CSA850360

Industria y Comercio
SUPERINTENDENCIA

PCT

DELEGATURA DE PROPIEDAD INDUSTRIAL

DIVISIÓN DE NUEVAS CREACIONES

09-135750

SOLICITUD DE PATENTE DE INVENCIÓN

TITULO: ANTAGONISTAS CÍCLICOS DE PÉPTIDO CXCR4

SOLICITANTE: ELI LILLY AND COMPANY

CIÓN: Lilly Corporate Center, Indianapolis, Indiana 46285 E.U.A.

DO: CARLOS R. OLARTE

BOGOTÁ 27 de Noviembre de 2009

P2 000180

En 30-12-2009

1/3

OLARTERAISBECK
OLARTE RAISBECK MOURE & CASTRO LTDA.

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 09-135750- -00000-0000
Fecha: 2009-11-27 16:32:24 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 465

PETITORIO - FORMULAR.
PATENTE PCT
ENTRADA EN FASE NACIONAL **P2009/000180**

SOLICITUD DE:

(1) [X] Patente de Invención. [ ] Patente de Modelo de Utilidad.

[X] Capítulo I. [ ] Capítulo II.

(2) SOLICITANTE (71)

Nombre: ELI LILLY AND COMPANY
Dirección: Lilly Corporate Center, Indianápolis, Indiana 46285
Nacionalidad o Domicilio: Estadounidense
Lugar de Constitución: Estados Unidos de América
Teléfono: _____ **Fax:** _____
E-mail: _____

IDENTIFICACIÓN
C.C. [ ] **NIT** [ ]
C.E. [ ] **Otro** [ ]
Cual _____
Número _____

REPRESENTANTE O APODERADO

Nombre: CARLOS R. OLARTE
Dirección: World Trade Center Torre A, Calle 100 No. 8A-37 Piso 10
Teléfono: 601-7700 **Fax:** 601-7799
E-mail: office@olarteraisbeck.com

IDENTIFICACIÓN
C.C. [X] **NIT** [ ]
C.E. [ ] **Otro** [ ]
Cual _____
Número 79.782.747Bta
TP 74.295

INVENTOR (ES) (72)

Nombre: **1)** Wayne David KOHN **2)** Sheng-bin PENG **3)** Liang Zeng YAN
Dirección: 1) 7905 Beechwood Farms Drive, Avon, Indiana 46123 2) 5400 Aivamar Place, Carmel, Indiana 46033 3) 12420 Springbrooke Run, Carmel, Indiana 46033
Nacionalidad o Domicilio: 1) y 3) Canadiense 2) Estadounidense

(5) **PCT (86)**

Solicitud Internacional No. PCT/US2008/064177 Fecha Mayo 20, 2008

Publicación Internacional No. WO 2008/150689 Fecha Diciembre 11, 2008

(6) **Título (54)** **ANTAGONISTAS CÍCLICOS DE PÉPTIDO CXCR4**

Clasificación Internacional (51) A61P 31/18, A61K 38/12, C07K 7/54.

Prioridad
SI [X] **NO** [ ]

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
US	Mayo 30, 2007	60/940,802
US	Mayo 31, 2007	60/940,996

(9) **Comprobante de pago No.** 09-87,681 **Fecha** Noviembre 23, 2009

(10) **ANEXOS**

[X]	Comprobante de pago de la tasa de presentación de la solicitud.
[X]	Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
[X]	Poderes, si fuere el caso. Adjunto copia simple ver expediente No. 07-128.249.
[ ]	Documento de cesión del inventor al solicitante o a su causante.
[ ]	Declaraciones.
[X]	Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).
[X]	Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).
[ ]	Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
[ ]	Traducción del examen preliminar internacional efectuado por la IPEA.
[ ]	De ser el caso, copia del contrato de acceso.
[ ]	De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
[ ]	De ser el caso, certificado de depósito del material biológico.
[ ]	Arte final 12 x 12.
[ ]	De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
[ ]	De ser el caso, modificaciones efectuadas a la solicitud internacional.
[X]	Otros. Búsqueda Internacional.

(11) **FIGURA CARACTERÍSTICA:**

(12) Solicito la concesión de la patente,

NOMBRE: CARLOS R. OLARTE FIRMA:

C.C. 79.782.747 Btá T.P. 74.295

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 8001760

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 09-135750- -00000-0000

Fecha: 2009-11-27 16:32:24 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 465

RECIBO OFICIAL DE CAJA : 09 - 87,681
FECHA : NOVIEMBRE 23 DE 2009

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
OLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	130533616	23/11/2009	4,446,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO		TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1	TRAMITES DE SOL. DE PATENTE DE IN	540,000.00
			TOTAL : $	540,000.00

SON: QUINIENTOS CUARENTA MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

WK 9397304

28 OCT. 2008

ESCRITURA PUBLICA NUMERO: (1227)
MIL DOSCIENTOS VEINTISIETE ---------

En la ciudad de Bogotá, Distrito Capital, República de Colombia, a los Veintiocho -- (28) días del mes de Octubre --- del año dos mil ocho (2008), ante mi **MARIA EUGENIA ROJAS DE URUETA** NOTARIA(O) VEINTISIETE (27) DEL CIRCULO DE BOGOTA, se otorgó la escritura pública que se consigna en los siguientes términos:----------

---

CLASE DE ACTO: PROTOCOLIZACION DE PODER.----------

JAMES IAN RAISBECK LLÍNAS Y CARLOS REINALDO OLARTE GARCÍA.----------

C.C. No. 79.376.996 Y 79.782.747.----------

Comparecieron **JAMES IAN RAISBECK LLÍNAS,** mayor de edad, vecino de ésta ciudad, identificado con la cédula de ciudadanía número 79.376.996 de estado civil casado con sociedad conyugal vigente y **CARLOS REINALDO OLARTE GARCIA**, mayor de edad, vecino de Bogotá, identificado con la cédula de ciudadanía número 79.782.747, de estado civil casado con sociedad conyugal vigente, obrando en sus propios nombres y dijeron: ----------

PRIMERO.- Que presentan para su protocolización y custodia en los archivos de ésta notaría, bajo el número y lugar que a ésta escritura corresponda, en cinco (5) folios autenticados, un poder y sus traducciones los cuales son del siguiente tenor:----------

PODER----------

El suscrito ELI LILLY AND COMPANY, domiciliado en Lilly Corporate Center, Indianápolis, Indiana 46285 US por medio del

presente otorga a Carlos R. Olarte, J. Ian Raisbeck y Ana María Frieri, poder especial, amplio y suficiente para presentar, tramitar, obtener, defender y hacer respetar todas y cualquiera de patentes, modelos de Utilidad y Diseños Industriales de la compañía en la República de Colombia, a cuyo efecto los faculta para dar todos los pasos necesarios ante cualquier autoridad administrativa o judicial de cualquier jerarquía para cumplir con lo encomendado, elevar solicitudes, apelaciones, respuestas y oposiciones, pagar impuestos y anualidades, solicitar testimonios, recibir documentos y valores, desistir y percibir. Se concede poder bastante para recibir notificaciones a nombre de la compañía, otorgar poderes a quienes vayan a representar a la compañía judicial o extrajudicialmente, responder en juicio a todas las reclamaciones y demandas que por motivo de encomendado se presentaren, dándoles asimismo facultades para sustituir el presente y revocar dichas sustituciones.----------

Firma (ilegible) ----------

Fecha 17 July 2003----------

City, Country/ Ciudad, País: Indianápolis, Indiana, United Status of America.- ----------

Traduccion oficial del Inglés por Marlén Neira Castro resolución 2117 del Ministerio de Justicia de Colombia.- ----------

APOSTILLA EN PODER DE DE REPRESENTACION DE ELI LILLY AND COMPANY.

Bilingüe inglés- español otorgado en Indianapoles, Indiana, Estados Unidos el 17 de julio de 2003 y firmado por Meter G. Stringer, Director Ejecutivo y patentes Internacionales de Ely Lilly & Company.- ----------

Todd Rokita Secretario de Estado----------

Secretario de Estado de Indiana Estado de Indiana----------

APOSTILLA

(Convencion de la Haya del 5 octubre 1961)

WK 9397305

1. País: Estados Unidos de América- este documento público ----------

2. Ha sido firmado por: Catherine Michel

3. Quien ejerce funciones de Secretario de la Corte del Circuito en y para el condado Jonson ----------

4 Lleva el sello del Secretario de la Corte del Circuito en y para el estado de Indiana ----------

Certificado

5. En: Indianapoles, Indiana ---------- ----------

6. El: 22 de julio de 2003.----------

7. Por el Secretario de Estado de Indiana 8 No. A2003-1279--------

9. Gran Sello del Estado de Indiana ------- ----------

10. Firma ilegible de Todd Rokita- Secretario de Estado de Indiana

* Vigente desde el 1 de mayo de 2003, todas las apostillas de Secretario y de Estado de Indiana tienen el sello impreso electrónicamente ----------

The Statehouse, Indianapoles, Indiana 46204, (307) 232-6531, fax (317) 233-3283- ----------

CERTIFICACION NOTARIAL

Bilingüe inglés- español, el 17 de julio de 2003 el suscrito notario Catherine Michel certifica que Meter G. Stringer, domiciliado en 7620 Castleton Faros, West Drive Indianápolis, Indiana- 46256 Estados Unidos ejercía funciones de Director Ejecutivo y Patentes Internacionales de Ely Lilly & Company, con sede en Indianápolis, Indiana, Estados Unidos..- ----------

MARLEN NEIRA CASTRO Traductor e Intérprete Oficial inglés-Español- Inglés----------

Resolución No. 2117 del 7 Oct 1987.- ----------

El otorgante fundamenta sus certificaciones en este documento: Delegación de Autoridad concerniente a Ciertas Materias de Patentes, suscrito el 20 de enero de 2003: conforme al poder

NOTARIA VEINTIUNO
María Eugenia Rojas de Urueta

otorgado al Asesor General de la Compañía por el Comité Ejecutivo de la Junta Directiva de Eli Lilly and Company.----------
Firma del notario público Catherine Michel certifica sus funciones vencen el 21 de octubre de 2008- residente en el condado Johnson
Sello de notario público de Indiana.----------
Es traducción fiel de inglés, con copia archivo para confrontación, Bogotá 28 de julio-2003.----------
MARLEN NEIRA CASTRO Traductor e Intérprete Oficial Inglés-Español- Inglés ----------
Resolución No. 2117 del 7 Oct 1987.- ----------
SEGUNDO.- Que solicita a la señora Notaria la incorporación de dichos documentos al protocolo de ésta notaría para que de su tenor se expidan las copias que más adelante se soliciten ----------
---
Se advierte que la protocolización de documentos no da mayor fuerza o autenticidad de la que tienen dichos documentos.- ---
---
Esta escritura pública fue firmada fuera del Despacho Notarial, de conformidad con el artículo 12 del Decreto 2.148 de 1.983. -------
---
Se advirtió a los otorgantes de esta escritura de la obligación que tienen de leer la totalidad de su texto, a fin de verificar la exactitud de todos los datos en ella consignados, con el fin de aclarar, modificar o corregir lo pertinente antes de firmarla, la firma de la misma demuestra su aprobación total del texto, en consecuencia, la notaria no asume ninguna responsabilidad por errores o inexactitudes establecidas con posterioridad a la firma de (el, los) otorgante(s) y de la notaría, en tal caso, este (os) debe(n) ser corregidos mediante el otorgamiento de una nueva escritura, suscrita por todos los que intervinieron en la inicial y sufragada por los mismos (articulo 35, decreto ley 960 de 1970).- ----------

Traducción oficial del Inglés por Marlén Neira Castro, resolución 2117 del Ministerio de Justicia de Colombia

APOSTILLA EN PODER DE REPRESENTACIÓN DE

ELI LILLY AND COMPANY

Bilingüe inglés- español otorgado en Indianapolis, Indiana, Estados Unidos el 17 de julio de 2003 y firmado por Peter G. Stringer, Director Ejecutivo y Patentes Internacionales de Ely Lilly & Company.

Todd Rokita — Secretario de Estado

Secretario de Estado de Indiana — Estado de Indiana

APOSTILLA

(Convención de la Haya del 5 de octubre de 196

1. País: Estados Unidos de América- Este documento Públ
2. ha sido firmado por Catherine Michel
3. Quien ejerce funciones de Secretario de la Corte del Circuito en y para el condado Johnson
4. Lleva el sello del Secretario de la Corte del Circuito en y para el estado de Indiana

CERTIFICADO

5. En Indianapolis, Indiana 6. El 22 de julio de 2003
7. Por el Secretario de Estado de Indiana 8. No. A2003- 1127
9. Gran Sello del Estado de Indiana
10. Firma legible de Todd Rokita- Secretario de Estado de Indiana

* Vigente desde el 1 mayo de 2003, todas las apostillas del Secretario de Estado de Indiana tienen el sello impreso electrónicamente.

The Statehouse, Indianapolis, Indiana 46204, (317) 232-6531, fax (317) 233-3283

CERTIFICACION NOTARIAL

Bilingüe inglés- español, el 17 de julio de 2003 el suscrito notario Catherine Michel certifica que Peter G. Stringer, domiciliado en 7620 Castleton Farms, West Drive, Indianapolis, Indiana - 46256 Estados Unidos ejercía funciones de Director Ejecutivo y Patentes Internacionales de Ely Lilly & Company, con sede en Indianapolis, Indiana, Estados Unidos.

MARLEN NEIRA CASTRO
Traductor e Intérprete Oficial
Inglés - Español - Inglés
Resolución No. 2117 del 7 Oct 1987

El otorgante fundamenta sus certificaciones en este documento: Delegación de Autoridad concerniente a Ciertas Materias de Patentes, suscrito el 20 de enero de 2003: conforme al poder otorgado al Asesor General de la Compañía por el Comité ejecutivo de la Junta Directiva de Eli Lilly and Company.

Firma del notario público Catherine Michel certifica

Sus funciones vencen el 21 de octubre de 2008 - residente en el condado Johnson

Sello de notario público de Indiana

--

Es traducción fiel del inglés, con copia archivo para confrontación. Bogotá 28 de julio-2003.

Marlen Neira C

Marlen Neira C

MARLEN NEIRA CASTRO
Traductor e Intérprete Oficial
Inglés - Español - Inglés
Resolución No. 2117 del 7 Oct 1987

OLARTERAISBECK

OLARTE RAISBECK & FRIERI, LTDA.
LEGAL & PROFESSIONAL SERVICES

WORLD TRADE CENTER – TORRE C
CALLE 100 NO. 8A-55 PISO 10
BOGOTA, COLOMBIA
TELEPHONE: +57-1 638-6200
Fax: +57-1 638-6201

www.olarteraisbeck.com

Power of Attorney

The undersigned,

domiciled in

by means of these presents hereby grants to **Carlos R. Olarte, J. Ian Raisbeck and Ana María Frieri** full and sufficient Power of Attorney to file, prosecute, obtain, defend and enforce any and all of the company's **Patents, Utility Models and Industrial Designs** in the Republic of Colombia, to which end it empowers them to take all steps necessary before any administrative or judicial authority of any class for the objects stated, to file petitions, appeals, replies and oppositions, pay taxes and annuities, request testimonies, receive documents and securities, abandon applications and collect refunds. Sufficient power is hereby granted to receive notifications on behalf of the Company, to confer powers on those who are to represent the company judicially and extrajudicially, to answer in court in the matter of all claims and demands that may be presented in connection with the objects stated, giving them likewise power to substitute these presents and revoke such substitutions.

Poder

El suscrito,

ELI LILLY AND COMPANY

domiciliado en

Lilly Corporate Center, Indianapolis, Indiana 46285 US

por medio del presente otorga a **Carlos R. Olarte, J. Ian Raisbeck y Ana María Frieri** poder especial, amplio y suficiente para presentar, tramitar, obtener, defender y hacer respetar todas y cualquiera de **Patentes, Modelos de Utilidad y Diseños Industriales** de la compañía en la República de Colombia, a cuyo efecto los faculta para dar todos los pasos necesarios ante cualquier autoridad administrativa o judicial de cualquier jerarquía para cumplir con lo encomendado, elevar solicitudes, apelaciones, respuestas y oposiciones, pagar impuestos y anualidades, solicitar testimonios, recibir documentos y valores, desistir y percibir. Se concede poder bastante para recibir notificaciones a nombre de la compañía, otorgar poderes a quienes vayan a representar a la compañía judicial y extrajudicialmente, responder en juicio a todas las reclamaciones o demandas que por motivo de lo encomendado se presentaren, dándoles asimismo facultad para sustituir el presente y revocar dichas sustituciones.

Signature/Firma

Date/Fecha: 17 July 2003

City,Country/Ciudad,País: Indianapolis, Indiana, United States of America

SECRETARY OF STATE
STATE OF INDIANA

Todd Rokita
Secretary of State

APOSTILLE

(Convention de la Haye du 5 Octobre 1961)

1. **Country: United States of America**

2. **This public document has been signed by** *Catherine Michel*

3. **acting in the capacity of circuit court clerk in & for** *Johnson* **County**

4. **and bears the seal/stamp of circuit court clerk in & for the State of Indiana**

Certified

5. **at Indianapolis, Indiana**

6. **this** *Twenty-second* **day of** *July, 2003*

7. **by Secretary of State of Indiana**

8. **No. A2003-** *11279*

9. **Seal/Stamp:**

10. **Signature:**

Todd Rokita

Indiana Secretary of State

*Effective May 1, 2003 all apostilles from the Indiana Secretary of State will have an electronically printed seal.

The Statehouse, Indianapolis, Indiana 46204, (317) 232-6531, Fax (317) 233-3283

OLARTERAISBECK
OLARTE RAISBECK & FRIERI, LTDA.
LEGAL & PROFESSIONAL SERVICES

WORLD TRADE CENTER – TORRE C
CALLE 100 NO. 8A-55 PISO 10
BOGOTA, COLOMBIA
TELEPHONE: +57-1 638-6200
Fax: +57-1 638-6201

www.olarteraisbeck.com

Notarial Certification

On this date,
17 July 2003
The undersigned Notary Public certifies:

1. That

Peter G. Stringer

of legal age and domiciled in

7620 Castleton Farms, West Drive, Indianapolis, Indiana 46256 US

signed the foregoing document.

2. That the grantor has executed the foregoing document as Executive Director, International Patents
of ELI LILLY AND COMPANY

3. That ELI LILLY AND COMPANY
having its principal place of business located in
Indianapolis, Indiana, United States of America
is duly incorporated, is currently in existence, and that the purposes for which the Power of Attorney is granted are within the scope of its corporate purposes.

4. That the grantor has the authority to execute the Power of Attorney and that his/her representation is legal.

5. The foundation for my certifications contained in points 2, 3 and 4 was the exhibition of the following authentic document(s):
Delegation of Authority Concerning Certain Patent Matters, executed January 20, 2003; pursuant to the authority granted to the General Counsel of the Company by the executive Committee of the Board of Directors of Eli Lilly and Company

NOTARY PUBLIC (Signature):

NOTARY PUBLIC SEAL INDIANA

Certificación Notarial

En esta fecha,

el Notario Público suscrito certifica:

1. Que
mayor de edad y domiciliado
suscribió el documento que antecede.

2. Que el otorgante ha suscrito el referido documento en su carácter de

de

4. Que
con sede principal ubicada en

está legalmente constituida, que tiene existencia legal vigente y que los propósitos para los cuales se otorga el poder se encuentra dentro de los que comprenden su objeto social.

4. Que el otorgante tiene la representación para suscribir el poder, y que ésta es legitima.

5. El fundamento de mis certificaciones contenidas en los puntos 2, 3 y 4 fue la exhibición de los (del) siguiente(s) documento(s) auténtico(s):

María Eugenia Rojas de Urueta

Catherine Michel
My Commission Expires October 21, 2008
Resident of Johnson County

WK 9397306

ADVERTENCIA, OTORGAMIENTO Y AUTORIZACION.- Leído el presente instrumento por el (los) compareciente (s) y advertido (s) sobre las formalidades legales, lo aprueban y firman conmigo y ante mí la(el) Notaria(o) quién lo autoriza. ---Se utilizaron las hojas de papel Notarial Nos. WK 9397304, WK 9397305 Y WK 9397306. ----------

DERECHOS LEGALES: $ 39.630.oo ----------

RESOLUCION 8850 DE 2007

IVA $ 10.781.oo ----------

JAMES IAN RAISBECK LLÍNAS

C.C.No. 79.376.996 BOGOTA

TEL: 601-7700

CARLOS REINALDO OLARTE GARCIA

C.C.No. 79.782.747 BOGOTA.

TEL: 601-7700

MARIA EUGENIA ROJAS DE URUETA

NOTARIA(O) VENTISIETE (27) DE BOGOTÁ

redb.

REPUBLICA DE COLOMBIA 27 María Eugenia Rojas NOTARIA

ESTE PAPEL NO TIENE COSTO PARA EL USUARIO

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fotocopia de la Escritura Pública No. 1227
de fecha 28 de Octubre de 2008
tomada de su original que expido en
seis (6) hojas útiles de papel
común autorizado ~~(Decreto 1348 de 1970) con~~ destino a
Interesado

Dado en Santafé de Bogotá D.C., a 28 OCT 2008
El Notario Veintisiete de Santafé de Bogotá

REPUBLICA DE COLOMBIA
27
María Eugenia Rojas
de Urueta
NOTARIA
Notaría Veintisiete de Santafé de Bogotá

ESPACIO EN BLANCO

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

PCT

(43) International Publication Date
11 December 2008 (11.12.2008)

(10) International Publication Number
WO 2008/150689 A1

(51) International Patent Classification:
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A61K 38/12 (2006.01)

(21) International Application Number:
PCT/US2008/064177

(22) International Filing Date: 20 May 2008 (20.05.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/940,802 30 May 2007 (30.05.2007) US
60/940,996 31 May 2007 (31.05.2007) US

(71) Applicant *(for all designated States except US)*: **ELI LILLY AND COMPANY** [US/US]; Lilly Corporate Center, Indianapolis. Indiana 46285 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KOHN, Wayne, David** [CA/US]; 7905 Beechwood Farms Drive, Avon, Indiana 46123 (US). **PENG, Sheng-bin** [US/US]; 5400 Alvamar Place, Carmel, Indiana 46033 (US). **YAN, Liang, Zeng** [CA/US]; 12420 Springbrooke Run, Carmel, Indiana 46033 (US).

(74) Agents: **COHEN, Charles, E.** et al.; Eli Lilly and Company, Patent Division, P. O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR. BW, BY, BZ, CA, CH, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) Title: CYCLIC PEPTIDE CXCR4 ANTAGONISTS

(57) Abstract: Provided are lactam-cyclized peptide CXCR4 antagonists useful in the treatment of cancers, rheumatoid arthritis, pulmonary fibrosis, and HIV infection.

WO 2008/150689 A1

Cyclic Peptide CXCR4 Antagonists

The present invention relates to novel cyclic peptide CXCR4 antagonist compounds and their use in treating diseases in which pathogenesis is mediated by CXCR4 and SDF-1.

CXCR4, a G-protein-coupled receptor, and its naturally occurring ligand, stromal cell-derived factor-1 (SDF-1; CXCL12), are a chemokine receptor-ligand pair. CXCR4 is constitutively- or over-expressed in a wide variety of human cancers. SDF-1, the only known ligand of CXCR4, is highly expressed in tumor microenvironments, as well as in bone marrow, lung, liver, and lymph nodes, i.e., organ sites most commonly involved in tumor metastasis. CXCR4/SDF-1 interaction plays important roles in multiple stages of tumorigenesis, including tumor growth, invasion, angiogenesis, and metastasis, as well as in rheumatoid arthritis, pulmonary fibrosis, and HIV infection (Tsutsumi et al. (2006) *Peptide Science* 88(2):279-289).

In view of the involvement of CXCR4/SDF-1 in these serious diseases, CXCR4 is an attractive therapeutic target.

AMD3100, a bicyclam CXCR4 antagonist, is currently in Phase III clinical trials for stem cell mobilization for transplantation of stem cells in patients with multiple myeloma and non-Hodgkins lymphoma. AMD070, another small molecule CXCR4 antagonist, is currently in Phase II clinical trials for HIV infection. CTCE9908, a bivalent (dimeric) peptide CXCR4 antagonist, is currently in Phase Ib/II clinical trials for cancer. FC131, a cyclic pentapeptide CXCR4 antagonist, inhibits ^{125}I-SDF-1 binding to CXCR4 transfectants with an IC_{50} of 4 nM (Fujii et al. (2003) *Angew. Chem. Int. Ed.* 42:3251-3253; Araki et al. (2003) *Peptide Science*. The Japanese Peptide Society (2004):207-210).

There exists a need for improved CXCR4 antagonists that are potent and selective, exhibiting little or no activity at other chemokine receptors. The compounds of the present invention are such potent and selective CXCR4 antagonists. Their high potency permits the use of low doses in therapeutic regimens, while their high selectivity minimizes non-target related adverse side effects. In addition, compounds disclosed herein possess other highly desirable pharmacologic properties, such as high bioavailability when administered subcutaneously, good *in vivo* metabolic stability, and pharmacokinetic/pharmacodynamic properties that permit convenient dosing.

Accordingly, in a first aspect, the present invention provides a lactam-cyclized peptide of formula I:

$$R_1 - \text{cyclo}[X_1 - \text{Tyr} - X_3 - \text{DArg} - \text{2Nal} - \text{Gly} - X_7] - X_8 - X_9 - X_{10} - R_2$$

(I)

(SEQ ID NO:1)

wherein:

a) said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

b) said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

c) said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

d) said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

e) said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7, wherein X_2 and X_7 are, respectively, a pair

selected from the group consisting of Tyr/Asp, Tyr/Glu, and Tyr/DGlu, and R_1 and X_1 are each absent;

R_1 is a substituent on the α-amino group of X_1 when X_1 contains an α-amino group and said α-amino group is not a constituent of said lactam amide bond, selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent, wherein X_1 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, and Glu;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent, and

R_2 is selected from the group consisting of NH_2 and NHEt, or

a pharmaceutically acceptable salt thereof.

Expressed alternatively, this is equivalent to a lactam-cyclized peptide of formula I:

$$R_1\text{ - cyclo}[X_1 - \text{Tyr} - X_3 - \text{DArg} - \text{2Nal} - \text{Gly} - X_7] - X_8 - X_9 - X_{10} - R_2$$

(I)

(SEQ ID NO:1)

wherein:

R_1 is a substituent on the α-amino group of X_1 when X_1 contains an α-amino group and said α-amino group is not a constituent of said lactam amide bond, selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent, wherein X_1 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, and Glu;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent, and

R_2 is selected from the group consisting of NH_2 and NHEt,

wherein:

a) said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

b) said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or when X_1 and X_7 are, respectively, a pair selected from the group consisting of

succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

c) said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

d) said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

e) said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7, when X_2 and X_7 are, respectively, a pair selected from the group consisting of Tyr/Asp, Tyr/Glu, and Tyr/DGlu, and R_1 and X_1 are each absent, or

a pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a lactam-cyclized peptide of formula I:

$$R_1\text{ - cyclo}[X_1 - Tyr - X_3 - DArg - 2Nal - Gly - X_7] - X_8 - X_9 - X_{10} - R_2$$

(I)

(SEQ ID NO:1)

or a pharmaceutically acceptable salt thereof,

wherein:

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent,

wherein when X_1 is (D/L)Agl, Dab, or Dap and the α-amino group of X_1 is not a constituent of the lactam amide bond, said α-amino group is substituted with R_1 which is selected from the group consisting of Ac and n-hexanoyl;

wherein when X_1 is Asp or Glu and the α-amino group of X_1 is not a constituent of the lactam amide bond, said α-amino group is substituted with R_1 which is selected from the group consisting of Ac and Bz; and

wherein when X_1 is Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Dap(Ac), Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe or succinyl, R_1 is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent,

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt,

and further wherein:

said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or wherein X_1 and X_7

are, respectively, a pair selected from the group consisting of succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

said lactam is formed by an amide bond between the α-amino group of Tyr at X_2 and the side chain carboxyl group of X_7, and X_7 is selected from the group consisting of Asp, Glu, and DGlu, and R_1 and X_1 are each absent.

A recurrent sequence motif in all the compounds of formula I is the presence of Tyr at position X_2, DArg at position X_4, 2Nal at position X_5, and Gly at position X_6.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of β-Ala, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, 2Nal, Phe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of Asp, Dab, Dap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of Arg and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of DAla, 5-aminovaleryl, 4-AMPA, Asp, Glu, Leu, Lys(Ac), Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, and DGlu;

X_8 is selected from the group consisting of Arg, DArg, and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, Asp, Dap, Glu, Gly, Lys, and Phe;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In a further aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of Ala, 5-aminovaleryl, Asp, Glu, Gly, Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr); and

X_7 is DGlu.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is absent;

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr);

X_7 is DGlu;

X_8 is selected from the group consisting of Arg and Lys(iPr), or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7;

R_1 is selected from the group consisting of Ac and n-hexanoyl;

X_1 is selected from the group consisting of (D/L)Agl, Dab, and Dap;

X_3 is selected from the group consisting of Arg and Lys(iPr);

X_7 is Glu;

X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_1 is (D/L)Agl or Dap.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7;

R_1 is selected from the group consisting of Ac and Bz;

X_1 is selected from the group consisting of Asp and Glu;

X_3 is selected from the group consisting of Arg and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Dab, Dap, DDap, and Lys;

X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_7 is (D/L)Agl, Dab, Dap, or DDap. In a more preferred embodiment, X_7 is (D/L)Agl or Dap.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7;

R_1 is absent;

X_1 is succinyl;

X_3 is Arg;

X_7 is selected from the group consisting of (D/L)Agl, Dab, Dap, Lys, and Orn;

X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_7 is (D/L)Agl or Dap.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7;

R_1 is absent;

X_1 is selected from the group consisting of Ala, DAla, Gly, Dap(Ac), Leu, Lys, Lys(Ac), 2Nal, Phe, and DPhe;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of Asp, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In a preferred embodiment of this aspect of the invention, X_1 is Ala, DAla, Gly, Leu, Lys, Lys(Ac), Phe, or DPhe. In a more preferred embodiment, X_1 is Ala, Gly, Lys, or Phe.

In a preferred embodiment of this aspect of the invention, X_3 is Arg, Lys, Lys(iPr), or Lys(Me_2). In a more preferred embodiment, X_3 is Arg.

In a preferred embodiment of this aspect of the invention, X_7 is Asp, Glu, or DGlu. In a more preferred embodiment, X_7 is Asp.

In a preferred embodiment of this aspect of the invention, X_8 is β-Ala, Arg, Gly, Lys, Lys(iPr), Orn, or is absent. In a more preferred embodiment, X_8 is β-Ala, Gly, Lys, Lys(iPr), Orn, or is absent.

In a preferred embodiment of this aspect of the invention, X_9 is Gly, 2Nal, D2Nal, DPhe, or is absent. In a more preferred embodiment, X_9 is Gly, 2Nal, D2Nal, or DPhe.

In a preferred embodiment of this aspect of the invention, X_{10} is 2Nal, or is absent. In a more preferred embodiment, X_{10} is 2Nal.

In a preferred embodiment of this aspect of the invention, R_2 is NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7;

R_1 is absent;

X_1 is selected from the group consisting of β-Ala, 4-AMB, 5-aminovaleryl, and 4-AMPA;

X_3 is Arg;

X_7 is selected from the group consisting of Asp and Glu;

X_8 is selected from the group consisting of Arg and DArg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_1 is β-Ala, 5-amino-valeryl, or 4-AMPA. In a more preferred embodiment, X_1 is β-Ala.

In a preferred embodiment of this aspect of the invention, X_7 is Asp.

In a preferred embodiment of this aspect of the invention, X_8 is Arg.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7;

R_1 is absent;

X_1 is absent;

X_3 is Arg;

X_7 is selected from the group consisting of Asp, Glu, and DGlu;

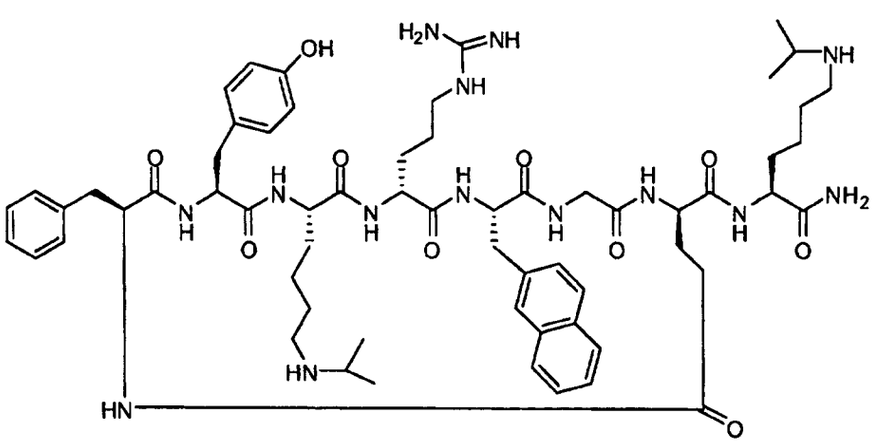
X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In another aspect, the present invention provides a lactam-cyclized peptide of the formula:



(SEQ ID NO:70)

or a pharmaceutically acceptable salt thereof. The lactam is formed by an amide bond between the α-amino group of Phe and the side chain carboxyl group of DGlu. The pharmaceutically acceptable salt can be an acetic acid salt.

In another aspect, the present invention provides a pharmaceutical composition, comprising a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, for use in therapy.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

In another aspect, the present invention provides the use of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, for the manufacture of a medicament for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

In another aspect, the present invention provides a method of treating rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia, comprising administering to a patient in need thereof an effective amount of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above.

In the macrocyclic peptidic compounds of the present invention (SEQ ID NO:1), amino acids X_1 through X_{10} are referred to herein by their commonly employed three letter symbols, shown left to right from amino-terminal end to carboxy-terminal end. D-

and L- (small capital letters) refer to absolute stereochemistry. Where neither designation is indicated in a particular formula, the L- form of the amino acid is present. X_1 can also be a dicarboxylic acid residue, i.e., a succinyl group. Amino acid or carboxylic acid residues within the brackets "[ ]" are within the cyclic structure; groups external to the brackets are outside the cyclized ring. In all cases, cyclization is via a lactam (amide) bond between X_1 (or X_2, i.e., Tyr) and X_7, which can be formed in several different ways, depending on the structures of X_1, X_2, and X_7.

When the lactam bond is formed between the side chain amino group of X_1 and the side chain carboxyl group of X_7 (Schemes 1 and 2; Examples 1-5), the α-amino group of X_1 is capped with Ac or n-hexanoyl.

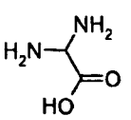
When the lactam bond is formed between the side chain carboxyl group of X_1 and the side chain amino group of X_7, the α-amino group of X_1 is capped with Ac or Bz (Schemes 3 and 4; Examples 6-19). X_1 also can be a bifunctional residue other than an α-amino acid, for example one with two carboxyl groups, i.e., a succinyl residue. In this case, one carboxyl group forms an amide bond with the α-amino group of Tyr, and the other forms the cyclic lactam structure through an amide bond with the side chain amino group of X_7 (Schemes 3 and 4; Examples 20-24). When X_1 is succinyl, R_1 is absent.

In most of the lactam-cyclized peptides disclosed herein (Schemes 5-15; Examples 25-28, 32-66, and 75-89), the α-amino group of X_1 forms the lactam structure via an amide bond with the side chain carboxyl group of X_7, and R_1 is absent. Also included in the synthetic schemes of this category are cyclic peptides containing X_1 residues, i.e., β-Ala, 4-AMB, 5-aminovaleryl, and 4-AMPA, wherein the amino group is a non-side chain, non-α-amino group (Schemes 5 and 6; Examples 67-74). R_1 is also absent in these cases.

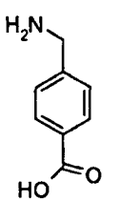
When both R_1 and X_1 are absent, the lactam structure is formed through an amide bond between the α-amino group of Tyr (X_2) and the side chain carboxyl group of X_7 (Schemes 5 and 6; Examples 29-31).

Structures of common amino acids, e.g., alanine, glycine, etc., are well known in the art. Structures of non-standard and substituted amino acids present in the instant invention compounds are shown below.

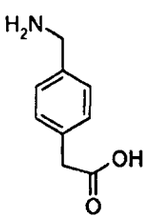
5



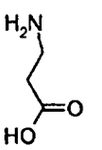
aminoglycine (Agl)



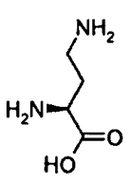
4-aminomethyl benzoic acid (4-AMB)



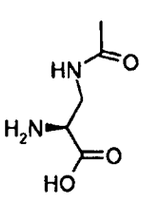
4-aminomethyl phenylacetic acid (4-AMPA)



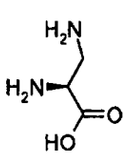
3-amino propanoic acid (β-Ala)



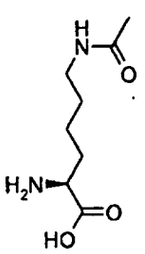
Dab



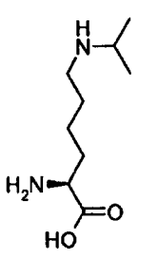
Dap(Ac)



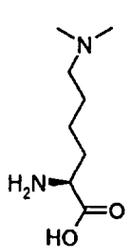
Dap



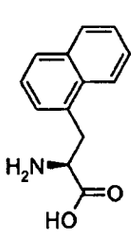
Lys(Ac)



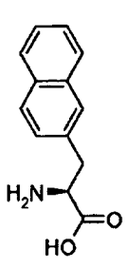
Lys(iPr)



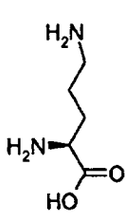
Lys(Me_2)



1Nal



2Nal



Orn

The lactam-cyclized peptides of the present invention can be prepared as pharmaceutically acceptable salts. Such salts, and common methodology for preparing them, are well known in the art. *See*, e.g., P. Stahl et al. (2002) *Handbook of Pharmaceutical Salts: Properties, Selection and Use*, VCHA/Wiley-VCH; Berge et al. (1977) "Pharmaceutical Salts," *Journal of Pharmaceutical Sciences* 66(1):1-19.

The compounds of the present invention are potent antagonists of CXCR4/SDF-1 interaction. Compounds of formula (I) and their pharmaceutically acceptable salts specifically exemplified herein exhibit an average K_i value of about 7.5 nM or less as determined by the CXCR4/^{125}I-SDF-1α binding assay described below. More preferred compounds of formula (I) and their pharmaceutically acceptable salts exhibit an average K_i value in the range of from about 0.2 nM to about 1 nM in this assay. Especially preferred compounds of formula (I) and their pharmaceutically acceptable salts exhibit an average K_i value less than about 0.2 nM in this assay.

In addition, the compounds and pharmaceutically acceptable salts of the present invention are preferably highly selective for the CXCR4 receptor, exhibiting little or no inhibitory activity against other chemokine receptors, including CCR1, CCR2, CXCR2, CXCR3, and other G-protein coupled receptors at the concentrations tested, and no significant activity against serotonin, dopamine, and opioid receptors. They also preferably exhibit good stability in blood and plasma, good subcutaneous bioavailability, desirable pharmacokinetic/pharmacodynamic properties, and potent *in vivo* efficacy in tumor growth inhibition, with a wide safety margin.

In view of these pharmacological properties, the compounds of the present invention are indicated for the treatment of disorders involving CXCR4/SDF-1 interaction, or receptor activity activity of CXCR4, such as in HIV infection. In particular, the present compounds are useful in treating malignancies in which angiogenic, growth, survival, and metastatic pathways mediated by CXCR4 and SDF-1 are implicated in pathogenesis, including breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia, as well as in rheumatoid arthritis, pulmonary fibrosis, and HIV infection (Tsutsumi et al. (2006) *Peptide Science* 88(2):279-289).

Agl (aminoglycine) is a pro-chiral building block. When this residue appears in a peptide formula herein, the α-carbon becomes a chiral center at which the two associated α-amino groups are each individually bonded to different moieties. In this case, the final peptide product contains two diastereomers that are unresolved, and that may be present in other than a 1:1 ratio. "(D/L)Agl" in a peptide formula denotes such a mixture of diastereomers. "(DL)Agl" denotes an Agl derivative that is racemic, for example Fmoc-(DL)Agl(Boc).

"K_i" values are calculated using IC_{50} values determined in the CXCR4/^{125}I-SDF-1α binding assay described below by employing equation 7.22 of *Enzymes, A Practical Introduction to Structure, Mechanism, and Data Analysis*, Robert A. Copeland, Wiley-VCH, New York, 1996, page 207.

The term "SDF-1" includes two isoforms, SDF-1α and SDF-1β, currently understood to exhibit similar functionality.

"Treatment" as used herein refers to curative treatment of disorders associated with CXCR4/SDF-1 interaction or CXCR4 receptor activity. Curative treatment refers to processes involving a slowing, interrupting, arresting, controlling, or stopping of disease progression, but does not necessarily involve a total elimination of all disease-related symptoms, conditions, or disorders.

The compounds of the present invention can be used as medicaments in human or veterinary medicine, administered by a variety of routes. Most preferably, such compositions are for parenteral administration. Such pharmaceutical compositions can be prepared by methods well known in the art. *See*, e.g., *Remington: The Science and Practice of Pharmacy*, 19th ed. (1995), A. Gennaro et al., Mack Publishing Co., and comprise one or more compounds of formula (I) or a pharmaceutically acceptable salt(s) thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

The effective amount of the present compounds is in the range of from about 1 mg to about 300 mg, more preferably from about 1 mg to about 200 mg, more preferably from about 1 mg to about 100 mg, and even more preferably from about 1 mg to about 50 mg, on a daily basis.

All lactam-cyclized peptides of the present invention can be synthesized either by solid-phase synthesis or solution phase synthesis, or a combination of both, with peptide

chain assembly on solid phase and cyclization or other modifications on resin or in solution. Such methods are well known in the art.

The following abbreviations used herein have the indicated meanings:

Ac: acetyl; **Agl:** aminoglycine; **AMB**: aminomethyl benzoic acid; **AMPA**: aminomethyl phenyl acetic acid; **Bn**: benzyl; **Boc**: tert-butyloxycarbonyl; **BOP**: (benzotriazol-1-yloxy)-tris(dimethylamino)phosphoniumhexafluorophosphate; **2-Br-Z**: 2-bromobenzyloxycarbonyl; **Bz**: benzoyl; **Bzl**: benzyl; **2-Cl-Z**: 2-chlorobenzyl-oxycarbonyl; **Dab**: 2,4-diaminobutyric acid; **Dap**: 2,3-diamino-propionic acid; **DCC**: dicyclohexyl-carbodiimide; **DCM**: dichloromethane; **DIC**: diisopropyl carbodiimide; **DIEA**: diisopropyl-ethylamine; **DMF**: *N,N*-dimethyl formamide; **DMSO**: dimethyl-sulfoxide; **EDT:** 1,2-ethane-dithiol; **Et**: ethyl; **Fm**: 9-fluorenylmethyl; **Fmoc**: 9-fluor-enylmethoxy carbonyl; **HATU**: N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide; **HBTU**: *O*-benzo-triazolyl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; **HCTU**: 1H-benzotriazo-lium 1-[bis(dimethylamino)methylene]-5-chloro-3-oxide hexafluorophosphate; **HF**: hydrogen fluoride; **HOBt**: hydroxybenzotriazole; **IBCF**: isobutyl chloroformate; **iPr**: isopropyl; **IPA**: isopropyl alcohol; **Me**: methyl; **2Nal**: 2-naphthylalanine; **NMM**: N-methylmorpholine; **NMP:** *N*-methyl-pyrrolidone; **OtBu:** tert-butyl ester; **Pbf**: 2,2,4,6,7-pentamethyl-dihydrobenzofurane-5-sulfonyl; **PBS:** phosphate buffered saline; **PyBOP**: (benzotriazol-1-yloxy)-tris(pyrrolidino)-phosphonium hexafluoro-phosphate; **PyBrOP**: bromotris(pyrrolidino)phosphonium hexafluorophosphate; **tBu:** tert-butyl; **TFA**: trifluoroacetic acid; **THF**: tetrahydrofuran; **TIS**: triisopropyl silane; **Tos**: p-toluene-sulfonyl; **Z**: benzyloxycarbonyl; **ZOSu**: N-(benzyloxycarbonyl-oxy) succinimide.

Preparation of compounds of the present invention as described in the following examples is meant to be illustrative rather than limiting. In each of these examples, the observed molecular weight is reported as a de-convoluted value. The de-convoluted value is derived from the formula MW(observed) = n(m/z)-n, where m/z represents the charged ion (positive mode) and n is the number of charges of the specific species. When multiple charged species are present in the mass spectrum, the observed molecular weight is reported as an average.

The synthetic processes disclosed in Examples 85-87, and isotopic labeling procedures disclosed in Example 89, for cyclic lactam peptides containing isopropyl

lysine side chains are equally applicable to other peptides disclosed herein containing lysine alkyl side chains, with appropriate modifications. The synthetic methods of Examples 86-88, which eliminate the need for toxic palladium catalysts, are also equally applicable to other peptides disclosed herein, with appropriate modifications.

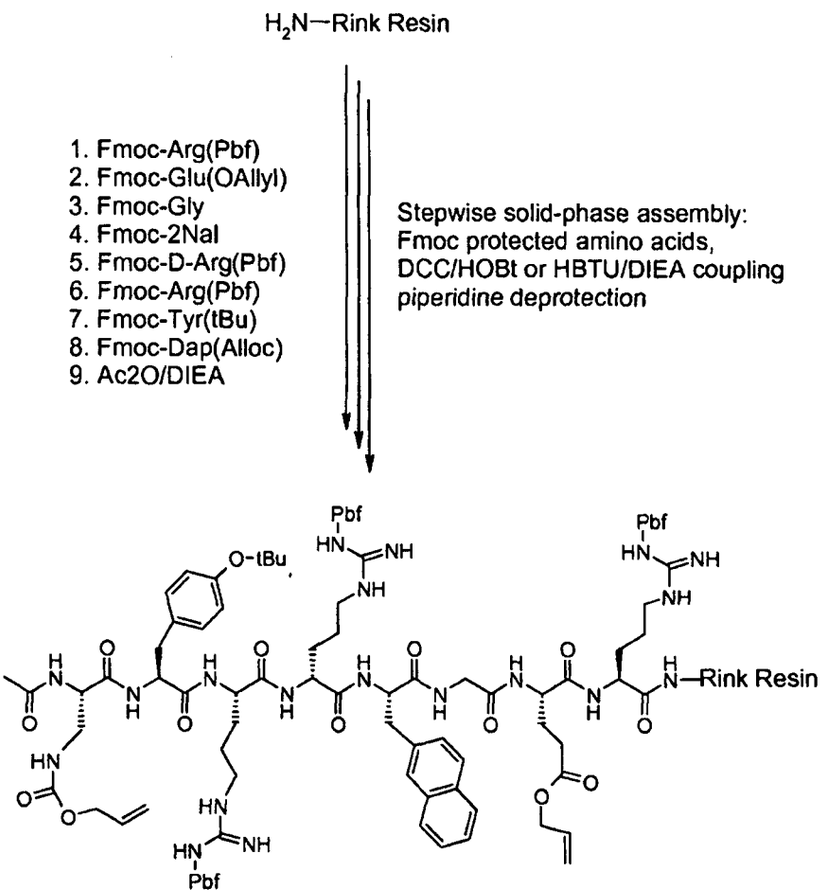
Example 1

Ac-cyclo[Dap-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:2)

The sequence Ac-Dap(Alloc)-Tyr(tBu)-Arg(Pbf)-DArg(Pbf)-2Nal-Gly-Glu(Oallyl)-Arg(Pbf) (SEQ ID NO:3) is assembled by standard Fmoc chemistry utilizing an ABI 431 Peptide Synthesizer (Applied Biosystems) as outlined in Scheme 1 below. The automated assembly is carried out by using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc chemistry HBTU/DIEA protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The solid support is Rink amide resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin) for C-terminal amides or indole resin [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin for C-terminal ethyl amides (NovaBiochem, EMD Biosciences, Inc., San Diego, CA). The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 9 major steps. In step 1, four equivalents of protected amino acid Fmoc-Arg(Pbf) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and coupled to deprotected Rink Amide resin using 20% piperidine. In step 2, four equivalents of Fmoc-Glu(Oallyl) are activated and coupled to the deprotected peptide resin from step 1. Appropriate steps are carried out until step 8, the coupling of Fmoc-Dap(Alloc). Then, Fmoc at the N-terminal end is removed using 20% piperidine in DMF and acetylation of the α-amino group is carried out off-line using 5 equivalents of acetic anhydride, 10 equivalents of DIEA in dry DMF or NMP, for 1 h at room temperature.

The allyl and Alloc side chain protection groups are removed with 0.1 equivalent of $Pd(Ph_3P)_4$ in the presence of 24 equivalents of phenylsilane in dichloromethane (Scheme 2). This process is repeated once for complete side chain deprotection. The deprotected carboxylic acid moiety of Glu is activated with PyBOP/DIEA and cyclized to the side chain amino group of Dap on the resin. The cyclized peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT

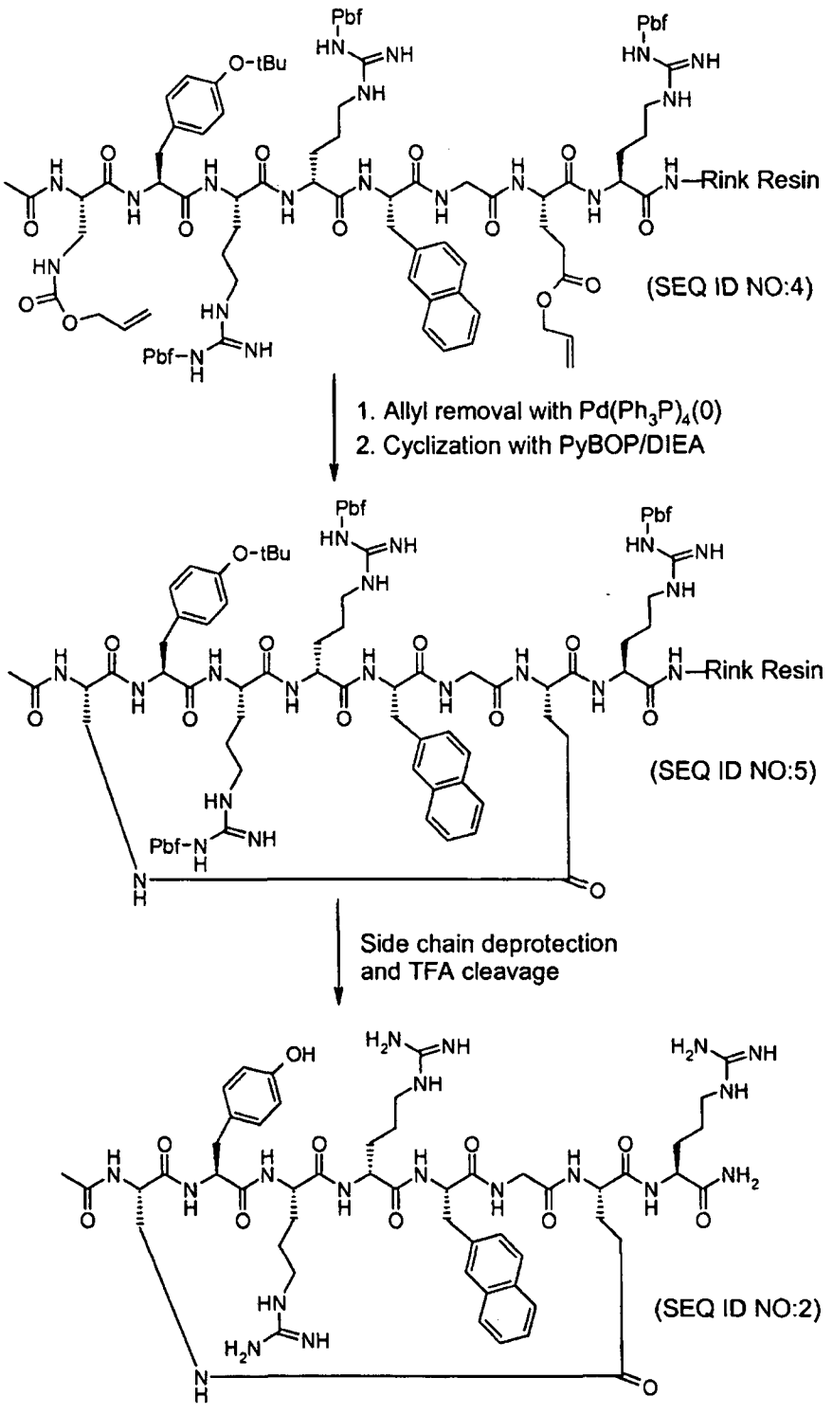
(95/2/1/2, v/v/v/v), or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature. The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers. Molecular weight calculated (MW cal.): 1142.30; MW observed (MW obs.): 1142.50.



(SEQ ID NO:4

Scheme 1. Amino side chain to carboxyl side chain peptide assembly

Peptide purification is accomplished using standard preparative HPLC techniques. Immediately following the cyclization, the peptide solution is diluted with water containing 0.1% (v/v) TFA, loaded onto a reversed phase C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile (v/v) gradient while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the final product is performed using analytical HPLC and mass spectral analysis by conventional techniques. For peptides with a basic side chain, the final lyophilized product is a TFA salt.



Scheme 2. Amino side chain to carboxyl side chain ring cyclization and cleavage

Example 2

Ac-cyclo[Dab-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:6)

Prepare as in Example 1, except that Fmoc-Dap(Alloc) in step 8 is replaced with Fmoc-Dab(Alloc). MW cal.:1156.33; MW obs.: 1156.10.

Example 3

Ac-cyclo[Dap-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:7)

Prepare as in Example 1, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(iPr)(Boc). MW cal.: 1156.37; MW obs.: 1156.78.

Example 4

n-Hexanoyl-cyclo[Dap-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:8)

Prepare as in Example 1, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(iPr)(Boc). Additionally, acetic anhydride in step 9 is replaced with hexanoic acid activated with PyBOP/DIEA. MW cal.: 1212.47; MW obs.: 1212.92.

Example 5

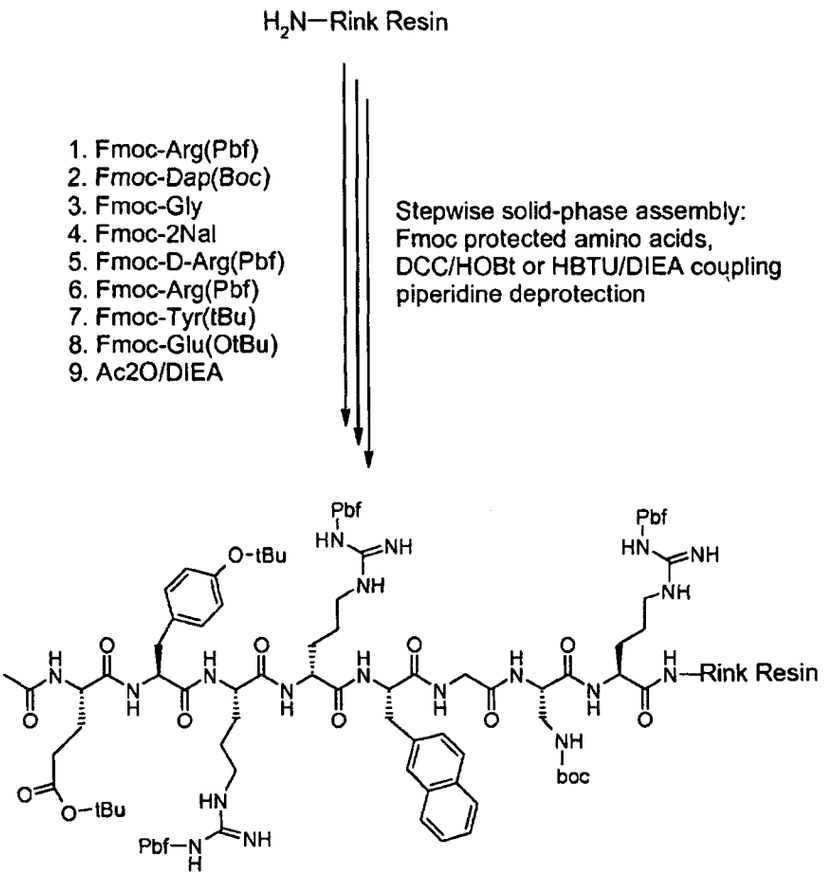
Ac-cyclo[(D/L)Agl-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:9)

Prepare as in Example 1, except that Fmoc-Glu(Oallyl) in step 2 is replaced with Fmoc-Glu(OtBu), and Fmoc-Dap(Alloc) in step 8 is replaced with Fmoc-(DL)Agl(Boc). After chain assembly, there is no $Pd(Ph_3P)_4$ treatment since no allyl protection is present. Instead, cyclization is carried out in solution after the linear peptide is cleaved from the solid support and deprotected. The crude linear peptide (0.25 mmol) from the cleavage is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following solution via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is then allowed to proceed at room temperature for 2 h. Solvents are then evaporated under vacuum, the residue is loaded onto a preparative reversed phase C18 HPLC column, and target cyclic peptide is isolated and characterized as described in Example 1. MW cal.: 1128.27; MW obs.: 1128.26.

