Industria y Comercio

SUPERINTENDENCIA

DELEGATURA DE PROPIEDAD INDUSTRIAL

DIRECCIÓN DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-252979- -00000-0000

Fecha: 2013-10-24 16:34:40 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 4

SOLICITUD DE PAT

INVENCIÓN

TITULO: PROCESOS PARA LA ELABORACIÓN DE DEPSIPÉPTIDOS MACROCÍCLICOS Y NUEVOS INTERMEDIARIOS

SOLICITANTE: NOVARTIS AG.

DIRECCIÓN: Lichtstrasse 35, CH-4056 Basel, Suiza

APODERADO: CARLOS R. OLARTE

BOGOTÁ: 24 de Octubre de 2013

P2013/000171

OLARTEMOURE

OLARTE MOURE & ASOCI/

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-252979- -00000-0000

Fecha: 2013-10-24 16:34:40 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 4

PETITORIO - FORMULARIO ÚN
PATENTE PCT
ENTRADA EN FASE NACIONAL **P2013/000171**

SOLICITUD DE:

(1)

[X] Patente de Invención. [ ] Patente de Modelo de Utilidad.

[X] Capítulo I. [ ] Capítulo II.

(2) **SOLICITANTE (71)**

Nombre: NOVARTIS AG
Dirección: Lichtstrasse 35, CH-4056 Basel, Suiza
Nacionalidad o Domicilio: Suiza
Lugar de Constitución: Suiza
Teléfono: **Fax:**
E-mail:

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

REPRESENTANTE O APODERADO

Nombre: CARLOS R. OLARTE
Dirección: Carrera 5 No 34-03 Bogotá D.C.
Teléfono: 601-7700 **Fax:** 601-7799
E-mail: office@olartemoure.com

IDENTIFICACIÓN
C.C. [X] **NIT** [ ]
C.E. [ ] **Otro** [ ]
Cual
Número 79782747Bta
TP 74.295

INVENTOR (ES) (72)

Nombre: VER PAGINA ADJUNTA
Dirección: VER PAGINA ADJUNTA
Nacionalidad o Domicilio: VER PAGINA ADJUNTA

(5) **PCT (86)**

Solicitud Internacional No. PCT/IB2012/051977 Fecha Abril 19, 2012

Publicación Internacional No. WO 2012/143888 A1 Fecha Octubre 26, 2012

(6) **Título (54)** **PROCESOS PARA LA ELABORACIÓN DE DEPSIPÉPTIDOS MACROCÍCLICOS Y NUEVOS INTERMEDIARIOS**

Clasificación Internacional (51) C07K 11/02

Prioridad

SI [X] **NO** [ ]

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
US	Abril 20,2011	61/477,319

(9) **Comprobante de pago No.** 13- **Fecha**

LISTA DE INVENTORES

PCT/IB2012/051977

Murat ACEMOGLU
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Suiza

Heribert HELLSTERN
Im Bachacker 9,
79423, Heitersheim
Alemania
Nacionalidad: Alemana

Felix KOLLMER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Alemana

John LOPEZ
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Colombiana

Robert SCHREIBER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Australiana

Christian SPRECHER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Suiza

Hans STETTLER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Suiza

ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud.
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- [x] Poderes, si fuere el caso. Adjunto copia simple.
- [ ] Documento de cesión del inventor al solicitante o a su causante.
- [X] Declaraciones. Declaración bajo la regla 4.17 (ii) del PCT.
- [x] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).
- [X] 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.
- [X] 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

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

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 107419

Bogotá D.C., Octubre 23 de 2013 - 16:31:55

RECIBIDO DE : OLARTE MOURE & ASOCIADOS NI 830.122.870

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	522591171	23/10/2013	8.388.000.00

*** Conceptos Pagados ***

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

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

Responsable: _____
-**-
Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-252979- -00000-0000
Fecha: 2013-10-24 16:34:40 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 4

Industria y Comercio SUPERINTENDENCIA	PLANILLA DE RADICACIÓN - DIGITALIZACIÓN DIARIA ***RELACIÓN DE ANEXOS***

Dependencia:	Fecha: 13 / 10 / 24 / (AAAA MM DD)	Recorrido:
2020		3

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

Radicador: | Planilla:

ID	No. Radicación	Consecutivo	Item
1	13- 252979 -	0	7
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			

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

Observaciones:

EL C.D ANEXO CONTIENE UN TOTAL DE 8 ARCHIVOS.
8 EN FORMATO P.D.F, LOS CUALES FUERON PROCESADOS.

Ce

Industria y Comercio
SUPERINTENDENCIA

LISTA DE CHEQUEO
ADMISIÓN A TRÁMITE – NUEVAS CREACIONES

PATENTE DE INVENCIÓN [✓] MODELO DE UTILIDAD [ ] Art 33 Decisión 486/00

- [✓] Indicación que se solicita una patente.
- [✓] Datos de identificación del solicitante o de la persona que presenta la solicitud
- [ ] Descripción de la invención
- [ ] Dibujos de ser estos pertinentes
- [ ] Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)

Completa [ ] Incompleta [ ]

PATENTE DE INVENCIÓN PCT [ ] MODELO DE UTILIDAD PCT [ ] Art.33 Decisión 486/00, Circular Única

- [ ] Indicación que se solicita una PCT
- [ ] Copia de la solicitud en español, tal como fue presentada inicialmente (capítulo descriptivo, reivindicatorio, resumen)
- [ ] Dibujos de ser estos pertinentes
- [ ] Comprobante de pago de las tasas establecidas (de ser el caso formato de descuento)

Completa [ ] Incompleta [ ]

DISEÑO INDUSTRIAL [ ] (Art. 119 Decisión 486/00)

- [ ] Indicación que se solicita 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 [ ] (Art. 92 Decisión 486/00)

- [ ] Indicación que se solicita un esquema de trazado
- [ ] Datos de identificación del solicitante o de la persona que presenta la solicitud
- [ ] Representación gráfica de un esquema de trazado
- [ ] Comprobante de pago de las tasas establecidas

Completa [ ] Incompleta [ ]

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-252979- -00000-0000

Fecha: 2013-10-24 16:34:40 Dep. 2020 DIR.NUEVASCR
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PI02-F08 (2009-11-18)

OLARTEMOURE

OLARTE MOURE & ASOCIADOS LTDA.

Industria y Comercio
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación.

PETITORIO - FORMULARIO ÚNICO DE SOLICITUD
PATENTE PCT
ENTRADA EN FASE NACIONAL **P2013/000171**

SOLICITUD DE:

1

[X] Patente de Invención. [ ] Patente de Modelo de Utilidad.

[ ] Capítulo I. [X] Capítulo II.

2

SOLICITANTE (71)

Nombre: NOVARTIS AG
Dirección: Lichtstrasse 35, CH-4056 Basel, Suiza

Nacionalidad o Domicilio: Suiza
Lugar de Constitución: Suiza

Teléfono: _____ **Fax:** _____
E-mail: _____

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

REPRESENTANTE O APODERADO

Nombre: CARLOS R. OLARTE
Dirección: Carrera 5 No 34-03 Bogotá D.C.
Teléfono: 601-7700 **Fax:** 601-7799
E-mail: office@olartemoure.com

IDENTIFICACIÓN
C.C. [X] **NIT** [ ]
C.E. [ ] **Otro** [ ]
Cual _____
Número 79782747Bta
TP 74.295

INVENTOR (ES) (72)

Nombre: VER PAGINA ADJUNTA
Dirección: VER PAGINA ADJUNTA
Nacionalidad o Domicilio: VER PAGINA ADJUNTA

5

PCT (86)

Solicitud Internacional No. PCT/IB2012/051977 Fecha Abril 19, 2012

Publicación Internacional No. WO 2012/143888 A1 Fecha Octubre 26, 2012

6

Título (54) **PROCESOS PARA LA ELABORACIÓN DE DEPSIPÉPTIDOS MACROCÍCLICOS Y NUEVOS INTERMEDIARIOS**

Clasificación Internacional (51) C07K 11/02

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

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
US	Abril 20,2011	61/477,319

9

Comprobante de pago No. 13- **Fecha** _____

ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud.
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
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- [ ] 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.
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- [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

LISTA DE INVENTORES

PCT/IB2012/051977

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Postfach, CH-4002 Basel
Suiza
Nacionalidad: Suiza

Heribert HELLSTERN
Im Bachacker 9,
79423, Heitersheim
Alemania
Nacionalidad: Alemana

Felix KOLLMER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Alemana

John LOPEZ
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Colombiana

Robert SCHREIBER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Australiana

Christian SPRECHER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Suiza

Hans STETTLER
Postfach, CH-4002 Basel
Suiza
Nacionalidad: Suiza

Nota del traductor:

El documento adjunto de poder de Novartis AG a Carlos R. Olarte y/o J. Ian Raisbeck y/o Ana Maria Frieri y/o Juan Guillermo Moure y/o Natalia Castro lleva los siguientes datos en inglés.

CERTIFICACIÓN NOTARIAL

1. En esta fecha, día 30 de Agosto de 2006, yo, el suscrito Notario Público certifico por medio del presente la autenticidad de las firmas del señor Arthur Canonica, ciudadano de Corticiasca, Suiza, residente en Wisen/SO, Suiza, y del Sr. Pascal Traub, ciudadano de Riehen, Suiza, residente en Basilea, Suiza, ambos personas mayores de edad y con dirección comercial en Lichstrasse 35, 4056, Basilea, Suiza. La autenticidad de la firmas fue establecida por medio de la comparación.
2. Además, yo, el Notario Público, certifico por el presente que las anteriores personas han ejecutado el anterior documento como apoderados de Novartis AG.
3. Además, yo, el Notario Público certifico por el presente que Novartis AG, con su principal lugar de negocios en Basilea, Suiza, está debidamente constituida, está actualmente vigente y que los propósitos para los cuales se otorgó el Documento de Poder están dentro del alcance de sus propósitos corporativos.

Traductor Oficial
JORGE E. SIERRA REYES
Licencia 376 - 1993 Minjusticia

4. Además, yo, el Notario Público, certifico por el presente que las anteriores personas tienen la autoridad para ejecutar el documento de Poder y que por este instrumento, su representación es legal.

La base para mis certificaciones de los puntos 2, 3 y 4 fue la exhibición de los siguientes documentos auténticos:

- Documento de Poder de Novartis AG fechado el 19 de junio de 2006 y
- Extracto del Registro de Comercio del Cantón de la ciudad de Basilea para Novartis AG, en Basilea, fechado el 25 de enero de 2006.

Basilea, Suiza, este día 30 de Agosto de 2006.

(Firmado) Dr. Matthias Staehelin, Notario Público del Cantón de la ciudad de Basilea, Suiza.

Mi licencia vence el 11 de Marzo de 2009.

Hay una Apostilla de Suiza, firmada por Heidi Fischer, bajo el No. 8049/13584.

Traductor Oficial
JORGE E. SIERRA REYES
Licencia 376 - 1993 Minjusticia

Certificación:

Certifico que la presente es una traducción verdadera y completa del documento adjunto: "Novartis"

Fecha: 12 de Septiembre de 2006.

Traductor: Jorge Enrique Sierra Reyes.

Licencia No. 376 de 1993, del Ministerio de Justicia.

Dirección: Carrera 15 No. 99-13 Oficina 411. Bogotá, Colombia.

Teléfono: (57-1) 2182308, (57-1) 6232925.

E-mail: treseser1@yahoo.com. No. 8346

Traductor Oficial
JORGE E. SIERRA REYES
Licencia 376 - 1993 Miniusticia

OLARTERAISBECK
OLARTE RAISBECK & FRIERI LTDA.

Carlos R. Olarte
J. Ian Raisbeck
Ana María Frieri
Juan Guillermo Moure
Natalia Castro

Zayde Chahin
Jacqueline Chaparro
José Luis Londoño
Felipe Mendoza
Simón Salinas

Of Counsel
Guillermo Chahin
Graciela Melo

Power of Attorney

The undersigned,

NOVARTIS AG

domiciled in

Lichtstrasse 35, CH-4056 Basel / Switzerland

by means of these presents hereby grants to **Carlos R. Olarte and/or J. Ian Raisbeck and/or Ana María Frieri and/or Juan Guillermo Moure and/or Natalia Castro** full and sufficient Power of Attorney to file, prosecute, obtain, defend and enforce any and all of the company's **Patents for Inventions, Utility Models, Layout-Designs (topographies) of Integrated Circuits, Industrial Designs, Trademarks, Advertising Slogans, Trade Names, Commercial Emblems, Geographical Indications, Protection of Trade Secrets, Obtainer's Certificates, Copyrights, Registration of Domain Names, Health Registrations, or any other intellectual property right** 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, recourses, 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,

NOVARTIS AG

domiciliado en

Lichtstrasse 35, CH-4056 Basel / Switzerland

por medio del presente otorga a **Carlos R. Olarte y/o J. Ian Raisbeck y/o Ana María Frieri y/o Juan Guillermo Moure y/o Natalia Castro** poder especial, amplio y suficiente para presentar, tramitar, obtener, defender y hacer respetar todas y cualquiera de las **Patentes de Invención, Modelos de Utilidad, Esquemas de Trazados de Circuitos Integrados, Diseños Industriales, Marcas, Lemas Comerciales, Nombres Comerciales, Enseñas Comerciales, Denominaciones de Origen, Protección de Secretos Empresariales, Certificados de Obtentor, Derechos de Autor, Registro de Nombres de Dominio, Registros Sanitarios, o cualquier otro bien de propiedad intelectual** 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, recursos, 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: Arthur Cimonica Pascal Traub

Date/Fecha: June 27, 2006

City,Country/Ciudad,País: Basel, Switzerland

World Trade Center - Torre A, Calle 100 No. 8A - 37, Piso 10, Bogotá D.C., Colombia
Tel + 57 1 601-7700, Fax + 57 1 601-7799, office@olarteraisbeck.com, www.olarteraisbeck.com

Attestation

1 On this date, the 30th (thirtieth) day of August 2006 (two thousand and six), I, the undersigned Notary Public certify herewith the authenticity of the signatures of **Mr. Arthur Canonica**, citizen of Corticiasca, Switzerland, residing in Wisen/SO, Switzerland and of **Mr. Pascal Traub**, citizen of Riehen, Switzerland, residing in Basel, Switzerland, both of legal age and with business address at, Lichtstrasse 35, 4056 Basel, Switzerland. The authenticity of the signatures was established by means of comparison.

2 Further, I, the Notary Public certify herewith that the foregoing persons have executed the foregoing document as proxy holders for Novartis AG.

3 Further, I, the Notary Public certify herewith that Novartis AG, having its principal place of business located in Basel / Switzerland, 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 Further, I, the Notary Public certify herewith that the foregoing persons have the authority to execute the Power of Attorney and that for this instrument their representation is legal.

The foundation for my certifications contained in points 2 (two), 3 (three) and 4 (four) was the exhibition of the following authentic documents:

- Power of Attorney by Novartis AG dated June 19 (nineteen), 2006 (two thousand and six); and
- Extract from the Register of Commerce of the Canton of Basel-City for Novartis AG, in Basel, dated January 25 (twenty five), 2006 (two thousand and six).

BASEL, Switzerland, this 30th (thirtieth) day of August 2006 (two thousand and six)

Dr. Matthias Staehelin, Notary Public
in the Canton of Basel-City, Switzerland

My license expires on March 11 (eleven), 2009 (two thousand and nine).

Leg.Prot.Nr. 915 /2006

APOSTILLE

(Hague Convention of October 5th, 1961)

1. Country: Switzerland (Schweiz / Suisse)

This official document

2. is signed by .....

.....

3. in his/her function as .....

4. and certified by the seal of the .....

.....

Certified [illegible] 2006

5. in Basel (Bâle) 6. on .....

7. by the State Chancery of the Canton of Basel-Stadt .....

8. no. .....

9. Seal/stamp: 10. Signature:

.....

Heidi Fischer

STATE CHANCERY * BASLE-CITY *

Sheet No. . . . . .

Box No. VIII (ii) DECLARATION: ENTITLEMENT TO APPLY FOR AND BE GRANTED A PATENT

The declaration must conform to the standardized wording provided for in Section 212; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (ii). If this Box is not used, this sheet should not be included in the request.

Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51*bis*.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate:

in relation to the international application PCT/IB2012/051977 ,

Novartis AG, is entitled to apply for and be granted a patent by virtue of the following:

an assignment from:

Murat ACEMOGLU, Novartis Pharma AG, Postfach, 4002 Basel, CH, dated 11.04.2012
Heribert HELLSTERN, Im Bachacker 9, 79423 Heitersheim, DE, dated 12.04.2012
Felix KOLLMER, Novartis Pharma AG, Postfach, 4002 Basel, CH, dated 11.04.2012
John LOPEZ, Novartis Pharma AG, Postfach, 4002 Basel, CH, dated 11.04.2012
Robert SCHREIBER, Novartis Pharma AG, Postfach, 4002 Basel, CH, dated 16.04.2012
Christian SPRECHER, Novartis Pharma AG, Postfach, 4002 Basel, CH, dated 10.04.2012
Hans STETTLER, Novartis Pharma AG, Postfach, 4002 Basel, CH, dated 11.04.2012

to Novartis Pharma AG.

and an assignment from:

Novartis Pharma AG, Lichtstrasse 35, 4056 Basel, Switzerland, dated 18.04.2012

to Novartis AG.

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (ii)".

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 October 2012 (26.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/143888 A1

(51) International Patent Classification: *C07K 11/02* (2006.01)

(21) International Application Number: PCT/IB2012/051977

(22) International Filing Date: 19 April 2012 (19.04.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/477,319 20 April 2011 (20.04.2011) US

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(72) Inventors; and

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(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, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

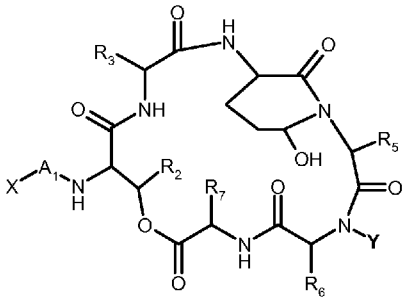
Declarations under Rule 4.17:

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

Published:

— *with international search report (Art. 21(3))*

(54) Title: PROCESSES FOR THE MANUFACTURE OF MACROCYCLIC DEPSIPEPTIDES AND NEW INTERMEDIATES



(I)

(57) Abstract: The invention relates to a method or process for the chemical manufacture of depsipeptides of the formula (I), wherein the symbols have the meaning defined in the description, to new intermediates and their manufacture, as well as related invention embodiments.

WO 2012/143888 A1

Processes for the Manufacture of Macrocyclic Depsipeptides and New Intermediates

Summary of the Invention

The invention relates to a method or process for the manufacture of macrocyclic depsipeptides, to new intermediates and their manufacture, as well as related invention embodiments.

Background of the Invention

Cyclic depsipeptides have numerous uses in pharmacology. As an example, the depsipeptides disclosed in WO2009/024527 are useful for treatment of various diseases. For example, the compound of formula II mentioned in WO2009/024527 is useful for the treatment and prevention of inflammatory and/or hyperpoliferative and pruritic skin diseases such as atopic dermatitis, psoriasis, pustular psoriasis, rosacea, keloids, hypertrophic scars, acne, Netherton's syndrome or other pruritic dermatoses such as prurigo nodularis, unspecified itch of the elderly as well as other diseases with epithelial barrier dysfunction such as aged skin.

Nostopeptin BN920, formerly isolated from the cyanobacterium Nostoc, was isolated also from Microcystis. Nostopeptin BN920 inhibited chymotrypsin with an IC50 value of 31 nM (see J. Nat. Prod. 68(9), 1324-7 (2005)).

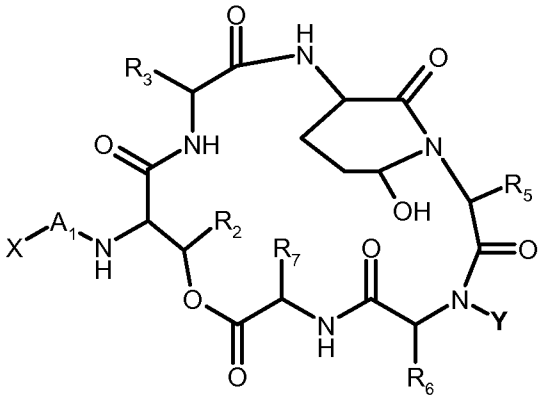
These compounds can be produced by fermentation (using chondromyces croactus, myxobacteria) along with other depsipeptides comprising the so-called ahp-substructure (ahp: 3-amino-6-hydroxy-piperidin-2-one) and the corresponding dehydro-ahp substructure (dehydro-ahp: 3-amino-3,4-dihydro-1H-pyridin-2-one), also called "dehydrate" herein, respectively. Therefore, the yield of fermentation with regard to any single of these compounds is rather low.

The present invention relates to processes or methods that allow obtaining such cyclic depsipeptides with increased yield and/or in good purity.

In view of the many risks, such as epimerization, tautomerization and the like in the synthesis of a complex molecule with many possible isomers, it has been possible to find

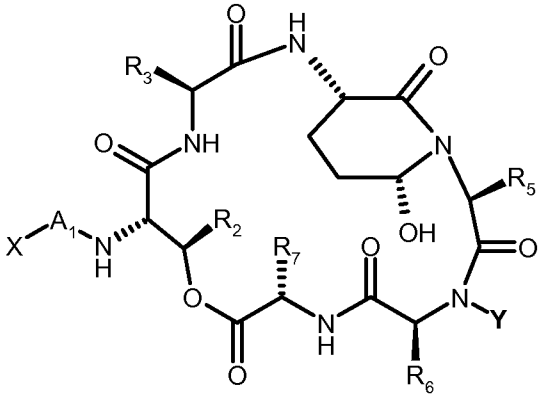
a manufacturing process, preferably comprising a mixture of solid phase peptide synthesis and reactions in solution, that allows to produce cyclic depsipeptides of formula I in good yield and/or the required stereoisomerical purity, especially both. It is possible to reduce the amount of by-products, and even to improve yield, by converting such by-products, especially the dehydro-ahp substructure and/or an analogue of the desired ahp-comprising products with a five-membered ring instead of the ahp, into the desired final products. This allows to further increase yield. No synthesis has so far come to our attention making use of solid phase peptide synthesis in this field.

(i/a) In a first embodiment, the invention relates to a method or process for the preparation of a cyclic depsipeptide compound of the formula I,



I

especially of the formula IA



IA

wherein

A_1 is a bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group, especially asparagine or glutamine, and is bound at its right hand side in formula I (corresponding to the C-terminus) via a carbonyl (preferably the carbonyl of an α-

carboxyl group thereof) to the rest of the molecule; or is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

X is bound via an N of A_1 and is acyl, or is absent if A_1 is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

R_2 is C_{1-8}-alkyl, especially methyl;

R_3 is the side chain of an amino acid, especially of leucine, isoleucine or valine;

R_5 is the side chain of an amino acid, preferably of phenylalanine, leucine, isoleucine or valine;

R_6 is the side chain of a hydroxy amino acid, especially of tyrosine;

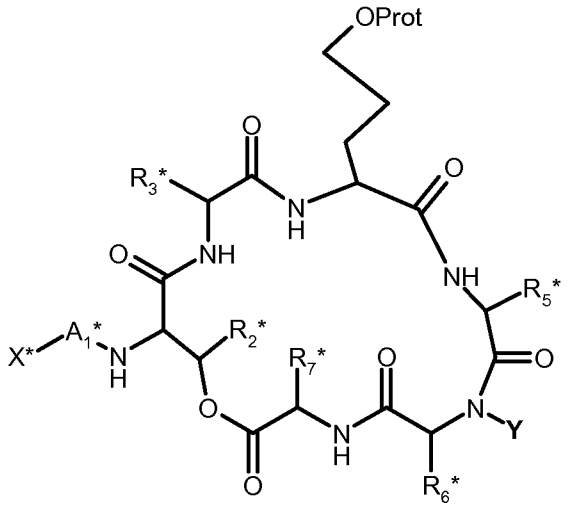
R_7 is the side chain of an amino acid, preferably of the amino acid leucine, isoleucine or valine; and

Y is hydrogen or C_{1-8}-alkyl;

or a salt thereof,

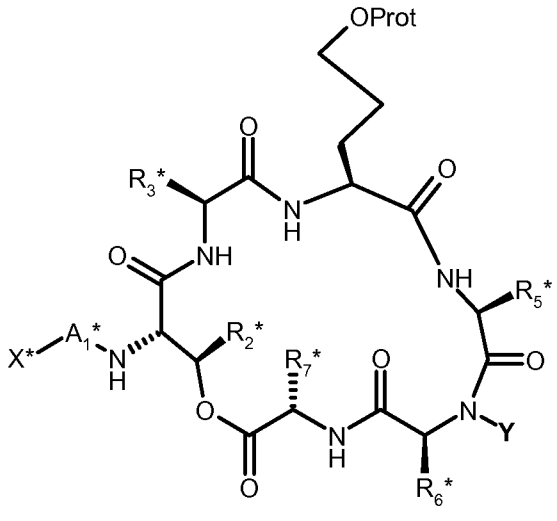
said method comprising

selectively deprotecting a compound of the formula II



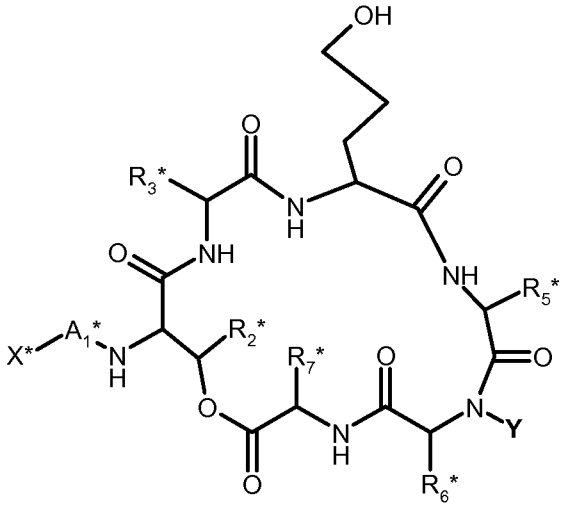
II

especially of the formula IIA



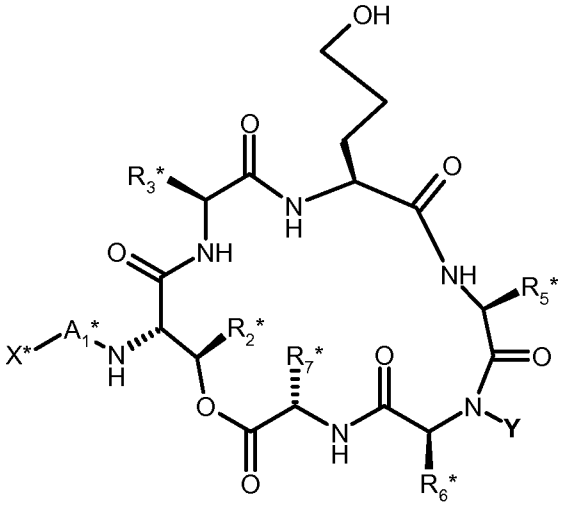
IIA

wherein Prot is a protecting group, Y is as defined for a compound of the formula I and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I, respectively, but with the proviso that reactive functional groups on these moieties (such as amino, imino, hydroxy, carboxy, sulfhydryl, amidino, guanidino, O-phosphono (-O-P(=O)$(OH)_2$) are present in protected form at least if they could participate in undesired side reactions, to result in a compound of the formula III,



(III)

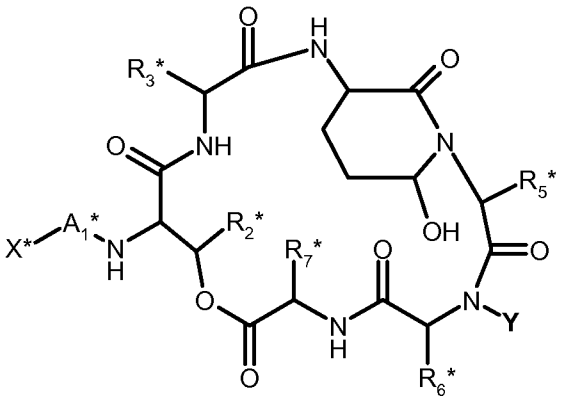
especially of the formula IIIA,



IIIA

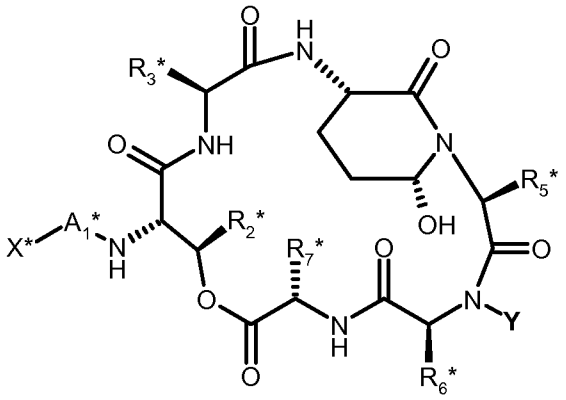
wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* have the meanings just defined,

reacting the free hydroxyl group under oxidizing conditions to form a compound of the formula IV



IV

especially IVA,



IVA

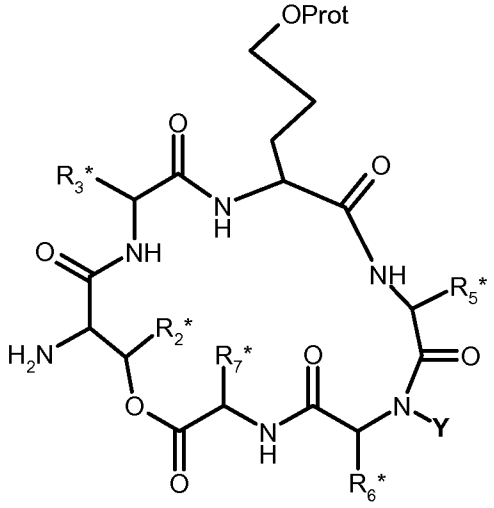
and removing remaining protecting groups to yield a compound of the formula I, or a salt thereof,

and, if desired, converting a free compound of the formula I, or especially IA, into a salt, a salt of a compound of the formula I into a different salt of a compound of the formula I, or especially IA, or into the free compound of the formula I, or especially IA, and/or converting a dehydrate analogue and/or five ring analogue of a compound of the formula I, or especially IA, into the corresponding compound of the formula I, or especially IA.

Suitable oxidizing conditions for the oxidation of a compound of the formula III or especially IIIA are usually using IBX in DMSO (J. Org. Chem. 1995, *60*, 7272-7276); Pyridinium dichromate or Pyridinium chlorochromate (Tetrahedron Lett. 1979, *5*, 399-402); oxalyl chloride, dimethyl sulfoxide and a tertiary amine (J. Peptide Sci. 2006, *12*, 140-146), oxoammonium salts (J. Org. Chem. 1985, *50*, 1332-1334); alkali hypochlorites catalyzed by oxoammonium salts (J Org. Chem. 1989, *54*, 2970-2972); oxoaminium salts (Tetrahedron Lett. 1988, *29*, 5671-5672), $RuCl_2(PPh_3)_3$ (Tetrahedron Lett. 1981, *22*, 1605-1608); TEMPO (1 mol%) in the presence of sodium hypochlorite (Tetrahedron Lett. 1990, *31*, 2177-2180); $NaIO_4$, TEMPO, NaBr (Tetrahedron 2006, *62*, 8928-8932); SiO_2 supported vanadium(IV)oxide and t-BuOOH (Advanced Synthesis & Catalysis 2007, *349*, 846-848). Preferably, the reaction is performed with IBX in DMSO or preferably in an inert solvent, such as tetrahydrofuran, in the presence of DMSO, at a temperature between 0-50°C, preferably between 20-25 °C.

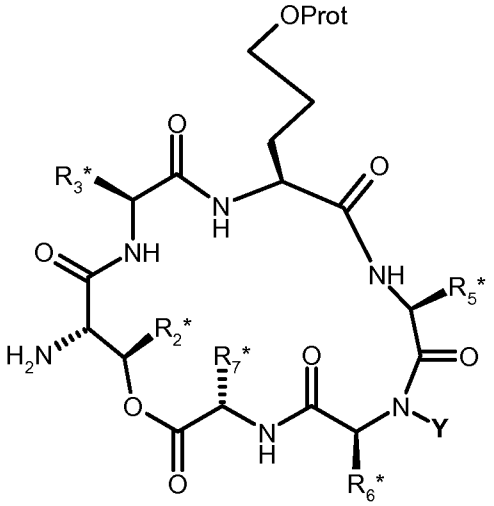
(ii/a) A further embodiment of the invention refers to the method or process described above, in addition comprising manufacturing the compound of the formula IV or especially IVA by a combination of Solid Phase Peptide Synthesis (especially For synthesis of the precursor XX or especially XXA given below for the oligopeptide precursor of the formula VIII or especially VIIIA given below, or of the oligopeptide precursor of the formula XXIV or especially XXIVA given below fot the oligopeptide precuros of the formula XXV or especially XXVA given below) and Solution Phase synthesis (especially from the compounds just mentioned to the final product) from the corresponding starting amino acids and side chain precursors.

(iii/a) Yet a further embodiment of the invention relates to a method or process as described above, further comprising, for the synthesis of a compound of the formula II above, reacting a compound of the formula VI,



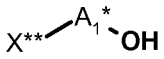
VI

especially VIA,



VIA

wherein Prot is a protecting group, Y is as defined for a compound of the formula I and R_2*, R_3*, R_5*, R_6*, and R_7* are as defined for a compound of the formula II above, with an acid of the formula VII



VII

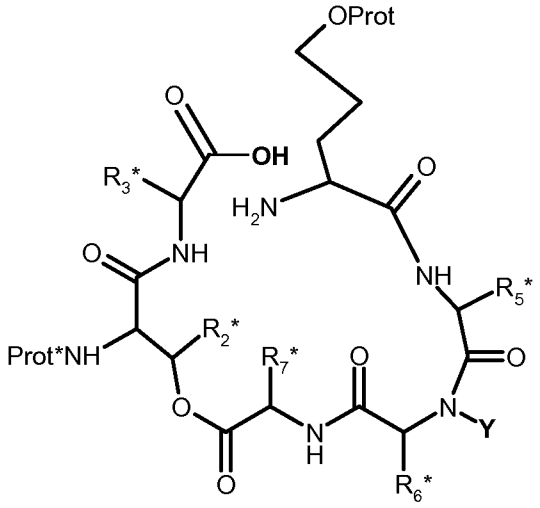
or a reactive derivative thereof,

wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined for a compound of the formula II above; and, if X** is an amino protecting group, removing said amino protecting group X** to yield the derivative of formula II (especially IIA) wherein, instead of X*, H (a hydrogen) is present and coupling the resulting amino group with an acyl group X* using the corresponding acid X*-OH wherein X* is as defined for a compound of the formula II defined above, or a reactive derivative thereof.

(iv/a) Another embodiment of the invention relates to the methods or processes described above, especially in the preceding paragraph, further comprising cyclization under lactamization of a linear, that is, not yet cyclic, precursor peptide of the compound of the formula VI, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, preferably using Solution Phase chemistry.

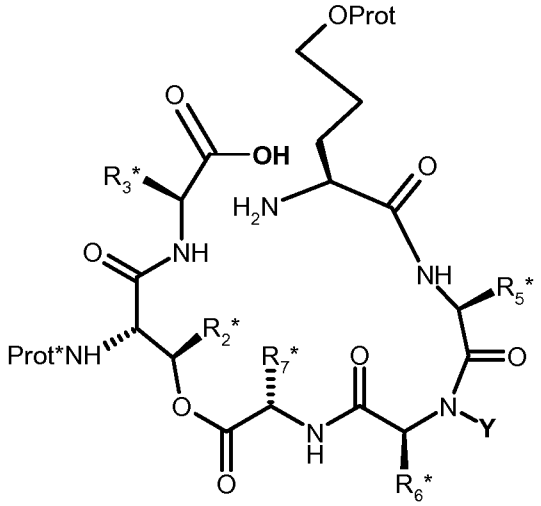
Lactamizations in solutions are usually carried out at very low concentrations of the substrate in order to avoid oligomerizations and polymerizations. This requires huge amounts of solvents and very large reactors to carry out the reactions. For example, the macrolactamization of an oligopeptide is performed at a concentration of 2 mMols/liter in reference Yokokawa et al., Tetrahedron 2005, *61*, 1459-1480. This difficulty can be circumvented by dissolving the tertiary base and the coupling reagent and, in a controlled way adding a solution of the oligopeptide to this solution. The controlled, especially slow, addition of the oligopeptide-solution generates permanently low concentrations of the activated oligopeptide in solution and thus prevents oligomerization and polymerization. The addition rate of the oligopeptide solution can be adjusted according to the reaction rate for the macrocyclization: if the macrocyclization is a fast reaction, the solution of the oligopeptide can be added fast. If the macrocyclization is slow, the addition of the solution must be slow to ensure permanent low concentration of the activated oligopeptide. Thus the controlled addition of the oligopeptide enables to work with much less solvent amounts and still maintaining the concentration of the activated oligopeptide below 10^{-3} mM,, e.g. in the range from 10^{-4} to 10^{-6} mM or even lower. This variant of controlled addition of the oligopeptide to the coupling reagent solution is an embodiment of the invention.

