN QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSN
2% hydrazine/DMF, RT, 10min
Fmoc Glu-OBzl, DEPBT/DMF, DIEA, 1hr
pip/DMF
dodecanoic acid, DEPBT/DMF, DIEA
TFA/DCM
HF/ p-cresol, 1hr
NH2-HN QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-N
SEQ 211
Molecular Weight =5202.94
Exact Mass =5197

FIGURE 5B

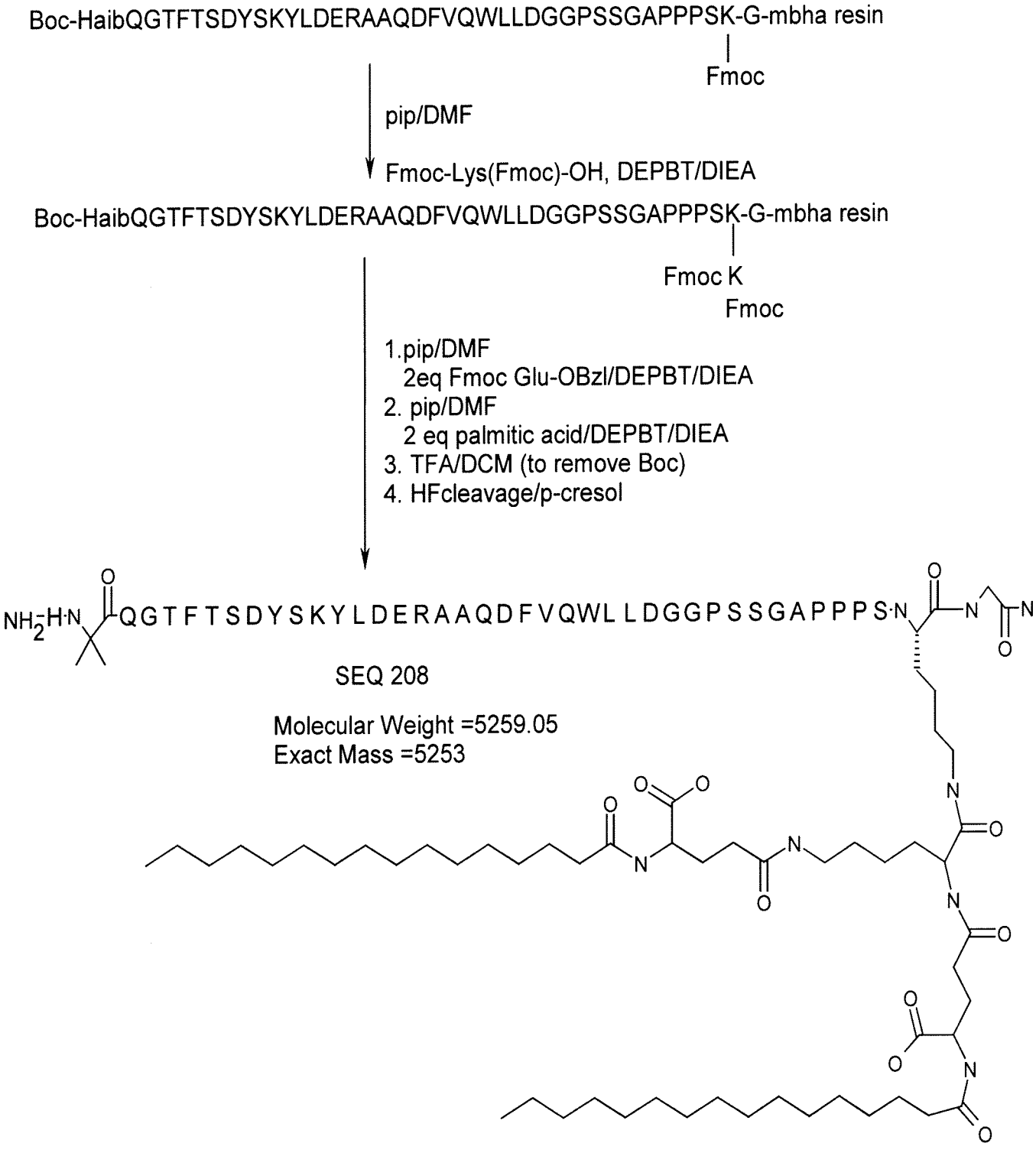

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK-G-mbha resin
Fmoc
pip/DMF
Fmoc-Lys(Fmoc)-OH, DEPBT/DIEA
Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK-G-mbha resin
Fmoc K
Fmoc
1.pip/DMF
2eq Fmoc Glu-OBzl/DEPBT/DIEA
2. pip/DMF
2 eq palmitic acid/DEPBT/DIEA
3. TFA/DCM (to remove Boc)
4. HFcleavage/p-cresol
NH2-H-N
QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-N
SEQ 208
Molecular Weight =5259.05
Exact Mass =5253

FIGURE 5C

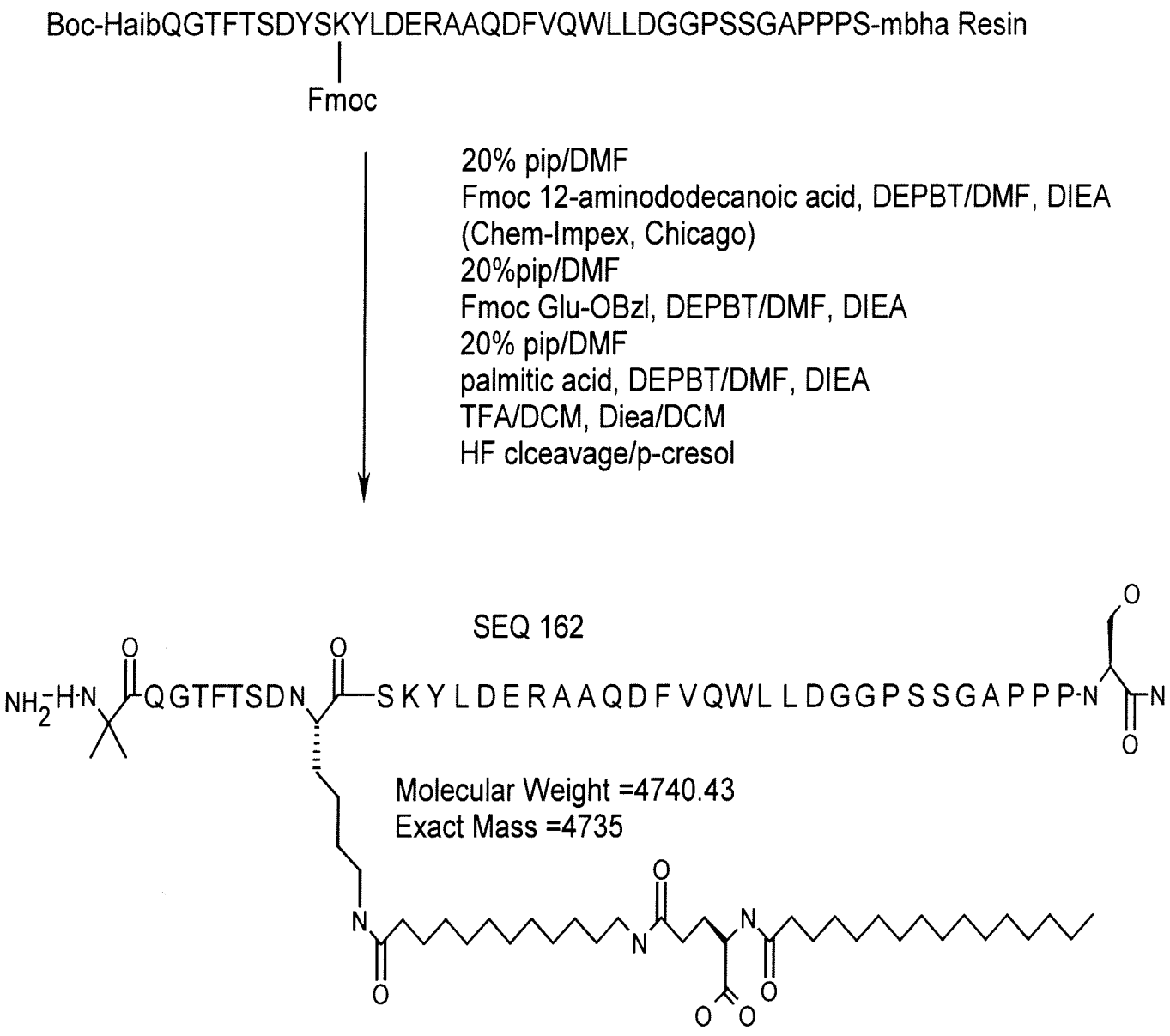

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-mbha Resin
Fmoc
20% pip/DMF
Fmoc 12-aminododecanoic acid, DEPBT/DMF, DIEA
(Chem-Impex, Chicago)
20%pip/DMF
Fmoc Glu-OBzl, DEPBT/DMF, DIEA
20% pip/DMF
palmitic acid, DEPBT/DMF, DIEA
TFA/DCM, Diea/DCM
HF clceavage/p-cresol
SEQ 162
NH2-H·N QGTFTSDN SKYLDERAAQDFVQWLLDGGPSSGAPPP·N N
Molecular Weight =4740.43
Exact Mass =4735

FIGURE 5D

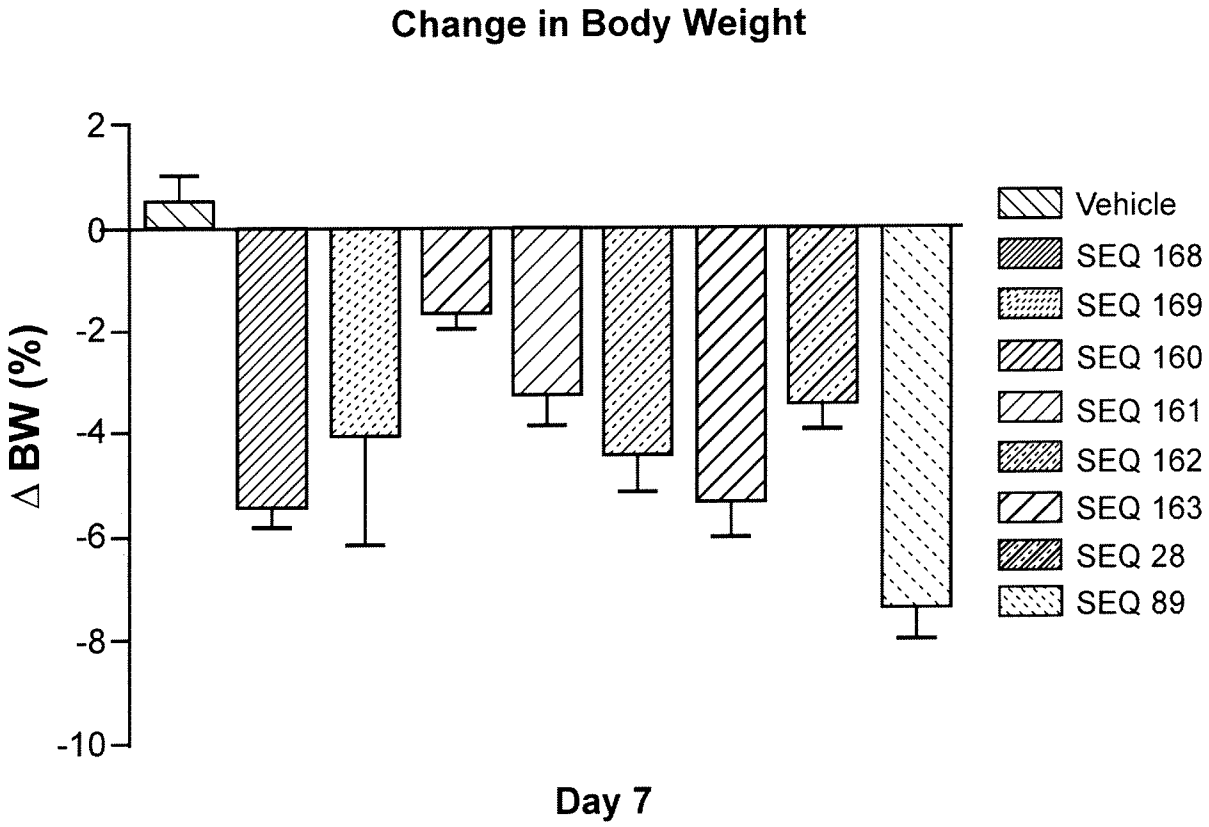

Change in Body Weight
2
0
-2
-4
-6
-8
-10
Δ BW (%)
Day 7
Vehicle
SEQ 168
SEQ 169
SEQ 160
SEQ 161
SEQ 162
SEQ 163
SEQ 28
SEQ 89

FIGURE 5E

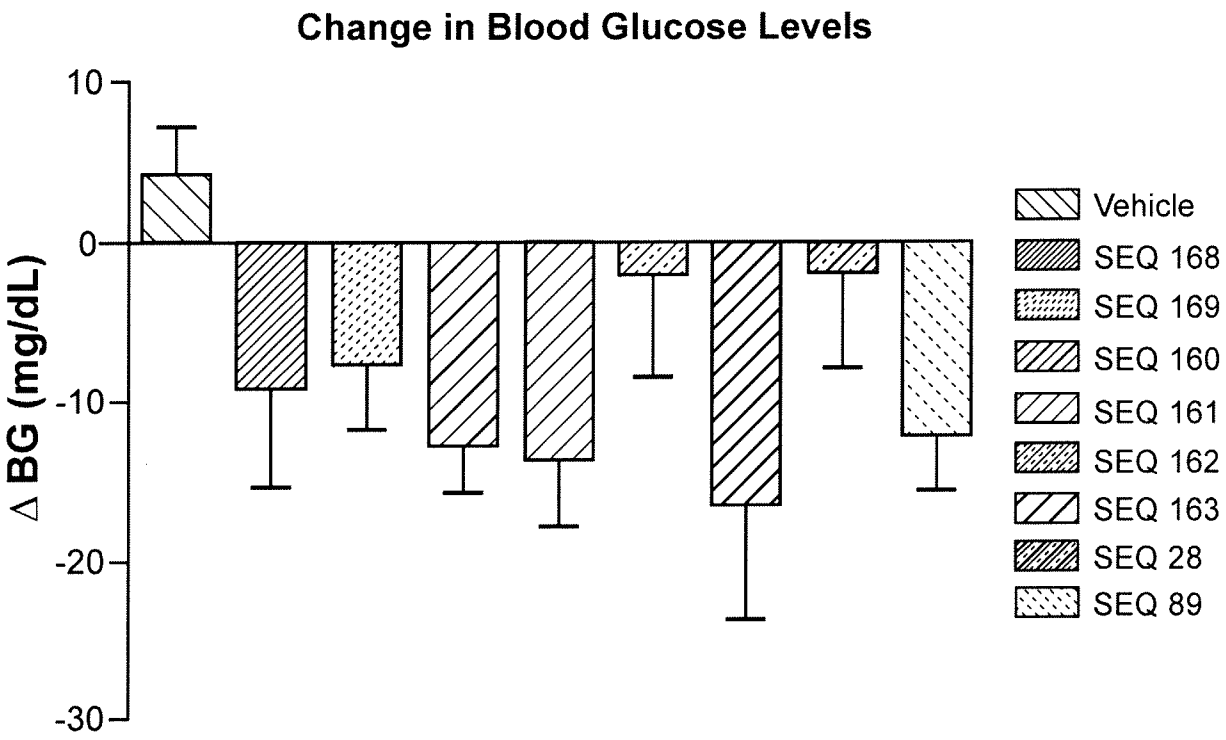

Change in Blood Glucose Levels
10
0
-10
-20
-30
Δ BG (mg/dL)
Vehicle
SEQ 168
SEQ 169
SEQ 160
SEQ 161
SEQ 162
SEQ 163
SEQ 28
SEQ 89
Day 7

FIGURE 5F

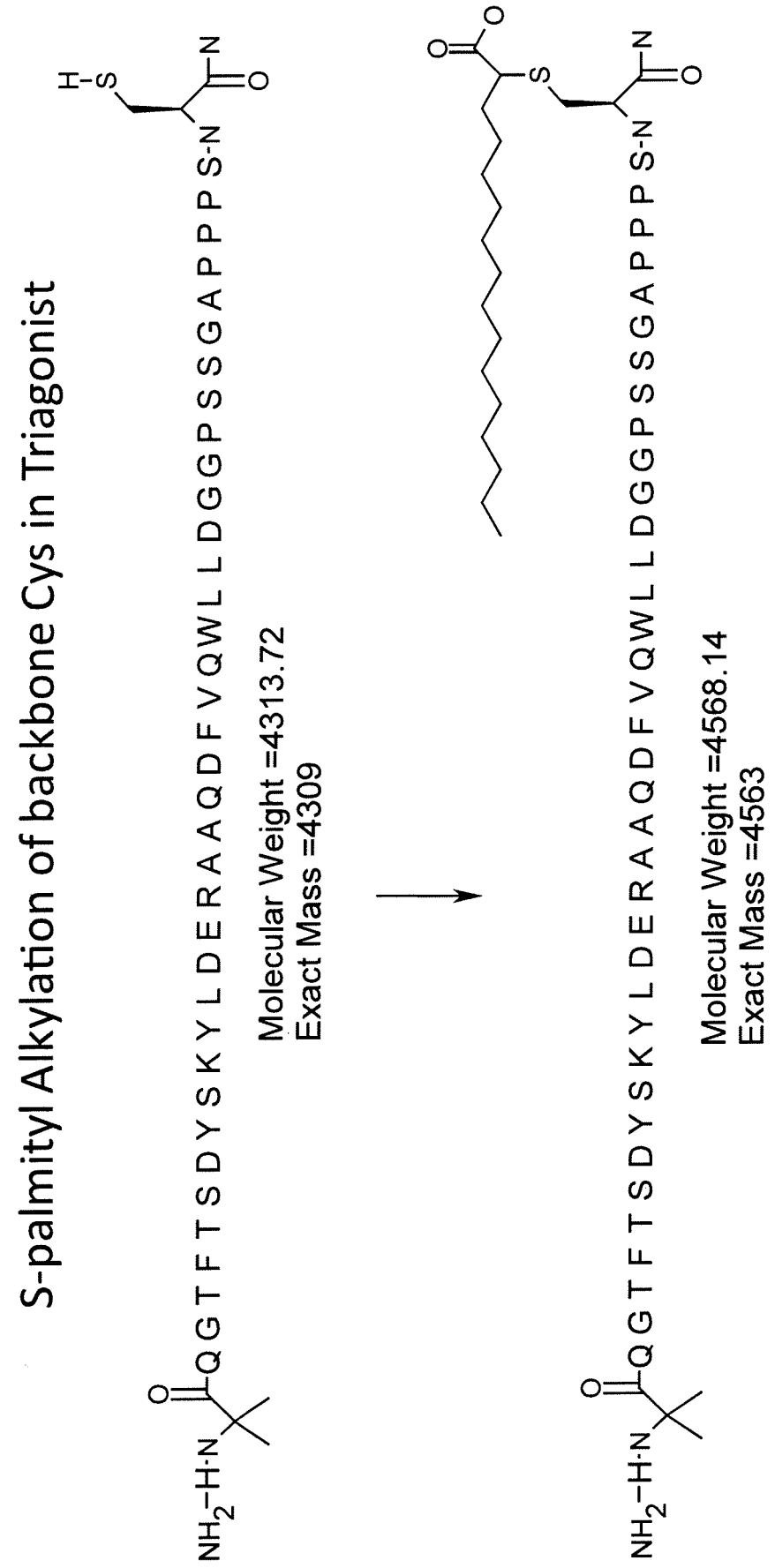

S-palmityl Alkylation of backbone Cys in Triagonist
Molecular Weight =4313.72
Exact Mass =4309
Molecular Weight =4568.14
Exact Mass =4563

FIGURE 6

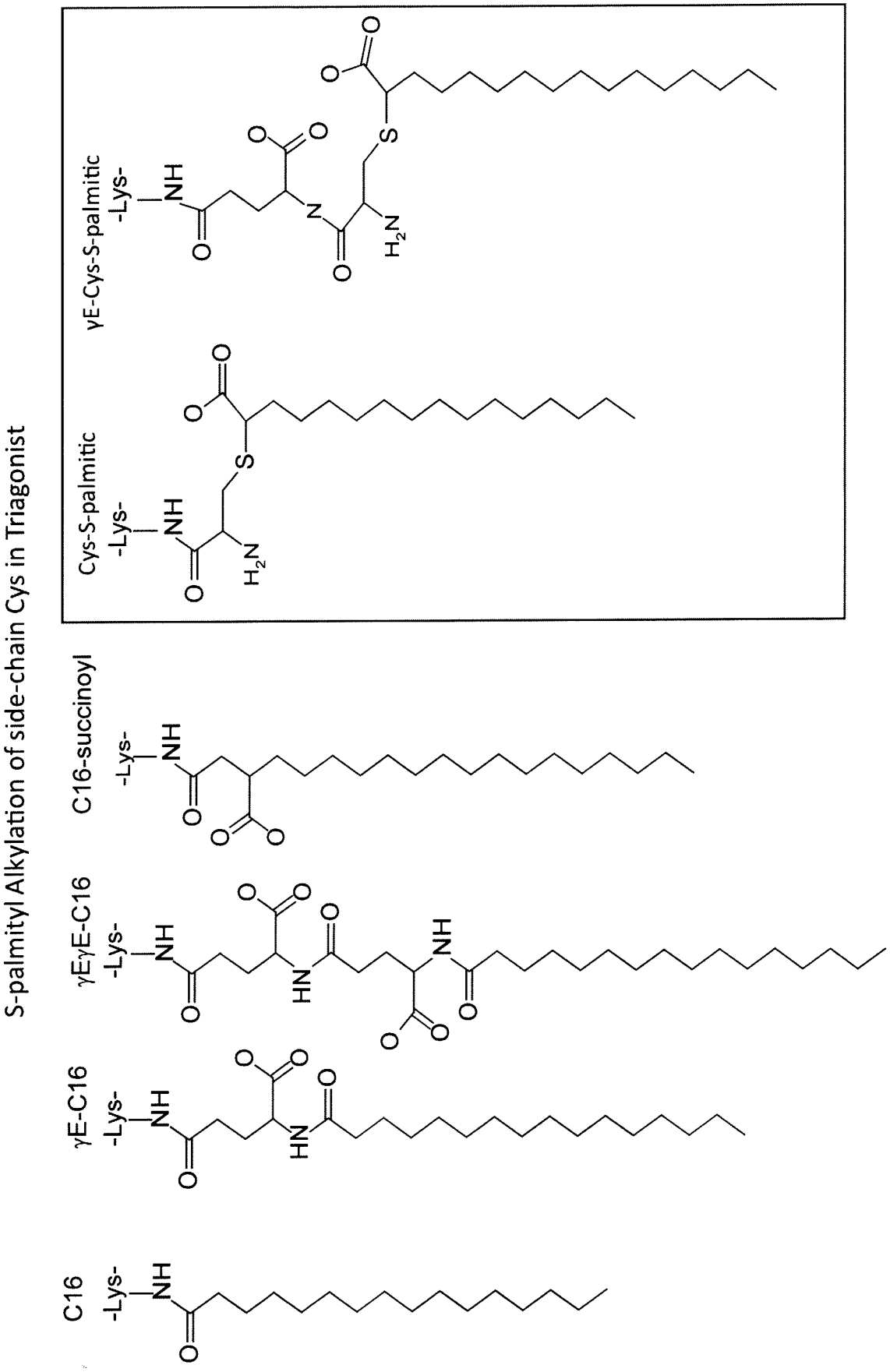

S-palmityl Alkylation of side-chain Cys in Triagonist
C16
-Lys-
γE-C16
-Lys-
γEγE-C16
-Lys-
C16-succinoyl
-Lys-
Cys-S-palmitic
-Lys-
γE-Cys-S-palmitic
-Lys-

FIGURE 7

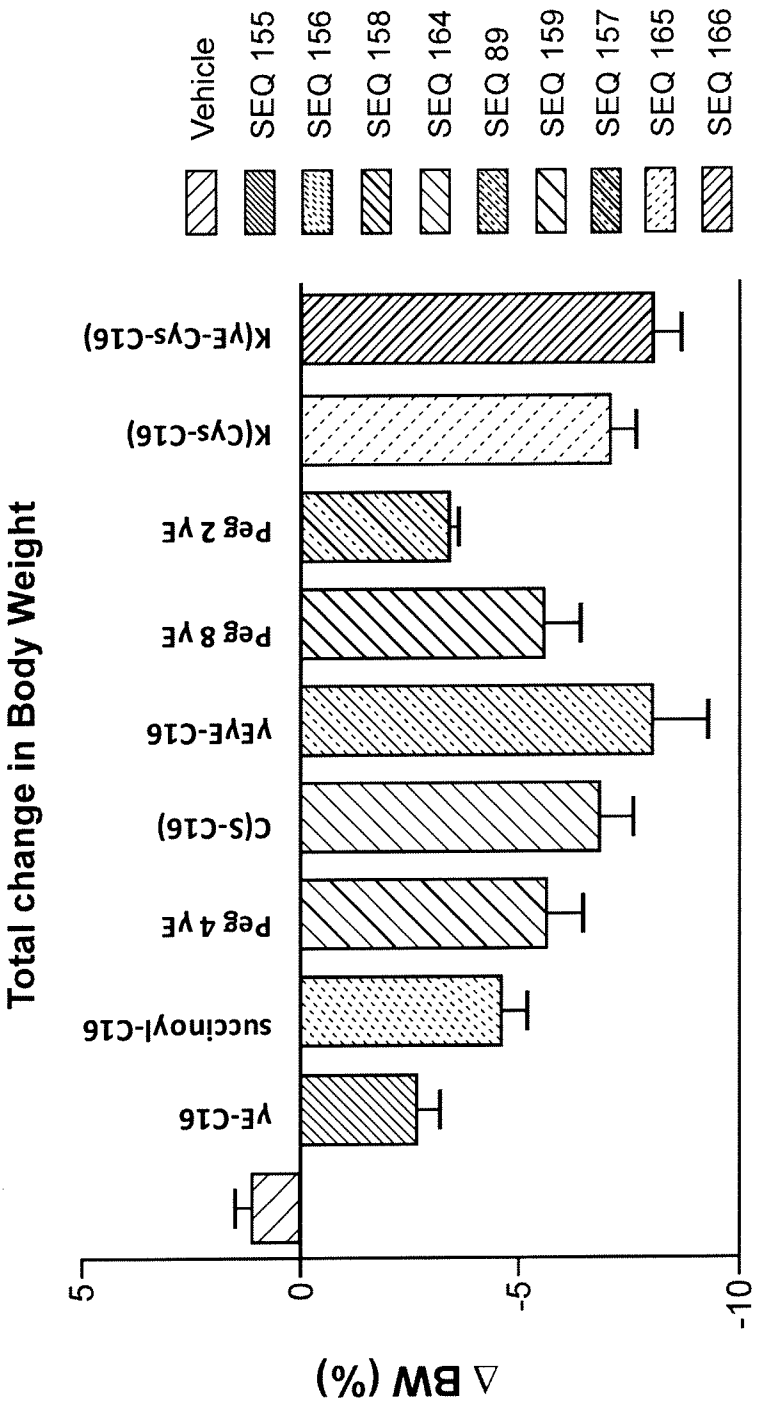

Vehicle
SEQ 155
SEQ 156
SEQ 158
SEQ 164
SEQ 89
SEQ 159
SEQ 157
SEQ 165
SEQ 166
Total change in Body Weight
K(yE-Cys-C16)
K(Cys-C16)
Peg 2 yE
Peg 8 yE
yEyE-C16
C(S-C16)
Peg 4 yE
succinoyl-C16
yE-C16
5
0
-5
-10
Δ BW (%)
Day 7

FIGURE 8

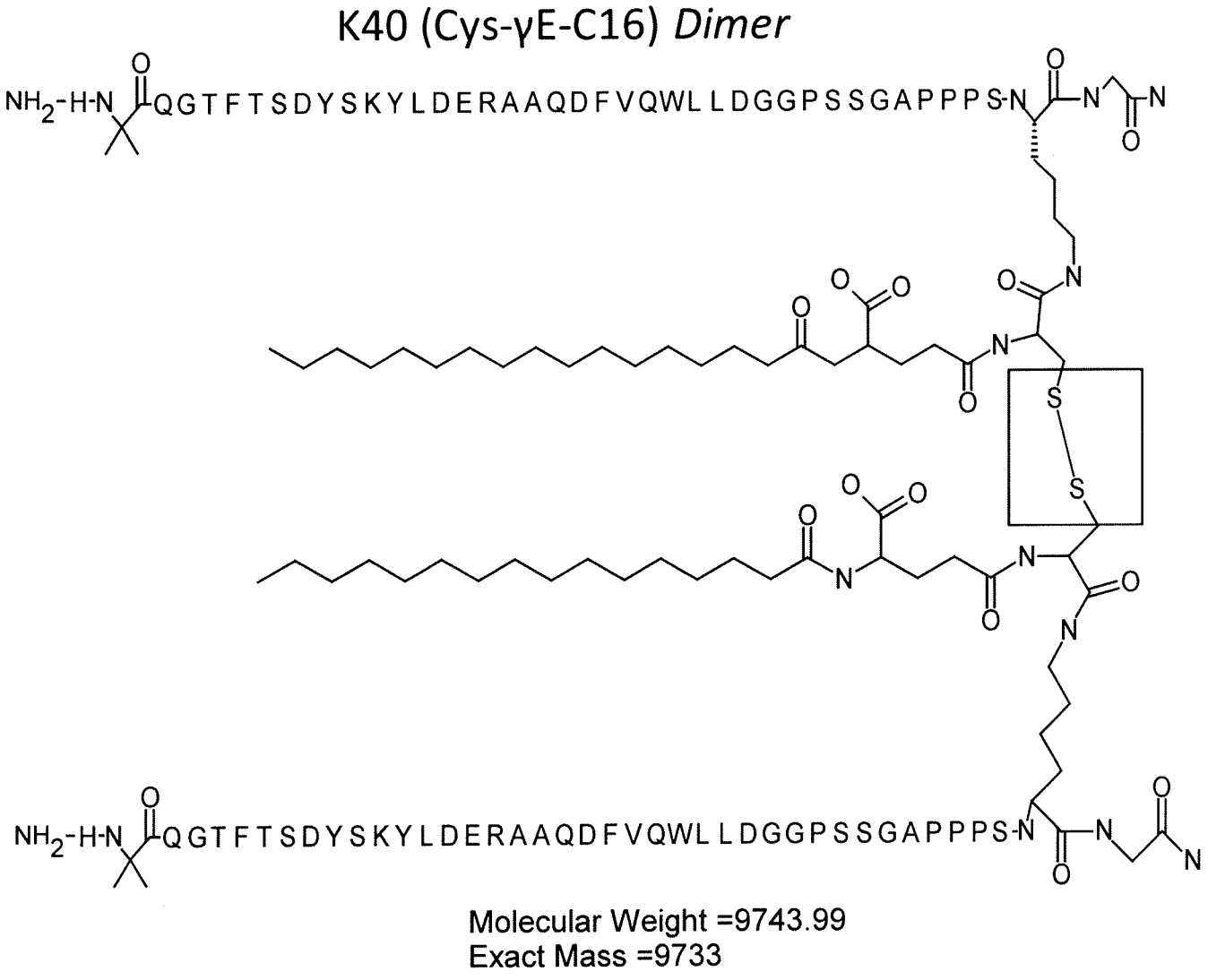

K40 (Cys-γE-C16) *Dimer*
NH2-H-N
QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-N
NH2-H-N
QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-N
Molecular Weight =9743.99
Exact Mass =9733

FIGURE 9A

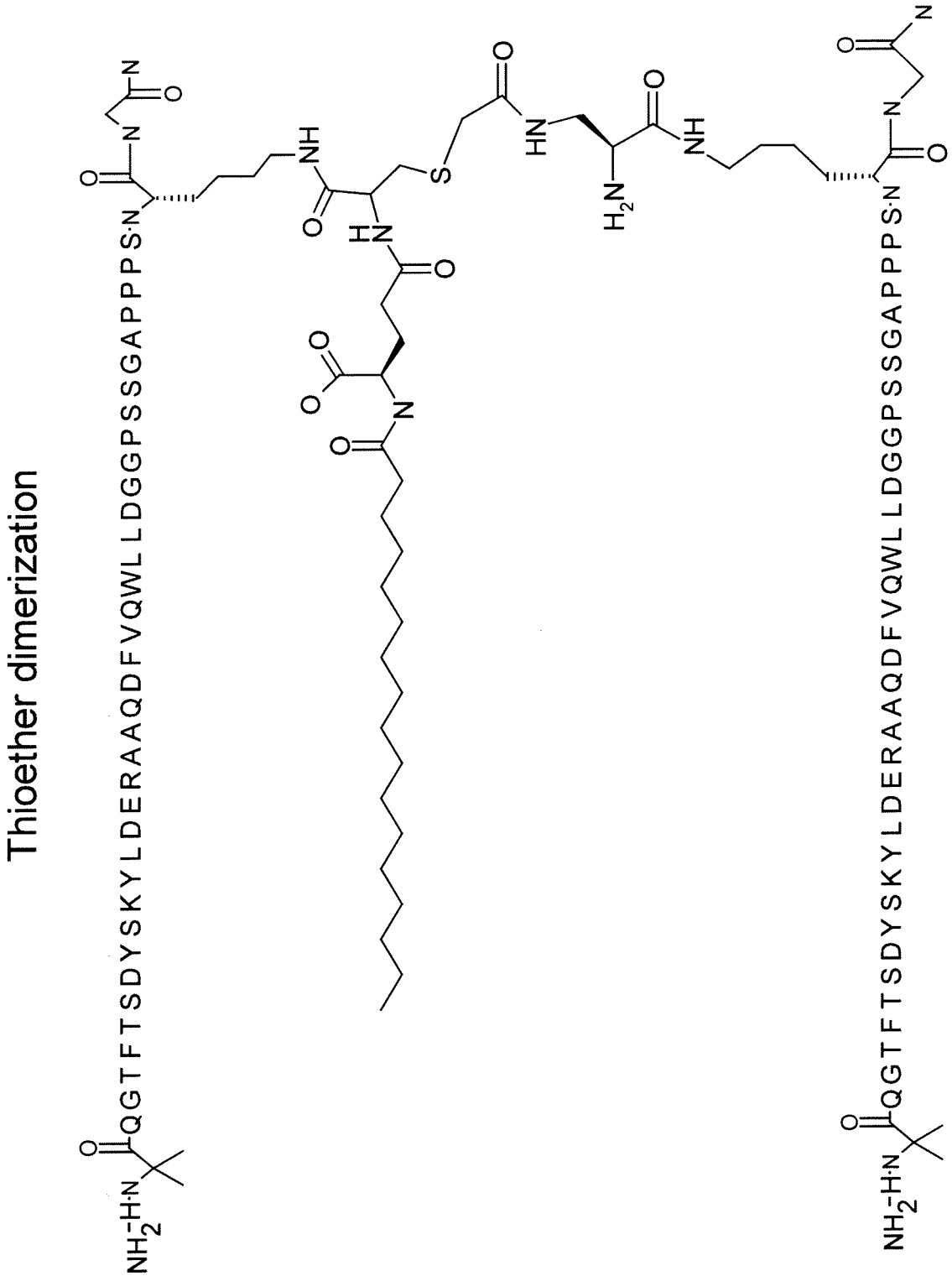

Thioether dimerization
QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS
QGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS

FIGURE 9B

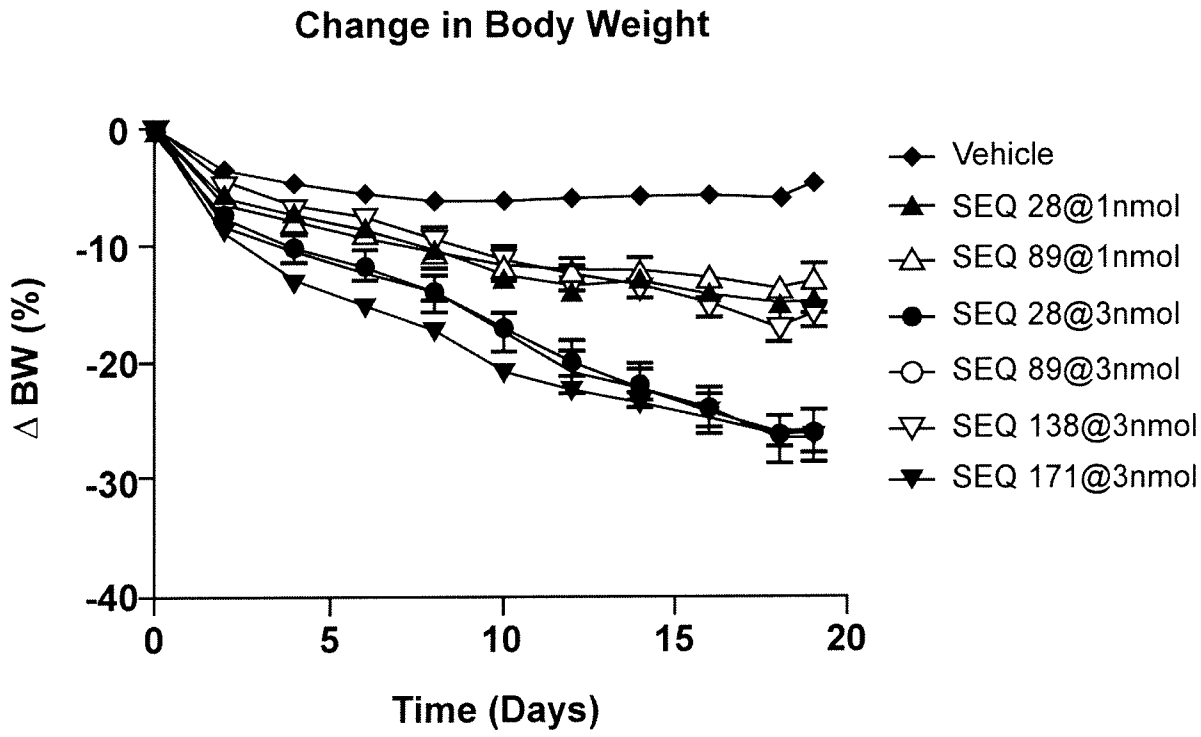

Change in Body Weight
0
-10
-20
-30
-40
Δ BW (%)
0
5
10
15
20
Time (Days)
Vehicle
SEQ 28@1nmol
SEQ 89@1nmol
SEQ 28@3nmol
SEQ 89@3nmol
SEQ 138@3nmol
SEQ 171@3nmol

FIGURE 10

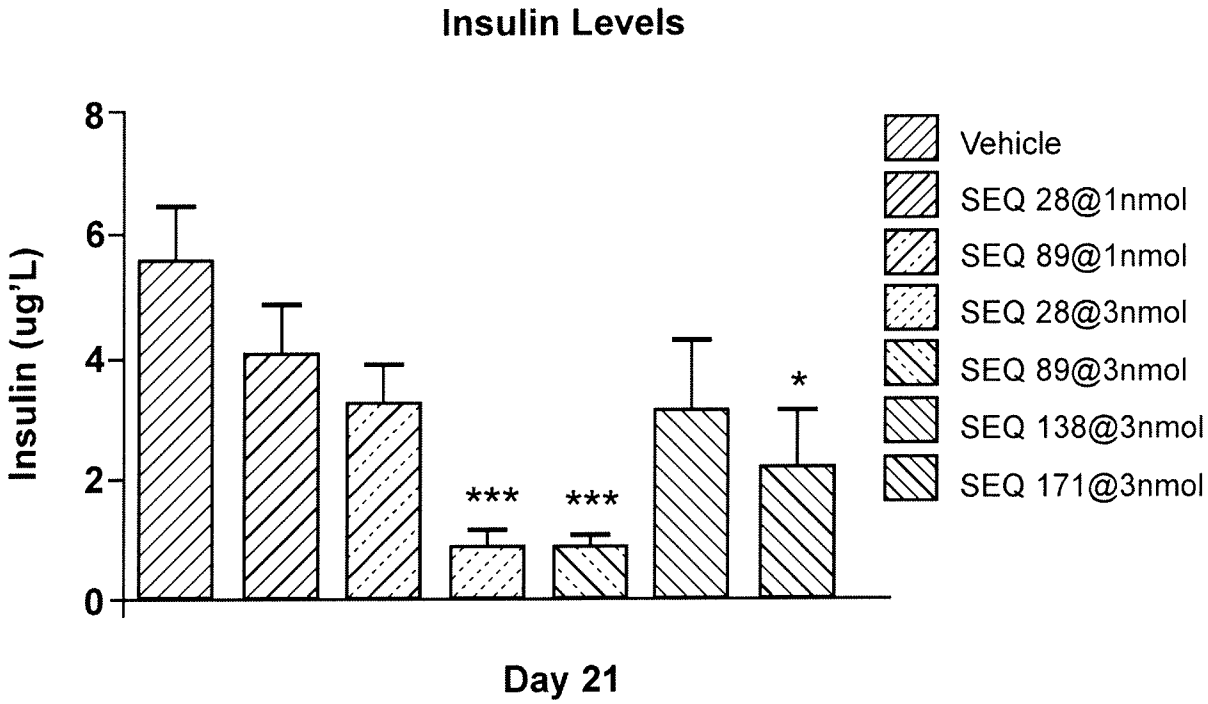

Insulin Levels
Insulin (ug'L)
8
6
4
2
0
***
***
*
Vehicle
SEQ 28@1nmol
SEQ 89@1nmol
SEQ 28@3nmol
SEQ 89@3nmol
SEQ 138@3nmol
SEQ 171@3nmol
Day 21

FIGURE 11

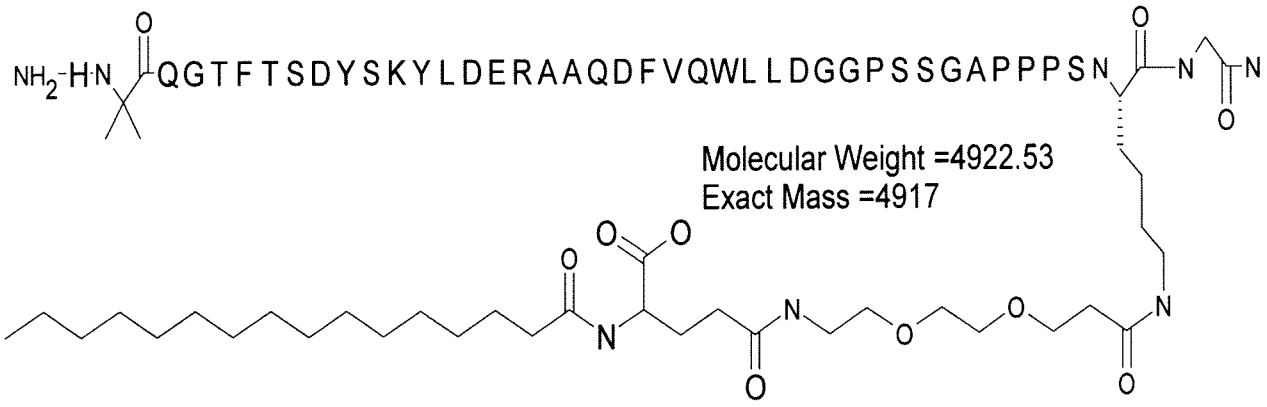


FIGURE 12A

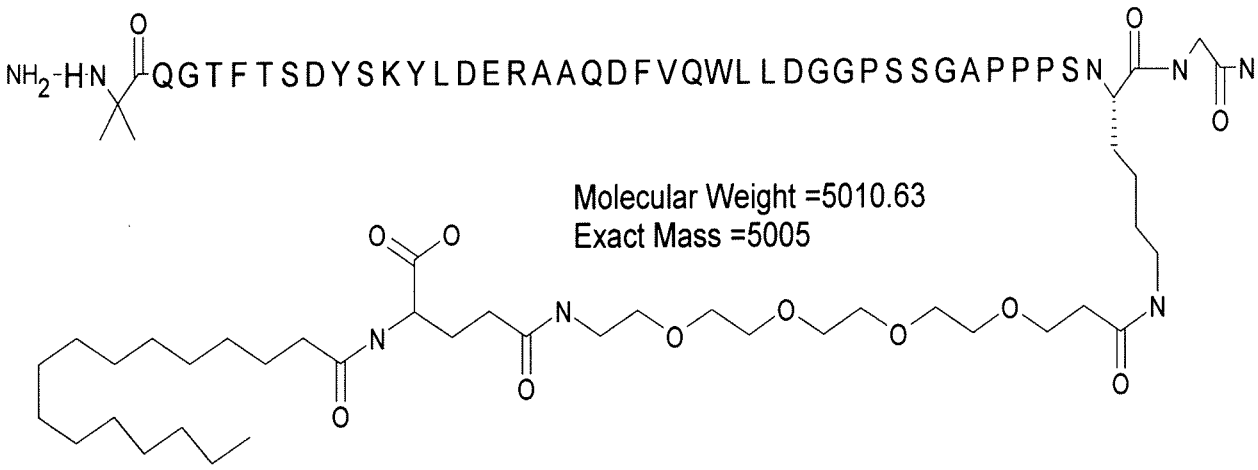


FIGURE 12B

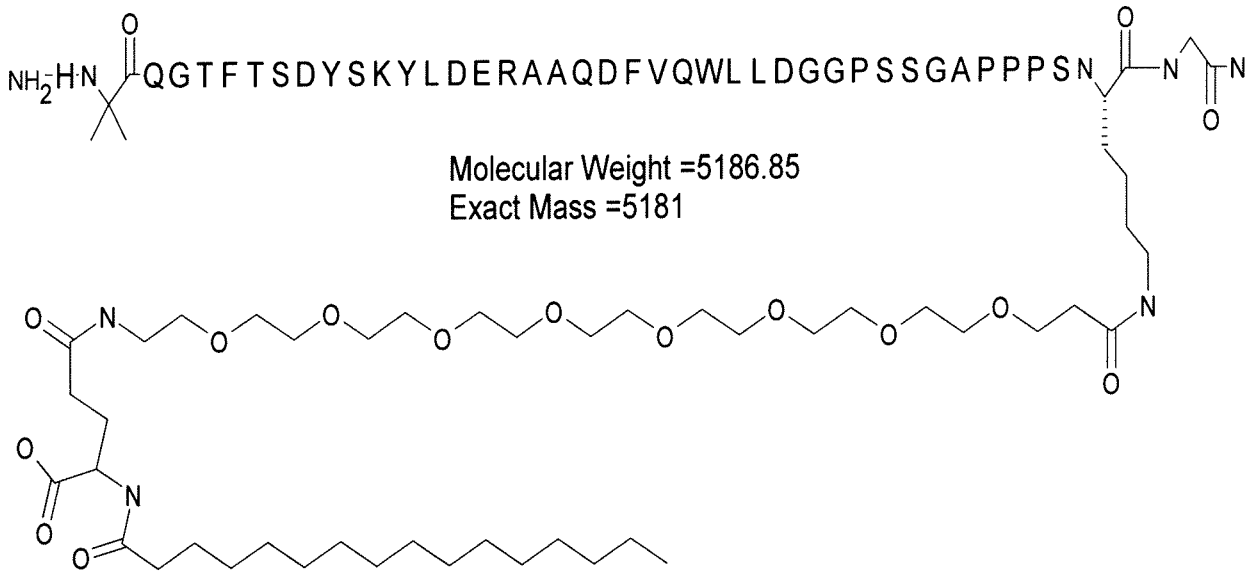


FIGURE 12C

SEQUENCE LISTING

<110> DiMarchi, et al.

<120> GLUCAGON ANALOGS EXHIBITING GIP RECEPTOR ACTIVITY

<130> 31135/45700A

<160> 262

<170> PatentIn version 3.5

<210> 1
<211> 29
<212> PRT
<213> Homo sapiens

<220>
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<223> Wild type glucagon

<400> 1

His Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Ser
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

<210> 2
<211> 42
<212> PRT
<213> Homo sapiens

<220>
<221> mat_peptide
<222> (1)..(42)
<223> Gastric Inhibitory Polypeptide

<400> 2

Tyr Ala Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Asp Trp Lys His Asn Ile Thr Gln
35 40

<210> 3
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<213> Artificial sequence

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<223> Synthetic polypeptide

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<222> (1)..(30)
<223> GLP-1(7-36) amidated

<220>
<221> MISC_FEATURE
<222> (30)..(30)
<223> C-terminal amidation

<400> 3

```
His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
            20                  25                  30
```

<210> 4
<211> 31
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (1)..(31)
<223> GLP-1(7-37)

<400> 4

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His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
            20                  25                  30
```

<210> 5
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
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<400> 5

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Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser
1               5                   10
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<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 6

Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser
1 5 10

<210> 7
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<400> 7

Lys Arg Asn Arg Asn Asn Ile Ala
1 5

<210> 8
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 8

Lys Arg Asn Arg
1

<210> 9
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
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<400> 9

Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser Lys
1 5 10

<210> 10
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<213> Artificial Sequence

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<223> Xaa is AIB

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<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 10

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1   5   10   15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
            20   25   30

Ser Gly Ala Pro Pro Pro Ser Lys
        35   40

<210> 11
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<223> Xaa can be any naturally occurring amino acid

<400> 11

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1   5   10   15

Lys Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
            20   25   30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 12
<211> 29
<212> PRT
<213> Artificial Sequence

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<223> mt-403

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<221> MISC_FEATURE
<222> (16)..(20)
<223> Lactam bridge between residues 16 and 20

<400> 12

His Ser Gln Gly Thr Phe Ile Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Glu Phe Ile Ala Trp Leu Met Asn Thr
20 25

<210> 13
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
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<223> mt-404

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<222> (16)..(20)
<223> Lactam bridge between residues 16 and 20

<400> 13

His Ser Gln Gly Thr Phe Ile Ser Asp Tyr Ser Lys Ala Leu Asp Glu
1 5 10 15

Ile His Gln Lys Glu Phe Ile Ala Trp Leu Met Asn Thr
20 25

<210> 14
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<212> PRT

<213> Artificial Sequence

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<400> 14

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1 5 10 15

Ile His Gln Lys Asp Phe Val Asn Trp Leu Leu Ala Gln
20 25

<210> 15
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
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<223> mt-395

<220>
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<222> (16)..(20)
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<400> 15

His Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Asp Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 16
<211> 39
<212> PRT
<213> Artificial Sequence

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<223> Synthetic peptide

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<223> mt-396

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<222> (16)..(20)
<223> Lactam bridge between residues 16 and 20

<400> 16

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Asp Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 17
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<212> PRT
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<223> mt-397

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<221> MISC_FEATURE
<222> (16)..(20)
<223> Lactam bridge between residues 16 and 20

<400> 17

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Glu Phe Val Asn Trp Leu Leu Ala Gln
20 25

<210> 18
<211> 39
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<213> Artificial Sequence

<220>
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<222> (16)..(20)
<223> Lactam bridge between residues 16 and 20

<400> 18

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 19
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<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<400> 19

Tyr Ala Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 20
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<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
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<223> mt-218

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<400> 20

Tyr Xaa Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 21
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<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> mt-219

<400> 21

Tyr Ala Pro Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 22
<211> 42
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

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<221> MISC_FEATURE
<223> mt-220

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<400> 22

Tyr Xaa Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Cys Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 23
<211> 40
<212> PRT
<213> Artificial Sequence

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<223> mt-225

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<220>
<221> MISC_FEATURE
<222> (16)..(20)
<223> Lactam bridge links Glu16 and Lys20

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Epsilon amine of Lys is attached to Ala-Ac-Cys(PEG), wherein the Ac-Cys(PEG) is a Cys residue comprising an alpha amino group capped with an acetyl group (CH3CO) and comprising a 40 kDa PEG covalently attached to its side chain

<400> 23

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 24
<211> 42
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> mt-226

<400> 24

Tyr Ala Pro Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Cys Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 25
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> mt-227

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (16)..(20)
<223> Lactam bridge links Glu16 and Lys20

<220>
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<222> (40)..(40)
<223> Epsilon amine of Lys is attached to Arg-Ac-Cys(PEG), wherein the Ac-Cys(PEG) is a Cys residue comprising an alpha amino group capped with an acetyl group (CH3CO) and comprising a 40 kDa PEG covalently attached to its side chain

<400> 25

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
        35   40

<210> 26
<211> 42
<212> PRT
<213> Artificial Sequence

<220>
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<220>
<221> MISC_FEATURE
<223> mt-228

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> PEG (40kDa) bound to Cysteine

<400> 26

Tyr Ala Pro Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1   5   10   15

Ile His Gln Gln Asp Phe Val Cys Trp Leu Leu Ala Gln Lys Gly Lys
            20   25   30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
        35   40

<210> 27
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Sythetic peptide

<220>
<221> MISC_FEATURE
<223> Analog #29

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> d-Ser

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Acylated with a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE

<222> (39)..(39)
<223> C-terminal amidation

<400> 27

His Ser Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 28
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> Analog #30

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Acylated with a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 28

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 29
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
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<220>
<221> MISC_FEATURE
<223> Analog #34

<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
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<222> (10)..(10)
<223> Acylated with a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (31)..(31)
<223> C-terminal amidation

<400> 29

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
20 25 30

<210> 30
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> Analog #36

<220>
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<223> d-Ser

<220>
<221> MISC_FEATURE
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<223> Acylated with a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>

<221> MISC_FEATURE
<222> (29)..(29)
<223> C-terminal amidation

<400> 30

His Ser Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> 31
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> Analog #37

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Acylated with a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> C-terminal amidation

<400> 31

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> 32
<211> 29
<212> PRT
<213> Artificial Sequence

<220>

<223> Sythetic peptide

<220>
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<223> Analog #44

<220>
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<223> Xaa is DMIA

<220>
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<222> (2)..(2)
<223> d-Ser

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Acylated with C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> C-terminal amidation

<400> 32

Xaa Ser Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> 33
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> Analog #49

<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
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<223> Acylated with a C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
<221> MISC_FEATURE

<222> (16)..(16)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 33

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Met Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 34

<400> 34
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<210> 35
<211> 39
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<213> Artificial Sequence

<220>
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<220>
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<223> Xaa is AIB

<220>
<221> MISC_FEATURE
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<223> C-terminal amidation

<400> 35

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Val Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 36
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> Analog #54

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Acylated with C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
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<222> (16)..(16)
<223> Xaa is AIB

<220>
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<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 36

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Lys Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 37
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
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<220>
<221> MISC_FEATURE
<223> Analog #61

<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
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<223> Acylated with a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 37

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 38
<211> 39
<212> PRT
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<220>
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<223> Analog #62

<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
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<223> Acylated with a C16 fatty acyl group via gamma-Glu spacer

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<221> MISC_FEATURE
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<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 38

His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 39
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<220>
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<220>
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<222> (39)..(39)
<223> C-terminal amidation

<400> 39

His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 40
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<223> Analog #64

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<222> (2)..(2)
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<223> Xaa is AIB

<220>
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<222> (29)..(29)
<223> C-terminal amidation

<400> 40

His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> 41
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<222> (20)..(20)
<223> Xaa is AIB

<220>
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<222> (39)..(39)
<223> C-terminal amidation

<400> 41

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His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1               5                   10                  15


Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser
        35
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<210> 42
<211> 29
<212> PRT
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<223> Xaa is AIB

<220>
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<222> (16)..(16)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> C-terminal amidation

<400> 42

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

<210> 43
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
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<222> (9)..(9)
<223> Acylated with a C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
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<223> Xaa is AIB

<220>
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<222> (29)..(29)
<223> C-terminal amidation

<400> 43

His Xaa Gln Gly Thr Phe Thr Ser Lys Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

<210> 44
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
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<223> Xaa is AIB

<220>
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<222> (10)..(10)
<223> Acylated with a C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

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<221> MISC_FEATURE
<222> (16)..(16)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> C-terminal amidation

<400> 44

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

<210> 45
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<223> Xaa is AIB

<220>
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<222> (12)..(12)
<223> Acylated with a C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
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<222> (16)..(16)
<223> Xaa is AIB

<220>
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<222> (29)..(29)
<223> C-terminal amidation

<400> 45

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr

20 25

<210> 46
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Sythetic peptide

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<222> (2)..(2)
<223> Xaa is AIB

<220>
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<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> C-terminal amidation

<400> 46

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Lys
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

<210> 47
<211> 29
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<220>
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<222> (20)..(20)
<223> Acylated with a C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> C-terminal amidation

<400> 47

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Arg Ala Lys Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

<210> 48
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
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<223> d-Ser

<220>
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<222> (20)..(20)
<223> Xaa is AIB

<220>
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<222> (40)..(40)
<223> C-terminal amidation

<400> 48

His Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 49

<400> 49
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<210> 50
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<222> (2)..(2)
<223> d-Ser

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 50

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 51
<211> 40
<212> PRT
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<222> (2)..(2)
<223> d-Ser

<220>
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<222> (20)..(20)
<223> Xaa is AIB

<220>
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<223> Covalently bound to PEG

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 51

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 52
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
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<222> (20)..(20)
<223> Xaa is AIB

<220>
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<223> C-terminal amidation

<400> 52

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 53
<211> 40
<212> PRT
<213> Artificial Sequence

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<223> Xaa is AIB

<220>
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<220>
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<223> Covalently bound to PEG

<220>
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<223> C-terminal amidation

<400> 53

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 54
<211> 40
<212> PRT
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<222> (24)..(24)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
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<223> C-terminal amidation

<400> 54

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys

1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Xaa Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 55

<400> 55
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<210> 56
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<223> Linked via lactam bridge

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 56

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Val Xaa Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 57

<400> 57
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<210> 58
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<223> Xaa is AIB

<220>
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<222> (17)..(21)
<223> Linked via lactam bridge

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 58

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20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Ser Gly Ala Pro Pro Pro Ser Cys
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Ser Gly Ala Pro Pro Pro Ser Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Ser Gly Ala Pro Pro Pro Ser Cys
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Ser Gly Ala Pro Pro Pro Ser Cys
35 40

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20 25 30

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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
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Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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20 25 30

Ser Gly Ala Pro Pro Pro Ser
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20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
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Ser Gly Ala Pro Pro Pro Ser Lys

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Ser Gly Ala Pro Pro Pro Ser Lys
35 40

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1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

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20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 90
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Ser Gly Ala Pro Pro Pro Ser
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<400> 91

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 92
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<223> Xaa is AIB

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<223> C-terminal amidation

<400> 92

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Asp Thr
20 25

<210> 93

<400> 93
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<210> 94
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<220>
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<220>
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<223> d-Ser

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<223> Acylated with a C16 fatty acyl group via acid amino acid spacer

<220>
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<222> (39)..(39)
<223> C-terminal amidation

<400> 94

His Ser Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 95
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<223> C-terminal amidation

<400> 95

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 96
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<400> 96

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
20 25 30

<210> 97
<211> 29
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<400> 97

His Ser Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> 98
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<400> 98

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
20 25

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<400> 99

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1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr

20 25

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<400> 100

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Met Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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<400> 102

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Val Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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<400> 103

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Lys Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

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<400> 104

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser

20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 105
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His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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<210> 106
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<400> 106

His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 107
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His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
20 25

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Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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<210> 109
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His Xaa Gln Gly Thr Phe Thr Ser Lys Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
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<400> 110

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Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
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<400> 111

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Lys
1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

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1 5 10 15

Arg Arg Ala Lys Asp Phe Val Gln Trp Leu Met Asn Thr
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<400> 114

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

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<400> 115

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 116
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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Cys
35 40

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<400> 118

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 119
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<223> Covalently bound to PEG

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<400> 119

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 120
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<220>
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<400> 120

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
20 25 30

<210> 121
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<212> PRT
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<400> 121

His Ser Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
20 25 30

<210> 122
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<400> 122

His Ser Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 123
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<222> (39)..(39)
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<400> 123

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 124

<211> 39
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<220>
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<223> Xaa is AIB

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<223> Acylated with C16 fatty acyl group via acidic amino acid spacer

<220>
<221> MISC_FEATURE
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<223> C-terminal amidation

<400> 124

His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 125
<211> 39
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<220>
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<223> Xaa is AIB

<220>
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<223> C-terminal amidation

<400> 125

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 126
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<223> Xaa is AIB

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<400> 126

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 127
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<220>
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<223> Xaa is AIB

<220>
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<400> 127

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 128
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<220>
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<223> C-terminal amidation

<400> 128

His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

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<223> C-terminal amidation

<400> 129

His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> 130
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<220>
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<223> C-terminal amidation

<400> 130

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 131
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<220>
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<223> C-terminal amidation

<400> 131

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 132
<211> 39
<212> PRT
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<220>
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<223> Xaa is AIB

<220>
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<222> (39)..(39)
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<400> 132

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 133
<211> 29
<212> PRT
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<222> (20)..(20)
<223> C-terminal amidation

<220>
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<222> (29)..(29)
<223> C-terminal amidation

<400> 133

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Asp Thr
20 25

<210> 134
<211> 40
<212> PRT
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<220>
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<223> mt-261

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<223> Xaa is Aib

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<223> 16 Carbon fatty acyl group covalently attached Lysine

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 134

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 135
<211> 40
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<213> Artificial Sequence

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<223> Xaa is Aib

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<220>
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<222> (40)..(40)
<223> 16 Carbon fatty acyl group covalently attached to Lysine

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 135

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 136
<211> 39
<212> PRT
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<220>
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<223> mt-384

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<223> Xaa is AIB

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<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
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<222> (39)..(39)
<223> C-terminal amidation

<400> 136

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 137
<211> 39
<212> PRT
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<220>
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<223> mt-384

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<223> Xaa is AIB

<220>

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<220>
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<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 137

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 138
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
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<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 138

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 139
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<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 139

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 140
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<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 140

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 141
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<212> PRT
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<223> Xaa is AIB

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<220>
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<222> (41)..(41)
<223> C-terminal amidation

<400> 141

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys

1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 142
<211> 41
<212> PRT
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<220>
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<222> (41)..(41)
<223> C-terminal amidation

<400> 142

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 143
<211> 39
<212> PRT
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<223> 4-amino-Phe covalently bound a C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
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<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 143

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 144
<211> 39
<212> PRT
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<220>
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<223> 4-amino-Phe is covalently bound to a C16 succinoyl

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
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<223> C-terminal amidation

<400> 144

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 145
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<220>
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<222> (40)..(40)
<223> Xaa is 4-amino-Phe

<220>
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<222> (40)..(40)
<223> 4-amino-Phe covalently bound to a C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 145

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Gly
35 40

<210> 146
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
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<223> Xaa is 4-amino Phe

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<223> 4-amino Phe covalently bound to C16 succinoyl

<220>
<221> MOD_RES
<222> (41)..(41)
<223> C-terminal amidation

<400> 146

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Gly
35 40

<210> 147
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<223> 4-amino-Phe covalently bound to C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 147

His Xaa Gln Gly Thr Xaa Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 148
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<212> PRT
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<220>
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<220>
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<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> 4-amino-Phe covalently bound to C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 148

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 149
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<212> PRT
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<220>
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<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 149

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Xaa Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 150
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<220>
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<220>
<221> MISC_FEATURE
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<400> 150

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 151
<211> 39
<212> PRT
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<220>
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<220>
<221> MOD_RES
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<223> C-terminal amidation

<400> 151

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Lys Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 152
<211> 39
<212> PRT
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<220>
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<223> Xaa is AIB

<220>
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<222> (14)..(14)
<223> Covalently bound to a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 152

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Lys Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 153
<211> 39
<212> PRT
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<220>
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<220>

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<223> Covalently bound to C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 153

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Lys
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 154
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (17)..(17)
<223> Covalently bound to C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 154

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Lys Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 155
<211> 41

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 155

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 156
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 succinoyl

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 156

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 157
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via a spacer comprising an amido miniPEG acid and a gamma-Glu, wherein the amido miniPEG acid has a structure {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, wherein n is 2

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 157

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 158
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE

<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising an amido miniPEG acid and a gamma-Glu, wherein the amido miniPEG acid has a structure {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, wherein n is 4

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 158

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 159
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising an amido miniPEG acid and a gamma-Glu, wherein the amido miniPEG acid has a structure {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, wherein n is 8

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 159

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 160
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to a Lys spacer, wherein the Lys spacer is attached to two C16 fatty acyl groups, wherein each C16 fatty acyl group is attached to the Lys spacer via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 160

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 161
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>

<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a Lys spacer, wherein the Lys spacer is attached to two C16 fatty acyl groups, wherein each C16 fatty acyl group is attached to the Lys spacer via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 161

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 162
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to a C12 fatty acyl group, which is covalently bound to a gamma-Glu spacer, which is covalently bound to a C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 162

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 163
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 163

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 164
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Sulfur of Cys is S-alkylated with -C(COO)H(CH2)13CH3

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 164

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys Gly
35 40

<210> 165
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Lys covalently bound to Cys, the sulfur of which is S-alkylated with -C(COO)H(CH2)13CH3

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 165

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 166
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to gamma-Glu, which is bound to Cys, the sulfur of which is S-alkylated with -C(COO)H(CH2)13CH3

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 166

```
His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1               5                   10                  15


Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser Lys Gly
        35                  40
```

<210> 167
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys, which is covalently bound to gamma-Glu, which is covalently bound to C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 167

```
His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
```

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 168
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys spacer, which is bound to gamma-Glu, which is bound to C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to SEQ ID NO 167 via Cys spacer which is disulfide bonded to a 2nd Cys, which the 2nd Cys is bound to Lys at position 40 of SEQ ID NO 167 and is bound to gamma-Glu, which, in turn is bound to C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 168

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 169
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys, which is bound to gamma-Glu, which is bound to C16 fatty acyl group and the sulfer of the Cys is thioether linked to a peptide of SEQ ID NO: 231 via Lys at position 40

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 169

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 170
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys, which is bound to gamma-Glu, which is bound to C16 fatty acyl group, and the sulfur of the Cys is disulfide bonded to a 2nd Cys, which the 2nd Cys is bound to a peptide of SEQ ID NO: 231 via Lys at position 40

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 170

```
His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1               5                   10                  15


Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser Lys Gly
        35                  40
```

<210> 171
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 171

```
His Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1               5                   10                  15


Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser Lys Gly
        35                  40
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<210> 172
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>

<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> Covalently bound to PEG

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C8 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 172

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 173
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> Covalently bound to PEG

<220>

<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C12 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 173

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 174
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> Covalently bound to PEG

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C14 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 174

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 175
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covantly bound to PEG

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 175

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 176
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)

<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C14 succinoyl

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 176

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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1               5                   10                  15


Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser Lys Gly
        35                  40
```

<210> 177
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 177

```
His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1               5                   10                  15


Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser Lys Gly
        35                  40
```

<210> 178
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Xaa is Tyr or an amino acid covalently bound to a C10 to C26 acyl or alkyl group

<220>
<221> MISC_FEATURE
<222> (18)..(18)
<223> Xaa is Arg or Ala

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB or Gln

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Xaa is Asp or Glu

<220>
<221> MISC_FEATURE
<222> (28)..(28)
<223> Xaa is Asp of Glu

<220>
<221> MISC_FEATURE
<222> (35)..(35)
<223> Xaa is Ala, Arg or Lys

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Xaa is absent or is Gly (e.g. when position 10 is not Tyr) or is an amino acid covalently bound to a C10-C26 acyl or alkyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> Optionally present, when residue at position 40 is an amino acid covalently bound to C10-C26 acyl or alkyl group, Xaa is Gly

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 178

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Xaa Gly Gly Pro Ser
20 25 30

Ser Gly Xaa Pro Pro Pro Ser Xaa Xaa
35 40

<210> 179
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Xaa is Tyr or an amino acid covalently bound to a C10 to C26 acyl or alkyl group

<220>
<221> MISC_FEATURE
<222> (18)..(18)
<223> Xaa is Arg or Ala

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB or Gln

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Xaa is Asp or Glu

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Xaa is absent or is Gly (e.g. when position 10 is not Tyr) or is an amino acid covalently bound to a C10-C26 acyl or alkyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> Optionally present, when residue at position 40 is an amino acid covalently bound to C10-C26 acyl or alkyl group then Xaa is Gly

<220>
<221> MISC_FEATURE

<222> (41)..(41)
<223> C-terminal amidation

<400> 179

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Xaa
35 40

<210> 180
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 180

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 181
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (1)..(29)
<223> At least one amino acid is an amino acid covalently bound to a C10 to C26 acyl or alkyl group

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (3)..(3)
<223> Xaa is Gln or Gln analog

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Xaa is Lys or Tyr

<220>
<221> MISC_FEATURE
<222> (18)..(18)
<223> Xaa is Arg or Ala

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa is AIB or Gln

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Xaa is Asp or Glu

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> Xaa is Thr or Gly

<220>
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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Asp Xaa
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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Asp Xaa
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Gly Pro Ser Ser Gly Arg Pro Pro Pro Ser
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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
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Ser Gly Xaa Pro Pro Pro Ser
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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
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20 25 30

Ser Gly Xaa Pro Pro Pro Ser
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20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

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Ser Gly Xaa Pro Pro Pro Ser
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20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
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Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
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Ser Gly Xaa Pro Pro Pro Ser
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<220>
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<223> Xaa can be any amino acid

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<223> Xaa can be any amino acid

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<223> Xaa can be any amino acid

<220>
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<223> C-terminal amidation

<400> 197

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 198
<211> 29
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<220>
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<220>
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<223> Xaa can be any amino acid

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<223> Xaa can be any amino acid

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<223> Xaa can be any amino acid

<220>
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<222> (29)..(29)
<223> C-terminal amidation

<400> 198

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

<210> 199
<211> 29
<212> PRT
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<223> Xaa can be any amino acid

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<223> Xaa can be any amino acid

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<223> C-terminal amidation

<400> 199

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

<210> 200
<211> 5
<212> PRT
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<220>
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<400> 200

Glu Arg Ala Ala Gln
1 5

<210> 201
<211> 6
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<213> Artificial Sequence

<220>
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<400> 201

Glu Arg Ala Ala Gln Asp
1 5

<210> 202

<400> 202
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<210> 203
<211> 41
<212> PRT
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<220>
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<220>
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<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 203

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 204
<211> 41
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<220>
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<222> (41)..(41)
<223> C-terminal amidation

<400> 204

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Ala Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 205
<211> 40
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<223> Covalently bound to C16 fatty acyl group via gamma-Glu spacer

<220>
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<223> C-terminal amidation

<400> 205

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly
35 40

<210> 206
<211> 39
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<223> Xaa is AIB

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<222> (10)..(10)
<223> Covalently bound to C16 fatty acyl group via gamma-Glu spacer

<220>
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<222> (39)..(39)
<223> C-terminal amidation

<400> 206

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Glu Gly Gly Pro Ser
20 25 30

Ser Gly Arg Pro Pro Pro Ser
35

<210> 207
<211> 40
<212> PRT
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<223> Xaa is AIB

<220>
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<223> Covalently bound to C16 fatty acyl group via gamma-Glu-gamma-Glu spacer

<220>
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<222> (40)..(40)
<223> C-terminal amidation

<400> 207

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly
35 40

<210> 208
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Lys spacer, wherein Lys spacer is bound via the alpha amine to a gamma-Glu spacer, which is bound to a C16 fatty acyl group and Lys spacer is bound via the epsilon amine to gamma-Glu which is bound to a C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 208

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 209

<400> 209
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<210> 210
<211> 41
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a Lys spacer, wherein the Lys spacer is bound to (1) C16 fatty acyl group via a gamma-Glu spacer and (2) C8 fatty acyl group via a gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 210

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 211
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a Lys spacer, wherein the Lys spacer is bound to (1) C16 fatty acyl group via a gamma-Glu spacer and (2) C12 fatty acyl group via a gamma-Glu spacer

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 211

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 212
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via side chain hydroxyl of Tyr

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 212

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Tyr Gly
35 40

<210> 213

<400> 213
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<210> 214

<400> 214
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<210> 215

<400> 215
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<210> 216

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<210> 219
<211> 40
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<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> MT-345

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<221> MOD_RES
<222> (2)..(2)
<223> Xaa is AIB

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<223> Acylated with a C14 fatty acyl group

<220>
<221> MOD_RES
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<223> C-terminal amidation

<400> 219

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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1               5                   10                  15


Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser Lys
        35                  40
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<210> 220

<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

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<221> MISC_FEATURE
<223> MT-346

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<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MOD_RES
<222> (40)..(40)
<223> C-terminal amidation

<400> 220

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 221
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<223> MT-347

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<223> Xaa is AIB

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<222> (16)..(16)
<223> Xaa is AIB

<220>
<221> MOD_RES
<222> (40)..(40)
<223> Acylated with a C14 fatty acyl group

<220>
<221> MOD_RES
<222> (40)..(40)
<223> C-terminal amidation

<400> 221

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 222
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
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<223> MT-348

<220>
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<223> Xaa is AIB

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<223> Xaa is AIB

<220>
<221> MOD_RES
<222> (40)..(40)
<223> C-terminal amidation

<400> 222

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 223

<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<223> MT-349

<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
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<222> (24)..(24)
<223> Covalently bound to 40kDa PEG via thioether made by reaction of peptide with haloacetyl-activated PEG

<220>
<221> MOD_RES
<222> (40)..(40)
<223> Acylated with a C14 fatty acyl group

<220>
<221> MOD_RES
<222> (40)..(40)
<223> C-terminal amidation

<400> 223

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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1               5                   10                  15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
            20                  25                  30

Ser Gly Ala Pro Pro Pro Ser Lys
        35                  40
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<210> 224
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

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<223> MT-350

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<223> Xaa is AIB

<220>
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<223> Covalently bound to 40kDa PEG via thioether made by reaction of peptide with haloacetyl-activated PEG

<220>
<221> MOD_RES
<222> (40)..(40)
<223> C-terminal amidation

<400> 224

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 225
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

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<223> MT-351

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<223> Xaa is AIB

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<223> Xaa is AIB

<220>
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<222> (24)..(24)
<223> Covalently bound to 40kDa PEG via thioether made by reaction of peptide with haloacetyl-activated PEG

<220>
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<222> (40)..(40)
<223> Acylated with a C14 fatty acyl group

<220>
<221> MOD_RES

<222> (40)..(40)
<223> C-terminal amidation

<400> 225

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 226
<211> 40
<212> PRT
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<223> Xaa is AIB

<220>
<221> MOD_RES
<222> (24)..(24)
<223> Covalently bound to 40kDa PEG via thioether made by reaction of peptide with haloacetyl-activated PEG

<220>
<221> MOD_RES
<222> (40)..(40)
<223> C-terminal amidation

<400> 226

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 227

<400> 227
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<210> 228
<211> 40
<212> PRT
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<223> Xaa is AIB

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<222> (16)..(16)
<223> Xaa is AIB

<220>
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<222> (40)..(40)
<223> C-terminal amidation

<400> 228

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Xaa
1   5   10   15

Gln Ala Ala Lys Glu Phe Val Asn Trp Leu Met Asn Gly Gly Pro Ser
            20   25   30

Ser Gly Ala Pro Pro Pro Ser Lys
        35   40

<210> 229
<211> 40
<212> PRT
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<221> MOD_RES
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MOD_RES
<222> (40)..(40)
<223> Acylated with a C16 fatty acyl group

<220>
<221> MOD_RES
<222> (40)..(40)
<223> C-terminal amidation

<400> 229

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 230
<211> 40
<212> PRT
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<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 230

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Glu
1 5 10 15

Lys Arg Ala Lys Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 231
<211> 41

<212> PRT
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<400> 231

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 232

<400> 232
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<210> 233
<211> 39
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<213> Artificial Sequence

<220>
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<220>
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<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Xaa is 4-amino-Phe

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> 4-amino-Phe covalently bound a C16 fatty acyl group via spacer comprising two acidic residues

<220>
<221> MISC_FEATURE

<222> (20)..(20)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 233

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 234
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<223> Xaa is AIB

<220>
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<222> (40)..(40)
<223> Xaa is 4-amino-Phe

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> 4-amino-Phe covalently bound to a C16 fatty acyl group via spacer comprising two acidic residues

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 234

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Gly

35 40

<210> 235
<211> 39
<212> PRT
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<220>
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<220>
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<223> 4-amino-Phe covalently bound to C16 fatty acyl group via spacer comprising acidic residue

<220>
<221> MISC_FEATURE
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<223> C-terminal amidation

<400> 235

His Xaa Gln Gly Thr Xaa Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 236
<211> 39
<212> PRT
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<220>
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<222> (10)..(10)
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<220>
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<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 236

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 237
<211> 39
<212> PRT
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<220>
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<220>
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<220>
<221> MISC_FEATURE
<222> (13)..(13)
<223> 4-amino-Phe covalently bound to C16 fatty acyl group via spacer comprising acidic residue

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 237

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Xaa Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 238
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MOD_RES
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MOD_RES
<222> (13)..(13)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising acidic residue

<220>
<221> MOD_RES
<222> (39)..(39)
<223> C-terminal amidation

<400> 238

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Lys Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 239
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>

<221> MISC_FEATURE
<222> (14)..(14)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising acidic residue

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 239

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Lys Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 240
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (16)..(16)
<223> Covalently bound to C16 fatty acyl group via spacer comprising acidic residue

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 240

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Lys
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 241
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (17)..(17)
<223> Covalently bound to C16 fatty acyl group via spacer comprising acidic residue

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 241

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Lys Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 242
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising acidic residue

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 242

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 243
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via a spacer comprising an amido miniPEG acid and an acidic amino acid, wherein the amido miniPEG acid has a structure {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, wherein n is 2

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 243

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 244
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising an amido miniPEG acid and an acidic amino acid, wherein the amido miniPEG acid has a structure {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, wherein n is 4

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 244

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 245
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising an amido miniPEG acid and an acidic amino acid, wherein the amido miniPEG acid has a structure {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, wherein n is 8

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 245

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 246
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to a Lys spacer, wherein the Lys spacer is attached to two C16 fatty acyl groups, wherein each C16 fatty acyl group is attached to the Lys spacer via spacer comprising an acidic residue

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 246

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 247
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>

<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a Lys spacer, wherein the Lys spacer is attached to two C16 fatty acyl groups, wherein each C16 fatty acyl group is attached to the Lys spacer via spacer comprising an acidic residue

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 247

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 248
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to a C12 fatty acyl group, which is covalently bound to a spacer comprising an acidic residue, which is covalently bound to a C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 248

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 249
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to a C16 fatty acyl group via acidic amino acid spacer

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to a C16 fatty acyl group via spacer comprising an acidic residue

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 249

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 250
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>

<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to an acidic residue, which is bound to Cys, the sulfur of which is S-alkylated with -C(COO)H(CH2)13CH3

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 250

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 251
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys, which is covalently bound to an acidic residue, which is covalently bound to C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 251

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 252
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys spacer, which is bound to an acidic residue, which is bound to C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to SEQ ID 251 via Cys spacer which is disulfide bonded to a 2nd Cys, which the 2nd Cys is bound to Lys at position 40 of SEQ ID 251 and is bound to an acidic residue, which, in turn is bound to C16 fatty acyl group

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 252

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 253
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE

<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys, which is bound to an acidic residue, which is bound to C16 fatty acyl group and the sulfer of the Cys is thioether linked to a peptide of SEQ ID NO: 231 via Lys at position 40

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 253

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 254
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Cys, which is bound to an acidic residue, which is bound to C16 fatty acyl group, and the sulfur of the Cys is disulfide bonded to a 2nd Cys, which the 2nd Cys is bound to a peptide of SEQ ID NO: 231 via Lys at position 40

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 254

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 255
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via spacer comprising two acidic residues

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 255

His Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 256
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE

<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via spacer comprising two acidic residues

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 256

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 257
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via spacer comprising two acidic residues

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 257

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 258

<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to C16 fatty acyl group via spacer comprising two acidic residues

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 258

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Ala Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 259
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to C16 fatty acyl group via spacer comprising an acidic residue

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 259

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly
35 40

<210> 260
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to C16 fatty acyl group via spacer comprising an acidic residue

<220>
<221> MISC_FEATURE
<222> (39)..(39)
<223> C-terminal amidation

<400> 260

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Glu Gly Gly Pro Ser
20 25 30

Ser Gly Arg Pro Pro Pro Ser
35

<210> 261
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>

<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Covalently bound to C16 fatty acyl group via spacer comprising two acidic residues

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> C-terminal amidation

<400> 261

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly
35 40

<210> 262
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa is AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)
<223> Covalently bound to Lys spacer, wherein Lys spacer is bound via the alpha amine to an acidic residue spacer, which is bound to a C16 fatty acyl group and Lys spacer is bound via the epsilon amine to an acidic residue which is bound to a C16 fatty acyl

<220>
<221> MISC_FEATURE
<222> (41)..(41)
<223> C-terminal amidation

<400> 262

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

REIVINDICACIONES

1. Un análogo de glucagón (SEQ ID NO: 1) caracterizado porque comprende:

a. un aminoácido que comprende una cadena lateral de imidazola en posición 1,

b. un aminoácido protector de DPP-IV en posición 2, opcionalmente, AIB,

c. un aminoácido acilado o un aminoácido alquilado en cualquiera de las posiciones 9, 10, 12, 16 0 37-43, opcionalmente en donde el grupo acilo o alquilo se unen al aminoácido mediante un separador,

d. un aminoácido alfa-alfa-disustituido en posición 20, y

e. hasta 10 modificaciones de aminoácidos adicionales relacionadas con la SEQ ID NO: 1;

en donde, cuando el análogo de glucagón carece de una partícula hidrofílica, el análogo de glucagón presenta un porcentaje de potencia de GIP de, al menos, 0,1%, en donde el análogo de glucagón tiene una selectividad 100 veces menor para el receptor GLP-1 humano en comparación con el receptor GIP.

2. El análogo de glucagón de acuerdo con la reivindicación 1, en donde el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde R1 y R2 son seleccionados de forma independiente del grupo que consiste en alquilo C1-C4, opcionalmente sustituido por hidroxilo, amida, tiol, halo o R1 y R2 junto con el carbono alfa al cual se encuentran unidos para formar un anillo.

3. El análogo de glucagón de acuerdo con la reivindicación 2 en donde aminoácido α,α-disustituido en posición 20 es AIB.

4. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 3, en donde el aminoácido en posición 16 es una hélice alfa que estabiliza a los aminoácidos que no sean AIB.

5. El análogo de glucagón de acuerdo con la reivindicación 4, en donde el aminoácido en posición 16 en un aminoácido con carga, opcionalmente un aminoácido con carga negativa o un aminoácido con carga positiva.

6. El análogo de glucagón de acuerdo con la reivindicación 5, en donde el aminoácido con carga es Glu o Lys.

7. Una análogo de glucagón (SEQ ID NO: 1), caracterizado porque comprende:

a. un aminoácido que comprende una cadena lateral de imidazola en la posición 1,

b. un aminoácido protector DPP-IV en posición 2, opcionalmente AIB,

c. un aminoácido acilado o un aminoácido alquilado en cualquiera de las posiciones 9, 10, 12, 16, 20 o 37-43, opcionalmente, en donde el grupo acilo o alquilo está ligado al aminoácido a través de un separador,

d. una hélice alfa que estabiliza el aminoácido en una o más de las posiciones 16-21, opcionalmente, posición 16, en donde el análogo no comprende un hélice alfa que estabiliza el aminoácido en posición 20 y

e. hasta 10 modificaciones de aminoácido relacionadas con SEQ ID NO: 1,

en donde, cuando el análogo de glucagón carece de una partícula hidrofílica, el análogo de glucagón muestra un porcentaje de potencia de GIP de, al menos, 0,2%, en donde el análogo de glucagón una selectividad 100 veces menor para el receptor de GLP-1 humano en comparación con el receptor de GIP.

8. El análogo de glucagon de acuerdo con la reivindicación 7, caracterizado porque comprende una hélice alfa que estabiliza el aminoácido en posición 16, opcionalmente, en donde la hélice alfa es un aminoácido con carga negativa o un aminoácido alfa, alfa-disustituido.

9. El análogo de glucagón de acuerdo con la reivindicación 8, en donde el aminoácido con carga negativa es Glu.

10. El análogo de glucagón de acuerdo con la reivindicación 8, en donde el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde R1 y R2 se seleccionan de forma independiente del grupo que consiste en alquilo C1-C4, opcionalmente sustituido con un hidroxilo, amida, tiol, halo o R1 y R2 junto con el carbono alfa al que están unidos para formar un anillo.

11. El análogo de glucagón de acuerdo con la reivindicación 10, caracterizado porque el aminoácido α,α-disustituido es AIB.

12. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 7 a 11, que comprende en la posición 18 un aminoácido alifático pequeño, opcionalmente, Ala, y un Arg en posición 17.

13. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 7 a 12, en donde el análogo no está modificado en la posición 20 en comparación con SEQ ID NO:1.

14. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 13, que comprende (i) una extensión de 1 a 21 aminoácidos de extremo C hasta el aminoácido en posición

29 o (ii) el aminoácido acilado o un aminoácido alquilado están en posición 10, 12 0 16.

15. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 14, en donde el aminoácido en posición 1 es His o un derivado de His.

16. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 15, en donde el aminoácido en posición 2 es un aminoácido alfa, alfa-disustituido.

17. El análogo de glucagón de acuerdo con la reivindicación 16, en donde el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde R1 y R2 se seleccionan de forma independiente del grupo que consiste en alquilo C1-C4, opcionalmente sustituido con hidroxilo, amida, tiol, halo o R1 y R2 junto con el carbono alfa al que están unidos forman un anillo.

18. El análogo de glucagón de acuerdo con la reivindicación 17, en donde el aminoácido α,α-disustituido en posición 2 es AIB.

19. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 18, caractirazo porque comprende una extensión de 1 a 21 aminoácidos de extremo C hasta el aminoácido en posición 29.

20. El análogo de glucagón de acuerdo con la reivindicación 19, en donde el aminoácido en posición 29 es Gly.

21. El análogo de acuerdo con las reivindicaciones 19 o 20, en donde la extensión comprende una secuencia de aminoácido que forma una estructura de jaula de Trp.

22. El análogo de glucagón de acuerdo con la reivindicación 21, en donde la extensión comprende una secuencia de aminoácido de GPSSGAPPPS (SEQ ID NO:5) o una secuencia de ella sustituida de forma conservadora.

23. El análogo de glucagón de acuerdo con las reivindicaciones 19 o 20, en donde la extensión comprende al menos un aminoácido con carga.

24. El análogo de glucagón de acuerdo con la reivindicación 23, en donde la extensión comprende una secuencia de aminoácido de X1-X2, en donde X1 es un aminoácido cargado y X2 es un aminoácido alifático pequeño.

25. El análogo de glucagón de acuerdo con la reivindicación 24, en donde X1 es un aminoácido con carga positiva.

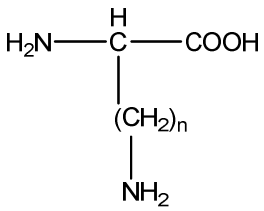
26. El análogo de glucagón de acuerdo con la reivindicación 25, en donde X1 es Arg.

27. El análogo de glucagón de acuerdo con la reivindicación 26 en donde la extensión comprende Arg-Gly.

28. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 27, excepto la 14, en donde (i) el aminoácido que comprende un grupo alquilo o acilo no nativo está localizado en posición 10 o (ii) el análogo de glucagón comprende una extensión de 1 a 21 aminoácidos de extremo C hasta el aminoácido en posición 29 y un aminoácido acilado o un aminoácido alquilado en cualquiera de las posiciones 37-43, opcionalmente 40.

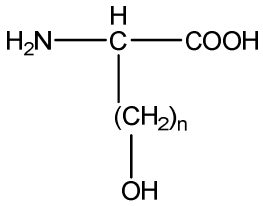
29. El análogo de glucagón de acuerdo con cualqueira de las reivindicaciones 1 a 27 en donde el aminoácido acilado o aminoácido alquilado está en posición 14.

30. El análogo de glucagón de acuerdo con cualqueira de las reivindicaciones 1 a 29, en donde el grupo acilo o el grupo alquilo está unido al amino ácido que comprende una estructura de Fórmula I (opcionalmente, Lys), Fórmula II (opcionalmente, Cys) o Fórmula III (opcionalmente, Ser), en donde las Fórmulas I, II y III es:



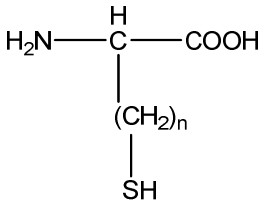
en donde n = 1 a 4

[Fórmula I]



en donde n = 1 a 4

[Fórmula II] y



en donde n = 1 a 4

[Fórmula III].

31. El análogo de glucagón de acuerdo con las reivindicaciones 1 a 29, en donde el aminoácido del grupo acilo o grupo alquilo está unido al amino-Phe o Tyr.

32. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 31, en donde el grupo acilo o grupo alquilo está unido a un aminoácido mediante a un separador de 3 a 10 átomos de longitud.

33. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 32, en donde el separador es un aminoácido o un dipéptido.

34. El análogo de glucagón de acuerdo con la reivindicación 33, en donde el separador comprende uno o dos residuos de aminoácido acídico.

35. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 34, en donde el aminoácido acilado o aminoácido alquilado está unido al grupo alquilo o acilo a través de un separador en donde la longitud total del separador y de grupo acilo es de una longitud aproximada de 14 a 28 átomos.

36. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 35, en donde el aminoácido acilado o aminoácido alquilado está unido a un grupo acilo graso de C12 a C18.

37. El análogo de glucagón de acuerdo con la reivindicación 36, en donde el grupo acilo es un grupo acilo graso de C14 a C16.

38. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 37, que comprende al menos un aminoácido con carga de extemo C al aminoácido en posición 27.

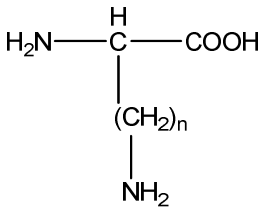
39. El análogo de glucagón de acuerdo con la reivindicación 38, que comprende un aminoácido con carga en posición 28.

40. El análogo de glucagón de acuerdo con las reivindicaciones 38 o 39, en donde el aminoácido con carga es un aminoácido con carga negativa.

41. El análogo de glucagón de acuerdo con la reivindicación 40, en donde el amino ácido con carga negativa es Asp.

42. El análogo de glucagón de acuerdo con las reivindicaciones 38 o 39, en donde el aminoácido con carga es un aminoácido con carga positiva.

43. El análogo de glucagón de acuerdo con la reivindicación 42, en donde el aminoácido con carga positiva es un aminoácido de Fórmula I:



en donde n = 1 a 4

[Fórmula I].

44. El análogo de acuerdo con la reivindicación 43, en donde en aminoácido de Fórmula I en posición 28 es Lys.

45. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 44, que comprende una modificación de aminoácido en posición 27, en posición 29 o en ambas posiciones 27 y 29.

46. El análogo de glucagón de acuerdo con la reivindicación 45, en donde el aminoácido en posición 27 es Leu, Nle, Val o Lys.

47. El análogo de glucagón de acuerdo con las reivindicaciones 45 o 46, en donde el aminoácido en posición 29 es Gly o Thr.

48. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 47, que comprende uno o más de:

i. un aminoácido protector DPP-IV en posición I,

ii. un aminoácido acídico, opcionalmente, Glu, en posición 3,

iii. un Ile en posición 7,

iv. un Ile o Arg en posición 12,

v. un aminoácido acídico, opcionalmente Glu, en posición 15,

vi. un aminoácido alifático, opcionalmente, Ala en posición 18,

vii. en aminoácidp acídico, opcionalmente, Glu en posición 21,

viii. un Asn, Ala o AIB en posición 24,

ix. un aminoácido alifático, opcionalmente, Ala, Leu o Nle, en posición 27,

x. un aminoácido acídico, opcionalmente, Glu o un aminoácido acídico alifático, opcionalmente, Ala en posición 28.

xi. en aminoácido alifático, opcionalmente, Ala en posición 29,

xii. amidación en el extremo C.

49. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 48, que comprende una secuencia de aminoácido de cualquiera de las SEQ ID Nos. 184 a 199.

50. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 1 a 48, que comprende cualquiera de las SEQ ID Nos: 28, 29, 31, 37–41, 79, 80, 89, 90, 95, 130, 145–152, 155–167, 171, 176, 177, 180, 203–207, 212 y 230.

51. El análogo de glucagón de acuerdo con la reivindicación 50, que comprende una secuencia de aminoácido de cualquiera de las SEQ ID Nos: 28, 37, 89, 95, 130, 171 y 180.

52. Un péptido que comprende la secuencia de

(a) SEQ ID No. 184,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEQ ID NO: 184)

en donde:

X_2 es AIB;

X_3 es Gln o análogo de Gln,

X_{10} es Tyr o un aminoácido unido de forma covalente a un grupo acilo o alguilo de C12 a C18,

X_{16} es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro y Ser,

X_{18} es Arg o Ala,

X_{20} es un aminoácido con carga negativa o un aminoácido con carga neutral, opcionalmente, AIB o Gln,

X_{21} es un aminoácido acídico, opcionalmente, Asp o Glu,

X_{27} es Leu, Ala, Nle o Met,

X_{28} es Ala o un aminoácido acídico (opcionalmente, Asp o Glu),

X_{29} es Ala o Gly,

X_{35} es Ala o un aminoácido básico (opcionalmente, Arg o Lys),

en donde, cuando X_{28} es un aminoácido acídico, X_{35} is es un aminoácido básico,

en donde, cuando X_{10} es Tyr, el péptido comprende en la posición 40 un aminoácido unido de forma covalente a un grupo alquilo o acilo de C12 a C18 y en donde, opcionalmente, el péptido comprende Gly en posición 41,

en donde, el aminoácido de extreme C del péptido está amidado o

(b) SEQ ID NO: 184 con hasta 3 modificaciones de aminoácido relacionadas con SEQ ID NO: 184, en donde el péptido presenta actividad agonista en el receptor de GIP humano, el receptor de GLP-1 y el receptor de glucagón humano.

53. El péptido de acuerdo con la reivindicación 52, en donde X_{10} es Tyr, el péptido comprende en la posición 40 un aminoácido unido de forma covalente a un grupo alquilo o acilo de C12 a C18 y el péptido opcionalmente comprende Gly en posición 41.

54. El péptido de acuerdo con las reivindicaciones 52 o 53, en donde X_{10} es un aminoácido unido de forma covalente a un grupo alquilo o acilo de C12 a C18.

55. El péptido de acuerdo con cualquiera de las reivindicaciones 52 a 54, en donde X_{20} es Gln.

56. El péptido de acuerdo con cualquiera de las reivindicaciones 55, en donde el aminoácido en posición 16 es un aminoácido con carga negativa, opcionalmente, Glu.

57. El péptido de acuerdo con la reivindicación 56, en donde X_{18} es Ala.

58. El péptido de acuerdo con cualquiera de las reivindicaciones 52 a 54, en donde X_{20} es AIB.

59. El péptido de acuerdo con la reivindicación 58, en donde X_{16} es un aminoácido distinto a AIB.

60. El péptido de acuerdo con cualquiera de las reivindicaciones 52 a 59, en donde (i) X_{28} es un aminoácido acídico, opcionalmente Asp o Glu y X_{35} es un aminoácido básico, opcionalmente Arg o Lys, (ii) sólo uno de X_{27} X_{28} y X_{29} es un Ala, (iii) el péptido comprende un Gly amidado en el extremo C.

61. El péptido de acuerdo con cualquiera de las reivindicaciones 52 a 60, en donde las modificaciones de hasta 3 aminoácidos son sustituciones conservadoras.

62. Un péptido que comprende la secuencia de

(a) SEQ ID NO: 185,

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGAPPPS$

(SEQ ID NO: 185)

en donde:

X_2 es AIB

X_{10} es Tyr o un aminoácido unido de forma covalente a un grupo alquilo o acilo de C12 a C18,

X_{16} es Glu, un aminoácido alfa, alfa disustituido, Lys o

X_{18} es Arg o Ala,

X_{20} es AIB o Gln,

X_{21} es Asp o Glu,

X_{27} es Leu, Nle o Met,

X_{28} es Ala, Asp o Glu,

X_{29} es Gly o Thr y

en donde, cuando X_{10} es Tyr, el peptide comprende en posición 40 un aminoácido uno de forma covalente a un grupo alquilo o acilo de C12 a C18 y en donde, opcionalmente, el péptido comprende Gly en posición 41,

en donde el aminoácido de extreme C del péptido está amidado o

(b) SEQ ID NO: 185 con hasta 3 modificaciones de aminoácido relacionadas con SEQ ID NO: 185, en donde el péptido presenta actividad agonista en el receptor de GIP humano, el receptor GLP-1 humano y el receptor de glucagón humano.

63. El péptido de acuerdo con la reivindicación 62, en donde hasta 3 modificaciones de aminoácidos son sustituciones conservadoras.

64. Un péptido que comprende:

(a) la secuencia de SEQ ID NO 186

$$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$$

(SEQ ID NO: 186)

en donde:

X_2 es AIB

X_3 es Gln o análogo de Gln,

X_{10} es Tyr o un aminoácido unido de forma covalente a un grupo alquilo o acilo de C10 a C26,

X_{16} es cualquier aminoácido, opcionalmente cualquier aminoácido diferente de Gly, Pro y Ser;

X_{18} es Arg o Ala,

X_{20} es un aminoácido con carga negativa o un aminoácido de carga neutra, opcionalmente AIB o Gln,

X_{21} es un aminoácido ácido, opcionalmente Asp o Glu,

X_{27} es Leu, Ala, Nle o Met,

X_{28} es Ala, o un aminoácido ácido (opcionalmente Asp o Glu),

X_{29} es Ala, Gly o Thr y

en donde el péptido comprende un aminoácido unido de manera covalente a un grupo acilo o alquilo de C10 a C26, opcionalmente en la posición 10, y el aminoácido del extremo de C del péptido está amidado, o

(b) SEQ ID NO: 186 con hasta 3 modificaciones de aminoácido relacionadas con SEQ ID NO: 186, en donde el péptido presenta actividad agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

65. El péptido de acuerdo con la reivindicación 64, en donde X_{20} es AIB.

66. El péptido de acuerdo con la reivindicación 65, en donde X_{29} es Thr y el péptido no comprende GPSSGAPPPS (SEQ ID NO: 5).

67. El péptido de acuerdo con las reivindicaciones 65 o 66, en donde X_{16} es un aminoácido diferente a AIB.

68. El péptido de acuerdo con la reivindicación 64, en donde X_{20} es Gln.

69. El péptido de acuerdo con la reivindicación 68, en donde el aminoácido en posición 16 es un aminoácido con carga negativa, opcionalmente, Glu.

70. El péptido de acuerdo con la reivindicación 69, en donde X_{18} es Ala.

71. El péptido de acuerdo con cualquiera de las reivindicaciones 64 a 70, excepto la 66, que comprende una extensión de 1 a 21 aminoácidos de extremo C hasta el aminoácido en posición 29.

72. El péptido de acuerdo con la reivindicación 71, en donde el aminoácido en posición 29 es Gly.

73. El péptido de acuerdo con las reivindicaciones 71 o 72, en donde la extensión comprende la secuencia de aminoácido GPSSGAPPPS (SEQ ID NO: 5) o una secuencia de ella sustituida de forma conservadora, o en donde la extensión comprende la secuencia X1-X2, en donde X1 es un aminoácido cargado y X2 es un aminoácido alifático pequeño, opcionalmente, en donde X1 es un aminoácido con carga positiva.

74. El péptido de acuerdo con la reivindicación 73, en donde el aminoácido con carga positiva es Arg.

75. El péptido de acuerdo con la reivindicación 74, en donde la extensión comprende o consiste en Arg-Gly.

76. El péptido de acuerdo con la reivindicación 73, en donde la extensión comprende la secuencia de aminoácido GPSSGAPPPS (SEQ ID NO:5), seguido de Lys o Lys-Gly, en donde Lys está unido de forma covalente a un grupo acilo de C10 a C26.

77. El péptido de acuerdo con la reivindicación 64, que comprede SEQ ID No. 186, en donde X_2 es AIB, X_3 es Gln, X_{10} es un aminoácido unido de forma covalente a un grupo alquilo o acilo de C10 a C26, X_{18} es Arg o Ala, X_{20} es AIB o Gln, X_{21} es Asp o Glu, X_{29} es Gly y el aminoácido de extremo C amidado, en donde Gly en posición 29 está fusionado con GPSSGAPPPS seguido de Lys o Lys-Gly, en donde el Lys es unido de forma covalente a un grupo acilo de C10 a C26.

78. El péptido de acuerdo con cualquiera de las reivindicaciones 64 a 77, en donde las modificaciones de hasta 3 aminoácidos son sustituciones conservadoras.

79. Un péptido que comprende

(a) la secuencia de SEQ ID NO: 187:

$$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$$

(SEQ ID NO: 187)

en donde:

X_2 es AIB,

X_{10} es Tyr o un aminoácido unido de forma covalente a un grupo alquilo o acilo de C10 a C 26,

X_{16} es Glu, aminoácido alfa, alfa-disustituido o Lys,

X_{18} es Arg o Ala,

X_{20} es un aminoácido con carga negatica o un aminoácido con carga neutral, opcionalmente, AIB o Gln,

X_{21} es Asp o Glu,

X_{27} es Leu, Ala, Nle o Met,

X_{28} is Ala, Asp o Glu,

X_{29} es Gly o Thr y

en donde el peptide comprende un aminoácido unido de forma covalente a un grupo alquilo o acilo de C12 a C18, opcionalmente, en posición 10 y el aminoácido de extremo C del péptido está amidado o

(b) SEQ ID NO: 187 con hasta 3 modificaciones de aminoácido relacionadas con SEQ ID NO: 187, en donde el péptido presenta actividad agonista en el receptor GIP humano, el receptor GLP-1 humano y el receptor de glucagón humano.

80. El péptido de acuerdo con la reivindicación 80, en donde las modificaciones de hasta 3 aminoácidos son sustituciones conservadoras.

81. Un péptido que comprende la secuencia SEQ ID No: 188 o una secuencia de SEQ ID No: 188 con hasta 3 modificaciones de aminoácido relacionadas con SEQ ID No: 188, en donde el péptido presente actividad agonista en el receptor GIP humano y en el receptor GLP-1 humano.

82. Un péptido que comprende la secuencia de SEQ ID No: 189 o una secuencia de SEQ ID No: 189 con hasta 3 modificaciones de aminoácidos relacionadas con SEQ ID No: 189, en donde el péptido presenta actividad agonista en el receptor GIP y el receptor GLP-1 humano.

83. Un péptido que comprende una secuencia de SEQ ID No: 190 o una secuencia de SEQ ID No: 190 con hasta 3 modificaciones de aminoácidos relacionadas con SEQ ID No: 190, en donde el péptido presenta actividad agonista en el receptor de GIP humano y el receptor de GLP-1 humano.

84. Un péptido que comprende una secuencia de SEQ ID No: 191 o una secuencia de SEQ ID No: 191 con hasta 3 modificaciones de aminoácidos relacionadas con SEQ ID No: 191, en donde el péptido presenta actividad agonista en el receptor de GIP humano y el receptor de GLP-1 humano.

85. Un péptido que comprende una secuencia de SEQ ID No: 192 o una secuencia de SEQ ID No: 192 con hasta 3 modificaciones de aminoácidos relacionadas con SEQ ID No: 192, en donde el péptido presenta actividad agonista en el receptor de GIP humano y el receptor de GLP-1 humano y, opcionalmente, en donde el péptido presenta actividad agonista en el receptor de glucagón humano.

86. Un péptido que comprende una secuencia de SEQ ID No: 193 o una secuencia de SEQ ID No: 193 con hasta 3 modificaciones de aminoácidos relacionadas con SEQ ID No: 193, en donde el péptido presenta actividad agonista en el receptor de GIP humano y el receptor de GLP-1 humano y, opcionalmente, en donde el péptido presenta actividad agonista en el receptor de glucagón humano.

87. Un péptido que comprende una secuencia de SEQ ID No: 194 o una secuencia de SEQ ID No: 194 con hasta 3 modificaciones de aminoácidos relacionadas con SEQ ID No: 194, en donde el péptido presenta actividad agonista en el receptor de GIP humano y el receptor de GLP-1 humano y, opcionalmente, en donde el péptido presenta actividad agonista en el receptor de glucagón humano.

88. Un péptido que comprende una secuencia de SEQ ID No: 195 o una secuencia de SEQ ID No: 195 con hasta 3 modificaciones de aminoácidos relacionadas con SEQ ID No: 195, en donde el

péptido presenta actividad agonista en el receptor de GIP humano y el receptor de GLP-1 humano y, opcionalmente, en donde el péptido presenta actividad agonista en el receptor de glucagón humano.

89. El péptido de acuerdo con cualquiera de las reivindicaciones 52 a 88, en donde el grupo alquilo o acilo graso de C12-C18 es un grupo alquilo o acilo graso de C14-C16, opcionalmente unido mediante un separador.

90. El péptido de acuerdo con cualquiera de las reivindicaciones 52 a 89, en donde el aminoácido unido de forma covalente a un grupo alquilo o acilo de C12 a C18 es un Lys o un amino-Phe o Tyr o Cys unido de forma covalente a un grupo alquilo o acilo de C12 a C 18.

91. El péptido de acuerdo con cualquiera de las reivindicaciones 52 a 90, en donde el separador es un aminoácido o un dipéptido, opcionalmente, en donde el separador comprende uno o dos residuos de aminoácido acídico.

92. Un péptido que comprende la secuencia de SEQ ID NO: 28.

93. Un péptido que comprende la secuencia de SEQ ID NO: 37.

94. Un péptido que comprende la secuencia de SEQ ID NO: 89.

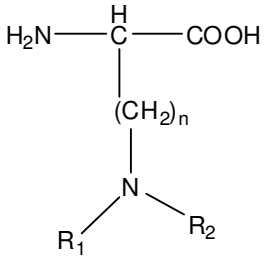
95. Un péptido que comprende la secuencia de SEQ ID NO: 171.

96. Un péptido que comprende la secuencia de SEQ ID NO: 180.

97. Un análogo de glucagón (SEQ ID NO: 1) que tiene actividad agonista de GIP y comprende:

(a) un aminoácido que comprende una cadena lateral de imidazola en posición 1,

(b) en posición 16, un aminoácido de Fórmula IV:



[Fórmula IV],

en donde n es 1 a 7, en donde R1 y R2 se selecciona de forma independiente del grupo que consiste en H, C1–C18 alquilo, (C1–C18 alquilo)OH, (C1–C18 alquilo)NH2, (C1–C18 alquilo)SH, (C0–C4 alquilo)(C3–C6)cicloalquilo, (C0–C4 alquilo)(C2–C5 heterocíclico), (C0–C4 alquilo)(C6–C10 arilo)R7 y (C1–C4 alquilo)(C3–C9 heteroarilo), en donde R7 es H o OH, en donde opcionalmente la cadena lateral del aminoácido de Fpormula IV comprende un grupo amino libre,

(c) un aminoácido α,α-disustituido amino acid en posición 20,

(d) hasta 10 modificaciones de aminoácido adicionales relacionadas con SEQ ID NO:1,

en donde, cuando el análogo carece de una partícula hidrofílica, el análogo de glucagón presenta una actividad de al menos 0,1% de GIP nativo en el receptor de GIP, en donde el análogo de glucagón tiene una selectividad 100 veces menor para el receptor de GLP-1 humano en comparación con el receptor de GIP.

98. El análogo de glucagón de acuerdo con la reivindicación 97, en donde el aminoácido en posición 1 es His o un derivado de His.

99. El análogo de glucagón de acuerdo con las reivindicaciones 97 o 98, en donde el aminoácido de Fórmula IV en (b) es homoLys, Lys, Orn o ácido 2,4-diaminobutírico (Dab).

100. El análogo de glucagón de acuerdo con las reivindicaciones 97 a 99, en donde el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde R1 y R2 son seleccionados de forma independiente del grupo que consiste en C1-C4 alquilo, opcionalmente sustituido con hidroxilo, amida, tiol, halo o R1 y R2 junto con el carbono alfa al cual está unido forman un anillo.

101. El análogo de glucagón de acuerdo con la reivindicación 100, en donde el aminoácido α,α-disustituido en posición 20 es AIB.

102. El análogo de glucagón de acuerdo con cualquiera de las reivindicaciones 97 a 101, que comprenden uno o más de:

a. un aminoácido protector DPP-IV en posición 2, opcionalmente AIB o D-Ser,

b. un aminoácido no-polar, alifático, grande, en posición 12, opcionalmente Ile,

c. un aminoácido distinto a Arg en posición 17, opcionalmente Gln,

d. un aminoácido alifático pequeño en posición 18, opcionalmente Ala,

e. un aminoácido distinto a Asp en posición 21, opcionalmente Glu,

f. un aminoácido distinto a Gln en posición 24, opcionalmente Asn o Ala,

g. un aminoácido distinto a Met en posición 27, opcionalmente, Leu,

h. un aminoácido distinto a Asn en posición 28, opcionalmente Ala,

i. un aminoácido distinto de Thr en posición 29, opcionalmente Gly,

j. una extensión de 1 a 21 aminoácidos de extremo C hasta el amino ácido en posición 29 y

k. la secuencia de aminoácido establecida como cualquiera de las SEQ ID Nos: 48, 52, 53 y 74.

103. Un análogo que comprende una secuencia madre con un total de hasta 3 modificaciones relacionadas con la secuencia madre, en donde la secuencia madre es cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124 y 127-131 o cualquiera de las SEQ ID NOs: 48, 52, 53 y 74 o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126 y 133, en donde las modificaciones de aminoácidos se seleccionan del grupo que consiste en:

a. aminoácido protector DPP-IV en posición 2, opcionalmente AIB o D-Ser,

b. un aminoácido no-polar, alifático, grande, en posición 12, opcionalmente Ile,

c. un aminoácido distinto de Arg en posición 17, opcionalmente Gln,

d. un aminoácido alifático pequeño en posición 18, opcionalmente Ala,

e. un aminoácido distinto de Asp en posición 21, opcionalmente Glu,

f. un aminoácido distinto de Gln en posición 24, opcionalmente Asn o Ala,

g. un aminoácido distinto de Met en posición 27, opcionalmente Leu,

h. un aminoácido distinto de Asn en posición 28, opcionalmente Ala,

i. un aminoácido distinto de Thr en posición 29, opcionalmente Gly,

j. una extensión de 1 a 21 aminoácidos de extremo C hasta el aminoácido en posición 29, y

k. la secuencia de aminoácido establecida en cualquiera de las SEQ ID Nos 48, 52, 53 y 74.

104. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el análogo de glucagón tiene una selectividad 100 veces menor para el receptor de GLP-1 humano en comparación con el receptor de GIP.

105. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el EC50 del análogo de glucagón en el receptor de GIP se

encuentra entre aproximadamente 50 veces de su EC50 en el receptor de GLP-1.

106. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el EC50 del análogo de glucagón en el receptor de GIP se encuentra entre aproximadamente 40 veces de su EC50 en el receptor de GLP-1.

107. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el EC50 del análogo de glucagón en el receptor de GIP se encuentra entre aproximadamente 30 veces de su EC50 en el receptor de GLP-1.

108. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el EC50 del análogo de glucagón en el receptor de GIP se encuentra entre aproximadamente 20 veces de su EC50 en el receptor de GLP-1.

109. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el EC50 del análogo de glucagón en el receptor de GIP se encuentra entre aproximadamente 10 veces de su EC50 en el receptor de GLP-1.

110. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el EC50 del análogo de glucagón en el receptor de GIP se encuentra entre aproximadamente 5 veces de su EC50 en el receptor de GLP-1.

111. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones precedentes, en donde el análogo de glucagón presenta un porcentaje de actividad de GIP de al menor 1%.

112. El análogo de glucagón de acuerdo con la reivindicación 111, en donde el porcentaje de potencia de GIP es del menos 10%.

113. El análogo de glucagón de acuerdo con la reivindicación 112, en donde el porcentaje de potencia de GIP es más del 15% o más del 20%.

114. El análogo de glucagón de acuerdo con la reivindicación 113, en donde el porcentaje de potencia de GIP es del menos 50%.

115. El análogo de glucagón de acuerdo con la reivindicación 114, en donde el porcentaje de potencia de GIP es mayor o aproximadamente del 100%.

116. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones anteriores, en donde el

análogo de glucagón presenta un porcentaje de potencia de GLP-1 de al menos 1%.

117. El análogo de glucagón o péptido de acuerdo con la reivindicación 116, en donde el porcentaje de potencia de GLP-1 es al menos 10%.

118. El análogo de glucagón o péptido de acuerdo con la reivindicación 117, en donde el porcentaje de potencia de GLP-1 es al menos 50%.

119. El análogo de glucagón o péptido de acuerdo con la reivindicación 118, en donde el porcentaje de potencia de GLP-1 es mayor o aproximadamente del 100%.

120. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones anteriores, en donde el porcentaje de potencia de GIP es 10 veces mayor o menor que el porcentaje de potencia de GLP-1.

121. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones anteriores en donde el porcentaje de potencia es al menos 1%.

122. El análogo de glucagón o péptido de acuerdo con la reivindicación 121, en donde el porcentaje de potencia de glucagón es al menos 10%.

123. El análogo de glucagón o péptido de acuerdo con la reivindicación 122, en donde el porcentaje de potencia de glucagón es al menos 50%.

124. El análogo de glucagón o péptido de acuerdo con la reivindicación 123, en donde el porcentaje de potencia de glucagón es mayor o aproximadamente 100%.

125. El análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones anteriores, en donde el porcentaje de potencia de GIP es 10 veces mayor o menor que el porcentaje de potencia del glucagón.

126. Un dímero o multidímero que comprende uno o más análogos de glucagón o péptidos de acuerdo con cualquiera de las reivindicaciones anteriores.

127. Un conjugado que comprende dos o más análogos de glucagón o péptidos de acuerdo con cualquiera de las reivindicaciones anteriores.

128. El conjugado de acuerdo con la reivindicación 127, en donde el análogo de glucagón está fusionado con un análogo de péptido heterólogo.

129. Un composición farmacéutica que comprende un análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones anteriores, un dímero o multidímero de acuerdo con la reivindicación 126, un conjugado de acuerdo con

la reivindicación 127 o 128 o una combinación de ellos y un excipiente, diluyente o transportador farmacéuticamente aceptable.

130. Un método para reducir el aumento de peso o inducir la pérdida de peso a un sujeto que lo necesite, que comprende la administración al paciente de una composición farmacéutica de acuerdo con la reivindicación 129 en una cantidad efectiva para reducir el aumento de peso o inducir la pérdida de peso.

131. El método de acuerdo con la reivindicación 130, en donde la cantidad efectiva para el tratamiento de la obesidad en un sujeto que la necesite.

132. Un método para el tratamiento de la diabetes que comprende la administración a un paciente de una composición farmacéutica de acuerdo con la reivindicación 129 en una cantidad efectiva para reducir los niveles de glucosa en sangre.

133. Un método para inducir parálisis temporaria del tracto intestinal, que comprende la administración a un paciente de una composición farmacéutica de acuerdo con la reivindicación 129 en una cantidad efectiva para inducir la parálisis temporaria del tracto instestinal.

134. El uso de un análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones anteriores, un dímero o

multidímero de acuerdo con la reivindicación 126, un conjugado de acuerdo con las reivindicaciones 127 o 128 o una combinación de ellas, en la fabricación de un medicamento para tratar la diabetes, reducir el aumento de peso o inducir la pérdida de peso o inducir parálisis temporaria del tracto intestinal.

135. El uso de un análogo de glucagón o péptido de acuerdo con cualquiera de las reivindicaciones anteriores, un dímero o multidímero de acuerdo con la reivindicación 126, un conjugado de acuerdo con las reivindicaciones 127 o 128 o una combinación de ellos para tratar la diabetes u obesidad, reducir el aumento de peso o inducir la pérdida de peso o inducir la parálisis temporaria del tracto intestinal.

136. Un péptido, opcionalmente un análogo de glucagón, según lo descripto en el presente.

RESUMEN

La presente invención proporciona análogos de glucagón que prestan actividad potente en el receptor de GIP y, como tal, son contemplados para el uso en el tratamiento de diabetes y obesidad. En realizaciones ejemplificadoras, el análogo de glucagón de la presenten invención muestran un EC50 en el receptor de GIP, que se encuentra en el rango nanomolar o picomolar.

TRATADO DE COOPERACIÓN EN MATERIA DE PATENTES

De la AUTORIDAD DE BÚSQUEDA INTERNACIONAL

Para:	**PCT** **NOTIFICACIÓN DE REMISIÓN DEL INFORME DE BÚSQUEDA INTERNACIONAL Y DE LA OPINIÓN ESCRITA DE LA AUTORIDAD DE BÚSQUEDA INTERNACIONAL, O LA DECLARACIÓN** **(Norma 44.1 del PCT)**
Breen, John P. BARNES & THORNBURG, LLP 11 South Meridian Street Indianapolis IN 46204 Estados Unidos de América	Fecha de remisión (día/mes/año) 26 de de julio de 2012 (26-07-2012
Referencia de archivo del Solicitante o Agente 32992-219935	**PARA OTROS TRAMITES** Véanse los párrafo 1 y 4 a continuación
Solicitud de Patente Internacional Nº PCT/US2011/066164	Fecha de presentación internacional (día/mes/año): 20 de diciembre de 2011
Solicitante: INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY…	

1. [x] Por la presente se notifica al solicitante que el informe de búsqueda internacional y la opinión escrita de la Autoridad de Búsqueda Internacional se han establecido y se transmiten con la presente:

Presentación de modificaciones y testimonio conforme al Artículo 19.

El solicitante tiene derecho, si lo desea, de modificar las reivindicaciones de la solicitud de patente internacional (véase la Norma 46):

¿Cuándo? El plazo para presentar dichas modificaciones es

normalmente de 2 meses desde la fecha de transmisión del Informe de Búsqueda Internacional.

¿Dónde? Directamente a la Oficina Internacional de la WIPO, 34, chemin de Colombettes, 1211 Ginebra 20, Suiza, Fax: (41-22)338-8270

Para obtener instrucciones más detalladas, véanse las notas en la página adjunta.

2. [] Por la presente se notifica al solicitante que no se establecerá informe de búsqueda internacional y que la declaración conforme al Artículo 17(2)(a) a tal efecto y la opinión escrita de la Autoridad de Búsqueda Internacional se transmiten con la presente.

3. [] Con respecto la protesta contra el pago de (un) arancel(es) adicionales conforme a la Norma 40.2, se notifica al solicitante que:

[] la protesta así como la resolución sobre ella ha sido transmitida a la Oficina Internacional junto con el pedido del solicitante de remitir los textos de la protesta y de la resolución sobre ella a las Oficinas designadas.

[] aún no ha habido resolución sobre la protesta; el solicitante será notificado en cuanto haya una resolución.

4. **Recordatorios**:

Poco después de los **18 meses** desde la fecha de prioridad, la Oficina Internacional publicará la solicitud de patente internacional. Si el solicitante desea impedir o postergar la publicación, un escrito de retiro de la solicitud de patente internacional, o de la reivindicación de prioridad, deberán ser presentados ante la Oficina Internacional según lo previsto en las Normas 90bis.1 y 90bis.3, respectivamente, antes de la finalización de los preparativos técnicos para la publicación internacional.

El solicitante podrá presentar ante la Oficina Internacional observaciones en forma informal acerca de la opinión escrita de la Autoridad de Búsqueda Internacional. La Oficina Internacional remitirá una copia de esas observaciones a todas las oficinas designadas a menos que se haya establecido o se deba establecer un informe del examen preliminar internacional. Estas observaciones también estarían disponibles para el público pero no con anterioridad al vencimiento del plazo de 30 meses des de la fecha de prioridad.

Dentro de los **19 meses** desde la fecha de prioridad, deberá presentarse un pedido de examen preliminar internacional si el solicitante desea postergar el ingreso en la fase nacional hasta los 30 meses desde la fecha de prioridad (en algunas Oficinas más); de lo contrario el solicitante deberá, dentro

de los 20 meses, realizar los trámites previstos para el ingreso en la fase nacional ante aquellas Oficinas designadas. Con respecto a las demás Oficinas designadas, el plazo de 30 meses (o con posterioridad) será aplicable aún cuando no se presente ningún pedido dentro de los 19 meses. Véase el Anexo al Formulario PCT/IB/301 y, para detalles acerca de los plazos aplicables, Oficina por Oficina, véase la Guía del Solicitante de PCT, Volumen II, Sedes Nacionales y el sitio de Internet de la WIPO.

Nombre y domicilio para correspondencia de la ISA/ Oficina de Patentes Europea, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Funcionario Autorizado: BUSSHOFF, Johannes Tel: +49 (0)89 2399-2897 OSP de PCT: 571-272-7774 Nº Telefónico

TRATADO DE COOPERACIÓN EN MATERIA DE PATENTES

PCT

INFORME DE BÚSQUEDA INTERNACIONAL

(Artículo 18 y Normas 43 y 44 del PCT)

<table>
<tr><td>Referencia de Archivo del Solicitante o agente:
32993-219935</td><td colspan="2">PARA REALIZAR OTROS TRAMITES
Véase Notificación de Transmisión de Informe de Búsqueda Internacional (Formulario PCT/ISA/220) y, si fuere aplicable, del punto 5 a continuación.</td></tr>
<tr><td>Solicitud Internacional No:
PCT/US2011/066164</td><td>Fecha de Presentación Internacional (día/mes/año): 20-12-2011</td><td>Fecha de Prioridad (Más antigua) (día/mes/año): 22-12-2010</td></tr>
<tr><td colspan="3">Solicitante:
INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY…</td></tr>
<tr><td colspan="3">Este informe de búsqueda internacional ha sido preparado por esta Autoridad de Búsqueda Internacional y es transmitido al solicitante conforme al Artículo 18. Se transmite una copia del mismo a la Oficina Internacional.

Este informe de búsqueda internacional consta de _10_ páginas.
[X] Está acompañado asimismo de una copia de cada documento de</td></tr>
</table>

arte previo citado en el informe

1. Fundamento de Informe

a. Con respecto al idioma, la búsqueda internacional se llevó a cabo basada en:

[x] la solicitud internacional en el idioma en que ésta se presentó.

[ ] una traducción de la solicitud internacional al _____, que es el idioma de la traducción provista para los fines de la búsqueda internacional (Normas 12.3(a) y 23.1(b)).

b. [] Este informe de búsqueda internacional se ha establecido tomando en cuenta la rectificación de un error obvio autorizada o notificada por a esta Autoridad conforme a la Norma 91 (Norma 43.6bis(a)).

c. [x] Con respecto a cualquier secuencia de nucleótidos y/o aminoácidos revelada en la solicitud internacional, véase el Casillero Nº I

2. [] Ciertas reivindicaciones se consideraron no buscables (Véase Casillero Nº II)

3. [x] Falta Unidad de Invención (Véase Casillero Nº III).

4. Con respecto al título:

[x] el texto se aprueba tal como lo presentó el solicitante

[] el texto ha sido establecido por esta Autoridad como sigue:

5. Con respecto al resumen:

[x] el texto es aprobado como lo presentó el solicitante

[] el texto ha sido establecido, conforme a la Norma 38.2(b), por esta Autoridad como aparece en el Casillero N° IV. El Solicitante puede, dentro de un mes desde la fecha de remisión de este informe de búsqueda internacional, presentar comentarios ante esta Autoridad.

6. Con respecto a los dibujos,

a. La Lámina de dibujos que se publicará con el resumen es: Figura No. 8.

[x] sugerida por el Solicitante

[] seleccionada por esta Autoridad, debido a que el Solicitante no sugirió una Figura.

[] seleccionada por esta Autoridad, debido a que ésta es la figura que mejor caracteriza a la invención

b. [] No se debe publicar ninguna de las figuras con el resumen.

INFORME DE BÚSQUEDA INTERNACIONAL	Solicitud de Patente Internacional N° PCT/EP2011/066164

Casillero Nº I. Secuencia de Nucleótidos y/o aminoácidos (Continuación del Punto 1.c de la página 1)

1. Con respecto a cualquier secuencia de nucleótidos y/o aminoácidos revelada en la solicitud internacional y necesaria para la invención reivindicada, la búsqueda internacional se llevó a cabo basado en:

(a) medios:

[ ] papel

[x] forma electrónica

(b) (tiempo)

[x] en la solicitud de patente internacional como se presentó

[] junto con la solicitud de patente internacional en forma electrónica

[] posteriormente ante esta Autoridad para los fines de la búsqueda

2. [] Además, en caso de que se haya presentado o provisto más de una versión o copia de un listado de secuencias y/o tabla relacionada con él, se prestaron las declaraciones juradas

exigidas de que la información de las copias posteriores o adicionales es idéntica a aquella de la solicitud de patente como se presentó o no va más allá de la solicitud de patente como se presentó, según corresponda.

3. Observaciones adicionales:

INFORME DE BÚSQUEDA INTERNACIONAL	Solicitud de Patente Internacional N° PCT/EP2011/006000

Casillero Nº II. Observaciones cuando ciertas reivindicaciones se consideraron no buscables (Continuación del punto 2 de la página 1).

El presente informe de búsqueda internacional no se ha establecido con respecto a ciertas reivindicaciones conforme a al Artículo 17(2)(a) por los siguientes motivos:

1. [] Reivindicaciones N°:

porque se relacionan con un objeto que no se requiere que busque esta Autoridad, a saber:

2. [] Reivindicaciones N°:

porque se relacionan con partes de la solicitud internacional que no cumplen con los requisitos establecidos en tal magnitud que no se puede realizar una búsqueda significativa, específicamente:

3. [] Reivindicaciones N°:

porque son reivindicaciones dependientes y no están redactadas de acuerdo con la segunda y tercera oraciones de la Norma 6.4(a).

Casillero Nº III. Observaciones cuando falta unidad de invención (Continuación del punto 3 de la página 1)

Esta Autoridad de Búsqueda Internacional encontró varias invenciones en esta solicitud de patente internacional, a saber:

Véase la página adicional

1. [] Como todos los aranceles de búsqueda adicionales se pagaron oportunamente por el solicitante, este informe de búsqueda internacional abarca todas las reivindicaciones buscables.

2. [] Como las reivindicaciones buscables pudieron buscarse sin esfuerzo que justifique un arancel adicional, esta Autoridad no intima al pago de ningún arancel adicional.

3. [x] Como ninguno de los aranceles de búsqueda adicionales requeridos se pagaron oportunamente por el solicitante, este informe de búsqueda internacional abarca solamente aquellas reivindicaciones para las cuales se pagaron aranceles, específicamente las reivindicaciones Nº:

1–13, 52–61, 92, 94 (completamente); 104–135 (parcialmente)

4. [] El solicitante no pagó ninguno de los aranceles de búsqueda adicionales. En consecuencia, este informe de búsqueda internacional se limita a la invención mencionada en primer lugar en las reivindicaciones; está abarcada por las reivindicaciones Nº:

Observación Bajo Protesta:

[] Los aranceles de búsqueda adicionales estuvieron acompañados por la protesta del solicitante y cuando correspondía por el arancel de protesta

[] Los aranceles de búsqueda adicionales estuvieron acompañados por la protesta del solicitante pero el arancel de protesta aplicable no se pagó dentro del plazo especificado en la invención.

[x] Ninguna protesta acompañó el pago de los aranceles de búsqueda adicionales.

Formulario PCT/ISA/210 (continuación de la página 1 (2))(abril de 2005)

INFORME DE BÚSQUEDA INTERNACIONAL	Solicitud Internacional N° PCT/US2011/066164

A. Clasificación del Objeto:

INV. A61K38/26 C07K14/605

ADD.

De acuerdo con la Clasificación Internacional de Patentes (IPC) o con la clasificación nacional e IPC.

B. CAMPOS DE BÚSQUEDA

Documentación mínima buscada (sistema de clasificación seguido por símbolos de clasificación)

A61K C07K

Documentación buscada además de la documentación mínima en la medida en que tales documentos están incluidos en los campos de búsqueda:

Base de datos electrónica consultada durante la búsqueda internacional (nombre de la base de datos y, si es posible, términos de búsqueda usados):

EPO-Internal, Búsqueda de Secuencias, WPI Data, BIOSIS

C. Documentos Considerados Relevantes		
Categoría*	Cita de documento, indicando, si corresponde, los pasajes relevantes	Relevante para la reivindicación N°
X	WO 2009/155257 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; SMILEY DAV) 23 de diciembre de 2009 (23-12-2009) Invención 1 página 6, línea 10 - página 9, línea 5 página 113; tabla 8 reivindicaciones 1-96	1-6, 14-51, 104-136
[x] Se mencionan otros documentos en la continuación del Casillero C. [x] Véase el anexo de familia de patentes		
* Categorías especiales de documentos citados: "A": documento que define el estado general del arte que no se considera de particular relevancia "E": documento anterior pero	"T": documento posterior publicado con posterioridad a la fecha de presentación internacional o a la fecha de prioridad y que no está en conflicto con la solicitud pero citado para comprender un	

publicado en la fecha de presentación internacional o con posterioridad a ella. "L": documento que puede arrojar dudas sobre la reivindicación de prioridad o que se cita para establecer la fecha de publicación de otra cita o por otra razón especial (especificada). "O": documento que refiere a una revelación oral, uso, exhibición u otro medio. "P": documento publicado antes de la fecha de presentación internacional pero con posterioridad a la fecha de prioridad reivindicada.	principio o teoría que sustenta la invención. "X": documento de particular relevancia: la invención reivindicada no puede considerarse nueva o no puede considerarse que implica un paso inventivo cuando el documento se toma solo. "Y": documento de particular relevancia: la invención reivindicada no puede considerarse que implica un paso inventivo cuando el documento se combina con otro u otros documentos, siendo dicha combinación obvia para un experto en el arte. "&": documento miembro de la misma familia de patentes.
Fecha de la finalización efectiva de la búsqueda internacional 17 de julio de 2012 (17-07-	Fecha de envío del informe de búsqueda internacional 26/07/2012

2012)	
Nombre y domicilio para correspondencia de la ISA/ Oficina de Patentes Europea, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040 Fax: (+31–70) 340–3016	Funcionario Autorizado Niebuhr–Ebel, K.

Formulario PCT/ISA/210 (página 2) (julio de 2009)

INFORME DE BÚSQUEDA INTERNACIONAL	Solicitud Internacional N° PCT/US2011/066164

C. Documentos Considerados Relevantes

Categoría*	Cita de documento, indicando, si corresponde, los pasajes relevantes	Relevante para la reivindicación N°
X	PAN CLARK Q ET AL: "Diseño de un péptido de acción prolongada que funciona como un agoonista de receptor de péptido similar a glucagón 1 y también como un antagonista de receptor de glucagón",	1–6, 14–51, 104–136

	DIARIO DE QUIMICA BIOLÓGICA, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US, volumen 281, Nº 18, 1 de mayo de 2006 (1-05-2006), páginas 12506-12515, XP002529523, ISSN: 0021-9258, DOI: 10.1074/JBC.M600127200 [recuperado el 27-02-2006] resumen página 12506, párrafo 3 - página 12507, párrafo 3 página 12508; tabla 1 página 12511; tabla 3 página 12513, párrafo 2 - párrafo 3	
X	WO 2010/071807 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; K0U BINBIN) 24 de junio de 2010 (24-06-2010) Invención 2 + 3 página 49, línea 27 - página 51, línea 6 página 59, línea 8 - página 60,	7-61, 104-136

	línea 22 página 66, línea 15 – página 67, línea 5	
X	PENGYUN LI ET AL: "Diseño, síntesis y cristalización de un análogo de glucagón como un agente terapéutico", ACTA CRYSTALLOGRAPHICA SECCIÓN F COMUNICACIONES DE BIOLOGÍA ESTRUCTURAL Y CRISTALIZACIÓN, volumen 63, N° 7, 1 de julio de 2007 (1-07-2007), páginas 599-601, XP55010072, ISSN: 1744-3091, D0I: 10.1107/S1744309107028655 Invención 3 resumen página 600; figura 1	52-61, 104-136
A	WO 2008/101017 A2 (INDIANA UNVERSITY RES AND TECH [US]; DAY JONATHAN [US]; PATTERSON JAME) 21 de agosto de 2008 (21-08-2008)	92, 94, 104-135

	Invención 15 + 17 reivindicación 65 secuencia 495 **-/--**	

INFORME DE BÚSQUEDA INTERNACIONAL	Solicitud Internacional N° PCT/US2011/066164

C. Documentos Considerados Relevantes

Categoría*	Cita de documento, indicando, si corresponde, los pasajes relevantes	Relevante para la reivindicación N°
A	-& DATABASE Geneseq [En línea] 27 de noviembre de 2008 (27-11-2008), "Péptido de GLP-1 humano modificado que presenta una potencia mejorada SEQ ID:495." XP002679991, recuperado de EBI acceso N° GSP:ATD14223 Base de Datos acceso N° ATD14223 secuencia	92, 94, 104-136
A	CHABENNE JOSEPH R ET AL:	92, 94,

	"Optimización de la secuencia de glucagón nativo para fines médicos.", DIARIO DE CIENCIA Y TECNOLOGÍA DE DIABETES NOV 2010 LNKD-PUBMED: 21129326, volumen 4, N° 6, noviembre de 2010 (11-2010), páginas 1322-1331, XP007920819, ISSN: 1932-2968 resumen página 1327, columna derecha, párrafo 2 - página 1328, columna derecha, párrafo 1 figura 5 página 1330, columna derecha, párrafo 2 tabla 1	104-136

INFORME DE BÚSQUEDA INTERNACIONAL	Solicitud Internacional N° PCT/US2011/066164

Documento de patente citado en el informe de búsqueda	Fecha de publicación	Miembro(s) de la familia de patentes	Fecha de publicación
WO 2009155257 Al	23-12-2009	AU 2009260301 Al	23-12-2009
		CA 2727161 Al	23-12-2009
		CN 102088989 A	08-06-2011
		EP 2307037 Al	13-04-2011
		JP 2011524418 A	01-09-2011
		KR 20110039230 A	15-04-2011
		TW 201010730 A	16-03-2010
		US 2011190200 Al	04-08-2011
		WO 2009155257 Al	23-12-2009
WO 2010071807 Al	24-06-2010	AR 074811 Al	16-02-2011
		AU 2009327418 Al	24-06-2010
		CA 2747499 Al	24-06-2010
		CN 102325539 A	18-01-2012
		EP 2376097 Al	19-10-2011
		KR 20110110174 A	06-10-2011
		PE 03322012 Al	14-04-2012
		SG 172291 Al	28-07-2011
		TW 201028162 A	01-08-2010
		US 2011288003 Al	24-11-2011
		WO 2010071807 Al	24-06-2010
W0 2008101017 A2	21-08-2008	AR 065349 Al	03-06-2009
		AR 080904 A2	16-05-2012
		AU 2008216265 Al	21-08-2008
		BR PI08O7728 A2	17-04-2012
		CA 2677932 Al	21-08-2008
		CL 4792008 Al	22-08-2008
		CN 101790538 A	28-07-2010
		CR 11028 A	29-10-2009
		DO P2009000194 A	30-06-2010
		EA 200901108 Al	26-02-2010
		EC SP099622 A	30-10-2009
		EP 2111414 A2	28-10-2009
		JP 2011511753 A	14-04-2011
		KR 20090119876 A	20-11-2009
		MA 31242 Bl	01-03-2010
		NZ 578948 A	30-03-2012
		PA 8769301 Al	18-12-2008
		PE 01082009 Al	15-02-2009
		TW 200848423 A	16-12-2008

US 2010190701 A1	29-07-2010
WO 2008101017 A2	21-08-2008

Formulario PCT/ISA/210 (anexo de familia de patentes) (Abril de 2005)

Solicitud de Patente Internacional N° PCT/US2011/066164

OTRA INFORMACIÓN CONTINUACIÓN DE PCT/ISA/210

Esta Autoridad de Búsqueda Internacional encontró varios (grupos de) invenciones en esta solicitud de patente internacional, a saber:

1. reivindicaciones: 1-6 (completamente); 14-51, 104-136 (parcialmente)

Un análogo de glucagón (SEQ ID NO: 1) caracterizado porque comprende: a. un aminoácido que comprende una cadena lateral de imidazola en posición 1; b. un aminoácido protector de DPP-IV en posición 2, opcionalmente, AIB; c. un aminoácido acilado o un aminoácido alquilado en cualquiera de las posiciones 9, 10, 12, 16 0 37-43, opcionalmente en donde el grupo acilo o alquilo se unen al aminoácido mediante un separador; d. un aminoácido alfa-alfa-disustituido en posición 20; y e. hasta 10 modificaciones de aminoácidos adicionales relacionadas con la SEQ ID NO: 1; en donde, cuando el análogo de glucagón carece de una partícula hidrofílica, el análogo de glucagón presenta un porcentaje de potencia de GIP de, al menos,

0,1%, en donde el análogo de glucagón tiene una selectividad 100 veces menor para el receptor GLP-1 humano en comparación con el receptor GIP.

2. reivindicaciones: 7-3 (completamente); 14-51, 104-136 (parcialmente)

como objeto, excepto d = un aminoácido que estabiliza la alfa hélice en una o más de las posiciones 16-21, opcionalmente la posición 16, en donde el análogo no comprende un aminoácido que estabiliza la alfa hélice en la posición 20.

3. reivindicaciones: 52-61 (completamente; 104-136 (parcialmente)

Un péptido que comprende la secuencia de $HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$ (SEQ ID NO: 184), en donde el péptido presenta actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano

4. reivindicaciones: 62, 63 (completamente); 104-136 (parcialmente)

Un péptido que comprende la secuencia de SEQ ID NO 185, en donde el péptido presenta actividad de agonista en

cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

5. reivindicaciones 64-78 (completamente); 104-136 (parcialmente)

Un péptido que comprende la secuencia de SEQ ID NO 186, en donde el péptido presenta actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

6. reivindicaciones 79, 80 (completamente); 104-136 (parcialmente)

Un péptido que comprende la secuencia de SEQ ID NO 187, en donde el péptido presenta actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

7-14. reivindicaciones 81-88 (completamente); reivindicaciones 104-136 (parcialmente)

Un péptido que comprende la secuencia de SEQ ID NO 188-195, en donde el péptido presenta actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

15-19. reivindicaciones: 92-96 (completamente); 104-135 (parcialmente)

Un péptido que comprende una de las secuencias de SEQ ID NO 28, 37, 89, 171, 180

20. reivindicaciones: 97-102 (completamente); 104-135 (parcialmente)

Un análogo de glucagón (SEQ ID NO: 1) que tiene actividad agonista de GIP y comprende: (a) un aminoácido que comprende una cadena lateral de imidazola en posición 1, (b) en posición 16, un aminoácido de Fórmula IV, en donde n es 1 a 7, en donde R1 y R2 se selecciona de forma independiente del grupo que consiste en H, C1-C18 alquilo, (C1-C18 alquilo)OH, (C1-C18 alquilo)NH2, (C1-C18 alquilo)SH, (C0-C4 alquilo)(C3-C6)cicloalquilo, (C0-C4 alquilo)(C2-C5 heterocíclico), (C0-C4 alquilo)(C6-C10 arilo)R7 y (C1-C4 alquilo)(C3-C9 heteroarilo), en donde R7 es H o OH, en donde opcionalmente la cadena lateral del aminoácido de Fpormula IV comprende un grupo amino libre, (c) un aminoácido α,α-disustituido amino acid en posición 20, (d) hasta 10 modificaciones de aminoácido adicionales relacionadas con SEQ ID NO:1,

en donde, cuando el análogo carece de una partícula hidrofílica, el análogo de glucagón presenta una actividad de al menos 0,1% de GIP nativo en el receptor de GIP, en donde el

análogo de glucagón tiene una selectividad 100 veces menor para el receptor de GLP-1 humano en comparación con el receptor de GIP.
21-109. reivindicaciones: 103 (completamente); 104-136 (parcialmente) Un análogo que comprende una secuencia madre con un total de hasta 3 modificaciones relacionadas con la secuencia madre, en donde la secuencia madre es cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124 y 127-131 o cualquiera de las SEQ ID NOs: 48, 52, 53 y 74 o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126 y 133, en donde las modificaciones de aminoácidos se seleccionan del grupo que consiste en varias modificaciones.

TRATADO DE COOPERACIÓN EN MATERIA DE PATENTES

De la

AUTORIDAD DE BÚSQUEDA INTERNACIONAL

Para: Véase el formulario PCT/ISA/220	**PCT** **OPINIÓN ESCRITA DE LA AUTORIDAD DE BÚSQUEDA INTERNACIONAL** **(Norma 43bis.1 del PCT)**
	Fecha de remisión (día/mes/año) Véase el formulario PCT/ISA/210 (página 2)
Referencia de archivo del Solicitante o Agente Véase el formulario PCT/ISA/220	**PARA OTROS TRAMITES** Véase el párrafo 2 a continuación

Solicitud Internacional No.	Fecha de Presentación internacional (día, mes, año)	Fecha de prioridad (día/mes/año)
PCT/US2011/066164	20-12-2011	22-12-2010

Clasificación Internacional de Patente (IPC) o tanto clasificación nacional como IPC INV. A61K38/26 C07K14/605
Solicitante: INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY…

1. Esta opinión escrita contiene indicaciones relacionadas con los siguientes puntos:

[x] Casillero Nº I. Fundamento del informe

[] Casillero Nº II. Prioridad

[X] Casillero Nº III. No establecimiento de opinión sobre la novedad, actividad inventiva y aplicabilidad industrial

[X] Casillero Nº IV. Falta de unidad de invención

[x] Casillero Nº V. Declaración fundamentada conforme al Artículo 35(2) sobre la novedad, actividad inventiva o aplicabilidad industrial; citas y explicaciones que fundamentan dicha declaración.

[] Casillero Nº VI. Ciertos documentos citados

[] Casillero Nº VII. Ciertos defectos en la solicitud de patente internacional

[x] Casillero Nº VIII. Ciertas Observaciones sobre la solicitud de patente internacional

2. OTRAS MEDIDAS

Si se presenta un pedido de informe preliminar internacional, esta opinión se considerará como la opinión escrita de la Autoridad de Examen de Búsqueda Internacional (“IPEA”) excepto que ésta no corresponda cuando el solicitante elige una Autoridad diferente de ésta para que sea la IPEA y la IPEA elegida ha notificado a la Oficina Internacional conforme a la

Norma 66.1bis(b) que la opinión escrita de esta Autoridad de Búsqueda Internacional no se considerará así. Si esta opinión, proporcionada anteriormente, se considera la opinión escrita de la IPEA, se invita al solicitante a presentar ante la IPEA una contestación escrita junto con, cuando corresponda, enmiendas, con anterioridad al vencimiento de tres meses desde la fecha de la remisión del Formulario PCT/ISA/220 o con anterioridad al vencimiento de 22 meses desde la fecha de la prioridad, la que sea posterior. Para más opciones, véase el Formulario PCT/ISA/220. 3. Para más detalles, véase las notas al Formulario PCT/ISA/220

Nombre y domicilio para correspondencia de la ISA/US: Oficina de Patentes Europea D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399-4465	Fecha de finalización de esta opinión Véase el formulario PCT/ISA/210	Funcionario autorizado Niebuhr-Ebel, K Nº Telefónico +49 89 2399-0 [sello] Oficina de Patentes Europea

OPINIÓN ESCRITA DE LA AUTORIDAD DE BÚSQUEDA INTERNACIONAL	Solicitud de Patente Internacional N° PCT/US2011/066164

Casillero Nº I. Fundamento de esta opinión.

1. Con respecto al **idioma**, esta opinión se ha establecido basada en:

[x] la solicitud de patente internacional en el idioma en que se presentó.

[] una traducción de la solicitud de patente internacional al _____, que es el idioma de la traducción provista para los propósitos de la búsqueda internacional (Normas 12.3 y 23.1(b)).

2. [] Esta opinión se ha establecido tomando en cuenta la **rectificación de un error evidente** autorizada o notificada por esta autoridad conforme a la Norma 91 (Norma 43bis.1(a)).

3. Con respecto a todas **las secuencias de nucleótidos y/o aminoácidos** que se revelan en la solicitud de patente internacional, esta opinión se ha establecido basado en un listado de secuencias presentado o provisto:

a. (medio)

[] en papel

[x] una forma electrónica

b. (tiempo)

[x] dentro de la solicitud de patente internacional presentada

[] junto con la solicitud de patente internacional en forma electrónica

[] con posterioridad ante esta Autoridad con propósitos de búsqueda.

4. [] Además, en caso de que se haya presentado o provisto más de una versión o copia de un listado de secuencias, se prestaron las declaraciones requeridas de que la información de las copias posteriores o adicionales es idéntica a aquella de la solicitud de patente presentada o no va más allá de la solicitud de patente presentada, según corresponda.

5. Comentarios adicionales

OPINIÓN ESCRITA DE LA AUTORIDAD DE BÚSQUEDA INTERNACIONAL	Solicitud de Patente Internacional N° PCT/US 11/37752

Casillero Nº III. No establecimiento de opinión sobre la novedad, paso inventivo y aplicabilidad industrial

Las cuestiones sobre si la invención reivindicada parece novedosa, involucra un paso inventivo (no es obvia) o tiene aplicabilidad industrial no se han examinado con respecto a:

[] la solicitud de patente internacional completa

[x] las reivindicaciones N° 62-91, 93, 95-103, 136 (completamente); 104-136 (parcialmente)

porque:

[] la solicitud de patente internacional, o las reivindicaciones mencionadas N° _____ se relacionan con el siguiente objeto que no requiere una búsqueda internacional *(especifique)*:

[] la memoria descriptiva, las reivindicaciones o los dibujos *(indique los elementos particulares a continuación)* o las reivindicaciones mencionadas N° _____ son tan poco claras

que no se pudo formar ninguna opinión sensata *(especifique)*:

[] las reivindicaciones, o las reivindicaciones mencionadas N° _____ están tan insuficientemente respaldadas por la memoria descriptiva que no se pudo formar ninguna opinión sensata *(especifique)*:

[x] no se ha establecido ningún informe de búsqueda internacional para la solicitud de patente internacional completa ni para las reivindicaciones mencionadas N° 62-91, 93, 95-103, 136 (completamente); 104-135 (parcialmente)

[] no se pudo formar ninguna opinión sensata sin el listado de secuencias, el solicitante, dentro del plazo establecido, no:

[] proporcionó un listado de secuencias en papel que cumpla con la norma provista en el Anexo C de las Instrucciones Administrativas, y dicho listado no estaba disponible para la Autoridad de Búsqueda Internacional en una forma y manera aceptable para ella.

[] proporcionó un listado de secuencias en forma electrónica que cumpla con la norma provista en el Anexo C de las Instrucciones Administrativas, y dicho listado no estuvo disponible para la Autoridad de Búsqueda Internacional en una forma y manera aceptable para ella.

[] pagó el arancel de presentación tardía establecido para la presentación de un listado de secuencias en respuesta a una invitación conforme a las Normas 13ter.1(a) o (b).

[x] Véase el Casillero Suplementario para ver más detalles.

OPINIÓN ESCRITA DE LA AUTORIDAD DE BÚSQUEDA INTERNACIONAL Solicitud de Patente Internacional N° PCT/US2011/066164

Casillero Nº IV. Falta de unidad de invención

1. [x] En respuesta a la invitación (Formulario PCT/ISA/206) para pagar aranceles adicionales, el solicitante, dentro del plazo aplicable, ha:

[x] pagado aranceles adicionales;

[] pagado aranceles adicionales bajo protesta y, cuando corresponde, el arancel de protesta.

[] pagado aranceles adicionales bajo protesta pero no se pagó el arancel de protesta aplicable

[] no pagó aranceles adicionales

2. [] La Autoridad consideró que el requisito de unidad de invención no se cumple y no elige invitar al solicitante a pagar los aranceles adicionales