Example 6

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:10)

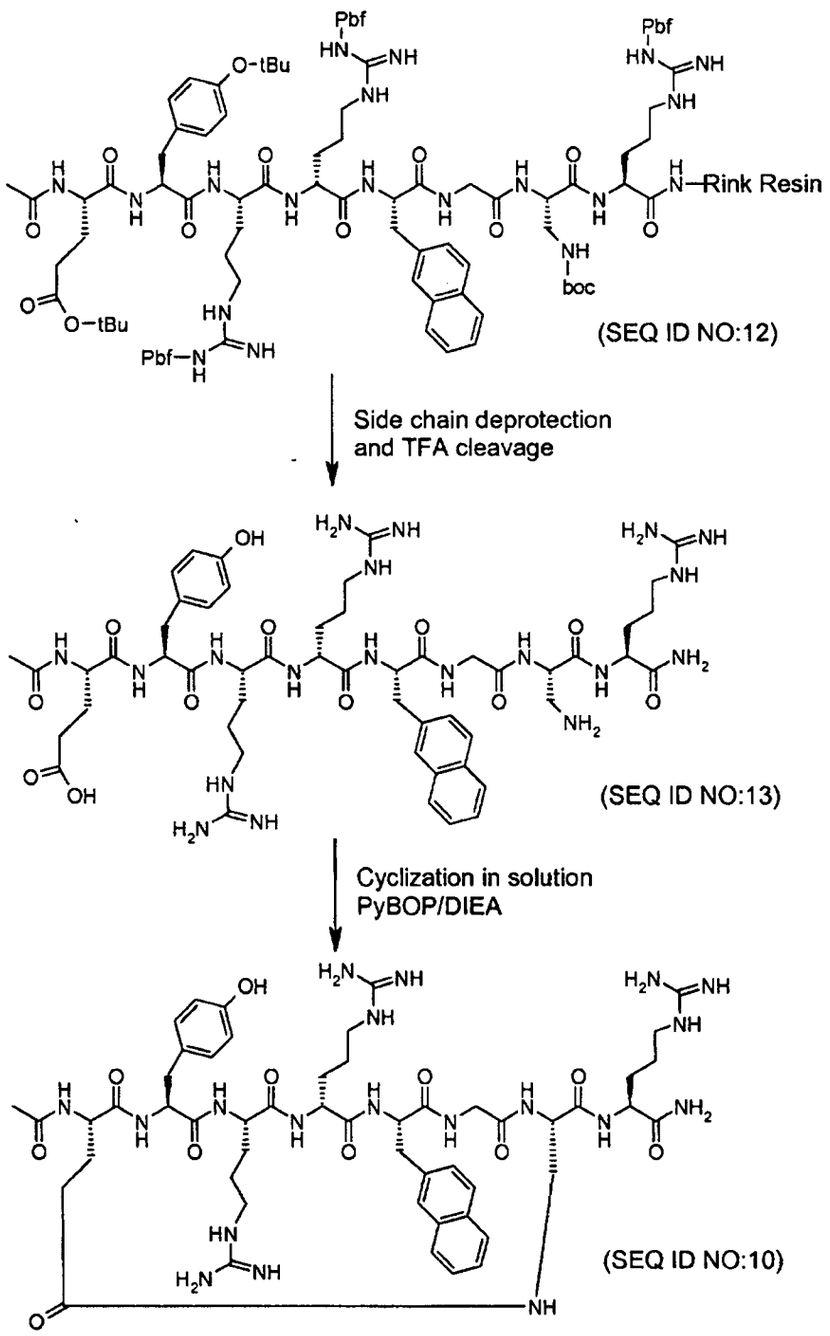
The sequence Glu(OtBu)-Tyr(tBu)-Arg(Pbf)-DArg(Pbf)-2Nal-Gly-Dap(Boc)-Arg(Pbf) (SEQ ID NO:11) is assembled by standard Fmoc chemistry utilizing an ABI 431 instrument as outlined in Scheme 3 below. The automated assembly is carried out by using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc chemistry (HBTU/DIEA) protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The solid support is Rink amide resin for C-terminal amides or indole resin [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin for C-terminal ethyl amides. Stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 9 major steps. In step 1, four equivalents of protected amino acid Fmoc-Arg(Pbf) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and coupled to deprotected Rink Amide resin. In step 2, four equivalents of Fmoc-Dap(Boc) are activated and coupled to the deprotected resin from step 1. Appropriate steps are carried out until step 8, the coupling of Fmoc-Glu(OtBu). For step 9, Fmoc at the N-terminal end is removed using 20% piperidine in DMF and acetylation of the α-amino group is carried out off-line with 5 equivalents acetic anhydride, 10 equivalents DIEA in dry DMF or NMP, for 1 h at room temperature. The finished peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v), or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature (Scheme 4). The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers. The crude product is used directly in the cyclization reaction. MW cal.: 1142.30; MW obs.: 1142.83.



(SEQ ID NO:12)

Scheme 3. Acid side chain to amino side chain peptide assembly

Cyclization is carried out in solution after the linear peptide is cleaved from the solid support with all the side chains deprotected (Scheme 4). The cleaved crude linear peptide (0.25 mmole) is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following reaction mixture via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is then allowed to proceed at room temperature for 2 h. Solvents are evaporated under vacuum, the residue is loaded onto a preparative reversed phase C18 HPLC column, and target cyclic peptide is isolated and characterized as described in Example 1.



Scheme 4. Acid side chain to amino side chain ring cyclization

Example 7

Bz-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:14)

Prepare as in Example 6, except that acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1204.37; MW obs.: 1204.87.

Example 8

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-DDap]-Arg-NH_2 (SEQ ID NO:15)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-DDap(Boc). MW cal.: 1142.30; MW obs.: 1142.73.

Example 9

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Lys]-Arg-NH_2 (SEQ ID NO:16)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Lys(Boc). MW cal.:1184.38; MW obs.: 1184.23.

Example 10

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:17)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc). MW cal.: 1156.33; MW obs.: 1156.07.

Example 11

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:18)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc). MW cal.: 1128.27; MW obs.: 1128.86.

Example 12

Bz-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:19)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1190.34; MW obs.: 1190.99.

Example 13

Bz-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:20)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1204.37; MW obs.: 1204.87.

Example 14

Ac-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:21)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1142.30; MW obs.: 1142.81.

Example 15

Ac-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:22)

Prepare as in Example 6, except that Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1128.27; MW obs.: 1128.78.

Example 16

Bz-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:23)

Prepare as in Example 6, except that Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1190.34; MW obs.: 1190.69.

Example 17

Ac-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:24)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1114.24; MW obs.: 1114.85.

Example 18

Ac-cyclo[Asp-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:25)

Prepare as in Example 6, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(Me_2), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1128.31; MW obs.: 1128.92.

Example 19

Bz-cyclo[Asp-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:26)

Prepare as in Example 6, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(Me_2), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1190.38; MW obs.: 1191.14.

Example 20

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:27)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1057.19; MW obs.: 1057.87.

Example 21

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:28)

Prepare as in Example 6, except that Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1071.22; MW obs.: 1071.85.

Example 22

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:29)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1085.25; MW obs.: 1085.87.

Example 23

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Orn]-Arg-NH_2 (SEQ ID NO:30)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Orn(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1099.27; MW obs.: 1100.23.

Example 24

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Lys]-Arg-NH_2 (SEQ ID NO:31)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Lys(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1113.30; MW obs.: 1114.25.

Example 25

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:32)

The sequence Gly-Tyr(tBu)-Lys(iPr)(Boc)-DArg(Pbf)-2Nal-Gly-DGlu(Oallyl)-Arg(Pbf) (SEQ ID NO:33) is assembled by standard Fmoc chemistry utilizing an ABI 431 instrument as outlined in Scheme 5 below. The automated assembly is carried out by using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc HBTU/DIEA chemistry protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The solid support is Rink amide resin for amides or indole resin [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin for C-terminal ethyl amides. Stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 major steps (Scheme 5). In step 1, four equivalents of protected amino acid Fmoc-Arg(Pbf) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and are coupled to deprotected Rink amide resin. In step 2, four equivalents of Fmoc-DGlu(Oallyl) are activated and coupled to the deprotected peptide resin from step 1. Appropriate steps are carried out until step 8, the coupling of Fmoc-Gly.

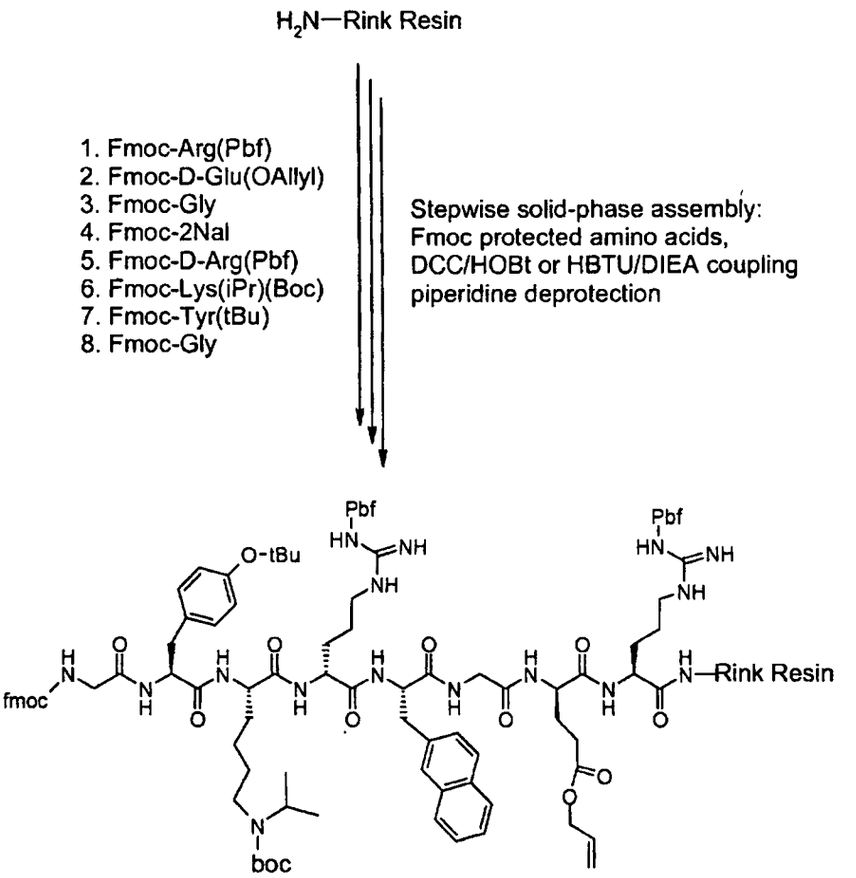
The allyl ester side chain protection group is removed with 0.1 equivalent of $Pd(Ph_3P)_4$ in the presence of 24 equivalents of phenylsilane in dichloromethane (Scheme

6). This process is repeated once for complete side chain deprotection. Then Fmoc at the N-terminal end is removed using 20% piperidine in DMF. The deprotected carboxylic acid moiety of DGlu is activated with PyBOP/DIEA, and cyclized to the α-amino group of glycine on the resin. The cyclized peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v), or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature. The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers. MW cal.: 1085.29; MW obs.: 1085.32.

Example 25a

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2·acetic acid salt (SEQ ID NO:34)

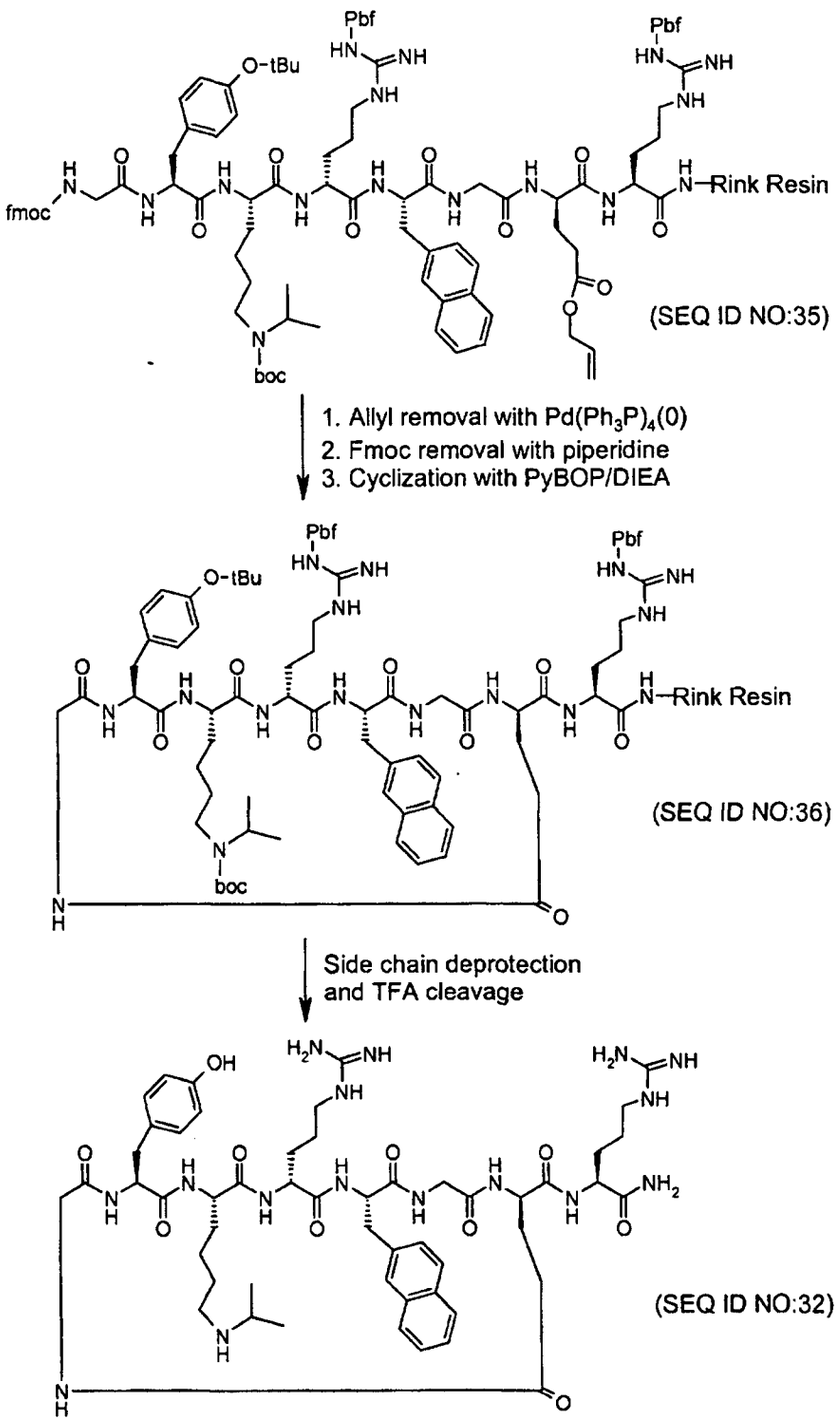
The TFA salt of the peptide of Example 25 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 1085.29; MW obs.: 1085.32.



(SEQ ID NO:35)

Scheme 5. α-amino group to carboxyl side chain peptide assembly

Peptide purification is accomplished using standard preparative HPLC techniques. Immediately following cyclization, the peptide solution is diluted with water containing 0.1% (v/v) TFA, loaded onto a reversed phase C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile (v/v) gradient while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the final product is performed using analytical HPLC and mass spectral analysis by conventional methods. For peptides with a basic side chain, the final lyophilized product is a TFA salt.



Scheme 6. α-amino group to carboxyl side chain ring cyclization and cleavage

Example 26

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:37)

Prepare as in Example 25, except that step 1 is omitted. MW cal.: 929.10; MW obs.: 929.39.

Example 26a

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2·acetic acid salt (SEQ ID NO:38)

The TFA salt of the peptide of Example 26 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 929.10; MW obs.: 929.39.

Example 27

cyclo[Gly-Tyr-Arg-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:39)

Prepare as in Example 25, except that Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf). MW cal.: 1071.22; MW obs.: 1071.02.

Example 28

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:40)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc). MW cal.: 1099.35; MW obs.: 1099.91.

Example 29

cyclo[Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:41)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), Fmoc-Gly in step 8 is not used, and step 8 is omitted. MW cal.: 1014.17; MW obs.: 1014.78.

Example 30

cyclo[Tyr-Arg-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:42)

Prepare as in Example 25, except that Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), Fmoc-Gly in step 8 is not used, and step 8 is omitted. MW cal.: 1014.17; MW obs.: 1014.65.

Example 31

cyclo[Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:43)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), Fmoc-Gly in step 8 is not used, and step 8 is omitted. MW cal.: 1000.14; MW obs.: 1000.63.

Example 32

cyclo[Gly-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:44)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf). MW cal.: 1057.19; MW obs.: 1057.35.

Example 33

cyclo[Gly-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:45)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Me_2). MW cal.: 1057.23; MW obs.: 1057.86.

Example 34

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:46)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 1071.26; MW obs.: 1071.76.

Example 35

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-NH_2 (SEQ ID NO:47)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 915.07; MW obs.: 915.38.

Example 36

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-Lys(iPr)-NH_2 (SEQ ID NO:48)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 1085.33; MW obs.: 1085.78.

Example 37

cyclo[Gly-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:49)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Me_2). MW cal.: 1071.26; MW obs.: 1071.05.

Example 38

cyclo[Gly-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:50)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf). MW cal.: 1071.22; MW obs.: 1071.50.

Example 39

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:51)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 1085.29; MW obs.: 1085.91.

Example 40

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:52)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 929.10; MW obs.: 929.39.

Example 41

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-NHEt (SEQ ID NO:53)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 943.13; MW obs.: 943.36.

Example 42

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:54)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 957.15; MW obs.: 957.50.

Example 43

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NHEt (SEQ ID NO:55)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, and step 1 is omitted. MW cal.: 957.15; MW obs.: 957.46.

Example 44

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NHEt (SEQ ID NO:56)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin. MW cal.: 1113.34; MW obs.: 1113.81.

Example 44a

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NHEt·acetic acid salt (SEQ ID NO:57)

The TFA salt of the peptide of Example 44 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v)

containing 2% acetic acid by volume, and lyophilized. MW cal.: 1113.34; MW obs.: 1113.81.

Example 45

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt (SEQ ID NO:58)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, and Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc). MW cal.: 1127.41; MW obs.: 1127.35.

Example 46

cyclo[Lys(Ac)-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:59)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(OtBu), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Me_2), and Fmoc-Gly in step 8 is replaced with Boc-Lys(Fmoc). After chain assembly, Fmoc on the side chain of the N-terminal Lys is removed with 20% piperidine in DMF and the Lys side chain is then acetylated using 10 equivalents of acetic anhydride/DIEA at room temperature for one hour. All side chain protection groups are removed and the linear peptide is cleaved from the solid support using a mixture of TFA/water/TIS/anisole (90/5/2.5/2.5, v/v/v/v) for 2 h at room temperature. Cyclization of the crude linear peptide is carried out in solution. The cleaved crude linear peptide (~0.25 mmole) is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following reaction mixture via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is then allowed to proceed at room temperature for 2 h. Solvents are then evaporated under vacuum, the residue is loaded onto a reversed phase C18 preparative HPLC column, and the target cyclic peptide is isolated and characterized as described in Example 1. MW cal.: 1184.42; MW obs.: 1184.06.

Example 47

cyclo[Dap(Ac)-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:60)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(OtBu), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with

Fmoc-Lys(Me_2), and Fmoc-Gly in step 8 is replaced with Boc-Dap(Fmoc). After chain assembly, Fmoc on the side chain of the N-terminal Dap is removed using 20% piperidine in DMF, and the Dap side chain is then acetylated with 10 equivalents of acetic anhydride/DIEA at room temperature for one hour. All side chain protection groups are then removed and the linear peptide is cleaved from the solid support using a mixture of TFA/water/TIS/anisole (90/5/2.5/2.5, v/v/v/v) for 2 h at room temperature. Cyclization of the crude linear peptide is carried out in solution. The cleaved crude linear peptide (~0.25 mmole) is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following reaction mixture via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is allowed to proceed at room temperature for 2 h. Solvents are then evaporated under vacuum, the residue is loaded onto a preparative HPLC column, and the target cyclic peptide is isolated and characterized as described in Example 1. MW cal.: 986.15; MW obs.: 985.97.

Example 48

cyclo[Ala-Tyr-Lys(iPr)-DArg -2Nal-Gly-Glu]-NH_2 (SEQ ID NO:61)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-Ala. MW cal.: 943.13; MW obs.: 942.92.

Example 49

cyclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:62)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DAla. MW cal.: 943.13; MW obs.: 943.44.

Example 50

cyclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:63)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-DAla. MW cal.: 943.13; MW obs.: 943.42.

Example 51

cyclo[Ala-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:64)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-Ala. MW cal.: 943.13; MW obs.: 943.48.

Example 52

cyclo[Leu-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:65)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-Leu. MW cal.: 985.21; MW obs.: 985.56.

Example 53

cyclo[Leu-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:66)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-Leu. MW cal.: 985.21; MW obs.: 985.49.

Example 54

cyclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:67)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DPhe. MW cal.: 1019.22; MW obs.: 1019.52.

Example 55

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:68)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1019.22; MW obs.: 1019.53.

Example 56

cyclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:69)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-DPhe. MW cal.: 1019.22; MW obs.: 1019.50.

Example 57

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1189.48; MW obs.: 1189.92.

Example 57a

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2·acetic acid salt (SEQ ID NO:71)

The TFA salt of the peptide of Example 57 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 1189.48; MW obs.: 1189.92.

Example 58

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:72)

Prepare as in Example 25, except that Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1175.41; MW obs.: 1175.81.

Example 59

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:73)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1019.22; MW obs.: 1019.56.

Example 60

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt (SEQ ID NO:74)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, Fmoc-Arg(Pbf) in step 1 is

replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1217.53; MW obs.: 1217.97.

Example 60a

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt·acetic acid salt (SEQ ID NO:75)

The TFA salt of the peptide of Example 60 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 1217.53; MW obs.: 1217.97.

Example 61

cyclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:76)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DAla. MW cal.: 971.18; MW obs.: 971.49.

Example 62

cyclo[2Nal-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:77)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-2Nal. MW cal.: 1097.34; MW obs.: 1097.53.

Example 63

cyclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:78)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-

DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DPhe. MW cal.: 1047.28; MW obs.: 1047.51.

Example 64

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:79)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc- Phe. MW cal.: 1047.28; MW obs.: 1047.57.

Example 65

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Gly-2Nal-NH_2 (SEQ ID NO:80)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-2Nal, and one step is added between steps 1 and 2 using Fmoc-Gly. MW cal.: 1183.39; MW obs.: 1183.26.

Example 66

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-β-Ala-D2Nal-NH_2 (SEQ ID NO:81)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-D2Nal, one step is added between steps 1 and 2 using Fmoc-β-Ala, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 1197.40; MW obs.: 1196.70.

Example 67

cyclo[β-Ala-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:82)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-β-Ala. MW cal.: 1085.25; MW obs.: 1085.05.

Example 68

cyclo[β-Ala-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:83)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-β-Ala. MW cal.: 1071.22; MW obs.: 1071.15.

Example 69

cyclo[5-aminovaleryl-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:84)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-5-aminovaleric acid. MW cal.: 1113.30; MW obs.: 1113.40.

Example 70

cyclo[5-aminovaleryl-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:85)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-5-amino valeric acid. MW cal.: 1099.27; MW obs.: 1100.25.

Example 71

cyclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:86)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl phenylacetic acid (4-AMPA). MW cal.: 1147.32; MW obs.: 1148.20.

Example 72

cyclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:87)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl phenylacetic acid (4-AMPA). MW cal.: 1161.34; MW obs.: 1161.99.

Example 73

cyclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Glu]-DArg-NH_2 (SEQ ID NO:88)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-DArg(Pbf), Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl phenylacetic acid (4-AMPA). MW cal.: 1161.34; MW obs.: 1161.83.

Example 74

cyclo[(4-AMB)-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:89)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl benzoic acid (4-AMB). MW cal.: 1147.32; MW obs.: 1147.66.

Example 75

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-Gly-2Nal-NH_2 (SEQ ID NO:90)

Prepare as in Example 25, except that step 1 is replaced with three sequential residue couplings: first Fmoc-2Nal, then Fmoc-Gly, and then Fmoc-Lys(iPr)(Boc). MW cal.: 1353.69; MW obs.: 1354.03.

Example 76

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-Gly-2Nal-NH_2 (SEQ ID NO:91)

Prepare as in Example 25, except that step 1 is replaced with three sequential residue couplings: first Fmoc-2Nal, then Fmoc-Gly, and then Fmoc-Lys(iPr)(Boc). In addition, Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1443.82; MW obs.: 1444.13.

Example 77

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Gly-DPhe-NH_2 (SEQ ID NO:92)

Prepare as in Example 25, except that step 1 is replaced with two sequential residue couplings: first Fmoc-DPhe, then Fmoc-Gly. MW cal.: 1133.36; MW obs.: 1133.73.

Example 78

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-DPhe-NH_2 (SEQ ID NO:93)

Prepare as in Example 25, except that step 1 is replaced with two sequential residue couplings: first Fmoc-DPhe, then Fmoc-Lys(iPr)(Boc). MW cal.: 1246.58; MW obs.: 1246.88.

Example 79

cyclo[Lys-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:94)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Lys(Boc). MW cal.: 1170.50; MW obs.: 1169.80.

Example 80

cyclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:95)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1147.40; MW obs.: 1146.70.

Example 81

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys-NH_2 (SEQ ID NO:96)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1147.40; MW obs.: 1146.70.

Example 82

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Orn-NH_2 (SEQ ID NO:97)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Orn(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1133.40; MW obs.: 1132.70.

Example 83

cyclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys-NH_2 (SEQ ID NO:98)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 and Fmoc-Lys(iPr)(Boc) in step 6 are each replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1105.37; MW obs.: 1105.40.

Example 84

cyclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys-NHEt (SEQ ID NO:99)

Prepare as in Example 25, except that Rink resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, Fmoc-Arg(Pbf) in step 1 and Fmoc-Lys(iPr)(Boc) in step 6 are each replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1133.36; MW obs.: 1133.82.

Example 85

Alternative Synthesis I of

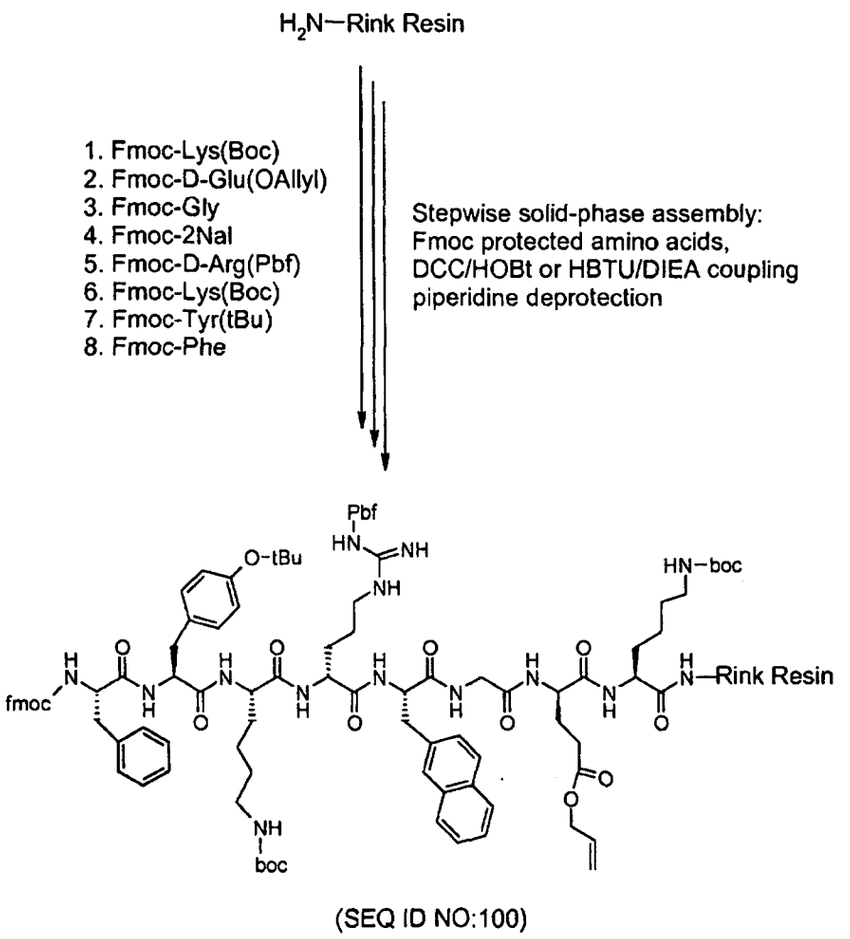
cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

Example 57 discloses the synthesis of SEQ ID NO:70 via Fmoc solid phase peptide synthesis chemistry employing the commercially available building block Fmoc-Lys(iPr)Boc, which is expensive and difficult to obtain in large quantity. The process

described in this example permits the synthesis of SEQ ID NO:70 using less expensive Fmoc-Lys(Boc), solution cyclization, and lysine alkylation through reductive amination using sodium cyanoboronhydride, providing a more economical route to this end product. Additional advantages are that the reaction media (acetic acid, acetone, and methanol) are relatively inexpensive, the reaction conditions are easily controlled, the ratio of solvents can vary significantly without affecting the alkylation reaction, and the recovery yield is 90% or higher.

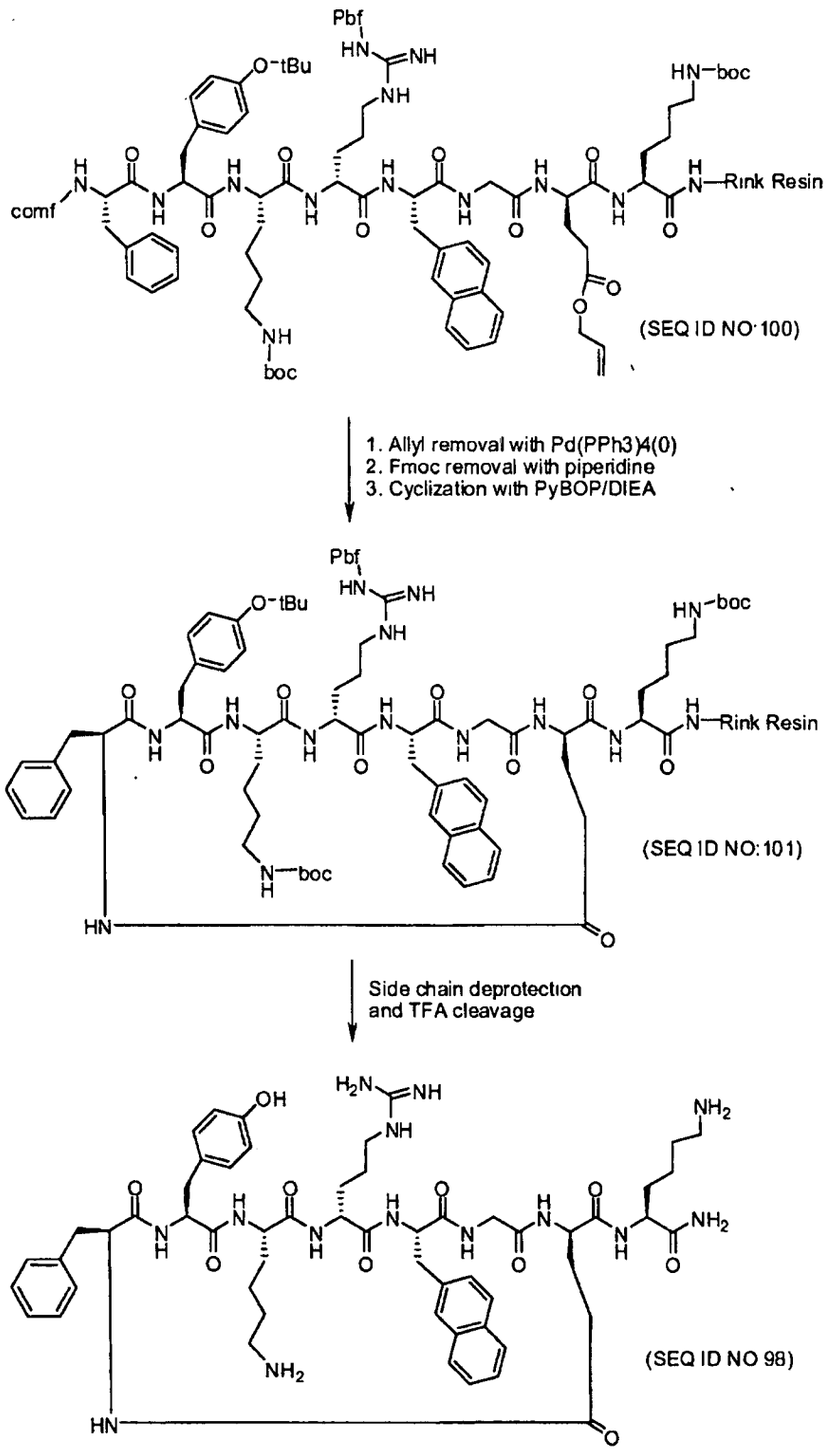
The sequence Phe-Tyr(tBu)-Lys(Boc)-DArg(Pbf)-2Nal-Gly-DGlu(Oallyl)-Lys(Boc) (SEQ ID NO:100) is assembled on Rink Amide Resin by standard Fmoc chemistry utilizing an ABI 431 Peptide Synthesizer as outlined in Scheme 7. The automated assembly is carried out using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc chemistry (HBTU/DIEA) protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The side chain protecting group scheme is: Lys(Boc), DGlu(Oallyl), DArg(Pbf), Tyr(tBu). The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 steps. In step 1, four equivalents of protected amino acid Fmoc-Lys(Boc) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and coupled to deprotected Rink amide resin. In step 2, four equivalents of Fmoc-DGlu(Oallyl) are activated and coupled to the deprotected peptide resin from step 1. These steps are repeated appropriately until step 8, the coupling of Fmoc-Phe.

The allyl ester side chain protecting group is removed with 0.1 equivalent of $Pd(Ph_3P)_4$ in the presence of 24 equivalents of phenylsilane in dichloromethane (Scheme 8). This process is repeated once for complete side chain deprotection. Fmoc at the N-terminal end is then removed using 20% piperidine in DMF. The deprotected carboxylic acid moiety of DGlu is activated with PyBOP/DIEA and cyclized to the α-amino group of Phe on the resin. The cyclized peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v) or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature. The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers.



(SEQ ID NO:100)

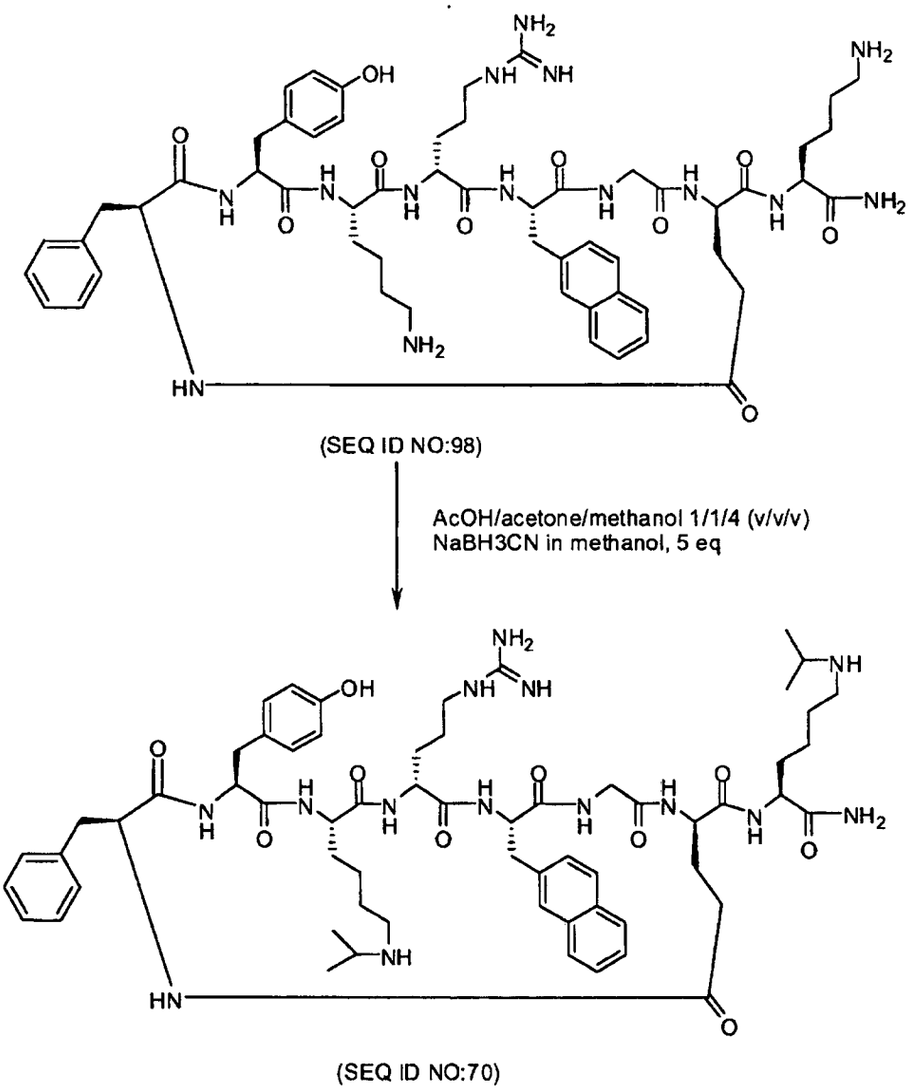
Scheme 7. Peptide chain assembly on solid phase



Scheme 8. Preparation of cyclic precursor peptide

Purification of the cyclic precursor peptide is accomplished using standard preparative HPLC techniques. The crude cleavage product is dissolved in a minimum amount of DMSO, loaded onto a reversed phased C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques.

The lyophilized precursor cyclic peptide is then alkylated in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride (Scheme 9). Peptide concentration is about 10mg/mL, and can vary significantly without affecting the results. Three to 5 equivalents of the reducing reagent sodium cyanoboronhydride are used, and the reaction is normally completed within 2 h at room temperature. The recovery yield is 90% or higher. For example, 20 mg of the precursor cyclic peptide are dissolved in 2 mL of methanol, to which 0.5 mL of acetic acid and 0.5 mL of acetone are added, mixed well, and then 5.6 mg of sodium cyanoboronhydride (2.5 equivalents in methanol) are added under stirring. The reaction mixture is stirred at room temperature for 30 min, upon which another 5.6 mg of sodium cyanoboronhydride are added. The reaction is monitored by HPLC and mass spectral analysis. After the reaction is complete, desalting of the reaction mixture and lyophilization yields 18.9 mg of final product (SEQ ID NO:70) with a purity of 97.5%. MW cal.: 1189.48; MW obs.: 1189.6.



Scheme 9. Reductive amination converting Lys to Lys(iPr)

The compound of Example 60 (SEQ ID NO:74) can also be prepared in an analogous manner, with appropriate modifications based on its constituent amino acids, using [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin. First, the precursor peptide (SEQ ID NO:99) is prepared as described in Example 84, and then reductive amination is carried out as shown in Scheme 9. This affords a final cyclic C-terminal ethyl amide peptide with a purity of 99.16%. MW cal.:1217.53; MW obs.: 1217.84.

Example 86

Alternative Synthesis II of

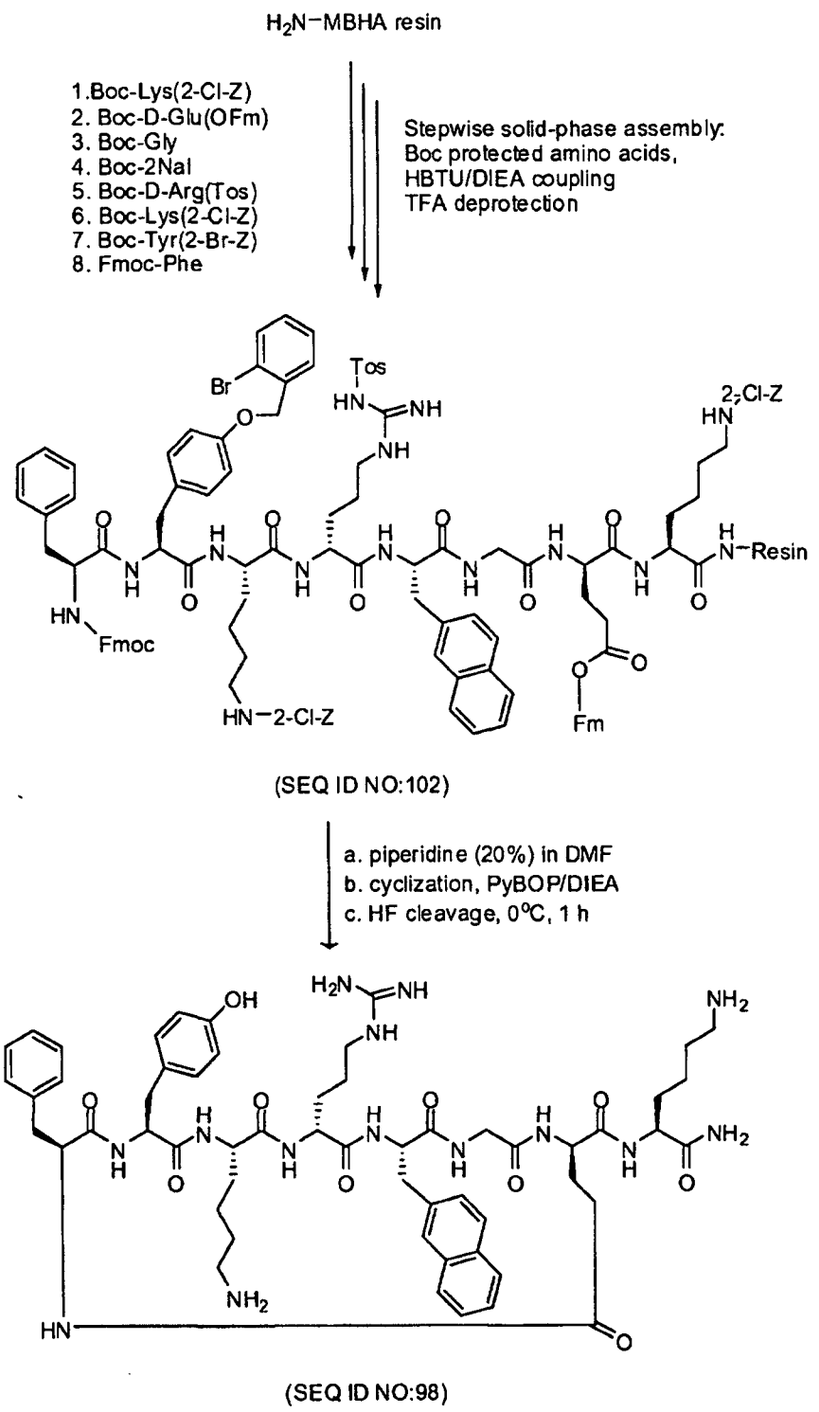
cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

Example 85 discloses a cost effective synthesis of SEQ ID NO:70 via Fmoc solid phase peptide synthesis chemistry employing relatively inexpensive Fmoc-Lys(Boc) and alkylation of lysine through reductive amination using sodium cyanoboronhydride in relatively inexpensive solvents (acetic acid, acetone, and methanol). However, this process involves the use of the heavy metal catalyst palladium for the removal of the allyl ester side chain protective group. Palladium is highly toxic, and quality control to insure complete removal of this element is complicated and difficult. The Boc solid phase peptide synthesis process with cyclization on the resin described in this example permits the production of SEQ ID NO:70 using relatively inexpensive Boc-Lys(2-Cl-Z) without the need for a toxic and expensive palladium catalyst, providing an even more economical, easily upscalable, less toxic route to an end product requiring a simpler quality control process.

The sequence Fmoc-Phe-Tyr(2-Br-Z)-Lys(2-Cl-Z)-DArg(Tos)-2Nal-Gly-DGlu(OFm)-Lys(2-Cl-Z) (SEQ ID NO:102) is manually assembled on MBHA (4-methyl-benzhydryl-amine) resin (Cat. No. D-2095, BaChem California Inc., Torrance, CA) using established solid phase peptide synthesis Boc chemistry (Schnölzer et al. (1992) *Int. J. Pept. Protein Res.* 40:180-193) as outlined in Scheme 10. The chain assembly is carried out using an *in situ* neutralization/HBTU/DIEA activation procedure as described in this reference. The side chain protecting group scheme is: Lys(2-Cl-Z), DGlu(OFm), DArg(Tos), and Tyr(2-Br-Z). The alpha-amino group of all the amino acid building blocks is protected with tert-butoxycarbonyl (Boc) except the N-terminal residue Phe, which is protected with Fmoc for efficiency of synthesis. The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 steps. In step 1, five equivalents of protected amino acid Boc-Lys(2-Cl-Z) are activated with HBTU/DIEA in DMF, and coupled to MBHA resin. In step 2, five equivalents of Boc-DGlu(OFm) are activated and coupled to the deprotected peptide resin from step 1 using neat TFA. These steps are repeated appropriately until step 8, the coupling of Fmoc-Phe.

The Fm side chain protecting group of DGlu, together with the Fmoc at the

N-terminal end, is removed using 20% piperidine in DMF. The deprotected carboxylic acid moiety of DGlu is activated with PyBOP/DIEA, HCTU/DIEA, or other appropriate activation reagents, and cyclized to the α-amino group of Phe on the resin. The cyclic peptide is simultaneously deprotected and cleaved from the resin using HF with 5% *m*-cresol or *p*-cresol as a scavenger for 1 hour at 0 °C. The solvents are then evaporated and the crude peptide is precipitated and washed three times with cold diethyl ether.



Scheme 10. Peptide chain assembly on solid phase using Boc chemistry

Purification of the cyclic precursor peptide (SEQ ID NO:98 as shown in Scheme 10) is accomplished using standard preparative HPLC techniques. The crude cleavage product is dissolved in a minimum amount of DMSO, loaded onto a reversed phased C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques. For SEQ ID NO:98, MW cal.: 1105.29; MW obs.: 1105.4.

The lyophilized precursor cyclic peptide (SEQ ID NO:98) is then alkylated in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride as in Scheme 9. Peptide concentration is about 10mg/mL, and can vary significantly without affecting the results. Five equivalents of the reducing reagent sodium cyanoboronhydride are used, and the reaction is normally completed within 2 h at room temperature. The recovery yield is 90% or higher. For example, 6.6 mg of the precursor cyclic peptide are dissolved in 0.8 mL of methanol, to which 0.2 mL of acetic acid and 0.2 mL of acetone are added, mixed well, and then 1.9 mg of sodium cyanoboronhydride (2.5 equivalents in methanol) are added under stirring in two equal portions. The reaction mixture is stirred at room temperature for 30 min, upon which another 1.9 mg of sodium cyanoboronhydride are added. The reaction is monitored by HPLC and mass spectral analysis. After the reaction is complete, desalting of the reaction mixture and lyophilization yields the final product (SEQ ID NO:70) with a purity of 96.5%. MW cal.: 1189.45; MW obs.: 1189.6.

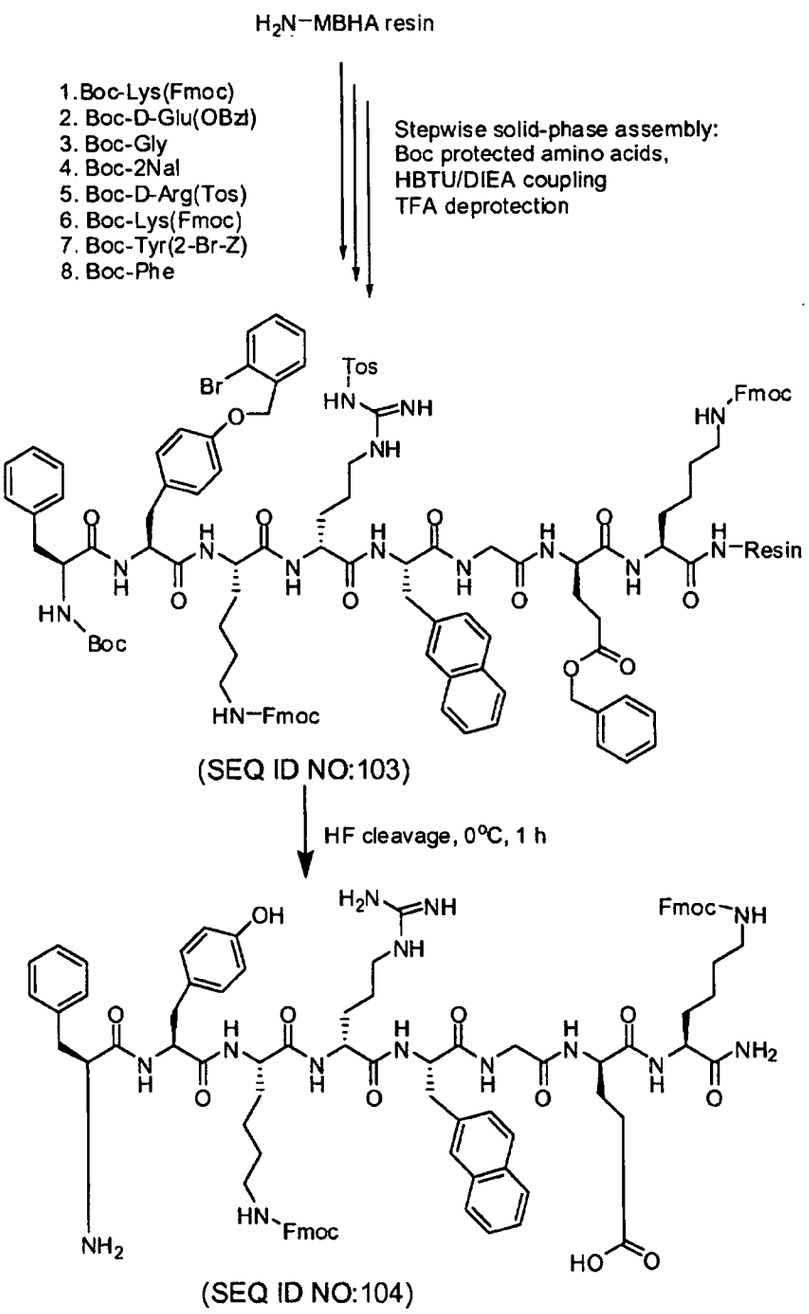
Example 87

Alternative Synthesis III of cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

The compound of Example 57 (SEQ ID NO:70) can also be prepared without the use of a palladium catalyst via solution cyclization, facilitating scaleup, as follows.

The sequence Boc-Phe-Tyr(2-Br-Z)-Lys(Fmoc)-DArg(Tos)-2Nal-Gly-DGlu(OBzl)-Lys(Fmoc) (SEQ ID NO:103) is manually assembled on MBHA resin using solid phase peptide synthesis Boc chemistry (Schnölzer et al. (1992) *Int. J. Pept. Protein Res.* 40:180-193) as outlined in Scheme 11. The chain assembly is carried out using the

in situ neutralization/HBTU/DIEA activation procedure as described in Schnölzer et al. The side chain protecting group scheme is: Lys(Fmoc), DGlu(OBzl), DArg(Tos), Tyr(2-Br-Z). The alpha-amino group of all the amino acid building blocks is protected with tert-butoxycarbonyl (Boc). The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 steps as shown in Scheme 11. In step 1, five equivalents of protected amino acid Boc-Lys(2-Cl-Z) are activated with HBTU (4 eq) /DIEA (10 eq) in DMF, and coupled to MBHA resin. In step 2, five equivalents of Boc-DGlu(OBzl) are activated and coupled to the deprotected peptide resin from step 1 using neat TFA. These steps are repeated appropriately until step 8, the coupling of Boc-Phe. The Boc protecting group is removed with neat TFA, the resin is neutralized with DIEA, and washed with DMF and methanol and dried in air before HF cleavage. The linear peptide is simultaneously deprotected and cleaved from the resin using HF with 5% *m*-cresol or *p*-cresol as a scavenger for 1 hour at 0 °C. The solvents are then evaporated and the crude peptide is precipitated and washed three times with cold diethyl ether.



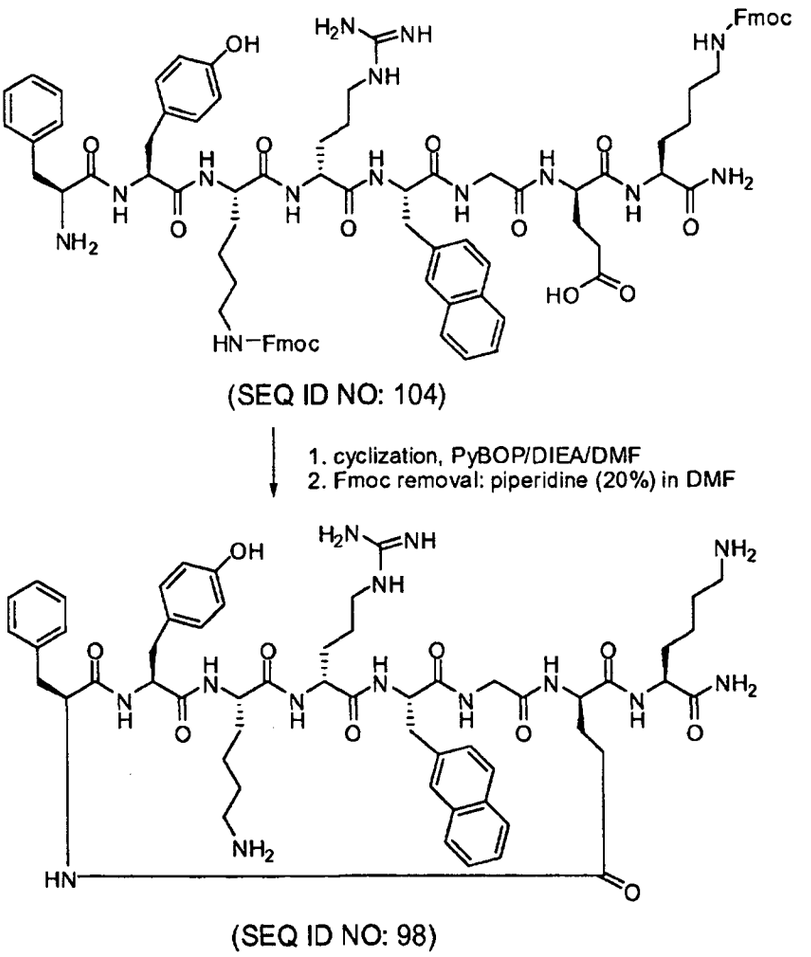
Scheme 11. Chain assembly using Boc chemistry

Purification of the linear precursor peptide (SEQ ID NO:104) is accomplished using standard preparative HPLC techniques. The crude cleavage product is dissolved in

a minimum amount of DMSO, loaded onto a reversed phased C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques. For SEQ ID NO:104, MW cal.: 1567.78; MW obs.: 1567.6.

Cyclization of the lyophilized precursor linear peptide (SEQ ID NO:104) is carried out in solution (Scheme 12). The linear peptide is dissolved in a small amount of dry DMF (~10 mg/mL). This peptide solution is slowly delivered via a syringe pump to the reaction mixture of PyBOP (2 eq, or other appropriate activation reagents, such as HCTU, BOP, HBTU, etc.) and DIEA (10 eq) in dry DMF under magnetic stirring. The reaction is then allowed to proceed at room temperature for 2 h. Neat piperidine is then added to the reaction mixture to a final concentration of 25% (v/v). The reaction mixture is kept under stirring for another 20 min to completely remove Fmoc protection. Solvents are evaporated under vacuum and the residue is loaded onto a preparative reversed phase C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized, and afford the cyclic precursor peptide (SEQ ID NO:98). Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques. For SEQ ID NO:98, MW cal.: 1105.29; MW obs.: 1105.4.

Alkylation of cyclic peptide SEQ ID NO:98 is carried out in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride as in Scheme 9 to generate the final product (SEQ ID NO:70). MW cal.: 1189.45; MW obs.: 1189.6.