(v/a) In yet a further embodiment, the invention relates to the method or process as described above, especially in the preceding paragraph, where the linear (this term where used meaning not yet cyclic) precursor peptide is of the formula VIII,



VIII,

especially VIIIA



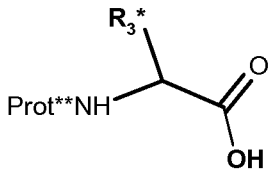
VIIIA

wherein Prot* is a protecting group that can be cleaved off selectively without affecting other protecting groups present and is stable during deprotection steps during synthesis of the linear precursor peptide (e.g. allyloxycarbonyl) and R_2*, R_3*, R_5*, R_6*, and R_7* are as defined for a compound of the formula VI as described above, further comprising, after cyclisation of the compound of the formula VIII, especially VIIIA, removing the protecting group Prot* *in situ* to yield the compound of the formula VI, especially VIA.

(vi/a) In another embodiment, the invention relates to the method or process described above, especially in the preceding paragraph, where the linear precursor peptide of the formula VIII, especially VIIIA, is synthesized from the corresponding amino acids by solid phase peptide synthesis and subsequent cleavage from the employed solid support.

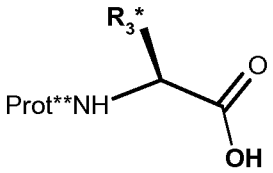
(vii/a) An embodiment of the invention further relates to the method or process as described above (especially in the preceding paragraph (vi/a)), further comprising either

in a variant a), coupling an amino acid of the formula IX,



IX

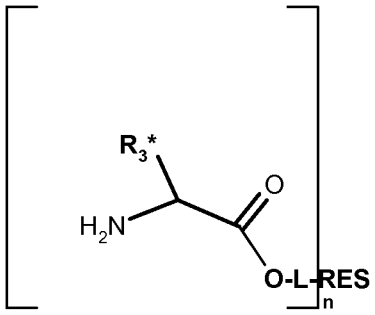
especially of the formula IXA,



IXA

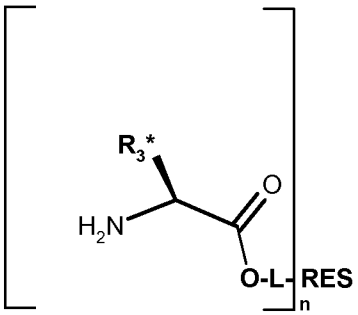
wherein R_3* is as defined for a compound of the formula II above and Prot** is an amino protecting group that can be removed on the resin without cleaving other bonds, or a reactive derivative of said amino acid, via an oxygen to a cleavable linker L which is bound to a solid resin RES (e.g. by reaction with a resin of the formula $(X\text{-}L)_z$-RES wherein L and RES are as just defined, X is e.g. halo, e.g. chloro, and z is a number larger than zero, e.g. a natural number)
and removing the protecting group Prot**;

coupling the obtainable resin bound amino acid symbolized by the formula X,



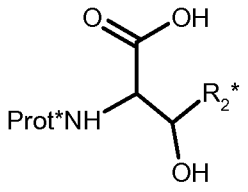
X

especially XA,



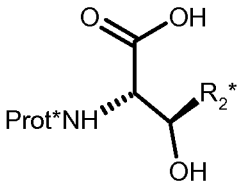
XA

in which RES and R_3* are as defined for a compound of the formula IX, n is a (e.g. natural)number larger than zero and L is a cleavable linker, with an amino acid of the formula XI,



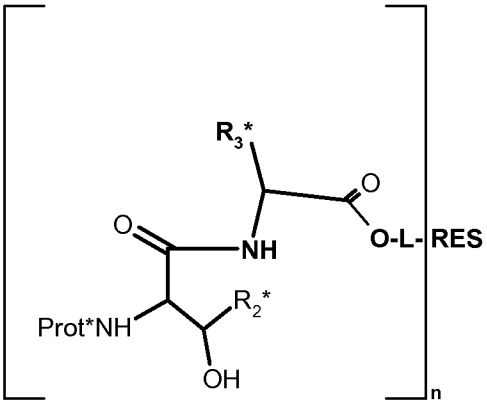
XI

especially XIA,



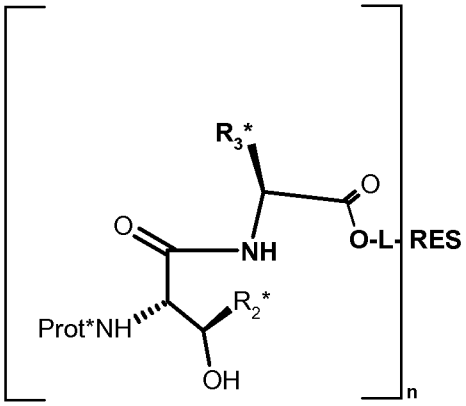
XIA

wherein Prot* is as defined for a compound of the formula VIII above and R_2* is as defined for a compound of the formula II above, or a reactive derivative of said amino acid, coupling the obtainable resin bound dipeptide symbolized by the formula XII,



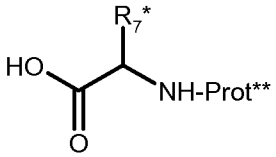
XII

especially XIIA;



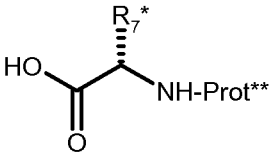
XIIA

in which Prot* is as defined for a compound of the formula VIII above, R_2* and R_3* are as defined for a compound of the formula II above, and n, L and RES are as defined for a compound of the formula X, via the free hydroxy group with an amino acid of the formula XIII,



XIII

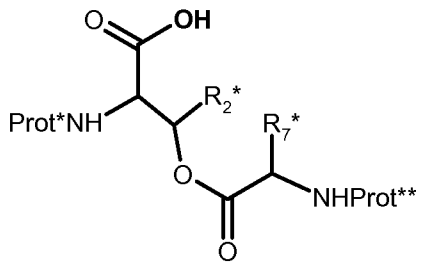
especially XIIIA,



XIIIA

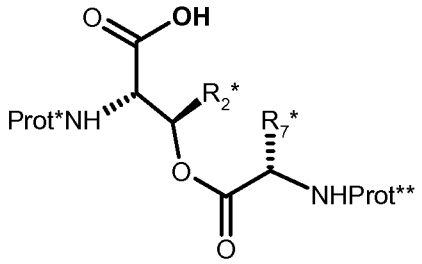
wherein Prot** is as defined for a compound of the formula IX and R_7* is as defined for a compound of the formula II above, or a reactive derivative of said amino acid, and removing the protecting group Prot**;

or, in a variant b), coupling a dipeptide of the formula XXVII,



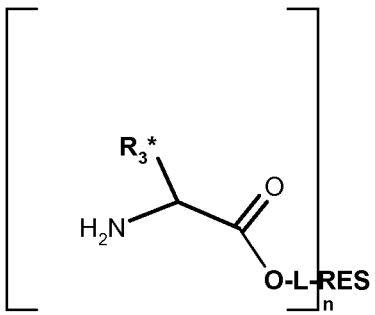
XXVII

especially of the formula XXVIIA,



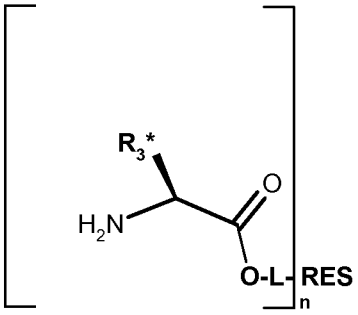
XXVIIA

wherein R_3* and Prot** are as described for a compound of the formula IX, especially IXA, and Prot* is as defined for a compound of the formula VIII above, or a reactive derivative of said dipeptide, to an amino acyl moiety, bound via an oxygen to a cleavable linker L which is bound to a solid resin RES, having the formula X,



X

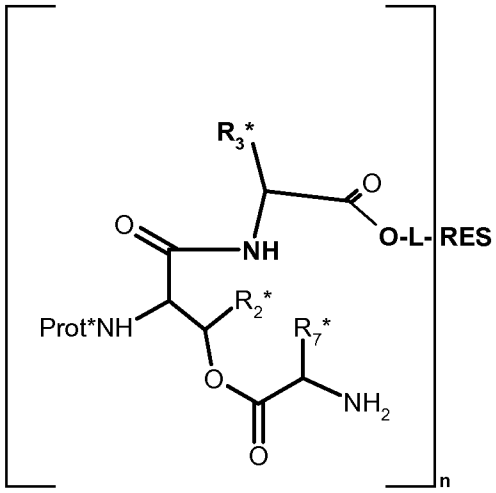
especially XA,



XA

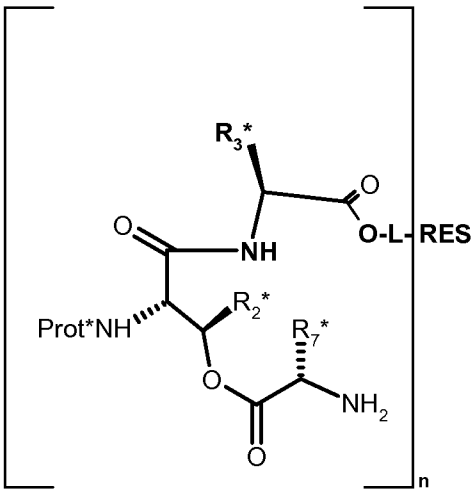
that can be obtained as described under variant a), in which RES, and R_3* are as defined for a compound of the formula IX and L and RES are as just defined; and removing the protecting group Prot**;

and, after the reactions of variant a) or of variant b),
(viii/a) coupling the obtainable compound of the formula XIV,



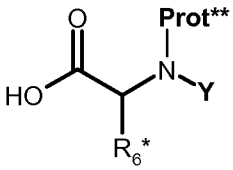
XIV

especially XIVA,



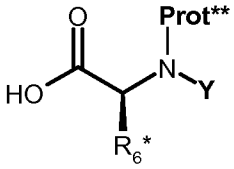
XIVA

wherein R_2*, R_3* and R_7* are as defined for a compound of the formula II above, Prot* is as defined for a compound of the formula VIII above and n, L and RES are as defined for a compound of the formula X, with an amino acid of the formula XV,



XV

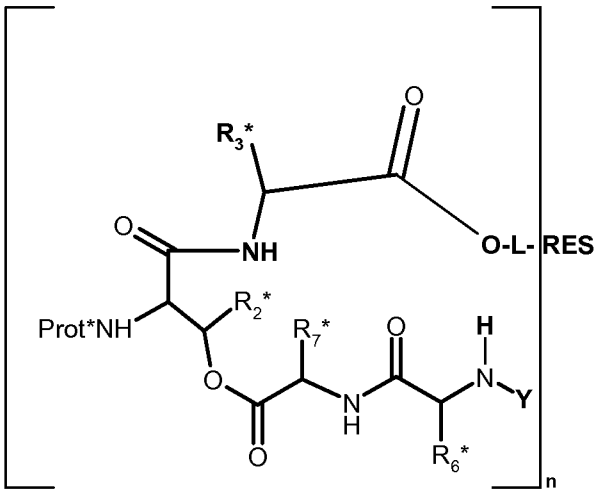
especially the formula XVA,



XVA

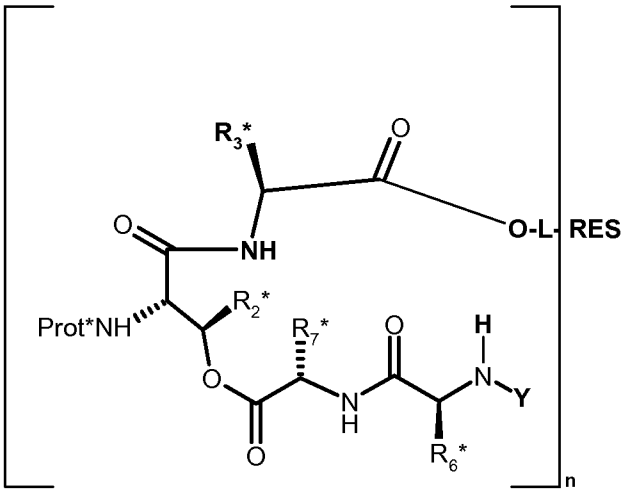
in which R_6* and Y are as defined for a compound of the formula II above and Prot** is as defined for a compound of the formula IX above, or a reactive derivative of said amino acid, and removing the protecting group Prot**;

(ix/a) preferably coupling the obtainable compound of the formula XVI



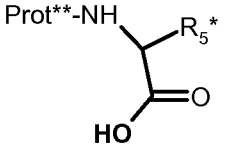
XVI

especially the formula XVIA,



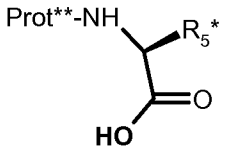
XVIA

wherein Y, R_2*, R_3*, R_7* and R_6* are as defined for a compound of the formula II above, Prot* is as defined for a compound of the formula VIII above and n, L and RES are as defined for a compound of the formula X, with an amino acid of the formula XVII,



XVII

especially formula XVIIA,

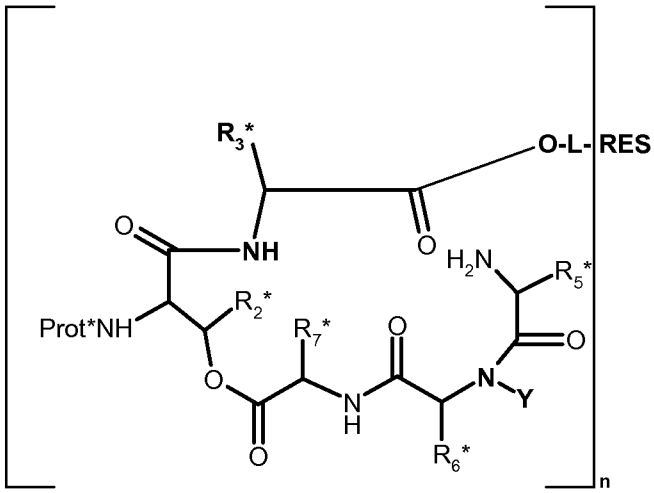


XVIIA

wherein R_5* is as defined for a compound of the formula II above and Prot** is as defined for a compound of the formula IX, or a reactive derivative of said amino acid, and removing the protecting group Prot**,

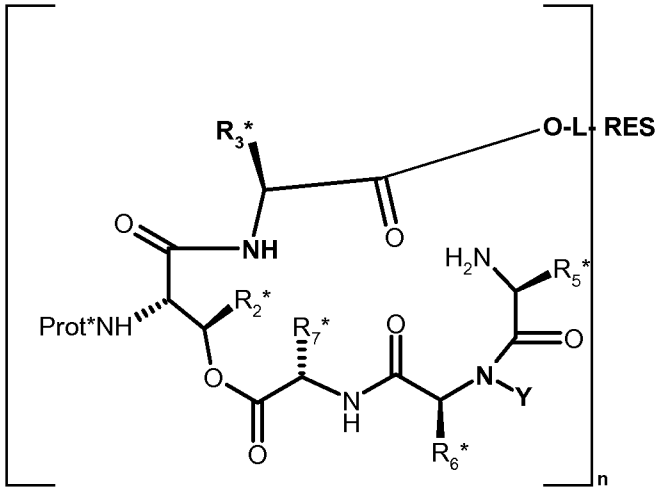
and preferably

(x/a) finally coupling the resulting compound of the formula XVIII,



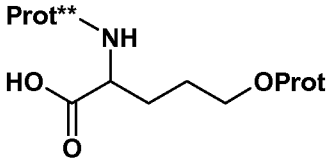
XVIII

especially XVIIIA,



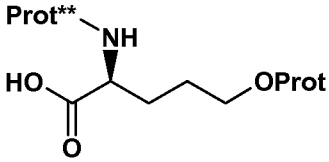
XVIIIA

wherein Y, R_2*, R_3*, R_7*, R_6* and R_5* are as defined for a compound of the formula II above, Prot* is as defined for a compound of the formula VIII above and n, L and RES are as defined for a compound of the formula X above, to an unnatural amino acid (= synthon) of the formula XIX,



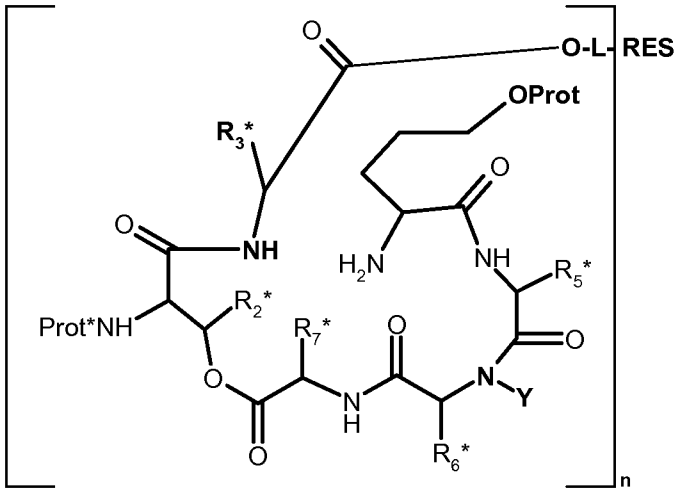
XIX

especially the formula XIXA,



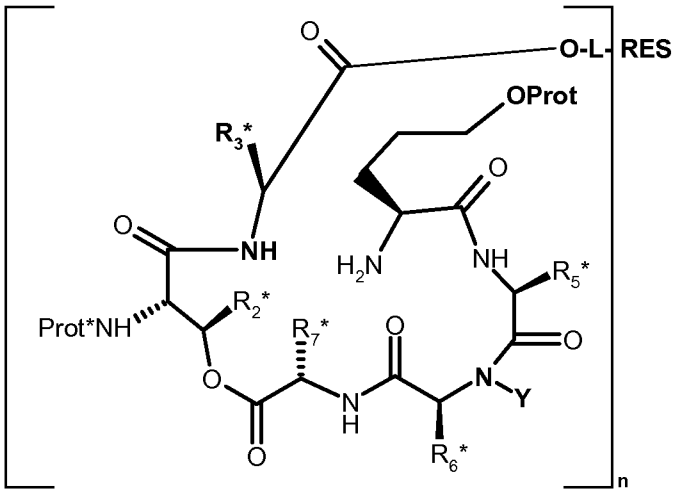
XIXA

wherein Prot is as defined for a compound of the formula II above and Prot** is as defined for a compound of the formula IX, or an activated derivative of said synthon, and removing the protecting group Prot** to yield a compound of the formula XX,



XX

especially XXA,



XXA

wherein Prot, Y, R_2*, R_3*, R_7*, R_6* and R_5* are as defined for a compound of the formula II above, Prot* is as defined for a compound of the formula VIII above and n, L and RES are as defined for a compound of the formula X,

and

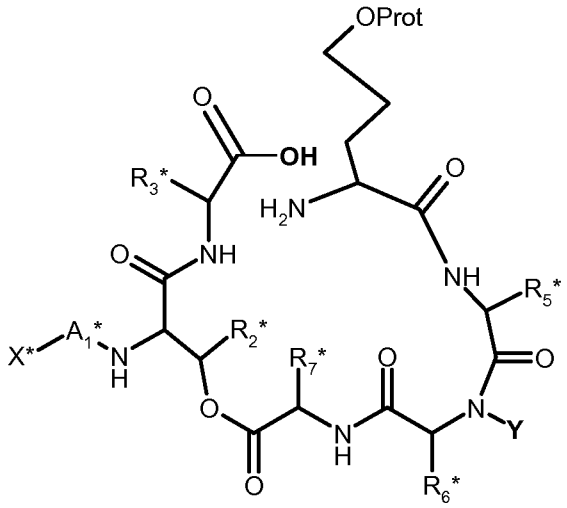
(xi/a) cleaving the solid phase bound peptide in formula XX off the solid phase L-RES to yield the corresponding compound of the formula VIII, especially VIIIA, as shown above.

Another embodiment of the invention relates to the synthesis of a compound of the formula II as given above according to section (i/a), preferably preceded by the reaction

according to section (ii/a) or more preferably according to section (iii/a); preferably preceded by the reaction according to section (iv/a) or preferably (v/a), preferably preceded by the reaction according to section (vi/a), preferably preceded by the reaction according to section (x/a), preferably preceded by the reaction according to section (ix/a), preferably preceded by the reaction according to section (viii/a), preferably preceded by the reaction according to section (vii/a).

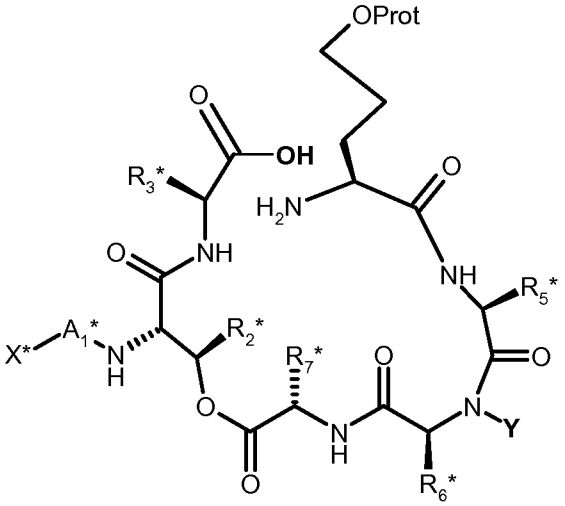
(i/b) Another embodiment of the invention relates to a method or process above, comprising, for the synthesis of the compound of the formula II given above, cyclization under lactamization of a linear, not yet cyclic, precursor peptide of the compound of the formula II, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, preferably using Solution Phase chemistry.

(ii/b) A further embodiment of the invention relates to the method or process according to the preceding paragraph (i/b), where the linear precursor peptide is of the formula XXV,



XXV

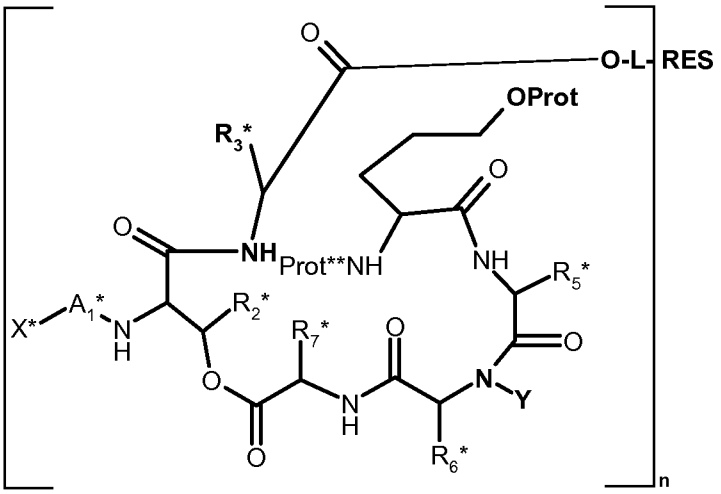
especially XXVA,



XXVA

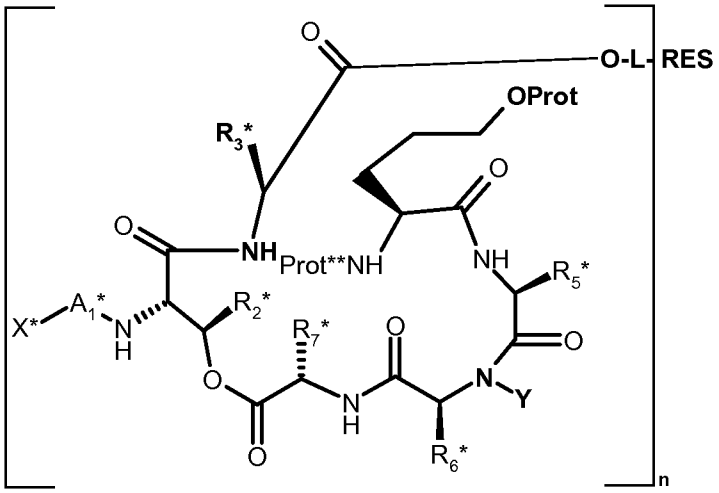
wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* and Prot are as defined for a compound of the formula II above.

(iii/b) Another embodiment refers to the method or process according to the preceding paragraph (ii/b), further comprising, for the synthesis of the compound of the formula XXV, cleaving a compound of the formula XXIV,



XXIV

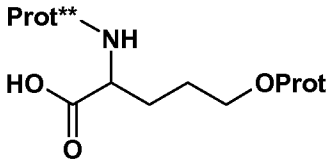
especially XXIVA,



XXIVA

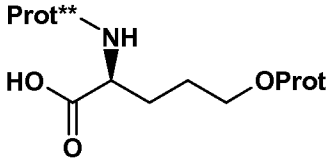
wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* and Prot are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, n is a natural number and Prot** is an amino protecting group that can be removed without parallel removal of the protecting group Prot and with the product remaining on the resin, and (before the cleavage, in parallel or subsequently to it) removing the protecting group Prot** to yield the compound of the formula XXV.

(iv/b) A further embodiment of the invention relates to the method or process according to the preceding paragraph (iii/b), further comprising, for the synthesis of the compound of the formula XXIV, coupling an amino acid of the formula XIX,



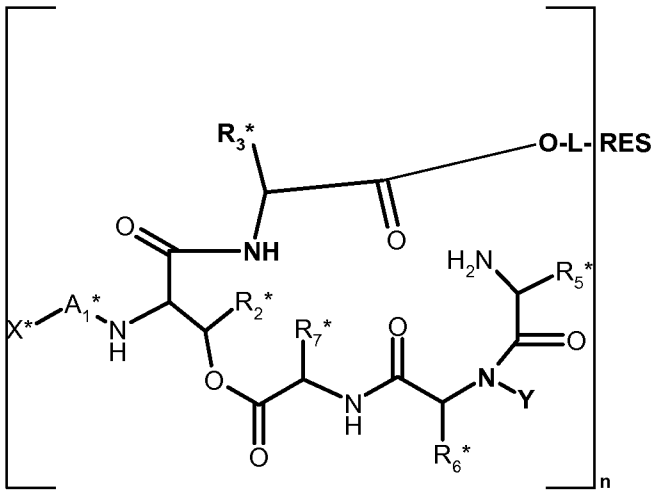
XIX,

especially XIXA,



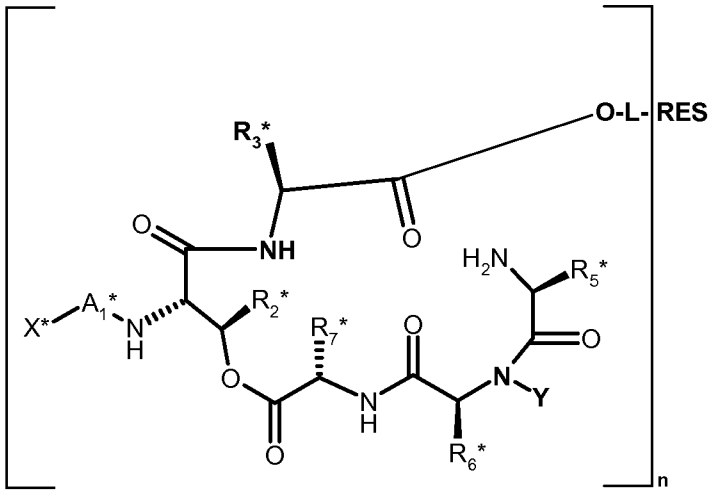
XIXA

wherein Prot is as defined for a compound of the formula II above and Prot** is as defined for a compound of the formula XXIV above, or an activated derivative of said amino acid, with a compound of the formula XXIII,



(XXIII)

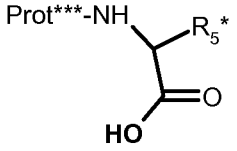
especially XXIIIA,



XXIIIA

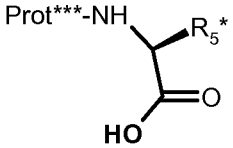
wherein X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number.

(v/b) Yet a further embodiment of the invention relates to the method or process according to the preceding paragraph (iv/b), further comprising, for the synthesis of the compound of the formula XXIII, coupling an amino acid of the formula XVII*



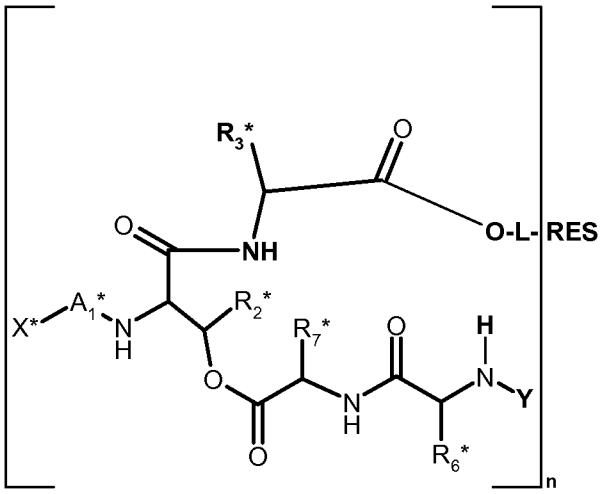
XVII*,

especially XVIIA*,



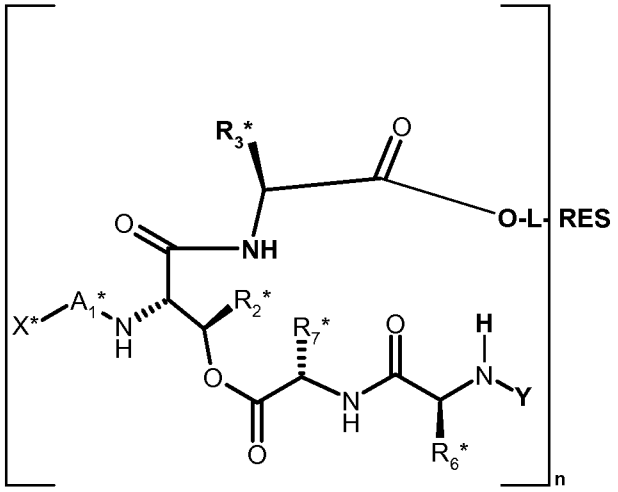
XVIIA*

wherein R_5* is as defined for a compound of the formula II above and Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin , or a reactive derivative of said amino acid, with a compound of the formula XXII,



XXII

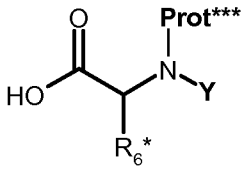
especially XXIIA



XXIIA

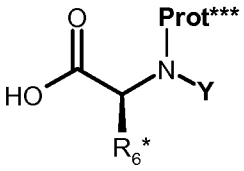
wherein X*, A_1*, R_2*, R_3*, R_6* and R_7* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number, and removing the protecting group Prot***.

(vi/b) In yet a further embodiment, the invention relates to the method or process according to the preceding paragraph (v/b), further comprising, for the synthesis of the compound of the formula XXII, coupling an amino acid of the formula XV*,



XV*

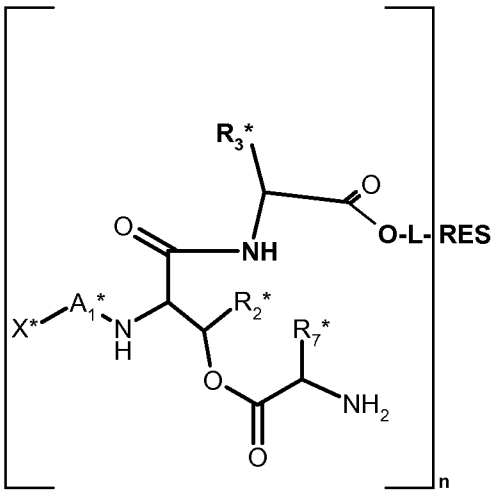
especially XVA*



XVA*

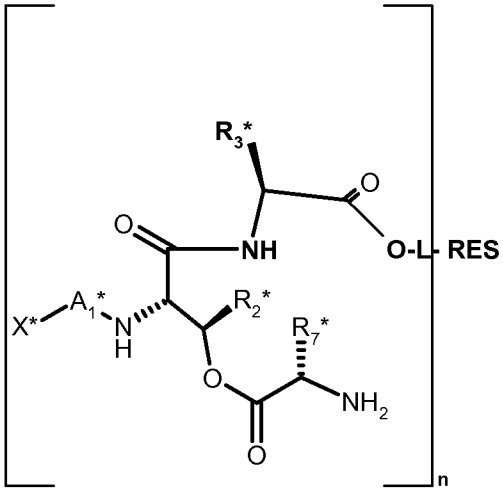
in which R_6* and Y are as defined for a compound of the formula IIabove and Prot*** is an amino protecting group that can be cleaved off selectively without affecting other

protecting groups present and with the product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula XXI,



XXI,

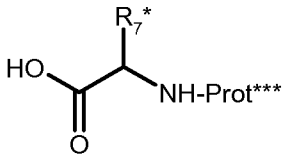
especially XXIA,



XXIA

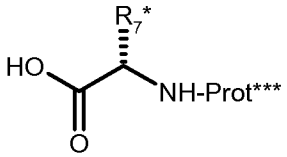
wherein X*, A_1*, R_2*, R_3* and R_7* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number, and removing the protecting group Prot***.

(vii/b) Another embodiment of the invention relates to the method or process according to the preceding paragraph (vi/b), further comprising, for the synthesis of a compound of the formula XXI, reacting an amino acid of the formula XIII*,



XIII*

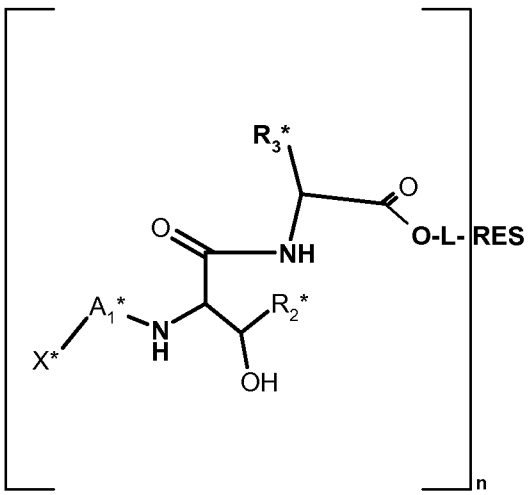
especially XIIIA*,



XIIIA*

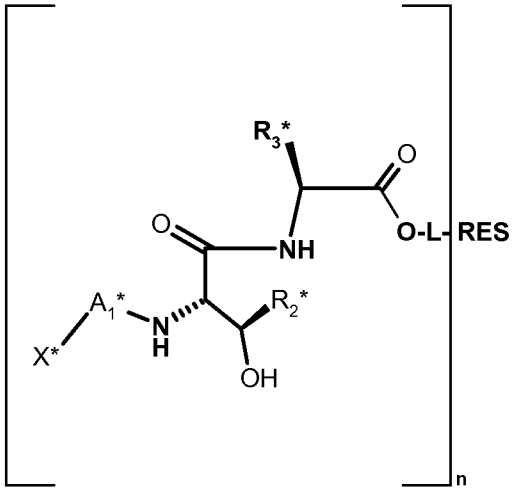
wherein Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R_7* is as defined for a compound of the formula II above, or a reactive derivative of said amino acid,

with the hydroxyl group of a compound of the formula XXVI,



XXVI

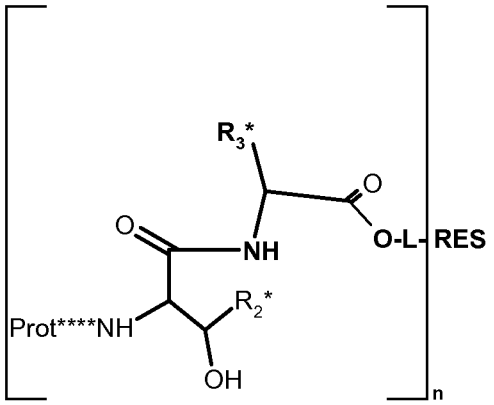
especially XXVIA,



XXVIA

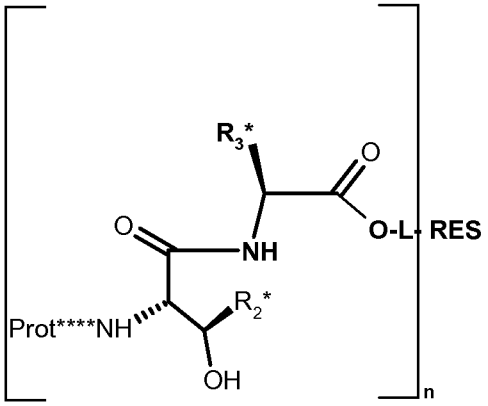
wherein X*, A_1*, R_2* and R_3* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number;
and removing the protecting group Prot***.