3. Esta Autoridad considera que el requisito de unidad de invención de acuerdo con la Norma 13.1, 13.2 y 13.3

[] se cumple

[x] no se cumple por los siguientes motivos:

Véase la página por separado

4. En consecuencia, este informe se ha establecido con

respecto a las siguientes partes de la solicitud de patente internacional

]] todas las partes

[x] las partes relacionadas con las reivindicaciones Nº 1-13, 52-61, 92, 94 (completamente); 104-135 (parcialmente)

Casillero Nº V. Declaración fundamentada conforme a la Cláusula 35(2) sobre la novedad, actividad inventiva o aplicabilidad industrial; citas y explicaciones que fundamentan dicha declaración.

1. Declaración

Novedad (N) Reivindicaciones 92, 94 SÍ

Reivindicaciones 1-61 (completamente); 104-135 (parcialmente NO

Actividad Inventiva (IS) Sí: Reivindicaciones: 92, 94

No: Reivindicaciones 1-61 (completamente); 104-135 (parcialmente) NO

Aplicabilidad industrial (IA) Sí: Reivindicaciones 1-61, 92, 94 (completamente); 104-135 (parcialmente)

No: Reivindicaciones:

2. Citas y explicaciones:

véase la página por separado

OPINIÓN ESCRITA DE LA AUTORIDAD DE BÚSQUEDA INTERNACIONAL	Solicitud de Patente Internacional N° PCT/US2011/066164

Casillero Nº VIII. Ciertas observaciones sobre la solicitud de patente internacional

Se efectúan las siguientes observaciones sobre la claridad de las reivindicaciones, la memoria descriptiva, y los dibujos o sobre la cuestión de si están completamente respaldadas por la memoria descriptiva:

Véase la página por separado

OPINIÓN ESCRITA DE LA AUTORIDAD DE BÚSQUEDA INTERNACIONAL (PÁGINA POR SEPARADO) Solicitud de Patente Internacional N° PCT/US2011/066164

Con referencia al Punto III

No establecimiento de opinión sobre la novedad, actividad inventiva y aplicabilidad industrial

1. Las reivindicaciones 130-133 y 135 se relacionan con un objeto que esta Autoridad considera que está cubierto por las disposiciones de la **Norma 39.1(iv) / 67.1(iv) del PCT.**

Para la evaluación de dichas reivindicaciones sobre la cuestión de si el objeto es patentable, no existe un criterio unificado en los Estados Firmantes del PCT. La patentabilidad puede depender de la formulación de las reivindicaciones. Si bien las reivindicaciones 130-133 y 135 se refieren a un método de tratamiento del cuerpo humano/animal o al uso de un compuesto en un tratamiento médico, la búsqueda y el examen se han llevado a cabo y se han basado en los supuestos efectos del compuesto/composición.

Con referencia al Punto IV

Falta de unidad de invención

2. La presente solicitud de patente no cumple con los requisitos de unidad de invención.

Hasta ahora, se han identificado 109 invenciones por separado. Cada una de ellas se caracteriza por una "característica técnica especial" individual, no hay una interacción técnica entre estas invenciones (véase a continuación). En consecuencia se solicita a los solicitantes que abonen aranceles adicionales.

3. En vista de la invención de la presente solicitud de patente, el problema técnico que se ha de resolver es la provisión de análogos de glucagón novedosos.

El arte previo disponible revela el concepto común y las soluciones a él. El concepto de unión en consecuencia no es ni novedoso ni inventivo y no puede proveer unidad de invención. el arte previo muestra las siguientes características técnicas.

- D1 (WO2009/155257, del solicitante) revela numerosos análogos de glucagón que retienen la actividad del receptor de glucagón y presentan solubilidad mejorada en relación con el péptido de glucagón nativo. El documento incluye un listado de secuencias de 115 análogos y las modificaciones adicionales incluyen acilación/alqulación, agregado de aminoácidos de

imidazol o [alfa] , [alfa] disustituidos y modificaciones de aminoácidos.

- D2 (Pan et al, 2006) revela una variedad de péptidos híbridos compuestos por glucagón y GLP-1 o exendina 4 que funcionan como un receptor de péptido 1 similar a glucagón y un antagonista de receptor de glucagón.

La solución en su forma general reivindicaa al problema planteado en la presente solicitud de patente en consecuencia no es novedosa.

4. En consecuencia no hay ninguna característica técnica especial común para el alcance completo de la presente solicitud de patente que defina un aporte apreciable (por ejemplo, novedoso y/o no trivial) por encima del arte previo. En vista del arte previo, cada variante reivindicada se considera una invención por separado.

5. Con eferencia a las invenciones 1, 2 y 3, cada una de las respectivas reivindicaciones independientes (1, 7 y 52) abarca innumerables análogos de glucagón. En el caso de un nuevo tratamiento de dichas invenciones se solicita al Solicitante que señale el supuesto concepto que unifica las invenciones

para evitar un rechazo por falta de unidad en una etapa posterior.

Con referencia al punto V

Declaración fundamentada conforme al Artículo 35(2) sobre la novedad, actividad inventiva o aplicabilidad industrial; citas y explicaciones que fundamentan dicha declaración.

La presente solicitud de patente se relaciona con análogos de glucagón. Después de un rechazo por falta de unidad, se abonaron cuatro aranceles de búsqueda adicionales. Se llevó a cabo una búsqueda para las invenciones 1–3, 15 y 17, que son el objeto de la presente opinión escrita.

Invención 1 (reivindicaciones 1–6 (completamente) y 14–51 y 104–136 (parcialmente):

Un análogo de glucagón que comprende un aminoácido que comprende una cadena lateral de imidazol en posición 1, un aminoácido protector de DPP–IV en posición 2, un aminoácido acilado o un aminoácido alquilado en cualquiera de las posiciones 9, 10, 12, 16 o 37–43, un aminoácido alfa–alfa–disustituido en posición 20, y hasta 10 modificaciones de aminoácidos adicionales en relación con el glucagón no modificado.

6. Se hace referencia a los siguientes documentos:

D1 WO 2009/155257 A1 (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; SMILEY DAV) 23 de diciembre de 2009 (23-12-2009)

D2 PAN CLARK Q ET AL: "Diseño de un péptido de acción prolongada que funciona como un agoonista de receptor de péptido similar a glucagón 1 y también como un antagonista de receptor de glucagón", DIARIO DE QUIMICA BIOLÓGICA, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US, volumen 281, Nº 18, 1 de mayo de 2006 (1-05-2006), páginas 12506-12515, XP002529523, ISSN: 0021-9258, DOI: 10.1074/JBC.M600127200 [recuperado el 27-02-2006].

7. Novedad (Art. 33 (2) del PCT)

7.1. **D1** (WO2009/155257) revela análogos de glucagón que retienen la actividad de receptor de glucagón y presentan solubilidad mejorada en relación con el péptido de glucagón. Estas modificaciones de glucagón incluyen acilación/alquilación, agregado de imidazol o aminoácidos [alfa], [alfa]-disustituidosy modificaciones de aminoácidos.

D2 (Pan et al, 1006) revela péptidos híbridos compuestos por glucagón y GLP-1 o exendina 4 que funcionan como un agonista

de receptor de péptido 1 similar a glucagón así como un antagonista de receptor de glucagón.

7.2. Las modificaciones que caracterizan al análogo de glucagón de la reivindicación 1 ya se revelan en D1 (p. 5, línea 14 – p. 9, línea 5). En consecuencia, el objeto de la reivindicación independiente 1 y de las reivindicaciones dependientes 2-6 (completamente) y 14-51 y 104-136 (parcialmente) no se consideran novedosos en el sentido del **Art. 33(2) del PCT.**

8. Actividad Inventiva (Art. 33(3) del PCT)

8.1. Aún cuando se pudiera establecer la novedad para las reivindicaciones, no se las consideraría inventivas por el siguiente motivo:

En vista de la invención de la presente solicitud de patente, el problema técnico que se ha de resolver es la provisión de análogos de glucagón novedosos. La reivindicación 1 comprende innumerables análogos de glucagón, pero a partir de los ejemplos no es obvio que alguno de dichos péptidos tenga alguna propiedad inesperada o ventajosa. Además, es cuestionable si todos los péptidos que están dentro el alcance de dicha reivindicación resuelven el problema planteado, es

decir tienen actividad de glucagón. En consecuencia, no se puede reconocer ninguna actividad inventiva y la reivindicación independiente 1 y las reivindicaciones dependientes 2-6 (completamente) y 14-51 y 104-136 (parcialmente) no se consideran inventivas en el sentido del **Art. 33(3) del PCT.**

Invención 2 (reivindicaciones 7-13 (completamente) y 14-51 y 104-136 (parcialmente):

Como la invención 1, excepto que contiene una hélice alfa que estabiliza el aminoácido en una o más de las posiciones 16-21 opcionalmente, la posición 16, en donde el análogo no comprende una alfa hélice que estabiliza el aminoácido en la posición 20.

9. Se hace referencia al siguiente documento:

D3 WO2010/071807 Al (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; K0U BINBIN) 24 de junio de 2010 (24-06-2010)

10. Novedad (Art. 33(2) del PCT)

10.1. **D3** (WO2010/071807) revela numerosos análogos de glucagón que tienen semividas extendidas. Las modificaciones de

glucagón incluyen acilación/alquilación, agregado de imidazol o aminoácidos [alfa], [alfa]-disustituidos, alfa hélice que estabiliza los aminoácidos y modificaciones de aminoácidos.

10.2 Las modificaciones que caracterizan a los análogos de glucagón de la reivindicación 7 ya se revelan en D3. En consecuencia, el objeto de las reivindicaciones 7-51 y 104-136 no se considera novedoso en el sentido el **Art. 33(2) del PCT.**

11. Actividad Inventiva (Art. 33(3) del PCT)

11.1. Aún cuando se pudiera establecer la novedad para las reivindicaciones, no se las consideraría inventivas por el siguiente motivo:

En vista de la invención de la presente solicitud de patente, el problema técnico que se ha de resolver es la provisión de análogos de glucagón novedosos. La reivindicación 7 comprende innumerables análogos de glucagón, pero a partir de los ejemplos no es obvio que alguno de dichos péptidos tenga alguna propiedad inesperada o ventajosa. Además, es cuestionable si todos los péptidos que están dentro del alcance de dicha reivindicación resuelven el problema planteado, es decir, tienen actividad de glucagón. En consecuencia, no se puede reconocer ninguna actividad

inventiva y las reivindicaciones 7-51 y 104-136 no se consideran inventivas en el sentido del **Art. 33(3) del PCT.**

Invención 3 (reivindicaciones 52-61 (completamente) y 104-136 (parcialmente):

Un péptido que comprende la secuencia de
HXXGTFTSDX SKYLDXRXAX XFVQWLXXXG PSSGXPPPS
(SEQ ID NO: 184) en donde el péptido presenta actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

12. Se hace referencia a los siguientes documentos:
D3 WO2010/071807 Al (UNIV INDIANA RES & TECH CORP [US]; DIMARCHI RICHARD D [US]; K0U BINBIN) 24 de junio de 2010 (24-06-2010)
D4 PENGYUN LI ET AL: "Diseño, síntesis y cristalización de un análogo de glucagón como un agente terapéutico", ACTA CRYSTALLOGRAPHICA SECCIÓN F COMUNICACIONES DE BIOLOGÍA ESTRUCTURAL Y CRISTALIZACIÓN, volumen 63, N° 7, 1 de julio de 2007 (1-07-2007), páginas 599-601, XP55010072, ISSN: 1744-3091, D0I: 10.1107/S1744309107028655

13. Novedad (Art. 33(2) del PCT)

13.1. El péptido representado por SEQ ID NO 184 contiene posiciones variables (X), a saber las posiciones 2, 3, 10, 16, 18, 20, 21, 27, 28, 29 y 35, por lo cual el residuo de tipo silvestre es aquel de las opciones en todas las posiciones, excepto 2, 28 y 29. En consecuencia, los péptidos reivindicados difieren del glucagón de tipo silvestre por lo menos en los siguientes aspectos: contiene ácido alfa-aminoisobutírico (AIB) en la posición 2, A o G en la posición 28 y A, R o K en la posición 29 y una extensión del extremo de C adicional GPSSGAPPPS.

13.2. **D4** describe la extensión del extremo de C basada en exendina del glucagón (GPSSGAPPPS), que "presenta solubilidad biofísica y estabilidad muy mejorada" (p. 600, columna izquierda, primer párrafo).

D3 describe que las "modificaciones en la posición 1 o 2 pueden aumentar la resistencia del péptido a la descomposición por dipeptidil peptidasa IV (DPP IV). Por ejemplo, el aminoácido en la posición 2 se puede sustituir con … aminoácido isobutírico" (p. 85, l. 1-4). Comenzando en la p. 86, l. 5 (Pasaje: Se enumeran "otras modificaciones y combinaciones") innumerables modificaciones posibles: "Por ejemplo, se pueden hacer sustituciones conservadoras o no conservadoras, agregados o eliminaciones en cualquiera de las

posiciones 2, 5, 7, 10, 11, 12, 13, 14, 17, 18, 19, 20, 21, 24, 27, 28 o 29". En la p. 88 de D3, se menciona una sustitución en la posición 28 con Glu y una sustitución de Thr en la posición 29 con un aminoácido con carga seleccionado del grupo formado por Lys y Arg. En consecuencia, se considera que las variantes reveladas en D3 están dentro del alcance de las reivindicaciones 52-61 y 104-136 y en consecuencia no se consideran novedosas en el sentido de **Art. 33 (2) PCT.**

14. Actividad Inventiva (Art. 33(3) del PCT)

14.1 Aún cuando se pudiera establecer la novedad para las reivindicaciones, no se las consideraría inventivas por el siguiente motivo:

En vista de la invención de la presente solicitud de patente, el problema técnico que se ha de resolver es la provisión de análogos de glucagón novedosos. La reivindicación 7 comprende innumerables análogos de glucagón, pero a partir de los ejemplos no es obvio que alguno de dichos péptidos tenga alguna propiedad inesperada o ventajosa. Además, es cuestionable si todos los péptidos que están dentro del alcance de dicha reivindicación resuelven el problema planteado, es decir, tienen actividad de glucagón. En consecuencia, no se puede reconocer ninguna actividad

inventiva y las reivindicaciones 7-51 y 104-136 no se consideran inventivas en el sentido del **Art. 33(3) del PCT.**

Invención 15 (reivindicación 92 (completamente) y 104-136 (parcialmente):

Un péptido que comprende SEQ ID NO 28:
HXQGTFTSDK SKYLDERAAQ DFVQWLLDGG PSSGAPPPS
en donde X en la posición 2 es ácido alfa-aminoisobutírico (AIB)

15. Se hace referencia a los siguientes documentos:
D5 WO 2008/101017 A2 (INDIANA UNVERSITY RES AND TECH [US]; DAY JONATHAN [US]; PATTERSON JAME) 21 de agosto de 2008 (21-08-2008)
-& DATABASE Geneseq [En línea]
27 de noviembre de 2008 (27-11-2008), "Péptido de GLP-1 humano modificado que presenta una potencia mejorada SEQ ID:495.", recuperado de EBI acceso N° GSP:ATD14223, Base de Datos acceso N° ATD14223

D6 CHABENNE JOSEPH R ET AL: "Optimización de la secuencia de glucagón nativo para fines médicos.", DIARIO DE CIENCIA Y TECNOLOGÍA DE DIABETES NOV 2010 LNKD- PUBMED: 21129326,

volumen 4, Nº 6, noviembre de 2010 (11-2010), páginas 1322-1331, XP007920819, ISSN: 1932-2968

16. Novedad (Art. 33(2) del PCT)

16.1. El péptido representado por SEQ ID NO 28 difiere del glucagón de tipo silvestre en los siguientes aspectos: contiene AIB en la posición 2, las sustituciones Y10K, S16E, R18A, M27L, N28D, T29G y la extensión del extremo de C GPSSGAPPPS.

16.2. **D5** (WO2008/101017) revela análogos de glucagón que funcionan como co-agonistas de receptor de glucagón/GLP-1. **D5** revela numerosas modificaciones de péptidos tales como AIB en la posición 2 y la extensión del extremo de C GPSSGAPPPS, pero SEQ ID NO: 28 difiere del péptido con la homología más alta, es decir #495 de **D5**, en cuatro residuos, a saber en las posiciones 10, 20, 27 y 28 y una cisteína del extremo de C.

16.3. **D6** revela la "optimización" de la secuencia de glucagón nativo introduciendo N28D o la extensión del extremo de GPSSGAPPPS.

16.4. La secuencia precisa de SEQ ID NO 28 no se revela en el arte previo, y en consecuencia, el objeto de la reivindicación

92 y sus reivindicaciones dependientes se considera novedoso en el sentido del **Art. 33(2) del PCT.**

17. Actividad Inventiva (Art. 33 (3) del PCT)

17.1. En vista del arte previo, el problema que se ha de resolver se puede formular como la provisión de análogos de glucagón alternativos. La solución propuesta es el péptido representado por SEQ ID NO 28.

17.2. Los Ejemplos de la solicitud de patente muestran que el péptido tiene propiedades ventajosas cuando se lo administra a ratones tales como la disminución el peso corporal (Fig. 1, 4, 5E y 10) y de los niveles de glucosa en sangre (Fig. 5F). En consecuencia, se reconoce actividad inventiva en el sentido del **Art. 33 (3) del PCT.**

Invención 17 (reivindicación 94 (completamente) y 104-136 (parcialmente):

Un péptido que comprende SEQ ID NO 89:
HXQGTFTSDY SKYLDERAAQ DFVQWLLDGG PSSGAPPPSK G
en donde X en la posición 2 es ácido alfa-aminoisobutírico (AIB).

18. Novedad (Art. 33(2) del PCT)

18.1. El péptido representado por SEQ ID NO SEQ ID NO: 89 difiere del glucagón de tipo silvestre en los siguientes aspectos: contiene AIB en la posición 2, las sustituciones S16E, R18A, M27L, N28D, T29G y la extensión del extremo de C GPSSGAPPPSKG.

18.2. **D3** (WO2008/101017) revela análogos de glucagón que funcionan como co-agonistas de receptor de glucagón/GLP-1. **D3** revela numerosas modificaciones de péptidos tales como AIB en la posición 2 y la extensión del extremo de C GPSSGAPPPS, pero SEQ ID NO: 89 difiere del péptido con la homología más alta es decir #495 de **D3**, en cuatro residuos, a saber en las posiciones 20, 27 y 28 y un extremo de C KG en lugar de una cisteína.

18.3. La secuencia precisa de SEQ ID NO: 89 no se revela en el arte previo y en consecuencia el objeto de la reivindicación 94 y sus reivindicaciones dependientes se consideran novedosas en el sentido del **Art. 33 (2) del PCT.**

19. Actividad inventiva (Art. 33(3) del PCT)

19.1. En vista del arte previo, el problema que se ha de resolver se puede formular como la provisión de análogos de glucagón alternativos. La solución propuesta es el péptido representado por SEQ ID NO 89.

19.2. Los Ejemplos de la solicitud de patente muestran que el péptido tiene propiedades ventajosas cuando se lo administra a ratones, tales como la disminución del peso corporal (Fig. 4, 5E, 8 y 10) y de los niveles de glucosa en sangre (Fig. 5F). En consecuencia, se reconoce una actividad inventiva en el sentido del **Art. 33(3) del PCT**.

Con referencia al Punto VIII

Ciertas Observaciones sobre la solicitud de patente internacional

20. Las reivindicaciones independientes 1, 7 y 52 no cumplen con los requisitos del **Artículo 6 del PCT**. Las características “en donde, cuando el análogo de glucagón carece de una partícula hidrofílica, el análogo de glucagón presenta un porcentaje de potencia de GIP de, al menos, 0,1%, en donde el análogo de glucagón tiene una selectividad 100 veces menor para el receptor GLP-1 humano en comparación con el receptor GIP” o “en donde el péptido presenta actividad agonista en el receptor de GIP humano, el receptor de GLP-1 y el receptor de

glucagón humano" son, de hecho, una simple citación del resultado que se ha de alcanzar. Por regla general, no se admiten las reivindicaciones que intentan definir la invención mediante el resultado que se ha de alcanzar.

21. Más aún, además de manifestaciones vagas y declaraciones no fundamentadas, la presente solicitud de patente no muestra ninguna actividad relacionada con los péptidos reivindicados en las reivindicaciones independientes 1, 7 y 52. En consecuencia, las reivindicaciones no están respaldadas por la memoria descriptiva (**Art. 6 del PCT**). En este contexto, el Solicitante debe ser consciente de que el alcance de una reivindicación debe reflejar el **aporte técnico real** de la solicitud de patente. Las manifestaciones o afirmaciones vagas que no tienen contenido técnico alguno no brindan ningún fundamento **(Art. 6 del PCT**, véase también **Pautas del PCT 5.44).**

ANÁLOGOS DE GLUCAGÓN QUE PRESENTAN ACTIVIDAD DE RECEPTOR DE GIP

Referencia Cruzada a Solicitudes de Patente de Referencia

Esta solicitud de patente reivindica prioridad de la Solicitud de Patente Provisoria Estadounidense N° 61/426.285, presentada el 22 de diciembre de 2010, y de la Solicitud de Patente provisoria Estadounidense N° 61/514.609, presentada el 3 de agosto de 2001, ambas solicitudes de patente se incorporan como referencia en su totalidad en la presente solicitud de patente.

Incorporación como referencia de Material presentado en forma electrónica

Se incorpora en su totalidad como referencia un listado de secuencias de nucleótidos/aminoácidos legible por computadora presentado simultáneamente con la presente y que se identifica a continuación: Archivo ACII (Texto) de 193 kilobytes con el nombre “DOCS-1756766-v1-45700A_SeqListing.txt”, creado el 16 de diciembre de 2011.

Antecedentes de la invención

Según los datos más recientes de la Planilla de Hechos Nacional de la Diabetes de la Asociación Estadounidense de Diabetes, 23,6 millones de niños y adultos de Estados Unidos están afectados por la diabetes. Cada año, se diagnostican 1,6 millones de casos nuevos de diabetes en personas de 20 años o mayores. Según un estudio publicado recientemente en el *Diario de la Asociación Médica Estadounidense*, más de dos tercios de los adultos de Estados Unidos tienen sobrepeso o son obesos (Flegal et al, *JAMA*303(3): 235-241 (2010)) y más de un tercio de esta población es obesa.

Las hormonas incretinas, el péptido 1 similar al glucagón (GLP-1) y el péptido insulinotrópico dependiente de la glucosa (GIP), son hormonas de péptido natural. Tanto el GLP-1 como el GIP estimulan la síntesis de la insulina y su secreción en una forma dependiente de la glucosa y no producen hipoglucemia (véanse, por ejemplo, Nauck et al., J. Clin. Endocrinol. Metab. 76:912-917 (1993) e Irwin et al., Regul. Pept. 153:70-76 (2009)).

Se ha demostrado que el GLP-1 es eficaz como terapia adyuvante para la diabetes y está asociado a la pérdida de peso. Lo que queda sin aclarar sobre la terapia dirigida al GIP, sin embargo, es si el tratamiento exitoso de la diabetes y la obesidad se logra antagonizando los efectos de esta hormona

(por ejemplo, a través del antagonismo del receptor de GIP) o a través de la imitación o el mejoramiento de los efectos del GIP.

Resumen de la invención

En la presente se proporcionan péptidos que son péptidos de agonistas de GIP contemplados para su uso en el tratamiento de sujetos que lo necesitan, por ejemplo, que tiene diabetes y obesidad.

El glucagón nativo no activa el receptor de GIP y normalmente tiene un 1% de la actividad del GLP-1 nativo en el receptor de GLP-1. En algunas realizaciones, los péptidos son análogos de glucagón que comprenden una estructura basada en la secuencia de aminoácidos del glucagón humano nativo (SEQ ID NO 1) pero que difieren en una o más posiciones comparada con SEQ ID NO 1, en donde las diferencias, o las modificaciones, aumentan la actividad de agonista del análogo en el receptor de GIP. Dichos análogos de glucagón tienen actividad de agonista en el receptor de GIP mayor que aquella del glucagón nativo y, en algunos aspectos, mayor que aquella del GIP nativo. En algunas o en todas las realizaciones, el agonista de GIP tiene un porcentaje de potencia del GIP de por lo menos el 0,1%. En algunos o en todos los aspectos de la presente invención, el

análogo de glucagón además presenta actividad de agonista en uno o ambos del receptor de glucagón y el receptor de GLP-1. Por consiguiente, en la presente se proporcionan agonistas de GIP, los co-agonistas de GIP y de GLP-1, los co-agonistas de GIP y de glucagón y los triagonistas de GIP, de GLP-1 y de glucagón.

En ejemplos de realizaciones, la selectividad del péptido de agonista de GIP de la presente invención para el receptor de GLP-1 humano contra el receptor de GIP es menor de 100 veces. En algunas o en todas las realizaciones, el péptido de agonista de GIP tiene un porcentaje de potencia del GIP dentro de 20 veces o 10 veces diferente (más alta o más baja) del porcentaje de potencia de glucagón y/o del porcentaje de potencia de GLP-1.

En algunas realizaciones de la presente invención, los análogos de glucagón comprenden (i) un aminoácido que comprende una cadena lateral de imidazol en la posición 1, (ii) un aminoácido protector de DPP-IV en la posición 2, (iii) un aminoácido que comprende un grupo acilo o alquilo no nativo opcionalmente en cualquiera de las posiciones 9, 10, 12, 16, 20 o 37-41 y opcionalmente en donde el grupo acilo o alquilo no nativo está unido a dicho aminoácido a través de un separador; (iv) un aminoácido estabilizador de la hélice alfa

en una o más de las posiciones 16, 17, 18, 19, 20 o 21 y (v) hasta diez (por ejemplo, hasta 1, 2, 3, 4, 5, 6, 7, 8 o 9) modificaciones de aminoácidos adicionales en relación con SEQ ID NO 1. En ejemplos de realizaciones, el análogo de glucagón comprende (i) un aminoácido que comprende una cadena lateral de aminoácido en la posición 1, (ii) un aminoácido protector de DPP-IV en la posición 2, opcionalmente ácido aminoisobutírico, (iii) un aminoácido que comprende un grupo acilo o alquilo no nativo, opcionalmente en cualquiera de las posiciones 9, 10, 12, 16, 20, o 37-41, opcionalmente en donde el grupo acilo o alquilo no nativo está unido a dicho aminoácido a través de un separador, (iv) un aminoácido alfa disustituido en la posición 20 y (v) hasta diez (por ejemplo, hasta 1, 2, 3, 4, 5, 6, 7, 8 o 9) modificaciones de aminoácidos adicionales en relación con la SEQ ID NO 1. En ejemplos alternativos de realizaciones, el análogo de glucagón comprende (i) un aminoácido que comprende una cadena lateral de imidazol en la posición 1, (ii) un aminoácido protector de DPP-IV en la posición 2, opcionalmente ácido aminoisobutírico, (iii) un aminoácido que comprende un grupo acilo o alquilo, opcionalmente en cualquiera de las posiciones 9, 10, 12, 16, 20 o 37-43, opcionalmente en donde el grupo acilo o alquilo no nativo está unido a dicho aminoácido a través de un separador, (iv) un aminoácido de estabilidad de la hélice alfa en una o más de las posiciones 16-21, opcionalmente, la posición 16, en

donde el análogo no comprende un aminoácido de estabilización de la hélice alfa en la posición 20 y (v) hasta diez (por ejemplo, hasta 1, 2, 3, 4, 5, 6, 7, 8 o 9) modificaciones de aminoácidos adicionales en relación con la SEQ ID NO 1. En ejemplos de realizaciones, cuando el análogo de glucagón carece de un grupo hidrófilo, el análogo de glucagón presenta un porcentaje de potencia de GIP de por lo menos el 0,1% (por ejemplo, por lo menos el 1%, por lo menos el 10%, por lo menos el 20%). En ejemplos de realizaciones, el análogo de glucagón tiene menos de 100 veces (por ejemplo, menos de 50 veces, menos de 25 veces, menos de 10 veces) la selectividad para el receptor de GLP-1 humano contra el receptor de GIP. En ejemplos de realizaciones, el análogo de glucagón presenta una EC_{50} en el receptor de GLP-1 que está dentro de 100 veces (por ejemplo, dentro de 50 veces, dentro de 25 veces, dentro de 10 veces) de su EC_{50} en el receptor de GIP.

En toda la solicitud de patente, todas las referencias a una posición de aminoácido particular por el número (por ejemplo, la posición 28) se refieren al aminoácido en esa posición en el glucagón nativo (SEQ ID NO 1) o la posición de aminoácido correspondiente en cualquier análogo de él. Por ejemplo, una referencia en la presente a “la posición 28” significa la posición 27 correspondiente para un análogo de glucagón en donde el primer aminoácido de SEQ ID NO 1 se ha eliminado. En

forma similar, una referencia en la presente a la “posición 28” significa la posición 29 correspondiente para un análogo de glucagón en donde se ha agregado un aminoácido antes del extremo de N de SEQ ID NO 1.

En algunas realizaciones, los péptidos de agonistas de GIP comprenden una secuencia de aminoácidos de cualquiera de SEQ ID NO: 27–33, 35–41, 43–46, 76–80, 83–87, 89, y 90, o cualquiera de SEQ ID NO: 48, 52, 53, y 74, o cualquiera de SEQ ID NO: 50, 51, 54, 56, 58–60, 62–66, 68–70, 72, 73, 75, 81, 82, 88, y 92. En algunas realizaciones, los péptidos de agonistas de GIP comprenden una estructura basada en una secuencia parental que comprende cualquiera de SEQ ID NOs: 27–33, 35–41, 43–46, 76–80, 83–87, 89, y 90, o cualquiera de SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de SEQ ID NOs: 50, 51, 54, 56, 58–60, 62–66, 68–70, 72, 73, 75, 81, 82, 88, y 92, pero difiere de la secuencia parental en una o más posiciones, como se describe también en la presente.

La invención por consiguiente proporciona un péptido que comprende, consiste esencialmente en, o consiste en la secuencia de SEQ ID NO 28. También se proporciona un péptido que comprende, consiste esencialmente en o consiste en la secuencia de SEQ ID NO 37. La invención también proporciona un péptido que comprende, que consiste esencialmente en o

consiste en la secuencia de SEQ ID NO 89. La invención además proporciona un péptido que comprende, que consiste esencialmente en o que consiste en la secuencia de SEQ ID NO 180. La invención además proporciona un péptido que comprende, consiste esencialmente en o consiste en la secuencia de SEQ ID NO 31.

La invención proporciona un péptido que comprende la secuencia de SEQ ID NO 184,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEQ ID NO: 184)

en donde:

X_2 es AIB;

X_3 es Gln o análogo de Gln;

X_{10} es Tyr o un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{12} a C_{18};

X_{16} es cualquier aminoácido, opcionalmente, cualquier aminoácido diferente de Gly, Pro, y Ser;

X_{18} es Arg o Ala;

X_{20} es un aminoácido con carga negativa o un aminoácido con carga neutra, opcionalmente, AIB o Gln;

X_{21} es un aminoácido ácido, opcionalmente, Asp o Glu;

X_{27} es Leu, Ala, Nle, o Met;

X_{28} es Ala o un aminoácido ácido (opcionalmente, Asp o Glu);

X_{29} es Ala o Gly;

X_{35} es Ala o un aminoácido básico (opcionalmente, Arg o Lys);

en donde, cuando X_{28} es aminoácido ácido, X_{35} es un aminoácido básico;

en donde, cuando X_{10} es Tyr, el péptido comprende en la posición 40 un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{12} a C_{18}, y, en donde, opcionalmente, el péptido comprende Gly en la posición 41, y

en donde el aminoácido del extremo de C del péptido se amida.

La invención también proporciona un péptido que comprende la secuencia de SEQ ID NO 184 con hasta 3 modificaciones de aminoácidos en relación con la SEQ ID NO 184, en donde el análogo presenta actividad de agonista de cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

La invención además proporciona un péptido que comprende la secuencia de SEQ ID NO 185,

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGAPPPS$

(SEQ ID NO: 185)

en donde:

X_2 es AIB;

X_{10} es Tyr o un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{12} a C_{18};

X_{16} es Glu, un ácido alfa, alfa disustituido, Lys o

X_{18} es Arg o Ala;

X_{20} es AIB o Gln;

X_{21} es Asp o Glu;

X_{27} es Leu, Nle, o Met;

X_{28} es Ala, Asp o Glu;

X_{29} es Gly o Thr;

y

en donde, cuando X_{10} es Tyr, el péptido comprende al menos en la posición 40 un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{12} a C_{18} y, en donde, opcionalmente, el péptido comprende Gly en la posición 41, y

en donde el aminoácido del extremo de C del péptido está amidado.

La invención también proporciona un péptido que comprende la secuencia de SEQ ID NO 185 con hasta 3 modificaciones de aminoácidos en relación con SEQ ID NO 185, en donde el análogo presenta la actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

Además se proporciona un péptido que comprende la secuencia de SEQ ID NO 186:

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SEQ ID NO: 186)

en donde:

X_2 es AIB;

X_3 es Gln o análogo de Gln;

X_{10} es Tyr o un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{10} a C_{26};

X_{16} es cualquier aminoácido, opcionalmente, cualquier aminoácido diferente de Gly, Pro, y Ser;

X_{18} es Arg o Ala;

X_{20} es un aminoácido con carga negativa o un aminoácido con carga neutra, opcionalmente AIB o Gln;

X_{21} es X_{21} es un aminoácido ácido, opcionalmente Asp o Glu;

X_{27} es Leu, Ala, Nle, o Met;

X_{28} es Ala o un aminoácido ácido (opcionalmente, Asp o Glu);

X_{29} es Ala, Gly o Thr; y

en donde el péptido comprende un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{10} a C_{26}, opcionalmente, en la posición 10, y el aminoácido de extremo de C del péptido está amidado.

Además, se proporciona un péptido que comprende la secuencia de SEQ ID NO 186 con hasta 3 modificaciones de aminoácidos en relación con SEQ ID NO 186, en donde el análogo presenta una actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

La invención proporciona un péptido que comprende la secuencia de SEQ ID NO 187:

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SEQ ID NO: 187)

en donde:

X_2 es AIB;

X_{10} es Tyr o un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{10} a C_{26};

X_{16} es Glu, aminoácido alfa, alfa-disustituido, o Lys;

X_{18} es Arg o Ala;

X_{20} es un aminoácido con carga negativa o aminoácido con carga neutra, opcionalmente, AIB o Gln;

X_{21} es Asp o Glu;

X_{27} es Leu, Ala, Nle, o Met;

X_{28} es Ala, Asp o Glu;

X_{29} es Gly o Thr; y

en donde el péptido comprende un aminoácido unido en forma covalente a un grupo acilo o alquilo de C_{12} a C_{18},

opcionalmente en la posición 10, y el aminoácido del extremo de C del péptido está amidado.

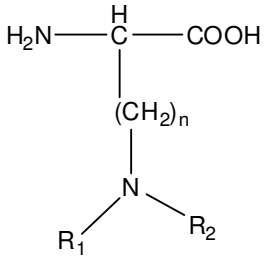
La invención también proporciona SEQ ID NO 187 con hasta 3 modificaciones de aminoácidos en relación con SEQ ID NO 187, en donde el análogo presenta una actividad de agonista en cada uno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano.

La invención además proporciona un análogo de cualquiera de SEQ ID NO 184, 185, 186 y 187, descritas en la presente, pero X_3 o el aminoácido de la posición 3 es Gln o un análogo de Gln o un aminoácido que reduce la actividad de glucagón, que incluye aquellos descritos en la presente. En ejemplos de realizaciones, el aminoácido que reduce la actividad de glucagón es un aminoácido ácido, básico o hidrófobo (por ejemplo, Glu, Orn o Nle). Opcionalmente, el aminoácido en la posición 3 es Glu.

Además se proporciona en la presente un análogo de glucagón (SEQ ID NO 1) que tiene actividad de agonista de GIP, que comprende:

(a) un aminoácido que comprende una cadena lateral de imidazol en la posición 1,

(b) en la posición 16, un aminoácido de la Fórmula IV:



[Fórmula IV],

en donde n es de 1 a 7, en donde cada uno de R_1 y R_2 se selecciona independientemente del grupo formado por H, alquilo de C_1–C_{18}, (alquilo de C_1–C_{18})OH, (alquilo de C_1–C_{18})NH_2, (alquilo de C_1–C_{18})SH, (alquilo de C_0–C_4)cicloalquilo (de C_3–C_6), (alquilo de C_0–C_4)(heterocíclico de C_2–C_5), (alquilo de C_0–C_4)(arilo de C_6–C_{10})R_7, y (alquilo de C_1–C_4)(heteroarilo de C_3–C_9), en donde R_7 es H o OH, en donde opcionalmente la cadena lateral del aminoácido de la Fórmula IV comprende un grupo amino libre,

(c) un aminoácido α,α-disustituido en la posición 20,

(d) hasta diez modificaciones de aminoácidos adicionales en relación con SEQ ID NO 1,

en donde, cuando el análogo carece de un grupo hidrófilo, el análogo de glucagón presenta por lo menos un 0,1% de actividad del GIP nativo en el receptor de GIP, en donde el análogo de

glucagón tiene menos de 100 veces la selectividad para el receptor de GLP-1 humano contra el receptor de GIP.

Además en la presente se proporcionan dímeros y multímeros que comprenden dos o más péptidos de agonistas de GIP de la presente invención. Los conjugados que comprenden un péptido de agonista de GIP de la presente invención y en la presente se proporciona un grupo conjugado. En algunos aspectos, el conjugado es un polipéptido de fusión que comprende el péptido de agonista de GIP de la presente invención fusionado a un péptido heterólogo. La presente invención también proporciona estuches que comprenden los péptidos de agonistas de GIP, dímeros, multímeros o conjugados de la presente invención (o una combinación de ellos).

Las composiciones farmacéuticas que comprenden cualquiera de los péptidos de agonistas de GIP, dímeros, multímeros, o conjugados de la presente invención (o una combinación de ellos) y un portador, diluyente, o excipiente farmacéuticamente aceptable se proporcionan también en la presente invención. Las composiciones farmacéuticas son preferentemente estériles y adecuadas para la administración parenteral. Las composiciones farmacéuticas están contempladas para su uso en métodos para tratar o prevenir la diabetes o la obesidad, o condiciones médicas asociadas a la diabetes o la

obesidad. Por consiguiente, en ejemplos de realizaciones la presente invención proporciona un método para reducir el aumento de peso o induce la pérdida de peso, un método para tratar o prevenir diarreas o la obesidad, y un método para inducir la parálisis temporal del tracto intestinal. Otras aplicaciones de los análogos de péptidos y composiciones farmacéuticas que comprenden las mismas se proporcionan en la siguiente descripción.

Breve Descripción de los Dibujos

La Figura 1 representa un gráfico del peso corporal (gramos) de ratones a los que se inyectaron 5 mmol/kg de un péptido de SEQ ID NO 28 (○), un péptido de SEQ ID NO: 37 (□), un péptido de SEQ ID NO: 38 ▽ o un péptido de SEQ ID NO: 39 (◊), o al que se inyectó un control excipiente (△), en función del tiempo después de la primera inyección.

La Figura 2 ilustra un gráfico del cambio total en el peso corporal (%), medido el Día 7 de ratones a los cuales se inyectó un control excipiente, o con uno de los siguientes péptidos: un péptido de SEQ ID NO 138 cuyo péptido está unido directamente a un grupo ácido graso de C_{16} sobre Lys en la posición 40, un péptido de SEQ ID NO 143 cuyo péptido está acilado a través de un separador de dipéptido de gamma-Glu-

gamma-Glu sobre un residuo de 4-amino-Phe en la posición 10, un péptido de SEQ ID NO 144 cuyo péptido está succinilado en C_{16} sobre un 4-amino-Phe en la posición 10, un péptido de SEQ ID NO 139 cuyo péptido está unido directamente a un grupo acilo graso de C_{16} sobre Lys en la posición 40 (que está seguido por un Gly en la posición 41), un péptido de SEQ ID NO 140 cuyo péptido está succinilado en C_{16} sobre una Lys en la posición 40 (que está seguido por un Gly en la posición 41), un péptido de SEQ ID NO 141 cuyo péptido está succinilado en C_{16} sobre una Lys en la posición 40 a través de un separador de beta-Ala (cuya Lys está seguida por una Gly en la posición 41), o un péptido de SEQ ID NO 142 cuyo péptido está unido directamente a un grupo acilo graso de C_{18} sobre una Lys en la posición 40 (que está seguida por una Gly en la posición 41).

Las Figuras 3A-3C se relacionan con péptidos acilados que comprenden un grupo succinoilo. La Figura 3A ilustra la estructuras químicas de diferentes residuos de aminoácidos acilados. De izquierda a derecha, (i) un residuo de Lys acilado directamente con un ácido graso de C_{16}, (ii) un residuo de Lys acilado con un ácido graso de C_{16} a través de un separador de ácido gamma-glutámico, (iii) un residuo de Lys acilado con un ácido graso de C_{16} a través de un separador de dipéptido (ácido gamma-glutámico-ácido gamma-glutámico), (iv) un residuo de Lys acilado con un succinoilo de C_{16}. La Figura

3B representa estructuras de tres ejemplos de anhídridos succínicos. La Figura 3C representa un esquema de síntesis de SEQ ID NO 156.

La Figura 4 es un gráfico del cambio en el peso corporal (%) de ratones a los que se inyectó con control excipiente (▽), o con un péptido de SEQ ID NO: 152 cuyo péptido comprende un residuo de Lys en la posición 16 unido en forma covalente a un grupo acilo graso de C_{16} a través de un separador de ácido gammaGlu (△), un péptido de SEQ ID NO: 28 cuyo péptido comprende un residuo de Lys en la posición 10 unido en forma covalente a un grupo acilo graso de C_{16} a través de un gammaGlu (hexágono abierto), un péptido de SEQ ID NO: 89 cuyo péptido comprende una Lys en la posición 40 unido en forma covalente a un grupo acilo graso de C_{16} a través de un separador de dipéptido de gammaGlu-gammaGlu (□), un péptido de SEQ ID NO: 145 cuyo péptido comprende una 4-aminoPhe en la posición 40 unida en forma covalente al grupo acilo graso de C_{16} a través de un separador de dipéptido de gammaGlu-gammaGlu (○), o un péptido de SEQ ID NO: 148 cuyo péptido comprende una 4-aminoPhe en la posición 10 unido en forma covalente a un grupo acilo graso de C_{16} a través de un separador de gammaGlu (◇).

Las Figura 5A-5F se relacionan con péptidos acilados dobles. La Figura 5A ilustra tres tipos de compuestos acilados dobles: (1) un sitio que comprende dos grupos acilo en una formación

ramificada, (2) un sitio que comprende dos grupos acilo en una formación lineal, y (3) dos sitios conectados cada uno a un grupo acilo graso a través de un separador de gammaGlu. La Figura 5B representa un esquema de síntesis de un péptido que comprende dos grupos acilo de diferentes tamaños en una formación ramificado. La Figura 5C representa un esquema de síntesis de un péptido que comprende dos grupos acilo del mismo tamaño en una formación ramificada. La Figura 5D representa un esquema de síntesis de un péptido que comprende dos grupos acilo en una formación lineal. La Figura 5E representa un gráfico del cambio en el peso corporal (%) medido el Día 7 del estudio de ratones que recibieron una inyección de péptido o una inyección de control excipiente. La Figura 5F representa un gráfico del cambio de los niveles de glucosa en sangre (mg/dL) medidos el Día 7 del estudio de ratones que recibieron una inyección de péptido o una inyección de control vehículo.

La Figura 6 ilustra la alquilación de S-palmitilo de un residuo de Cys que forma parte de una cadena principal de péptido.

La Figura 7 ilustra dos tipos de alquilación de S-palmitilo de un residuo de Cys, en donde el residuo de Cys forma parte de un separador de acilación. En un tipo de (estructura de la

izquierda dentro del casillero), una Cys se une a un residuo de Lys de la cadena principal del péptido y la Cys se alquila con S-palmitilo. En otro tipo (estructura de la derecha dentro del casillero), una Cys forma parte de un separador de dipéptido (gammaGlu-Cys) cuya gamma Glu está unida a una Lys de la cadena principal de péptido y la Cys está alquilada con S-palmitilo.

La Figura 8 representa un gráfico del cambio de peso corporal (%) medido el Día 7 de ratones a los que se inyectó control excipiente o con uno de nueve péptidos acilados diferentes: SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 164, SEQ ID NO: 89, SEQ ID NO: 159, SEQ ID NO: 157, SEQ ID NO: 165, SEQ ID NO: 166.

Las Figuras 9A y 9B se relacionan con dímeros que comprenden dos péptidos en donde por lo menos uno comprende un grupo acilo. La Figura 9A ilustra una estructura de un homodímero, en donde cada péptido comprende un residuo de Lys en la posición 40ª. Cada residuo de Lys está unido en forma covalente a través del grupo épsilon NH_2 al residuo de Cys. Cada residuo de Cys tiene un enlace de péptido a un residuo de gammaGlu, que, a su vez, está unido a un grupo acilo graso de C_{16}. Los átomos de azufre de cada residuo de Cys forman un puente de disulfuro. La Figura 9B ilustra una estructura de un

homodímero que está unido a través de un enlace de tioéter. Cada péptido comprende un residuo de Lys en la posición 40ª. L Lys del péptido superior está unida a un residuo de Cys, que tiene un enlace de péptido a un residuo de gammaGlu, que, a su vez, está unido a un grupo acilo graso de C_{16}. El azufre del residuo de Cys está unido a través de enlace de de tioéter a un grupo químico que, a su vez, está unido al residuo de Lys del péptido inferior.

La Figura 10 representa un gráfico del cambio en el peso corporal (%) de ratones a los que se inyectó un control excipiente o un péptido de SEQ ID NO 28 (a 1 o 3 nmol/kg), un péptido de SEQ ID NO 89 (a 1 o 3 nmol/kg) un péptido de SEQ ID NO 138 (a 3 nmol/kg), o un péptido de SEQ ID NO 171 (a 3 nmol/kg) en función del tiempo después de la inyección.

La Figura 11 representa un gráfico de los niveles de insulina, medidos el Día 21, de ratones a los que se inyectó un control excipiente o un péptido de SEQ ID NO 28 (a 1 o 3 nmol/kg), un péptido de SEQ ID NO: 89 (a 1 o 3 nmol/kg), un péptido de SEQ ID NO: 138 (a 3 nmol/kg), o un péptido de SEQ ID NO: 171 (a 3 nmol/kg).

Las Figuras 12A–12C representan una colección de esquemas de péptidos acilados que comprenden cada uno, un aminoácido acilado unido en forma covalente a un grupo acilo a través de

un separador de “miniPEG”. La Figura 12A representa el péptido acilado de SEQ ID NO 157, la Figura 12B representa el péptido acilado de SEQ ID NO 158 y la Figura 12C representa el péptido acilado de SEQ ID NO 159.

Descripción Detallada

Las presentes invenciones proporcionan péptidos de agonistas de GIP (por ejemplo, análogos de glucagón humano nativo (SEQ ID NO 1) (también denominados “análogos de glucagón”), análogos de cualquiera de las SEQ ID NOs: 27–33, 35–41, 43–46, 76–80, 83–87, 89, y 90, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58–60, 62–66, 68–70, 72, 73, 75, 81, 82, 88, y 92, análogos de cualquiera de las SEQ ID NOs: 184–199) que presentan la actividad de agonista en el receptor de GIP. Como se usa en la presente, el término “péptido” comprende una secuencia de 2 o más aminoácidos y normalmente menos de 100, o menos de 50 aminoácidos. Los aminoácidos normalmente pueden ser naturales o codificados o no naturales o no codificados. Los aminoácidos no naturales se refieren a aminoácidos que no aparecen naturalmente in vivo pero que, de todos modos, se pueden incorporar en las estructuras de péptidos descritas en la presente. “No codificado” como se usa en la presente se refiere a un aminoácido que no es un isómero de L de ninguno

de los siguientes 20 aminoácidos: Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr. El término “péptido de agonista de GIP” se refiere a un compuesto que se une y activa la señalización corriente abajo del receptor de GIP. El péptido de agonista de GIP puede tener cualquiera de los niveles de actividad en el receptor de GIP (por ejemplo, nivel de actividad absoluta o nivel de actividad relativa), selectividad para el receptor de GIP, o porcentaje de potencia de GIP, descrita en la presente. Véase, por ejemplo, la sección titulada *Actividad del Receptor de GIP*. Sin embargo, no se debe interpretar que este término limita el compuesto a tener actividad solamente en el receptor de GIP. En cambio, los péptidos de agonistas de GIP de la presente invención pueden presentar actividades adicionales en otros receptores, como se discute también en la presente. Los péptidos de agonistas de GIP, por ejemplo, pueden presentar actividad de agonista en el receptor de GLP-1 y/o el receptor de glucagón.

ACTIVIDAD DE LOS PÉPTIDOS REVELADOS ACTUALMENTE

Actividad del Receptor de GIP

En algunas o todas las realizaciones, los péptidos de la presente invención presentan una EC_{50} para la activación del receptor de GIP que está en la gama nanomolar. En ejemplos de

realizaciones, la EC_{50} del péptido del receptor de GIP es menor de 1000 nM, menor de 900 nM, menor de de 800 nM, menor de 700 nM, menor de 600 nM, menor de 500 nM, menor de 400 nM, menor de 300 nM, menor de 200 nM. En algunas realizaciones, la EC_{50} del péptido en el receptor de GIP es de 100 nM o menos, por ejemplo de 75 nM o menos, 50 nM o menos, 25 nM o menos, 10 nM o menos, 5 nM o menos, o 1 nM o menos. En algunas o todas las realizaciones, el péptido de la presente invención presenta una EC_{50} para la activación del receptor de GIP que está en la gama picomolar. En ejemplos de realizaciones, la EC_{50} del péptido de agonista de GIP en el receptor de GIP es menor de 1000 pM, menor de 900 pM, menor de 800 pM, menor de 700 pM, menor de 600 pM, menor de 500 pM, menor de 400 pM, menor de 300 pM, menor de 200 pM. En algunas realizaciones, la EC_{50} del péptido en el receptor de GIP es de 100 pM o menos, por ejemplo, de 75 pM o menos, de 50 pM o menos, de 25 pM o menos, de 10 pM o menos, de 5 pM o menos, o de 1 pM o menos. El término “aproximadamente” como se usa en la presente significa más o menos que el valor o la gama de valores expresadas por el 10 por ciento, pero no se desea designar ningún valor o gama de valores a solamente esta definición más amplia. Cada valor o gama de valores precedido por el término “aproximadamente” también comprende la realización del valor o la gama de valores absolutos expresados.

Los métodos adecuados para determinar la EC_{50} de un péptido para la activación de un receptor, por ejemplo, el receptor de GIP son conocidos en el arte. Un ejemplo de ensayo in Vitro, en donde la inducción de cAMP representada por la actividad de luciferasa se mide en las células HEK293 que sobreexpresan el receptor de GIP, se describe en la presente en el Ejemplo 2.

En algunas o en todas las realizaciones, los péptidos (por ejemplo, análogos de glucagón, análogos de la totalidad de SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, y 90, o cualquiera de SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, y 92) de las presentes invenciones presentan actividad aumentada en el receptor de GIP, comparado con el glucagón humano nativo. El glucagón nativo (SEQ ID NO 1) no activa el receptor de GIP, el glucagón nativo presenta esencialmente 0% (por ejemplo, menos del 0,001%, menos del 0,0001%) de la actividad de GIP nativo en el receptor de GIP. La actividad relativa de un péptido en el receptor de GIP en relación con el glucagón nativo se calcula como la relación inversa de (EC_{50} del péptido de las presentes invenciones / EC_{50} del glucagón nativo) x 100%.

La actividad relativa de un péptido en el receptor de GIP comparado con el GIP nativo se calcula como la relación inversa de (EC_{50} del péptido de las presentes invenciones / EC_{50}

del GIP nativo) x 100% (un valor también denominado en la presente “porcentaje de potencia de GIP”).

En algunas o en todas las realizaciones de las presentes invenciones, los péptidos de las presentes invenciones presentan el porcentaje de potencia de GIP que es por lo menos 0,1%. En ejemplos de realizaciones, los péptidos presentan por lo menos el 0,5%, por lo menos el 1%, por lo menos el 5%, por lo menos el 10%, por lo menos el 20%, por lo menos el 30%, por lo menos el 40%, por lo menos el 50%, por o menos el 60%, por lo menos el 70%, por lo menos el 90%, por lo menos el 90%, por lo menos el 100% de la actividad del GIP nativo en el receptor de GIP.

En algunas realizaciones de las presentes invenciones, los péptidos de las presentes invenciones presentan actividad en el receptor de GIP que es mayor que aquella del GIP nativo. En ejemplos de realizaciones, el péptido de agonista de GIP presenta un porcentaje de potencia de GIP de por lo menos el 125%, por lo menos el 150%, por lo menos el 175%, por lo menos el 200%, por lo menos el 300%, por lo menos el 400%, por lo menos el 500%, por lo menos el 600%, por lo menos el 700%, por lo menos el 800%, por lo menos el 900%, o por lo menos el 1000%. En algunas realizaciones, los péptidos de agonistas de

GIP descritos en la presente presentan un porcentaje de potencia de GIP no mayor del 1000% o no mayor del 10.000%.

En algunos aspectos, los péptidos de las presentes invenciones presentan un porcentaje de potencia de GIP dentro de la gama del 0,001 al 10.000 por ciento, o del 0,01 al 10.000 por ciento, o del 0,1 al 10.000 por ciento, o del 1 al 10.000 por ciento, o del 0,001 al 5000 por ciento, o del 0,01 al 5000 por ciento, o del 0,1 al 5000 por ciento, o del 0,1 al 1000 por ciento.

Co-agonistas

En algunas o en la totalidad de las realizaciones, el péptido de las presentes invenciones es un péptido de co-agonista siempre que active el receptor de GIP y un segundo receptor diferente del receptor de GIP.

Co-agonistas de GIP/GLP-1

A modo de ejemplo, el péptido de las presentes invenciones en algunos aspectos presenta actividad tanto en el receptor de GIP como en el receptor de GLP-1 (co-agonistas de receptor de GLP-1/GIP"). En algunos aspectos, los péptidos presentan actividad en los receptores de GLP-1 y de GIP, pero la

actividad de glucagón se ha reducido significativamente o destruido, por ejemplo, por una modificación de aminoácidos en la posición 3. Por ejemplo, la sustitución de esta posición con un aminoácido ácido, básico o hidrófobo (ácido glutámico, ornitina, norleucina) reduce la actividad de glucagón. En algunas o en todas las realizaciones, el péptido de agonista de GIP es un péptido que presenta un 10% o menos (por ejemplo, el 5% o menos, o el 1% o menos, o el 0,1% o menos) de la actividad del glucagón nativo en el receptor de glucagón.

En algunas o todas las realizaciones, la EC_{50} del péptido de las presentes invenciones en el receptor de GIP está dentro de 50 veces o menos, de 40 veces o menos, de 30 veces o menos, de 20 veces o menos, o de 10 veces o menos, o de 5 veces o menos diferente (más alta o más baja) de su EC_{50} en el receptor de GLP-1. Por ejemplo, la EC_{50} en el receptor de GIP puede ser 10 veces más alta o 10 veces más baja que la EC_{50} en el receptor de GLP-1. En algunas o en todas las realizaciones, el porcentaje de potencia de GIP del péptido de las presentes invenciones es menor de 50, 40, 30, 20, 10, o 5 veces diferente (más alto o más bajo) del porcentaje de potencia de GLP-1.

Por consiguiente, el péptido de las presentes invenciones tiene menos de 10 veces la selectividad para el receptor de

GLP-1 humano contra el receptor de GIP. Como se usa en la presente, el término “selectividad” de una molécula para un primer receptor en relación con un segundo receptor se refiere a la siguiente relación: EC_{50} de la molécula en el segundo receptor dividido por la EC_{50} de la molécula en el primer receptor. Por ejemplo, una molécula que tiene una EC_{50} de 1 nM en un primer receptor y una EC_{50} de 100 nM en el segundo receptor tiene 100 veces la selectividad para el primer receptor en relación con el segundo receptor. En ejemplos de realizaciones, la selectividad del péptido de las presentes invenciones para el receptor de GLP-1 humano contra el receptor de GIP es menor de 100 veces (por ejemplo, menos de 90 veces, menos de 80 veces, menos de 70 veces, menos de 60 veces, menos de 50 veces, menos de 40 veces, menos de 30 veces, menos de 20 veces, menos de 10 veces, menos de 5 veces).

En algunas o en todas las realizaciones, los péptidos de las presentes invenciones presentan actividad mejorada en el receptor de GLP-1, comparado con el glucagón humano nativo. El glucagón nativo tiene el 1% de la actividad de GLP-1 nativo en el receptor de GLP-1. La actividad relativa del péptido en el receptor de GLP-1 en relación con el glucagón nativo se calcula como la relación inversa de (EC_{50} del péptido de las presentes invenciones / EC_{50} del glucagón nativo) x 100%.

La actividad relativa de un péptido en el receptor de GLP-1 comparado con el GLP-1 nativo se calcula como la relación inversa de (EC_{50} del péptido de las presentes invenciones / EC_{50} de GLP-1 nativo) x 100% (un valor denominado en la presente "porcentaje de potencia de GLP-1").

En algunas o en todas las realizaciones de las presentes invenciones, los péptidos de las presentes invenciones presentan un porcentaje de potencia de potencia de GLP-1 de por lo menos 0,1%. En ejemplos de realizaciones, los péptidos presentan un porcentaje de potencia de GLP-1 de por lo menos 0,5%, por lo menos 1%, por lo menos 5%, por lo menos 10%, por lo menos 20%, por lo menos 30%, por lo menos 40%, por lo menos 50%, por lo menos 60%, por lo menos 70%, por lo menos 80%, por lo menos 90%, o por lo menos 100%.

En algunas realizaciones de las presentes invenciones, los péptidos de las presentes invenciones presentan actividad en el receptor de GLP-1 que es mayor que la de GLP-1 nativo. En ejemplos de realizaciones, el péptido de las presentes invenciones presenta un porcentaje de potencia de GLP-1 de por lo menos 125%, por lo menos 150%, por lo menos 175%, por lo menos 200%, por lo menos 300%, por lo menos 400%, por lo menos 500%, por lo menos 600%, por lo menos 700%, por lo menos 800%, por lo menos 900%, o por lo menos 1000%. En algunas

realizaciones, los péptidos de las presentes invenciones presentan un porcentaje de potencia de GLP-1 no mayor de 1000% o no mayor de 10.000%.

Co-agonistas de GIP/Glucagón

A modo de otro ejemplo, el péptido de las presentes invenciones en algunos aspectos presenta actividad en el receptor de GIP y en el receptor de glucagón ("co-agonistas de receptores de glucagón/GIP"). En algunas realizaciones, la actividad de GLP-1 se ha reducido significativamente o se ha destruido, por ejemplo, por una modificación de aminoácido en la posición 7, por ejemplo, la sustitución con Ile, una eliminación del extremo de C del aminoácido(s) al aminoácido en la posición 27 o 28, que produce un péptido de 27 o 28 aminoácidos o una combinación de ellos. En algunas realizaciones, el péptido de las presentes invenciones es un péptido que presenta un 10% o menos (por ejemplo, 5% o menos, o 1% o menos, o 0,1% o menos de la actividad de GLP-1 nativo en el receptor de GLP-1.

En algunas o en todas las realizaciones, la EC_{50} del péptido de las presentes invenciones en el receptor de GIP está dentro de 50 veces o menos, 40 veces o menos, 30 veces o menos, 20 veces o menos, o 10 veces o menos, o 5 veces o menos diferente (más

alta o más baja) de la EC_{50} en el receptor de glucagón. En algunas o todas las realizaciones, el porcentaje de potencia de GIP del péptido de las presentes invenciones es menor de 50, 40, 30, 20, 10, o 5 veces diferente (mayor o menor) de su porcentaje de potencia de glucagón.

En algunas realizaciones, los péptidos de las presentes invenciones presentan una actividad aumentada en el receptor de glucagón, comparado con el glucagón humano nativo. La actividad relativa de un péptido en el receptor de glucagón comparado con el glucagón nativo se calcula como la relación inversa de (EC_{50} del péptido de las presentes invenciones / EC_{50} del glucagón nativo) x 100% (un valor denominado en la presente “porcentaje de potencia de glucagón”).

En algunas realizaciones de las presentes invenciones, los péptidos de las presentes invenciones presentan un porcentaje de potencia de glucagón de por lo menos 0,1%. En ejemplos de realizaciones, los péptidos presentan un porcentaje de potencia de glucagón de por lo menos 0,5%, por lo menos 1%, por lo menos 5%, por lo menos 10%, por lo menos 20%, por lo menos 30%, por lo menos 40%, por lo menos 50%, por lo menos 60%, por lo menos 70%, por lo menos 80%, por lo menos 90%, o por lo menos 100%.

En algunas realizaciones de las presentes invenciones, los péptidos de las presentes invenciones presentan una actividad en el receptor de glucagón que es mayor que aquella del glucagón nativo. En ejemplos de realizaciones, el péptido de las presentes invenciones, presenta un porcentaje de potencia del glucagón, de por lo menos 125%, por lo menos 150%, por lo menos 175%, por lo menos 200%, por lo menos 300%, por lo menos 400%, por lo menos 500%, por lo menos 600%, por lo menos 700%, por lo menos 800%, por lo menos 900%, o por lo menos 1000%. En algunas realizaciones, los péptidos de las presentes invenciones presentan un porcentaje de potencia de glucagón no mayor de 1000% o no mayor de 10.000%.

Triagonistas

En algunas realizaciones, los péptidos de las presentes invenciones presentan actividad en dos o más receptores, diferentes del receptor de GIP. Por consiguiente, las presentes invenciones proporcionan en algunos aspectos péptidos de triagonistas de GIP. En algunos aspectos, los péptidos de las presentes invenciones presentan actividad en cada uno de los receptores de glucagón, GIP y GLP-1 (“tri-agonistas de glucagón/GIP/GLP-1”).

En algunas realizaciones, la EC_{50} del péptido de las presentes invenciones del receptor de GIP está dentro de 50 veces o menos 20 veces o menos, o 10 veces o menos diferente (mayor o menor) de la EC_{50} del péptido de las presentes invenciones en (a) el receptor de GLP-1, (b) el receptor de glucagón, o ambos. En algunas realizaciones, la EC_{50} del péptido de las presentes invenciones en el receptor de GIP está dentro de 40 veces, 30 veces, 20 veces diferente (mayor o menor) de la EC_{50} en el receptor de GLP-1, y opcionalmente dentro de 50 veces diferente de la EC_{50} en el receptor de glucagón. En algunas realizaciones, el porcentaje de potencia de GIP del péptido de las presentes invenciones es menor de 50 veces, 20 veces o 10 veces diferente (mayor o menor) de (a) el porcentaje de potencia de GLP-1, (b) su porcentaje de potencia de glucagón., o ambos. En algunas realizaciones, el porcentaje de potencia de GP del péptido de las presentes invenciones está dentro de 40 veces, 30 veces, 20 veces diferente (mayor o menos) del porcentaje de potencia de GLP-1 y opcionalmente dentro de 50 veces diferente del porcentaje de potencia de glucagón. En algunas realizaciones, el péptido de las presentes invenciones no tiene por lo menos 100 veces la selectividad para el receptor de GLP-1 humano contra el receptor de GIP. En ejemplos de realizaciones, la selectividad del péptido de las presentes invenciones para el receptor de GLP-1 humano contra el receptor de GIP se menor de 100 veces (por ejemplo, menor

de 90 veces, menor de 80 veces, menor de 70 veces, menor de 60 veces, menor de 50 veces, menor de 40 veces, menor de 30 veces, menor de 20 veces, menor de 10 veces, menor de 5 veces).

Agonismo de GIP en la ausencia de agonismo de GLP-1 y de agonismo de glucagón

En algunas realizaciones, el péptido de las presentes invenciones presenta actividad en solamente el receptor de GIP, y no en ningún otro receptor, por ejemplo, el receptor de GLP-1, el receptor de glucagón. En ejemplos de realizaciones, el péptido de las presentes invenciones presenta actividad en el receptor de GIP, y la actividad de glucagón y de GLP-1 se ha reducido significativamente o destruido, por ejemplo mediante modificaciones de aminoácidos en las posiciones 3 y 7. En algunas realizaciones, el péptido de las presentes invenciones es un péptido que presenta un porcentaje de potencia de GLP-1 del 10% o menos (por ejemplo, 5% o menos, o 1% o menos, o 0,1% o menos). En algunas realizaciones, el péptido de las presentes invenciones es un péptido que presenta un porcentaje de potencia de glucagón del 10% o menos (por ejemplo, el 5% o menos, o el 1% o menos, o el 0,1% o menos).

Actividad de Conjugados

En algunas o en todas las realizaciones, cuando el péptido de las presentes invenciones está conjugado a un grupo heterólogo (por ejemplo, un grupo hidrófilo), como se describe también en la presente, el péptido de las presentes invenciones presentan una actividad reducida (por ejemplo, un porcentaje de potencia menor o una EC_{50} más alta) que cuando el péptido de las presentes invenciones está en una forma libre o no conjugada. Por lo tanto, está contemplado que cuando cualquiera de los niveles de actividad absoluta precedentes (por ejemplo, el porcentaje de potencia de GIP, el porcentaje de potencia de GLP-1 o el porcentaje de potencia de glucagón, pero no relaciones relativas) se aplica a un péptido en la forma conjugada, por ejemplo, pegilada, dichos niveles de actividad absoluta se reducen en 10 veces, 20 veces, 30 veces, 40 veces, 50 veces, o 100 veces y que dichas veces de reducción de los niveles de actividad están contempladas dentro del alcance de la invención. A la inversa, cuando está conjugado, el péptido de las presentes invenciones presenta un porcentaje de potencia de GIP que es 10 veces, 20 veces, 30 veces, 40 veces, 50 veces, 100 veces o mayor que la potencia del péptido de las presentes invenciones cuando está conjugado a un grupo heterólogo.

ESTRUCTURA DE LOS PÉPTIDOS REVELADOS ACTUALMENTE

Análogos de Glucagón

En algunas realizaciones, los péptidos de las presentes invenciones son estructuralmente similares al glucagón humano nativo (SEQ ID NO 1), por ejemplo, es un análogo de glucagón humano nativo (también denominado en la presente “análogo de glucagón”, o “análogo de péptido de glucagón”). Como se usan en la presente, los términos “análogo de glucagón” y “análogo de péptido de glucagón” y similares, se refieren a péptidos que son estructuralmente similares al glucagón humano nativo y estos términos no necesariamente implican que los péptidos activan el receptor de glucagón.

En algunas o en todas las realizaciones, el péptido de la presente invención revelado es un análogo del glucagón humano nativo (SEQ ID NO 1) que comprende una secuencia de aminoácidos basada en una secuencia de aminoácidos de SEQ ID NO 1 pero difiere de SEQ ID NO 1 siempre que la secuencia de aminoácidos del análogo de glucagón comprende uno o más (por ejemplo, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, y en algunos casos, 16 o más (por ejemplo, 17, 18, 19, 20, 21, 22, 23, 24, 25, etc.), modificaciones de aminoácidos especificadas u opcionales. En algunas o en todas las realizaciones, el péptido de las presentes invenciones comprende un total de 1, hasta 2, hasta 3, hasta 4, hasta 5,

hasta 6, hasta 7, hasta 8, hasta 9, o hasta 10 modificaciones de aminoácidos adicionales (por ejemplo, además de las modificaciones de aminoácidos especificadas), en relación con la secuencia de glucagón humano nativo (SEQ ID NO: 1). Por ejemplo, con respecto a un análogo de glucagón (SEQ ID NO: 1) que comprende (a) un aminoácido que comprende una cadena lateral de aminoácido en la posición 1, (b) un aminoácido protector contra DPP-IV en la posición 2, (c) un aminoácido acilado o un aminoácido alquilado en cualquiera de las posiciones 9, 10, 12, 16, 20, o 37-43, (d) un aminoácido de estabilización de la hélice de alfa en una o más de las posiciones 16, 17, 18, 19, 20, y 21, y (e) hasta diez modificaciones de aminoácidos adicionales en relación con SEQ ID NO: 1, las presentes invenciones proporcionan un análogo de glucagón que comprende (a)-(d) con hasta 10 modificaciones de aminoácidos adicionales además de las modificaciones de aminoácidos especificadas en (a)-(d). En algunas o en todas las realizaciones, las modificaciones son cualquiera de aquellas descritas en la presente, por ejemplo, acilación, alquilación, pegilación, truncamiento en el extremo de C, sustitución del aminoácido en una o más de las posiciones 1, 2, 3, 7, 10, 12, 15, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, y 29.

Como se usa en la presente, una “modificación de aminoácido” se refiere a (i) una sustitución de un aminoácido de SEQ ID NO

1 con un aminoácido diferente (aminoácido natural o codificado o no codificado o no natural), (ii) un agregado de un aminoácido (un aminoácido natural o codificado o no codificado o no natural), a SEQ ID NO 1 o (iii) una eliminación de uno o más aminoácidos de SEQ ID NO 1.

En algunas o en todas las realizaciones, la sustitución de aminoácido es una sustitución de aminoácido conservadora, por ejemplo, una sustitución conservadora del aminoácido en una o más de las posiciones 2, 5, 7, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 27, 28 o 29. Como se usa en la presente, el término “sustitución de aminoácido conservadora” se define en la presente como la sustitución de un aminoácido que tiene propiedades similares, por ejemplo, el tamaño, la carga, la hidrofobicidad, la hidrofilicidad, y/o la aromaticidad e incluye los intercambios dentro de uno de los siguientes cinco grupos:

I. Residuos pequeños, alifáticos, no polares o ligeramente polares:

Ala, Ser, Thr, Pro, Gly;

II. Residuos polares, con carga negativa y sus amidas y ésteres:

Asp, Asn, Glu, Gln, ácido cisteico y ácido homocisteico;

III. Residuos polares, con carga positiva:

His, Arg, Lys; Ornitina (Orn)

IV. Residuos grandes, alifáticos, no polares:

Met, Leu, Ile, Val, Cys, Norleucina (Nle), homocisteína

V. Residuos grandes, aromáticos:

Phe, Tyr, Trp, acetil fenilalanina

En realizaciones alternativas, la sustitución de aminoácido no se una sustitución de aminoácidos conservadora, por ejemplo, es una sustitución de aminoácidos no conservadora.

Como se usa en la presente, “aminoácido con una carga” se refiere a un aminoácido que comprende una cadena lateral que tiene una carga negativa (es decir, desprotonado) o con carga positiva (es decir, protonado) en una solución acuosa al pH fisiológico. Por ejemplo, los aminoácidos con carga negativa incluyen ácido aspártico, ácido glutámico, ácido cisteico, ácido homocisteico, ácido homoglutámico, mientras que los aminoácidos con una carga positiva incluyen arginina, lisina e histidina. Los aminoácidos con una carga incluyen los aminoácidos con una carga entre los 20 aminoácidos codificados, así como lo aminoácidos atípicos o no naturales o no codificados. Como se usa en la presente, el término “aminoácido ácido” se refiere a un aminoácido que comprende un segundo grupo ácido (diferente del ácido alfa carboxílico del

aminoácido), que incluye por ejemplo, un grupo ácido carboxílico o ácido sulfónico de la cadena lateral).

En algunas realizaciones, el péptido de las presentes realizaciones comprende una secuencia de aminoácido que tiene por lo menos un 25% de identidad de secuencia a la secuencia de aminoácido del glucagón humano nativo (SEQ ID NO 1). En algunas realizaciones, el péptido de las presentes realizaciones comprende una secuencia de aminoácido que es por lo menos 30%, por lo menos 40%, por lo menos 50%, por lo menos 60%, por lo menos 70%, por lo menos 80%, por lo menos 85%, por lo menos 90% o tiene más del 90% de identidad de secuencia a SEQ ID NO 1. En algunas realizaciones, la secuencia de aminoácido del péptido revelado en la actualidad que tiene el % de identidad de secuencia citado anteriormente es la secuencia de aminoácido de longitud completa del péptido revelado actualmente. En algunas realizaciones, la secuencia de aminoácido del péptido de las presentes invenciones que tiene el % de identidad de secuencia citado anteriormente es solamente una parte de la secuencia de aminoácido del péptido revelado actualmente. En algunas realizaciones, el péptido revelado actualmente comprende una secuencia de aminoácido que tiene un A% o mayor identidad de secuencia de proteínas a una secuencia de aminoácido de referencia de por lo menos 5 aminoácidos contiguos (por ejemplo, por lo menos 6, por lo

menos 7, por lo menos 8, por lo menos 9, por lo menos 10 aminoácidos) de SEQ ID NO 1, en donde la secuencia de aminoácidos de referencia empieza con el aminoácido en la posición C de SEQ ID NO 1 y termina con el aminoácido en la posición D de SEQ ID NO 1, en donde A es 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99; C es 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, o 28 y D es 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 o 29. Todas y cada una de las combinaciones de los parámetros precedentes están previstas, incluyendo en forma no taxativa, por ejemplo, en donde A es 90% y C y D son 1 y 27, o 6 y 27, o 8 y 27, o 10 y 27, o 12 y 27, o 16 y 27.

Los análogos del glucagón humano nativo (SEQ ID NO 1) descritos en la presente pueden comprender una cadena principal del péptido de cualquier cantidad de aminoácidos, es decir, puede tener cualquier longitud de péptido. En algunas realizaciones, los péptidos descritos en la presente tienen la misma longitud que SEQ ID NO 1, es decir, tienen 29 aminoácidos de longitud. En algunas realizaciones, el péptido revelado actualmente tiene más de 29 aminoácidos de longitud, por ejemplo, el péptido revelado actualmente comprende una longitud del extremo de C de 1-20 aminoácidos como se describe

también en la presente. Por consiguiente, el péptido de las presentes invenciones en algunas realizaciones, tiene 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, o 50 aminoácidos de longitud. En algunas realizaciones, el péptido revelado actualmente tiene hasta 50 aminoácidos de longitud. En algunas realizaciones, el péptido revelado actualmente tiene más de 29 aminoácidos de longitud (por ejemplo, más de 50 aminoácidos, (por ejemplo, por lo menos 60, por lo menos 70, por lo menos 80, por lo menos 90, por lo menos 100, por lo menos 150, por lo menos 200, por lo menos 250, por lo menos 300, por lo menos 350, por lo menos 400, por lo menos 450, por lo menos 500 aminoácidos de longitud debido a la fusión con otro péptido. En otras realizaciones, el péptido revelado actualmente tiene menos de 29 aminoácidos de longitud, por ejemplo, 28, 27, 26, 25, 24, 23, aminoácidos.

De acuerdo con lo antedicho, en algunos aspectos, el péptido de las presentes invenciones es un análogo de glucagón humano nativo (SEQ ID NO 1) que comprende una secuencia de aminoácidos basada en SEQ ID NO 1, cuya secuencia comprende una o más modificaciones de aminoácidos que afectan la actividad de GIP, la actividad de glucagón y/o la actividad de GLP-1, aumentan la estabilidad, por ejemplo, reduciendo la degradación del péptido (por ejemplo, mejorando la resistencia a la DPP-IV proteasa), aumenta la solubilidad, aumentan la

semivida, retardan el inicio de acción, prolongan la duración de la acción en el receptor de GIP, glucagón o GLP-1, o una combinación de cualquiera de las precedentes. Dichas modificaciones de aminoácidos, además de otras modificaciones, se describen también a continuación, y cualquiera de estas modificaciones es puede aplicar individualmente o en combinación.

AMINOÁCIDOS QUE COMPRENDEN UN GRUPO ACILO NO NATIVO

De acuerdo con algunas o todas las realizaciones, los péptidos de agonistas de GIP que son análogos de glucagón (SEQ ID NO 1) comprenden un aminoácido que comprende un grupo acilo no nativo denominado en la presente un “aminoácido acilado”, independientemente de cómo se prepare, por ejemplo, mediante la incorporación de un aminoácido acilado previamente dentro del péptido, o acilación del péptido después de la síntesis. En algunas o en todas las realizaciones, el aminoácido acilado está ubicado en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, 37, 38, 39, 40, 41, 42, o 43 del análogo de glucagón. En ejemplos de aspectos, el aminoácido acilado está ubicado en cualquiera de las posiciones 9, 10, 12, 16, 20, o 40 del análogo de glucagón o en cualquiera de las posiciones 10, 13, 14, 16, 17, o 40 del análogo de glucagón. En ejemplos de aspectos, el aminoácido acilado está ubicado en una o más

cualquiera de las posiciones 10, 14, y 40. En ejemplos de aspectos, el aminoácido acilado está ubicado en cualquiera de las posiciones 10, 12, o 16 del análogo de péptido.

El aminoácido acilado en algunas de las realizaciones hace que el péptido de agonista de GIP tenga uno o más de (i) una semivida prolongada en circulación, (ii) un inicio de acción retardado, (iii) una duración de acción prolongada, (iv) una resistencia mejorada a las proteasas, tales como DPP-IV, y (v) potencia aumentada en uno o más cualquiera del receptor de GIP, el receptor de GLP-1, y el receptor de glucagón.

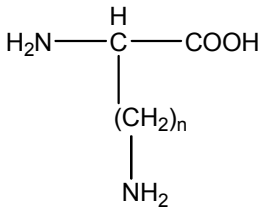
Acilación Directa

En algunas realizaciones, el grupo acilo está unido directamente a un aminoácido del péptido de agonista de GIP. De acuerdo con una realización, el péptido de agonista de GIP comprende un grupo acilo que está unido al péptido a través de una unión de éster, tioéster o amida.

En aspectos específicos, el péptido de agonista de GIP comprende un grupo acilo luego de la acilación directa de una amina, hidroxilo, o tiol de una cadena lateral de un aminoácido del péptido de agonista de GIP. En algunas realizaciones, la acilación en la posición 9, 10, 12, 13, 14,

16, 17, 20, o 40 (por ejemplo, en cualquiera de las posiciones 10, 14, y 40) del péptido de agonista de GIP. Al respecto, el péptido de agonista de GIP comprende la secuencia de aminoácidos de SEQ ID NO: 1, o una secuencia de aminoácidos modificada de ella que comprende una o más de las modificaciones de aminoácidos descritas en la presente, en donde por lo menos uno de los aminoácidos en las posiciones 9, 10, 12, 13, 14, 16, 17, 20, y 40 (por ejemplo, en cualquiera de las posiciones 10, 14, y 40) del péptido de agonista de GIP es un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral.

En algunas realizaciones, el aminoácido que comprende una amina de la cadena lateral es un aminoácido de la Fórmula I:



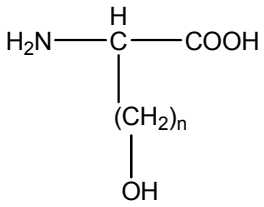
en donde n = 1 a 4

[Fórmula I]

En algunas realizaciones, el aminoácido de la Fórmula I es el aminoácido en donde n es 4 (Lys) o n es 3 (Orn). En algunas realizaciones, el aminoácido que comprende una amina de la cadena lateral es un aminoácido que comprende una amina de la

cadena lateral. En ejemplos de aspectos, el aminoácido aromático que comprende una amina de la cadena lateral es 4-amino-fenilalanina (4-aminoPhe), p-amino fenilglicina, p-amino homofenilalanina, o 3-amino tirosina. En ejemplos de aspectos, el aminoácido aromático que comprende una amina de la cadena lateral es 4-amino-Phe.

En otras realizaciones, el aminoácido que comprende un hidroxilo de la cadena lateral es un aminoácido de la Fórmula II:



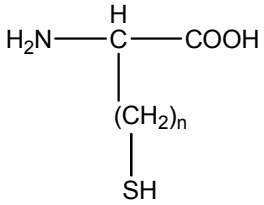
en donde n = 1 a 4

[Fórmula II]

En algunos ejemplos de realizaciones, el aminoácido de la Fórmula II es el aminoácido en donde n es 1 (Ser). En ejemplos de aspectos, el aminoácido de la Fórmula II es el aminoácido en donde n es 2 (homoserina). En ejemplos similares de realizaciones, el aminoácido que comprende un hidroxilo de la cadena lateral es una Thr u homotreonina. En ejemplos similares de realizaciones, el aminoácido que comprende un hidroxilo de la cadena lateral es un aminoácido aromático que

comprende un hidroxilo de la cadena lateral. Ejemplos de aspectos, el aminoácido aromático que comprende un hidroxilo de cadena lateral es tirosina, homotirosina, metil-tirosina, o 3-amino tirosina.

En aún otras realizaciones, el aminoácido que comprende un tiol de la cadena lateral es un aminoácido de la Fórmula III:



en donde n = 1 a 4

[Fórmula III]

En algunos ejemplos de realizaciones, el aminoácido de la Fórmula III es el aminoácido en donde n es 1 (Cys).

En aún otras realizaciones, el aminoácido que comprende una amina, hidroxilo, o tiol de la cadena lateral, es un aminoácido disustituido que comprende la misma estructura de la Fórmula I, de la Fórmula II o de la Fórmula III, excepto que el enlace de hidrógeno al carbono alfa del aminoácido de la Fórmula I, de la Fórmula II o de la Fórmula III se reemplaza con una segunda cadena lateral.

Separadores de Acilación

En realizaciones alternativas, el grupo acilo está unido a través de un separador a un aminoácido del péptido de agonista de GIP, en donde el separador está posicionado entre el aminoácido del péptido de agonista de GIP y el grupo acilo. En algunas realizaciones, el péptido de agonista de GIP comprende un separador entre el péptido y el grupo acilo. En algunas realizaciones, el péptido de agonista de GIP está unido en forma covalente al separador, que está unido en forma covalente al grupo acilo.

En algunas realizaciones, el separador es un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral o un dipéptido o tripéptido que comprende un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral. El aminoácido al cual está unido el separador (por ejemplo, un aminoácido con una sustitución única o doble) que comprende un grupo que permite la unión al separador. Por ejemplo, un aminoácido que comprende un NH_2, –OH, o –COOH de la cadena lateral (por ejemplo, Lys, Orn, Ser, Asp, o Glu) es adecuado. Al respecto, el péptido de agonista de GIP en algunos aspectos comprende la secuencia de aminoácidos de SEQ ID NO 1, o una secuencia de aminoácidos modificada de ella que comprende una o más de las modificaciones de aminoácidos descritas en la

presente, en donde por lo menos uno de los aminoácidos en las posiciones 9, 10, 12, 13, 14, 16, 17, 20, y 37-43 (por ejemplo, la posición 10, 14, o 40) es un aminoácido que comprende una amina, hidroxilo o carboxilato de la cadena lateral.

En algunas realizaciones, el separador es un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral, o un dipéptido o tripéptido que comprende un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral.

Cuando el grupo acilo está unido a través de un grupo amino de un separador, el grupo acilo en algunos aspectos está unido a través de la amina alfa o a través de una amina de la cadena lateral del aminoácido del separador. En el caso de que el grupo acilo esté unido a través de una amina alfa, el aminoácido del separador puede ser cualquier aminoácido. Por ejemplo, el aminoácido del separador puede ser un aminoácido hidrófobo, por ejemplo, Gly, Ala, Val, Leu, Ile, Trp, Met, Phe, Tyr, ácido 6-amino hexanoico, ácido 5-aminovalérico, ácido 7-aminoheptanoico y ácido 8-aminooctanoico. Alternativamente, en algunos aspectos, el aminoácido del separador es un residuo ácido, por ejemplo, Asp, Glu, ácido homoglutámico, ácido homocisteico, ácido cisteico, ácido gamma-glutámico.

En el caso de que el grupo acilo esté unido a través de una amina de la cadena lateral del separador del aminoácido, el separador es un aminoácido que comprende una amina de la cadena lateral, por ejemplo, un aminoácido de la Fórmula I (por ejemplo, Lys u Orn). En este caso, es posible que tanto la amina alfa como la amina de la cadena lateral del aminoácido del separador que se debe unir a un grupo acilo, de manera tal que el péptido de agonista de GIP está diacilado. Las realizaciones de la invención incluyen dichas moléculas diaciladas. En algunas realizaciones, el grupo acilo está unido a una 4-amino-Phe, p-amino fenilglicina, p-amino homofenilalanina, o 3-amino tirosina.

Cuando el grupo acilo está unido a través de un grupo hidroxilo de un separador, el aminoácido o uno de los aminoácidos del dipéptido o tripéptido puede ser un aminoácido de la Fórmula II. En un ejemplo específico de la realización, el aminoácido es una Ser. En ejemplos similares de realizaciones, el grupo acilo está unido a un Thr u homotreonina. Ejemplos similares de realizaciones, el grupo acilo está unido a través del hidroxilo de un aminoácido aromático que comprende un hidroxilo, de la cadena lateral, por ejemplo, tirosina, homotirosina, metil-tirosina o 3-amino tirosina.

Cuando el grupo acilo está unido a través de un grupo tiol de un separador, el aminoácido o uno de los aminoácidos del dipéptido o tripéptido puede ser un aminoácido de la Fórmula III. En un ejemplo de realización específico, el aminoácido es Cys.

En algunas realizaciones, el separador es un separador bifuncional hidrófilo. En ejemplos de realizaciones, el separador bifuncional hidrófilo comprende dos o más grupos reactivos, por ejemplo, una amina, un hidroxilo, un tiol y un grupo carboxilo o cualquier combinación de ellos. En ejemplos de realizaciones, el separador bifuncional hidrófilo comprende un grupo hidroxilo y un carboxilato. En otras realizaciones, el separador bifuncional hidrófilo comprende un grupo amina y un carboxilato. En otras realizaciones, el separador bifuncional hidrófilo comprende un grupo tiol y un carboxilato. En una realización específica, el separador comprende un amino polialquiloxicarboxilato. Al respecto, el separador puede comprender, por ejemplo, $NH_2(CH_2CH_2O)_n(CH_2)_mCOOH$, en donde m es cualquier número entero de 1 a 6 y n es cualquier número entero de 2 a 12, tal como, por ejemplo, ácido 8-amino-3,6-dioxaoctanoico, que está comercialmente disponible en Peptides International, Inc. (Louisville, KY).

En ejemplos de realizaciones, el separador comprende un grupo polietilenglicol pequeño (PEG) que comprende una estructura [-$O-CH_2-CH_2-]_n$, en donde n es un número entero de entre 2 y 16, (por ejemplo, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Dichos PEG pequeños se denominan en la presente "miniPEG." En ejemplos de aspectos, el miniPEG es un miniPEG con una funcionalidad que comprende uno o más grupos funcionales. Los grupos funcionales adecuados incluyen, en forma no taxativa, una amina, un hidroxilo, un tiol y un grupo carboxilo o cualquier combinación de ellos. En ejemplos de aspectos, el miniPEG es un ácido de miniPEG que comprende una estructura $\{[-O-CH_2-CH_2-]_n-COO-\}$, en donde n es lo definido anteriormente. En ejemplos de aspectos, el miniPEG es un amido miniPEG que comprende una estructura $\{-N-CH_2-CH_2-[-O-CH_2-CH_2-]_n\}$, en donde n es lo definido anteriormente. En ejemplos de aspectos, el miniPEG es un ácido de amido miniPEG que comprende una estructura $\{-N-CH_2-CH_2-[-O-CH_2-CH_2-]_n-COO-\}$, en donde n es lo definido anteriormente. Los reactivos adecuados para su uso en la acilación de un aminoácido con un miniPEG están comercialmente disponibles en vendedores, tales como Peptides International (Louisville, KY). Además, se describen en la presente técnicas adecuadas para acilar un aminoácido con miniPEG (véase el Ejemplo 1).

En algunas realizaciones, el separador es un separador bifuncional hidrófobo. Los separadores bifuncionales hidrófobos son conocidos en el arte. Véase, por ejemplo, *Técnicas de Bioconjugados,* G. T. Hermanson (Academic Press, San Diego, CA, 1996), que se incorpora en la presente como referencia en su totalidad. En ejemplos de realizaciones, el separador bifuncional hidrófobo comprende dos o más grupos reactivos, por ejemplo, una amina, un hidroxilo, un tiol, y un grupo carboxilo o cualquier combinación de ellos. En ejemplos de realizaciones, el separador bifuncional hidrófobo comprende un grupo hidroxilo y un carboxilato. En otras realizaciones, el separador bifuncional hidrófobo comprende un grupo amina y un carboxilato. En otras realizaciones, el separador bifuncional hidrófobo comprende un grupo tiol y un carboxilato. Los separadores bifuncionales hidrófobos adecuados que comprenden un carboxilato y un grupo hidroxilo o un grupo tiol son conocidos en el arte e incluyen, por ejemplo, ácido 8-hidroxioctanoico y ácido 8-mercaptooctanoico.

En algunas realizaciones, el separador bifuncional no es un ácido dicarboxílico que comprende un metileno no ramificado de 1-7 átomos de carbono entre los grupos carboxilato. En algunas realizaciones, el separador bifuncional es ácido dicarboxílico que comprende un metileno no ramificado de 1-7 átomos de carbono entre los grupos carboxilato.

El separador (por ejemplo, aminoácido, dipéptido, tripéptido, separador bifuncional hidrófilo o separador bifuncional hidrófobo) en realizaciones específicas tiene de 3 a 10 átomos (por ejemplo, de 6 a 10 átomos, (por ejemplo, 6, 7, 8, 9, o 10 átomos) de longitud. En algunas realizaciones específicas, el separador tiene de 3 a 10 átomos (por ejemplo, de 6 a 10 átomos) de longitud y el grupo acilo es un grupo acilo de C_{12} a C_{18} (por ejemplo, un grupo acilo graso de C_{14}, un grupo acilo graso de C_{16}, de manera tal que la longitud total del separador y el grupo acilo sea de 14 a 18 átomos, por ejemplo, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, o 28 átomos. En algunas realizaciones, la longitud del separador y el grupo acilo es de 17 a 28 (por ejemplo, de 19 a 26, de 19 a 21) átomos.

De acuerdo con algunas o la totalidad de las realizaciones precedentes, el separador bifuncional puede ser un aminoácido sintético o natural (que incluye, en forma no taxativa, cualquiera de aquellos descritos en la presente) que comprende una cadena principal de aminoácido que tiene de 3 a 10 átomos de longitud (por ejemplo, ácido 6-amino hexanoico, ácido 5-aminovalérico, ácido 7-aminoheptanoico y ácido 8-aminootanoico). Alternativamente, el separador puede ser un separador de dipéptido o de tripéptido que tiene una cadena

principal de péptido que tiene de 5 a 10 átomos (por ejemplo, de 6 a 10 átomos) de longitud. Cada aminoácido del separador de dipéptido o tripéptido puede ser el mismo o diferente del otro aminoácido(s) del dipéptido o tripéptido y se puede seleccionar independientemente del grupo formado por: aminoácidos naturales o codificados y/o aminoácidos no codificados o no naturales, que incluyen, por ejemplo, cualquiera de los isómeros D o L de los aminoácidos naturales (Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, Tyr), o cualquiera de los isómeros D o L de los aminoácidos no naturales o no codificados seleccionados del grupo formado por: β–alanina (β–Ala), N–α–metil–alanina (Me–Ala), ácido aminobutírico (Abu), ácido γ–aminobutírico (γ–Abu), ácido aminohexanoico (ε–Ahx), ácido aminoisobutírico (Aib), ácido aminometilpirrol carboxílico, ácido aminopiperidinacarboxílico, aminoserina (Ams), ácido aminotetrahidropiran–4–carboxílico, arginina N–metoxi–N–metil amida, ácido β–aspártico (β–Asp), ácido azetidina carboxílico, 3–(2–benzotiazolil)alanina, α–*terc*–butilglicina, ácido 2–amino–5–ureido–n–valérico (citrulina, Cit), β–Ciclohexilalanina (Cha), acetamidometil–cisteína, ácido diaminobutanoico (Dab), ácido diaminopropiónico (Dpr), dihidroxifenilalanina (DOPA), dimetiltiazolidina (DMTA), ácido γ–Glutámico (γ–Glu), homoserina (Hse), hidroxiprolina (Hyp),

isoleucina N-metoxi-N-metil amida, metil-isoleucina (MeIle), ácido isonipecótico (Isn), metil-leucina (MeLeu), metil-lisina, dimetil-lisina, trimetil-lisina, metanoprolina, sulfóxido de metionina (Met(O)), metionina-sulfona (Met(O_2)), norleucina (Nle), metil-norleucina (Me-Nle), norvalina (Nva), ornitina (Orn), ácido para-aminobenzoico (PABA), penicilamina (Pen), metilfenilalanina (MePhe), 4-clorofenilalanina (Phe(4-Cl)), 4-fluorofenilalanina (Phe(4-F)), 4-nitrofenilalanine (Phe(4-NO_2)), 4-cianofenilalanina ((Phe(4-CN)), fenilglicina (Phg), piperidinilalanina, piperidinilglicina, 3,4-deshidroprolina, pirrolidinilalanina, sarcosina (Sar), selenocisteína (Sec), O-Bencil-fosfoserina, ácido 4-amino-3-hidroxi-6-metilheptanoico (Sta), ácido 4-amino-5-ciclohexil-3-hidroxipentanoico (ACHPA), ácido 4-amino-3-hidroxi-5-fenilpentanoico (AHPPA), ácido 1,2,3,4,-tetrahidro-isoquinolina-3-carboxílico (Tic), tetrahidropiranglicina, tienilalanina (Thi) , O-bencil-fosfotirosina, O-fosfotirosina, metoxitirosina, etoxitirosina, O-(bis-dimetilamino-fosfono)-tirosina, tetrabutilamina de sulfato de tirosina, metil-valina (MeVal), y ácido 3-mercaptopropiónico alquilado. En ejemplos de aspectos, el separador es un residuo de Cys o un residuo de Lys.