Scheme 12. Cyclization and Fmoc removal

Example 88

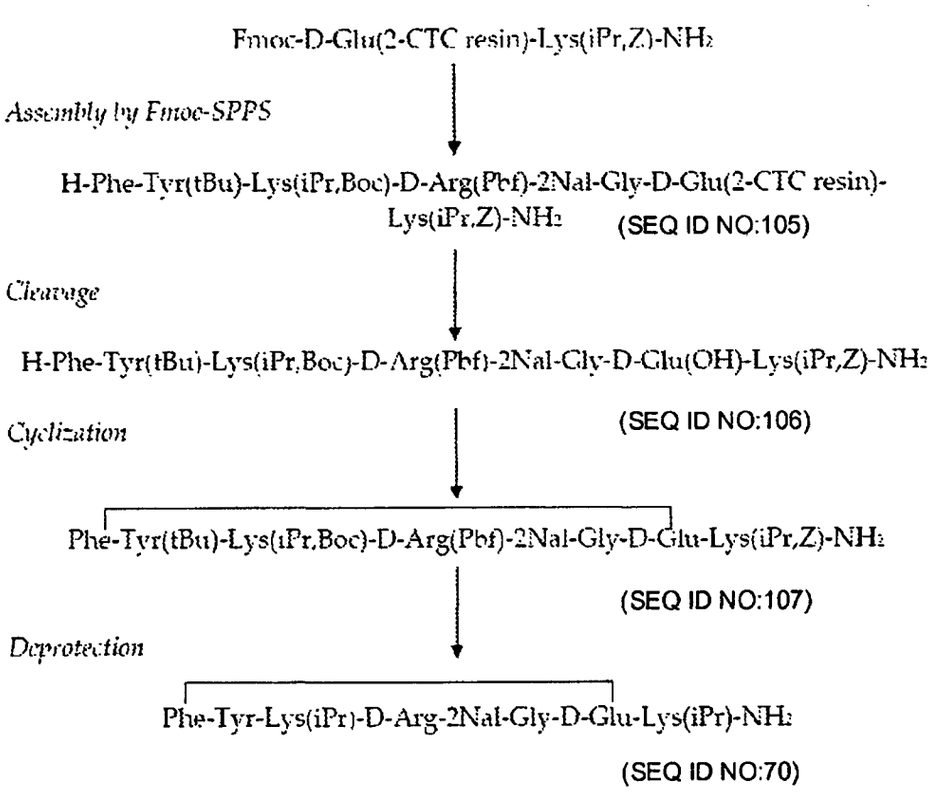
Alternative Synthesis IV of

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

The compound of Example 57 (SEQ ID NO:70) can also be prepared without the use of a palladium catalyst by the synthetic process summarized in Scheme 13 below.

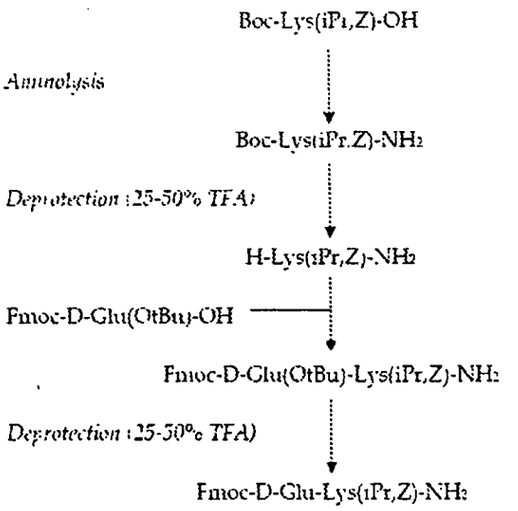
The dipeptide Fmoc-DGlu-Lys(iPr,Z)-NH_2 is first prepared in solution with an exposed DGlutamic acid side chain. The dipeptide is linked to a hyper-acid labile CTC (2-chlorotritylchloride PS resin, 1% DVB (100-200 mesh) resin (Senn Chemicals USA Inc., San Diego, CA; catalog number 40207), and the final peptide product is then

synthesized on this resin via standard Fmoc synthesis as described above. Selective removal of the peptide from the CTC resin allows only the DGlutamic acid side chain to react with the N-terminus of the peptide in solution and generate the cyclic peptide product. Subsequently, the remaining side chains are cleaved with 95% TFA or other strong acid.



Scheme 13. Overall synthetic scheme from Fmoc-DGlu-Lys(iPr,Z)-NH_2

The dipeptide Fmoc-DGlu-Lys(iPr,Z)-NH_2 is first prepared as shown below (Scheme 14):



Scheme 14. Preparation of Fmoc-DGlu-Lys(iPr,Z)-NH_2

Boc-Lys(iPr,Z)-OH is reacted with NMM and IBCF in THF. After addition of NH_4OH, the solvents are removed by rotary evaporation and the product is taken up in ethyl acetate. The ethyl acetate phase is washed extensively with 5% $NaHCO_3$ and then with 0.1 N HCl, and then dried over anhydrous sodium sulfate. Sodium sulfate is removed by filtration and the ethyl acetate is removed by evaporation at reduced pressure. The resulting Boc-Lys(iPr,Z)-NH_2 is dissolved in DCM, and TFA is added. Once the reaction has proceeded to completion, the solvents are removed by rotary evaporation. H-Lys(iPr,Z)-NH_2 is then dissolved in DMF. The pH is adjusted to 8 with DIEA. In a separate vessel, Fmoc-DGlu(OtBu)-OH, HBTU, and HOBt are dissolved in DMF; DIEA is added to adjust the pH to 8. The two solutions are mixed, and the reaction is monitored by C18 reversed phase HPLC. The pH is monitored and adjusted, where necessary, with DIEA. The solvents are removed by rotary evaporation, and the product dissolved in ethyl acetate. The ethyl acetate phase is washed extensively with 5% $NaHCO_3$ and then with 0.1 N HCl, and then dried over anhydrous sodium sulfate. The sodium sulfate is removed by filtration and the ethyl acetate is removed by evaporation at reduced pressure. Rotary evaporation is continued until a dry residue is formed. The resulting Fmoc-DGlu(OtBu)-Lys(iPr,Z)-NH_2 is dissolved in DCM, and TFA is added. Once the reaction has proceeded to completion, the solvents are removed by rotary evaporation. The solid

product is obtained by trituration with diethyl ether. After the precipitate is washed with ether, the product is dried in a vacuum oven.

Solid phase peptide synthesis of the final product is performed as follows. Fmoc-DGlu(OtBu)-Lys(iPr,Z)-NH_2 is dissolved in DCM and reacted with CTC resin in the presence of DIEA in a reaction vessel. After 3 h, the peptide-resin is washed free of reagents with DCM and Z-OSu is added. The pH is monitored and adjusted to pH 8-9 by adding DIEA if necessary. After 8 h, the peptide-resin is washed free of reagents with DCM, transferred to a polypropylene container, and dried in a vacuum oven.

The protected peptide-resin is assembled using Fmoc-chemistry as follows. The coupling cycle used is: 1) De-blocking: treatment with 25% piperidine in DMF; 2) Washing cycles with DMF, IPA and DMF again; 3) Ninhydrin test (qualitative: if positive, proceed to coupling Step 4); 4) Coupling with 2 equivalents Fmoc-amino acid in presence of HOBt /DIC in DMF; 5) Washing cycles with DMF; 6) Ninhydrin test (qualitative: if negative, proceed to next de-blocking/coupling cycle; if positive, proceed to re-coupling Step 7; if slightly positive, proceed to acetylation Step 10); 7) Re-coupling (if required), with 1 equivalent Fmoc-amino acid in the presence of HOBt, HBTU/DIEA in DMF; 8) Washing cycles with DMF; 9) Ninhydrin test (qualitative: if negative, proceed to next de-blocking/coupling cycle; if positive, proceed to acetylation Step 10); 10) Acetylation (if required) with 2% acetic anhydride in 4% DIEA in DMF; 11) Washing cycles (with DMF, IPA, and DMF again); 12) Ninhydrin test (qualitative: if positive, proceed to next de-blocking/coupling cycle). After the final coupling cycle, the peptide-resin (SEQ ID NO:105) is washed with ether and dried under vacuum.

The protected peptide-resin is washed with DCM. Cleavage of the fully protected linear peptide from the resin is performed with 2% TFA in DCM followed by filtration. The solvents are removed by rotary evaporation, and the fully linear protected peptide (SEQ ID NO:106) is precipitated by trituration with ether. The fully protected linear peptide is transferred to a polypropylene container and dried in a vacuum oven.

The fully protected linear peptide is cyclized in the presence of PyBOP, HOBt, and DIEA in DMF. The pH is maintained between pH 7-8 by addition of DIEA, if necessary. After the reaction has proceeded to completion, the solvents are removed by rotary evaporation, and the product is taken up in ethyl acetate. The ethyl acetate phase is washed extensively with 5% $NaHCO_3$ and then with 0.1 N HCl and saturated NaCl

solution. It is then dried over anhydrous sodium sulfate. The sodium sulfate is removed by filtration and the ethyl acetate is removed by rotary evaporation at reduced pressure. The protected cyclic peptide (SEQ ID NO:107) is precipitated by trituration with ether and dried in a vacuum oven.

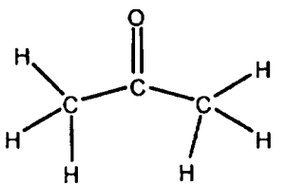
Deprotection is performed in TFA:H_2O:TIS. When the reaction is complete, the solvents are removed by rotary evaporation and the cyclic peptide (SEQ ID NO:70) is precipitated by trituration with ether and dried in a vacuum oven. MW cal.: 1189.48; MW obs.: 1189.50.

Example 89

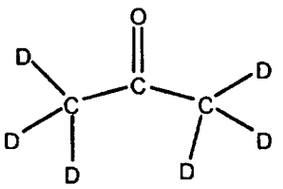
Incorporation of Isotopic Labels:

Synthesis of cyclo[Phe-Tyr-Lys(iPr-d_6)-DArg-2Nal-Gly-DGlu]-Lys(iPr-d_6)-NH_2 (SEQ ID NO:108)

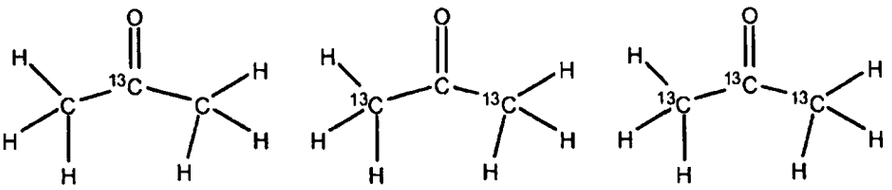
Starting with isotopically labeled acetone such as ^{13}C-, ^{14}C-, deuterium-, or tritium-labeled acetones as shown below, the processes of Examples 85-87 permit site-specific isotopic labeling of cyclic peptide CXCR4 antagonists for various pharmacological and imaging studies. The isotopically labeled acetones are commercially available from various sources. An example is given below using acetone-d_6 to prepare a peptide containing 12 deuterium atoms. The resulting compound, differing in molecular weight by 12 Da compared to the non-labeled counterpart, is easily differentiated in mass spectra and exhibits identical target receptor affinity.



Acetone



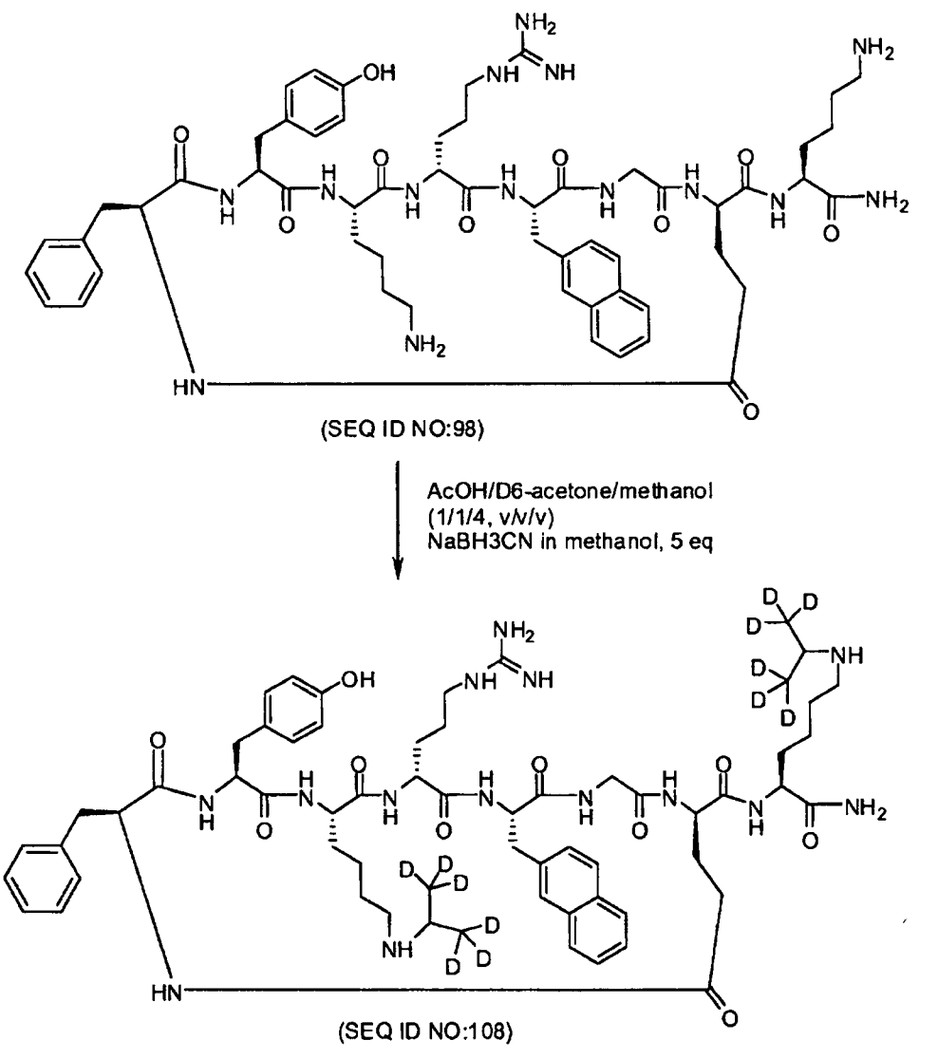
Acetone-d_6



Acetone-^{13}C Acetone-2-^{13}C Acetone-3-^{13}C

Using any of the methods in Examples 85-87, one can prepare and purify cyclic peptide precursors (SEQ ID NO:98, SEQ ID NO:99, etc.). Alkylation is carried out in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride as in Scheme 9, with the exception that standard acetone is replaced with the desired isotopically labeled acetone. In the present example, deuterium acetone-d6 is used.

Peptide (97 mg) is dissolved in 15 mL of acetic acid/acetone-d_6/methanol (1:1:4, v/v/v). Peptide concentration can vary significantly without affecting the results. Five equivalents of sodium cyanoboronhydride are used, and the reaction is normally completed within 2 h at room temperature. The reaction is monitored by HPLC and mass spectral analysis. After the reaction is complete, desalting of the reaction mixture and lyophilization yields 90.5 mg of final product (SEQ ID NO:108) with a purity of 99.9%. MW cal.: 1201.48; MW obs.: 1201.7.



Scheme 15. Site-specific deuterium labeling of CXCR4 antagonist

Use of isotopically labeled sodium cyanoboronhydride (such as NaBD3CN, NaBT3C N, etc.) permits incorporation of additional variations to the site-specific labeling patterns.

The pharmacological properties of the present compounds can be determined by employing the assays described below.

Human CXCR4/^{125}I-SDF-1α Binding Inhibition Assay

SDF-1 binding to CXCR4 is the first step in activating the CXCR4 intracellular signalling pathway. To determine if a compound can block the interaction of SDF-1 and CXCR4, human leukemia CCRF-CEM cells (ATCC CCL 119) expressing endogenous CXCR4 are employed in an ^{125}I-labeled SDF-1α binding assay. The assay is performed in a 96-well U-bottom, non-treated polystyrene plate (Corning Incorporated, Costar, No. 3632). The binding assay buffer is prepared with RPMI 1640 medium (Gibco, Grand Island, NY) containing 10 mM HEPES, pH 7.5, and 0.2% BSA. Briefly, 200 μL reaction mixtures containing 300 pM SDF ligand (60 pM ^{125}I-SDF-1α (Perkin Elmer) and 240 pM cold SDF-1α (R&D Systems), different concentrations of the test compound in assay buffer, 100,000 human CCRF-CEM cells, and 0.5 mg SPA beads (Wheatgerm agglutinin beads; Amersham) are incubated at room temperature for 2 hr. Plates are then counted in a 1450 Microbeta Liquid Scintillation and Luminescence Counter (Wallac) in SPA mode. CXCR4 antagonists decrease the bound radioactivity in this assay in a dose-dependent manner. The inhibitory potency (K_i or IC_{50}) of a test compound is calculated using GraphPad Prism software, based on the dose-dependent decrease of bound radioactivity.

All compounds exemplified above exhibit an average K_i value of about 7.5 nM or less in this assay. For example, the compound of Example 1 exhibits an average K_i of 3.45 nM in this assay. Many of these compounds exhibit an average K_i value between about 0.2 nM and about 1 nM. For example, the compound of Example 50 exhibits an average K_i value of 0.285 nM in this assay. Other compounds exhibit an average K_i value less than about 0.2 nM. For example, the compound of Example 75 exhibits an average K_i value of 0.096 nM in this assay.

Chemotaxis Assay

CXCR4/SDF-1 interaction regulates migration (chemotaxis) of cells bearing CXCR4 on their surface. To determine the antagonist and cellular activities of a test

compound, a chemotaxis assay using human histiocytic lymphoma U937 cells (ATCC CRL 1593) that express endogenous CXCR4 are employed. Briefly, U937 cells, grown in DMEM medium (Gibco, Grand Island, NY) containing 10% FBS, 1% MEM sodium pyruvate (Gibco), 1% MEM nonessential amino acids (Gibco), and 1% GlutaMAX I (Gibco), are harvested and washed once with chemotaxis assay buffer prepared with 1x RPMI medium (Gibco) containing 10 mM HEPES, pH 7.5, and 0.3% BSA. After washing, cells are resuspended in assay buffer at a concentration of 5 x 10^6 cells/mL. The assay is performed in a 96-well ChemoTx plate (NeuroProbe) according to the manufacturer's directions. Generally, 50 µL of cell mixture with or without test compound are plated on the upper chamber, and 30 µL of SDF-1α (R&D Systems, 10 ng/mL) prepared in 1 x chemotaxis buffer are added to the lower chamber. After assembly, the plate is incubated for 2.5 hr at 37°C under 5% CO_2. Following the incubation, 5 µL of CellTiter 96 AQ (Promega, Madison, WI) are added into the lower chamber. The plate is then incubated for 60 min at 37°C, and the migrated cells are detected by measuring the absorbance at 492 nm with a Tecan Spectrafluor Plus Microplate Reader (Salzburg, Austria). CXCR4 antagonists inhibit cell migration, reducing the absorbance reading. The inhibitory potency (IC_{50}) of a test compound in this assay is calculated using GraphPad Prism software, based on the dose-dependent decrease of absorbance at 492 nm.

Most of the compounds exemplified above exhibit an average IC_{50} value of about 60 nM or less in this assay. Many of these compounds exhibit an average IC_{50} value of about 6 nM or less, e.g., the compound of Example 19 exhibits an average IC_{50} value of 2.05 nM in this assay. Many of these compounds exhibit an average IC_{50} value of about 0.6 nM or less, e.g., the compound of Example 50 exhibits an average IC_{50} value of 0.171 nM in this assay.

Chemokine Receptor Binding Selectivity Assays

The binding selectivity of the present compounds for the CXCR4 receptor compared to that for other chemokine receptors, such as human CCR1, CCR2, CXCR2, or CXCR3, and other G-protein-coupled receptors, can be assessed in cells transfected with nucleic acid encoding and expressing such receptors, or in cells in which such

receptors are endogenously expressed. Whole cells or membrane fragments can be used to assess competition of test compounds with the respective ligands for these receptors in a manner similar to that described above for the CXCR4/^{125}I-SDF-1α binding inhibition assay.

For example, the compound of Example 57a exhibits a K_i value greater than 73,000 nM in a ligand binding assay using human chemokine receptor CXCR2.

Compound-Induced White Blood Cell and Neutrophil Mobilization in C57BL/6 Mice

Stem cells within the bone marrow actively maintain continuous production of all mature blood cell lineages throughout life. Bone marrow is the primary site for white blood cell (WBC)/neutrophil production and release into the circulation. The CXCR4/SDF-1 axis appears to be critical for the retention and release of WBCs, neutrophils, and hematopoietic progenitor cells in the bone marrow, and interruption of CXCR4/SDF-1 interaction in bone marrow leads to an increase of these cells in peripheral blood. A short-term mouse WBC/neutrophil mobilization model can be used to determine the *in vivo* target-modulating activity of a test compound. Briefly, pathogen-free 5-6 week old female C57BL/6 mice (Taconic) are housed for at least one week prior to assay. Animals are allowed continuous access to sterilized rodent chow and acidified water. Groups of 5 mice are injected subcutaneously with test compounds in saline, or with saline control, and then sacrificed by CO_2 asphyxiation and cervical dislocation at various time points post compound administration. Peripheral blood is collected by cardiac puncture using EDTA-coated syringes and tubes. Complete blood cell analysis is performed on a Hemavet Mascot hematology analyzer (Drew Scientific Group, Dallas, TX). Total WBCs, neutrophils, and lymphocytes in the peripheral blood are recorded. Effective CXCR4 antagonists administered subcutaneously to mice increase the neutrophil and WBC counts in peripheral blood compared to saline control.

A significant number of compounds exemplified above exhibit an average neutrophil ratio (ratio of neutrophil increase in treatment group vs. neutrophil increase in saline control group), measured 3 hours after compound administration, greater than about 2 in this assay. For example, the compound of Example 39 exhibits an average neutrophil ratio of 4.6 at a dose of 5 mg/kg in this assay.

Anti-Tumor Activity in a SCID/Namalwa Xenograft Model

SDF-1/CXCR4 interaction appears to play an important role in multiple stages of tumorigenesis, including tumor growth, invasion, angiogenesis, and metastasis. To evaluate *in vivo* anti-tumor activity of a test compound, a tumor xenograft model using NOD/SCID micc (Jackson Laboratorics) and human non-Hodgkin's lymphoma Namalwa cells (ATCC CRL 1432) are employed. Briefly, 200,000 Namalwa cells mixed with matrigel (1:1) are implanted subcutaneously into the rear flank of the animals. The implanted tumor cells grow as solid tumors, the dimensions of which can be continuously monitored and measured using a caliper. To determine the *in vivo* efficacy of a test compound in this model, one can treat animals (10/group) with different doses of test compounds dissolved in saline or PBS, beginning 48 hours post tumor cell implantation. Compounds are dosed subcutaneously, and tumor volume and body weight are determined every 2 or 3 days. Studies generally last 3-4 weeks, depending on tumor growth. The anti-tumor growth activity of a test compound is determined by the percent reduction in tumor volume in treatment groups compared to tumor volume in control groups treated with vehicle alone.

Several compounds exemplified above, for example the compound of Example 26, significantly inhibit tumor growth in this assay when administered at 1 mg/kg BID.

Pharmacologic properties such as compound bioavailability, *in vivo* metabolic stability, and pharmacokinetic/pharmacodynamic properties can be determined by methods well known in the art of drug development. Preferred compounds of the present invention exhibit high bioavailability when administered subcutaneously. Some compounds exemplified herein exhibit bioavailability near 100% in rats, for example the compound of Example 44. Preferred compounds also exhibit good *in vivo* metabolic stability. For example, no detectable metabolites are observed in dog and monkey plasma and urine up to 24 hours after administration of the compound of Example 57a. Preferred compounds also exhibit favorable pharmacokinetic/pharmacodynamic properties that permit convenient dosing. For example, in mice, the half-life (T1/2) of the compound of Example 58 is about 3 hours. With respect to pharmacodynamic properties, preferred compounds induce prolonged neutrophil and white blood cell mobilization in mice. For example, the compound of Example 25 induces a significant increase of

neutrophils and white blood cells in peripheral blood for at least 6 hours after single dose subcutaneous administration at 5 mg/kg in mice.

What Is Claimed Is:

1. A lactam-cyclized peptide of formula I:

R_1 - cyclo[X_1 – Tyr – X_3 – DArg – 2Nal – Gly – X_7] – X_8 – X_9 – X_{10} – R_2

(I)

(SEQ ID NO:1)

wherein:

a) said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

b) said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

c) said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

d) said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

c) said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7, wherein X_2 and X_7 are, respectively, a pair selected from the group consisting of Tyr/Asp, Tyr/Glu, and Tyr/DGlu, and R_1 and X_1 are each absent;

R_1 is a substituent on the α-amino group of X_1 when X_1 contains an α-amino group and said α-amino group is not a constituent of said lactam amide bond, selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent, wherein X_1 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, and Glu;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and $Lys(Me_2)$;

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent, and

R_2 is selected from the group consisting of NH_2 and NHEt, or

a pharmaceutically acceptable salt thereof.

2. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of β-Ala, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, 2Nal, Phe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of Asp, Dab, Dap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of Arg and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

3. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of DAla, 5-aminovaleryl, 4-AMPA, Asp, Glu, Leu, Lys(Ac), Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, and DGlu;

X_8 is selected from the group consisting of Arg, DArg, and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

4. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, Asp, Dap, Glu, Gly, Lys, and Phe;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

5. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of Ala, 5-aminovaleryl, Asp, Glu, Gly, Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

6. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, 4, or 5, wherein:

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr); and

X_7 is DGlu.

7. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 5, wherein:

R_1 is absent;

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr);

X_7 is DGlu;

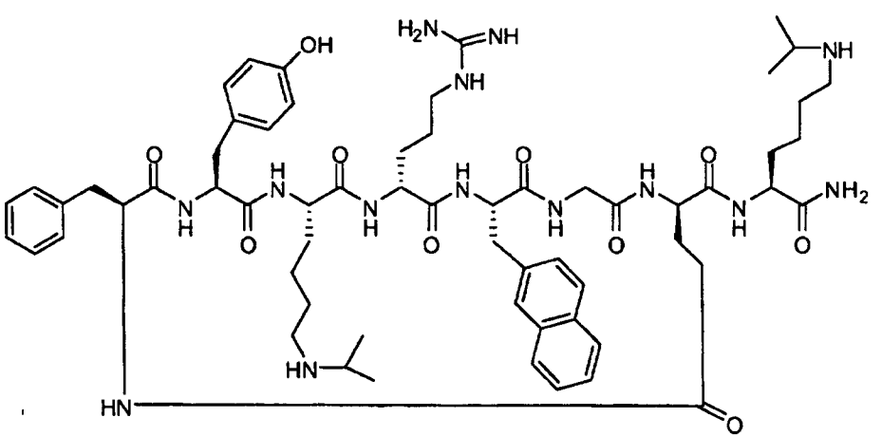
X_8 is selected from the group consisting of Arg and Lys(iPr), or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

8. A lactam-cyclized peptide of the formula:



(SEQ ID NO:70)

or a pharmaceutically acceptable salt thereof.

9. The lactam-cyclized peptide of claim 8, wherein said pharmaceutically acceptable salt is an acetic acid salt.

10. A pharmaceutical composition, comprising a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, and a pharmaceutically acceptable carrier, diluent, or excipient.

11. A lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, for use in therapy.

12. A lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

13. Use of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, for the manufacture of a medicament for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

14. A method of treating rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia, comprising administering to a patient in need thereof an effective amount of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/064177

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P31/18 A61K38/12 C07K7/54

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)
A61P 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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	UEDA SATOSHI ET AL: "Structure-activity relationships of cyclic peptide-based chemokine receptor CXCR4 antagonists: disclosing the importance of side-chain and backbone functionalities." JOURNAL OF MEDICINAL CHEMISTRY 25 JAN 2007, vol. 50, no. 2, 25 January 2007 (2007-01-25), pages 192-198, XP009107403 ISSN: 0022-2623 the whole document	
A	JP 2004 196769 A (ORPHAN LINK KK; FUJII NOBUTAKA; STEPHAN C PIPER) 15 July 2004 (2004-07-15) page 3; claims	

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[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
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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
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90

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAMAMURA HIROKAZU ET AL: "DEVELOPMENT OF ANTI-HIV AGENTS TARGETING DYNAMIC SUPRAMOLECULAR MECHANISM: ENTRY AND FUSION INHIBITORS BASED ON CXCR4/CCR5 ANTAGONISTS AND GP41-C34-REMODELING PEPTIDES" CURRENT HIV RESEARCH, BENTHAM SCIENCE PUBLISHERS, HILVERSUM, NL, vol. 3, no. 4, 1 October 2005 (2005-10-01), pages 289-301, XP008077509 ISSN: 1570-162X the whole document	
P,A	WO 2007/096662 A (TU MUENCHEN [DE]; JONES NICHOLAS ANDREW [GB]; WESTER HANS JURGEN [DE];) 30 August 2007 (2007-08-30) claims	
P,A	TAMAMURA HIROKAZU ET AL: "Development of low molecular weight CXCR4 antagonists by exploratory structural tuning of cyclic tetra- and pentapeptide-scaffolds towards the treatment of HIV infection, cancer metastasis and rheumatoid arthritis." CURRENT MEDICINAL CHEMISTRY 2007, vol. 14, no. 1, 2007, pages 93-102, XP009107441 ISSN: 0929-8673	
P,A	GRANDE FEDORA ET AL: "Small molecules anti-HIV therapeutics targeting CXCR4." CURRENT PHARMACEUTICAL DESIGN 2008, vol. 14, no. 4, 2008, pages 385-404, XP009107439 ISSN: 1873-4286	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2004196769 A	15-07-2004	NONE	
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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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(54) Title: CYCLIC PEPTIDE CXCR4 ANTAGONISTS

(57) Abstract: Provided are lactam-cyclized peptide CXCR4 antagonists useful in the treatment of cancers, rheumatoid arthritis, pulmonary fibrosis, and HIV infection.

WO 2008/150689 A1

Cyclic Peptide CXCR4 Antagonists

The present invention relates to novel cyclic peptide CXCR4 antagonist compounds and their use in treating diseases in which pathogenesis is mediated by CXCR4 and SDF-1.

CXCR4, a G-protein-coupled receptor, and its naturally occurring ligand, stromal cell-derived factor-1 (SDF-1; CXCL12), are a chemokine receptor-ligand pair. CXCR4 is constitutively- or over-expressed in a wide variety of human cancers. SDF-1, the only known ligand of CXCR4, is highly expressed in tumor microenvironments, as well as in bone marrow, lung, liver, and lymph nodes, i.e., organ sites most commonly involved in tumor metastasis. CXCR4/SDF-1 interaction plays important roles in multiple stages of tumorigenesis, including tumor growth, invasion, angiogenesis, and metastasis, as well as in rheumatoid arthritis, pulmonary fibrosis, and HIV infection (Tsutsumi et al. (2006) *Peptide Science* 88(2):279-289).

In view of the involvement of CXCR4/SDF-1 in these serious diseases, CXCR4 is an attractive therapeutic target.

AMD3100, a bicyclam CXCR4 antagonist, is currently in Phase III clinical trials for stem cell mobilization for transplantation of stem cells in patients with multiple myeloma and non-Hodgkins lymphoma. AMD070, another small molecule CXCR4 antagonist, is currently in Phase II clinical trials for HIV infection. CTCE9908, a bivalent (dimeric) peptide CXCR4 antagonist, is currently in Phase Ib/II clinical trials for cancer. FC131, a cyclic pentapeptide CXCR4 antagonist, inhibits ^{125}I-SDF-1 binding to CXCR4 transfectants with an IC_{50} of 4 nM (Fujii et al. (2003) *Angew. Chem. Int. Ed.* 42:3251-3253; Araki et al. (2003) *Peptide Science*. The Japanese Peptide Society (2004):207-210).

There exists a need for improved CXCR4 antagonists that are potent and selective, exhibiting little or no activity at other chemokine receptors. The compounds of the present invention are such potent and selective CXCR4 antagonists. Their high potency permits the use of low doses in therapeutic regimens, while their high selectivity minimizes non-target related adverse side effects. In addition, compounds disclosed herein possess other highly desirable pharmacologic properties, such as high bioavailability when administered subcutaneously, good *in vivo* metabolic stability, and pharmacokinetic/pharmacodynamic properties that permit convenient dosing.

Accordingly, in a first aspect, the present invention provides a lactam-cyclized peptide of formula I:

$$R_1 - \text{cyclo}[X_1 - \text{Tyr} - X_3 - \text{DArg} - \text{2Nal} - \text{Gly} - X_7] - X_8 - X_9 - X_{10} - R_2$$

(I)

(SEQ ID NO:1)

wherein:

a) said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

b) said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

c) said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

d) said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

e) said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7, wherein X_2 and X_7 are, respectively, a pair

selected from the group consisting of Tyr/Asp, Tyr/Glu, and Tyr/DGlu, and R_1 and X_1 are each absent;

R_1 is a substituent on the α-amino group of X_1 when X_1 contains an α-amino group and said α-amino group is not a constituent of said lactam amide bond, selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent, wherein X_1 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, and Glu;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and $Lys(Me_2)$;

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent, and

R_2 is selected from the group consisting of NH_2 and NHEt, or

a pharmaceutically acceptable salt thereof.

Expressed alternatively, this is equivalent to a lactam-cyclized peptide of formula I:

$$R_1 - \text{cyclo}[X_1 - \text{Tyr} - X_3 - \text{DArg} - 2\text{Nal} - \text{Gly} - X_7] - X_8 - X_9 - X_{10} - R_2$$

(I)

(SEQ ID NO:1)

wherein:

R_1 is a substituent on the α-amino group of X_1 when X_1 contains an α-amino group and said α-amino group is not a constituent of said lactam amide bond, selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent, wherein X_1 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, and Glu;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and $Lys(Me_2)$;

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent, and

R_2 is selected from the group consisting of NH_2 and NHEt,

wherein:

a) said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

b) said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or when X_1 and X_7 are, respectively, a pair selected from the group consisting of

succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

c) said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

d) said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, when X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

e) said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7, when X_2 and X_7 are, respectively, a pair selected from the group consisting of Tyr/Asp, Tyr/Glu, and Tyr/DGlu, and R_1 and X_1 are each absent, or

a pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a lactam-cyclized peptide of formula I:

$$R_1\text{ - cyclo}[X_1 - \text{Tyr} - X_3 - \text{DArg} - \text{2Nal} - \text{Gly} - X_7] - X_8 - X_9 - X_{10} - R_2$$

(I)

(SEQ ID NO:1)

or a pharmaceutically acceptable salt thereof,

wherein:

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent,

wherein when X_1 is (D/L)Agl, Dab, or Dap and the α-amino group of X_1 is not a constituent of the lactam amide bond, said α-amino group is substituted with R_1 which is selected from the group consisting of Ac and n-hexanoyl;

wherein when X_1 is Asp or Glu and the α-amino group of X_1 is not a constituent of the lactam amide bond, said α-amino group is substituted with R_1 which is selected from the group consisting of Ac and Bz; and

wherein when X_1 is Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Dap(Ac), Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe or succinyl, R_1 is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent,

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt,

and further wherein:

said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or wherein X_1 and X_7

are, respectively, a pair selected from the group consisting of succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, and X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

said lactam is formed by an amide bond between the α-amino group of Tyr at X_2 and the side chain carboxyl group of X_7, and X_7 is selected from the group consisting of Asp, Glu, and DGlu, and R_1 and X_1 are each absent.

A recurrent sequence motif in all the compounds of formula I is the presence of Tyr at position X_2, DArg at position X_4, 2Nal at position X_5, and Gly at position X_6.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of β-Ala, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, 2Nal, Phe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of Asp, Dab, Dap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of Arg and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of DAla, 5-aminovaleryl, 4-AMPA, Asp, Glu, Leu, Lys(Ac), Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, and DGlu;

X_8 is selected from the group consisting of Arg, DArg, and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, Asp, Dap, Glu, Gly, Lys, and Phe;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In a further aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of Ala, 5-aminovaleryl, Asp, Glu, Gly, Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr); and

X_7 is DGlu.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

R_1 is absent;

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr);

X_7 is DGlu;

X_8 is selected from the group consisting of Arg and Lys(iPr), or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7;

R_1 is selected from the group consisting of Ac and n-hexanoyl;

X_1 is selected from the group consisting of (D/L)Agl, Dab, and Dap;

X_3 is selected from the group consisting of Arg and Lys(iPr);

X_7 is Glu;

X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_1 is (D/L)Agl or Dap.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7;

R_1 is selected from the group consisting of Ac and Bz;

X_1 is selected from the group consisting of Asp and Glu;

X_3 is selected from the group consisting of Arg and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Dab, Dap, DDap, and Lys;

X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_7 is (D/L)Agl, Dab, Dap, or DDap. In a more preferred embodiment, X_7 is (D/L)Agl or Dap.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7;

R_1 is absent;

X_1 is succinyl;

X_3 is Arg;

X_7 is selected from the group consisting of (D/L)Agl, Dab, Dap, Lys, and Orn;

X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_7 is (D/L)Agl or Dap.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7;

R_1 is absent;

X_1 is selected from the group consisting of Ala, DAla, Gly, Dap(Ac), Leu, Lys, Lys(Ac), 2Nal, Phe, and DPhe;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of Asp, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

In a preferred embodiment of this aspect of the invention, X_1 is Ala, DAla, Gly, Leu, Lys, Lys(Ac), Phe, or DPhe. In a more preferred embodiment, X_1 is Ala, Gly, Lys, or Phe.

In a preferred embodiment of this aspect of the invention, X_3 is Arg, Lys, Lys(iPr), or Lys(Me_2). In a more preferred embodiment, X_3 is Arg.

In a preferred embodiment of this aspect of the invention, X_7 is Asp, Glu, or DGlu. In a more preferred embodiment, X_7 is Asp.

In a preferred embodiment of this aspect of the invention, X_8 is β-Ala, Arg, Gly, Lys, Lys(iPr), Orn, or is absent. In a more preferred embodiment, X_8 is β-Ala, Gly, Lys, Lys(iPr), Orn, or is absent.

In a preferred embodiment of this aspect of the invention, X_9 is Gly, 2Nal, D2Nal, DPhe, or is absent. In a more preferred embodiment, X_9 is Gly, 2Nal, D2Nal, or DPhe.

In a preferred embodiment of this aspect of the invention, X_{10} is 2Nal, or is absent. In a more preferred embodiment, X_{10} is 2Nal.

In a preferred embodiment of this aspect of the invention, R_2 is NHEt.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7;

R_1 is absent;

X_1 is selected from the group consisting of β-Ala, 4-AMB, 5-aminovaleryl, and 4-AMPA;

X_3 is Arg;

X_7 is selected from the group consisting of Asp and Glu;

X_8 is selected from the group consisting of Arg and DArg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In a preferred embodiment of this aspect of the invention, X_1 is β-Ala, 5-amino-valeryl, or 4-AMPA. In a more preferred embodiment, X_1 is β-Ala.

In a preferred embodiment of this aspect of the invention, X_7 is Asp.

In a preferred embodiment of this aspect of the invention, X_8 is Arg.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of formula I (SEQ ID NO:1), wherein:

said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7;

R_1 is absent;

X_1 is absent;

X_3 is Arg;

X_7 is selected from the group consisting of Asp, Glu, and DGlu;

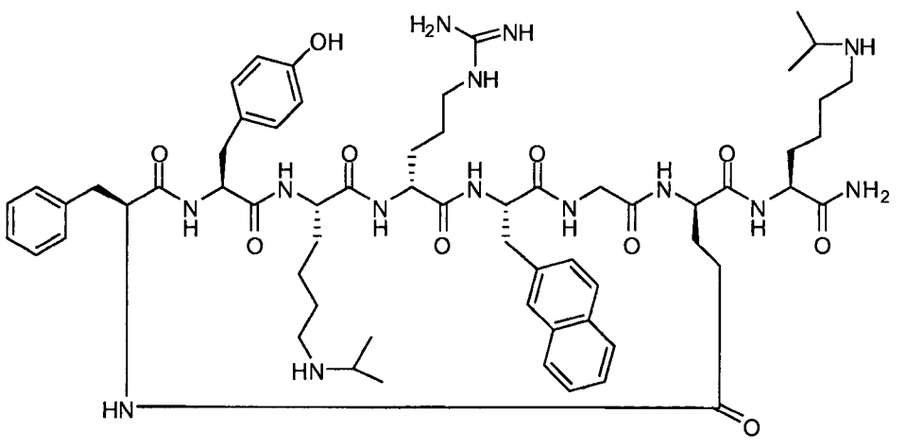
X_8 is Arg;

X_9 is absent;

X_{10} is absent; and

R_2 is NH_2.

In another aspect, the present invention provides a lactam-cyclized peptide of the formula:



(SEQ ID NO:70)

or a pharmaceutically acceptable salt thereof. The lactam is formed by an amide bond between the α-amino group of Phe and the side chain carboxyl group of DGlu. The pharmaceutically acceptable salt can be an acetic acid salt.

In another aspect, the present invention provides a pharmaceutical composition, comprising a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, for use in therapy.

In another aspect, the present invention provides a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

In another aspect, the present invention provides the use of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above, for the manufacture of a medicament for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

In another aspect, the present invention provides a method of treating rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia, comprising administering to a patient in need thereof an effective amount of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof as variously described above.

In the macrocyclic peptidic compounds of the present invention (SEQ ID NO:1), amino acids X_1 through X_{10} are referred to herein by their commonly employed three letter symbols, shown left to right from amino-terminal end to carboxy-terminal end. D-

and L- (small capital letters) refer to absolute stereochemistry. Where neither designation is indicated in a particular formula, the L- form of the amino acid is present. X_1 can also be a dicarboxylic acid residue, i.e., a succinyl group. Amino acid or carboxylic acid residues within the brackets "[ ]" are within the cyclic structure; groups external to the brackets are outside the cyclized ring. In all cases, cyclization is via a lactam (amide) bond between X_1 (or X_2, i.e., Tyr) and X_7, which can be formed in several different ways, depending on the structures of X_1, X_2, and X_7.

When the lactam bond is formed between the side chain amino group of X_1 and the side chain carboxyl group of X_7 (Schemes 1 and 2; Examples 1-5), the α-amino group of X_1 is capped with Ac or n-hexanoyl.

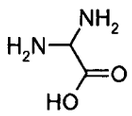
When the lactam bond is formed between the side chain carboxyl group of X_1 and the side chain amino group of X_7, the α-amino group of X_1 is capped with Ac or Bz (Schemes 3 and 4; Examples 6-19). X_1 also can be a bifunctional residue other than an α-amino acid, for example one with two carboxyl groups, i.e., a succinyl residue. In this case, one carboxyl group forms an amide bond with the α-amino group of Tyr, and the other forms the cyclic lactam structure through an amide bond with the side chain amino group of X_7 (Schemes 3 and 4; Examples 20-24). When X_1 is succinyl, R_1 is absent.

In most of the lactam-cyclized peptides disclosed herein (Schemes 5-15; Examples 25-28, 32-66, and 75-89), the α-amino group of X_1 forms the lactam structure via an amide bond with the side chain carboxyl group of X_7, and R_1 is absent. Also included in the synthetic schemes of this category are cyclic peptides containing X_1 residues, i.e., β-Ala, 4-AMB, 5-aminovaleryl, and 4-AMPA, wherein the amino group is a non-side chain, non-α-amino group (Schemes 5 and 6; Examples 67-74). R_1 is also absent in these cases.

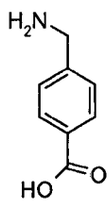
When both R_1 and X_1 are absent, the lactam structure is formed through an amide bond between the α-amino group of Tyr (X_2) and the side chain carboxyl group of X_7 (Schemes 5 and 6; Examples 29-31).

Structures of common amino acids, e.g., alanine, glycine, etc., are well known in the art. Structures of non-standard and substituted amino acids present in the instant invention compounds are shown below.

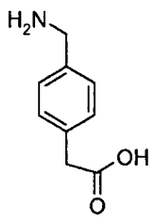
5



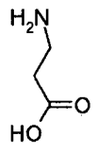
aminoglycine (Agl)



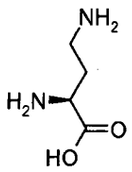
4-aminomethyl benzoic acid (4-AMB)



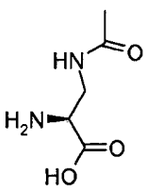
4-aminomethyl phenylacetic acid (4-AMPA)



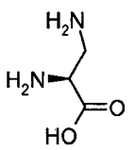
3-amino propanoic acid (β-Ala)



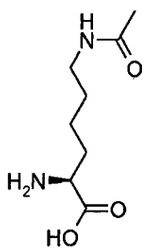
Dab



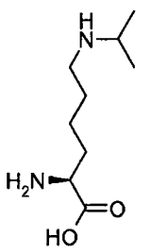
Dap(Ac)



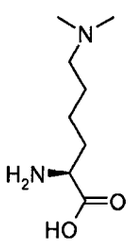
Dap



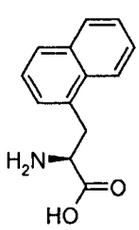
Lys(Ac)



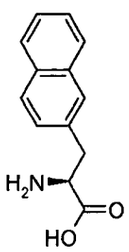
Lys(iPr)



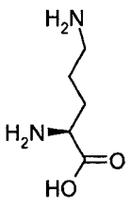
Lys(Me_2)



1Nal



2Nal



Orn

The lactam-cyclized peptides of the present invention can be prepared as pharmaceutically acceptable salts. Such salts, and common methodology for preparing them, are well known in the art. *See*, e.g., P. Stahl et al. (2002) *Handbook of Pharmaceutical Salts: Properties, Selection and Use*, VCHA/Wiley-VCH; Berge et al. (1977) "Pharmaceutical Salts," *Journal of Pharmaceutical Sciences* 66(1):1-19.

The compounds of the present invention are potent antagonists of CXCR4/SDF-1 interaction. Compounds of formula (I) and their pharmaceutically acceptable salts specifically exemplified herein exhibit an average K_i value of about 7.5 nM or less as determined by the CXCR4/^{125}I-SDF-1α binding assay described below. More preferred compounds of formula (I) and their pharmaceutically acceptable salts exhibit an average K_i value in the range of from about 0.2 nM to about 1 nM in this assay. Especially preferred compounds of formula (I) and their pharmaceutically acceptable salts exhibit an average K_i value less than about 0.2 nM in this assay.

In addition, the compounds and pharmaceutically acceptable salts of the present invention are preferably highly selective for the CXCR4 receptor, exhibiting little or no inhibitory activity against other chemokine receptors, including CCR1, CCR2, CXCR2, CXCR3, and other G-protein coupled receptors at the concentrations tested, and no significant activity against serotonin, dopamine, and opioid receptors. They also preferably exhibit good stability in blood and plasma, good subcutaneous bioavailability, desirable pharmacokinetic/pharmacodynamic properties, and potent *in vivo* efficacy in tumor growth inhibition, with a wide safety margin.

In view of these pharmacological properties, the compounds of the present invention are indicated for the treatment of disorders involving CXCR4/SDF-1 interaction, or receptor activity activity of CXCR4, such as in HIV infection. In particular, the present compounds are useful in treating malignancies in which angiogenic, growth, survival, and metastatic pathways mediated by CXCR4 and SDF-1 are implicated in pathogenesis, including breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia, as well as in rheumatoid arthritis, pulmonary fibrosis, and HIV infection (Tsutsumi et al. (2006) *Peptide Science* 88(2):279-289).

Agl (aminoglycine) is a pro-chiral building block. When this residue appears in a peptide formula herein, the α-carbon becomes a chiral center at which the two associated α-amino groups are each individually bonded to different moieties. In this case, the final peptide product contains two diastereomers that are unresolved, and that may be present in other than a 1:1 ratio. "(D/L)Agl" in a peptide formula denotes such a mixture of diastereomers. "(DL)Agl" denotes an Agl derivative that is racemic, for example Fmoc-(DL)Agl(Boc).

"K_i" values are calculated using IC_{50} values determined in the CXCR4/^{125}I-SDF-1α binding assay described below by employing equation 7.22 of *Enzymes, A Practical Introduction to Structure, Mechanism, and Data Analysis*, Robert A. Copeland, Wiley-VCH, New York, 1996, page 207.

The term "SDF-1" includes two isoforms, SDF-1α and SDF-1β, currently understood to exhibit similar functionality.

"Treatment" as used herein refers to curative treatment of disorders associated with CXCR4/SDF-1 interaction or CXCR4 receptor activity. Curative treatment refers to processes involving a slowing, interrupting, arresting, controlling, or stopping of disease progression, but does not necessarily involve a total elimination of all disease-related symptoms, conditions, or disorders.

The compounds of the present invention can be used as medicaments in human or veterinary medicine, administered by a variety of routes. Most preferably, such compositions are for parenteral administration. Such pharmaceutical compositions can be prepared by methods well known in the art. *See*, e.g., *Remington: The Science and Practice of Pharmacy*, 19th ed. (1995), A. Gennaro et al., Mack Publishing Co., and comprise one or more compounds of formula (I) or a pharmaceutically acceptable salt(s) thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

The effective amount of the present compounds is in the range of from about 1 mg to about 300 mg, more preferably from about 1 mg to about 200 mg, more preferably from about 1 mg to about 100 mg, and even more preferably from about 1 mg to about 50 mg, on a daily basis.

All lactam-cyclized peptides of the present invention can be synthesized either by solid-phase synthesis or solution phase synthesis, or a combination of both, with peptide

chain assembly on solid phase and cyclization or other modifications on resin or in solution. Such methods are well known in the art.

The following abbreviations used herein have the indicated meanings:

Ac: acetyl; **Agl:** aminoglycine; **AMB**: aminomethyl benzoic acid; **AMPA**: aminomethyl phenyl acetic acid; **Bn**: benzyl; **Boc**: tert-butyloxycarbonyl; **BOP**: (benzotriazol-1-yloxy)-tris(dimethylamino)phosphoniumhexafluorophosphate; **2-Br-Z**: 2-bromobenzyloxycarbonyl; **Bz**: benzoyl; **Bzl**: benzyl; **2-Cl-Z**: 2-chlorobenzyl-oxycarbonyl; **Dab**: 2,4-diaminobutyric acid; **Dap**: 2,3-diamino-propionic acid; **DCC**: dicyclohexyl-carbodiimide; **DCM**: dichloromethane; **DIC**: diisopropyl carbodiimide; **DIEA**: diisopropyl-ethylamine; **DMF**: *N,N*-dimethyl formamide; **DMSO**: dimethyl-sulfoxide; **EDT:** 1,2-ethane-dithiol; **Et**: ethyl; **Fm**: 9-fluorenylmethyl; **Fmoc**: 9-fluor-enylmethoxy carbonyl; **HATU**: N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide; **HBTU**: *O*-benzo-triazolyl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; **HCTU**: 1H-benzotriazo-lium 1-[bis(dimethylamino)methylene]-5-chloro-3-oxide hexafluorophosphate; **HF**: hydrogen fluoride; **HOBt**: hydroxybenzotriazole; **IBCF**: isobutyl chloroformate; **iPr**: isopropyl; **IPA**: isopropyl alcohol; **Me**: methyl; **2Nal**: 2-naphthylalanine; **NMM**: N-methylmorpholine; **NMP:** *N*-methyl-pyrrolidone; **OtBu:** tert-butyl ester; **Pbf**: 2,2,4,6,7-pentamethyl-dihydrobenzofurane-5-sulfonyl; **PBS:** phosphate buffered saline; **PyBOP**: (benzotriazol-1-yloxy)-tris(pyrrolidino)-phosphonium hexafluoro-phosphate; **PyBrOP**: bromotris(pyrrolidino)phosphonium hexafluorophosphate; **tBu:** tert-butyl; **TFA**: trifluoroacetic acid; **THF**: tetrahydrofuran; **TIS**: triisopropyl silane; **Tos**: p-toluene-sulfonyl; **Z**: benzyloxycarbonyl; **ZOSu**: N-(benzyloxycarbonyl-oxy) succinimide.

Preparation of compounds of the present invention as described in the following examples is meant to be illustrative rather than limiting. In each of these examples, the observed molecular weight is reported as a de-convoluted value. The de-convoluted value is derived from the formula MW(observed) = n(m/z)-n, where m/z represents the charged ion (positive mode) and n is the number of charges of the specific species. When multiple charged species are present in the mass spectrum, the observed molecular weight is reported as an average.

The synthetic processes disclosed in Examples 85-87, and isotopic labeling procedures disclosed in Example 89, for cyclic lactam peptides containing isopropyl

lysine side chains are equally applicable to other peptides disclosed herein containing lysine alkyl side chains, with appropriate modifications. The synthetic methods of Examples 86-88, which eliminate the need for toxic palladium catalysts, are also equally applicable to other peptides disclosed herein, with appropriate modifications.

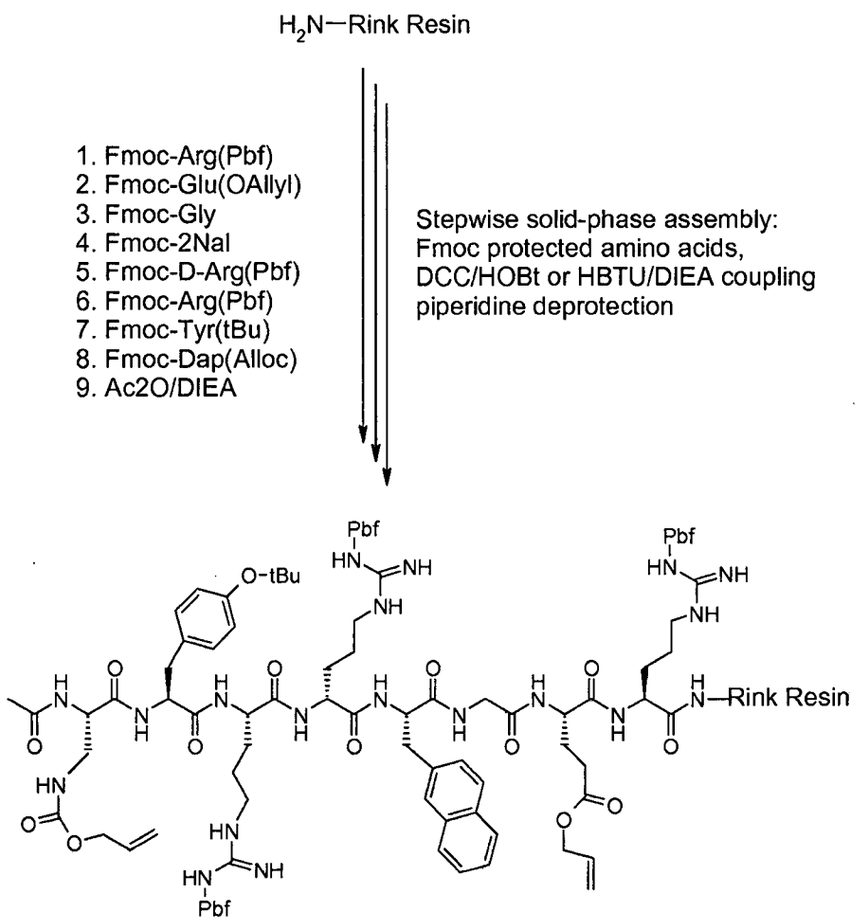
Example 1

Ac-cyclo[Dap-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:2)

The sequence Ac-Dap(Alloc)-Tyr(tBu)-Arg(Pbf)-DArg(Pbf)-2Nal-Gly-Glu(Oallyl)-Arg(Pbf) (SEQ ID NO:3) is assembled by standard Fmoc chemistry utilizing an ABI 431 Peptide Synthesizer (Applied Biosystems) as outlined in Scheme 1 below. The automated assembly is carried out by using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc chemistry HBTU/DIEA protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The solid support is Rink amide resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin) for C-terminal amides or indole resin [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin for C-terminal ethyl amides (NovaBiochem, EMD Biosciences, Inc., San Diego, CA). The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 9 major steps. In step 1, four equivalents of protected amino acid Fmoc-Arg(Pbf) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and coupled to deprotected Rink Amide resin using 20% piperidine. In step 2, four equivalents of Fmoc-Glu(Oallyl) are activated and coupled to the deprotected peptide resin from step 1. Appropriate steps are carried out until step 8, the coupling of Fmoc-Dap(Alloc). Then, Fmoc at the N-terminal end is removed using 20% piperidine in DMF and acetylation of the α-amino group is carried out off-line using 5 equivalents of acetic anhydride, 10 equivalents of DIEA in dry DMF or NMP, for 1 h at room temperature.