(viii/b) In a further embodiment, the invention relates to the method or process according to the preceding paragraph (vii/b) , further comprising, for the synthesis of a compound of the formula XXVI, especially XXVIA, coupling a resin bound dipeptide symbolized by the formula XII*,



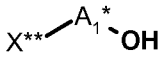
XII*

especially XIIA*



XIIA*

in which Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present in a compound of the formula II as defined above and with the product remaining on the resin, R_2* and R_3* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number, after removal of the protecting group Prot**** via the thus obtainable free amino group, with.an acid of the formula VII

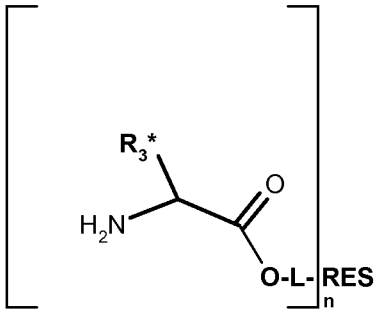


VII

wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined for a compound of the formula II above, or a reactive derivative of said acid;

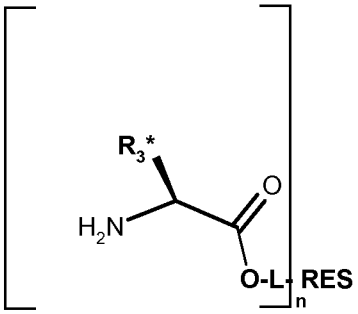
and, if X** is an amino protecting group, removing said amino protecting group X** to yield the derivative of formula II wherein, instead of X*, H is present and coupling the resulting amino group with an acyl group X* using the corresponding acid X*-OH wherein X* is as defined for a compound of the formula II above, or a reactive derivative of said acid.

(ix/b) A yet further embodiment of the invention relates to the method or process according to the preceding paragraph (viii/b), further comprising, for the synthesis of a compound of the formula XII, coupling a resin bound amino acid symbolized by the formula X,



X

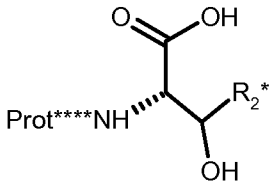
especially XA,



XA

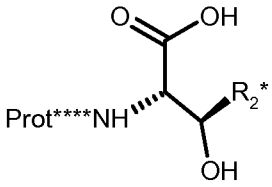
wherein R_3* is as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number,

with an amino acid of the formula XI*,



XI*,

especially XIA*,

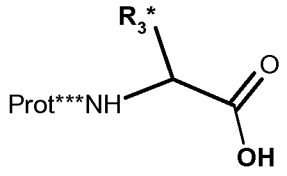


XIA*

wherein Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin , and R_2* is

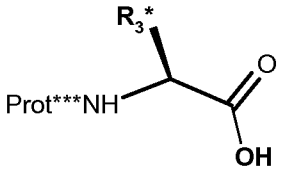
as defined for a compound of the formula II above, or a reactive derivative of said amino acid.

(x/b) A further embodiment of the invention relates to the method or process according to the preceding paragraph (ix/b), further comprising, for obtaining the resin bound amino acid of the formula X, coupling an amino acid of the formula IX*,



IX*

especially IXA*,



IXA*

wherein R_3* is as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin ; or a reactive derivative of said amino acid of the formula IX, to a cleavable linker L which is bound to a solid resin RES, and removing the protecting group Prot***.

(i/c) Another embodiment of the invention relates to the method or process according to any one of the preceding paragraphs (i/a) to (x/b) where the symbols A_1, R2, R3, R5, R6, R7, X and Y or the corresponding unprotected or protected moieties R_2*, R_3*, R_5*, R_6*, R_7*, X* and Y3 are selected so that, in the resulting compound of the formula I, or a salt thereof,

A_1 is the bivalent radical of L-glutamine bound via the carbonyl of its α-carboxy group to the amino group at the right of A_1 in formula I and via its α-amino group to X, or is 2S-(2-hydroxy-3-phosphonooxy)-propionyl;

R_2 is methyl;

R_3 is isopropyl, isobutyl (2-methyl-n-propyl wherever used) or benzyl, especially isobutyl;

R_5 is sec-butyl or benzyl, especially sec-butyl;

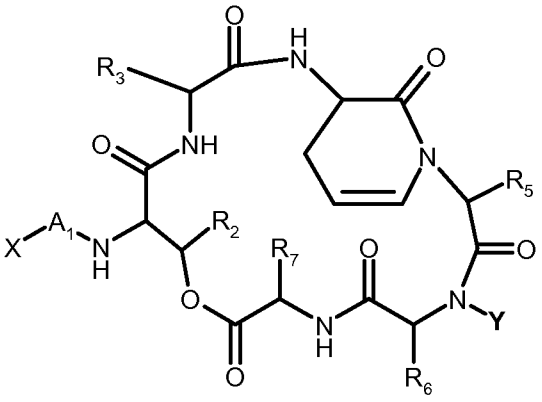
R_6 is 4-hydroxybenzyl;

R_7 is isopropyl or sec-butyl (1-methyl-n-propyl wherever used), especially sec-butyl;

X is acetyl or isobutyryl, or is absent if A_1 is 2S-(2-hydroxy-3-phosphonooxy)-propionyl and

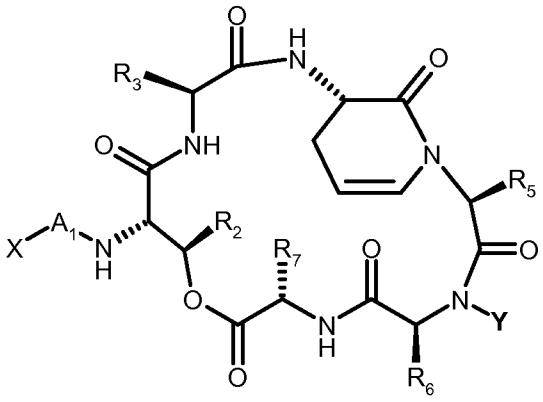
Y is methyl. This paragraph is also named paragraph a) below.

(i/d) In another particular embodiment, the invention relates to a method or process for converting a dehydrate of a compound of the formula I given above or in particular with the substituents as defined in the preceding paragraph (i/c) into the corresponding compound of the formula I, where the dehydrate has the formula V,



V,

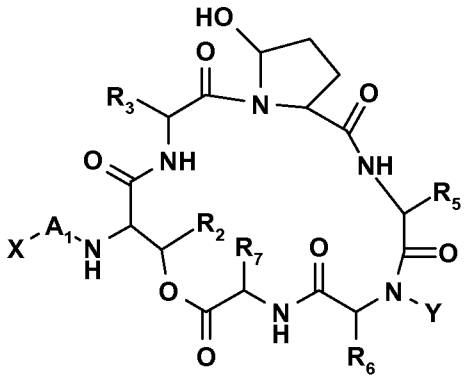
especially VA,



VA

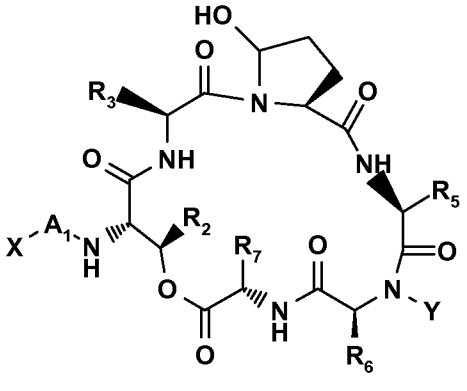
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I above;

or especially a method or process for shifting the equilibrium of a mixture of a compound of the formula I and its corresponding dehydrate, and/or its corresponding hemiaminal analogue with a five-ring instead of the ahp structure in formula I which may also be formed as byproduct and has the formula V*,



V*

especially the formula VA*,



VA*,

in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I above, respectively;

in favor of the compound of the formula I,

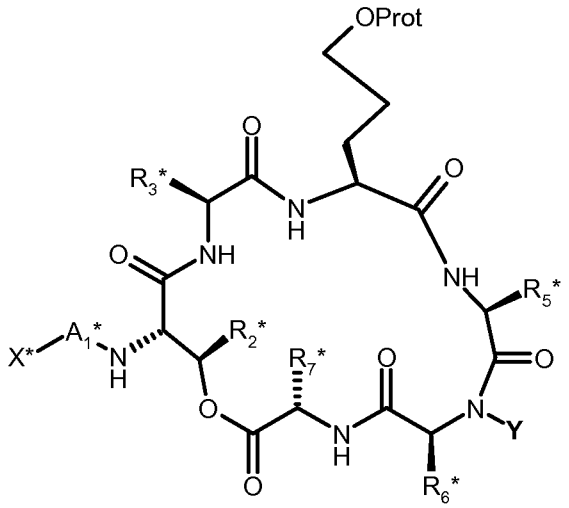
said method or process comprising using an aqueous acid as reactive solvent to drive the reaction. This method can be used independently (e.g. also for the product of fermentation or biosynthesis) or in addition to the other processes or methods described above and below to increase the yield or to re-convert a compound of the formula V,

especially VA, and/or the analogue with a five-membered ring instead of the ahp structure in formula I, into the corresponding compound of the formula I.

The method described for the conversion of the dehydrate and /or the five ring analogue (always regarding the desired ahp ring) into the desired compound of the formula I or especially IA, e.g. of Compound A -dehydrate from Example 3 B into Compound A, enables a straight-forward synthesis of this class of compounds. Up to now, an acidic treatment as final step had to be circumvented in order to avoid the dehydration of the product.

(i/e) A further embodiment of the invention relates to the method according to the preceding paragraph (i/d), wherein the acid is a carboxylic acid, especially a halo substituted C_{1-8}alkanoic acid, more especially trifluoroacetic acid or trichloroacetic acid.

(i/f) The invention, in yet a further embodiment, relates to a compound of the formula II,



II

wherein Prot is a protecting group, Y is as defined for a compound of the formula I in the first instance above or in particular as or claim 17 and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I as defined in claim 1 or in paragraph (ia) given above, respectively, however with the proviso that reactive functional groups on these moieties are present in protected form.

(i/g) In a further embodiment, the invention relates to a novel compound selected from the group consisting of compounds of the formula II, III, IV, V, VI, VIII, X, XII, XIV, XVI,

XVIII, XIX, XX, XXI, XXII, XXIII, XXIV, XXV, XXVI and XXVIII, and especially of the formula IIA, IIIA, IVA, VA, VIA, VIIIA, XA, XIIA, XIVA, XVIA, XVIIIA, XIXA, XXA, XXIA, XXIIA, XXIIIA, XXIVA, XXVA, XXVIA and XXVIIIA, yet more especially to the group consisting of the following compounds given in the examples: From Scheme 1: , compound 2, compound 3, compound 4, synthon 1; from Scheme 2: compound 5; Fmoc-Leu-Linker-Resin according to Example 1B(2); Fmoc-Thr-Leu-Linker-Resin according to Example 1B(3); Fmoc-Gln(Trt)-Thr-Leu-Linker-Resin according to Example 1B(4); Isobutyryl-Gln(Trt)-Thr-Leu-Linker-Resin according to Example 1B(5); Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Linker-Resin according to Example 1B(6); the product of Example 1B(7) = Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Linker-Resin (previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Fmoc)-Leu-Linker-Resin); Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Linker-Resin (previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Ile-Fmoc)-Leu-Linker-Resin) according to Example 1B(8); Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-Linker-Resin (previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-Me-Tyr(tBu)-Ile-Synthon 1-H)-Leu-Linker-Resin) according to example 1B(9). Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-OH (previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-Me-Tyr(tBu)-Ile-Synthon 1-H)-Leu-OH)according to example 1B(10) and Scheme 3; H-Thr-Leu-Resin according to example 1B(12); H-Gln(Trt)-Thr-Leu-Resin according to example 1B(13); Isobutyryl-Gln(Trt)-Thr-Leu-Resin according to example 1B(14); Isobutyryl-Gln(Trt)-Thr(Ile-H)-Leu-Resin according to example 1B(15); Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-Resin according to example 1B(16); Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resin according to example 1B(17); Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-Resin according to example 1B(18); from Scheme 3, Compound 6 and/or 7 (the latter preferred); from Scheme 4: Compound 8 and the 5-ring hemiaminal-isomer; from Scheme 5, precursor peptide 2, compound 9, compound 10 (this one being preferred in the present enumeration regarding Scheme 5), and/or compound 11; Fmoc-Thr-Leu-Trt-Tentagel-S from Example 2A(1); Fmoc-Gln(Trt)-Thr-Leu-Trt-Tentagel-S according to Example 2A(2); Ac-Gln(Trt)-Thr-Leu-Trt-Tentagel-S according to Example 2A(3); Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S according to Example 2A(4); Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Fmoc)-Leu-Trt-Tentagel-S (previously named: Ac-Gln(Trt)-Thr(Val-N-Me-Tyr(tBu)-Fmoc)-Leu-Trt-Tentagel-S) according to Example 2A(5); Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Fmoc)-Leu-Trt-Tentagel-S (previously named: Ac-Gln(Trt)-Thr(Val-N-Me-Tyr(tBu)-Phe-Fmoc)-Leu-Trt-Tentagel-S)

according to Example 2A(6); and Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Synthon1-H)-Leu-OH (previously named: Ac-Gln(Trt)-Thr(Val-N-Me-Tyr(tBu)-Phe-Synthon1-H)-Leu-OH) (precursor peptide 2) according to Example 2A(7).

The following definitions (or also definitions already included above) can replace more general terms used in invention embodiments above and below in order to define further embodiments of the invention, with either one, two or more or all general terms being replaceable by the more specific terms in order to define such invention embodiments:

A bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group is preferably an alpha-carbamoyl or carboxyl-C_{1-8}-substituted amino acid, especially the bivalent moiety of asparagine or glutamine, and is bound at its right hand side in formula I via a carbonyl (preferably the carbonyl of its α-carboxyl group) to the rest of the molecule.

C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl (C_{1-8}-alkanoyl carrying both a hydroxyl and a phosphono (-O-P(=O)$(OH)_2$) group) A_1 is e.g. 2,3-dihydroxy-propanoyl (preferably in S-form) or 2-hydroxy-3-phosphono-propanoyl (preferably in S-form).

R_2 and R_2* are C_{1-8}-alkyl, especially methyl wherever mentioned.

R_3 is the side chain of an amino acid, especially of a natural amino acid. Preferably, it is C_{1-8}alkyl which may be branched or linear. Most especially, C_{1-8}alkyl is n-(2-methyl)propyl (isobutyl), n-(1-methylpropyl (sec-butyl) or methyl, that is, the amino acid carrying the moiety is leucine, isoleucine or valine.

R_3* is the corresponding side chain in protected form if a functional group is present that has to be hindered to participate in a reaction. Preferably, it is C_{1-8}alkyl which may be branched or linear, especially as defined in the preceding paragraph.

A “side chain of an amino acid” may be selected from any moiety, e.g. a mono- or polycyclic, linear, saturated, unsaturated (e.g. with conjugated double bonds) or partially saturated organic moiety, e.g. with up to 20 carbon atoms and 0 to 5 heteroatoms in the basis structure independently selected from N, O and S replacing the corresponding

number of carbon atoms, and may be substituted by up to three moieties selected from amino, imino, hydroxy, carboxy, carbamoyl, sulfhydryl, amidino, guanidino, O-phosphono ($-O-P(=O)(OH)_2$). Preferably, the side chains are selected from those of the 20 standard alpha-amino acids arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparagine, glutamine, cysteine, glycine, alanine, leucine, isoleucine, methionine, phenylalanine, tryptophan, tyrosine, valine and further proline (then with internal cylization including the alpha-amino group).

For the amino acids, either their names or the customary three letter codes are used in the present disclosure, in accordance with the following table:

Amino acid	**Three letter code**
Alanine	Ala
Arginine	Arg
Asparagine	Asn
Aspartic acid	Asp
Asparagine or aspartic acid	Asx
Cysteine	Cys
Glutamic acid	Glu
Glutamine	Gln
Glutamine or glutamic acid	Glx
Glycine	Gly
Histidine	His
isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Phenylalanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tryptophan	Try
Tyrosine	Tyr
Valine	Val

R_5 is the side chain of an amino acid, preferably a standard amino acid. Preferably, it is C_{1-8}alkyl which may be branched or linear and which is unsubstituted or substituted by phenyl. Most especially it is benzyl, n-(2-methyl)propyl, isobutyl or methyl, that is, the amino acid carrying the moiety is phenylalanine, leucine, isoleucine or valine.

R_6 is the side chain of a hydroxy amino acid, especially of tyrosine.

R_7 is the side chain of an amino acid, especially of a natural amino acid. Preferably, it is C_{1-8}alkyl which may be branched or linear. Most especially it is n-(2-methyl)propyl (isobutyl), n-(1-methyl)propyl (sec-butyl) or methyl, that is, the amino acid carrying the moiety is leucine, isoleucine or valine.

C_{1-8}-alkyl can be linear or branched one or more times; for example, it can be n-(2-methyl)propyl, n-(1-methyl)propyl or methyl.

All of the compounds can, where salt-forming groups such as basic groups, e.g. amino or imino, or acidic groups, e.g. carboxyl or phenolic hydroxyl, are present, be used in free form or as salts or as mixtures of salts and free forms. Thus where ever a compound is mentioned, this includes all these variants. For example, basic groups may form salts with acids, such as hydrohalic acids, e.g. HCl, sulfuric acid or organic acids, such as acetic acid, while acidic groups may form salts with positive ions, e.g. ammonium, alkylammonium, alkali or alkaline-earth metal salt cations, e.g. Ca, Mg, Na, K or Li cations, or the like.

“Or the like” or “and the like”, wherever used in this disclosure, refers to the fact that other alternatives to those mentioned preceding such expression are known to the person skilled in the art and may be added to those expressions specifically mentioned; in other embodiments, “or the like” and ”and the like” may be deleted in one or more or all invention embodiments.

The protecting groups Prot, Prot*, Prot**, Prot***, Prot**** and any further protecting groups present on the moieties A*, R_2*, R_3*, R_5*, R_6*, R_7*, X*, where ever mentioned throughout the present description and claims, are selected so that they allow for orthogonal protection.

Orthogonal protection is a strategy allowing the deprotection of multiple protective groups one (or more but not all) at the time where desired each with a dedicated set of reaction conditions without affecting the other protecting group(s) or bonds to resins, e.g. via linkers on solid synthesis resins. In other terms: The strategy uses different classes of protecting groups that are removed by different chemical mechanisms, also using appropriate linkers in the case of solid phase peptide synthesis (where the linker-resin bond might together be considered as a carboxy protecting group).

Preferably, the protecting groups are selected as follows:

The protecting group Prot is preferably selected so as to resist removal of any other protecting groups used or present during the synthesis according to the invention of a depsipeptide, e.g. able to resist mild bases (see Prot*), but removable with fluoride ion (especially under anhydrous conditions), e.g. $Bu_4N^+F^-$ (also if created *in situ*, e.g. using $Bu_4N^+Cl^-$ with KF • H_2O, KF with 18-crown-6, LiBr with 18-crown-6, BF_3 • diethylether, pyridine-HF, HF in urea, $Et_3N(HF)_3$ (wherein Et is ethyl) or the like, where the solvent is e.g. selected from the group consisting of N,N-dimethylformamide, acetonitrile, chloroform and tetrahydrofurane.

Preferably, Prot is an ether protecting group, especially selected from the group consisting of silyl protecting groups in which the silyl moiety carries up to three organic moieties bound via a carbon (optionally via a further Si atom), such as tert-butyldiphenylsilyl, trimethylsilyl, triisopropylsilyl, tert-butyldimethylsilyl, triphenylsilyl, diphenylmethylsilyl, ti-tert-butyldimethylsilyl, tert-butylmethoxyphenylsilyl, tris(trimethylsilyl)silyl or the like.

Prot* is a protecting group that can be cleaved off selectively without affecting other protecting groups present, and also without affecting the depsipeptide forming ester bond or a linker to a resin RES, and is stable during deprotection steps during synthesis of the linear precursor peptide (e.g. removal of allyloxycarbonyl); it is preferably a protecting group removable by specific triphenylphosphin complexes in the presence of metal hydrides or other reductants, e.g. $(PH_3P)_4Pd$ preferably in combination with di-n-butyl tin hydride or tri-n-butyl tin hydride, phenylsilane, sodium borohydride or dimedone, in an appropriate solvent, e.g. tetrahydrofurane, and is preferably not cleavable under

conditions that allow for the removal of a protecting group Prot**; for example, and Prot* is selected from the group consisting of C_3-C_8alk-2-enyloxycarbonyl moieties, e.g. allyl-oxycarbonyl (Alloc), 1-isopropylallyloxycarbonyl, 4-nitrocinnamyloxycarbonyl and 3-(3'-pyridyl)prop-2-enyloxycarbonyl.

Prot** is a protecting group that can be removed on the resin without cleaving other bonds (no cleavage of an amino acid or peptide bound via the carbonyl of its (especially α-carboxyl group to the binding via a linker L mentioned below; also without cleaving off the protecting group Prot once present), especially a protecting group removable without cleavage of an ester (instead of an amide) bond in a depsipeptide or depsipeptide precursor and under conditions other than those for the protecting groups Prot* and Prot, while preserving the binding via the linker to a resin RES where present; it is preferably removable by a mild base e.g. piperidine, morpholine, dicyclohexylamine, p-dimethyl-amino-pyridine, diisopropylamine, piperazine, tris-(2-aminoethyl)amine in an appropriate solvent, e.g. N,N-dimethylformamide, methylene chloride; Prot** is, e.g., selected from the group consisting of fluoren-9-ylmethoxycarbonyl (Fmoc); 2-(2' or 4'-pyridyl)ethoxy-carbonyl and 2,2-bis(4'nitrophenyl)ethoxycarbonyl.

Prot *** (an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin) and Prot**** (a protecting group that can be cleaved off selectively without affecting other protecting groups present in a compound of the formula II as defined above and below and with the product remaining on the resin) are preferably protecting groups that can be removed as Prot** and are, e.g., selected from those mentioned for R**, e.g. fluoren-9-ylmethoxycarbonyl (Fmoc).

The preferred orthogonal synthesis method in this case makes use of the Fmoc method known in general for peptide synthesis using solid phase peptide synthesis combined with solution phase macrolactamization and further chemical conversions.

Alternatively, e.g. the Boc protecting group might be used instead of Fmoc Prot**, Prot*** and Prot****.

However, this will require different side chain protecting groups and also the hydroxy-group of N-methyl-Tyr will then have to be protected in a different way to maintain orthogonality of the protection groups.

Other protecting groups present as well as the binding linker to a resin RES where present are preferably not removable under conditions under which Prot* and Prot** can be removed, e.g. in A*, the amide can be N-protected e.g. with trityl (triphenylmethyl) (cleavage e.g. with trifluoro acetic acid (TFA); in R_6* a tyrosine hydroxy can be protected as t-butyl-ether, or protected by tert-butyldimethylsilyl, methoxymethyl, Boc (tert-butoxycarbonyl) or arylacetate (cleavage with TFA).

Appropriate protecting groups are known in the art, as well methods for their introduction and removal. For example, the protecting groups, their introduction and removal methods may be selected from those described in standard textbooks such as "Protective Groups in Organic Synthesis", 3rd ed., T.W. Green and P.G.M. Wuts (Eds.). J. Wiley & Sons, Inc., New York etc. 1999.

The protecting groups Prot, Prot*, Prot**, Prot***, Prot**** and other protecting groups are thus not limited to those mentioned above – rather they should fulfill conditions that make them appropriate for orthogonal protection, e.g. as described above or below.

It is recommended to avoid too basic conditions (though the bases described for Fmoc cleavage, such as piperidine, are usually allowable) to avoid cleavage of the depsipeptide (ester) bond.

Among the possible solid support for Solid Phase Peptide Synthesis (SPPS), the following may be mentioned:

- Gel-type supports without or with spacer: These are highly solvated polymers with an equal distribution of functional groups. This type of support is the most common, and includes:
Polystyrene: Styrene cross-linked with e.g. 1-2% divinylbenzene; Polyacrylamide or polymethacrylamide: as hydrophilic alternative to polystyrene; Polyethylene glycol (PEG): PEG-Polystyrene (PEG-PS) is more stable than polystyrene and spaces the site

of synthesis from the polymer backbone; PEG-based supports: Composed of a PEG-polypropylene glycol network or PEG with polyamide or polystyrene (these already include a spacer, PEG);
- Surface-type supports: Materials developed for surface functionalization, including controlled pore glass, cellulose fibers, and highly cross-linked polystyrene.
- Composites: Gel-type polymers supported by rigid matrices.

Usually these gels carry reactive groups to which a linker L as mentioned for various precursors above and below can be bound. For example, such groups include aminomethyl groups, polyethyleneglycol groups with a terminal hydroxy, and the like.

Any such support can be used in the embodiments of the present invention.

Gel type supports are used in another special embodiment of the invention, Among these, polystyrene (divinylbenzene crosslinked); polyacrylamide and polymethacrylamide resins are especially preferred.

Among the possible linkers, all commonly known and appropriate may be used.

Examples in possible embodiments of the invention are the 2-methoxy-4-benzyloxy-benzyl alcohol linker (a Sasrin-Linker, Sasrin stands for superacid sensitive resin, binds the amino acids or peptides via alcoholic OH); the trityl linker family (e,g, Trityl, 2Cl-Trityl, which bind the amino acids or peptides via OH); the 4-(2,4-dimethoxyphenylhydroxy-methyl)phenoxymethyl-Linker (Rink-Acid-Linker, binds the amino acids or peptides via OH); or tris(alkoxy)benzyl ester linkers (HAL-Linker, binds the amino acids or peptides via OH).

Where reactive derivatives of acids, especially amino acids, or peptides, e.g. dipeptides, are mentioned, they may be formed *in situ* or may be used as such.

Reactive (or active) derivatives used as such include the acyl-halides, e.g. acyl-chlorides, -fluorides or -nitrophenyl esters, e.g. the 2,4-dinitrophenyl esters, or acid anhydrides (symmetric or e.g. with acetic acid) of the carboxy groups of the acids to be reacted.

For *in situ* amino acid activation, customary coupling agents may be applied. Such reagents are known to the person skilled in the art and can be purchased conveniently from many sources, e.g. Aldrich ChemFiles – Peptide Synthesis (Aldrich Chemical Co., Inc., Sigma-Aldrich Corporation, Milwaukee, WI, USA) Vol. 7 No. 2, 2007 (see http://www.sigmaaldrich.com/etc/medialib/docs/Aldrich/Brochure/al_chemfile_v7_n2.Par.0001.File.tmp/al_chemfile_v7_n2.pdf). Among the possible coupling agents for amide and ester bond synthesis the following may be mentioned:

Triazoles, uronium or hexafluorophosponium derivatives, e.g. 1-hydroxy-benzotriazole (HOBt), 1-hydroxy-7-aza-benzotriazole (HOAt), ethyl 2-cyano-2-(hydroxyimino)acetate, 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate methanaminium (HATU), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP), 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate (HBTU), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluoroborate (TBTU), 2-succinimido-1,1,3,3-tetramethyluronium-tetrafluoroborate (TSTU), 2-(5-norbornen-2,3-dicarboximido)-1,1,3,3-tetramethyluronium -tetrafluoroborate (TNTU), O-[(cyano(ethoxycarbonyl)-methyliden)amino]-1,1,3,3-tetrameth yluronium-tetrafluoroborate (TOTU), O-(benzotriazol-1-yl)-1,3-dimethyl-1,3-dimethylene uronium hexafluorophosphate (HBMDU), O-(benzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate (HBPyU), O-(benzotriazol-1-yl)-1,1,3,3-bis(pentamethylene)uronium hexafluorophosphate (HBPipU), 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HODhbt), 1-hydroxy-7-aza-benzotriazole and its corresponding uronium or phosphonium salts, designated HAPyU and AOP, 1-cyano-2-ethoxy-2-oxoethylideneaminooxy-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), chlorotripyrrolidinophosphonium hexafluorophosphate (PyCloP), or the like;

Carbodiimides, e.g. dicyclohexylcarbodiimide, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, 1-tert-butyl-3-ethylcarbodiimide, N-cyclohexyl-N'-2-morpholinoethyl)carbodiimide or diisopropylcarbodiimide (especially for ester formation via O-acyl urea formation of the carboxylic group); or

active ester forming agents, e.g. 2-mercaptobenzothiazole (2-MBT),

azide forming agents, e.g. diphenyl phosphoryl azide,

acid anhydrides, such as propane phosphonic acid anhydride,

acid halogenation agents, e.g. 1-chloro-N,N,2-trimethyl-1-propenylamine, chloro-N,N,N',N'-bis(tetramethylene)formamidinium tetrafluoroborate or hexafluorophosphate, chloro-N,N,N',N'-tetramethlformamidinium hexafluorophosphate, fluoro-N,N,N',N'-tetrametylformamidinium hexafluorophosphate, fluoro-N,N,N',N'-bis(tetramethylene)formamidinium hexafluorophosphate,

or the like, or mixtures of two or more such agents.

Also for the ester coupling of compounds of the formula XII or XIIA with those of the formula XIII or XIIIA, respectively, or of compounds of the formula XIII* or XIIIA* with those of the formula XXVI or XXVIA, respectively, the corresponding reactive carboxyl compounds can be used or formed *in situ*. Here, especially MSNT is preferred as coupling agent as this allows for the maintenance of high stereospecificity.

The reaction may, where appropriate, be conducted in the presence of a mild base (e.g. N-methylmorpholine, a trialkylamine, e.g. ethyldiisopropylamine, a di-(alkyl)aminopyridine, such as N,N-dimethylaminopyridine, or the like (taking care that the conditions are not so basic as to allow for the hydrolysis of ester groups, e.g. the depsipeptide ester group, present in precursors of the compound of the formula I), where appropriate or required in the presence of an appropriate solvent or solvent mixture, e.g. an N,N dialkylformamide, such as dimethylformamide, a halogenated hydrocarbon, e.g. dichloromethane, N-alkylpyrrolidones, such as N-methylpyrrolidone, nitriles, e.g. acetonitrile, or further an aromatic hydrocarbon, e.g. toluene, or mixtures of two or more, where, provided an excess of coupling agent is present, also water may be present. The temperatures may be ambient temperature or lower or higher, e.g. in the range from -20 °C to 50 °C.

The amino acids of the formula IX, IXA, XI, XIA, XIII, XIIIA, XV, XVA, XVII, XVIIA, XXVII (obtainable e.g. by Solution Phase synthesis), XVII*, XVIIA*, XV*, XVA*, XIII*, XIIIA*, XI*, XIA*, IX* and IXA* are known or they can be synthesized according to methods known in the art, they are commercially available, and/or they can be synthesized in analogy to methods known in the art.

Also the remaining starting materials, e.g. the acid of the formula XIX or VII, or the dipeptide of the formula XXVII or XXVIIA, are known or they can be synthesized according to methods known in the art, they are commercially available, and/or they can be synthesized in analogy to methods known in the art.

For example, the synthon of the formula XIX can be prepared as described in Example 1 A(4) (which is a specific embodiment of the invention) or in analogy thereto. The synthesis of the intermediate compound 1 (Scheme 1) is described in Tetrahedron 61, 1459-1480 (2005).

The coupling reactions for dipeptides make use of the corresponding carboxylic groups of amino acids in free form or in activated form.

Examples: The following examples illustrate the invention without limiting its scope.

Abbreviations

aq.	aqueous
Boc/BOC	tert-Butoxycarbonyl
brine	sodium chloride solution in water (saturated at RT)
Bzl	benzyl
COMU	1-cyano-2-ethoxy-2-oxoethylideneaminooxy-dimethylamino-morpholino-carbenium hexafluorophosphate
DCM	dichloromethane
DIPEA	N,N-diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-dimethylformamide
Fmoc/FMOC	9-fluorenymethoxycarbonyl
Et	ethyl
HATU	2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyluronium hexafluorophosphate Methanaminium
HFIP	Hexafluoroisopropanol
HPLC	High Performance Liquid Chromatography
HR-MS	High Resolution Mass Spectroscopy
IPA	Isopropylacetate
IPC	In-Process Control
IR	Infrared Spectroscopy
IT	internal temperature
Kaiser test	Ninhydrin-based test to monitor deprotection in SPPS (see E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, Analytical Biochemistry **1970**, *34* 595); if mentioned to be OK, this means successful deprotection.
me	methyl
MS	Mass Spectroscopy
MSNT	1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole
NMR	Nuclear Magnetic Resonance Spetroscopy
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

BOP	Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
RP	Reversed Phase
RT/rt	room temperature
SPPS	Solid Phase Peptide Synthesis
TBME	tert-Butyl-methylether
TFA	trifluoroacetic acidTEMPO 2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical.
RBF	Round bottomed flask

For amino acid abbreviations see the table above.

Compounds Names...

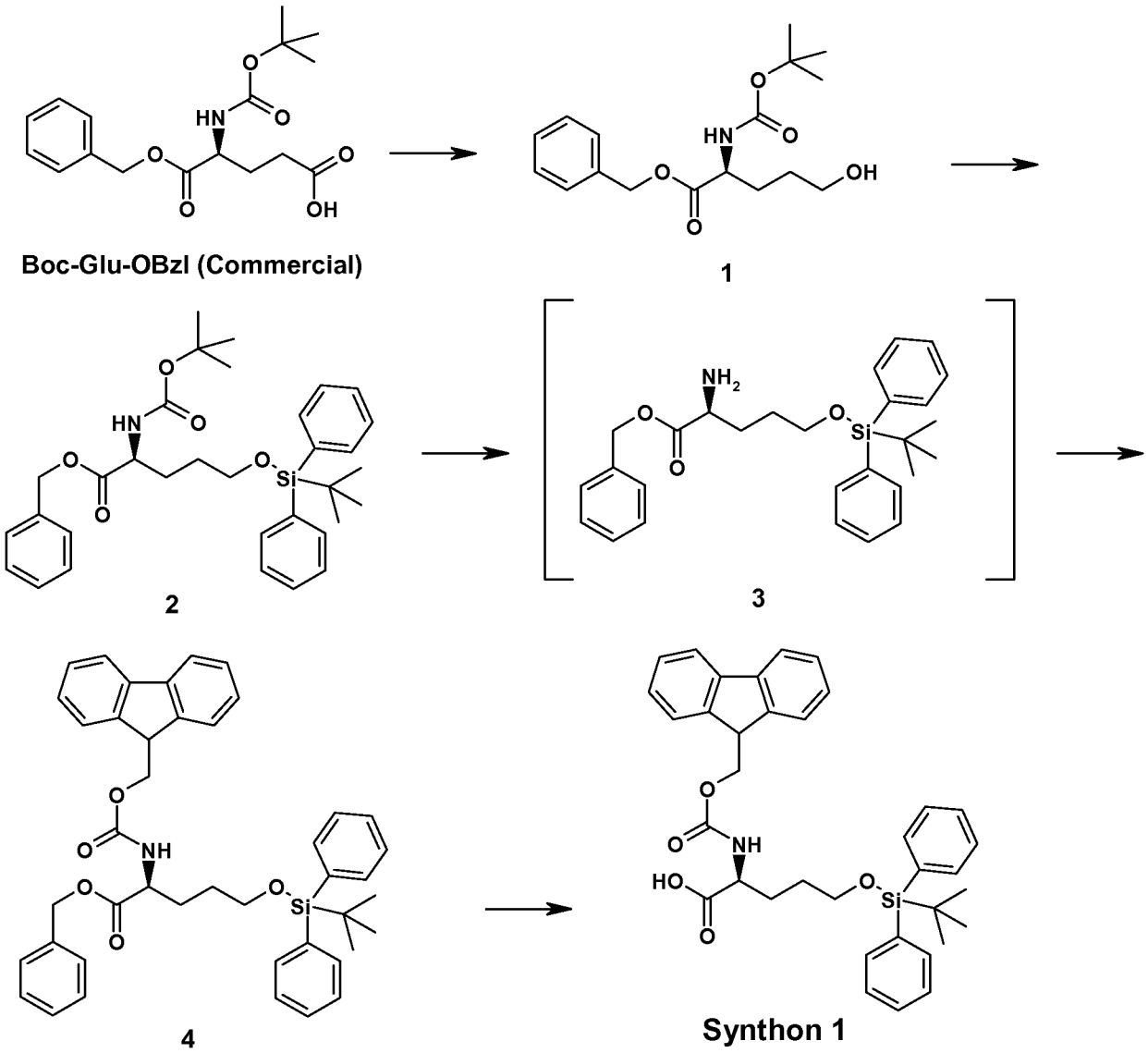
The names of the open chain oligopeptides have been derived according to recommendations of the Joint Commission on Biochemical Nomenclature (“International Union of Pure and Applied Chemistry” and “International Union of Biochemistry”) published in Pure & Appl. Chem. 1984, *56*, 595-624. Previously, a simple peptide naming convention was used for these compounds. The previous names are given in parenthesis.