En algunas realizaciones, el separador comprende una carga negativa total, por ejemplo, comprende uno o dos aminoácidos con una carga negativa. En algunas realizaciones, el dipéptido

no es ninguno de los dipéptidos de la estructura general A-B, en donde A se selecciona del grupo formado por Gly, Gln, Ala, Arg, Asp, Asn, Ile, Leu, Val, Phe, y Pro, en donde B se selecciona de Lys, His, Trp. En algunas realizaciones, los aminoácidos del separador de dipéptido se seleccionan del grupo formado por: Ala, β-Ala, Leu, Pro, ácido γ-aminobutírico, Glu y γ-Glu.

En algunos ejemplos de realizaciones, el péptido de agonista de GIP comprende un grupo acilo luego de la acilación de una amina, hidroxilo, o tiol de un separador, cuyo separador está unido a una cadena lateral de un aminoácido en la posición 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40), o en el aminoácido del extremo de C del péptido de agonista de GIP.

En aún más realizaciones específicas, el grupo acilo está unido al aminoácido en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40) del análogo de péptido y la longitud del separador y el grupo acilo es de 14 a 28 átomos. El aminoácido de cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40), en algunos aspectos, es un aminoácido de la Fórmula I, por ejemplo, Lys,

o un aminoácido de la Fórmula I. En realizaciones más específicas, el análogo de péptido carece de un puente intramolecular, por ejemplo, un puente intramolecular covalente. El análogo de glucagón, por ejemplo, puede ser un análogo de glucagón que comprende uno o más aminoácidos alfa, alfa-disustituidos, por ejemplo, AIB, para estabilizar la hélice alfa del análogo.

Grupos Acilo

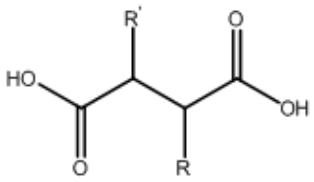
El grupo acilo del aminoácido acilado puede ser una cadena de carbono de cualquier tamaño, por ejemplo de cualquier longitud y puede ser lineal o ramificada. En algunas realizaciones específicas, el grupo acilo es un ácido graso de C_4 a C_{30}. Por ejemplo, el grupo acilo puede ser cualquiera de un ácido graso de C_4, ácido graso de C_6, ácido graso de C_8, ácido graso de C_{10}, ácido graso de C_{12}, ácido graso de C_{14}, ácido graso de C_{16}, ácido graso de C_{18}, ácido graso de C_{20}, ácido graso de C_{22}, ácido graso de C_{24}, ácido graso de C_{26}, ácido graso de C_{28}, o un ácido graso de C_{30}. En algunas realizaciones, el grupo acilo es un ácido graso de C_8 a C_{20}, por ejemplo, un ácido graso de C_{14} o un ácido graso de C_{16}.

En una realización alternativa, el grupo acilo es un ácido biliar. El ácido biliar puede ser cualquier ácido biliar adecuado, que incluye, en forma no taxativa, ácido cólico,

ácido quenodesoxicólico, ácido desoxicólico, ácido litocólico, ácido taurocólico, ácido glicocólico y ácido de colesterol.

En ejemplos de realizaciones, el grupo acilo es un ácido succínico o un derivado del ácido succínico. Por "derivado del ácido succínico" como se usa en la presente se entiende un compuesto que comprende un ácido succínico sustituido o un ácido succínico cíclico sustituido (es decir, anhídrido succínico), o un anhídrido succínico de anillo expandido sustituido (es decir, un anillo de 6-8 miembros que comprende el grupo -C(O)-O-C(O)- y de 3 a 5 átomos de carbono adicionales), en donde el ácido succínico sustituido, el ácido succínico cíclico sustituido (es decir, el anhídrido succínico), o el anhídrido succínico de anillo expandido sustituido se sustituye con una o más cadenas de alquilo o una o más cadenas de carbono con una funcionalidad.

En ejemplos de aspectos, el derivado del ácido succínico comprende una estructura de la Fórmula V:

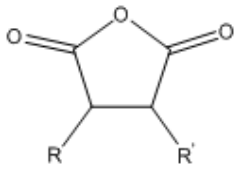


[Fórmula V]

en donde cada uno de R y R' es independientemente H, una cadena de carbono de C_4-C_{30} lineal o ramificada, o una cadena

de carbono con una funcionalidad de C_4–C_{30} lineal o ramificada. En ejemplos de realizaciones, R y/o R’ es una cadena de carbono que comprende una cadena de alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de un alquilo de C_4, un alquilo de C_6, un alquilo de C_8, un alquilo de C_{10}, un alquilo de C_{12}, un alquilo de C_{14}, un alquilo de C_{16}, un alquilo de C_{18}, un alquilo de C_{20}, un alquilo de C_{22}, un alquilo de C_{24}, un alquilo de C_{26}, un alquilo de C_{28}, o un alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo, un alquilo de C_{14} o un alquilo de C_{16}. En ejemplos de aspectos, la cadena de carbono con una funcionalidad comprende un grupo funcional, que incluye, en forma no taxativa, carboxilo, sulfhidrilo, amina, cetilo, sulfoxilo o amido.

En ejemplos de aspectos, el derivado del ácido succínico comprende un anhídrido succínico que comprende una estructura de la Fórmula VI:

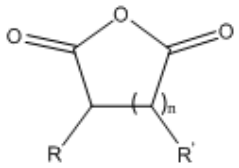


[Fórmula VI]

en donde cada uno de R y R’ es independientemente H, una cadena de carbono de C_4–C_{30} lineal o ramificada, o una cadena de carbono con una funcionalidad de C_4–C_{30} lineal o ramificada.

En ejemplos de realizaciones, R y/o R' es una cadena de carbono que comprende una cadena de alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de un alquilo de C_4, alquilo de C_6, alquilo de C_8, alquilo de C_{10}, alquilo de C_{12}, alquilo de C_{14}, alquilo de C_{16}, alquilo de C_{18}, alquilo de C_{20}, alquilo de C_{22}, alquilo de C_{24}, alquilo de C_{26}, alquilo de C_{28}, o un alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo, un alquilo de C_{14} o un alquilo de C_{16}. En ejemplos de aspectos, la cadena de carbono con una funcionalidad comprende un grupo funcional, que incluye, en forma no taxativa, carboxilo, sulfhidrilo, amina, cetilo, sulfoxilo o amido.

En ejemplos de aspectos, el derivado del ácido succínico es un derivado del anhídrido succínico, que incluye aquellos de la Fórmula VII:



[Fórmula VII]

en donde n es 1–4, y

en donde cada uno de R y R' es independientemente H, una cadena de carbono de C_4–C_{30} lineal o ramificada, o una cadena de carbono con una funcionalidad de C_4–C_{30} lineal o ramificada. En ejemplos de realizaciones, R y/o R' es una cadena de

carbono que comprende una cadena de alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de alquilo de C_4, alquilo de C_6, alquilo de C_8, alquilo de C_{10}, alquilo de C_{12}, alquilo de C_{14}, alquilo de C_{16}, alquilo de C_{18}, alquilo de C_{20}, alquilo de C_{22}, alquilo de C_{24}, alquilo de C_{26}, alquilo de C_{28}, o un alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo un alquilo de C_{14} o un alquilo de C_{16}. En ejemplos de aspectos, la cadena de carbono con una funcionalidad comprende un grupo funcional que incluye, en forma no taxativa, carboxilo, sulfhidrilo, amina, cetilo, sulfoxilo o amido.

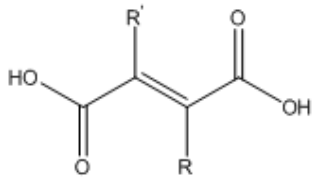
Cuando solamente uno de R y R’ de las Fórmulas V–VI es H, el aminoácido acilado se denomina “Succinoilo de C_x”. Como se usa en la presente, el término “Succinoilo de C_x”, en donde x es un número entero, se refiere a una estructura en donde R es una cadena de alquilo de y carbonos e y = x–1, e y no incluye los carbonos del grupo succinoilo. Por ejemplo, una estructura de la Fórmula VI en donde R es un grupo alquilo de C_{15} y R’ es H se denomina Succinoilo de C_{16}. Por ejemplo, la Figura 3A ilustra un Succinoilo de C_{16} Lys, en donde una Lys se ha succinoilado en la amina. Cuando ni R ni R’ de las Fórmulas V–VI es H, entonces el aminoácido acilado se denomina “Succinoilo de C_x,$C_{x'}$”. Como se usa en la presente, el término “Succinoilo de C_x,$C_{x'}$”, en donde x y x` son números enteros, se

refiere a una estructura en donde R es una cadena de alquilo de y carbonos y R' es una cadena de alquilo de y' carbonos, e y' = x'-1. Por ejemplo, una estructura de la Fórmula VI en donde R es un grupo alquilo de C_{15} y R' es un grupo alquilo de C_{13} se denomina succinoilo de C_{16},C_{14}. Cuando el derivado del ácido succínico es un anhídrido succínico del anillo expandido sustituido y ni R ni R' de la Fórmula VII es H, entonces el aminoácido acilado se denomina "C_x,$C_{x'}$-n-Succinoilo". Como se usa en la presente, el término "C_x,$C_{x'}$-n-Succinoilo", en donde x, x' y n son números enteros, se refiere a una estructura en donde R es una cadena de alquilo de y carbonos, R' es una cadena de alquilo de y' carbonos y el anillo del anhídrido succínico se prolonga en n carbonos. Por ejemplo, una estructura de la Fórmula VII en donde R y R' son grupos alquilo de C_{15} y n = 2 se denomina C_{16},C_{16}-2-Succinoilo.

En ejemplos de realizaciones, el grupo acilo es un ácido maleico o un derivado del ácido maleico. Por "derivado del ácido maleico" como se usa en la presente se entiende un compuesto que comprende un ácido maleico sustituido o un ácido maleico cíclico sustituido (es decir, anhídrido maleico) o un anhídrido maleico de anillo expandido sustituido que comprende el grupo -C(O)-O-C(O)- y de 3 a 5 carbonos adicionales), en donde el ácido maleico sustituido, el ácido maleico cíclico sustituido (es decir, el anhídrido maleico), o el anhídrido

maleico expandido sustituido se sustituye con una o más cadenas de alquilo o una o más cadenas de carbono con una funcionalidad.

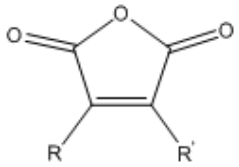
En ejemplos de aspectos, el derivado del ácido maleico comprende una estructura de la Fórmula VIII:



[Fórmula VIII]

en donde cada uno de R y R' es independientemente H, una cadena de carbono de C_4–C_{30} lineal o ramificada, o una cadena de carbono con una funcionalidad de C_4–C_{30} lineal o ramificada. En ejemplos de realizaciones, R y/o R' es una cadena de carbono que comprende una cadena de alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de un alquilo de C_4, alquilo de C_6, alquilo de C_8, alquilo de C_{10}, alquilo de C_{12}, alquilo de C_{14}, alquilo de C_{16}, alquilo de C_{18}, alquilo de C_{20}, alquilo de C_{22}, alquilo de C_{24}, alquilo de C_{26}, alquilo de C_{28}, o alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo, un alquilo de C_{14} o un alquilo de C_{16}. En ejemplos de aspectos, la cadena de carbono con una funcionalidad comprende un grupo funcional, que incluye, en forma no taxativa, carboxilo, sulfhidrilo, amina, cetilo, sulfoxilo o amido.

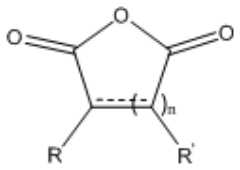
En ejemplos de aspectos, el derivado del ácido maleico comprende un anhídrido maleico que comprende una estructura de la Fórmula IX:



[Fórmula IX]

en donde cada uno de R y R’ es independientemente H, una cadena de carbono de C_4–C_{30} lineal o ramificada, o una cadena de carbono con una funcionalidad de C_4–C_{30} lineal o ramificada. En ejemplos de realizaciones, R y/o R’ es una cadena de carbono que comprende una cadena de alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de un alquilo de C_4, alquilo de C_6, alquilo de C_8, alquilo de C_{10}, alquilo de C_{12}, alquilo de C_{14}, alquilo de C_{16}, alquilo de C_{18}, alquilo de C_{20}, alquilo de C_{22}, alquilo de C_{24}, alquilo de C_{26}, alquilo de C_{28}, o un alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo, un alquilo de C_{14} o un alquilo de C_{16}. En ejemplos de aspectos, la cadena de carbono con una funcionalidad comprende un grupo funcional, que incluye, en forma no taxativa, carboxilo, sulfhidrilo, amina, cetilo, sulfoxilo o amido.

En ejemplos de realizaciones, el derivado del ácido maleico es un derivado del anhídrido maleico, que incluye aquellos de la Fórmula X:



[Formula X]

en donde n es 1-4, hay por lo menos un enlace doble de C=C entre dos carbonos no carbonilo, y

en donde cada uno de R y R' es independientemente H, una cadena de carbono de C_4-C_{30} lineal o ramificada, o una cadena de carbono con una funcionalidad de C_4-C_{30} lineal o ramificada. En ejemplos de realizaciones, R y/o R' es una cadena de carbono que comprende una cadena de alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de un alquilo de C_4, alquilo de C_6, alquilo de C_8, alquilo de C_{10}, alquilo de C_{12}, alquilo de C_{14}, alquilo de C_{16}, alquilo de C_{18}, alquilo de C_{20}, alquilo de C_{22}, alquilo de C_{24}, alquilo de C_{26}, alquilo de C_{28}, o un alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo un alquilo de C_{14} o un alquilo de C_{16}. En ejemplos de aspectos, la cadena de carbono con una funcionalidad comprende un grupo funcional, que incluye, en forma no taxativa, carboxilo, sulfhidrilo, amina, cetilo, sulfoxilo o amido.

Cuando solamente uno de R y R' de las Fórmulas VIII–IX es H, el aminoácido acilado se denomina "Maleoilo de C_x". Como se usa en la presente, el término "Maleoilo de C_x", e donde x es un número entero, se refiere a una estructura en donde R es una cadena de alquilo de y carbonos e y = x–1, e y no incluye los carbonos del grupo de maleoilo. Por ejemplo, una estructura de la Fórmula IX en donde R es un grupo alquilo de C_{15} y R' es un H se denomina Maleoilo de C_{16}. Cuando ni R ni R' de las Fórmulas VIII–IX es H, entonces el aminoácido acilado se denomina "Maleoilo de C_x,$C_{x'}$". Como se usa en la presente, el término "Maleoilo de C_x,$C_{x'}$", en donde x y x' son números enteros, se refiere a una estructura en donde R es una cadena de alquilo de y carbonos y R' es una cadena de alquilo de y' carbonos e y' = x'–1. Por ejemplo, una estructura de la Fórmula IX en donde R es un grupo alquilo de C_{15} y R' es un grupo alquilo de C_{13} se denomina maleoilo de C_{16},C_{14}. Cuando el derivado del ácido maleico es un anhídrido maleico de anillo expandido sustituido y ni R ni R' de la Fórmula X es H, entonces el aminoácido acilado se denomina "C_x,$C_{x'}$–n–Maleoilo". Como se usa en la presente, el término "C_x,$C_{x'}$–n–Maleoilo", en donde x, x' y n son números enteros, se refiere a una estructura en donde R es una cadena de alquilo de y carbonos, R' es una cadena de alquilo de y' carbonos y el anillo de anhídrido maleico se extiende en n carbonos. Por ejemplo, una

estructura de la Fórmula en donde R y R' son grupos alquilo de C_{15} y n = 2 se denomina C_{16},C_{16}-2-Maleoilo.

Métodos de Unión de un grupo acilo

Los métodos adecuados para unir grupos acilo a péptidos a través de aminas, hidroxilos y tioles de los péptidos son conocidos en el arte. Véanse, por ejemplo, el Ejemplo 1 (para métodos de acilación a través de una amina), Miller, *Biochem Biophys Res Commun* 218: 377-382 (1996); Shimohigashi y Stammer, *Int J Pept Protein Res* 19: 54-62 (1982); y Previero et al., *Biochim Biophys Acta* 263: 7-13 (1972) (para métodos de acilación a través de un hidroxilo); y San y Silvius, J Pept Res 66: 169-180 (2005) (para métodos de acilación a través de un tiol); Bioconjugate Chem. "Modificaciones Químicas de Proteínas: Historia y Aplicaciones" páginas 1, 2-12 (1990); Hashimoto et al., Pharmacuetical Res. "Síntesis de Derivados de Palmitoilo de Insulina y su Actividad Biológica" Volumen 6, N° 2 páginas 171-176 (1989).

En algunas realizaciones, el péptido de agonista de GIP comprende un aminoácido acilado mediante la acilación de un alcano de cadena larga por un péptido de agonista de GIP. En aspectos específicos, el alcano de cadena larga comprende un grupo amina, hidroxilo, o tiol (por ejemplo, octadecilamina,

tetradecanol, y hexadecanotiol) que reacciona con un grupo carboxilo, o una forma activada de él, del péptido de agonista de GIP. El grupo carboxilo, o la forma activada de él, del péptido de agonista de GIP pueden formar parte de una cadena lateral de un aminoácido (por ejemplo, ácido glutámico, ácido aspártico) del péptido de agonista de GIP o puede formar parte de la cadena principal del péptido.

En ejemplos de realizaciones, el péptido de agonista de GIP comprende un grupo acilo mediante la acilación del alcano de cadena larga por un separador que se une al péptido de agonista de GIP. En aspectos específicos, el alcano de cadena larga comprende un grupo amina, hidroxilo, o tiol que reacciona con un grupo carboxilo, o una forma activada de él, del separador. Los separadores adecuados que comprenden un grupo carboxilo, o una forma activada de él, se describen en la presente e incluyen, por ejemplo, separadores bifuncionales, por ejemplo, aminoácidos, dipéptidos, tripéptidos, separadores bifuncionales hidrófilos, y separadores bifuncionales hidrófobos.

Como se usa en la presente, el término “forma activada de un grupo carboxilo” se refiere a un grupo carboxilo con la fórmula general R(C=O)X, en donde X es un grupo saliente y R es el análogo de glucagón o el separador. Por ejemplo, las

formas activadas de un grupo carboxilo pueden incluir, en forma no taxativa, cloruros de acilo, anhídridos, y ésteres. En algunas realizaciones, el grupo carboxilo activado es un éster con un grupo saliente éster de N-hidroxisuccinimida (NHS).

Con respecto a estos aspectos, en donde un alcano de cadena larga está acilado por el análogo de glucagón o el separador, el alcano de cadena larga puede ser de cualquier tamaño y puede comprender cualquier longitud de cadena de carbono. El alcano de cadena larga puede ser lineal o ramificada. En ejemplos de aspectos, el alcano de cadena larga es un alcano de C_4 a C_{30}. Por ejemplo, el alcano de cadena larga puede ser cualquiera de un alcano de C_4, alcano de C_6, alcano de C_8, alcano de C_{10}, alcano de C_{12}, alcano de C_{14}, alcano de C_{16}, alcano de C_{18}, alcano de C_{20}, alcano de C_{22}, alcano de C_{24}, alcano de C_{26}, alcano de C_{28}, o un alcano de C_{30}. En algunas realizaciones, el alcano de cadena larga comprende un alcano de C_8 a C_{20}, por ejemplo, un alcano de C_{14}, un alcano de C_{16}, o un alcano de C_{18}.

Además, en algunas realizaciones, un grupo amina, hidroxilo o tiol del péptido de agonista de GIP está acilado con un ácido de colesterol. En una realización específica, el péptido de agonista de GIP está unido al ácido de colesterol a través de

un separador de des-amino Cys alquilada, es decir un separador de ácido 3-mercaptopropiónico alquilado.

Cuando el grupo acilo es un ácido succínico, un derivado del ácido succínico, un ácido maleico o un derivado del ácido maleico, el péptido está succinoilado/maleoilado por la reacción de un grupo amina, hidroxilo o tiol del péptido de agonista de GIP, o separador, con un ácido succínico, derivado del ácido succínico, ácido maleico o derivado del ácido maleico de la Fórmula V, Fórmula VI, Fórmula VII, Fórmula VIII, Fórmula IX, o Fórmula X. Los métodos de succinoilación se describen en la presente.

Grupos Acilo Adicionales

El péptido en algunas o en todas las realizaciones comprende un aminoácido acilado en una posición diferente de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo en una o más cualquiera de las posiciones 10, 14, y 40). La ubicación del aminoácido acilado puede ser cualquier posición dentro del péptido de agonista de GIP, que incluye cualquiera de las posiciones 1-29, una posición del extremo de C al 29° aminoácido (por ejemplo, el aminoácido en la posición 30, 31, 32, 33, 34, 35, 36, 44, 45, 46, 47, etc., en una posición dentro de una prolongación del extremo de C o en el extremo de

C), opcionalmente junto con un segundo aminoácido acilado en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40), siempre que se retenga si no se mejora la actividad del agonista de GIP del análogo de péptido. Ejemplos no taxativos incluyen las posiciones 5, 7, 11, 13, 14, 17, 18, 19, 21, 24, 27, 28, o 29.

Consistentemente con lo antedicho, el análogo de glucagón, en ejemplos de aspectos, comprende dos (o más) aminoácidos acilados y se lo puede considerar un péptido acilado doble o un péptido diacilado, cuando hay dos grupos acilo. En ejemplos de aspectos, la totalidad de los aminoácidos acilados están ubicados en dos posiciones de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40). En ejemplos de aspectos, las acilaciones ocurren en dos de las posiciones 10, 14, y 40. En ejemplos de aspectos, el péptido comprende un primer aminoácido acilado en la posición 10 y un segundo aminoácido acilado en la posición 40.

En aún otros ejemplos de realizaciones, el análogo de glucagón comprende dos (o más) grupos acilo unidos a un solo aminoácido de la cadena principal del péptido. El péptido puede se puede considerar como un péptido acilado doble o un péptido

diacilado, cuando hay dos grupos acilo. Los dos (o más) grupos acilo pueden ser el mismo grupo acilo o diferentes grupos acilo, dispuestos en una formación ramificada o lineal. Por ejemplo, para lograr una formación ramificada, el análogo de glucagón puede comprender un aminoácido acilado (que forma parte de la cadena principal del péptido) unido a un separador que comprende por lo menos tres grupos funcionales, por lo menos dos de los cuales están unidos cada uno en forma covalente a un grupo acilo y uno de los cuales está unido al aminoácido acilado de la cadena principal del péptido. En ejemplos de aspectos, una formación ramificada se puede lograr a través, por ejemplo, de un residuo de Lys que comprende dos grupos amino (una amina de cadena lateral y una alfa amina) para la unión directa a un grupo acilo graso o la unión indirecta a un grupo acilo graso a través de un separador. En ejemplos de aspectos, se puede colocar un separador adicional entre el aminoácido de la cadena principal del péptido y el separador que comprende por lo menos tres grupos funcionales. Por ejemplo, el aminoácido de la cadena principal del péptido puede estar unido (por ejemplo, a través de una cadena lateral) a un primer separador, que, a su vez, está unido a un segundo separador, en donde el segundo separador comprende por lo menos tres grupos funcionales, por lo menos dos de los cuales están unidos en forma covalente a un grupo alquilo y uno de los cuales está unido al primer separador.

En ejemplos de aspectos, en donde los grupos alquilo están dispuestos en una formación lineal, el análogo de glucagón comprende un aminoácido acilado (que forma parte de la cadena principal del péptido) unido directamente a un primer grupo acilo, que, a su vez, está unido a un separador, que a su vez, está unido a un segundo grupo acilo.

Ejemplos de estructuras de compuestos acilados dobles se ilustran en la Figura 5A.

Grupos Hidrófilos y Grupos Acilo

Los péptidos de agonistas de GIP que comprenden un aminoácido acilado opcionalmente también comprenden un grupo hidrófilo. En algunas o en todas las realizaciones, el grupo hidrófilo comprende una cadena de polietilenglicol (PEG). La incorporación de un grupo hidrófilo se logra a través de cualquier medio adecuado, tal como cualquiera de los métodos descritos en la presente. Al respecto, el péptido de agonista de GIP puede comprender la SEQ ID NO 1, que incluye cualquiera de las modificaciones descritas en la presente, en donde por lo menos uno de los aminoácidos en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17 20, y 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40) es un

aminoácido acilado y por lo menos uno de los aminoácidos en la posición 16, 17, 21, 24, o 29, una posición dentro de una prolongación del extremo de C o el aminoácido del extremo de C, es una Cys, Lys, Orn, homo-Cys, o Ac-Phe, cuya cadena lateral está unida en forma covalente a un grupo hidrófilo (por ejemplo, PEG). En algunas realizaciones, el aminoácido en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, y 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40) del péptido de agonista de GIP (opcionalmente a través de un separador) al grupo acilo y ese aminoácido está unido también a un grupo hidrófilo o está unido además a una Cys, Lys, Orn, homo-Cys, o Ac-Phe, que está unida al grupo hidrófilo.

Alternativamente, el análogo de glucagón acilado puede comprender un separador, en donde el separador está acilado y modificado para comprender el grupo hidrófilo. Ejemplos no taxativos de separadores adecuados incluyen un separador que comprende uno o más aminoácidos seleccionados del grupo formado por Cys, Lys, Orn, homo-Cys y Ac-Phe.

En algunos aspectos, el péptido de agonista de GIP en algunas realizaciones está acilado en la misma posición de aminoácido donde está unido un grupo hidrófilo, o en una posición de aminoácido diferente. Ejemplos no taxativos incluyen la

acilación en la posición 9, 10, 12, 13, 14, 16, 17, 20, o 40 (por ejemplo, en una o más de las posiciones 10, 14, y 40) y la pegilación en una o más posiciones en la parte del extremo de C del análogo de glucagón, por ejemplo, en la posición 24, 28 o 29, dentro de la prolongación del extremo de C (por ejemplo, 37-43), o en el extremo de C (por ejemplo, agregando una Cys del extremo de C).

En algunas realizaciones específicas, el péptido de agonista de GIP que comprende un aminoácido acilado carece de un puente intramolecular, por ejemplo un puente intramolecular covalente (por ejemplo, un puente de lactama).

AMINOÁCIDOS QUE COMPRENDEN UN GRUPO ALQUILO NO NATIVO

De acuerdo con algunas o todas las realizaciones, los péptidos de agonistas de GIP que son análogos de glucagón (SEQ ID NO 1) comprenden un aminoácido que comprende un grupo alquilo no nativo (denominado en la presente un “aminoácido alquilado”, independientemente de cómo se prepare, por ejemplo, mediante la incorporación de un aminoácido alquilado previamente en el péptido, o la alquilación del péptido después de la síntesis). En algunos o en todos los aspectos, el aminoácido alquilado está ubicado en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, 37, 38, 39, 40, 41, 42, o 43 (por ejemplo, en una

o más cualquiera de las posiciones 10, 14, y 40). En ejemplos de aspectos, el aminoácido alquilado está ubicado en cualquiera de las posiciones 9, 10, 12, 16, 20, o 40 o en cualquiera de las posiciones 10, 13, 14, 16, 17, o 40. En ejemplos de aspectos, el aminoácido alquilado está ubicado en cualquiera de las posiciones 10, 12, 16, o 40 o en cualquiera de las posiciones 10, 12, o 16. En ejemplos de aspectos, el aminoácido alquilado está ubicado en una o más cualquiera de las posiciones 10, 14, y 40.

El aminoácido alquilado en algunas realizaciones hace que el péptido de agonista de GIP tenga uno o más de (i) una semivida prolongada en circulación, (ii) un inicio de acción retardado, (iii) una duración de acción prolongada, (iv) una resistencia mejorada a las proteasas, tales como DPP-IV, y (v) una potencia aumentada en uno o más cualquiera del receptor de GIP, el receptor de GLP-1 y el receptor de glucagón.

Älquilación Directa

En algunas realizaciones, el grupo alquilo está unido directamente a un aminoácido del péptido de agonista de GIP. De acuerdo con una realización, el péptido de agonista de GIP comprende un grupo alquilo que está unido al péptido a través de una unión de éter, tioéter o amino.

En ejemplos específicos, el péptido de agonista de GIP comprende un grupo alquilo después de la alquilación directa de una amina, hidroxilo o tiol de una cadena lateral de un aminoácido del péptido de agonista de GIP. En algunas o en todas las realizaciones, el grupo alquilo está unido al aminoácido del péptido de agonista de GIP mediante la reacción de la amina, hidroxilo o tiol con un grupo alquilo activado. Los grupos alquilo en algunos aspectos están activados con un grupo saliente, por ejemplo, un halógeno, un éster de sulfonato, piridiltiol, sal de amonio o fenoxilo.

En algunas o en todas las realizaciones, el aminoácido alquilado está ubicado en una de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 40 (por ejemplo, en una cualquiera de las posiciones 10, 14 y 40) o en una de las posiciones 10, 12 o 16. Al respecto, el péptido de agonista de GP comprende la secuencia de aminoácido de SEQ ID NO 1, o una secuencia de aminoácidos modificada de ella, que comprende una o más de las modificaciones de aminoácidos descritas en la presente, en donde por lo menos uno de los aminoácidos en las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 40 (por ejemplo, en una cualquiera de las posiciones 10, 14 y 40) es un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral.

En algunas realizaciones, el aminoácido que comprende una amina de la cadena lateral es un aminoácido de la Fórmula I. En algunas realizaciones, el aminoácido de la Fórmula I es el aminoácido en donde n es 4 (Lys) o n es 3 (Orn). En algunas realizaciones, el aminoácido que comprende una amina de la cadena lateral es un aminoácido aromático que comprende una amina de la cadena lateral. En ejemplos de aspectos, el aminoácido aromático que comprende una amina de la cadena lateral es una 4-amino-fenilalanina (4-aminoPhe), p-amino fenilglicina, p-amino homofenilalanina, o 3-amino tirosina. En ejemplos de aspectos, el aminoácido aromático que comprende una amina de la cadena lateral es 4-amino-Phe.

En otras realizaciones, el aminoácido que comprende un hidroxilo de la cadena lateral es un aminoácido de la Fórmula II. En algunos ejemplos de realizaciones, el aminoácido de la Fórmula II es un aminoácido en donde n es 1 (Ser). En ejemplos de aspectos, el aminoácido de la Fórmula II es el aminoácido en donde n es 2 (homoserina). En ejemplos de realizaciones similares, el aminoácido que comprende un hidroxilo de la cadena lateral es una Thr u homotreonina. En ejemplos similares de realizaciones, el aminoácido que comprende un hidroxilo de la cadena lateral es un aminoácido aromático que comprende un hidroxilo de la cadena lateral. En ejemplos de aspectos, el aminoácido aromático que comprende un hidroxilo

de la cadena lateral es tirosina, homotirosina, metil-tirosina o 3-amino tirosina.

En aún otras realizaciones, el aminoácido que comprende un tiol de la cadena lateral es un aminoácido de la Fórmula III. En algunos ejemplos de realizaciones, el aminoácido de la Fórmula III es el aminoácido en donde n es 1 (Cys). En algunos o en todos los ejemplos de realizaciones, el aminoácido de la Fórmula III está unido en forma covalente a un grupo alquilo, por ejemplo una cadena de carbono sin una funcionalidad o con una funcionalidad. En ejemplos de aspectos, el aminoácido está S-palmitilo-alquilado (es decir, S-palmitato-alquilado) en donde el átomo de azufre de un residuo de Cys está unido en forma covalente al carbono β del palmitato. En otras realizaciones, el aminoácido de la Fórmula III está unido en forma covalente al carbono β de un acetato de Cn a través del átomo de azufre del residuo de Cys, en donde n es un número entero de 4 a 30. Ejemplos de formas diferentes de alquilato de S-palmitilo se muestran en las Figuras 6 y 7.

En aún otras realizaciones, el aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral es un aminoácido disustituido que comprende la misma estructura de la Fórmula I, de la Fórmula II o de la Fórmula III, excepto que el hidrógeno

unido al carbono alfa del aminoácido de la Fórmula I, de la Fórmula II o de la Fórmula III se reemplaza con una segunda cadena lateral.

Separadores de Alquilación

En realizaciones alternativas, el grupo alquilo no nativo está unido a través de un separador a un aminoácido del péptido de agonista de GIP, en donde el separador está posicionado entre el aminoácido del péptido de agonista de GIP y el grupo alquilo no nativo. En algunas realizaciones, el péptido de agonista de GIP está unido en forma covalente al separador, que está unido en forma covalente al grupo alquilo no nativo.

En ejemplos de realizaciones, el análogo de glucagón está modificado para comprender un grupo alquilo no nativo mediante la alquilación de una amina, hidroxilo o tiol de un separador, cuyo separador está unido por una cadena lateral a un aminoácido en una de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo en cualquiera de las posiciones 10, 14, y 40). En ejemplos de realizaciones, el grupo alquilo está unido al separador mediante la reacción de la amina, el hidroxilo o el tiol del separador con un grupo alquilo que tiene un grupo saliente (por ejemplo, halógeno, éster de sulfonato, piridiltiol, sal de amonio, fenoxilo).

El aminoácido al cual está unido el separador puede ser cualquier aminoácido que comprende un grupo que permite la unión al separador. Por ejemplo, el aminoácido que comprende un NH_2, –OH, o –COOH (por ejemplo, Lys, Orn, Ser, Asp, o Glu) de la cadena lateral es adecuado. Al respecto, el análogo de glucagón alquilado puede comprender una secuencia de aminoácido modificada de SEQ ID NO 1, que comprende una o más de las modificaciones de aminoácido descritas en la presente, con por lo menos uno de los aminoácidos en las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37–43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40) modificado hacia cualquier aminoácido que comprende una amina, hidroxilo o carboxilato de la cadena lateral.

En algunas realizaciones, el separador es un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral, o un dipéptido o un tripéptido que comprende un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral. El aminoácido al cual está unido el separador puede ser cualquier aminoácido (por ejemplo, un aminoácido α–sustituido único o doble) que comprende un grupo que permite la unión al separador. Por ejemplo, un aminoácido que comprende un NH_2, –OH, o –COOH (por ejemplo, Lys, Orn, Ser, Asp, o Glu) de la cadena lateral es adecuado. Al respecto, el péptido de agonista de

GIP en algunos aspectos comprende la secuencia de aminoácidos de SEQ ID NO 1, o una secuencia de aminoácidos modificada de ella que comprende una o más de las modificaciones de aminoácidos descritas en la presente, en donde por lo menos uno de los aminoácidos en las posiciones 9, 10, 12, 13, 14, 16, 17, 20, y 37-43 (por ejemplo, en cualquiera de las posiciones 10, 14, y 40) es un aminoácido que comprende una amina, hidroxilo o carboxilato de la cadena lateral.

En algunos aspectos, el separador es un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral, o un dipéptido o tripéptido que comprende un aminoácido que comprende una amina, hidroxilo o tiol de la cadena lateral.

Cuando el grupo alquilo está unido a través de un grupo amina de un separador, el grupo alquilo en algunos aspectos está unido a través de la alfa amina o a través de una amina de la cadena lateral del aminoácido del separador. En el caso de que el grupo alquilo esté unido a través de una alfa amina, el aminoácido del separador puede ser cualquier aminoácido. Por ejemplo, el aminoácido del separador puede ser un aminoácido hidrófobo, por ejemplo, Gly, Ala, Val, Leu, Ile, Trp, Met, Phe, Tyr, ácido 6-amino hexanoico, ácido 5-aminovalérico, ácido 7-aminoheptanoico, y ácido 8-aminooctanoico. Alternativamente, en algunos aspectos, el aminoácido del separador es un residuo

ácido, por ejemplo, Asp, Glu, ácido homoglutámico, ácido homocisteico, ácido cisteico, ácido gamma-glutámico.

En el caso de que el grupo alquilo esté unido a través de una amina de la cadena lateral del separador de aminoácido, el separador es un aminoácido que comprende una amina de la cadena lateral, por ejemplo, un aminoácido de la Fórmula I (por ejemplo, Lys u Orn). Al respecto, es posible que tanto la alfa amina como la amina de la cadena lateral del aminoácido del separador esté unida a un grupo alquilo, de manera tal que el péptido de agonista de GIP esté dialquilado. Las realizaciones de la invención incluyen dichas moléculas dialquiladas. En algunas realizaciones, el grupo alquilo está unido a una 4-amino-Phe, p-amino fenilglicina, p-amino homofenilalanina, o 3-amino tirosina.

Cuando el grupo alquilo está unido a través de un grupo hidroxilo del separador, el aminoácido o uno de los aminoácidos del dipéptido o tripéptido puede ser un aminoácido de la Fórmula II. En un ejemplo específico de realización, el aminoácido es Ser. En ejemplos similares de realizaciones, el grupo alquilo está unido a una Thr u homotreonina. En ejemplos similares de realizaciones, el grupo alquilo está unido a través del hidroxilo de un aminoácido aromático que comprende

un hidroxilo de la cadena lateral, por ejemplo, tirosina, homotirosina, metil-tirosina o 3-amino tirosina.

Cuando el grupo alquilo está unido a través de un grupo tiol de un separador, el aminoácido o uno de los aminoácidos del dipéptido o tripéptido puede ser un aminoácido de la Fórmula III. En un ejemplo específico de realización, el aminoácido es Cys. Cuando el grupo alquilo está unido a través de un separador, el aminoácido o uno de los aminoácidos del dipéptido o tripéptido puede ser un aminoácido de la Fórmula III. En un ejemplo específico de realización, el aminoácido es Cys. En ejemplos de realizaciones, el separador es un residuo de Cys, que está unido en forma covalente a un grupo alquilo, por ejemplo, una cadena de carbono sin funcionalidad o con funcionalidad. En ejemplos de aspectos, el residuo de Cys está alquilado con S-palmitilo (es decir, alquilado con S-palmitato), opcionalmente en donde el residuo de Cys está unido a un residuo de Lys que forma parte de la cadena principal del péptido. En realizaciones alternativas, el separador es un dipéptido que comprende un residuo de Cys, que está unido en forma covalente a un grupo alquilo. En ejemplos de aspectos, la Cys está alquilada con S-palmitilo, y la Cys está unida a otro aminoácido del separador, que, a su vez, está unido, por ejemplo, a un residuo de Lys que forma parte de la cadena principal del péptido. Otro ejemplo de

alquilación de S-palmitilo se proporciona en la presente en el Ejemplo 20.

En otros ejemplos de realizaciones, el separador es un separador bifuncional que comprende (i) un primer extremo que comprende un grupo saliente que reacciona con un grupo alquilo que comprende una amina, hidroxilo o tiol y (ii) un segundo extremo que comprende un grupo funcional que reacciona con la cadena lateral del aminoácido del análogo de glucagón. En ejemplos de aspectos, el aminoácido del análogo de glucagón es un aminoácido de la Fórmula I (por ejemplo, Lys) o de la Fórmula II (por ejemplo, Ser) y el aminoácido tiene una funcionalidad con un ácido carboxílico o derivado del ácido carboxílico. En ejemplos alternativos de aspectos, el aminoácido del análogo de glucagón es un aminoácido de la Fórmula III y el aminoácido tiene una funcionalidad con una haloacetamida, un maleimido, o disulfuro. En algunas realizaciones, el aminoácido del análogo de glucagón es un aminoácido que comprende un carboxilato de la cadena lateral, por ejemplo, Glu, Asp, con una funcionalidad de amina, hidroxilo o tiol.

En algunas o en todas las realizaciones, el separador es un separador bifuncional hidrófilo. En ejemplos de realizaciones, el separador bifuncional hidrófilo comprende dos o más grupos

reactivos, por ejemplo, una amina, un hidroxilo, un tiol y un grupo carboxilo o cualquier combinación de ellos. En ejemplos de realizaciones, el separador bifuncional hidrófilo comprende un grupo hidroxilo y un carboxilato. En otras realizaciones, el separador bifuncional hidrófilo comprende un grupo amina y un carboxilato. En otras realizaciones, el separador bifuncional hidrófilo comprende un grupo tiol y un carboxilato. En una realización específica, el separador comprende un amino polialquiloxicarboxilato. Al respecto, el separador puede comprender, por ejemplo, $NH_2(CH_2CH_2O)_n(CH_2)_mCOOH$, en donde m es cualquier número entero de 1 a 6 y n es cualquier número entero de 2 a 12, tal como, por ejemplo, ácido 8-amino-3,6-dioxaoctanoico, que está comercialmente disponible en Peptides International, Inc. (Louisville, KY).

En ejemplos de realizaciones, el separador comprende un grupo polietilenglicol pequeño (PEG) que comprende una estructura $[-O-CH_2-CH_2-]_n$, en donde n es un número entero entre 2 y 16, (por ejemplo, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Dichos PEG pequeños se denominan en la presente “miniPEG”. En ejemplos de aspectos, el miniPEG es un miniPEG con una funcionalidad que comprende uno o más grupos funcionales. Los grupos funcionales adecuados incluyen, en forma no taxativa, una amina, un hidroxilo, un tiol y un grupo carboxilo o cualquier combinación de ellos. En ejemplos de aspectos, el

miniPEG es un ácido de miniPEG que comprende una estructura {[-O-CH_2-CH_2-]$_n$-COO-}, en donde n es lo definido anteriormente. En ejemplos de aspectos, el miniPEG es un amido miniPEG que comprende una estructura {-N- CH_2- CH_2-[-O-CH_2-CH_2-]$_n$}, en donde n es lo definido anteriormente. En ejemplos de aspectos, la miniPEG es un ácido de amido miniPEG que comprende una estructura {-N- CH_2- CH_2-[-O-CH_2-CH_2-]$_n$-COO-}, en donde n es lo definido anteriormente. Los reactivos adecuados para su uso en la alquilación de un aminoácido con un miniPEG están comercialmente disponibles en vendedores, tales como Peptides International (Louisville, KY).

En algunas realizaciones, el separador es un separador bifuncional hidrófobo. Los separadores bifuncionales hidrófobos son conocidos en el arte. Véase, por ejemplo, *Técnicas de Bioconjugados*, G. T. Hermanson (Academic Press, San Diego, CA, 1996), que se incorpora como referencia en su totalidad. En ejemplos de realizaciones, el separador bifuncional hidrófobo comprende dos o más grupos reactivos, por ejemplo, una amina, un hidroxilo, un tiol y un grupo carboxilo o cualquier combinación de ellos. En ejemplos de realizaciones, el separador bifuncional hidrófobo comprende un grupo hidroxilo y un carboxilato. En otras realizaciones, el separador bifuncional hidrófobo comprende un grupo amino y un carboxilato. En otras realizaciones, el separador bifuncional

hidrófobo comprende un grupo tiol y un carboxilato. Los separadores bifuncionales hidrófobos adecuados que comprenden un carboxilato y un grupo hidroxilo o un grupo tiol son conocidos en el arte e incluyen, por ejemplo, ácido S-hidroxioctanoico y ácido S-mercaptooctanoico.

En algunas realizaciones, el separador bifuncional no es un ácido dicarboxílico que comprende un metileno no ramificado de 1-7 átomos de carbono entre los grupos carboxilato. En algunas realizaciones, el separador bifuncional es un ácido dicarboxílico que comprende un metileno no ramificado de 1-7 átomos de carbono entre los grupos carboxilato.

El separador (por ejemplo, aminoácido, dipéptido, tripéptido, separador bifuncional hidrófilo o separador bifuncional hidrófobo) en realizaciones específicas tiene de 3 a 10 átomos (por ejemplo, de 6 a 10 átomos, (por ejemplo, 6, 7, 8, 9 o 10 átomos) de longitud. En realizaciones más específicas, el separador tiene de 3 a 10 átomos (por ejemplo, de 6 a 10 átomos) de longitud y el grupo alquilo no nativo es un alquilo de C_{12} a C_{18}, por ejemplo, alquilo de C_{14}, alquilo de C_{16}, de manera tal que la longitud total del separador y el grupo alquilo sea de 14 a 28 átomos, por ejemplo 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, o 28 átomos. En algunas

realizaciones, la longitud del separador y el grupo alquilo tiene de 17 a 28 (por ejemplo, de 19 a 26, de 19 a 21) átomos.

De acuerdo con algunas o la totalidad de las realizaciones precedentes, el separador bifuncional puede ser un aminoácido sintético o natural (que incluye, en forma no taxativa, cualquiera de aquellos descritos en la presente) que comprende una cadena principal de aminoácido que tiene de 3 a 10 átomos de longitud (por ejemplo, ácido 6-amino hexanoico, ácido 5-aminovalérico, ácido 7-aminoheptanoico y ácido 8-aminooctanoico. Alternativamente, el separador puede ser un separador de dipéptido o tripéptido que tiene una cadena principal de péptido que tiene de 3 a 10 átomos (por ejemplo, de 6 a 10 átomos) de longitud. Cada aminoácido del separador de dipéptido o de tripéptido puede ser igual o diferente del aminoácido(s) del dipéptido o tripéptido y se puede seleccionar independientemente del grupo formado por: aminoácidos naturales o codificados o no codificados o no naturales, que incluyen, por ejemplo, cualquiera de los isómeros D o L de los aminoácidos naturales (Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, Tyr), o cualquiera de los isómeros D o L de los aminoácidos no naturales o no codificados seleccionados del grupo formado por: β-alanina (β-Ala), N-α-metil-alanina (Me-Ala), ácido aminobutírico (Abu), ácido γ-aminobutírico (γ-Abu),

ácido aminohexanoico (ε-Ahx), ácido aminoisobutírico (Aib), ácido aminometilpirrol carboxílico, ácido aminopiperidinacarboxílico, aminoserina (Ams), ácido aminotetrahidropiran-4-carboxílico, arginina N-metoxi-N-metil amida, ácido β-aspártico (β-Asp), ácido azetidina carboxílico, 3-(2-benzotiazolil)alanina, α-*terc*-butilglicina, ácido 2-amino-5-ureido-n-valérico (citrulina, Cit), β-Ciclohexilalanina (Cha), acetamidometil-cisteína, ácido diaminobutanoico (Dab), ácido diaminopropiónico (Dpr), dihdidroxifenilalanina (DOPA), dimetiltiazolidina (DMTA), ácido γ-Glutámico (γ-Glu), homoserina (Hse), hidroxiprolina (Hyp), isoleucina N-metoxi-N-metil amida, metil-isoleucina (MeIle), ácido isonipecótico (Isn), metil-leucina (MeLeu), metil-lisina, dimetil-lisina, trimetil-lisina, metanoprolina, sulfóxido de metionina (Met(O)), metionina-sulfona (Met(O_2)), norleucina (Nle), metil-norleucina (Me-Nle), norvalina (Nva), ornitina (Orn), ácido para-aminobenzoico (PABA), penicilamina (Pen), metilfenilalanina (MePhe), 4-Clorofenilalanina (Phe(4-Cl)), 4-fluorofenilalanina (Phe(4-F)), 4-nitrofenilalanina (Phe(4-NO_2)), 4-cianofenilalanina ((Phe(4-CN)), fenilglicina (Phg), piperidinilalanina, piperidinilglicina, 3,4-dehidroprolina, pirrolidinilalanina, sarcosina (Sar), selenocisteina (Sec), O-Bencil-fosfoserina, ácido 4-amino-3-hidroxi-6-metilheptanoico (Sta), ácido 4-amino-5-ciclohexil-3-hidroxipentanoico (ACHPA),

ácido 4-amino-3-hidroxi-5-fenillpentanoico (AHPPA), ácido 1,2,3,4,-tetrahidro-isoquinolina-3-carboxílico (Tic), tetrahidropiranglicina, tienilalanina (Thi) , O-bencil-fosfotirosina, O-fosfotirosina, metoxitirosina, etoxitirosina, O-(bis-dimetilamino-fosfono)-tirosina, tetrabutilamina de sulfato de tirosina, metil-valina (MeVal), y ácido 3-mercaptopropiónico alquilado. En ejemplos de aspectos, el separador es un residuo de Cys.

En algunas realizaciones, el separador comprende una carga negativa general, por ejemplo, comprende uno o dos aminoácidos con carga negativa. En algunas realizaciones, el dipéptido no es ninguno de los dipéptidos de la estructura general A-B, en donde A se selecciona del grupo formado por Gly, Gln, Ala, Arg, Asp, Asn, Ile, Leu, Val, Phe, y Pro, en donde B se selecciona del grupo formado por Lys, His, Trp. En algunas realizaciones, los aminoácidos del separador de dipéptido se seleccionan del grupo formado por: Ala, β-Ala, Leu, Pro, ácido γ-aminobutírico, Glu, y γ-Glu.

En algunos ejemplos de realizaciones, el péptido de agonista de GIP comprende un grupo alquilo después de la alquilación de una amina, hidroxilo, o tiol de un separador, cuyo separador está unido a una cadena lateral de un aminoácido en la posición 9, 10, 12, 13, 14, 16, 17, 20, o 37-43, (por ejemplo,

en una o más cualquiera de las posiciones 10, 14, y 40) o el aminoácido del extremo de C del péptido de agonista de GIP.

En aún más realizaciones específicas, el grupo alquilo está unido al aminoácido en cualquiera de las realizaciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40), del análogo de péptido y la longitud del separador y el grupo alquilo tiene de 14 a 28 átomos. El aminoácido en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43, (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40), en algunos aspectos, es un aminoácido de la Fórmula I, por ejemplo, Lys, o un aminoácido disustituido relacionado con la Fórmula I. En muchas realizaciones específicas, el análogo de péptido carece de un puente intramolecular, por ejemplo, un puente intramolecular covalente. El análogo de glucagón, por ejemplo, puede ser un análogo de glucagón que comprende uno o más aminoácido alfa,alfa-disustituido, por ejemplo, AIB, para estabilizar la hélice alfa del análogo.

Grupos Alquilo

El grupo alquilo no nativo del aminoácido alquilado puede ser una cadena de carbono de cualquier tamaño, de cualquier tamaño y puede ser lineal o ramificada. En algunas realizaciones

específicas, el grupo alquilo es un alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de un alquilo de C_4, alquilo de C_6, alquilo de C_8, alquilo de C_{10}, alquilo de C_{12}, alquilo de C_{14}, alquilo de C_{16}, alquilo de C_{18}, alquilo de C_{20}, alquilo de C_{22}, alquilo de C_{24}, alquilo de C_{26}, alquilo de C_{28}, o alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo, un alquilo de C_{14} o un alquilo de C_{16}.

En ejemplos de realizaciones, el grupo alquilo no nativo del aminoácido alquilado es una cadena de carbono lineal o ramificado con una funcionalidad de cualquier longitud. En algunas realizaciones específicas, la cadena de carbono es un alquilo de C_4 a C_{30}. Por ejemplo, el grupo alquilo puede ser cualquiera de un alquilo de C_4, alquilo de C_6, alquilo de C_8, alquilo de C_{10}, alquilo de C_{12}, alquilo de C_{14}, alquilo de C_{16}, alquilo de C_{18}, alquilo de C_{20}, alquilo de C_{22}, alquilo de C_{24}, alquilo de C_{26}, alquilo de C_{28}, o un alquilo de C_{30}. En algunas realizaciones, el grupo alquilo es un alquilo de C_8 a C_{20}, por ejemplo, un alquilo de C_{14} o n alquilo de C_{16}. En ejemplos de aspectos, la cadena de carbono con una funcionalidad comprende un grupo funcional, que incluye, en forma no taxativa, carboxi, sulfhidrilo, amina, cetilo, sulfoxilo o amido.

En ejemplos de realizaciones, el grupo alquilo no nativo es una cadena de carbono con funcionalidad de carboxi de la estructura $-C_x-COOH$, en donde x es un número entero, opcionalmente un número entero entre 4 y 30 (por ejemplo, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30), en donde el carbono de carboxi es el alfa carbono y cada uno de los carbonos de C_x está designado beta, gamma, delta, épsilon, etc., en donde el beta carbono está unido al alfa carbono. Por ejemplo, en donde cuando x es 4, el grupo alquilo no nativo está designado de la siguiente manera: $C_\varepsilon-C_\delta-C_\gamma-C_\beta-C_\alpha OOH$. En ejemplos de realizaciones, la cadena de carbono con funcionalidad de carboxi está unida a través de un carbono diferente del carbono de carboxi, es decir, uno de los carbonos de C_x. En ejemplos de aspectos, la cadena de carbono con funcionalidad de carboxi está unida a través de un beta, gamma, delta, o épsilon carbono de la cadena de carbono con funcionalidad de carboxi a la cadena lateral del aminoácido alquilado. En realizaciones alternativas, la cadena de carbono con funcionalidad de carboxi está unida a través del beta, gamma, delta, o épsilon carbono de la cadena de carbono con funcionalidad de carboxi a la cadena lateral de un separador cuyo separador está unido al aminoácido alquilado. En ejemplos de aspectos, la cadena de carbono con una funcionalidad de carboxi está unida a través del beta carbono de la cadena de

carbono con funcionalidad de carboxi a la cadena lateral del aminoácido alquilado. En realizaciones alternativas, la cadena de carbono con la funcionalidad de carboxi está unida a través del beta carbono de la cadena de carbono con la funcionalidad de carboxi a la cadena lateral de un separador cuyo separador está unido al aminoácido alquilado.

Métodos de Unión de un grupo Alquilo

Los métodos para unir un grupo alquilo a un aminoácido son conocidos en el arte. Por ejemplo, un grupo alquilo activado con un grupo saliente puede reaccionar con un aminoácido que comprende una cadena lateral nucleófila, por ejemplo, una cadena lateral que comprende una amina, hidroxilo, o tiol. El grupo saliente en ejemplos de aspectos es un halógeno, un éster de sulfonato, piridiltiol, sal de amonio o fenoxilo.

En ejemplos de realizaciones, el aminoácido se ha de unir a un grupo alquilo es un residuo de Cys y el átomo de azufre está alquilado, por ejemplo, “S-alquilado”. En ejemplos de realizaciones, el sulfato de la Cys reacciona con el grupo saliente de un grupo acilo que comprende una cadena de carbono con una funcionalidad de carboxi de la estructura $-C_x-COOH$, en donde x es un número entero, opcionalmente un número entero entre 4 y 30 (por ejemplo, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30), en donde el carbono de carboxi es el alfa carbono y cada uno de los carbonos de C_x está designado beta, gamma, delta, épsilon, etc., en donde el beta carbono está unido al alfa carbono. Por ejemplo, en donde cuando x es 4, el grupo alquilo no nativo se designa de la siguiente manera: C_ε–C_δ–C_γ–C_β–C_αOOH. En ejemplos de realizaciones, la cadena de carbono con una funcionalidad de carboxi está unida a través de un carbono diferente del carbono de carboxi, es decir uno de los carbonos de C_x. En ejemplos de aspectos, la cadena de carbono con una funcionalidad de carboxi está unida a través del beta, gamma, delta, o épsilon carbono de la cadena de carbono con una funcionalidad de carboxi a la cadena lateral del aminoácido alquilado. En realizaciones alternativas, esta cadena de carbono con una funcionalidad de carboxi está unida a través del beta, gamma, delta, o épsilon carbono de la cadena de carbono con una funcionalidad de carboxi a la cadena lateral de un separador cuyo separador está unido al aminoácido alquilado. En ejemplos de aspectos, la cadena con una funcionalidad de carboxi está unida a través del beta carbono de la cadena de carbono con funcionalidad de carboxi a la cadena lateral del aminoácido alquilado. En realizaciones alternativas, la cadena de carbono con una funcionalidad de carboxi está unida a través del beta carbono de la cadena de carbono con una funcionalidad de carboxi a la cadena lateral

de un separador cuyo separador está unido al aminoácido alquilado.

En ejemplos de aspectos, el grupo saliente es un halógeno, tal como yodo, bromo, cloro, o flúor, ésteres de sulfonato tales como tosilato, triflatos, o fluorosulfonatos, piridiltiol, sal de amonio, sales de diazonio, nitratos, fosfatos o fenoxilo.

En aspectos específicos, el grupo alquilo comprende un grupo saliente de yodo y una cadena de carbono con una funcionalidad de carboxi que comprende un total de 16 carbonos (que incluyen el carbono del carboxilato). La alquilación con dicho ácido yodo-carboxílico se puede denominar “alquilación de S-palmitilo” que es sinónimo con “alquilación de S-palmitato”. Otro ejemplo de alquilación de palmitilo se proporciona en la presente en los Ejemplos 1 y 20.

Grupos Alquilo Adicionales

El péptido en algunos aspectos comprende un aminoácido alquilado en una posición diferente de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40). La ubicación del aminoácido alquilado puede ser cualquier posición dentro del péptido de agonista de GIP, que incluye cualquiera de las posiciones 1-29, un extremo de C al aminoácido de la posición

29ª (por ejemplo, el aminoácido en la posición 30, 31, 32, 33, 34, 35, 36, 44, 45, 46, 47, etc., en una posición dentro de una prolongación del extremo de C o en el extremo de C), opcionalmente junto con un segundo aminoácido alquilado en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40), siempre que se retenga, si no mejora, la actividad de agonista de GIP del análogo de péptido. Ejemplos no taxativos incluyen las posiciones 5, 7, 11, 13, 14, 17, 18, 19, 21, 24, 27, 28, o 29.

Consistentemente con lo antedicho, el análogo de glucagón, en ejemplos de aspectos, comprende dos (o más) aminoácidos alquilados. En ejemplos de aspectos, la totalidad de los aminoácidos alquilados están ubicados en dos posiciones de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, o 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40). En ejemplos de aspectos, el péptido que comprende un primer aminoácido alquilado en la posición 10 y un segundo aminoácido alquilado en la posición 40.

En aún otros ejemplos de realizaciones, el análogo de glucagón comprende grupos alquilo adicionales unidos a un aminoácido de la cadena principal de péptido. Los dos (o más) grupos alquilo pueden ser el mismo grupo alquilo o grupos alquilo diferentes,

dispuestos en una formación ramificada o lineal. Por ejemplo, para lograr una formación ramificada, el análogo de glucagón puede comprender un aminoácido alquilado (que forma parte de la cadena principal de péptido) unido a un separador que comprende por lo menos tres grupos funcionales, por lo menos dos de los cuales están unidos cada uno a un grupo alquilo y uno de los cuales está unido al aminoácido alquilado de la cadena principal de péptido. En ejemplos de aspectos, se puede lograr una formación ramificada, por ejemplo, a través de un residuo de Lys que comprende dos grupos amina (una amina de la cadena lateral y una alfa amina) para la unión directa a un grupo alquilo graso o la unión indirecta a un grupo alquilo graso a través de un separador. En ejemplos de aspectos, se puede colocar un separador adicional entre el aminoácido de la cadena principal del péptido y el separador que comprende por lo menos tres grupos funcionales. Por ejemplo, el aminoácido de la cadena principal del péptido se puede unir (por ejemplo, a través de su cadena lateral) a un primer separador, que, a su vez, está unido a un segundo separador, en donde el segundo separador comprende por lo menos tres grupos funcionales, por lo menos dos de los cuales están unidos cada uno en forma covalente a un grupo alquilo y uno de los cuales está unido al primer separador.

En ejemplos de aspectos, en donde los grupos alquilo están dispuestos en una formación lineal, el análogo de glucagón comprende un aminoácido alquilado (que forma parte de la cadena principal del péptido) directamente unido a un primer grupo alquilo que, a su vez, está unido a un separador, que a su vez, está unido a un segundo grupo alquilo.

Ejemplos de estructuras de los compuestos alquilados dobles son los derivados de los compuestos acilados dobles que se ilustran en la Figura 5A.

Grupos Hidrófilos y Grupos Alquilo

Los péptidos de agonistas de GIP que comprenden un aminoácido alquilado opcionalmente además comprenden un grupo hidrófilo. En algunas realizaciones específicas el grupo hidrófilo comprende una cadena de polietilenglicol (PEG). La incorporación de un grupo hidrófilo en algunos aspectos se realiza por cualquier medio adecuado, tal como cualquiera de los métodos descritos en la presente. Al respecto, el péptido de agonista de GIP puede comprender SEQ ID NO 1, que incluye cualquiera de las modificaciones descritas en la presente, en donde por lo menos uno de los aminoácidos en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, y 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40) del

péptido de agonista de GIP comprende un grupo alquilo y por lo menos uno de los aminoácidos en la posición 16, 17, 21, 24, o 29, una posición dentro de una prolongación del extremo de C, o el aminoácido del extremo de C es una Cys, Lys, Orn, homo-Cys, o Ac-Phe, cuya cadena lateral está unida en forma covalente a un grupo hidrófilo (por ejemplo, PEG). En algunas realizaciones, el aminoácido en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17, 20, y 37-43 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40) del péptido de agonista de GIP está unido (opcionalmente a través de un separador) al grupo alquilo y ese aminoácido también está unido a un grupo hidrófilo o está unido a una Cys, Lys, Orn, homo-Cys, o Ac-Phe, que está unida al grupo hidrófilo.

Alternativamente, el análogo de glucagón alquilado puede comprender un separador, en donde el separador está alquilado y modificado para comprender el grupo hidrófilo. Ejemplos no taxativos de separadores adecuados incluyen un separador que comprende uno o más aminoácidos seleccionados del grupo formado por Cys, Lys, Orn, homo-Cys, y Ac-Phe.

En algunos aspectos, el péptido de agonista de GIP en algunas realizaciones está alquilado en la misma posición de aminoácido donde está unido un grupo hidrófilo, o en una posición de aminoácido diferente. Ejemplos no taxativos

incluyen la alquilación en la posición 9, 10, 12, 13, 14, 16, 17, 20, o 40 (por ejemplo, en una o más cualquiera de las posiciones 10, 14, y 40) y la pegilación en una o más posiciones de la parte del extremo de C del análogo de glucagón, por ejemplo, en la posición 24, 28 o 29, dentro de una prolongación del extremo de C (por ejemplo, 37-43), o en el extremo de C (por ejemplo, agregando una Cys del extremo de C).

Realización de Grupo Alquilo Adicional

En realizaciones específicas, el péptido de agonista de GIP que comprende un aminoácido alquilado carece de un puente intramolecular, por ejemplo, un puente intramolecular covalente (por ejemplo, un puente de lactama).

ESTABILIZACIÓN DE LA HÉLICE ALFA Y AMINOÁCIDOS QUE ESTABILIZAN LA HÉLICE ALFA

Sin estar atados por ninguna teoría particular, los péptidos de agonistas de GIP que son análogos de glucagón comprenden una estructura de hélice, por ejemplo, una hélice alfa. En algunas o en todas las realizaciones, el péptido de agonista de GIP comprende aminoácidos que estabilizan la estructura de la hélice alfa. Por consiguiente, en algunos aspectos, el

péptido de agonista de GIP comprende uno o más aminoácidos que estabilizan la hélice alfa. Como se usa en la presente, el término “aminoácido que estabiliza la hélice alfa” se usa en forma intercambiable con el término “aminoácido que estabiliza la hélice alfa” y se refiere a un aminoácido que proporciona la estabilidad aumentada a una hélice alfa de los péptidos de agonistas de GIP de los cuales forma parte. Véanse, por ejemplo, Lyu et al., *Proc Natl Acad Sci U.S.A.* 88: 5317-5320 (1991); Branden & Tooze, *Introducción a la Estructura de Proteína*, Garland Publishing, Nueva York, NY, 1991; Fasman, *Predicción de la Estructura de Proteína y los Principios de la Conformación de Proteína*, ed. Fasman, Plenum, NY, 1989). Los aminoácidos que promueven la hélice alfa con los propósitos de la presente incluyen, en forma no taxativa, alanina, norvalina, norleucina, ácido alfa aminobutírico, ácido alfa-aminoisobutírico (AIB), leucina, isoleucina, valina, y similares. En algunas realizaciones, el aminoácido que promueve la hélice alfa es cualquier aminoácido que forma parte de la hélice alfa hallado en una proteína natural, por ejemplo, Leu, Phe, Ala, Met, Gly, Ile, Ser, Asn, Glu, Asp, Lys, Arg.

En ejemplos de realizaciones, el aminoácido que promueve la hélice alfa proporciona más estabilidad a la hélice alfa comparado con glicina o alanina. En ejemplos de realizaciones,

el aminoácido que promueve la hélice alfa es un ácido alfa, alfa di-sustituido, por ejemplo AIB.

Hélice alfa: Posición de los aminoácidos que promocionan la hélice alfa

En algunas o en todas las realizaciones de las presentes invenciones, el péptido de agonista de GIP comprende una secuencia de aminoácido que es similar al glucagón nativo (SEQ ID NO 1) y el péptido de agonista de GIP comprende por lo menos un aminoácido que promueve la hélice alfa en una o más de las posiciones 16-21 del análogo de péptido (por ejemplo, una o más de las posiciones 16, 17, 18, 19, 20, 21). En algunas o en todas las realizaciones, el análogo de péptido comprende un aminoácido que promueve la hélice alfa en una, dos, tres o todas las posiciones 16, 17, 20 y 21.

Hélice alfa: Aminoácidos Alfa, Alfa Di-Sustituidos

En algunas o todas las realizaciones, el aminoácido que promueve la hélice alfa es un aminoácido alfa, alfa di-sustituido. En realizaciones específicas, el aminoácido alfa, alfa di-sustituido comprende R^1 y R^2, cada uno de los cuales está unido al alfa carbono, en donde cada uno de R^1 y R^2 se selecciona independientemente del grupo formado por alquilo de

C_1–C_4, opcionalmente sustituido con un hidroxilo, amida, tiol, halo, o R^1 y R^2 junto con el alfa carbono al cual están unidos forman un anillo (por ejemplo, un anillo de C_3–C_8). En ejemplos de realizaciones, cada uno de R^1 y R^2 se selecciona del grupo formado por metilo, etilo, propilo y n-butilo, o R^1 y R^2 juntos forman un ciclooctano o cicloheptano (por ejemplo, ácido 1-aminocicloctano-1-carboxílico). En ejemplos de realizaciones, R^1 y R^2 son iguales. En otras realizaciones, R^1 es diferente de R^2. En ejemplos de aspectos, cada uno de R^1 y R^2 es un alquilo de C_1–C_4. En algunos aspectos, cada uno de R^1 y R^2 es un alquilo de C_1 o de C_2. En ejemplos de realizaciones, cada uno de R^1 y R^2 es metilo, de manera tal que el aminoácido alfa, alfa disustituido es ácido alfa-aminoisobutírico (AIB). En otros ejemplos de realizaciones, el aminoácido alfa,alfa disustituido es ACPC.

En algunos aspectos, el péptido de agonista de GIP descrito en la presente comprende uno o más aminoácidos alfa, alfa di-sustituidos y el péptido de agonista de GIP específicamente carece de un puente intramolecular covalente (por ejemplo, una lactama), ya que el aminoácido alfa, alfa disustituido es capaz de estabilizar la hélice alfa sin un puente covalente. En algunos aspectos, el péptido de agonista de GIP comprende uno o más aminoácidos alfa, alfa di-sustituidos en el extremo de C (alrededor de las posiciones 12-29). En algunas o en

todas las realizaciones, una, dos, tres, cuatro o más de las posiciones 16, 17, 18, 19, 20, 21, 24 o 29 o, una, dos, tres, cuatro, cinco o la totalidad de las posiciones 16, 17, 18, 19, 20, o 21 del péptido de agonista de GIP está sustituida con un aminoácido α, α-disustituido, por ejemplo, un ácido amino iso-butírico (AIB), un aminoácido disustituido con el mismo o un grupo diferente seleccionado de metilo, etilo, propilo, y n-butilo, o con un ciclooctano o cicloheptano (por ejemplo, ácido 1-aminociclooctano-1-carboxílico). Por ejemplo, la sustitución de la posición 20 con AIB mejora la actividad de GIP, sin un puente intramolecular, por ejemplo, un puente intramolecular no covalente (por ejemplo un puente de sal) o un puente intramolecular covalente (por ejemplo, una lactama). En algunas o todas las realizaciones, una, dos, tres o más de las posiciones 16, 20, 21 o 24 están sustituidas con AIB. En ejemplos de realizaciones, una, dos o la totalidad de los aminoácidos que corresponden a las posiciones 2, 16 y 20 del glucagón nativo (SEQ ID NO 1) están sustituidas con un aminoácido alfa, alfa disustituido tal como AIB. En ejemplos de realizaciones, el análogo de glucagón comprende un AIB en las posiciones 2 y 16 o en las posiciones 2 y 20. En otros ejemplos de aspectos, el análogo de glucagón comprende una D-Ser en la posición 2 y un AIB en la posición 16 o en la posición 20.

Hélice Alfa: Puentes Intramoleculares

En algunos ejemplos de realizaciones, el aminoácido que promueve la hélice alfa es un aminoácido que está unido a otro aminoácido del péptido de agonista de GIP a través de un puente intramolecular. En dichas realizaciones, cada uno de estos dos aminoácidos unidos a través de un puente intramolecular se considera un aminoácido que promueve la hélice alfa. En ejemplos de realizaciones, el péptido de agonista de GIP comprende uno o dos puentes intramoleculares. En ejemplos de realizaciones, el péptido de agonista de GIP comprende un puente intramolecular en combinación con por lo menos otro aminoácido que promueve la hélice alfa, por ejemplo, un aminoácido alfa, alfa-disustituido.

En algunas realizaciones, el puente intramolecular es un puente que conecta dos partes del péptido de agonista de GIP a través de enlaces monovalentes, que incluyen, por ejemplo, las interacciones de Van der Waals, enlaces de hidrógeno, enlaces de iónicos, interacciones hidrófobas, interacciones de dipolo-dipolo, y similares. Al respecto, el análogo de glucagón comprende un puente intramolecular no covalente. En algunas realizaciones, el puente intramolecular no covalente es un puente de sal.

En algunas realizaciones, el puente intramolecular es un puente que conecta dos partes del péptido de agonista de GIP a

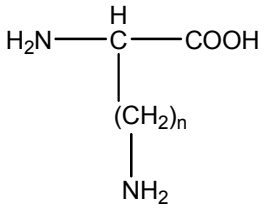
través de enlaces covalentes. Al respecto, el péptido de agonista de GIP comprende un puente intramolecular covalente. En algunas realizaciones, el puente intramolecular (por ejemplo, el puente intramolecular no covalente, puente intramolecular covalente) se forma entre dos aminoácidos que están separados a 3 aminoácidos, por ejemplo, aminoácidos en las posiciones i e i+4, en donde i es cualquiera número entero entre 12 y 25 (por ejemplo, 12 y 25 (por ejemplo, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, y 25). Más particularmente, las cadenas laterales de los pares de aminoácidos 12 y 16, 16 y 20, 20 y 24 o 24 y 28 (pares de aminoácidos en donde i = 12, 16, 20, o 24) están unidos uno a otro y por lo tanto estabilizan la hélice alfa. Alternativamente, i puede ser 17. En algunas realizaciones específicas, el péptido de agonista de GIP comprende un puente intramolecular entre los aminoácidos 17 y 21. En algunas realizaciones, el péptido de agonista de GIP comprende un puente intramolecular entre los aminoácidos en las posiciones 16 y 20 o 12 y 16 y un segundo puente intramolecular entre los aminoácidos en las posiciones 17 y 21. Los péptidos de agonistas de GIP que comprenden uno o más puentes intramoleculares están contemplados en la presente. En realizaciones específicas, en donde los aminoácidos en las posiciones i e i+4 están unidos por un puente intramolecular, el tamaño de la unión es de 8 átomos, o de 7–9 átomos.

En otras realizaciones, el puente intramolecular se forma entre dos aminoácidos que están separados a dos aminoácidos de distancia, por ejemplo, los aminoácidos en las posiciones j y j+3, en donde j es cualquier número entero entre 12 y 26 (por ejemplo, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, y 26). En algunas realizaciones específicas, j es 17. En realizaciones específicas, en donde los aminoácidos de las posiciones j y j+3 están unidos por un puente intramolecular, el tamaño de la unión es de 6 átomos o de 5 a 7 átomos.

En aún otras realizaciones, el puente intramolecular se forma entre dos aminoácidos que están separados a 6 aminoácidos de distancia, por ejemplo, los aminoácidos en las posiciones k y k+7, en donde k es cualquier número entero entre 12 y 22 (por ejemplo, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, y 22). En algunas realizaciones específicas, k es 12, 13, o 17.

Hélice Alfa: Aminoácidos involucrados en puentes intramoleculares

Ejemplos de pares de aminoácidos que son capaces de unirse (en forma covalente o no covalente) para formar un puente de unión de seis átomos incluyen Orn y Asp, Glu y un aminoácido de la Fórmula I, en donde n es 2, y el ácido homoglutámico y un aminoácido de la Fórmula I, en donde n es 1, en donde la Fórmula I:



en donde n = 1 a 4

[Fórmula I]

Ejemplos de pares de aminoácidos que son capaces del enlace para formar un puente de unión de siete átomos incluyen Orn-Glu (anillo de lactama); Lys-Asp (lactama); u Homoser-Homoglu (lactona). Ejemplos de pares de aminoácidos que pueden formar una unión de ocho átomos incluyen Lys-Glu (lactama); Homolys-Asp (lactama); Orn-Homoglu (lactama); 4-aminoPhe-Asp (lactama); o Tyr-Asp (lactona). Ejemplos de pares de aminoácidos que pueden formar una unión de nueve átomos incluyen Homolys-Glu (lactama); Lys-Homoglu (lactama); 4-aminoPhe-Glu (lactama); o Tyr-Glu (lactona). Cualquiera de las cadenas laterales sobre estos aminoácidos puede además sustituirse con otros grupos químicos adicionales, siempre que la estructura tridimensional de la hélice alfa no esté interrumpida. Un experto en el arte puede prever pares alternativos o análogos de aminoácidos alternativos, que incluyen derivados modificados químicamente, que crean una estructura de estabilización de un tamaño similar o con un efecto deseado. Por ejemplo, un puente de homocisteína-

disulfuro de homocisteína se puede modificar además para proporcionar el efecto deseado.

Aún sin una unión covalente, los pares de aminoácidos descritos anteriormente (o pares similares que un experto en el arte puede prever) también proporcionan estabilidad agregada a la hélice alfa a través de enlaces no covalentes, por ejemplo, a través de la formación de puentes de sal o interacciones de enlace de hidrógeno. Por consiguiente, se pueden formar puentes de sal entre: Orn y Glu; Lys y Asp; Homo-serina y Homo-glutamato; Lys y Glu; Asp y Arg; Homo-Lys y Asp; Orn y Homo-Glutamate; 4-aminoPhe y Asp; Tyr y Asp; Homo-Lys y Glu; Lys y Homo-Glu; 4-aminoPhe y Glu; o Tyr y Glu. En algunas realizaciones, el análogo comprende un puente de sal entre cualquiera de los siguientes pares de aminoácidos: Orn y Glu; Lys y Asp; Lys y Glu; Asp y Arg; Homo-Lys y Asp; Orn y Homo-Glutamate; Homo-Lys y Glu; y Lys y Homo-Glu. Se pueden formar puentes de sal entre otros pares de cadenas laterales con cargas opuestas. Véanse, por ejemplo, Kallenbach et al., *Función del Enlace de Péptido en la Estructura y el Plegamiento de las Proteínas*, en La Unión de Amida: Importancia Estructural en las Ciencias Químicas, Bioquímicas y de los Materiales, John Wiley & Sons, Inc. (2000).

En algunas realizaciones, el puente intramolecular no covalente es un puente hidrófobo. De acuerdo con una realización, el hélice alfa del análogo se estabiliza por medio de la incorporación de aminoácidos hidrófobos en las posiciones j y j+3 o i y i+4. Por ejemplo, i puede ser Tyr y i+4 puede ser ya sea Val o Leu; i puede ser Phey i+4 puede ser Met; o i puede ser Phe y i+4 puede ser Ile. Debe entenderse que, a efectos de la presente, los acoplamientos de aminoácidos previamente mencionados pueden revertirse, de manera tal que el amino ácido que se indicó que se encontraba en la posición i puede alternativamente ubicarse en i+4, mientras que el amino ácido i+4 puede ubicarse en la posición i. Debe comprenderse también que se pueden realizar acoplamientos de aminoácidos adecuados para j y j+3.

Hélice alfa: Puente Covalente Intramolecular

En algunas realizaciones, el puente covalente intramolecular es un anillo de lactama o un puente de lactama. El tamaño del anillo de lactama puede variar, dependiendo de la longitud de las cadenas laterales de aminoácidos, y en una realización la lactama se forma a través del enlace de las cadenas laterales de una ornitina con una cadena lateral de ácido aspártico. Los puentes de lactama y los métodos para hacerlos son conocidos en el estado de la técnica. Véase, por ejemplo, Houston, Jr., et al., *J PeptideSci* 1: 274-282 (2004), y el Ejemplo 1 de la

presente. En algunas realizaciones, el análogo comprende una secuencia modificada de SEC ID NO: 1 y un puente de lactama entre i y i+4, en donde i es como se lo define precedentemente. En algunas realizaciones, el péptido agonista de GIP comprende dos puentes de lactama: uno entre aminoácidos en posiciones 16 y 20 y otro entre amino ácidos en las posiciones 17 y 21. En algunas realizaciones, el péptido agonista de GIP comprende un puente de lactama y un puente de sal. En la presente se describen otras realizaciones ejemplares, en la sección titulada “EJEMPLOS”. Otras realizaciones ejemplares incluyen los siguientes acoplamientos, opcionalmente con un puente de lactama: Glu en la posición 12 con Lys en la posición 16; Lys nativo en la posición 12 con Glu en la posición 16; Glu en la posición 16 con Lys en la posición 20; Lys en la posición 16 con Glu en la posición 20; Glu en la posición 20 con Lys en la posición 24; Lys en la posición 20 con Glu en la posición 24; Glu en la posición 24 con Lys en la posición 28; Lys en la posición 24 con Glu en la posición 28.

En algunas realizaciones, el puente intramolecular covalente es una lactona. En el estado de la técnica se conocen métodos adecuados para hacer puentes de lactona. Véase, por ejemplo, Sheehan et al., *J Am ChemSoc* 95: 875-879 (1973).

En algunos aspectos, la metátesis de olefinas se usa para realizar enlaces cruzados de uno o dos giros del hélice alfa del análogo a través de un sistema de enlace cruzado de todos los hidrocarburos. El péptido agonista de GIP en esta instancia comprende amino ácidos α-metilados que llevan cadenas laterales olefínicas de diferentes longitudes y configuradas ya sea con estereoquímica R o Sen las posiciones de j y j+3 o i y i+4. En algunas realizaciones, el lado olefínico comprende (CH_2)n, en donde n es un número entero entre 1 y 6. En algunas realizaciones, n es 3 para un enlace cruzado de una longitud de 8 átomos. En algunas realizaciones, n es 2 para un enlace cruzado de una longitud de 6 átomos. Un péptido agonista de GIP ejemplar que comprende un enlace cruzado olefínico se describe en la presente como SEC ID NO: 17. En el estado de la técnica se conocen métodos adecuados para formar tales puentes intramoleculares. Véase, por ejemplo, Schafmeister et al., *J. Am. Chem. Soc.* 122: 5891-5892 (2000) y Walenski et al., *Science* 305: 1466-1470 (2004). En realizaciones alternativas, el análogo comprende residuos Ser O-alílico ubicados en giros adyacentes helicoidales, que forman un puente juntos a través de metátesis de cierre de anillo por rutenio catalizado. Tales procedimientos de enlace cruzado se describen, por ejemplo, en Blackwell et al., *Angew, Chem.*, Int. Ed. 37: 3281-3284 (1998).

En aspectos específicos, el uso de amino ácido tio-dialanina no natural, lantionina, que ha sido ampliamente adoptado como un péptido mimético de cistina, se usa para un enlace cruzado de un giro dela alfa hélice. En el estado del arte se conocen métodos de ciclación basados en lantionina. Véase, por ejemplo, Matteucci et al., *TetrahedronLetters* 45: 1399-1401 (2004); Mayer et al., *J. Peptide Res*. 51: 432-436 (1998); Polinsky et al., J. Med. Chem. 35: 4185-4194 (1992); Osapay et al., *J. Med. Chem.* 40: 2241-2251 (1997); Fukase et al., *Bull. Chem. Soc. Jpn.* 65: 2227-2240 (1992); Harppwr L., *J. Org. Chem.* 36: 73-80 (1971); Goodman y Shao, *Pure Appl. Chem*. 68: 1303-1308 (1996); y Osapay y Goodman, J. *Chem. Soc. Chem. Commun.* 1599-1600 (1993).

En algunas realizaciones, se usan ataduras de α, ω-diaminoalcano, por ejemplo, 1,4-diaminopropano y 1,5-diaminopentano, entre dos residuos Glu en las posiciones i y i+7 para estabilizar el alfa hélice del análogo. Dichas ataduras conducen a la formación de un puente de 9 átomos o más de longitud, dependiendo de la longitud de la atadura de diaminoalcano. En el arte previo se describen métodos adecuados para producir péptidos con enlace cruzado con dichas ataduras. Véase, porejemplo, Phelan et al., *J. Am. Chem. Soc*. 119: 455-460 (1997).

En aún otras realizaciones, se usa un puente de disulfuro para generar un enlace cruzado de uno o dos giros del alfa hélice del análogo. Alternativamente, un puente de disulfuro modificado en uno o ambos átomos de sulfuro se reemplazan con un grupo metileno que resulta de una macrociclaciónisostérica se usa para estabilizar el alfa hélice del análogo. Métodos adecuados para modificar péptidos con puentes de disulfuro o ciclaciones basadas en sulfuro se describen, por ejemplo, en Jackson et al., *J. Am. Chem. Soc.* 113: 9391-9392 (1991) y Rudinger y Jost, *Experientia* 20: 570-571 (1964).

En aún otras realizaciones, el alfa hélice del análogo se estabiliza a través de la unión de un átomo de metal con dos residuos His o un par His y Cys ubicado en j y j+3 y i+4. El átomo de metal puede ser, por ejemplo, Ru(III), Cu(II), Zn(II), o Cd(II). Tales métodos para la estabilización del alfa hélice basada en la unión con metal se conocen en el arte previo. Veáse, por ejemplo, Andrews y Tabor, *Tetrahedron* 55: 11711-11743 (1999); Ghadiri et al., *J. Am. Chem. Soc.* 112: 1630-1632 (1990); y Ghadiri et al., *J. Am. Chem. Soc.* 119: 9063-9064 (1997).

El alfa hélice del péptido agonista de GIP puede alternativamente ser estabilizada por otros medios de ciclación de péptidos, los cuales se analizan en Davies, J.

PeptideSci. 9: 471-501 (2003). El alfa hélice se puede estabilizar por medio de la formación de un puente amida, puente tioéter, puente tioéster, puente urea, puente carbamato, puente sulfonamida, y similares. Por ejemplo, un puente tioéster se puede formar entre el extremo de C y la cadena lateral de un residuo de Cys. Alternativamente, un tioéster se puede formar a través de cadenas laterals de aminoácidos que tienen un tiol (Cys) y un ácido carboxílico (por ejemplo, Asp, Glu). En otro método, un agente de enlace cruzado, por ejemplo ácido dicarboxílico, por ejemplo, ácido subérico (ácido octanodioico), etc. puede introducir un enlance entre dos grupos funcionales de una cadena lateral de aminoácido, como por ejemplo un amino libre, hidroxilo, grupo tiol, y combinaciones de éstos.

DESCRIPCIONES ADICIONALES

A continuación se proporcionan descripciones adicionales de los análogos de glucagón de las presents divulgaciones. Como se discute en la presente, la posición del aminoácido en las descripciones que figuran a continuación se referencia según la numeración del amino ácido en la SEC ID NO: 1. Además, mientras que las descripciones hacen referencia a glucagón humano nativo (SEC ID NO: 1), por ejemplo, modificaciones de SEC ID NO: 1, estas descripciones no necesariamente implican (i) que dichas modificaciones están presentes en todos los

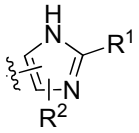
péptidos divulgados en la presente y (ii) que el único método para hacer los péptidos aquí divulgados es comenzar con glucagón humano nativo y modificar esa secuencia. En cambio, estas descripciones se proporcionan a efectos de describir algunas realizaciones de los análogos de glucagon divulgados en la presente, y los péptidos de estas divulgaciones se pueden realizar *de novo* sin utilizar glucagón humano nativo como material de partida, como se describe más detalladamente en la sección titulada “MÉTODOS PARA HACER PÉPTIDOS”.

Posición 1

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en la posición 1, con relación a la SEC ID NO: 1, por ejemplo, el análogo de glucagón comprende un aminoácido que no es His en la posición 1. En aspectos ejemplares, el análogo de glucagón comprende un aminoácido aromático, grande en la posición 1. En realizaciones ejemplares, el amino ácido aromático, grandees Tyr, Phe, Trp, amino-Phe (por ejemplo, 4-amino-Phe), cloro-Phe, sulfo-Phe, 4-Piridil-Ala, metil-Tyr, o 3-amino Tyr.

En otras realizaciones ejemplares, el análogo de glucagón comprende un aminoácido que comprende una cadena lateral de imidazol en la posición 1. En aspectos ejemplares, el

aminoácido en la posición 1 comprende una estructura de la Fórmula A



[Fórmula A]

en donde cada uno de R1 y R2 se selecciona independientemente del grupo formado por H, alquilo(C1-6), O(C1-6)alquilo, (C1-6)alquilo-OH, F, y (C1-6)alquilo, de los cuales al menos un H se reemplaza por F.

En aspectos ejemplares, el aminoácido en la posición 1 es el residuo nativo de glucagón (SEC ID NO: 1) L-histidina (His), o es un derivado de His (derivado de His), por ejemplo, un derivado de His en donde los alfa átomos se modifican. El derivado de His en aspectos ejemplares es D-histidina, desaminohistidina, hidroxil-histidina, acetil-histidina, homo-histidina, N-metil histidina, alfa-metil histidina, ácido imidazol acético, o ácido alfa, alfa-dimetilimidazol acético (DMDA).

En aún otros aspectos, el amino ácido en la posición 1 es un aminoácido protector contra la DPP-IV, como se describe en la

presente. En algunos aspectos, el aminoácido protector contra la DPP-IV es un derivado de His.

Posición 2

En algunas realizaciones, los péptidos actualmente divulgados comprenden un aminoácido protector contra DPP-IV en la posición 2. Como se utiliza en la presente, el término "aminoácido protector contra DPP-IV" se refiere a un aminoácido que logra una resistencia sustancial respecto de los péptidos actualmente divulgados contra la segmentación de dipeptidilpeptidasa IV (DPP IV). En algunos aspectos, el aminoácido protector contra DPP-IV es uno de D-serina, D-alanina, valina, glicina, N-metilserina, N-metilalanina, o ácido alfaaminoisobutírico (AIB).En algunos aspectos, el aminoácido protector contra DPP-IV es un aminoácido disustituido alfa, alfa. En algunos aspctos, el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde cada uno de R1 y R2 se selecciona independientemente del grupo formado por alquilo C1-C4, opcionalmente sustituido con un hidroxilo, amida, tiol, halo, o R1 y R2 junto con el carbono de alfa al cual están unidos forman un anillo.En algunos aspectos, el aminoácido α,α-disustituido es AIB o 1-aminociclopropano-1-carboxilato (ACPC).

El término "alquilo C_1-C_n" en donde n puede ser de 1 a 6, como se usa en la presente, representa un grupo alquilo ramificado o lineal que tiene entre uno y el número especificado de átomos de carbono. Grupos alquilo C_1-C_6 típicos incluyen, entre otros, metilo, etilo, n-propilo, iso-propilo, butilo, iso-butilo, sec-butilo, terc-butilo, pentilo, hexilo y similares.

En realizaciones ejemplares, el aminoácido protector contra DPP=IV es el D-isomero de Ser (D-Ser), o una sustitución de este con un aminoacido conservador. Por ejemplo, el aminoácido protector contra DPP-IV puede comprender una estructura de cadena lateral de -(alquilo C1-C4)OH. Opcionalmente, cuando la estructura de cadena lateral comprende -(alquilo C3)OH o -(alquilo C4)OH, la cadena de carbono puede ser una cadena recta o ramificada.

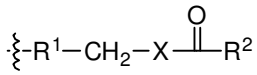
En aspectos ejemplares, cuando el aminoácido protector contra DPP-IV es D-Ser y el aminoácido en la posición 1 es His, el péptido agonista del receptor de GIP no es conjugado a un sector heterólogo, por ejemplo, un sector hidrófilo (por ejemplo, PEG). En otros aspectos de las actuales divulgaciones, el aminoácido protector contra DPP-IV no es D-Serina.

Posición 3

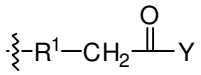
En algunas realizaciones, el análogo de glucagón comprende en la posición 3 un residuo aminoácido acidico, básico, o hidrófobo. Sin ceñirse a ninguna teoría en particular, tales análogos de glucagón exhiben una actividad del receptor de glucagón reducida. En algunas realizaciones, el aminoácido acidico, básico, o el aminoácido hidrófobo es ácido glutámico, ornitina, norleucina. Los análogos de glucagón que se sustituyen, por ejemplo, con acido glutámico, ornitina o norleucina en algunos aspectos tienen alrededor de 10% o menos de la actividad del glucagón nativo en el receptor de glucagón, por ejemplo, alrededor de 1-10%, o alrededor de 0,1-10%, o más de aproximadamente 0,1% pero menos de aproximadamente 10%. En algunas realizaciones, los análogos de glucagón exhiben alrededor de 0,5%, alrededor de 1%, o alrededor de 7% de la actividad del glucagón nativo.

En algunas realizaciones, el análogo de glucagón comprende el aminoácido nativo de SEC ID NO: 1 en la posición 3, por ejemplo, glutamina, o comprende un análogo de glutamina. Sin ceñirse a ninguna teoría en particular, tales análogos de glucagón que comprenden un análogo de glutamina no exhiben una pérdida sustancial de actividad en el receptor de glucagón, y en algunos casos, el análogo de glucagón que comprende el análogo de glutamina mejora la actividad del receptor de glucagón. En algunas realizaciones, el análogo de glutamina

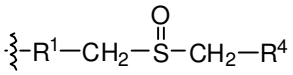
comprende en la posición 3 un aminoácido que comprende una cadena lateral de la Estructura I, II o III:



Estructura I



Estructura II



Estructura III

en donde R^1 es alquilo C_{0-3} o heteroalquilo C_{0-3}; R^2 es NHR^4 o alquilo C_{1-3}; R^3 es alquilo C_{1-3}; R^4 es H o alquilo C_{1-3}; X es NH, O, o S; y Y es NHR^4, SR^3, o OR^3. En algunas realizaciones, X es NH o Y es NHR^4. En algunas realizaciones, R^1 es alquilo C_{0-2} o heteroalquilo C_1.En algunas realizaciones, R^2 es NHR^4 o alquilo C_1.En algunas realizaciones, R^4 es H o alquilo C^1l. En realizaciones ejemplares, se proporciona un aminoácido que comprende una cadena lateral de la Estructura I donde, R^1 es CH_2–S, X es NH, y R^2 es CH_3 (acetamidometil-cisteina, C(Acm)); R^1es CH_2, X es NH, y R^2es CH_3 (acidoacetildiaminobutanóico, Dab(Ac)); R^1es alquilo C_0, X es NH, R^2es NHR^4, y R^4es H (acidocarbamoildiaminopropanoico, Dap(urea)); o R^1es CH_2–CH_2, X es NH, y R^2es CH_3 (acetilornitina, Orn(Ac)). Enrealizacionesejemplares, se proveeun aminoácido que comprende una cadena lateral de laEstructura II donde, R^1es

CH_2, Y es NHR^4, y R^4es CH_3 (metilglutamina, Q(Me)); en realizaciones ejemplares, se proporciona un aminoácido que comprende una cadena lateral de la Estructura III donde R^1 es CH_2 y R^4 es H (metionina-sulfoxido, M(O)); en realizaciones específicas, el aminoácido en la posición 3 se sustituye con Dab(Ac).

Posición 7

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en la posición 7, con relación a SEC ID NO: 1, por ejemplo, el análogo de glucagón comprende un aminoácido diferente de Thr en la posición 7. En algunos aspectos, el aminoácido en la posición 7 es un aminoácido alifático, grande, por ejemplo, Ile, Leu, Ala, y similares. Sin ceñirse a ninguna teoría en particular, se cree que los análogos de glucagón que comprenden dicho aminoácido en la posición 7 exhiben una actividad reducida drásticamente en el receptor de GLP-1.

Posición 9

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en la posición 9, con relación a SEC ID NO: 1, por ejemplo, el análogo de glucagón comprende un aminoácido que no es Asp en la posición 9. En algunas realizaciones, el análogo de glucagón comprende un aminoácido

con carga negativa que no es Asp, por ejemplo, Glu, ácido homoglutámico, ácido cistéico, ácido homocistéico. En algunos aspectos, el aminoácido en la posición 9 es un aminoácido acilado o un aminoácido alquilado, como se discute en la presente.

Posición 10