The allyl and Alloc side chain protection groups are removed with 0.1 equivalent of $Pd(Ph_3P)_4$ in the presence of 24 equivalents of phenylsilane in dichloromethane (Scheme 2). This process is repeated once for complete side chain deprotection. The deprotected carboxylic acid moiety of Glu is activated with PyBOP/DIEA and cyclized to the side chain amino group of Dap on the resin. The cyclized peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT

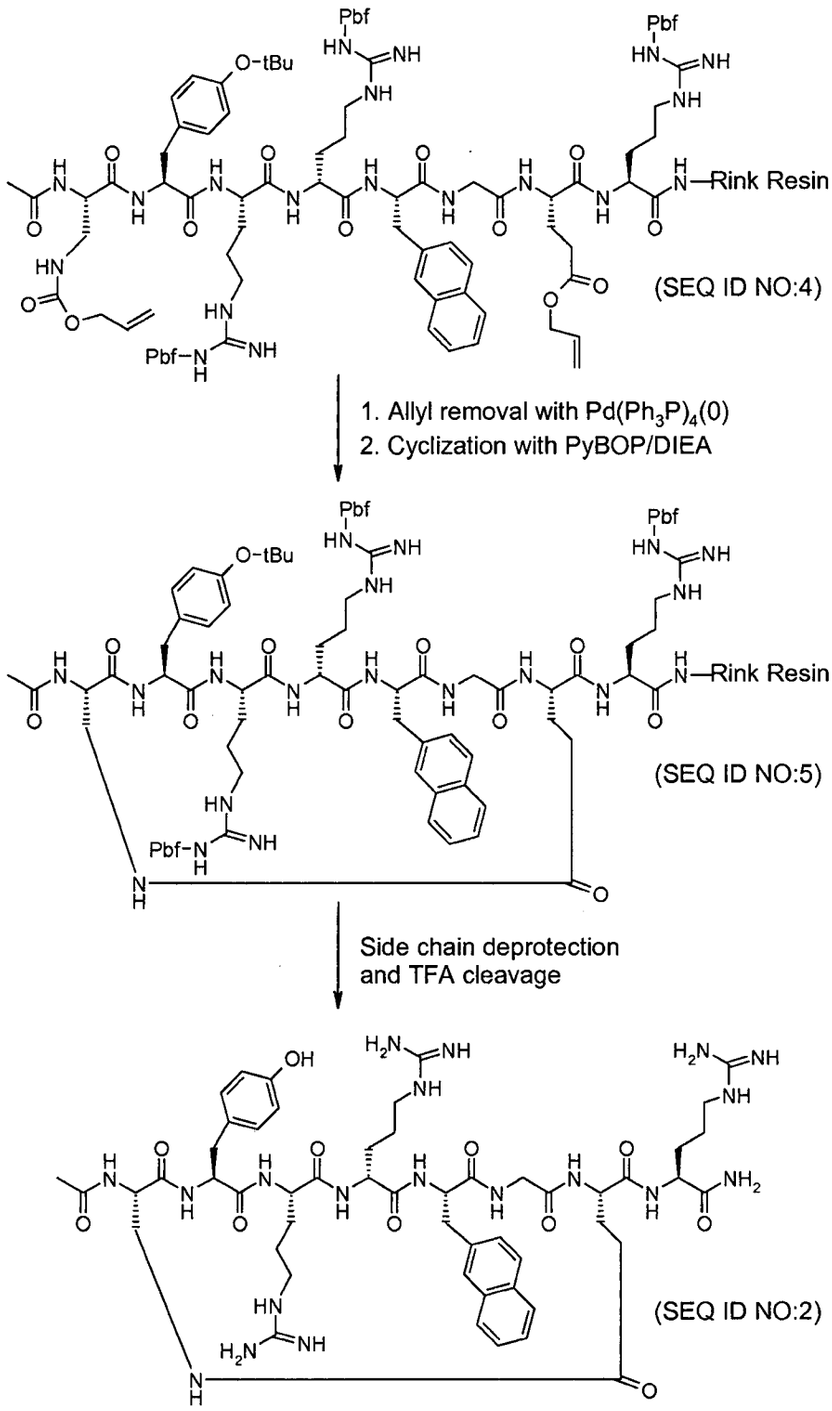
(95/2/1/2, v/v/v/v), or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature. The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers. Molecular weight calculated (MW cal.): 1142.30; MW observed (MW obs.): 1142.50.



(SEQ ID NO:4

Scheme 1. Amino side chain to carboxyl side chain peptide assembly

Peptide purification is accomplished using standard preparative HPLC techniques. Immediately following the cyclization, the peptide solution is diluted with water containing 0.1% (v/v) TFA, loaded onto a reversed phase C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile (v/v) gradient while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the final product is performed using analytical HPLC and mass spectral analysis by conventional techniques. For peptides with a basic side chain, the final lyophilized product is a TFA salt.



Scheme 2. Amino side chain to carboxyl side chain ring cyclization and cleavage

Example 2

Ac-cyclo[Dab-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:6)

Prepare as in Example 1, except that Fmoc-Dap(Alloc) in step 8 is replaced with Fmoc-Dab(Alloc). MW cal.:1156.33; MW obs.: 1156.10.

Example 3

Ac-cyclo[Dap-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:7)

Prepare as in Example 1, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(iPr)(Boc). MW cal.: 1156.37; MW obs.: 1156.78.

Example 4

n-Hexanoyl-cyclo[Dap-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:8)

Prepare as in Example 1, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(iPr)(Boc). Additionally, acetic anhydride in step 9 is replaced with hexanoic acid activated with PyBOP/DIEA. MW cal.: 1212.47; MW obs.: 1212.92.

Example 5

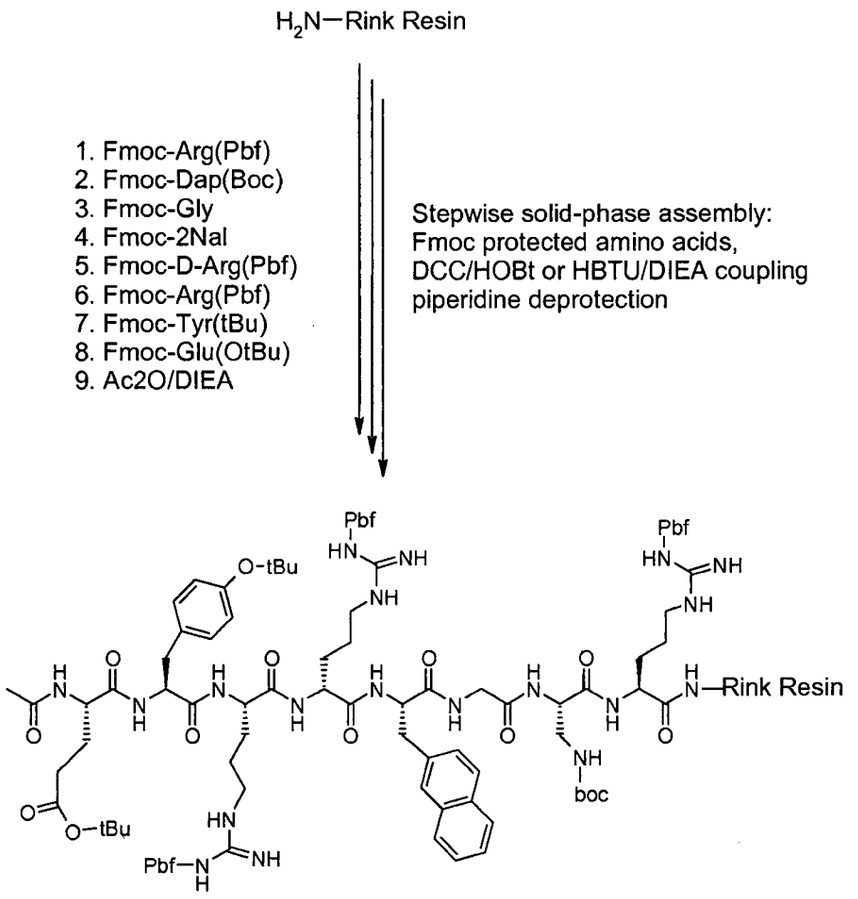
Ac-cyclo[(D/L)Agl-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:9)

Prepare as in Example 1, except that Fmoc-Glu(Oallyl) in step 2 is replaced with Fmoc-Glu(OtBu), and Fmoc-Dap(Alloc) in step 8 is replaced with Fmoc-(DL)Agl(Boc). After chain assembly, there is no $Pd(Ph_3P)_4$ treatment since no allyl protection is present. Instead, cyclization is carried out in solution after the linear peptide is cleaved from the solid support and deprotected. The crude linear peptide (0.25 mmol) from the cleavage is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following solution via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is then allowed to proceed at room temperature for 2 h. Solvents are then evaporated under vacuum, the residue is loaded onto a preparative reversed phase C18 HPLC column, and target cyclic peptide is isolated and characterized as described in Example 1. MW cal.: 1128.27; MW obs.: 1128.26.

Example 6

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:10)

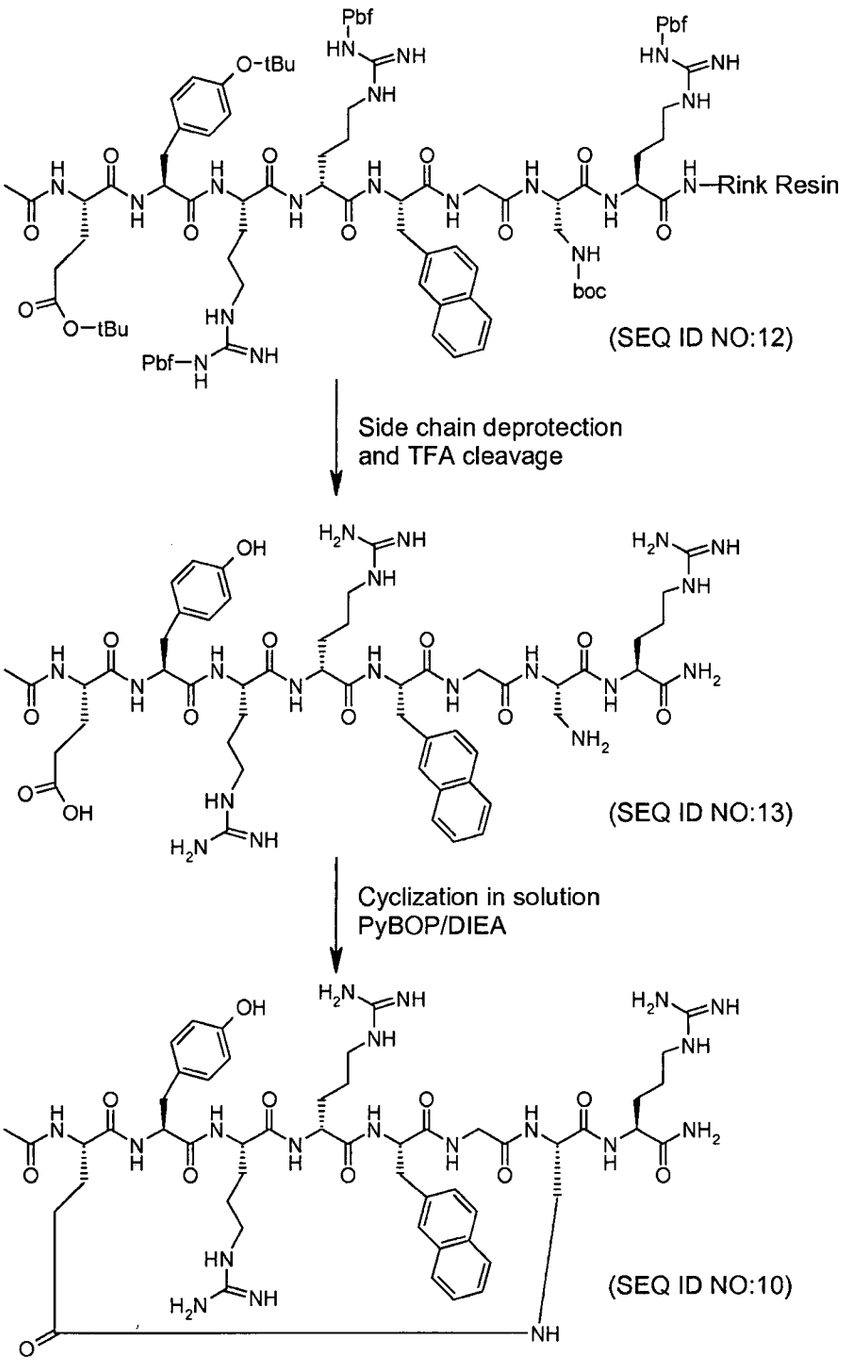
The sequence Glu(OtBu)-Tyr(tBu)-Arg(Pbf)-DArg(Pbf)-2Nal-Gly-Dap(Boc)-Arg(Pbf) (SEQ ID NO:11) is assembled by standard Fmoc chemistry utilizing an ABI 431 instrument as outlined in Scheme 3 below. The automated assembly is carried out by using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc chemistry (HBTU/DIEA) protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The solid support is Rink amide resin for C-terminal amides or indole resin [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin for C-terminal ethyl amides. Stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 9 major steps. In step 1, four equivalents of protected amino acid Fmoc-Arg(Pbf) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and coupled to deprotected Rink Amide resin. In step 2, four equivalents of Fmoc-Dap(Boc) are activated and coupled to the deprotected resin from step 1. Appropriate steps are carried out until step 8, the coupling of Fmoc-Glu(OtBu). For step 9, Fmoc at the N-terminal end is removed using 20% piperidine in DMF and acetylation of the α-amino group is carried out off-line with 5 equivalents acetic anhydride, 10 equivalents DIEA in dry DMF or NMP, for 1 h at room temperature. The finished peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v), or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature (Scheme 4). The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers. The crude product is used directly in the cyclization reaction. MW cal.: 1142.30; MW obs.: 1142.83.



(SEQ ID NO:12)

Scheme 3. Acid side chain to amino side chain peptide assembly

Cyclization is carried out in solution after the linear peptide is cleaved from the solid support with all the side chains deprotected (Scheme 4). The cleaved crude linear peptide (0.25 mmole) is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following reaction mixture via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is then allowed to proceed at room temperature for 2 h. Solvents are evaporated under vacuum, the residue is loaded onto a preparative reversed phase C18 HPLC column, and target cyclic peptide is isolated and characterized as described in Example 1.



Scheme 4. Acid side chain to amino side chain ring cyclization

Example 7

Bz-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:14)

Prepare as in Example 6, except that acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1204.37; MW obs.: 1204.87.

Example 8

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-DDap]-Arg-NH_2 (SEQ ID NO:15)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-DDap(Boc). MW cal.: 1142.30; MW obs.: 1142.73.

Example 9

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Lys]-Arg-NH_2 (SEQ ID NO:16)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Lys(Boc). MW cal.:1184.38; MW obs.: 1184.23.

Example 10

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:17)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc). MW cal.: 1156.33; MW obs.: 1156.07.

Example 11

Ac-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:18)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc). MW cal.: 1128.27; MW obs.: 1128.86.

Example 12

Bz-cyclo[Glu-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:19)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1190.34; MW obs.: 1190.99.

Example 13

Bz-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:20)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1204.37; MW obs.: 1204.87.

Example 14

Ac-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:21)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1142.30; MW obs.: 1142.81.

Example 15

Ac-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:22)

Prepare as in Example 6, except that Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1128.27; MW obs.: 1128.78.

Example 16

Bz-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:23)

Prepare as in Example 6, except that Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1190.34; MW obs.: 1190.69.

Example 17

Ac-cyclo[Asp-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:24)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1114.24; MW obs.: 1114.85.

Example 18

Ac-cyclo[Asp-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:25)

Prepare as in Example 6, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(Me_2), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). MW cal.: 1128.31; MW obs.: 1128.92.

Example 19

Bz-cyclo[Asp-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:26)

Prepare as in Example 6, except that Fmoc-Arg(Pbf) in step 6 is replaced with Fmoc-Lys(Me_2), and Fmoc-Glu(OtBu) in step 8 is replaced with Fmoc-Asp(OtBu). In addition, acetic anhydride in step 9 is replaced with benzoic acid anhydride. MW cal.: 1190.38; MW obs.: 1191.14.

Example 20

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:27)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-(DL)Agl(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1057.19; MW obs.: 1057.87.

Example 21

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:28)

Prepare as in Example 6, except that Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1071.22; MW obs.: 1071.85.

Example 22

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:29)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Dab(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1085.25; MW obs.: 1085.87.

Example 23

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Orn]-Arg-NH_2 (SEQ ID NO:30)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Orn(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1099.27; MW obs.: 1100.23.

Example 24

cyclo[Succinyl-Tyr-Arg-DArg-2Nal-Gly-Lys]-Arg-NH_2 (SEQ ID NO:31)

Prepare as in Example 6, except that Fmoc-Dap(Boc) in step 2 is replaced with Fmoc-Lys(Boc), Fmoc-Glu(OtBu) in step 8 is not used, and this step is omitted. In addition, acetic anhydride in step 9 is replaced with succinic anhydride. MW cal.: 1113.30; MW obs.: 1114.25.

Example 25

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:32)

The sequence Gly-Tyr(tBu)-Lys(iPr)(Boc)-DArg(Pbf)-2Nal-Gly-DGlu(Oallyl)-Arg(Pbf) (SEQ ID NO:33) is assembled by standard Fmoc chemistry utilizing an ABI 431 instrument as outlined in Scheme 5 below. The automated assembly is carried out by using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc HBTU/DIEA chemistry protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The solid support is Rink amide resin for amides or indole resin [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin for C-terminal ethyl amides. Stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 major steps (Scheme 5). In step 1, four equivalents of protected amino acid Fmoc-Arg(Pbf) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and are coupled to deprotected Rink amide resin. In step 2, four equivalents of Fmoc-DGlu(Oallyl) are activated and coupled to the deprotected peptide resin from step 1. Appropriate steps are carried out until step 8, the coupling of Fmoc-Gly.

The allyl ester side chain protection group is removed with 0.1 equivalent of $Pd(Ph_3P)_4$ in the presence of 24 equivalents of phenylsilane in dichloromethane (Scheme

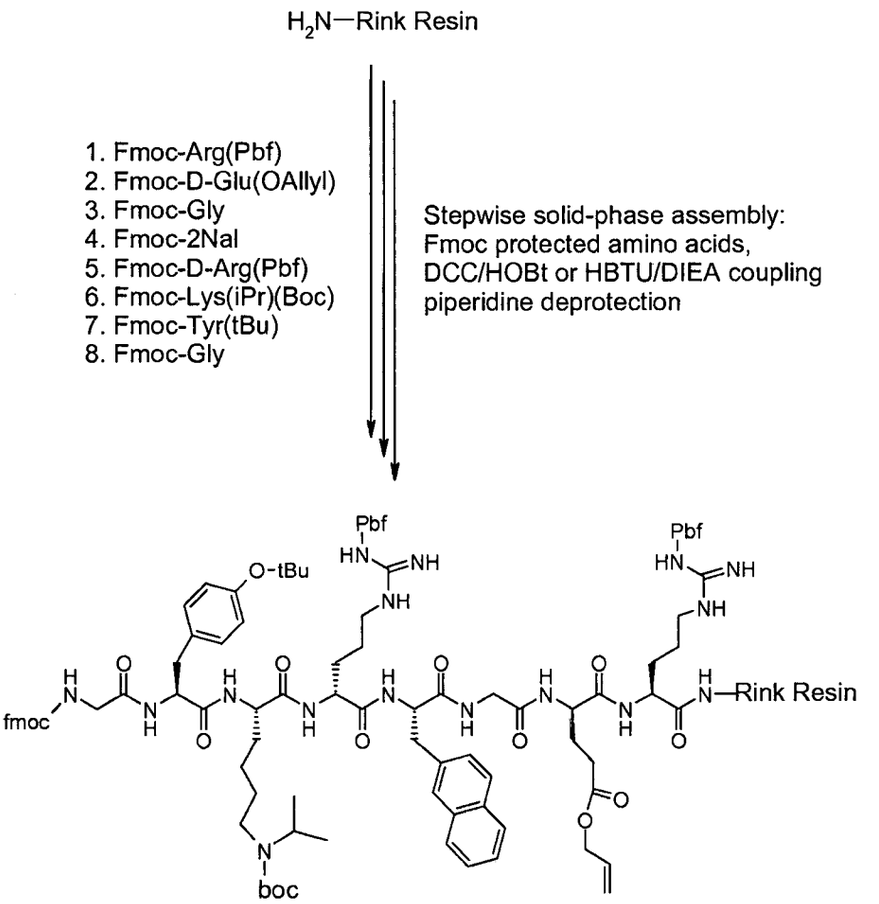
6). This process is repeated once for complete side chain deprotection. Then Fmoc at the N-terminal end is removed using 20% piperidine in DMF. The deprotected carboxylic acid moiety of DGlu is activated with PyBOP/DIEA, and cyclized to the α-amino group of glycine on the resin. The cyclized peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v), or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature. The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers. MW cal.: 1085.29; MW obs.: 1085.32.

Example 25a

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2·acetic acid salt

(SEQ ID NO:34)

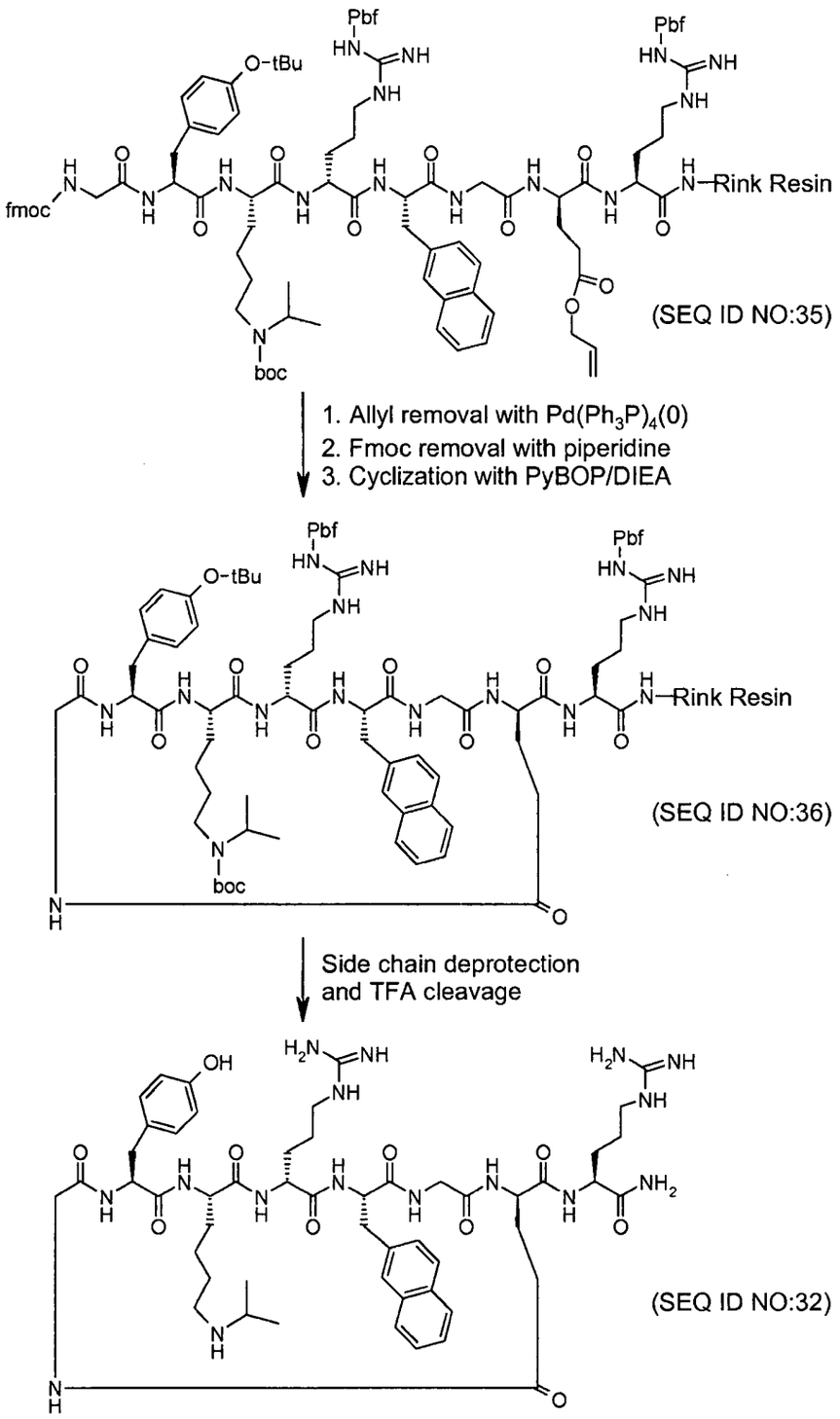
The TFA salt of the peptide of Example 25 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 1085.29; MW obs.: 1085.32.



(SEQ ID NO:35)

Scheme 5. α-amino group to carboxyl side chain peptide assembly

Peptide purification is accomplished using standard preparative HPLC techniques. Immediately following cyclization, the peptide solution is diluted with water containing 0.1% (v/v) TFA, loaded onto a reversed phase C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile (v/v) gradient while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the final product is performed using analytical HPLC and mass spectral analysis by conventional methods. For peptides with a basic side chain, the final lyophilized product is a TFA salt.



Scheme 6. α-amino group to carboxyl side chain ring cyclization and cleavage

Example 26

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:37)

Prepare as in Example 25, except that step 1 is omitted. MW cal.: 929.10; MW obs.: 929.39.

Example 26a

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2·acetic acid salt (SEQ ID NO:38)

The TFA salt of the peptide of Example 26 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 929.10; MW obs.: 929.39.

Example 27

cyclo[Gly-Tyr-Arg-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:39)

Prepare as in Example 25, except that Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf). MW cal.: 1071.22; MW obs.: 1071.02.

Example 28

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:40)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc). MW cal.: 1099.35; MW obs.: 1099.91.

Example 29

cyclo[Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:41)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), Fmoc-Gly in step 8 is not used, and step 8 is omitted. MW cal.: 1014.17; MW obs.: 1014.78.

Example 30

cyclo[Tyr-Arg-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:42)

Prepare as in Example 25, except that Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), Fmoc-Gly in step 8 is not used, and step 8 is omitted. MW cal.: 1014.17; MW obs.: 1014.65.

Example 31

cyclo[Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:43)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), Fmoc-Gly in step 8 is not used, and step 8 is omitted. MW cal.: 1000.14; MW obs.: 1000.63.

Example 32

cyclo[Gly-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:44)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf). MW cal.: 1057.19; MW obs.: 1057.35.

Example 33

cyclo[Gly-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:45)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Me_2). MW cal.: 1057.23; MW obs.: 1057.86.

Example 34

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:46)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 1071.26; MW obs.: 1071.76.

Example 35

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-NH_2 (SEQ ID NO:47)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 915.07; MW obs.: 915.38.

Example 36

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-Lys(iPr)-NH_2 (SEQ ID NO:48)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 1085.33; MW obs.: 1085.78.

Example 37

cyclo[Gly-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:49)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Me_2). MW cal.: 1071.26; MW obs.: 1071.05.

Example 38

cyclo[Gly-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:50)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf). MW cal.: 1071.22; MW obs.: 1071.50.

Example 39

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:51)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 1085.29; MW obs.: 1085.91.

Example 40

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:52)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 929.10; MW obs.: 929.39.

Example 41

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-NHEt (SEQ ID NO:53)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(allyl). MW cal.: 943.13; MW obs.: 943.36.

Example 42

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:54)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 957.15; MW obs.: 957.50.

Example 43

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NHEt (SEQ ID NO:55)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, and step 1 is omitted. MW cal.: 957.15; MW obs.: 957.46.

Example 44

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NHEt (SEQ ID NO:56)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin. MW cal.: 1113.34; MW obs.: 1113.81.

Example 44a

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NHEt·acetic acid salt (SEQ ID NO:57)

The TFA salt of the peptide of Example 44 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v)

containing 2% acetic acid by volume, and lyophilized. MW cal.: 1113.34; MW obs.: 1113.81.

Example 45

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt (SEQ ID NO:58)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, and Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc). MW cal.: 1127.41; MW obs.: 1127.35.

Example 46

cyclo[Lys(Ac)-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:59)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(OtBu), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Me_2), and Fmoc-Gly in step 8 is replaced with Boc-Lys(Fmoc). After chain assembly, Fmoc on the side chain of the N-terminal Lys is removed with 20% piperidine in DMF and the Lys side chain is then acetylated using 10 equivalents of acetic anhydride/DIEA at room temperature for one hour. All side chain protection groups are removed and the linear peptide is cleaved from the solid support using a mixture of TFA/water/TIS/anisole (90/5/2.5/2.5, v/v/v/v) for 2 h at room temperature. Cyclization of the crude linear peptide is carried out in solution. The cleaved crude linear peptide (~0.25 mmole) is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following reaction mixture via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is then allowed to proceed at room temperature for 2 h. Solvents are then evaporated under vacuum, the residue is loaded onto a reversed phase C18 preparative HPLC column, and the target cyclic peptide is isolated and characterized as described in Example 1. MW cal.: 1184.42; MW obs.: 1184.06.

Example 47

cyclo[Dap(Ac)-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:60)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(OtBu), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with

Fmoc-Lys(Me_2), and Fmoc-Gly in step 8 is replaced with Boc-Dap(Fmoc). After chain assembly, Fmoc on the side chain of the N-terminal Dap is removed using 20% piperidine in DMF, and the Dap side chain is then acetylated with 10 equivalents of acetic anhydride/DIEA at room temperature for one hour. All side chain protection groups are then removed and the linear peptide is cleaved from the solid support using a mixture of TFA/water/TIS/anisole (90/5/2.5/2.5, v/v/v/v) for 2 h at room temperature. Cyclization of the crude linear peptide is carried out in solution. The cleaved crude linear peptide (~0.25 mmole) is dried under vacuum and dissolved in 10 mL of dry DMF. This peptide solution is delivered to the following reaction mixture via a syringe pump during a 2 h period: 15 mL of dry dichloromethane and 15 mL of dry DMF containing 1.0 mmole of PyBOP and 4.0 mmoles of DIEA. The reaction is allowed to proceed at room temperature for 2 h. Solvents are then evaporated under vacuum, the residue is loaded onto a preparative HPLC column, and the target cyclic peptide is isolated and characterized as described in Example 1. MW cal.: 986.15; MW obs.: 985.97.

Example 48

cyclo[Ala-Tyr-Lys(iPr)-DArg -2Nal-Gly-Glu]-NH_2 (SEQ ID NO:61)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-Ala. MW cal.: 943.13; MW obs.: 942.92.

Example 49

cyclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:62)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DAla. MW cal.: 943.13; MW obs.: 943.44.

Example 50

cyclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:63)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-DAla. MW cal.: 943.13; MW obs.: 943.42.

Example 51

cyclo[Ala-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:64)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-Ala. MW cal.: 943.13; MW obs.: 943.48.

Example 52

cyclo[Leu-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:65)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-Leu. MW cal.: 985.21; MW obs.: 985.56.

Example 53

cyclo[Leu-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:66)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-Leu. MW cal.: 985.21; MW obs.: 985.49.

Example 54

cyclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:67)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DPhe. MW cal.: 1019.22; MW obs.: 1019.52.

Example 55

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:68)

Prepare as in Example 25, except that step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1019.22; MW obs.: 1019.53.

Example 56

cyclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:69)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-DPhe. MW cal.: 1019.22; MW obs.: 1019.50.

Example 57

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1189.48; MW obs.: 1189.92.

Example 57a

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2·acetic acid salt (SEQ ID NO:71)

The TFA salt of the peptide of Example 57 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 1189.48; MW obs.: 1189.92.

Example 58

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:72)

Prepare as in Example 25, except that Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1175.41; MW obs.: 1175.81.

Example 59

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:73)

Prepare as in Example 25, except that step 1 is omitted, and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1019.22; MW obs.: 1019.56.

Example 60

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt (SEQ ID NO:74)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, Fmoc-Arg(Pbf) in step 1 is

replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1217.53; MW obs.: 1217.97.

Example 60a

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt·acetic acid salt (SEQ ID NO:75)

The TFA salt of the peptide of Example 60 is converted to an acetic acid salt by adsorbing the material onto a preparative C18 column of suitable size, equilibrated with 2% acetic acid/H_2O (v/v). The column is then washed with three to five column volumes of 2% aqueous acetic acid (v/v). The peptide is eluted using 1:1 water/acetonitrile (v/v) containing 2% acetic acid by volume, and lyophilized. MW cal.: 1217.53; MW obs.: 1217.97.

Example 61

cyclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:76)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DAla. MW cal.: 971.18; MW obs.: 971.49.

Example 62

cyclo[2Nal-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:77)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-2Nal. MW cal.: 1097.34; MW obs.: 1097.53.

Example 63

cyclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:78)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-

DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc-DPhe. MW cal.: 1047.28; MW obs.: 1047.51.

Example 64

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:79)

Prepare as in Example 25, except that Rink amide resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, step 1 is omitted, Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), and Fmoc-Gly in step 8 is replaced with Fmoc- Phe. MW cal.: 1047.28; MW obs.: 1047.57.

Example 65

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Gly-2Nal-NH_2 (SEQ ID NO:80)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-2Nal, and one step is added between steps 1 and 2 using Fmoc-Gly. MW cal.: 1183.39; MW obs.: 1183.26.

Example 66

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-β-Ala-D2Nal-NH_2 (SEQ ID NO:81)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-D2Nal, one step is added between steps 1 and 2 using Fmoc-β-Ala, and Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl). MW cal.: 1197.40; MW obs.: 1196.70.

Example 67

cyclo[β-Ala-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:82)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-β-Ala. MW cal.: 1085.25; MW obs.: 1085.05.

Example 68

cyclo[β-Ala-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:83)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-β-Ala. MW cal.: 1071.22; MW obs.: 1071.15.

Example 69

cyclo[5-aminovaleryl-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:84)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-5-aminovaleric acid. MW cal.: 1113.30; MW obs.: 1113.40.

Example 70

cyclo[5-aminovaleryl-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:85)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-5-amino valeric acid. MW cal.: 1099.27; MW obs.: 1100.25.

Example 71

cyclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:86)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Asp(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl phenylacetic acid (4-AMPA). MW cal.: 1147.32; MW obs.: 1148.20.

Example 72

cyclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:87)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl phenylacetic acid (4-AMPA). MW cal.: 1161.34; MW obs.: 1161.99.

Example 73

cyclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Glu]-DArg-NH_2 (SEQ ID NO:88)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-DArg(Pbf), Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl phenylacetic acid (4-AMPA). MW cal.: 1161.34; MW obs.: 1161.83.

Example 74

cyclo[(4-AMB)-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:89)

Prepare as in Example 25, except that Fmoc-DGlu(Oallyl) in step 2 is replaced with Fmoc-Glu(Oallyl), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Arg(Pbf), and Fmoc-Gly in step 8 is replaced with Fmoc-4-aminomethyl benzoic acid (4-AMB). MW cal.: 1147.32; MW obs.: 1147.66.

Example 75

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-Gly-2Nal-NH_2 (SEQ ID NO:90)

Prepare as in Example 25, except that step 1 is replaced with three sequential residue couplings: first Fmoc-2Nal, then Fmoc-Gly, and then Fmoc-Lys(iPr)(Boc). MW cal.: 1353.69; MW obs.: 1354.03.

Example 76

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-Gly-2Nal-NH_2 (SEQ ID NO:91)

Prepare as in Example 25, except that step 1 is replaced with three sequential residue couplings: first Fmoc-2Nal, then Fmoc-Gly, and then Fmoc-Lys(iPr)(Boc). In addition, Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1443.82; MW obs.: 1444.13.

Example 77

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Gly-DPhe-NH_2 (SEQ ID NO:92)

Prepare as in Example 25, except that step 1 is replaced with two sequential residue couplings: first Fmoc-DPhe, then Fmoc-Gly. MW cal.: 1133.36; MW obs.: 1133.73.

Example 78

cyclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-DPhe-NH_2 (SEQ ID NO:93)

Prepare as in Example 25, except that step 1 is replaced with two sequential residue couplings: first Fmoc-DPhe, then Fmoc-Lys(iPr)(Boc). MW cal.: 1246.58; MW obs.: 1246.88.

Example 79

cyclo[Lys-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:94)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Lys(Boc). MW cal.: 1170.50; MW obs.: 1169.80.

Example 80

cyclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:95)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(iPr)(Boc), Fmoc-Lys(iPr)(Boc) in step 6 is replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1147.40; MW obs.: 1146.70.

Example 81

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys-NH_2 (SEQ ID NO:96)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1147.40; MW obs.: 1146.70.

Example 82

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Orn-NH_2 (SEQ ID NO:97)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 is replaced with Fmoc-Orn(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1133.40; MW obs.: 1132.70.

Example 83

cyclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys-NH_2 (SEQ ID NO:98)

Prepare as in Example 25, except that Fmoc-Arg(Pbf) in step 1 and Fmoc-Lys(iPr)(Boc) in step 6 are each replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1105.37; MW obs.: 1105.40.

Example 84

cyclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys-NHEt (SEQ ID NO:99)

Prepare as in Example 25, except that Rink resin is replaced with [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin, Fmoc-Arg(Pbf) in step 1 and Fmoc-Lys(iPr)(Boc) in step 6 are each replaced with Fmoc-Lys(Boc), and Fmoc-Gly in step 8 is replaced with Fmoc-Phe. MW cal.: 1133.36; MW obs.: 1133.82.

Example 85

Alternative Synthesis I of

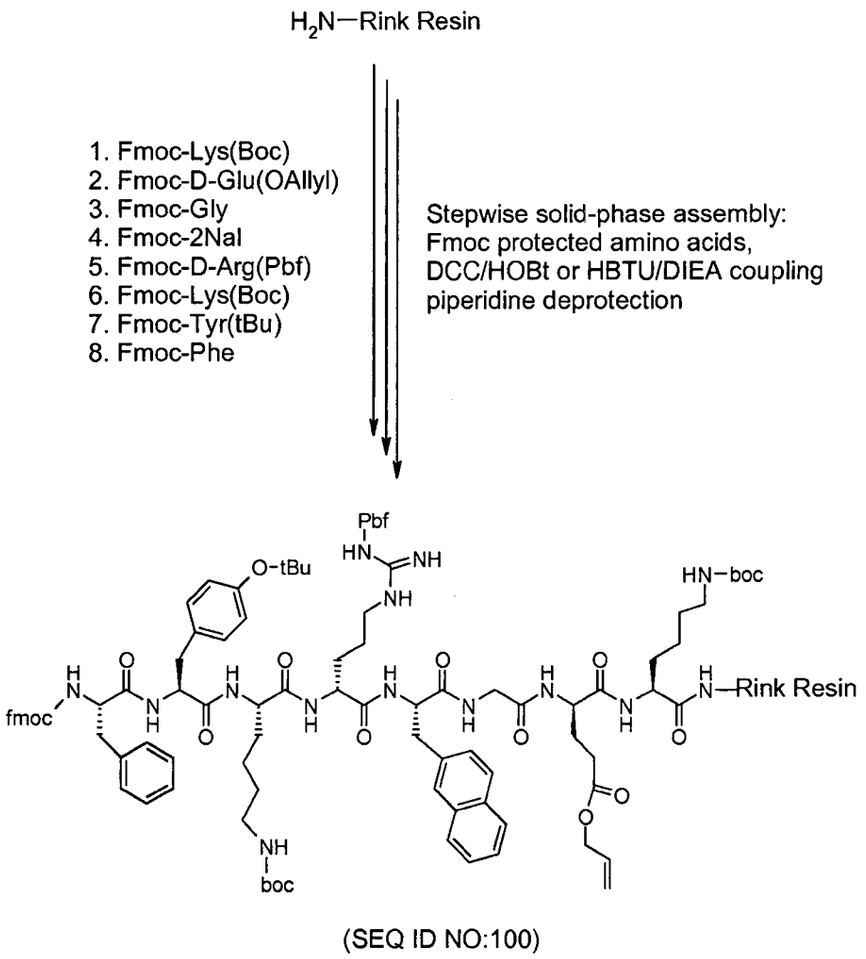
cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

Example 57 discloses the synthesis of SEQ ID NO:70 via Fmoc solid phase peptide synthesis chemistry employing the commercially available building block Fmoc-Lys(iPr)Boc, which is expensive and difficult to obtain in large quantity. The process

described in this example permits the synthesis of SEQ ID NO:70 using less expensive Fmoc-Lys(Boc), solution cyclization, and lysine alkylation through reductive amination using sodium cyanoboronhydride, providing a more economical route to this end product. Additional advantages are that the reaction media (acetic acid, acetone, and methanol) are relatively inexpensive, the reaction conditions are easily controlled, the ratio of solvents can vary significantly without affecting the alkylation reaction, and the recovery yield is 90% or higher.

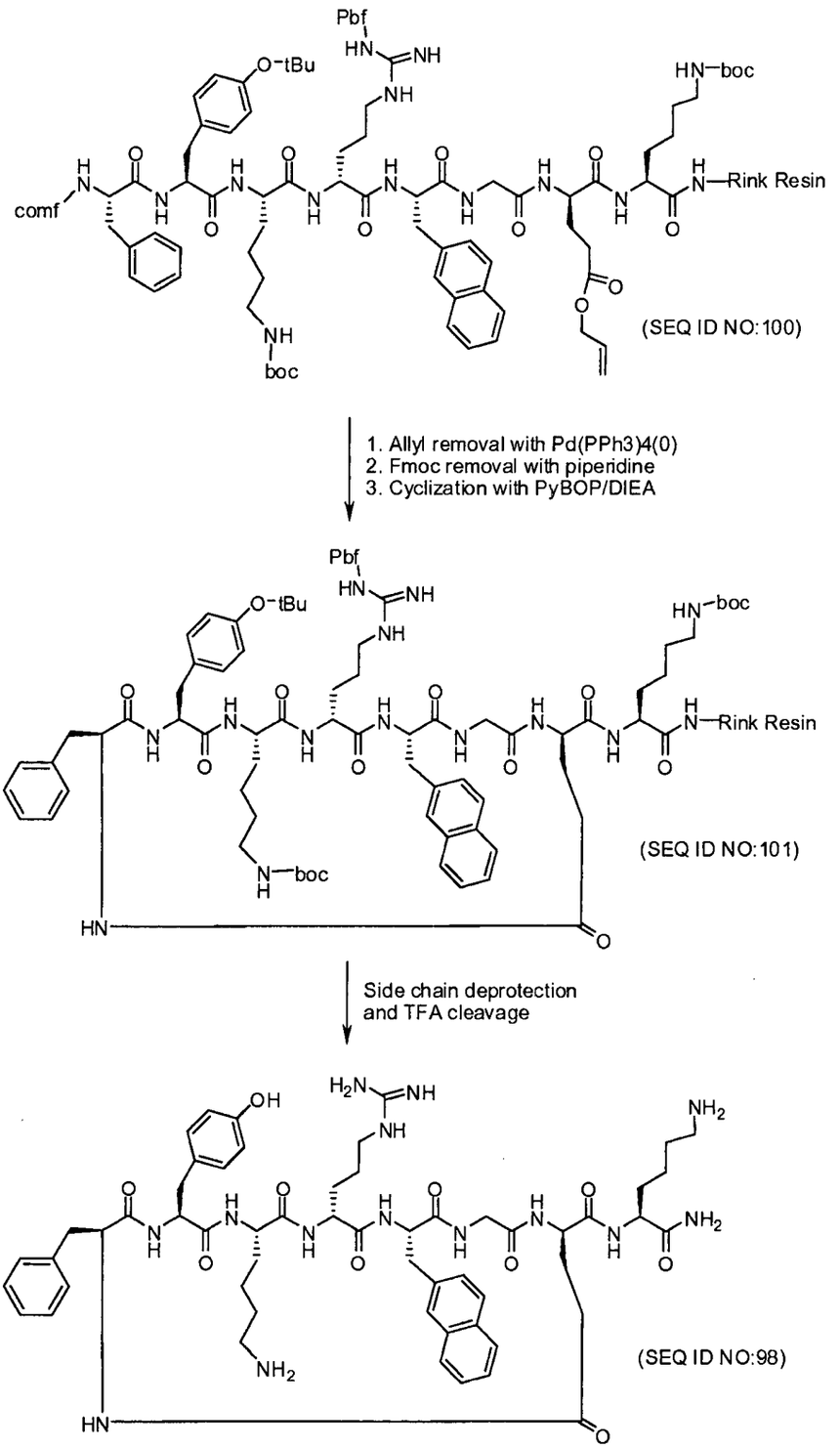
The sequence Phe-Tyr(tBu)-Lys(Boc)-DArg(Pbf)-2Nal-Gly-DGlu(Oallyl)-Lys(Boc) (SEQ ID NO:100) is assembled on Rink Amide Resin by standard Fmoc chemistry utilizing an ABI 431 Peptide Synthesizer as outlined in Scheme 7. The automated assembly is carried out using the standard Applied Biosystems DCC/HOBt chemistry protocol or FastMoc chemistry (HBTU/DIEA) protocol following the supplier's directions (PE Applied Biosystems Inc., Foster City, CA). The side chain protecting group scheme is: Lys(Boc), DGlu(Oallyl), DArg(Pbf), Tyr(tBu). The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 steps. In step 1, four equivalents of protected amino acid Fmoc-Lys(Boc) are activated with DCC/HOBt (or HBTU/DIEA for FastMoc chemistry) in NMP, and coupled to deprotected Rink amide resin. In step 2, four equivalents of Fmoc-DGlu(Oallyl) are activated and coupled to the deprotected peptide resin from step 1. These steps are repeated appropriately until step 8, the coupling of Fmoc-Phe.

The allyl ester side chain protecting group is removed with 0.1 equivalent of $Pd(Ph_3P)_4$ in the presence of 24 equivalents of phenylsilane in dichloromethane (Scheme 8). This process is repeated once for complete side chain deprotection. Fmoc at the N-terminal end is then removed using 20% piperidine in DMF. The deprotected carboxylic acid moiety of DGlu is activated with PyBOP/DIEA and cyclized to the α-amino group of Phe on the resin. The cyclized peptide is simultaneously deprotected and cleaved from the resin using a scavenger cocktail of TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v) or TFA/H_2O/TIS/anisole (92/2/4/2, v/v/v/v) for 2 hours at room temperature. The solvents are then evaporated under vacuum, and the peptide is precipitated and washed three times with cold diethyl ether to remove the scavengers.



(SEQ ID NO:100)

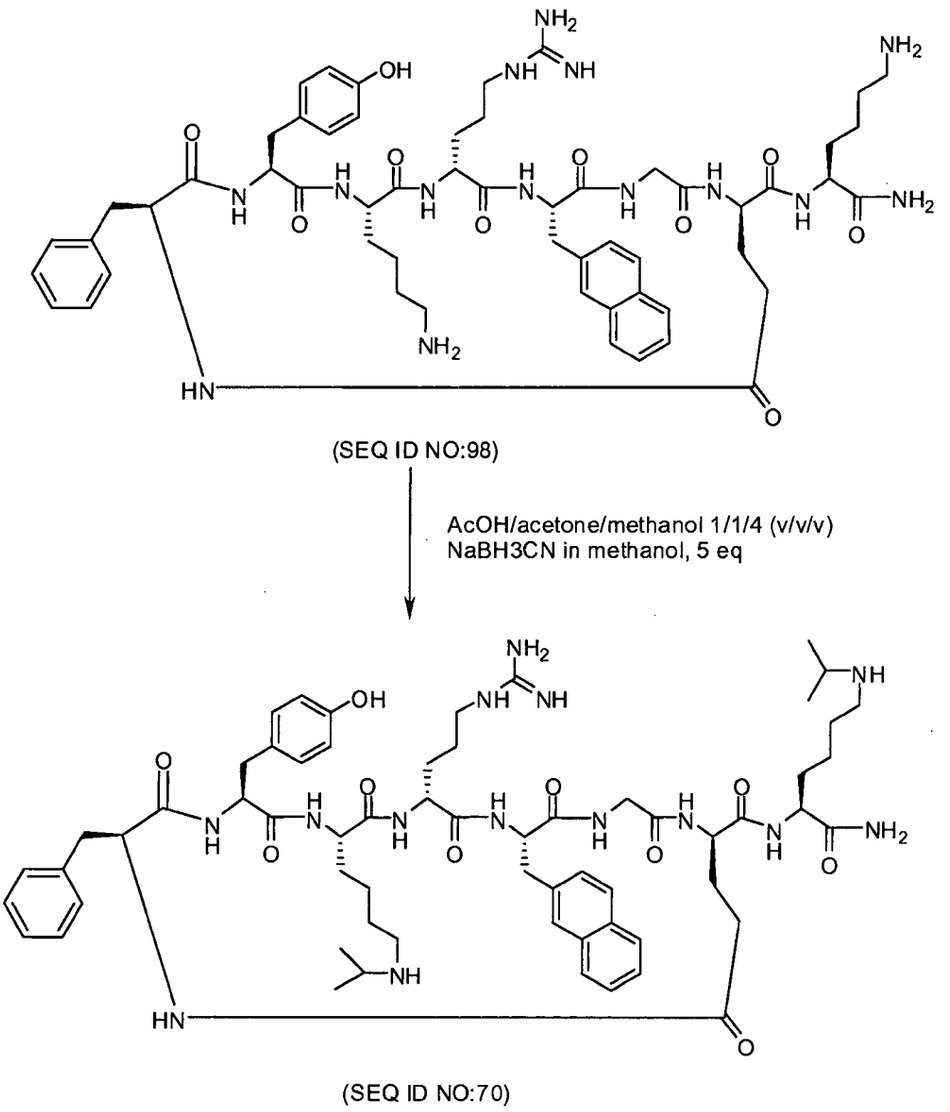
Scheme 7. Peptide chain assembly on solid phase



Scheme 8. Preparation of cyclic precursor peptide

Purification of the cyclic precursor peptide is accomplished using standard preparative HPLC techniques. The crude cleavage product is dissolved in a minimum amount of DMSO, loaded onto a reversed phased C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques.

The lyophilized precursor cyclic peptide is then alkylated in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride (Scheme 9). Peptide concentration is about 10mg/mL, and can vary significantly without affecting the results. Three to 5 equivalents of the reducing reagent sodium cyanoboronhydride are used, and the reaction is normally completed within 2 h at room temperature. The recovery yield is 90% or higher. For example, 20 mg of the precursor cyclic peptide are dissolved in 2 mL of methanol, to which 0.5 mL of acetic acid and 0.5 mL of acetone are added, mixed well, and then 5.6 mg of sodium cyanoboronhydride (2.5 equivalents in methanol) are added under stirring. The reaction mixture is stirred at room temperature for 30 min, upon which another 5.6 mg of sodium cyanoboronhydride are added. The reaction is monitored by HPLC and mass spectral analysis. After the reaction is complete, desalting of the reaction mixture and lyophilization yields 18.9 mg of final product (SEQ ID NO:70) with a purity of 97.5%. MW cal.: 1189.48; MW obs.: 1189.6.



Scheme 9. Reductive amination converting Lys to Lys(iPr)

The compound of Example 60 (SEQ ID NO:74) can also be prepared in an analogous manner, with appropriate modifications based on its constituent amino acids, using [3-({ethyl-Fmoc-amino}-methyl)-indol-1-yl]-acetyl AM resin. First, the precursor peptide (SEQ ID NO:99) is prepared as described in Example 84, and then reductive amination is carried out as shown in Scheme 9. This affords a final cyclic C-terminal ethyl amide peptide with a purity of 99.16%. MW cal.:1217.53; MW obs.: 1217.84.

Example 86

Alternative Synthesis II of

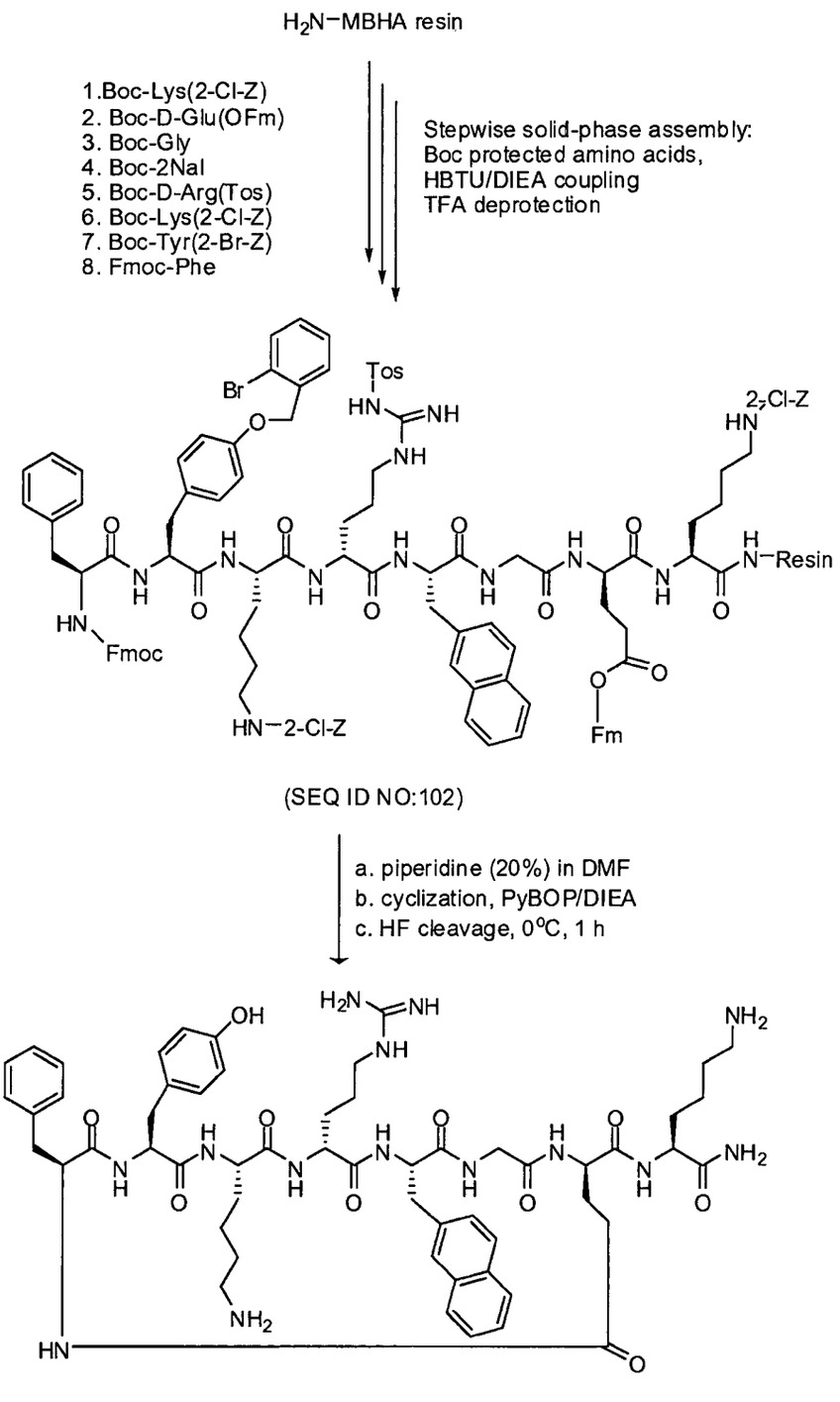
cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

Example 85 discloses a cost effective synthesis of SEQ ID NO:70 via Fmoc solid phase peptide synthesis chemistry employing relatively inexpensive Fmoc-Lys(Boc) and alkylation of lysine through reductive amination using sodium cyanoboronhydride in relatively inexpensive solvents (acetic acid, acetone, and methanol). However, this process involves the use of the heavy metal catalyst palladium for the removal of the allyl ester side chain protective group. Palladium is highly toxic, and quality control to insure complete removal of this element is complicated and difficult. The Boc solid phase peptide synthesis process with cyclization on the resin described in this example permits the production of SEQ ID NO:70 using relatively inexpensive Boc-Lys(2-Cl-Z) without the need for a toxic and expensive palladium catalyst, providing an even more economical, easily upscalable, less toxic route to an end product requiring a simpler quality control process.

The sequence Fmoc-Phe-Tyr(2-Br-Z)-Lys(2-Cl-Z)-DArg(Tos)-2Nal-Gly-DGlu(OFm)-Lys(2-Cl-Z) (SEQ ID NO:102) is manually assembled on MBHA (4-methyl-benzhydryl-amine) resin (Cat. No. D-2095, BaChem California Inc., Torrance, CA) using established solid phase peptide synthesis Boc chemistry (Schnölzer et al. (1992) *Int. J. Pept. Protein Res.* 40:180-193) as outlined in Scheme 10. The chain assembly is carried out using an *in situ* neutralization/HBTU/DIEA activation procedure as described in this reference. The side chain protecting group scheme is: Lys(2-Cl-Z), DGlu(OFm), DArg(Tos), and Tyr(2-Br-Z). The alpha-amino group of all the amino acid building blocks is protected with tert-butoxycarbonyl (Boc) except the N-terminal residue Phe, which is protected with Fmoc for efficiency of synthesis. The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 steps. In step 1, five equivalents of protected amino acid Boc-Lys(2-Cl-Z) are activated with HBTU/DIEA in DMF, and coupled to MBHA resin. In step 2, five equivalents of Boc-DGlu(OFm) are activated and coupled to the deprotected peptide resin from step 1 using neat TFA. These steps are repeated appropriately until step 8, the coupling of Fmoc-Phe.

The Fm side chain protecting group of DGlu, together with the Fmoc at the

N-terminal end, is removed using 20% piperidine in DMF. The deprotected carboxylic acid moiety of DGlu is activated with PyBOP/DIEA, HCTU/DIEA, or other appropriate activation reagents, and cyclized to the α-amino group of Phe on the resin. The cyclic peptide is simultaneously deprotected and cleaved from the resin using HF with 5% *m*-cresol or *p*-cresol as a scavenger for 1 hour at 0 °C. The solvents are then evaporated and the crude peptide is precipitated and washed three times with cold diethyl ether.



Scheme 10. Peptide chain assembly on solid phase using Boc chemistry

Purification of the cyclic precursor peptide (SEQ ID NO:98 as shown in Scheme 10) is accomplished using standard preparative HPLC techniques. The crude cleavage product is dissolved in a minimum amount of DMSO, loaded onto a reversed phased C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques. For SEQ ID NO:98, MW cal.: 1105.29; MW obs.: 1105.4.

The lyophilized precursor cyclic peptide (SEQ ID NO:98) is then alkylated in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride as in Scheme 9. Peptide concentration is about 10mg/mL, and can vary significantly without affecting the results. Five equivalents of the reducing reagent sodium cyanoboronhydride are used, and the reaction is normally completed within 2 h at room temperature. The recovery yield is 90% or higher. For example, 6.6 mg of the precursor cyclic peptide are dissolved in 0.8 mL of methanol, to which 0.2 mL of acetic acid and 0.2 mL of acetone are added, mixed well, and then 1.9 mg of sodium cyanoboronhydride (2.5 equivalents in methanol) are added under stirring in two equal portions. The reaction mixture is stirred at room temperature for 30 min, upon which another 1.9 mg of sodium cyanoboronhydride are added. The reaction is monitored by HPLC and mass spectral analysis. After the reaction is complete, desalting of the reaction mixture and lyophilization yields the final product (SEQ ID NO:70) with a purity of 96.5%. MW cal.: 1189.45; MW obs.: 1189.6.