Example 1: Synthesis of Compound A

1A Synthesis of Synthon 1

(i) Alternative 1:

Reaction Scheme 1:



1A(1) Preparation of compound 1

(*S*)-Benzyl 2-((*tert*-butoxycarbonyl)amino)-5-hydroxypentanoate

Compound 1 was prepared by a procedure similar to the procedure reported in R. K. Olson, K. Ramasamy, T. Emery, *J. Org. Chem.* **1984**, *49*, 3527. BOC-Glu-OBzl (50g, 148.2 mmol) was dissolved in tetrahydrofuran (800 mL) and triethylamine (47.3g, 467.4 mmol) was added. The solution was cooled down to IT= -10 °C. Ethyl-chloroformate (51.8g, 98% purity, 467.8 mmol) was added slowly, maintaining the temperature at IT = -10 to -15 °C. The suspension thus obtained was stirred for an additional hour. IPC (HPLC) indicated the disappearance of the starting material. The reaction mixture was allowed to warm-up to 0 °C and water (800 mL) was added at 0-5 °C within 25-30 minutes. Separation of the mixture into 2-phases became visible. Under intensive stirring, sodium-borohydride (11.8g, 299.4 mmol) was added in 10 portions at 0-5 °C. Caution was exercised as hydrogen gas evolved during addition of sodium-borohydride. The reaction mixture was stirred for additional 5 minutes at 0-5 °C and the temperature was increased to 20-25 °C within 30 minutes. Caution was required as hydrogen-gas continued to evolve during temperature increase. The reaction mixture was stirred for 15 minutes at 20-25 °C before work-up.
For work-up, water (1250 mL) was added to the reaction mixture, followed by the addition of ethyl acetate (1250 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (600 mL). The organic layers were combined and were washed with brine (2x 600 mL). The organic layer was dried over magnesium sulfate, the solvent was evaporated at reduced pressure and 40-45 °C to obtain 50.8g crude product. HPLC purity: 94 a%. The crude product was purified by flash chromatography on silica gel with hexane fraction/ethyl acetate (7:3 to 1:1) as mobile phase.
Yield: 40.85g (85.2%). Purity: 98% (HPLC). MS and NMR confirmed the proposed structure.

1A(2) Preparation of compound 2:(*S*)-Benzyl 2-((*tert*-butoxycarbonyl)amino)5-((*tert*-butyldiphenylsilyl)oxy)pentanoate

The alcohol (compound **1**) from the previous step (40.3g, 124.6 mmol) was dissolved in dimethyl formamide (200 mL) and imidazole (12.8g, 99.5% purity, 186.9 mmol) was added. The mixture was stirred at room temperature, until a solution was formed (5-10

min). tert.-Butyl-diphenyl-silyl-chloride (41.9g, 98% purity, 149.5 mmol) was added dropwise within 10 minutes and stirring was continued for additional 15 minutes at room temperature. IPC (HPLC) indicated disappearance of the starting material (alcohol). For work-up, isopropyl acetate (400 mL) was added to the reaction mixture, followed by the slow addition of a half-saturated aq. sodium bicarbonate solution (400 mL). Caution was exercised as the addition was exothermic., gas evolution takes place. The phases were separated and the aqueous phase was extracted with isopropyl acetate (400 mL). The organic layers were combined and extracted with water (400 mL). The organic layer was dried on magnesium sulfate and the solvent was evaporated at 40-45 °C under reduced pressure. The residue was dried over night *in vacuo* at 25 °C to obtain 83.25g crude product as colorless oil. HPLC analysis indicated the presence of 81 a% desired product and 18.6 a% of the corresponding silanole. The crude product was used without further purification for the next step. NMR and HR-MS of a purified sample confirmed the proposed structure. HR-MS: calculated for $C_{33}H_{43}NO_5Si$: $[M+H]^+$: 562.29833; $[M+NH_4]^+$: 579.32488; $[M+Na]^+$: 584.28027. Found: $[M+H]^+$: 562.29848; $[M+NH_4]^+$: 579.32489; $[M+Na]^+$: 584.27995.

1A(3) Preparation of compound 4:

(*S*)-Benzyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-((*tert*-butyldiphenylsilyl)oxy)pentanoate

83.25g crude product from the previous step (corresponding to 70g theoretical yield of compound **2**, 124.6 mmol) was dissolved in dichloromethane (650 mL) and trifluoroacetic acid (358.8g, 99% purity, 3115 mmol) was added dropwise to the intensively stirred solution. IPC (HPLC) after 30 minutes indicated complete cleavage of the BOC protecting group. The reaction mixture (clear solution) was transferred into a 6 L 4-bottomed flask with mechanical stirrer and was diluted with 1000 mL of dichloromethane. Aqueous half saturated sodium carbonate solution (1800 mL) was slowly added to the intensively stirred solution. Caution was exercised as strong gas evolution was observed during addition of sodium carbonate. pH of the aqueous phase after completion of the addition: 9-10. The reaction mixture was stirred for additional 15 minutes and the phases were separated. The aqueous phase was extracted with dichloromethane (1000 mL) and the organic layers were combined to obtain a solution of the product in dichloromethane.

The solution was concentrated at 40-45 °C under reduced pressure to a final volume of ca. 650 mL. for the next step. HPLC indicated the presence of 80.5 % of compound **3** and 19.5 a% silanole in the solution.
For FMOCylation, saturated aq. sodium bicarbonate solution (650 mL) was slowly added to the intensively stirred solution of compound **3**, followed by the addition of FMOC-chloride (36.55g, 97% purity, 137 mmol). Caution was exercised as gas evolution was observed during the addition of FMOC-Cl. Stirring was continued for 15 minutes at room temperature. An IPC (HPLC) of the organic layer indicated disappearance of the intermediate compound 3 and complete conversion into compound 4. For work-up, the layers were separated and the aqueous phase was extracted with dichloromethane (650 mL). The organic layers were combined and were extracted with water (650 mL). The organic layer was dried over magnesium sulfate and the solvent was removed at 40-45 °C under reduced pressure to obtain 112.6g crude product.
The crude product was suspended in ethanol/isopropanol/water (89:5:6; 2400 mL) and the suspension was heated to IT 50 °C to obtain a solution. The solution was cooled down to 45 °C, seed crystals were added and the temperature was continued to cool down to 20-25 °C within 1 hour. Crystallization started at ca. 40 °C. The suspension was stirred at 20-25 °C over night, then cooled down to IT 0-5 °C within 30 minutes and stirring was continued for additional 2 hours at 0-5 °C. The product was isolated by filtration, the filter cake was washed with ethanol/isopropanol/water (89:5:6; 240 mL) and dried under reduced pressure to obtain pure compound **4** (100 a% purity according to HPLC). Yield: 66g (77.4%). The product was fully characterized by MS and NMR HR-MS: Calculated for $C_{43}H_{45}NO_5Si$: $[M+H]^+$: 684.31398; $[M+NaH_4]^+$: 701.34053; $[M+Na]^+$: 706.29592. Found: $[M+H]^+$: 684.31392; $[M+NH_4]^+$: 701.34021; $[M+Na]^+$: 706.29539. The mother liquor gave 30.6g of a foam comprising 30 a% product and 29 a% silanole according to HPLC.

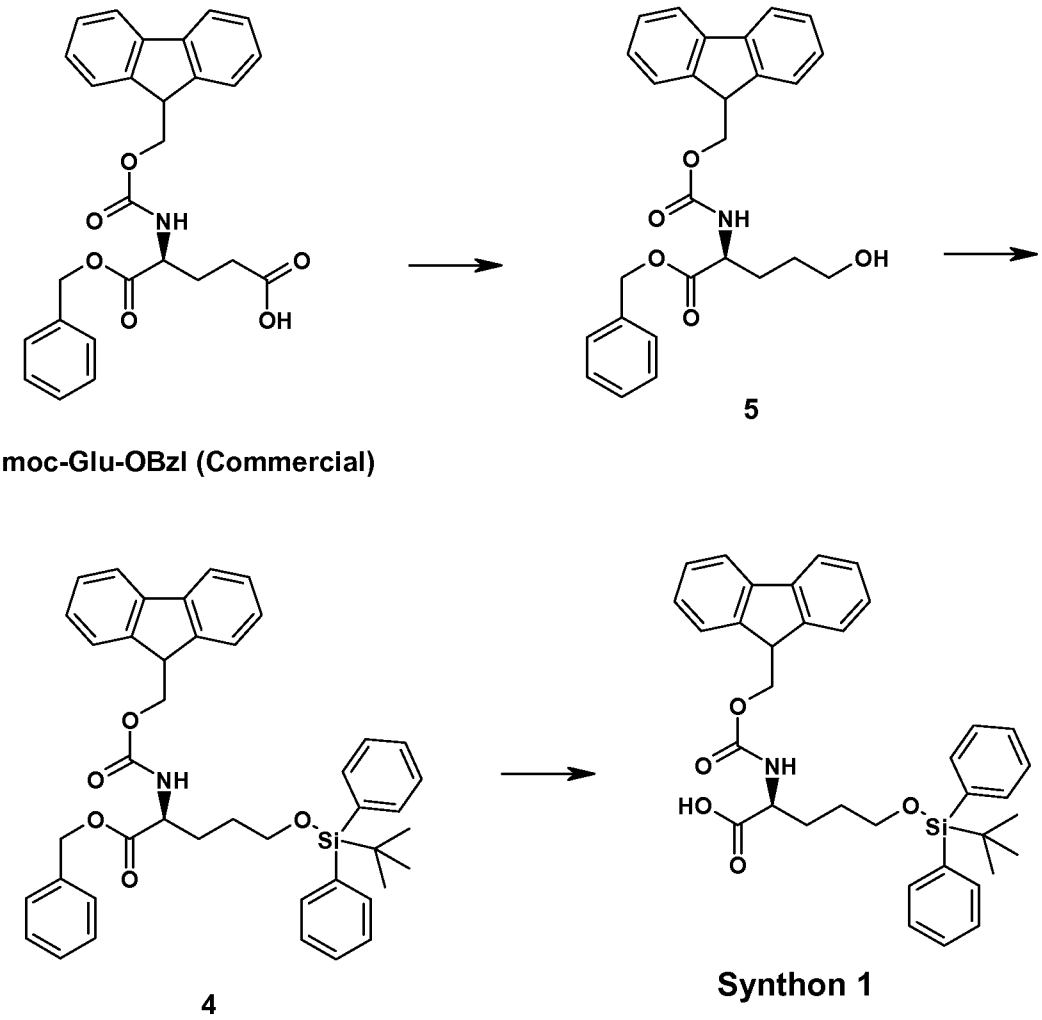
1A(4) Preparation of Synthon 1 from compound 4:
(S)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-((*tert*-butyldiphenylsilyl)oxy)pentanoic acid

Compound **4** (66g, 96.6 mmol) was suspended in ethanol/isopropylalcohol/water (89:5:6; 3000 mL) and the suspension was heated to IT 45 °C to obtain a solution. The solution was cooled down to IT 30 °C. After inertization with Argon, palladium-catalyst (10% on

barium sulfate; 6.6g) was added to the solution under an argon stream. The product was then hydrogenated under a hydrogen pressure slightly above the atmospheric pressure at 30-35 °C. The hydrogenation was completed after 1.5h according to HPLC. The reaction mixture was filtered over a cellulose based filter aid (Cellflock 40; cellulose based filtering aid) and the filter aid was washed with ethanol/isopropanol/water (89:5:6; 600 mL). Evaporation of the solvent under reduced pressure at 45-50 °C gave 59.58g foam as crude product. The crude product was purified by chromatography on silica gel in 2 portions (2x1 kg silica gel 60) using dichloromethane/methanol 95:5 to 80:20 as mobile phase. Yield: 52g (90.6%). The product was fully characterized by MS and NMR. HR-MS: Calculated for $C_{36}H_{39}NO_5Si$ $[M+H]^+$: 594.26703; $[M+NH_4]^+$: 611.29358; $[M+Na]^+$: 616.24897; Found: $[M+H]^+$: 594.26743; $[M+NH_4]^+$: 611.29385; $[M+Na]^+$: 616.24900.

(ii) Alternative 2:

Reaction Scheme 2:



1A(5) One-pot Preparation of compound 4 from Fmoc-Glu-OBzl via compound 5:

Fmoc-Glu-OBzl (5g, 10.88 mmol) was dissolved in tetrahydrofurane (80 mL) and triethylamine (3.3g, 32.6 mmol) was added. The solution was cooled down to -15 °C and ethyl chloroformate (7.3g, 67.27 mmol) was added to the solution over 30 minutes at -12 °C. The suspension thus obtained was stirred for an additional hour at -10 to -15 °C and the temperature was elevated to 0 °C. Water (80 mL) was added dropwise to the reaction mixture, maintaining the temperature at 0 °C. Sodium borohydride (total 0.805 g, 21.27 mmol) was added in 3 portions (1 portion every 10 minutes) at 0 °C and the reaction mixture was stirred for additional 1h at 0 °C. Caution was exercised as

hydrogen gas evolved. The reaction mixture was diluted with water (100 mL) and extracted with isopropyl acetate (150 mL) and the layers were separated. The aqueous phase was extracted again with isopropyl acetate (100 mL) and the organic layers were combined. The combined organic phase was washed with half saturated sodium chloride solution (2x 50 mL) and the solution was concentrated at reduced pressure to a final volume of ca. 50 mL. The concentrated solution was clear-filtered and the filter residue was washed with isopropyl acetate (20 mL). The solution of compound **5** thus obtained was transferred into a round bottomed flask and imidazole (1.49g, 21.89 mmol) was added. The mixture was stirred for 15 min at Rt and tert.-butyl-diphenyl-silyl-chloride (4.45g, 16.19 mmol) was added. The reaction mixture was stirred for 15 h at rt. For work-up, the suspension was diluted with isopropyl acetate (20 mL) and extracted with water (3x50 mL). The organic layer was separated and the solvent was evaporated under reduced pressure to obtain 9.95g crude product. The crude product was purified by column chromatography on silicagel with isopropyl acetate/hexane (2:8) as mobile phase to obtain 6.5g of a solid, which was suspended in hexane and stirred for 3h at rt. The precipitate was isolated by filtration and dried at 50 °C under reduced pressure to obtain 5.6g of compound **4**. Yield: 75% over two steps. HR-MS: calculated for $C_{43}H_{45}NO_5Si$: $[M+H]^+$: 684.31398; $[M+NH_4]^+$: 701.34053; $[M+Na]^+$: 706.29592. Found: $[M+H]^+$: 684.31430; $[M+NH_4]^+$: 701.34073; $[M+Na]^+$: 706.29577.

1A(6) Alternative one-pot preparation of compound 4 from Fmoc-Glu-OBzl via compound 5 using isopropyl-chloroformate instead of ethyl-chloroformate:
Fmoc-Glu-OBzl (60g, 130.579 mmol) was dissolved in tetrahydrofuran (550 mL) and triethylamine (40.8g, 403.202 mmol) was added. A cloudy solution was obtained with some precipitate. This cloudy solution/suspension was transferred into a dropping funnel and was added to a pre-cooled solution of isobutyl-chloroformate (54.96g, 402.41 mmol) in tetrahydrofuran (300 mL) in a 4.5L reactor at -35 to -30 °C, maintaining this temperature during the addition. Residuals in the dropping funnel were washed with additional tetrahydrofuran (50mL) and the reaction mixture was stirred at -35 to -30 °C for another 2 hours. Water (960 mL) was added to the reaction mixture within 45 minutes, allowing the temperature to increase until 0 °C. A suspension was formed. Sodium borohydride (14.4g, 380.625 mmol) was added in 20 portions within 1h at 0°C and the reaction mixture was stirred for an additional hour at 0°C. Caution was exercised as hydrogen gas evolved.

The suspension was poured onto t-butyl-methylether (600 mL) and the reaction flask was washed with water (600 mL), which was added to the product mixture (2-phases). The phases were separated, the water phase was extracted with t-butyl-methylether (600 mL) and the organic phases were combined. The organic phase was washed with water (2x600 mL), dried over anhydrous magnesium sulfate (200g) and the solvent was removed under reduced pressure until a final volume of 1L was achieved. The solution was diluted with dimethyl-formamide (600g) and the solvent was evaporated under reduced pressure, until a final volume of 400 mL is achieved. The solution of compound **5** thus obtained was transferred into a round bottomed flask. Imidazole (14.4g, 211.524 mmol) was added to the DMF solution of compound **5** and the mixture was stirred for 5 minutes at rt. Finally, TBDPS-Cl (39.6g, 144.07 mmol) is added dropwise during 20 minutes at 20-25 °C and the reaction mixture is stirred for an additional hour at this temperature.

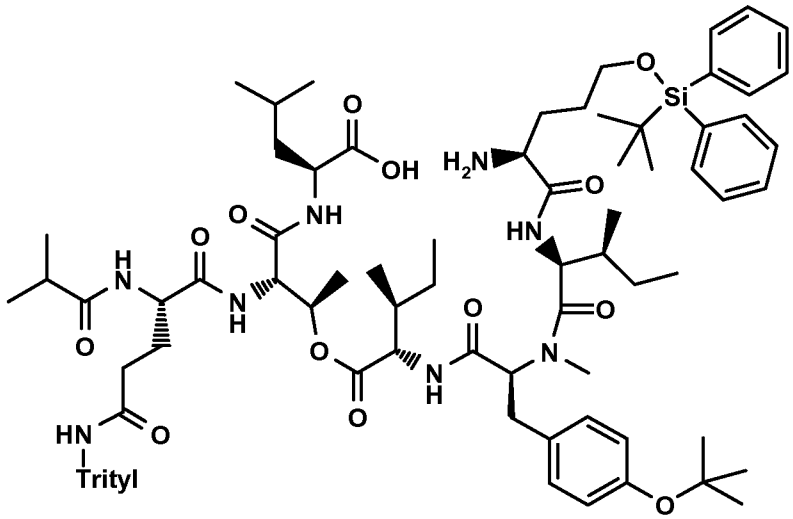
The reaction mixture was then poured onto ethyl acetate (1200 mL) and the mixture was extracted with water(700 mL). The layers were separated and the organic layer was washed with water (3x300 mL). Evaporation of the solvent under reduced pressure gave 106g crude product.

The crude product (106g) was dissolved in Ethanol/Isopropanol/Water (89:5:6; 1200 mL) at 40-50 °C and seed crystals (0.5g compound **4**) were added. The mixture was allowed to cool down to room temperature and stirred for 17 hrs at rt. The suspension was cooled to -20 °C and stirred for 2 hrs at -20 °C. The product was isolated by filtration, the filter cake was washed with the solvent mixture Ethanol/Isopropanol/Water (89:5:6; 3x200 mL) and dried at 40 °C under reduced pressure to obtain 66.5g of compound **4** (74.6% yield over 2 steps). HPLC indicated >99 a% purity for the product.

Additional product can be isolated from the mother liquor (34g after evaporation of the solvent), which contains ca. 30 a% compound **4** according to HPLC.

1B Synthesis of Precursor peptide 1 by SPPS

Precursor peptide 1: Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-OH



Precursor peptide 1

Precursor peptide 1 has been prepared using 2 different Solid Supports

Equipment:

Solid phase synthesis reactor with a filter cloth or sintered glass filter plate at the bottom. A nitrogen manifold allows to drain the reactor contents via filter cloth or sintered glass filter plate and bottom valve.

1B(1) Coupling of the trityl-linker to the solid support:

200g of Aminomethyl-polystyrene resin (crosslinked with 1% divinyl benzene, loading of aminomethyl groups 1mmol/g) (supplier: Senn Chemicals AG, Dielsdorf/Switzerland) were stirred alternately with several portions of dimethylformamide (1600mL) and isopropanol (1600mL) After two final washes with dimethlyformamide, the resin was treated with a previously prepared solution of 4-hydroxy-diphenylmethyl-benzoic acid (91.3g 300mmol), 1-hydroxy-benzotriazole monohydrate (45.9g, 300mmol) and diisopropylcarbodiimide (75.7g, 600mmol) in dimethylformamide (1600mL). The reaction mixture was stirred for 1.5h and a Ninhydrin test was performed. The test still showed free amino groups and thus diisopropylcarbodiimide (7.6g, 60mmol) was added and the

reaction stirred over night. A further ninhydrin test in the morning was negative and the reaction mixture was filtered off. The resin was washed with dimethylformamide and isopropanol alternatingly. The resin was dried in vacuo and yielded 257g of dry linker-resin. The material was used for the next synthesis step without further analysis.

1B(2) Coupling of Fmoc-Leu-OH

Preparation of Fmoc-Leu-Linker-Resin

Linker-Resin (190g, 147.8mmol) was swollen by stirring in toluene (1400mL). The solvent was filtered off and replaced by a solution of toluene (1400mL) and acetyl chloride (53mL, 1478mmol). This mixture was stirred for 2h, filtered off, replaced by an identical mixture which was stirred for another 2h before filtering off. The chlorinated resin was washed twice with toluene and three times with dichloromethane.

In a round bottom flask, a solution of Fmoc-Leu-OH (104.8g, 296mmol) and of N-Methyl-morpholine (49mL, 444mmol) in dichloromethane (600mL) was prepared. This solution was added to the resin and stirred over night. In the morning, the solution was filtered of and the resin was washed with dichloromethane and isopropanol alternatingly. The resin was dried *in vacuo* and yielded 234.7g of dry Fmoc-Leu-linker-Resin. The loading with Fmoc-groups was determined at 0.787mmol/g what led to a yield of 185mmol (125% of theory). Amino acid analysis at an external contractor confirmed <0.1% D-Leu enantiomer.

1B(3) Coupling of Fmoc-Thr-OH

Preparation of Fmoc-Thr-Leu-Linker-Resin

Fmoc-Leu-Linker-Resin (140g, 109mmol) was swollen by stirring in two successive portions of dimethylformamide (1100mL) for 30min each.

Fmoc protecting group was cleaved by two subsequent washings of 20% piperidine in dimethylformamide for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylfomamide and isopropanol. Phenolphtalein and water were added to a sample of the final wash solution. The absence of pink colour confirmed successful removal of piperidine.

The resin was washed with tetrahydrofurane (1200mL) three times to prepare for the following coupling step.

In a round bottomed flask a solution of Fmoc-Thr-OH (112.1g, 328mmol), hydroxybenzotriazole monohydrate (51.25g, 334mmol) and diisopropylcarbodiimide (51mL, 655mmol) in tetrahydrofuran (600mL) was prepared
The solution was added to the resin and the pH checked immediatelly (pH= 6.5). The reaction mixture was stirred for 1.5h until a ninhydrin test showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly. A small sample of the resin was dried and sent for amino acid analysis(0.13% D-Leu, <0.1% D-Thr, <0.1% L-allo-Thr, <0.1% D-allo-Thr), the bulk of the material was subjected to the next step without further drying.

1B(4) Coupling of Fmoc-Gln(Trt)-OH

Preparation of Fmoc-Gln(Trt)-Thr-Leu-Linker-Resin

The Fmoc-Thr-Leu-Linker-Resin from the previous step was swollen by stirring in two subsequent portions of dimethylformamide (1100mL) for 30min each.
Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylfomamide and isopropanol. Phenolphtalein and water were added to a sample of the final wash solution. The absence of pink colour proved successful removal of piperidine.
The resin was washed with dimethylformamide (1100mL) three times to prepare for the following coupling step.
In a round bottomed flask a solution of Fmoc-Gln(Trt)-OH (138.6g, 226mmol), HATU (86.2g, 226mmol) and Ethyldiisopropylamine (58.4g, 452mmol) in dimethylformamide (400mL) was prepared.
The solution was added to the resin and the pH checked immediately (pH= 10). The reaction mixture was stirred for 3h until a ninhydrin test showed complete reaction. The solution was filtered of and the resin was washed with dimethylformamide and isopropanol alternatingly.
The resin was dried in vacuo and yielded 170g of dry Fmoc-Gln(Trt)-Thr-Leu-Linker-Resin. The loading with Fmoc-groups was determined at 0.60 mmol/g indicating a yield of 102mmol (94% of theory over the last two steps). Amino acid analysis at an external contractor led to the following values: (0.13% D-Leu, <0.1% D-Thr, <0.1% L-allo-Thr, <0.1% D-allo-Thr, <0.8% D-Gln).

1B(5) Coupling of Isobutyric acid

Preparation of Isobutyryl-Gln(Trt)-Thr-Leu-Linker-Resin

Fmoc-Gln(Trt)-Thr-Leu-Linker-Resin (169g, 101mmol) was swollen by stirring in two subsequent portions of dimethylformamide (1300mL) for 30min each.

Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide (1300mL) for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylfomamide and isopropanol. Phenolphtalein and water were added to a sample of the final wash solution. The absence of pink colour proved successful removal of piperidine.

The resin was washed with dimethylformamide (1100mL) three times to prepare for the following coupling step.

In a round bottom flask a solution of Isobutyric acid (17.9g, 203mmol), PyBOP (105.5g, 203mmol) and Ethyldiisopropylamine (52.4g, 406mmol) in dimethylformamide (550mL) was prepared.

The solution was added to the resin and the pH checked immediatelly (pH= 9.5). The reaction mixture was stirred for 2.5h until a ninhydrin test showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly.

The batch was directly subjected to the next step without drying and further analysis.

1B(6) Coupling of Fmoc-Ile-OH (Esterification)

Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Linker-Resin

Isobutyryl-Gln(Trt)-Thr-Leu-Linker-Resin (wet from step above, 101mmol) was swollen by stirring in three subsequent portions of dichloromethane (1200mL) for 20min each. The solvent was filtered off and MSNT (88g, 297mmol) and Fmoc-Ile-OH (105g, 297mmol) were added as solids. Dichloromethane (500mL) was added as well as a solution of N-methyl imidazole (18.2g, 223mmol) and ethyldiisopropyamine (51.2g, 396mmol) in dichloromethane (100mL) The reaction mixture was stirred for 2h until HPLC in process control showed complete reaction. The solution was filtered off and the resin was washed with three portions of dichloromethane, three portions of dimethylformamide and three portions of isopropanol subsequently. The resin was dried in vacuo and yielded 172g of dry Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Linker-Resin. Fmoc loading was determined to be 0.418mmol/g thus indicating a yield of 72mmol (71% over the last two steps).

1B(7) Coupling of Fmoc-N-methyl-Tyr(tBu)-OH

Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Linker-Resin (Previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Fmoc)-Leu-Linker-Resin)

Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Linker-Resin (172g, 72mmol) was swollen by stirring in two subsequent portions of dimethylformamide (1300mL) for 30min each. Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide (1400mL) for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylfomamide and isopropanol. Phenolphtalein and water were added to a sample of the final wash solution. The absence of pink colour proofed successful removal of piperidine.

The resin was washed with dimethylformamide (1100mL) three times to prepare for the following coupling step.

A solution of Fmoc-N-methyl-Tyr(tBu)-OH (68.7g, 144mmol) and HATU (55.1g, 144mmol) in dimethylformamide (700mL) was prepared and added to the peptide-resin, followed by the addition of a solution of Ethyl-diisopropylamine (37.5g 289mmol) in dimethylformamide (100mL)under stirring. pH checks immediately after addition of the coupling solution and after 1h of reaction gave the same result (pH 10) The solution was stirred for 2h until a ninhydrin test showed complete reaction. The solution was filtered of and the resin was washed with dimethylformamide and isopropanol alternatingly.

The batch was directly subjected to the next step without drying and further analysis.

1B(8) Coupling of Fmoc-Ile-OH

Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-IleFmoc)-Leu-Linker-Resin. (Previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Ile-Fmoc)-Leu-Linker-Resin.)

The wet Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Linker-Resin (72mmol) was swollen by stirring in two subsequent portions of dimethylformamide (1200mL and 1300mL) for 30min each. Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide (1400mL) for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylfomamide and isopropanol. Phenolphtalein and water were added to a sample of the final wash solution. The absence of pink colour proved successful removal of piperidine.

The resin was washed with dimethylformamide (1100mL) three times to prepare for the following coupling step.
In a round bottom flask a solution of Fmoc-Ile-OH (103.9g, 294mmol) COMU (125.9g, 294mmol) and Ethyldiisopropylamine (76g, 588mmol) in dichloromethane (440mL) and dimethylformamide (440mL) was prepared.
The solution was added to the resin and the reaction mixture stirred for 20h. After that time a ninhydrin test was done, showing complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly.
The resin was dried in vacuo and yielded 185.5g of dry Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Linker-Resin. The loading with Fmoc groups was determined to be 0.40mmol/g. Thus a quantitative yield of 74mmol resulted.

1B(9) Coupling of Synthon 1 and cleavage of final Fmoc protecting group: Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-Linker-Resin
(Previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-me-Tyr-Ile-Synthon 1-H)-Leu-Linker-Resin)
Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Linker-Resin (30g, 12mmol) was swollen by stirring in two subsequent portions of dimethylformamide (240mL and 250mL) for 30min each.
Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide (250mL) for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylfomamide and isopropanol. Phenolphtalein and water were added to a sample of the final wash solution. The absence of pink colour proved successful removal of piperidine.
The resin was washed with dimethylformamide (250mL) three times to prepare for the following coupling step.
In a round bottom flask, a solution of of synthon 1 (14.6g, 24.6mmol) PyBOP (12.85g, 24.6mmol) and Ethyldiisopropylamine (6.4g, 49.2mmol) in dimethylformamide (120mL) was prepared.
The solution was added to the resin and the reaction mixture stirred for 3h. After that time a ninhydrin test confirmed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly.

The resulting peptide-resin was swollen by stirring in two subsequent portions of dimethylformamide (250mL) for 30min each.
Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide (250mL) for 5min and 15min respectively. The peptide-resin was washed by several alternating washes with dimethylfomamide and isopropanol.
Finally the peptide-resin was washed three times with dichloromethane (250mL) to prepare for the cleavage of the peptide.

1B(10) Cleavage of Precursor peptide 1 from solid Support
Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-OH (= Precursor peptide 1)
(Previously named: Isobutyryl-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Ile-Synthon 1-H)-Leu-OH)
To the wet Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-Linker-Resin, a mixture of acetic acid (125mL) and dichloromethane (125mL) was added and the suspension stirred for 2h. The suspension was filtered and the filtrate collected in a round bottomed flask (filtrate 1). The resin was washed twice with dichloromethane (250mL) and the washes were combined with filtrate 1.
The resin was treated with a fresh portion of acetic acid (125mL) and dichloromethane (125mL) for another 2h. The suspension was filtered and the filtrate collected in a round bottomed flask (filtrate 2). The resin was washed twice with dichloromethane (250mL) and the washes combined with filtrate 2.
Acetic acid (200mL) and dichloromethane (50mL) were added to the resin and the suspension stirred over night. The suspension was filtered and the filtrate collected as filtrate 3.
The three filtrates were worked up separately to assess the effectiveness of the cleavage method. The filtrates were concentrated in a rotary evaporator and the residual acetic acid removed by azeotropic distillation with three portions of toluene (100mL). The oily residues were then dried in high vacuo at a lyophilisator. Yield: filtrate 1: 12.5g, filtrate 2: 2.1g, filtrate 3: 0.3g.
The crude material, Precursor peptide 1, was purified by RP-chromatography, the fractions concentrated in a rotary evaporator and the concentrate freeze dried. Purity: 98.8% Yield: 8.5g (48% for the last two steps). The product was characterized by, ^{1}H-

NMR, ^{13}C-NMR and HR-MS. The spectra confirmed the proposed structure. The NMR spectra indicated the presence of several conformations.
HR-MS: calculated for $C_{85}H_{116}N_8O_{13}Si$: $[M+H]^+$: 1485.85039. Found: $[M+H]^+$: 1485.84929.

Second SPPS-synthesis of Precursor peptide 1 using a chlorinated resin

1B(11) Immobilization of the first amino acid (Fmoc-Leu-OH)
2-Chlorotrityl chloride resin (30 g, L= 1.2 mmol/g; Merck Novabiochem 855017) was swelled with 240 ml of dried DCM for 10 min and then the solvent was drained, the solid phase reactor was kept close under a soft nitrogen stream. In the meantime, in a round bottom flask were mixed Fmoc-Leu-OH (42.6g, 120.8 mmol) and 100 ml of dry 1,4-Dioxane. The solvent was evaporated under vacuum at 45°C, then a second portion of 1,4 dioxane was added. The solvent was evaporated again under vacuum at 45°C until a colorless oil residue was obtained. To this oily residue were then added sequentially dry DCM (155 mL) and DIPEA (40.4 mL), the solution was added in one single portion to the pre-swollen resin and then 5 min later a second portion of DIPEA (16.5 mL) was added. The suspension was stirred for 2h and the reaction medium was drained. In order to quench potential non-reacted chloride, 240 ml of a quenching solution of DCM/MeOH/DIPEA (70/15/15, v/v) was added and the mixture was stirred for 10 min. A loading of 0.95 mmol/g was measured.
General protocol for Fmoc cleavage:
To the pre-swollen resin were added 240 ml of a solution of 25% piperidine in DMF, the suspension was stirred for 5 minutes and then the reaction mixture was removed by filtration, then a second portion of the same solution of piperidine was added, the suspension was stirred for 15 minutes and then the solvent was removed by filtration.
Resin washing with drying down:
The resin was washed as follows:
250 ml of DMF (6x2 min)
250 ml of IPA (3x2 min)
250 ml of TBME (6x2 min)
The resin was dried overnight under vacuum at 40 C° to obtain 35.7 g of H-Leu-Resin.

1B(12): SPPS synthesis of H-Thr-Leu-ResinH-Leu-Resin (30g) from the previous step was swollen with 270 ml of DMF for 30 min then the solvent was drained.
In a round bottomed flask (RBF) were mixed Fmoc-Thr-OH (25.0g, 73.2 mmol), BOP (40.4g, 91.5 mmol) and DMF (250 mL), the mixture was stirred for 2 min and then DIPEA (18.9g, 146.4 mmol) was added. The mixture thus obtained was added in one portion to the pre-swollen peptide-resin and the reaction mixture was stirred for 2.5h. The Kaiser test was positive and therefore a second coupling was done according to the conditions previously described. After 1.0h the Kaiser test was negative and the reaction was considered to be complete and the reaction medium was removed via filtration.
The resin was washed as follows:
250 ml DMF (4x2 min)
250 ml IPA (3x2min)
250 ml TBME (5x2 min).
The resin was dried over night under vacuum at 40 C° yielding 52.2 g of Fmoc-Thr-Leu-Resin ready for the Fmoc-cleaving step.
Fmoc cleavage:
The resin (52.2g) was suspended in 272 ml of DMF and stirred for 1.0h then the solvent was removed by filtration.
The Fmoc protecting group was cleaved in the same manner as described for 1B(11) and the peptide-resin was washed as follows:
250 ml DMF (6x2 min)
250 ml IPA (3x2min)
250 ml TBME (6x2 min)
The resin was dried overnight under vacuum at 40 C° yielding 39.9 g of H-Thr-Leu-Resin

1B(13): SPPS synthesis of H-Gln(Trt)-Thr-Leu-Resin
H-Thr-Leu-Resin from the previous step (39.9g) was swollen with 270 ml of DMF for 30 min then the solvent was drained.
In an RBF were mixed Fmoc-Gln(Trt)-OH (44.7g g, 73.2 mmol), BOP (40.4g, 91.5 mmol) and 250 ml of DMF. The mixture was stirred for 2 min and then DIPEA (18.9g, 146.4 mmol) was added. The resulting mixture was added in one portion to the pre-swollen H-Thr-Leu-Resin and the reaction mixture was stirred for 2.5h, the Kaiser test was negative, then the reaction was considered to be complete and the reaction medium was removed via filtration.

The resin was washed as follows:
250 ml DMF (4x2 min)
250 ml IPA (3x2min)
250 ml DMF (4x2min)
The wet peptide-resin was used for the Fmoc-cleaving step without any further manipulation.

Fmoc cleavage:

The fmoc protecting group was cleaved according to the procedure described in step 1B(11). Then the resin was washed as follows:
250 ml DMF (4x2 min)
250 ml IPA (3x2min)
250 ml TBME (5x2 min)
The resin was dried overnight under vacuum at 40 C° yielding 54.4 g of H-Gln(Trt)-Thr-Leu-Resin.