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en la posición 10, con relación a SEC ID NO: 1, por ejemplo, el análogo de glucagón comprende un aminoácido que no es Tyr en la posición 10. En algunos aspectos, el aminoácido en la posición 10 es Trp. Sin ceñirse a ninguna teoría en particular, se cree que los análogos de glucagón que comprenden dicho aminoácido en la posición 10 exhiben actividad en el receptor de GIP, el receptor de GLP-1, y/o el receptor de glucagón, la cual no es reducida, en comparación con el péptido correspondiente con Tyr en la posición 10.

En algunas realizaciones, el análogo de glucagón comprende un aminoácido acilado o un aminoácido alquilado, como se discute en la presente.

Posición 12

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en la posición 12, con relación a SEC ID NO: 1, por ejemplo, el análogo de glucagón comprende un aminoácido que no es Lys en la posición 10. En algunos aspectos, el aminoácido en la posición 12 es un aminoácido no polar, alifático, grande, opcionalmente, isoleucina. En algunos aspectos, el aminoácido en la posición 12 es Arg. Sin ceñirse a ninguna teoría en particular, se cree que los análogos de glucagón que comprenden aminoácido no polar, alifático, grande, por ejemplo, Ile, exhiben una actividad mejorada en el receptor de GIP. En algunas realizaciones, el análogo de glucagón comprende un aminoácido acilado o un aminoácido alquilado, como se discute en la presente.

Posición 15

En algunas realizaciones, los análogos de glucagón comprenden una modificación de aminoácido en la posición 15, con relación a SEC ID NO: 1, por ejemplo, el análogo de glucagón comprende un aminoácido que no es Asp en la posición 15. En algunos aspectos, el aminoácido en la posición 15 se elimina o es ácido glutámico, ácido homoglutámico, ácido cistéico o ácido homocistéico. Sin ceñirse a ninguna teoría en particular, tales análogos de glucagón exhiben una estabilidad mejorada, por ejemplo, por medio de la reducción de la degradación o clivaje del análogo a lo largo del tiempo, especialmente en

soluciones reguladoras acídicas o alcalinas; por ejemplo, soluciones reguladoras con un pH dentro del rango de 5,5 a 8. En algunas realizaciones, los análogos de glucagón que comprenden esta modificación retienen por lo menos 75%, 80%, 90%, 95%, 96%, 97%, 98% o 99% del análogo original luego de 24 horas a 25°C.

Posición 16

En algunas realizaciones, los análogos de glucagón comprenden una modificación de aminoácido en la posición 16, con relación a SEC ID NO: 1, por ejemplo, el análogo de glucagón comprende un aminoácido que no es Ser en la posición 16. En algunos aspectos, el aminoácido en la posición 16 es ácido glutámico o con otro aminoácido con carga negativa que tenga una cadena lateral de una longitud de 4 átomos, o alternativamente, con cualquiera de glutamina, ácido homoglutámico, o ácido homocistéico, o un aminoácido con carga que tenga una cadena lateral que contenga por lo menos un heteroátomo (por ejemplo, N, O, S, P) y con una cadena lateral de una longitud de aproximadamente 4 (o 3–5) átomos. En algunas realizaciones, el análogo de glucagón comprende en la posición 16 un aminoácido que se selecciona del grupo formado por ácido glutámico, glutamina, ácido homoglutámico, ácido homocistéico, treonina o glicina, o es un aminoácido seleccionado del grupo formado por

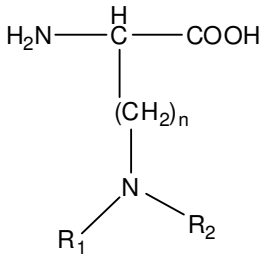
ácido glutámico, glutamine, ácido homoglutámico y ácido homocistéico.

Sin ceñirse a ninguna teoría en particular, tales análogos de glucagón exhiben una estabilidad mejorada, por ejemplo, a través de la reducción de la degradación del péptido a lo largo del tiempo, especialmente, en soluciones reguladoras ácidas o alcalinas, por ejemplo, soluciones reguladoras con un pH dentro del rango de 5,5 a 8. Tales análogos de glucagón son menos susceptibles al clivaje de la union de péptido Asp15-Ser16.

En algunas realizaciones, el aminoácido en la posición 16 es un aminoácido con carga negativa, opcionalmente en combinación con un aminoácido que promueve el alfa hélice (por ejemplo, un aminoácido disustituido alfa, alfa o AIB) en la posición 20. Tales análogos de glucagón exhiben actividad GIP.

En realizaciones alternativas, el análogo de glucagón comprende en la posición 16 un Thr o un aminoácido que promueve elalfa hélice, como se describe precedentemente. En algunas realizaciones, el aminoácido que promueve elalfa hélice es AIB o Glu.

En algunos aspectos, el aminoácido en la posición 16 es un aminoácido con carga positiva, por ejemplo, Fórmula IV:



[Fórmula IV]

en donde n es 1 a 16, o 1 a 10, o 1 a 7, o 1 a 6, o 2 a 6, o 2 o 3 o 4 o 5, cada uno de R_1y R_2seselecciona independientemente del grupo formado por H, alquilo C_1–C_{18}, (alquilo C_1–C_{18})OH, (alquiloC_1–C_{18})NH_2, (alquiloC_1–C_{18})SH, (alquiloC_0–C_4), cicloalquilo(C_3–C_6), (alquiloC_0–C_4)(heterocíclicoC_2–C_5), (alquiloC_0–C_4)(ariloC_6–C_{10})R_7, y (alquiloC_1–C_4)(heteroariloC_3–C_9), en donde R_7es H o OH, Lys.

En algunas realizaciones, el análogo de glucagón comprende un aminoácido acilado o un aminoácido alquilado, como se describe en la presente.

En aún otras realizaciones, el aminoácido en la posición 16 es un aminoácido que comprende una cadena lateral que es conjugada a un sectorheterólogo, como se describe en la presente en el capítulo titulado “CONJUGADOS”.

Posiciones 17 y 18

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en una o ambas de las posiciones 17 y 18, con relación a SEC ID NO: 1, de modo tal que el lugar de Arg-Argdibásico en las posiciones 17 y 18 se elimina. En algunas realizaciones, el análogo de glucagón comprende un aminoácido que no es Arg en las posiciones 17 y 18, o en una de ellas. Sin ceñirse a ninguna teoría en particular, se cree que la eliminación del lugar de dibásico mejora la eficiencia *in vivo* del análogo de glucagón. En algunos aspectos, el aminoácido en la posición 17 no es un aminoácido básico.

En algunos aspectos, el aminoácido en la posición 17 es un aminoácido alifático. En algunas realizaciones, el aminoácido en la posición 17 es sustituido por otro aminoácido como se describe en la presente, por ejemplo, un aminoácido que comprende un sector hidrófilo, un aminoácido que promueve elalfa hélice. En algunas realizaciones, el aminoácido que promueve elalfa hélice forma un puente intramolecular no covalente con un aminoácido en j+3 o i+4. En algunos aspectos, el aminoácido en la posición 17 es Gln.

En algunos aspectos, el aminoácido en la posición 18 no es un aminoácido básico. En algunos aspectos, el aminoácido en la posición 18 es un aminoácido alifático. En algunas

realizaciones, el aminoácido en la posición 18 es un aminoácido alifático pequeño, por ejemplo, Ala.

En algunos aspectos específicos, el aminoácido en la posición 18 es uno aminoácido alifático pequeño, por ejemplo, Ala, y el aminoácido en la posición 17 es Arg. En otros aspectos, el aminoácido en la posición 18 es un aminoácido alifático pequeño, por ejemplo, Ala, y el aminoácido en la posición 17 es Gln.

En algunos aspectos, el aminoácido en la posición 17 es un aminoácido que comprende una cadena lateral conjugada a una sección heteróloga, como se describe en la presente en el capítulo que se titula “CONJUGADOS”.

Posición 20

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en la posición 20, con relación a SEC ID NO: 1, por ejemplo, el aminoácido en la posición 20 es un aminoácido diferente de Gln. En algunos aspectos, el aminoácido en la posición 20 es un aminoácido que promueve la alfa hélice, por ejemplo como se describe precedentemente. En algunos aspectos, el aminoácido en la posición 20 es un aminoácido disustituido alfa, alfa por ejemplo, AIB, ACPC. En algunas realizaciones, el aminácido que promueve la alfa

hélice forma un puente intramolecular no covalente con un aminoácido en j-3 o i-4.

En algunas realizaciones específicas, el aminoácido es un aminoácido hidrófilo que tiene una cadena lateral que tiene carga o que puede formar unión con hidrógeno, y tiene una longitud de por lo menos alrededor de 5 (o alrededor de 4-6) átomos, por ejemplo, lisina, citrulina, arginina, u ornitina. En otros aspectos, el aminoácido en la posición 20 es Ser, Thr, Ala o AIB.

En algunos aspectos, el aminoácido en la posición 20 es un aminoácidoacilado o un aminoácido alquilado, como se discute en la presente.

En algunos aspectos, el aminoácido en la posición 20 es un aminoácido que comprende una cadena lateral conjugada a una sección heteróloga, como se describe en la presente en el capítulo que se titula “CONJUGADOS”.

Sin ceñirse a ninguna teoría en particular, tales análogos de glucagón exhiben una actividad mejorada del receptor de GLP-1 y/o del receptor de GIP o exhiben una degradación reducida que ocurre por la desamidación de Gln y/o exhiben una estabilidad incrementada.

Posición 21

En algunas realizaciones, el análogo de glucagón comprende una modificación de aminoácido en la posición 21, con relación a SEC ID NO: 1, por ejemplo, el aminoácido en la posición 21 es un aminoácido diferente de Asp. En aspectos ejemplares, el aminoácido en la posición 21 es Ser, Thr, Ala o AIB.En otros aspectos, el aminoácido en la posición 21 es Lys, Arg, Orn o Citrulina.En algunos aspectos, el aminoácido en la posición 21 es Glu, ácido homoglutámico, o ácido homocistéico. En algunos aspectos, el aminoácido en la posición 21 es un aminoácido que comprende una cadena lateral que se conjuga a una sección heteróloga, como se describe en la presente en el capítulo que se titula “CONJUGADOS”.

En algunas realizaciones, el aminoácido en la posición 21 es un aminoácido que promueve la alfa hélice. En algunas realizaciones, el aminoácido que promueve la alfa hélice forma un puente intramolecular no covalente con un aminoácido en j-3 o i-4.

Sin ceñirse a ninguna teoría en particular,tales análogos de glucagón exhiben una degradación reducida que ocurre por la degradación por deshidratación de Asp para formar un

intermediario succinimida cíclico seguido por isomerización de iso-aspartato y/o exhiben estabilidad aumentada.

Posición 23

En algunos aspectos, el análogo de glucagón comprende una modificación de aminoácido en la posición 23, con relación a SEC ID NO: 1. En algunos aspectos, el aminoácido en la posición 23 es un aminoácido diferente de Val, por ejemplo Ile.

Posición 24

En algunos aspectos, el análogo de glucagón comprende una modificación de aminoácido en la posición 24, con relación a SEC ID NO: 1. En algunos aspectos, el aminoácido en la posición 24 es un aminoácido diferente de Gln, por ejemplo, Ala, Asn, Cys. En algunos aspectos, el aminoácido en la posición 24 es un aminoácido que comprende una cadena lateral que se conjuga a una sección heteróloga, como se describe en la presente en el capítulo que se titula “CONJUGADOS”.

Posición 27

En algunos aspectos, el análogo de glucagón comprende una modificación de aminoácido en la posición 27, con relación a SEC ID NO: 1. En algunos aspectos, el aminoácido en la posición 27 es un aminoácido diferente de Met. En algunas

realizaciones, el análogo de glucagón comprende en la posición 27 un aminoácido que previene la degradación oxidativa del péptido. En algunos aspectos, el aminoácido en la posición 27 es sulfóxido de metionina, leucina, isoleucina o norleucina.En algunas realizaciones específicas, el aminoácido en la posición 27 es leucina o norleucina.

En otros aspectos, el aminoácido en la posición 27 es un aminoácido alifático (por ejemplo, Gly, Ala, Val, Leu, Ile) o un aminoácido de la Fórmula IV, como se describe en la presente, por ejemplo, Lys. En realizaciones ejemplares, el aminoácido de la posición 27 es Val o Lys. Sin ceñirse a ninguna teoría en particular, tal modificación de aminoácido reduce la actividad de glucagón.

Posición 28

En algunos aspectos, el análogo de glucagón comprende una modificación de aminoácido en la posición 28, con relación a SEC ID NO: 1. En algunos aspectos, el aminoácido en la posición 28 es un aminoácido diferente de Asn. En algunos aspectos, el aminoácido en la posición 28 es un aminoácido diferente de Asn. En algunos aspectos, el aminoácido en la posición 28 es Ala, Ser, Thr o AIB. En algunos aspectos, el aminoácido en la posición 28 es un aminoácido con carga, por ejemplo, un aminoácido con carga negativa, como se describe en

la presente. Véase la sección que se titula *"Extremo de C con carga"*.En algunos aspectos, el aminoácido en la posición 28 es Asp.

En aspectos ejemplares, el aminoácido en la posición 28 es un aminoácido de la Fórmula IV como se describe en la presente. El aminoácido en realizaciones ejemplares es Lys. Sin ceñirse a ninguna teoría en particular, tal modificación de aminoácido reduce la actividad de glucagón.

Posición 29

En algunos aspectos, el análogo de glucagón comprende una modificación de aminoácido en la posición 29, con relación a SEC ID NO: 1. En algunos aspectos, el aminoácido en la posición 29 es un aminoácido diferente de Thr. En algunos aspectos, el aminoácido en la posición 29 es Gly. En algunos aspectos, el aminoácido en la posición 29 es Ala.

En algunos aspectos, el aminoácido en la posición 29 es un aminoácido que comprende una cadena lateral que se conjuga con una sección heteróloga, como se describe en la presente en el capítulo que se titula "CONJUGADOS".

Extremo de C con carga

En algunas realizaciones, el análogo de glucagón comprende una o más sustituciones de aminoácidos y/o agregados que introducen un aminoácido con carga en una porción del extremo de C del análogo. En algunas realizaciones, tales modificaciones mejoran la estabilidad y solubilidad. Como se usa en la presente, el término “aminoácido con carga” o “residuo con carga” se refiere a un aminoácido que comprende una cadena lateral que es de carga positiva o de carga negativa (es decir, de-protonizado) o de carga positiva(es decir, protonizado) en una solución acuosa en pH fisiológico. En algunos aspectos, estas sustituciones de aminoácidos y/o agregados que introducen modificaciones en un aminoácido con carga están en la posición del extremo de C a la posición 27 de SEC ID NO: 1. En algunas realizaciones, uno, dos o tres (y en algunos casos, más de tres) aminoácidos con carga se introducen dentro de la sección de extremo de C (por ejemplo, posición de extremo de C a posición 27). De acuerdo con algunas realizaciones, el aminoácido nativo en las posiciones 28 y/o 29 se sustituye con aminoácidos con carga, y/o en otra realización de uno a tres aminoácidos con carga también se agregan al extremo de C del análogo. En realizaciones ejemplares, uno, dos o todos los aminoácidos con carga tienen carga negativa. El aminoácido con carga negativa en algunas realizaciones es ácido aspártico, ácido glutámico, ácido cistéico, ácido homocistéico o ácido homoglutámico. En algunos

aspectos, estas modificaciones aumentan la solubilidad o la estabilidad. En algunas realizaciones, la posición 30 no es un aminoácido con carga. Sin ceñirse a ninguna teoría en particular, un aminoácido con carga, por ejemplo, un aminoácido con carga negativa, por ejemplo, Glu, redujo la actividad de GIP.

Truncación del Extremo de C

De acuerdo con algunas realizaciones, los análogos de glucagón que se divulgan en la presente se modifican por truncación del extremo de C por uno o dos residuos de aminoácido. Tales péptidos de glucagón modificados retienen actividad y potencia similares en el receptor de glucagón y el receptor de GLP-1. Con relación a esto, los péptidos de glucagón pueden comprender aminoácidos 1-27 o 1-28 del análogo de glucagón nativo (SEC ID NO: 1), opcionalmente con cualquiera de las modificaciones adicionales que se describen en la presente.

Extremo de C con carga neutral

En algunas realizaciones, el análogo de glucagón comprende un grupo que posee carga neutral, como por ejemplo una amida o un ester, en el extremo de C, en lugar del alfa carboxilato, con relación a SEC ID NO: 1. Sin ceñirse a ninguna teoría en particular, tales modificaciones en aspectos ejemplares aumentan la actividad del análogo de glucagón en el receptor

de GLP-1. En esta misma línea, en algunas realizaciones, el análogo de glucagón es un péptido amidado, de tal forma que el residuo del extremo de C comprende una amida en lugar del alfa carboxilato de un aminoácido. Como se usa en la presente, una referencia general a un péptido o análogo comprende péptidos que tienen un extremo amino modificado, extremo carboxi, o extremos tanto amino como carboxi. Por ejemplo, una cadena de aminoácido que compone un grupo amida en lugar del ácido carboxílico de extremo se entiende que comprende una secuencia de aminoácido que designa aminoácidos estándar.

Extensiones del Extremo de C

En algunas realizaciones de las presentes divulgaciones, los análogos de glucagón comprenden una extensión del extremo de C de 1-21 aminoácidos fusionados con el aminoácido en la posición 29. La extensión del extremo de C puede comprender 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 o 21 aminoácidos. En algunos aspectos, la extensión del extremo de C es cualquiera de los péptidos heterólogosque se describen debajo en la sección denominada “CONJUGADOS”. Por ejemplo, el algunos aspectos, la extensión comprende una secuencia de aminoácido que forma una estructura de jaula Trp, por ejemplo, la extensión comprende una secuencia de aminoácido de GPSSGAPPPS (SEC ID NO: 5) o una secuencia sustituida conservativamente de ésta. En aspectos

alternativos, la extensión de 1 a 21 aminoácidos comprende por lo menos un aminoácido con carga. En aspectos ejemplares, la extensión comprende una secuencia de aminoácido de: X1-X2, en donde X1 es un aminoácido con carga y X2 es un aminoácido alifático pequeño. En algunos aspectos, X1 es un aminoácido con carga positiva, por ejemplo, Arg. En algunos aspectos, la extensión comprende Arg-Gly.

En algunas realizaciones, la extensión comprende una secuencia de aminoácido de SEC ID NO: 5 (GPSSGAPPPS), SEC ID NO: 6 (GGPSSGAPPPS), SEC ID NO: 7 (KRNRNNIA), o SEC ID NO: 8 (KRNR). En algunos aspectos específicos, la secuencia de aminoácido se adjunta mediante el aminácido del extremo de C del análogo de glucagón, por ejemplo, aminoácido en la posición 29. En algunas realizaciones, la secuencia de aminoácidos de cualquiera de SEC ID Nos: 5-8 está unida del aminoácido 29 del análogo de glucagón a través de una union de péptido. En algunas realizaciones específicas, el aminoácido en la posición 29 del análogo de glucagón es un Gly y el Gly se fusiona con una de las secuencias de aminoácidos de SEC ID NOs: 5-8.

En aspectos ejemplares, el análogo de glucagón comprende una extensión que forma una estructura conocida en el estado de la técnica como Jaula de Trp (véase, por ejemplo, Paschek et al.,

ProcNatlAcadSci USA 105 (46): 17754-17759 (2008). En algunos aspectos, la extensión comprende la secuencia de aminoácidos GPSSGAPPPS (SEC ID NO: 5) o GGPSSGAPPPS (SEC ID NO: 6) o GPSSGRPPPS (SEC ID NO: 183) o una secuencia de uno de los anteriores con 1, 2 o 3 sustituciones de aminoácidos conservadoras. En aspectos ejemplares, cuando la extensión comprende la secuencia de aminoácido de SEC ID NO: 183, el aminoácido en la posición 28 es un aminoácido con carga negativa, por ejemplo, Asp o Glu.

Otras modificaciones

Se proporcionan descripciones de otras modificaciones relativas a SEC ID NO: 1 de los análogos de glucagón de la presente divulgación a lo largo de la presente descripción. El listado precedente no es taxativo, sino simplemente se proporciona a modo de ejemplo.

En algunas realizaciones, los análogos de glucagón descriptos en la presente son glicosilados, amidados, carboxilados, fosforilados, esterificados, N-acilados, ciclizados a través de, por ejemplo, un puente disulfuro, o convertidos en una sal (por ejemplo, una sal con agregado ácido, una sal con agregado básico) y/o opcionalmente dimerizados, multimerizados o polimerizados, o conjugados.

PÉPTIDOS EXCLUIDOS

Los análogos de glucagón de las presentes divulgaciones son estructuralmente diferentes de los análogos de glucagón que exhiben actividad agonista del receptor de GIP descriptos en la Solicitud Internacional de Patente No. PCT US2009/47447 (presentada el 16 dejunio de 2009), Solicitud de Patente Estadounidense No. 61/073.274 (presentada el 17 de junio de 2008); Solicitud de Patente Estadounidense No. 61/078.171 (presentada el 3 de julio de 2008); Solicitud de Patente Estadounidense No. 61/090.448 (presentada el 20 deagostode 2008), Solicitud de Patente Estadounidense No. 61/151.349 (presentada el 10 defebrero de 2009), Solicitud de Patente Estadounidense No. 61/187.578 (presentada el 16 dejunio de 2009), la Solicitud Internacional de Patente No. PCT/US2010/038825 (presentada el 16 dejunio de 2010); cuyos contenidos se incorporan en la presente por referencia en su totalidad.De esta manera, en cualquiera o en todas las realizaciones, el análogo de glucagón de las presentes divulgaciones no es ninguno de las análogos de glucagón o péptidos descriptos en la Solicitud Internacional de Patente No.PCT/US2009/47447 (presentada el 16 dejunio de 2009, ypublicada bajo el número WO 2010/011439), Solicitud de Patente Estadounidense No. 61/073.274 (presentada el 17 dejunio de 2008); Solicitud de Patente Estadounidense No. 61/078.171 (presentada el 3 de julio de 2008); Solicitud de

Patente Estadounidense No. 61/090.448 (presentada el 20 deagostode 2008), Solicitud de Patente Estadounidense No. 61/151.349 (presentada el 10 defebrerode 2009), Solicitud de Patente Estadounidense No. 61/187.578 (presentada el 16 dejunio de 2009), la Solicitud Internacional de Patente No. PCT/US2010/038825 (presentada el 16 dejunio de 2010 y publicada bajo el número WO 2010/148089), Solicitud de Patente Estadounidense No. 61/298.812 (presentada el 27 de enero de 2010),o la Solicitud de Patente Internacional No.PCT/US2011/022608 (presentada el 26 de enero de 2011, y publicadabajo el número WO 2011/094337).En realizaciones ejemplares, los péptidos, péptidos de glucagón, o los análogos de glucagón de las presentes divulgaciones no son (es decir, excluyen) cualquiera de los péptidos de SEC ID NOs: 1–262 de WO 2010/011439; SEC ID NOs: 1–680 de WO 2010/148089, o SEC ID NOs: 1–1318 de PCT/US2011/022608.

En realizaciones ejemplares, los péptidos, péptidos de glucagón, o análogos de glucagón de las presentes divulgaciones no son (es decir, excluyen) todos y cada uno de los péptidos de SEC ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71,

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En realizaciones ejemplares, los péptidos, péptidos de glucagón, o análogos de glucagón de las presentes divulgaciones no son (es decir, excluyen) todos y cada uno de los péptidos de SEC ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56,

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666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, y/o 680 de la Solicitud Internacional dePatentepublicada con el No. WO 2010/148089En realizaciones ejemplares, los péptidos, péptidos de glucagón, o análogos de glucagón de las presentes divulgaciones no son (es decir, excluyen) todos y cada uno de los péptidos de SEC ID NOs: 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669 de la Solicitud Internacional de Patentepublicada con el No. WO 2010/148089, que se presentan en la presente como SEC ID NOs: 219-229, respectivamente.

En realizaciones ejemplares, los péptidos, péptidos de glucagón, o análogos de glucagón de las presentes divulgaciones no son (es decir, excluyen) todos y cada uno de los péptidos de SEC ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149,

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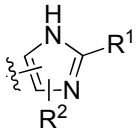
REALIZACIONES EJEMPLARES

En realizaciones ejemplares, el péptido de las presentes divulgaciones es un análogo de glucagón (SEC ID NO: 1) que comprende (i) un aminoácido que comprende una cadena lateral de imidazol en la posición 1, (ii) un aminoácido protector contra DPP-IV en la posición 2, (iii) un aminoácido acilado o aminoácido alquilado, opcionalmente en cualquiera de las posiciones 9, 10, 12, 16, 20 o 37-43, en donde opcionalmente el grupo acilo o alquilo se enlaza con el aminoácido por medio de un separador; (iv) un aminoácido estabilizador del alfa hélice en una o más de las posiciones 16-21, y (v) hasta diez modificaciones de aminoácidos adicionales relacionadas con SEC ID NO: 1, en donde cuando en análogo de glucagón no es conjugado a una sección heteróloga, por ejemplo, una sección hidrófila (por ejemplo, PEG), el análogo de glucagón exhibe por lo menos o alrededor de 0,1% (por ejemplo, al menos o alrededor de 1% o al menos o alrededor de 10%, al menos o alrededor de 50%, al menos alrededor del 80%, al menos o

alrededor del 100%, al menos o alrededor del 500%) de actividad de GIP nativo en el receptor de GIP.

Los análogos de glucagón descriptos en la presente pueden comprender cualquier perfil de actividad descripto en la presente.Véase, por ejemplo, la sección que se titula "*ACTIVIDAD DE LOS PÉPTIDOS DIVULGADOS EN LA PRESENTE*". En aspectos ejemplares, el análogo de glucagón exhibe un porcentaje de potencia de GIP de por lo menos o alrededor de 1%, por lo menos o alrededor de 10%, por lo menos o alrededor de 50%, por lo menos o alrededor de 90%,por lo menos o alrededor de 100%,por lo menos o alrededor de 300% o por lo menos o alrededor de 500%. En algunos aspectos, en análogo de glucagón también exhibe un porcentaje de potencia de GLP-1 de por lo menos o alrededor de 1%, por lo menos o alrededor de 10%, por lo menos o alrededor de 50%, por lo menos o alrededor de 90%,por lo menos o alrededor de 100%,por lo menos o alrededor de 300% o por lo menos o alrededor de 500%. En aspectos alternativos o adicionales, el análogo de glucagón exhibe un porcentaje de potencia de glucagón de por lo menos o alrededor de 1%, por lo menos o alrededor de 10%, por lo menos o alrededor de 50%, por lo menos o alrededor de 90%, o por lo menos o alrededor de 100%. De esta manera, mientras que los análogos de glucagón pueden ser considerados como péptidos

agonistas de GIP, en algunos aspectos, los análogos de glucagón pueden también ser considerados como co-agonista de GIP-GLP-1, co-agonista de GIP-glucagón, o triagonista de GIP-GLP-1-glucagón. Por ejemplo, el péptido puede exhibir actividad agonista en cada uno de los receptores de GIP humanos, receptores de GLP-1 humanos y receptores de glucagón humanos, en donde el péptido exhibe una concentración efectiva media en el receptor de GIP que es dentro de 100 veces (por ejemplo, 50 veces, 40 veces, 30 veces, 20 veces, 15 veces, 10 veces, o menos) su concentración efectiva media en el receptor de GLP-1 y es dentro de 100 veces (por ejemplo, 50 veces, 40 veces, 30 veces, 20 veces, 15 veces, 10 veces, o menos) su concentración efectiva media en el receptor de glucagón. En realizaciones ejemplares, el análogo de glucagón comprende un aminoácido que comprende una cadena lateral de imidazol en la posición 1. En aspectos ejemplares, el aminoácido en la posición 1 comprende la estructura de la Fórmula A



[Fórmula A]

en donde cada uno de R1 y R2 se seleccionan independientemente del grupo formado por H, alquilo(C1-6), O alquilo(C1-6),

alquilo(C1-6)OH, F, y alquilo (C1-6) de los cuales por lo menos un H se reemplaza por F.

En aspectos ejemplares, el aminoácidoen la posición 1 es el residuo nativode glucagón (SEC ID NO: 1) L-histidina (His), o es un derivadode His (derivado de His), por ejemplo, un derivado de His en donde los alfa átomos son modificados. Según se usa en la presente, eltérmino “derivado de His” se refierea una secciónquímica que comprendeun imidazol, por ejemplo, comprende unaestructurade la Fórmula A, o un imidazol sustituido, adjunto a por lo menos un átomo de carbono. En realizaciones ejemplares, el derviado deHis comprendeunaestructura similar alaestructurade histidina, excepto quela alfa amina, alfa carbono, o alfa carboxilatose reemplaza con otra sección química.En realizaciones ejemplares, el derviado deHises una histidina alfa sustituida en la cual elátomo de hidrógenoenlazado al carbono alfa se sustituye por otra sección química, por ejemplo, metil, etil, propil, isopropil, hidroxil, metoxi, etoxi, ysimilares.El derivado deHisen algunos aspectos es D-histidina, desaminohistidina, hidroxil-histidina, acetil-histidina, homo-histidina, N-metil histidina, alfa-metil histidina, ácido imidazol acético, o ácido alfa dimetilimidazol acético (DMIA).

En algunos aspectos, el aminoácido protector contra DPP-IV en la posición 2 es uno de D-serina, D-alanina, valina, glicina, N-metilserina, M-metilalanina o alfa, ácido aminoisobutírico (AIB). En algunos aspectos, el aminoácido protector contra DPP-IV es D-Ser, o una sustitución de este con un aminoácido conservador, o un aminoácido α,α-disustituido. En algunos aspectos, el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde cada uno de R1 y R2 se selecciona independientemente del grupo formado por alquilo C1-C4, opcionalmente sustituido con un hidroxilo, amida, tiol, halo o R1 y R2 junto con el carbono alfa al cual están unidos forman un anillo. En algunos aspectos, el aminoácido α,α-disustituido es AIB. En aspectos ejemplares, cuando el aminoácido protector contra DPP-IV es D-Ser, el péptido agonista de GIP es no conjugado a una sección heteróloga, por ejemplo, una sección hidrófila (por ejemplo, PEG). En otros aspectos de estas divulgaciones, el aminoácido protector contra DPP-IV no es D-Serina.

En algunos aspectos, el análogo de glucagón comprende un aminoácido estabilizador de la alfa hélice en cualquiera de las posiciones 16, 17, 18, 19, 20 o 21. En algunos aspectos, el análogo de glucagón comprende un aminoácido estabilizador de la alfa hélice en uno, dos, tres, cuatro, cinco o todas las posiciones 16, 17, 18, 19, 20 o 21. En aspectos ejemplares, el

análogo de glucagón comprende un aminoácido estabilizador de la alfa hélice en las posiciones 16, 17, 20 y 21. En algunos aspectos, el análogo de glucagón comprende un aminoácido estabilizador de la alfa hélice en las posiciones 16 y 20. En aspectos alternativos o adicionales, el análogo de glucagón comprende un aminoácido estabilizador de la alfa hélice en las posiciones 17 y 21.

En algunos aspectos, cuando el análogo de glucagón comprende un aminoácido estabilizador de la alfa hélice en la posición 20, el aminoácido enla posición 20 es un aminoácido alfa disustituido, alfa. En aspectos ejemplares, el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido a un carbono alfa, en donde cada uno de R1 y R2 se selecciona independientemente del grupo formado por alquilo C1-C4, opcionalmente sustituido por un hidroxilo, amida, tiol, halo o R1 y R2 junto con el carbono alfa al cual está unidos forman un anillo. En algunas realizaciones, el aminoácido α,α-disustituidoes 1-aminociclopropano-1-carboxilato (ACPC). En algunos aspectos, el α,α-disustituido en la posición 20 es AIB. Opcionalmente, en algunas realizaciones, cuando el aminoácido en la posición 20 es un aminoácido disustituido alfa, alfa, el aminoácido en la posición 16 es un aminoácido estabilizador de la alfa hélice diferente de AIB. En realizaciones ejemplares, el aminoácido en la posición 16 es

un aminoácido con carga, por ejemplo, un aminoácido con carga positiva, un aminoácido con carga negativa. En algunos aspectos, cuando el aminoácido en la posición 20 es un aminoácido disustituido alfa, alfa, el aminoácido en la posición 16 es un aminoácido de la Fórmula IV con carga positiva, por ejemplo, Lys, o es un aminoácido con carga negativa, por ejemplo, Glu. En algunos aspectos, cuando el aminácido en la posición 20 es un aminoácido disustituido alfa, alfa, el aminoácido en la posición 16 es un aminoácido con carga neutral, por ejemplo, Ser, Ala, Gly.

De esta manera, en realizaciones ejemplares, el análogo de glucagón comprende (i) un aminoácido que comprende una cadena lateral de imidazol en la posición 1, (ii) un aminoácido protector contra DPP-IV en la posición 2, opcionalmente, ácido aminoisobutírico, (iii) un aminoácido que comprende un grupo acilo o alquilo no nativo, opcionalmente en cualquiera de las posiciones 9, 10, 12, 16, 20 o 37-43, opcionalmente, donde el grupo acilo o alquilo no nativo se enlaza a dicho aminoácido por medio de un separador; (iv) un aminoácido disustituido alfa, alfa en la posición 20, y (v) hasta diez (por ejemplo, hasta 1, 2, 3, 4, 5, 6, 7, 8 o 9) modificaciones adicionales de aminoácido con relación a SEC ID NO: 1.

En realizaciones ejemplares, cuando el análogo de glucagón comprende un aminoácido disustituido alfa, alfa en la posición 20, y cuando en análogo de glucagón carece de una sección hidrófila, el análogo de glucagón exhibe un porcentaje de potencia de GIP de por lo menos 0,1% (por ejemplo, por lo menos 1%, por lo menos 10%, por lo menos 20%). En realizaciones ejemplares, el análogo de glucagón tiene menos de 100 veces (por ejemplo, menos de o alrededor de 90 veces, menos de o alrededor de 80 veces,menos de o alrededor de 70 veces,menos de o alrededor de 60 veces,menos de o alrededor de 50 veces,menos de o alrededor de 40 veces,menos de o alrededor de 30 veces,menos de o alrededor de 20 veces,menos de o alrededor de 15 veces,menos de o alrededor de 10 veces,menos de o alrededor de 5 veces) de selectividad para el receptor de GLP-1 humano respecto del receptor de GIP. En aspectos ejemplares, el péptido tiene una concentración efectiva media en el receptor de GIP que es menor a 100 veces (por ejemplo, menora o alrededor de 90 veces, menora o alrededor de 80 veces,menora o alrededor de 70 veces,menora o alrededor de 60 veces,menora o alrededor de 50 veces,menora o alrededor de 40 veces,menora o alrededor de 30 veces,menora o alrededor de 20 veces,menora o alrededor de 15 veces,menora o alrededor de 10 veces,menora o alrededor de 5 veces) de diferencia respecto de su concentración efectiva media en el receptor de GLP-1, que opcionalmente es menor de 100 veces (por ejemplo, menor a o

alrededor de 90 veces, menor a o alrededor de 80 veces,menor a o alrededor de 70 veces,menor a o alrededor de 60 veces,menor a o alrededor de 50 veces,menor a o alrededor de 40 veces,menor a o alrededor de 30 veces,menor a o alrededor de 20 veces,menor a o alrededor de 15 veces,menor a o alrededor de 10 veces,menor a o alrededor de 5 veces) la concentración efectiva media en el receptor de glucagón.

En realizaciones ejemplares, cuando el análogo de glucagón comprende un aminoácido disustituido alfa, alfa en la posición 20, el aminoácido el α,α-disustituido comprende R1 y R2, cada uno de los cuales se encuentra unido al carbono alfa, en donde cada uno de R1 y R2 se selecciona independientemente del grupo formado por alquilo C1-C4, opcionalmente sustituido por hidroxilo, amida, tiol, halo, o R1 y R2 junto con el carbono alfa al cual están unidos forman un anillo. En realizaciones ejemplares, el aminoácido el α,α-disustituido en la posición 20 es AIB. También, en realizaciones ejemplares, cuando el análogo de glucagón comprende un aminoácido disustituido alfa, alfa en la posición 20, el aminoácido en la posición 16 es un aminoácido estabilizador del alfa hélice diferente de AIB. En aspectos ejemplares, el aminoácido en la posición 16 es un aminoácido con carga, opcionalmente, un aminoácido con carga negativa (por ejemplo, Glu o Asp) o un aminoácido con carga positiva (por ejemplo, LusoOrn).

En realizaciones alternativas, el análogo de glucagón no comprende un aminoácido estabilizador del alfa hélice en la posición 20, y una o más de las posiciones 16, 17, 18, 19 o 21 es un aminoácido estabilizador del alfa hélice. En algunos aspectos, el aminoácido estabilizador del alfa hélice se ubica en la posición 16. En algunas realizaciones, el aminoácido estabilizador del alfa hélice es un aminoácido con carga negativa (por ejemplo, Glu), o un aminoácido con carga positiva (por ejemplo, que comprende una estructura de la Fórmula IV (por ejemplo, Lys)) o un aminoácido disustituido alfa, alfa. En algunos aspectos, el aminoácido α,α-disustituido comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde cada uno de R1 y R2 se selecciona independientemente del grupo formado por alquilo C1-C4, opcionalmente sustituido por un hidroxilo, amida, tiol, halo, o R1 y R2 junto con el carbono alfa al cual están unidos forman un anillo. En aspectos específicos, el aminoácido α,α-disustituido en la posición 16 es AIB.

En realizaciones adicionales, cuando el análogo de glucagón no comprende un aminoácido estabilizador del alfa hélice en la posición 20, y cuando una o más de las posiciones 16, 17, 18, 19 9 21 es un aminoácido estabilizador del alfa hélice, el análogo de glucagón comprende (i) una prolongación de 1 a 21

aminácidos del extremo de C al aminoácido en la posición 29 del péptido análogo o (ii) el aminoácido acilado o el aminoácido alquilado se ubica en la posición 10, 12 o 16. En algunos aspectos, el análogo de glucagón comprende una extensión de 1 a 21 aminoácidos del extremo de C al aminoácido en la posición 29 del péptido análogo, y opcionalmente el aminoácido en la posición 29 es Gly. La extensión de 1 a 21 aminoácidos en algunos aspectos es cualquiera de las que se describen en la presente – véase, por ejemplo, la sección que se titula "*Extensiones del Extremo de C*". En algunos aspectos, la extensión comprende una secuencia de aminoácidos que forma una estructura de jaula Trp, por ejemplo, la extensión comprende la secuencia de aminoácidos de GPSSGAPPPS (SEC ID NO: 5); o una secuencia de ésta sustituida conservadoramente. En aspectos alternativos, la extensión de 1 a 21 aminoácidos comprende por lo menos un aminoácido con carga. En aspectos ejemplares, la extensión comprende una secuencia de aminoácidos de: X1–X2, en donde X1 es un aminoácido con carga y X2 es un aminoácido alifático pequeño. En algunos aspectos, X1 es un aminoácido con carga positiva, por ejemplo, Arg. En algunos aspectos, la extensión comprende Arg–Gly.

De esta manera, en realizaciones ejemplares alternativas, el análogo de glucagón comprende (i) un aminoácido que comprende una cadena lateral de imidazol en la posición 1, (ii) un

aminoácido protector contra DPP-IV en la posición 2, opcionalmente, ácido aminoisobutírico, (iii) un aminoácido que comprende un grupo acilo o alquilo no nativo, opcionalmente en cualquiera de las posiciones 9, 10, 12, 16, 20 o 37-43, opcionalmente en donde el grupo alquilo o acilo no nativo se enlaza a dicho aminoácido por medio de un separador, (iv) un aminoácido estabilizador del alfa hélice en una o más de las posiciones 16-21, opcionalmente, posición 16, en donde el análogo no comprende un aminoácido estabilizador del alfa hélice en la posición 20, y (v) hasta diez (por ejemplo, hasta 1, 2, 3, 4, 5, 6, 7, 8 o 9) modificaciones adicionales de aminoácidos con relación a SEC ID NO: 1. En aspectos ejemplares, el análogo no comprende un aminoácido disustituido alfa, alfa en la posición 20, opcionalmente, AIB, o un aminoácido estabilizador del alfa hélice seleccionado del grupo formado por: Leu, Phe, Ala, Met, Gly, Ile, Ser, Asn, Glu, Asp, Lys y Arg. En aspectos ejemplares, el análogo de glucagón no se modifica en la posición 20 en comparación con SEC ID NO: 1 y, por lo tanto, tiene un residuo Gln que es el aminoácido nativo de glucagón en esta posición.

En realizaciones ejemplares, cuando el análogo de glucagón comprende un aminoácido estabilizador del alfa hélice en una o más de las posiciones 16-21, opcionalmente, posición 16, y el análogo no comprende un aminoácido estabilizador del alfa

hélice en la posición 20, y cuando el análogo de glucagón carece de una sección hidrófila, el análogo de glucagón exhibe un porcentaje de potencia de GIP de por lo menos 0,1% (por ejemplo, por lo menos 10%, por lo menos 20%). En realizaciones ejemplares, el análogo de glucagón tiene menos de 100 veces (por ejemplo, menos de o aproximadamente 90 veces, menos de o aproximadamente 80 veces, menos de o aproximadamente 70 veces, menos de o aproximadamente 60 veces, menos de o aproximadamente 50 veces, menos de o aproximadamente 40 veces, menos de o aproximadamente 30 veces, menos de o aproximadamente 20 veces, menos de o aproximadamente 15 veces, menos de o aproximadamente 10 veces, menos de o aproximadamente 5 veces) la selectividad para el receptor de GLP-1 humano respecto del receptor de GIP. En aspectos ejemplares, el péptido tiene una concentración media efectiva en el receptor de GIP que es menos de 100 veces (por ejemplo, menos de o aproximadamente 90 veces, menos de o aproximadamente 80 veces, menos de o aproximadamente 70 veces, menos de o aproximadamente 60 veces, menos de o aproximadamente 50 veces, menos de o aproximadamente 40 veces, menos de o aproximadamente 30 veces, menos de o aproximadamente 20 veces, menos de o aproximadamente 15 veces, menos de o aproximadamente 10 veces, menos de o aproximadamente 5 veces) su concentración efectiva media en el receptor de GLP-1, que, opcionalmente, es menos de 100 veces

(por ejemplo, menos de o aproximadamente 90 veces, menos de o aproximadamente 80 veces, menos de o aproximadamente 70 veces, menos de o aproximadamente 60 veces, menos de o aproximadamente 50 veces, menos de o aproximadamente 40 veces, menos de o aproximadamente 30 veces, menos de o aproximadamente 20 veces, menos de o aproximadamente 15 veces, menos de o aproximadamente 10 veces, menos de o aproximadamente 5 veces) su concentración efectiva media en el receptor de glucagón.

En aspectos ejemplares, cuando el análogo de glucagón comprende un aminoácido estabilizador del alfa hélice en una o más de las posiciones 16-21 y el análogo no comprende un aminoácido estabilizador del alfa hélice en la posición 20, el análogo de glucagón comprende un aminoácido estabilizador del alfa hélice en la posición 16, opcionalmente, en donde el aminoácido estabilizador del alfa hélice es un aminoácido con carga negativa (por ejemplo, Glu o Asp) o un aminoácido disustituido alfa, alfa. El aminoácido disustituido alfa, alfa en la posición 16 puede comprender R1 y R2, cada uno de los cuales está unido al carbono alfa, en donde cada uno de R1 y R2 se seleccionan independientemente del grupo formado por alquilo C1-C4, opcionalmente sustituido por hidroxilo, amida, tiol, halo, o R1 y R2 junto con el carbono alfa al cual están unidos forman un anillo. En aspectos ejemplares, el aminoácido en la posición 16 es AIB. En aspectos ejemplares, el análogo

de glucagón comprende en la posición 18 un aminoácido alifático pequeño, que es opcionalmente Ala, y un Arg en la posición 17. En aspectos ejemplares, el análogo de glucagón comprende la secuencia ERAAQ (SEC ID NO: 200) como posiciones 16 a 20 o ERAAQD (SEC ID NO:201) como las posiciones 16 a 21.

En aspectos ejemplares, el análogo de glucagón comprende un aminoácido que comprende un grupo acilo o alquilo no nativo en cualquiera de las posiciones 9, 10, 12, 16, 20. En aspectos ejemplares, el análogo de glucagón comprende un aminoácido unido en forma covalente a un grupo acilo o alquilo C12 a C18 en cualquiera de las posiciones 9, 10, 12, 13, 14, 16, 17 y 20. En aspectos ejemplares, el análogo de glucagón comprende un aminoácido unido en forma covalente al grupo acilo o alquilo C12 a C18, en cualquiera de las posiciones 10, 14. En algunos aspectos, el análogo de glucagón comprende un aminoácido acilado o un aminoácido alquilado en la posición 10, 12, o 16. En aspectos ejemplares, el aminoácido acilado o el aminoácido alquilado se ubica en la posición 14. En algunos aspectos, el análogo de glucagón comprende una extensión de 1 a 21 aminoácidos de extremo de C con respecto al aminoácido en la posición 29 y comprende un aminoácido que comprende un agrupo acilo o alquilo no nativo en cualquiera de las posiciones 37-43 (por ejemplo, 37, 38, 39, 40, 41, 42, 43). En

algunos aspectos, el aminoácido que comprende un grupo acilo o alquilo no nativo se ubica en la posición 40.

En realizaciones ejemplares, el acilo

En aspectos ejemplares, el análogo de glucagón comprende una extensión de 1 a 21 aminoácidos de extremo de C respecto del aminoácido en la posición 29 y, en algunos aspectos, la extensión forma una estructura que se conoce en el estado de la técnica como JanulaTrp. En algunos aspectos, la extensión comprende la secuencia de aminoácidos GPSSGAPPPS (SEC ID NO: 5) o GGPSSGAPPPS (SEC ID NO: 6) o GPSSGRPPPS (SEC ID NO: 183) o una secuencia de alguno de los que anteceden con 1, 2 o 3 sustituciones conservadoras de aminoácidos. En aspectos alternativos, la extensión comprende al menos un aminoácido con carga, por ejemplo, la extensión comprende una secuencia de aminoácidos de: X1-X2, en donde X1 es un aminoácido con carga (por ejemplo, un aminoácido con carga positiva (por ejemplo, Arg)) y X2 es un aminoácido alifático pequeño. En algunos aspectos, la extensión comprende Arg-Gly.

En aspectos ejemplares, el aminoácido acilado o el aminoácido alquilado comprende una estructura de la Fórmula I (opcionalmente, Lys), Fórmula II (opcionalmente, Cys) o Fórmula III (opcionalmente, Ser). Opcionalmente, en algunos

aspectos, el aminoácido acilado o el aminoácido alquilado comprenden una estructura de la Fórmula I, por ejemplo, Lys.

En algunas realizaciones, el aminoácido acilado o el aminoácido alquilado es un aminoácido aromático que comprende una cadena lateral de amina. En aspectos ejemplares, el aminoácido aromático que comprende una cadena lateral de amina es 4-amino-fenilalanina(4-aminoPhe), p-amino fenilglicina, p-amino homofenilalanina, o 3-amino tirosina. En aspectos ejemplares, el aminoácido aromático que comprende una cadena lateral es 4-aminoPhe. En aspectos ejemplares, el aminoácido acilado o alquilado es un aminoácido de la Fórmula II, n es 2 (homoserina). En aspectos ejemplares, el aminoácido acilado o alquilado es Thr o homotreonina. En realizaciones ejemplares, el aminoácido acilado o alquilado es un aminoácido aromático que comprende una cadena lateral hidroxilo, que incluye de modo no taxativo tirosina, homotirosina, metil-tirosina o 3-aminotirosina.

En aspectos ejemplares, el análogo de glucagón comprende un aminoácido unido en forma covalente a Cx-succinil, en donde x es un número entero entre 10 y 26, opcionalmente, entre 12 y 18. En aspectos ejemplares, el Cx-succinil se une al péptido o al análogo de glucagón por medio de un separador. El separador puede ser cualquiera de los que se describe en la presente.

En algunos aspectos, el aminoácido acilado o el aminoácido alquilado se enlazan con el grupo acilo o con el grupo alquilo mediante un separador. En algunos aspectos, se separador tiene una longitud de 3 a 10 átomos. En algunos aspectos, el separador es un aminoácido o dipéptido, y, en algunos aspectos, el separador comprende uno o dos residuos de aminoácido acídico, por ejemplo, Glu. En algunos aspectos, el grupo acilo o alquilo se enlaza con el aminoácido a través en donde la longitud total del separador y del grupo acilo es de alrededor de 14 a alrededor de 28 átomos. En algunos aspectos, el separador comprende un Cys. En algunos aspectos, el separador comprende uno o dos gamma-Glu. En algunos aspectos, el separador comprende un Lys. En algunos aspectos, el separador comprende una combinación de dos de Cys, gamma-Glu y Lys, o dos residuos gamma-Glu.

En aspectos particulares, el separador es un residuo Cys, que se une en forma covalente a un grupo alquilo, por ejemplo, una cadena de carbono funcionalizada o no funcionalizada. En aspectos ejemplares, el residuo Cys es S-palmitoil alquilado (es decir, S-palmitato alquilado), opcionalmente, en donde el residuo Cys se une al residuo Lys que es parte de la columna del péptido.

En aspectos ejemplares, el separador comprende una sección de polietilenglicol pequeña (PEG) que comprende una estructura $[-O-CH_2-CH_2-]_n$, en donde n es un número entero entre 2 y 16 (por ejemplo, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16).

Con relación al aminoácido acilado, el grupo acilo en algunos aspectos es un grupo acilo graso de C12 a C18 (por ejemplo, C12, C13, C14, C15, C16, C17, C18). En algunos aspectos, el grupo acilo es un grupo acilo graso de C14 o C16. En aspectos alternativos, el grupo acilo es ácido succínico o derivado de ácido succínico (por ejemplo, un derivado de ácido succínico de la Fórmula V, VI o VII). En aspectos alternativos, el grupo acilo es un ácido maléico o un derivado de ácido maléico (por ejemplo, un derivado de ácido maléico de la Fórmula VIII, IX o X).

Con relación al aminoácido alquilado, el grupo alquilo no nativo en algunos aspectos es una cadena de carbono carboxifuncionalizada de la estructura –Cx–COOH, en donde x es un número entero, opcionalmente un número entero entre 4 – 30 (por ejemplo, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30).

En aspectos ejemplares, el péptido o análogo de glucagón comprende dos o más grupos acilo o alquilo. En esta línea, el

péptido o análogo de glucagón puede ser un péptido diacilado o diacilado doble. Los dos o más grupos acilo o alquilo pueden estar dispuestos en forma lineal, opcionalmente con separadores intervinientes. Los dos o más grupos acilo o alquilo pueden estar dispuestos en formación ramificada, como se describe en la presente. En aspectos ejemplares, los dos grupos acilo o alquilo están unidos a un residuo separador Lys.

En algunos aspectos de las presentes divulgaciones, el análogo de glucagón comprende por lo menos un aminoácido del extremo de C con carga al aminoácido en la posición 27. Por ejemplo, en algunos aspectos, el análogo de glucagón comprende un aminoácido con carga (por ejemplo, un aminoácido con carga negativa) en la posición 28. El aminoácido con carga negativa en algunas realizaciones es Asp. En aspectos alternativos, el aminoácido en la posición 28 es un aminoácido con carga positiva, por ejemplo, un aminoácido con carga positiva es un aminoácido de la Fórmula I, por ejemplo, Lys.

En aspectos alternativos o adicionales, el análogo de glucagón comprende una modificación de aminoácido en la posición 27, en la posición 29, o en ambas posiciones. Por ejemplo, el aminoácido en la posición 27 es en algunos aspectos Leu, Nle,

Val o Lys y/o el aminoácido en la posición 29 es en algunos aspectos GlyorThr.

Los análogos de glucagón descriptos en la presente pueden comprender modificaciones adicionales de aminoácido, por ejemplo, hasta diez (por ejemplo, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) modificaciones adicionales, con relación a SEC ID NO: 1, como se enseña en la presente. En aspectos ejemplares, el análogo de glucagón comprende uno o más de:

a) un aminoácido protector contra DPP-IV en la posición 1 del análogo de péptido;
b) un aminoácido acídico, opcionalmente, Glu, en la posición 3;
c) un Ile en la posición 7;
d) un Ile o Arg en la posición 12;
e) un aminoácido acídico, opcionalmente, Glu, en la posición 15;
f) un aminoácido alifático, opcionalmente, Ala, en la posición 18;
g) un aminoácido acídico, opcionalmente Glu, en la posición 21;
h) un Asn, Ala o AIB en la posición 24;
i) un aminoácido alifático, opcionalmente, Ala o Leu, o Nle, en la posición 27;

j) un aminoácido acídico, opcionalmente Glu, o un aminoácido alifático, opcionalmente Ala, en la posición 28;

k) un aminoácido alifático, opcionalmente Ala, en la posición 29;

l) amidación en el extremo de C.

De acuerdo con lo que antecede, el análogo de glucagón en aspectos ejemplares comprende cualquier secuencia de aminoácidos entre SEC ID Nos: 27–33, 35–41, 43–46, 76–80, 83–87, 89 y 90, o cualquiera de SEC ID Nos: 94–100, 102–112, 120–124, 127–131. En aspectos ejemplares, el análogo de glucagón comprende o consiste de cualquiera de SEC ID Nos: 94–100, 102–112, 120–124, 127–131. En aspectos ejemplares, el análogo de glucagón comprende o consiste en cualquiera de SEC ID Nos: 28, 29, 31, 37–41, 43–46, 76–80, 83–87, 89 y 90. En aspectos ejemplares, el análogo de glucagón consiste en cualquiera de SEC ID NOs: 28, 29, 31, 37–41, 79, 80, 89, 90, 95, 130, 145–152, 155–167, 171, 176, 177, 180, 203–207, 212 y 230. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 27. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 30. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 32. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 33. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 35.

En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 36. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 37. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 89. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 31. En aspectos ejemplares, el análogo de glucagón comprende o consiste en SEC ID NO: 180.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 28. En aspectos ejemplares, el péptido consiste en SEC ID NO: 31.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 31. En aspectos ejemplares, el péptido consiste en SEC ID NO: 31.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 37. En aspectos ejemplares, el péptido consiste en SEC ID NO: 37.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 89. En aspectos ejemplares, el péptido consiste en SEC ID NO: 89.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 95. En aspectos ejemplares, el péptido consiste en SEC ID NO: 95.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 130. En aspectos ejemplares, el péptido consiste en SEC ID NO: 130.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 31. En aspectos ejemplares, el péptido consiste en SEC ID NO: 171.

La invención también contempla un péptido que comprende la secuencia SEC ID NO: 180. En aspectos ejemplares, el péptido consiste en SEC ID NO: 180.

La invención proporciona un péptido que comprende la secuencia de SEC ID NO: 184,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEC ID NO: 184)

en donde:

X_2 es AIB;

X_3es Gln o análogo deGln;

X_{10}es Tyr o un aminoácido unido en forma covalente a un grupo acilo o alquilo C12 a C18;

X_{16}es cualquier aminoácido, opcionalmente, cualquier aminoácido que no seaGly, Pro, y Ser;

X_{18}es Arg o Ala;

X_{20}es un aminoácido con carga negativa o un aminoácido con carga neutral, opcionalmente, AIB o Gln;

X_{21}es un aminoácidoacídico, opcionalmente, AspoGlu;

X_{27}es Leu, Ala, Nle, o Met;

X_{28}es Ala o unaminoácido acídico (opcionalmente, Asp o Glu);

X_{29}es Ala o Gly;

X_{35}es Ala o un aminoácido básico (opcionalmente, Arg o Lys);

en donde, cuando X_{28}es un aminoácidoacídico, X_{35}es un aminoácido básico;

en donde, cuando X_{10}es Tyr, el péptidocomprendeen la posición 40 un aminoácido unido en forma covalente a un grupo acilo o alquilo de C12 a C18, y, en donde, opcionalmente, el péptido comprendeGlyen la posición 41, y

en donde el aminoácido del extremo de C del péptidoes amidado.

En aspectos ejemplares, X10 de SEC ID NO: 84 es Tyr, el péptido comprende en la posición 40 un aminoácido unido en forma covalente a un grupo acilo o alquilo de C12 a C18, y el péptido opcionalmente comprende Gly en la posición 41. En aspectos ejemplares, X10 de SEC ID NO: 184 es un aminoácido unido en forma covalente con un grupo acilo o alquilo C12-C18.

En aspectos ejemplares, X20 de SEC ID NO: 184 es Gln, y, opcionalmente, el aminoácido en la posición 16 es un aminoácido con carga negativa (por ejemplo, Glu). En aspectos ejemplares, X18 de SEC ID NO: 184 es Ala y el péptido comprende E16, R17, A18, A19, y Q20.

En aspectos ejemplares alternativos, X20 de SEC ID NO: 184 es AIB. Opcionalmente, X_{16} de SEC ID NO: 184 es cualquier aminoácido que no sea AIB.

Además, en aspectosejemplares, (i) X_{28} de SEC ID NO: 184 es un aminoácidoacídico, opcionalmente Asp o Glu, y X_{35} de SEQ ID NO: 184 es un aminoácidobásico, opcionalmenteArg o Lys, (ii) únicamenteuno de X_{27}, X_{28} y X_{29} de SEC ID NO: 184 es un Ala, (iii) el péptidocomprende un Glyamidado en el extremo de C.
La invencióntambiénproporcionaunpéptidoquecomprende la secuencia de SEC ID NO: 184 con hasta 3 modificaciones de aminoácido (porejemplo, sustitucionesconservadoras) con relación a SEC ID NO: 184, en donde el análogoexhibeactividadagonista en cadauno de: el receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagónhumano. En realizacionesejemplares, la actividad (porejemplo, la concentraciónefectiva media) en cadauno de el receptor de GIP humano, el receptor de GLP-1 humano y el

receptor de glucagónhumano del análogo de glucagon estádentro de las 100 veces (porejemplo, dentro de 50 veces, dentro de 25 veces, dentro de 10 veces) el uno del otro.

La invencióntambiénproporcionaunpéptidoquecomprende la secuencia de SEC ID NO: 185,

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGAPPPS$

(SEQ ID NO: 185)

en donde:

X_2 es AIB;

X_{10} es Tyr o un aminoácido covalentemente unido a un grupo acilo o alquilo C12 a C18;

X_{16} es Glu, un aminoácido disustituido alfa, alfa, Lys o

X_{18} es Arg o Ala;

X_{20} es AIB o Gln;

X_{21} es Asp o Glu;

X_{27} es Leu, Nle, o Met;

X_{28} es Ala, Asp o Glu;

X_{29} es Gly de Thr;

y

en donde, cuando X_{10}es Tyr, el péptido comprende en la posición 40 un aminoácido covalentemente unido a un grupo acilo o alquilo C12 a C18 y, en donde, opcionalmente, el péptido comprendeGlyen la posición 41, y

en donde el aminoácido del extremo de C del péptido es amidado.

La invenciónambiénproporcionaunpéptidoquecomprende la secuencia de SEC ID NO: 185 con hasta 3 modificaciones de aminoácido (porejemplo, sustitucionesconservadoras) con relación a SEC ID NO: 185, en donde el análogoexhibeactividadagonista en cadauno de: el receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagónhumano. En realizacionesejemplares, la actividad (porejemplo, la concentraciónefectiva media) en cadauno del receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagónhumano del análogo de glucagon estádentro de las 100 veces (porejemplo, dentro de 50 veces, dentro de 25 veces, dentro de 10 veces) el uno del otro.

La invenciónambiénproporcionaunpéptidoquecomprende la secuencia de SEC ID NO: 196,

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}GPSSGX_{35}PPPS$

(SEC ID NO: 196)

en donde:

X_2 es AIB;

X_3 es análogo de Gln o Gln o un aminoácido que reduce la actividad de glucagón (opcionalmente Glu);

X_{10} es Tyr o un aminoácido covalentemente unido a un grupo acilo o alquilo de C12 a C18;

X_{16} es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro, y Ser;

X_{18} es Arg o Ala;

X_{20}es un aminoácido con carga negativao un aminoácido con carga neutral, opcionalmente, AIB o Gln;

X_{21}es un aminoácido acídico, opcionalmente, Asp o Glu;

X_{27}es Leu, Ala, Nle, o Met;

X_{28}es Ala o un aminoácido acídico (opcionalmente, Asp o Glu);

X_{29}es Ala o Gly;

X_{35}es Ala o un aminoácido básico (opcionalmente, Arg o Lys);

en donde, cuando X_{28}es un aminoácidoacídico, X_{35}es un aminoácido básico;

en donde, cuando X_{10}es Tyr, el péptido comprende en la posición 40 un aminoácido covalentemente unido a un grupo acilo o alquilo de C12 a C18, y, en donde, opcionalmente, el péptido comprendeGlyen la posición 41, y

en donde el aminoácido del extremo de C del péptido es amidado.

En la presente se describen otros aminoácidos que reducen la actividad de glucagón. Véase la sección que se titula “Posición 3”. En aspectos ejemplares, X_3 es un aminoácido hidrófobo, básico o acídico (por ejemplo, ácido glutámico, ornitin, norleucina). En aspectos ejemplares, X_3 es Glu.

Además, se provee un péptido que comprende la secuencia de SEC ID NO: 18:

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SECID NO: 186)

en donde:

X_2 es AIB;

X_3es Gln o análogo deGln;

X_{10}es Tyr o un aminoácido covalentemente unido a un grupo acilo o alquilo de C10a C26;

X_{16} es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro, y Ser;

X_{18}es Arg o Ala;

X_{20}es un aminoácido con carga negativa o un aminoácido con carga neutral, opcionalmente, AIB o Gln;

X_{21} es X_{21}es un aminoácido acídico, opcionalmente, AspoGlu;

X_{27}es Leu, Ala, Nle, o Met;

X_{28}es Ala o un aminoácidoacídico (opcionalmente, Asp o Glu);

X_{29}es Ala, Gly o Thr; y

en dondeel péptido comprendeun aminoácido unido en forma covalente a un grupo acilo o alquilo de C10 a C26, opcionalmente, en posición 10, y el aminoácido del extremo de C del péptido es amidado.

En aspectos ejemplares, X20 de SEC ID NO: 186 es AIB. En aspectos ejemplares, X29 es Thr y el péptido no comprende

GPSSGAPPPS (SEC ID NO: 5). En algunos aspectos, X20 de SEC ID NO: 186 es AIB y X16 es un aminoácido diferente de AIB.

En aspectos ejemplares, X20 es Gln. En algunos aspectos, X16 es un aminoácido con carga negativa, opcionalmente, Glu. En aspectos ejemplares, X18 es Ala, y opcionalmente, el péptido comprende E16, R17, A19 y Q20.

En algunos aspectos, el péptido de SEQ ID NO: 186 comprende una extensión de extremo C de 1 a 21 aminoácidos al aminoácido en posición 29, y opcionalmente el aminoácido en posición 29 es Gly. En los aspectos ejemplares, la extensión comprende la secuencia GPSSGAPPPS (SEQ ID NO: 5) del aminoácido, o una secuencia sustituida de manera conservadora, o donde la extensión comprende la secuencia X1-X2, donde X1 es un aminoácido con carga positiva y X2 es un pequeño aminoácido alifático, opcionalmente, donde X1 aminoácido con carga positiva. En algunos aspectos, el aminoácido con carga positiva es Arg y opcionalmente el péptido comprende o constituye Arg-Gly. En ciertos aspectos, la extensión comprende la secuencia GPSSGAPPPS (SEQ ID NO: 5) del aminoácido seguida por Lys o Lys-Gly, donde el Lys está covalentemente unido a un grupo acilo de C10 a C26.

En los aspectos ejemplares, el péptido comprende la SEQ ID NO: 186, donde X_2 es AIB, X_3 es Gln, X_{10} es un aminoácido covalentemente unido a un grupo de acilo o alquilo de C10 a C26, X_{18} es Arg o Ala, X_{20} es AIB o Gln, X_{21} es Asp o Glu, X_{29} es Gly, y el aminoácido de extremo C es amidado, donde Gly en posición 29 se funde a GPSSGAPPPS seguido por Lys o Lys-Gly, donde el Lys está covalentemente unido a un grupo acilo de C10-C26.

La invención también proporciona un péptido que comprende la secuencia de SEQ ID NO: 186 con hasta 3 modificaciones de

aminoácidos (por ejemplo, sustituciones conservadoras)relativas a la SEQ ID NO: 186, donde el análogo presenta la actividad de agonista en cada receptor de de GIP humano, el receptor de de GLP-1 humano y el receptor de de glucagón humano. En las representaciones ejemplares, la actividad (por ejemplo, el EC50) en cada receptor de de GIP humano, el receptor de de GLP-1 humano y el receptor de de glucagón humano del análogo de glucagón es en 100 veces (por ejemplo, en 50 veces, en 25 veces, en 10 veces) entre sí.

La invención proporciona un péptido que comprende la secuencia de SEQ ID NO: 187:

$HX_2QGTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SEQ ID NO: 187)

donde:

X_2 es AIB;

X_{10} es Tyr o un aminoácido covalentemente unido a un grupo acilo o alquilo de C10 to C26;

X_{16} es Glu, alfa, aminoácido alfa disustituido, o Lys;

X_{18} es Arg o Ala;

X_{20} es un aminoácido con carga negativa o un aminoácido con carga neutral, opcionalmente, AIB o Gln;

X_{21} es Asp o Glu;

X_{27} es Leu, Ala, Nle, o Met;

X_{28} es Ala, Asp o Glu;

X_{29} es Gly o Thr; y

donde el péptido comprende un aminoácido covalentemente unido a un grupo acilo o alquilo C12 a C18, opcionalmente, en posición 10, y el aminoácido extremo C del péptido es amidado.

La invención también proporciona un péptido que comprende la secuencia de SEQ ID NO: 187 con hasta 3 modificaciones de aminoácidos (por ejemplo, sustituciones conservadoras) relativas a la SEQ ID NO: 187, donde el análogo presenta la actividad de agonista en cada receptor de de GIP humano, el receptor de de GLP-1 humano y el receptor de de glucagón humano. En las representaciones ejemplares, la actividad (por ejemplo, el EC50) en cada receptor de GIP humano, el receptor de GLP-1 humano y el receptor de glucagón humano del análogo de glucagón es en 100 veces (por ejemplo, en 50 veces, en 25 veces, en 10 veces) entre sí.

La invención proporciona un péptido que comprende la secuencia de SEQ ID NO: 198:

$HX_2X_3GTFTSDX_{10}SKYLDX_{16}RX_{18}AX_{20}X_{21}FVQWLX_{27}X_{28}X_{29}$

(SEQ ID NO: 198)

donde:

X_2 es AIB;

X_3 es análogo de Gln o Gln o un aminoácido que reduce la actividad del glucagón(por ejemplo, Glu);

X_{10} es Tyr o un aminoácido covalentemente unido a un grupo acilo o alquilo de C10 a C26;

X_{16} es cualquier aminoácido, opcionalmente, cualquier aminoácido distinto de Gly, Pro, y Ser;

X_{18} es Arg o Ala;

X_{20} es un aminoácido con carga negativa o un aminoácido con carga neutral, opcionalmente, AIB o Gln;

X_{21} es X_{21} es un aminoácido acídico, opcionalmente, Asp o Glu;

X_{27} es Leu, Ala, Nle, o Met;

X_{28} es Ala o un aminoácido acídico(opcionalmente, Asp o Glu);

X_{29} es Ala, Gly o Thr; y donde el péptido comprende un aminoácido covalentemente unido a un grupo acilo o alquilo de C10 a C26, opcionalmente, en posición 10, y el aminoácido extremo C del péptido es amidado.

Los aminoácidos adicionales que reducen la actividad de glucagón se describen en la presente. Ver sección denominada “Posición 3”. En los aspectos ejemplares, X_3 es un aminoácido acídico, básico o hidrofóbico (por ejemplo, ácido glutámico, ornitina, norleucina). En los aspectos ejemplares, X_3 es Glu.

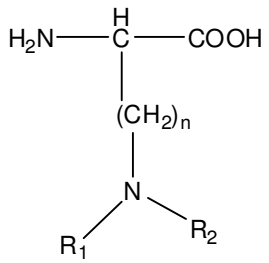
La invención proporciona un péptido que comprende la SEQ ID NO: 184. La invención proporciona un péptido que comprende la SEQ ID NO: 185. La invención proporciona un péptido que comprende

la SEQ ID NO: 196. La invención proporciona un péptido que comprende la SEQ ID NO: 186. La invención proporciona un péptido que comprende la SEQ ID NO: 187. La invención proporciona un péptido que comprende la SEQ ID NO: 198.

Además según la presente es un análogo de glucagón (SEQ ID NO: 1) que tiene actividad de agonista GIP, que comprende:

(a) un aminoácido que comprende la cadena lateral de imidazol en posición 1,

(b) en posición 16, un aminoácido de Fórmula IV:



[Fórmula IV],

donde n es 1 a 7, donde cada R1 y R2 es independientemente seleccionado del grupo que constituye H, alquilo C1-C18, (alquilo C1-C18)OH, (alquilo C1-C18)NH2, (alquilo C1-C18)SH, (alquilo C0-C4)(C3-C6)cicloalquilo, (alquilo C0-C4)(heterocíclico C2-C5), (alquilo C0-C4)(arilo C6-C10)R7, y (alquilo C1-C4)(heteroarilo C3-C9), donde R7 es H u OH, donde opcionalmente la cadena lateral del aminoácido de la Fórmula IV comprende un grupo de amino libre,

(c) un aminoácido α,α-disustituido en posición 20,

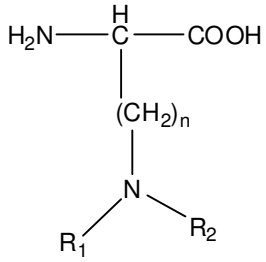
(d) hasta diez modificaciones adicionales de aminoácidos relativas a la SEQ ID NO: 1,

donde, cuando el análogo carece de un grupo hidrofílico, el análogo de glucagón presenta al menos 0.1% de actividad del GIP nativo en el receptor de GIP, donde el análogo de glucagón tiene una selectividad inferior a 100 veces para el receptor de GLP-1 humano versus el receptor de GIP.

En las representaciones ejemplares, el análogo de glucagón (SEQ ID NO: 1) que tiene actividad de agonista GIP, comprende uno o más de:

(a) un aminoácido que comprende la cadena lateral de imidazol en posición 1,

(b) en posición 16, un aminoácido de Fórmula IV:



[Fórmula IV],

donde n es 1 a 7, donde cada R1 y R2 es independientemente seleccionado del grupo que constituye H, alquilo C1-C18, (alquilo C1-C18)OH, (alquilo C1-C18)NH2, (alquilo C1-C18)SH, cicloalquilo (alquilo C0-C4)(C3-C6), (alquilo C0-C4)(heterocíclico C2-C5), (alquilo C0-C4)(arilo C6-C10)R7, y

(alquilo C1-C4)(heteroarilo C3-C9), donde R7 es H u OH, donde opcionalmente la cadena lateral del aminoácido de la Fórmula IV comprende un grupo de amino libre,

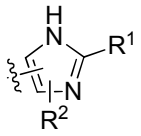
(c) un aminoácido α,α-disustituido en posición 20,

(d) hasta diez modificaciones adicionales de aminoácidos relativas a la SEQ ID NO: 1,

donde, cuando el análogo carece de un grupo heterólogo, por ejemplo, un grupo hidrofílico (por ejemplo, PEG), el análogo de glucagón presenta al menos el 0,1% (por ejemplo, al menos el 1%, al menos el 10%, al menos el 50%, al menos el 80%, al menos el 100%, al menos el 500%) de actividad del GIP nativo en el receptor de GIP. En los aspectos ejemplares, el péptido tiene un EC50 en el receptor de GIP que es inferior a a 100 veces (por ejemplo, inferior a o aproximadamente 90 veces, inferior a o aproximadamente 80 veces, inferior a o aproximadamente 70 veces, inferior a o aproximadamente 60 veces, inferior a o aproximadamente 50 veces, inferior a o aproximadamente 40 veces, inferior a o aproximadamente 30 veces, inferior a o aproximadamente 20 veces, inferior a o aproximadamente 15 veces, inferior a o aproximadamente 10 veces, inferior a o aproximadamente 5 veces), diferente de su EC50 en el receptor de GLP-1, que, opcionalmente, es inferior a 100 veces (por ejemplo, inferior a o aproximadamente 90 veces, inferior a o aproximadamente 80 veces, inferior a o aproximadamente 70 veces, inferior a o aproximadamente 60 veces, inferior a o

aproximadamente 50 veces, inferior a o aproximadamente 40 veces, inferior a o aproximadamente 30 veces, inferior a o aproximadamente 20 veces , inferior a o aproximadamente 15 veces, inferior a o aproximadamente 10 veces, inferior a o aproximadamente 5 veces), diferente de su EC50 en el receptor de glucagón.

En las representaciones ejemplares, el análogo glucagón comprende en posición 1 un aminoácido que comprende una estructura de Fórmula A



[Fórmula A],

donde cada R1 y R2 es independientemente seleccionado del grupo que constituye H, alquilo (C1-6), alquilo O(C1-6), (C1-6)alquilo-OH, F, y alquilo (C1-C6)del cual al menos un H es reemplazado por F.

En los aspectos ejemplares, el aminoácido en posición 1 es el residuo nativo de glucagón (SEQ ID NO: 1) L-histidina (His), o es un derivado de His (derivado de His), por ejemplo, D-histidina, desaminohistidina, hidróxilo-histidina, acetilo-histidina, homo-histidina, N-metilo histidina, alfa-metilo histidina, ácido acético de imidazol, o alfa, ácido acético de imidazol alfa-dimetilo (DMIA).

En los aspectos ejemplares, el análogo de glucagón comprende el aminoácido de Fórmula IV en posición 16 en (b) es homoLys, Lys, Orn, o ácido 2,4-diaminobutírico (Dab).

En los aspectos ejemplares, el aminoácido en posición 20, por ejemplo, el aminoácido α,α-disustituido, comprende R1 y R2, cada uno de los cuales está unido al carbono alfa, donde cada R1 y R2 es independientemente seleccionado del grupo que constituye el alquilo C1-C4, opcionalmente sustituido con un hidróxilo, amida, tiol, halo, o R1 y R2 junto con el carbono alfa al cual se unen para formar un anillo. En los aspectos ejemplares, la α,α-disustituida en posición 20 es AIB. En otros aspectos ejemplares, la α,α-disustituida en posición 20 es ACPC.

En los aspectos ejemplares, el análogo de glucagón comprende hasta diez modificaciones adicionales relativas a la SEQ ID NO: 1. En los aspectos ejemplares, el análogo de glucagón comprende una sustitución de aminoácido, relativa a la SEQ ID NO: 1, en una o más de las posiciones 2, 12, 17, 18, 21, 24, 27, 28, y 29. En los aspectos ejemplares, el análogo de glucagón comprende uno o más de:

i. un aminoácido DPP-IV protector en posición 2; opcionalmente AIB o D-Ser;

ii. un aminoácido grande, alifático, no polar en posición 12, opcionalmente Ile;

iii. un aminoácido diferente de Arg en posición 17, opcionalmente Gln;

iv. un aminoácido pequeño alifático en posición 18, opcionalmente Ala;

v. un aminoácido diferente de Asp en posición 21, opcionalmente Glu;

vi. un aminoácido diferente de Gln en posición 24, opcionalmente Asn o Ala;

vii. un aminoácido diferente de Met en posición 27, opcionalmente Leu;

viii. un aminoácido diferente de Asn en posición 28, opcionalmente Ala;

ix. un aminoácido diferente de Thr en posición 29, opcionalmente Gly; y

x. una extensión de extremo C de aminoácidos de 1 a 21 en posición 29.

En los aspectos ejemplares, el análogo de glucagón comprende una extensión de GPSSGAPPPS o GPSSGAPPPSC.

En los aspectos ejemplares, el análogo de glucagón que comprende un His en posición 1, Lys en posición 16 y AIB en posición 20 no comprende Gln-Ala en posiciones 17-18.

En otras representaciones ejemplares, el análogo de glucagón comprende una secuencia de aminoácidos de cualquiera de las SEQ ID NOs: 48, 52, 53, y 74. Dichos análogos de glucagón son similares en estructura a aquellas SEQ ID NOs: 27-33, 35-41,

43-46, 76-80, 83-87, 89, y 90, excepto que los análogos de glucagón antes mencionados (SEQ ID NOs: 48, 52, 53, y 74) no comprenden un aminoácido acídico o alquilado.

En incluso otras representaciones ejemplares, el análogo de glucagón comprende una secuencia de aminoácidos de cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, y 92 o cualquiera de las SEQ ID NOs: 114-119, 125, 126, y 133, o cualquiera de las SEQ ID NOs: 139-144, 150-153, 208, 210, y 211. Dichos análogos de glucagón comprenden un aminoácido grande, aromático en posición 1, por ejemplo, Tyr.

En algunas representaciones, los péptidos agonistas de GIP comprenden una secuencia de aminoácidos de cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, y 127-131, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, y 133. En algunas representaciones, el péptido agonista de GIP comprende una secuencia de aminoácidos de cualquiera de las SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, y 230.

En algunas representaciones, los péptidos agonistas de GIP comprenden una estructura basada en una secuencia parental que comprende cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46,

76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, y 127-131, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, y 133, o cualquiera de las SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, y 230, pero difiere de la secuencia parental en una o más posiciones.

En algunas o cualquiera de las representaciones, el péptido de las presentes divulgaciones es un análogo de una secuencia parental que comprende cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, y 127-131, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, y 133, o cualquiera de las SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, y 230 que comprende una secuencia de aminoácidos basada en la secuencia de aminoácidos de la secuencia parental pero difiere de la secuencia parental puesto que la secuencia de aminoácidos del análogo comprende uno o más (por ejemplo, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, y en algunas instancias, 16 o más (por ejemplo, 17, 18, 19, 20, 21, 22, 23, 24, 25, etc.), modificaciones especificadas u opcionales de aminoácidos. En algunas o cualquiera de las representaciones, el péptido de las presentes divulgaciones comprende un total de

1, hasta 2, hasta 3, hasta 4, hasta 5, hasta 6, hasta 7, hasta 8, hasta 9, o hasta 10 modificaciones adicionales de aminoácidos relativas a la secuencia parental que comprende cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, y 127-131, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, y 133, o cualquiera de las SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, y 230. En algunas o cualquiera de las representaciones, las modificaciones son cualquiera de aquellas que fueron descritas en la presente invención con respecto a los análogos de glucagón, por ejemplo, acilación, alquilación, pegilación, truncamiento en extremo C, substitución del aminoácido en una o más posiciones 1, 2, 3, 7, 10, 12, 15, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, y 29.

En algunas o cualquiera de las representaciones, la modificación es una substitución o reemplazo de aminoácidos, por ejemplo, una sustitución de aminoácidos conservadora. En algunos aspectos, la sustitución conservadora es un reemplazo del aminoácido en una o más posiciones 2, 5, 7, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 27, 28 o 29. En representaciones alternativas, la sustitución de aminoácidos no es una sustitución de aminoácidos conservadora, por ejemplo, es una sustitución de aminoácidos no conservadora.

En algunas representaciones, el péptido de las presentes divulgaciones comprende una secuencia de aminoácidos que tiene al menos el 25% de la identidad de la secuencia a la secuencia de aminoácidos de la secuencia parental, que comprende cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, y 127-131, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, y 133, o cualquiera de las SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, y 230. En algunas representaciones, el péptido de las presentes divulgaciones comprende una secuencia de aminoácidos que es al menos el 30%, al menos el 40%, al menos el 50%, al menos el 60%, al menos el 70%, al menos el 80%, al menos el 85%, al menos el 90% o supera el 90% de la identidad de la secuencia a la secuencia parental. En algunas representaciones, la secuencia de aminoácidos del péptido ahora divulgado que tiene el % de identidad de secuencia antes mencionado es la secuencia de aminoácidos completa del péptido ahora divulgado. En algunas representaciones, la secuencia de aminoácidos del péptido de las presentes divulgaciones que tiene el % de identidad de secuencia antes mencionado es solo una parte de la secuencia de aminoácidos del péptido ahora divulgado. En algunas representaciones, el péptido ahora divulgado comprende una

secuencia de aminoácidos que tiene aproximadamente A% o mayor identidad de secuencia a una secuencia de aminoácidos de referencia de al menos 5 aminoácidos contiguos (por ejemplo, al menos 6, al menos 7, al menos 8, al menos 9, al menos 10 aminoácidos) de la secuencia parental, donde la secuencia de aminoácidos de referencia comienza con el aminoácido en posición C de la SEQ ID NO: 1 y finaliza con el aminoácido en posición D de la SEQ ID NO: 1, donde A es 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99; C es 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, o 28 y D es 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 o 29. Todas y cada una de las posibles combinaciones de los parámetros antes mencionados son previstos, que incluye pero no taxativamente, por ejemplo, donde A es 90% y C y D son 1 y 27, o 6 y 27, o 8 y 27, o 10 y 27, o 12 y 27, o 16 y 27.

Los análogos de la secuencia parental que comprenden cualquiera de las SEQ ID NOs: 27–33, 35–41, 43–46, 76–80, 83–87, 89, y 90, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58–60, 62–66, 68–70, 72, 73, 75, 81, 82, 88, y 92, o cualquiera de las que comprende las SEQ ID NOs: 28, 29, 31, 37–41, 79, 80, 89, 90, 95, 130, 145–152, 155–167, 171, 176, 177, 180, 203–207, 212, y 230 descritas en la presente invención pueden comprender una columna vertebral

del péptido de cualquier cantidad de aminoácidos, es decir, pueden ser de cualquier longitud peptídica. En algunas representaciones, los péptidos descritos en la presente invención tienen la misma longitud que la SEQ ID NO: 1, es decir, son 29 aminoácidos de longitud. En algunas representaciones, el péptido ahora divulgado es más largo que 29 aminoácidos de longitud, por ejemplo, el péptido ahora divulgado comprende una extensión de extremo C de 1-21 aminoácidos, tal como será descrito más adelante en la presente invención. Por lo tanto, el péptido de las presentes divulgaciones en algunas representaciones, es 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, o 50 aminoácidos de longitud. En algunas representaciones, el péptido ahora divulgado es hasta 50 aminoácidos de longitud. En algunas representaciones, el péptido ahora divulgado es más largo que 29 aminoácidos de longitud (por ejemplo, superior a 50 aminoácidos, (por ejemplo, al menos o aproximadamente 60, al menos o aproximadamente 70, al menos o aproximadamente 80, al menos o aproximadamente 90, al menos o aproximadamente 100, al menos o aproximadamente 150, al menos o aproximadamente 200, al menos o aproximadamente 250, al menos o aproximadamente 300, al menos o aproximadamente 350, al menos o aproximadamente 400, al menos o aproximadamente 450, al menos o aproximadamente 500 aminoácidos de longitud) debido a la fusión con otro péptido. En otras representaciones, el péptido ahora divulgado es

inferior a 29 aminoácidos de longitud, por ejemplo, 28, 27, 26, 25, 24, 23, aminoácidos.

De acuerdo con lo antes mencionado, en algunos aspectos, el péptido de las presentes divulgaciones es un análogo de una secuencia parental que comprende cualquiera de las SEQ ID NOs: 27-33, 35-41, 43-46, 76-80, 83-87, 89, 90, 94-100, 102-112, 120-124, y 127-131, o cualquiera de las SEQ ID NOs: 48, 52, 53, y 74, o cualquiera de las SEQ ID NOs: 50, 51, 54, 56, 58-60, 62-66, 68-70, 72, 73, 75, 81, 82, 88, 92, 114-119, 125, 126, y 133, o cualquiera de las SEQ ID NOs: 28, 29, 31, 37-41, 79, 80, 89, 90, 95, 130, 145-152, 155-167, 171, 176, 177, 180, 203-207, 212, y 230 cuya secuencia del análogo comprende una o más modificaciones de aminoácidos que afectan la actividad de GIP, la actividad de glucagón, y/o actividad de GLP-1, mejoran la estabilidad, por ejemplo, la reducción de la degradación del péptido (por ejemplo, la mejora de la resistencia a las proteasas de DPP-IV), mejoran la solubilidad, incrementan la semivida, retrasan el comienzo de la acción, extienden la duración de la acción en el GIP, glucagón, o receptor de GLP-1, o una combinación de cualquiera de las antes mencionadas. Dichas modificaciones de aminoácidos, junto con otras modificaciones, están descritas más adelante en la presente invención con respecto a los análogos de glucagón, y cualquiera de estas modificaciones se pueden aplicar de manera individual o en combinación.

MÉTODOS PARA CREAR PÉPTIDOS

Los métodos conocidos en el arte se pueden obtener mediante los análogos de glucagón de la divulgación. Los métodos adecuados de los péptidos sintetizadores de novo son descriptos en la presente invención, por ejemplo, Chan et al., *Fmoc Solid Phase Peptide Synthesis*, *Oxford University Press*, Oxford, Reino Unido, 2005; *Peptide and Protein Drug Analysis*, ed. Reid, R., *Marcel Dekker, Inc.*, 2000; *Epitope Mapping*, ed. Westwood et al., *Oxford University Press*, Oxford, Reino Unido, 2000; y Patente U.S. No. 5.449.752. Los métodos ejemplares adicionales para crear los péptidos de las presentes divulgaciones se encuentran en el Ejemplo 1.

En algunas representaciones, los péptidos descriptos en la presente invención son comercialmente sintetizados por las empresas, como *Synpep* (Dublin, CA), *Peptide Technologies Corp.* (Gaithersburg, MD), y *Multiple Peptide Systems* (San Diego, CA). En este sentido, los péptidos pueden ser sintéticos , recombinantes, aislados, y/o purificados.

También, en las instancias donde los análogos de la divulgación no comprende n los aminoácidos no-codificados o no-naturales, el análogo de glucagón puede ser recombinantemente creado mediante el uso de un ácido nucleico que codifica la secuencia de aminoácidos del análogo mediante el uso de métodos recombinantes estándar. Ver, por ejemplo, Sambrook et al., *Molecular Cloning: A Laboratoy Manual*. 3rd ed., *Cold Spring*

Harbor Press, *Cold Spring Harbor*, NY 2001; y Ausubel et al., Current Protocols in Molecular Biology, *Greene Publishing Associates* y *John Wiley & Sons*, NY, 1994.

En algunas representaciones, el análogo de glucagones de la divulgación está aisladas. El término "aislado" como se utiliza en la presente invención significa haber sido quitado de su medio natural. En las representaciones ejemplares, se crea el análogo a través de métodos recombinantes y se aísla el análogo de la célula huésped.

En algunas representaciones, el análogo de glucagón de la divulgación son purificados. El término "purificados," como se utiliza en la presente invención se relaciona al aislamiento de una molécula o compuesto en una forma que está esencialmente libre de contaminantes que en algunos aspectos están normalmente relacionados con la molécula o compuesto en un medio nativo o natural y significa que se ha aumentado en pureza como resultado de estar separado de otros componentes de la composición original. El péptido o compuesto purificado incluyen, por ejemplo, péptidos esencialmente libre de moléculas de ácido nucleico, lípidos, y carbohidratos, u otras materias primas o intermediarios que son utilizados o formados durante la síntesis química de los péptidos. Se reconoce que "pureza" es un término relativo, y que no se interpreta necesariamente como pureza absoluta o enriquecimiento absoluto. En algunos aspectos, la pureza es al menos o aproximadamente

50%, es al menos o aproximadamente 60%, al menos o aproximadamente 70%, al menos o aproximadamente 80%, o al menos o aproximadamente 90% (por ejemplo, al menos o aproximadamente 91%, al menos o aproximadamente 92%, al menos o aproximadamente 93%, al menos o aproximadamente 94%, al menos o aproximadamente 95%, al menos o aproximadamente 96%, al menos o aproximadamente 97%, al menos o aproximadamente 98%, al menos o aproximadamente 99% o es aproximadamente 100%).

CONJUGADOS

La invención además proporciona conjugados que comprenden uno o más de los análogos de glucagón descritos en la presente a un grupo heterólogo. Tal como se utiliza en la presente, el término “grupo heterólogo” es sinónimo con el término “grupo conjugado” y se refiere a cualquier molécula (química o bioquímica, de origen natural o no-codificada) que es diferente de los análogos de glucagón descriptos en la presente invención. Los grupos conjugados ejemplares que pueden estar unidos a cualquiera de los análogos descriptos en la presente invención incluyen pero no de manera taxativa a un péptido heterólogo o polipéptido (que incluyen por ejemplo, una proteína plasmática), un agente objetivo, una inmunoglobulina o porción(por ejemplo, región variable, CDR, o región Fc), una etiqueta de diagnóstico como una etiqueta radioisótopica, fluoróforica o enzimática, un polímero que incluye polímeros solubles en agua, u otros agentes terapéuticos o de

diagnóstico. En algunas representaciones se proporciona un conjugado que comprende un análogo de la presente invención y una proteína plasmática, donde se selecciona la proteína plasmática del grupo que constituye albúmina, transferina, fibrinógeno y globulinas. En algunas representaciones el grupo de proteínas plasmáticas del conjugado es albúmina o transferina. El conjugado en algunas representaciones comprende uno o más de los análogos de glucagón descritos en la presente invención y uno o más de: un péptido (que es distinto del glucagón y/o de los análogos de glucagón activos del receptor de GLP-1 descritos en la presente invención, un polipéptido, una molécula de ácido nucleico, un anticuerpo o fragmento, un polímero, un punto cuántico, una molécula pequeña, una toxina, un agente de diagnóstico, un carbohidrato, un aminoácido.

En algunas representaciones, el grupo heterólogo es un péptido que es distinto de los análogos de glucagón descritos en la presente invención y el conjugado es un péptido fusionado o un péptido quimérico. En algunas representaciones, el grupo heterólogo es una extensión peptídica de 1-21 aminoácidos. En representaciones específicas, la extensión está unida al extremo C del análogo de glucagón, por ejemplo, al aminoácido en posición 29.

En algunos aspectos específicos, la extensión es un único aminoácido o dipéptido. En representaciones específicas, la extensión comprende un aminoácido seleccionado del grupo que constituye: un aminoácido con carga (por ejemplo, un aminoácido con carga negativa (por ejemplo, Glu), un aminoácido con carga positiva), un aminoácido que comprende un grupo hidrofílico. En algunos aspectos, la extensión es Gly, Glu, Cys, Gly-Gly, Gly-Glu.

En algunas representaciones, la extensión comprende una secuencia de aminoácidos de las SEQ ID NO: 5 (GPSSGAPPPS), SEQ ID NO: 6 (GGPSSGAPPPS), SEQ ID NO: 7 (KRNRNNIA), o SEQ ID NO: 8 (KRNR). En aspectos específicos, la secuencia de aminoácidos está unida a través de aminoácidos de extremo C del análogo de glucagón, por ejemplo, aminoácido en posición 29. En algunas representaciones, la secuencia de aminoácidos de cualquiera de las SEQ ID NOs: 5-8 está unida al aminoácido 29 del análogo de glucagón a través de una unión peptídica. En algunas representaciones específicas, el aminoácido en posición 29 del análogo de glucagón es un Gly y el Gly está fusionado a una de las secuencias de aminoácidos de cualquiera de las SEQ ID NOs: 5-8.

En algunas representaciones, el grupo heterólogo es un polímero. En algunas representaciones, el polímero es seleccionado del grupo que constituye: poliamidas, policarbonatos, polialquilenos y derivados que incluyen,

glicoles de polialquileno, óxidos de polialquileno, tereptalates de polialquileno, polímeros de ésteres acrílicos y metacrílicos, que incluyen poli(metilo metacrilato), poli(metacrilato etílico), poli(metacrilato butílico), poli(metacrilato isobutílico), poli(metacrilato hexílico), poli(metacrilato isodecílico), poli(metacrilato laurílico), poli(metacrilato fenílico), poli(acrilato metílico), poli(acrilato isopropílico), poli(acrilato isobutílico), y poli(acrilato octadecílico), polímeros polivinílicos que incluyen alcoholes polivinílicos, éteres polivinílicos, ésters polivinílicos, haluros polivinílicos, poli(acetato vinílico), y polivinilpirrolidona, poliglicólidos, polisiloxanos, poliuretanos y co-polímeros, celulosas que incluyen celulosa de alquilo, celulosas de hidroxialquilo, éteres de celulosas, ésteres de celulosa,celulosas de nitro, celulosa de metilo, celulosa de etilo, celulosa de hidroxipropilo, celulosa de metilo hidroxi-propílica, celulosa de metilo hidroxibutílica, acetato de celulosa, propionato de celulosa, butirato acetato de celulosa, ftalate acetato de celulosa, celulosa de carboxiletilo, triacetato de celulosa, y sal de sodio sulfato de celulosa, polipropileno, polietilenos que incluyen poli(glicol etilínico glycol), poli(óxido etilínico), y poli(tereftalate etilínico), y poliestireno.

En algunos aspectos, el polímero es un polímero biodegradable, que incluye un polímero sintético biodegradable (por ejemplo,