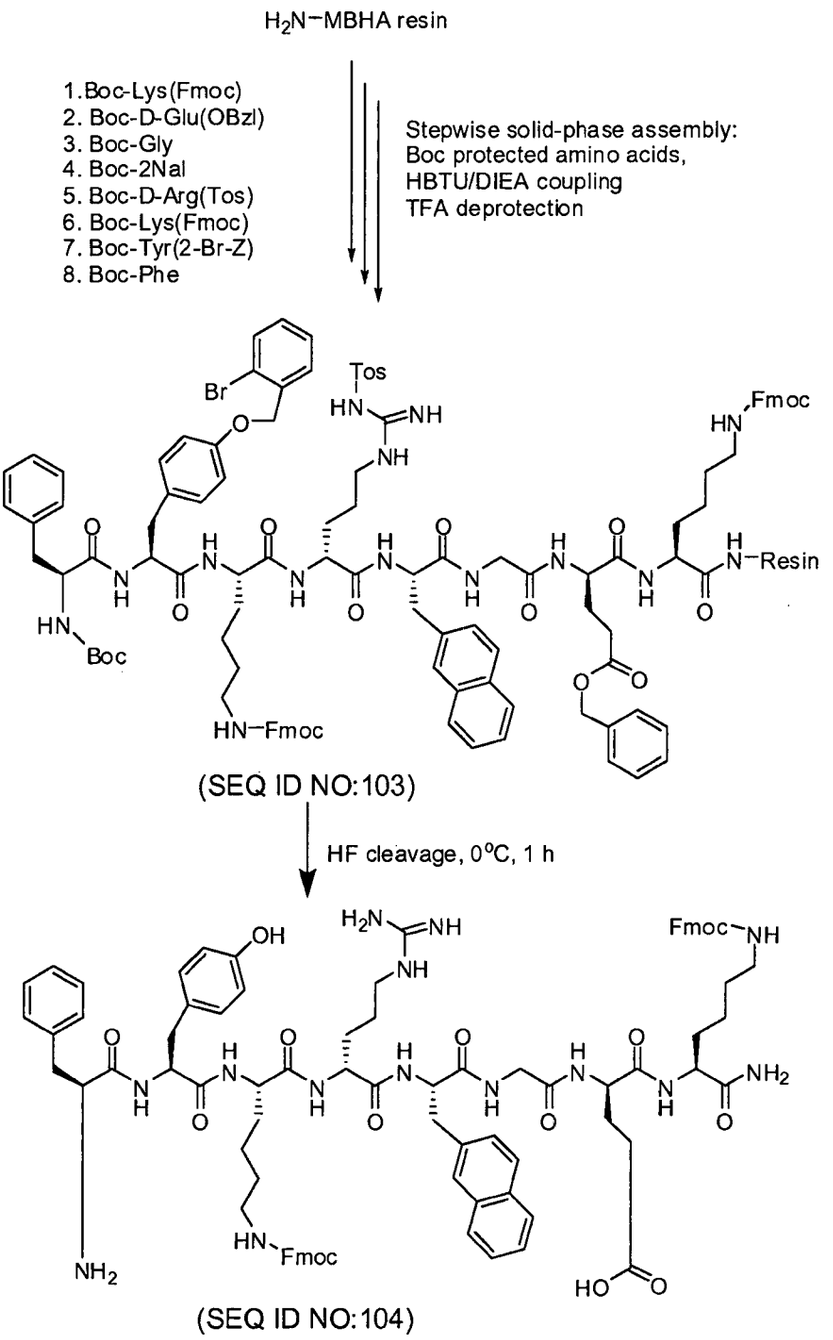
Example 87

Alternative Synthesis III of cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

The compound of Example 57 (SEQ ID NO:70) can also be prepared without the use of a palladium catalyst via solution cyclization, facilitating scaleup, as follows.

The sequence Boc-Phe-Tyr(2-Br-Z)-Lys(Fmoc)-DArg(Tos)-2Nal-Gly-DGlu(OBzl)-Lys(Fmoc) (SEQ ID NO:103) is manually assembled on MBHA resin using solid phase peptide synthesis Boc chemistry (Schnölzer et al. (1992) *Int. J. Pept. Protein Res.* 40:180-193) as outlined in Scheme 11. The chain assembly is carried out using the

in situ neutralization/HBTU/DIEA activation procedure as described in Schnölzer et al. The side chain protecting group scheme is: Lys(Fmoc), DGlu(OBzl), DArg(Tos), Tyr(2-Br-Z). The alpha-amino group of all the amino acid building blocks is protected with tert-butoxycarbonyl (Boc). The stepwise chain assembly starts from the C-terminal end of the linear peptide and is accomplished in 8 steps as shown in Scheme 11. In step 1, five equivalents of protected amino acid Boc-Lys(2-Cl-Z) are activated with HBTU (4 eq) /DIEA (10 eq) in DMF, and coupled to MBHA resin. In step 2, five equivalents of Boc-DGlu(OBzl) are activated and coupled to the deprotected peptide resin from step 1 using neat TFA. These steps are repeated appropriately until step 8, the coupling of Boc-Phe. The Boc protecting group is removed with neat TFA, the resin is neutralized with DIEA, and washed with DMF and methanol and dried in air before HF cleavage. The linear peptide is simultaneously deprotected and cleaved from the resin using HF with 5% *m*-cresol or *p*-cresol as a scavenger for 1 hour at 0 °C. The solvents are then evaporated and the crude peptide is precipitated and washed three times with cold diethyl ether.



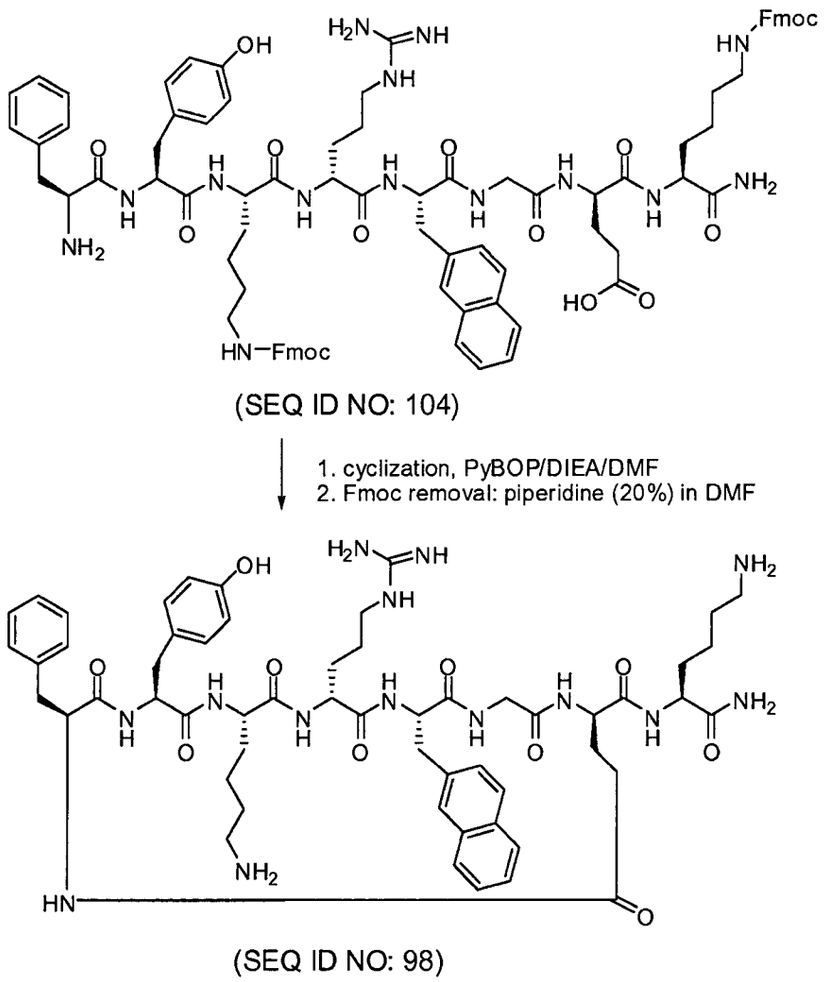
Scheme 11. Chain assembly using Boc chemistry

Purification of the linear precursor peptide (SEQ ID NO:104) is accomplished using standard preparative HPLC techniques. The crude cleavage product is dissolved in

a minimum amount of DMSO, loaded onto a reversed phased C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized. Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques. For SEQ ID NO:104, MW cal.: 1567.78; MW obs.: 1567.6.

Cyclization of the lyophilized precursor linear peptide (SEQ ID NO:104) is carried out in solution (Scheme 12). The linear peptide is dissolved in a small amount of dry DMF (~10 mg/mL). This peptide solution is slowly delivered via a syringe pump to the reaction mixture of PyBOP (2 eq, or other appropriate activation reagents, such as HCTU, BOP, HBTU, etc.) and DIEA (10 eq) in dry DMF under magnetic stirring. The reaction is then allowed to proceed at room temperature for 2 h. Neat piperidine is then added to the reaction mixture to a final concentration of 25% (v/v). The reaction mixture is kept under stirring for another 20 min to completely remove Fmoc protection. Solvents are evaporated under vacuum and the residue is loaded onto a preparative reversed phase C18 HPLC column, and eluted with an aqueous 0.1% trifluoroacetic acid/acetonitrile gradient (v/v) while monitoring at 214 nm. The appropriate fractions are pooled and lyophilized, and afford the cyclic precursor peptide (SEQ ID NO:98). Further characterization of the intermediate precursor cyclic peptide is performed using analytical HPLC and mass spectral analysis by conventional techniques. For SEQ ID NO:98, MW cal.: 1105.29; MW obs.: 1105.4.

Alkylation of cyclic peptide SEQ ID NO:98 is carried out in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride as in Scheme 9 to generate the final product (SEQ ID NO:70). MW cal.: 1189.45; MW obs.: 1189.6.



Scheme 12. Cyclization and Fmoc removal

Example 88

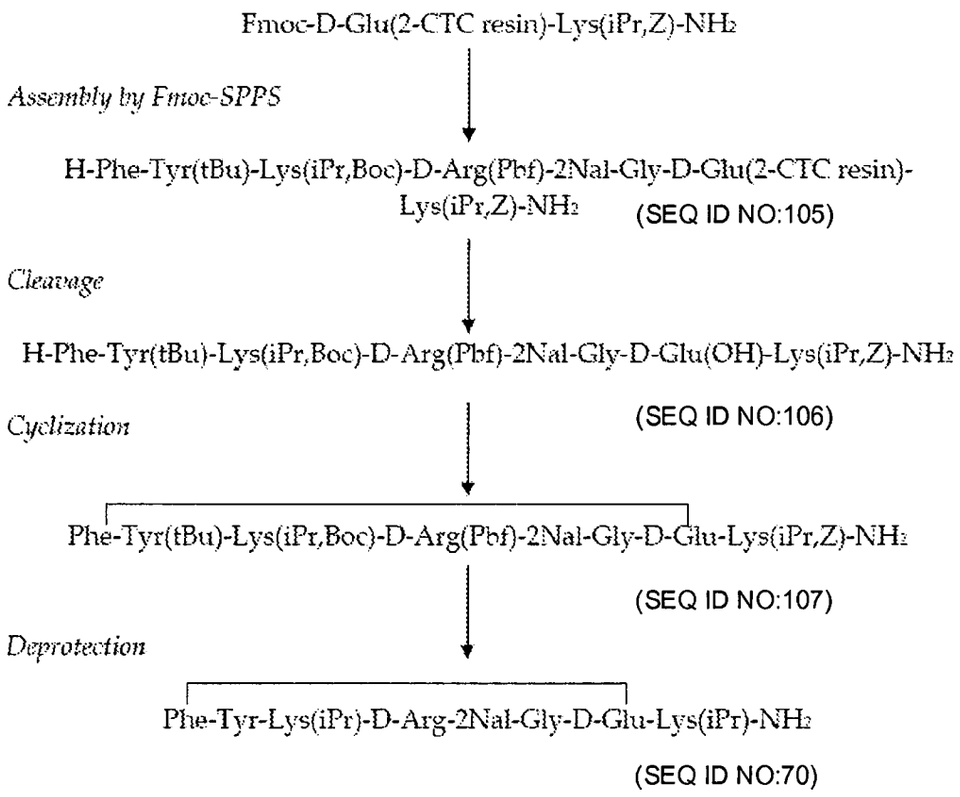
Alternative Synthesis IV of

cyclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

The compound of Example 57 (SEQ ID NO:70) can also be prepared without the use of a palladium catalyst by the synthetic process summarized in Scheme 13 below.

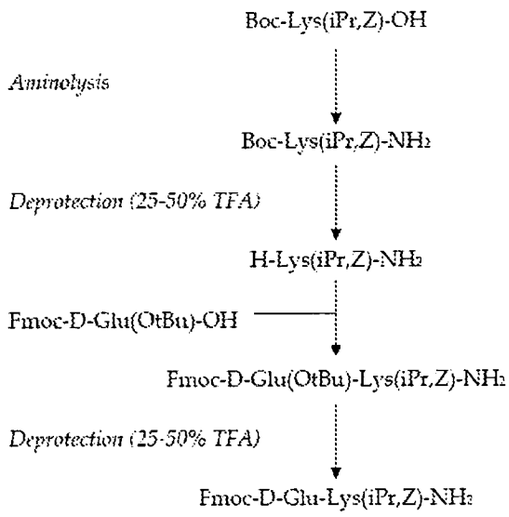
The dipeptide Fmoc-DGlu-Lys(iPr,Z)-NH_2 is first prepared in solution with an exposed DGlutamic acid side chain. The dipeptide is linked to a hyper-acid labile CTC (2-chlorotritylchloride PS resin, 1% DVB (100-200 mesh) resin (Senn Chemicals USA Inc., San Diego, CA; catalog number 40207), and the final peptide product is then

synthesized on this resin via standard Fmoc synthesis as described above. Selective removal of the peptide from the CTC resin allows only the DGlutamic acid side chain to react with the N-terminus of the peptide in solution and generate the cyclic peptide product. Subsequently, the remaining side chains are cleaved with 95% TFA or other strong acid.



Scheme 13. Overall synthetic scheme from Fmoc-DGlu-Lys(iPr,Z)-NH_2

The dipeptide Fmoc-DGlu-Lys(iPr,Z)-NH_2 is first prepared as shown below (Scheme 14):



Scheme 14. Preparation of Fmoc-DGlu-Lys(iPr,Z)-NH_2

Boc-Lys(iPr,Z)-OH is reacted with NMM and IBCF in THF. After addition of NH_4OH, the solvents are removed by rotary evaporation and the product is taken up in ethyl acetate. The ethyl acetate phase is washed extensively with 5% $NaHCO_3$ and then with 0.1 N HCl, and then dried over anhydrous sodium sulfate. Sodium sulfate is removed by filtration and the ethyl acetate is removed by evaporation at reduced pressure. The resulting Boc-Lys(iPr,Z)-NH_2 is dissolved in DCM, and TFA is added. Once the reaction has proceeded to completion, the solvents are removed by rotary evaporation. H-Lys(iPr,Z)-NH_2 is then dissolved in DMF. The pH is adjusted to 8 with DIEA. In a separate vessel, Fmoc-DGlu(OtBu)-OH, HBTU, and HOBt are dissolved in DMF; DIEA is added to adjust the pH to 8. The two solutions are mixed, and the reaction is monitored by C18 reversed phase HPLC. The pH is monitored and adjusted, where necessary, with DIEA. The solvents are removed by rotary evaporation, and the product dissolved in ethyl acetate. The ethyl acetate phase is washed extensively with 5% $NaHCO_3$ and then with 0.1 N HCl, and then dried over anhydrous sodium sulfate. The sodium sulfate is removed by filtration and the ethyl acetate is removed by evaporation at reduced pressure. Rotary evaporation is continued until a dry residue is formed. The resulting Fmoc-DGlu(OtBu)-Lys(iPr,Z)-NH_2 is dissolved in DCM, and TFA is added. Once the reaction has proceeded to completion, the solvents are removed by rotary evaporation. The solid

product is obtained by trituration with diethyl ether. After the precipitate is washed with ether, the product is dried in a vacuum oven.

Solid phase peptide synthesis of the final product is performed as follows. Fmoc-DGlu(OtBu)-Lys(iPr,Z)-NH_2 is dissolved in DCM and reacted with CTC resin in the presence of DIEA in a reaction vessel. After 3 h, the peptide-resin is washed free of reagents with DCM and Z-OSu is added. The pH is monitored and adjusted to pH 8-9 by adding DIEA if necessary. After 8 h, the peptide-resin is washed free of reagents with DCM, transferred to a polypropylene container, and dried in a vacuum oven.

The protected peptide-resin is assembled using Fmoc-chemistry as follows. The coupling cycle used is: 1) De-blocking: treatment with 25% piperidine in DMF; 2) Washing cycles with DMF, IPA and DMF again; 3) Ninhydrin test (qualitative: if positive, proceed to coupling Step 4); 4) Coupling with 2 equivalents Fmoc-amino acid in presence of HOBt /DIC in DMF; 5) Washing cycles with DMF; 6) Ninhydrin test (qualitative: if negative, proceed to next de-blocking/coupling cycle; if positive, proceed to re-coupling Step 7; if slightly positive, proceed to acetylation Step 10); 7) Re-coupling (if required), with 1 equivalent Fmoc-amino acid in the presence of HOBt, HBTU/DIEA in DMF; 8) Washing cycles with DMF; 9) Ninhydrin test (qualitative: if negative, proceed to next de-blocking/coupling cycle; if positive, proceed to acetylation Step 10); 10) Acetylation (if required) with 2% acetic anhydride in 4% DIEA in DMF; 11) Washing cycles (with DMF, IPA, and DMF again); 12) Ninhydrin test (qualitative: if positive, proceed to next de-blocking/coupling cycle). After the final coupling cycle, the peptide-resin (SEQ ID NO:105) is washed with ether and dried under vacuum.

The protected peptide-resin is washed with DCM. Cleavage of the fully protected linear peptide from the resin is performed with 2% TFA in DCM followed by filtration. The solvents are removed by rotary evaporation, and the fully linear protected peptide (SEQ ID NO:106) is precipitated by trituration with ether. The fully protected linear peptide is transferred to a polypropylene container and dried in a vacuum oven.

The fully protected linear peptide is cyclized in the presence of PyBOP, HOBt, and DIEA in DMF. The pH is maintained between pH 7-8 by addition of DIEA, if necessary. After the reaction has proceeded to completion, the solvents are removed by rotary evaporation, and the product is taken up in ethyl acetate. The ethyl acetate phase is washed extensively with 5% $NaHCO_3$ and then with 0.1 N HCl and saturated NaCl

solution. It is then dried over anhydrous sodium sulfate. The sodium sulfate is removed by filtration and the ethyl acetate is removed by rotary evaporation at reduced pressure. The protected cyclic peptide (SEQ ID NO:107) is precipitated by trituration with ether and dried in a vacuum oven.

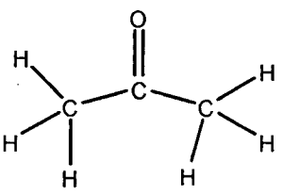
Deprotection is performed in TFA:H_2O:TIS. When the reaction is complete, the solvents are removed by rotary evaporation and the cyclic peptide (SEQ ID NO:70) is precipitated by trituration with ether and dried in a vacuum oven. MW cal.: 1189.48; MW obs.: 1189.50.

Example 89

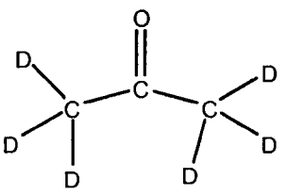
Incorporation of Isotopic Labels:

Synthesis of cyclo[Phe-Tyr-Lys(iPr-d_6)-DArg-2Nal-Gly-DGlu]-Lys(iPr-d_6)-NH_2 (SEQ ID NO:108)

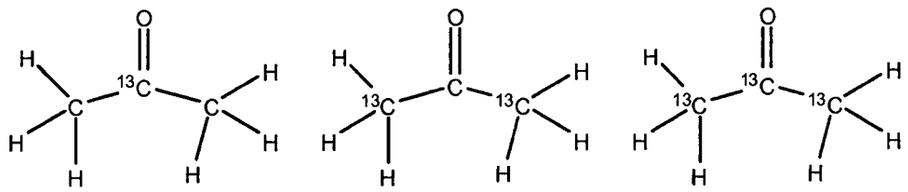
Starting with isotopically labeled acetone such as ^{13}C-, ^{14}C-, deuterium-, or tritium-labeled acetones as shown below, the processes of Examples 85-87 permit site-specific isotopic labeling of cyclic peptide CXCR4 antagonists for various pharmacological and imaging studies. The isotopically labeled acetones are commercially available from various sources. An example is given below using acetone-d_6 to prepare a peptide containing 12 deuterium atoms. The resulting compound, differing in molecular weight by 12 Da compared to the non-labeled counterpart, is easily differentiated in mass spectra and exhibits identical target receptor affinity.



Acetone



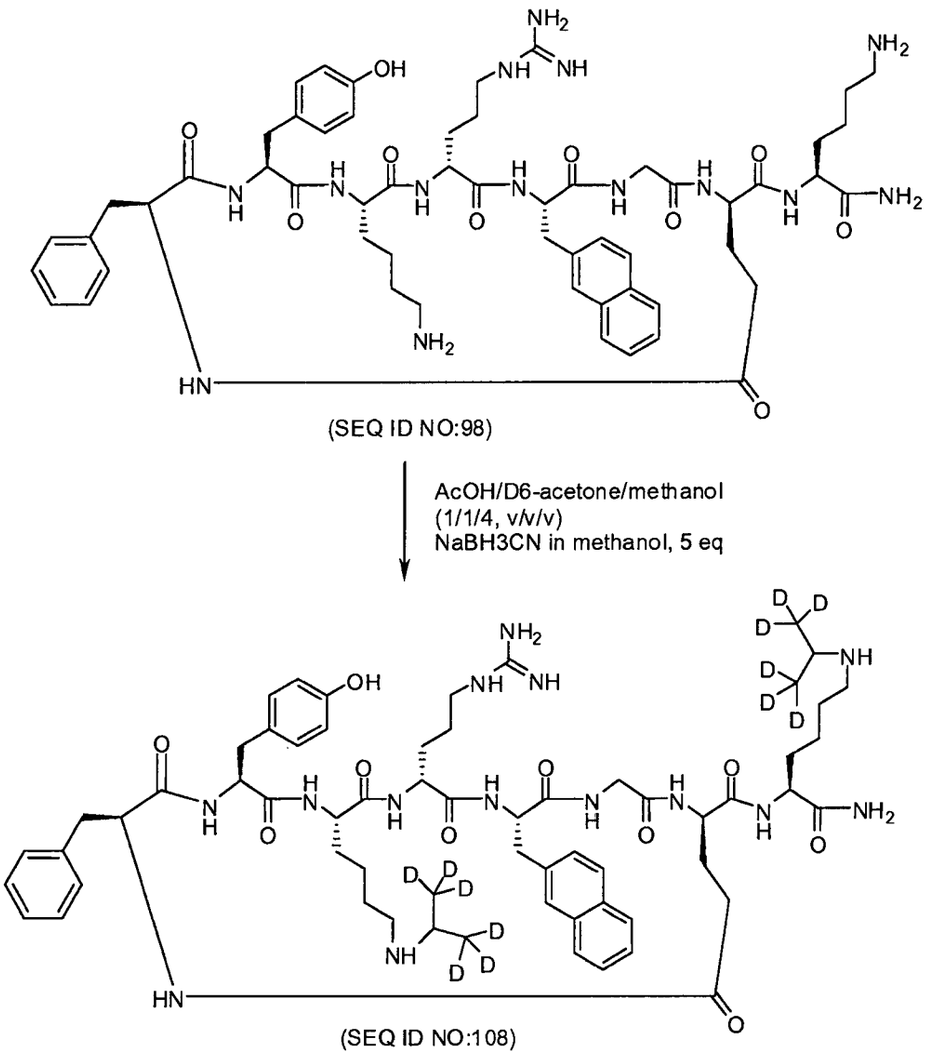
Acetone-d_6



Acetone-^{13}C Acetone-2-^{13}C Acetone-3-^{13}C

Using any of the methods in Examples 85-87, one can prepare and purify cyclic peptide precursors (SEQ ID NO:98, SEQ ID NO:99, etc.). Alkylation is carried out in a solution of acetic acid/acetone/methanol (1:1:4, v/v/v) through reductive amination using sodium cyanoboronhydride as in Scheme 9, with the exception that standard acetone is replaced with the desired isotopically labeled acetone. In the present example, deuterium acetone-d6 is used.

Peptide (97 mg) is dissolved in 15 mL of acetic acid/acetone-d_6/methanol (1:1:4, v/v/v). Peptide concentration can vary significantly without affecting the results. Five equivalents of sodium cyanoboronhydride are used, and the reaction is normally completed within 2 h at room temperature. The reaction is monitored by HPLC and mass spectral analysis. After the reaction is complete, desalting of the reaction mixture and lyophilization yields 90.5 mg of final product (SEQ ID NO:108) with a purity of 99.9%. MW cal.: 1201.48; MW obs.: 1201.7.



Scheme 15. Site-specific deuterium labeling of CXCR4 antagonist

Use of isotopically labeled sodium cyanoboronhydride (such as NaBD3CN, NaBT3C N, etc.) permits incorporation of additional variations to the site-specific labeling patterns.

The pharmacological properties of the present compounds can be determined by employing the assays described below.

Human CXCR4/^{125}I-SDF-1α Binding Inhibition Assay

SDF-1 binding to CXCR4 is the first step in activating the CXCR4 intracellular signalling pathway. To determine if a compound can block the interaction of SDF-1 and CXCR4, human leukemia CCRF-CEM cells (ATCC CCL 119) expressing endogenous CXCR4 are employed in an ^{125}I-labeled SDF-1α binding assay. The assay is performed in a 96-well U-bottom, non-treated polystyrene plate (Corning Incorporated, Costar, No. 3632). The binding assay buffer is prepared with RPMI 1640 medium (Gibco, Grand Island, NY) containing 10 mM HEPES, pH 7.5, and 0.2% BSA. Briefly, 200 μL reaction mixtures containing 300 pM SDF ligand (60 pM ^{125}I-SDF-1α (Perkin Elmer) and 240 pM cold SDF-1α (R&D Systems), different concentrations of the test compound in assay buffer, 100,000 human CCRF-CEM cells, and 0.5 mg SPA beads (Wheatgerm agglutinin beads; Amersham) are incubated at room temperature for 2 hr. Plates are then counted in a 1450 Microbeta Liquid Scintillation and Luminescence Counter (Wallac) in SPA mode. CXCR4 antagonists decrease the bound radioactivity in this assay in a dose-dependent manner. The inhibitory potency (K_i or IC_{50}) of a test compound is calculated using GraphPad Prism software, based on the dose-dependent decrease of bound radioactivity.

All compounds exemplified above exhibit an average K_i value of about 7.5 nM or less in this assay. For example, the compound of Example 1 exhibits an average K_i of 3.45 nM in this assay. Many of these compounds exhibit an average K_i value between about 0.2 nM and about 1 nM. For example, the compound of Example 50 exhibits an average K_i value of 0.285 nM in this assay. Other compounds exhibit an average K_i value less than about 0.2 nM. For example, the compound of Example 75 exhibits an average K_i value of 0.096 nM in this assay.

Chemotaxis Assay

CXCR4/SDF-1 interaction regulates migration (chemotaxis) of cells bearing CXCR4 on their surface. To determine the antagonist and cellular activities of a test

compound, a chemotaxis assay using human histiocytic lymphoma U937 cells (ATCC CRL 1593) that express endogenous CXCR4 are employed. Briefly, U937 cells, grown in DMEM medium (Gibco, Grand Island, NY) containing 10% FBS, 1% MEM sodium pyruvate (Gibco), 1% MEM nonessential amino acids (Gibco), and 1% GlutaMAX 1 (Gibco), are harvested and washed once with chemotaxis assay buffer prepared with 1x RPMI medium (Gibco) containing 10 mM HEPES, pH 7.5, and 0.3% BSA. After washing, cells are resuspended in assay buffer at a concentration of 5 x 10^6 cells/mL. The assay is performed in a 96-well ChemoTx plate (NeuroProbe) according to the manufacturer's directions. Generally, 50 µL of cell mixture with or without test compound are plated on the upper chamber, and 30 µL of SDF-1α (R&D Systems, 10 ng/mL) prepared in 1 x chemotaxis buffer are added to the lower chamber. After assembly, the plate is incubated for 2.5 hr at 37°C under 5% CO_2. Following the incubation, 5 µL of CellTiter 96 AQ (Promega, Madison, WI) are added into the lower chamber. The plate is then incubated for 60 min at 37°C, and the migrated cells are detected by measuring the absorbance at 492 nm with a Tecan Spectrafluor Plus Microplate Reader (Salzburg, Austria). CXCR4 antagonists inhibit cell migration, reducing the absorbance reading. The inhibitory potency (IC_{50}) of a test compound in this assay is calculated using GraphPad Prism software, based on the dose-dependent decrease of absorbance at 492 nm.

Most of the compounds exemplified above exhibit an average IC_{50} value of about 60 nM or less in this assay. Many of these compounds exhibit an average IC_{50} value of about 6 nM or less, e.g., the compound of Example 19 exhibits an average IC_{50} value of 2.05 nM in this assay. Many of these compounds exhibit an average IC_{50} value of about 0.6 nM or less, e.g., the compound of Example 50 exhibits an average IC_{50} value of 0.171 nM in this assay.

Chemokine Receptor Binding Selectivity Assays

The binding selectivity of the present compounds for the CXCR4 receptor compared to that for other chemokine receptors, such as human CCR1, CCR2, CXCR2, or CXCR3, and other G-protein-coupled receptors, can be assessed in cells transfected with nucleic acid encoding and expressing such receptors, or in cells in which such

receptors are endogenously expressed. Whole cells or membrane fragments can be used to assess competition of test compounds with the respective ligands for these receptors in a manner similar to that described above for the CXCR4/^{125}I-SDF-1α binding inhibition assay.

For example, the compound of Example 57a exhibits a K_i value greater than 73,000 nM in a ligand binding assay using human chemokine receptor CXCR2.

Compound-Induced White Blood Cell and Neutrophil Mobilization in C57BL/6 Mice

Stem cells within the bone marrow actively maintain continuous production of all mature blood cell lineages throughout life. Bone marrow is the primary site for white blood cell (WBC)/neutrophil production and release into the circulation. The CXCR4/SDF-1 axis appears to be critical for the retention and release of WBCs, neutrophils, and hematopoietic progenitor cells in the bone marrow, and interruption of CXCR4/SDF-1 interaction in bone marrow leads to an increase of these cells in peripheral blood. A short-term mouse WBC/neutrophil mobilization model can be used to determine the *in vivo* target-modulating activity of a test compound. Briefly, pathogen-free 5-6 week old female C57BL/6 mice (Taconic) are housed for at least one week prior to assay. Animals are allowed continuous access to sterilized rodent chow and acidified water. Groups of 5 mice are injected subcutaneously with test compounds in saline, or with saline control, and then sacrificed by CO_2 asphyxiation and cervical dislocation at various time points post compound administration. Peripheral blood is collected by cardiac puncture using EDTA-coated syringes and tubes. Complete blood cell analysis is performed on a Hemavet Mascot hematology analyzer (Drew Scientific Group, Dallas, TX). Total WBCs, neutrophils, and lymphocytes in the peripheral blood are recorded. Effective CXCR4 antagonists administered subcutaneously to mice increase the neutrophil and WBC counts in peripheral blood compared to saline control.

A significant number of compounds exemplified above exhibit an average neutrophil ratio (ratio of neutrophil increase in treatment group vs. neutrophil increase in saline control group), measured 3 hours after compound administration, greater than about 2 in this assay. For example, the compound of Example 39 exhibits an average neutrophil ratio of 4.6 at a dose of 5 mg/kg in this assay.

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Anti-Tumor Activity in a SCID/Namalwa Xenograft Model

SDF-1/CXCR4 interaction appears to play an important role in multiple stages of tumorigenesis, including tumor growth, invasion, angiogenesis, and metastasis. To evaluate *in vivo* anti-tumor activity of a test compound, a tumor xenograft model using NOD/SCID mice (Jackson Laboratories) and human non-Hodgkin's lymphoma Namalwa cells (ATCC CRL 1432) are employed. Briefly, 200,000 Namalwa cells mixed with matrigel (1:1) are implanted subcutaneously into the rear flank of the animals. The implanted tumor cells grow as solid tumors, the dimensions of which can be continuously monitored and measured using a caliper. To determine the *in vivo* efficacy of a test compound in this model, one can treat animals (10/group) with different doses of test compounds dissolved in saline or PBS, beginning 48 hours post tumor cell implantation. Compounds are dosed subcutaneously, and tumor volume and body weight are determined every 2 or 3 days. Studies generally last 3-4 weeks, depending on tumor growth. The anti-tumor growth activity of a test compound is determined by the percent reduction in tumor volume in treatment groups compared to tumor volume in control groups treated with vehicle alone.

Several compounds exemplified above, for example the compound of Example 26, significantly inhibit tumor growth in this assay when administered at 1 mg/kg BID.

Pharmacologic properties such as compound bioavailability, *in vivo* metabolic stability, and pharmacokinetic/pharmacodynamic properties can be determined by methods well known in the art of drug development. Preferred compounds of the present invention exhibit high bioavailability when administered subcutaneously. Some compounds exemplified herein exhibit bioavailability near 100% in rats, for example the compound of Example 44. Preferred compounds also exhibit good *in vivo* metabolic stability. For example, no detectable metabolites are observed in dog and monkey plasma and urine up to 24 hours after administration of the compound of Example 57a. Preferred compounds also exhibit favorable pharmacokinetic/pharmacodynamic properties that permit convenient dosing. For example, in mice, the half-life (T1/2) of the compound of Example 58 is about 3 hours. With respect to pharmacodynamic properties, preferred compounds induce prolonged neutrophil and white blood cell mobilization in mice. For example, the compound of Example 25 induces a significant increase of

neutrophils and white blood cells in peripheral blood for at least 6 hours after single dose subcutaneous administration at 5 mg/kg in mice.

What Is Claimed Is:

1. A lactam-cyclized peptide of formula I:

R_1 - cyclo[X_1 – Tyr – X_3 – DArg – 2Nal – Gly – X_7] – X_8 – X_9 – X_{10} – R_2

(I)

(SEQ ID NO:1)

wherein:

a) said lactam is formed by an amide bond between the side chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of (D/L)Agl/Glu, Dab/Glu, and Dap/Glu, and R_1 is Ac or n-hexanoyl; or

b) said lactam is formed by an amide bond between the side chain carboxyl group of X_1 and the side chain amino group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, and Glu/Lys, and R_1 is Ac or Bz, or wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of succinyl/(D/L)Agl, succinyl/Dab, succinyl/Dap, succinyl/Lys, and succinyl/Orn, and R_1 is absent; or

c) said lactam is formed by an amide bond between the α-amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, and DPhe/DGlu, and R_1 is absent; or

d) said lactam is formed by an amide bond between a non-α, non-side-chain amino group of X_1 and the side chain carboxyl group of X_7, wherein X_1 and X_7 are, respectively, a pair selected from the group consisting of β-Ala/Asp, β-Ala/Glu, 5-amino-valeryl/Asp, 5-aminovaleryl/Glu, 4-AMB/Glu, 4-AMPA/Asp, and 4-AMPA/Glu, and R_1 is absent; or

e) said lactam is formed by an amide bond between the α-amino group of X_2 and the side chain carboxyl group of X_7, wherein X_2 and X_7 are, respectively, a pair selected from the group consisting of Tyr/Asp, Tyr/Glu, and Tyr/DGlu, and R_1 and X_1 are each absent;

R_1 is a substituent on the α-amino group of X_1 when X_1 contains an α-amino group and said α-amino group is not a constituent of said lactam amide bond, selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent, wherein X_1 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, and Glu;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, DAla, 5-aminovaleryl, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and $Lys(Me_2)$;

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent;

wherein when X_8 is absent, X_9 and X_{10} are each absent, and when X_9 is absent, X_{10} is absent, and

R_2 is selected from the group consisting of NH_2 and NHEt, or

a pharmaceutically acceptable salt thereof.

2. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of β-Ala, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, 2Nal, Phe, and succinyl, or is absent;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of Asp, Dab, Dap, Glu, DGlu, Lys, and Orn;

X_8 is selected from the group consisting of Arg and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

3. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of DAla, 5-aminovaleryl, 4-AMPA, Asp, Glu, Leu, Lys(Ac), Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dab, Dap, DDap, Glu, and DGlu;

X_8 is selected from the group consisting of Arg, DArg, and Lys, or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

4. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac, Bz, and n-hexanoyl, or is absent;

X_1 is selected from the group consisting of (D/L)Agl, Ala, β-Ala, Asp, Dap, Glu, Gly, Lys, and Phe;

X_3 is selected from the group consisting of Arg, Lys, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, 2Nal, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

5. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, wherein:

R_1 is selected from the group consisting of Ac and Bz, or is absent;

X_1 is selected from the group consisting of Ala, 5-aminovaleryl, Asp, Glu, Gly, Phe, DPhe, and succinyl;

X_3 is selected from the group consisting of Arg, Lys(iPr), and Lys(Me_2);

X_7 is selected from the group consisting of (D/L)Agl, Asp, Dap, Glu, and DGlu;

X_8 is selected from the group consisting of β-Ala, Arg, Gly, Lys, Lys(iPr), and Orn, or is absent;

X_9 is selected from the group consisting of Gly, D2Nal, and DPhe, or is absent;

X_{10} is 2Nal, or is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

6. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 1, 4, or 5, wherein:

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr); and

X_7 is DGlu.

7. The lactam-cyclized peptide or pharmaceutically acceptable salt thereof of claim 5, wherein:

R_1 is absent;

X_1 is selected from the group consisting of Gly and Phe;

X_3 is Lys(iPr);

X_7 is DGlu;

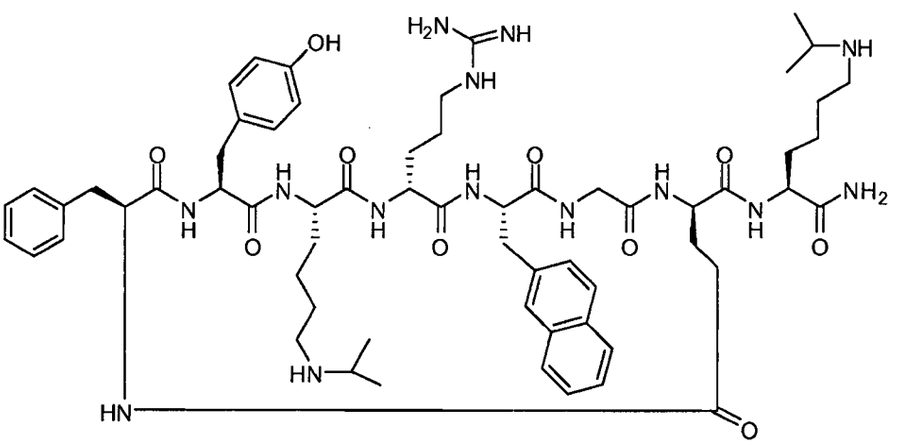
X_8 is selected from the group consisting of Arg and Lys(iPr), or is absent;

X_9 is absent;

X_{10} is absent; and

R_2 is selected from the group consisting of NH_2 and NHEt.

8. A lactam-cyclized peptide of the formula:



(SEQ ID NO:70)

or a pharmaceutically acceptable salt thereof.

9. The lactam-cyclized peptide of claim 8, wherein said pharmaceutically acceptable salt is an acetic acid salt.

10. A pharmaceutical composition, comprising a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, and a pharmaceutically acceptable carrier, diluent, or excipient.

11. A lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, for use in therapy.

12. A lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

13. Use of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9, for the manufacture of a medicament for the treatment of rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia.

14. A method of treating rheumatoid arthritis, pulmonary fibrosis, HIV infection, or a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and chronic lymphocytic leukemia, comprising administering to a patient in need thereof an effective amount of a lactam-cyclized peptide or pharmaceutically acceptable salt thereof of any one of claims 1-9.

SEQUENCE LISTING

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<150> 60/940996
<151> 2007-05-31

<150> 60/940802
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Glu Tyr Arg Arg Xaa Gly Xaa Arg
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Asp Tyr Arg Arg Xaa Gly Xaa Arg
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Asp Tyr Arg Arg Xaa Gly Xaa Arg
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Asp Tyr Xaa Arg Xaa Gly Xaa Arg
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<400> 31

Tyr Arg Arg Xaa Gly Lys Arg
1 5

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<400> 32

Gly Tyr Xaa Arg Xaa Gly Glu Arg
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<223> Xaa = d isomer Glu(Oallyl)

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<223> Xaa = Arg(Pbf)

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Gly Xaa Xaa Xaa Xaa Gly Xaa Xaa
1 5

<210> 34
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192

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Gly Tyr Xaa Arg Xaa Gly Glu Arg
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<400> 35

Gly Xaa Xaa Xaa Xaa Gly Xaa Xaa
1 5

<210> 36
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<400> 36

Gly Xaa Xaa Xaa Xaa Gly Glu Xaa
1 5

<210> 37
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<400> 37

Gly Tyr Xaa Arg Xaa Gly Glu
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<400> 38

Gly Tyr Xaa Arg Xaa Gly Glu
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<400> 39

Gly Tyr Arg Arg Xaa Gly Glu Arg
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Gly Tyr Xaa Arg Xaa Gly Glu Xaa
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<223> Xaa = 2Nal

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<400> 41

Tyr Arg Arg Xaa Gly Glu Arg
1 5

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<400> 42

Tyr Arg Arg Xaa Gly Glu Arg
1 5

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<400> 43

Tyr Arg Arg Xaa Gly Asp Arg
1 5

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<400> 44

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1 5

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<400> 45

Gly Tyr Xaa Arg Xaa Gly Asp Arg
1 5

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<223> Xaa = Lys(iPr)

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<223> Xaa = 2Nal

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<223> AMIDATION

<400> 46

Gly Tyr Xaa Arg Xaa Gly Asp Arg
1 5

<210> 47
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<223> AMIDATION

<400> 47

Gly Tyr Xaa Arg Xaa Gly Asp
1 5

<210> 48
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<223> Xaa = Lys(iPr)

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<400> 48

Gly Tyr Xaa Arg Xaa Gly Asp Xaa
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<223> AMIDATION

<400> 49

Gly Tyr Xaa Arg Xaa Gly Glu Arg
1 5

<210> 50
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<400> 50

Gly Tyr Arg Arg Xaa Gly Glu Arg
1 5

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<400> 51

Gly Tyr Xaa Arg Xaa Gly Glu Arg
1 5

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<400> 52

Gly Tyr Xaa Arg Xaa Gly Glu
1 5

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<400> 53

Gly Tyr Xaa Arg Xaa Gly Asp
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<400> 54

Gly Tyr Xaa Arg Xaa Gly Glu
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Gly Tyr Xaa Arg Xaa Gly Glu

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<400> 56

Gly Tyr Xaa Arg Xaa Gly Glu Arg
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<400> 57

Gly Tyr Xaa Arg Xaa Gly Glu Arg
1 5

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<400> 58

Gly Tyr Xaa Arg Xaa Gly Glu Xaa
1 5

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<400> 59

Xaa Tyr Xaa Arg Xaa Gly Glu Arg
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<400> 60

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<400> 61

Ala Tyr Xaa Arg Xaa Gly Glu
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<223> AMIDATION

<400> 62

Ala Tyr Xaa Arg Xaa Gly Glu
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<400> 63

Ala Tyr Xaa Arg Xaa Gly Glu
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Ala Tyr Xaa Arg Xaa Gly Glu
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<223> AMIDATION

<400> 65

Leu Tyr Xaa Arg Xaa Gly Glu
1 5

<210> 66
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<400> 66

Leu Tyr Xaa Arg Xaa Gly Glu
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<210> 67
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<400> 67

Phe Tyr Xaa Arg Xaa Gly Glu
1 5

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<400> 68

Phe Tyr Xaa Arg Xaa Gly Glu
1 5

<210> 69
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<400> 69

Phe Tyr Xaa Arg Xaa Gly Glu
1 5

<210> 70
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<400> 70

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1 5

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<400> 71

Phe Tyr Xaa Arg Xaa Gly Glu Xaa
1 5

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<400> 72

Phe Tyr Xaa Arg Xaa Gly Glu Arg
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<400> 73

Phe Tyr Xaa Arg Xaa Gly Glu
1 5

<210> 74
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<400> 74

Phe Tyr Xaa Arg Xaa Gly Glu Xaa
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<400> 75

Phe Tyr Xaa Arg Xaa Gly Glu Xaa
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<400> 76

Ala Tyr Xaa Arg Xaa Gly Glu
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<210> 77
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Xaa Tyr Xaa Arg Xaa Gly Glu
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<210> 78
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<400> 78

Phe Tyr Xaa Arg Xaa Gly Glu
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Phe Tyr Xaa Arg Xaa Gly Glu
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<210> 80
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<400> 81

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<400> 82

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Xaa Tyr Arg Arg Xaa Gly Asp Arg
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Tyr Arg Arg Xaa Gly Glu Arg
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Phe Tyr Xaa Arg Xaa Gly Glu Xaa Gly Xaa
1 5 10

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Phe Xaa Xaa Xaa Xaa Gly Xaa Xaa
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Phe Tyr Xaa Arg Xaa Gly Glu Xaa
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Phe Xaa Xaa Xaa Xaa Gly Xaa Xaa
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<400> 106

Phe Xaa Xaa Xaa Xaa Gly Xaa Xaa
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<220>
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<400> 108

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1 5

ANTAGONISTAS CÍCLICOS DE PÉPTIDO CXCR4

La presente invención se refiere a compuestos antagonistas CXCR4 de péptidos cíclicos novedosos y su uso en el tratamiento de enfermedades en las cuales la patogénesis está mediada por CXCR4 y SDF-1.

CXCR4, un receptor acoplado a la proteína G, y su ligando que se presenta naturalmente, el factor 1 derivado de células estromales (SDF-1; CXCL12), son un par ligando-receptor de quimiocinas. CXCR4 se expresa constitutivamente o se sobreexpresa en una amplia variedad de cánceres humanos. SDF-1, el único ligando conocido de CXCR4, se expresa ampliamente en los microambientes de tumor, así como en la médula ósea, pulmón, hígado, y ganglios linfáticos, esto es, los sitios de órganos más comúnmente involucrados en metástasis de tumor. La interacción de CXCR4/SDF-1 juega papeles importantes en multiples etapas de la tumorigénesis, incluyendo crecimiento de tumores, invasión, angiogénesis, y metástasis, así como en artritis reumatoide, fibrosis pulmonar, e infección por VIH (Tsutsumi et al. (2006) *Peptide Science* 88(2):279-289).

En vista de la participación de CXCR4/SDF-1 en estas enfermedades serias, CXCR4 es un objetivo terapéutico atractivo.

AMD3100, un antagonista de biciclama CXCR4, está actualmente en ensayos clínicos de Fase III para la movilización de células madre para el transplante de células madre en pacientes con mieloma múltiple y linfoma que no es de Hodgkins. AMD070, otro antagonista de molécula pequeña de CXCR4, está actualmente en ensayos clínicos de Fase II para la infección por VIH. CTCE9908, un antagonista péptido bivalente (dimérico) CXCR4, está actualmente en ensayos clínicos de Fase Ib/II para el cáncer. FC131, un antagonista de pentapéptido cíclico de CXCR4, inhibe el enlace de ^{125}I-SDF-1 a los transfectantes de CXCR4 con un IC_{50} de 4 nM (Fujii et al. (2003) *Angew. Chem. Int. Ed.* 42:3251-3253; Araki et al. (2003) *Peptide Science*. The Japanese Peptide Society (2004):207-210).

Existe la necesidad de antagonistas mejorados de CXCR4 que sean potentes y selectivos, que muestren poca o ninguna actividad en otros receptores de quimiocina. Los compuestos de la presente invención aon tales antagonistas potentes y selectivos de CXCR4. Su alta potencia permite el uso de dosis bajas en regímenes terapéuticos, mientras que su alta selectividad minimiza los efectos colaterales adversos no relacionados con el objetivo. Además, los compuestos descritos en la presente poseen otras propiedades farmacológicas altamente deseables, tales como una alta biodisponibilidad cuando se administran subcutáneamente,

buena estabilidad metabólica *in vivo*, y propiedades farmacocinéticas/farmacodinámicas que permiten una dosificación conveniente.

De esta manera, en un primer aspecto, la presente invención proporciona un péptido ciclizado por lactamas de la fórmula I:

R_1 - ciclo[X_1 - Tyr - X_3 -DArg - 2Nal - Gly - X_7] - X_8 - X_9 - X_{10} - R_2

(I)

(SEQ ID NO:1)

en donde:

a) la lactama se forma por un enlace amida entre el grupo amino de cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de (D/L)Agl/Glu, Dab/Glu, y Dap/Glu, y R_1 es Ac o n-hexanoilo; o

b) la lactama se forma por un enlace amida entre el grupo carboxilo de cadena lateral de X_1 and el grupo amino de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, y Glu/Lys, y R_1 es Ac o Bz, o en donde X_1 y X_7 son,

respectivamente, un par seleccionado del grupo que consiste de succinilo/(D/L)Agl, succinilo/Dab, succinilo/Dap, succinilo/Lys, y succinilo/Orn, y R_1 está ausente; o

c) la lactama se forma por un enlace amida entre el grupo α-amino de X_1 y el grupo carboxilo de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, y DPhe/DGlu, y R_1 está ausente; o

d) la lactama se forma por un enlace amida entre un grupo amino que no es α, sin cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de β-Ala/Asp, β-Ala/Glu, 5-amino-valerilo/Asp, 5-aminovalerilo/Glu, 4-AMB/Glu, 4-AMPA/Asp, y 4-AMPA/Glu, y R_1 está ausente; o

e) la lactama se forma por un enlace amida entre el grupo α-amino de X_2 y el grupo carboxilo de cadena lateral de X_7, en donde X_2 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Tyr/Asp, Tyr/Glu, y Tyr/DGlu, y R_1 y X_1 están cada uno ausentes;

R_1 es un sustituyente sobre el grupo α-amino de X_1 cuando X_1 contiene un grupo α-amino y el grupo α-amino no es un constituyente del enlace de lactama y amida, seleccionado del grupo que consiste de Ac, Bz, y n-hexanoilo, o está ausente, en donde X_1 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, y Glu;

X_1 es seleccionado del grupo que consiste de (D/L)Agl, Ala, β-Ala, DAla, 5-aminovalerilo, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, y succinilo, o está ausente;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, y Orn;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, 2Nal, D2Nal, y DPhe, o está ausente;

X_{10} is 2Nal, o está ausente;

en donde cuando X_8 está ausente, X_9 y X_{10} están cada uno ausentes, y cuando X_9 está ausente, X_{10} está ausente, y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt,

o

una sal farmacéuticamente aceptable del mismo.

Expresado de forma alternativa, esto es equivalente al péptido ciclizado por lactamas de fórmula I:

R_1 - ciclo[X_1 - Tyr - X_3 -DArg - 2Nal - Gly - X_7] - X_8 - X_9 - X_{10} - R_2

(I)

(SEQ ID NO:1)

en donde:

R_1 es un sustituyente sobre el grupo α-amino de X_1 cuando X_1 contiene un grupo α-amino y el grupo α-amino no es un constituyente del enlace de lactama y amida, seleccionado del grupo que consiste de Ac, Bz, y n-hexanoilo, o está ausente, en donde X_1 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, y Glu;

X_1 es seleccionado del grupo que consiste de (D/L)Agl, Ala, β-Ala, DAla, 5-aminovalerilo, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, y succinilo, o está ausente;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, y Orn;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, 2Nal, D2Nal, y DPhe, o está ausente;

X_{10} is 2Nal, o está ausente;

en donde cuando X_8 está ausente, X_9 y X_{10} están cada uno ausentes, y cuando X_9 está ausente, X_{10} está ausente, y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt,

en donde:

a) la lactama se forma por un enlace amida entre el grupo de cadena lateral amino de X_1 y el grupo carboxilo de cadena lateral de X_7, cuando X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de (D/L)Agl/Glu, Dab/Glu, y Dap/Glu, y R_1 is Ac o n-hexanoilo; o

b) la lactama se forma por un enlace amida entre el grupo carboxilo de cadena lateral de X_1 y el grupo amino de cadena lateral de X_7, cuando X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, y Glu/Lys, y R_1 es Ac o Bz, o cuando X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste

de succinilo/(D/L)Agl, succinilo/Dab, succinilo/Dap, succinilo/Lys, y succinilo/Orn, y R_1 está ausente; o

c) la lactama se forma por un enlace amida entre el grupo α-amino de X_1 y el grupo carboxilo de cadena lateral de X_7, cuando X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, y DPhe/DGlu, y R_1 está ausente; o

d) la lactama se forma por un enlace amida entre un grupo amino que no es α, sin cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7, cuando X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de β-Ala/Asp, β-Ala/Glu, 5-amino-valerilo/Asp, 5-aminovalerilo/Glu, 4-AMB/Glu, 4-AMPA/Asp, y 4-AMPA/Glu, y R_1 está ausente; o

e) la lactama se forma por un enlace amida entre el grupo α-amino de X_2 y el grupo carboxilo de cadena lateral de X_7, cuando X_2 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Tyr/Asp, Tyr/Glu, y Tyr/DGlu, y R_1 y X_1 están cada uno ausentes, o

una sal farmacéuticamente aceptable del mismo.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas de fórmula I:

R_1 - ciclo[X_1 - Tyr - X_3 - DArg - 2Nal - Gly - X_7] - X_8 - X_9 - X_{10} - R_2

(I)

(SEQ ID NO:1)

o una sal farmacéuticamente aceptable del mismo,

en donde:

X_1 es seleccionado del grupo que consiste de (D/L)Agl, Ala, β-Ala, DAla, 5-aminovalerilo, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, y succinilo, o está ausente,

en donde cuando X_1 is (D/L)Agl, Dab, o Dap y el grupo α-amino de X_1 no es un constituyente del enlace amida de lactama, el grupo α-amino se sustituye con R_1 el cual es seleccionado del grupo que consiste de Ac y n-hexanoilo;

en donde cuando X_1 es Asp o Glu y el grupo α-amino de X_1 no es un constituyente del enlace amida de lactama, el grupo α-amino se sustituye con R_1 el cual es seleccionado del grupo que consiste de Ac y Bz; y

en donde cuando X_1 es Ala, β-Ala, DAla, 5-aminovalerilo, 4-AMB, 4-AMPA, Dap(Ac), Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe o succinilo, R_1 está ausente;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, y Orn;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, 2Nal, D2Nal, y DPhe, o está ausente;

X_{10} es 2Nal, o está ausente,

en donde cuando X_8 está ausente, X_9 y X_{10} están cada uno ausentes, y cuando X_9 está ausente, X_{10} está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt,

y además en donde:

la lactama se forma por un enlace amida entre el grupo amino de cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7, y X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de (D/L)Agl/Glu, Dab/Glu, y Dap/Glu, y R_1 is Ac o n-hexanoilo; o

la lactama se forma por un enlace amida entre el grupo carboxilo de cadena lateral de X_1 y el grupo amino de cadena lateral de X_7, y X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Asp/(D/L)Agl, Asp/Dab,

Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, y Glu/Lys, y R_1 is Ac o Bz, o en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de succinilo/(D/L)Agl, succinilo/Dab, succinilo/Dap, succinilo/Lys, y succinilo/Orn, y R_1 está ausente; o

la lactama se forma por un enlace amida entre el grupo α-amino de X_1 y el grupo carboxilo de cadena lateral de X_7, y X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, y DPhe/DGlu, y R_1 está ausente; o

la lactama se forma por un enlace amida entre un grupo amino que no es α, sin cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7, y X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de β-Ala/Asp, β-Ala/Glu, 5-amino-valerilo/Asp, 5-aminovalerilo/Glu, 4-AMB/Glu, 4-AMPA/Asp, y 4-AMPA/Glu, y R_1 está ausente; o

la lactama se forma por un enlace amida entre el grupo α-amino de Tyr en X_2 y el grupo carboxilo de cadena lateral de X_7, y X_7 es seleccionado del grupo que consiste de Asp, Glu, y DGlu, y R_1 y X_1 están cada uno ausentes.