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1B(14): SPPS synthesis of Isobutyryl-Gln(Trt)-Thr-Leu-Resin

The peptide-resin from the previous step (54.4g) was swollen with 270 ml of DMF for 30 min, then the solvent was drained.
In a RBF were mixed Isobutyric acid (6.4 g, 73.2 mmol), PyBop (38.1 g, 73.2 mmol) and 230 ml of DMF, the mixture was stirred for 2 min and then DIPEA (28.3 g, 219.6 mmol) was added. The solution was added in one portion to the pre-swollen peptide-resin and the reaction mixture was stirred for 1.5h. The Kaiser test was negative, then the reaction was considered to be complete and the reaction medium was removed via filtration.
The resin was washed as follows:
250 ml DMF (4x2 min)
250 ml IPA (3x2min)
250 ml TBME (5x2 min).
The resin was dried overnight under vacuum at 40 C° yielding 55.8 g of Isobutyryl-Gln(Trt)-Thr-Leu-Resin.

1B(15): SPPS synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-H)-Leu-Resin

The peptide-resin coming from the previous step (55.8g) was suspended in 250 ml of dry DCM for 1.0h. Then the solvent was removed by filtration and the wet peptide-resin was kept under a soft stream of nitrogen and the temperature was adjusted to 0-5°C.

In a RBF were mixed Fmoc-Ile-OH (51.7 g, 146.4 mmol), and 150 ml of dry dioxane. The solvent was evaporated under vacuum at 45°C until an oily residue was observed. The distillation process was repeated and then to the residue were added 150 ml of dried DCM, the solution was cooled to -10°C and MSNT (43.3 g, 146.4 mmol) was added, the suspension was stirred for 3 min and then N-methyl-imidazole (14.2 g, 173 mmol) was added, the mixture was stirred for 2 min and the solution was added dropwise in 10 min to the above prepared peptide-resin. After the addition was finished the suspension was stirred under a nitrogen atmosphere for 2.0h

The resin was washed as follows:

250 ml DMC (4x2 min)

250 ml DMF (3x2min)

The wet peptide-resin was used for the Fmoc-cleaving step without any further manipulation.

Fmoc cleavage:

The Fmoc protecting group was cleaved according to the general protocol described in 1B(11)

After the Fmoc cleavage was done, the resin was washed as follows:

250 ml DMF (5x2 min)

250 ml IPA (3x2min)

250 ml TBME (5x2 min)

The resin was dried overnight under vacuum at 40 C° yielding 57.2 g of Isobutyryl-Gln(Trt)-Thr(O-Ile-H)-Leu-Resin.

1B(16): SPPS synthesis of Isobutyryl-Gln(Trt)-Thr(O-Ile-Tyr(tBu)MeN)-Leu-Resin

The peptide-resin coming from the previous step (57.2g) was suspended in 250 ml DMF for 30 min. for swelling, then the solvent was removed by filtration.

In a RBF were mixed Fmoc-NMeTyr(tBu)-OH (52.0 g, 109.8 mmol), HATU (41.7 g, 109.8 mmol) and 230 ml of DMF and the mixture was stirred for 2 min. Then DIPEA (28.3 g, 219.6 mmol) was added and the solution was stirred for 2.0 min. The resulting solution was then added to the pre-swollen peptide-resin.

The reaction mixture was stirred for 1.0 h and a Kaiser-test was performed. The Kaiser test was negative. The reaction was considered to be complete and the reaction medium was removed via filtration.

The peptide-resin was washed as follows:

250 ml DMF (4x2 min)
250 ml IPA (1x2min)
The wet peptide-resin was used for the Fmoc-cleaving step without any further manipulation.

Fmoc cleavage:

The Fmoc protecting group was cleaved according to the general protocol used in 1B(11).
After the Fmoc cleavage was done, the resin was washed as follows:
250 ml DMF (5x2 min)
250 ml IPA (1x2min)
250 ml DMF (5x2 min)
The obtained isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-Resin was used for the next step without drying.

1B(17): SPPS synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resin

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-Resin from the previous step was swollen with 270 ml of DMF for 30 min, then the solvent was drained.
In a round bottomed flask were mixed Fmoc-Ile-OH (38.8 g, 109.8 mmol), HATU (41.7 g, 109.8 mmol) and DMF (250 mL), the mixture was stirred for 2 min and then DIPEA (28.3 g, 219.6 mmol) was added. The solution was added in one portion to the pre-swollen peptide-resin and the reaction mixture was stirred for 2.0h, The Chloranil test was positive and then a second coupling with Fmoc-Ile-OH was performed. 3h after the second coupling, the peptide-resin was isolated by filtration.
The peptide-resin was washed as follows:
250 ml DMF (4x2 min)
250 ml IPA (3x2 min)
250 ml DMF (4x2 min)
The wet peptide-resin was used for the Fmoc-cleaving step without any further manipulation.

Fmoc cleavage:

The Fmoc protecting group was removed in the same manner as described in 1B(11), and the peptide-resin was washed as follows.
250 ml DMF (5x2 min)
250 ml IPA (3x2 min)

250 ml TBME (4x2 min).
The peptide-resin was dried overnight under vacuum at 40 C° yielding 67.0 g of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resin.

1B(18): SPPS synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-Resin

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resin from the previous step (33.5 g) was suspended in 150 ml of DMF for 30 min then the solvent was drained. In a RBF were mixed Fmoc-Synthon 1-OH (34.3 g, 57.8 mmol), PyBop (30.0 g, 57.7 mmol) and 113 ml of DMF, the mixture was stirred for 2 min and then DIPEA (14.9g, 115.2 mmol) was added. The solution was added in one portion to the pre-swollen peptide-resin and the reaction mixture was stirred for 2.0h.The reaction medium was removed by filtration.
The peptide-resin was washed as follows:
150 ml DMF (4x2 min)
150 ml IPA (3x2min)
150 ml DMF (4x2 min)
The wet peptide-resin was used for the Fmoc-cleaving step without any further manipulation.

Fmoc cleavage:

The Fmoc protecting group was removed in the same way as in 1B(11) but using 150 ml of the piperidin solution,and the peptide-resin was washed as follows.
150 ml DMF (5x2 min)
150 ml IPA (3x2min)
150 ml TBME (4x2 min)
The peptide-resin was dried overnight under vacuum at 40 C° yielding 36.0 g of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-Resin.

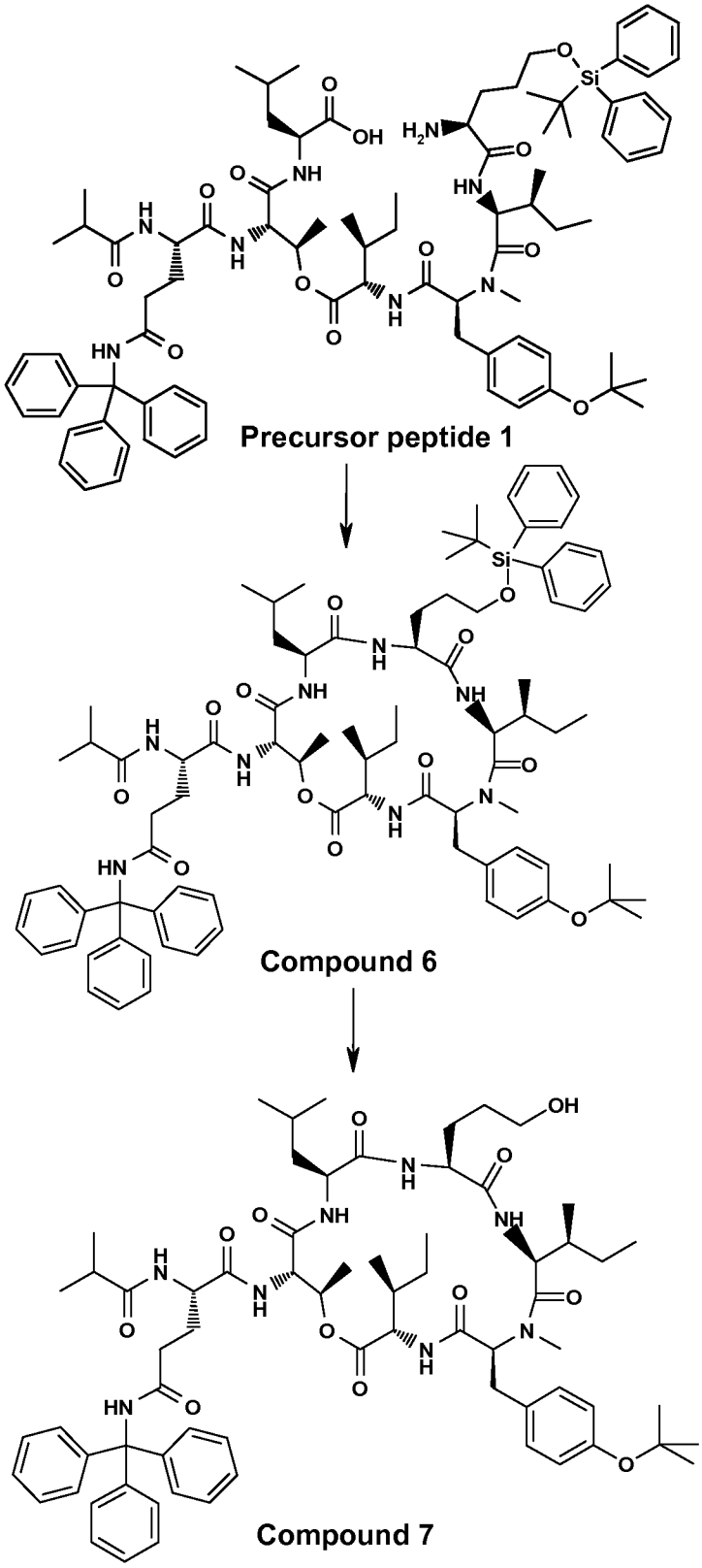
1B(19): Alternative SPPS synthesis: cleavage of peptide from solid support

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-Resin from the previous step (29.75g) was treated with 350 ml of dried DCM for 1.0h, the solvent was removed by filtration and then 350 ml of a solution of 30% v/v HFIP in DCM was added. The mixture was stirred for 10 min and then the solvent was removed by filtration and kept aside. To the wet resin was added a second portion of the same HFIP solution and the solution was stirred for 10 min, then the solvent was removed by filtration and pooled with the

previous solution. The resin was washed 3 times with DCM (350 ml) and the washes were combined with the cleavage solutions.
The combined solution was concentrated under vacuum until an oily residue was observed and then 200 ml of toluene was added, the solvent was evaporated under reduced pressure at 45°C until an oily residue was obtained. 350 ml of hexane was added and the suspension was stirred for 2h. The solvent was removed by filtration and the filter cake was washed with hexane (50 ml), the wet cake was dried overnigh under vacuum at 35°C to yield 19.24g of crude **precursor peptide 1** (= Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon 1-H)-Leu-OH).
Part of the crude precursor peptide 1 (6.0g) was purified by RP-chromatography, the product fractions were concentrated in a rotary evaporator and the concentrate was freeze dried to obtain 2.2g of pure precursor peptide 1 in 99.3 %a purity. Product containing side fractions were treated similarly to obtain additional 1.25g of less pure precursor peptide in 71.4 %a purity. Extrapolated for the whole amount of crude precursor peptide 1 (19.24g), this would correspond to 7.05g pure **precursor peptide 1** from major fractions and additional 4.01g **precursor peptide 1** (in 71.4 %a purity) from side fractions.

1C Solution Phase Synthesis of Compound 8

Reaction Scheme 3


Precursor peptide 1
Compound 6
Compound 7

1C(1) Synthesis of Compound 6

(*S*)-N^1-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-6-(4-(*tert*-butoxy)benzyl)-3,9-di((*S*)-*sec*-butyl)-12-(3-((*tert*-butyldiphenylsilyl)oxy)propyl)-15-isobutyl-7,19-dimethyl-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl)-2-isobutyramido-N^5-tritylpentanediamide

A cloudy solution/suspension of Precursor peptide 1 (1.9 g, 1.28 mmol) in acetonitrile (120 mL) was added during 90 min to a stirred mixture of HATU (972 mg, 2.56 mmol) and DMAP (468.6 mg, 3.84 mmol) in acetonitrile (100 mL) at 35 °C. The dropping funnel was washed with acetonitrile (30 mL). An IPC (HPLC) after the addition of the suspension indicated the absence of the precursor peptide and the completion of the cyclization. For work-up, the solvent was evaporated under reduced pressure until a final volume of ca. 50 mL and the residue was diluted with isopropyl acetate (250). The organic phase was extracted with water (2x100 mL) and the solvent was evaporated under reduced pressure to obtain 2.3 g of crude product. The crude product was purified by flash-chromatography on silicagel with ethyl acetate as mobile phase to obtain 1.79 g (1.22 mmol) compound 6 as a foam. Yield: 95%. The product was characterized by, ^{1}H-NMR ^{13}C-NMR and HR-MS. The spectra confirmed the proposed structure.NMR spectra indicated the presence of several conformations.
HR-MS: calculated for $C_{85}H_{114}N_8O_{12}Si$: $[M+H]^+$:1467.83983; $[M+NH_4]^+$:1484.86637; $[M+Na]^+$: 1489.82177. Found: $[M+H]^+$:1467.83984; $[M+NH_4]^+$:1484.86555; $[M+Na]^+$: 1489.82073.

Second example for the synthesis of Compound 6 (15g scale)

A solution of the precursor peptide **1** (15.0g) in t-butyl-methyl-ether (750 mL) was added slowly during 1.5h to a pre-cooled solution of DMAP (2.80g) and HATU (5.87g) in acetonitrile (375mL) at 0°C. The reaction mixture was stirred for additional 30 min at room temperature. The reaction mixture was then diluted with t-butyl-methyl-ether (750mL) and poured onto half-saturated aq. NaCl-solution (1500 mL). The phases were separated and the organic phase was extracted again with half-saturated aq. NaCl-solution (1500 mL). The organic layer was separated and the solvent was partly evaporated under reduced pressure to a final volume of ca. 175 mL. This solution was filtered over silica gel (column with 225g silica gel) using t-butyl-methyl-ether as mobile

phase. Evaporation of the solvent and drying in vacuo at 40-45 °C gave compound 6 in 97.53% purity according to HPLC. Yield: 14.19g (95.7%).

1C(2) Synthesis of compound 7

(*S*)-N^1-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-6-(4-(*tert*-butoxy)benzyl)-3,9-di((*S*)-*sec*-butyl)-12-(3-hydroxypropyl)-15-isobutyl-7,19-dimethyl-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl)-2-isobutyramido-N^5-tritylpentanediamide

Compound 6 (1.79 g, 1.22 mmol) was dissolved in tetrahydrofurane (60 mL) and $Et_3N(HF)_3$ (6.62 g, 41.1 mmol) was added at room temperature. The reaction mixture was stirred at room temperature for 8.5 hours, then diluted with isopropyl acetate (200 mL), and the resulting solution/suspension was slowly added to an intensively stirred saturated aq. $NaHCO_3$-solution. The organic phase was separated and extracted with water (100 mL). Evaporation of the solvent under reduced pressure gave 1.86g crude product, which was purified by flash-chromatography on silica gel with ethyl acetate/isopropanol (95:5) as mobile phase to obtain 1.24 g (1.00 mmol) of compound 7. Yield: 82%.

The product was fully characterized by IR, NMR and MS. The spectra confirmed the proposed structure.

HR-MS: calculated for $C_{69}H_{96}N_8O_{12}$: $[M+H]^+$:1229.72205; $[M+NH_4]^+$: 1246.74860; $[M+Na]^+$: 1251.70399. Found: $[M+H]^+$: 1229.72128; $[M+NH_4]^+$:1246.74780; $[M+Na]^+$:1251.70310.

Second example for the preparation of compound 7 (14g scale)

Compound **6** (14.0g, 9.537 mmol) was dissolved in tetrahydrofuran (220 mL) and t-butyl-methyl-ether (116 mL) was added. The solution was treated with $Et_3N(HF)_3$ (23.05g) by slow addition within 10 minutes. The reaction mixture was stirred for 24h at room temperature. For work-up the reaction mixture was diluted with t-butyl-methyl-ether (570 mL) and the mixture was poured onto half saturated aq. $NaHCO_3$-solution (632 mL). The biphasic mixture was stirred for 30 min and the phases were separated. The organic phase was extracted with water (280 mL). Both aqueous phases were extracted with t-butyl-methyl-ether (380 mL) and the organic phases were combined. The organic phase was dried over anhydrous $MgSO_4$ (8.0g) and the solvent was partly evaporated under reduced pressure to a final volume of ca. 100 mL. Toluene (140 mL) was added and the

solvent was evaporated again to a final volume of 80 mL. This solution was diluted with t-butyl-methyl-ether (70 mL) and the product was precipitated by slow addition of heptanes (140 mL) during 30 min. The formed suspension was heated to 50-55 °C and stirred for 30 min at this temperature. The suspension was then cooled down to 0°C within 30 min, stirred at 0 °C for 2h and the product was isolated by filtration. The product, a white precipitate, was dried in vacuo to obtain 11.08g of compound **7**. (94.5% yield). HPLC of the product indicated 97 a% purity.

1C(3) Synthesis of Compound 8 (see Reaction scheme 4 for structure)

(*S*)-N^1-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-6-(4-(*tert*-butoxy)benzyl)-3,9-di((*S*)-*sec*-butyl)-15-isobutyl-7,19-dimethyl-2,5,8,11,14,17-hexaoxo-12-(3-oxopropyl)-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl)-2-isobutyramido-N^5-tritylpentanediamide

Compound **7** (1.2g, 0.98 mmol) was dissolved in a mixture of tetrahydrofuran (190 mL) and dimethyl sulfoxide (62 mL). 1-Hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX) (2.42g, 45% g/g, 3.9 mmol) was added and the solution was stirred for ca. 4.5 hours, after which time HPLC indicated disappearance of the starting material (compound 7). The reaction mixture was then poured onto saturated, aq. $NaHCO_3$-solution (300 mL) and was extracted with dichloromethane (2x300 mL). The organic layers were combined and were washed with water (2x300 mL). Evaporation of the solvent under reduced pressure gave2.31g of crude product as a foam. The crude product was purified by flash-chromatography on silicagel with ethyl acetate/isopropanol (95:5) to obtain 1.16g of a product mixture, comprising at least 2 products with the desired mass in LC-MS. The product mixture was used as such for the next step.
HR-MS (major isomer): calculated for $C_{69}H_{94}N_8O_{12}$: $[M+H]^+$: 1227.70640; $[M+NH_4]^+$: 1244.73295; $[M+Na]^+$: 1249.68834 . Found: $[M+H]^+$: 1227.70599; $[M+NH_4]^+$: 1244.73200; $[M+Na]^+$: 1249.68733.
HR-MS (minor isomer): calculated for $C_{69}H_{94}N_8O_{12}$: $[M+H]^+$: 1227.70640; $[M+NH_4]^+$: 1244.73295; $[M+Na]^+$: 1249.68834 . Found: $[M+H]^+$: 1227.70598; $[M+NH_4]^+$: 1244.73198; $[M+Na]^+$: 1249.68751.

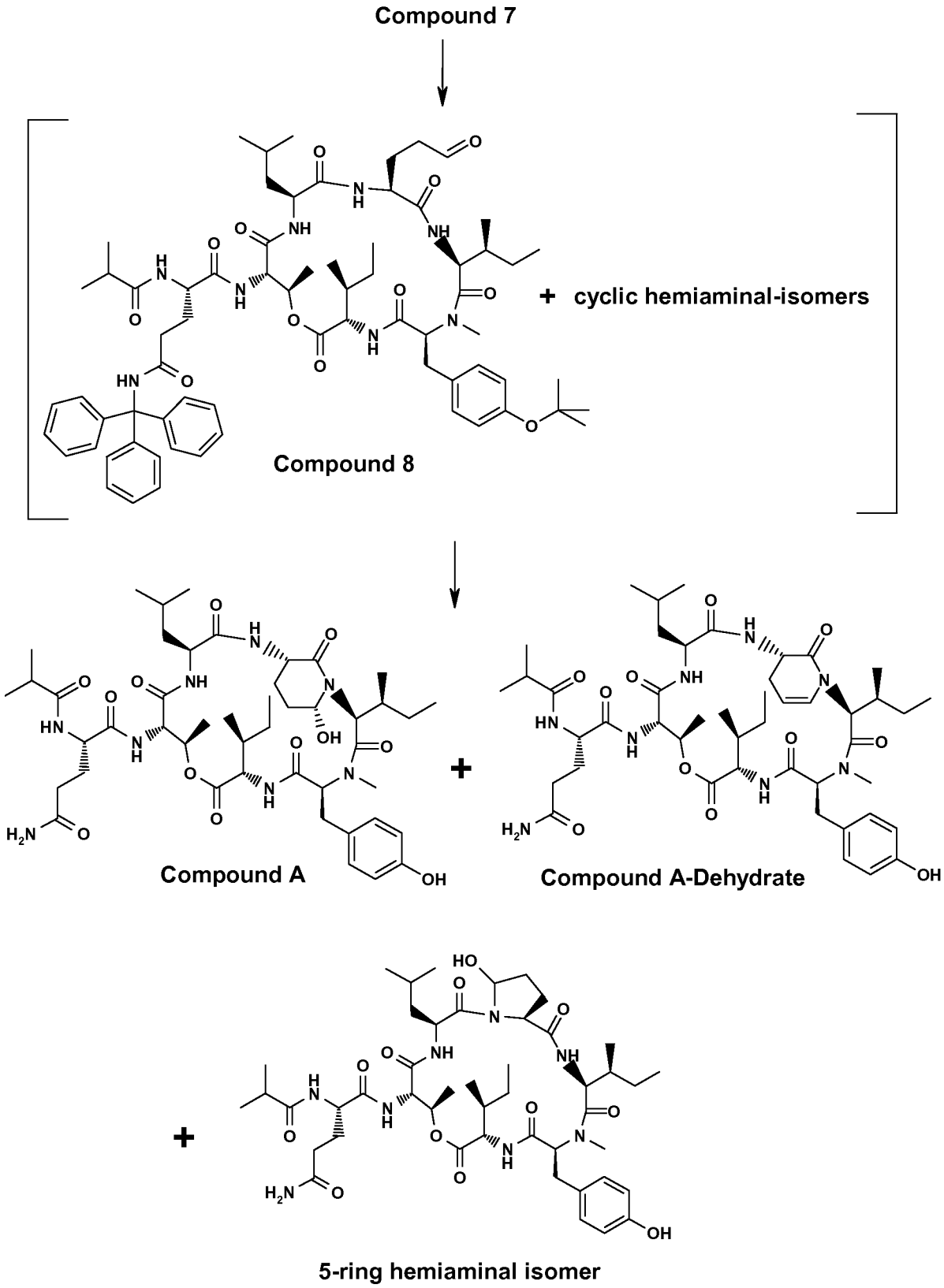
Second example for the synthesis of compound 8 (10g scale)
Compound **7** (10.0g, 8.13 mmol) was dissolved in tetrahydrofuran (127 mL) and dimethylsulfoxide (42 mL). To this solution, 1-Hydroxy-1,2-benziodoxol-3(1*H*)-one 1-

oxide (IBX) (15.18g, 45 %m/m, 24.4 mmol) was added under intense stirring at room temperature. The reaction mixture was stirred for 16h at room temperature. The reaction mixture was then poured onto aq. $NaHCO_3$-solution (500 mL) and ethyl acetate (250 mL) was added. The biphasic mixture was stirred for 30 minutes and the layers were separated. The organic phase was washed with half-saturated aq. NaCl-solution (250 mL) and the layers were separated. The water layers were extracted with ethyl acetate ((250 mL) and the organic phases were combined. The combined organic phase was dried on anhydrous magnesium sulfate and the solvent was partly evaporated to obtain ca. 50g solution. This solution was flashed over a silica gel column (100g silica gel) using ethyl acetate/methanol (98:2 v/v) as mobile phase. The product solution (537.4g) thus obtained was treated with toluene (135 mL) and the resulting solution was concentrated to 112g final weight by partly evaporation of the solvent at 45°C under reduced pressure. The resulting solution was cooled to 0 °C and heptanes (135 mL) was added during 30 min. The suspension thus obtained was stirred for 1h at 0°C, the product was isolated by filtration and washed with heptanes (2x30 mL).. Finally the product was dried in vacuo at 45 °C over night to obtain 8.824g of a mixture of compound **8** and its hemiaminal-isomers. Yield: 88.4%.

1D Synthesis of compound A

(S)-N^1-((2S,5S,8S,11R,12S,15S,18S,21R)-2,8-di-(S)-*sec*-butyl-21-hydroxy-5-(4-hydroxybenzyl)-15-isobutyl-4,11-dimethyl-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl)-2-isobutyramidopentanediamide

Reaction Scheme 4:


Compound 7
+ cyclic hemiaminal-isomers
Compound 8
Compound A
Compound A-Dehydrate
5-ring hemiaminal isomer

Compound 8 (2.0g) was dissolved in dichloromethane (400 mL) and the solution was cooled down to 0 °C. Trifluoroacetic acid (115.9g) was added to the stirred solution at 0 °C and the reaction mixture was stirred for 4h at 0 °C. Dichloromethane (400 mL) was added at this temperature, followed by the addition of water (20g). The reaction mixture was allowed to warm-up to room temperature and stirring was continued for additional 5 hours at room temperature. For work-up, the reaction mixture was poured onto a stirred solution of sodium acetate (165.1g) in water (800 mL) and ethyl acetate (400 mL) was added to obtain a solution. The upper layer (aqueous phase) was removed and the lower organic phase (dichloromethane phase) was washed with water (2x200 mL). The water layers were extracted with ethyl acetate (200 mL) and the organic layers were combined. The solvent was removed under reduced pressure to obtain crude Compound A as a mixture of 5- and 6-ring isomers (see Reaction Scheme 5), accompanied by trityl alcohol and other byproducts of the reaction.

The crude product was purified by RP-chromatography on Silica Kromasil 100-10-C8 with a gradient of acetonitrile/water as mobile phase. The product containing fractions were evaporated to remove acetonitrile, the precipitate was dissolved in ethyl acetate and the solvent was removed under reduced pressure to obtain 0.895g of compound A; yield: 59.1%. The product was fully characterized by NMR and MS and the spectra of the product were identical to Compound A from fermentation.

HR-MS: Calculated for $C_{46}H_{72}O_{12}N_8$: $[M+H]^+$: 929.53425; $[M+NH_4]^+$: 946.56080; $[M+Na]^+$: 951.51619. Found: $[M+H]^+$: 929.53445; $[M+NH_4]^+$: 946.56129; $[M+Na]^+$: 951.51624.

^{1}H-NMR (600 MHz, d_6-DMSO) δ_H: -0.11 (3H, d, J= 6.2Hz), 0.64 (4H, m), 0.77 (3H, d, J= 6.2Hz), 0.81 (3H, t, J= 7.3Hz), 0.84 (3H, d, J= 7.0 Hz), 0.88 (3H, d, J= 6.6Hz), 1.02 (3H, d, J= 6.7Hz), 1.02 (1H, m), 1.03 (3H, d, J= 6.7Hz), 1.09 (1H, m), 1.20 (3H, d, J= 6.2Hz), 1.24 (1H, m), 1.39 (1H, m), 1.51 (1H, m), 1.75 (6H, m), 1.83 (1H. m), 1.92 (1H, m), 2.12 (2H, m), 2.47 (1H, m), 2.58 (1H, m), 2.67 (1H, m), 2.71 (3H, s), 3.16 (1H, d, J= 14.2Hz), 4.30 (1H, m), 4.34 (1H, m), 4.42 (1H, d, J= 10.6Hz), 4.45 (1H, m), 4.61 (1H, d, J= 9.2Hz), 4.71 (1H, dd, J= 9.5, 5.5Hz), 4.93 (1H, s), 5.05 (1H, dd, J= 11.4, 2.6 Hz), 5.48 (1H, m), 6.07 (1H, d, J= 2.6Hz), 6.64 (2H, d, J= 8.4Hz), 6.73 (1H, s), 6.99 (2H, d, J= 8.4Hz), 7.25 (1H, s), 7.35 (1H, d, J= 9.2Hz), 7.64 (1H, d, J= 9.5Hz), 7.73 (1H, d, J= 9.2Hz), 8.01 (1H, d, J= 7.7Hz), 8.42 (1H, d, J= 8.8Hz), 9.17 (1H, s).

^{13}C-NMR (150 MHz, d_6-DMSO) δ_C: 10.35, CH_3; 11.21 CH_3; 13.85, CH_3; 16.00, CH_3; 17.68, CH_3; 19.52, 2 x CH_3; 20.89, CH_3; 21.75, CH_2; 23.30, CH_3; 23.74, CH_2; 24.21, CH; 24.48, CH_2; 27.35, CH_2; 29.78, CH_2; 30.08, CH_3; 31.49, CH_2; 33.18, CH; 33.24, CH_2;

33.76, CH, 37.41, CH; 39.23, CH_2; 48.84, CH; 50.69, CH; 52.11, CH; 54.17 CH; 54.70, CH; 55.31, CH; 60.66, CH; 71.89, CH; 73.97, CH; 115.32, 2 x CH; 127.34, Cq; 130.37, 2 x CH; 156.27, Cq; 169.12, Cq; 169.29, Cq; 169.37, Cq; 169.79 Cq; 170.65, Cq; 172.40, Cq; 172.53, Cq; 173.87 Cq; 176.38, Cq.

Second example for the synthesis of compound A (8.5g scale)

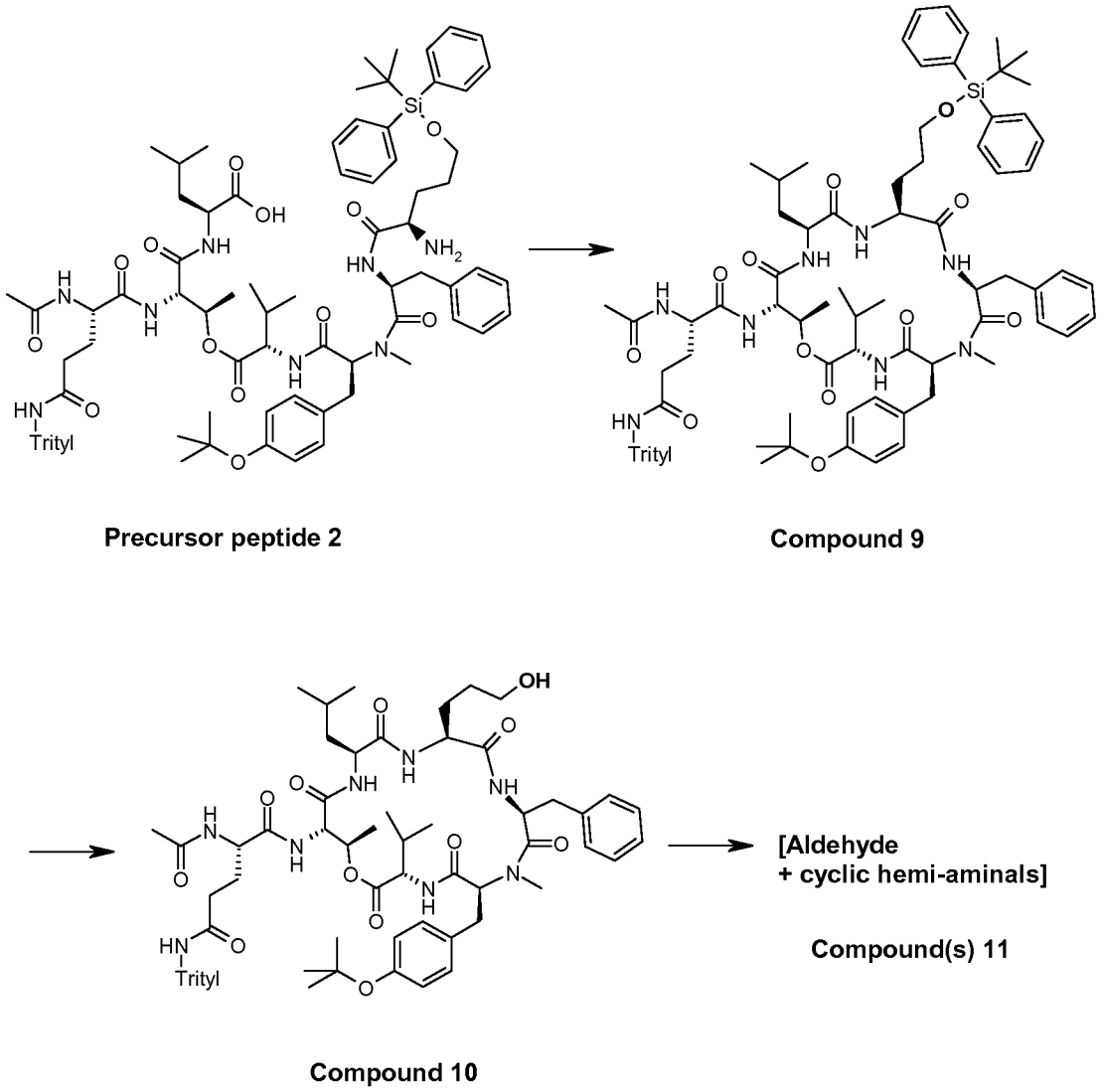
Compound **8** (8.5g, 6.924 mmol) was dissolved in dichloromethane (595 mL) and the solution was cooled to 0 °C. A solution of trifluoroacetic acid (127.5 mL) in dichloromethane (127.5 mL) was added to the cooled solution, maintaining the temperature at 0-5 °C. The reaction mixture was stirred for 5.5 h at 0 °C and was diluted with dichloromethane (850 mL), followed by addition of water (42.5 mL). The reaction mixture was allowed to warm up to room temperature and was stirred for 17.5h at room temperature. For work-up, the reaction mixture was poured onto a stirred, biphasic mixture of aqueous sodium acetate (169g sodium acetate in 722g water) and ethyl acetate (700 mL). The phases were separated and the organic phase was washed again with aqueous sodium acetate solution (169g sodium acetate in 722g water). The layers were separated and the organic phase was washed with water (2x850 mL). The individual aqueous phases were extracted with ethyl acetate (700 mL) and the organic layers were combined. The organic layer was dried over anhydrous magnesium sulfate and the solvent was partly evaporated at 40-45 °C under reduced pressure to obtain 187g of a thin suspension. Heptane (187g) was added within 15 minutes and the suspension formed was cooled to 0 °C. The suspension was stirred for 1h at 0 °C and the product was isolated by filtration. The crude product was dried in vacuo at 40 °C to obtain 5.65g crude compound **A**. Crude yield: 87.8%.

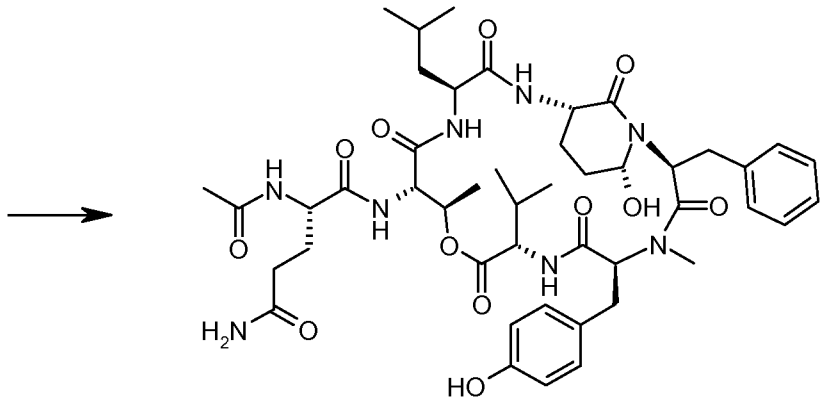
0.96g of the crude product was purified by RP-chromatography on Silica Kromasil 100-10-C8 with a gradient of acetonitrile/water as mobile phase. The product containing fractions were evaporated to remove acetonitrile, the precipitate was dissolved in ethyl acetate and the solvent was partly removed under reduced pressure to obtain 40g of a solution. Heptane (40g) was added to the stirred solution during 30 min at room temperature and the resulting suspension was stirred at room temperature for additional 2h. The product was isolated by filtration and washed with ethyl acetate/heptane (1:1, 2x10mL). The product was dried in vacuo at 40-45 °C for 16h to obtain 0.702g of compound **A**. Yield after RP-chromatography and precipitation: 64%.

Example 2: Synthesis of Compound B (Nostopeptin BN920)

(*S*)-2-acetamido-N^1-((1*S*,2*S*,5*S*,8*S*,11*R*,12*S*,15*S*,18*S*,21*R*)-2-benzyl-21-hydroxy-5-(4-hydroxybenzyl)-15-isobutyl-8-isopropyl-4,11-dimethyl-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl)pentanediamide

Reaction Scheme 5:





Compound B (Nostopeptin BN920)

2A Synthesis of Precursor Peptide 2 by SPPS:
Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Synthon1-H)-Leu-OH

Equipment:
Peptide-synthesizer equipped with a 250 ml glass-reactor with frit and manifold for automatic solvent delivery, shaking and sucking off reagents.