polímeros de ácido láctico y ácido glicólico, polianhídridos, poli(oto)ésteres, poliuretanos, poli(ácido butírico), poli(ácido valérico), y poli(láctido-cocaprolactona)), y un polímero natural biodegradable (por ejemplo, alginato y otros polisacáridos que incluyen dextrano y celulosa, colágeno, derivados químicos(sustituciones, adiciones de grupos químicos, por ejemplo, alquilo, alquileno, hidroxilaciones, oxidaciones, y otras modificaciones de rutina realizadas por aquellos expertos en el arte), albúmina y otras proteínas hidrofílicas(por ejemplo, ceína y otras prolaminas y proteínas hidrofóbicas)), así también como cualquier copolímero o mezcla. En general, estos materiales se degradan por hidrólisis enzimática o por exposición al agua *in vivo*, por erosión superficial o masiva.

En algunos aspectos, el polímero es un polímero bioadhesivo, como un hidrogel bioerosionable descrito por H. S. Sawhney, C. P. Pathak y J. A. Hubbell en *Macromolecules*, 1993, 26, 581-587, las enseñanzas del cual se incorporan en la presente invención, ácidos polihialurónicos, caseína, gelatina, glutina, polianhídridos, ácido poliacrílico, alginato, quitosano, poli(metacrilatos metilícos), poli(metacrilatos etílicos), poli(metacrilato butílico), poli(metacrilato isobutílico), poli(metacrilato hexílico), poli(metacrilato isodecílico), poli(metacrilato laurílico), poli(metacrilato fenílico),

poli(acrilato metílico), poli(acrilato isopropílico), poli(acrilato isobutílico), y poli(acrilato octadecílico).

En algunas representaciones, el polímero es un polímero soluble en agua o un polímero hidrofílico. Los polímeros hidrofílicos son descritos más adelante en la presente invención bajo el nombre de “Grupos hidrofílicos.” Se conocen en el arte los polímeros adecuados solubles en agua e incluyen, por ejemplo, polivinilpirrolidona, celulosa de hidroxipropilo(HPC; Klucel), metilocelulosa de hidroxipropilo (HPMC; Methocel), nitrocelulosa, etilocelulosa de hidroxipropilo, butilcelulosa de hidroxipropilo, pentilcelulosa de hidroxipropilo, celulosa de metilo, etilocelulosa (Ethocel), celulosa de hidroxietilo, varias celulosas de alquilo y celulosas de hidroxialquilo, varios éteres de celulosa, acetato de celulosa, celulosa de carboximetilo, celulosa de carboximetilo de sodio, celulosa de carboximetilo de calcio, copolímeros de ácido de acetato/crotónico vinílico, metacrilato de poli-hidroxialquilo, metacrilato de hidroximetilo, copolímeros de ácido metacrílico, ácido polimetacrílico, polimetilometacrilato, copolímeros de éteres vinílicos anhídrido/metilo maleico, alcohol poli vinílico, ácido poliacrílico de sodio y calcio, ácido poliacrílico, polímeros carboxi-acídicos, carboxipolimetileno, polímeros de carboxivinílicos, copolímero de polioxipropileno polioxetileno, anhídrido co-maleico polimetilovinleter, carboximetilamida, copolímero de divinilbenceno metacrílico de

potasio, polioxietilenoglicoles, óxido de polietileno, y derivados, sales, y combinaciones.

En representaciones específicas, el polímero es un glicol de polialquileno, que incluye, por ejemplo, glicol de polietileno (PEG).

En algunas representaciones, el grupo heterólogo es un carbohidrato. En algunas representaciones, el carbohidrato es un monosacárido (por ejemplo, glucosa, galactosa, fructosa), un disacárido (por ejemplo, sucrosa, lactosa, maltosa), un oligosacárido (por ejemplo, rafinosa, estaquiosa), o un polisacárido (por ejemplo, almidón, amilasa, amilopectina, celulosa, quitina, callosa, laminarina, xilano, manano, fucoidán, o galactomanano).

En algunas representaciones, el grupo heterólogo es un lípido. El lípido, en algunas representaciones, es un ácido graso, eicosanoide , prostaglandina, leucotrieno, tromboxano, etanolamina N-acilo), glicerolípido (por ejemplo, mono-, di-, tri- gliceroles sustituidos), glicerofosfolípido (por ejemplo, fosfatidilcolina, fosfatidilinositol, fosfatidiletanolamina, fosfatidilserina), esfingolípido (por ejemplo,esfingosina, ceramida), lípido esterol(por ejemplo, esteroide, colesterol), lípido prenol, sacarolípido, o un policétido, aceite, cera, colesterol, esterol, vitamina soluble en grasa, monoglicérido, diglicérido, triglicérido, un fosfolípido.

En algunas representaciones, el grupo heterólogo está unido vía enlace no-covalente o covalente al análogo de la presente divulgación. En los aspectos ejemplares, el grupo heterólogo está unido al análogo de la presente divulgación vía un conector. Se puede realizar el enlace mediante uniones químicas covalentes, fuerzas físicas como interacciones electrostáticas, de hidrógenos, iónicas, van der Waals, o hidrofílicas. Se puede utilizar una variedad de sistemas de acoplamiento no covalente, incluyendo biotina-avidina, ligando/receptor, enzima/sustrato, ácido nucleico/proteína de unión de ácido nucleico, lípido/proteína de unión de lípido, asociaciones moleculares de adhesión celular; o cualquier asociación o fragmento que tenga afinidad entre sí.

El análogo de glucagón en algunas representaciones está unido a grupos conjugados vía enlace covalente directo mediante la reacción de los residuos de los aminoácidos objetivos del análogo con un agente de derivación orgánico que es capaz de reaccionar con cadenas laterales seleccionadas o con los residuos de extremo C o N de estos aminoácidos objetivos. Los grupos reactivos en el análogo o grupo conjugado incluyen, por ejemplo, un aldehído, amino, éster, tiol, α-haloacetilo, maleimido o grupo hidrazino. Los agentes de derivación incluyen, por ejemplo, éster de sulfosuccinimida maleimidobenzoil (conjugación a través de residuos de cisteína), N-hidroxisuccinimida (a través de residuos de

lisina), glutaraldehído, anhídrido succínico u otros agentes conocidos en el arte. Alternativamente, los grupos conjugados pueden estar unidos al análogo de manera indirecta a través de los portadores intermediarios, como portadores de polisacáridos o polipéptidos. Los ejemplos de portadores de polisacáridos incluyen al aminodextrano. Los ejemplos de portadores adecuados de polipéptidos incluyen polilisina, ácido poliglutámico, ácido poliaspártico, co-polímeros, y polímeros mezclados de estos aminoácidos y otros, por ejemplo, serinas, para conferir propiedades de solubilidad deseables en el portador cargado resultante.

Los residuos de cisteinilo usualmente reaccionan con α-haloacetatos (y aminas correspondientes), como ácido cloracético, cloroacetamida para proporcionar derivados de carboximetilo o carboxiamidometilo. Los residuos de cisteinilo también son derivados mediante la reacción con bromotrifluoroacetona, alfa-bromo-β-(5-imidozoil) ácido propiónico, fosfato de cloroacetilo, N-alquilomaleimidas, disulfuro de 3-nitro-2-piridilo, disulfuro de 2-piridilo metílico, p-cloromercuribenzoato, 2-cloromercuri-4-nitrofenol, o cloro-7-nitrobenzo-2-oxa-1,3-diazole.

Los residuos de histidilo son derivados mediante la reacción con dietilpirocarbonato en pH 5,5-7,0 porque este agente es relativamente específico para la cadena lateral de histidilo. El bromido de para-bromofenacil también es útil; se realiza la

reacción preferentemente en cacodilato de sodio 0.1 M en pH 6.0.

Los residuos de lisinilo y amino-extremo reaccionan con anhídridos de ácido succínico u otro ácido carboxílico. La derivatización con estos agentes tiene el efecto de invertir la carga de los residuos de lisinilo. Otros reagentes adecuados para la derivatización de residuos que contengan alfa-amino incluyen imidoésteres como picolinimidato, fosfato piridoxal, piridoxal, cloroborohídrido, ácido trinitrobencenosulfónico, O-metilisourea, 2-4-pentanodiona, y reacción de transaminasa-catalizada con glioxilato.

Los residuos de arginilo se modifican mediante la reacción con uno o varios reagentes convencionales, entre ellos fenilglioxal, 2,3-butanodiona, 1,2-ciclohexanodiona, y ninhidrina. La derivatización de residuos de arginina requiere que se realice la reacción en condiciones alcalinas debido al alto pKa del grupo funcional de guanidina. Además, estos reagentes pueden reaccionar con los grupos de lisina así como con el grupo amino de épsilon de arginina.

Se puede realizar la modificación específica de residuos de tirosil, con interés particular en introducir etiquetas espectrales en los residuos de tirosil mediante la reacción con compuestos aromáticos de diazonio o tetranitrometano. Usualmente, se utilizan N-acetilimidizol y tetranitrometano

para formar especies de tirosilo O-acetílico y derivados de 3-nitro, respectivamente.

Los grupos colaterales de carboxilo (aspartilo o glutamilo) se modifican selectivamente mediante la reacción con carbodiimidas (R-N=C=N-R'), donde R y R' son grupos diferentes de alquilo, como carbodiimida 1-ciclohexilo-3-(2-morfolinilo-4-etilo) o carbodiimida 1-etilo-3-(4-azonia-4,4-dimetilopentilo. Además, los residuos de aspartilo y glutamilo son convertidos a residuos de asparaginilo y glutaminilo mediante la reacción con iones de amonio.

Otras modificaciones incluyen hidroxilación de prolina y lisina, fosforilación de grupos de hidróxilo de residuos de serilo o treonil, metilación de los grupos amino alfa de lisina, arginina, y cadenas laterales de histidina (T. E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), deamidación de asparagina o glutamina, acetilación de la amina de extremo N, y/o amidación o esterificación del grupo de ácido carboxílico de extremo C.

Otro tipo de modificación covalente implica glicósidos que se acoplan química o enzimáticamente al análogo. Se pueden unir los azúcar(es) a (a) arginina e histidina, (b) grupos libres de carboxilo, (c) grupos libres de sulfhidrilo como aquellos de cisteina, (d) grupos libres de hidróxilo como aquellos de serina, treonina, o hidroxiprolina, (e) residuos aromáticos

como aquellos de tirosina, o triptófano, o (f) el grupo amida de glutamina. Estos métodos están descritos en WO87/05330 publicado 11 Sep. 1987, y en *Aplin* y *Wriston*, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

En algunas representaciones, el análogo de glucagón está conjugado a un grupo heterólogo vía enlace covalente entre una cadena lateral de un aminoácido del análogo de glucagón y el grupo heterólogo. En algunas representaciones, el análogo de glucagón está conjugado a un grupo heterólogo vía la cadena lateral de un aminoácido en posición 16, 17, 21, 24, o 29, una posición en una extensión de extremo C, o el aminoácido de extremo C, o una combinación de estas posiciones. En algunos aspectos, el aminoácido covalentemente unido a un grupo heterólogo (por ejemplo, el aminoácido que comprende un grupo heterólogo) es un Cys, Lys, On, homo-Cys, o Ac-Phe, y la cadena lateral del aminoácido está covalentemente unida a un grupo heterólogo.

En algunas representaciones, el conjugado comprende un conector que une el análogo de glucagón al grupo heterólogo. En algunos aspectos, el conector comprende una cadena de átomos de 1 a aproximadamente 60, o 1 a 30 átomos o más, 2 a 5 átomos, 2 a 10 átomos, 5 a 10 átomos, o 10 a 20 átomos de largo. En algunas representaciones, los átomos en cadena son todos átomos carbónicos. En algunas representaciones, los átomos en cadena en la parte principal del conector son seleccionados del grupo

que constituyen C, O, N, y S. Los átomos en cadena y los conectores se pueden seleccionar según la solubilidad esperada (hidrofilia) para proporcionar un conjugado más soluble. En algunas representaciones, el conector proporciona un grupo funcional que está sujeto a una división mediante una enzima u otras condiciones catalizadoras o hidrolíticas encontradas en el tejido u órgano célula meta. En algunas representaciones, la longitud del conector es lo suficientemente larga para reducir el potencial para el impedimento estérico. Si el conector es una unión covalente o una unión de peptidilo y el conjugado es un polipéptido, todo el conjugado puede ser una proteína fusionada. Dichos conectores de peptidilo pueden ser de cualquier longitud. Los conectores ejemplares son de aproximadamente 1 a 50 aminoácidos de longitud, 5 a 50, 3 a 5, 5 a 10, 5 a 15, o 10 a 30 aminoácidos de longitud. Dichas proteínas fusionadas se pueden crear de manera alternativa mediante los métodos de ingeniería genética recombinante conocidos por una de las técnicas comunes en el arte.

Conjugados: Fusiones de Fc

Como se menciona antes, en algunas representaciones, los análogos están conjugados, por ejemplo, fusionados a una inmunoglobulina o parte (por ejemplo, región variable, CDR, o región Fc). Los tipos conocidos de inmunoglobulinas (Ig) incluyen IgG, IgA, IgE, IgD o IgM. La región Fc es una region de extremo C de una cadena pesada de Ig, que es responsable de

unir a los receptores de Fc que llevan a cabo actividades como reciclaje (que producen semivida prolongada), citotoxicidad mediada por la célula dependiente del anticuerpo (ADCC), y citotoxicidad dependiente complementaria (CDC).

Por ejemplo, según algunas definiciones la región Fc de la cadena pesada de IgG humano se extiende desde Cys226 al extremo C de la cadena pesada. La "región bisagra" generalmente se extiende desde Glu216 a Pro230 del IgG1 humano (las regiones bisagras de otros isotipos de IgG se pueden alinear con la secuencia de IgG1 mediante la alineación de los cisteinas en la unión cisteínica). La region Fc de un IgG incluye dos dominios constantes , CH2 y CH3. El dominio CH2 de una región Fc del IgG humano usualmente se extiende de 231 aminoácidos a 341 aminoácidos. El dominio CH3 de una región Fc del IgG humano usualmente se extiende de 342 a 447 aminoácidos. Las referencias realizadas a la numeración de aminoácidos de inmunoglobulinas o fragmentos de inmunoglobulina, o regiones, están todas basadas en Kabat et al. 1991, *Sequences of Proteins of Immunological Interest*, U.S. Departmento de Salud Pública, *Bethesda, Md*. En una de las representaciones relacionadas, la región Fc puede comprender una o más regiones constantes nativas o modificadas de una cadena pesada de inmunoglobulina, diferente de CH1, por ejemplo, las regiones CH2 y CH3 de IgG e IgA, o las regiones CH3 y CH4 de IgE.

Los grupos conjugados adecuados incluyen partes de la secuencia de inmunoglobulina que incluyen el sitio de unión de FcRn. FcRn, un receptor de rescate, es responsable de reciclar inmunoglobulinas y regresarlas a circulación en la sangre. Se ha descrito la región de la parte Fc de IgG que une al receptor de FcRn basada en cristalografía de rayos X (*Burmeister et al.* 1994, *Nature 372:379*). La mayor área de contacto del Fc con el FcRn se encuentra cerca de la unión de los dominios CH2 y CH3. Los contactos Fc-FcRn están todos en una única cadena pesada de Ig. Los mayores sitios de contacto incluyen residuos de aminoácido 248, 250-257, 272, 285, 288, 290-291, 308-311, y 314 del dominio CH2 y residuos de aminoácidos 385-387, 428, y 433-436 del dominio CH3.

Algunos grupos conjugados pueden o no incluir sitio (s) de unión de FcγR. Los FcγR son responsables de ADCC y CDC. Los ejemplos de las posiciones en la región Fc que crean un contacto directo con FcγR son aminoácidos 234-239 (región bisagra más baja), aminoácidos 265-269 (circuito B/C l), aminoácidos 297-299 (circuito C'/E), y aminoácidos 327-332 circuito(F/G)(*Sondermann et al., Nature 406: 267-273, 2000*). La región bisagra más baja de IgE se ha implicado también en la unión de FcRI (*Henry, et al., Biochemistry 36, 15568-15578, 1997*). Los residuos en la unión del receptor de IgA están descritos en *Lewis et al., (J Immunol. 175:6694-701, 2005*). Los residuos de aminoácidos en la unión del receptor de IgE

están descritos en *Sayers et al. (J Biol Chem. 279(34):35320-5, 2004)*.

Se pueden realizar modificaciones de aminoácidos a la región Fc de una inmunoglobulina. Dichas regiones Fc variantes comprenden al menos una modificación de aminoácido en el dominio CH3 de la región Fc (residuos 342-447) y/o al menos una modificación de aminoácido en el dominio CH2 de la región Fc (residuos 231-341). Las mutaciones creyeron impartir una afinidad creciente para FcRn que incluye T256A, T307A, E380A, y N434A (*Shields et al. 2001, J. Biol. Chem. 276:6591*). Otras mutaciones pueden reducir la unión de la región Fc a FcγRI, FcγRIIA, FcγRIIB, y/o FcγRIIIA sin reducir de manera significativa la afinidad para FcRn. Por ejemplo, la sustitución del Asn en posición 297 de la región Fc con Ala u otro aminoácido quita un sitio de N-glicoslación altamente conservado y puede producir inmunogenicidad reducida con prolongada concomitante de la región Fc, así también como unión reducida a FcγRs (*Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et al. 1995, J. Biol. Chem. 276:6591*). Las modificaciones de aminoácids en posiciones 233-236 de IgG1 se han realizado para que reduzcan la unión a FcγRs (*Ward y Ghetie 1995, Therapeutic Immunology 2:77 y Armour et al. 1999, Eur. J. Immunol. 29:2613)*. Algunas sustituciones ejemplares de aminoácidos están descritas en las Patentes US 7.355.008 y 7.381.408, cada una

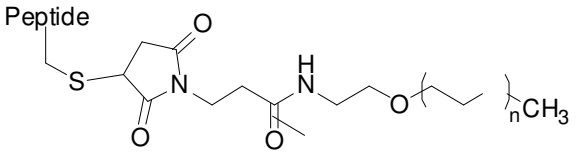
incorporada como referencia de la presente invención en su totalidad.

Conjugados: Grupos hidrofílicos

Los análogos de glucagón descritos en la presente invención pueden ser posteriormente modificados para mejorar su solubilidad y estabilidad en soluciones acuosas en pH fisiológico, cuando retiene la alta actividad biológica relativa al glucagón nativo. Los grupos hidrofílicos como los grupos PEG pueden ser unidos a los análogos bajo cualquier condición adecuada utilizada para reaccionar una proteína con una molécula de polímero activado. Se puede utilizar cualquier medio conocido en el arte, incluyendo vía acilación, alquiloación reductora, adición de *Michael*, alquiloación de tiol u otros métodos de conjugación/ligación quimioselectiva a través de un grupo reactivo en el grupo PEG(por ejemplo, un grupo aldehído, amino, éster, tiol, α-haloacetilo, maleimido o hydrazino) a un grupo reactivo compuesto meta(por ejemplo, un grupo aldehído, amino, éster, tiol, α-haloacetilo, maleimido o hydrazino). La activación de los grupos que se pueden utilizar para unir el polímero soluble en agua a una o más proteínas incluye pero no taxativamente a sulfona, maleimida, sulfhidrilo, tiol, triflato, tresilato, azidirina, oxirano, 5-piridilo, y grupo acílico alfa-halogenado (por ejemplo, ácido acético alfa-iodo, ácido alfa-bromoacético, ácido alfa-cloroacético). Si se uniera al análogo mediante alquiloación

reductora, el polímero seleccionado debería tener un único aldehído reactivo para que se controle el grado de polimerización. Ver, por ejemplo, *Kinstler et al., Adv. Drug. Delivery Rev. 54: 477-485 (2002); Roberts et al., Adv. Drug Delivery Rev. 54: 459-476 (2002); y Zalipsky et al., Adv. Drug Delivery Rev. 16: 157-182 (1995).*

En aspectos específicos, un residuo de aminoácidos del análogo que tiene un tiol se modifica con un grupo hidrofílico como PEG. En algunas representaciones, se modifica el tiol con PEG activado con maleimida en una reacción de adición *Michael* para producir un análogo PEGilado que comprende el enlace de tioeter como aparece debajo:



En algunas representaciones, se modifica el tiol con un PEG activado por haloacetilo en una reacción de sustitución nucleofílica para producir un análogo PEGilado que comprende un enlace tioeter.

Los grupos hidrofílicos adecuados incluyen glicol de polietileno (PEG), glicol de polipropileno, polioles polioxietilenados (por ejemplo, POG), sorbitol polioxietilenado, glucosa polioxietilenada, glicerol polioxietilenado (POG), polioxialquilenos, propionaldehído

glicólico de polietileno, copolímeros de glicol de glicol/propileno etilénico, glicol de polietileno- monometoxi, glicol de mono-(C1-C10) alkoxi- o polietileno-arloxi, carboximetilcelulosa, poliacetales, alcohol polivinílico(PVA),pirrolidona polivinílica, poli-1, 3-dioxolana, poli-1,3,6-trioxano, copolímero de anhídrido etileno/maleico, poli (β-aminoácidos) (ya sean homopolímeros o copolímeros al azar), glicol de polietileno poli(n-vinilo pirrolidona), homopolímeros de glicol de polietileno (PPG) y otros óxidos poliaquilenos, copolímeros de óxido óxido/etileno polipropileno, ácidos colónicos y otros polímeros de polisacáridos, Ficoll o dextrano y sus mezclas. Los dextranos son polímeros de polisacáridos de subunidades de glucosa, predominantemente unidos por enlaces □1-6. El dextrano se encuentra en muchos rangos de peso molecular, por ejemplo, aproximadamente 1 kD to aproximadamente 100 kD, o de aproximadamente 5, 10, 15 o 20 kD a aproximadamente 20, 30, 40, 50, 60, 70, 80 o 90 kD. Se contemplan los polímeros lineales o ramificados. Las preparaciones resultantes de los conjugados pueden estar básicamente monodispersas o polidispersas, y pueden tener aproximadamente 0.5, 0.7, 1, 1.2, 1.5 o 2 grupos de polímeros por análogo.

En algunas o cualquiera de las representaciones, el análogo de glucagón está conjugado a un grupo hidrofílico vía enlace covalente entre una cadena lateral de un aminoácido del análogo

de glucagón y el grupo hidrofílico. En algunas o cualquiera de las representaciones, el análogo de glucagón está conjugado a un grupo hidrofílico vía la cadena lateral de un aminoácido en posición 16, 17, 21, 24, o 29, una posición en la extensión del extremo C, o el aminoácido de extremo C, o una combinación de estas posiciones. En algunos aspectos, el aminoácido covalentemente unido a un grupo hidrofílico (por ejemplo, el aminoácido que comprende un grupo hidrofílico) es un Cys, Lys, On, homo-Cys, o Ac-Phe, y la cadena lateral del aminoácido está covalentemente unida a un grupo hidrofílico (por ejemplo, PEG).

Conjugados: rPEG

En algunas o cualquiera de las representaciones, el conjugado de la presente divulgación comprende el análogo de glucagón que tiene actividad de agonista en el receptor de GIP fusionado a un análogo accesorio que es capaz de formar una conformación extendida similar al PEG químico (por ejemplo, una molécula de PEG recombinante (rPEG)), como aquellas que están descritas en la Publicación Internacional de la Solicitud de Patente No. WO2009/023270 y Publicación de la Solicitud de Patente U.S. No. US20080286808. La molécula rPEG en algunos aspectos es un polipéptido que comprende uno o más de glicina, serina, ácido glutámico, ácido aspártico, alanina, o prolina. En algunos aspectos, la rPEG es un homopolímero, por ejemplo, poli-glicina, poli-serina, ácido poli-glutámico, ácido poli-aspártico, poli-alanina, o poli-prolina. En otras

representaciones, la rPEG comprende dos tipos de aminoácidos repetidos, por ejemplo, poli(Gly-Ser), poli(Gly-Glu), poli(Gly-Ala), poli(Gly-Asp), poli(Gly-Pro), poli(Ser-Glu), etc. En algunos aspectos, la rPEG comprende tres tipos diferentes de aminoácidos, por ejemplo, poli(Gly-Ser-Glu). En aspectos específicos, the rPEG incrementa la semivida del Glucagón y/o análogo de agonista de GLP-1. En algunos aspectos, la rPEG comprende una carga positiva neta o carga negativa neta. La rPEG en algunos aspectos carece de estructura secundaria. En algunas representaciones, la rPEG es superior a o igual a 10 aminoácidos de longitud y en algunas representaciones es aproximadamente 40 a aproximadamente 50 aminoácidos de longitud. El péptido accesorio en algunos aspectos se fusiona al extremo N o C del análogo de la presente divulgación a través de una unión peptídica bond o un sitio de división de proteinasa, o se inserta en los circuitos del análogo de la presente divulgación. La rPEG en algunos aspectos comprende un marcador de afinidad o está unido a un PEG que es superior a 5 kDa. En algunas representaciones, la rPEG confiere el análogo de la presente divulgación con un radio hidrodinámico incrementado, semivida del suero, resistencia a la proteasa, o solubilidad y en algunos aspectos confiere el análogo con inmunogenicidad descendiente.

Conjugados: Multímeros

La invención también proporciona multímeros o dímeros de los análogos divulgados en la presente invención, incluyendo homo- o hetero- multímeros u homo- hetero- dímeros. Dos o más de los análogos se pueden unir utilizando agentes de enlances estándar y procedimientos conocidos por aquellos expertos en el arte. Por ejemplo, se pueden formar dímeros entre dos péptidos a través del uso de conectores cruzados bifuncionales de tiol y conectores cruzados bifuncionales de amina, particularlmente para los análogos que han sido sustituidos con cisteína, ornitina de lisina, residuos de homocisteína o fenilalanina acetílica. El dímero puede ser un homodímero o alternativamente puede ser un heterodímero. En representaciones ejemplares, el conector que enlaza los dos (o más) análogos es PEG, por ejemplo, un PEG de 5 kDa PEG, 20 kDa. En algunas representaciones, el conector es un enlace disulfúrico. Por ejemplo, cada monómero del dímero puede comprender un residuo de Cys(por ejemplo, un Cys posicionado en el interior o en el extremo) y el átomo sulfúrico de cada residuo de Cys participa en la formación del enlace disulfúrico. En los aspectos ejemplares, cada monómero del dímero está unido vía un enlace tioeter. En los aspectos ejemplares, una amina de épsilon de un residuo de Lys de un monómero está unido a un residuo de Cys, el cual, a su vez, está conectedo vía un grupo químico a la amina de épsilon de un residuo de Lys del otro monómero. Los métodos para crear dichos

dímeros enlazados por tioeter están descritos más adelante en la presente invención. En algunos aspectos, los monómeros están conectados vía aminoácidos de extremo (por ejemplo, extremo N o extremo C), vía aminoácidos internos, o vía un aminoácido de extremo de al menos un monómero y un aminoácido interno de al menos un monómero diferente. En aspectos específicos, los monómeros no están conectados vía un aminoácido de extremo N. En algunos aspectos, los monómeros del multímero están unidos en una orientación “cola a cola” donde los aminoácidos de extremo C cada monómero están unidos.

PRODROGAS

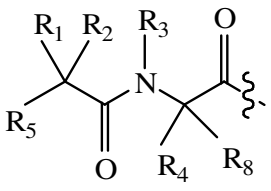
Además de lo proporcionado por la invención son prodrogas de los péptidos y análogos descriptos en la presente. Como se utiliza en la presente invención, se define el término "prodroga" como cualquier compuesto que realiza modificación química antes de presentar todos sus efectos farmacológicos.

En representaciones ejemplares, la prodroga es una prodroga peptídica basada en amida, similar a aquellas descritas en la Publicación Internacional de la Solicitud de Patente No. WO/2010/071807, que se publicó el 24 de junio, 2010. El propósito de dichas prodrogas es retrasar el comienzo de la acción y extender la semivida de la droga. El comienzo retrasado de la acción favorece en que permite distribución sistemática de la prodroga antes de su activación. Por

consiguiente, la administración de prodrogas elimina complicaciones causadas por actividades de mucha demanda al momento de la administración e incrementa el índice terapéutico de la droga parental.

En los aspectos ejemplares, la prodroga comprende la estructura: A-B-Q; donde Q es un péptido o análogo descriptos en la presente invención ; A es un aminoácido o un ácido hidroxi; B es un aminoácido N-alquilado unido a Q a través de un enlace de amida entre A-B y una amina de Q; donde A, B, o el aminoácido de Q al cual A-B está unido es un aminoácido no-codificado, además donde la semivida (t1/2) de la división química de A-B de Q es al menos aproximadamente 1 hora a aproximadamente 1 semana en PBS bajo condiciones fisiológicas. Como se utiliza en la presente invención el término "ácido hidroxi" se refiere a un aminoácido que se ha modificado para reemplazar el grupo amino alfa carbónico con un grupo de hidróxilo.

En algunas representaciones el elemento dipeptídico de la prodroga tiene la estructura general de la Fórmula I:



donde

R_1, R_2, R_4 y R_8 son independientemente seleccionados del grupo que constituye H, alquilo C_1–C_{18}, alquenilo C_2–C_{18}, (alquilo C_1–C_{18})OH, (alquilo C_1–C_{18})SH, (alquilo C_2–C_3)SCH_3, (alquilo C_1–C_4)$CONH_2$, (alquilo C_1–C_4)COOH, (alquilo C_1–C_4)NH_2, (alquilo C_1–C_4)$NHC(NH_2^+)NH_2$, (alquilo C_0–C_4)(cicloalquilo C_3–C_6), (alquilo C_0–C_4)(heterocíclico C_2–C_5), (alquilo C_0–C_4)(arilo C_6–C_{10})R_7, (alquilo C_1–C_4)(heteroarilo C_3–C_9), y alquilo C_1–C_{12}(A)(W_1)C_1–C_{12} alquilo, donde W_1 es un heteroátomo seleccionado del grupo que constituye N, S y O, o R_1 y R_2 junto con los átomos a los cuales están unidos para formar un cicloalquilo o arilo C_3–C_{12}; o R_4 y R_8 junto con los átomos a los cuales están unidos forman un cicloalquilo C_3–C_6;

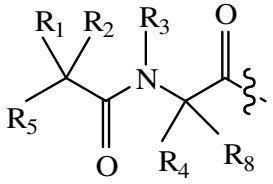
R_3 es seleccionado del grupo que constituye alquilo C_1–C_{18}, (alquilo C_1–C_{18})OH, (alquilo C_1–C_{18})NH_2, (alquilo C_1–C_{18})SH, (alquilo C_0–C_4)(C_3–C_6)cicloalquilo, (alquilo C_0–C_4)(heterocíclico C_2–C_5), (alquilo C_0–C_4)(arilo C_6–C_{10})R_7, y (alquilo C_1–C_4)(heteroarilo C_3–C_9) o R_4 y R_3 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros;

R_5 es NHR_6 o OH;

R_6 es H, alquilo C_1–C_8 o R_6 y R_2 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros; y

R_7 es seleccionado del grupo que constituye H y OH.

En otras representaciones el elemento dipeptídico de la prodroga tiene la estructura general de la Fórmula I:



donde

R_1, R_2, R_4 y R_8 son independientemente seleccionados del grupo que constituye H, alquilo C_1–C_{18}, alquenilo C_2–C_{18}, (alquilo C_1–C_{18})OH, (alquilo C_1–C_{18})SH, (alquilo C_2–C_3)SCH_3, (alquilo C_1–C_4)$CONH_2$, (alquilo C_1–C_4)COOH, (alquilo C_1–C_4)NH_2, (alquilo C_1–C_4)$NHC(NH_2^+)NH_2$, (alquilo C_0–C_4)(C_3–C_6 cicloalquilo), (alquilo C_0–C_4)(C_2–C_5 heterocíclico), (alquilo C_0–C_4)(arilo C_6–C_{10})R_7, (alquilo C_1–C_4)(C_3–C_9 heteroarilo), y C_1–C_{12} alquilo(W_1)C_1–C_{12} alquilo, donde W_1 es un heteroátomo seleccionado del grupo que constituye N, S y O, o R_1 y R_2 junto con los átomos a los cuales están unidos forman un C_3–C_{12} cicloalquilo; o R_4 y R_8 junto con los átomos a los cuales están unidos forman un C_3–C_6 cicloalquilo;

R_3 es seleccionado del grupo que constituye C_1–C_{18} alquilo, (C_1–C_{18} alquilo)OH, (C_1–C_{18} alquilo)NH_2, (C_1–C_{18} alquilo)SH, (C_0–C_4 alquilo)(C_3–C_6)cicloalquilo, (C_0–C_4 alquilo)(C_2–C_5 heterocíclico), (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7, y (C_1–C_4 alquilo)(C_3–C_9 heteroarilo) o R_4 y R_3 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros;

R_5 es NHR_6 o OH;

R_6 es H, C_1–C_8 alquilo o R_6 y R_1 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros; y

R_7 es seleccionado del grupo que constituye hidrógeno, C_1–C_{18} alquilo, C_2–C_{18} alquenilo, (C_0–C_4 alquilo)$CONH_2$, (C_0–C_4 alquilo)COOH, (C_0–C_4 alquilo)NH_2, (C_0–C_4 alquilo)OH, y halo.

En algunas representaciones R_8 es H y R_5 es NHR_6.

En algunas representaciones el elemento dipiptédico de la prodroga tiene la estructura de la Fórmula I, donde

R_1 y R_8 son independientemente H o C_1–C_8 alquilo;

R_2 y R_4 son independentemente seleccionados del grupo que constituye H, C_1–C_8 alquilo, C_2–C_8 alquenilo, (C_1–C_4 alquilo)OH, (C_1–C_4 alquilo)SH, (C_2–C_3 alquilo)SCH_3, (C_1–C_4 alquilo)$CONH_2$, (C_1–C_4 alquilo)COOH, (C_1–C_4 alquilo)NH_2, (C_1–C_4 alquilo)$NHC(NH_2+)NH_2$, (C_0–C_4 alquilo)(C_3–C_6 cicloalquilo), (C_0–C_4 alquilo)(C_2–C_5 heterocíclico), (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7, y CH_2(C_3–C_9 heteroarilo), o R_1 y R_2 junto con los átomos a los cuales están unidos forman un C_3–C_{12} cicloalquilo o arilo;

R_5 es NHR_6; y

R_6 es H o C_1–C_8 alquilo.

En otras representaciones el elemento dipeptídico de la prodroga tiene la estructura de la Fórmula I, donde

R_1 y R_8 son independientemente alquilo H o C_1–C_8;

R_2 y R_4 son independientemente seleccionados del grupo que constituye alquilo H, C_1–C_8, alquenilo C_2–C_8, (alquilo C_1–C_4)OH, (alquilo C_1–C_4)SH, (alquilo C_2–C_3)SCH_3, (alquilo C_1–C_4)$CONH_2$, (alquilo C_1–C_4)COOH, (alquilo C_1–C_4)NH_2, (alquilo C_1–C_4)$NHC(NH_2+)NH_2$, (alquilo C_0–C_4)(cicloalquilo C_3–C_6), (alquilo C_0–C_4)(heterocíclico C_2–C_5), (alquilo C_0–C_4)(arilo C_6–C_{10})R_7, y CH_2(heteroarilo C_3–C_9), o R_1 y R_2 junto con los átomos a los cuales están unidos forman un cicloalquilo C_3–C_{12};

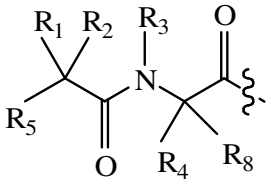
R_3 es alquilo C_1–C_{18};

R_5 es NHR_6;

R_6 es alquilo H o C_1–C_8; y

R_7 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_{18}, alquenilo C_2–C_{18}, (alquilo C_0–C_4) $CONH_2$, (alquilo C_0–C_4)COOH, (alquilo C_0–C_4)NH_2, (alquilo C_0–C_4)OH, y halo.

La semivida de la prodroga formada de acuerdo con la presente divulgación se determina mediante los sustituyentes del elemento dipeptíco de la prodroga, su ubicación, y el aminoácido al cual está unido. Por ejemplo, la prodroga puede comprender un péptido o análogo descriptos en la presente invención, donde el elemento dipeptíco de la prodroga está unido a través del grupo alfa amino del aminoácido de extremo N del péptido o análogo descriptos en la presente invención. En esta representación las prodrogas que tienen un $t_{1/2}$ de, por ejemplo, aproximadamente 1 hora comprenden un elemento dipeptídico de la prodroga con la estructura:



donde

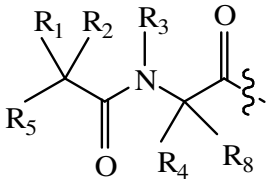
R_1 y R_2 son independientemente alquilo o arilo C_1–C_{18}; o R_1 y R_2 están unidos a través de $-(CH_2)_p$, donde p es 2–9;

R_3 es alquilo C_1–C_{18};

R_4 y R_8 son cada uno hidrógeno; y

R_5 es una amina.

En otras representaciones, las prodrogas que tienen un $t_{1/2}$ de, por ejemplo, aproximadamente 1 hora comprenden un elemento dipeptídico de la prodroga con la estructura:



donde

R_1 y R_2 son independientemente C_1–C_{18} alquilo o (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7; o R_1 y R_2 están unidas a través de $-(CH_2)_p$, donde p es 2–9;

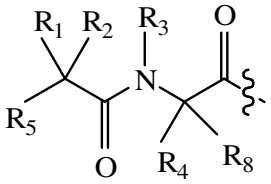
R_3 es C_1–C_{18} alquilo;

R_4 y R_8 son cada uno hidrógeno;

R_5 es NH_2; y

R_7 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_{18}, alquenilo C_2–C_{18}, (alquilo C_0–C_4)$CONH_2$, (alquilo C_0–C_4)COOH, (alquilo C_0–C_4)NH_2, (alquilo C_0–C_4)OH, y halo.

Además, las prodrogas que tienen el elemento dipeptídico de la prodroga unido al aminoácido alfa extremo N del péptido o análogo descritos en la presente invención y que tienen un $t_{1/2}$, por ejemplo, entre aproximadamente 6 a aproximadamente 24 horas, comprenden un elemento dipeptídico de la prodroga con la estructura:



donde R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo y arilo C_1–C_{18}, o R_1 y R_2 están unidos a través de $(CH_2)_p$, donde p es 2–9;

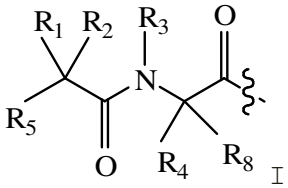
R_3 es alquilo C_1–C_{18} o R_3 y R_4 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4–12;

R_4 y R_8 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo y arilo C_1–C_8; y R_5 es una amina;

con la condición que tanto R_1 y R_2 no sean hidrógeno y siempre que R_4 o R_8 sea hidrógeno.

En algunas representaciones, las prodrogas que tienen el elemento dipeptídico de la prodroga unidos al aminoácido alfa extremo N del péptido o análogo descritos en la presente invención y que tienen un $t_{1/2}$, por ejemplo, entre

aproximadamente 12 a aproximadamente 72 horas, o en algunas representaciones entre aproximadamente 12 a aproximadamente 48 horas, comprenden un elemento dipeptídico de la prodroga con la estructura:



I

donde R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo C_1–C_{18}, (alquilo C_1–C_{18})OH, (alquilo C_1–C_4)NH_2, y (alquilo C_0–C_4)(arilo C_6–C_{10})R_7, o R_1 y R_2 están unidos a través de $(CH_2)_p$, donde p es 2–9;

R_3 es alquilo C_1–C_{18} o R_3 y R_4 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4–12;

R_4 y R_8 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo C_1–C_8 y (alquilo C_0–C_4)(arilo C_6–C_{10})R_7;

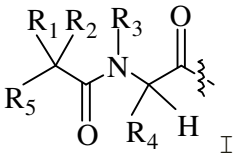
R_5 es NH_2; y

R_7 es seleccionado del grupo que constituye H, alquilo C_1–C_{18}, alquenilo C_2–C_{18}, (alquilo C_0–C_4)$CONH_2$, (alquilo C_0–C_4)COOH, (alquilo C_0–C_4)NH_2, (alquilo C_0–C_4)OH, y halo;

con la condición que tanto R_1 y R_2 no sean hidrógeno y siempre que al menos R_4 o R_8 sean hidrógeno.

En algunas representaciones, las prodrogas que tienen el elemento dipeptídico de la prodroga unidos al aminoácido

extremo N del péptido o análogo descritos en la presente invención y que tienen un $t_{1/2}$, por ejemplo, entre aproximadamente 12 a aproximadamente 72 horas, o en algunas representaciones entre aproximadamente 12 a aproximadamente 48 horas, comprenden un elemento dipeptídico de la prodroga con la estructura:



I

donde R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo C_1–C_8 y (alquilo C_1–C_4)NH_2, o R_1 y R_2 están unidos a través de $(CH_2)_p$, donde p es 2–9;

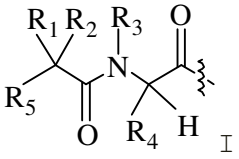
R_3 es alquilo C_1–C_8 o R_3 y R_4 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4–6;

R_4 es seleccionado del grupo que constituye hidrógeno y alquilo C_1–C_8; y

R_5 es NH_2;

con la condición que tanto R_1 y R_2 no sean hidrógeno.

En otras representaciones, las prodrogas que tienen el elemento dipeptídico de la prodroga unidos al aminoácido extremo N del péptido o análogo descritos en la presente invención y que tienen un $t_{1/2}$, por ejemplo, entre aproximadamente 12 a aproximadamente 72 horas, o en algunas representaciones entre aproximadamente 12 a aproximadamente 48 horas, comprenden un elemento dipeptídico de la prodroga con la estructura:



donde

R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo C_1–C_8 y (alquilo C_1–C_4)NH_2;

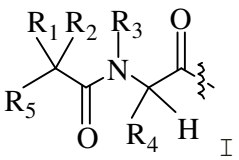
R_3 es alquilo C_1–C_6;

R_4 es hidrógeno; y

R_5 es NH_2;

con la condición que tanto R_1 y R_2 no sean hidrógeno.

En algunas representaciones, las prodrogas que tienen el elemento dipeptídico de la prodroga unidos al aminoácido extremo N del péptido o análogo descritos en la presente invención y que tienen un $t_{1/2}$, por ejemplo, entre aproximadamente 12 a aproximadamente 72 horas, o en algunas representaciones entre aproximadamente 12 a aproximadamente 48 horas, comprenden un elemento dipeptídico de la prodroga con la estructura:



donde

R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno y alquilo C_1–C_8, (alquilo C_1–C_4)NH_2, o R_1 y R_2 están unidos a través de $(CH_2)_p$, donde p es 2–9;

R_3 es alquilo C_1–C_8;

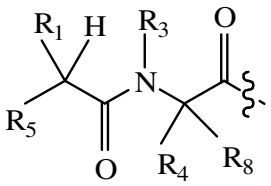
R_4 es (alquilo C_0–C_4)(arilo C_6–C_{10})R_7;

R_5 es NH_2; y

R_7 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_8 y (alquilo C_0–C_4)OH;

con la condición que tanto R_1 y R_2 no sean hidrógeno.

Además de que una prodroga que tiene el elemento dipeptídico de la prodroga unidos al aminoácido alfa extremo N del péptido y análogo descritos en la presente invención y que tienen un $t_{1/2}$, por ejemplo, de aproximadamente 72 a aproximadamente 168 horas se proporciona donde el elemento dipeptídico de la prodroga tiene la estructura:



donde R_1 es seleccionado del grupo que constituye hidrógeno, alquilo y arilo C_1–C_8;

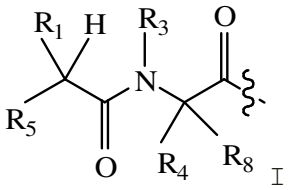
R_3 es alquilo C_1–C_{18};

R_4 y R_8 son cada uno hidrógeno; y

R_5 es una amina o amina N–sustituida o un hidróxilo;

Con la condición que, si R_1 es alquilo o arilo, entonces R_1 y R_5 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4–11.

En algunas representaciones, el elemento dipeptídico de la prodroga del dipéptido tiene la estructura:



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donde R_1 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_8 y (alquilo C_0–C_4)(arilo C_6–C_{10})R_7;

R_3 es alquilo C_1–C_{18};

R_4 y R_8 son cada uno hidrógeno;

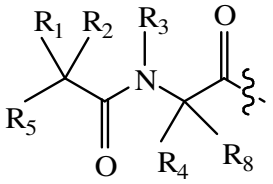
R_5 es NHR_6 o OH;

R_6 es H, alquilo C_1–C_8, o R_6 y R_1 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros; y

R_7 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_{18}, alquenilo C_2–C_{18}, (alquilo C_0–C_4)$CONH_2$, (alquilo C_0–C_4)COOH, (alquilo C_0–C_4)NH_2, (alquilo C_0–C_4)OH, y halo;

Con la condición que, si R_1 es alquilo o (alquilo C_0–C_4)(arilo C_6–C_{10})R_7, entonces R_1 y R_5 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4–11.

En algunas representaciones el elemento dipeptídico de la prodroga está unido a una amina de cadena lateral de un aminoácido interno del péptido o análogo descritos en la presente invención. En esta representación las prodrogas que tienen un $t_{1/2}$, por ejemplo, de aproximadamente 1 hora tienen la estructura:



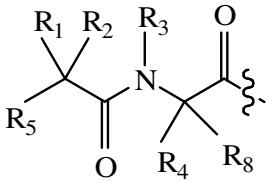
donde

R_1 y R_2 son independientemente alquilo o arilo C_1–C_8; o R_1 y R_2 están unidos a través de $(CH_2)_p$, donde p es 2–9;

R_3 es alquilo C_1–C_{18};

R_4 y R_8 son cada uno hidrógeno; y R_5 es una amina.

En algunas representaciones, las prodrogas que tienen un $t_{1/2}$, por ejemplo, de aproximadamente 1 hora tienen la estructura:



donde

R_1 y R_2 son independientemente alquilo C_1–C_8 o (alquilo C_0–C_4)(arilo C_6–C_{10})R_7; o R_1 y R_2 están unidos a través de –$(CH_2)_p$–, donde p es 2–9;

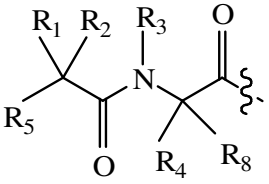
R_3 es alquilo C_1–C_{18};

R_4 y R_8 son cada uno hidrógeno;

R_5 es NH_2; y

R_7 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_{18}, alquenilo C_2–C_{18}, (alquilo C_0–C_4)$CONH_2$, (alquilo C_0–C_4)COOH, (alquilo C_0–C_4)NH_2, (alquilo C_0–C_4)OH, y halo.

Además, las prodrogas que tienen un $t_{1/2}$, por ejemplo, entre aproximadamente 6 a aproximadamente 24 horas y que tienen el elemento dipeptídico de la prodroga unido a una cadena lateral de aminoácido interno comprenden un elemento dipeptídico de la prodroga con la estructura:



donde R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo y arilo C_1–C_8, o R_1 y R_2 están unidos a través de $-(CH_2)_p$, donde p es 2–9;

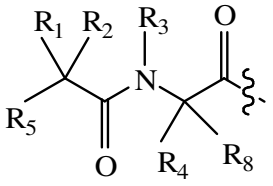
R_3 es alquilo C_1–C_{18} o R_3 y R_4 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4–12;

R_4 y R_8 son independientemente alquilo o arilo C_1–C_{18};y

R_5 es una amina o amina N–sustituida;

con la condición que tanto R_1 y R_2 no sean hidrógeno y siempre qu R_4 o R_8 sea hidrógeno.

En algunas representaciones, las prodrogas que tienen un $t_{1/2}$, por ejemplo, entre aproximadamente 12 a aproximadamente 72 horas, o en algunas representaciones entre aproximadamente 12 a aproximadamente 48 horas, y que tienen el elemento dipeptídico de la prodroga unido a una cadena lateral de aminoácido interno comprenden un elemento dipeptídico de la prodroga con la estructura:



donde R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo C_1–C_8, y (alquilo C_0–C_4)(alquilo C_6–C_{10})R_7, o R_1 y R_2 están unidos a través de $-(CH_2)_p-$ donde p es 2–9;

R_3 es alquilo C_1–C_{18} o R_3 y R_4 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4–12;

R_4 y R_8 son independientemente hidrógeno, alquilo C_1–C_{18} o (alquilo C_0–C_4)(arilo C_6–C_{10})R_7;

R_5 es NHR_6;

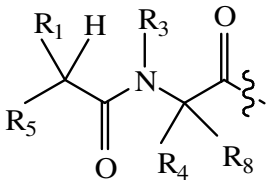
R_6 es alquilo H o C_1–C_8, o R_6 y R_2 junto con los átomos a los cuales están unidos forman un anillo heterocíclico 4, 5 o 6 miembros; y

R_7 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_{18}, alquenilo C_2–C_{18},(alquilo C_0–C_4)$CONH_2$, (alquilo C_0–C_4)COOH,(alquilo C_0–C_4)NH_2,(alquilo C_0–C_4)OH,y halo;

con la condición que tanto R_1 y R_2 no sean hidrógeno y siempre que al menos R_4 o R_8 sean hidrógeno.

Además una prodroga que tiene un $t_{1/2}$, por ejemplo, de aproximadamente 72 a aproximadamente 168 horas y que tiene el elemento dipeptídico de la prodroga unido a una cadena lateral de aminoácido interno del péptido o análogo descritos en la

presente invención se proporciona donde el elemento dipeptídico de la prodroga tiene la estructura:



donde R_1 y R_2 son independientemente seleccionados del grupo que constituye hidrógeno, alquilo y arilo C_1–C_{18};

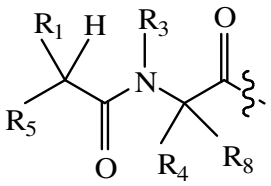
R_3 es alquilo C_1–C_{18};

R_4 y R_8 son cada uno hidrógeno;

R_5 es una amina o amina N-sustituida o un hidróxilo;

con la condición que, si R_1 y R_2 ambos son independientemente un alquilo o arilo, R_1 o R_2 están unidos a través de $(CH2)_p$ a R_5, donde p es 2–9.

En algunas representaciones, una prodroga que tiene un $t_{1/2}$, por ejemplo, de aproximadamente 72 a aproximadamente 168 horas y que tiene el elemento dipeptídico de la prodroga unido a una cadena lateral de aminoácido interna del péptido o análogo descritos en la presente invención se proporciona donde el elemento dipeptídico de la prodroga tiene la estructura:



donde R_1 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_{18} y (alquilo C_0–C_4)(arilo C_6–C_{10})R_7;

R_3 es alquilo C_1–C_{18};

R_4 y R_8 son cada uno hidrógeno;

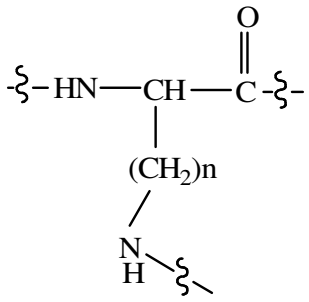
R_5 es NHR_6 u OH;

R_6 es alquilo H o C_1–C_8, o R_6 y R_1 junto con los átomos a los cuales están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros; y

R_7 es seleccionado del grupo que constituye hidrógeno, alquilo C_1–C_{18}, alquenilo C_2–C_{18},(alquilo C_0–C_4)$CONH_2$, (alquilo C_0–C_4)COOH,(alquilo C_0–C_4)NH_2,(alquilo C_0–C_4)OH, y halo;

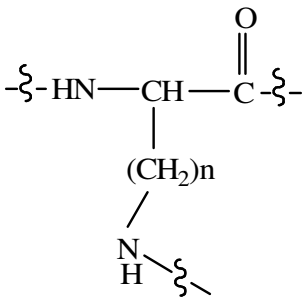
Con la condición que, si R_1 y R_2 son ambos independientemente un alquilo o (alquilo C_0–C_4)(arilo C_6–C_{10})R_7, R_1 o R_2 está unido a través de $(CH_2)_p$ a R_5, donde p es 2–9.

En algunas representaciones, el elemento del profármaco del dipéptido está relacionado con un amino de la cadena lateral de un aminoácido interno del dipéptido o análogo como se describe en la presente, en donde el aminoácido interno está compuesto por la estructura de la Fórmula II:



en donde

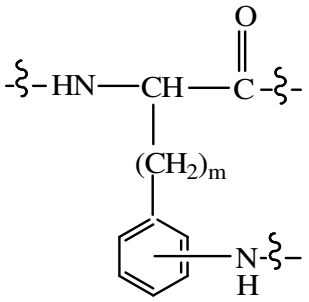
n es un entero que se selecciona de 1 a 4. En algunas representaciones, n es 3 ó 4 y, en otras, el aminoácido interno es lisina. En ciertas manifestaciones, el elemento del profármaco del dipéptido está relacionado con un grupo amino primario en una cadena lateral de un aminoácido ubicada en la posición 12, 16, 17, 18, 20, 28, ó 29 del péptido o análogo que se describe en la presente. En otras declaraciones, el aminoácido en posición 12, 16, 17, 18, 20, 28, ó 29 comprende la estructura de la Fórmula II:



en donde n es un entero que se selecciona de 1 a 4, y el elemento del profármaco del dipéptido está relacionado con la cadena lateral de un aminoácido a través de un enlace de amida. En varias representaciones, n es 4 y el aminoácido se ubica en la posición 20.

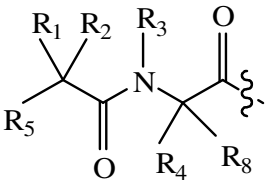
En otra representación, el elemento del profármaco del dipéptido está relacionado con el péptido o análogo del mismo a través de una amina presente en un grupo arilo de un aminoácido aromático. En algunas manifestaciones, el aminoácido aromático es un aminoácido interno del péptido o análogo que aquí se

describe. Sin embargo, el aminoácido aromático también puede ser el aminoácido en el extremo N. En algunas declaraciones el aminoácido aromático se selecciona del grupo que consiste de un amino-Phe, alanina amino-naftil, amino triptófano, amino-fenil-glicina, amino-homo-Phe, y amino tirosina. En otras representaciones, el amino primario que forma un enlace amida con el elemento del profármaco del dipéptido se encuentra en una paraposición del grupo arilo. En otras manifestaciones, el aminoácido aromático está compuesto por la estructura de la Fórmula III:



en donde m es un entero de 1 a 3.

En aquellas representaciones, en donde el elemento del pro fármaco del di péptido está relacionado con el péptido o análogo que aquí se describe a través de una amina presente en un grupo arilo de un aminoácido aromático, los pro fármacos que poseen un $t_{1/2}$, por ejemplo, de aproximadamente 1 hora poseen un di péptido con la siguiente estructura:

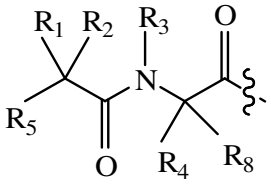


En donde R_1 y R_2 son de forma independiente C_1–C_{18} alquilo o arilo;

R_3 es C_1–C_{18} alquilo o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico 4–12;

R_4 y R_8 se seleccionan independientemente del grupo que consiste de hidrógeno, C_1–C_{18} alquilo y arilo; y R_5 es un amino o un hidróxilo.

En algunas representaciones, en donde el elemento del profármaco del dipéptido está relacionado con el péptido o análogo que aquí se describe a través de una amina presente en un grupo arilo de un aminoácido aromático, los profármacos que poseen un $t_{1/2}$, por ejemplo, de aproximadamente 1 hora poseen un dipéptido con la siguiente estructura:



en donde R_1 y R_2 son independientemente C_1–C_{18} alquilo o (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7;

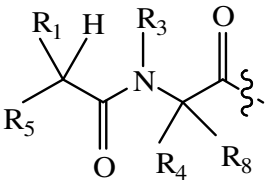
R_3 es C_1–C_{18} alquilo o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico 4–12;

R_4 y R_8 se seleccionan independientemente del grupo que consiste de hidrógeno, C_1–C_{18} alquilo y (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7;

R_5 es NH_2 o OH; y

R_7 se selecciona del grupo que consiste de hidrógeno, C_1–C_{18} alquilo, C_2–C_{18} alquenilo, (C_0–C_4 alquilo)$CONH_2$, (C_0–C_4 alquilo)COOH, (C_0–C_4 alquilo)NH_2, (C_0–C_4 alquilo)OH, y halo.

Asimismo, se representa el profármaco que contiene el elemento del profármaco del dipéptido que está relacionado a través de un aminoácido aromático y que posee un $t_{1/2}$, por ejemplo, de aproximadamente 6 a 24 horas en donde un dipéptido comprende la siguiente estructura:



en donde

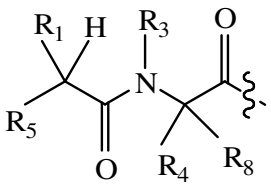
R_1 se selecciona del grupo que consiste de hidrógeno, C_1–C_{18} alquilo y arilo, o R_1 y R_2 están unidos a través de $-(CH_2)_p$, en donde p es 2–9;

R_3 es C_1–C_{18} alquilo o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico 4–6;

R_4 y R_8 se seleccionan independientemente del grupo que consiste de hidrógeno, C_1–C_{18} alquilo y arilo; y R_5 es un amino o amino N–substituido.

En algunas representaciones, se representa el profármaco que contiene el elemento del profármaco del dipéptido que está relacionado a través de un aminoácido aromático y que posee un

$t_{1/2}$, por ejemplo, de aproximadamente 6 a 24 horas en donde un dipéptido comprende la siguiente estructura:



en donde

R_1 se selecciona del grupo que consiste de hidrógeno, C_1–C_{18} alquilo, (C_1–C_{18} alquilo)OH, (C_1–C_4 alquilo)NH_2, y (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7;

R_3 es C_1–C_{18} alquilo o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico 4-6;

R_4 y R_8 se seleccionan independientemente del grupo que consiste de hidrógeno, C_1–C_{18} alquilo y (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7;

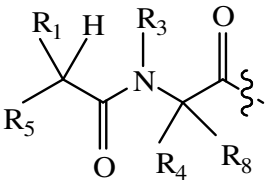
R_5 es NHR_6;

R_6 es H, C_1–C_8 alquilo, o R_6 y R_1 R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros; y

R_7 se selecciona del grupo que consiste de hidrógeno, C_1–C_{18} alquilo, C_2–C_{18} alquenilo, (C_0–C_4 alquilo)$CONH_2$, (C_0–C_4 alquilo)COOH, (C_0–C_4 alquilo)NH_2, (C_0–C_4 alquilo)OH, y halo.

Además, se representa el profármaco que contiene el elemento del profármaco del dipéptido que está relacionado a través de un aminoácido aromático y que posee un $t_{1/2}$, por ejemplo, de

aproximadamente 72 a 168 horas en donde un dipéptido está compuesto por la siguiente estructura:



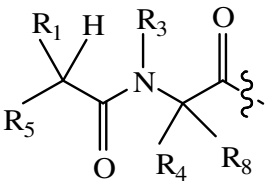
en donde R_1 y R_2 se seleccionan independientemente del grupo que consiste de hidrógeno, C_1–C_8 alquilo y arilo;

R_3 es C_1–C_{18} alquilo o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico 4–6;

R_4 y R_8 son hidrógeno; y

R_5 se selecciona del grupo que consiste de amino, amino N-substituido e hidróxilo.

En algunas manifestaciones, se representa el profármaco que contiene el elemento del profármaco del dipéptido que está relacionado a través de un aminoácido aromático y que posee un $t_{1/2}$, por ejemplo, de aproximadamente 72 a 168 horas en donde un dipéptido está compuesto por la siguiente estructura:



en donde R_1 y R_2 se selecciona del grupo que consiste de hidrógeno, C_1–C_8 alquilo, (C_1–C_4 alquilo)COOH, y (C_0–C_4

alquilo)(C_6–C_{10} arilo)R_7, o R_1 y R_5 junto con los átomos a los que están unidos forman un anillo heterocíclico 4–11;

R_3 es C_1–C_{18} alquilo o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico 4–6;

R_4 es hidrógeno o forma un anillo heterocíclico 4–6 con R_3;

R_8 es hidrógeno;

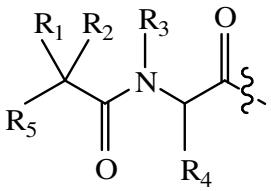
R_5 es NHR_6 o OH;

R_6 es H o C_1–C_8 alquilo, o R_6 y R_1 junto con los átomos a los que están unidos forman un anillo heterocíclico de 4, 5 o 6 miembros; y

R_7 se selecciona del grupo que consiste de hidrógeno, C_1–C_{18} alquilo, C_2–C_{18} alquenilo, (C_0–C_4 alquilo)$CONH_2$, (C_0–C_4 alquilo)COOH, (C_0–C_4 alquilo)NH_2, (C_0–C_4 alquilo)OH, y halo.

En algunas manifestaciones, el elemento del profármaco del dipéptido está relacionado con un aminoácido aromático a través de un grupo amino primario presente como un sustituyente de arilo del aminoácido aromático, en donde el aminoácido aromático está ubicado en posición 10, 13, 22, o 25 del péptido o análogo aquí descripto (sobre la base de la numeración para glucagón nativo, ver ejemplo SEQ ID N°: 1). En otras manifestaciones, el elemento del profármaco del dipéptido que está relacionado con un aminoácido aromático se ubica en posición 22 del péptido o análogo que aquí se describe.

De acuerdo con ciertas representaciones, el elemento del profármaco del dipéptido está relacionado con el amino en el extremo N del péptido o análogo que aquí se describe, y que incluye por ejemplo un péptido relacionado al glucagón, u osteocalcina, así como análogos, derivados y conjugados de la presente, en donde el elemento del profármaco del dipéptido está compuesto por la siguiente estructura:



en donde R_1 se selecciona del grupo que consiste de H y C_1–C_8 alquilo;

R_2 y R_4 se seleccionan independientemente del grupo que consiste de H, C_1–C_8 alquilo, C_2–C_8 alquenilo, (C_1–C_4 alquilo)OH, (C_1–C_4 alquilo)SH, (C_2–C_3 alquilo)SCH_3, (C_1–C_4 alquilo)$CONH_2$, (C_1–C_4 alquilo)COOH, (C_1–C_4 alquilo)NH_2, (C_1–C_4 alquilo)$NHC(NH_2^+)$ NH_2, (C_0–C_4 alquilo)(C_3–C_6 cicloalquilo), (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7, CH_2(C_5–C_9 heteroarilo), o R_1 y R_2 R_4 junto con los átomos a los que están unidos forman C_3–C_6 cicloalquilo;

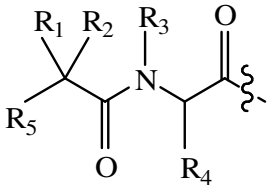
R_3 se seleccionan del grupo que consiste de C_1–C_8 alquilo, (C_3–C_6)cicloalquilo o R_4 y R_3 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 o 6 miembros;

R_5 es NHR_6 o OH;

R_6 es H, o R_6 y R_2 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 o 6 miembros; y

R_7 se seleccionan del grupo que consiste de H y OH. En algunas representaciones, R_1 es H o C_1–C_8 alquilo, R_2 se seleccionan del grupo que consiste de H, C_1–C_6 alquilo, CH_2OH, (C_1–C_4 alquilo)NH_2, (C_3–C_6 cicloalquilo) y CH_2(C_6 arilo)R_7 o R_6 y R_2 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 miembros, R_3 es C_1–C_6 alquilo, y R_4 se seleccionan del grupo que consiste de H, C_1–C_4 alquilo, (C_3–C_6)cicloalquilo, (C_1–C_4 alquilo)OH, (C_1–C_4 alquilo)SH y (C_0–C_4 alquilo)(C_6 arilo)R_7, o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 miembros. En otra manifestación, R_3 es CH_3, R_5 es NHR_6, y en otra representación alternativa, R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 miembros y R_5 es NHR_6.

De acuerdo con otras representaciones, el elemento del profármaco del dipéptido está relacionado con el amino en el extremo N del péptido o análogo que aquí se describe, en donde el elemento del profármaco del dipéptido está compuesto por la siguiente estructura:



en donde R_1 se selecciona del grupo que consiste de H y C_1–C_8 alquilo;

R_2 y R_4 se seleccionan independientemente del grupo que consiste de H, C_1–C_8 alquilo, C_2–C_8 alquenilo, (C_1–C_4 alquilo)OH, (C_1–C_4 alquilo)SH, (C_2–C_3 alquilo)SCH_3, (C_1–C_4 alquilo)$CONH_2$, (C_1–C_4 alquilo)COOH, (C_1–C_4 alquilo)NH_2, (C_1–C_4 alquilo)$NHC(NH_2^+)$ NH_2, (C_0–C_4 alquilo)(C_3–C_6 cicloalquilo), (C_0–C_4 alquilo)(C_6–C_{10} arilo)R_7, CH_2(C_5–C_9 heteroarilo), o R_1 y R_2 junto con los átomos a los que están unidos forman un C_3–C_6 cicloalquilo;

R_3 se selecciona del grupo que consiste de C_1–C_8 alquilo, (C_3–C_6)cicloalquilo o R_4 y R_3 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 o 6 miembros;

R_5 es NHR_6 o OH;

R_6 es H, o R_6 y R_2 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 o 6 miembros; y

R_7 se selecciona del grupo que consiste de hidrógeno, C_1–C_{18} alquilo, C_2–C_{18} alquenilo, (C_0–C_4 alquilo)$CONH_2$, (C_0–C_4 alquilo)COOH, (C_0–C_4 alquilo)NH_2, (C_0–C_4 alquilo)OH, y halo. En algunas manifestaciones, R_1 es H o C_1–C_8 alquilo, R_2 se selecciona del grupo que consiste de H, C_1–C_6 alquilo, CH_2OH,

(C_1-C_4 alquilo)NH_2, (C_3-C_6 cicloalquilo) y CH_2(C_6 arilo)R_7 o R_6 y R_2 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 miembros, R_3 es C_1-C_6 alquilo, y R_4 se selecciona del grupo que consiste de H, C_1-C_4 alquilo, (C_3-C_6)cicloalquilo, (C_1-C_4 alquilo)OH, (C_1-C_4 alquilo)SH y (C_0-C_4 alquilo)(C_6 arilo)R_7, o R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 miembros. En otra representación, R_3 es CH_3, R_5 es NHR_6, y en una manifestación alternativa R_3 y R_4 junto con los átomos a los que están unidos forman un anillo heterocíclico de 5 miembros y R_5 es NHR_6.

COMPOSICIONES FARMACÉUTICAS, USOS Y KITS

Sales

En algunas representaciones, el análogo de glucagón se manifiesta en forma de sal, por ejemplo, una sal farmacéuticamente aceptable. Como se utiliza en la presente invención, el término “sal farmacéuticamente aceptable” remite a las sales de los compuestos que retienen la actividad biológica del compuesto original, y que no son biológicamente o de otro modo indeseados. Dichas sales pueden prepararse *in situ* durante el último aislamiento y purificación del análogo, o se pueden preparar de forma separada si se hace reaccionar una función de base libre con un ácido adecuado. La mayor parte de los compuestos que aquí se revelan tienen la capacidad de

formar ácidos y/o sales base gracias a la presencia de grupos carboxilo y/o amino o grupos similares.

Las sales con adición de ácido, que son farmacéuticamente aceptables, pueden prepararse con ácidos orgánicos o inorgánicos. Entre las sales con adición de ácido representativas se encuentran, pero no están limitadas a acetato, adipato, citrato, aspartato, benzoato, bencenosulfonato, bisulfato, butirato, canforato, canfor sulfonato, digluconato, glicerofosfato, hemisulfato, heptanoato, hexanoato, fumarato, hidrocloruro, hidrobromida, hidroyoduro, 2-hidroxietansulfonato (isotionato), lactato, maleato, metano sulfonato, nicotinato, 2-naftaleno sulfonato, oxalato, palmitato, pectinato, persulfato, 3-fenilpropinato, picrato, pivalato, propinato, succinato, tartrato, tiocianato, fosfato, glutamato, bicarbonato, p-toluenosulfonato y undecanoato. Las sales derivadas de los ácidos inorgánicos incluyen ácido hidroclórico, ácido hidrobrómico, ácido sulfúrico, ácido nítrico, ácido fosfórico, etc. Las sales derivadas de los ácidos orgánicos comprenden ácido acético, ácido propiónico, ácido glicólico, ácido pirúvico, ácido oxálico, ácido málico, ácido malónico, ácido succinico, ácido maleico, ácido fumárico, ácido tartárico, ácido cítrico, ácido benzoico, ácido cinámico, ácido mandélico, ácido metanosulfónico, ácido etanosulfónico, ácido p-tolueno-sulfónico, ácido salicílico, etc. Entre los ejemplos de los

ácidos que pueden utilizarse para formar sales con adición de ácido, que sean farmacéuticamente aceptables, se encuentran, por ejemplo, un ácido inorgánico, como ácido hidroclórico, ácido hidrobrómico, ácido sulfúrico y ácido fosfórico y una ácido orgánico, como por ejemplo, ácido oxálico, ácido maleico, ácido succínico y ácido cítrico.

Las sales de adición básicas también pueden prepararse *in situ* durante el aislamiento final y purificación de la fuente del ácido salicílico, o a través de la reacción de un grupo que contiene ácido carboxílico con una base adecuada, tal como el hidróxido, carbonato o bicarbonato de un catión metálico farmacéuticamente aceptable o con amoníaco o un amino primario, secundario o terciario. Entre las sales farmacéuticamente aceptables se encuentran, pero no están limitadas a cationes basados en metales alcalinos o metales alcalinotérreos como litio, sodio, potasio, calcio, magnesio y sales de aluminio, entre otras, y amonio cuaternario no tóxico y cationes de amida que incluyen amonio, tetrametilamonio, tetraetilamonio, metilamonio, dimetilamonio, trimetilamonio, trietilamonio, dietilamonio y etilamonio, entre otros. Otros aminos orgánicos representativos que son útiles para la formación de sales de adición básicas incluyen por ejemplo etilenodiamida, etanolamida, diethanolamina, piperidina, piperazinaetilendiamida, etanolamina, dietanolamina,

piperidina, piperazina, entre otras. Aquellas sales que están derivadas de bases orgánicas comprenden, pero no están limitadas a sales de aminos primarios, secundarios y terciarios.

Además, los grupos básicos que contienen nitrógeno pueden ser cuaternizados con el análogo de la presente representación como haluros de baja alcalinidad, por ejemplo metilo, etilo, propilo y cloruro de butilo, bromuros, yoduros; los haluros de cadena larga como yoduros, bromuros y cloruros de estearilo, miristilo, lauril, decil; haluros arilaquilo como bromuros de fenetilo y bencilo y otros. Por lo tanto, se obtienen productos que pueden dispersarse o son solubles en agua o aceite.

Formulaciones

De acuerdo con algunas representaciones, una composición farmacéutica se obtiene cuando la composición comprende un análogo de glucagón de la presente manifestación, o una sal sales farmacéuticamente aceptable del mismo, y un vehículo farmacéuticamente aceptable. Como se utiliza en la presente, el término “vehículo farmacéuticamente aceptable” incluye cualquier vehículo farmacéutico estándar, tal como un tapón fosfato salino, agua, emulsiones como por ejemplo emulsiones compuestas por aceite/ agua o agua/ aceite y distintos tipos de agentes humectantes. El término además comprende todos los

agentes aprobados por un organismo regulador del gobierno federal de los Estados Unidos o que estén incluidos en la Farmacopea de los Estados Unidos destinada a utilizarse en animales y humanos.

La composición farmacéutica puede incluir cualquier ingrediente farmacéuticamente aceptable como agentes acidificantes, aditivos, adsorbentes, propelente en aerosol, agentes de desplazamiento de aire, agentes alcalinizantes, agentes antiglomerantes, anticoagulantes, conservantes antimicróbicos, antioxidantes, antisépticos, bases, aglutinantes, agentes de tapón, agentes quelatos, agentes de recubrimiento, agentes de coloración, desecantes, detergentes, diluentes, desinfectantes, desintegrantes, agentes de dispersión, agentes mejoradores de disolución, colorantes, emolientes, agentes emulsionantes, estabilizantes de emulsión, rellenos, agentes formadores de películas, resaltadores de sabor, agentes saborizantes, mejoradores del flujo, agentes gelificantes, agentes de granulación, humectantes, lubricantes, mucoadhesivos, bases de ungüentos, ungüentos, excipientes oleaginosos, bases orgánicas, bases de pastillas, pigmentos, plastificadotes, agentes de pulido, conservantes, agentes de secuestro, penetrantes de la piel, agentes de solubilización, solventes, agentes de estabilización, bases de supositorios, agentes tensoactivos, surfactantes, agentes de suspensión, agentes edulcorantes, agentes terapéuticos, agentes espesantes,

agentes de tonicidad, agentes de toxicidad, agentes que aumentan la viscosidad, agentes de absorción de agua, cosolventes miscibles con agua, ablandadores de agua, o agentes de humedecimiento.

En algunas representaciones, la composición farmacéutica puede incluir cualquier componente o una combinación de los mismos, a saber: acacia, potasio de acesulfamo, citrato de acetiltributilo, citrato de acetiltrietilo, agar, albúmina, alcohol, alcohol deshidratado, alcohol desnaturalizado, alcohol diluido, ácido aleurítico, ácido algínico, poliésteres alifáticos, alúmina, hidróxido de aluminio, estearato de aluminio, amilopectina, α-amilosa, ácido ascórbico, palmitato de ascorbilo, aspartamo, agua bacteriostática para la inyección, bentonita, magma de bentonita, cloruro de benzalconio, cloruro de bencetonio, ácido benzoico, alcohol bencílico, benzoato de bencilo, bronopol, hidroxianisol butilado, hidroxitolueno butilado, butilparabeno, sodio de butilparabeno, alginato de calcio, ascorbato de calcio, carbonato de calcio, ciclamato de calcio, fosfato de calcio anhidro dibásico, fosfato de calcio deshidratado dibásico, fosfato de calcio tribásico, propionato de calcio, silicato de calcio, sorbato de calcio, estearato de calcio, sulfato de calcio, hemihidrato de sulfato de calcio, aceite de canola, carbómero, dióxido de carbono, calcio de carboximetilcelulosa,

sodio de carboximetilcelulosa, □-caroteno, carragenina, aceite de castor, aceite de castor hidrogenado, cera emulsionante catiónica, acetato de celulosa, ftalato de acetato de celulosa, etil celulosa, celulosa microcristalina, celulosa en polvo, celulosa microcristalina silicificada, carboximetilcelulosa de sodio, alcohol cetoestearílico, cetrimida, alcohol cetílica, clorhexidina, clorobutanol, clorocresol, colesterol, acetato de clorhexidina, gluconato de clorhexidina, clorhidrato de clorhexidina, clorodifluoroetano (HCFC), clorodifluorometano, clorofluorocarbonos (CFC)clorofenoxietanol, cloroxilenol, sólidos de jarabe de maíz, ácido cítrico anhidro, monohidrato de ácido cítrico, manteca de cacao, agentes colorantes, aceite de maíz, aceite de semilla de algodón, cresol, m-cresol, o-cresol, p-cresol, sodio de croscarmelosa, crospovidona, ácido ciclámico, ciclodextrinas, dextratos, dextrina, dextrosa, dextrosa anhidra, diazolidinil urea, ftalato de dibutilo, sebacato de dibutilo, dietanolamina, ftalato de dietilo, difluoroetano (HFC), dimetil-β-ciclodextrina, compuestos de tipo de ciclodextrina tales como Captisol®, éter de dimetilo, ftalato de dimetilo, edentato de dipotasio, edentato de disodio, fosfato de hidrógeno de disodio, calcio de docusato, potasio de docusato, sodio de docusato, galato de dodecilo, bromuro de dodeciltrimetilamonio, edentato de calcio y disodio, ácido edtico, eglumina, alcohol etílico, etilcelulosa, galato de etilo, laurato de etilo, etil maltol, oleato de etilo,

etilparabeno, potasio de etilparabeno, sodio de etilparabeno, etil vanillina, fructosa, fructosa líquida, fructosa molida, fructosa libre de pirógenos, fructosa en polvo, ácido fumárico, gelatina, glucosa, glucosa líquida, mezclas de glicéridos de ácidos grasos vegetales saturados, glicerina, behenato de glicerilo, monooleato de glicerilo, monoestearato de glicerilo, monoestearato de glicerilo autoemulsionante, palmitoestearato de glicerina, glicina, glicoles, glicofurol, goma guar, heptafluoropropano (HFC), bromuro de hexadeciltrimetilamonio, jarabe de alta fructosa, albúmina de suero humano, hidrocarburos (HC), ácido clorhídrico diluido, aceite vegetal hidrogenado tipo II, hidroxietilcelulosa, 2-hidroxietil-β-ciclodextrina, hidroxipropilcelulosa, hidroxipropilcelulosa poco hidrogenada, 2-hidroxipropil-β-ciclodextrina, hidroxipropilmetilcelulosa poco sustituida, ftalato de hidroxipropilmetilcelulosa, imidurea, índigo carmín, intercambiadores de iones, óxidos de hierro, alcohol isopropílico, miristato de isopropilo, palmitato de isopropilo, solución salina isotónica, caolín, ácido láctico, lactitol, lactosa, lanolina, alcoholes de lanolina, lanolina anhidra, lecitina, silicato de magnesio y aluminio, carbonato de magnesio, carbonato de magnesio normal, carbonato de magnesio anhidro, hidróxido de carbonato de magnesio, hidróxido de magnesio, sulfato de laurilo de magnesio, óxido de magnesio, silicato de magnesio, estearato de magnesio, trisilicato de

magnesio, trisilicato de magnesio anhidro, ácido málico, malta, maltitol, solución de maltitol, maltodextrina, maltol, maltosa, manitol, triglicéridos de cadena mediana, meglumina, mentol, metilcelulosa, metacrilato de metilo, oleato de metilo, metilparabeno, potasio de metilparabeno, sodio de metilparabeno, celulosa microcristalina y sodio de carboximetilcelulosa, aceite mineral, aceite mineral liviano, aceite mineral y alcoholes de lanolina, aceite, aceite de oliva, monoetanolamina, montmorilonita, galato de octilo, ácido oleico, ácido palmítico, parafina, aceite de maní, petrolato, petrolato y alcoholes de lanolina, barniz farmacéutico, fenol, fenol licuado, fenoxietanol, fenoxipropanol, alcohol feniletílico, acetato fenilmercúrico, borato fenilmercúrico, nitrato fenilmercúrico, polacrilina, potasio de polacrilina, poloxámero, polidextrosa, polietilenglicol, óxido de polietileno, poliacrilatos, polímeros de bloquea de polietileno y de polioxipropileno, polimetacrilatos, éteres de alquilo de polioxietileno, derivados de aceite de castor de polioxietileno, ésteres de ácidos grasos de sorbitol de polioxietileno, estearatos de polioxietileno, alcohol polivinílico, polivinil pirrolidona, alginato de potasio, benzoato de potasio, bicarbonato de potasio, bisulfito de potasio, cloruro de potasio, citrato de potasio, citrato de potasio anhidro, fosfato de hidrógeno de potasio, metabisulfito de potasio, fosfato de potasio monobásico, propionato de

potasio, sorbato de potasio, povidona, propanol, ácido propiónico, carbonato de propileno, propilenglicol, alginato de propilenglicol, galato de propilo, propilparabeno, potasio de propilparabeno, sodio de propilparabeno, sulfato de protamina, aceite de semilla de colza, solución de Ringer, sacarina, amonio de sacarina, calcio de sacarina, sodio de sacarina, aceite de cártamo, saponita, proteínas del suero, aceite de sésamo, sílice coloidal, dióxido de silicio coloidal, alginato de sodio, ascorbato de sodio, benzoato de sodio, bicarbonato de sodio, bisulfito de sodio, cloruro de sodio, citrato de sodioanhidro, citrato de sodio deshidratado, cloruro de sodio, ciclamato de sodio, edentato de sodio, sulfato de dodecilo de sodio, sulfato de laurilo de sodio, metabisulfito de sodio, fosfato de sodio, fosfato de sodio dibásico, fosfato de sodio monobásico, propioanto de sodio anhidro tribásico, propionato de sodio, sorbato de sodio, glicolato de almidón de sodio, fumarato de estearilo de sodio, sulfito de sodio, ácido sórbico, ésteres de sorbitán (ácidos grasos de sorbitán), sorbitol, solución de sorbitol 70%, aceite de soja, cera de esperma de ballena, almidón, almidón de maíz, almidón de papa, almidón pregelatinizado, almidón de maíz esterilizable, ácido esteárico, ácido esteárico purificado, alcohol estearílico, sacarosa, azúcares, azúcar comprimible, azúcar de confitura, esferas de azúcar, azúcar invertida, *Sugartab*, Amarillo Ocaso FCF, parafina sintética, talco, ácido tartárico, tartrazina,

tetrafluoroetano (HFC), aceite de teobroma, timerosal, dióxido de titanio, alfa tocoferol, acetato de tocoferilo, succinato de ácido de alfa tocoferilo, beta-tocoferol, delta-tocoferol, gamma-tocoferol, tragacanto, triacetina, citrato de tributilo, trietanolamina, citrato de trietilo, trimetil-β-ciclodextrina, bromuro de trimetiltetradecilamonio, tris tampón, edentato de trisodio, vanillina, aceite vegetal hidrogenado de tipo I, agua, agua blanda, agua dura, agua sin dióxido de carbono, agua sin pirógenos, agua para inyección, agua estéril para inhalación, agua estéril para inyección, agua estéril para irrigación, ceras, cera emulsionante aniónica, cera de carnauba, cera emulsionante catiónica, cera de éster de cetilo, cera microcristalina, cera emulsionante no iónica, cera para supositorios, cera blanca cera amarilla, petrolato blanco, grasa de lana, goma xantana, xilitol, zeína, propionato de zinc, sales de zinc, estearato de zinc o cualquier excipiente en *Manual de Excipientes Farmacéuticos*, Tercera edición, A. H. Kibbe (Pharmaceutical Press, London, UK, 2000), que se incorpora como referencia en su totalidad. *Remington's Pharmaceutical Sciences*, Sexta edición, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), que se incorpora como referencia en su totalidad, revela varios componentes que se utilizan para formular composiciones farmacéuticamente aceptables y técnicas conocidas para preparar las mismas. Excepto que a medida que cualquier agente convencional sea

incompatible con las composiciones farmacéuticas, se contempla su uso en las composiciones farmacéuticas. Los ingredientes activos suplementarios también pueden incorporarse a las composiciones.

En algunas representaciones, el o los siguiente(s) componente(s) puede(n) estar presente(s) en la composición farmacéutica en cualquier concentración, tal como, por ejemplo, por lo menos, A, en donde A es 0.0001% w/v, 0.001% w/v, 0.01% w/v, 0.1% w/v, 1% w/v, 2% w/v, 5% w/v, 10% w/v, 20% w/v, 30% w/v, 40% w/v, 50% w/v, 60% w/v, 70% w/v, 80% w/v, ó 90% w/v. En algunas manifestaciones, el o los siguiente(s) componente(s) puede(n) estar presente(s) en la composición farmacéutica en cualquier concentración, tal como, por ejemplo, en la mayor parte de B, en donde B es 90% w/v, 80% w/v, 70% w/v, 60% w/v, 50% w/v, 40% w/v, 30% w/v, 20% w/v, 10% w/v, 5% w/v, 2% w/v, 1% w/v, 0.1% w/v, 0.001% w/v, ó 0.0001%. En algunas representaciones, el o los siguiente(s) componente(s) puede(n) estar presente(s) en la composición farmacéutica en cualquier rango de concentración, por ejemplo aproximadamente de A a B. En algunas manifestaciones, A es 0,0001% y B es 90%.

En algunas representaciones, el ingrediente farmacéuticamente aceptable se selecciona del grupo que consiste en un azúcar (por ejemplo, glucose, sacarosa, trehalosa, lactosa, fructosa, maltosa, dextrano, glicerina, dextrano, melibiosa, melecitosa,

rafinosa, manotriosa, estaquiosa, maltosa, lactulosa, maltulosa, o isomaltulosa, o combinación de estos azúcares), alcohol de azúcar (por ejemplo, glicol, glicerol, eritritol, treitol, arabitol, xilitol, ribitol, manitol, sorbitol, dulcitol, iditol, isomalt, maltitol, lactitol, o glucitol, o combinaciones de estos tres alcoholes de azúcar), una sal (por ejemplo, cloruro de sodio), un emulsionante o surfactante (por ejemplo, polisorbatos, tales como monooleato de sorbitano 20 polioxietileno , u otro copolímeros en bloque de óxido de etileno y óxido de propileno), lioprotectantes, y mezclas de los mismos. Por ejemplo, los excipientes como los azúcares o alcoholes de azúcar están presents en, por ejemplo, una concentración de alrededor de 20 mg/mL a aproximadamente 40 mg/mL, ó 25 a 45 mg/mL, tal como 35 mg/mL.

La composición farmacéutica puede formularse para lograr un pH fisiológicamente compatible. En algunas representaciones, el pH de una composición farmacéutica puede ser por lo menos 5, por lo menos 5,5, por lo menos 6, por lo menos 6,5, por lo menos 7, por lo menos 7,5, por lo menos 8, por lo menos 8,5, por lo menos 9, por lo menos 9,5, por lo menos 10, o por lo menos 10,5 hasta e incluyendo pH 11, que depende de la formulación y la vía de administración, por ejemplo entre 4 y 7, o 4,5 y 5,5. En representaciones ejemplares, las composiciones farmacéuticas pueden incluir agentes de tapón para lograr un pH fisiológicamente compatible. Los agentes de

tapón pueden comprender cualquier compuesto que sea capaz de actuar como tapón en el pH deseado, tales como, por ejemplo, tapones de fosfato(por ejemplo, PBS), trietanolamina, Tris, bicina, TAPS, tricina, HEPES, TES, MOPS, PIPES, cacodilato, MES, acetato, citrato, succinato, histidina u otros tapones farmacéuticamente aceptables. En representaciones ejemplares, la resistencia del tapón es de por lo menos 0,5 mM, por lo menos 1 mM, por lo menos 5 mM, por lo menos 10 mM, por lo menos 20 mM, por lo menos 30 mM, por lo menos 40 mM, por lo menos 50 mM, por lo menos 60 mM, por lo menos 70 mM, por lo menos 80 mM, por lo menos 90 mM, por lo menos 100 mM, por lo menos 120 mM, por lo menos 150 mM, o por lo menos 200 mM. En algunas representaciones, la resistencia del tapón no es más que 300 mM (por ejemplo, como máximo 200 mM, como máximo 100 mM, como máximo 90 mM, como máximo 80 mM, como máximo 70 mM, como máximo 60 mM, como máximo 50 mM, como máximo 40 mM, como máximo 30 mM, como máximo 20 mM, como máximo 10 mM, como máximo 5 mM, como máximo 1 mM). Por ejemplo, la concentración del tapón puede ser de alrededor de 2 mM a aproximadamente 100 mM, o alrededor de 10 mM a 50 mM.

Vías de Administración

El siguiente debate sobre las vías de administración se brinda simplemente para ilustrar las representaciones ejemplares y no

deben interpretarse de forma tal que limite el alcance de la presente.

Las formulaciones que son adecuadas para la administración vía oral pueden constar de (a) soluciones líquidas, tales como una cantidad eficaz del análogo de la presente representación disuelta en diluentes como agua, solución salina, o jugo de naranja; (b) cápsulas, sachets, comprimidos y pastillas, en donde cada una contiene una cantidad predeterminada de ingrediente activo, como sólidos o gránulos; (c) polvos; (d) suspensiones en un líquido apropiado; y (e) emulsiones adecuadas. Las formulaciones líquidas pueden incluir diluentes, como agua y alcoholes, por ejemplo, etanol, alcohol bencílico y alcoholes de polietilenos, ya sea con o sin el agregado de un surfactante farmacéuticamente aceptado. Las presentaciones en cápsulas pueden ser con un recubrimiento gelatinoso común, duro o blando, que contenga, por ejemplo, surfactantes, lubricantes y rellenos inertes, como lactosa, sacarosa, fosfato de calcio y maicena. Por su parte, las presentaciones en tabletas pueden incluir uno o más excipientes como lactosa, sacarosa, manitol, maicena, almidón de papa, ácido algínico, celulosa microcristalina, acacia, gelatina, goma guar, dióxido de silicio coloidal, sodio croscarmeloso, talco, estearato de magnesio, estearato de calcio, estearato de zinc, ácido esteárico y otros excipientes, colorantes, diluente, agentes de tapón, agentes de humectación, conservantes, agentes

saborizantes y excipientes farmacológicamente compatibles. Las presentaciones en pastillas pueden incluir el análogo de la presente representación con un sabor, por lo general sacarosa y acacia o goma tragacanto, como también pastillas que comprenden el análogo de la presente representación en una base inerte, tal como gelatina y glicerina, o sacarosa y acacia, emulsiones, geles, y otros, que contengan, además, dichos excipientes como son conocidos en el arte.

El análogo de la representación, solo o en combinación con otros componentes adecuados, puede suministrarse vía administración pulmonar y pueden hacerse formulaciones en aerosol para ser administradas por inhalación. Estas formulaciones en aerosol pueden colocarse en propelentes presurizados, tales como diclorodifluorometano, propano, nitrógeno y otros. También pueden formularse como medicamentos para preparaciones sin presión, tales como un nebulizador o un atomizador. Dichas formulaciones en atomizadores también pueden utilizarse para pulverizar la mucosa. En algunas representaciones, el análogo se formula en una combinación de polvo o en micropartículas o nanopartículas. Las formulaciones pulmonares se conocen en el arte. Véase, por ejemplo, Qian et al., Int J Pharm 366: 218-220 (2009); Adjei y Garren, Pharmaceutical Research, 7(6): 565-569 (1990); Kawashima et al., J Controlled Release 62(1-2): 279-287 (1999); Liu et al., Pharm Res 10(2): 228-232 (1993); Solicitud de Patente

Internacional, N° de publicación WO 2007/133747 y WO 2007/141411.

Las formulaciones adecuadas para la administración parenteral incluyen soluciones para inyecciones estériles e isotónicas, ya sean acuosas o no, que puedan contener anti-oxidantes, tapones, bacteriostáticos, y solutos que convierten a la formulación en isotónica con la sangre del receptor determinado y las suspensiones estériles acuosas y no acuosas que pueden incluir agentes de suspensión, solubilizado res, agentes espesantes, estabilizadores y conservantes. El término "parenteral" no significa que sea a través del canal alimenticio sino a través de otra vía tal como la subcutánea, intramuscular, intraespinal o intravenosa. El análogo de la presente representación puede administrarse a través de un diluente fisiológicamente aceptable en un vehículo farmacéutico, tal como un líquido estéril o una mezcla de líquidos, que incluye agua, solución salina, dextrosa en solución acuosa y soluciones de azúcar relacionadas, un alcohol, tal como etanol o alcohol hexadecil, un glicol, tal como glicol de propileno o glicol de polietileno, dimetilsulfóxido, glicerol, cetales como 2,2- dimetil-1,3-dioxolano-4-metanol, éter, poli (etilenglicol) 400, aceites, ácidos grasos, esteres de ácido graso o glicéridos, o glicéridos de ácidos grasos acetilados con o sin la adición de un surfactante farmacéuticamente aceptable, tal como jabón o un

detergente, un agente de suspensión, tal como pectina, carbómeros, metilcelulosa, hidroxipropilmetilcelulosa, o carboximetilcelulosa, o agentes emulsionantes y otros adyuvantes farmacéuticos.

Los aceites, que pueden ser utilizados en las formulaciones parenterales incluyen petróleo, aceites de origen animal, vegetal o sintéticos. Entre los ejemplos específicos de aceites se incluyen maní, soja, sésamo, semilla de algodón, maíz, oliva, petrolatum y minerales. Los ácidos grasos adecuados para utilizar en las formulaciones parenterales incluyen ácido oleico, ácido esteárico, y ácido isosteárico. El oleato de etilo y miristato de isopropilo son ejemplos de esteres de ácidos grasos adecuados.

Los jabones adecuados, que pueden ser utilizados en las formulaciones parenterales incluyen metal alcalino graso, amonio y sales de trietanolamina, y detergentes adecuados que incluyen (a) detergentes catiónicos tal como, por ejemplo, *Haluros* de dialquil dimetil *amonio*, y haluros de piridinio alquilo, (b) detergentes aniónicos como por ejemplo alquilo, arilo y sulfonatos de olefina, alquilo, olefina, éter, y sulfatos de monoglicéridos y sulfosuccinatos, (c) detergentes no iónicos como óxidos de amina grasa, alcanolamidas de ácidos grasos, y copolímeros de polioxietilenopolipropileno, (d) detergentes anfotéricos tales como, por ejemplo alquilo-β-

aminopropionatos, y sales de amonio cuaternario 2-alquilo - imidazolina y (e) mezclas de estos.

Las formulaciones parenterales por lo general contienen aproximadamente de 0,5% a 25% en peso del análogo de la presente en la solución. Se pueden utilizar conservantes y tapones. Con el fin de minimizar o eliminar la irritación en el lugar de la inyección, tales composiciones pueden contener uno o más surfactantes no iónicos con un balance hidrófilo-lipófilo (HLB) de aproximadamente 12 a 17. La cantidad de surfactante en dichas formulaciones normalmente variará de aproximadamente 5% a 15% en peso. Los surfactantes adecuados incluyen esteres de ácido graso de sorbitán glicol polietileno, tales como monooleato de sorbitán y los aductos de alto peso molecular de óxido de etileno con una base hidrofóbica, formados por la condensación de óxido de propileno con el propileno glicol. Las formulaciones parenterales pueden presentarse en dosis de unidad o recipientes multi-dosis sellados, tales como ampollas y viales, y se pueden almacenar en una condición de congelado (liofilizado), que requiere solo de la adición del excipiente líquido estéril, por ejemplo, agua, en el caso de las inyecciones, inmediatamente antes de usar. Las soluciones y suspensiones de inyección extemporánea pueden prepararse a partir de gránulos, comprimidos y polvos estériles de la forma anteriormente descripta.

Las formulaciones inyectables son apropiadas en el contexto de la presente invención. Los requisitos que debe cumplir el vehículo farmacéutico efectivo para las composiciones inyectables son bien conocidos por los expertos en el arte. Véase, por ejemplo, *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., páginas 238-250 (1982), y *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pág. 622-630 (1986)).

Además, el análogo de la presente divulgación puede presentarse en supositorios, para la administración rectal, gracias a la mezcla con una variedad de bases, tales como bases emulsificantes o bases solubles en agua. Las formulaciones apropiadas para la administración vaginal pueden presentarse como pesarios, tampones, cremas, geles, pastas, espumas, o fórmulas en aerosol que contengan, además del ingrediente activo, tales vehículos que se conocen en el arte por ser apropiados.

Será apreciado por un experto en el arte que, además de las composiciones farmacéuticas descritas anteriormente, el análogo de la presente pueda formularse como complejos de inclusión, tales como complejos de inclusión de ciclodextrina, o liposomas.

Dosis

Los análogos de la representación son considerados útiles en métodos utilizados para tratar una enfermedad o afección en la que juegan un papel importante el agonista del receptor GIP, co- agonista del receptor GIP/GLP-1, co- agonista del receptor GIP/glucagón, o tri-agonista del receptor GIP/GLP-1/glucagón. Por razones de la presente invención, la cantidad o dosis del análogo administrado debe ser suficiente para lograr, por ejemplo, una respuesta profiláctica o terapéutica en el sujeto o animal, durante un plazo de tiempo razonable. Por ejemplo, la dosis del análogo de la presente divulgación debe ser suficiente para estimular la secreción cAMP desde las células como aquí se describe o suficiente para reducir los niveles de glucosa en sangre, los niveles de grasa, los niveles de ingesta de alimentos o el peso corporal de un mamífero, en un periodo que oscila entre 1 a 4 minutos, 1 a 4 horas o 1 a 4 semanas o más, por ejemplo, 5 a 20 semanas o más, desde el tiempo de administración. En una representación ejemplar, el periodo de tiempo puede ser aún mayor. La dosis estará determinada por la eficacia del análogo determinado de la presente divulgación y la condición del animal (por ejemplo, un humano), como también el peso corporal del animal (por ejemplo, un humano) a ser tratado.

Muchos ensayos utilizados para determinar una dosis administrada son conocidos en el arte. Por motivos que aquí se especifican, un ensayo, que consiste en comparar el alcance al

que los niveles de glucosa en sangre son reducidos cuando se administra una dosis suministrada del análogo de la presente representación a un mamífero entre un grupo de mamíferos, a los que a cada uno se les suministró una dosis diferente del análogo, podría utilizarse para determinar una dosis inicial para ser administrada a un mamífero. El alcance al que los niveles de glucosa en sangre son reducidos cuando se administra una dosis determinada puede ser ensayado con métodos conocidos en el arte y que incluyen, por ejemplo, aquellos descriptos aquí en el Ejemplo11.

La dosis del análogo de la presente divulgación también está determinado por la existencia, naturaleza y extensión de cualquier efecto secundario adverso que pueda acompañar la administración de un análogo particular de la presente divulgación. Por lo general, el médico responsable determinará la dosis del análogo de la presente divulgación con la que se tratará a cada paciente y tendrá que considerar varios factores tales como edad, peso corporal, salud general, dieta, sexo, análogo de la presente divulgación que será administrado, vía de administración y la gravedad de la condición a ser tratada. A modo de ejemplo y sin intención de limitar la invención, la dosis del análogo de la presente divulgación puede ser alrededor de 0,0001 a aproximadamente 1 g/kg por peso corporal del sujeto a ser tratado por día, desde alrededor de 0,0001 a aproximadamente 0,001 g/kg por peso corporal por día, o

alrededor de 0,01 mg a aproximadamente 1 g/kg por peso corporal por día. En representaciones ejemplares, la dosis puede ser una dosis total por semana de alrededor de 1 mg a aproximadamente 40 mg, o alrededor de 4 mg a aproximadamente 30 mg, o alrededor de 4 a aproximadamente 20 mg, o alrededor de 10 a aproximadamente 20 mg, o alrededor de 12 mg a aproximadamente 30 mr.

En algunas representaciones, la composición farmacéutica comprende cualquiera de los análogos aquí revelados en un nivel de pureza adecuado para ser administrado a un paciente. En algunas manifestaciones, el análogo tiene un nivel de pureza de por lo menos alrededor del 90%, alrededor del 91%, alrededor del 92%, alrededor del 93%, alrededor del 94%, alrededor del 95%, alrededor del 96%, alrededor del 97%, alrededor del 98% o alrededor del 99%, y un excipiente, vehículo y diluente farmacéuticamente aceptable. La composición farmacéutica en algunos aspectos comprende el análogo de la presente divulgación en una concentración de por lo menos A, en donde A es aproximadamente 0,001 mg/ml, aproximadamente 0,01 mg/ml, 0 aproximadamente 1 mg/ml, aproximadamente 0,5 mg/ml, aproximadamente 1 mg/ml, aproximadamente 2 mg/ml, aproximadamente 3 mg/ml, aproximadamente 4 mg/ml, aproximadamente 5 mg/ml, aproximadamente 6 mg/ml, aproximadamente 7 mg/ml, aproximadamente 8 mg/ml,

aproximadamente 9 mg/ml, aproximadamente 10 mg/ml, aproximadamente 11 mg/ml, aproximadamente 12 mg/ml, aproximadamente 13 mg/ml, aproximadamente 14 mg/ml, aproximadamente 15 mg/ml, aproximadamente 16 mg/ml, aproximadamente 17 mg/ml, aproximadamente 18 mg/ml, aproximadamente 19 mg/ml, aproximadamente 20 mg/ml, aproximadamente 21 mg/ml, aproximadamente 22 mg/ml, aproximadamente 23 mg/ml, aproximadamente 24 mg/ml, aproximadamente 25 mg/ml o más. En algunas representaciones, la composición farmacéutica incluye el análogo en una concentración en la mayor parte de B, en donde B es alrededor de 30 mg/ml, alrededor de 25 mg/ml, alrededor de 24 mg/ml, alrededor de 23, mg/ml, alrededor de 22 mg/ml, alrededor de 21 mg/ml, alrededor de 20 mg/ml, alrededor de 19 mg/ml, alrededor de 18 mg/ml, alrededor de 17 mg/ml, alrededor de 16 mg/ml, alrededor de 15 mg/ml, alrededor de 14 mg/ml, alrededor de 13 mg/ml, alrededor de 12 mg/ml, alrededor de 11 mg/ml, alrededor de 10 mg/ml, alrededor de 9 mg/ml, alrededor de 8 mg/ml, alrededor de 7 mg/ml, alrededor de 6 mg/ml, alrededor de 5 mg/ml, alrededor de 4 mg/ml, alrededor de 3 mg/ml, alrededor de 2 mg/ml, alrededor de 1 mg/ml, o alrededor de 0.1 mg/ml. En algunas manifestaciones, las composiciones pueden contener un análogo en un rango de concentración de A B mg/ml, por ejemplo, alrededor de 0,001 a alrededor de 30,0 mg/ml.