Una porción de secuencia recurrente en todos los compuestos de fórmula I es la presencia de Tyr en la posición X_2, DArg en la posición X_4, 2Nal en la posición X_5, y Gly en la posición X_6.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

R_1 es seleccionado del grupo que consiste de Ac y Bz, o está ausente;

X_1 es seleccionado del grupo que consiste de β-Ala, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, 2Nal, Phe, y succinilo, o está ausente;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de Asp, Dab, Dap, Glu, DGlu, Lys, y Orn;

X_8 es seleccionado del grupo que consiste de Arg y Lys, o está ausente;

X_9 está ausente;

X_{10} está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

R_1 es seleccionado del grupo que consiste de Ac y Bz, o está ausente;

X_1 es seleccionado del grupo que consiste de DAla, 5-aminovalerilo, 4-AMPA, Asp, Glu, Leu, Lys(Ac), Phe, DPhe, y succinilo;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, DDap, Glu, y DGlu;

X_8 es seleccionado del grupo que consiste de Arg, DArg, y Lys, o está ausente;

X_9 está ausente;

X_{10} está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

R_1 es seleccionado del grupo que consiste de Ac, Bz, y n-hexanoilo, o está ausente;

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X_1 es seleccionado del grupo que consiste de (D/L)Agl, Ala, β-Ala, Asp, Dap, Glu, Gly, Lys, y Phe;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dap, Glu, y DGlu;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, 2Nal, D2Nal, y DPhe, o está ausente;

X_{10} is 2Nal, o está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

En un aspecto adicional, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

R_1 es seleccionado del grupo que consiste de Ac y Bz, o está ausente;

X_1 es seleccionado del grupo que consiste de Ala, 5-aminovalerilo, Asp, Glu, Gly, Phe, DPhe, y succinilo;

X_3 es seleccionado del grupo que consiste de Arg, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dap, Glu, y DGlu;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, D2Nal, y DPhe, o está ausente;

X_{10} is 2Nal, o está ausente; and

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

X_1 es seleccionado del grupo que consiste de Gly y Phe;

X_3 es Lys(iPr); and

X_7 es DGlu.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

R_1 está ausente;

X_1 es seleccionado del grupo que consiste de Gly y Phe;

X_3 es Lys(iPr);

X_7 es DGlu;

X_8 es seleccionado del grupo que consiste de Arg y Lys(iPr), o está ausente;

X_9 está ausente;

X_{10} está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

la lactama se forma por un enlace amida entre el grupo amino de cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7;

R_1 es seleccionado del grupo que consiste de Ac y n-hexanoilo;

X_1 es seleccionado del grupo que consiste de (D/L)Agl, Dab, y Dap;

X_3 es seleccionado del grupo que consiste de Arg y Lys(iPr);

X_7 es Glu;

X_8 es Arg;

X_9 está ausente;

X_{10} está ausente; y

R_2 es NH_2.

En una modalidad preferida de este aspecto de la invención, X_1 es (D/L)Agl o Dap.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

la lactama se forma por un enlace amida entre el grupo carboxilo de cadena lateral de X_1 y el grupo amino de cadena lateral de X_7;

R_1 es seleccionado del grupo que consiste de Ac y Bz;

X_1 es seleccionado del grupo que consiste de Asp y Glu;

X_3 es seleccionado del grupo que consiste de Arg y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Dab, Dap, DDap, y Lys;

X_8 is Arg;

X_9 está ausente;

X_{10} está ausente; and

R_2 es NH_2.

En una modalidad preferida de este aspecto de la invención, X_7 es (D/L)Agl, Dab, Dap, o DDap. En una modalidad más preferida, X_7 es (D/L)Agl o Dap.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

la lactama se forma por un enlace amida entre el grupo carboxilo de cadena lateral de X_1 y el grupo amino de cadena lateral de X_7;

R_1 está ausente;

X_1 es succinilo;

X_3 es Arg;

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Dab, Dap, Lys, y Orn;

X_8 es Arg;

X_9 está ausente;

X_{10} está ausente; y

R_2 es NH_2.

En una modalidad preferida de este aspecto de la invención, X_7 es (D/L)Agl o Dap.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

la lactama se forma por un enlace amida entre el grupo α-amino de X_1 y el grupo carboxilo de cadena lateral de X_7;

R_1 está ausente;

X_1 es seleccionado del grupo que consiste de Ala, DAla, Gly, Dap(Ac), Leu, Lys, Lys(Ac), 2Nal, Phe, y DPhe;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de Asp, Glu, y DGlu;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, 2Nal, D2Nal, y DPhe, o está ausente;

X_{10} is 2Nal, o está ausente;

en donde cuando X_8 está ausente, X_9 y X_{10} están cada uno ausentes; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

En una modalidad preferida de este aspecto de la invención, X_1 es Ala, DAla, Gly, Leu, Lys, Lys(Ac), Phe, o DPhe. En una modalidad más preferida, X_1 es Ala, Gly, Lys, o Phe.

En una modalidad preferida de este aspecto de la invención, X_3 es Arg, Lys, Lys(iPr), o Lys(Me_2). En una modalidad más preferida, X_3 es Arg.

En una modalidad preferida de este aspecto de la invención, X_7 es Asp, Glu, o DGlu. En una modalidad más preferida, X_7 es Asp.

En una modalidad preferida de este aspecto de la invención, X_8 es β-Ala, Arg, Gly, Lys, Lys(iPr), Orn, o está ausente. En una modalidad más preferida, X_8 es β-Ala, Gly, Lys, Lys(iPr), Orn, o está ausente.

En una modalidad preferida de este aspecto de la invención, X_9 es Gly, 2Nal, D2Nal, DPhe, o está ausente. En una modalidad más preferida, X_9 es Gly, 2Nal, D2Nal, o DPhe.

En una modalidad preferida de este aspecto de la invención, X_{10} es 2Nal, o está ausente. En una modalidad más preferida, X_{10} es 2Nal.

En una modalidad preferida de este aspecto de la invención, R_2 es NHEt.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

la lactama se forma por un enlace amida entre un grupo amino que no es α, sin cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7;

R_1 está ausente;

X_1 es seleccionado del grupo que consiste de β-Ala, 4-AMB, 5-aminovalerilo, y 4-AMPA;

X_3 es Arg;

X_7 es seleccionado del grupo que consiste de Asp y Glu;

X_8 es seleccionado del grupo que consiste de Arg y DArg;

X_9 está ausente;

X_{10} está ausente; y

R_2 es NH_2.

En una modalidad preferida de este aspecto de la invención, X_1 es β-Ala, 5-amino-valerilo, o 4-AMPA. En una modalidad más preferida, X_1 es β-Ala.

En una modalidad preferida de este aspecto de la invención, X_7 es Asp.

En una modalidad preferida de este aspecto de la invención, X_8 es Arg.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de fórmula I (SEQ ID NO:1), en donde:

la lactama se forma por un enlace amida entre el grupo α-amino de X_2 y el grupo carboxilo de cadena lateral de X_7;

R_1 está ausente;

X_1 está ausente;

X_3 es Arg;

X_7 es seleccionado del grupo que consiste de Asp, Glu, y DGlu;

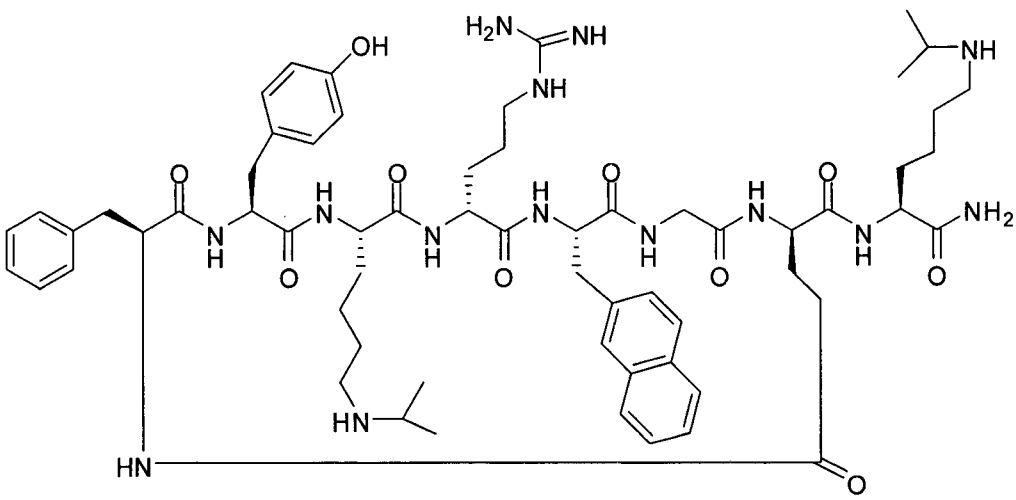
X_8 es Arg;

X_9 está ausente;

X_{10} está ausente; y

R_2 es NH_2.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas de la fórmula:



(SEQ ID NO:70)

o una sal farmacéuticamente aceptable del mismo. La lactama se forma por un enlace amida entre el grupo α-amino de Phe y el grupo carboxilo de cadena lateral de DGlu. La sal farmacéuticamente acceptable puede ser una sal de ácido acético.

En otro aspecto, la presente invención proporciona una composición farmacéutica, que comprende un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo como se describe diversamente arriba, y un portador, diluyente, o excipiente farmacéuticamente aceptable.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo como se describe diversamente arriba, para uso en terapia.

En otro aspecto, la presente invención proporciona un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo como se describe diversamente arriba, para el tratamiento de artritis reumática, fibrosis pulmonar, infección por VIH, o un cáncer seleccionado del grupo que consiste de cáncer de mama, cáncer pancreático, melanoma, cáncer de próstata, cáncer de riñón, neuroblastoma, linfoma que no es Hodgkin, cáncer de pulmón, cáncer ovárico, cáncer colorectal, mieloma múltiple, glioblastoma multiforme, y leucemia linfocítica crónica.

760

En otro aspecto, la presente invención proporciona el uso de un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo como se describe diversamente arriba, para la manufactura de a medicament para el tratamiento de artritis reumática, fibrosis pulmonar, infección por VIH, o un cáncer seleccionado del grupo que consiste de cáncer de mama, cáncer pancreático, melanoma, cáncer de próstata, cáncer de riñón, neuroblastoma, linfoma que no es Hodgkin, cáncer de pulmón, cáncer ovárico, cáncer colorectal, mieloma múltiple, glioblastoma multiforme, y leucemia linfocítica crónica.

En otro aspecto, la presente invención proporciona un método de tratamiento de artritis reumática, fibrosis pulmonar, infección por VIH, o un cáncer seleccionado del grupo que consiste de cáncer de mama, cáncer pancreático, melanoma, cáncer de próstata, cáncer de riñón, neuroblastoma, linfoma que no es Hodgkin, cáncer de pulmón, cáncer ovárico, cáncer colorectal, mieloma múltiple, glioblastoma multiforme, y leucemia linfocítica crónica, que comprende administrar a un paciente que necesite del mismo una cantidad efectiva de péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo como se describe diversamente arriba.

En los compuestos peptídicos macrocíclicos de la presente invención (SEQ ID NO:1), los aminoácidos X_1 hasta el

X_{10} se refieren en la presente por sus símbolos comúnmente empleados de tres letras, que se muestran de izquierda a derecha desde el extremo de la terminal amino al extremo de la terminal carboxi. D- y L- (letras mayúsculas pequeñas) se refieren a la estereoquímica absoluta. Donde no se indica ninguna designación en una fórmula en particular, la forma L- del aminoácido está presente. X_1 también puede ser un residuo de ácido dicarboxílico, es decir, un grupo succinilo. Los residuos de aminoácidos o de ácido carboxílico dentro de los paréntesis "[ ]" están dentro de la estructura cíclica; los grupos externos a los paréntesis están fuera del anillo ciclizado. En todos los casos, la ciclización es por medio del enlace de lactama (amida) entre X_1 (o X_2, es decir, Tyr) y X_7, el cual se puede formar en varias formas diferentes, dependiendo de las estructuras de X_1, X_2, y X_7.

Cuando el enlace de lactama se forma entre el grupo amino de cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7 (Esquemas de Reacción 1 y 2; Ejemplos 1-5), el grupo α-amino de X_1 se cierra con Ac o n-hexanoilo.

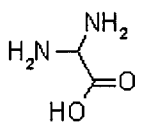
Cuando el enlace de lactama se forma entre el grupo carboxilo de cadena lateral de X_1 y el grupo amino de cadena lateral de X_7, el grupo α-amino de X_1 se cierra con Ac o Bz (Esquemas de Reacción 3 y 4; Ejemplos 6-19). X_1 también

puede ser una residuo bifuncional diferente al α-aminoácido, por ejemplo uno con dos grupos carboxilo, es decir, un residuo de succinilo. En este caso, un grupo carboxilo forma un enlace amida con el grupo α-amino de Tyr, y las otras formas de la estructura cíclica de lactama a través de un enlace amida con el grupo amino de cadena lateral de X_7 (Esquemas de Reacción 3 y 4; Ejemplos 20-24). Cuando X_1 is succinilo, R_1 está ausente.

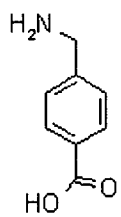
En la mayoría de los péptidos ciclizados por lactamas aquí descritos (Esquemas de Reacción 5-15; Ejemplos 25-28, 32-66, y 75-89), el grupo α-amino de X_1 forma la estructura de lactama por medio de un enlace de amida con el grupo carboxilo de cadena lateral de X_7, y R_1 está ausente. También se incluyen en los Esquemas de Reacción sintéticos de esta categoría, los péptidos cíclicos que contienen residuos X_1, es decir, β-Ala, 4-AMB, 5-aminovalerilo, y 4-AMPA, en donde el grupo amino es un grupo que no es α-amino sin cadena lateral (Esquemas de Reacción 5 y 6; Ejemplos 67-74). R_1 también está ausente en estos casos.

Cuando ambos R_1 y X_1 están ausentes, la estructura de lactama se forma a través ed un enlace amida entre el grupo α-amino de Tyr (X_2) y el grupo carboxilo de cadena lateral de X_7 (Esquemas de Reacción 5 y 6; Ejemplos 29-31).

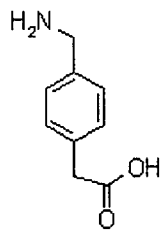
Las estructuras de aminoácidos comunes, por ejemplo, alanina, glicina, etc., son bien conocidas en la técnica. Las estructuras de aminoácidos no estándar y substituidos presentes en los compuestos de la invención actual se muestran a continuación.



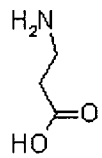
Aminoglicina (Agl)



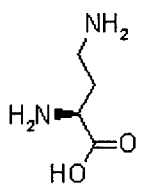
Acido 4-aminometil benzoico (4-AMB)



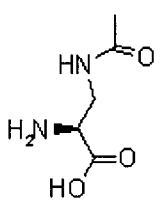
Acido 4-aminometil fenilacético (4-AMPA)



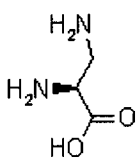
Acido 3-amino propanoico (β-Ala)



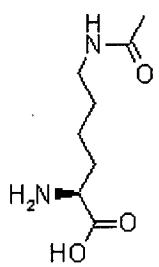
Dab



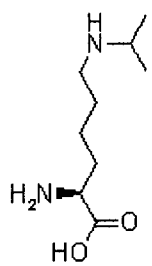
Dap(Ac)



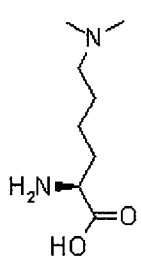
Dap



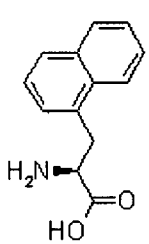
Lys(Ac)



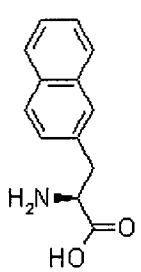
Lys(iPr)



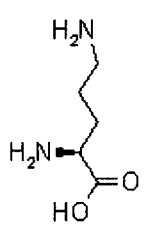
Lys(Me_2)



1Nal



2Nal



Orn

Los péptidos ciclizados por lactamas de la presente invención se pueden preparar como sales farmacéuticamente aceptables. Tales sales, y la metodología común para preparar los mismos, son bien conocidas en la técnica. Ver, por ejemplo, P. Stahl et al. (2002) *Handbook de Pharmaceutical Salts: Properties, Selection y Use*, VCHA/Wiley-VCH; Berge et al. (1977) "Pharmaceutical Salts," *Journal de Pharmaceutical Sciences* 66(1):1-19.

Los compuestos de la presente invención son antagonistas potentes de la interacción CXCR4/SDF-1. Los compuestos de la fórmula (I) y sus sales farmacéuticamente aceptables especificamente ejemplificados en la presente muestran un valor K_i promedio de alrededor de 7.5 nM o menos como se determina por el ensayo de enlace CXCR4/^{125}I-SDF-1α decrito a continuación. Los compuestos más preferidos de la fórmula (I) y sus sales farmacéuticamente aceptables muestran un valor K_i promedio en el rango de desde alrededor de 0.2 nM hasta alrededor de 1 nM en este ensayo. Los compuestos especialmente preferidos de la fórmula (I) y sus sales farmacéuticamente aceptables muestran un valor K_i promedio menos de alrededor de 0.2 nM en este ensayo.

Además, los compuestos y sales farmacéuticamente aceptables de la presente invención son preferiblemente

selectivos altamente para el receptor CXCR4, muestra poca o no muestra actividad inhibitoria contra otros receptor de quimiocina, incluyendo CCR1, CCR2, CXCR2, CXCR3, y otros receptores acoplados a la proteína G en las concentraciones probadas, y sin actividad importante contra receptores de serotonina, dopamina, y opioide. También preferiblemente muestra buena estabilidad en la sangre y plasma, buena biodisponibilidad subcutánea, propiedades farmacocinéticas/farmacodinámicas deseables, y eficacia in vitro potente en la inhibición del crecimiento del tumor, como un margen de seguridad amplio.

En vista de estas propiedades farmacológicas, los compuestos de la presente invención se indican para el tratamiento de trastornos que involucran la interacción de CXCR4/SDF-1, o actividad del receptor, actividad de CXCR4, tal como en infección por VIH. En particular, los compuestos actuales son útiles en el tratamiento de malignidades en las cuales las trayectorias angiogénicas, de crecimiento, de sobrevivencia y y metastáticos mediadas por CXCR4 y SDF-1 se implican en la patogenesis, incluyendo cáncer de mama, cáncer pancreático, melanoma, cáncer de próstata, cáncer de riñón, neuroblastoma, linfoma que no es Hodgkin, cáncer de pulmón, cáncer ovárico, cáncer colorectal, mieloma múltiple, glioblastoma multiforme, y leucemia linfocítica crónica, así como en artritis reumática, fibrosis pulmonar, e infección

por VIH (Tsutsumi et al. (2006) *Peptide Science* 88(2):279-289).

El Ag1 (aminoglicina) es un bloque de construcción proquiral. Cuando este residuo aparece en una fórmula de péptido en la presente, el α-carbono se vuelve un centro quiral en el cual los dos grupos α-amino asociados son cada uno individualmente enlazados a porciones diferentes. En este caso, el producto final de péptidos contiene dos diaestereómeros que no son resueltos, y que pueden presentarse diferenta a una relación 1:1. "(D/L)Ag1" en una fórmula de péptido denota tal mezcla de diaestereómeros. "(DL)Ag1" denota un derivado Ag1 que es racémico, por ejemplo Fmoc-(DL)Ag1(Boc).

Los valores "K_i" se calculan usando valores IC50 determinados en el ensayo de enlace CXCR4/125I-SDF-1α descrito a continuación al emplear la ecuación 7.22 de Enzymes, A Practical Introduction to Structure, Mechanism, and Data Analysis, Robert A. Copeland, Wiley-VCH, New York, 1996, página 207.

El término "SDF-1" incluye dos isoformas, SDF-1α y SDF-1β, se entiende actualmente para mostrar una funcionalidad similar.

"Tratamiento" como se usa en la presente se refiere al tratamiento curativo de trastornos asociados con la

interacción de CXCR4/SDF-1 o la actividad del receptor CXCR4. el tratamiento curativo se refiere a procesos que involucran retardar, interrumpir, detener, controlar o detener el progreso de la enfermedad, pero no necesariamente involucra una eliminación total de todos los síntomas, condiciones, o trastornos relacionados a la enfermedad.

Los compuestos de la presente invención se pueden usar como medicamentos en la medicina humana o veterinaria, administrados por una variedad de rutas. Más preferiblemente, tales composiciones son para administración parenteral. Tales composiciones farmacéuticas se pueden preparar por métodos bien conocidos en la técnica. Ver, por ejemplo, *Remington: The Science y Practice de Pharmacy*, 19a ed. (1995), A. Gennaro et al., Mack Publishing Co., y comprende uno o más compuestos de la fórmula (I) o unas sales farmacéuticamente aceptables de los mismos, y un portador, diluyente, o excipiente farmacéuticamente aceptable.

La cantidad efectiva de los compuestos actuales está en el rango de desde alrededor de 1 mg hasta alrededor de 300 mg, más preferiblemente desde alrededor de 1 mg hasta alrededor de 200 mg, más preferiblemente desde alrededor de 1 mg hasta alrededor de 100 mg, y aún más preferiblemente desde alrededor de 1 mg hasta alrededor de 50 mg, en una base diaria.

Todos los péptidos ciclizados por lactamas de la presente invención se pueden sintetizar ya sea por síntesis de fase sólida o síntesis de fase de solución, o una combinación de ambos, con la cadena de péptido ensamblada en la fase sólida y ciclización u otras modificaciones en resina o en solución. Tales métodos son bien conocidos en la técnica.

Las siguientes abreviaturas usadas en la presente tienen los significados indicados:

Ac: acetilo; **Agl**: aminoglicina; **AMB**: ácido aminometil benzoico; **AMPA**: ácido aminometil fenil acético; **Bn**: bencilo; **Boc**: tert-butiloxicarbonilo; **BOP**: (benzotriazol-1-iloxi)-tris(dimetilamino)fosfoniohexafluorofosfato; **2-Br-Z**: 2-bromobencilooxicarbonilo; **Bz**: benzoilo; **Bzl**: bencilo; **2-Cl-Z**: 2-clorobencil-oxicarbonilo; **Dab**: ácido 2,4-diaminobutírico; **Dap**: ácido 2,3-diamino-propionico; **DCC**: diciclohexil-carbodiimida; **DCM**: diclorometano; **DIC**: diisopropil carbodiimida; **DIEA**: diisopropil-etilamina; **DMF**: *N,N*-dimetil formamida; **DMSO**: dimetil-sulfóxido; **EDT**: 1,2-etan-ditiol; **Et**: etilo; **Fm**: 9-fluorenilmetilo; **Fmoc**: 9-fluor-enilmetoxi carbonilo; **HATU**: N-óxido de N-[(dimetilamino)-1H-1,2,3-triazolo[4,5-b]piridin-1-ilmetilen]-N-metilmetanaminio hexafluorofosfato; **HBTU**: *O*-benzo-triazolil-*N,N,N',N'*-tetrametiluronio hexafluorofosfato; **HCTU**: 1-[bis(dimetilamino)metilen]-5-

cloro-3-óxido hexafluorofosfato 1H-benzotriazo-lio; **HF**: fluoruro de hidrógeno; **HOBt**: hidroxibenzotriazol; **IBCF**: cloroformiato de isobutilo; **iPr**: isopropilo; **IPA**: alcohol de isopropilo; **Me**: metilo; **2Nal**: 2-naftilalanina; **NMM**: N-metilmorfolina; **NMP**: *N*-metil-pirrolidona; **OtBu**: éster de tert-butilo; **Pbf**: 2,2,4,6,7-pentametil-dihidrobenzofuran-5-sulfonilo; **PBS**: **solución amortiguadora salina de fosfato**; **PyBOP**: (benzotriazol-1-iloxi)-tris(pirrolidino)-fosfonio hexafluoro-fosfato; **PyBrOP**: bromotris(pirrolidino)fosfonio hexafluorofosfato; **tBu**: tert-butilo; **TFA**: ácido trifluoroacético; **THF**: tetrahidrofurano; **TIS**: triisopropil silano; **Tos**: p-toluen-sulfonilo; **Z**: benciloxicarbonilo; **ZOSu**: N-(benciloxicarbonil-oxi) succinimida.

La preparación de los compuestos de la presente invención como se describe en los siguientes ejemplos significa para ilustrar en vez de limitar. En cada uno de estos ejemplos, el peso molecular observado se reporta como un valor sin lóbulos. El valor sin lóbulos se deriva de la fórmula PM(observado) = n(m/z)-n, donde m/z representa el ión cargado (modo positivo) y n es el número de cargas de las especies especificas. Cuando las especies cargadas múltiples se presentan en el espectro de masas, el peso molecular observado se reporta como un promedio.

Los procesos sintéticos descritos en los Ejemplos 85-87, y procedimientos etiquetados isotópicos descritos en el

Ejemplo 89, para péptidos ciclizados por lactamas que contiene cadenas laterales de isopropil lisina son igualmente aplicables a otros péptidos descritos en la presente que contienen cadenas laterales de lisina alquilo, con modificaciones apropiadas. Los métodos sintéticos de los Ejemplos 86-88, los cuales eliminan la necesidad para catalizadores de paladio tóxicos, también son igualmente aplicables a otros péptidos descritos en la presente, con modificaciones apropiadas.

Ejemplo 1

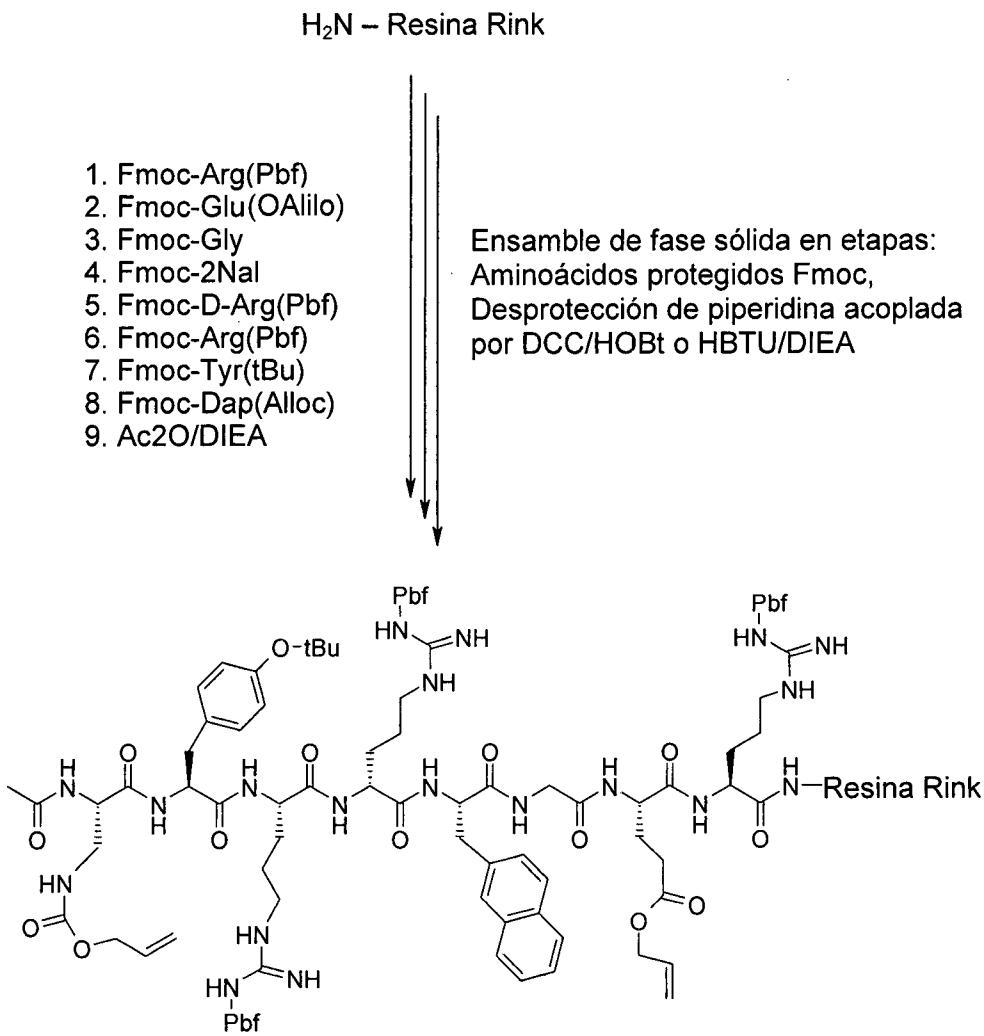
Ac-ciclo[Dap-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:2)

La secuencia Ac-Dap(Alloc)-Tyr(tBu)-Arg(Pbf)-DArg(Pbf)-2Nal-Gly-Glu(Oalilo)-Arg(Pbf) (SEQ ID NO:3) se ensambla por química estándar Fmoc utilizando un Sintetizador de péptido ABI 431 (Applied Biosystems) como se resume en el Esquema de reacción 1 a continuación. El ensamble automatizado se lleva a cabo al usar el protocolo de química Applied Biosystems DCC/HOBt o protocolo HBTU/DIEA de química FastMoc siguiendo las instrucciones del proveedor (PE Applied Biosystems Inc., Foster City, CA). El soporte sólido es la resina de amida Rink (resina 4-(2',4'-dimetoxifenil-Fmoc-aminometil)-fenoxi) para amidas de terminal C o resina indol, resina AM [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo para amidas etilo de terminal C

(NovaBiochem, EMD Biosciences, Inc., San Diego, CA).El ensamble de cadenas por etapas inicia del extremo terminal C del péptido lineal y se realiza en 9 etapas principales. En la etapa 1, cuatro equivalentes de aminoácido protegido Fmoc-Arg(Pbf) se activan con DCC/HOBt (o HBTU/DIEA para química FastMoc) en NMP, y se acoplan para desproteger la resina de amida Rink usando 20% de piperidina. En la etapa 2, cuatro equivalentes de Fmoc-Glu(Oalilo) se activan y acoplan a la resina de péptido desprotegida de la etapa 1. Se llevan a cabo las etapas apropiadas hasta la etapa 8, el acoplamiento de Fmoc-Dap(Alloc). Luego, Fmoc en el extremo de terminal N se remueve usando 20% de piperidina en DMF y la acetilación del grupo α-amino se lleva a cabo la línea completa usando 5 equivalentes de anhídrido acético, 10 equivalentes de DIEA en DMF o NMP seco, durante 1 h a temperatura ambiente.

Los grupos protegidos de cadena lateral alilo y Alloc se remueven con 0.1 equivalente de $Pd(Ph_3P)_4$ en la presencia de 24 equivalentes de fenilsilano en diclorometano (Esquema de reacción 2). Este proceso se repite una vez para la desprotección de cadena lateral completa. La porción de ácido carboxílico desprotegida de Glu se activa con PyBOP/DIEA y se cicliza para el grupo amino de cadena lateral de Dap en la resina. El péptido ciclizado se desprotege y escinde simultáneamente de la resina al usar un cóctel de

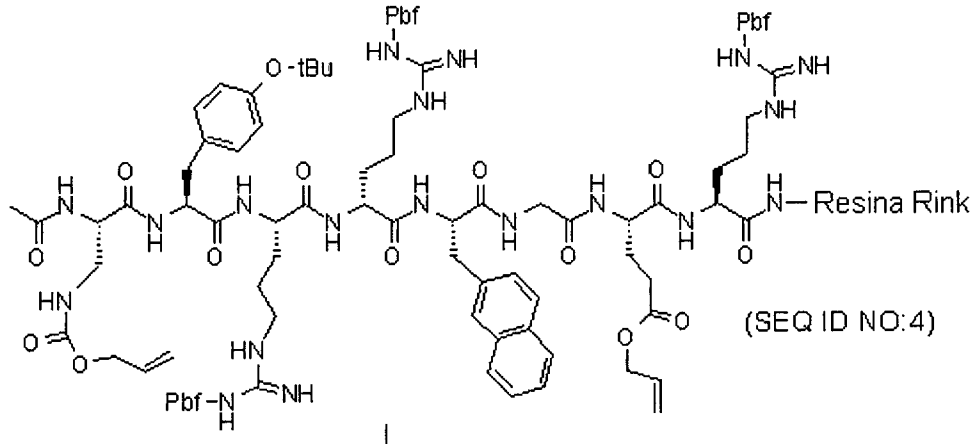
secuestrantes de TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v), o TFA/H_2O/TIS/anisol (92/2/4/2, v/v/v/v) durante 2 horas a temperatura ambiente. Luego se evaporan los solventes bajo vacío, y el péptido se precipita y se lava tres veces con éter dietílico frío para retirar los secuestrantes. Peso molecular calculado (PM cal.): 1142.30; PM observado (PM obs.): 1142.50.



(SEQ ID NO:4)

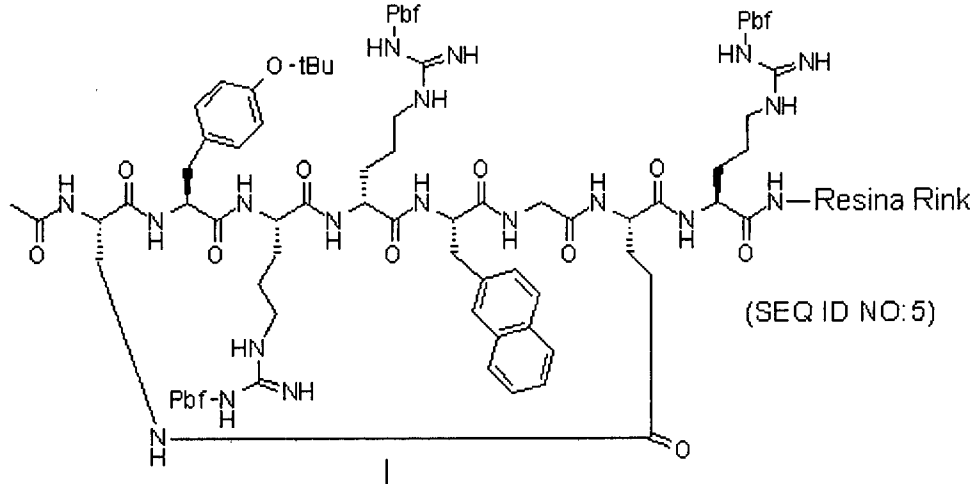
Esquema de reacción 1. Cadena lateral amino para ensamble de péptido de cadena lateral carboxilo

La purificación de péptidos se efectúa al usar técnicas CLAR preparativas estándar. Inmediatamente después de la ciclización, la solución de péptidos se diluye con agua que contiene 0.1% (v/v) TFA, cargada en una columna C18 CLAR de fase inversa, y eluida con un gradiente de ácido trifluoroacético al 0.1% acuoso/acetonitrilo (v/v) mientras se monitorea a 214 nm. Las fracciones apropiadas se acumulan y liofilizan. La caracterización adicional del producto final se efectúa al usar CLAR analítica y análisis espectral de masas por técnicas convencionales. Para los péptidos con una cadena lateral básica, el producto final liofilizado es una sal de TFA.



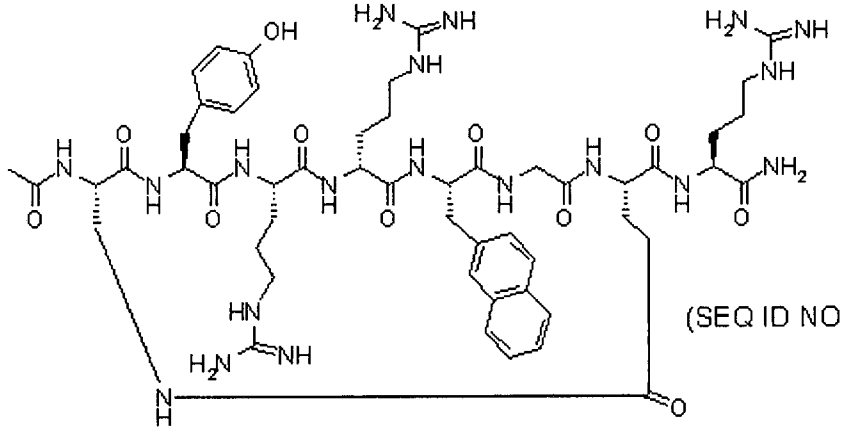
(SEQ ID NO:4)

1. Eliminación de alilo con $Pd(Ph_3P)_4(0)$
2. Ciclización con PyBOP/DIEA



(SEQ ID NO:5)

Desprotección de cadena lateral y desdoblamiento TFA



(SEQ ID NO:2)

Esquema de reacción 2. Cadena lateral amino para ciclización y desdoblamiento del anillo de cadena lateral carboxilo

Ejemplo 2

Ac-ciclo[Dab-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:6)

Preparar como en el Ejemplo 1, excepto que Fmoc-Dap(Alloc) en la etapa 8 se reemplaza con Fmoc-Dab(Alloc). PM cal.:1156.33; PM obs.: 1156.10.

Ejemplo 3

Ac-ciclo[Dap-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:7)

Preparar como en el Ejemplo 1, excepto que Fmoc-Arg(Pbf) en la etapa 6 se reemplaza con Fmoc-Lys(iPr)(Boc). PM cal.: 1156.37; PM obs.: 1156.78.

Ejemplo 4

n-Hexanoil-ciclo[Dap-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:8)

Preparar como en el Ejemplo 1, excepto que Fmoc-Arg(Pbf) en la etapa 6 se reemplaza con Fmoc-Lys(iPr)(Boc). Adicionalmente, anhídrido acético en la etapa 9 se reemplaza con ácido hexanoico activado con PyBOP/DIEA. PM cal.: 1212.47; PM obs.: 1212.92.

Ejemplo 5

Ac-ciclo[(D/L)Agl-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:9)

Preparar como en el Ejemplo 1, excepto que Fmoc-Glu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(OtBu), y Fmoc-Dap(Alloc) en la etapa 8 se reemplaza con Fmoc-(DL)Agl(Boc). Después del ensamble de cadenas, no hay tratamiento $Pd(Ph_3P)_4$ ya que la protección de alilo no está presente. En lugar de eso, la ciclización se lleva a cabo en solución después de que el péptido lineal se desdobla del soporte sólido y se desprotege. El péptido lineal crudo (0.25 mmol) de la escisión se seca bajo vacío y se disuelve en 10 mL de DMF seco. Esta solución de péptidos se suministra a la siguiente solución por medio de una bomba de jeringa durante un periodo de 2 h: 15 mL de diclorometano seco y 15 mL de DMF seco que contiene 1.0 mmol de PyBOP y 4.0 mmoles de DIEA. La reacción luego se permite que avance a temperatura ambiente durante 2 h. Luego se evaporan los solventes bajo vacío, el residuo se carga sobre una columna CLAR C18 de fase inversa preparativa, y se aisla y caracteriza el péptido cíclico objetivo como se describe en Ejemplo 1. PM cal.: 1128.27; PM obs.: 1128.26.

Ejemplo 6

Ac-ciclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:10)

La secuencia Glu(OtBu)-Tyr(tBu)-Arg(Pbf)-DArg(Pbf)-2Nal-Gly-Dap(Boc)-Arg(Pbf) (SEQ ID NO:11) se ensambla por

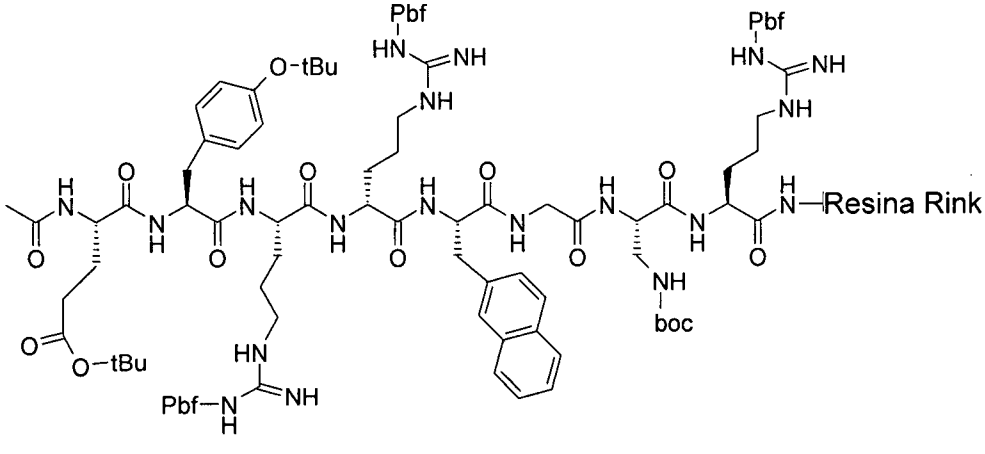
química estándar Fmoc utilizando un instrumento ABI 431 como se resume en el Esquema de reacción 3 a continuación. El ensamble automatizado se lleva a cabo al usar el protocolo de química Applied Biosystems DCC/HOBt o protocolo de química FastMoc (HBTU/DIEA) siguiendo las instrucciones del proveedor (PE Applied Biosystems Inc., Foster City, CA). El soporte sólido es la resina de amida Rink para amidas de terminal C o resina indol, resina AM [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo para amidas de etilo de terminal C. El ensamble de cadenas por etapas inicia del extremo de terminal C del péptido lineal y se realiza en 9 etapas principales. En la etapa 1, cuatro equivalentes de Fmoc-Arg(Pbf) de aminoácido protegido se activan con DCC/HOBt (o HBTU/DIEA para química FastMoc) en NMP, y se acopla para desproteger la resina de amida Rink. En la etapa 2, cuatro equivalentes de Fmoc-Dap(Boc) se activan y acoplan a la resina desprotegida de la etapa 1. Se llevan a cabo las etapas apropiadas hasta la etapa 8, el acoplamiento de Fmoc-Glu(OtBu). Para la etapa 9, Fmoc en el extremo de terminal N se remueve usando 20% de piperidina en DMF y la acetilación del grupo α-amino se lleva a cabo fuera de línea con 5 equivalentes de anhídrido acético, 10 equivalentes DIEA en DMF seco o NMP, durante 1 h a temperatura ambiente. El péptido terminado se desprotege y escinde simultáneamente de la resina al usar un cóctel de

secuestrantes de TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v), o TFA/H_2O/TIS/anisol (92/2/4/2, v/v/v/v) durante 2 horas a temperatura ambiente (Esquema de reacción 4). Luego se evaporan los solventes bajo vacío, y el péptido se precipita y se lava tres veces con éter dietílico frío para retirar los secuestrantes. El producto crudo se usa directamente en la reacción de ciclización. PM cal.: 1142.30; PM obs.: 1142.83.

H_2N-Resina Rink

1. Fmoc-Arg(Pbf)
2. Fmoc-Dap(Boc)
3. Fmoc-Gly
4. Fmoc-2Nal
5. Fmoc-D-Arg(Pbf)
6. Fmoc-Arg(Pbf)
7. Fmoc-Tyr(tBu)
8. Fmoc-Glu(OtBu)
9. Ac2O/DIEA

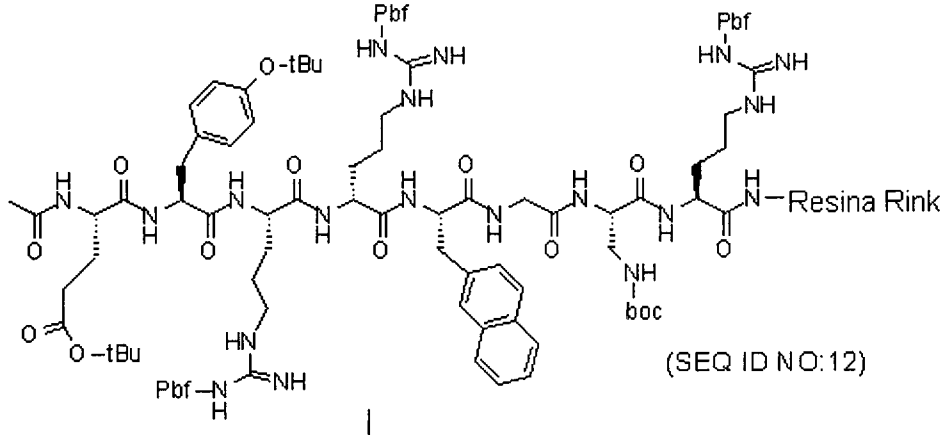
Ensamble de fase sólida en etapas:
Aminoácidos protegidos Fmoc,
Desprotección de piperidina acoplada
por DCC/HOBt o HBTU/DIEA



(SEQ ID NO:12)

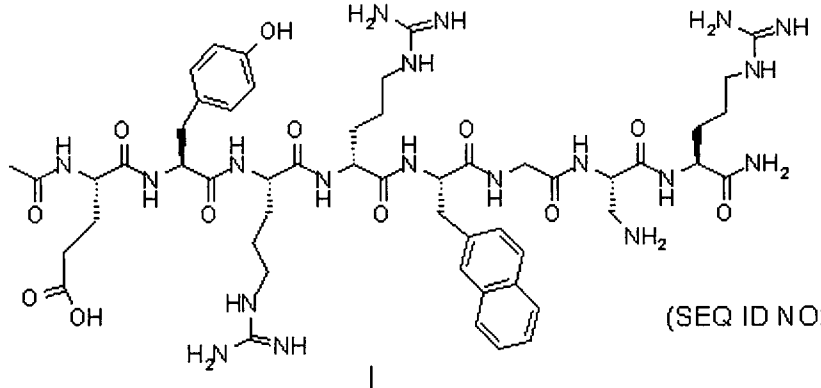
Esquema de reacción 3. Cadena lateral de ácido para ensamble de péptido de cadena lateral amino

La ciclización se lleva a cabo en solución después de que el péptido lineal se desdobla del soporte sólido con todas las cadenas laterales desprotegidas (Esquema de reacción 4). El péptido lineal crudo escindido (0.25 mmol) se seca bajo vacío y se disuelve en 10 mL de DMF seco. Esta solución de péptidos se suministra a la siguiente mezcla de reacción por medio de una bomba de jeringa durante un periodo de 2 h: 15 mL de diclorometano seco y 15 mL de DMF seco que contiene 1.0 mmol de PyBOP y 4.0 mmoles de DIEA. La reacción luego se permite que avance a temperatura ambiente durante 2 h. Los solventes se evaporan bajo vacío, el residuo se carga sobre una columna CLAR C18 de fase inversa preparativa, y se aisla y caracteriza el péptido cíclico objetivo como se describe en Ejemplo 1.



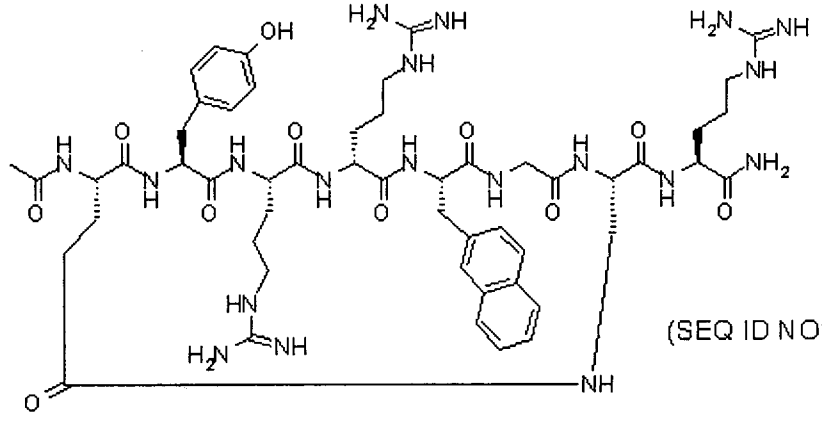
(SEQ ID NO:12)

Desprotección de cadena lateral y desdoblamiento TFA



(SEQ ID NO:13)

Ciclización en solución PyBOP/DIEA



(SEQ ID NO:10)

Esquema de reacción 4. Cadena lateral de ácido para ciclización de anillo de cadena lateral amino

Ejemplo 7

Bz-ciclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:14)

Preparar como en el Ejemplo 6, excepto que anhídrido acético en la etapa 9 se reemplaza con anhídrido del ácido benzoico. PM cal.: 1204.37; PM obs.: 1204.87.

Ejemplo 8

Ac-ciclo[Glu-Tyr-Arg-DArg-2Nal-Gly-DDap]-Arg-NH_2 (SEQ ID NO:15)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-DDap(Boc). PM cal.: 1142.30; PM obs.: 1142.73.

Ejemplo 9

Ac-ciclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Lys]-Arg-NH_2 (SEQ ID NO:16)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-Lys(Boc). PM cal.:1184.38; PM obs.: 1184.23.

Ejemplo 10

Ac-ciclo[Glu-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:17)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-Dab(Boc). PM cal.: 1156.33; PM obs.: 1156.07.

Ejemplo 11

Ac-ciclo[Glu-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:18)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-(DL)Agl(Boc). PM cal.: 1128.27; PM obs.: 1128.86.

Ejemplo 12

Bz-ciclo[Glu-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:19)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-(DL)Agl(Boc). Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido del ácido benzoico. PM cal.: 1190.34; PM obs.: 1190.99.

Ejemplo 13

Bz-ciclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:20)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-Dab(Boc), y Fmoc-Glu(OtBu) en la etapa 8 se reemplaza con Fmoc-Asp(OtBu). Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido del ácido benzoico. PM cal.: 1204.37; PM obs.: 1204.87.

Ejemplo 14

Ac-ciclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:21)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-Dab(Boc), y Fmoc-Glu(OtBu) en la etapa 8 se reemplaza con Fmoc-Asp(OtBu). PM cal.: 1142.30; PM obs.: 1142.81.

Ejemplo 15

Ac-ciclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:22)

Preparar como en el Ejemplo 6, excepto que Fmoc-Glu(OtBu) en la etapa 8 se reemplaza con Fmoc-Asp(OtBu). PM cal.: 1128.27; PM obs.: 1128.78.

Ejemplo 16

Bz-ciclo[Asp-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:23)

Preparar como en el Ejemplo 6, excepto que Fmoc-Glu(OtBu) en la etapa 8 se reemplaza con Fmoc-Asp(OtBu). Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido del ácido benzoico. PM cal.: 1190.34; PM obs.: 1190.69.

Ejemplo 17

Ac-ciclo[Asp-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:24)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-(DL)Agl(Boc), y Fmoc-Glu(OtBu) en la etapa 8 se reemplaza con Fmoc-Asp(OtBu). PM cal.: 1114.24; PM obs.: 1114.85.

Ejemplo 18

Ac-ciclo[Asp-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:25)

Preparar como en el Ejemplo 6, excepto que Fmoc-Arg(Pbf) en la etapa 6 se reemplaza con Fmoc-Lys(Me_2), y Fmoc-Glu(OtBu) en la etapa 8 se reemplaza con Fmoc-Asp(OtBu). PM cal.: 1128.31; PM obs.: 1128.92.

Ejemplo 19

Bz-ciclo[Asp-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:26)

Preparar como en el Ejemplo 6, excepto que Fmoc-Arg(Pbf) en la etapa 6 se reemplaza con Fmoc-Lys(Me_2), y Fmoc-Glu(OtBu) en la etapa 8 se reemplaza con Fmoc-Asp(OtBu). Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido del ácido benzoico. PM cal.: 1190.38; PM obs.: 1191.14.

Ejemplo 20

ciclo[Succinilo-Tyr-Arg-DArg-2Nal-Gly-(D/L)Agl]-Arg-NH_2 (SEQ ID NO:27)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-(DL)Agl(Boc), Fmoc-Glu(OtBu) en la etapa 8 no se usa, y se omite esta etapa. Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido succínico. PM cal.: 1057.19; PM obs.: 1057.87.

Ejemplo 21

ciclo[Succinilo-Tyr-Arg-DArg-2Nal-Gly-Dap]-Arg-NH_2 (SEQ ID NO:28)

Preparar como en el Ejemplo 6, excepto que Fmoc-Glu(OtBu) en la etapa 8 no se usa, y se omite esta etapa. Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido succínico. PM cal.: 1071.22; PM obs.: 1071.85.

Ejemplo 22

ciclo[Succinilo-Tyr-Arg-DArg-2Nal-Gly-Dab]-Arg-NH_2 (SEQ ID NO:29)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-Dab(Boc), Fmoc-Glu(OtBu) en la etapa 8 no se usa, y se omite esta etapa. Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido succínico. PM cal.: 1085.25; PM obs.: 1085.87.

Ejemplo 23

ciclo[Succinilo-Tyr-Arg-DArg-2Nal-Gly-Orn]-Arg-NH_2 (SEQ ID NO:30)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-Orn(Boc), Fmoc-Glu(OtBu) en la etapa 8 no se usa, y se omite esta etapa. Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido succínico. PM cal.: 1099.27; PM obs.: 1100.23.

Ejemplo 24

ciclo[Succinilo-Tyr-Arg-DArg-2Nal-Gly-Lys]-Arg-NH_2 (SEQ ID NO:31)

Preparar como en el Ejemplo 6, excepto que Fmoc-Dap(Boc) en la etapa 2 se reemplaza con Fmoc-Lys(Boc), Fmoc-Glu(OtBu) en la etapa 8 no se usa, y se omite esta etapa. Además, anhídrido acético en la etapa 9 se reemplaza con anhídrido succínico. PM cal.: 1113.30; PM obs.: 1114.25.

Ejemplo 25

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:32)

La secuencia Gly-Tyr(tBu)-Lys(iPr)(Boc)-DArg(Pbf)-2Nal-Gly-DGlu(Oalilo)-Arg(Pbf) (SEQ ID NO:33) se ensambla por química estándar Fmoc utilizando un instrumento ABI 431 como se resume en el Esquema de reacción 5 a continuación. El ensamble automatizado se lleva a cabo al usar el protocolo de química Applied Biosystems DCC/HOBt o protocolo de química FastMoc HBTU/DIEA siguiendo las instrucciones del proveedor (PE Applied Biosystems Inc., Foster City, CA). El soporte sólido es la resina de amida Rink para amidas o resina indol, resina AM [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo para amidas de etilo de terminal C. El ensamble de cadenas por etapas inicia del extremo de terminal C del péptido lineal y se realiza en 8 etapas principales (Esquema de reacción 5). En la etapa 1, cuatro equivalentes de Fmoc-Arg(Pbf) de aminoácido protegido se activan con DCC/HOBt (o HBTU/DIEA para química FastMoc) en NMP, y se acoplan para desproteger la resina de amida Rink. En la etapa 2, cuatro equivalentes de Fmoc-DGlu(Oalilo) se activan y acoplan a la resina de péptido desprotegida de la etapa 1. Se llevan a cabo las etapas apropiadas hasta la etapa 8, el acoplamiento de Fmoc-Gly.

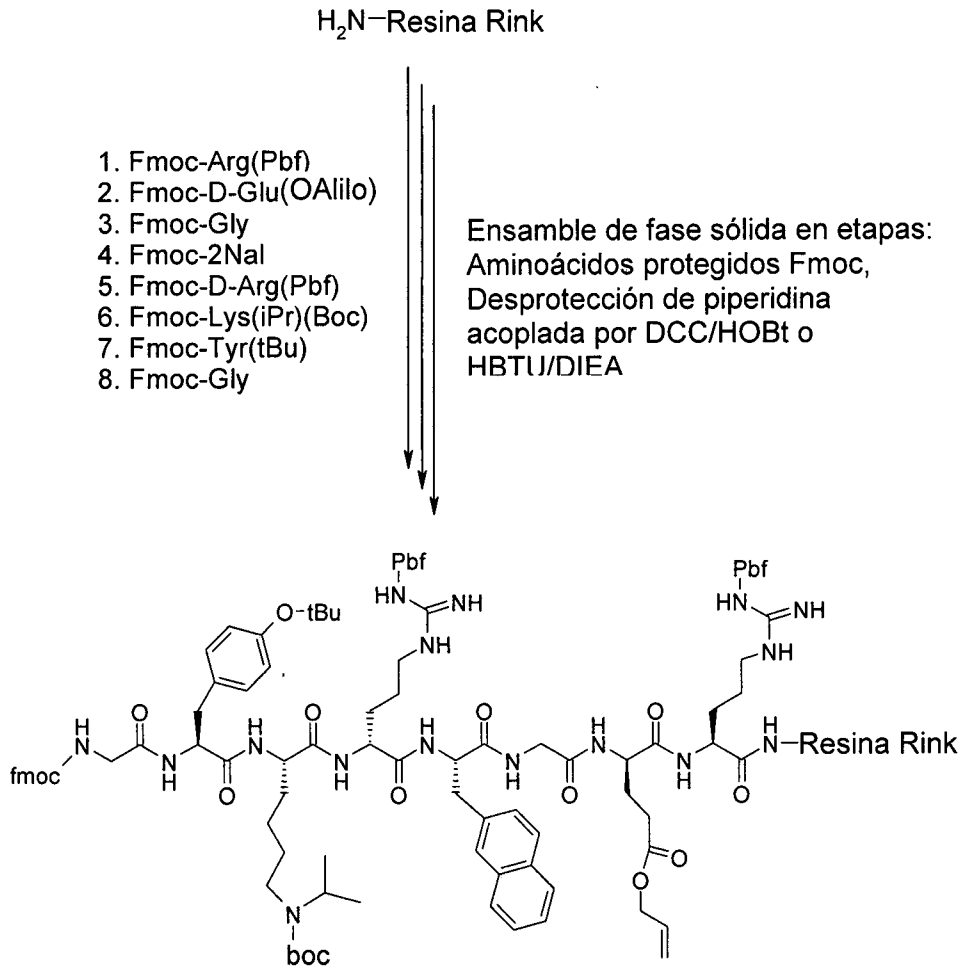
El grupo de protección de cadena lateral de éster de alilo se remueve con 0.1 equivalente de $Pd(Ph_3P)_4$ en la presencia de 24 equivalents de fenilsilano en diclorometano

(Esquema de reacción 6). Este proceso se repite una vez para la desprotección de cadena lateral completa. Luego Fmoc en el extremo de terminal N se remueve usando 20% de piperidina en DMF. La porción de ácido carboxílico desprotegida de DGlu se activa con PyBOP/DIEA, y se cicliza al grupo α-amino de glicina en la resina. El péptido ciclizado se desprotege y escinde simultáneamente de la resina al usar un cóctel de secuestrantes de TFA/H_2O/TIS/EDT (95/2/1/2, v/v/v/v), o TFA/H_2O/TIS/anisol (92/2/4/2, v/v/v/v) durante 2 horas a temperatura ambiente. Luego se evaporan los solventes bajo vacío, y el péptido se precipita y se lava tres veces con éter dietílico frío para retirar los secuestrantes. PM cal.: 1085.29; PM obs.: 1085.32.