2A(1) Synthesis of Fmoc-Thr-Leu-Trt-Tentagel-S
Fmoc-Leu-Trt-Tentagel-S-Resin (18.7g loading 0.37mmol/g (supplied by Rapp Polymere GmBH, Tübingen/Germany)) were swollen in DMF by shaking for 30 min.
The Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in DMF for 5min and 15min respectively. After resin washing by several alternating washes with DMF and isopropanol the complete removal of bases was checked with the absence of a pink color after Phenolphthalein and water addition to the last washing step.
4.7g of Fmoc-Thr-OH, 5.26g of HATU and 1.8g of DIPEA were dissolved in 50ml of DMF. After 5min of stirring additional 1.8g of DIPEA were added. After checking the pH (>11) the mixture was added to the deprotected resin and shaken for 2h. The performed Kaiser test was OK and the resin was washed by several alternating washes with DMF and isopropanol. After drying the resin weight was 19.01g. A small sample was cleaved and checked by HPLC. A single main peak showed a successful conversion.

2A(2) Synthesis of Fmoc-Gln(Trt)-Thr-Leu-Trt-Tentagel-S

19.01g Fmoc-Thr-Leu-Trt-Tentagel-S-Resin (6.6 mmol) were pre-swollen in DMF and the Fmoc protecting group was cleaved by two subsequent treatments of 20% piperidine in DMF for 5min and 15min respectively. After resin washing by several alternating washes with DMF and isopropanol the complete removal of bases was checked with the absence of a pink color after Phenolphthalein and water addition to the last washing step.

8.07g of Fmoc-Gln(Trt)-OH, 5.01g of HATU and 3.4g of DIPEA were dissolved in 50ml of DMF. After checking the pH (>11) the mixture was added to the deprotected resin and shaken for 1.5h. The performed Kaiser test was OK and the resin was washed by several alternating washes with DMF and isopropanol. The resulting resin was directly used in the following step below, only a small sample was cleaved and checked by HPLC. A single main peak showed successful conversion.

2A(3) Synthesis of Ac-Gln(Trt)-Thr-Leu-Trt-Tentagel-S

The Fmoc-Gln(Trt)-Thr-Leu-Trt-Tentagel-S resin from above was re-swollen in DMF by shaking in DMF for 10min. The Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in DMF for 5min and 15min respectively. The resin was washed by several alternating washes with DMF and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink color proved successful removal of piperidine.

0.774g of acetic-acid, 6.707g of PyBOP and 3.33g of DIPEA were dissolved in 50ml of DMF. After checking the pH (>11) the mixture was added to the deprotected resin and shaken for 2.5h. The performed Kaiser test was OK and the resin was washed by several alternating washes with DMF and isopropanol. The resulting resin was directly used in the following step below, only a small sample was cleaved and checked be HPLC. A single main peak showed successful conversion.

2A(4) Synthesis of Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S (Previously named: Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S) (side-chain esterification)

The Ac-Gln(Trt)-Thr-Leu-Trt-Tentagel-S resin from above was re-swollen in DMF by shaking in DMF for 10min.

8.7g of Fmoc-Val-OH and 8.3g of DIPEA were dissolved in 25ml of DCM. In parallel 2.76g of MSNT were dissolved in another 25ml of DCM. Both solutions were combined

and after 3min pre-activation put to the peptide resin and shaken for 2h. The resin was washed by several alternating washes with DMF and isopropanol. The resulting resin was directly used in the following step below, only a small sample was cleaved and checked be HPLC. A single main peak showed successful conversion.

2A(5) Synthesis of Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Fmoc)-Leu-Trt-Tentagel-S (Previouly named: Ac-Gln(Trt)-Thr(Val-N-me-Tyr(tBu)-Fmoc)-Leu-Trt-Tentagel-S)

The Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S resin from above was re-swollen in DMF by shaking in DMF for 10min.

The Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in DMF for 5min and 15min respectively. The resin was washed by several alternating washes with DMF and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink color proved successful removal of piperidine.

6.1g of Fmoc-N-Me-Tyr(tBu)-OH, 4.8g of HATU and 3.3g of DIPEA were dissolved in 50ml of DMF. After checking the pH (>11) the mixture was added to the deprotected resin and shaken for 2.5h. The performed Kaiser test was OK and the resin was washed by several alternating washes with DMF and isopropanol. The resulting resin was directly used in the following step below, only a small sample was cleaved and checked be HPLC. A single main peak showed successful conversion.

2A(6) Synthesis of Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Fmoc)-Leu-Trt-Tentagel-S (Previously named: Ac-Gln(Trt)-Thr(Val-N-me-Tyr(tBu)-Phe-Fmoc)-Leu-Trt-Tentagel-S)

Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Fmoc)-Leu-Trt-Tentagel-S resin from above was re-swollen in DMF by shaking in DMF for 10min.

The Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in DMF for 5min and 15min respectively. The resin was washed by several alternating washes with DMF and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink color proved successful removal of piperidine.

9.66g of Fmoc-Phe-OH, 9.48g of HATU and 6.4g of DIPEA were dissolved in 100ml of DMF. After checking the pH (>11) the mixture was added to the deprotected resin and shaken for 2h. The performed Kaiser test was OK and the resin was washed by several

alternating washes with DMF and isopropanol. The resulting resin was directly used in the following step below, only a small sample was cleaved and checked be HPLC. A single main peak showed successful conversion.

2A(7) Synthesis of Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Synthon1-H)-Leu-OH (Previously named: Ac-Gln(Trt)-Thr(Val-N-me-Tyr(tBu)-Phe-Synthon1-H)-Leu-OH) (= Precursor peptide 2)

Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Fmoc)-Leu-Trt-Tentagel-S resin from above was re-swollen in DMF by shaking in DMF for 10min.

The Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in DMF for 5min and 15min respectively. The resin was washed by several alternating washes with DMF and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink color proved successful removal of piperidine.

6.77g of Synthon1, 5.9g of PyBOP and 2.95g of DIPEA were dissolved in 100ml of DMF. After checking the pH (>11) the mixture was added to the deprotected resin and shaken for 2h. The performed Kaiser test was OK and the resin washed by several alternating washes with DMF and isopropanol. After taking a small sample for a HPLC check, the Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in DMF for 5min and 15min respectively. The resin was washed by several alternating washes with DMF and isopropanol. Finally the resin was washed two times with DCM. Cleavage of the synthesized peptide from the resin was achieved by shaking in a mixture of 80 % of acetic acid in DCM over night. The resulting peptide solution was filtered off and the resin was washed two times with DCM. The combined filtrates were evaporated and finally purified by RP-chromatography using a gradient system. The collected fractions were analyzed by HPLC and the pure fractions were pooled, evaporated and finally lyophilized.

Yield: 3.54g (42%) Precursor peptide 2.

HR-MS: Calculated for $C_{85}H_{108}N_8O_{13}Si$: $[M+H]^+$ = 1477.78779; Found: $[M+H]^+$ = 1477.78691.

2B Synthesis of Compound B

(*S*)-2-acetamido-N^1-((1*S*,2*S*,5*S*,8*S*,11*R*,12*S*,15*S*,18*S*,21*R*)-2-benzyl-21-hydroxy-5-(4-hydroxybenzyl)-15-isobutyl-8-isopropyl-4,11-dimethyl-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl)pentanediamide

2B(0) Synthesis of Compound 9

(*S*)-2-acetamido-N^1-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-9-benzyl-6-(4-(*tert*-butoxy)benzyl)-12-(3-((*tert*-butyldiphenylsilyl)oxy)propyl)-15-isobutyl-3-isopropyl-7,19-dimethyl-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl)-N^5-tritylpentanediamide

The lactamization of Precursor peptide 2 to obtain Compound B was performed under similar conditions described for the preparation of Compound 6. The product, Compound 9, was fully characterized by IR, NMR and MS. The spectra confirmed the proposed structure.

HR-MS: Calculated for $C_{85}H_{106}N_8O_{12}Si$: $[M+H]^+$: 1459.77723; $[M+NH_4]^+$: 1476.80377. Found: $[M+H]^+$: 1459.77719; $[M+NH_4]^+$: 1476.80291.

2B(1) Synthesis of Compound 10

(*S*)-2-acetamido-N^1-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-9-benzyl-6-(4-(*tert*-butoxy)benzyl)-12-(3-hydroxypropyl)-15-isobutyl-3-isopropyl-7,19-dimethyl-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl)-N^5-tritylpentanediamide

The de-silylation reaction to obtain compound 10 was performed under similar conditions described for the preparation of compound 7. The product was fully characterized by IR, NMR and MS. The spectra confirmed the proposed structure.

HR-MS: Calculated for $C_{69}H_{88}N_8O_{12}$: $[M+H]^+$: 1221.65945; $[M+NH_4]^+$: 1238.6860; $[M+Na]^+$: 1243.64139. Found: $[M+H]^+$: 1221.65894; $[M+NH_4]^+$: 1238.68518; {$M+Na]^+$: 1243.64001.

2B(2) Synthesis of Compound 11 (Aldehyde)

(*S*)-2-acetamido-N^1-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-9-benzyl-6-(4-(*tert*-butoxy)benzyl)-15-isobutyl-3-isopropyl-7,19-dimethyl-2,5,8,11,14,17-hexaoxo-12-(3-oxopropyl)-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl)-N^5-tritylpentanediamide

The oxidation of Compound 10 to obtain Compound 11 was performed under similar

conditions described for the preparation of Compound 8. LC-MS analysis showed several peaks with the desired mass, indicating the presence of a mixture of the aldehyde, 5-ring-hemiaminale and 6-ring-hemiaminale. The mixture was used as such for the next step. HR-MS (major peak): Calculated for $C_{69}H_{86}N_8O_{12}$: $[M+H]^+$: 1219.64380; $[M+NH_4]^+$: 1236.67035; $[M+Na]^+$: 1241.62574. Found: $[M+H]^+$: 1219.64404; $[M+NH_4]^+$: 1236.67053; $\{M+Na]^+$: 1241.62524.

2B(3) Synthesis of Compound B (Nostopeptin BN920) from Compound 11

(*S*)-2-acetamido-N^1-((1*S*,2*S*,5*S*,8*S*,11*R*,12*S*,15*S*,18*S*,21*R*)-2-benzyl-21-hydroxy-5-(4-hydroxybenzyl)-15-isobutyl-8-isopropyl-4,11-dimethyl-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl)pentanediamide

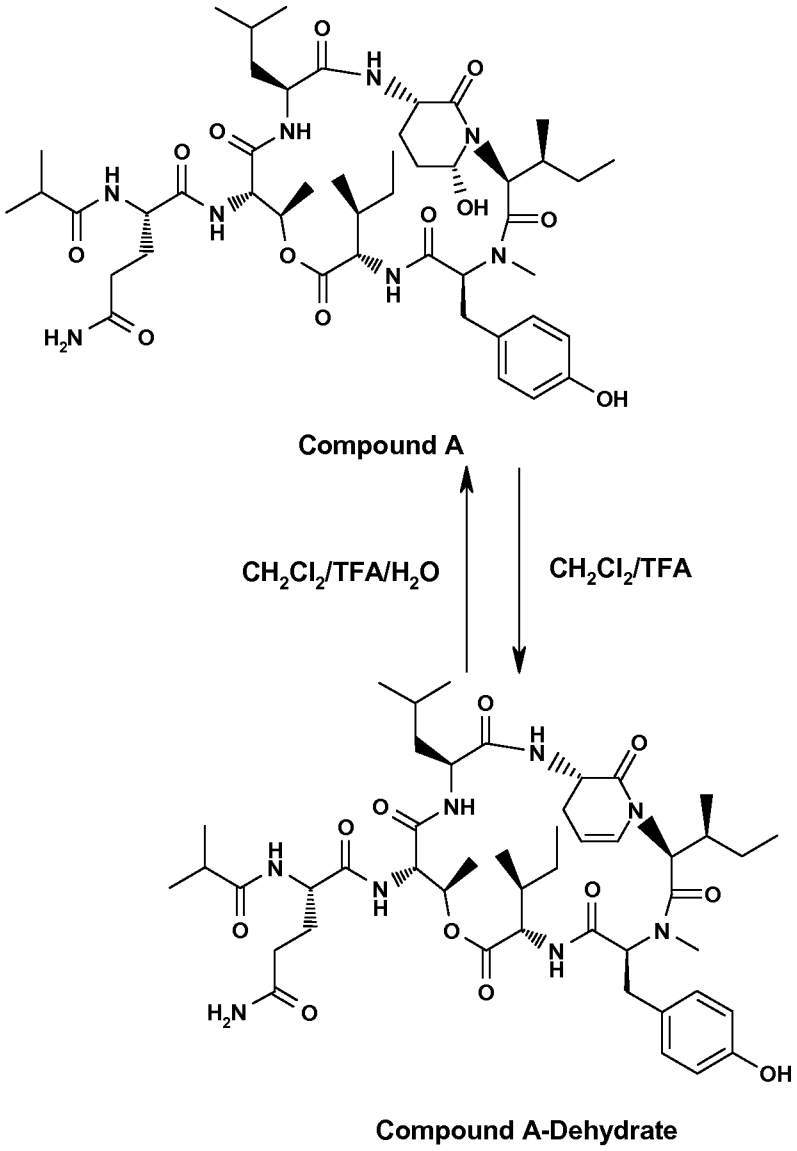
Compound(s) 11 (1.0g, 0.82 mmol) was dissolved in dichloromethane (200 mL). Trifluoroacetic acid (57.8g) was added at 15 to 25 °C during 10 minutes and the reaction mixture was stirred for 45 hours at room temperature. The reaction mixture was then diluted with dichloromethane (200 mL) and water (10 mL) was added. Stirring was continued for additional 24 hours at room temperature. The reaction mixture was poured onto a saturated aq. $NaHCO_3$-solution (700 mL) within 20 minutes and dichloromethane (500 mL), isopropanol (50 mL) and water (500 mL) were added sequentially. The layers were separated and the aqueous layer was extracted with a solution of isopropanol (50 mL) in dichloromethane (500 mL). The layers were again separated and the aqueous layer was extracted several times with dichloromethane (5x300 mL). The organic layers were combined and the solvent was evaporated under reduced pressure to obtain crude product mixture (1.0 g), comprising the desired Nostopeptin BN920 along with trityl alcohol and other byproducts of the reaction. The crude product was purified by silica gel chromatography with dichloromethane/isopropanol as mobile phase to obtain pure Nostopeptin BN920 (212 mg, 97 %(A) purity). Collection of less pure fractions gave additional 402 mg Product with 90 %(A) purity. Yield from all fractions: 614 mg (81%). The product was fully characterized by IR, NMR and MS.The spectra confirmed the proposed structure.

HR-MS: Calculated for $C_{46}H_{64}N_8O_{12}$: $[M+H]^+$: 921.47165; $[M+NH_4]^+$: 938.49820; $[M+Na]^+$: 943.45359. Found: $[M+H]^+$: 921.47167; $[M+NH_4]^+$: 938.49861; $[M+Na]^+$: 943.45337.

Example 3: Shift of Equilibrium

Reaction Scheme 4 shows the presence of the dehydrate form of Compound A. It has now been discovered that this can be converted easily (back) into Compound A using a simple procedure for hydration of the dehydrate form depicted in the following reaction scheme:

Reaction Scheme 6:



This allows to improve the yield of Compound A in any type of synthesis (be it chemical as in the present disclosure or by use of fermentation as in WO2009/024527).

For example, during cleavage of acid sensitive protecting groups in a compound comprising the ahp-subunit, e.g. Compound A in Example 1, the formation of large amounts of the corresponding dehydrated byproduct is observed. This byproduct is usually separated e.g. by chromatography and disposed. This leads to loss of valuable product and to low yield for this step. For example, if the oxidation product(s) of Compound 8 are subjected to acidic conditions to cleave the trityl- and t-butyl protecting groups (scheme 4), significant amounts of Compound-A-dehydrate are formed as byproduct. Depending on the acid concentration and reaction conditions, Compound-A-dehydrate might be formed even as major product in this product mixture.

For example, a ratio Compound A/Compound A-dehydrate (1:2) was observed when trifluoroacetic acid/dichloromethane (5:95 v/v) was used to cleave the protecting groups after the oxidation step (Example 1).

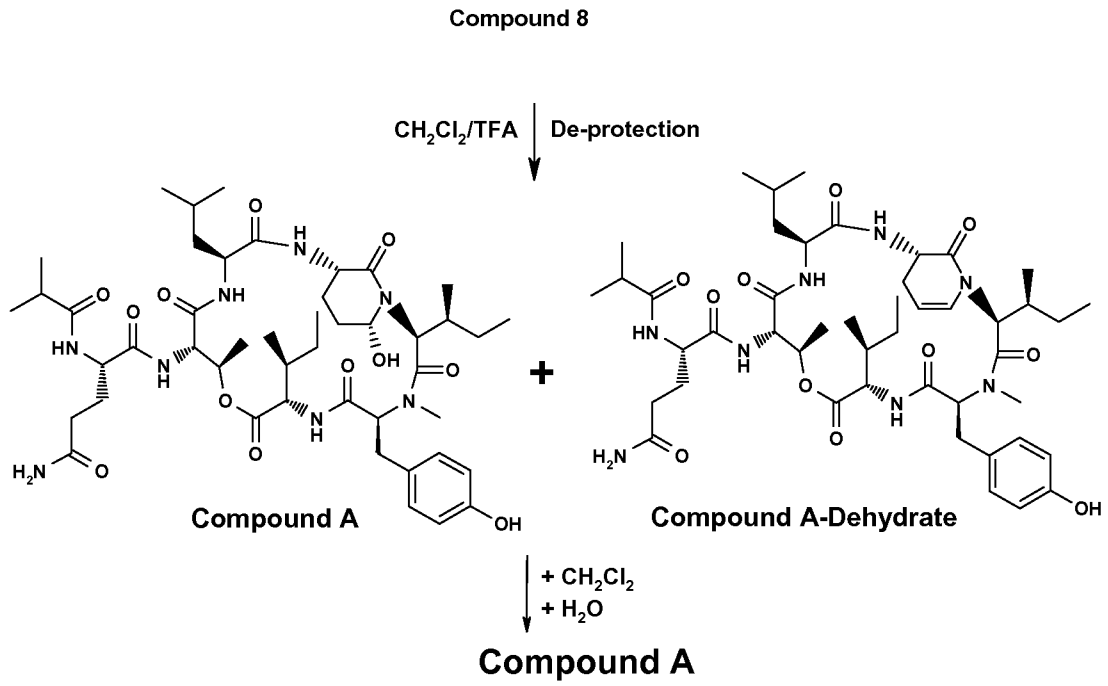
It was therefore searched for ways to convert the dehydrate byproduct into the desired product. It has now been found that this can be achieved by acid catalyzed equilibration of the product mixture in the presence of water under well defined conditions. Addition of water to the reaction mixture of example 1 and subsequent stirring at room temperature for 19h gave a product mixture with a ratio Compound A/Compound A-dehydrate of ca. 96:4. Thus, addition of water to the reaction mixture after the acid-catalyzed de-protection step (scheme 4) changed the ratio of Compound A / Compound A -dehydrate from (1:2) under water free conditions to (96:4) after water addition and equilibration.

The formation of Compound A -dehydrate from Compound A under acidic de-protection conditions was confirmed by conversion of pure Compound A into Compound A -dehydrate using trifluoroacetic acid in DCM. Treatment of Compound A with 33% (v/v) TFA in DCM for 2h at room temperature gave a product mixture of Compound A -dehydrate/ Compound A in a ratio of 78:22 according to HPLC. The dehydration could be driven to >95% conversion, when water absorbing agents, such as molecular sieves were added to the reaction mixture. Thus, stirring of pure Compound A in a 1:2 mixture of TFA/DCM in the presence of molecular sieves gave the dehydrated-product in

quantitative crude yield and ca. 96 area% HPLC purity (example 3 B). There were still ca. 4 area% of Compound A present in the crude product.

Conversion of Compound A -dehydrate from Example 3 B into Compound A was demonstrated by stirring Compound A-dehydrate in dichloromethane in the presence of trifluoroacetic acid and water (Example 3 C). The product thus obtained comprised 95.6 area% Compound A and only 4.4 area% Compound A -dehydrate according to HPLC.

Reaction Scheme 7:



Experimental details for Example 3

3A:

23 mg product mixture from oxidation (compound 8 and cyclic aminals derived from it) was dissolved in DCM (5.7 mL) and TFA (0.3 mL) was added to the solution under intensive stirring. The reaction mixture was stirred for 5h at room temperature, until an IPC (HPLC) indicated complete cleavage of the protecting groups. Compound A and Compound A-dehydrate were present in the reaction mixture in a ratio of 1:2, along with additional byproducts. The reaction mixture was diluted with DCM (5.7 mL) and the

intensively stirred solution was treated with water (0.23 mL). HPLC after 19h stirring time at room temperature indicated a ratio of 96:4 for Compound A / Compound A -dehydrate.

For workup, the reaction mixture was poured onto a solution of sodium acetate (1.62g) in water (23 mL) and ethyl acetate (35 mL) was added. The phases were separated and the organic layer was extracted with water (2x25 mL). The water layers were extracted with ethyl acetate (35 mL) and the organic layers were combined. The solvent was evaporated at reduced pressure to obtain 22 mg of crude product as a foam. The crude product was purified by flash chromatography on silica gel with ethyl acetate/methanol 95:5 to 90:10 to obtain Compound A in 96.8 area% purity, accompanied by 1.8 area% of the 5-ring isomer.

3B:

Compound A (250 mg) was dissolved in dichloromethane (10 mL) and trifluoroacetic acid (5 mL) was added, followed by the addition of molecular sieves (1g). IPC (HPLC) after 1h indicated a ratio of 17:83 for Compound A /Compound A -dehydrate. The reaction mixture was stirred for a total of 72h at room temperature. For workup, the molecular sieves were removed by filtration and the solution was poured onto saturated aq. $NaHCO_3$-solution. The aqueous layer was extracted with ethyl acetate (50 mL). The organic phase was washed with water (20 mL) and the solvent was evaporated at reduced pressure and the residue was dried in vacuo to obtain quantitative yield (245 mg) crude product as a foam. HPLC analysis of the crude product indicated the presence of Compound A / Compound A-dehydrate at a ratio of ca. 4:96 area%.
HR-MS: calculated for $C_{46}H_{70}N_8O_{11}$ $[M+H]^+$= 911.52368, $[M+NH4]^+$= 928.55023, [M+Na]+= 933.50563; found $[M+H]^+$= 911.52372, $[M+NH4]^+$= 928.55029, $[M+Na]^+$= 933.50538. The structure of Compound A-dehydrate was confirmed by ^{1}H-NMR.
^{1}H-NMR (600 MHz, d_6-DMSO) δ_H: 0.06 (3H, d, J= 6.6 Hz), 0.67 (3H, t, J= 7.3 Hz), 0.70 (3H, d, J= 7.0 Hz), 0.77 (3H, d, J= 6.6 Hz), 0.81 (1H, m), 0.87 (6H, m), 1.00 (3H, d, J= 7.0 Hz), 1.02 (3H, d, J= 7.0 Hz), 1.06 (1H, m), 1.16 (3H, d, J= 6.5 Hz), 1.17 (1H, m), 1.30 (1H, m), 1.41 (1H, m), 1.53 (1H, m), 1.74 (2H, m), 1.91 (2H, m), 2.01 (1H, m), 2.11 (2H, m), 2.45 (3H, m), 2.73 (3H, s), 2.74 (1H, m), 3.18 (1H, m), 4.32 (2H, m), 4.50 (1H, m), 4.54 (1H, m), 4.64 (1H, d, J= 9.5 Hz), 4.77 (1H, d, J= 11.0 Hz), 5.18 (1H, m), 5.26 (1H, m), 5.42 (1H, q, J= 6.6 Hz), 6.25 (1H, d, J= 7.3 Hz), 6.32 (1H, d, J= 7.7 Hz), 6.67 (2H, d,

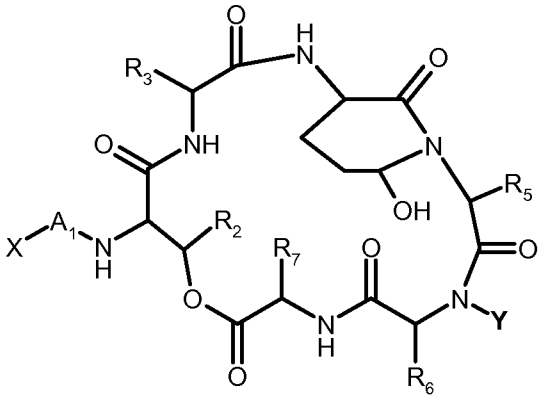
J= 8.4 Hz), 6.75 (1H, s), 7.04 (2H, d, J= 8.4 Hz), 7.25 (1H, s), 7.30 (1H, d, J= 8.8 Hz), 7.89 (1H, d, J= 9.2 Hz), 7.98 (1H, d, J= 8.1 Hz), 8.50 (1H, d, J= 8.4 Hz), 9.24 (1H, s).

3C:

Compound A-dehydrate (110 mg) from example 2 was dissolved in DCM (20 mL). TFA (1g) and water (0.2 mL) were added and the reaction mixture was stirred for 20h at room temperature. For workup, the reaction mixture was poured onto ethyl acetate (50 mL) and the ethyl acetate solution was extracted with a saturated aq. $NaHCO_3$-solution (50 mL). The organic layer was extracted with water (20 mL) and the solvent was evaporated. The residue was dissolved in ethyl acetate/isopropanol 9:1 (20 mL) and the solution was clear filtered over a 0.45 micrometer filter. The solvent was evaporated under reduced pressure and the residue was dried in vacuo at 45 °C to obtain 100mg crude product. HPLC analysis indicated the presence of ca. 95.6 area% Compound A and ca. 4.4 area% Compound A -dehydrate.

Claims

1. A method or process for the preparation of a cyclic depsipeptide compound of the formula I,



I

wherein

A_1 is a bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group, especially asparagine or glutamine, and is bound at its right hand side in formula I via a carbonyl to the rest of the molecule; or is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

X is bound via an N of A_1 and is acyl, or is absent if A_1 is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

R_2 is C_{1-8}-alkyl, especially methyl;

R_3 is the side chain of an amino acid, especially of leucine, isoleucine or valine;

R_5 is the side chain of an amino acid, preferably of phenylalanine, leucine, isoleucine or valine;

R_6 is the side chain of a hydroxy amino acid, especially of tyrosine;

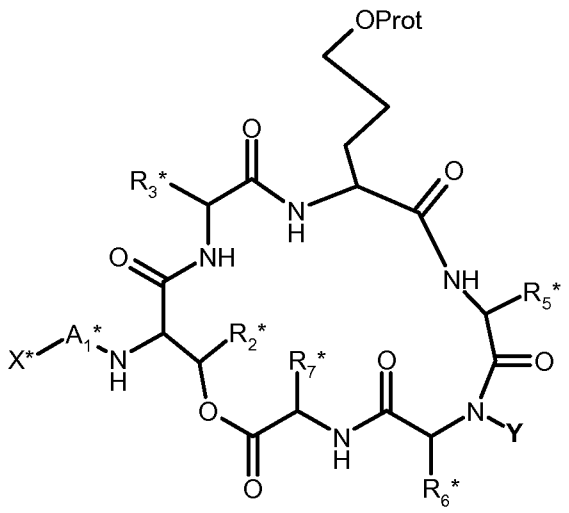
R_7 is the side chain of an amino acid, preferably of the amino acid leucine, isoleucine or valine; and

Y is hydrogen or C_{1-8}-alkyl;

or a salt thereof,

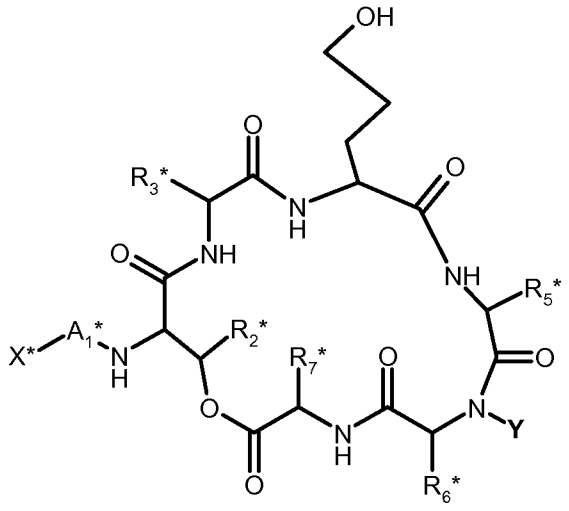
said method comprising

selectively deprotecting a compound of the formula II



II

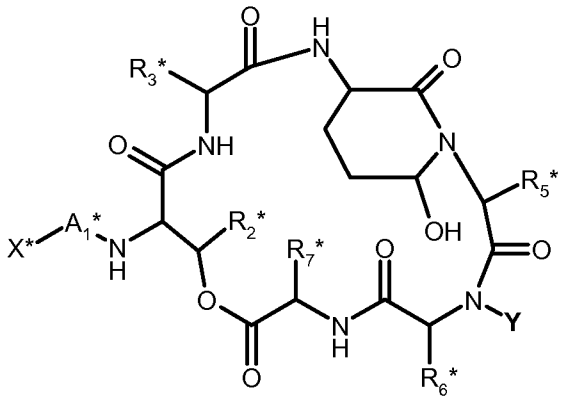
wherein Prot is a protecting group, Y is as defined for a compound of the formula I and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I, respectively, but with the proviso that reactive functional groups on these moieties are present in protected form at least if they could participate in undesired side reactions, to result in a compound of the formula III,



(III)

wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* have the meanings defined above,

reacting the free hydroxyl group under oxidizing conditions to form a compound of the formula IV



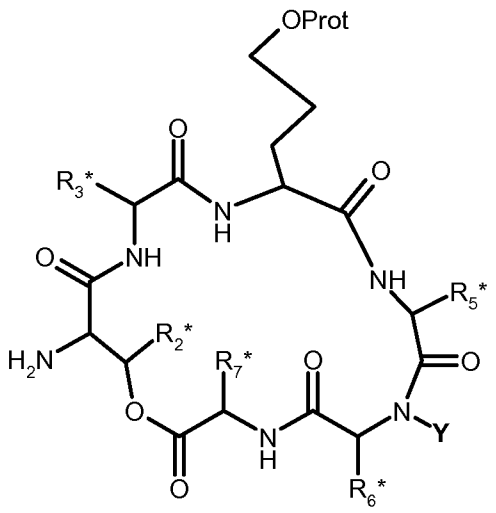
IV

and removing remaining protecting groups to yield a compound of the formula I, or a salt thereof,

and, optionally, converting a free compound of the formula I into a salt, a salt of a compound of the formula I into a different salt of a compound of the formula I or into the free compound of the formula I and/or converting a dehydrated analogue of a compound of the formula I into the corresponding compound of the formula I.

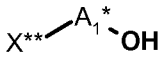
2. The method or process according to claim 1, wherein the compound of the formula IV is synthesized by a combination of Solid Phase Peptide Synthesis and Solution Phase synthesis from the corresponding starting amino acids and side chain precursors.

3. The method or process according to any one of the preceding claims, further comprising, for the synthesis of a compound of the formula II shown in claim 1, reacting a compound of the formula VI,



VI

wherein Prot is a protecting group, Y is as defined for a compound of the formula I and R_2*, R_3*, R_5*, R_6*, and R_7* are as defined for a compound of the formula II in claim 1, with an acid of the formula VII

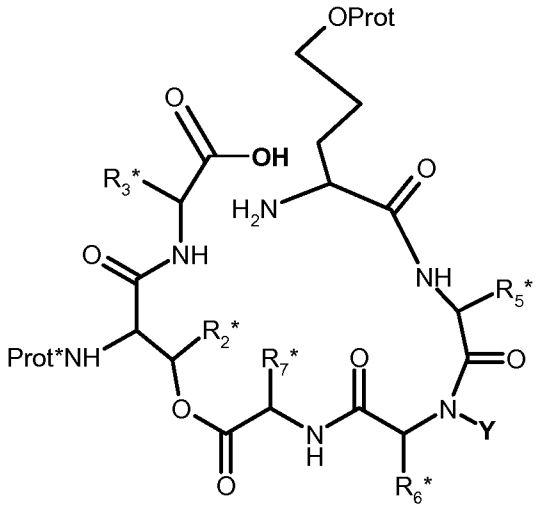


VII

or a reactive derivative thereof,
wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined for a compound of the formula II in claim 1; and, if X** is an amino protecting group, removing said amino protecting group X** to yield H instead of X* and coupling the resulting amino group with an acyl group X* using the corresponding acid X*-OH wherein C* is as defined for a compound of the formula II in claim 1, or a reactive derivative thereof.

4. The method or process according to claim 3, further comprising cyclization under lactamization of a linear precursor peptide of the compound of the formula VI, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, preferably using Solution Phase chemistry.

5. The method or process according to claim 4, where the linear precursor peptide is of the formula VIII,



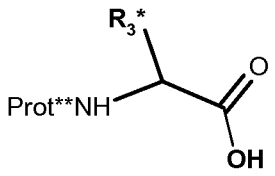
VIII,

wherein Prot* is a protecting group that can be cleaved off selectively without affecting other protecting groups present and is stable during deprotection steps during synthesis of the linear precursor peptide (e.g. allyloxycarbonyl) and R_2*, R_3*, R_5*, R_6*, and R_7* are as defined for a compound of the formula VI in claim 4, further comprising, after cyclisation of the compound of the formula VIII, especially VIIIA, removing the protecting group Prot* *in situ* to yield the compound of the formula VI.

6. The method or process according to claim 5, where the linear precursor peptide of the formula VIII, especially VIIIA, is synthesized from the corresponding amino acids by solid phase peptide synthesis and subsequent cleaving-off from the employed solid phase.