Formas objetivo

Alguien con habilidad común en el arte podrá apreciar inmediatamente que los análogos de la divulgación pueden modificarse de distintas formas, de manera tal que la eficacia profiláctica o terapéutica del análogo de la presen te divulgación aumenta gracias a la modificación. Por ejemplo, el análogo de la presente divulgación puede conjugarse de forma directa o indirecta a través de un enlazador a un grupo objetivo. En el arte se conoce la práctica en la que se conjugan compuestos, por ejemplo análogos de glucagón como aquí se describen, con grupos objetivo. Véase, por ejemplo, Wadhwa et al., *J Drug Targeting*, 3, 111-127 (1995) y Patente estadounidense N° 5.087.616. El término "grupo objetivo " como aquí se utiliza, se refiere a toda molécula o agente que reconoce y se une específicamente a un receptor de superficie celular, de modo tal que el grupo objetivo dirige la entrega del análogo de la presente divulgación a una población de células en donde se expresa la superficie del receptor (el receptor glucagón, el receptor GLP-1). Entre los grupos objetivos se encuentran, pero no están limitados a anticuerpos o fragmentos de los mismos, péptidos, hormonas, factores de crecimiento, citocinas y cualquier otro ligando natural o no natural, que se une a los receptores de superficie celular (por ejemplo, ***Receptor*** del ***Factor de Crecimiento*** Epitelial (EGFR), receptor T-celular (TCR), receptor B-celular (BCR), CD28,

receptor del factor de crecimiento derivado de plaquetas (PDGF), receptor de acetilcolina del tipo nicotínico (nAChR), etc.). Como se utiliza en la presente invención, el término "enlazador" es un enlace, molécula o grupo de moléculas que ligan dos entidades separadas. Los enlazadores pueden brindar un espacio óptimo de las dos entidades o pueden además brindar una conexión lábil que permita que las dos entidades estén separadas entre ellas. Las conexiones lábiles incluyen a los grupos fotoescindibles, grupos lábiles al ácido, grupos lábiles y grupos escindibles por enzimas. El término "enlazador" en algunas representaciones hace referencia a todo agente o molécula que une al análogo de la presente divulgación con el grupo objetivo. Alguien con habilidad común en el arte reconoce que los sitios del análogo de la presente divulgación, que no son necesarios para desempeñar la función del análogo, son ideales para sujetar un enlazador y/o un grupo objetivo, siempre que el enlazador y/o grupo objetivo, una vez que se sujetan al análogo, no interfiere(n) con la función del análogo de la presente divulgación, es decir, la habilidad de estimular la secreción cAMP desde las células para tratar diabetes u obesidad.

Formulaciones de liberación controlada

De forma alternativa, los análogos de glucagón que aquí se describen pueden modificarse en forma de depósito, de manera

tal que la forma en que el análogo de la presente divulgación se libera en el cuerpo en el que es administrado se controla en cuanto al tiempo y ubicación dentro del cuerpo (Véase, por ejemplo, Patente estadounidense N° 4.450.150). Las formas de depósito del análogo de la presente divulgación puede ser, por ejemplo, una composición que puede implantarse y que comprende el análogo y un material poroso o no poroso, tal como un polímero, en donde el análogo de la presente divulgación se encapsula o difunde a través del material y/o degradación del material no poroso. Luego el depósito se implanta en la ubicación deseada dentro del cuerpo y el análogo se libera del implante en un ritmo predeterminado.

La composición farmacéutica en aspectos ejemplares se modifica para poseer todo tipo de perfil de liberación *in vivo.* En algunos aspectos, la composición farmacéutica es una liberación inmediata, una liberación controlada, una liberación sostenida, una liberación extendida, una liberación retardada o una formulación de liberación bifásica. Los métodos para formular los péptidos que controlan la liberación son conocidos en el arte. Véase, por ejemplo, Qian et al., *J Pharm* 374: 46-52 (2009) y las publicaciones internacionales de patentes N° WO 2008/130158, WO2004/033036; WO2000/032218; y WO 1999/040942. Las composiciones instantáneas además, pueden comprender, por ejemplo micelas o liposomas, o alguna otra forma encapsulada, o

puede administrarse durante una liberación extendida para brindar un almacenamiento prolongado y/o un efecto de entrega. Las formulaciones farmacéuticas divulgadas pueden administrarse de acuerdo con todo régimen que comprende, por ejemplo, un régimen diario (1 vez por día, 2 veces por día, 3 veces por día, 4 veces por día, 5 veces por día, 6 veces por día), tres veces por semana, dos veces por semana, cada dos días, cada tres días, cada cuatro días, cada cinco días, cada seis días, semanalmente, cada dos semanas, cada tres semanas, mensualmente, una o dos veces por mes.

Combinaciones

Los análogos de glucagón que aquí se describen pueden administrarse solos o en combinación con otro agente terapéutico que esté destinado a tratar o prevenir cualquier enfermedad o afección médica que se describe en la presente. Por ejemplo, los análogos de glucagón que aquí se describen pueden co-administrarse con un agente anti- diabético o anti-obesidad (de manera simultánea o consecutiva). Los agentes anti- diabéticos conocidos en el arte o bajo investigación comprenden insulina, leptina, Péptido YY (PYY), Péptido pancreático (PP), Factor de crecimiento fibroblasto 21 (FGF21), agonista receptor Y2Y4, sulfonilureas, tales como tolbutamida (Orinase), acetohexamida (Dymelor), tolazamida (Tolinase), clorpropamida (Diabinese), glipizida (Glucotrol), gliburida

(Diabeta, Micronase, Glynase), glimepirida (Amaryl), o gliclazida (Diamicron); meglitinidas, tales como repaglinida (Prandin) o nateglinida (Starlix); biguanidas tales como metformina (Glucophage) o fenformina; tiazolidinadionas tales como rosiglitazona (Avandia), pioglitazona (Actos), o troglitazona (Rezulin), u otros inhibidores de PPARγ; inhibidores de alfa glucosidasa que inhiben la digestión de carbohidratos, tales como miglitol (Glyset), acarbosa (Precose/Glucobay); exenatida (Byetta) o pramlintida; inhibidores de Dipeptidil peptidasa-4 (DPP-4) tales como vildagliptina o sitagliptina; inhibidores de SGLT (transportador de glucosa que depende de sodio 1); activadores de glucocinasa (GKA); antagonistas de receptor de glucagón (GRA); o inhibidores de FBPasa (fructosa 1,6-bisfosfatasa).

Los agentes anti- obesidad conocidos en el arte o bajo investigación comprenden supresores del apetito, que incluyen estimulantes de tipo de fenetilamina, fentermina (opcionalmente con fenfluramina o dexfenfluramina), dietilpropion (Tenuate®), fendimetrazina (Prelu-2®, Bontril®), benzfetamina (Didrex®), sibutramina (Meridia®, Reductil®); rimonabant (Acomplia®), otros antagonistas de receptor de canabinoides; oxintomodulina; clorhidrato de fluoxetina (Prozac); Qnexa (topiramato y fentermina), Excalia (bupropión y zonisamida) o Contrave (bupropión y naltrexona); o inhibidores de lipasa,

similares a XENICAL (Orlistat) o Cetilistat (también conocido como ATL-962), o GT 389-255.

Los péptidos que se describen en algunas representaciones son co- administrados con un agente utilizado para el tratamiento de la enfermedad de higado graso no alcoholico o NASH. Entre los agentes utilizados para tratar la enfermedad de hígado graso no alcohólico se incluyen ácido ursodeoxicólico (a.k.a., Actigall, URSO, y Ursodiol), Metformina (Glucophage), rosiglitazona (Avandia), Clofibrato, Gemfibrozil, Polymixina B, y Betaina.

Los péptidos que se describen en algunas representaciones son co- administrados con un agente para el tratamiento de una enfermedad neurodegenerativa, por ejemplo, la enfermedad de Parkinson. Los agentes que actúan en contra de la enfermedad de Parkinson además son conocidos en el arte e incluyen pero no están limitados a levodopa, carbidopa, anticolinérgicos, bromocriptina, pramipexol, y ropinirol, amantadina, y rasagilina.

En vista de lo anteriormente expuesto, la invención además ofrece composiciones farmacéuticas y kits que también comprenden uno de estos otros agentes terapéuticos. El agente terapéutico adicional puede administrarse simultáneamente o de

manera consecutiva con el análogo de la presente divulgación. En algunos aspectos, el análogo se administra antes del agente terapéutico adicional, mientras que en otros aspectos, el análogo se administra después de un agente terapéutico adicional.

Usos

De acuerdo con la información aquí brindada por primera vez, se contempla que las composiciones (por ejemplo, las composiciones farmacéuticas relacionadas) de la presente divulgación son útiles en el tratamiento de una enfermedad o afección médica, en la que por ejemplo, la falta de actividad del receptor GIP, el receptor GLP-1 o ambos es un factor en la aparición y/o progresión de la enfermedad o afección médica. De este modo, la presente divulgación brinda un método para tratar o prevenir una enfermedad o afección médica en un paciente, en donde la enfermedad o afección médica presenta una falta de activación del receptor GIP y/o receptor GLP-1 que es asociada con la aparición y/o progresión de la enfermedad. En el método se brinda al paciente una composición o conjugado de acuerdo con todos aquellos descriptos en la presente, en una cantidad eficaz para el tratamiento o prevención de la enfermedad o afección médica.

En algunas representaciones, la enfermedad o afección médica es un síndrome metabólico. El Síndrome metabólico, también

conocido como Síndrome X, Síndrome de insulinorresistencia o Síndrome de Reaven es una enfermedad que afecta a más de 50 millones de norteamericanos. El Síndrome metabólico por lo general se caracteriza por la agrupación de por lo menos tres o más de los siguientes factores de riesgo: (1) obesidad abdominal (tejido adiposo excesivo en o alrededor del abdomen), (2) dislipidemia aterogénica (problema de grasa en sangre que incluye triglicéridos elevados, colesterol HDL bajo y colesterol LDL alto que aumenta la acumulación de placa en las paredes de las arterias), (3) presión sanguínea alta, (4) resistencia a la insulina o intolerancia a la glucosa, (5) estado protrombótico (por ejemplo, fibrinógeno alto o inhibidor del activador del plasminógeno-1 en sangre), y (6) estado proinflamatorio (por ejemplo, proteína C reactiva elevada en sangre). También se encuentran otros factores de riesgo como envejecimiento, desequilibrio hormonal y predisposición genética.

El síndrome metabólico se asocia con un incremento en el riesgo de enfermedad coronaria y otras afecciones relacionadas con la acumulación de placa vascular, tales como ataque cerebral y enfermedades vasculares periféricas, que se refieren como enfermedad cardiovascular aterosclerótica (ASCVD). Los pacientes que presentan Síndrome metabólico pueden avanzar desde un estado de resistencia a la insulina en la etapa

inicial a una diabetes de tipo II con un aumento de riesgo adicional de padecer ASCVD. Sin intención de asociarse a una teoría en particular, la relación entre la resistencia a la insulina, el Síndrome metabólico y la enfermedad vascular puede abarcar uno o más mecanismos patogénicos concurrentes que incluyen vasodilatación afectada e inducida por la insulina, reducción de la insulina asociada a la resistencia SIN disponibilidad debido al estrés oxidativo incrementado y anomalías en las hormonas derivadas de adiositos tal como adiponectina (Lteif y Mather, Can. J. Cardiol. 20 (apéndice. B):66B-76B (2004)).

De acuerdo con el panel para el tratamiento en adultos del Programa nacional de educación sobre el colesterol 2001 (ATP III), cualquiera de las tres siguientes características presentes en el mismo individuo cumplen con el criterio del Síndrome metabólico: (a) obesidad abdominal (con una circunferencia de la cintura mayor a 102 cm en los hombres y mayor a 88 cm en las mujeres); (b) triglicéridos en suero (150 mg/dl o más); (c) colesterol HDL (40 mg/dl o menos en los hombres y 50 mg/dl o menos en las mujeres); (d) presión sanguínea (130/85 o más); y (e) glucosa en sangre en ayunas (110 mg/dl o más). De acuerdo con la Organización Mundial de la Salud (OMS), un individuo que posee niveles de insulina altos (prueba de glucosa en ayunas elevada o glucosa elevada luego de una comida) con por lo menos dos de los siguientes

criterios cumple con el criterio del Síndrome metabólico: (a) obesidad abdominal (el radio de la cintura a la cadera mayor de 0,9, índice de masa corporal de por lo menos 30 kg/m2, o medida de la cintura superior a 94 cm); (b) un panel de colesterol que demuestra un nivel de triglicéridos de por lo menos 150 mg/dl o un nivel de colesterol HDL inferior a 35 mg/dl; (c) presión sanguínea de 140/90 o más, o en tratamiento por presión alta). (Mathur, Ruchi, “Metabolic Syndrome,” ed. Shiel, Jr., William C., MedicineNet.com, May 11, 2009).

Por motivos aquí descriptos, si un individuo cumple con el criterio de uno o ambos criterios expuestos por el panel para el tratamiento en adultos del Programa nacional de educación sobre el colesterol o la OMS, se considera a dicho individuo como afectado por el Síndrome metabólico.

Sin intención de asociarse a una teoría en particular, las composiciones y conjugados aquí descriptos son útiles para el tratamiento del Síndrome metabólico. De este modo, la invención brinda un método de prevención o tratamiento del Síndrome metabólico o la forma de reducir uno, dos, tres o más factores de riesgo del mismo en un sujeto, y comprende la posibilidad de brindar al sujeto una composición aquí descripta, en una cantidad efectiva, a fin de prevenir o tratar el Síndrome metabólico o los factores de riesgo del mismo.

En algunas representaciones, el método trata una condición médica hiperglucémica. En aspectos ejemplares, la condición

médica hiperglucémica es diabetes, diabetes melitus tipo I, diabetes melitus tipo II, diabetes gestacional, ya sea insulino dependiente o no insulino dependiente. En algunos aspectos, el método trata la condición médica hiperglucémica gracias a la reducción de uno o más complicaciones que presenta la diabetes que incluye nefropatía, retinopatía y enfermedad vascular.
En algunos aspectos, la enfermedad o afección médica es obesidad. En algunos aspectos, la obesidad es inducida por fármacos. En algunos aspectos, el método trata a la obesidad gracias a la prevención o reducción del peso adquirido o a través del aumento de pérdida de peso en el paciente. En algunos aspectos, el método trata a la obesidad gracias a la reducción del apetito, disminución de la ingesta de alimentos, disminución de los niveles de grasa en pacientes o reducción del rango de movimiento de la comida a través del sistema gastrointestinal.

Debido a que la obesidad está asociada a la aparición o progresión de otras enfermedades, los métodos de tratamiento de la obesidad son útiles en métodos para reducir aquellas complicaciones relacionadas con la obesidad, que incluyen la enfermedad vascular (enfermedad de la arteria coronaria, ataque cerebral, enfermedad vascular periférica, reperfusión isquémica, etc.), hipertensión, aparición de diabetes tipo II, hiperlipidemia y enfermedades musculoesqueletal. De este modo,

la presente divulgación otorga métodos de tratamiento o prevención de estas complicaciones asociadas a la obesidad.

En algunas representaciones, la enfermedad o afección médica es ***Enfermedad*** hepática grasa no alcohólica (NAFLD). Las siglas NAFLD hacen referencia a un amplio espectro de enfermedades del hígado que oscilan desde hígado graso simple (esteatosis) hasta esteatohepatitis no alcohólica (NASH), hasta cirrosis (cicatrización irreversible y avanzada del hígado). Todas las etapas de NAFLD tienen en común la acumulación de grasa (infiltración adiposa) en las células del hígado (hepatocitos). El hígado graso simple es la acumulación anormal de un cierto tipo de grasa, triglicéridos, en las células del hígado sin presentar inflamación o cicatrices. En NASH, la acumulación de grasa está asociada con distintos niveles de inflamación (hepatitis) y cicatrización (fibrosis) del hígado. Las células inflamatorias pueden destruir las células del hígado (necrosis hepatocelular). En los términos "esteatohepatitis" y "esteatonecrosis", esteato se refiere a la infiltración grasa, hepatitis se refiere a la inflamación en el hígado, y necrosis hace hincapié en las células del hígado destruidas. NASH finalmente puede conducir a la cicatrización del hígado (fibrosis) y luego a la cicatrización irreversible y avanzada (cirrosis). La cirrosis que es causada por NASH es la última etapa y la más severa dentro del espectro NAFLD. (Mendler, Michel, “Fatty Liver: Nonalcoholic Fatty Liver Disease (NAFLD)

and Nonalcoholic Steatohepatitis (NASH),” ed. Schoenfield, Leslie J., MedicineNet.com, 29 de agosto, 2005).

La enfermedad de hígado alcohólico o la enfermedad de hígado inducido por alcohol comprende tres enfermedades de hígado patológicamente distintas que están relacionadas o producidas por el consumo excesivo de alcohol: hígado graso (esteatosis), hepatitis aguda o crónica y cirrosis. La hepatitis alcohólica puede variar desde una hepatitis moderada, con pruebas de laboratorio anormales que son las únicas indicaciones de la enfermedad, a disfunción hepática severa con complicaciones tales como ictericia (pigmento amarillo causado por retención de la bilirrubina), encefalopatía hepática (disfunción neurológica causada por insuficiencia hepática), ascitis (acumulación de fluidos en el abdomen), várices esofágicas sangrantes (várices en el esófago), coagulación sanguínea anormal y coma. Histológicamente, la hepatitis alcohólica tiene una apariencia característica con la degeneración de balonaje de hepatocitos, inflamación con neutrófilos y a veces cuerpos de Mallory (agregaciones anormales de proteínas de filamentos intermedios celulares). La cirrosis se caracteriza, de forma anatómica, gracias a los nódulos generalizados en el hígado que están combinados con fibrosis. (Worman, Howard J., “Alcoholic Liver Disease”, Columbia University Medical Center website).

Sin intención de asociarse a una teoría en particular, las composiciones y conjugados que aquí se describen son útiles

para el tratamiento de la enfermedad de hígado alcohólico, NAFLD, o cualquier etapa de la misma, que incluye, por ejemplo, esteatosis, esteatohepatitis, hepatitis, inflamación hepática, NASH, cirrosis o complicaciones de las mismas. En consecuencia, la presente divulgación brinda un método para prevenir o tratar la enfermedad de hígado alcohólico, NAFLD o cualquier etapa de la misma, en un sujeto y esto consiste en proporcional al sujeto una composición aquí descripta, en una cantidad efectiva para prevenir o tratar la enfermedad de hígado alcohólico, NAFLD, o una etapa de la misma. Dichos métodos de tratamiento incluyen uno, dos, tres o más de lo siguiente: contenido graso en el hígado, incidencia o progresión de la cirrosis, incidencia de carcinoma hepatocelular, signos de inflamación, por ejemplo, niveles de enzimas hepáticas anormales (por ejemplo, aspartato aminotransaminasa (AST) y/o alanina aminotransferasa ALT, o LDH), ferritina en suero elevada, bilirrubina en suero elevada, y/o signos de fibrosis, por ejemplo, niveles de TGF-beta elevados. En una representación ejemplar, las composiciones se utilizan para tratar a pacientes que han superado una afección como hígado graso simple (esteatosis) y presentan signos de inflamación o hepatitis. Dichos métodos pueden tener como resultado, por ejemplo, una reducción de los niveles de AST y/o ALT.

GLP-1 y exendina-4 demostraron tener un efecto neuroprotector. La presente divulgación también proporciona los usos de las

composiciones aquí descriptas en el momento de tratar enfermedades, que incluyen pero no están limitadas a la Enfermedad de Alzheimer, la Enfermedad de Parkinson, Esclerosis Múltiple, Esclerosis Lateral Amiotrófica, otros desórdenes relacionados con demielinación, demencia senil, demencia subcortical, demencia arterosclerótica, demencia asociada al VIH, u otras demencias, un cáncer en el sistema nervioso central, lesión traumática en el cerebro, lesión en la médula espinal, ataque o isquemia cerebral, vasculitis cerebral, epilepsia, enfermedad de Huntington, Síndrome de Tourette, Síndrome de Guillain Barre, Enfermedad de Wilson, Enfermedad de Pick, desórdenes neuroinflamatorios, encefalitis, encefalomielitis o meningitis de origen viral, micótico o bacterial, u otras infecciones en el sistema nervioso central, enfermedad priónicas, ataxias cerebelar, degeneración cerebelar, síndromes de degeneración espinocerebelar, ataxia de Friedreichs, ataxia telangiectasia, dismiotrofia espinal, Parálisis supranuclear progresiva, distonia, espasticidad muscular, tremor, retinitis pigmentosa, degeneración estriatonigral, encelofalo-miopatías mitochondrial, ***lipofuscinosis ceroides neuronales***, encefalopatías hepáticas, encefalopatías renales, encefalopatías metabólicas, encefalopatías inducidas por toxinas, y daño cerebral inducido por radiación.

En algunas representaciones, las composiciones se utilizan en conjunción con la administración parenteral de nutrientes a pacientes que no presentan diabetes, en un hospital, por ejemplo, a pacientes que reciben nutrición parenteral o nutrición parenteral total. Entre los ejemplos que no son restrictivos se encuentran los pacientes de cirugía, pacientes en coma, pacientes con enfermedades en el tracto digestivo o que presentan un tracto gastrointestinal no funcional (por ejemplo, por extracción quirúrgica, capacidad de absorción afectada o bloqueo, Enfermedad de Crohn, colitis ulcerativa, obstrucción en el tracto gastrointestinal, fístula en el tracto gastrointestinal, pancreatitis aguda, intestino isquémico, cirugía mayor del tracto gastrointestinal, ciertas anomalías del tracto gastrointestinal congénitas, diarrea prolongada o síndrome de intestino corto debido a una cirugía), pacientes en shock y pacientes bajo un proceso de curación que a menudo reciben una administración parenteral de carbohidratos junto con varias combinaciones de lípidos, electrolitos, minerales, vitaminas y amino ácidos. Las composiciones que comprenden el péptido agonista GIP y el péptido antagonista glucagón, como aquí se describen, y la composición de nutrición parenteral pueden administrarse al mismo tiempo, en momentos distintos, antes o después de cada uno, siempre que la composición logre el efecto biológico deseado en el momento en el que la composición de nutrición parenteral sea asimilada. Por ejemplo,

la nutrición parenteral puede administrarse 1, 2 o 3 veces por día, mientras que la composición se administre una vez día por medio, tres veces por semana, dos veces por semana, una vez por semana, una vez cada dos semanas, una vez cada tres semanas y una vez al mes.

Como se utiliza en la presente, el término “tratamiento” incluye la profilaxis de la condición o desorden específico o el alivio de los síntomas que están asociados con una condición o desorden específico y/o la prevención o eliminación de dichos síntomas. Por ejemplo, como se utiliza en la presente, el término “diabetes”, en general, se refiere a la alteración de los niveles de glucosa en sangre comparada con niveles normales y puede incluir el aumento o disminución de los niveles de glucosa en sangre dependiendo de la situación determinada.

Como se utiliza en la presente, los términos cantidad “efectiva” o “una cantidad terapéuticamente efectiva” de un péptido de glucagón se refiere a una cantidad no tóxica y suficiente de péptido para obtener el efecto deseado. Por ejemplo, un efecto deseado sería la prevención o tratamiento de hipoglucecemia, que se mediría, por ejemplo, a través de un aumento en el nivel de glucosa en sangre. Un efecto deseado y alternativo del péptido de glucagón de la presente divulgación incluiría el tratamiento de hiperglucemia, por ejemplo, que se mediría a través de un cambio en el nivel de glucosa en sangre cercano al normal, o inducción de la pérdida de peso o

prevención de la obtención de peso, por ejemplo, como se mide a través de la reducción del peso corporal, o prevención o reducción del aumento del peso corporal, o normalización de la distribución de la grasa corporal. La cantidad que se considera "eficaz" variará en cada sujeto, dependiendo de la edad y las condiciones generales del individuo, forma de administración, etc. Por lo tanto, no siempre es posible especificar una "cantidad efectiva" exacta. Sin embargo, una cantidad "efectiva" adecuada, en un caso particular, puede estar determinada por alguien con conocimiento en el arte que utilice una experimentación de rutina.

SUJETO

Con respecto a los métodos de tratamiento anteriormente mencionados, el paciente es cualquier huésped. En algunas representaciones, el huésped es un mamífero. Como se utiliza en la presente, el término "mamífero" se refiere a cualquier animal vertebrado de la clase de los mamíferos, que además incluye, pero no está limitado a cualquier monotrema, marsupial y taxas placentario. En algunas representaciones, el mamífero es uno de los mamíferos del orden de Rodentia, como ratones o hámsters y mamíferos del orden de Lagomorfos, como conejos. En representaciones ejemplares, los mamíferos son del orden de los Carnívoros, que incluyen Felinos (gatos) y Caninos (perros). En representaciones ejemplares, los mamíferos son del

orden de Artiodáctilos, que incluyen Bovinos (vacas) y Porcinos (cerdos) o del orden de Perisodáctilos, que incluyen Equinos (caballos). En algunos ejemplos, los mamíferos son del orden de los Primates, Cébidos, o Simios (monos) o del orden de los Antropoide (humanos y simios). En representaciones particulares, el mamífero es humano.

KITS

Los análogos de glucagón de la presente divulgación se pueden brindar de acuerdo con una representación como parte de un kit. En consecuencia, en algunas representaciones, se brinda un kit para administrar un análogo de glucagón al paciente que lo necesita, en donde el kit incluye un análogo de glucagón como se describe en la presente.

En una representación, el kit se brinda con un dispositivo que administra el análogo de glucagón al sujeto. Este dispositivo, en algunos aspectos, es una aguja de una jeringa, un dispositivo de una lapicera, un inyector de tinta o cualquier otro inyector sin aguja. El kit puede incluir adicional o alternativamente uno o más recipientes, por ejemplo, ampollas, tubos, botellas, jeringas solas o pre- rellenadas, cartuchos, bombas de infusión (externas o implantables), inyectores de tinta, dispositivos pre- llenados, etc. Como opción, pueden contener un análogo de glucagón en una forma liofilizada o en una solución acuosa. El kit en algunas representaciones incluye

las instrucciones de uso. De acuerdo con una representación, el dispositivo del kit es un dispositivo que expende aerosol, en donde la composición se envasa previamente en un dispositivo en aerosol. En otra representación, el kit comprende una jeringa y una aguja, y en otra representación, la composición de glucagón estéril se envasa previamente en una jeringa.

Los kits en algunas representaciones incluyen las instrucciones de uso. Estas en algunos aspectos incluyen instrucciones para su uso de acuerdo con todos los métodos aquí descriptos. Además, estas pueden comprender instrucciones para mantener una dieta saludable y/o una rutina de ejercicios físicos. Las instrucciones pueden presentarse como folleto o en forma electrónica, por ejemplo, un dispositivo de almacenamiento que incluya las instrucciones.

Los siguientes ejemplos se brindan simplemente para ilustrar la presente invención y, de ninguna manera, limitar su alcance.

EJEMPLO

EJEMPLO 1

A continuación se brindan métodos ejemplares de la síntesis de los análogos de péptidos incluidos en las representaciones.

Síntesis de fragmentos de péptidos del glucagón

Materiales:

Todos los péptidos descriptos en los EJEMPLOS fueron amidados a menos que se especifique lo contrario.

Durante la síntesis de péptidos se utilizó la resina MBHA, resina de 4-metilbenzhidrilamina en base de poliestireno. Se adquirieron de Midwest Biotech la resina MBHA, 100-180 mesh, poliestireno reticulado con 1% DVB; carga de 0,7-1,0 mmol/g), amino ácidos protegidos por BOC y Fmoc. Se desarrolló la síntesis de péptidos de la fase sólida con aminoácidos protegidos por BOC con un sintetizador de péptidos Applied Biosystems 430A. Se llevó a cabo la síntesis de amino ácidos protegidos por Fmoc con a un sintetizador de péptido Applied Biosystems Modelo 433.

Síntesis de péptidos (Decomposición de HF/ Aminoácidos Boc):
La síntesis de estos análogos se desarrolló en un sintetizador de péptidos Applied Biosystems modelo 430A. Los péptidos sintéticos se elaboraron a través de la adición secuencial de aminoácidos en un cartucho que contiene 2 mmol de aminoácidos protegidos por Boc. Específicamente, en el desarrollo de la síntesis se utilizaron acoplamientos simples de DEPBT activados. Al finalizar la etapa de acoplamiento, la resina de peptidilo se trató con TFA para remover el grupo de protección Boc en el extremo N. Se lavó varias veces con dimetilfornamida (DMF) y este ciclo repetitivo se repitió por el número deseado

de etapas de acoplamiento. Luego del montaje, la protección de la cadena lateral, Fmoc, se eliminó con un 20% de tratamiento de piperidina y se realizó la acilación utilizando DIC. Al finalizar toda la síntesis, la resina de peptidilo se secó utilizando diclometanos (DMC), y se dividió el péptido de la resina con un anhídrido HF.

Para la lactamización, los grupos de protección ortogonal se seleccionaron para Glu y Lys (Por ejemplo, Glu (Fm), Lys (Fmoc)). Luego de extraer los grupos protectores y antes de la decomposición de HF, se realizó la ciclización como se describe anteriormente. (Véase, por ejemplo, Solicitud Internacional de Patente, publicación N° WO2008/101017).

Tratamiento HF de la resina de peptidilo

La resina de peptidilo se trató con fluoruro de hidrógeno anhídrido (HF) y esto, en general, presentó aproximadamente 350 mg (~50% rendimiento) del péptido desprotegido crudo. Específicamente, la resina de peptidilo (30mg a 200mg) se colocó en el recipiente con reacción HF para producir la descomposición. Al recipiente se agregó 500 µL of p-cresol como un depurador de ion de carbono. Se montó el recipiente al sistema HF y se sumergió en la mezcla de hielo seco/ metanol. Se evacuó el recipiente con una bomba de vacío y se extrajeron 10 ml de HF. Se revolvió esta mezcla de reacción de la resina de peptidilo y el HF durante una hora a 0° C, luego se

estableció el vacio y HF se evacuó rápidamente (10-15 min). Se removió cuidadosamente el recipiente y se llenó con aproximadamente 35 ml de éter para precipitar el péptido y extraer el p-cresol y los grupos orgánicos de moléculas pequeñas que se obtuvieron del tratamiento HF. Se filtró la mezcla mediante un filtro de teflón y se repitió dos veces para remover cualquier exceso de cresol. Se desechó este líquido filtrado. El péptido precipitado se disolvió en aproximadamente 20 ml de un 10% ácido acético (aq). Se recolectó y liofilizó este líquido filtrado, que contiene el péptido deseado.

Se realizó un análisis de HPLC analítica del péptido solubilizado en las siguientes condiciones [4,6 X 30 mm Xterra C8, 1,50 mL/minuto, 220 nm, tampón A 0,1% ácido trifluoroacético (TFA)/ 10% acrilonitrilo (CAN), tampón B buffer 0,1% TFA/100% ACN, gradiente 5-95%B durante 15 minutos]. El extracto se diluyó dos veces con agua y se cargó en una columna de fase invertida preparativa de C4 Vydac de 2,2 X 25 cm y se eluyó usando un gradiente de acetonitrilo sobre un sistema de HPLC de Waters (tampón A de 0,1% TFA/10% ACN, tampón B de 0,1% TFA/10% ACN y un gradiente de 0-100% B durante 120 minutos a un flujo de 15,00 ml/minuto. El análisis de HPLC del péptido purificado demostró más del 95% de pureza y el análisis de espectro de masa por ionización de electrospray confirmó la identidad del péptido.

Acilación de Péptidos

Se prepararon péptidos acilados de la siguiente manera. Se sintetizaron péptidos sobre una resina de soporte sólida usando un Sintetizador de Péptidos CS Bio 4886 o un Sintetizador de Péptidos Applied Biosystems 430A. Se usó la química de neutralización in situ que se describió en Schnolzer et al., *Int. J. Peptide Protein Res.* 40: 180-193 (1992). Para los péptidos acilados, el residuo de aminoácido objetivo que se debe acilar (por ejemplo, la posición 10, relativa al número de posición del aminoácido SEQ ID N°: 3) se sustituyó con un residuo de N ε -FMOC lisina. El tratamiento del péptido protegido por BOC en el extremo de N con 20% de piperidina en DMF durante 30 minutos para extraer grupos FMOC/formilo. El acoplamiento al residuo de ε-amino Lys se logró acoplando un exceso del aminoácido de separador protegido por FMOC (ex. FMOC-Glu-Otbu) o cadena de acilo (ex. $CH_3(CH_2)_{14}$-COOH) y PyBOP o reactivo de acoplamiento de DEPBT en DMF/*N,N*-diisopropiletilamina /DIEA. La eliminación posterior del grupo FMOC del aminoácido está seguida por la repetición del acoplamiento con una cadena de acilo. El tratamiento final con 100% TFA derivó en la eliminación de todos los grupos protectores de cadena lateral y el grupo BOC del extremo de N. Las resinas de péptidos se hicieron neutras con 5% DIEA/DMF, se secaron y luego se descompusieron desde el soporte usando HF/p-cresol, 95:5, a 0°C durante una hora. Después de la extracción

con éter, se usó 5% solución de HOAc para el solvato del péptido crudo. Una muestra de la solución se verificó luego que contuviera el péptido de peso molecular correcto mediante ESI-MS. Los péptidos correctos se purificaron mediante RP-HPLC usando un gradiente lineal de 10% acetonitrilo (CH_3CN) /0,1% TFA a 0,1% TFA en 100% CH_3CN. Se usó una columna de proteína de C18 22 mm x 250 mm para la purificación. Los análogos de péptidos acilados generalmente terminaron la elución por una relación de tampón de 20:80. Las partes se mezclaron juntas y se controló la pureza sobre una RP-HPLC analítica. Las fracciones puras se liofilizaron y dieron péptidos sólidos, blancos.

Si un péptido comprende un puente de lactama y residuos objetivos que se deben acilar, la acilación se lleva a cabo como se describió anteriormente al agregar ese aminoácido a la cadena principal del péptido.

Se prepararon acilaciones duales o di-acilaciones de la siguiente manera. Se sintetizaron péptidos sobre una resina de soporte sólida usando un Sintetizador de Péptidos CS Bio 4886 o un Sintetizador de Péptidos Applied Biosystems 430A. Se usó la química de neutralización in situ que se describió en Schnolzer et al., *Int. J. Peptide Protein Res.* 40: 180-193 (1992). Para los péptidos acilados dobles, el residuo de aminoácido blanco que se debe acilar (por ejemplo, la posición 10 y 40, relativa

al número de posición del aminoácido SEQ ID N°: 3) se sustituyó con un residuo de N ε –FMOC lisina. El tratamiento del péptido protegido por BOC en el extremo de N con 20% de piperidina en DMF durante 30 minutos para extraer grupos FMOC/formilo. El acoplamiento al residuo de ε–amino Lys se logró acoplando el exceso del aminoácido de separador protegido por FMOC (ex. FMOC–Glu–OtBu) o cadena de acilo (ex. $CH_3(CH_2)_{14}$–COOH) y PyBOP o reactivo de acoplamiento de DEPBT en DMF/DIEA. La eliminación posterior del grupo FMOC del aminoácido está seguida por la repetición del acoplamiento con una cadena de acilo. El tratamiento final con 100% TFA derivó en la eliminación de todos los grupos protectores de cadena lateral y el grupo BOC del extremo de N. Las resinas de péptidos se hicieron neutras con 5% DIEA/DMF, se secaron y luego se descompusieron desde el soporte usando HF/p–cresol, 95:5, a 0°C durante una hora. Después de la extracción con éter, se usó 5% solución de HOAc para el solvato del péptido crudo. Una muestra de la solución se verificó luego que contuviera el péptido de peso molecular correcto mediante ESI–MS. Los péptidos correctos se purificaron mediante RP–HPLC usando un gradiente lineal de 10% CH_3CN/0,1% TFA a 0,1% TFA en 100% CH_3CN. Se usó una columna de proteína de C18 22 mm x 250 mm para la purificación. Los análogos de péptidos acilados generalmente terminaron la elución por una relación de tampón de 20:80. Las partes se mezclaron juntas y se controló la pureza sobre una RP–HPLC analítica. Las

fracciones puras se liofilizaron y dieron péptidos sólidos, blancos.

Se prepararon acilaciones dobles ramificadas en un sitio de la siguiente manera. Se sintetizaron péptidos sobre una resina de soporte sólida usando un Sintetizador de Péptidos CS Bio 4886 o un Sintetizador de Péptidos Applied Biosystems 430A. Se usó la química de neutralización in situ que se describió en Schnolzer et al., *Int. J. Peptide Protein Res.* 40: 180-193 (1992). Para los péptidos acilados, el residuo de aminoácido objetivo que se debe acilar (por ejemplo, la posición 10, relativa al número de posición del aminoácido SEQ ID N°: 3) se sustituyó con un residuo de N ε -FMOC lisina. El tratamiento del péptido protegido por BOC en el extremo de N con 20% de piperidina en DMF durante 30 minutos para extraer grupos FMOC/formilo. El acoplamiento al residuo Lys a través del extremo de C se logró acoplando el exceso de aminoácidos de separador protegido por FMOC del grupo ε-amino y α-amino (ej. FMOC-Lys(FMOC)-OH) y PyBOP o reactivo de acoplamiento de DEPBT en DMF/DIEA. La eliminación posterior del grupo FMOC del aminoácido está seguida por el acoplamiento de cada grupo ε-amino y α-amino con una cadena de acilo. El tratamiento final con 100% TFA derivó en la eliminación de todos los grupos protectores de cadena lateral y el grupo BOC del extremo de N. Las resinas de

péptidos se hicieron neutras con 5% DIEA/DMF, se secaron y luego se descompusieron desde el soporte usando HF/p-cresol, 95:5, a 0°C durante una hora. Después de la extracción con éter, se usó 5% solución de HOAc para el solvato del péptido crudo. Una muestra de la solución se verificó luego que contuviera el péptido de peso molecular correcto mediante ESI-MS. Los péptidos correctos se purificaron mediante RP-HPLC usando un gradiente lineal de 10% CH_3CN/0,1% TFA a 0,1% TFA en 100% CH_3CN. Se usó una columna de proteína de C18 22 mm x 250 mm para la purificación. Los análogos de péptidos acilados generalmente terminaron la elución por una relación de tampón de 20:80. Las partes se mezclaron juntas y se controló la pureza sobre una RP-HPLC analítica. Las fracciones puras se liofilizaron y dieron péptidos sólidos, blancos.

Se prepararon acilaciones dobles, lineales en un sitio de la siguiente manera. Se sintetizaron péptidos sobre una resina de soporte sólida usando un Sintetizador de Péptidos CS Bio 4886 o un Sintetizador de Péptidos Applied Biosystems 430A. Se usó la química de neutralización in situ que se describió en Schnolzer et al., *Int. J. Peptide Protein Res.* 40: 180-193 (1992). Para los péptidos acilados, el residuo de aminoácido objetivo que se debe acilar (por ejemplo, la posición 10, relativa al número de posición del aminoácido SEQ ID N°: 3) se sustituyó con un residuo de N ε -FMOC lisina. El tratamiento del péptido protegido por BOC en el extremo de N con 20% de piperidina en

DMF durante 30 minutos para extraer grupos FMOC/formilo. El acoplamiento al residuo libre Lys ε-amino se logró acoplando el exceso de aminoácidos de separador protegido por FMOC (ej. FMOC-Glu-OtBu) o uma cadena de acilo (ej. $CH_3(CH_2)_{14}$-COOH) y PyBOP o reactivo de acoplamiento de DEPBT en DMF/DIEA. La eliminación posterior del grupo FMOC del aminoácido está seguida por el acoplamiento de la cadena de acilo. La eliminación posterior del grupo FMOC del aminoácido está seguida por el acoplamiento de una cadena de acilo funcionalizada al final de la cola del ácido graso con un aminoácido protegido como FmocNH-$(CH_2)_{11}$-COOH. El aminoácido acilado simple que deriva se trató con un 20% de piperidina en DMF para extraer al grupo protegido por FMOC, seguido de un acoplamiento con la cadena de acilo. El tratamiento final con 100% TFA derivó en la eliminación de todos los grupos protectores de cadena lateral y el grupo BOC del extremo de N. Las resinas de péptidos se hicieron neutras con 5% DIEA/DMF, se secaron y luego se descompusieron desde el soporte usando HF/p-cresol, 95:5, a 0°C durante una hora. Después de la extracción con éter, se usó 5% solución de HOAc para el solvato del péptido crudo. Una muestra de la solución se verificó luego que contuviera el péptido de peso molecular correcto mediante ESI-MS. Los péptidos correctos se purificaron mediante RP-HPLC usando un gradiente lineal de 10% CH_3CN/0,1% TFA a 0,1% TFA en 100% CH_3CN. Se usó una columna de proteína de C18 22 mm x 250

mm para la purificación. Los análogos de péptidos acilados generalmente terminaron la elución por una relación de tampón de 20:80. Las partes se mezclaron juntas y se controló la pureza sobre una RP-HPLC analítica. Las fracciones puras se liofilizaron y dieron péptidos sólidos, blancos.

En los ejemplos de las acilaciones dobles en un sitio o dos, las dos cadenas de acilo que se van a acoplar al péptido pueden ser iguales o diferentes. En el caso de dos cadenas de acilo distintas, los aminoácidos objetivo que se van a acilar son sustituidas con dos grupos de protección diferentes. Por ejemplo, un residuo de lisina N ε -FMOC y un residuo de lisina N ε -ivDde (acilación doble en dos sitios) o un FMOC Lys (ivDde)-OH (acilación doble en un sitios, 852082 Novabiochem). El tratamiento del péptido protegido por BOC del extremo de N con 20% de piperidina en DMF durante 30 minutos para extraer al grupo FMOC/ formilo. El residuo libre ε-amino Lys se acopló con un grupo de acilo o un aminoácido de separador y continúo un acoplamiento a un grupo de acilo. El péptido acildo derivado se trata con un 2% de hidracina/ DMF para extraer al grupo de protección (ivDde).

El residuo libre ε-amino Lys se acopló con un grupo de acilo o un aminoácido de separador y continúo una acoplación a un grupo de acilo. El Péptido acilado doble se aísla como se describió anteriormente.

A continuación se describe una síntesis de ejemplo del péptido acilado dual (Glucagón Aib2 E16 A18 L27 D28 Cex K40(C16γE-K-γEC12)G41-amida, que posee la secuencia de aminoácido SEQ ID NO: 211). Además, esto se demuestra en la Figura 5B.

Se colocaron 0,28gm (0,2mmolar) de resina mbha (Midwest Biotech) en un recipiente de reacción y se montó la siguiente secuencia en un sintetizador CSBio336, con acoplamientos simples activados por DEPBT/DIEA de aminoácidos BOC.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha resina

La resina del péptido protegido por BOC se transfirió a un recipiente de reacción manual y se trató con 20% de piperidina en DMF a temperatura ambiente, durante 10 minutos. Se filtró la resina, se lavó tres veces con DMF y se agregó una solución activada de Fmoc Lys(ivDde)-OH (EMD-Novabiochem)(que anteriormente se preparó mediante la disolución de 2mmolar Fmoc Lys(ivDde) en 4,0ml 0,5M DEPBT/DMF y se agregó 0,35ml (2mmolar) DIEA.

La resina del péptido se mezcló a temperatura ambiente durante 16 horas, luego se filtró, se lavó con DMF y se trató con 20% de piperidina en DMF durante 10 minutos. La resina se filtró, se lavó varias veces con DMF y se agregó una solución activada

de Fmoc Glu-OBzl (se preparó mediante la disolución de 2mmolar Fmoc Glu-OBzl en 4,0ml 0,5M DEPBT/DMF y se agregó 0,35ml DIEA). Se mezcló la reacción durante 1 hora a temperatura ambiente, luego se filtró y se lavó con DMF. Luego, se realizó otro tratamiento con 20% pip/DMF, se lavó la resina varias veces con DMF y se agregó una solución activada de ácido palmítico para completar la acilación de α-amine de la cadena lateral Lys en la posición 40 (se preparó anteriormente mediante la disolución de 2mmolar de ácido palmítico en 4.0ml 0.5M DEPBT/DMF y se agregó 0,35ml DIEA). La reacción se mezcló durante 1 hora.

La resina de péptido se filtró, se lavó con DMF y se trató con 2% hidracina/DMF a temperatura ambiente durante 15 minutos, luego se filtró y se lavó con DMF varias veces. Se agregó una solución activada de Fmoc Glu-OBzl (igual que se mencionó anteriormente) y se mezcló la reacción durante 1 hora a temperatura ambiente. La resina se dividió en tres partes para realizar la acilación final en el amino ε-lysine. En este caso, se agregó una porción de una solución activada de ácido dodecanoico. Se preparó anteriormente mediante la disolución de 1mmolar de ácido dodecanoico (Aldrich) en 2,0ml 0,5M DEPBT/DMF y se agregó 0,175ml DIEA. La reacción se mezcló durante 1 hora, se filtró, se lavó con DMF y luego con diclorometano. Se extrajo el grupo Boc en el extremo N con 50%TFA/DCM, y luego de la neutralización con 5%DIEA/DCM, una decomposición de HF se

colocó en un baño de hielo durante 1 hora mediante p-cresol como depurador. Luego de la evaporación de HF, se suspendió el residuo en etil éster y la mezcla resina/ péptido se filtro y lavó con éster. Se extrajo el péptido en un ácido acético acuoso y se analizó sobre HPLC. (4,6x50mm Zorbax SB-C8, 1ml/min, 45˚C, 214nm (0,5A) A=0,1%TFA, B=0,1%TFA/90%ACN. El extracto de decomposición restante se cargó en una columna de Amberchrom XT20 de 21,2 x 250mm y un gradiente de 0,1%TFA/acetonitrilo se corrió sobre un FPLC de Pharmacia para su purificación.

Las fracciones 83-86 se combinaron, se congelaron y liofilizaron para obtener 22,3mg del péptido de la secuencia SEQ ID NO: 211 con una pureza del 90%+. En forma teórica, mol.wt.= 5202.9, masa observada ESI = 5202.0

Acilación del *Péptido a través de la Succinoilación*

Los péptidos succinoilados se preparan de la siguiente manera. Los péptidos se sintetizan sobre una resina de apoyo sólido con un sintetizador de péptidos CS Bio 4886 o Applied Biosystems 430A. Se usó la química de neutralización in situ que se describió en Schnolzer et al., *Int. J. Peptide Protein Res.* 40: 180-193 (1992). Para los péptidos succinoilados, el residuo de aminoácido objetivo que se debe acilar (por ejemplo, la posición 10, relativa al número de posición del aminoácido SEQ

ID N°: 3) se sustituyó con un residuo de N ε -FMOC lisina. El tratamiento del péptido protegido por BOC en el extremo de N con 20% de piperidina en DMF durante 10 minutos extrajo a los grupos FMOC/formilo. La resina se filtró, se lavó con DMF/DCM y se re suspendió en DCM. El acoplamiento al residuo libre de ε-amino Lys se logró acoplando el exceso del n-hexadecilsucciníco anídrido (TCl) junto con 4-dimetilaminopiridina. La resina se mezcló durante la noche, se filtró, se lavó con DCM y se trató con un 50% TFA/DCM para extraer todo grupo de protección de la cadena lateral y los grupos BOC en el extremo N. Las resinas del péptido se neutralizaron con un 5% DIEA/DMF, se secaron y luego se abrieron del soporte utilizando HF/p-cresol, 95:5, a 0°C durante una hora. A continuación de la extracción del éster, se utilizó una solución de 5% HOAc para disolver el péptido crudo. Una muestra de la solución se verifica posteriormente a través del análisis HPLC. La solución de ácido acético que resulta se carga en una columna de Amberchrom XT20 de 10x250mm para su purificación. Así corre un gradiente acuoso TFA/ACN mientras se recolecta la fracción y se monitorea el UV a 214nm. Las fracciones puras son liofilizadas y producen péptidos sólidos.

A continuación se describe un ejemplo de la síntesis del péptido succinilado (Glucagón Aib2 E16 A18 L27 D28 Cex K40(C16

succinoil)G41-amida, con una secuencia de aminoácido SEQ ID NO: 156). Además, esto se demuestra en la Figura 3C.

Se colocaron 0,28gm (0,2mmolar) de resina mbha (Midwest Biotech) en un recipiente de reacción y se montó la siguiente secuencia en un sintetizador CSBio336 utilizando DEPBT/DIEA, con acoplamientos simples.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha resina

Aproximadamente, un tercio de la resina del péptido protegido por BOC se transfirió a un recipiente de reacción manual y se trató con 20% de piperidina en DMF a temperatura ambiente, durante 10 minutos. Se filtró la resina, se lavó tres veces con DMF, dos veces con diclorometano y se re suspendió en 10ml de DCM. Se agregó 324mg (1mmolar) n-hexadecilsuccinicoanídrido (TCI) y 2-3mg de 4-dimetilaminopiridina (Aldrich).

Se mezcló la resina durante la noche a temperatura ambiente antes del filtrado, se efectuó un doble lavado con DCM y se trató con 50% de ácido trifluoroacético (TFA) en cloruro de metileno (DCM) durante 1 a 2 minutos. Se filtró la resina, se efectuaron lavados múltiples con DCM, se realizó la neutralización mediante el lavado con 5% de N,N-diisopropiletilamina (DIEA) en DCM y se transfirió al recipiente de reacción de HF. Se corrió una decomposición de HF utilizando 5 ml de fluoruro de

hidrógeno líquido y 0,5 ml de un depurador p-cresol. Después de agitar durante 1 hora en un baño de hielo, el HF se removió en vacío y el residuo se suspendió en eter etílico. Se filtró la suspensión con un embudo de vidrio sinterizado, los sólidos se lavaron con éter y el péptido se extrajo dentro de 15 ml de ácido acético acuoso al 50%. Después del análisis por HPLC (4,6x50mm Zorbax SB-C8, 1ml/min, 45˚C,214nm, A=0,1%TFA, B=0,1%TFA/90%ACN, usando un gradiente de 30%B a 90%B durante 10 minutos), el extracto de la decomposición se cargó en una columna Amberchrom de 21,2 x 250 mm para su purificación. Se corrió un gradiente de TFA acuoso en acetonitrilo mientras se recolectaron las fracciones y se monitoreó la absorvancia de UV. Se identificaron las fracciones 55-56 como fracciones de componente único, se congelaron y liofilizaron. Se recuperaron 32,5 mg con una pureza de 90%+ (DLS-027-68B) Peso molecular teórico = 4720,3, masa observada por ionización por electrospray (ESI) = 4717,0.

Alquilación del Péptido (p.ej., S-alquilación)

A continuación se describe y en la Figura 6 se muestra un ejemplo de la síntesis de una amida de péptido (Glucagon Aib2 E16 A18 L27 D29 Cex Cys40(S-2 palmitil) alquilado (p. ej. S-alquilado) que posee una secuencia de aminoácidos de SEQ ID NO 164:

se colocaron 0,28 gm 0,2 mmol de resina de mbha (Midwest Biotech) en un recipiente de reacción CSBio y la siguiente secuencia se ensambló en un sintetizador CSBio336 utilizando aminoácidos Boc y agentes de acoplamiento únicos activados de DEPBT/DIEA.

HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSC-amida

La resina del péptido se transfirió a un recipiente de reacción de HF y se corrió la decomposición de HF utilizando 10 ml de fluoruro de hidrógeno líquido y 1 ml de un depurador p-cresol en un baño de hielo durante 1 hora. Después de la evaporación del HF, el residuo se suspendió en éter etílico y el péptido y la resina se filtraron con un embudo de vidrio sinterizado. Después del lavado con éter etílico y el secado rápidamente al aire el péptido se extrajo dentro de 50% de ácido acético acuoso. Después del análisis por HPLC (4,6x50mm Zorbax SB-C8, 1ml/min, 45˚C,214nm, A=0,1%TFA, B=0,1%TFA/90%ACN con un gradiente de 30%B a 90%B durante 10 minutos) el extracto de la decomposición se diluyó 3-4 con agua y se cargó en una columna Amberchrom XT20 de 22,2 x 250 mm para su purificación utilizando un gradiente de TFA acuoso en CAN. La colonia de partida se purificó nuevamente en la misma columna para obtener 79 mg con 95% de pureza. Peso molecular teórico = 4313,7, masa observada por ESI = 4312,0.

Se suspendieron 16 mg (3,7µmol) del mencionado péptido de tiol libre en 1 ml de metanol con agitación en una corriente de nitrógeno, se agregaron 1,5ul de tetrametilguanidina en 1ml de metanol (MeOH) seguidos de 3mg (7,8µmol) de ácido 2-iodo hexadecanoico (ácido 2-iodo palmítico) en 0,5mg de tetrahidrofurano (THF). El ácido 2-iodo hexadecanoico fue preparado previamente por tratamiento de ácido 2-bromo hexadecanoico con yoduro de potasio en acetona (DLS-027-52). La reacción de alquilación se calentó en un baño por 10 min. durante los cuales la mayor parte del solvente se evaporó. El residuo se disolvió en ácido acético acuoso, fue analizado por HPLC y resultó ser más hidrófobo que el péptido de partida. La solución de ácido acético restante se cargó en una columna Amberchrom XT20 de 10x250mm para la purificación. Se corrió un gradiente de TFA acuoso en acetonitrilo mientras se recolectaban las fracciones y se monitoreaba la absorvancia de UV a 214nm. Se combinaron las fracciones 64-68, se congelaron y liofilizaron para obtener 6,1mg de material (el péptido de una SEQ ID NO: 164) con una pureza de 90%+. Peso molecular teórico = 4568,1, masa observada por MALDI = 4568,79.

Cuando el péptido comprende un residuo de Lys en lugar de un residuo de Cys en el extremo de C, el péptido puede

obtenerse con el esqueleto peptídico de Lys en primer lugar. El residuo de aminoácido de llegada que será N-alquilado se sustituye con un residuo de N ε -FMOC lisina. Un tratamiento del péptido protegido por BOC en el extremo de N completo con 20% de piperidina en DMF durante 30 minutos remueve los grupos FMOC/formilo del residuo de Lys. Un residuo de Cys se acopla con el residuo de ε-amino Lys libre. El residuo de Cys es alquilado por reacción con ácido 2-iodo palmítico como se describió anteriormente.

Acilación del Péptido con separadores "MiniPEG"

A continuación se describe un ejemplo de la síntesis de un péptido acilado con un separador miniPEG (el péptido posee una secuencia de aminoácidos de SEQ ID NO: 89):

Se colocaron 0,28gm (0,2mmol) de resina de 4-metilbenzidrilamina (mbha)(Midwest Biotech, Inc., Fishers, IN) en un recipiente de reacción y la siguiente secuencia se ensambló en un sintetizador CSBio336 con aminoácidos Boc y agentes de acoplamiento únicos activados de 3-(dietoxifosforiloxi)-3H-benzo[d][1,2,3] triazin-4-ona /N,N-Diisopropiletilamina (DEPBT/DIEA).

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha

Aproximadamente, un tercio de la resina de péptido protegido por Boc se transfirió a un recipiente de reacción manual y se trató con 20% de piperidina en dimetilformamida a temperatura ambiente por 10 minutos. Se filtró la resina, se lavó varias veces con dimetilformamida (DMF) y se agregó una solución activada de Fmoc amidoPEG4 (preparada previamente por disolución de 487,5mg 1,0mmol de Ácido N-Fmoc Amido dPEG4 (Peptides International, Louisville, KY) en 2,0ml 0,5M de DEPBT/DMF y agregado de 0,175ml 1mmol de N,N-diisopropiletilamina). La reacción se mezcló a temperatura ambiente por aprox. 1 hora.

Se filtró la resina de péptido, se lavó con DMF, y se trató nuevamente con 20% de piperidina/DMF por 10min. Se filtró la resina, se lavó con DMF varias veces, y se agregó una solución activada de Fmoc Glu-OBzl (previamente preparada por disolución de 470mg 1mmol de Fmoc Glu-γ-OBzl (Aapptec, Louisville, KY) en 2,0ml 0,5M de DEPBT/DMF y agregado de 0,175ml 1mmol de DIEA). La reacción se mezcló a temperatura ambiente durante 1 hora.

Se filtró nuevamente la resina del péptido, se lavó con DMF, y trató con 20% de piperidina/DMF a temperatura ambiente por 10 min. Se filtró, y se lavó con DMF varias veces y se agregó una solución activada

de ácido palmítico (preparada previamente por disolución de 256mg 1mmol de ácido palmítico (Sigma-Aldrich, St. Louis, MO) en 2,0ml 0,5M de DEPBT/DMF y agregado de 0,175ml 1mmol de N,N-diisopropiletilamina (DIEA)). La reacción se mezcló a temperatura ambiente durante 1 hora.

Finalmente, la resina del péptido se filtró, se lavó con DMF seguido de diclorometano y se trató con 50% de TFA en DCM para remover el grupo Boc en el extremo de N. Después de 1-2 min. se filtró la resina, se lavó varias veces con DCM seguida de una solución de 5% de DIEA en DCM. La resina del péptido seca fue transferida a un recipiente de reacción de HF y se realizó una decomposición de HF utilizando 5ml de fluoruro de hidrógeno líquido y 0,5ml de un depurador p-cresol. Después de mezclar durante 1 hora en un baño de hielo, el HF se removió en vacío y el residuo fue suspendido en éter etílico. La mezcla péptido/resina se filtró en un embudo de vidrio sinterizado, se lavó con éter etílico y fue rápidamente secada. El péptido se extrajo dentro de 10ml de ácido acético acuoso al 50% que fue analizado por HPLC (4,6x50mm Zorbax SB-C8, 1ml/min, 214nm, A=0,1%TFA, B=0,1%TFA/90%ACN, con gradiente de 30%B to 90%B durante 10min). El extracto crudo restante se

diluyó 3X con agua y se cargó en una columna Amberchrom XT20 de 22,2x250mm para la purificación utilizando un gradiente de TFA acuoso en ACN. Una colonia de purificación de partida se purificó nuevamente en la misma columna para obtener 19mg con una pureza de 90%+. Peso molecular teórico = 5010,6; masa observada por ESI = 5008,0.

El mismo procedimiento se repitió utilizando diferentes ácidos N-Fmoc Amido dPEG. En una instancia, se utilizó el ácido N-Fmoc Amido dPEG8 (Peptides International, Louisville, KY) y en otra, el ácido N-Fmoc Amido dPEG2 (Peptides International, Louisville, KY). Las estructuras de los péptidos acilados se muestran en las Figuras 12A-12C.

Dimerización de péptidos - Dimerización con disulfuro.

A continuación se describe un ejemplo de la síntesis de un péptido con un dímero de disulfuro:

se colocaron 0,28 gm (0,2 mmol) de resina de mbha (Midwest Biotech) en un recipiente de reacción CSBio y la siguiente secuencia se ensambló en un sintetizador CSBio336 utilizando aminoácidos Boc y agentes de acoplamiento únicos activados de DEPBT/DIEA.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-amida

La resina de péptido protegido por Boc se transfirió a un recipiente de reacción manual y se trató con 20% de

piperidina en DMF a temperatura ambiente por 10 min. Se filtró la resina, se lavó con DMF, luego se aciló con una solución activada de Fmoc Cys(Trt) (previamente preparada por disolución de 1,17gm 2 mmol de Fmoc Cys(Trt)-OH (Aapptec) en 4,0ml 0,5M de DEPBT/DMF y agregado de 0,35ml 2mmol de N,N-diisopropiletilamina (DIEA)). La reacción se mezcló a temperatura ambiente durante 1 hora, se filtró, se lavó con DMF y el mismo procedimiento se utilizó para agregar Fmoc Glu-OBzl seguido de ácido palmítico. Finalmente, se filtró la resina, se lavó con DMF, con diclorometano y se trató con 50% de TFA en DCM por 1-2 min. Después de la neutralización con 50% de DIEA en DCM, la resina del péptido se transfirió a un recipiente de reacción de HF y se corrió la decomposición de HF utilizando 10ml de fluoruro de hidrógeno líquido y 1 ml de un depurador p-cresol en un baño de hielo durante 1 hora. Después de la evaporación del HF, el residuo se suspendió en éter etílico y el péptido y la resina se filtraron con un embudo de vidrio sinterizado. Después del lavado con éter etílico y un secado rápido, el péptido se extrajo dentro 50% de ácido acético acuoso. Después del análisis por HPLC (4,6x50mm Zorbax SB-C8, 1ml/min, 45˚C,214nm, A=0,1%TFA, B=0,1%TFA/90%ACN, con gradiente

de 30%B a 90%B durante 10 min) el extracto de la decomposición se diluyó 3-4 con agua y se cargó en una columna Amberchrom XT20 de 21,2 x 250 mm para su purificación utilizando un gradiente de TFA acuoso en acetonitrilo. Se combinaron las fracciones de purificación 64-67, se congelaron y liofilizaron para obtener 58,7mg de material con una pureza analizada por HPLC de 90%+.

DLS-027-97A Peso molecular teórico = 4866,48, masa observada por ESI = 4864,0.

HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Cys(SH) γE-C16)G-amida

Se disolvieron 18,6mg (3,8µmol) del mencionado péptido en 2,0ml 3M de guanidina en 0,05M de tris (pH8,5) y se agregó 1ml de dimetilsulfoxida. La reacción fue agitada a temperatura ambiente y expuesta al aire. Después de 6 horas, por HPLC analítico se comparó el péptido de partida y se demostró la presencia de un pico mayor de hidrofobia.

La mezcla de la reacción se diluyó en 20ml de TFA al 0,1% y se cargó en una columna Amberchrom XT20 de 10x250mm. Se corrió una purificación con un gradiente de 0.1% de TFA en acetonitrilo mientras se monitoreaba la absorbancia de UV a 20nm. Se combinaron las

fracciones 68-72 y liofilizaron para obtener 5,5mg de dímero purificado.

La pureza analizada por HPLC fue de 90%+. Peso molecular teórico = 9743,99, masa observada por MALDI = 9745,1. DLS-027-98B. La estructura del dímero resultante se muestra en la Figura 9A.

Dimerización de péptidos - Dimerización con tioéter.

A continuación se describe un ejemplo de la síntesis de un dímero de tioéter:

se colocaron 0,28 gm (0,2 mmol) de resina de mbha (Midwest Biotech) en un recipiente de reacción y la siguiente secuencia se ensambló en un sintetizador CSBio336 utilizando agentes de acoplamiento únicos activados de DEPBT/DIEA.

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Fmoc)G-mbha

La resina de péptido protegido por Boc fue transferida a un recipiente de reacción manual y fue tratada con 20% piperidina en DMF a temperatura ambiente por 10 min. Después de lavados múltiples con DMF, se agregó una solución activada de Boc Dap(Fmoc) (previamente preparada por disolución de 426mg 1mmol de Boc Dap(Fmoc)-OH (Chem-Impex) en 2,0ml 0,5M de DEPBT/DMF y se agregaron 0,175ml 1mmol de DIEA. La reacción fue mezclada a temperatura ambiente por 2 horas, filtrada y lavada con DMF y tratada nuevamente con 20% de

piperidina en DMF, como se mencionó anteriormente. Después de lavados con DMF, la resina fue acilada con una solución activada de ácido bromoacético (previamente preparada por disolución de 139mg 1mmol de ácido bromoacético (Aldrich) en 2,0ml de 0,5M de DEPBT/DMF y se agregaron 0,175ml de DIEA. La reacción se mezcló a temperatura ambiente por 1-2 horas, se filtró y la resina se lavó con DMF seguido de DCM. La resina del péptido fue tratada con 50% de TFA en DCM a temperatura ambiente por 2 min., luego se filtró, se lavó con DCM y se neutralizó con 5% de DIEA en DCM. La resina del péptido completa fue transferida a un recipiente de reacción de HF y se corrió una decomposición de HF utilizando 5ml de fluoruro de hidrógeno líquido/ 0,5ml de p-cresol. Después de revolver durante 1 hora en un baño de hielo, el HF fue evaporado y el residuo fue suspendido en eter etílico. La mezcla resina /péptido fue filtrada en un embudo de vidrio sinterizado y lavada con eter etílico. El péptido se extrajo en 50% de ácido acético acuoso y el producto crudo se analizó por HPLC: 4,6x50mm Zorbax SB-C8, 1ml/min, 45˚C,214nm, A=0,1%TFA, B=0,1%TFA/90%ACN, con gradiente= 30%B to 90%B por 10min. El extracto de la decomposición se cargó en una columna Amberchrom XT20 de 21,2 x 250 mm para su

purificación utilizando un gradiente de TFA acuoso en acetonitrilo mientras se monitoreaba la absorvancia de UV a 220nm. Se combinaron las fracciones 48-53, se congelaron y liofilizaron para obtener 18mg de péptido de una SEQ ID NO: 89 con K40 (Dap-BrAcetyl) con una pureza de 90%+. Peso molecular teórico = 4602,8, masa observada por ESI = 4616,0.

HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Dap-BrAcetyl)G-amida

Se disolvieron 15mg (3,2µmol) del mencionado péptido K40(Dap-BrAcetyl) y 15mg del péptido de SEQ ID NO: 89 K40(Cys γE-C16) en 3,0ml de 7M urea/0,05M tris (pH8,6) y se mezclaron a temperatura ambiente mientras se monitoreaba el progreso de la reacción por HPLC. Después de 30 minutos, se redujo la altura de pico de la mayoría de los materiales de partida mientras que un nuevo pico era el componente predominante. La mezcla de la reacción se diluyó en 25ml de TFA al 0,1% y se cargó en una columna Amberchrom XT20 de 10x250mm para purificación. Se utilizó un gradiente de TFA acuoso en acetonitrilo mientras se monitoreaba la absorvancia de UV a 220nm. Se combinaron las fracciones 57-61, congelaron y liofilizaron para obtener 7,1mg de dímero de tioéter. Pureza por HPLC = 90%+; peso molecular teórico=9401,4; masa observada

por MALDI=9402,8. La estructura del dímero resultante se muestra en la Figura 9B.

PEGilación del Péptido

Para la pegilación del péptido, 40 kDa de metoxi poli(etilenglicol) idoacetamida (NOF) reaccionó con un equivalente molar del péptido en 7M Urea, 50mM tampón de Tris-HCl usando la cantidad mínima necesaria de solvente para disolver tanto el péptido como PEG en una solución transparente (generalmente menos de 2 mL para una reacción usando 2-3 mg de péptido) La agitación vigorosa a temperatura ambiente menzó durante 4-6 horas y la reacción se analizó mediante RP-HPLC analítica. Los productos PEGilados aparecieron en forma distinguible del material de partida con tiempos de retención reducidos. La purificación se realizó sobre una columna de C4 Vydac con condiciones similares a aquellas usadas para la purificación de péptido inicial. La elución ocurrió alrededor de las relaciones de tampón de 50:50. Las fracciones de péptido PEGilado se hallaron y se liofilizaron. Los rendimientos fueron superiores al 50%, y variaron según la reacción

Análisis mediante espectrometría de masas

El espectro de masas se obtuvo con un espectrómetro de masas cuadrupolo Sciex API-III con fuente de iones ESI

estándar. Las condiciones de ionización utilizadas fueron las siguientes: Ionización por electrospray ESI en modo de ion positivo; voltaje de spray de iones, 3.9 kV; orificio potencial, 60 V. El gas nebulizante y de cono utilizado fue una corriente de nitrógeno de .9 L/min. El espectro de masas se registró desde 600-1800 Thompsons a 0,5 Th por paso y 2 msec de intervalo (*dwell time*). La muestra (aproximadamente (1mg/mL) se disolvió en 50% de acetonitrilo acuoso con 1% de ácido acético y se introdujo con una bomba de inyección externa a razón de 5 µL/min.

Cuando los péptidos fueron analizados en una solución de PBS por ESI MS, fueron desalinizados utilizando un dispositivo de extracción de fase sólida ZipTip que contenía 0,6 µL de resina C4, de acuerdo con las instrucciones del fabricante (Millipore Corporation, Billerica, MA, visite la página de Millipore en millipore.com/catalogue.nsf/docs/C5737).

Análisis por Cromatrografía Líquida de Alto Rendimiento (HPLC):

Los análisis preliminares se realizaron con péptidos crudos para obtener una aproximación a sus tasas relativas de conversión en Solución Salina con tampón de Fosfato (PBS) (ph 7,2) mediante el análisis por cromatografía líquida de alto rendimiento (HPLC) y por

MALDI (deserción/ionización láser asistida por una matriz). Las muestras de péptido crudo se disolvieron en el tampón de PBS en una concentración de 1mg/ml. 1 ml de la solución resultante se almacenó en un recipiente de HPLC de 1,5 ml que fue posteriormente sellado e incubado a 37°C. Las alícuotas de 100µl fueron extraídas con varios intervalos de tiempo, enfriadas a temperatura ambiente y analizadas por HPLC.

Los análisis por HPLC se realizaron con un sistema de Cromatografía Beckman System Gold utilizando un detector de UV a 214nm. Los análisis por HPLC se realizaron en una columna C18 Vydac de 150 mm x 4.6 mm. La tasa de flujo fue de 1ml/min. El solvente A contenía 0,1% de TFA en agua destilada y el solvente B contenía 0,1 % de TFA en 90% de CH3CN. Se empleó un gradiente lineal (40% a 70%B en 15 minutos). La información se recolectó y analizó utilizando un software Peak Simple Chromatography.

Los coeficientes iniciales de hidrólisis se utilizaron para medir la constante de velocidad de disociación de las respectivas prodrogas. Las concentraciones de una prodroga y de una droga se calcularon por sus áreas máximas, respectivamente. Las constantes de velocidad de disociación de las prodrogas de la primera orden se

determinaron mediante el ploteo del logaritmo de la concentración de la prodroga en varios intervalos de tiempo. La pendiente de este ploteo da como resultado la constante de velocidad “k”. Las semividas de la degradación de varias prodrogas se calcularon utilizando la fórmula t1/2 = .693/k.

EJEMPLO 2

Este ejemplo describe un método para analizar la actividad biológica de los péptidos en las presentes divulgaciones, método que incluye ensayos por síntesis de cAMP.

La capacidad de los análogos de glucagón de inducir cAMP se midió en un ensayo de informante de luciferasa de luciérnaga. Células HEK293 co-transfectadas con un receptor (receptor de glucagón o de GLP-1 o GIP) y gen de luciferasa unido a un elemento sensible a cAMP se privaron del suero cultivando durante 16 horas en DMEM (Invitrogen, Carlsbad, CA) complementado con 0,25% de suero de crecimiento bovino (HyClone, Logan, UT) y luego se incubaron con diluciones en serie de otros análogos de glucagón, de GLP-1, GIP o análogos de glucagón novedosos durante 5 horas a 37°C, 5% CO_2 en 96 placas de “Biorrecubrimiento” recubiertas con poli-D-Lisina de 96 receptáculos (BD Biosciences, San José, CA) Al cabo de la incubación se agregaron 100

microlitros de reactivo de sustrato de luminiscencia LucLite (Perkin-Elmer, Wellesley, MA) a cada receptáculo. La placa se batió brevemente, se incubó durante 10 minutos a la sombra y la salida de luz se midió sobre el contador de centelleo de líquido MicroBeta-1450 (Perkin-Elmer, Wellesley, MA) Se calcularon las concentraciones del 50% eficaces usando el software Origin (OriginLab, Northampton, MA.

EJEMPLO 3

Este ejemplo describe un método para analizar la estabilidad de los péptidos de las presentes divulgaciones.

Cada análogo de glucagón se disolvió en agua o PBS y se realizó un análisis de HPLC inicial. Después de ajustar el pH (4, 5, 6, 7), las muestras se incubaron durante un período de tiempo especificado a 37°C y se analizaron nuevamente mediante HPLC para determinar la integridad del péptido. Se determinó la concentración del péptido específico de interés y el porcentaje que quedó intacto se calculó comparado con el análisis inicial.

EJEMPLO 4

Este ejemplo describe un método para analizar la solubilidad de los péptidos.

Se prepara una solución (1 mg/ml o 3 mg/ml) de glucagón (o un análogo) en 0,01N HCl. 100 µl de solución de materia prima se diluye en 1ml con 0,01N HCl y se determina la absorbancia de UV (276 nm). El pH de la solución de materia prima remanente se ajusta a pH 7 usando 200-250 µl 0,1M Na_2HPO_4 (pH 9,2). La solución se deja en reposo toda la noche a 4°C y luego se centrifuga. 100 µl de sobrenadante se diluyen en 1ml con 0,01N HCl y se determina la absorbancia de UV (por duplicado).

La lectura de la absorbancia inicial se compensa por el aumento en el volumen y se usa el siguiente cálculo para establecer el porcentaje de solubilidad:

$$\frac{\text{Absorbancia Final}}{\text{Absorbancia Inicial}} \times 100 = \text{porcentaje soluble}$$

EJEMPLO 5

Este ejemplo describe un método para analizar la unión de los péptidos a un receptor.

La afinidad de los péptidos al receptor de glucagón se midió como un ensayo de competencia de unión utilizando la tecnología del ensayo de proximidad de centelleo. Diluciones de 3 veces en serie de los péptidos hechas en el tampón del ensayo de proximidad

de centelleo (0,05 M Tris-HCl, pH 7,5, 0,15 M NaCl, 0,1% p/v de albúmina de suero bovino) se mezclaron en una placa de fondo blanco/transparente de 96 receptáculos (Corning Inc., Acton, MA) con 0,05 nM (3-[^{125}I]-yodotirosil) Tyr10 glucagón (Amersham Biosciences, Piscataway, NJ), 1-6 microgramos por receptáculo, fragmentos de membrana de plasma preparados a partir de células que sobreexpresan el receptor de glucagón humano, y 1 mg/receptáculo de perlas de ensayo de proximidad de centelleo de tipo A de aglutinina de germen de trigo tratada con polietilenimina (Amersham Biosciences, Piscataway, NJ). Después de batir durante 5 minutos a 800 rpm sobre un batidor giratorio, la placa se incubó durante 12 horas a temperatura ambiente y luego se leyó sobre un contador de centelleo líquido MicroBeta1450 (Perkin-Elmer, Wellesley, MA). Se midió la radioactividad unida no específicamente (NSB) en los receptáculos con una concentración 4 veces mayor de ligando nativo “frío” que la concentración más alta en las muestras de ensayo y la radioactividad unida total no se detectó en los receptáculos con ningún competidor. El porcentaje de unión específica se calculó de la siguiente manera: % Unión Específica = ((NSB Unida)/(NSB Unida total)) X 100. Los valores de

IC_{50} se determinaron usando el software Origin (OriginLab, Northampton, MA).

EJEMPLO 6

Los siguientes péptidos que comprenden al menos Tyr en la posición 1, AIB en la posición 2 (para resistencia DPP-IV), Lys en la posición 16, AIB en la posición 20, y Leu, Ala y Gly en las posiciones 27-29, se realizaron de la manera descripta básicamente en el Ejemplo 1 y su actividad agonista fue sometida a ensayo in vitro en cada receptor de GLP-1, receptor de glucagón, y receptor de GIP, de la manera descripta básicamente en el Ejemplo 2:

La EC50 en el receptor de GLP-1 (GLP-1R), receptor de glucagón (GR) y el receptor de GIP (GIPR) se presenta en la Tabla 1.

TABLA 1

Péptido	SEQ ID NO:	Receptor de GLP-1		Receptor de Glucagón		Receptor de GIP	
		EC_{50} (nM) [Desv. Estándar]	% actividad relativa*	EC_{50} (nM) [Desv. Estándar]	% actividad relativa*	EC_{50} (nM) [Desv. Estándar]	% Actividad relativa*
mt-263	10	0,0100 [0,0154]	154,20%	4,0450 [0,0762]	1,88%	0,0054 [0,0166]	305,91%
mt-402	11	0,0070 [0,0154]	220,60%	0,0298 [0,0762]	256,00%	0,0185 [0,0166]	89,46%
mt-403	12	0 0027 [0,154]	581,29%	0,0077 [0,0762]	987,18%	0,0061 [0,0166]	273,10%
mt-404	13	0,0060 [0,0154]	258,29%	0,1076 [0,0762]	70,80%	0,0327 [0,0166]	50,57%
mt-405	14	0,0022 [0,0154]	717,21%	0,0096 [0,0762]	797,18%	0,0039 [0,0166]	425,45%

La actividad relacionada es la actividad del péptido relacionada con la actividad de la hormona nativa

EJEMPLO 7

Los péptidos adicionales mt-395 (SEQ ID NO: 15), mt-396 (SEQ ID NO: 16), mt-397 (SEQ ID NO: 17) y mt-398 (SEQ ID NO: 18) basados en la estructura de mt-263 se prepararon como se describe básicamente en el Ejemplo 1 y se sometieron a ensayo in vitro como se describe en el Ejemplo 2: La EC50 en GLP-1R, GR y GIPR se presenta en la Tabla 2.

TABLA 2

Péptido	SEQ ID NO:						
		Receptor de GLP-1		Receptor de Glucagon		Receptor de GIP	
		EC_{50},nM [Desv. Estándar]	Actividad relativa	EC_{50},nM [Desv. Estándar]	Actividad relativa	EC_{50},nM [Desv. Estándar]	Actividad relativa

mt-263	10	0,0081 [0,0245]	300,61 %	3,1371 [0,0298]	0,95%	0,0033 [0,0135]	403,89 %
mt-395	15	0,0076 [0,0245]	321,55 %	3,4095 [0,0298]	0,87%	0,0025 [0,0135]	537,45 %
mt-396	16	0,0093 [0,0245]	262,27 %	2,9033 [0,0298]	1,03%	0,0034 [0,0135]	402,69 %
mt-397	17	0,0085 [0,0245]	287,88 %	5,3528 [0,0298]	0,56%	0,0029 [0,0135]	470,03 %
mt-398	18	0,0078 [0,0245]	314,93 %	3,7352 [0,0298]	0,80%	0,0031 [0,0135]	433,76 %

La actividad relacionada es la actividad del péptido relacionada con la actividad de la hormona nativa

EJEMPLO 8

Los péptidos adicionales que comprenden Tyr en la posición 1, AIB en la posición 2 (para resistencia DPP-IV), Glu en la posición 16, Lys en la posición 20 (unidas por un puente de lactama entre las posiciones 16 y 20), y Leu-Ala-Gly en las posiciones 27-29, y

GPSSGAPPPS en las posiciones 30-39 se prepararon de la manera descripta básicamente en el Ejemplo 1 y su actividad agonista fue sometida a ensayo in vitro en cada receptor de GLP-1, receptor de glucagón, y receptor de GIP, de la manera descripta básicamente en el Ejemplo 2. Se preparon y ensayaron otros péptidos sin puente de lactama. Los resultados de los ensayos de actividad se presentan en la Tabla 3.

TABLA 3

Péptido	SEQ ID NO:	GLP-1R			GR			GIPR		
		EC_{50}, nM	Desv. Estándar	Actividad relativa	EC_{50}, nM	Desv. Estándar	Actividad relativa	EC_{50}, nM	Desv. Estándar	Actividad relativa
mt-217	19	0,222	0,025	11,26%	13,886	0,114	0,82%	9,574	0,019	0,20%
mt-218	20	0,338	0,025	7,40%	16,298	0,114	0,70%	14,283	0,019	0,13%
mt-219	21	0,151	0,025	16,56%	17,628	0,114	0,65%	6,165	0,019	,31%

mt-220	22	0,180	0,025	13,89%	9,670	0,114	1,18%	10,268	0,019	0,19%
mt-225	23	0,098	0,029	29,59%	2,712	0,054	1,99%	1,899	0,017	0,90%
mt-226	24	0,097	0,029	29,90%	3,462	0,054	1,56%	1,467	0,017	1,16%
mt-227	25	0,080	0,029	36,25%	4,244	0,054	1,27%	1,320	0,017	1,29%
mt-228	26	0,146	0,029	19,86%	5,364	0,054	1,01%	2,266	0,017	0,75%

La actividad relacionada es la actividad del péptido relacionada con la actividad de la hormona nativa

EJEMPLO 9

Se prepararon ejemplos de análogos de los péptidos de las presentes divulgaciones como se describe básicamente en el Ejemplo 1. Cada uno de los análogos de péptidos comprende una secuencia de aminoácidos basada en glucagón nativo (SEQ ID NO: 1) con His nativo en posición1, un aminoácido protector de DPP-IV en posición 2, un aminoácido acilado en posición 10, uno o más aminoácidos estabilizadores de la hélice alfa dentro de las posiciones 16-21 del análogo de péptido y otras

modificaciones. Todos los péptidos fueron amidados en el extremo de C. Los péptidos se sometieron a ensayos de actividad para cada receptor de glucagón, GLP-1 y GIP, como se describe en el Ejemplo 2. La reunión de los resultados de los diferentes ensayos de actividad se presenta en la Tabla 4.

TABLA 4

SEQ ID NO:	**Análogo N°**	**Breve anotación de modificaciones en aminoácidos**	**EC50 (nM) en Receptor de Glucagon**	**EC50 (nM) en Receptor de GLP-1**	**EC50 (nM) en Receptor de GIP**
1		Glucagón nativo	0,025	ND	ND
		GLP-1 nativo	ND	0,025	ND
2		GIP nativo	ND	ND	0,01
27	29	dSer2, K10acyl, E16, A18, L27, D28, G29 +CEX	0,003	0,002	0,085
28	30	**Aib2, K10acyl, E16, A18, L27, D28, G29+CEX**	0,002	0,002	0,003

29	34	Aib2, K10acyl, E16, A18, L27, D28, GRG29-31	0,007	0,003	0,064
30	36	dSer2, K10acyl, E16, Aib20, E21, L27, D28	0,008	0,0031	0,25
31	37	Aib2, K10acyl, E16, Aib20, E21, L27, D28	0,008	0,0044	0,008
32	44	DMIA1, dSer2, K10acyl, E16, A18, L27, D28	0,004	0,003	0,103
33	49	Aib2, K10acyl, Aib16, A10, D28, G29+CEX	0,005	0,004	0,248
35	53	Aib2, K10acyl, Aib16, A18, V27, K28, G29+CEX	0,011	0,005	0,073
36	54	Aib2, K10acyl, Aib16, A18, K27, D28, G29+CEX	0,008	0,004	0,171

37	61	Aib2, K10acyl, E16, Aib20, E21, L27, D28, G29 +CEX	0,004	0,006	0,002
38	62	Aib2, E3, K10acyl, E16, Aib20, E21, L27, D28, G29 +CEX	0,127	0,008	0,012
39	63	Aib2, E3, K10acyl, E16, L27, D28, G29 +CEX	1,276	0,008	0,026
40	64	Aib2, E3, K10acyl, E16, Aib20, E21, L27, D28	0,042	0,006	0,024
41	65	Aib2, E3, K10acyl, E16, Aib20, L27, D28, G29+CEX	0,221	0,006	0,013

CEX = GPSSGAPPPS (SEQ ID NO: 5)

Acyl = C16 acyl

Cada uno de los análogos de péptido de la Tabla 4 demostraron una potente actividad en cada receptor de

glucagón, GLP-1 y GIP. Notablemente, cada uno de los análogos de péptido exhibieron una EC50 en cada receptor de GIP <0.5 nM, y la mayoría de los análogos de péptido exhibieron una EC50 en el receptor de GIP < 0.1 nM. Estos análogos de péptido exhibieron además una potente actividad en el receptor de GLP-1, cada análogo de péptido exhibió una EC50 ≤0.008 nM. Los análogos de péptido que carecen de Glu en la posición 3 demostraron una potente actividad en el receptor de glucacón, cada análogo de péptido exhibió una EC50 < 0.03 nM.

En general, los análogos de péptido que comprenden un AIB en la posición 20 o una combinación de Glu en la posición 16 y una extensión en el extremo de C exhibieron una actividad altamente potente en el receptor de GIP. Además, los análogos de péptido que comprenden un AIB en la posición 2 exhibieron una mayor actividad en el receptor de GIP, en comparación con el análogo de péptido correspondiente que comprende d-Ser en la posición 2 (por ejemplo, comparar las EC50 en el receptor de GIP entre las SEQ ID NO: 2 y 3 o las SEQ ID NO: 5 y 6)

EJEMPLO 10

Se prepararon ejemplos de análogos de los péptidos de las presentes divulgaciones como se describe básicamente

en el Ejemplo 1. Cada uno de los análogos de péptidos es similar en estructura, y comprende una secuencia de aminoácidos basada en glucagón nativo (SEQ ID NO: 1) con His nativo en posición 1, un aminoácido protector de DPP-IV en posición 2, un aminoácido estabilizador de la hélice alfa en las posición 16, salvo que la posición en la que se encuentra un aminoácido acilado varía en este conjunto de análogos de péptido. Todos los péptidos fueron amidados en el extremo de C. Los péptidos se sometieron a ensayos de actividad para cada receptor de glucagón, GLP-1 y GIP, como se describe en el Ejemplo 2. La reunión de los resultados de los diferentes ensayos de actividad de algunos de los péptidos preparados y ensayados se presenta en la Tabla 5.

TABLA 5

SEQ ID NO:	**Breve anotación de modificaciones en aminoácidos**	**EC50 (nM) en Receptor de Glucagón**	**EC50 (nM) en Receptor de GLP-1**	**EC50 (nM) en Receptor de GIP**
1	Glucagón nativo	0,10048	ND	ND
	GLP-1 nativo	ND	0,01593	ND
2	GIP nativo	ND	ND	0,05665

42	Glucagon Aib2, Aib16, amida	0,30892	0,43695	103,93715
43	Glucagon Aib2,K9(rErEC16),Aib16, amida	1,15223	0,23172	15,14376
45	Glucagon Aib2, K12(rErEC16),Aib16, amida	0,04254	0,02049	0,65062

Como se demostró en la Tabla 5, los análogos de péptidos que comprenden un aminoácido acilado en alguna de las posiciones 9 a 12, demostraron una mejora en la actividad de GIP, en comparación con el análogo de péptido no acilado de SEQ ID NO: 42. Sin embargo, no todos los análogos de péptidos acilados preparados y ensayados demostraron una actividad de GIP mejorada. Uno de los análogos de péptido acilado en una posición diferente a la posición 9 y 12 demostró una actividad reducida en el receptor de GIP.

EJEMPLO 11

Este ejemplo describe la actividad *in vivo* de los ejemplos de análogos de glucagón de las presentes divulgaciones.

Los efectos *in vivo* de los análogos de glucagón activo de receptor de GIP que poseen una secuencia de aminoácidos de una de las SEQ ID NO: 28, 37-39 y 134 se ensayaron en ratones con obesidad inducida por una dieta (DIO) y comparados con los efectos *in vivo* en los ratones a los cuales se les administró un análogo agonista de glucagón o un control de excipiente (*vehicle*). Cada grupo de ratones del ensayo estaba formado por diecinueve ratones y cada ratón había sido subcutáneamente inyectado con una dosis de 10nmol/kg de péptido o un control de excipiente (*vehicle*). Se midieron el peso corporal y la ingesta de alimentos los días 0, 1, 3, 5 y 7 posteriores a la administración, mientras que los niveles de glucosa en sangre en ayunas se midieron los días 0 y 7. Los ratones presentaban un ayuno de 6 horas previo a la medición de la glucosa en sangre en los días 0 y 7. Los niveles de glucosa en sangre se midieron ad lib, además, en el día 5 posterior a la administración.

Todos los ratones inyectados con un análogo de glucagón activo de receptor de GIP demostraron una reducción considerable (una reducción de alrededor del 11% y el 27%) del peso corporal 7 días después de la administración, en comparación con los ratones a los que

se les administró un control de excipiente (*vehicle*). Además, todos los ratones inyectados con un análogo de glucagón activo de receptor de GIP presentaron una reducción importante (una reducción de alrededor del 43% y el 65%) en los niveles de glucosa en sangre 7 días después de la administración.

Otro estudio se realizó en ratones macho db/db para analizar los efectos *in vivo* de los análogos de glucagón activo de receptor de GIP. En este estudio, se administraron 20nmol/kg de un análogo de glucagón activo de receptor de GIP con una secuencia de aminoácidos de una de las SEQ ID NO: 28, 31, 37, 135 y 136 a los ratones por inyección subcutánea. Se administró un control de excipiente (*vehicle*) a un grupo de ratones de control. Se midieron los niveles de glucosa en sangre antes de la administración y 1, 2, 4, 8, 24, 48, y 72 horas después de la misma. Se midió el peso corporal antes de la administración y 72 horas después de la misma. En consonancia con los resultados observados en el estudio con ratones DIO, todos los ratones a los que se les administró un análogo de glucagón activo de receptor de GIP demostraron una reducción importante del peso corporal (una reducción de alrededor del 2% y el 5%) en comparación con el grupo que recibió el control

de excipiente (*vehicle*). Además, todos los ratones que recibieron un análogo de glucagón activo de receptor de GIP presentaron una reducción en los niveles de glucosa en sangre.

EJEMPLO 12

Este ejemplo describe las estructuras de los ejemplos de péptidos adicionales.

Los análogos de glucagón se prepararon de la manera descripta básicamente en el Ejemplo 1 y en la Tabla 6 se describe la estructura de dichos análogos. Se analizó la actividad de los análogos de glucagón en cada receptor de glucagón, receptor de GLP-1 y receptor de GIP de la manera descripta básicamente en el Ejemplo2.

TABLA 6

SEQ ID NO:	**Estructura**	**EC50 (nM) en Receptor de Glucagón**	**EC50 (nM) en Receptor de GLP-1**	**EC50 (nM) en Receptor de GIP**

48	H(D-Ser)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(-SH)-amida	0,026	0,01	0,433
50	Y(D-ser)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC-amida	0,041	0,108	0,072
51	Y(D-ser)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-TE)-amida	0,255	0,572	0,433
52	H(aib)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC-amida	0,2	0,005	0,006
53	H(aib)QGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-TE)-amida	3,511	0,027	0,232
54	Y(aib)QGTFTSDYSIYLDKQAA(aib)EFV(aib)WLLAGGPSSGAPPPSC-amida	0,566	0,091	0,467

56	Y(aib)QGTFTSDYSIYLDEQAAKEFV(aib)WLLAGGPSSGAPPPSC-amida, (los residuos subrayados se unen por puente de lactama)	1,266	0,096	1,981
58	Y(aib)QGTFTSDYSIYLDKEAA(aib)KFVNWLLAGGPSSGAPPPSC-amida, (los residuos subrayados se unen por puente de lactama)	0,063	0,014	0,005
59	Y(aib)QGTFTSDYSIYLDKEAA(aib)KFVNWLLAGGPSSGAPPPSC(40K-TE)-amida, (los residuos subrayados se unen por puente de lactama)	0,432	0,063	0,122
60	Y(aib)QGTFTSDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC-amida	0,047	0,0028	0,025

68	Y(aib)QGTFTSD(K(γE-C14-acyl))SIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-ME)-amida	50,1	0,612	0,862
69	Y(aib)QGTFTSD(K(γE-C16-acyl))SIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-ME)-amida	0,055	0,02	0,015
70	Y(aib)QGTFTSDYSIYLDKQAA(aib)EFVC(40K-ME)WLLAGGPSSGAPPPS(K(C16-acyl))-amida	0,018	0,007	0,014
72	Y(aib)EGTFISDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	1,676	2,686	0,035
73	Y(aib)EGTFTSDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	~10	0,91	1,56
74	H(aib)QGTFISDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	2,519	6,42	2,541

75	Y(aib)QGTFISDYSIYLDKQAA(acpc)EFVNWLLAGGPSSGAPPPSC(40K-TE)-NH2	3,116	17,7	2,438
79	H(aib)QGTFTSDK(γE-C16)SKYLDERRA(aib)EFVQWLLDGGPSSGAPPPS-NH_2	0,004	0,006	0,003
80	H(aib)EGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-NH2	1,276	0,008	0,026
81	Y(aib)QGTFTSDK(γE-γE-C16)SIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPS-NH2	0,073	0,008	0,005
175	Y(aib)EGTFTSDYSIYLDKQAA(aib)EFVNWLLAGGPSSGAPPPSC-NH_2, en el que C en el extremo de C está ligado por unión covalente a 40 kDa PEG	0,62	0,066	0,100
172	YaibQGTFTSDYSIYLDKQAAaibEFVC(40K-TE PEG)WLLAGGPSSGAPPPSK(C8)-amida	3,06	0,108	0,487

173	YaibQGTFTSDYSIYLDKQAAaibEFVC(40K-TEPEG)WLLAGGPSSGAPPPSK(C12)-amida	0,190	0,011	0,089
174	YaibQGTFTSDYSIYLDKQAAaibEFVC(40K-TEPEG)WLLAGGPSSGAPPPSK(C14)-amida	0,025	0,010	0,019

EJEMPLO 13

Los análogos de glucagón se prepararon de la manera descripta básicamente en el Ejemplo 1 y en la Tabla 7 se describe la estructura de dichos análogos. Se analizó la actividad de los análogos de glucagón en cada receptor de glucagón, receptor de GLP-1 y receptor de GIP de la manera descripta básicamente en el Ejemplo2.

TABLA 7

SEQ ID NO:	Secuencia de Aminoácidos	EC50 (nM) en Receptor de Glucagón	EC50 (nM) en Receptor de GLP-1	EC50 (nM) en Receptor de GIP
28	HAibQGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amida	0,002	0,002	0,003
89	HAibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSKG(γEγE-C16)-amida	0,0041	0,0021	0,024
90	H Aib QGTFTSDKSK(γEγE-C16)YLDERAAQDFVQWLLDGGPSSGAPPPS-amida	0,0056	0,0027	0,017
91	YAibQGTFTSDK(γEγE-C16)SIYLDKQAAAibEFVNWLLDGGPSSGAPPPS-amida	0,073	0,008	0,005
92	YAibQGTFTSDK(γEγE-C16)SIYLDKQAAAibEFVNWLLDT-amida	0,0105	0,0031	0,021
31	HAibQGTFTSDK(γE-C16)SKYLDERRAAibEFVQWLLDT-amida	0,008	0,0044	0,008

EJEMPLO 14

Los análogos de glucagón se prepararon de la manera descripta básicamente en el Ejemplo 1 y en la Tabla 8 se describe la estructura de dichos análogos. La descripción completa de dichos análogos se presenta en la lista de secuencias y la SEQ ID NO: para cada uno se presenta en la Tabla 8. Se analiza la actividad de los análogos de glucagón en cada receptor de glucagón, receptor de GLP-1 y receptor de GIP de la manera descripta básicamente en el Ejemplo 2.

TABLA 8				
		Glu (0.025)	GLP (0.025)	GIP (0.01)
SEQ ID NO: 28	Péptido tri-agonista de GIP/GLP-1/glucagón: HaibQGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-NH_2	0,002	0,002	0,003
SEQ ID NO: 39	Péptido co-agonista de GIP/GLP-1: HaibEGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-NH_2	1,276	0,008	0,026
SEQ ID NO: 37	Péptido tri-agonista de GIP/GLP-1/glucagón: HaibQGTFTSDK(γE-C16)SKYLDERRAaibEFVQWLLDGGPSSGAPPPS-NH_2	0,004	0,006	0,003

SEQ ID NO: 262	Péptido co-agonista de GIP/GLP-1: HaibEGTFTSDK(γE-C16)SKYLDERRAaibEFVQWL LDGGPSSGAPPPS-NH_2	0,127	0,008	0,012

Para poder analizar los efectos *in vivo* de estos péptidos, los péptidos de la Tabla 8 (excluyendo los péptidos nativos) se inyectaron a ratones DIO (ratones C57B66) por vía subcutánea con dosis de 5 o 10 nmol/kg todos los lunes, miércoles y viernes, durante 4 semanas. Los ratones recibieron una dieta diabetógena, elevada en grasas. Como se muestra en la Figura 1, el peso corporal de los ratones que recibieron inyecciones de unos de estos péptidos disminuyó, en comparación con los que recibieron el control de excipiente (*vehicle*).

EJEMPLO 15