Ejemplo 25a

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2·sal de ácido acético (SEQ ID NO:34)

La sal de TFA del péptido de Ejemplo 25 se convierte a una sal de ácido acético al adsorber el material sobre una columna preparativa C18 de tamaño adecuado, equilibrada con ácido acético al 2%/H_2O (v/v). La columna luego se lava con tres a cinco volúmenes de columna de ácido acético acuoso al 2% (v/v). El péptido se eluye al usar 1:1 agua/acetonitrilo (v/v) que contiene ácido acético al 2% en volumen, y se liofiliza. PM cal.: 1085.29; PM obs.: 1085.32.



(SEQ ID NO:35)

Esquema de reacción 5. Grupo α-amino para ensamble de péptido de cadena lateral carboxilo

La purificación de péptidos se efectúa al usar técnicas CLAR preparativas estándar. Inmediatamente después de la ciclización, la solución de péptidos se diluye con agua que contiene 0.1% (v/v) TFA, cargada en una columna C18 CLAR de fase inversa, y se eluye con un gradiente de ácido trifluoroacético al 0.1% acuoso/acetonitrilo (v/v) mientras se monitorea a 214 nm. Las fracciones apropiadas se acumulan y liofilizan. La

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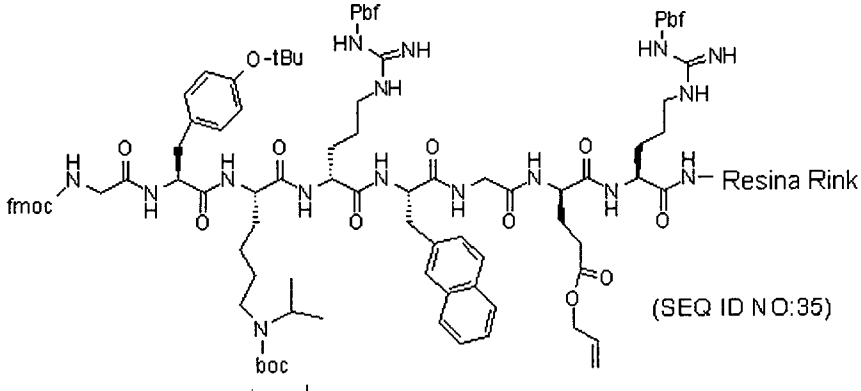
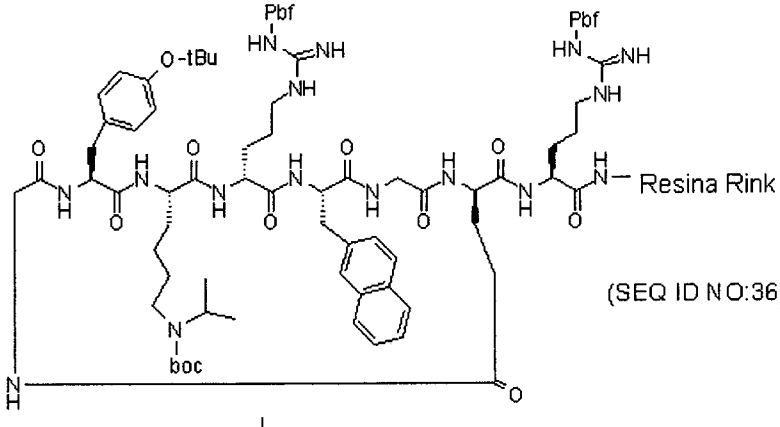
caracterización adicional del producto final se efectúa al usar CLAR analítica y análisis espectral de masas por métodos convencionales. Para los péptidos con una cadena lateral básica, el producto final liofilizado es una sal de TFA.



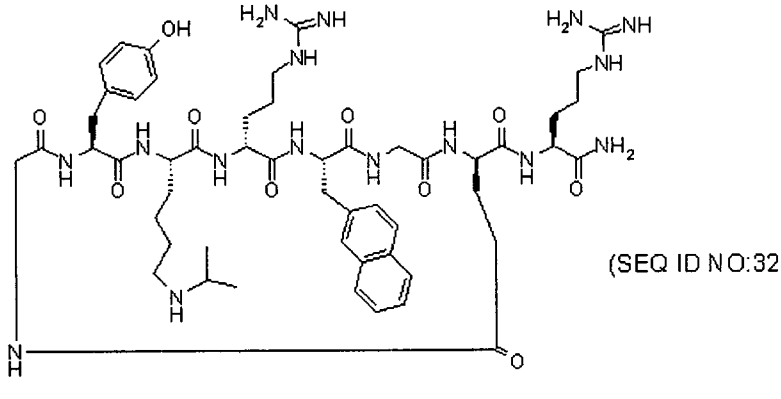
(SEQ ID NO:35)

1. Eliminación de alilo con $Pd(Ph_3P)_4(0)$
2. Eliminación Fmoc con piperidina
3. Ciclización con PyBOP/DIEA



(SEQ ID NO:36)

Desprotección de cadena lateral y desdoblamiento TFA



(SEQ ID NO:32)

Esquema de reacción 6. Grupo α-amino para ciclización y desdoblamiento del anillo de cadena lateral carboxilo

Ejemplo 26

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:37)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1. PM cal.: 929.10; PM obs.: 929.39.

Ejemplo 26a

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2·sal de ácido acético (SEQ ID NO:38)

La sal de TFA del péptido de Ejemplo 26 se convierte a una sal de ácido acético al adsorber el material sobre una columna preparativa C18 de tamaño adecuado, equilibrada con ácido acético al 2%/H_2O (v/v). La columna luego se lava con tres a cinco volúmenes de columna de ácido acético acuoso al 2% (v/v). El péptido se eluye al usar 1:1 agua/acetonitrilo (v/v) que contiene ácido acético al 2% en volumen, y se liofiliza. PM cal.: 929.10; PM obs.: 929.39.

Ejemplo 27

ciclo[Gly-Tyr-Arg-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:39)

Preparar como en el Ejemplo 25, excepto que Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf). PM cal.: 1071.22; PM obs.: 1071.02.

Ejemplo 28

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:40)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(iPr)(Boc). PM cal.: 1099.35; PM obs.: 1099.91.

Ejemplo 29

ciclo[Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:41)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), Fmoc-Gly en la etapa 8 no se usa, y se omite la etapa 8. PM cal.: 1014.17; PM obs.: 1014.78.

Ejemplo 30

ciclo[Tyr-Arg-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:42)

Preparar como en el Ejemplo 25, excepto que Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf),

Fmoc-Gly en la etapa 8 no se usa, y se omite la etapa 8. PM cal.: 1014.17; PM obs.: 1014.65.

Ejemplo 31

ciclo[Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:43)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(alilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), Fmoc-Gly en la etapa 8 no se usa, y se omite la etapa 8. PM cal.: 1000.14; PM obs.: 1000.63.

Ejemplo 32

ciclo[Gly-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:44)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(alilo), y Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf). PM cal.: 1057.19; PM obs.: 1057.35.

Ejemplo 33

ciclo[Gly-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:45)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(alilo), y Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Lys(Me_2). PM cal.: 1057.23; PM obs.: 1057.86.

Ejemplo 34

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:46)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(alilo). PM cal.: 1071.26; PM obs.: 1071.76.

Ejemplo 35

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-NH_2 (SEQ ID NO:47)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, y Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(alilo). PM cal.: 915.07; PM obs.: 915.38.

Ejemplo 36

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-Lys(iPr)-NH_2 (SEQ ID NO:48)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(iPr)(Boc), y Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(alilo). PM cal.: 1085.33; PM obs.: 1085.78.

Ejemplo 37

ciclo[Gly-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:49)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo),

y Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Lys(Me_2). PM cal.: 1071.26; PM obs.: 1071.05.

Ejemplo 38

ciclo[Gly-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:50)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf). PM cal.: 1071.22; PM obs.: 1071.50.

Ejemplo 39

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:51)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo). PM cal.: 1085.29; PM obs.: 1085.91.

Ejemplo 40

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:52)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, y Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo). PM cal.: 929.10; PM obs.: 929.39.

Ejemplo 41

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Asp]-NHEt (SEQ ID NO:53)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, se omite la etapa 1, y Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(alilo). PM cal.: 943.13; PM obs.: 943.36.

Ejemplo 42

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:54)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, se omite la etapa 1, y Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo). PM cal.: 957.15; PM obs.: 957.50.

Ejemplo 43

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NHEt (SEQ ID NO:55)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, y se omite la etapa 1. PM cal.: 957.15; PM obs.: 957.46.

Ejemplo 44

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NHEt (SEQ ID NO:56)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo. PM cal.: 1113.34; PM obs.: 1113.81.

Ejemplo 44a

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NHEt·sal de ácido acético (SEQ ID NO:57)

La sal de TFA del péptido del Ejemplo 44 se convierte a una sal de ácido acético al adsorber el material sobre una columna preparativa C18 de tamaño adecuado, equilibrada con ácido acético al 2%/H_2O (v/v). La columna luego se lava con tres a cinco volúmenes de columna de ácido acético acuoso al 2% (v/v). El péptido se eluye al usar 1:1 agua/acetonitrilo (v/v) que contiene ácido acético al 2% en volumen, y se liofiliza. PM cal.: 1113.34; PM obs.: 1113.81.

Ejemplo 45

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt (SEQ ID NO:58)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-

amino}-metil)-indol-1-il]-acetilo, y Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(iPr)(Boc). PM cal.: 1127.41; PM obs.: 1127.35.

Ejemplo 46

ciclo[Lys(Ac)-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:59)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(OtBu), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Lys(Me_2), y Fmoc-Gly en la etapa 8 se reemplaza con Boc-Lys(Fmoc). Después del ensamble de cadenas, Fmoc en la cadena lateral de la terminal N, Lys se remueve con 20% de piperidina en DMF y la cadena lateral Lys luego es acetilada usando 10 equivalentes de anhídrido acético/DIEA a temperatura ambiente durante una hora. Se remueven todos los grupos protegidos de cadena lateral y el péptido lineal se desdoble del soporte sólido usando una mezcla de TFA/agua/TIS/anisol (90/5/2.5/2.5, v/v/v/v) durante 2 h a temperatura ambiente. La ciclización del péptido lineal crudo se lleva a cabo en solución. El péptido lineal crudo escindido (~0.25 mmol) se seca bajo vacío y se disuelve en 10 mL de DMF seco. Esta solución de péptido se suministra a la siguiente mezcla de reacción por medio de una bomba de jeringa durante un periodo de 2 h: 15 mL de diclorometano

seco y 15 mL de DMF seco que contiene 1.0 mmol de PyBOP y 4.0 mmoles de DIEA. La reacción luego se permite que avance a temperatura ambiente durante 2 h. Luego se evaporan los solventes bajo vacío, el residuo se carga en una columna CLAR preparativa C18 de fase inversa, y se aisla y caracteriza el péptido cíclico objetivo como se describe en Ejemplo 1. PM cal.: 1184.42; PM obs.: 1184.06.

Ejemplo 47

ciclo[Dap(Ac)-Tyr-Lys(Me_2)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:60)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(OtBu), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Lys(Me_2), y Fmoc-Gly en la etapa 8 se reemplaza con Boc-Dap(Fmoc). Después del ensamble de cadenas, Fmoc en la cadena lateral de la terminal N, Dap se remueve usando 20% de piperidina en DMF, y la cadena lateral Dap luego es acetilada con 10 equivalentes de anhídrido acético/DIEA a temperatura ambiente durante una hora. Todos los grupos protegidos de cadena lateral luego se remueven y el péptido lineal se desdobla del soporte sólido usando una mezcla de TFA/agua/TIS/anisol (90/5/2.5/2.5, v/v/v/v) durante 2 h a temperatura ambiente. La ciclización del péptido lineal crudo se lleva a cabo en solución. El péptido lineal crudo

escindido (~0.25 mmole) se seca bajo vacío y se disuelve en 10 mL de DMF seco. Esta solución de péptidos se suministra a la siguiente mezcla de reacción por medio de una bomba de jeringa durante un periodo de 2 h: 15 mL de diclorometano seco y 15 mL de DMF seco que contiene 1.0 mmole de PyBOP y 4.0 mmoles de DIEA. La reacción se permite procesar a temperatura ambiente durante 2 h. Luego se evaporan los solventes bajo vacío, el residuo se carga sobre una columna CLAR preparativa, y se aisla y caracteriza el péptido cíclico objetivo como se describe en Ejemplo 1. PM cal.: 986.15; PM obs.: 985.97.

Ejemplo 48

ciclo[Ala-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:61)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Ala. PM cal.: 943.13; PM obs.: 942.92.

Ejemplo 49

ciclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:62)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-DAla. PM cal.: 943.13; PM obs.: 943.44.

Ejemplo 50

ciclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:63)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-DAla. PM cal.: 943.13; PM obs.: 943.42.

Ejemplo 51

ciclo[Ala-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:64)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Ala. PM cal.: 943.13; PM obs.: 943.48.

Ejemplo 52

ciclo[Leu-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:65)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Leu. PM cal.: 985.21; PM obs.: 985.56.

Ejemplo 53

ciclo[Leu-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:66)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Leu. PM cal.: 985.21; PM obs.: 985.49.

Ejemplo 54

ciclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:67)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-DPhe. PM cal.: 1019.22; PM obs.: 1019.52.

Ejemplo 55

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NH_2 (SEQ ID NO:68)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1019.22; PM obs.: 1019.53.

Ejemplo 56

ciclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:69)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-DPhe. PM cal.: 1019.22; PM obs.: 1019.50.

Ejemplo 57

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:70)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(iPr)(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1189.48; PM obs.: 1189.92.

Ejemplo 57a

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2·sal de ácido acético (SEQ ID NO:71)

La sal de TFA del péptido de Ejemplo 57 se convierte a una sal de ácido acético al adsorber el material sobre una columna preparativa C18 de tamaño adecuado, equilibrada con ácido acético al 2%/H_2O (v/v). La columna luego se lava con tres a cinco volúmenes de columna de ácido acético acuoso al 2% (v/v). El péptido se eluye al usar 1:1 agua/acetonitrilo (v/v) que contiene ácido acético al 2% en volumen, y se liofiliza. PM cal.: 1189.48; PM obs.: 1189.92.

Ejemplo 58

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Arg-NH_2 (SEQ ID NO:72)

Preparar como en el Ejemplo 25, excepto que Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1175.41; PM obs.: 1175.81.

Ejemplo 59

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-NH_2 (SEQ ID NO:73)

Preparar como en el Ejemplo 25, excepto que se omite la etapa 1, y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1019.22; PM obs.: 1019.56.

Ejemplo 60

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt (SEQ ID NO:74)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(iPr)(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1217.53; PM obs.: 1217.97.

Ejemplo 60a

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NHEt·sal de ácido acético (SEQ ID NO:75)

La sal de TFA del péptido de Ejemplo 60 se convierte a una sal de ácido acético al adsorber el material sobre una columna preparativa C18 de tamaño adecuado, equilibrada con ácido acético al 2%/H_2O (v/v). La columna luego se lava con tres a cinco volúmenes de columna de ácido acético acuoso

al 2% (v/v). El péptido se eluye al usar 1:1 agua/acetonitrilo (v/v) que contiene ácido acético al 2% en volumen, y se liofiliza. PM cal.: 1217.53; PM obs.: 1217.97.

Ejemplo 61

ciclo[DAla-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:76)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-DAla. PM cal.: 971.18; PM obs.: 971.49.

Ejemplo 62

ciclo[2Nal-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:77)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-2Nal. PM cal.: 1097.34; PM obs.: 1097.53.

Ejemplo 63

ciclo[DPhe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:78)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-DPhe. PM cal.: 1047.28; PM obs.: 1047.51.

Ejemplo 64

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-NHEt (SEQ ID NO:79)

Preparar como en el Ejemplo 25, excepto que la resina de amida Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, se omite la etapa 1, Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc- Phe. PM cal.: 1047.28; PM obs.: 1047.57.

Ejemplo 65

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Gly-2Nal-NH_2 (SEQ ID NO:80)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-2Nal, y una

etapa se agrega entre las etapas 1 y 2 usando Fmoc-Gly. PM cal.: 1183.39; PM obs.: 1183.26.

Ejemplo 66

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-Glu]-β-Ala-D2Nal-NH_2 (SEQ ID NO:81)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-D2Nal, una etapa se agrega entre las etapas 1 y 2 usando Fmoc-β-Ala, y Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo). PM cal.: 1197.40; PM obs.: 1196.70.

Ejemplo 67

ciclo[β-Ala-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:82)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-β-Ala. PM cal.: 1085.25; PM obs.: 1085.05.

Ejemplo 68

ciclo[β-Ala-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:83)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(Oalilo),

Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-β-Ala. PM cal.: 1071.22; PM obs.: 1071.15.

Ejemplo 69

ciclo[5-aminovalerilo-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:84)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con ácido Fmoc-5-aminovalérico. PM cal.: 1113.30; PM obs.: 1113.40.

Ejemplo 70

ciclo[5-aminovalerilo-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:85)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con ácido Fmoc-5-amino valérico. PM cal.: 1099.27; PM obs.: 1100.25.

Ejemplo 71

ciclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Asp]-Arg-NH_2 (SEQ ID NO:86)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Asp(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con ácido Fmoc-4-aminometil fenilacético (4-AMPA). PM cal.: 1147.32; PM obs.: 1148.20.

Ejemplo 72

ciclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:87)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con ácido Fmoc-4-aminometil fenilacético (4-AMPA). PM cal.: 1161.34; PM obs.: 1161.99.

Ejemplo 73

ciclo[(4-AMPA)-Tyr-Arg-DArg-2Nal-Gly-Glu]-DArg-NH_2 (SEQ ID NO:88)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-DArg(Pbf), Fmoc-

DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con ácido Fmoc-4-aminometil fenilacético (4-AMPA). PM cal.: 1161.34; PM obs.: 1161.83.

Ejemplo 74

ciclo[(4-AMB)-Tyr-Arg-DArg-2Nal-Gly-Glu]-Arg-NH_2 (SEQ ID NO:89)

Preparar como en el Ejemplo 25, excepto que Fmoc-DGlu(Oalilo) en la etapa 2 se reemplaza con Fmoc-Glu(Oalilo), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Arg(Pbf), y Fmoc-Gly en la etapa 8 se reemplaza con ácido Fmoc-4-aminometil benzoico (4-AMB). PM cal.: 1147.32; PM obs.: 1147.66.

Ejemplo 75

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-Gly-2Nal-NH_2 (SEQ ID NO:90)

Preparar como en el Ejemplo 25, excepto que la etapa 1 se reemplaza con tres acoplamientos de residuo secuencial: primero Fmoc-2Nal, luego Fmoc-Gly, y luego Fmoc-Lys(iPr)(Boc). PM cal.: 1353.69; PM obs.: 1354.03.

Ejemplo 76

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-Gly-2Nal-NH_2 (SEQ ID NO:91)

Preparar como en el Ejemplo 25, excepto que la etapa 1 se reemplaza con tres acoplamientos de residuo secuencial: primero Fmoc-2Nal, luego Fmoc-Gly, y luego Fmoc-Lys(iPr)(Boc). Además, Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1443.82; PM obs.: 1444.13.

Ejemplo 77

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Gly-DPhe-NH_2 (SEQ ID NO:92)

Preparar como en el Ejemplo 25, excepto que la etapa 1 se reemplaza con dos acoplamientos de residuos secuenciales: primero Fmoc-DPhe, luego Fmoc-Gly. PM cal.: 1133.36; PM obs.: 1133.73.

Ejemplo 78

ciclo[Gly-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-DPhe-NH_2 (SEQ ID NO:93)

Preparar como en el Ejemplo 25, excepto que la etapa 1 se reemplaza con dos acoplamientos de residuos secuenciales: primero Fmoc-DPhe, luego Fmoc-Lys(iPr)(Boc). PM cal.: 1246.58; PM obs.: 1246.88.

Ejemplo 79

ciclo[Lys-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:94)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(iPr)(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Lys(Boc). PM cal.: 1170.50; PM obs.: 1169.80.

Ejemplo 80

ciclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys(iPr)-NH_2 (SEQ ID NO:95)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(iPr)(Boc), Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplaza con Fmoc-Lys(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1147.40; PM obs.: 1146.70.

Ejemplo 81

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Lys-NH_2 (SEQ ID NO:96)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Lys(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1147.40; PM obs.: 1146.70.

Ejemplo 82

ciclo[Phe-Tyr-Lys(iPr)-DArg-2Nal-Gly-DGlu]-Orn-NH_2 (SEQ ID NO:97)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 se reemplaza con Fmoc-Orn(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1133.40; PM obs.: 1132.70.

Ejemplo 83

ciclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys-NH_2 (SEQ ID NO:98)

Preparar como en el Ejemplo 25, excepto que Fmoc-Arg(Pbf) en la etapa 1 y Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplazan cada uno con Fmoc-Lys(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1105.37; PM obs.: 1105.40.

Ejemplo 84

ciclo[Phe-Tyr-Lys-DArg-2Nal-Gly-DGlu]-Lys-NHEt (SEQ ID NO:99)

Preparar como en el Ejemplo 25, excepto que la resina Rink se reemplaza con resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo, Fmoc-Arg(Pbf) en la etapa 1 y Fmoc-Lys(iPr)(Boc) en la etapa 6 se reemplazan cada uno con Fmoc-Lys(Boc), y Fmoc-Gly en la etapa 8 se reemplaza con Fmoc-Phe. PM cal.: 1133.36; PM obs.: 1133.82.

Ejemplo 85

Síntesis Alternativa I de ciclo[Phe-Tyr-Lys(iPr)-dArg-2Nal-Gly-dGlu]-Lys(iPr)-NH2 (SEQ ID NO:70)

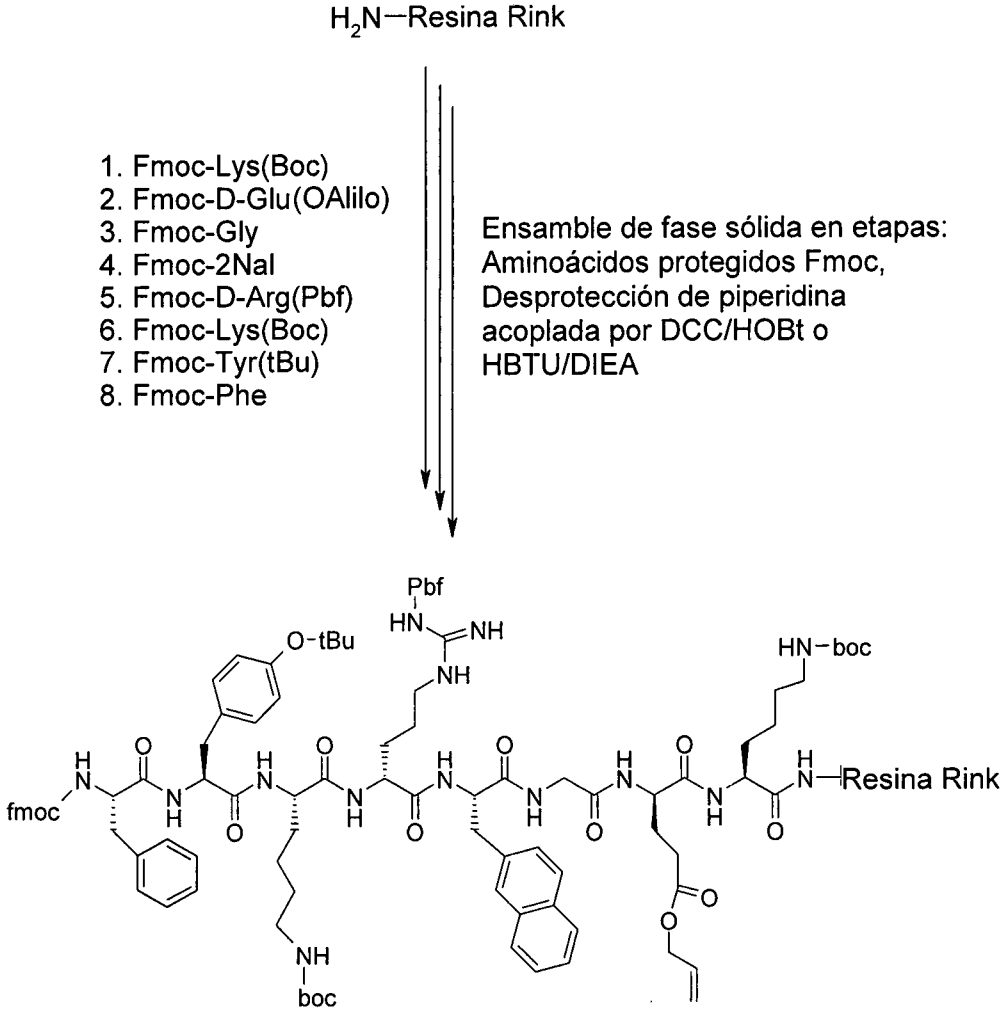
El ejemplo 57 describe la síntesis de SEQ ID NO:70 por medio de química de síntesis de péptidos en fase sólida Fmoc empleando el Fmoc-Lys(iPr)Boc que bloquea la construcción comercialmente disponible, el cual es costoso y difícil de obtener en cantidad grande. El proceso descrito en este ejemplo permite la síntesis de SEQ ID NO:70 usando menos Fmoc-Lys(Boc) costoso, ciclización de solución, y alquilación de lisina a través de aminación reductora usando cianoborohidruro de sodio, proporcionando una ruta más economica para este producto final. Las ventajas adicionales son que el medio de reacción (ácido acético, acetona, y metanol) son relativamente barato, las condiciones de reacción se controlan fácilmente, la relación de solventes puede variar de manera importante sin afectar la reacción de alquilación, y el rendimiendo recuperado es 90% o mayor.

La secuencia Phe-Tyr(tBu)-Lys(Boc)-DArg(Pbf)-2Nal-Gly-DGlu(Oalilo)-Lys(Boc) (SEQ ID NO:100) se ensambla en la resina de amida Rink por química Fmoc estándar utilizando un Sintetizador de péptido ABI 431 como se resume en el Esquema de reacción 7. El ensamble automatizado se lleva a cabo usando el protocolo de química Applied Biosystems

DCC/HOBt o protocolo de química FastMoc (HBTU/DIEA) siguiendo las instrucciones del proveedor (PE Applied Biosystems Inc., Foster City, CA). El esquema de grupos protectores de cadenas laterales es: Lys(Boc), DGlu(Oalilo), DArg(Pbf), Tyr(tBu). El ensamble de cadenas por etapas inicia del extremo de terminal C del péptido lineal y se realiza en 8 steps. En la etapa 1, cuatro equivalentes de Fmoc-Lys(Boc) de aminoácido protegido se activan con DCC/HOBt (o HBTU/DIEA para química FastMoc) en NMP, y se acopla para desproteger la resina de amida Rink. En la etapa 2, cuatro equivalentes de Fmoc-DGlu(Oalilo) se activan y acoplan a la resina de péptido desprotegida de la etapa 1. Estas etapas se repiten apropiadamente hasta la etapa 8, el acoplamiento de Fmoc-Phe.

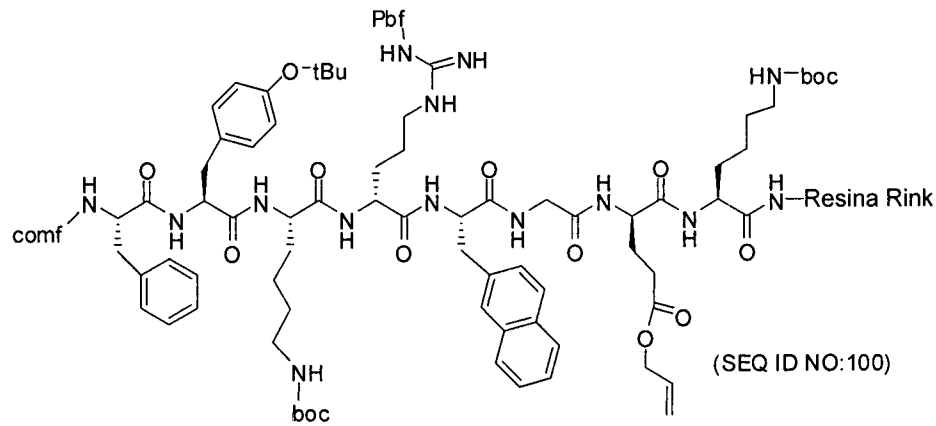
El grupo protegido de cadena lateral de éster de alilo se remueve con 0.1 equivalente de $Pd(Ph_3P)_4$ en la presencia de 24 equivalentes de fenilsilano en diclorometano (Esquema de reacción 8). Este proceso se repite una vez para la desprotección de cadena lateral completa. El Fmoc en el extremo de terminal N luego se remueve usando 20% de piperidina en DMF. La porción de ácido carboxílico desprotegida de DGlu se activa con PyBOP/DIEA y se cicliza al grupo α-amino de Phe en la resina. El péptido ciclizado se desprotege y escinde simultáneamente de la resina al usar un cóctel de secuestrantes de $TFA/H_2O/TIS/EDT$

(95/2/1/2, v/v/v/v) o TFA/H_2O/TIS/anisol (92/2/4/2, v/v/v/v) durante 2 horas a temperatura ambiente. Luego se evaporan los solventes bajo vacío, y el péptido se precipita y se lava tres veces con éter dietílico frío para retirar los secuestrantes.



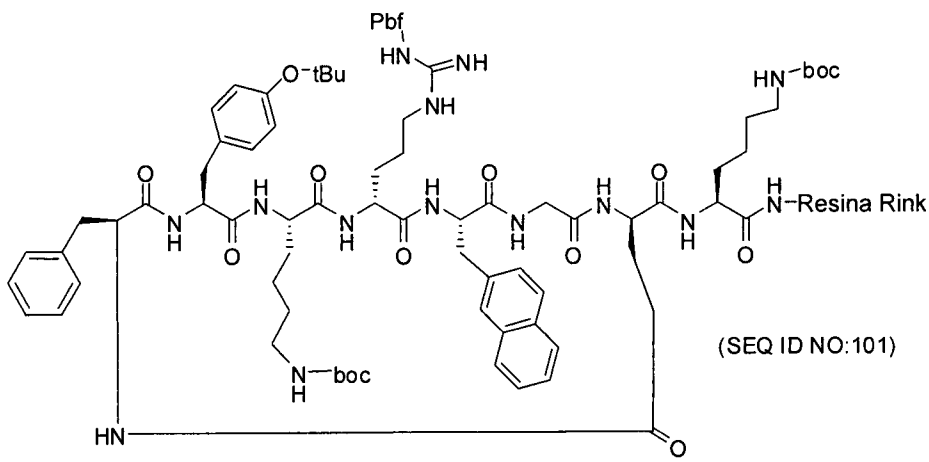
(SEQ ID NO:100)

Esquema de reacción 7. Ensamble de cadena de péptido en fase sólida



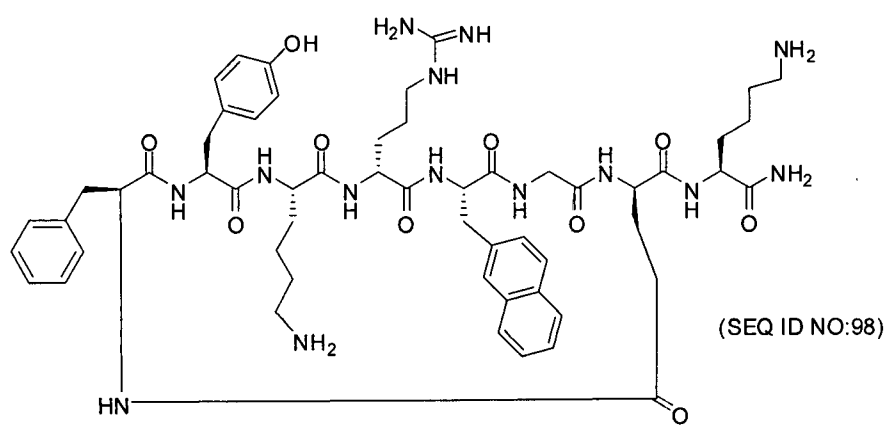
(SEQ ID NO:100)

1. Eliminación de alilo con Pd(PPh3)4(0)
2. Eliminación Fmoc con piperidina
3. Ciclización con PyBOP/DIEA



(SEQ ID NO:101)

Desprotección de cadena lateral y desdoblamiento TFA



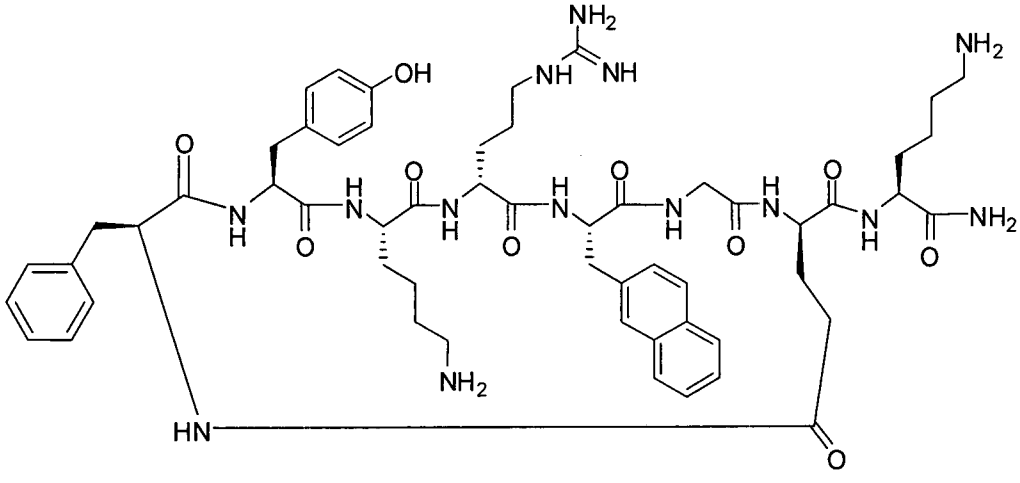
(SEQ ID NO:98)

Esquema de reacción 8. Preparación de péptido precursor cíclico

La purificación del péptido precursor cíclico se realiza al usar técnicas CLAR preparativas estándar. El producto escindido crudo se disuelve en una cantidad mínima de DMSO, cargado en una columna CLAR C18 de fase inversa, y se eluye con un gradiente de ácido trifluoroacético acuoso al 0.1%/acetonitrilo (v/v) mientras se monitorea a 214 nm. Las fracciones apropiadas se acumulan y liofilizan. La caracterización adicional del péptido precursor cíclico intermediario se efectúa al usar CLAR analítica y análisis espectral de masas por técnicas convencionales.

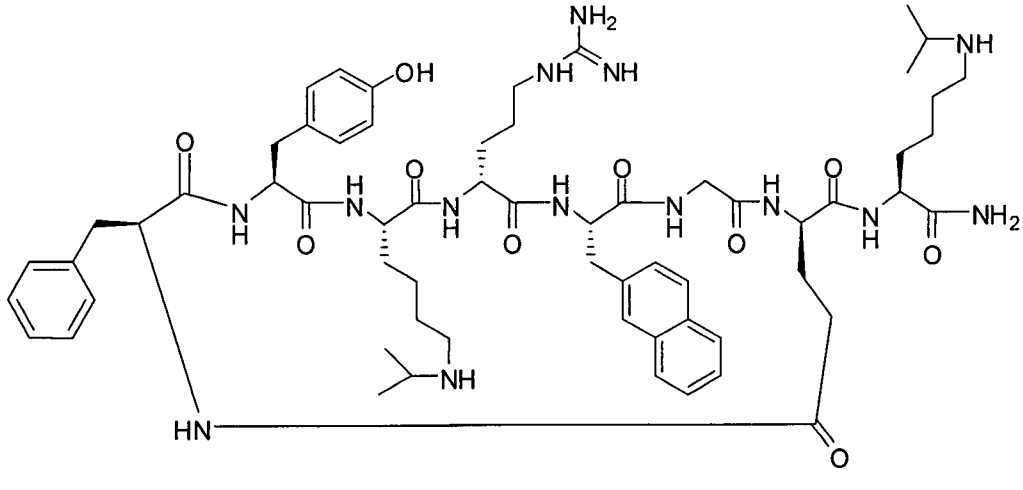
El péptido cíclico precursor liofilizado luego se alquila en una solución de ácido acético/acetona/metanol (1:1:4, v/v/v) a través de aminación reductora usando cianoborohidruro de sodio (Esquema de reacción 9). La concentración de péptidos es alrededor de 10mg/mL, y puede variar de manera importante sin afectar los resultados. Tres hasta 5 equivalentes se usan del reactivo reductor cianoborohidruro de sodio, y la reacción se completa normalmente dentro de 2 h a temperatura ambiente. El rendimiento recuperado es de 90% o mayor. Por ejemplo, 20 mg del péptido precursor cíclico se disuelven en 2 mL de metanol, para el cual 0.5 mL de ácido acético y 0.5 mL de acetona se agregan, se mezclan bien, y luego 5.6 mg de cianoborohidruro de sodio (2.5 equivalentes en metanol) se agregan bajo agitación. La mezcla de reacción se agita a temperatura ambiente durante

30 min, durante lo cual otros 5.6 mg de cianoborohidruro de sodio se agregan. La reacción se monitorea por CLAR y análisis espectral de masas. Después de que se termina la reacción, la desalación de la mezcla de reacción y la liofilización proporciona 18.9 mg de producto final (SEQ ID NO:70) con una pureza de 97.5%. PM cal.: 1189.48; PM obs.: 1189.6.



(SEQ ID NO:98)

AcOH/acetona/metanol 1/1/4 (v/v/v)
NaBH3CN en metanol, 5 eq



(SEQ ID NO:70)

320

Esquema de reacción 9. Aminación reductora que convierte Lys hasta Lys(iPr)

El compuesto del Ejemplo 60 (SEQ ID NO:74) también se puede preparar en una manera análoga, con modificaciones apropiadas con base en sus aminoácidos constituyentes, usando resina AM de [3-({etil-Fmoc-amino}-metil)-indol-1-il]-acetilo. Primero, el péptido precursor (SEQ ID NO:99) se prepara como se describe en el Ejemplo 84, y luego la aminación reductora se lleva a cabo como se muestra en el Esquema de reacción 9. Esto proporciona un péptido de amina etilo de terminal C cíclico final con una pureza de 99.16%. PM cal.:1217.53; PM obs.: 1217.84.

Ejemplo 86

Síntesis Alternativa II de ciclo[Phe-Tyr-Lys(iPr)-dArg-2Nal-Gly-dGlu]-Lys(iPr)-NH2 (SEQ ID NO:70)

El ejemplo 85 describe una síntesis eficiente en costo de SEQ ID NO:70 por medio de química de síntesis de péptidos en fase sólida Fmoc empleando Fmoc-Lys(Boc) relativamente barato y alquilación de lisina a través de aminación reductora usando cianoborohidruro de sodio en solventes relativamente baratos (ácido acético, acetona, y metanol). Sin embargo, este proceso involucra el uso del paladio catalizador de metal pesado para la eliminación del grupo

protegido de cadena lateral de éster de alilo. El paladio es altamente tóxico, y el control de calidad para eliminación completa segura de este elemento se complica y dificulta. El proceso de síntesis de péptido de fase sólida Boc con ciclización en la resina descrita en este ejemplo permite la producción de SEQ ID NO:70 usando Boc-Lys(2-Cl-Z) relativamente barato sin la necesidad de un catalizador de paladio costoso y tóxico, proporcionando una ruta menos tóxica, se pueden escalar hacia arriba fácilmente, menos ruta tóxia para un producto final que requiere un proceso de control de calidad simple.

La secuencia Fmoc-Phe-Tyr(2-Br-Z)-Lys(2-Cl-Z)-DArg(Tos)-2Nal-Gly-DGlu(OFm)-Lys(2-Cl-Z) (SEQ ID NO:102) se ensambla manualmente en MBHA resina (4-metil-benzhidril-amina) (Cat. No. D-2095, BaChem California Inc., Torrance, CA) usando química Boc de síntesis de péptido de fase sólida establecida (Schnölzer et al. (1992) *Int. J. Pept. Protein Res.* 40:180-193) como se resume en el Esquema de reacción 10. El ensamble de las cadenas se lleva a cabo usando un procedimiento de neutralización *in situ*/HBTU/activación DIEA como se describe en esta referencia. El esquema de grupos protectores de cadenas laterales es: Lys(2-Cl-Z), DGlu(OFm), DArg(Tos), y Tyr(2-Br-Z). el grupo alfa-amino de todos los bloques de construcción de aminoácido se protege con tert-butoxicarbonil (Boc) excepto el Phe de residuo de terminal N, el cual se

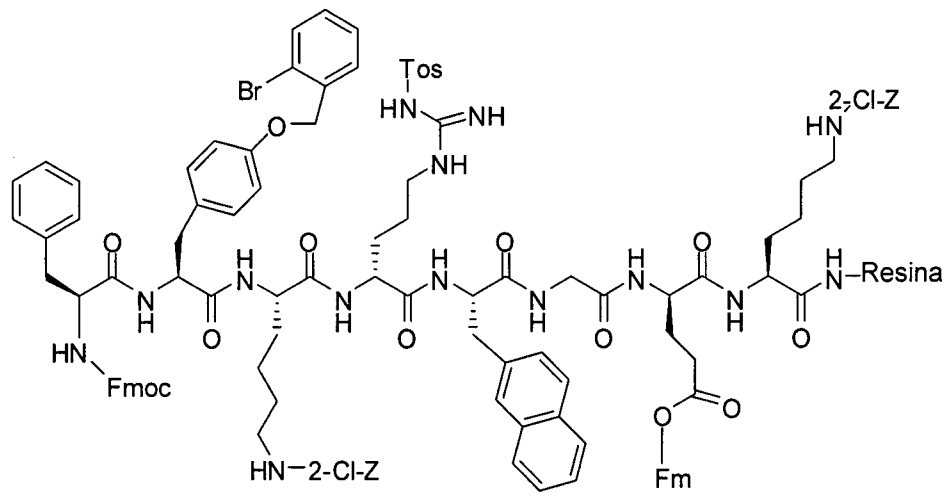
protege con Fmoc para eficiencia de la síntesis. El ensamble de cadenas por etapas inicia del extremo de terminal C del péptido lineal y se realiza en 8 etapas. En la etapa 1, cinco equivalentes de Boc-Lys(2-Cl-Z) de aminoácido protegido se activan con HBTU/DIEA en DMF, y se acoplan para la resina MBHA. En la etapa 2, cinco equivalentes de Boc-DGlu(OFm) se activan y acoplan a la resina de péptido desprotegida de la etapa 1 usando TFA puro. Estas etapas se repiten apropiadamente hasta la etapa 8, el acoplamiento de Fmoc-Phe.

El grupo protector de cadena lateral Fm de DGlu, junto con el Fmoc en el extremo de terminal N, se remueve usando 20% de piperidina en DMF. La porción de ácido carboxílico desprotegida de DGlu se activa con PyBOP/DIEA, HCTU/DIEA, u otros reactivos de activación apropiados, y se cicliza para el grupo α-amino de Phe en la resina. El péptido cíclico se desprotege y escinde simultáneamente de la resina usando HF con 5% de *m*-cresol o *p*-cresol como un depurador durante 1 hora a 0°C. Los solventes luego se evaporan y el péptido crudo se precipita y se lava tres veces con éter dietílico frío.

H_2N-resina MBHA

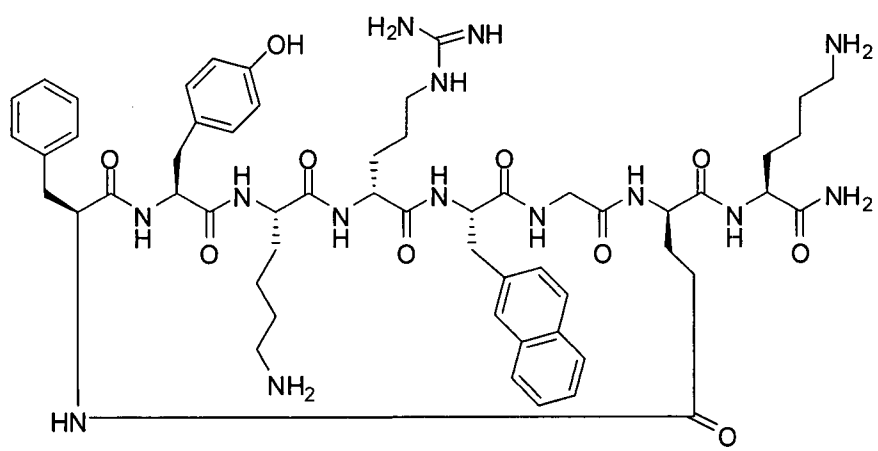
1.Boc-Lys(2-Cl-Z)
2. Boc-D-Glu(OFm)
3. Boc-Gly
4. Boc-2Nal
5. Boc-D-Arg(Tos)
6. Boc-Lys(2-Cl-Z)
7. Boc-Tyr(2-Br-Z)
8. Fmoc-Phe

Ensamble de fase sólida en etapas: aminoácidos protegidos por Boc, desprotección TFA acoplada a HBTU/DIEA



(SEQ ID NO:102)

a. piperidina (20%) en DMF
b. ciclización, PyBOP/DIEA
c. desdoblamiento HF, 0°C, 1 h



(SEQ ID NO:98)

Esquema de reacción 10. Ensamble de la cadena de péptido en fase sólida usando química Boc

La purificación del péptido precursor cíclico (SEQ ID NO:98 como se muestra en el Esquema de reacción 10) se realiza al usar técnicas CLAR preparativas estándar. El producto escindido crudo se disuelve en una cantidad mínima de DMSO, cargado en una columna CLAR C18 de fase inversa, y se eluye con un gradiente de ácido trifluoroacético acuoso al 0.1%/acetonitrilo (v/v) mientras se monitorea a 214 nm. Las fracciones apropiadas se acumulan y liofilizan. La caracterización adicional del péptido precursor cíclico intermediario se efectúa al usar CLAR analítica y análisis espectral de masas por técnicas convencionales. Para SEQ ID NO:98, PM cal.: 1105.29; PM obs.: 1105.4.

El péptido cíclico precursor liofilizado (SEQ ID NO:98) luego se alquila en una solución de ácido acético/acetona/metanol (1:1:4, v/v/v) a través de aminación reductora usando cianoborohidruro de sodio como en el Esquema de reacción 9. La concentración de péptidos es alrededor de 10mg/mL, y puede variar de manera importante sin afectar los resultados. Cinco equivalentes del reactivo reductor cianoborohidruro de sodio se usaron, y la reacción se completa normalmente dentro de 2 h a temperatura ambiente. El

rendimiento recuperado es 90% o mayor. Por ejemplo, 6.6 mg del péptido precursor cíclico se disuelven en 0.8 mL de metanol, para el cual 0.2 mL de ácido acético y 0.2 mL de acetona se agregaron, mezclaron bien, y luego 1.9 mg de cianoborohidruro de sodio (2.5 equivalentes en metanol) se agregaron bajo agitación en dos porciones iguales. La mezcla de reacción se agita a temperatura ambiente durante 30 min, durante lo cual otros 1.9 mg de cianoborohidruro de sodio se agregan. La reacción se monitorea por CLAR y análisis espectral de masas. Después de que se termina la reacción, la desalación de la mezcla de reacción y la liofilización proporciona el producto final (SEQ ID NO:70) con una pureza de 96.5%. PM cal.: 1189.45; PM obs.: 1189.6.

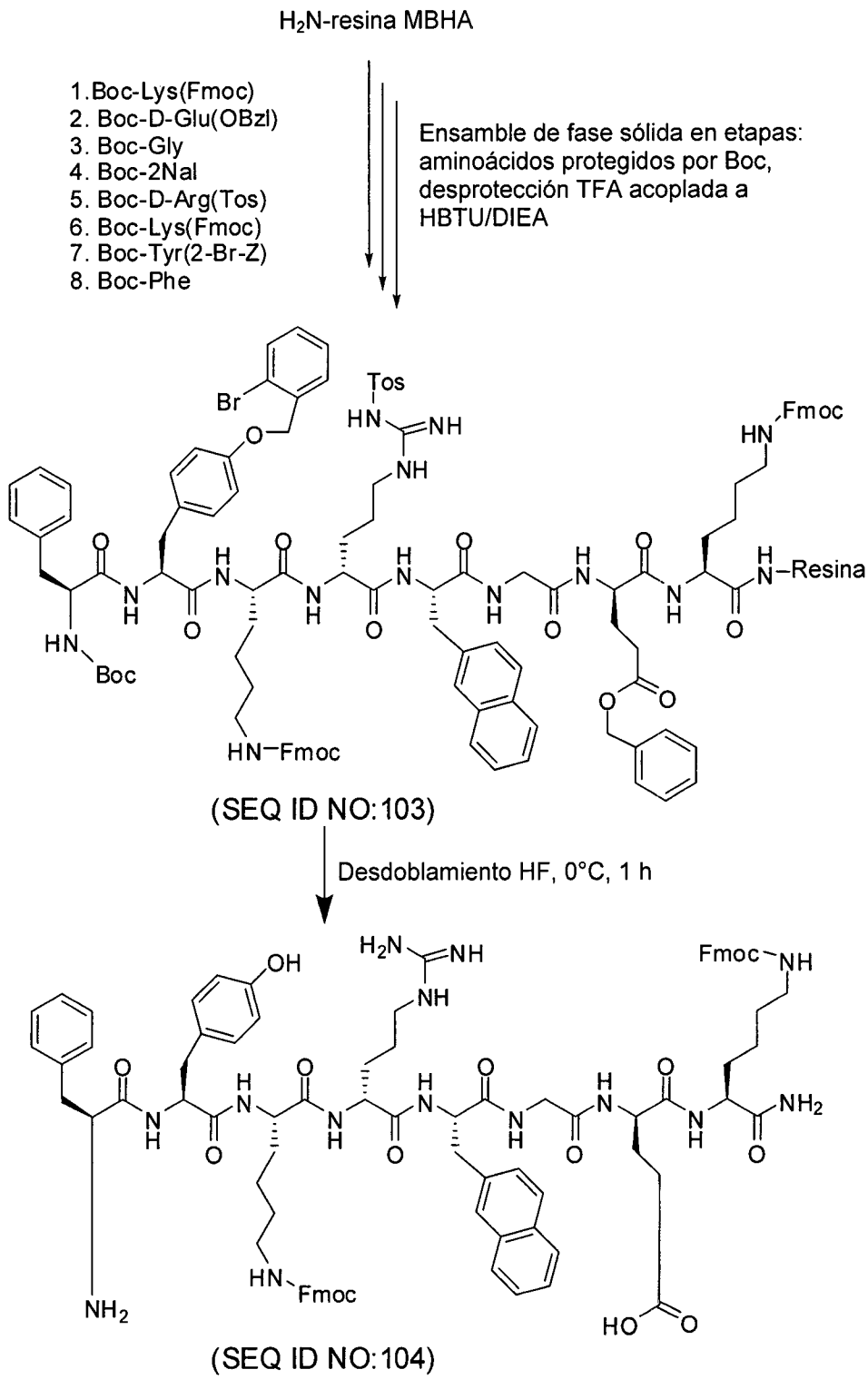
Ejemplo 87

Síntesis Alternativa III de ciclo[Phe-Tyr-Lys(iPr)-dArg-2Nal-Gly-dGlu]-Lys(iPr)-NH2 (SEQ ID NO:70)

El compuesto del Ejemplo 57 (SEQ ID NO:70) también se puede preparar sin el uso de un catalizador de paladio por medio de ciclización de solución, que facilita el aumento progresivo, como sigue.

La secuencia Boc-Phe-Tyr(2-Br-Z)-Lys(Fmoc)-DArg(Tos)-2Nal-Gly-DGlu(OBzl)-Lys(Fmoc) (SEQ ID NO:103) se ensambla manualmente en resina MBHA usando química Boc de síntesis de péptido de fase sólida (Schnölzer et al. (1992) *Int. J.*

Pept. Protein Res. 40:180-193) como se resume en el Esquema de reacción 11. El ensamble de las cadenas se lleva a cabo usando el procedimiento de neutralización *in situ*/HBTU/activación DIEA como se describe en Schnölzer et al. El esquema de grupos protectores de cadenas laterales es: Lys(Fmoc), DGlu(OBzl), DArg(Tos), Tyr(2-Br-Z). El grupo alfa-amino de todos los bloques de construcción de aminoácido se protege con tert-butoxicarbonilo (Boc). El ensamble de cadenas por etapas inicia del extremo de terminal C del péptido lineal y se realiza en 8 etapas como se muestra en el Esquema de reacción 11. En la etapa 1, cinco equivalentes de Boc-Lys(2-Cl-Z) de aminoácido protegido se activan con HBTU (4 eq) /DIEA (10 eq) en DMF, y acoplan para resina MBHA. En la etapa 2, cinco equivalentes de Boc-DGlu(OBzl) se activan y acoplan a la resina de péptido desprotegida de la etapa 1 usando TFA puro. Estas etapas se repiten apropiadamente hasta la etapa 8, el acoplamiento de Boc-Phe. El grupo protector Boc se remueve con TFA puro, la resina se neutraliza con DIEA, y se lava con DMF y metanol y se seca en aire antes del desdoblamiento de HF. El péptido lineal se desprotege y escinde simultáneamente de la resina usando HF con 5% de *m*-cresol o *p*-cresol como un depurador durante 1 hora a 0°C. Los solventes luego se evaporan y el péptido crudo se precipita y se lava tres veces con éter dietílico frío.



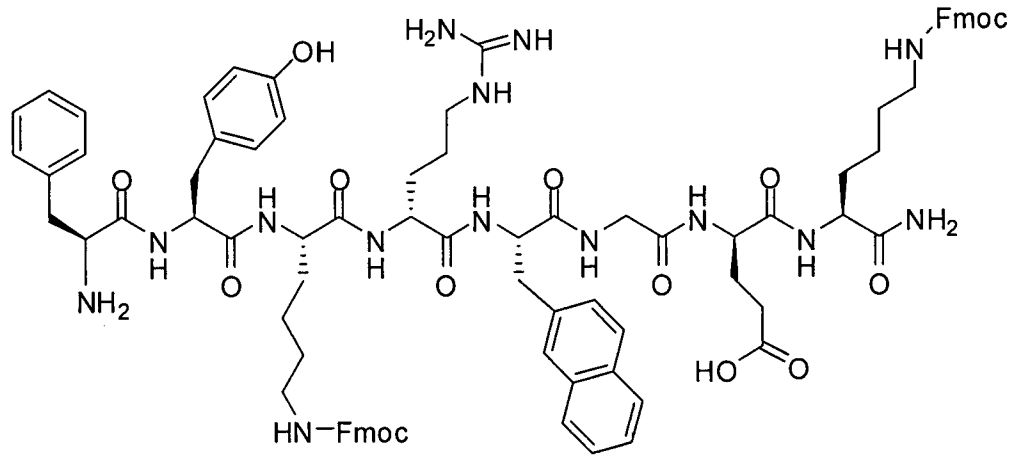
Esquema de reacción 11. Ensamble de cadena usando química Boc

La purificación del péptido precursor lineal (SEQ ID NO:104) se realiza al usar técnicas CLAR preparativas estándar. El producto escindido crudo se disuelve en una cantidad mínima de DMSO, cargado en una columna CLAR C18 de fase inversa, y se eluye con un gradiente de ácido trifluoroacético acuoso al 0.1%/acetonitrilo (v/v) mientras se monitorea a 214 nm. Las fracciones apropiadas se acumulan y liofilizan. La caracterización adicional del péptido precursor cíclico intermediario se efectúa al usar CLAR analítica y análisis espectral de masas por técnicas convencionales. Para SEQ ID NO:104, PM cal.: 1567.78; PM obs.: 1567.6.