7. The method according to claim 6, comprising either

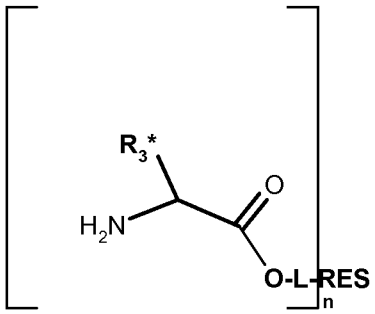
in a variant a), coupling an amino acid of the formula IX,



IX

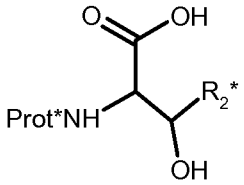
wherein R_3* is as defined for a compound of the formula II in claim 1 and Prot** is an amino protecting group that can be removed on the resin without cleaving other bonds, or a reactive derivative of said amino acid, via an oxygen to a cleavable linker L which is bound to a solid resin RES,
and removing the protecting group Prot**;

coupling the obtained resin bound amino acid symbolized by the formula X,



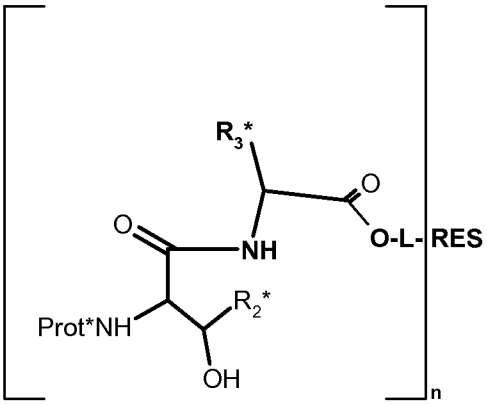
X

in which RES and R_3* are as defined for a compound of the formula IX, n is a natural number and L is a cleavable linker, with an amino acid of the formula XI,



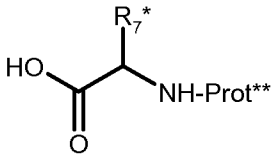
XI

wherein Prot* is as defined for a compound of the formula VIII in claim 5 and R_2* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid, coupling the obtained resin bound dipeptide symbolized by the formula XII,



XII

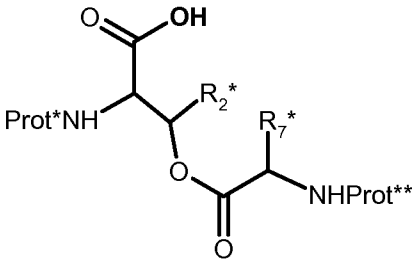
in which Prot* is as defined for a compound of the formula VIII in claim 5, R_2* and R_3* are as defined for a compound of the formula II in claim 1, and n, L and RES are as defined for a compound of the formula X, via the free hydroxy group with an amino acid of the formula XIII,



XIII

wherein Prot** is as defined for a compound of the formula IX and R_7* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid, and removing the protecting group Prot**;

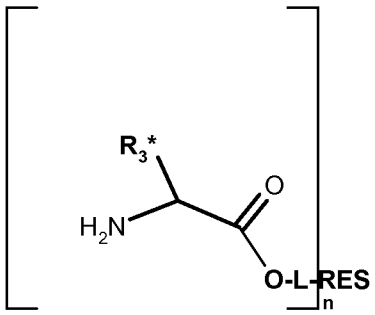
or, in a variant b), coupling a dipeptide of the formula XXVII,



XXVII

wherein R_3* and Prot** are as described for a compound of the formula IX, especially IXA, and Prot* is as defined for a compound of the formula VIII in claim 5, or a reactive

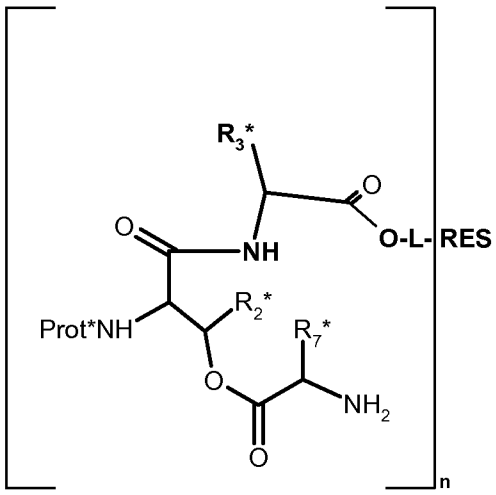
derivative of said dipeptide, to an amino acyl moiety, bound via an oxygen to a cleavable linker L which is bound to a solid resin RES, having the formula X,



X

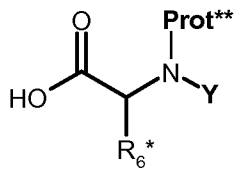
that can be obtained as described under variant a), in which RES, and R_3* are as defined for a compound of the formula IX and L and RES are as just defined;
and removing the protecting group Prot**;

and, after the reactions of variant a) or of variant b), coupling the obtained compound of the formula XIV,



XIV

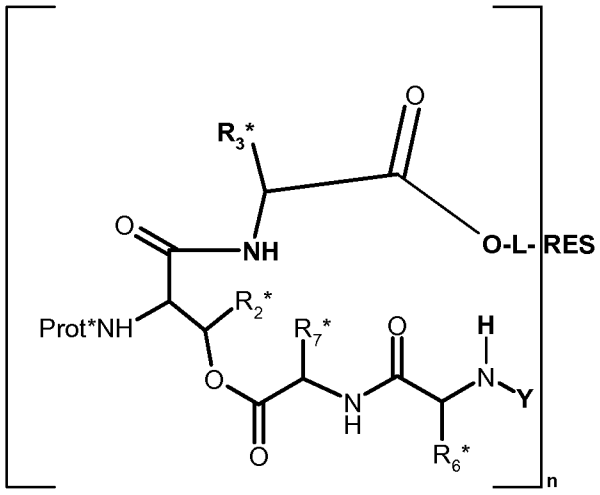
wherein R_2*, R_3* and R_7* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X, with an amino acid of the formula XV,



XV

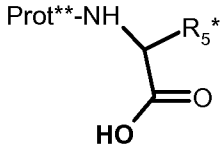
in which R_6* and Y are as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula IX, or a reactive derivative of said amino acid, and removing the protecting group Prot**;

coupling the obtained compound of the formula XVI



XVI

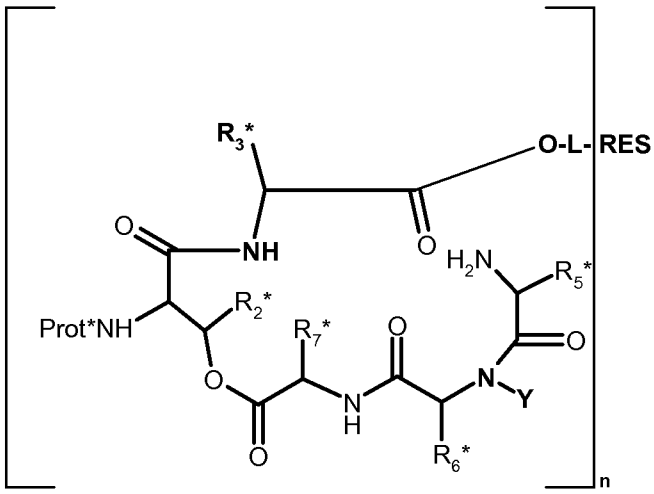
wherein Y, R_2*, R_3*, R_7* and R_6* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X, with an amino acid of the formula XVII,



XVII

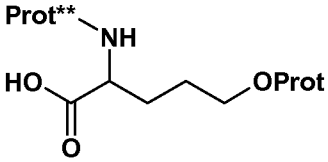
wherein R_5* is as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula IX, or a reactive derivative of said amino acid, and removing the protecting group Prot**,

and finally coupling the resulting compound of the formula XVIII,



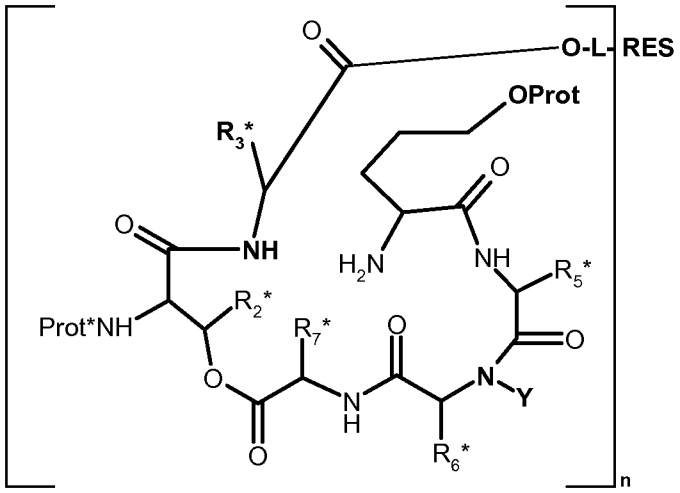
XVIII

wherein Y, R_2*, R_3*, R_7*, R_6* and R_5* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X, to a synthon of the formula XIX,



XIX

wherein Prot is as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula IX, or an activated derivative of said synthon, and removing the protecting group Prot** to yield a compound of the formula XX,



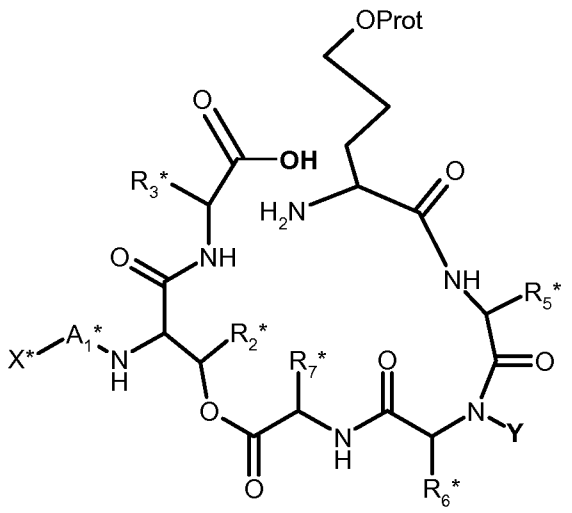
XX

wherein Prot, Y, R_2*, R_3*, R_7*, R_6* and R_5* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X,

and cleaving the solid phase bound peptide in formula XX off the solid phase L-RES to yield the corresponding compound of the formula VIII, especially VIIIA, as shown in claim 5.

8. The method or process according to claim 1 or claim 2, comprising, for the synthesis of the compound of the formula II of claim 1, cyclizing under lactamization of a linear precursor peptide of the compound of the formula II, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, preferably using Solution Phase chemistry.

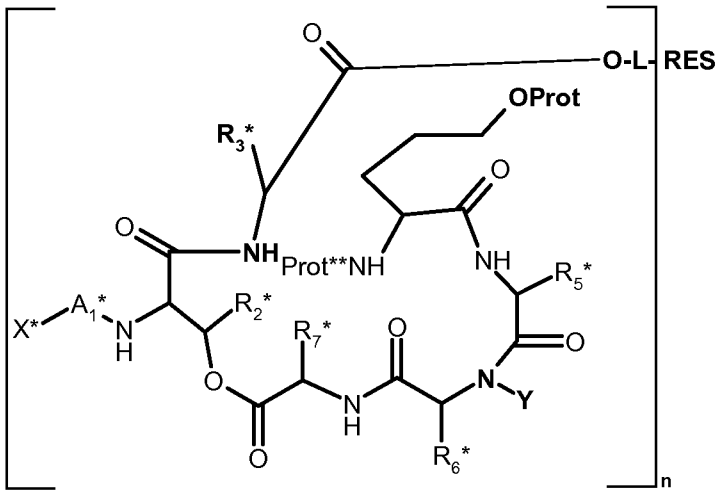
9. The method or process according to claim 8, where the linear precursor peptide is of the formula XXV,



XXV

wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* and Prot are as defined for a compound of the formula II in claim 1.

10. The method or process according to claim 9, further comprising, for the synthesis of the compound of the formula XXV, cleaving a compound of the formula XXIV,

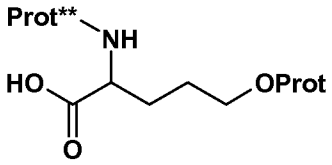


XXIV

wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* and Prot are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, n is a natural number and Prot** is an amino protecting group that can be removed without parallel removal of the protecting group Prot and with the product remaining on the resin, and (before the

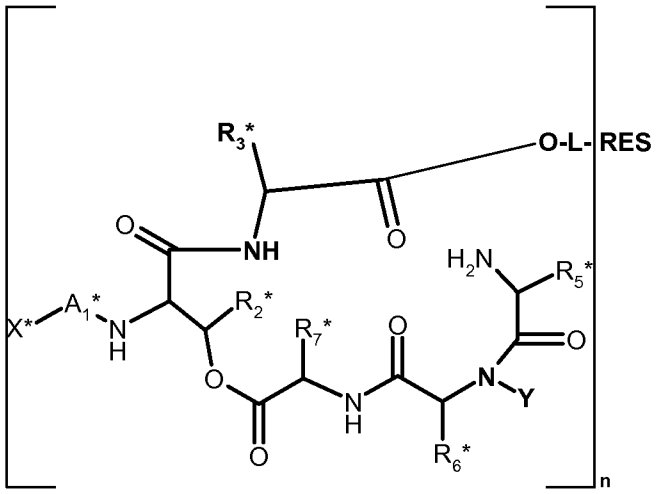
cleavage, in parallel or subsequently to it) removing the protecting group Prot** to yield the compound of the formula XXV.

11. The method or process according to claim 10, further comprising, for the synthesis of the compound of the formula XXIV, coupling an amino acid of the formula XIX,



XIX,

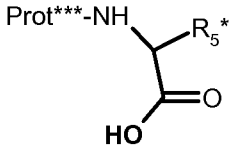
wherein Prot is as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula XXIV in claim 10, or an activated derivative of said amino acid, with a compound of the formula XXIII,



(XXIII)

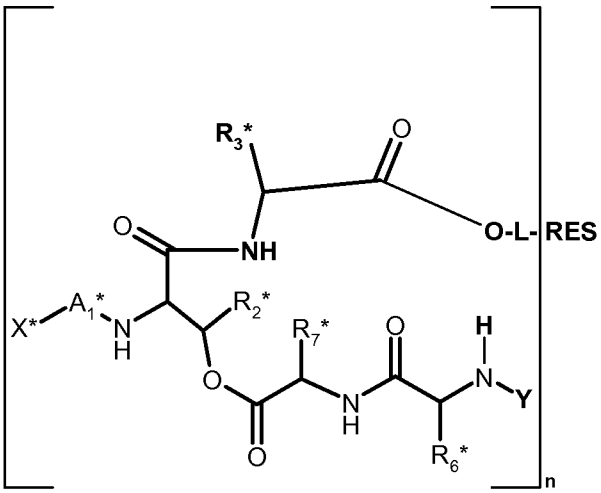
wherein X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number.

12. The method or process according to claim 11, further comprising, for the synthesis of the compound of the formula XXIII, coupling an amino acid of the formula XVII*



XVII*,

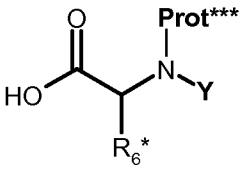
wherein R_5* is as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula XXII,



XXII

wherein X*, A_1*, R_2*, R_3*, R_6* and R_7* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number, and removing the protecting group Prot***.

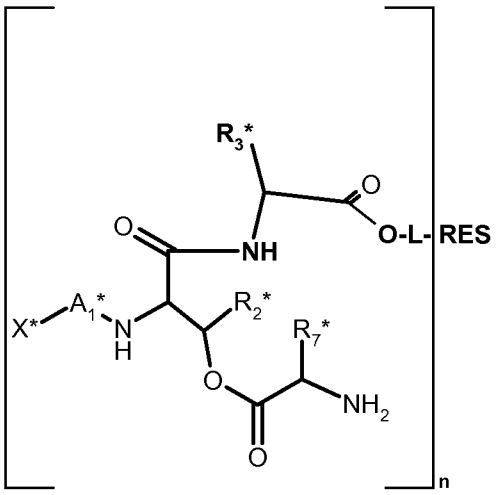
13. The method or process according to claim 12, further comprising, for the synthesis of the compound of the formula XXII, coupling an amino acid of the formula XV*,



XV*

in which R_6* and Y are as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group that can be cleaved off selectively without affecting other

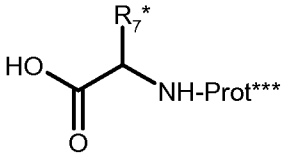
protecting groups present and with the product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula XXI,



XXI,

wherein X*, A_1*, R_2*, R_3* and R_7* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number, and removing the protecting group Prot***.

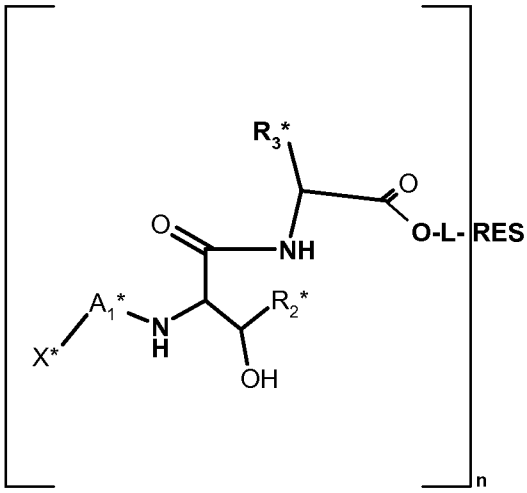
14. The method or process according to claim 13, further comprising, for the synthesis of a compound of the formula XXI, reacting an amino acid of the formula XIII*,



XIII*

wherein Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R_7* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid,

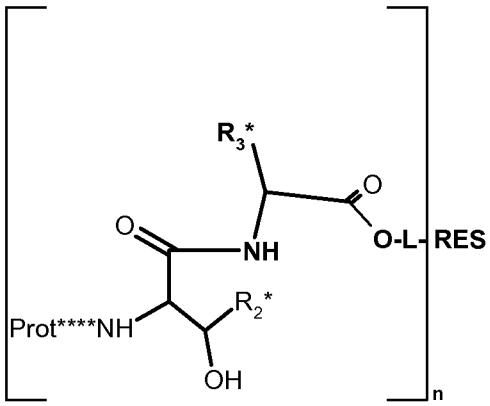
with the hydroxyl group of a compound of the formula XXVI,



XXVI

wherein X*, A_1*, R_2* and R_3* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number;
and removing the protecting group Prot***.

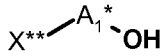
15. The method or process according to claim 14, further comprising, for the synthesis of a compound of the formula XXVI, coupling a resin bound dipeptide symbolized by the formula XII*,



XII*

in which Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present in a compound of the formula II as defined in claim 1 and with the product remaining on the resin, R_2* and R_3* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n

is a natural number, after removal of the protecting group Prot**** via the thus obtained free amino group, with an acid of the formula VII

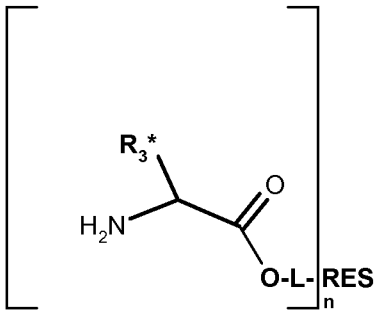


VII

wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined for a compound of the formula II in claim 1, or a reactive derivative of said acid;

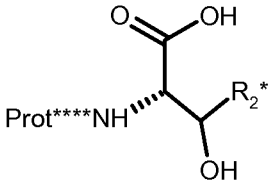
and, if X** is an amino protecting group, removing said amino protecting group X** to yield H instead of X* and coupling the resulting amino group with an acyl group X* using the corresponding acid X*-OH wherein X* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said acid.

16. The method or process according to claim 15, further comprising, for the synthesis of a compound of the formula XII, coupling a resin bound amino acid symbolized by the formula X,



X

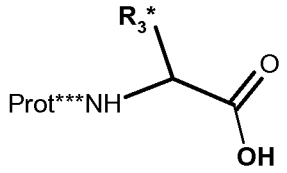
wherein R_3* is as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number,
with an amino acid of the formula XI*,



XI*,

wherein Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R_2* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid.

17. The method or process according to claim 16, further comprising, for obtaining the resin bound amino acid of the formula X, coupling an amino acid of the formula IX*,



IX*

wherein R_3* is as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin; or a reactive derivative of said amino acid of the formula IX, to a hydroxy group bound via a cleavable linker L which is bound to a solid resin RES, and removing the protecting group Prot***.

18. The method or process according to any one of claims 1 to 16, where the symbols A_1, R_2, R_3, R_5, R_6, R_7, X and Y or the corresponding unprotected or protected moieties R_2*, R_3*, R_5*, R_6*, R_7*, X* and Y3 are selected so that, in the resulting compound of the formula I, or a salt thereof,

A_1 is the bivalent radical of L-glutamine bound via the carbonyl of its α-carboxyl group to the amino group at the right of A_1 in formula I and via its α-amino group to X, or is 2S-(2-hydroxy-3-phosphonooxy)-propionyl;

R_2 is methyl;

R_3 is isopropyl, isobutyl or benzyl, especially isobutyl;

R_5 is sec-butyl or benzyl, especially sec-butyl;

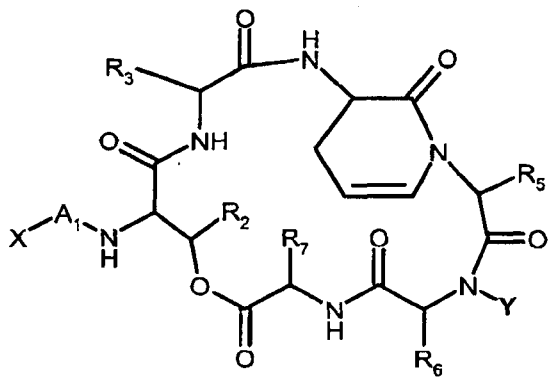
R_6 is 4-hydroxybenzyl;

R_7 is isopropyl or sec-butyl, especially sec-butyl;

X is acetyl or isobutyryl, or is absent if A_1 is 2S-(2-hydroxy-3-phosphonooxy)-propionyl and

Y is methyl.

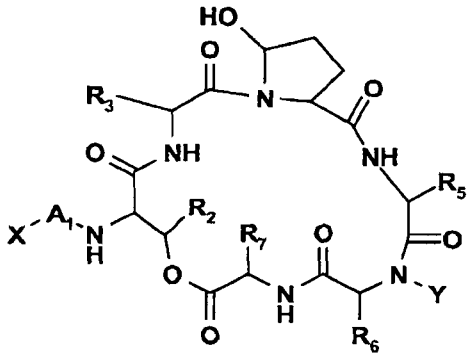
19. A method or process for converting a dehydrate of a compound of the formula I given in claim 1 or with the substituents as defined in claim 18 into the corresponding compound of the formula I, where the dehydrate has the formula V,



V,

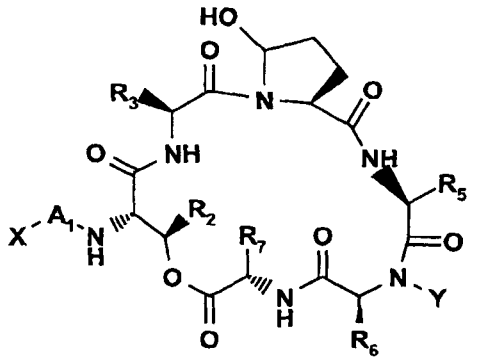
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I in any one of claims 1 and 18;

and/or its corresponding hemiaminal analogue with a five-ring instead of the ahp structure in formula I which may also be formed as byproduct and has the formula V*,



V*

especially the formula VA*,



VA*,

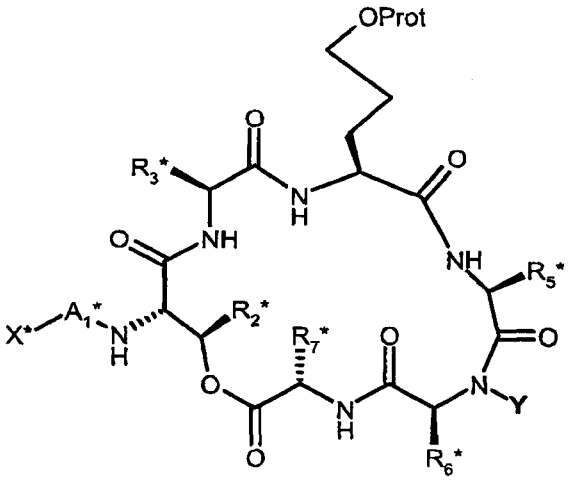
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I in any one of claims 1 and 18, respectively;

or a method or process for shifting the equilibrium of a mixture of a compound of the formula I and its corresponding dehydrate and/or hemiaminal in favor of the compound of the formula I,

said method or process comprising using an aqueous acid as reactive solvent to drive the reaction.

20. The method according to claim 19, wherein the acid is a carboxylic acid, especially a halo substituted C_{1-8}alkanoic acid, more especially trifluoroacetic acid or trichloroacetic acid.

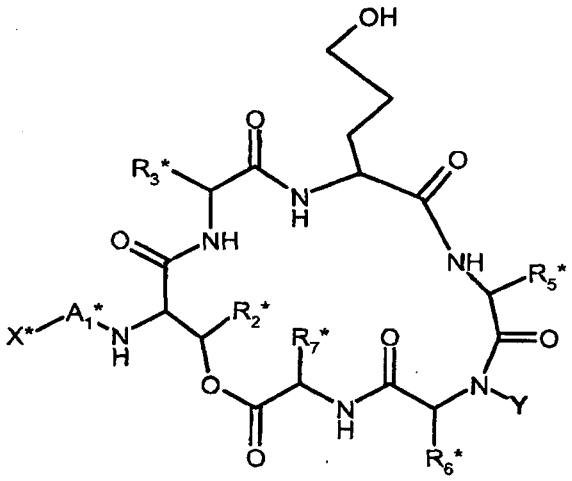
21. A compound of the formula II,



II

wherein Prot is a protecting group, Y is as defined for a compound of the formula I in claim 1 or claim 18 and X^*, A_1^*, R_2^*, R_3^*, R_5^*, R_6^*, and R_7^* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I as defined in claim 1 or in claim 18, respectively, however with the proviso that reactive functional groups on these moieties are present in unprotected or protected form.

22. A compound of the formula III,



(III)

wherein Y is as defined for a compound of the formula I in claim 1 or claim 18 and X^*, A_1^*, R_2^*, R_3^*, R_5^*, R_6^*, and R_7^* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I as defined in claim 1 or in claim 18, respectively, however with the proviso that reactive functional groups other than the hydroxy represented at the top in formula III on these moieties are present in unprotected or protected form.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/051977

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K11/02
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BEILSTEIN Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YOKOKAWA F ET AL: "Synthetic studies of the cyclic depsipeptides bearing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit. Total synthesis of the proposed structure of micropeptin T-20", TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 61, no. 6, 7 February 2005 (2005-02-07), pages 1459-1480, XP027860763, ISSN: 0040-4020 [retrieved on 2005-02-07] Scheme: 1, 6, 9, 10,11,12; 2. Synthetic strategy;compounds 2,32-34,44,45,46,51 ----- -/--	1-21

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2012	07/08/2012

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/051977

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YOKOKAWA F ET AL: "Total synthesis of somamide A, an Ahp (3-amino-6-hydroxy-2-piperidone)-containin g cyclic depsipeptide", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 43, no. 48, 25 November 2002 (2002-11-25), pages 8673-8677, XP004390459, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(02)02178-0 Scheme 5compounds 1,4, 24,25 -----	1-21
X	YOKOKAWA F ET AL: "Synthetic studies of micropeptin T-20, a novel 3-amino-6-hydroxy-2-piperidone (Ahp)-containing cyclic depsipeptide", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 42, no. 34, 20 August 2001 (2001-08-20), pages 5903-5908, XP004295953, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(01)01136-4 Schemes;compounds 15,16,20,21,28 -----	1-21
A	SARA C. STOLZE ET AL: "Solid phase total synthesis of the 3-amino-6-hydroxy-2-piperidone (Ahp) cyclodepsipeptide and protease inhibitor Symplocamide A", CHEMICAL COMMUNICATIONS, vol. 46, no. 46, 1 January 2010 (2010-01-01), page 8857, XP55033621, ISSN: 1359-7345, DOI: 10.1039/c0cc02889d the whole document -----	1-21
A,P	SARA C. STOLZE ET AL: "Development of a Solid-Phase Approach to the Natural Product Class of Ahp-Containing Cyclodepsipeptides", EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, vol. 2012, no. 8, 23 January 2012 (2012-01-23), pages 1616-1625, XP55033623, ISSN: 1434-193X, DOI: 10.1002/ejoc.201101757 the whole document -----	1-21

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/051977

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/024527 A1 (NOVARTIS AG [CH]; KRASTEL PHILIPP [DE]; LIECHTY BRIGITTA-MARIA [CH]; M) 26 February 2009 (2009-02-26)	18
A	claims	1-17, 19-21

Y	LINE BOUREL-BONNET ET AL: "Solid-Phase Total Synthesis of Kahalalide A and Related Analogues", JOURNAL OF MEDICINAL CHEMISTRY, vol. 48, no. 5, 1 March 2005 (2005-03-01), pages 1330-1335, XP55033736, ISSN: 0022-2623, DOI: 10.1021/jm049841x Scheme 1	1-17

Y	STAWIKOWSKI M ET AL: "A novel strategy for the solid-phase synthesis of cyclic lipodepsipeptides", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 47, no. 48, 27 November 2006 (2006-11-27), pages 8587-8590, XP025003510, ISSN: 0040-4039, DOI: 10.1016/J.TETLET.2006.09.116 [retrieved on 2006-11-27] Scheme 1; page 8578, left-hand column, paragraph 3.	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2012/051977

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009024527 A1	26-02-2009	AR 068343 A1	11-11-2009
		AU 2008290567 A1	26-02-2009
		CA 2696570 A1	26-02-2009
		CO 6260099 A2	22-03-2011
		CR 11251 A	08-03-2010
		EA 201000329 A1	30-08-2010
		EC SP109968 A	31-03-2010
		EP 2183273 A1	12-05-2010
		JP 2010536731 A	02-12-2010
		KR 20100044893 A	30-04-2010
		MA 31701 B1	01-09-2010
		NZ 583284 A	27-04-2012
		PE 10072009 A1	14-08-2009
		SV 2010003485 A	23-03-2011
		TW 200922613 A	01-06-2009
		US 2009156472 A1	18-06-2009
		WO 2009024527 A1	26-02-2009
		ZA 201000776 A	27-10-2010

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2012/051977

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-17, 20, 21

   Synthesis of an AHP containing cyclic depsipeptide.

   ---

2. claim: 18

   A method of converting a dehydrate or hemiaminal of the compound of formula I, or modiffying the equilibrium of a reaction comprising using an aqueous acid as reactive solvent.

PROCESOS PARA LA ELABORACIÓN DE DEPSIPÉPTIDOS MACROCÍCLICOS Y NUEVOS INTERMEDIARIOS

Breve Descripción de la Invención

La invención se refiere a un método o proceso para la elaboración de depsipéptidos macrocíclicos, a nuevos intermediarios y su elaboración, así como a modalidades de la invención relacionadas.

Antecedentes de la Invención

Los depsipéptidos cíclicos tienen numerosos usos en farmacología. Como un ejemplo, los depsipéptidos que se dan a conocer en la Publicación Internacional Número WO2009/024527 son útiles para el tratamiento de diferentes enfermedades. Por ejemplo, el compuesto de la fórmula II mencionado en la Publicación Internacional Número WO2009/024527 es útil para el tratamiento y la prevención de enfermedades inflamatorias y/o hiperproliferativas y pruríticas de la piel, tales como dermatitis atópica, soriasis, soriasis pustular, rosácea, queloides, escaras hipertróficas, acné, síndrome de Netherton u otras dermatosis pruríticas, tales como prurigo nodularis, comezón no especificada de los ancianos, así como otras enfermedades con disfunción de la barrera epitelial, tales como piel envejecida.

La Nostopeptina BN920, anteriormente aislada a partir de la cianobacteria Nostoc, se aisló también a partir de Microcistis. La Nostopeptina BN920 inhibió la quimiotripsina con un valor de IC_{50} de

31 nM (véase J. Nat. Prod. 68(9), 1324-7 (2005)).

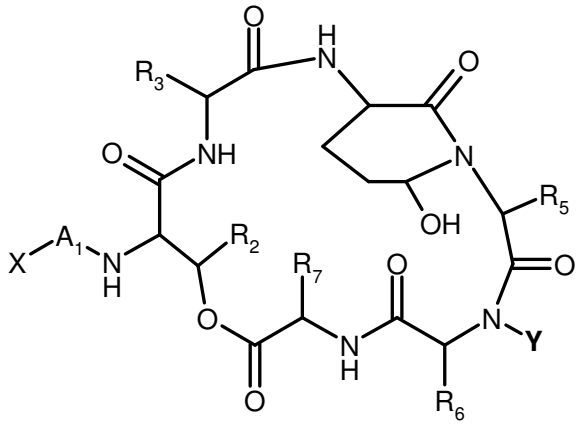
Estos compuestos se pueden producir mediante fermentación (usando condromyces croactus, mixobacterias) junto con otros depsipéptidos, los cuales comprenden la denominada como subestructura-ahp (ahp: 3-amino-6-hidroxi-piperidin-2-ona), y la subestructura deshidro-ahp correspondiente (deshidro-ahp: 3-amino-3,4-dihidro-1H-piridin-2-ona), también denominada como “deshidrato” en la presente, respectivamente. Por consiguiente, el rendimiento de la fermentación con respecto a cualquiera de estos compuestos es más bien bajo.

La presente invención se refiere a procesos o métodos que permiten obtener estos depsipéptidos cíclicos con un mayor rendimiento y/o en una buena pureza.

En vista de los muchos riesgos, tales como epimerización, tautomerización y similares, en la síntesis de una molécula compleja con muchos isómeros posibles, ha sido posible encontrar un proceso de manufacturación que comprende de preferencia una mezcla de síntesis de péptidos en fase sólida y reacciones en solución, el cual permite producir los depsipéptidos cíclicos de la fórmula I en un buen rendimiento y/o la pureza estereoisomérica requerida, en especial ambos. Es posible reducir la cantidad de subproductos, e incluso mejorar el rendimiento, mediante la conversión de estos subproductos, en especial la subestructura deshidro-ahp y/o un análogo de los productos que comprenden ahp deseados con un anillo de cinco miembros en lugar de la ahp, en los productos finales

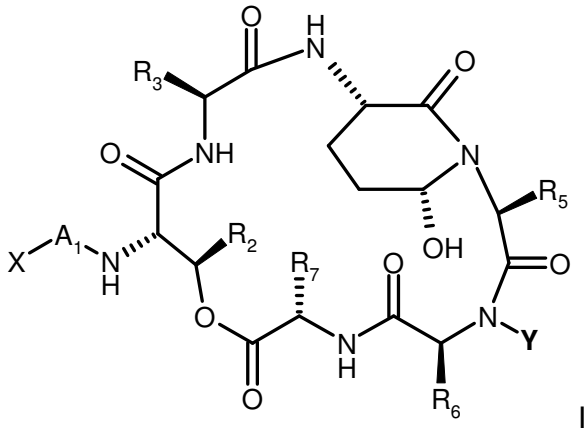
deseados. Esto permite aumentar adicionalmente el rendimiento. Hasta ahora, no ha llegado a nuestra atención ninguna síntesis que haga uso de la síntesis de péptidos en fase sólida en este campo.