Los péptidos acilados que comprenden la secuencia de aminoácidos SEQ ID NO: 1 (glucagón nativo) con Tyr en la posición 1, AIB e la posición 2, Glu en la posición 3, Ile en la posición 12, Lys en la posición 16, Gln en la posición 17, Ala en la posición 18, AIB en la posición 20, Glu en la posición 21, Asn en la posición

24, Leu en la posición 27, Ala en la posición 28, Gly en la posición 29, seguidos de la secuencia de aminoácidos GPSSGSPPPS (SEQ ID NO: 5) y amidación en el extremo de C se prepararon de la manera descripta básicamente en el Ejemplo 1. Los péptidos difieren por el tipo de acilación, agente separador de acilación, y/o posición del aminoácido acilado. Los diferentes residuos acilados se describen en la Figura 3. Se analizó los efectos *in vitro* de los péptidos en cada receptor de glucagón, receptor de GLP-1. y receptor de GIP de la manera básicamente descripta en el Ejemplo 2. La Tabla 9 resume la estructura y los efectos de cada péptido.

TABLA 9

Aminoácido acilado (posición del mismo)	Tipo de acilación	Agente separador	Aminoácido en posición 41	**Estructura (SEQ ID NO):**	**EC50 (nM) en Receptor de Glucagón**	**EC50 (nM) en Receptor de GLP-1**	**EC50 (nM) en Receptor de GIP**

Lys(40)	C16	Ninguno	n/a	**Y aib E GTFTSDYSIYLDKQA A aib EFVNWLL AGGPSSGAPPPS K(-C16)- NH_2 (SEQ ID NO: 138)**	**0,085**	**0,002**	**0,001**
Lys(40)	C16	Ninguno	Gly	**Y aib E GTFTSDYSIYLDKQA A aib EFVNWLL AGGPSSGAPPPS K(-C16)G -NH_2 (SEQ ID NO: 139)**	**0,203**	**0,005**	**0,001**
Lys(40)	SuccinoylC16	Ninguno	Gly	**Y aib E GTFTSDYSIYLDKQA A aib EFVNWLL AGGPSSGAPPPS K(-SuccinoylC16) G -NH_2 (SEQ ID NO: 140)**	**0,077**	**0,001**	**0,001**

Lys(40)	Succinoyl C16	β-Ala	Gly	**Y aib E GTFTSDYSIYLDKQA A aib EFVNWLL AGGPSSGAPPPS K(-βAlaSuccinoylC16) G $-NH_2$ (SEQ ID NO: 141)**	**0,058**	**0,001**	**0,002**
Lys(40)	SuccinoylC18	Ningu no	Gly	**Y aib E GTFTSDYSIYLDKQA A aib EFVNWLL AGGPSSGAPPPS K(-SuccinoylC18) G $-NH_2$ (SEQ ID NO: 142)**	**0,121**	**0,004**	**0,001**

4-aminoPhe(10)	C16	γE γE	n/a	**Yaib E GTFTSDF(4-aminoγEγEC16)SI YLDKQAA aib EFVNWLLAGGPSSGA PPPS- NH_2 (SEQ ID NO: 143)**	**0,165**	**0,002**	**0,003**
4-aminoPhe(10)	SuccinoYl C16	Ningu no	n/a	**Yaib E GTFTSDF(4amino-succinoylC16)SI YLDKQAA aib EFVNWLLAGGPSSGA PPPS- NH_2 (SEQ ID NO: 144)**	**>0,500**	**0,004**	**0,013**

Para poder analizar los efectos *in vivo* de los péptidos enumerados en la Tabla 9, se inyectaron a ratones DIO (cada uno de los cuales tenía un peso corporal promedio de 49,0 g) con una dosis de 10 nmol/kg todos los lunes, miércoles y viernes, durante 1 semana. Se midió el peso corporal y la ingesta de alimentos de los ratones los Días 0, 2, 4, 6 y 7, mientras que los niveles de glucosa en sangre se midieron los Días 0 y 7.

Como se muestra en la Figura 2, el peso corporal de los ratones que recibieron inyecciones con estos péptidos acilados demostró una reducción de al menos 5% en el peso corporal total al Día 7.

EJEMPLO 16

Los péptidos acilados que comprenden la secuencia de glucagón nativo SEQ ID NO: 1) con AIB en la posición 2, Glu en la posición 16 (excepto cuando la posición 16 está acilada), Ala en la posición 18, Leu en la posición 27, Asp en la posición 28, Gly en la posición 29, seguidos de la secuencia de aminoácidos GPSSGSPPPS (SEQ ID NO: 5), y amidación en el extremo de C se prepararon de la manera descripta básicamente en en Ejemplo 1: Los péptidos difieren por el tipo de acilación, agente separador de acilación, y/o posición del aminoácido acilado. Se analizó la actividad *in vitro* de los péptidos en cada receptor de glucagón, receptor de GLP-1. y receptor de GIP de la manera

básicamente descripta en el Ejemplo 2. La Tabla 10 resume la estructura y la actividad de cada péptido.

TABLA 10

Estructura					**Receptor de Glucagón**		**Receptor de GLP-1**		**Receptor de GIP-1**	
Aminoácido acilado (posición del mismo)	Tipo de acilación	Agente separador	Aminoácido en posición 41	Secuencia de Aminoácidos (SEQ ID NO:)	**EC_{50} Desv. Estándar**	**n***	**EC_{50} Desv. Estándar**	**n***	**EC_{50} Desv. Estándar**	**n***
Lys(10)	C16	γE	n/a	**HaibQGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 28)**	**0,006 (0,003)**	**1**	**0,007 (0,0020)**	**1**	**0.003 (0.001)**	**1**

4-amino Phe(40)	C16	γEγE	Gly	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(C16)G-amida (SEQ ID NO: 177)**	**0,010 (0,001)**		**0,014 (0,002)**		**0.021 (0.002)**	
4-amino Phe(40)	succinoyl C16	n/a	Gly	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(SuccinoylC14)G-amida (SEQ ID NO: 176)**	**0,012 (0,002)**		**0,014 (0,011)**		**0.101 (0.002)**	
4-amino Phe(6)	C16	γE	n/a	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSaF(γEγE-C16)G-amida (SEQ ID NO: 145)**	**0,002 (0,001)**	**2**	**0,002 (0,011)**	**2**	**0.017 (0.057)**	**2**
4-amino Phe(10)	C16	γE	n/a	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSaF(C16succinoyl)G-amida (SEQ ID NO: 146)**	**0,0070,001)**	**2**	**0,007 (0,003)**	**2**	**0.052 (0.205)**	**2**

4-amino Phe(13)	C16	γE	n/a	**HaibQGTaF(γE-C16)TSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 147)**	**0,246 (0,079)**	**2**	**0,004 (0,013)**	**2**	**3,131 (N/A)**	**1**
4-amino Phe(10)	succinoyl C16	n/a	n/a	**HaibQGTFTSDaF(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 148)**	**0,008 0,008)**	**2**	**0,009 (0,036)**	**2**	**0.009 (0.020)**	**2**
Lys(13)	C16	γE	n/a	**HaibQGTFTSDYSKaF(γE-C16)LDERAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 149)**	**0,011 (0,004)**	**2**	**0,007 (0,036)**	**2**	**0.395 (0.2630)**	**2**
Lys(14)	C16	γE	n/a	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSaF(C16succinoyl)G-amida (SEQ ID NO: 150)**	**0,006 (0,002)**	**2**	**0,004 (0,018)**	**2**	**0.141 (0.046)**	**2**

Lys(16)	C16	γE	n/a	**HaibQGTFTSDYSKK(γE-C16)LDERAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 151)**	**0,0080,001)**	**2**	**0,004 (0,013)**	**2**	**0.309 (0.0884)**	**2**
Lys(17)	C16	γE	n/a	**HaibQGTFTSDYSKYK(γE-C16)DERAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 152)**	**0,004 (0,001)**	**2**	**0,004 (0,013)**	**2**	**0.011**	**2**
Lys(10)	C16	γE	n/a	**HaibQGTFTSDYSKYLDK(γE-C16)RAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 153)**	**0,012 (0,006)**	**2**	**0,007 (0,006)**	**2**	**0.276**	**2**
4-amino Phe(40)	C16	γEγE	Gly	**HaibQGTFTSDYSKYLDEK(γE-C16)AAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 154)**	**0,008 (0,001)**	**2**	**0,004 (0,019)**	**2**	**0.175**	**2**

* número de experimentos, diferentes fracciones de SEQ ID NO: 147 y 149 se utilizaron en los experimentos. La información puede provenir de diferentes experimentos.

EJEMPLO 17

Los péptidos seleccionados de la Tabla 10 (péptidos con SEQ ID NO: 145, 148, y 152) y los péptidos con SEQ ID NO: 28 y 29 fueron sometidos a ensayos para analizar la actividad *in vivo* en ratones DIO (con un peso corporal inicial promedio de 58,0 g) con una dieta diabetógena, elevada en grasas. Los péptidos en una dosis de 10 nmol/kg (del peso corporal inicial promedio) se inyectaron por vía subcutánea los Días 0 a 3. El peso corporal y la ingesta de alimentos de los ratones se midieron los Días 0, 1, 3, 5 y 7 mientras que los niveles de glucosa en sangre se midieron los Días 0 y 7. Como se muestra en la Figura 2, los ratones que recibieron inyecciones con uno de estos péptidos presentaron una reducción del peso corporal durante el transcurso de los 7 días, en comparación con los que recibieron el control de excipiente (*vehicle*). Los ratones que recibieron inyecciones con estos péptidos presentaron una reducción de al menos 5% en el peso corporal total al Día 7, de los cuales dos presentaron

una reducción de al menos 10% del peso corporal total al Día 7.

EJEMPLO 18

Los péptidos acilados que comprenden un separador “mini-PEG” se prepararon de la manera descripta básicamente en el Ejemplo 1. La Figura 12 representa un esquema de las estructuras de estos péptidos acilados. Los péptidos que comprenden un separador mini-PEG fueron sometidos a ensayo para analizar la actividad *in vitro* de cada receptor de glucagón, receptor de GLP-1 y receptor de GIP de la manera descripta básicamente en el Ejemplo 2, y comparar las actividades de los péptidos acilados que no comprenden un separador o que comprenden un γE separador. Los péptidos de este experimento que comprenden la secuencia de aminoácidos de glucagón nativo SEQ ID NO: 1) con AIB en la posición 2, Glu en la posición 16, Ala en la posición 18, Leu en la posición 28, Asp en la posición 28, Gly en la posición 29, seguidos de la secuencia de aminoácidos GPSSGSPPPSK (SEQ ID NO: 9) donde K es un aminoácido acilado, y una amidación en el extremo de C. La Tabla 11 resume la estructura y las actividades de cada péptido.

TABLA 11										
Estructura					**Receptor de Glucagon**		**Receptor de GLP-1**		**Receptor de GIP**	
Aminoácido acilado (posición del mismo)	Tipo de acilación	Agente separador	Aminoácido en posición 41	Secuencia de Aminoácidos	**EC_{50} Desv. Estándar**	**n***	**EC_{50} Desv. Estándar**	**n***	**EC_{50} Desv. Estándar**	**n***

Lys (40)	C16	γE	Gly	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGSPPPSK(γE-C16)G-amida (SEQ ID NO: 155)**	**0,004 (0,0004)**	**2**	**0,003 (0,0003)**	**2**	**0,008 (0,001)**	**1**
Lys (40)	Succinoyl C16	Ninguno	Gly	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(C16succinoyl)G-amida (SEQ ID NO: 156)**	**0,003 (0,0003)**	**2**	**0,003 (0,0008)**	**2**	**0,006 (0,0009)**	**1**
Lys (40)	C16	PEG2-γE	Gly	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGASSGAPPPSK(Peg2-γE-C16)G-amida (SEQ ID NO: 157)**	**0,011 (0,001)**	**1**	**0,009 (0,0005)**	**1**	**0,021 (0,004)**	**1**

Lys (40)	C16	PEG4-γE	Gly	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Peg4-γE-C16)G-amida (SEQ ID NO: 158)**	**0,004 (0,0005)**	**2**	**0,003 (0,0005)**	**2**	**0,0050,0005)**	**2**
Lys (40)	C16	PEG8-γE	Gly	**HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(Peg8-γE-C16)G-amida (SEQ ID NO: 159)**	**0,006 (0,0009)**	**1**	**0,005 (0,001)**	**1**	**0,002 (0,0004)**	**2**

EJEMPLO 19

Se prepararon péptidos de acilación doble. Dos péptidos comprenden un residuo de un aminoácido acilado simple que transporta dos acilaciones en una formación ramificada. El primer péptido posee una acilación ramificada en la posición 10, y el segundo péptido posee la acilación ramificada en la posición 40. La Figura 5 (en la parte superior) muestra la estructura de un residuo Lys acilado simple que transporta dos acilaciones en una formación ramificada. En otra realización, un péptido comprende un residuo de aminoácido acilado que transporta dos acilaciones en una formación lineal. La Figura 5 (en el medio) describe la estructura de un residuo Lys acilado simple que transporta una acilación de C12 ligada a una acilación de C16 mediante un separador γE en una cadena lineal. En otra realización, un péptido comprende dos residuos de aminoácidos acilados: El primero en la posición 10 y el segundo en la posición 40. La Figura 5 (al pie) describe la estructura de cada residuo Lys acilado que transporta una acilación de C16 mediante un separador γE. Los paréntesis representan que los residuos de Lys

están conectados a través de los esqueletos de los aminoácidos en las posiciones 11-39.

Se analizó la actividad *in vitro* de los péptidos que transportan dos acilaciones en cada receptor de glucagón, receptor de GLP-1. y receptor de GIP de la manera básicamente descripta en el Ejemplo 2. La Tabla 12 presenta los resultados.

TABLA 12

Péptidos con acilación doble	EC_{50} (cAMP, nmol)*		
	GCGR	GLPR	GIPR
HaibQGTFTSD K*(rEC16) SKYLDERAAQDFVQ WLLDGGPSSGAPPPS-amida **(SEQ ID NO: 28)**	0,004	0,003	0,007
HaibQGTFTSD K*K(rEC16)$_2$ SKYLDERAAQDFVQ WLLDGGPSSGAPPPS-amida **(SEQ ID NO: 160)**	0,024	0,007	0,015

HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPS SGAPPPS K*K(rEC16)$_2$-amida **(SEQ ID NO: 161)**	0,011	0,007	0,010
HaibQGTFTSD K*(C12-rEC16) SKYLDERAAQDFVQWLLDGGPSSGAPPPS - amida **(SEQ ID NO: 162)**	0,008	0,003	0,033
HaibQGTFTSD K*(rEC16) YSKYLDERAAQDFVQWLLDGGPSSGAPPPS K*(rEC16)-amida **(SEQ ID NO: 163)**	0,018	0,011	0,024

El Día 0, los péptidos de la Tabla 12, así como dos dímeros (descriptos en el Ejemplo 22 y en la Figura 9) y dos péptidos adicionales (un péptido de SEQ ID NO: 28 y un péptido de SEQ ID NO: 89) se inyectaron por la vía subcutánea una vez en ratones DIO (en una dosis de 10 nmol/kg) A los ratones DIO se les suministró una dieta diabetógena con anterioridad a la administración de la inyección y el peso corporal promedio de los ratones era de 60 g. Se midió el peso corporal y la ingesta de alimentos de los ratones los Días 0, 1, 3, 5 y 7,

mientras que los niveles de glucosa en sangre en ayunas se midieron los Días 0 y 7.

Como muestra la Figura 5E, los ratones que recibieron inyecciones con estos péptidos presentaron una reducción del peso corporal al Día 7, en comparación con los ratones que fueron inyectados con el control de excipiente (*vehicle*).

Como muestra la Figura 5E, los niveles de glucosa en sangre se redujeron en los los ratones que recibieron inyecciones con estos péptidos, en comparación con los ratones que fueron inyectados con el control de excipiente (*vehicle*).

EJEMPLO 20

Los péptidos se S-alquilaron de tres maneras diferentes. En la primera realización, un residuo de Cys alquilado S-palmitilo formaba parte del esqueleto peptídico. El residuo de Cys alquilado estaba ubicado en la posición 40. La estructura es la que se muestra en la Figura 6 (al pie) y se detalla como SEQ ID NO: 164 en la lista de secuencias. En una segunda realización, el residuo de Cys alquilado S-palmitilo se une al residuo de Lys, el que se ubica en la posición 40 del péptido. La estructura resultante se presenta en la Figura 7 (Cys-S—Palmítico) y se incluye como SEQ ID NO: 165 en la lista

de secuencias. En una tercera realización, el residuo de Cys alquilado S-palmitilo se une a un residuo de separador (ácido gamma-glutámico) el que se une al residuo de Lys ubicado en la posición 40 del péptido. La estructura resultante se presenta en la Figura 7 (γE-Cys-S—Palmítico) y se incluye como SEQ ID NO: 166 en la lista de secuencias. Los péptidos S-alquilados se prepararon como se describe en el Ejemplo 1.

Los péptidos fueron sometidos a ensayo para analizar la actividad *in vitro* en cada receptor de glucagón, receptor de GLP-1 y receptor de GIP de la manera descripta básicamente en el Ejemplo 2. La EC50 en cada receptor para cada péptido se enumera en la Tabla 13.

TABLA 13

Estructura	Receptor de Glucagón			Receptor de GLP-1			Receptor de GIP		
HaibQGTFTSDYSKYLDERAAQDFVQW LLDGGPSSGAPPPSC(S-palmityl)**G-amida (SEQ ID NO: 163)**	0,006	**0,0008**	**2**	0,003	**0,0003**	**2**	0,051	**0,007**	**1**

HaibQGTFTSDYSKYLDERAAQDFVQW LLDGGPSSGAPPPSK(Cys S-palmityl)G**-amida (SEQ ID NO: 164)**	0,006	**0,0013**	**1**	0,003	**0,0003**	**1**	0,028	**0,001**	**1**
HaibQGTFTSDYSKYKLDERAAQDFVQ WLLDGGPSSGAPPPSK(γE-Cys S-palmityl)G**amida (SEQ ID NO: 165)**	0,009	**0,0013**	**1**	0,003	**0,0003**	**1**	0,043	**0,001**	**1**

EJEMPLO 21

La actividad *in vivo* de los péptidos seleccionados de los anteriores Ejemplos se sometió a ensayos mediante la administración de dichos péptidos a ratones DIO por la vía subcutánea en una dosis de 10nmol/kg de peso corporal. El peso corporal promedio de los ratones fue de 60 g y cada grupo tenía 8 ratones.

Entre los péptidos sometidos a ensayo se encontraban un péptido acilado con un separador de ácido gamma-glutámico, un péptido acilado con un separador dipeptídico de dos ácidos gamma-glutámico, un péptido succinilado de C16, un péptido acilado con un separador miniPEG que comprende la estructura $(-O-CH_2-CH_2-)_n$, donde n es 2, un péptido acilado con un separador miniPEG que

comprende la estructura $(-O-CH_2-CH_2-)_n$, donde n es 4, un péptido acilado con un separador miniPEG que comprende la estructura $(-O-CH_2-CH_2-)_n$, donde n es 8, un péptido alquilado S-palmityl, donde Lys es el residuo de esqueleto y Cys es un separador entre Lys y el grupo acilo, y un péptido alquilado S-palmitilo, donde Lys es el residuo de esqueleto y Cys de ácido gamma-glutámico es un separador dipeptídico entre Lys y el grupo acilo. Se midió el peso corporal y la ingesta de alimentos los Días 0, 1, 3, 5 y 7, mientras que los niveles de glucosa en sangre se midieron los Días 0 y 7.

Como lo muestra la Figura 8, muchos de los péptidos del ensayo demostraron una reducción de al menos 5% en el cambio total de peso corporal medido al Día 7.

EJEMPLO 22

Se prepararon tres homodímeros, cada uno de los cuales comprendía dos péptidos de SEQ ID NO: 167. El residuo de Lys en el extremo de C (en la posición 40) de cada péptido de SEQ ID NO: 167 fue amidado (en vez de contener un carboxilato alfa) y el grupo Epsilon NH2 del residuo de Lys estaba enlazado al residuo de Cys, que a su vez estaba unido al residuo del ácido gamma-glutámico. El residuo del ácido gamma-glutámico estaba unido al grupo acilo de C16. Cada mitad de dímero

estaba unida a la otra mitad mediante unión de disulfuro o de tíoeter. El Ejemplo 1 muestra la síntesis de los homodímeros.

Las estructuras de los productos resultantes se muestran en las Figuras 9A-9B.

Los homodímeros fueron sometidos a ensayo para analizar la actividad *in vitro* en cada receptor de glucagón, receptor de GLP-1 y receptor de GIP de la manera descripta básicamente en el Ejemplo 2. La EC50 en cada receptor para cada péptido se enumera en la Tabla 14.

TABLA 14									
Estructura de cada monómero	Receptor de Glucagon			Receptor de GLP-1			Receptor de GIP		
	EC_{50}	**Desv. Estándar**	**n***	**EC_{50}**	**Desv. Estándar**	**n***	**EC_{50}**	**Desv. Estándar**	**n***
Dímero de Disulfuro (disulfuro con dos acilaciones de C16) **HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(CysrE-C16)G-amida (SEQ ID NO: 168)**	0,004	**0,0007**	**2**	0,0022	**0,0003**	**2**	0,0044	**0,0002**	**2**

S-acetylDap K40 dímero *(tioéter con una acilación de C16)* **HaibQGTFTSDYSKYLDERAAQDFVQWLLDFGGPSSGAPPPSK(CysrE-C16)G-amida SEQ ID NO: 169)**	0,003	**0,0003**	**2**	0,002	**0,0003**	**2**	0,009	**0,0005**	**2**
S-acetylDap K40 dímero *(disulfuro con una acilación de C16)* **HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK(CysrEC16)G-amida SEQ ID NO: 170)**	0,003	**0,0003**	**1**	0,001	**0,0003**	**1**	0,014	**0,001**	**1**

EJEMPLO 23

Los ratones DIO (8 individuos por grupo, peso corporal promedio 54 g) fueron inyectados por la vía subcutánea con uno de los 4 péptidos descritos en la tabla que aparece a continuación, con una dosis de 1 nmol/kg/día o 3 nmol/kg/día, o el control de excipiente (*vehicle*) todos los días. Las estructuras y la actividad in vitro de cada receptor de glucagón, receptor de GLP-1 y receptor de GIP se presentan en la Tabla 15.

TABLA 15

Estructura	EC50 (nM) en Receptor de Glucagón [Desv. Estándar]	EC50 (nM) en Receptor de GLP-1 [Desv. Estándar]	EC50 (nM) en Receptor de GIP [Desv. Estándar]
HaibQGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPS-amida (SEQ ID NO: 28)	0,002 [0,001]	0,002 [0,001]	0,002 [0,001]

HaibQGTFTSDYSKYLDERAAQDFQVWLLDGGPSSG APPPSK(γEγE-C16)G-amida (SEQ ID NO: 89)	0,004 [0,001]	0,004 [0,001]	0,020 [0,006]
YaibEGTFTSDYSIYLDKQAAaibEFVNWLLAGGPS SGAPPPSK(C16)-amida (SEQ ID NO: 138)	0,053 [0,015]	0,003 [0,001]	0,002 [0,001]
HaibEGTFTSDYSKYLDERAAQDFVQWLLDGGPSSG APPPSK(γEγE-C16)G-amida (SEQ ID NO: 171)	0,455 [0,137]	0,005 [0,001]	0,038 [0,005]

El peso corporal y la ingesta de alimentos se midieron día por medio, desde el Día 0 (el día de la primera administración). La composición corporal se midió los Días 0 y 9, mientras que los niveles de glucosa en sangre se midieron ad lib los Días 0, 7, 14, y 20. ipGTT (1 g/kg) se midió el Día 20 a 0, 15, 20, 60, y 120 minutos de la inyección post-glucosa. La necropsia (en hígado y páncreas) y el sangrado final se midieron el día 22 (después de un ayuno durante la noche)

Como lo muestra la Figura 10, el peso corporal se redujo en todos los animales a los que se les suministró un péptido de la Tabla 15. La ingesta de alimentos medida el Día 19 se redujo en los animales a los que se les suministró un péptido de la Tabla 15, en comparación con

los ratones a los que se les suministró el control de excipiente (*vehicle*). Como lo muestra la Figura 11, los niveles de insulina (medidos el Día 21) también se redujeron en los animales a los que se les suministró un péptido, en comparación con el control de excipiente (*vehicle*). Notablemente, los ratones que presentaron la mayor reducción de peso fueron también los ratones que presentaron los menores niveles de insulina.

EJEMPLO 24

Los Péptidos que presentaron una capacidad de reducción del peso corporal, según el ensayo del Ejemplo 21, fueron seleccionados para estudios adicionales sobre mutación en los que los aminoácidos dentro de la mitad de extremo de C del péptido se modificaron de una o varias maneras. En la primera realización, el péptido que posee una secuencia de SEQ ID NO: 89 se altera de manera tal que el aminoácido en la posición 27, 28, o 29 se sustituyó con un residuo de alanina. En otra realización, el péptido que posee una secuencia de SEQ ID NO: 28 se modificó para tener una Glu en la posición 28 y una Arg en la posición 35. Se consideró que se formaría un puente de sal entre los dos aminoácidos para estabilizar la estructura de Trp en el extremo C del péptido. En otra realización, el péptido que posee una

secuencia de SEQ ID NO: 28 se modificó para incluir a Gly como aminoácido de extremo de C. Los péptidos, así como otros péptidos de similar apariencia al péptido de SEQ ID NO: 89 o un péptido de SEQ ID NO: 28, fueron sometidos a ensayo para analizar la actividad *in vitro* de cada receptor de glucagón, receptor de GLP-1. y receptor de GIP de la manera básicamente descripta en el Ejemplo 2. La Tabla 16 presenta los resultados.

TABLA 16

Estructura	EC50 (nM) en Receptor de Glucagón	EC50 (nM) en Receptor de GLP-1	EC50 (nM) en Receptor de GIP
HaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPSK(γEC16)G- NH_2 SEQ ID NO: 89)	0,006	0,005	0,028
HaibQGTFTSDK(γE-C16ac)SKYLDERAAQDFVQWLLDGGPSSGAPPP S-amida (SEQ ID NO: 28)	0,003	0,004	0,004

HaibQGTFTSDYSKYLDERAAQDFVQWL LAGGPSSGAPPPSK(γEγE-C16)G-NH_2 SEQ ID NO: 203)	0,003	0,001	0,007
HaibQGTFTSDYSKYLDERAAQDFVQWL LDA GPSSGAPPPSK(γEγE-C16)G-NH_2 SEQ ID NO: 204)	0,003	0,001	0,069
HaibQGTFTSDK(γE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPSG-NH_2 **(**SEQ ID NO: 205)	0,005	0,002	0,004
YaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPSK(K-bisγEC16)G-NH_2 (SEQ ID NO: 208)	0,031	0,025	0,079
YaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPSK(K-γEC16,γEC8)G-NH_2 (SEQ ID NO: 210)	0,010	0,005	0,359
YaibQGTFTSDYSKYLDERAAQDFVQWLL DGGPSSGAPPPSK(K-γEC16,γEC12)G-NH_2 (SEQ ID NO: 211)	0,010	0,006	0,038
HaibQGTFTSDYSKYLDERAAQDFVQWLLDG GPSSGAPPPSY(O-ácido palmítico)G-NH_2 SEQ ID NO: 212)	0,007	0,004	0,339

HaibQGTFTSDK(γEγE-C16)SKYLDERAAQDFVQWLLDGGPSSGAPPPSG-NH_2 SEQ ID NO: 207)	0,005	0,002	0,015

EJEMPLO 25

Los péptidos se prepararon de la manera básicamente descripta en el Ejemplo 1 y se sometieron a ensayos para analizar la actividad in vitro de cada receptor de glucagón, de GLP-1 y de GIP de la manera básicamente descripta en el presente. Las estructuras y los valores de la EC50 (nM) de cada péptido se presentan en la Tabla 17.

TABLA 17

Péptido	EC50 (nM) de Glucagon [Desv. Estándar]	EC50 (nM) de GLP-1 [Desv. Estándar]	EC50 (nM) de GIP [Desv. Estándar]

Glucagon	0.025 [0.003]		
GLP-1		0.025 [0.003]	
GIP			0.0065 [0.0001]
H**aib**QGTFTSDYSIYLDEKRAKEF VCWLLAGGPSSGAPPPSK-**amida** (SEQ ID NO: 230)	1.644 [0.302]	0.222 [0.034]	0.164 [0.010]

Todas las referencias, que incluyen las publicaciones, las solicitudes de patentes, y las patentes, citadas en la presente se incorporan por la presente como referencia en la misma medida que si cada referencia se indicara individual y específicamente que fueron incorporadas como referencia y se expusieron en su totalidad en la presente.

El uso de los términos “un” y “una” y “el” y referencias similares en el contexto de la descripción de la invención (especialmente en el contexto de las siguientes reivindicaciones) se debe interpretar que abarcan tanto el singular como el plural, a menos que se

indique de otro modo en la presente o que estén contradichas claramente por el contexto. Los términos “que comprende”, “que tiene”, “que incluye” y “que contiene” se debe interpretar que son términos de extremos abiertos (es decir, que significa “que incluye, pero en forma no taxativa”) a menos que se indique de otro modo.

La cita de gamas de valores de la presente simplemente se desea que sirvan como un método taquigráfico de referirse a cada valor por separado que están dentro de la gama y cada punto final, a menos que se indique de otro modo, y cada valor por separado y punto final se incorpora en la memoria descriptiva como si se citara individualmente en la presente.

Todos los métodos descritos en la presente se pueden realizar en cualquier orden adecuado a menos que se indique de otro modo en la presente o que esté claramente contradicho de otro modo por el contexto. El uso de todos y cada uno de los ejemplos, o expresiones de ejemplo (por ejemplo, “tal como”) dadas en la presente, simplemente iluminan mejor la invención y no plantean un límite sobre el alcance de la invención a menos que se indique de otro modo. No se debe interpretar que ninguna expresión en la memoria

descriptiva indica ningún elemento no reivindicado como esencial para la práctica de la invención.

En la presente se describen realizaciones preferidas de esta invención, que incluyen la mejor manera que conocen los inventores para realizar la invención. Las variaciones de estas realizaciones preferidas pueden hacerse evidentes para los expertos en el arte al leer la descripción precedente. Los inventores esperan que los expertos en el arte empleen dichas variaciones como apropiadas, y los inventores desean que la invención se practique de un modo diferente de aquel descrito específicamente en la presente. Por consiguiente, esta invención incluye todas las modificaciones y equivalentes del objeto citado en las reivindicaciones adjuntas a la presente como lo permite la ley aplicable. Además, todas las combinaciones de los elementos descritos anteriormente en todas las variaciones posibles de ellas están comprendidas por la invención a menos que se indique de otro modo en la presente o que esté contradicho de otro modo por el contexto.

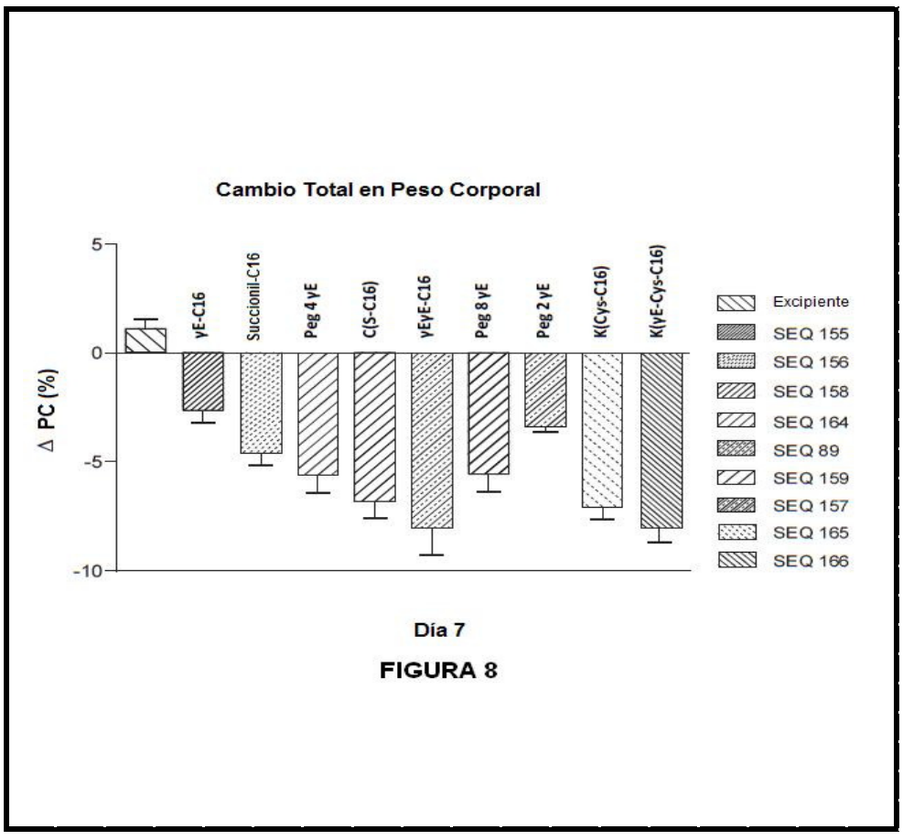

Cambio Total en Peso Corporal
5
0
-5
-10
Δ PC (%)
γE-C16
Succionil-C16
Peg 4 γE
C(S-C16)
γEγE-C16
Peg 8 γE
Peg 2 γE
K(Cys-C16)
K(γE-Cys-C16)
Excipiente
SEQ 155
SEQ 156
SEQ 158
SEQ 164
SEQ 89
SEQ 159
SEQ 157
SEQ 165
SEQ 166
Día 7

FIGURA 8

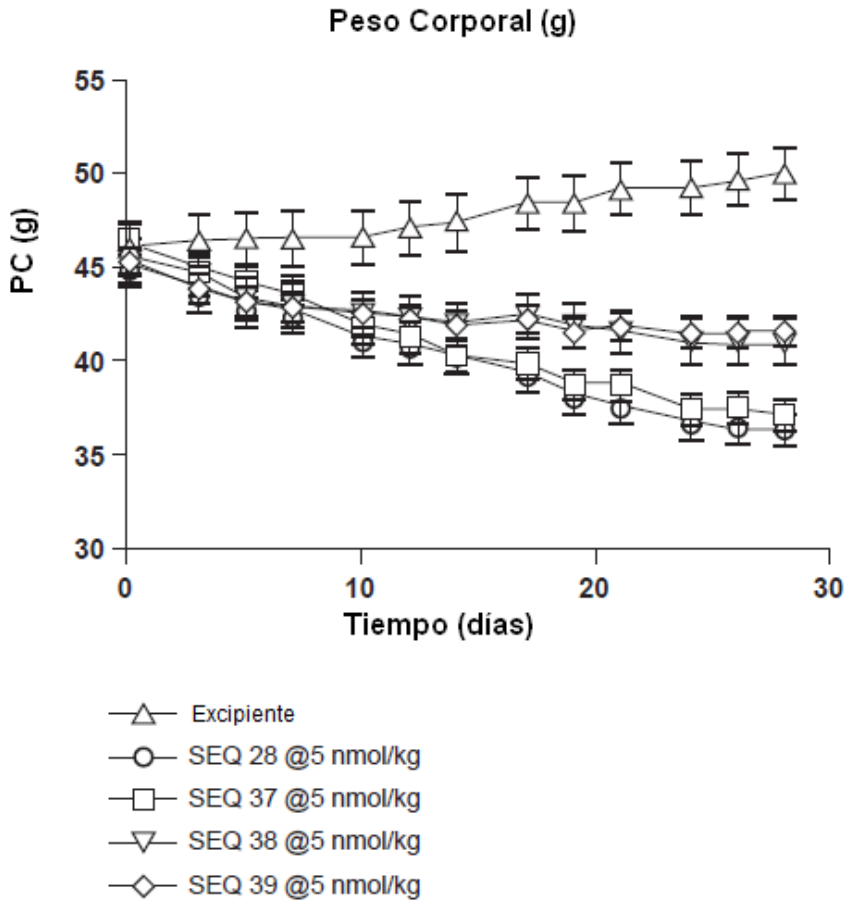

Peso Corporal (g)
PC (g)
55
50
45
40
35
30
0
10
20
30
Tiempo (días)
Excipiente
SEQ 28 @5 nmol/kg
SEQ 37 @5 nmol/kg
SEQ 38 @5 nmol/kg
SEQ 39 @5 nmol/kg

FIGURA 1

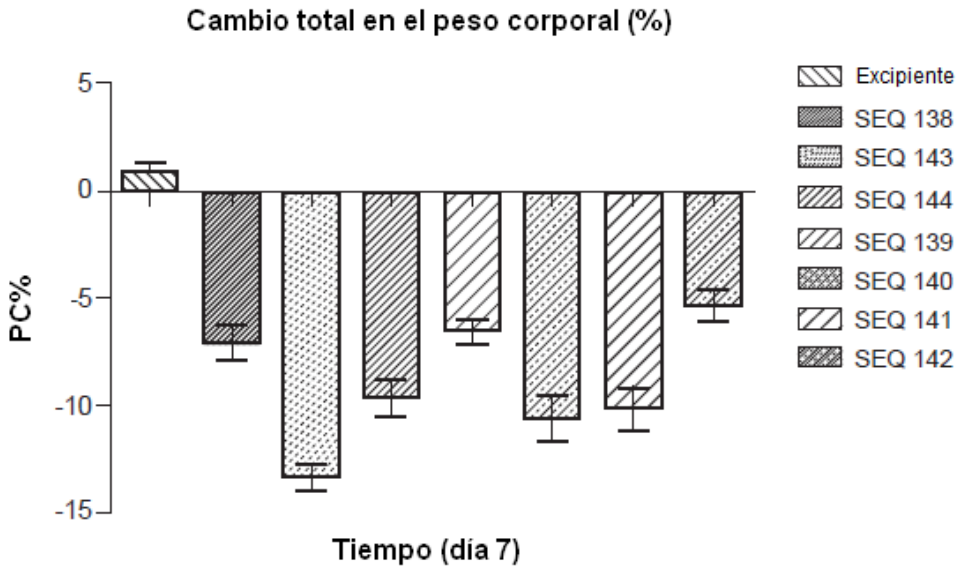

Cambio total en el peso corporal (%)
PC%
5
0
-5
-10
-15
Tiempo (día 7)
Excipiente
SEQ 138
SEQ 143
SEQ 144
SEQ 139
SEQ 140
SEQ 141
SEQ 142

FIGURA 2

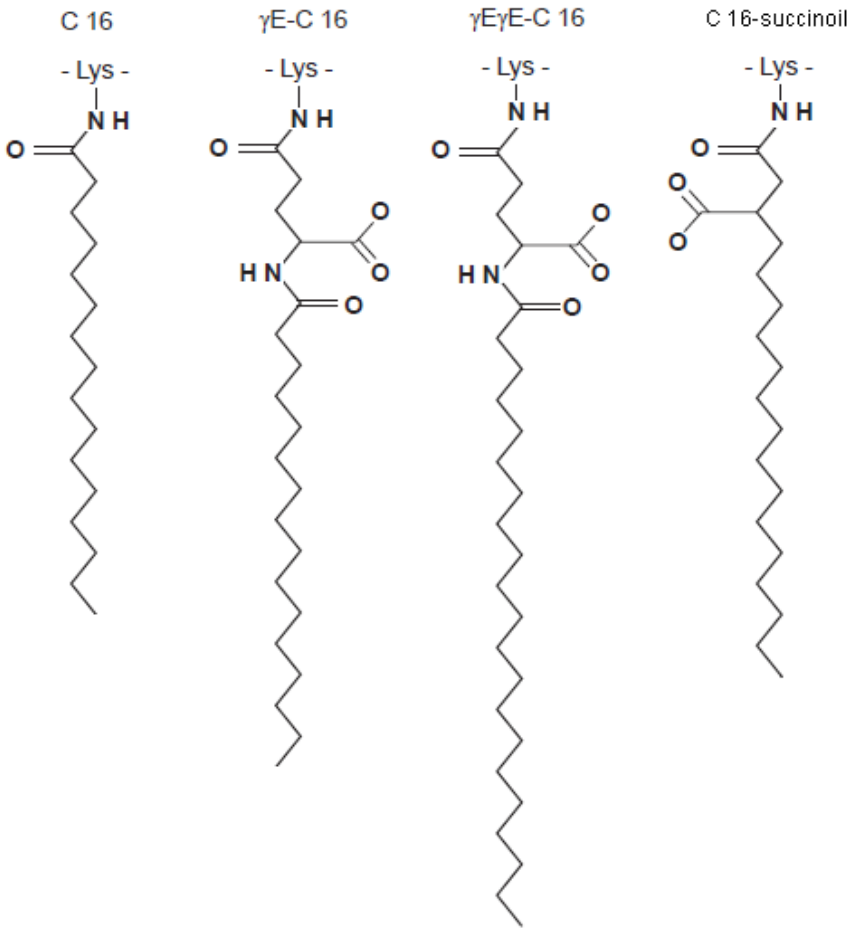
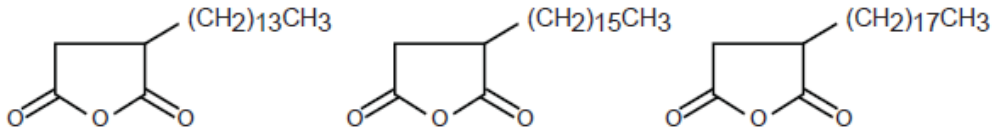

C 16
- Lys -
NH
O
γE-C 16
- Lys -
NH
O
HN
O
O
O
γEγE-C 16
- Lys -
NH
O
HN
O
O
O
C 16-succinoil
- Lys -
NH
O
O
O

FIGURA 3A



Tetradecilo Hexadecilo Octadecilo

FIGURA 3B

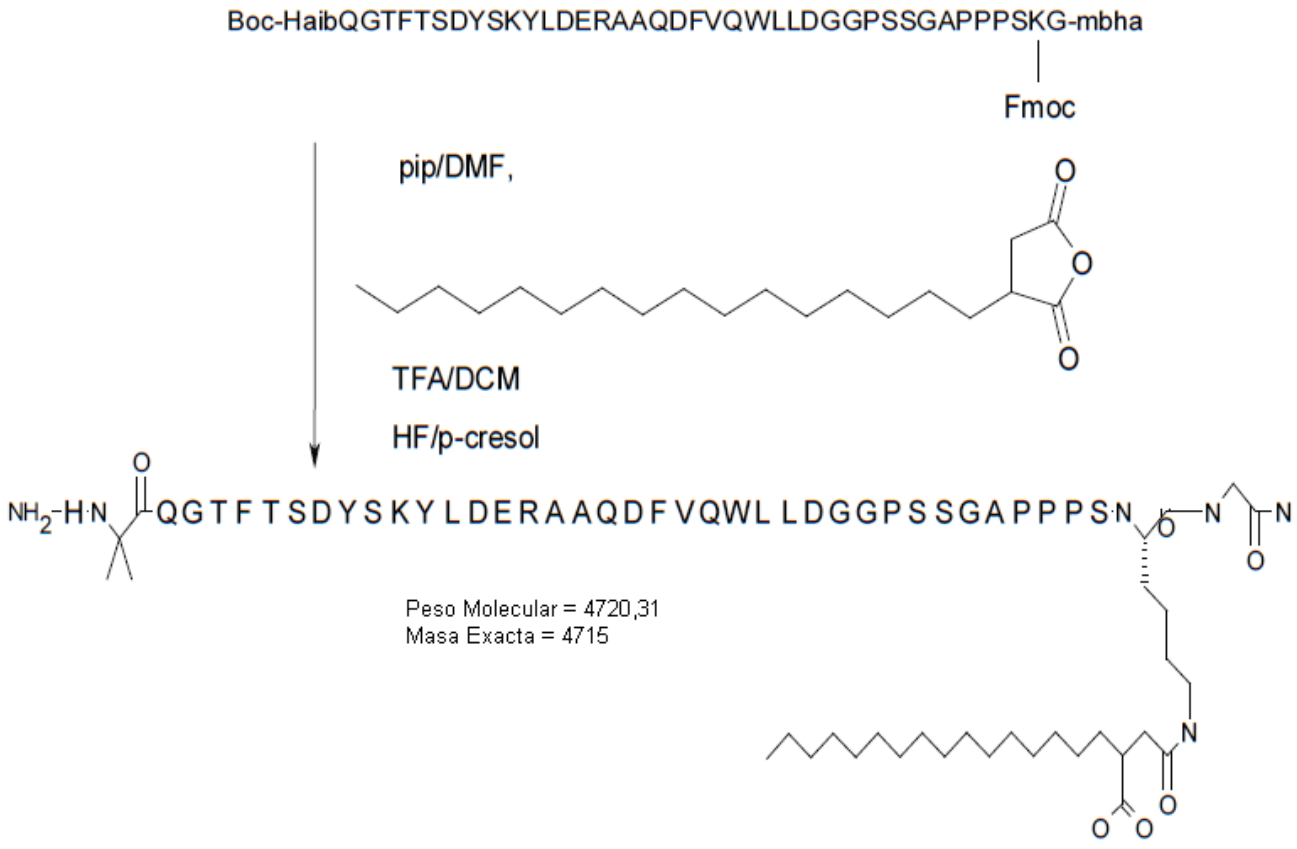

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSKG-mbha
Fmoc
pip/DMF,
TFA/DCM
HF/p-cresol
Peso Molecular = 4720,31
Masa Exacta = 4715

FIGURA 3C

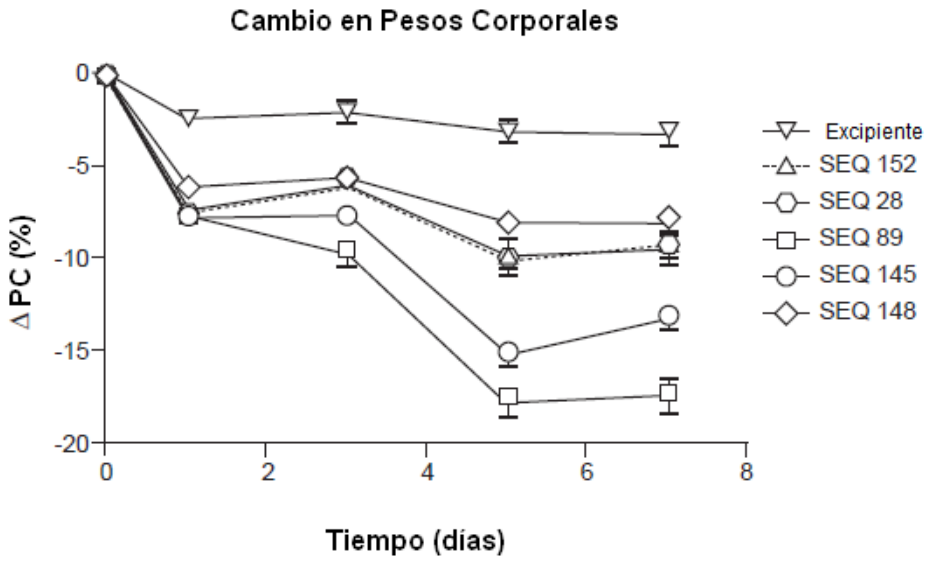

Cambio en Pesos Corporales
0
-5
-10
-15
-20
Δ PC (%)
0
2
4
6
8
Tiempo (días)
Excipiente
SEQ 152
SEQ 28
SEQ 89
SEQ 145
SEQ 148

FIGURA 4

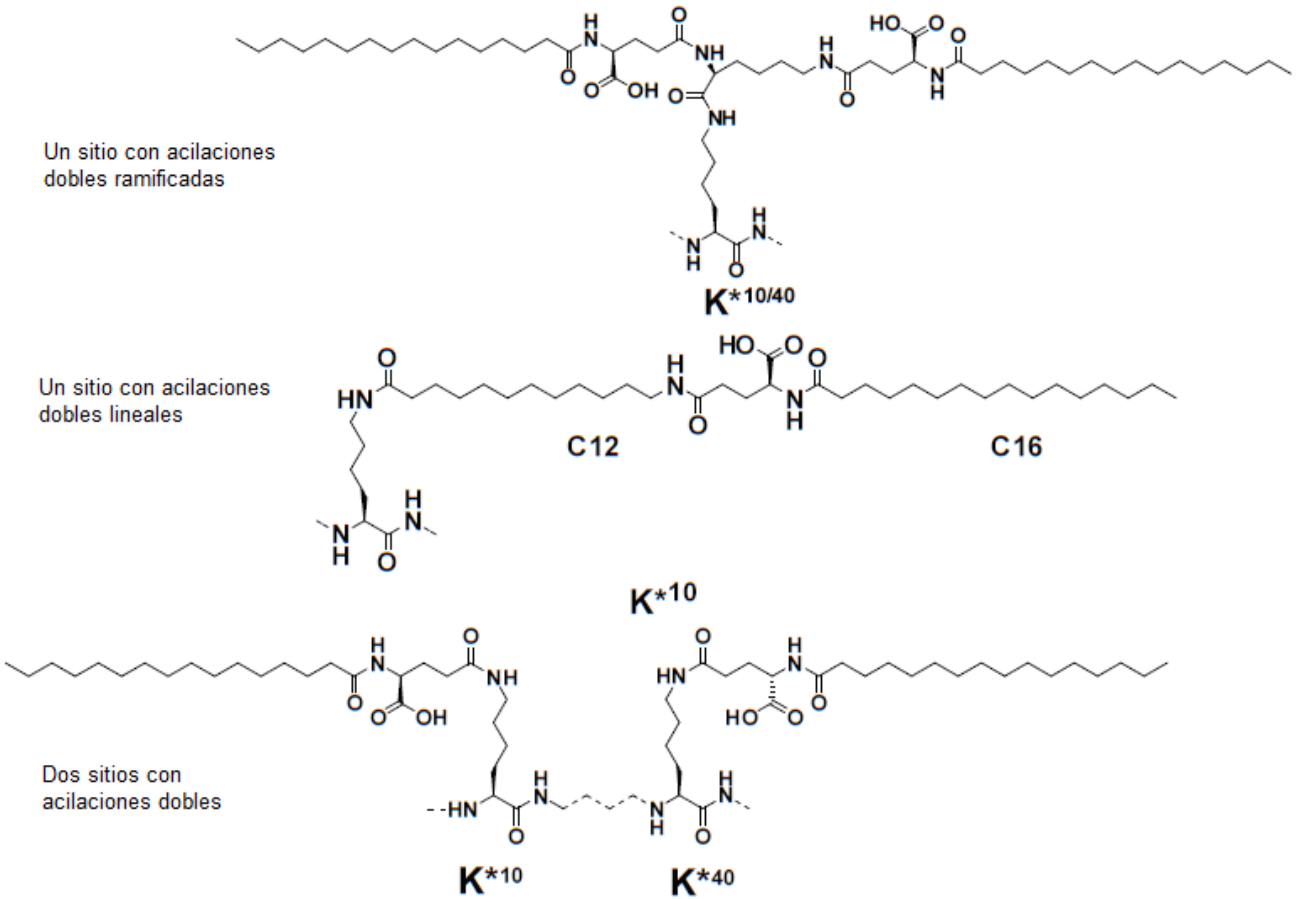

Un sitio con acilaciones dobles ramificadas
K*10/40
Un sitio con acilaciones dobles lineales
C12
C16
K*10
Dos sitios con acilaciones dobles
K*10
K*40

FIGURA 5A

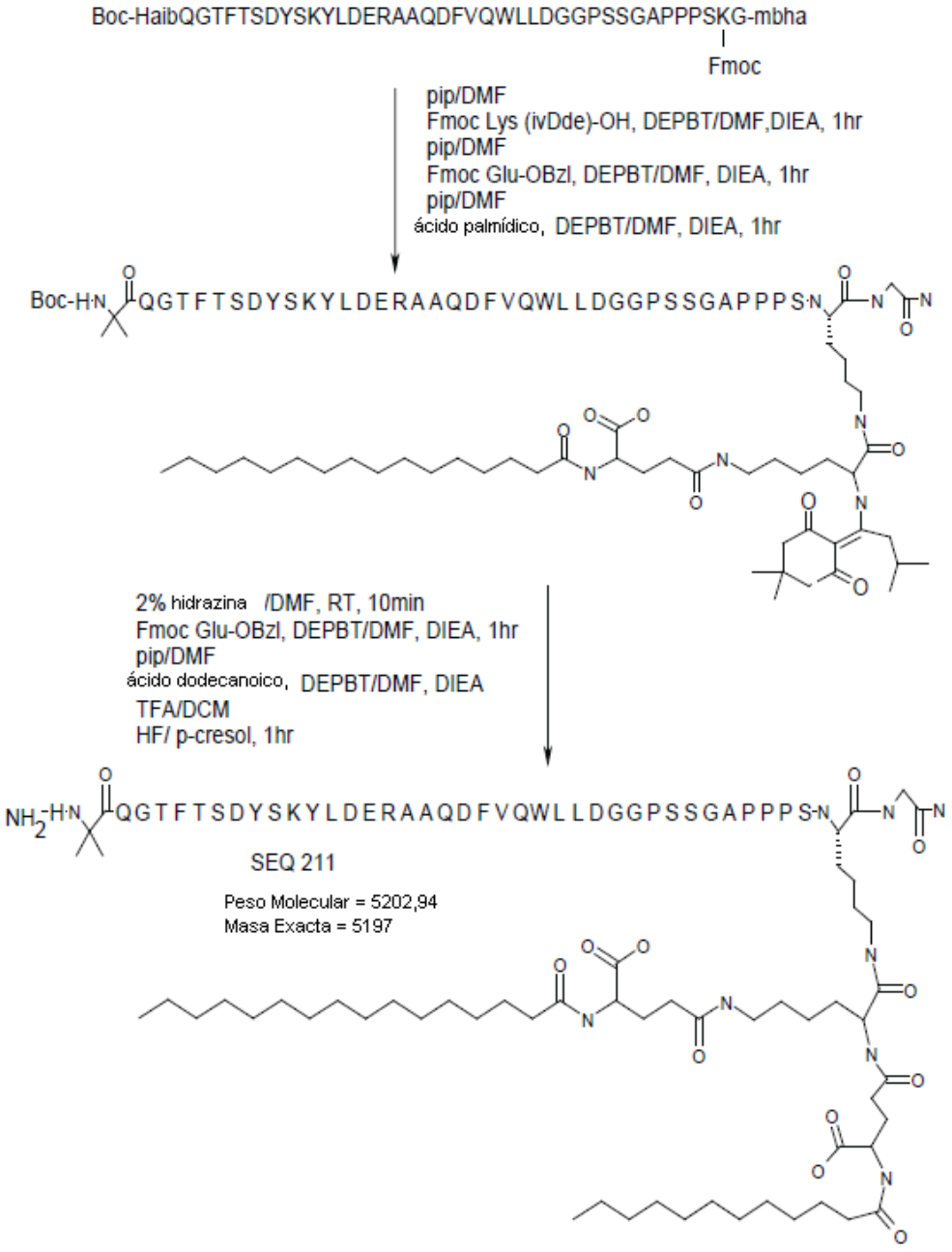

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSKG-mbha
Fmoc
pip/DMF
Fmoc Lys (ivDde)-OH, DEPBT/DMF,DIEA, 1hr
pip/DMF
Fmoc Glu-OBzl, DEPBT/DMF, DIEA, 1hr
pip/DMF
ácido palmídico, DEPBT/DMF, DIEA, 1hr
2% hidrazina /DMF, RT, 10min
Fmoc Glu-OBzl, DEPBT/DMF, DIEA, 1hr
pip/DMF
ácido dodecanoico, DEPBT/DMF, DIEA
TFA/DCM
HF/ p-cresol, 1hr
SEQ 211
Peso Molecular = 5202,94
Masa Exacta = 5197

FIGURA 5B

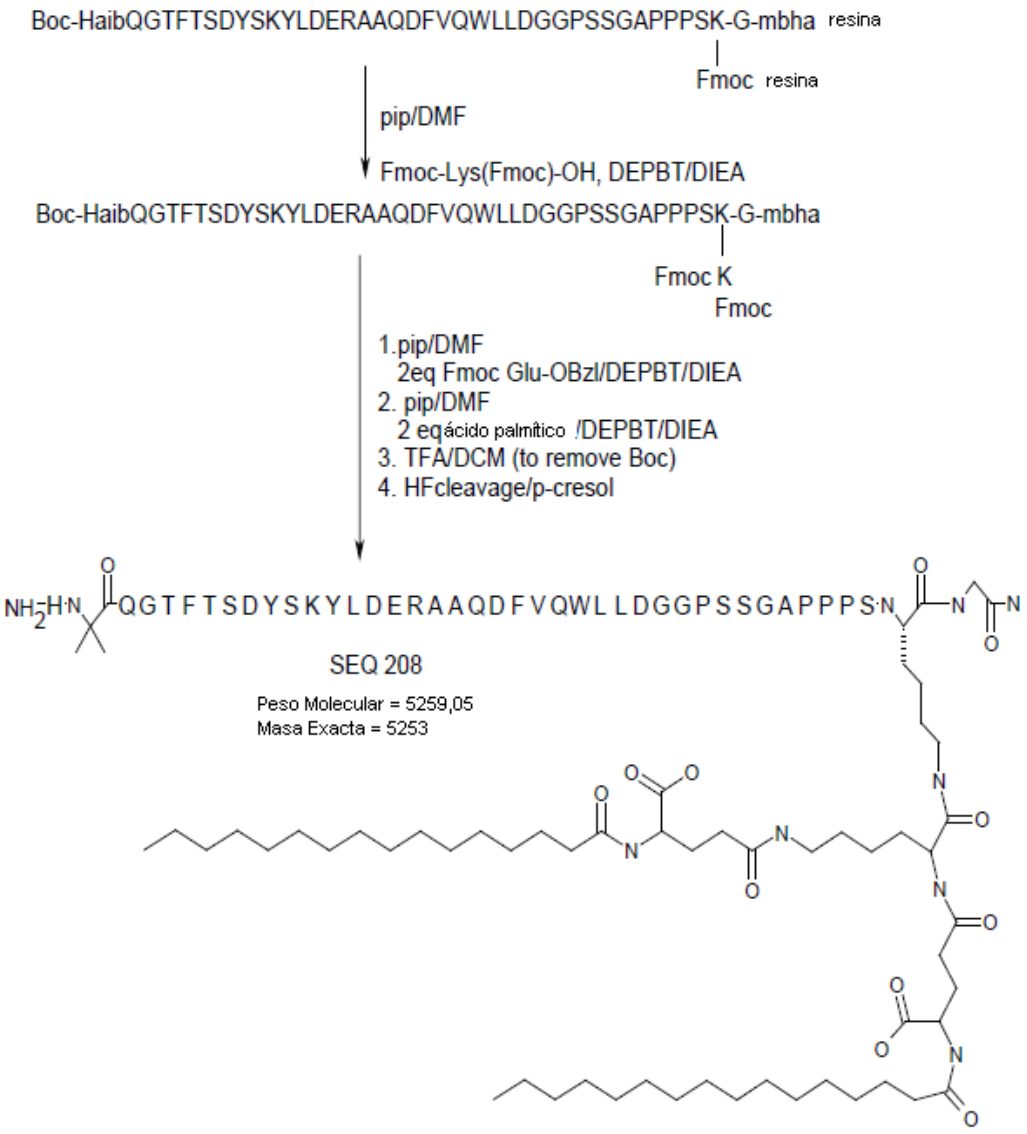

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK-G-mbha resina
Fmoc resina
pip/DMF
Fmoc-Lys(Fmoc)-OH, DEPBT/DIEA
Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPSK-G-mbha
Fmoc K
Fmoc
1.pip/DMF
2eq Fmoc Glu-OBzl/DEPBT/DIEA
2. pip/DMF
2 eq ácido palmítico /DEPBT/DIEA
3. TFA/DCM (to remove Boc)
4. HFcleavage/p-cresol
SEQ 208
Peso Molecular = 5259,05
Masa Exacta = 5253

FIGURA 5C

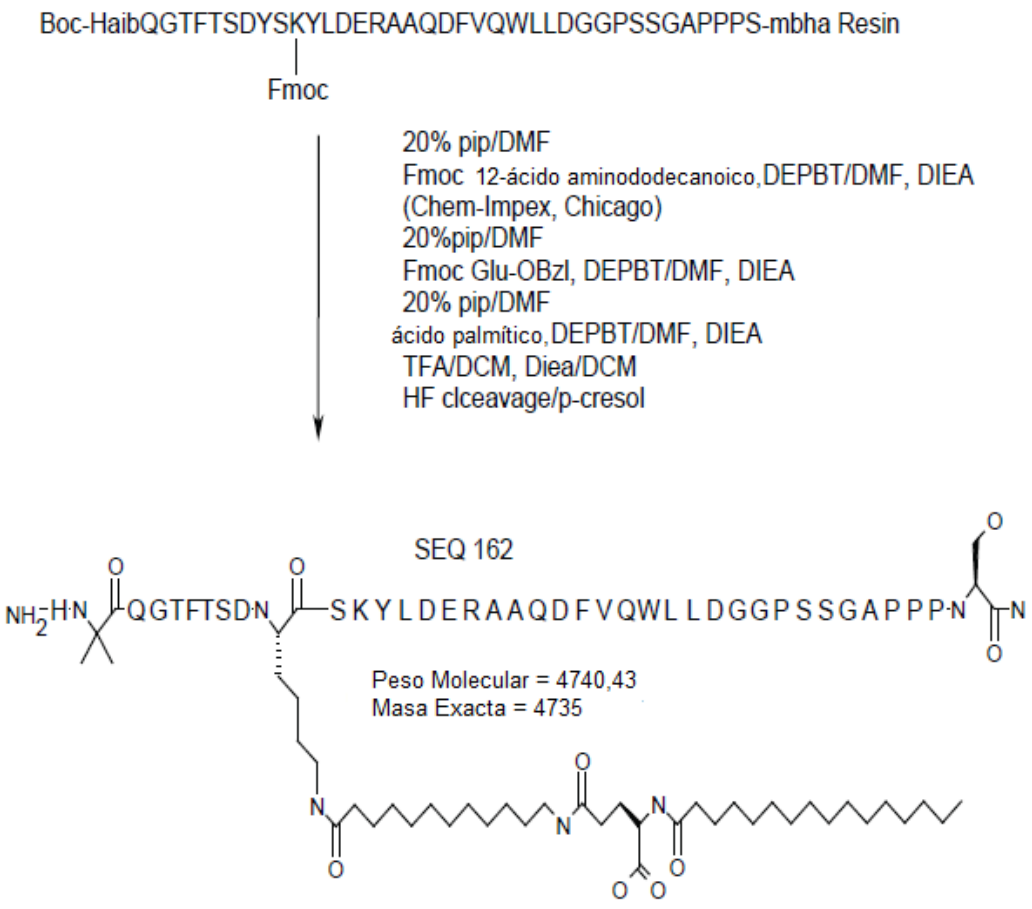

Boc-HaibQGTFTSDYSKYLDERAAQDFVQWLLDGGPSSGAPPPS-mbha Resin
Fmoc
20% pip/DMF
Fmoc 12-ácido aminododecanoico,DEPBT/DMF, DIEA
(Chem-Impex, Chicago)
20%pip/DMF
Fmoc Glu-OBzl, DEPBT/DMF, DIEA
20% pip/DMF
ácido palmítico,DEPBT/DMF, DIEA
TFA/DCM, Diea/DCM
HF clceavage/p-cresol
SEQ 162
Peso Molecular = 4740,43
Masa Exacta = 4735

FIGURA 5D

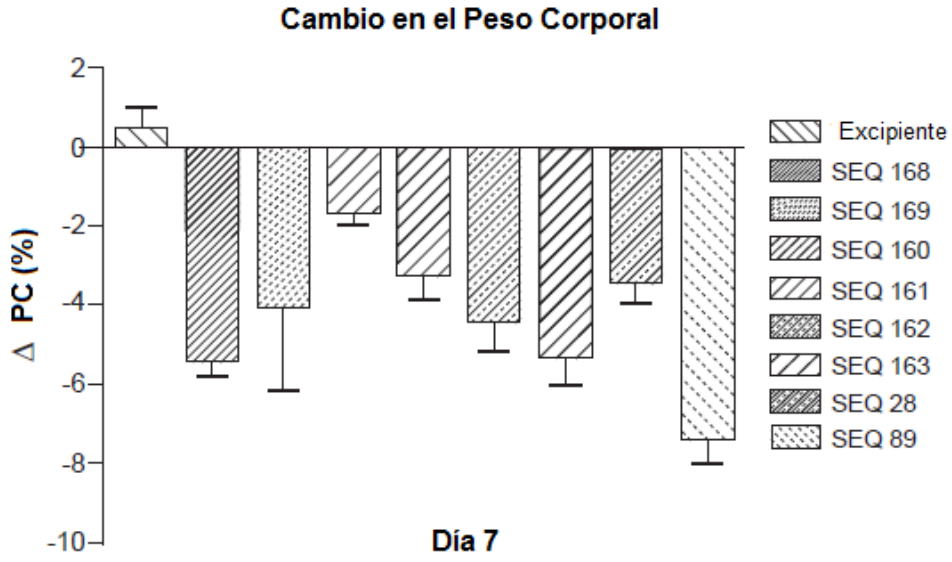

Cambio en el Peso Corporal
2
0
-2
-4
-6
-8
-10
Δ PC (%)
Día 7
Excipiente
SEQ 168
SEQ 169
SEQ 160
SEQ 161
SEQ 162
SEQ 163
SEQ 28
SEQ 89

FIGURA 5E

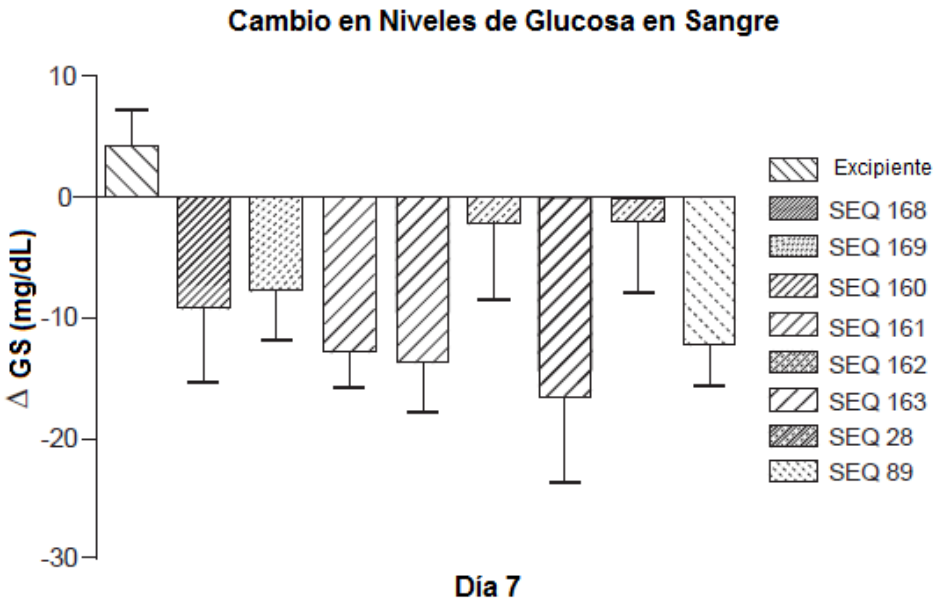
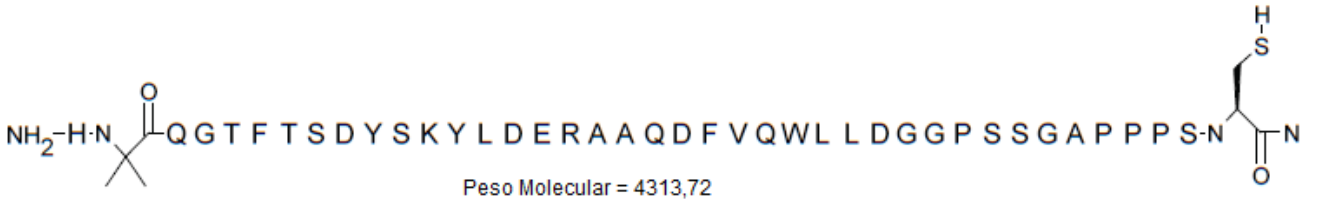
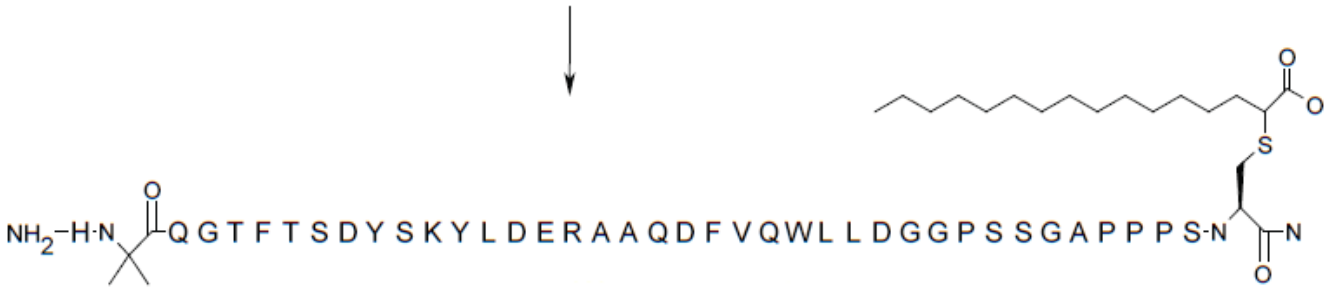

Cambio en Niveles de Glucosa en Sangre
10
0
-10
-20
-30
Δ GS (mg/dL)
Excipiente
SEQ 168
SEQ 169
SEQ 160
SEQ 161
SEQ 162
SEQ 163
SEQ 28
SEQ 89
Día 7

FIGURA 5F

Alquilación de S-palmitilo de la Cadena Principal de Cys en Triagonista



Peso Molecular = 4313,72
Masa Exacta = 4309



Peso Molecular = 4568,14
Masa Exacta = 4563

FIGURA 6

Alquilación de S-palmitilo de Cadena Lateral de Cys en Triagonista

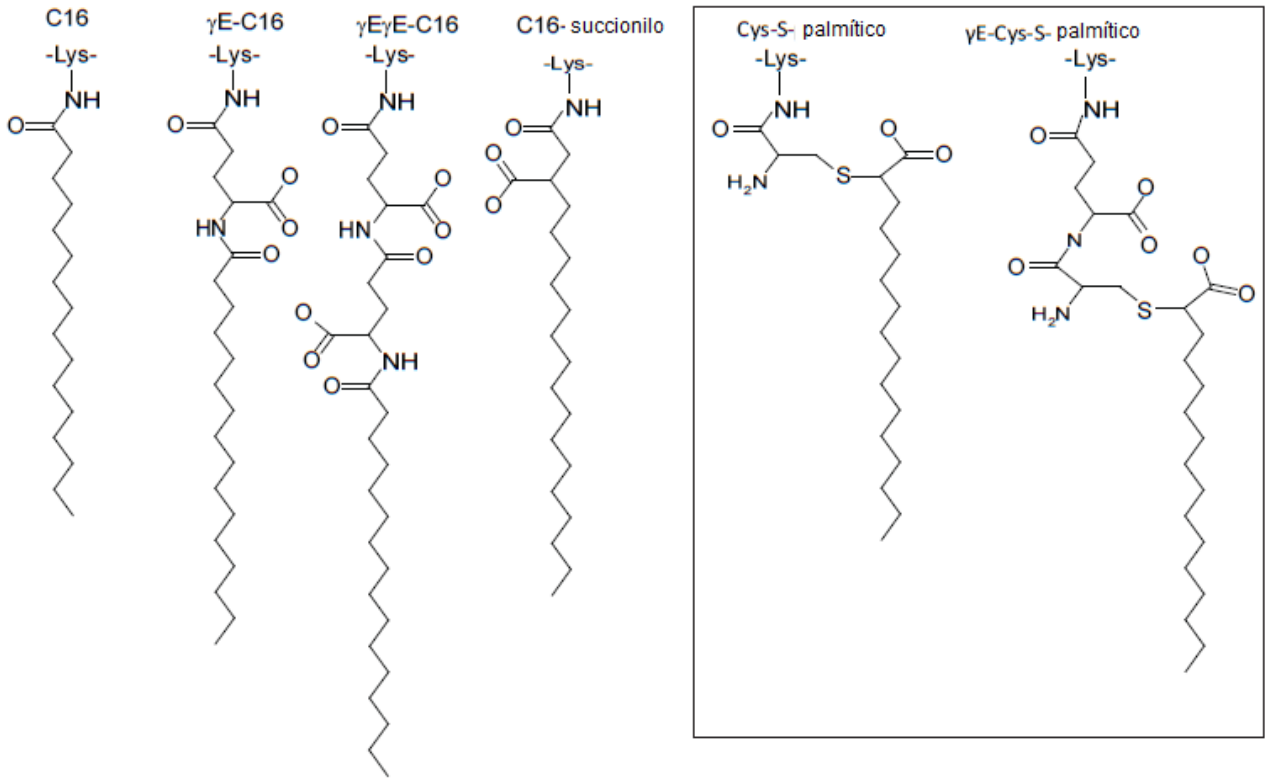


FIGURA 7

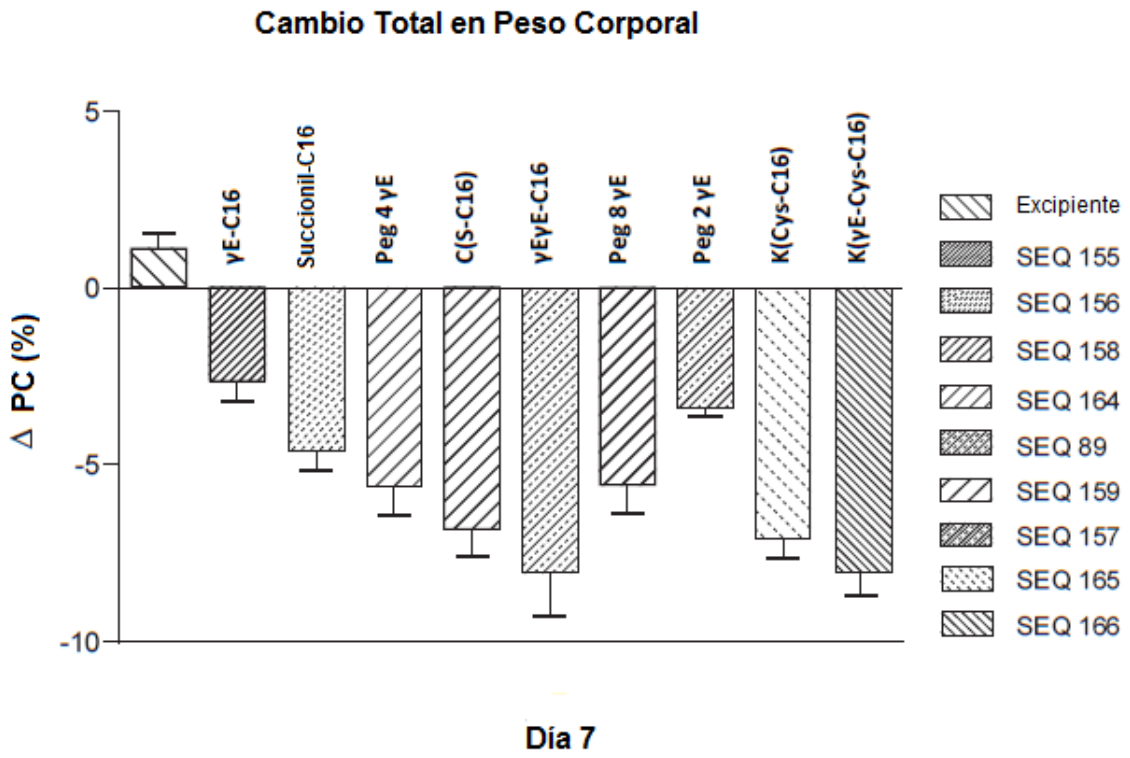
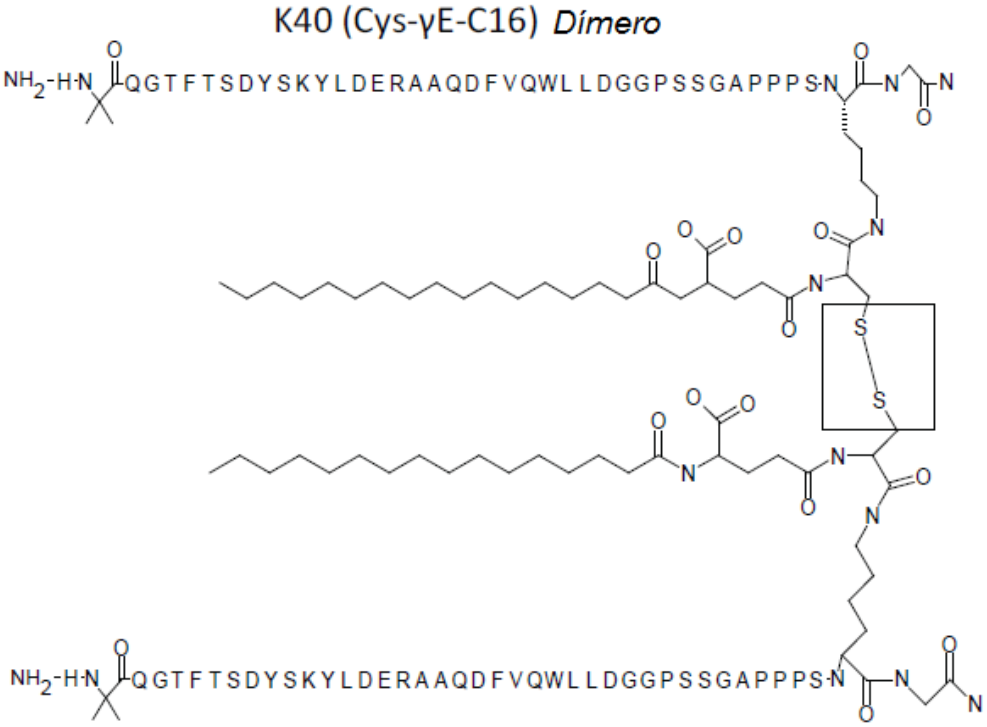

Cambio Total en Peso Corporal
5
0
-5
-10
Δ PC (%)
γE-C16
Succionil-C16
Peg 4 γE
C(S-C16)
γEγE-C16
Peg 8 γE
Peg 2 γE
K(Cys-C16)
K(γE-Cys-C16)
Excipiente
SEQ 155
SEQ 156
SEQ 158
SEQ 164
SEQ 89
SEQ 159
SEQ 157
SEQ 165
SEQ 166
Día 7

FIGURA 8



Peso Molecular = 4743,99
Masa Exacta = 9733

FIGURA 9A

Dimerización de Tioéter

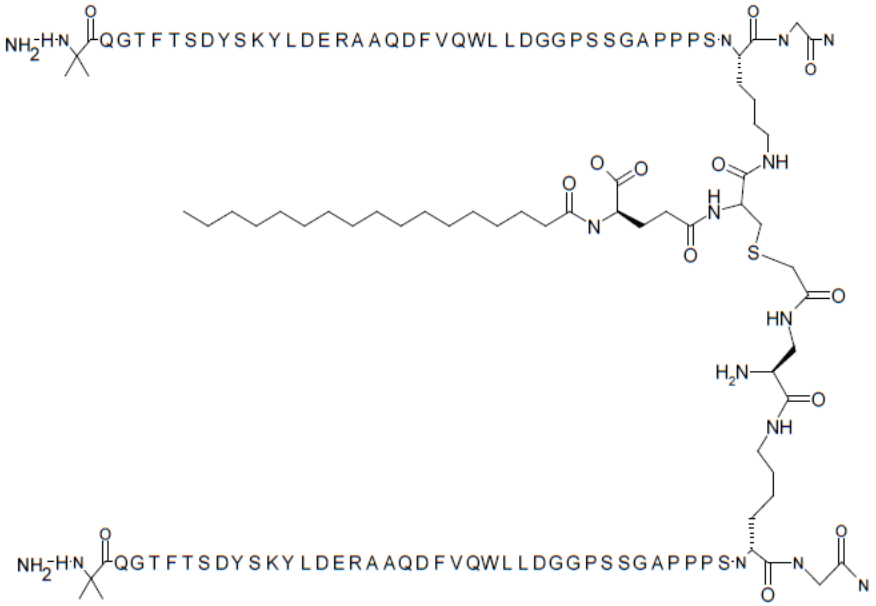


FIGURA 9B

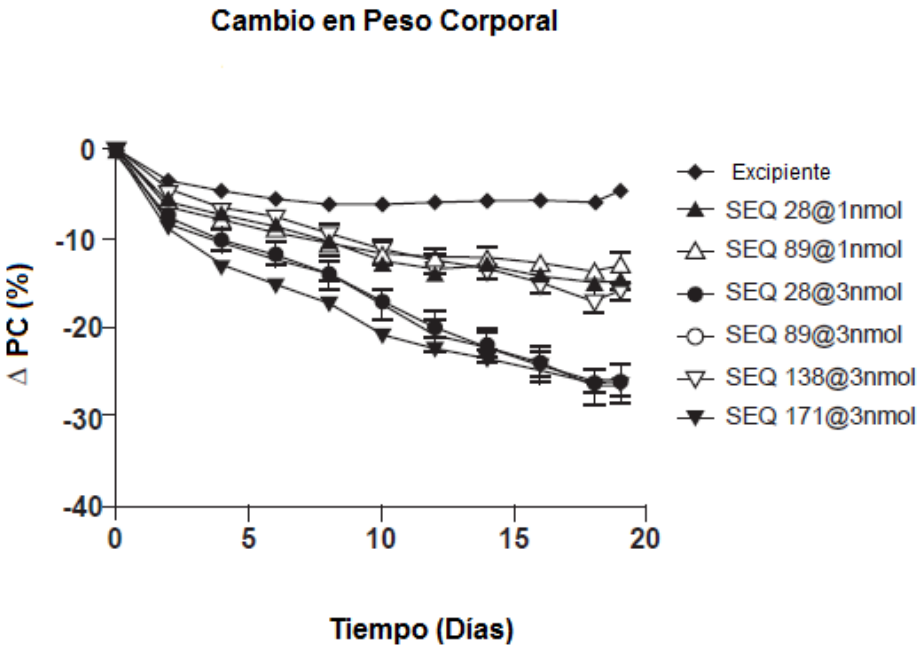

Cambio en Peso Corporal
0
-10
-20
-30
-40
Δ PC (%)
0
5
10
15
20
Tiempo (Días)
Excipiente
SEQ 28@1nmol
SEQ 89@1nmol
SEQ 28@3nmol
SEQ 89@3nmol
SEQ 138@3nmol
SEQ 171@3nmol

FIGURA 10

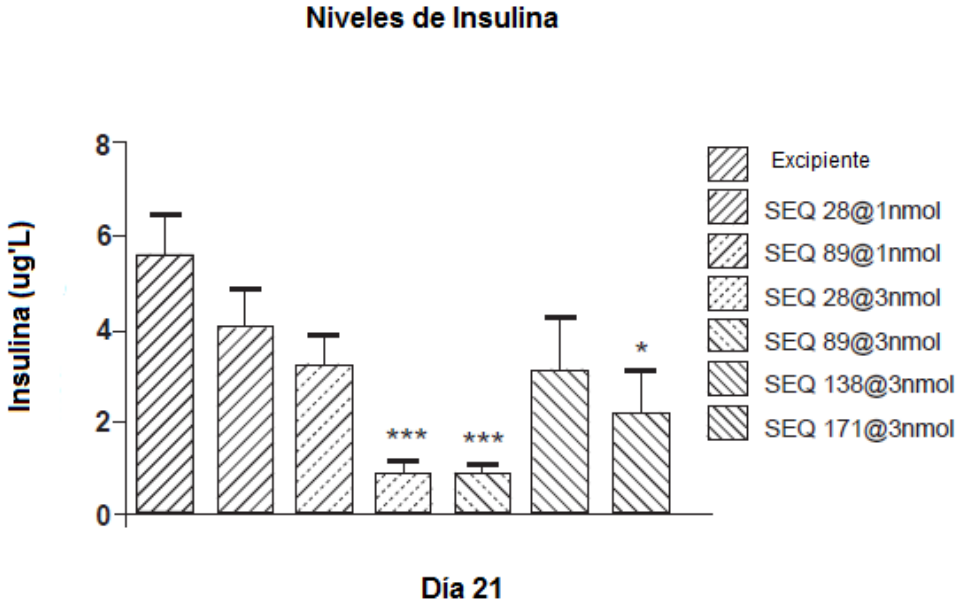

Niveles de Insulina
Insulina (ug'L)
8
6
4
2
0
***
***
*
Excipiente
SEQ 28@1nmol
SEQ 89@1nmol
SEQ 28@3nmol
SEQ 89@3nmol
SEQ 138@3nmol
SEQ 171@3nmol
Día 21

FIGURA 11

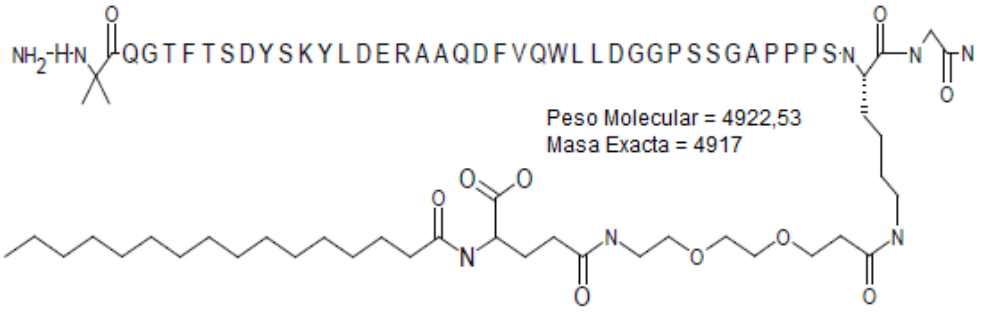


FIGURA 12A

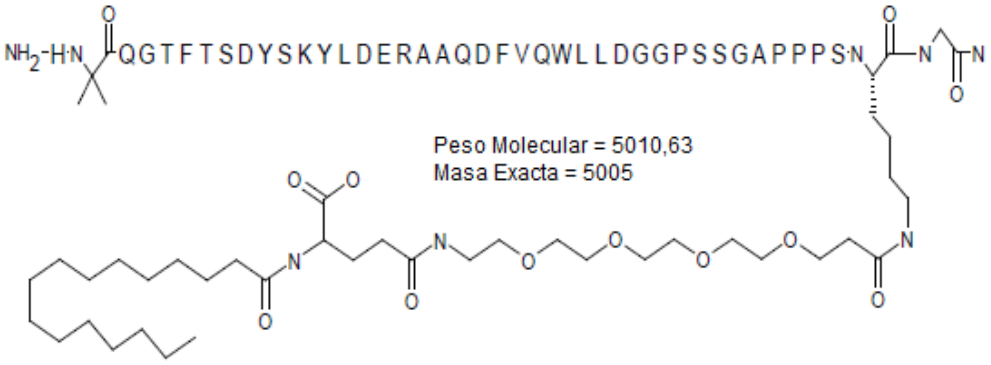


FIGURA 12B

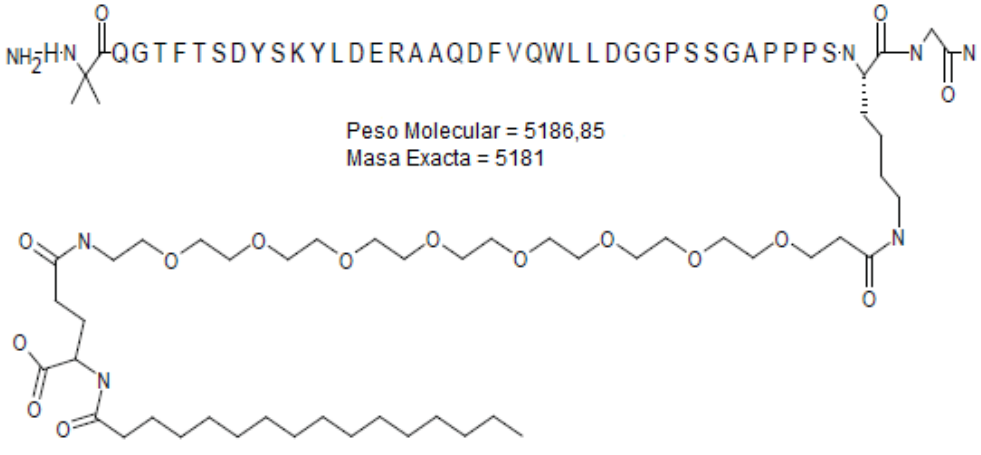


FIGURA 12C

LISTADO DE SECUENCIAS

<110> DiMarchi, et al.

<120> ANÁLOGOS DE GLUCAGÓN QUE PRESENTAN ACTIVIDAD DE RECEPTOR DE GIP

<130> 31135/45700A

<160> 262

<170> PatentIn version 3.5

<210> 1

<211> 29

<212> PRT

<213> Homo sapiens

<220>

<221> MISC_FEATURE

<223> Glucagon tipo salvaje

<400> 1

His Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Ser

1 5 10 15

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
20 25

<210> 2

<211> 42

<212> PRT

<213> Homo sapiens

<220>

<221> CADENA

<222> (1)..(42)

<223> Polipéptido inhibidor gástrico

<400> 2

Tyr Ala Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Asp Trp Lys His Asn Ile Thr Gln
35 40

<210> 3

<211> 30

<212> PRT

<213> Secuencia artificial

<220>

<223> Polipéptido sintético

<220>

<221> MISC_FEATURE

<222> (1)..(30)

<223> GLP-1(7-36) amidado

<220>

<221> MISC_FEATURE

<222> (30)..(30)

<223> Amidación de terminal C

<400> 3

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
20 25 30

<210> 4

<211> 31

<212> PRT

<213> Homo sapiens

<220>

<221> MISC_FEATURE

<222> (1)..(31)

<223> GLP-1(7-37)

<400> 4

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
20 25 30

<210> 5

<211> 10

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<400> 5

Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser
1 5 10

<210> 6
<211> 11
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<400> 6

Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser
1 5 10

<210> 7
<211> 8
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<400> 7

Lys Arg Asn Arg Asn Asn Ile Ala
1 5

<210> 8
<211> 4
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<400> 8

Lys Arg Asn Arg
1

<210> 9
<211> 11
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<400> 9

Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser Lys
1 5 10

<210> 10
<211> 40
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<223> mt-263

<220>
<221> MISC_FEATURE
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<223> Xaa es AIB

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 10

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 11

<211> 40

<212> PRT

<213> Secuencia artificial

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<220>

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<220>

<221> misc_feature

<222> (2)..(2)

<223> Xaa puede ser un aminoácido que sucede naturalmente

<220>

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<222> (20)..(20)

<223> Xaa puede ser un aminoácido que sucede naturalmente

<400> 11

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Lys Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 12

<211> 29

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-403

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> Puente de lactam entre residuos 16 y 20

<400> 12

His Ser Gln Gly Thr Phe Ile Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Glu Phe Ile Ala Trp Leu Met Asn Thr
20 25

<210> 13

<211> 29

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-404

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> Puente de lactam entre residuos 16 y 20

<400> 13

His Ser Gln Gly Thr Phe Ile Ser Asp Tyr Ser Lys Ala Leu Asp Glu
1 5 10 15

Ile His Gln Lys Glu Phe Ile Ala Trp Leu Met Asn Thr
20 25

<210> 14

<211> 29

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-405

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> Puente de lactam entre residuos 16 y 20

<400> 14

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Asp Phe Val Asn Trp Leu Leu Ala Gln
20 25

<210> 15

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-395

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> Puente de lactam entre residuos 16 y 20

<400> 15

His Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Asp Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser

35

<210> 16

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-396

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> Puente de lactam entre residuos 16 y 20

<400> 16

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Ile His Gln Lys Asp Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 17

<211> 29

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-397

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> Puente de lactam entre residuos 16 y 20

<400> 17

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Ile His Gln Lys Glu Phe Val Asn Trp Leu Leu Ala Gln
20 25

<210> 18

<211> 39

<212> PRT

<213> Secuencia artificial

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<223> Péptido sintético

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<221> MISC_FEATURE

<223> mt-398

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> Puente de lactam entre residuos 16 y 20

<400> 18

Tyr Ser Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 19
<211> 42
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<223> mt-217

<400> 19

Tyr Ala Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 20
<211> 42
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<223> mt-218

<220>
<221> MISC_FEATURE
<222> (2)..(2)

<223> Xaa es AIB

<400> 20

Tyr Xaa Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 21
<211> 42
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<223> mt-219

<400> 21

Tyr Ala Pro Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 22
<211> 42
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<223> mt-220

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<400> 22

Tyr Xaa Glu Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Cys Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 23

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-225

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> El puente de lactam une Glu16 y Lys20

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> La amina Epsilon de Lys se une a Ala-Ac-Cys(PEG), caracterizado porque Ac-Cys(PEG) es un residuo Cys que comprende un grupo amino alfa cubierto con un grupo acetilo (CH3CO) y que comprende 40 kDa PEG unido covalentemente a su cadena lateral

<400> 23

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Glu

1 5 10 15

Gln Ala Ala Lys Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 24

<211> 42

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-226

<400> 24

Tyr Ala Pro Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Cys Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 25

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-227

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (16)..(20)

<223> El puente de lactam une Glu16 y Lys20

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> La amina Epsilon de Lys se une a Arg-Ac-Cys(PEG), caracterizado porque Ac-Cys(PEG) es un residuo Cys que comprende un grupo amino alfa cubierto con un grupo acetilo (CH3CO) y que comprende 40 kDa PEG unido covalentemente a su cadena lateral

<400> 25

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 26

<211> 42

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<223> mt-228

<220>

<221> MISC_FEATURE

<222> (24)..(24)

<223> PEG (40kDa) unido a Cisteína

<400> 26

Tyr Ala Pro Gly Thr Phe Ile Ser Asp Tyr Ser Ile Ala Met Asp Lys
1 5 10 15

Ile His Gln Gln Asp Phe Val Cys Trp Leu Leu Ala Gln Lys Gly Lys
20 25 30

Lys Asn Trp Leu Lys His Asn Ile Thr Gln
35 40

<210> 27

<211> 39

<212> PRT

<213> Secuencia artificial

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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr

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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Met Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
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Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr

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Arg Arg Ala Lys Asp Phe Val Gln Trp Leu Met Asn Thr
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Ser Gly Ala Pro Pro Pro Ser Cys
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Ser Gly Ala Pro Pro Pro Ser Cys
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Glu Ala Ala Xaa Lys Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Ser Gly Ala Pro Pro Pro Ser Cys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Lys Gly
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Lys Cys
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Ser Gly Ala Pro Pro Pro Ser Cys

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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Gln Ala Ala Xaa Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Cys
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
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Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser

35

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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
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His Xaa Glu Gly Thr Phe Ile Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
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Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Met Asn Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Lys
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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
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Arg Arg Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Lys Gly
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Val Lys Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Lys Asp Gly Gly Pro Ser
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu

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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
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Ser Gly Ala Pro Pro Pro Ser
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His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
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Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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His Xaa Gln Gly Thr Phe Thr Ser Lys Tyr Ser Lys Tyr Leu Asp Xaa
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Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
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Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa

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Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr

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Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
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Arg Arg Ala Lys Asp Phe Val Gln Trp Leu Met Asn Thr
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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly

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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Cys
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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
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His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Arg Gly
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Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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His Xaa Glu Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser
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Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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1 5 10 15

Arg Arg Ala Xaa Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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1 5 10 15

Arg Arg Ala Xaa Glu Phe Val Gln Trp Leu Leu Asp Thr
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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Lys Gly
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1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Asp Thr
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Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
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Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser

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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
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Ser Gly Ala Pro Pro Pro Ser Lys
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Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
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Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys

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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
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Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
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Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys

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Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
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Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Gly
35 40

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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Gly
35 40

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<400> 147

His Xaa Gln Gly Thr Xaa Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
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His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

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<400> 149

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Xaa Leu Asp Glu
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Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

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His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser

35

<210> 151

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MOD_RES

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MOD_RES

<222> (13)..(13)

<223> Covalentemente unido a un grupo graso acilo C16 mediante gamma-Glu

<220>

<221> MOD_RES

<222> (39)..(39)

<223> Amidación de terminal C

<400> 151

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Lys Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 152
<211> 39
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
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<222> (2)..(2)
<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (14)..(14)

<223> Covalentemente unido a un grupo graso acilo C16 mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 152

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Lys Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 153

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Covalentemente unido a grupo acilo graso C16 mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 153

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Lys

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 154

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (17)..(17)

<223> Covalentemente unido a grupo acilo graso C16 mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 154

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Lys Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 155

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a un grupo graso acilo C16 mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 155

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 156

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Succinoil C16

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 156

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 157

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 que comprende un aminoácido miniPEG y un gamma- glu, caracterizado por el aminoácido miniPEG tiene una estructura {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, caracterizado porque n es 2

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 157

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 158

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a un grupo graso acilo C16 que comprende un aminoácido miniPEG y un gamma-Glu, caracterizado porque el amino ácido tiene la estructura {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, caracterizado porque n es 4

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 158

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser

20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly

35 40

<210> 159

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a un grupo graso acilo C16 que comprende un aminoácido miniPEG y un gamma-Glu, caracterizado porque el amino ácido tiene la estructura {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, caracterizado porque n es 8

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 159

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 160
<211> 39
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a Lys , caracterizado porque Lys se une a dos grupos acilos grasos C16, caracterizado porque acada grupo acilo graso C 16 se une a Lys mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 160

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 161

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Lys , caracterizado porque Lys se une a dos grupos acilos grasos C16, caracterizado porque acada grupo acilo graso C 16 se une a Lys mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 161

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 162
<211> 39
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa es AIB

<220>
<221> MISC_FEATURE
<222> (10)..(10)

<223> Covalentemente unido a un grupo acilo graso C12 que es covalentemente unido a un gamma- glu, que es covalentemente unido a un grupo acilo graso C16.

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 162

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 163

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a un grupo graso acilo C16 mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a un grupo graso acilo C16 mediante gamma-Glu

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 163

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 164
<211> 41
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa es AIB

<220>
<221> MISC_FEATURE
<222> (40)..(40)

<223> Sulfuro de Cys es S-alquilatado con -C(COO)H(CH2)13CH3

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 164

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys Gly
35 40

<210> 165

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Lys Covalentemente unido a Cys, el sulfurodel cual es S-alquilatado con -C(COO)H(CH2)13CH3

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 165

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 166

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a gamma-Glu, que se une a Cys, cuyo sulfuro es S-alquilatado con -C(COO)H(CH2)13CH3

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 166

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 167

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que es Covalentemente unido a gamma-Glu, que es covalentemente unido a un grupo acilo graso C16

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 167

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 168

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que se une a gamma-Glu, que se une a Grupo acilo graso C16

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a SEC ID NO 167 mediante Cys que es bisulfito unido al 2do Cys unido a Lys en posición 40 de la secuencia 167 y es unido a gamma-Glu, que, en cambio se une al Grupo acilo graso C16

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 168

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 169
<211> 41
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa es AIB

<220>
<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que se une a gamma-Glu, que se une a Grupo acilo graso C16 y el sulfuro de Cys es tioéter unido al péptido de Secuencia NO: 231 mediante Lys en posición 40

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 169

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 170

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que se une a gamma-Glu, que se une a Grupo acilo graso C16 y el sulfuro de Cys es bisufito unido al 2do Cys que se une al péptido de Secuencia NO: 231 mediante Lys en posición 40

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 170

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 171

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 mediante gamma-Glu-gamma-Glu

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 171

His Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 172

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (24)..(24)

<223> Covalentemente unido a PEG

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Grupo acilo graso C8

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 172

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 173
<211> 40
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa es AIB

<220>
<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (24)..(24)

<223> Covalentemente unido a PEG

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Grupo acilo graso C12

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 173

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 174

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (24)..(24)

<223> Covalentemente unido a PEG

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Grupo acilo graso C14

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 174

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 175

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> covalentemente se une a PEG

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 175

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Cys
35 40

<210> 176

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a C14 succinoil

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 176

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 177

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Grupo acilo graso C16

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 177

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly

35 40

<210> 178

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Xaa es es Tyr o un aminoácido covalentemente unido a acilo C10 a C26 o un grupo alquilo

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es Asp o Glu

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Asp of Glu

<220>

<221> MISC_FEATURE

<222> (35)..(35)

<223> Xaa es Ala, Arg o Lys

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Xaa está ausente o es Gly (por ejemplo cuando la posición 10 no es Tyr) o es un aminoácido covalentemente unido a acilo C10-C26 o grupo alquilo

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Opcionalmente presente cuando el residuo en posición 40 es un aminoácido covalentemente unido a grupo acilo o alquilo C10-C26, Xaa es Gly

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 178

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Xaa Gly Gly Pro Ser
20 25 30

Ser Gly Xaa Pro Pro Pro Ser Xaa Xaa
35 40

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<211> 41

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<222> (2)..(2)

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<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un grupo alquilo o acilo C10 a C26

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<222> (18)..(18)

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<223> Xaa es AIB o Gln

<220>

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<222> (21)..(21)

<223> Xaa es Asp o Glu

<220>

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<222> (40)..(40)

<223> Xaa está ausente o es Gly (por ejemplo cuando la posición 10 no es Tyr) o es un aminoácido covalentemente unido a grupo acilo o alquilo C10-C26

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<222> (41)..(41)

<223> Opcionalmente presente, cuando el residuo en posición 40 es un aminoácido covalentemente unido a un grupo alquilo o acilo C10-C26 entonces Xaa es Gly

<220>

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<222> (41)..(41)

<223> Amidación de terminal C

<400> 179

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Xaa
35 40

<210> 180

<211> 41

<212> PRT

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<222> (2)..(2)

<223> Xaa es AIB

<220>

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<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 mediante gamma-Glu-gamma-Glu

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 180

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 181

<211> 29

<212> PRT

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<223> Péptido sintético

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<222> (1)..(29)

<223> Al menos un aminoácido es un aminoácido covalentemente unido a grupo alquilo o acilo C10 a C26

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<222> (2)..(2)

<223> Xaa es AIB

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<223> Xaa es Gln o Gln Análogo

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<222> (10)..(10)

<223> Xaa es Lys o Tyr

<220>

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<223> Xaa es Asp o Glu

<220>

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<222> (29)..(29)

<223> Xaa es Thr o Gly

<220>

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<222> (29)..(29)

<223> Amidación de terminal C

<400> 181

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Asp Xaa
20 25

<210> 182

<211> 29

<212> PRT

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<220>

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<222> (10)..(10)

<223> Xaa es Lys Covalentemente unido a grupo alquilo o acilo C10 a C26

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<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

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<223> Xaa es AIB o Gln

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<223> Xaa es Asp o Glu

<220>

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<222> (29)..(29)

<223> Xaa es Gly o Thr

<220>

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<222> (29)..(29)

<223> Amidación de terminal C

<400> 182

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Leu Asp Xaa
20 25

<210> 183

<211> 10

<212> PRT

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<223> Péptido sintético

<400> 183

Gly Pro Ser Ser Gly Arg Pro Pro Pro Ser
1 5 10

<210> 184

<211> 39

<212> PRT

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<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa es Gln o Gln Análogo

<220>

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<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un grupo alquilo o acilo C12 aC18

<220>

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<222> (16)..(16)

<223> Xaa es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro, y Ser

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es un aminoácido de carga negativa o un aminoácido de carga neutral, opcionalmente, AIB o Gln

<220>

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<222> (21)..(21)

<223> Xaa es un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala o es un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Ala o Gly

<220>

<221> MISC_FEATURE

<222> (35)..(35)

<223> Xaa es Ala o es un aminoácido básico, opcionalmente, Arg o Lys

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 184

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Xaa Pro Pro Pro Ser
35

<210> 185

<211> 39

<212> PRT

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<223> Xaa es AIB

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<222> (10)..(10)

<223> Xaa es Tyr o es un aminoácido ácido covalentemente unido a un grupo alquilo o acilo C12 to C18

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa es Glu, un alfa, aminoácido alfa disustituido o Lys.

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Gly o Thr

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 185

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 186
<211> 29
<212> PRT
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<220>
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<223> Xaa es AIB

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<222> (3)..(3)

<223> Xaa es Gln o Gln Análogo

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un grupo alquilo o acilo C10 a C26

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro, y Ser

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es un aminoácido de carga negativa o un aminoácido de carga neutral, opcionalmente, AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala o un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Ala, Gly o Thr

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Amidación de terminal C

<400> 186

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa

1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa

20 25

<210> 187

<211> 29

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

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<223> Xaa es AIB

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<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a a acilo C10 a C26 o grupo alquilo

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa es Glu, alfa, aminoácido alfa- disustituido, o Lys

<220>

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<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es a un aminoácido de carga negativa o un aminoácido de carga neutral opcionalmente, AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Gly o Thr

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Amidación de terminal C

<400> 187

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

<210> 188

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

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<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa es un aminoácido ácido, básico o hidrofóbico, opcionalmente, Glu

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un acilo C12 a C18 o grupo alquilo

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa puede ser cualquier aminoácido, opcionalmente cualquier aminoácido que no sea Gly, Pro o Ser

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es a un aminoácido de carga negativa o un aminoácido de carga neutral, opcionalmente, AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala o un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Ala o Gly

<220>

<221> MISC_FEATURE

<222> (35)..(35)

<223> Xaa es Ala o un aminoácido básico, opcionalmente, Arg o Lys

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 188

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Xaa Pro Pro Pro Ser
35

<210> 189

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

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<223> Xaa es AIB

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<221> MISC_FEATURE

<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un acilo C12 a C18 o un grupo alquilo

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<222> (16)..(16)

<223> Xaa es Glu, un alfa, aminoácido alfa- disustituido o Lys

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es Asp o Glu

<220>

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<222> (27)..(27)

<223> Xaa es Leu, Nle, o Met

<220>

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<222> (28)..(28)

<223> Xaa es Ala, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Gly o Thr

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 189

His Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa

1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 190

<211> 29

<212> PRT

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<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

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<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa es un aminoácido ácido, básico o hidrofóbico, opcionalmente, es Glu

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un acilo C10 a C26 o grupo alquilo

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro, y Ser

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es a un aminoácido de carga negativa o un aminoácido de carga neutral, opcionalmente, AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala o un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Ala, Gly o Thr

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Amidación de terminal C

<400> 190

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

<210> 191

<211> 29

<212> PRT

<213> Secuencia artificial

<220>

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<223> Xaa es Tyr o un aminoácido covalentemente unido a un acilo C10 a C26 o grupo alquilo

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa es Glu, alfa, aminoácido alfa- disustituido, o Lys

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es a un aminoácido de carga negativa o un aminoácido de carga neutral opcionalmente, AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Gly o Thr

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Amidación de terminal C

<400> 191

His Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

<210> 192

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa es Gln o Gln Análogo o aminoácido ácido, básico o hidrofóbico, opcionalmente, es Glu o Gln

<220>

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<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un acilo C12 a C18 o grupo alquilo

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro, y Ser

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es un aminoácido de carga negativa o un aminoácido de carga neutral, opcionalmente, AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala o un aminoácido ácido, opcionalmente, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Ala o Gly

<220>

<221> MISC_FEATURE

<222> (35)..(35)

<223> Xaa es Ala o un aminoácido básico, opcionalmente, Arg o Lys

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 192

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Xaa Pro Pro Pro Ser
35

<210> 193
<211> 39
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
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<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa es Gln o Gln Análogo o aminoácido ácido, básico o hidrofóbico, opcionalmente, es Glu o Gln

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Xaa es Tyr o un aminoácido covalentemente unido a un acilo C12 a C18 o grupo alquilo

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa es Glu, un alfa, aminoácido alfa disustituidoo Lys

<220>

<221> MISC_FEATURE

<222> (18)..(18)

<223> Xaa es Arg o Ala

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB o Gln

<220>

<221> MISC_FEATURE

<222> (21)..(21)

<223> Xaa es Asp o Glu

<220>

<221> MISC_FEATURE

<222> (27)..(27)

<223> Xaa es Leu, Nle, o Met

<220>

<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa es Ala, Asp o Glu

<220>

<221> MISC_FEATURE

<222> (29)..(29)

<223> Xaa es Gly o Thr

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 193

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 194
<211> 29
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa es AIB

<220>
<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa es Gln o Gln Análogo o aminoácido ácido, básico o hidrófico, opcionalmente, es Glu o Gln

<220>

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<222> (10)..(10)

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<222> (16)..(16)

<223> Xaa es cualquier aminoácido, opcionalmente, cualquier aminoácido que no sea Gly, Pro, y Ser

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<223> Xaa es Arg o Ala

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<223> Xaa es a un aminoácido de carga negativa o un aminoácido de carga neutral, opcionalmente, AIB o Gln

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<222> (21)..(21)

<223> Xaa es un aminoácido ácido, opcionalmente, Asp o Glu

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<222> (27)..(27)

<223> Xaa es Leu, Ala, Nle, o Met

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<222> (28)..(28)

<223> Xaa es Ala o un aminoácido ácido, opcionalmente, Asp o Glu

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<222> (29)..(29)

<223> Xaa es Ala, Gly o Thr

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<223> Amidación de terminal C

<400> 194

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa

1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa

20 25

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<223> Xaa es AIB

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<223> Xaa es Gln o Gln Análogo o aminoácido ácido, básico o hidrófico, opcionalmente, es Glu o Gln

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<223> Xaa es Tyr o un aminoácido covalentemente unido a un acilo C10 a C26 o grupo alquilo

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<223> Xaa es Glu, alfa, aminoácido alfa- disustituido, o Lys

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<223> Xaa es a un aminoácido de carga negativa o un aminoácido de carga neutral, opcionalmente, AIB o Gln

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<223> Xaa es Asp o Glu

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<223> Xaa es Gly o Thr

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<223> Amidación de terminal C

<400> 195

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

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<223> Amidación de terminal C

<400> 196

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Xaa Pro Pro Pro Ser
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<222> (39)..(39)

<223> Amidación de terminal C

<400> 197

His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser

35

<210> 198

<211> 29

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His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
20 25

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<223> Amidación de terminal C

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His Xaa Xaa Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Arg Xaa Ala Xaa Xaa Phe Val Gln Trp Leu Xaa Xaa Xaa
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Glu Arg Ala Ala Gln
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Glu Arg Ala Ala Gln Asp
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<223> Xaa es AIB

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<222> (40)..(40)

<223> Covalentemente unido a un grupo acilo graso C16 mediante gamma-Glu-gamma-Glu

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<222> (41)..(41)

<223> Amidación de terminal C

<400> 203

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 204

<211> 41

<212> PRT

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<220>

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<223> Amidación de terminal C

<400> 204

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Ala Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

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<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 205

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly
35 40

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<211> 39

<212> PRT

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<400> 206

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Glu Gly Gly Pro Ser
20 25 30

Ser Gly Arg Pro Pro Pro Ser
35

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<211> 40

<212> PRT

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<223> Amidación de terminal C

<400> 207

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly

35 40

<210> 208

<211> 41

<212> PRT

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<223> Xaa es AIB

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<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Lys , caracterizado porque se une mediante Alfa amina a gamma-Glu , que se une un grupo acilo graso C16 y Lys se une mediante amina epsilon a gamma-Glu que se une a un Grupo acilo graso C16

<220>

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<222> (41)..(41)

<223> Amidación de terminal C

<400> 208

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

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<222> (2)..(2)

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<221> MISC_FEATURE

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<223> Covalentemente unido a Lys, caracterizado porque Lys se une a (1) Grupo acilo graso C16 mediante gamma-Glu y (2) C8 grupo acilo graso mediante gamma-Glu

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<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 210

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 211

<211> 41

<212> PRT

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<222> (2)..(2)

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<223> Covalentemente unido a Lys , caracterizado porque Lys se une a (1) Grupo acilo graso C16 mediante gamma-Glu y (2) C12 grupo acilo graso mediante gamma-Glu

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<222> (41)..(41)

<223> Amidación de terminal C

<400> 211

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 212
<211> 41
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<223> Covalentemente unido a grupo acilo graso C16 medianteside chain hydroxyl of Tyr

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 212

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Tyr Gly
35 40

<210> 213

<400> 213

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<220>

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<223> Amidación de terminal C

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His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 220

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<212> PRT

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<222> (40)..(40)

<223> Amidación de terminal C

<400> 220

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 221

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<212> PRT

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<222> (40)..(40)

<223> Acilado con un Grupo acilo graso C14

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<223> Amidación de terminal C

<400> 221

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa

1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 222

<211> 40

<212> PRT

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<223> Xaa es AIB

<220>

<221> MOD_RES

<222> (40)..(40)

<223> Amidación de terminal C

<400> 222

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 223

<211> 40

<212> PRT

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<220>

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<220>

<221> MOD_RES

<222> (40)..(40)

<223> Acilado con un Grupo acilo graso C14

<220>

<221> MOD_RES

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<223> Amidación de terminal C

<400> 223

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 224
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<223> Covalentemente unido a 40kDa PEG mediante tioéter realizado mediante reacción de péptido con PEG activado con haloacetilo

<220>

<221> MOD_RES

<222> (40)..(40)

<223> Amidación de terminal C

<400> 224

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 225

<211> 40

<212> PRT

<213> Secuencia artificial

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<223> Acilado con un Grupo acilo graso C14

<220>

<221> MOD_RES

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<223> Amidación de terminal C

<400> 225

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 226

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<212> PRT

<213> Secuencia artificial

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<223> Péptido sintético

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<223> Covalentemente unido a 40kDa PEG mediante tioéter realizado mediante reacción de péptido con PEG activado con haloacetilo

<220>

<221> MOD_RES

<222> (40)..(40)

<223> Amidación de terminal C

<400> 226

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Cys Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 227

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<223> Amidación de terminal C

<400> 228

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Xaa
1 5 10 15

Gln Ala Ala Lys Glu Phe Val Asn Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 229

<211> 40

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<223> MT-364

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<221> MOD_RES

<222> (2)..(2)

<223> Xaa es AIB

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<221> MOD_RES

<222> (40)..(40)

<223> Acilado con un grupo acilo graso C16

<220>

<221> MOD_RES

<222> (40)..(40)

<223> Amidación de terminal C

<400> 229

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Met Asn Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 230

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

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<222> (2)..(2)

<223> Xaa es AIB

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<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 230

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Ile Tyr Leu Asp Glu
1 5 10 15

Lys Arg Ala Lys Glu Phe Val Cys Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 231

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

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<220>

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<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 231

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 232

<400> 232

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<210> 233

<211> 39

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<222> (10)..(10)

<223> Xaa es 4-amino-Phe

<220>

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<222> (10)..(10)

<223> 4-amino-Phe covalentemente unido a un grupo acilo graso C16 que comprende dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (20)..(20)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 233

Tyr Xaa Glu Gly Thr Phe Thr Ser Asp Xaa Ser Ile Tyr Leu Asp Lys
1 5 10 15

Gln Ala Ala Xaa Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 234

<211> 41

<212> PRT

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<220>

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<223> Xaa es AIB

<220>

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<223> Xaa es 4-amino-Phe

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> 4-amino-Phe covalentemente unido a un grupo graso acilo C16 a través de un espaciador que comprende dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 234

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Xaa Gly
35 40

<210> 235
<211> 39
<212> PRT
<213> Secuencia artificial

<220>
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<220>
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<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> Xaa es 4-amino-Phe

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> 4-amino-Phe Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 235

His Xaa Gln Gly Thr Xaa Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser

35

<210> 236

<211> 39

<212> PRT

<213> Secuencia artificial

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<223> Xaa es AIB

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<223> Xaa es 4-amino-Phe

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<222> (10)..(10)

<223> 4-amino-Phe Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 236

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 237

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

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<220>

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<220>

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<222> (13)..(13)

<223> 4-amino-Phe Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 237

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Xaa Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 238

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MOD_RES

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MOD_RES

<222> (13)..(13)

<223> Covalentemente unido a un grupo graso acilo C16 a través de un espaciador que comprende un residuo ácido

<220>

<221> MOD_RES

<222> (39)..(39)

<223> Amidación de terminal C

<400> 238

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Lys Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 239

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

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<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (14)..(14)

<223> Covalentemente unido a un grupo graso acilo C16 a través de un espaciador que comprende un residuo ácido

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 239

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Lys Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 240

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

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<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (16)..(16)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende un residuo ácido

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 240

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Lys

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 241

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

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<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (17)..(17)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende un residuo ácido

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 241

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Lys Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 242

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a un grupo graso acilo C16 a través de un espaciador que comprende un residuo ácido

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 242

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 243

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende un aminoácido miniPEG acid y un aminoácido ácido, caracterizado porque el aminoácido miniPEG tiene una estructura {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, caracterizado porque n es 2

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 243

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 244
<211> 41
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa es AIB

<220>
<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende un aminoácido miniPEG y un aminoácido ácido, caracterizado porque el aminoácido miniPEG tiene una estructura {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, caracterizado porque n es 4

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 244

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 245

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprende un aminoácido miniPEG acid y un aminoácido ácido, caracterizado porque el aminoácido miniPEG tiene una estructura {-N- CH2- CH2-[-O-CH2-CH2-]n-COO-}, caracterizado porque n es 8

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 245

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 246
<211> 39
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Xaa es AIB

<220>
<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a a Lys, caracterizado porque Lys se une a dos grupos acilo graso C16, caracterizado porque cada grupo acilo graso C16 se une a Lys a través de un espaciador que comprende un residuo ácido

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 246

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 247

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Lys, caracterizado porque Lys se une a dos grupos acilo graso C16, caracterizado porque cada grupo acilo graso C16 se une a Lys a través de un espaciador que comprende un residuo ácido

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 247

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 248

<211> 39

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a un grupo acilo graso C12, que se une a un espaciador que comprende un residuo ácido que se une covalentemente a un grupo graso acilo C16

<220>

<221> MISC_FEATURE

<222> (39)..(39)

<223> Amidación de terminal C

<400> 248

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 249

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a un grupo graso acilo C16 mediante aminoácido ácido

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a un grupo graso acilo C16 a través de un espaciador que comprende un residuo ácido

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 249

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys
35 40

<210> 250

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a un residuo ácido, que se une a Cys, cuyo sulfuro es S-alquilatado con -C(COO)H(CH2)13CH3

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 250

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 251

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que es covalentemente unido a un residuo ácido que es covalentemente unido a Grupo acilo graso C16

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 251

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 252

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que se une a un residuo ácido, que se une a Grupo acilo graso C16

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a SEQ ID 251 mediante un espaciador de Cys que es disulfuro unido a un segundo Cys, cuyo segundo Cys se une a Lys en la posición 40 de SEQ ID 251 y se une a un residuo ácido, que a su vez se une a un grupo acilo graso C16

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 252

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 253

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que se une a un residuo ácido, que se une a grupo acilo graso C16 y el sulfuro de Cys es tioéter unido a un peptide de SEQ ID NO: 231 mediante Lys en posición 40

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 253

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly

35 40

<210> 254

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a Cys, que se une a un residuo ácido, que se une a Grupo acilo graso C16, y el sulfuro de Cys es disulfuro unido al 2do Cys que se une a un péptio de SEQ ID NO: 231 mediante Lys en posición 40

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 254

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 255
<211> 41
<212> PRT
<213> Secuencia artificial

<220>
<223> Péptido sintético

<220>
<221> MISC_FEATURE
<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprede dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 255

His Xaa Glu Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 256

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 a través de un separdor que comprede dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 256

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu

1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 257

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprede dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 257

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Ala Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 258

<211> 41

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprede dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 258

His Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Ala Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

<210> 259

<211> 40

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprede dos residuos ácidos

<220>

<221> MISC_FEATURE

<222> (40)..(40)

<223> Amidación de terminal C

<400> 259

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly
35 40

<210> 260

<211> 39

<212> PRT

<213> Secuencia artificial

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<223> Péptido sintético

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprede un residuo ácido

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<222> (39)..(39)

<223> Amidación de terminal C

<400> 260

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Glu Gly Gly Pro Ser
20 25 30

Ser Gly Arg Pro Pro Pro Ser
35

<210> 261

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<213> Secuencia artificial

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<223> Péptido sintético

<220>

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<222> (2)..(2)

<223> Xaa es AIB

<220>

<221> MISC_FEATURE

<222> (10)..(10)

<223> Covalentemente unido a grupo acilo graso C16 a través de un espaciador que comprede dos residuos ácidos

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<222> (40)..(40)

<223> Amidación de terminal C

<400> 261

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Gly

35 40

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<212> PRT

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<223> Xaa es AIB

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<222> (40)..(40)

<223> Covalentemente unido al espaciador de Lys, caracterizado porque el espaciador de Lys se une mediante Alfa amina a un espaciador de residuo ácido, que se une a un grupo acilo graso C16 y espaciador de Lys se une mediante amina epsilon a un residuo ácido que se une a un acilo graso C16

<220>

<221> MISC_FEATURE

<222> (41)..(41)

<223> Amidación de terminal C

<400> 262

Tyr Xaa Gln Gly Thr Phe Thr Ser Asp Tyr Ser Lys Tyr Leu Asp Glu
1 5 10 15

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser Lys Gly
35 40

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(81) Estados Designados *(salvo que se indique de otro modo, para todos los tipos de protección nacional existentes):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW**.**

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-- de carácter de inventor (Norma 4.17(iv))

[Continúa en la página siguiente]

(56) Título: ANÁLOGOS DE GLUCAGÓN QUE PRESENTAN ACTIVIDAD DE RECEPTOR DE GIP

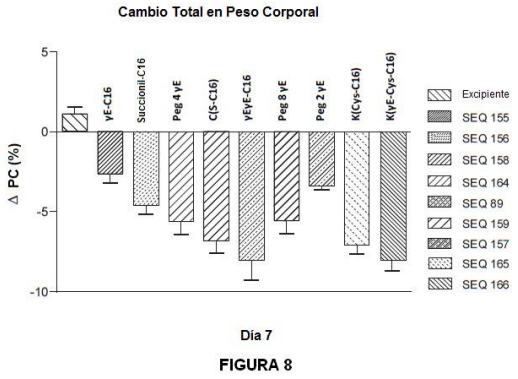

FIGURA 8

(57) Resumen de la invención: La presente invención proporciona análogos de glucagón que prestan actividad potente en el receptor de GIP y, como tal, son contemplados para el uso en el tratamiento de diabetes y obesidad. En realizaciones ejemplificadoras, el análogo de glucagón de la presenten invención muestran un EC50 en el receptor de GIP, que se encuentra en el rango nanomolar o picomolar.

Publicada:
-- sin informe de búsqueda internacional y se publicará nuevamente cuando se reciba dicho informe (Norma 48.2(g)).

-- con la parte de listado de secuencias de la memoria descriptiva (Norma 5.2(a)).