La ciclización del péptido lineal precursor liofilizado (SEQ ID NO:104) se lleva a cabo en solución (Esquema de reacción 12). El péptido lineal se disuelve en una cantidad pequeña de DMF seco (~10 mg/mL). Esta solución de péptidos se administra lentamente por medio de una bomba de jeringa a la mezcla de reacción de PyBOP (2 eq, u otros reactivos de activación apropiados, tales como HCTU, BOP, HBTU, etc.) y DIEA (10 eq) en DMF seco bajo agitación magnética. La reacción luego se permite que avance a temperatura ambiente durante 2 h. La piperidina pura luego se agrega a la mezcla de reacción a una concentración final de 25% (v/v). La mezcla de

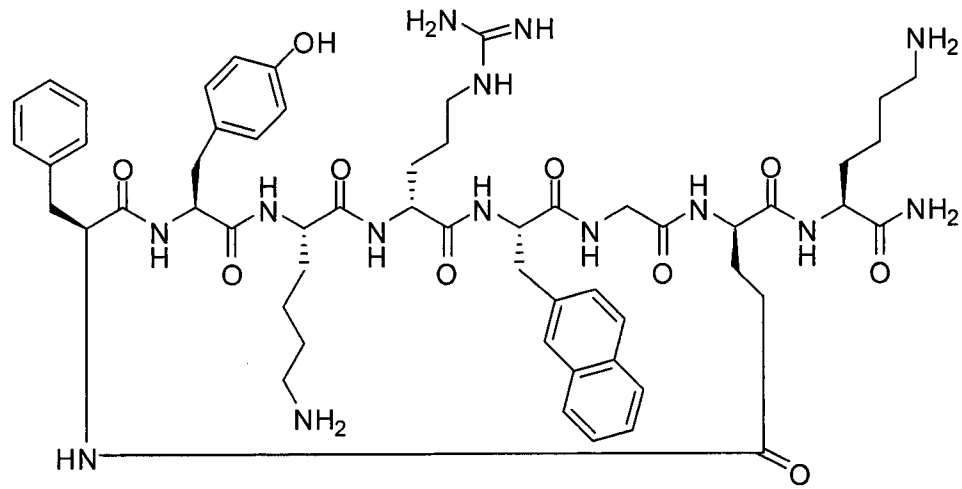
reacción se mantiene bajo agitación durante otros 20 min para remover completamente la protección Fmoc. Los solventes se evaporan bajo vacío y el residuo se carga sobre una columna CLAR C18 de fase inversa preparativa, y se eluyen con un gradiente de ácido trifluoroacético acuoso al 0.1%/acetonitrilo (v/v) mientras se monitorea a 214 nm. Las fracciones apropiadas se acumulan y liofilizan, y proporcionan el péptido precursor cíclico (SEQ ID NO:98). La caracterización adicional del péptido precursor cíclico intermediario se efectúa al usar CLAR analítica y análisis espectral de masas por técnicas convencionales. Para SEQ ID NO:98, PM cal.: 1105.29; PM obs.: 1105.4.

La alquilación de péptido cíclico SEQ ID NO:98 se lleva a cabo en una solución de ácido acético/acetona/metanol (1:1:4, v/v/v) a través de aminación reductora usando cianoborohidruro de sodio como en el Esquema de reacción 9 para generar el producto final (SEQ ID NO:70). PM cal.: 1189.45; PM obs.: 1189.6.



(SEQ ID NO: 104)

1. ciclización, PyBOP/DIEA/DMF
2. Eliminación Fmoc: piperidina (20%) en DMF



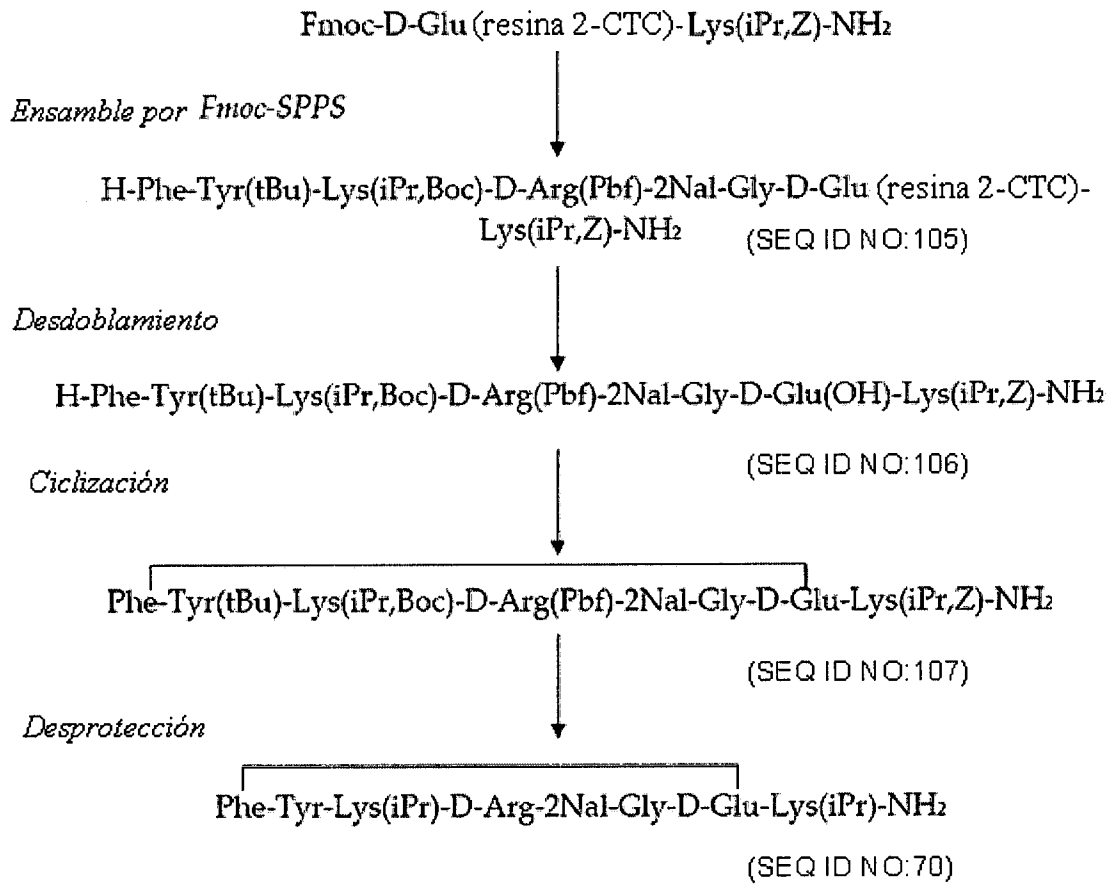
(SEQ ID NO: 98)

Esquema de reacción 12. Ciclización y eliminación Fmoc

Ejemplo 88

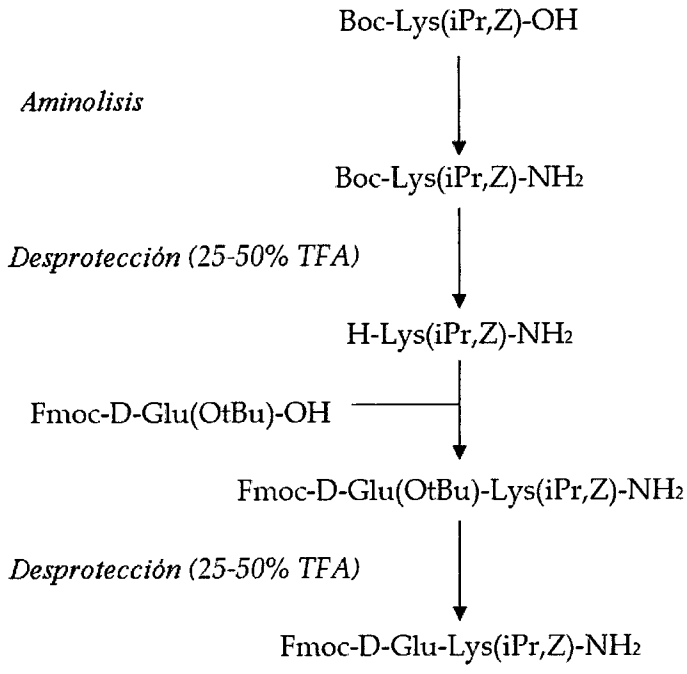
Síntesis Alternativa IV de ciclo[Phe-Tyr-Lys(iPr)-dArg-2Nal-Gly-dGlu]-Lys(iPr)-NH2 (SEQ ID NO:70)

El compuesto del Ejemplo 57 (SEQ ID NO:70) también se puede preparar sin el uso de un catalizador de paladio por el proceso sintético resumido en el Esquema de reacción 13 a continuación. El dipéptido Fmoc-DGlu-Lys(iPr,Z)-NH_2 se prepara primero en solución con una cadena lateral de ácido DGlutámico expuesto. El dipéptido se liga a una resina (resina PS de 2-clorotritilcloruro, 1% DVB (100-200 malla) CTC inestable de hiper-ácido (Senn Chemicals USA Inc., San Diego, CA; número de catalogo 40207), y el producto final de péptidos luego se sintetiza en esta resina por medio de síntesis Fmoc estándar como se describe arriba. La eliminación selectiva del péptido de la resina CTC permite solamente la cadena lateral de ácido DGlutámico para reaccionar con la terminación N del péptido en solución y generar el producto de péptido cíclico. Posteriormente, las cadenas laterales restantes se desdoblan con 95% de TFA u otro ácido grande.



Esquema de reacción 13. Esquema de reacción sintético general de Fmoc-DGlu-Lys(iPr,Z)-NH_2

El dipéptido Fmoc-DGlu-Lys(iPr,Z)-NH_2 primero se prepara como se muestra a continuación (Esquema de reacción 14):



Esquema de reacción 14. Preparación de Fmoc-DGlu-Lys(iPr,Z)-NH_2

El Boc-Lys(iPr,Z)-OH se hace reaccionar con NMM y IBCF en THF. Después de la adición de NH_4OH, los solventes se remueven por evaporación rotatoria y el producto se toma en acetato de etilo. La fase de acetato de etilo se lava ampliamente con 5% $NaHCO_3$ y luego con HCl 0.1 N, y luego se seca sobre sulfato de sodio anhidro. El sulfato de sodio se remueve por filtración y el acetato de etilo se remueve por evaporación a presión reducida. El Boc-Lys(iPr,Z)-NH_2 resultante se disuelve en DCM, y TFA se agrega. Una vez que la reacción ha avanzado hasta terminación, los solventes se remueven por evaporación rotatoria. El H-Lys(iPr,Z)-NH_2 luego se disuelve en DMF. El pH se ajusta hasta 8 con DIEA. En un recipiente separado, Fmoc-DGlu(OtBu)-OH, HBTU, y

HOBt se disuelven en DMF; DIEA se agrega para ajustar el pH hasta 8. Las dos soluciones se mezclan, y la reacción se monitorea por CLAR de fase inversa C18. El pH se monitorea y ajusta, donde sea necesario, con DIEA. Los solventes se remueven por evaporación rotatoria, y el producto se disuelve en acetato de etilo. La fase de acetato de etilo se lava ampliamente con $NaHCO_3$ al 5% y luego con HCl0.1 N, y luego se seca sobre sulfato de sodio anhidro. El sulfato de sodio se remueve por filtración y el acetato de etilo se remueve por evaporación a presión reducida. La evaporación rotatoria se continúa hasta que se forma un residuo seco. El Fmoc-DGlu(OtBu)-Lys(iPr,Z)-NH_2 resultante se disuelve en DCM, y TFA se agrega. Una vez que la reacción ha avanzado hasta terminación, los solventes se remueven por evaporación rotatoria. El producto sólido se obtiene por trituración con éter de dietilo. Después el precipitado se lava con éter, el producto se seca en un horno al vacío.

La síntesis del péptido de fase sólida del producto final se lleva a cabo como sigue. El Fmoc-DGlu(OtBu)-Lys(iPr,Z)-NH_2 se disuelve en DCM y se hace reaccionar con resina CTC en la presencia de DIEA en un recipiente de reacción. Después de 3 h, la resina-péptido se lava libre de reactivos con DCM y Z-OSu se agrega. El pH se monitorea y ajusta hasta pH 8-9 al agregar DIEA si es necesario. Después de 8 h, la resina-péptido se lava libre de reactivos con DCM, se transfiere a un contenedor de polipropileno, y se seca en un horno al vacío.

La resina-péptido protegida se ensambla usando química Fmoc como sigue. El ciclo de acoplamiento usado es: 1) Desbloqueo: tratamiento con 25% de piperidina en DMF; 2) ciclos de lavado con DMF, IPA y DMF nuevamente; 3) prueba Ninhidrina (cualitativo: si es positivo, se procesa para acoplar la etapa 4); 4) acoplamiento con 2 equivalentes de aminoácido Fmoc en presencia de HOBt /DIC en DMF; 5) ciclos de lavado con DMF; 6) prueba Ninhidrina (cualitativo: si es negativo, se procesa para el siguiente desbloqueo/ciclo de acoplamiento; si es positivo, se procesa para re-acoplar la etapa 7; si es positivo ligeramente, se procesa para acetilación de la etapa 10); 7) Re-acoplamiento (si se requiere), con 1 equivalente de aminoácido Fmoc en la presencia de HOBt, HBTU/DIEA en DMF; 8) ciclos de lavado con DMF; 9) prueba Ninhidrina (cualitativo: si es negativo, se procesa para el siguiente desbloqueo/ciclo de acoplamiento; si es positivo, se procesa para acetilación de la etapa 10); 10) acetilación (si se requiere) con 2% de anhídrido acético en 4% de DIEA en DMF; 11) ciclos de lavado (con DMF, IPA, y DMF nuevamente); 12) prueba Ninhidrina (cualitativo: si es positivo, se procesa para el siguiente desbloqueo/ciclo de acoplamiento). Después del ciclo de acoplamiento final, la resina-péptido (SEQ ID NO:105) se lava con éter y se seca bajo vacío.

La resina-péptido protegida se lava con DCM. El desdoblamiento del péptido lineal protegido completamente de

la resina se lleva a cabo con 2% de TFA en DCM seguido por filtración. Los solventes se remueven por evaporación rotatoria, y el péptido protegido completamente lineal (SEQ ID NO:106) se precipita por trituración con éter. El péptido lineal completamente protegido se transfiere a un contenedor de polipropileno y se seca en un horno al vacío.

El péptido lineal completamente protegido se cicliza en la presencia de PyBOP, HOBt, y DIEA en DMF. El pH se mantiene entre pH 7-8 por la adición de DIEA, si es necesario. Después la reacción se procesa para completarse, los solventes se remueven por evaporación rotatoria, y el producto se toma en acetato de etilo. La fase de acetato de etilo se lava ampliamente con $NaHCO_3$ al 5% y luego con HCl 0.1 N y solución NaCl saturada. Luego se seca sobre sulfato de sodio anhidro. El sulfato de sodio se remueve por filtración y el acetato de etilo se remueve por evaporación rotatoria a presión reducida. El péptido cíclico protegido (SEQ ID NO:107) se precipita por trituración con éter y se seca en un horno al vacío.

La desprotección se lleva a cabo en TFA:H_2O:TIS. Cuando la reacción se completa, los solventes se remueven por evaporación rotatoria y el péptido cíclico (SEQ ID NO:70) se precipita por trituración con éter y se seca en un horno al vacío. PM cal.: 1189.48; PM obs.: 1189.50.

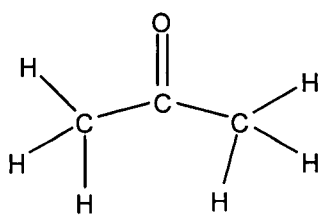
Ejemplo 89

Incorporación de las Etiquetas Isotópicas:

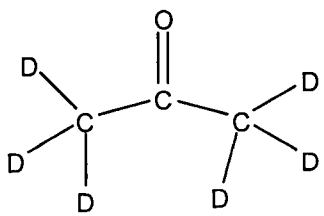
Síntesis de ciclo[Phe-Tyr-Lys(iPr-d_6)-DArg-2Nal-Gly-DGlu]-Lys(iPr-d_6)-NH_2

(SEQ ID NO:108)

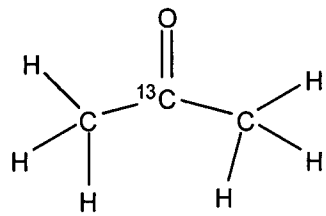
Partiendo de la acetona isotópicamente etiquetada tales como las acetonas etiquetadas con ^{13}C, ^{14}C, deuterio, o tritio como se muestran a continuación, los procesos de los Ejemplos 85-87 permiten un etiquetado isotópico específico del sitio de antagonistas del péptido cíclico CXCR4 para diversos estudios de formación de imágenes y farmacológicos. Las acetonas isotópicamente etiquetadas están comercialmente disponibles de diversas fuentes. Un ejemplo se da a continuación al usar acetona-d_6 para preparar un péptido que contiene 12 átomos de deuterio. El compuesto resultante, que difiere en peso molecular por 12 Da comparado con la contraparte no etiquetada, se diferencia fácilmente en espectros de masas y muestra una afinidad de receptor objetivo i´dentica.



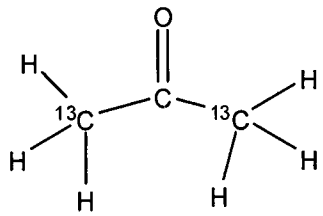
Acetona



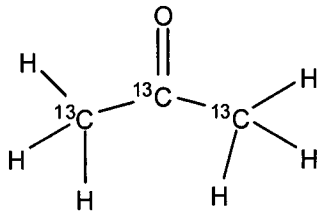
Acetona-d_6



Acetona-^{13}C



Acetona-2-^{13}C

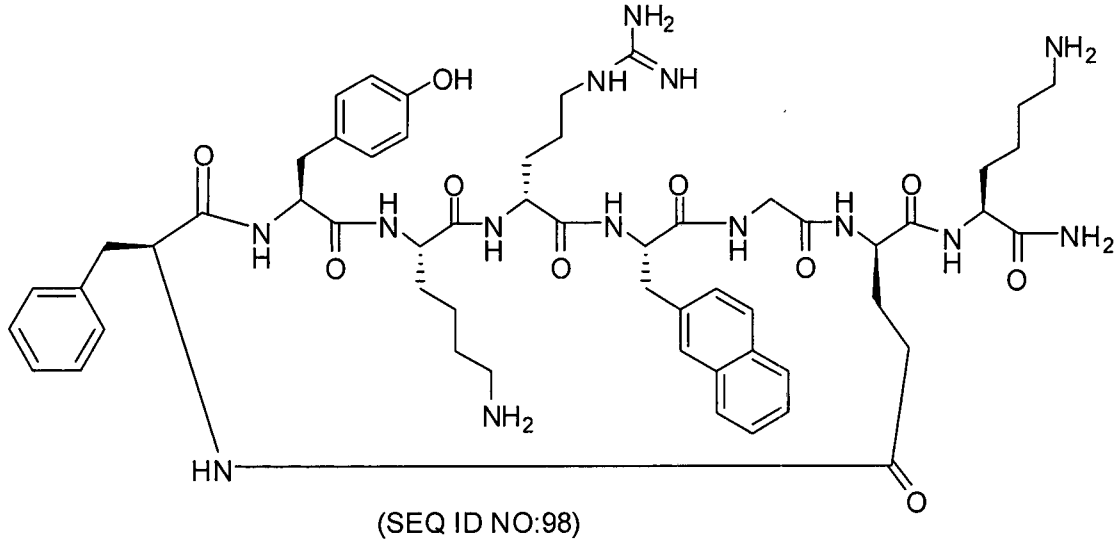


Acetona-3-^{13}C

Al usar cualquiera de los métodos en los Ejemplos 85-87, se pueden preparar y purificar precursores de péptidos cíclicos (SEQ ID NO:98, SEQ ID NO:99, etc.). La alquilación se lleva a cabo en una solución de ácido acético /acetona/metanol (1:1:4, v/v/v) a través de aminación reductora al usar cianoborohidruro de sodio como en el Esquema de Reacción 9, con la excepción de que la acetona estándar se reemplaza con la acetona etiquetada isotópicamente deseada. En el presente ejemplo, se usa deuterio acetona-d6.

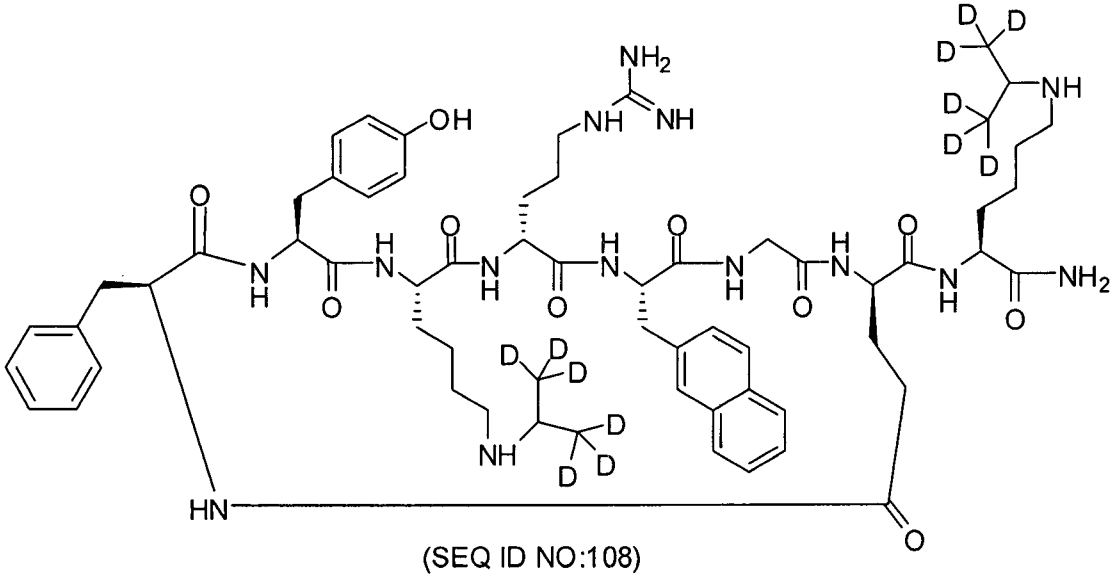
El péptido (97 mg) se disuelve en 15 mL de ácido acético /acetona-d_6/metanol (1:1:4, v/v/v). La concentración de péptidos puede variar significativamente sin afectar los resultados. Cinco equivalentes de cianoborohidruro de sodio se usan, y la reacción se completa normalmente dentro de 2 h a temperatura ambiente. La reacción se observa por CLAR y análisis espectral de masas. Después de que se termina la reacción, el desalado de la mezcla de reacción y la liofilización produce 90.5 mg de producto final (SEQ ID

NO:108) con una pureza de 99.9%. PM cal.: 1201.48; PM obs.: 1201.7.



(SEQ ID NO:98)

AcOH/D6-acetona/metanol
(1/1/4, v/v/v),
NaBH3CN in methanol, 5 eq



(SEQ ID NO:108)

Esquema de Reacción 15. Etiquetado con deuterio específico del sitio del antagonista CXCR4

El uso de cianoborohidruro de sodio isotópicamente etiquetado (tal como NaBD3CN, NaBT3C N, etc.) permite la incorporación de variaciones adicionales a los patrones de etiquetado específicos del sitio.

Las propiedades farmacológicas de los compuestos actuales se pueden determinar al emplear los ensayos abajo descritos.

Ensayo de Inhibición de Enlace de CXCR4 Humano/^{125}I-SDF-1α

El enlace de SDF-1 a CXCR4 es la primera etapa de activación de la trayectoria de señalización intracelular de CXCR4. Para determinar si un compuesto puede bloquear la interacción de SDF-1 y CXCR4, se emplean células de leucemia humana CCRF-CEM (ATCC CCL 119) que expresan el CXCR4 endógeno, en un ensayo de enlace de SDF-1α etiquetado con ^{125}I. El ensayo se efectúa en una placa de poliestireno sin tratar, con fondo en "U" de 96 pozos (Corning Incorporated, Costar, No. 3632). La solución amortiguadora del ensayo de enlace se prepara con medio RPMI 1640 (Gibco, Grand Island, NY) que contiene 10 mM HEPES, pH 7.5, y 0.2% BSA. Brevemente, 200 µL de mezclas de reacción que contienen 300

pM del ligando SDF (60 pM de ^{125}I-SDF-1α (Perkin Elmer) y 240 pM de SDF-1α frío (R&D Systems), concentraciones diferentes del compuesto de prueba en la solución amortiguadora de ensayo, 100,000 células humanas CCRF-CEM, y 0.5 mg de perlas SPA (perlas de aglutinina de germen de trigo; Amersham) se incuban a temperatura ambiente por 2 hr. Se cuentan luego las placas en un contador de luminiscencia y escintilación líquida 1450 (Wallac) en el modo SPA. Los antagonistas CXCR4 disminuyen la radioactividad enlazada en este ensayo en una forma dependiente de la dosis. La potencia inhibidora (K_i o IC_{50}) de un compuesto de prueba se calcula al usar el software GraphPad Prism, con base en la disminución dependiente de la dosis de la radioactividad de enlace.

Todos los compuestos antes ejemplificados muestran un valor promedio de K_i de alrededor de 7.5 nM o menos en este ensayo. Por ejemplo, el compuesto del Ejemplo 1 muestra un K_i promedio de 3.45 nM en este ensayo. Muchos de estos compuestos muestran un valor promedio K_i entre alrededor de 0.2 nM y alrededor de 1 nM. Por ejemplo, el compuesto del Ejemplo 50 muestra un valor promedio de K_i de 0.285 nM en este ensayo. Otros compuestos muestran un valor promedio K_i menor de alrededor de 0.2 nM. Por ejemplo, el compuesto del

Ejemplo 75 muestra un valor promedio de K_i de 0.096 nM en este ensayo.

Ensayo de Quimiotaxis

La interacción CXCR4/SDF-1 regula la migración (quimiotaxis) de células que llevan CXCR4 sobre su superficie. Para determinar las actividades antagonista y celular de un compuesto de prueba, se emplea un ensayo de quimiotaxis que usa células U937 de linfoma histiocítico humano (ATCC CRL 1593) que expresan CXCR4 endógeno. Brevemente, células U937, que crecen en medio DMEM (Gibco, Grand Island, NY) que contiene 10% FBS, 1% MEM de piruvato de sodio (Gibco), 1% MEM de aminoácidos no esenciales (Gibco), y 1% GlutaMAX 1 (Gibco), se cosechan y se lavan una vez con solución amortiguadora de ensayo de quimiotaxis preparada con medio 1x RPMI (Gibco) que contiene 10 mM HEPES, pH 7.5, y 0.3% BSA. Después del lavado, las células se vuelven a suspender en solución anmortiguadora de ensayo a una concentración de 5 x 10^6 células/mL. El ensayo se efectuó en una placa de 96 pozos ChemoTx (NeuroProbe) según las instrucciones del fabricante. Generalmente, 50 µL de mezcla de células con o sin compuestos de prueba se colocan en placas sobre la cámara superior, y 30 µL de SDF-1α (R&D Systems, 10 ng/mL) preparados en solución amortiguadora de quimiotaxis 1 x se agregan a la cámara inferior. Después del

ensamble, se incuba la placa durante 2.5 hr a 37°C bajo 5% de CO_2. Después de la incubación, se agregan 5 µL de CellTiter 96 AQ (Promega, Madison, WI) dentro de la cámara inferior. La placa luego se incuba durante 60 min a 37°C, y se detectan las células migradas al medir la absorbancia a 492 nm con un Lector de Microplacas Tecan Spectrafluor Plus (Salzburg, Austria). Los antagonistas CXCR4 inhiben la migración celular, al reducir la lectura de absorbancia. La potencia inhibidora (IC_{50}) de un compuesto de prueba en este ensayo se calcula al usar el software GraphPad Prism, con base en la disminución dependiente de la dosis de absorbancia a 492 nm.

La mayoría de los compuestos antes ejemplificados muestran un valor promedio de IC_{50} de alrededor de 60 nM o menos en este ensayo. Muchos de estos compuestos muestran un valor promedio de IC_{50} de alrededor de 6 nM o menos, por ejemplo, el compuesto de Ejemplo 19 muestra un valor promedio IC_{50} de 2.05 nM en este ensayo. Muchos de estos compuestos muestran un valor promedio IC_{50} de alrededor de 0.6 nM o menos, por ejemplo, el compuesto de Ejemplo 50 muestra un valor promedio IC_{50} de 0.171 nM en este ensayo.

Ensayos de Selectividad de Enlace del Receptor de Quimiocinas

La selectividad de enlace de los compuestos actuales para el receptor CXCR4 comparada con aquella de los otros

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receptores de quimiocina, tales como los CCR1, CCR2, CXCR2, o CXCR3 humanos, y otros receptores acoplados a la proteína G, se puede evaluar en células transfectadas con ácido nucleicos que codifica y expresa tales receptores, o en células en las cuales tales receptores se expresan endógenamente. Las células enteras o fragmentos de membrana se pueden usar para evaluar la competencia de compuestos de prueba con los ligandos respectivos para estos receptores en una forma similar a aquella antes descrita para el ensayo de inhibición de enlace CXCR4/^{125}I-SDF-1α.

Por ejemplo, el compuesto del Ejemplo 57a muestra un valor K_i mayor que 73,000 nM en un ensayo de enlace de ligandos al usar el receptor de quimiocina humana CXCR2.

Movilización de Neutrófilos y de Células de Glóbulos Blancos Inducida por el Compuesto en Ratones C57BL/6

Las células madre dentro de la médula ósea mantienen activamente la producción continua de todos los linajes de células maduras sanguíneas a lo largo de la vida. La médula ósea es el sitio primario para la producción de células de glóbulos blancos (WBC)/neutrófilos y liberarlas a la circulación. El eje CXCR4/SDF-1 parece ser crítico para la retención y liberación de WBCs, neutrófilos, y células hematopoyéticas progenitoras en la médula ósea, y la

interrupción de la interacción de CXCR4/SDF-1 en la médula ósea conduce a un incremento de estas células en la sangre periférica. Se puede usar un modelo de movilización de neutrófilos/WBC de ratón de corto plazo para determinar la actividad de modulación del objetivo *in vivo* de un compuesto de prueba. Brevemente, ratones hembras C57BL/6 libres de patógenos de 5-6 semanas de edad (Taconic) se alojan durante al menos una semana previo al ensayo. Se les permitió un acceso continuo a los animales a alimento esterilizado para roedores y agua acidificada. Grupos de 5 ratones se inyectaron subcutáneamente con compuestos de prueba en solución salina, o con el control en solución salina, y luego se sacrificaron por asfixia con CO_2 y dislocación cervical en diversos puntos de tiempo posteriores a la administración del compuesto. La sangre periférica se recolecta por punción cardiaca al usar jeringas y tubos recubiertos con EDTA. Se efectúa un análisis completo de glóbulos en un analizador de hematología Hemavet Mascot (Drew Scientific Group, Dallas, TX). Se registran los WBCs, neutrófilos, y linfocitos totales en la sangre periférica. Los antagonistas efectivos de CXCR4 administrados subcutáneamente a ratones se incrementan con los conteos de neutrófilos y WBC en la sangre periférica en comparación con el control salino.

Un número importante de compuestos arriba ejemplificados muestra una relación promedio de neutrófilo (relación del

incremento de neutrófilo en el grupo de tratamiento vs. incremento de neutrófilo en el grupo de control de solución salina), medido 3 horas después de la administración del compuesto, mayor que alrededor de 2 en este ensayo. Por ejemplo, el compuesto de Ejemplo 39 muestra una relación de valor promedio de neutrófilo de 4.6 a una dosis de 5 mg/kg en este ensayo.

Actividad Anti-Tumor en un Modelo de Xenoinjerto SCID/Namalwa

La interacción SDF-1/CXCR4 parece jugar un papel importante en las etapas múltiples de la tumorigénesis, incluyendo el crecimiento de tumor, invasión, angiogénesis, y metástasis. Para evaluar la actividad anti-tumor *in vivo* de un compuesto de prueba, se emplea un modelo de xenoinjerto de tumor al usar ratones NOD/SCID (Jackson Laboratories) y células Namalwa humanas de linfoma que no es Hodgkin (ATCC CRL 1432). Brevemente, 200,000 células Namalwa mezcladas con matrigel (1:1) se implantan subcutáneamente dentro del flanco trasero de los animales. Las células de tumor crecen como tumores sólidos, las dimensiones de las cuales se pueden monitorear y medir usando un calibrador. Para determinar la eficacia *in vivo* de un compuesto de prueba en este modelo, se pueden tratar animales (10/grupo) con dosis diferentes de los compuestos de prueba disueltas en solución salina o PBS, comenzando 48 horas después de la implantación de células de

tumor. Los compuestos se dosifican subcutáneamente, y volumen del tumor y peso corporal se determinan cada 2 o 3 días. Los estudios generalmente duran 3-4 semanas, dependiendo del crecimiento del tumor. La actividad de crecimiento anti-tumor de un compuesto de prueba se determina por la reducción en porcentaje del volumen del tumor en grupos de tratamiento en comparación con el volumen del tumor en grupos de control tratados solo con el vehículo.

Varios compuestos antes ejemplificados, por ejemplo el compuesto de Ejemplo 26, inhiben significativamente el crecimiento del tumor en este ensayo cuando se administran a 1 mg/kg de BID.

Las propiedades farmacológicas tales como la biodisponibilidad del compuesto, estabilidad metabólica *in vivo*, y propiedades farmacocinéticas/farmacodinámicas se pueden determinar por métodos bien conocidos en el arte del desarrollo de fármacos. Los compuestos preferidos de la presente invención muestran una alta biodisponibilidad cuando se administran subcutáneamente. Tales compuestos ejemplificados en la presente, muestran una biodisponibilidad cercana a 100% en ratas, por ejemplo el compuesto del Ejemplo 44. Los compuestos preferidos también muestran una buena estabilidad metabólica *in vivo*. Por ejemplo, no se observan metabolitos detectables en el plasma y orina de perro y mono hasta las 24 horas después de la administración del compuesto

del Ejemplo 57a. Los compuestos preferidos también muestran propiedades farmacocinéticas/farmacodinámicas favorables que permiten una dosificación conveniente. Por ejemplo, en ratones, la vida media (T1/2) del compuesto del Ejemplo 58 es alrededor de 3 horas. Con respecto a las propiedades farmacodinámicas, los compuestos preferidos inducen una movilización prolongada de neutrófilos y células de glóbulos blancos en ratones. Por ejemplo, el compuesto del Ejemplo 25 induce un incremento importante de neutrófilos y células de glóbulos blancos en sangre periférica por al menos 6 horas después de una administración subcutánea de dosis sencilla a 5 mg/kg en ratones.

REIVINDICACIONES

1. Un péptido ciclizado por lactamas de fórmula I:

R_1 - ciclo[X_1 - Tyr - X_3 - DArg - 2Nal - Gly - X_7] - X_8 - X_9 - X_{10} - R_2

(I)

(SEQ ID NO:1)

caracterizado porque:

a) la lactama se forma por un enlace amida entre el grupo amino de cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de (D/L)Agl/Glu, Dab/Glu, y Dap/Glu, y R_1 es Ac o n-hexanoilo; o

b) la lactama se forma por un enlace amida entre el grupo carboxilo de cadena lateral de X_1 y el grupo amino de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Asp/(D/L)Agl, Asp/Dab, Asp/Dap, Glu/(D/L)Agl, Glu/Dab, Glu/Dap, Glu/DDap, y Glu/Lys, y R_1 es Ac o Bz, o en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de succinilo/(D/L)Agl, succinilo/Dab, succinilo/Dap, succinilo/Lys, y succinilo/Orn, y R_1 está ausente; o

c) la lactama se forma por un enlace amida entre el grupo α-amino de X_1 y el grupo carboxilo de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Ala/Glu, Ala/DGlu, DAla/Glu, DAla/DGlu, Dap(Ac)/Glu, Gly/Asp, Gly/Glu, Gly/DGlu, Leu/Glu, Leu/DGlu, Lys/DGlu, Lys(Ac)/Glu, 2Nal/Glu, Phe/Glu, Phe/DGlu, DPhe/Glu, y DPhe/DGlu, y R_1 está ausente; o

d) la lactama se forma por un enlace amida entre un grupo amino no α, sin cadena lateral de X_1 y el grupo carboxilo de cadena lateral de X_7, en donde X_1 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de β-Ala/Asp, β-Ala/Glu, 5-amino-valerilo/Asp, 5-aminovalerilo/Glu, 4-AMB/Glu, 4-AMPA/Asp, y 4-AMPA/Glu, y R_1 está ausente; o

e) la lactama se forma por un enlace amida entre el grupo α-amino de X_2 y el grupo carboxilo de cadena lateral de X_7, en donde X_2 y X_7 son, respectivamente, un par seleccionado del grupo que consiste de Tyr/Asp, Tyr/Glu, y Tyr/DGlu, y R_1 y X_1 están cada uno ausentes;

R_1 es un sustituyente sobre el grupo α-amino de X_1 cuando X_1 contiene un grupo α-amino y el grupo α-amino no es un constituyente del enlace amida de la lactama, seleccionado del grupo que consiste de Ac, Bz, y n-hexanoilo, o está

ausente, en donde X_1 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, y Glu;

X_1 es seleccionado del grupo que consiste de (D/L)Agl, Ala, β-Ala, DAla, 5-aminovalerilo, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, Gly, Leu, Lys, Lys(Ac), 2Nal, Phe, DPhe, y succinilo, o está ausente;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, DDap, Glu, DGlu, Lys, y Orn;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, DArg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, 2Nal, D2Nal, y DPhe, o está ausente;

X_{10} es 2Nal, o está ausente;

en donde cuando X_8 está ausente, X_9 y X_{10} están cada uno ausentes, y cuando X_9 está ausente, X_{10} está ausente, y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt, o

una sal farmacéuticamente aceptable del mismo.

2. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de la reivindicación 1, caracterizado porque:

R_1 es seleccionado del grupo que consiste de Ac y Bz, o está ausente;

X_1 es seleccionado del grupo que consiste de β-Ala, 4-AMB, 4-AMPA, Asp, Dab, Dap, Dap(Ac), Glu, 2Nal, Phe, y succinilo, o está ausente;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de Asp, Dab, Dap, Glu, DGlu, Lys, y Orn;

X_8 es seleccionado del grupo que consiste de Arg y Lys, o está ausente;

X_9 está ausente;

X_{10} está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

3. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de la reivindicación 1, caracterizado porque:

R_1 es seleccionado del grupo que consiste de Ac y Bz, o está ausente;

X_1 es seleccionado del grupo que consiste de DAla, 5-aminovalerilo, 4-AMPA, Asp, Glu, Leu, Lys(Ac), Phe, DPhe, y succinilo;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dab, Dap, DDap, Glu, y DGlu;

X_8 es seleccionado del grupo que consiste de Arg, DArg, y Lys, o está ausente;

X_9 está ausente;

X_{10} está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

4. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de la reivindicación 1, caracterizado porque:

R_1 es seleccionado del grupo que consiste de Ac, Bz, y n-hexanoilo, o está ausente;

X_1 es seleccionado del grupo que consiste de (D/L)Agl, Ala, β-Ala, Asp, Dap, Glu, Gly, Lys, y Phe;

X_3 es seleccionado del grupo que consiste de Arg, Lys, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dap, Glu, y DGlu;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, 2Nal, D2Nal, y DPhe, o está ausente;

X_{10} es 2Nal, o está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

5. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de la reivindicación 1, caracterizado porque:

R_1 es seleccionado del grupo que consiste de Ac y Bz, o está ausente;

X_1 es seleccionado del grupo que consiste de Ala, 5-aminovalerilo, Asp, Glu, Gly, Phe, DPhe, y succinilo;

X_3 es seleccionado del grupo que consiste de Arg, Lys(iPr), y Lys(Me_2);

X_7 es seleccionado del grupo que consiste de (D/L)Agl, Asp, Dap, Glu, y DGlu;

X_8 es seleccionado del grupo que consiste de β-Ala, Arg, Gly, Lys, Lys(iPr), y Orn, o está ausente;

X_9 es seleccionado del grupo que consiste de Gly, D2Nal, y DPhe, o está ausente;

X_{10} is 2Nal, o está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

6. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de la reivindicación 1, 4, o 5, caracterizado porque:

X_1 es seleccionado del grupo que consiste de Gly y Phe;

X_3 es Lys(iPr); and

X_7 es DGlu.

7. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de la reivindicación 5, caracterizado porque:

R_1 está ausente;

X_1 es seleccionado del grupo que consiste de Gly y Phe;

X_3 es Lys(iPr);

X_7 es DGlu;

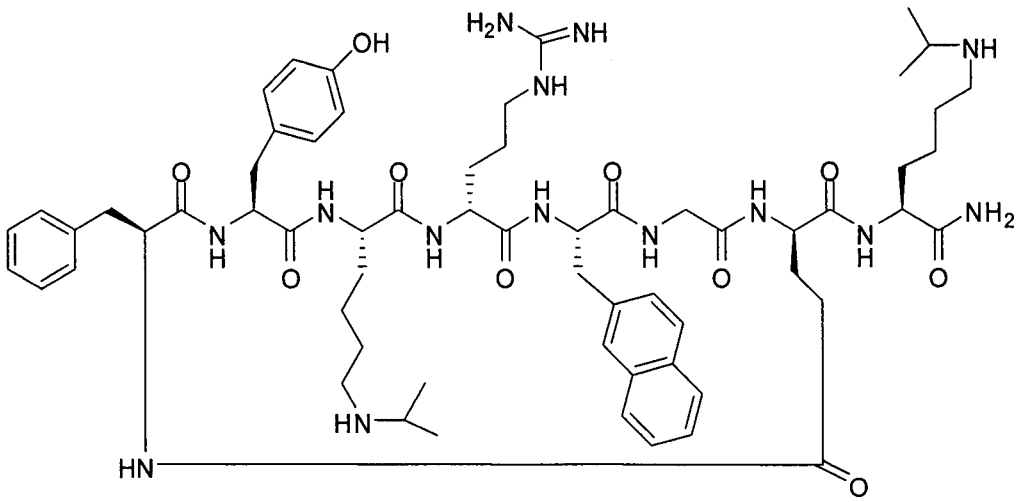
X_8 es seleccionado del grupo que consiste de Arg y Lys(iPr), o está ausente;

X_9 está ausente;

X_{10} está ausente; y

R_2 es seleccionado del grupo que consiste de NH_2 y NHEt.

8. El péptido ciclizado por lactamas de la fórmula:



(SEQ ID NO:70)

o una sal farmacéuticamente aceptable del mismo.

9. El péptido ciclizado por lactamas de la reivindicación 8, caracterizado porque la sal farmacéuticamente aceptable es una sal de ácido acético.

10. Una composición farmacéutica, caracterizada porque comprende un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de cualesquiera de las reivindicaciones 1-9, y un portador, diluyente, o excipiente farmacéuticamente aceptable.

11. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de cualesquiera de las reivindicaciones 1-9, para uso en terapia.

12. El péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de cualesquiera de las reivindicaciones 1-9, para el tratamiento de artritis reumática, fibrosis pulmonar, infección por VIH, o un cáncer seleccionado del grupo que consiste de cáncer de mama, cáncer pancreático, melanoma, cáncer de próstata, cáncer de riñón, neuroblastoma, linfoma que no es Hodgkin, cáncer de pulmón, cáncer ovárico, cáncer colorectal, mieloma múltiple, glioblastoma multiforme, y leucemia linfocítica crónica.

13. El uso de un péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de cualesquiera de las reivindicaciones 1-9, para la manufactura de un medicamento para el tratamiento de artritis reumática, fibrosis pulmonar, infección por VIH, o un cáncer seleccionado del grupo que

consiste de cáncer de mama, cáncer pancreático, melanoma, cáncer de próstata, cáncer de riñón, neuroblastoma, linfoma que no es Hodgkin, cáncer de pulmón, cáncer ovárico, cáncer colorectal, mieloma múltiple, glioblastoma multiforme, y leucemia linfocítica crónica.

14. Un método para el tratamiento de artritis reumática, fibrosis pulmonar, infección por VIH, o un cáncer seleccionado del grupo que consiste de cáncer de mama, cáncer pancreático, melanoma, cáncer de próstata, cáncer de riñón, neuroblastoma, linfoma que no es Hodgkin, cáncer de pulmón, cáncer ovárico, cáncer colorectal, mieloma múltiple, glioblastoma multiforme, y leucemia linfocítica crónica, caracterizado porque comprende administrar a un paciente que necesita del mismo, una cantidad efectiva de péptido ciclizado por lactamas o sal farmacéuticamente aceptable del mismo de cualesquiera de las reivindicaciones 1-9.

RESUMEN DE LA INVENCION

Se proporcionan antagonistas CXCR4 de péptidos ciclizados por lactamas útiles en el tratamiento de cánceres, artritis reumática, fibrosis pulmonar, e infección por VIH.

LISTADO DE SECUENCIAS

<110> Eli Lilly y Company

<120> Antagonistas cíclicos de péptido CXCR4

<130> X17256_WO

<150> 60/940996
<151> 2007-05-31

<150> 60/940802
<151> 2007-05-30

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<220>
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<222> (8)..(8)
<223> Xaa = beta-Ala, Arg, d isómero Arg, Gly, Lys, Lys(iPr), Orn o está ausente, y cuando ausente, Xaa en las posiciones 9 y 10 también están
ausentes

<220>
<221> ASPECTO_DIVERSO
<222> (9)..(9)
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<223> Amidado como NHEt

<400> 58

Gly Tyr Xaa Arg Xaa Gly Glu Xaa
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<400> 59

Xaa Tyr Xaa Arg Xaa Gly Glu Arg
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<400> 60

Xaa Tyr Xaa Arg Xaa Gly Glu
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<223> AMIDACIÓN

<400> 61

Ala Tyr Xaa Arg Xaa Gly Glu
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Ala Tyr Xaa Arg Xaa Gly Glu
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<400> 63

Ala Tyr Xaa Arg Xaa Gly Glu

1 5

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Ala Tyr Xaa Arg Xaa Gly Glu
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Leu Tyr Xaa Arg Xaa Gly Glu
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<400> 66

Leu Tyr Xaa Arg Xaa Gly Glu
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<400> 67

Phe Tyr Xaa Arg Xaa Gly Glu
1 5

<210> 68
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<400> 68

Phe Tyr Xaa Arg Xaa Gly Glu
1 5

<210> 69
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<400> 69

Phe Tyr Xaa Arg Xaa Gly Glu
1 5

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<400> 70

Phe Tyr Xaa Arg Xaa Gly Glu Xaa
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<210> 71
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<400> 71

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1 5

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<400> 72

Phe Tyr Xaa Arg Xaa Gly Glu Arg
1 5

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<400> 73

Phe Tyr Xaa Arg Xaa Gly Glu
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<400> 74

Phe Tyr Xaa Arg Xaa Gly Glu Xaa
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<400> 75

Phe Tyr Xaa Arg Xaa Gly Glu Xaa
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<400> 76

Ala Tyr Xaa Arg Xaa Gly Glu
1 5

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<400> 77

Xaa Tyr Xaa Arg Xaa Gly Glu
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<400> 78

Phe Tyr Xaa Arg Xaa Gly Glu
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<400> 79

Phe Tyr Xaa Arg Xaa Gly Glu
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Gly Tyr Xaa Arg Xaa Gly Glu Gly Xaa
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<400> 81

Gly Tyr Xaa Arg Xaa Gly Glu Xaa Xaa

1 5

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<400> 82

Xaa Tyr Arg Arg Xaa Gly Glu Arg
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<223> Xaa = 2Nal

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<400> 83

Xaa Tyr Arg Arg Xaa Gly Asp Arg
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<223> Xaa = 2Nal

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<400> 84

Tyr Arg Arg Xaa Gly Glu Arg
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Tyr Arg Arg Xaa Gly Asp Arg
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<210> 86
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PATENT COOPERATION TREATY

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ELI LILLY AND COMPANY
Patent Division

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
ELI LILLY AND COMPANY
Attn. Cohen, Charles E.
Patent Division
P.O. Box 6288
Indianapolis IN 46206-6288
ETATS-UNIS D'AMERIQUE

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

(PCT Rule 44.1)

Applicant's or agent's file reference X17256	Date of mailing *(day/month/year)* 12/11/2008
International application No. PCT/US2008/064177	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant ELI LILLY AND COMPANY	International filing date *(day/month/year)* 20/05/2008

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 the notes on the accompanying sheet.

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 the 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**

   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 as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   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. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   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.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Katrin Sommermeyer

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Volume I/A, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1–10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

If a demand for international preliminary examination is made, the written opinion of the International Searching Authority will, except in certain cases where the International Preliminary Examining Authority did not act as International Searching Authority and where it has notified the International Bureau under Rule 66.1*bis*(b), be considered to be a written opinion of the International Preliminary Examining Authority. If a demand is made, the applicant may submit to the International Preliminary Examining Authority a reply to the written opinion 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 (Rule 43*bis*.1(c)).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide*, Volume II.

PATENT COOPERATION TREATY

PCT

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.
X17256		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2008/064177	20/05/2008	30/05/2007
Applicant		
ELI LILLY AND COMPANY		

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 5 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. [ ] **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(b), 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. _____
      [ ] 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/US2008/064177

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b 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. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] on paper

   [X] in electronic form

   c. time of filing/furnishing

   [X] contained in the international application as filed

   [X] filed together with the international application in electronic form

   [ ] furnished 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/US2008/064177

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P31/18 A61K38/12 C07K7/54

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)
A61P 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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	UEDA SATOSHI ET AL: "Structure-activity relationships of cyclic peptide-based chemokine receptor CXCR4 antagonists: disclosing the importance of side-chain and backbone functionalities." JOURNAL OF MEDICINAL CHEMISTRY 25 JAN 2007, vol. 50, no. 2, 25 January 2007 (2007-01-25), pages 192-198, XP009107403 ISSN: 0022-2623 the whole document	
A	JP 2004 196769 A (ORPHAN LINK KK; FUJII NOBUTAKA; STEPHAN C PIPER) 15 July 2004 (2004-07-15) page 3; claims	

-/--

[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 document 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
21 October 2008	12/11/2008

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	Vogt, Titus

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/064177

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAMAMURA HIROKAZU ET AL: "DEVELOPMENT OF ANTI-HIV AGENTS TARGETING DYNAMIC SUPRAMOLECULAR MECHANISM: ENTRY AND FUSION INHIBITORS BASED ON CXCR4/CCR5 ANTAGONISTS AND GP41-C34-REMODELING PEPTIDES" CURRENT HIV RESEARCH, BENTHAM SCIENCE PUBLISHERS, HILVERSUM, NL, vol. 3, no. 4, 1 October 2005 (2005-10-01), pages 289-301, XP008077509 ISSN: 1570-162X the whole document	
P,A	WO 2007/096662 A (TU MUENCHEN [DE]; JONES NICHOLAS ANDREW [GB]; WESTER HANS JURGEN [DE];) 30 August 2007 (2007-08-30) claims	
P,A	TAMAMURA HIROKAZU ET AL: "Development of low molecular weight CXCR4 antagonists by exploratory structural tuning of cyclic tetra- and pentapeptide-scaffolds towards the treatment of HIV infection, cancer metastasis and rheumatoid arthritis." CURRENT MEDICINAL CHEMISTRY 2007, vol. 14, no. 1, 2007, pages 93-102, XP009107441 ISSN: 0929-8673	
P,A	GRANDE FEDORA ET AL: "Small molecules anti-HIV therapeutics targeting CXCR4." CURRENT PHARMACEUTICAL DESIGN 2008, vol. 14, no. 4, 2008, pages 385-404, XP009107439 ISSN: 1873-4286	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/064177

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2004196769 A	15-07-2004	NONE	
WO 2007096662 A	30-08-2007	NONE	

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

X17256

PCT

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)

30 MAR 2009

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/US2008/064177	20.05.2008	30.05.2007

International Patent Classification (IPC) or both national classification and IPC
INV. A61P31/18 A61K38/12 C07K7/54

Applicant
ELI LILLY AND COMPANY

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 or 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 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Vogt, Titus Telephone No. +49 89 2399-8477

Form PCT/ISA/237 (Cover Sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2008/064177

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 and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in electronic form.

   ☐ furnished 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 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.

5. Additional comments:

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	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

V Reasoned Statement.

Subject matter of the present application.

The present application is directed to the provision of cyclic peptide antagonists of the CXC receptor 4, and the pharmaceutical use thereof for the treatment of CXCR4 related diseases, such as cancer and HIV.

Cited prior art documents (Rule 64(1) PCT).

D1: UEDA ET AL. (Jan. 2007) J. MED. CHEM. 50, 192-198.
D2: JP 2004 196769 A.
D3: TAMAMURA ET AL. (2005) CURR. HIV RESEARCH 3, 289-301.
D4: WO 2007/096662 A.
D5: TAMAMURA ET AL. (2007) CURR. MED. CHEM. 14, 93-102.
D6: GRANDE ET AL. (2008) CURR. PHARM. DESIGN 14, 385-404.

D4, D5 and D6 do not form part of the prior art under Rule 64(1) PCT.

Novelty and Inventive step (Art. 33(2,3) PCT).

The novelty and inventive step of the presently claimed subject matter are acknowledged for the following reasons.

The presently claimed subject matter appears to be based on the cyclic pentapeptide FC131 (cyclo[D-Tyr^1-Arg^2-Arg^3-Nal^4-Gly^5]), and differs therefrom in that it comprises two more amino acids in the ring and in that it comprises a L-Tyr^1 and a D-Arg^3.

The addition of two amino acids to the ring to create a cyclic lactam appears standard practice in the art and can in itself not confer an inventive step to the claimed subject matter. The presently claimed subject matter however appears to deviate from the general line followed in the prior art, since these documents all disclose the importance of the D-Tyr^1 and L-Arg^3 (see for instance: Table 1 of D1). In other words the prior art seems to point away from the present solution. Moreover, the applicant indicated that preliminary tests showed good results for some of the prepared compounds (cf. p. 68-72).

Industrial applicability (Art. 33(4) PCT).

The subject matter of claims 1-14 meets the requirement of industrial applicability.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/064177

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P31/18 A61K38/12 C07K7/54

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)
A61P 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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	UEDA SATOSHI ET AL: "Structure-activity relationships of cyclic peptide-based chemokine receptor CXCR4 antagonists: disclosing the importance of side-chain and backbone functionalities." JOURNAL OF MEDICINAL CHEMISTRY 25 JAN 2007, vol. 50, no. 2, 25 January 2007 (2007-01-25), pages 192-198, XP009107403 ISSN: 0022-2623 the whole document	
A	JP 2004 196769 A (ORPHAN LINK KK; FUJII NOBUTAKA; STEPHAN C PIPER) 15 July 2004 (2004-07-15) page 3; claims	

-/--

[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 document 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
21 October 2008	12/11/2008

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	Vogt, Titus

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/064177

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAMAMURA HIROKAZU ET AL: "DEVELOPMENT OF ANTI-HIV AGENTS TARGETING DYNAMIC SUPRAMOLECULAR MECHANISM: ENTRY AND FUSION INHIBITORS BASED ON CXCR4/CCR5 ANTAGONISTS AND GP41-C34-REMODELING PEPTIDES" CURRENT HIV RESEARCH, BENTHAM SCIENCE PUBLISHERS, HILVERSUM, NL, vol. 3, no. 4, 1 October 2005 (2005-10-01), pages 289-301, XP008077509 ISSN: 1570-162X the whole document	
P,A	WO 2007/096662 A (TU MUENCHEN [DE]; JONES NICHOLAS ANDREW [GB]; WESTER HANS JURGEN [DE];) 30 August 2007 (2007-08-30) claims	
P,A	TAMAMURA HIROKAZU ET AL: "Development of low molecular weight CXCR4 antagonists by exploratory structural tuning of cyclic tetra- and pentapeptide-scaffolds towards the treatment of HIV infection, cancer metastasis and rheumatoid arthritis." CURRENT MEDICINAL CHEMISTRY 2007, vol. 14, no. 1, 2007, pages 93-102, XP009107441 ISSN: 0929-8673	
P,A	GRANDE FEDORA ET AL: "Small molecules anti-HIV therapeutics targeting CXCR4." CURRENT PHARMACEUTICAL DESIGN 2008, vol. 14, no. 4, 2008, pages 385-404, XP009107439 ISSN: 1873-4286	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/064177

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2004196769 A	15-07-2004	NONE	
WO 2007096662 A	30-08-2007	NONE	

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 09-135750- -00000-0000

Fecha: 2009-11-27 16:32:24 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 465

dustria y Comercio
UPERINTENDENCIA

REQUISITOS MÍNIMOS PARA ADMISIÓN A TRAMITE PCT

PATENTE DE INVENCIÓN [X] MODELO DE UTILIDAD [ ]

- Indicación de que se solicita la concesión de una patente [X]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [X]
- Descripción de la invención [X]
- Dibujos de ser estos pertinentes [X]
- Comprobante de pago de las tasas establecidas [X]

COMPLETA [X] INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- Indicación de que se solicita el registro de Diseño Industrial [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica del esquema trazado [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

Eliana Ubon
Firma Responsable

Fecha 27-11-09

Carrera 13 No. 27-00 Piso 5,7y10
Conmutador: 3 82 0840
Fax: 3505220
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá D.C. - Colombia

Industria y Comercio
SUPERINTENDENCIA

Bogotá,
2020

Doctor(a)
OLARTE GARCIA CARLOS REYNALDO
Apoderado(a) y/o Representante de
ELI LILLY COMPANY

REFERENCIA	Radicación No.	9 135750
	Solicitud internacional	PCT/US2008/064177
	Fecha inicio fase nacional	30/12/2009
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	398

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante, conforme a lo establecido por el artículo 26 literal k) de la Decisión 486 de la Comisión de la Comunidad Andina. Cuando se trate de documentos otorgados en el extranjero, estos deberán legalizarse de conformidad con lo dispuesto por los artículos 259 y 260 del Código de Procedimiento Civil. Cuando se trate de las declaraciones a que hace referencia la Regla 4.17 ii) del Tratado de Cooperación en Materia de Patentes PCT., el alcance de las tales declaraciones se determinará según indiquen claramente una cesión de derechos del inventor al solicitante o su causante, de conformidad con lo dispuesto en el numeral 5.2.15 del Capítulo Quinto, Título X de la Circular Única de Superintendencia de Industria y Comercio.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

JESUS FERNEL GARCIA GARCIA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista de fecha 15 ENE. 2010
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