(i/a) En una primera modalidad, la invención se refiere a un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:



I

en especial de la fórmula IA:



IA

en donde:

A_1 es una fracción bivalente de un aminoácido con un grupo carboxilo o carbamoílo terminal, en especial asparagina o glutamina, y se enlaza en su lado derecho en la fórmula I (que corresponde al término C) por medio de un carbonilo (de preferencia el carbonilo de un grupo α-carboxilo del mismo) con el resto de la molécula; o es alcanoílo de 1 a 8 átomos de carbono o hidroxi-alcanoílo de 1 a 8 átomos de carbono fosforilado;

X se enlaza por medio de un N de A_1 y es acilo, o está ausente si A_1 es alcanoílo de 1 a 8 átomos de carbono o hidroxi-alcanoílo de 1 a 8 átomos de carbono fosforilado;

R_2 es alquilo de 1 a 8 átomos de carbono, en especial metilo;

R_3 es la cadena lateral de un aminoácido, en especial de leucina, isoleucina o valina;

R_5 es la cadena lateral de un aminoácido, de preferencia de fenilalanina, leucina, isoleucina o valina;

R_6 es la cadena lateral de un hidroxi-aminoácido, en especial de tirosina;

R_7 es la cadena lateral de un aminoácido, de preferencia del aminoácido leucina, isoleucina o valina; e

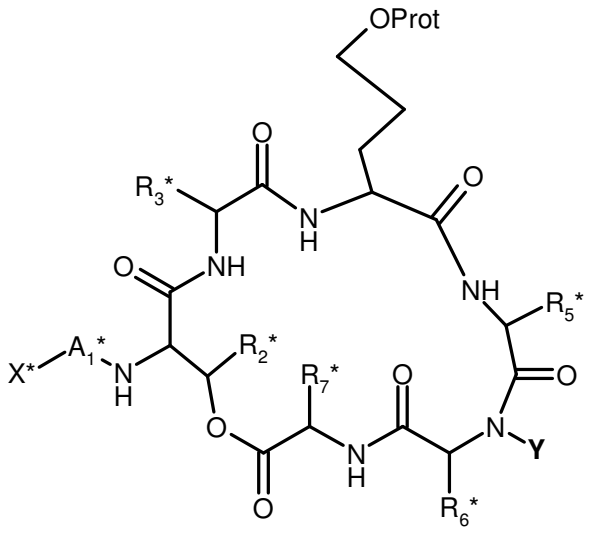
Y es hidrógeno o alquilo de 1 a 8 átomos de carbono;

o una sal del mismo,

comprendiendo este método:

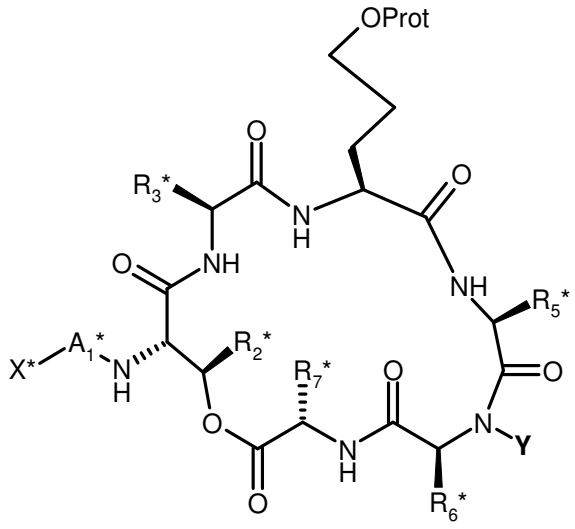
desproteger selectivamente un compuesto de la fórmula

II:



II

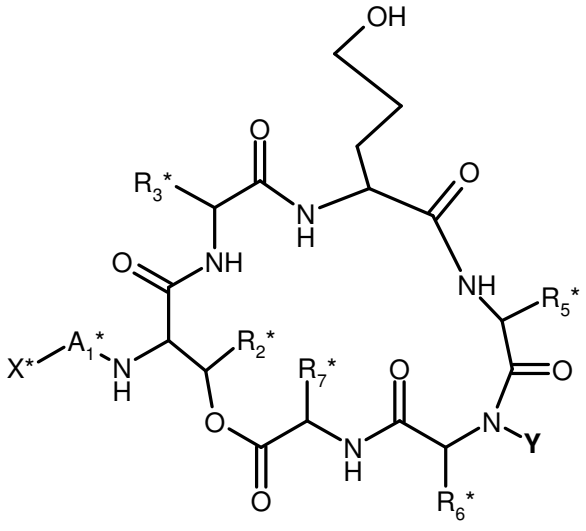
en especial de la fórmula IIA:



IIA

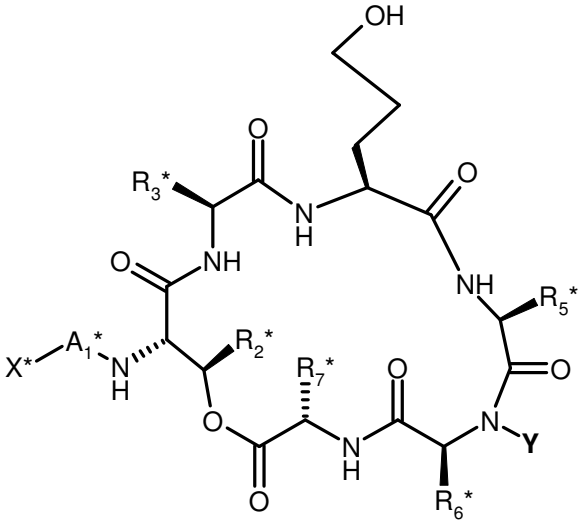
en donde Prot es un grupo protector, Y es como se define para un compuesto de la fórmula I, y X*, A_1*, R_2*, R_3*, R_5*, R_6*, y R_7* corresponden a X, A_1, R_2, R_3, R_5, R_6, y R_7 en la fórmula I,

respectivamente, pero con la condición de que los grupos funcionales reactivos sobre estas fracciones (tales como amino, imino, hidroxilo, carboxilo, sulfhidrilo, amidino, guanidino, O-fosfono (-O-P(=O)-$(OH)_2$) están presentes en una forma protegida cuando menos si podrían participar en reacciones secundarias indeseadas, para dar como resultado un compuesto de la fórmula III:



III

en especial de la fórmula IIIA:

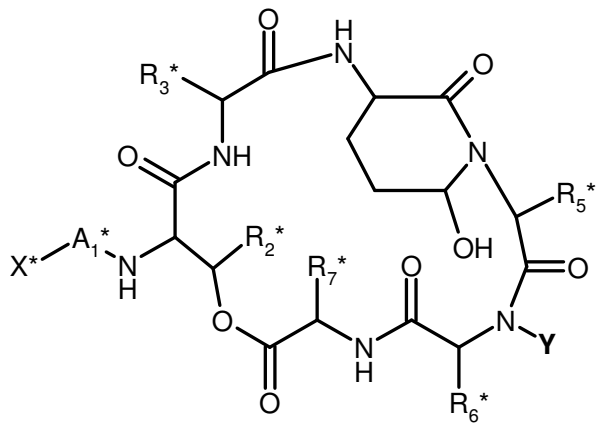


IIIA

en donde X*, A_1*, R_2*, R_3*, R_5*, R_6*, y R_7* tienen los

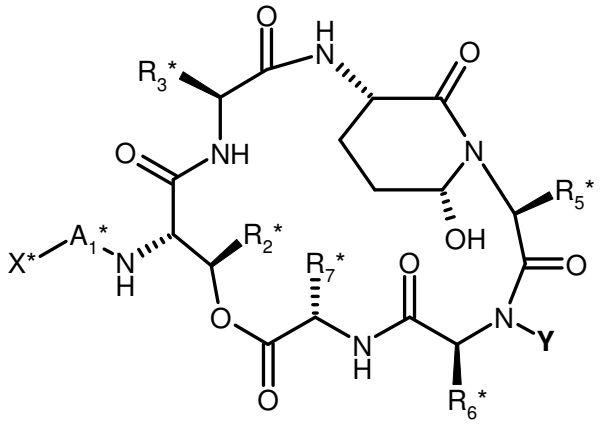
significados recién definidos,

hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula IV:



IV

en especial IVA:



IVA

y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula I, o una sal del mismo,

y, si se desea, convertir un compuesto libre de la fórmula I, o en especial IA, en una sal, una sal de un compuesto de la

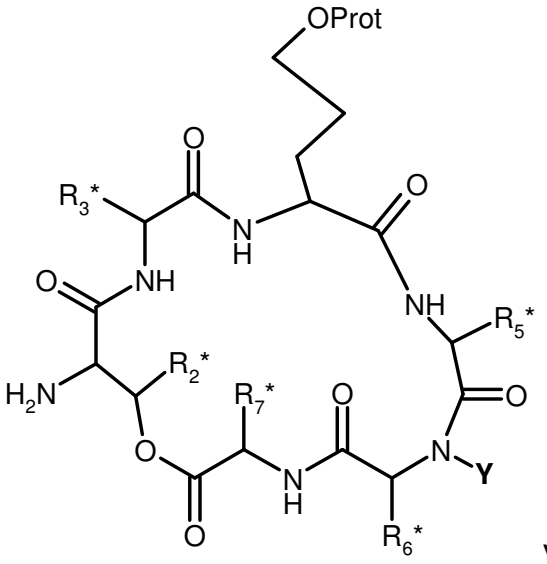
fórmula I en una sal diferente de un compuesto de la fórmula I, o en especial IA, o en el compuesto libre de la fórmula I, o en especial IA, y/o convertir un análogo de deshidrato y/o un análogo de cinco anillos de un compuesto de la fórmula I, o en especial IA, en el compuesto correspondiente de la fórmula I, o en especial IA.

Las condiciones de oxidación adecuadas para la oxidación de un compuesto de la fórmula III o en especial IIIA son usualmente utilizando IBX en sulfóxido de dimetilo (J. Org. Chem. 1995, *60*, 7272-7276); Dicromato de piridinio o Clorocromato de piridinio (Tetrahedron Lett. 1979, *5*, 399-402); cloruro de oxalilo, sulfóxido de dimetilo y una amina terciaria (J. Peptide Sci. 2006, *12*, 140-146), sales de oxoamonio (J. Org. Chem. 1985, *50*, 1332-1334); hipocloritos alcalinos catalizados por sales de oxoamonio (J Org. Chem. 1989, *54*, 2970-2972); sales de oxoaminio (Tetrahedron Lett. 1988, *29*, 5671-5672), $RuCl_2(PPh_3)_3$ (Tetrahedron Lett. 1981, *22*, 1605-1608); TEMPO (1 por ciento molar) en la presencia de hipoclorito de sodio (Tetrahedron Lett. 1990, *31*, 2177-2180); $NaIO_4$, TEMPO, NaBr (Tetrahedron 2006, *62*, 8928-8932); óxido de vanadio(IV) soportado por SiO_2 y t-BuOOH (Advanced Synthesis & Catalysis 2007, *349*, 846-848). De preferencia, la reacción se lleva a cabo con IBX en sulfóxido de dimetilo, o de preferencia en un solvente inerte, tal como tetrahidrofurano, en la presencia de sulfóxido de dimetilo, a una temperatura de entre 0 °C y 50 °C, de preferencia de entre 20 °C y 25 °C.

(ii/a) Una modalidad adicional de la invención se refiere al

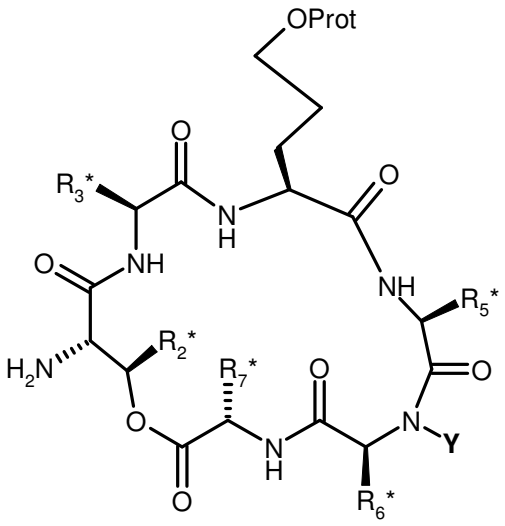
método o proceso descrito anteriormente, el cual además comprende elaborar el compuesto de la fórmula IV o en especial IVA mediante una combinación de síntesis de péptidos en fase sólida (en especial para la síntesis del precursor XX o en especial XXA que se da más adelante para el precursor de oligopéptido de la fórmula VIII o en especial VIIIA que se da más adelante, o del precursor de oligopéptido de la fórmula XXIV o en especial XXIVA que se da más adelante para el precursor de oligopéptido de la fórmula XXV o en especial XXVA que se da más adelante) y síntesis en fase de solución (en especial a partir de los compuestos recién mencionados hasta el producto final) a partir de los aminoácidos de partida y precursores de cadena lateral correspondientes.

(iii/a) Todavía una modalidad adicional de la invención se refiere a un método o proceso como se describe anteriormente, el cual además comprende, para la síntesis de un compuesto de la fórmula II anterior, hacer reaccionar un compuesto de la fórmula VI:



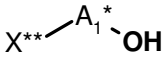
VI

en especial VIA:



VIA

en donde Prot es un grupo protector, Y es como se define para un compuesto de la fórmula I, y R_2*, R_3*, R_5*, R_6*, y R_7* son como se definen para un compuesto de la fórmula II anterior, con un ácido de la fórmula VII:



VII

o un derivado reactivo del mismo,

en donde X** es un grupo protector de amino, o es X*, y en donde X* y A_1* son como se definen para un compuesto de la fórmula II anterior; y, si X** es un grupo protector de amino, remover el grupo protector de amino X** para proporcionar el derivado de la fórmula II (en especial IIA) en donde, en lugar de X*,está presente H (un hidrógeno) y acoplar el grupo amino resultante con un grupo acilo X* utilizando el ácido correspondiente X*-OH, en donde X* es como se define para un compuesto de la fórmula II definido

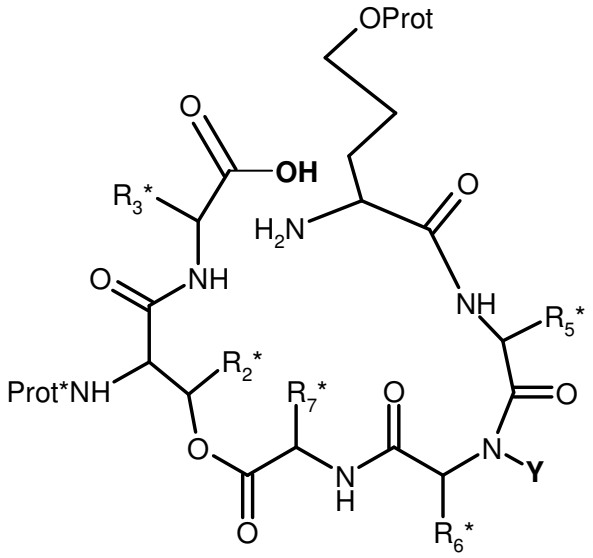
anteriormente, o un derivado reactivo del mismo.

(iv/a) Otra modalidad de la invención se refiere a los métodos o procesos descritos anteriormente, en especial en el párrafo anterior, los cuales además comprenden la ciclación bajo lactamización de un péptido precursor lineal, es decir, todavía no cíclico, del compuesto de la fórmula VI, que lleve un grupo amino N-terminal y un grupo carboxilo C-terminal, en condiciones de reacción que permitan la formación de un enlace de amida a partir del grupo amino y el grupo carboxilo, de preferencia utilizando la química en fase de solución.

Las lactamizaciones en soluciones usualmente se llevan a cabo en concentraciones muy bajas del sustrato con el objeto de evitar las oligomerizaciones y polimerizaciones. Esto requiere de enormes cantidades de solventes y reactores muy grandes para llevar a cabo las reacciones. Por ejemplo, la macrolactamización de un oligopéptido se lleva a cabo en una concentración de 2 milimoles/litro en la referencia de Yokokawa y colaboradores, Tetrahedron 2005, *61*, 1459-1480. Esta dificultad se puede circunvenir mediante la disolución de la base terciaria y el reactivo de acoplamiento y, de una manera controlada, agregar una solución del oligopéptido a esta solución. La adición controlada, en especial lenta, de la solución de oligopéptido genera concentraciones permanentemente bajas del oligopéptido activado en solución y, por consiguiente, previene la oligomerización y polimerización. La velocidad de adición de la solución de oligopéptido se puede ajustar de acuerdo con la velocidad de reacción para la macrociclación: si la macrociclación es

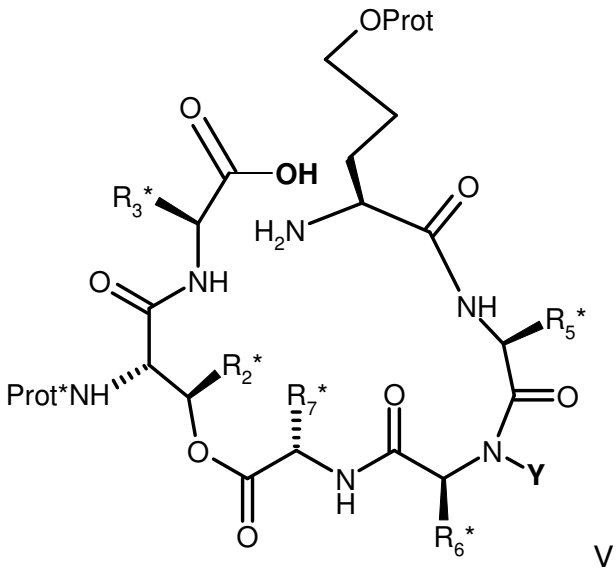
una reacción rápida, la solución del oligopéptido se puede agregar rápido. Si la macrociclación es lenta, la adición de la solución debe ser lenta para asegurar la baja concentración permanente del oligopéptido activado. Por consiguiente, la adición controlada del oligopéptido hace posible trabajar con cantidades mucho menores de solvente y todavía mantener la concentración del oligopéptido activado por debajo de 10^{-3} mM, por ejemplo, en el intervalo de 10^{-4} a 10^{-6} mM o incluso más baja. Esta variante de adición controlada del oligopéptido a la solución del reactivo de acoplamiento es una modalidad de la invención.

(v/a) En todavía una modalidad adicional, la invención se refiere al método o proceso como se describe anteriormente, en especial en el párrafo anterior, en donde el péptido precursor lineal (significando este término, donde se utilice, que todavía no es cíclico) es de la fórmula VIII:



VIII,

en especial VIIIA:



VIIIA

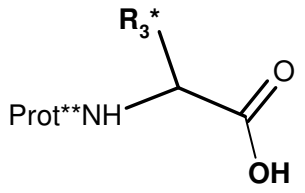
en donde Prot* es un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y es estable durante los pasos de desprotección durante la síntesis del péptido precursor lineal (por ejemplo, aliloxi-carbonilo), y R_2*, R_3*, R_5*, R_6*, y R_7* son como se definen para un compuesto de la fórmula VI como se describe anteriormente, el cual además comprende, después de la ciclación del compuesto de la fórmula VIII, en especial VIIIA, remover el grupo protector Prot* *in situ* para proporcionar el compuesto de la fórmula VI, en especial VIA.

(vi/a) En otra modalidad, la invención se refiere al método o proceso descrito anteriormente, en especial en el párrafo anterior, en donde el péptido precursor lineal de la fórmula VIII, en especial VIIIA, se sintetiza a partir de los aminoácidos correspondientes

mediante la síntesis de péptidos en fase sólida, y la disociación subsiguiente a partir del soporte sólido empleado.

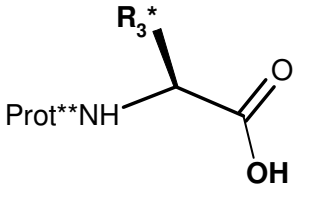
(vii/a) Una modalidad de la invención se refiere además al método o proceso como se describe anteriormente (en especial en el párrafo anterior (vi/a)), el cual además comprende cualquiera de:

en una variante a), acoplar un aminoácido de la fórmula IX:



IX

en especial de la fórmula IXA:



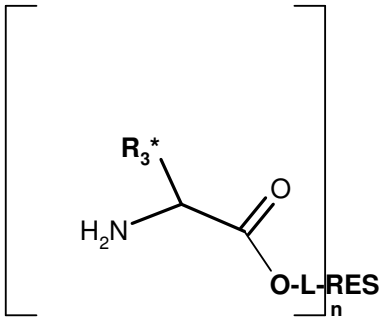
IXA

en donde R_3* es como se define para un compuesto de la fórmula II anterior, y Prot** es un grupo protector de amino que se puede remover de la resina sin disociar otros enlaces, o un derivado reactivo de este aminoácido, por medio de un oxígeno, a un enlazador disociable L, el cual se enlaza a una resina sólida RES (por ejemplo, mediante la reacción con una resina de la fórmula $(X\text{-}L)_z$-RES, en donde L y RES son como justamente se definieron, X es,

por ejemplo, halógeno, por ejemplo, cloro, y Z es un número mayor que cero, por ejemplo, un número natural)

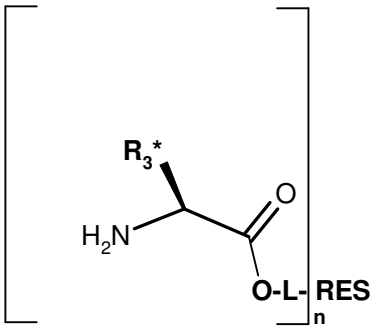
y remover el grupo protector Prot**;

acoplar el aminoácido enlazado con resina que se puede obtener, simbolizado por la fórmula X:



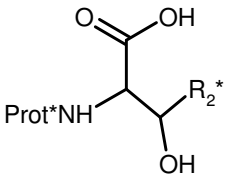
X

en especial XA:



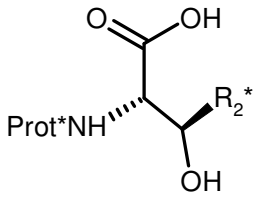
XA

en donde RES y R_3* son como se definen para un compuesto de la fórmula IX, n es un número (por ejemplo, natural) mayor que cero, y L es un enlazador disociable, con un aminoácido de la fórmula XI:



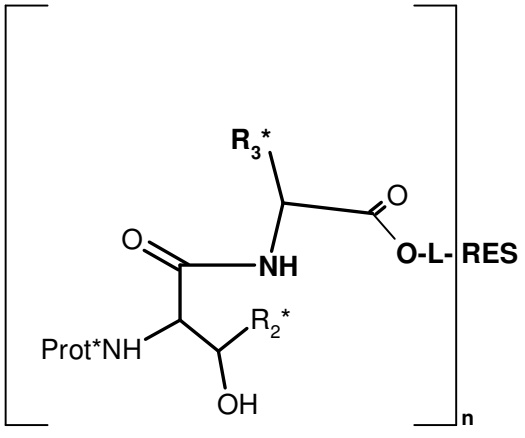
XI

en especial XIA:



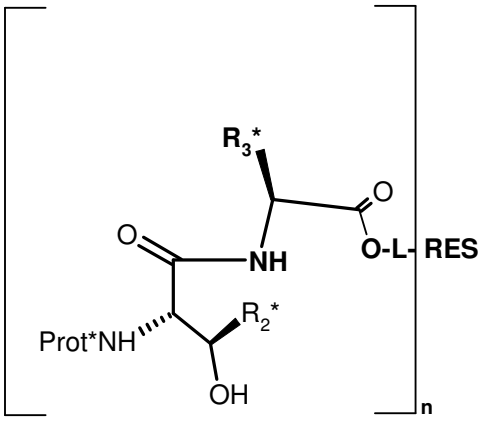
XIA

en donde Prot* es como se define para un compuesto de la fórmula VIII anterior, y R_2* es como se define para un compuesto de la fórmula II anterior, o un derivado reactivo de este aminoácido, acoplar el dipéptido enlazado con resina que se puede obtener, simbolizado por la fórmula XII:



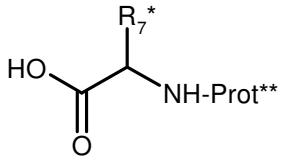
XII

en especial XIIA:



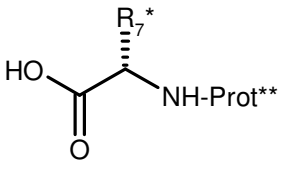
XIIA

en donde Prot* es como se define para un compuesto de la fórmula VIII anterior, R_2* y R_3* son como se definen para un compuesto de la fórmula II anterior, y n, L y RES son como se definen para un compuesto de la fórmula X, por medio del grupo hidroxilo libre, con un aminoácido de la fórmula XIII:



XIII

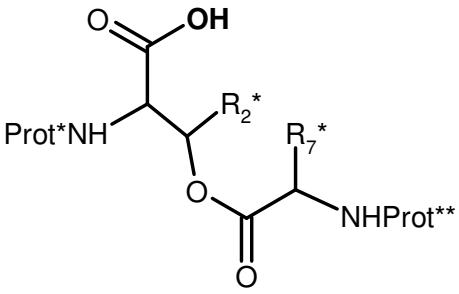
en especial XIIIA,



XIIIA

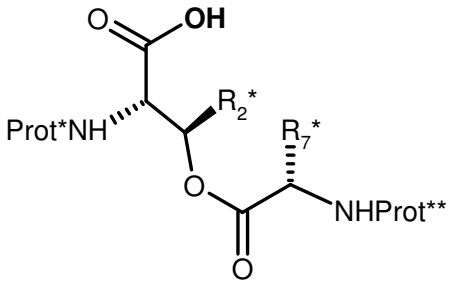
en donde Prot** es como se define para un compuesto de la fórmula IX, y R_7* es como se define para un compuesto de la fórmula II anterior, o un derivado reactivo de este aminoácido, y remover el grupo protector Prot**;

o, en una variante b), acoplar un dipéptido de la fórmula XXVII:



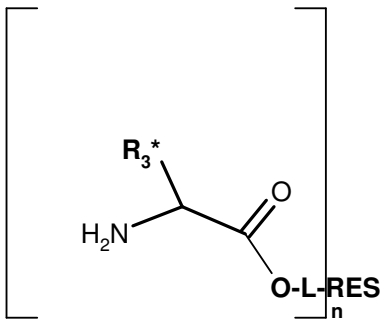
XXVII

en especial de la fórmula XXVIIA:



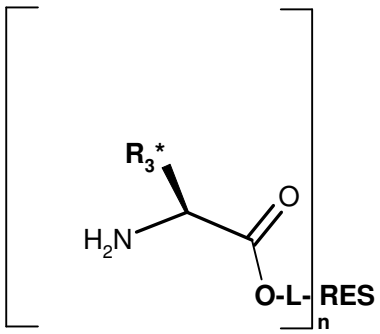
XXVIIA

en donde R_3* y Prot** son como se describen para un compuesto de la fórmula IX, en especial IXA, y Prot* es como se define para un compuesto de la fórmula VIII anterior, o un derivado reactivo de este dipéptido, con una fracción de amino-acilo, enlazada por medio de un oxígeno, a un enlazador disociable L, el cual se enlaza a una resina sólida RES, que tiene la fórmula X:



X

en especial XA:



XA

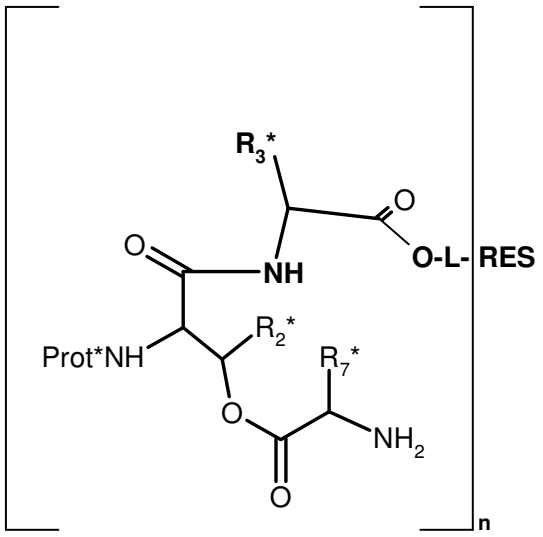
que se puede obtener como se describe bajo la variante

a), en donde RES y R_3* son como se definen para un compuesto de la fórmula IX, y L y RES son como justamente se definieron;

y remover el grupo protector Prot**;

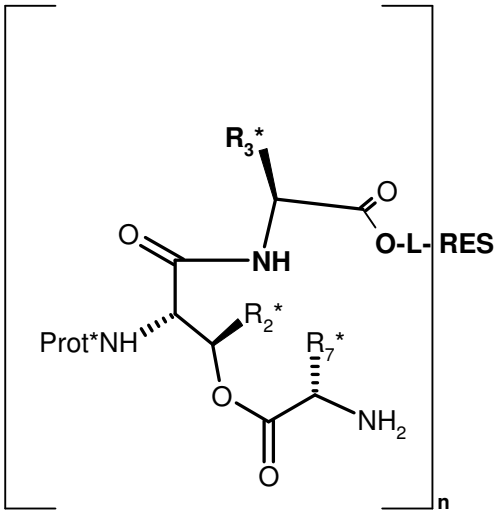
y, después de las reacciones de la variante a) o de la variante b),

(viii/a) acoplar el compuesto que se puede obtener de la fórmula XIV:



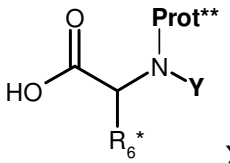
XIV

en especial XIVA:



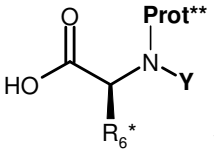
XIVA

en donde R_2*, R_3* y R_7* son como se definen para un compuesto de la fórmula II anterior, Prot* es como se define para un compuesto de la fórmula VIII anterior, y n, L y RES son como se definen para un compuesto de la fórmula X, con un aminoácido de la fórmula XV:



XV

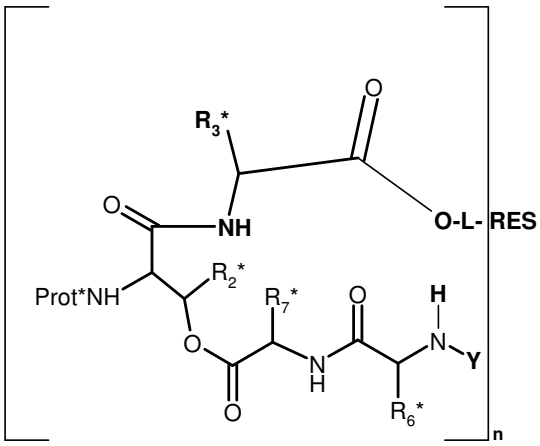
en especial la fórmula XVA:



XVA

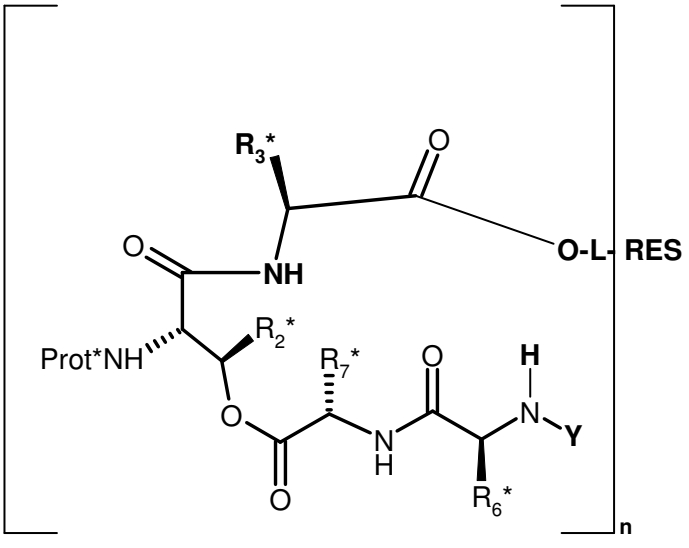
en donde R_6* e Y son como se definen para un compuesto de la fórmula II anterior, y Prot** es como se define para un compuesto de la fórmula IX anterior, o un derivado reactivo de este aminoácido, y remover el grupo protector Prot**;

(ix/a) de preferencia acoplar el compuesto que se puede obtener de la fórmula XVI:



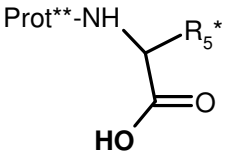
XVI

en especial la fórmula XVIA:



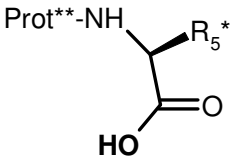
XVIA

en donde Y, R_2*, R_3*, R_7* y R_6* son como se definen para un compuesto de la fórmula II anterior, Prot* es como se define para un compuesto de la fórmula VIII anterior, y n, L y RES son como se definen para un compuesto de la fórmula X, con un aminoácido de la fórmula XVII:



XVII

en especial fórmula XVIIA:



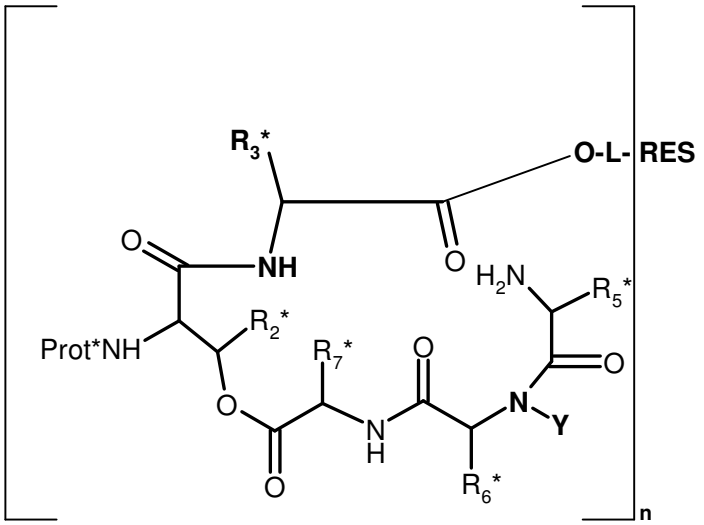
XVIIA

en donde R_5* es como se define para un compuesto de la fórmula II anterior, y Prot** es como se define para un compuesto de la fórmula IX, o un derivado reactivo de este aminoácido, y remover

el grupo protector Prot**,

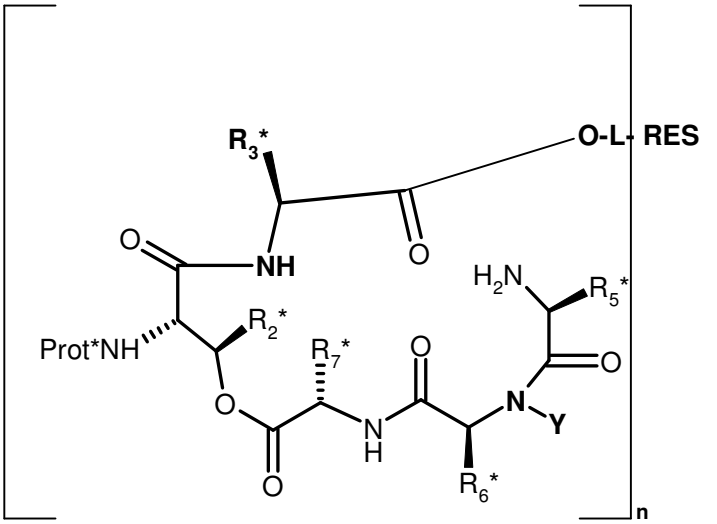
y de preferencia,

(x/a) finalmente acoplar el compuesto resultante de la fórmula XVIII:



XVIII

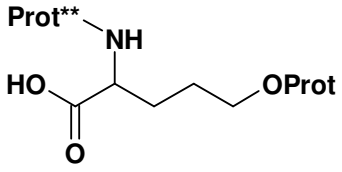
en especial XVIIIA:



XVIIIA

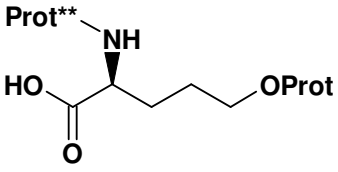
en donde Y, R_2*, R_3*, R_7*, R_6* y R_5* son como se definen para un compuesto de la fórmula II anterior, Prot* es como se define para

un compuesto de la fórmula VIII anterior, y n, L y RES son como se definen para un compuesto de la fórmula X anterior, con un aminoácido no natural (= sintón) de la fórmula XIX:



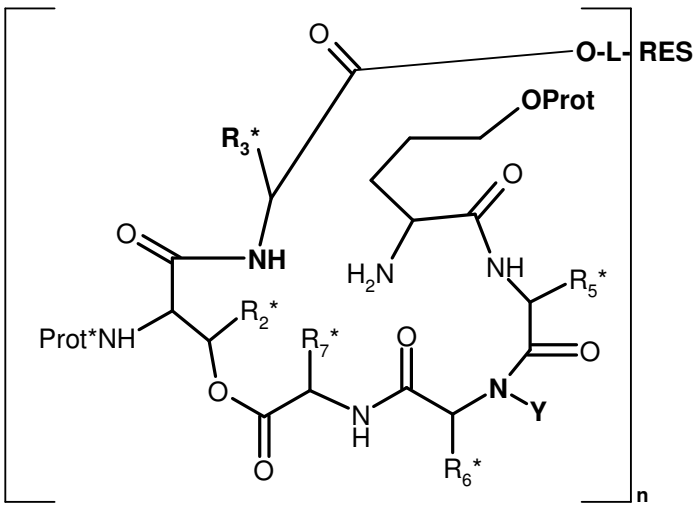
XIX

en especial la fórmula XIXA:



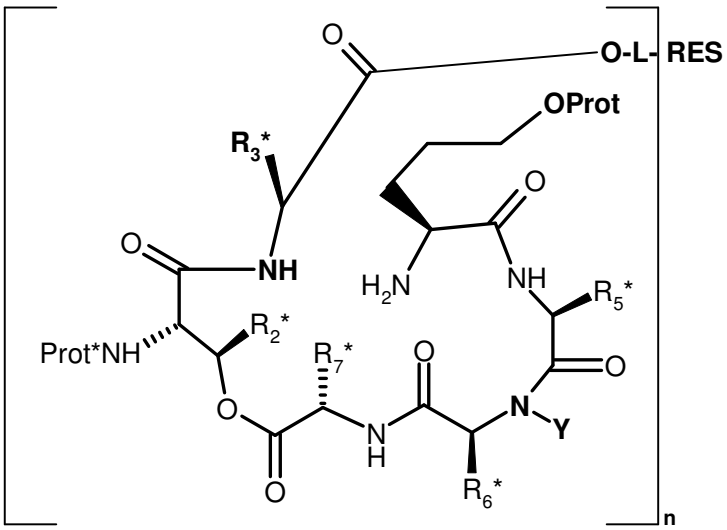
XIXA

en donde Prot es como se define para un compuesto de la fórmula II anterior, y Prot** es como se define para un compuesto de la fórmula IX, o un derivado activado de dicho sintón, y remover el grupo protector Prot**, para proporcionar un compuesto de la fórmula XX:



XX

en especial XXA:



XXA

en donde Prot, Y, R_2*, R_3*, R_7*, R_6* y R_5* son como se definen para un compuesto de la fórmula II anterior, Prot* es como se define para un compuesto de la fórmula VIII anterior, y n, L y RES son como se definen para un compuesto de la fórmula X,

y

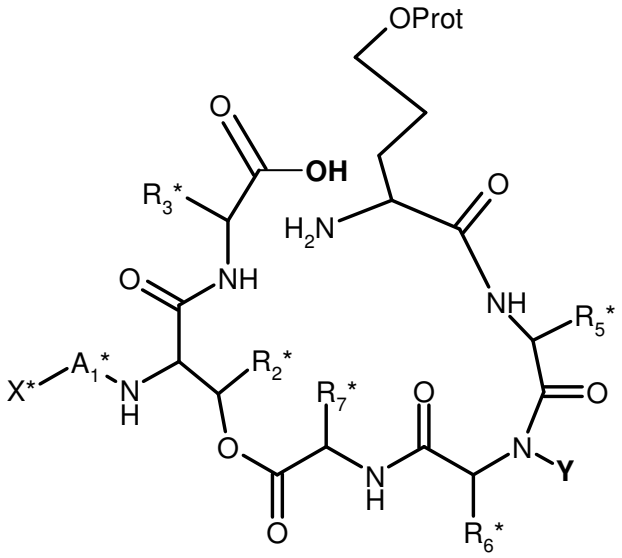
(xi/a) disociar el péptido enlazado en fase sólida en la fórmula XX del L-RES en fase sólida, para proporcionar el compuesto correspondiente de la fórmula VIII, en especial VIIIA, como se muestra anteriormente.

Otra modalidad de la invención se refiere a la síntesis de un compuesto de la fórmula II como se da anteriormente de acuerdo con la sección (i/a), de preferencia precedida por la reacción de acuerdo con la sección (ii/a), o más preferiblemente de acuerdo con la sección (iii/a); de preferencia precedida por la reacción de acuerdo con la sección (iv/a) o de preferencia (v/a), de preferencia precedida por la reacción de acuerdo con la sección (vi/a), de

preferencia precedida por la reacción de acuerdo con la sección (x/a), de preferencia precedida por la reacción de acuerdo con la sección (ix/a), de preferencia precedida por la reacción de acuerdo con la sección (viii/a), de preferencia precedida por la reacción de acuerdo con la sección (vii/a).

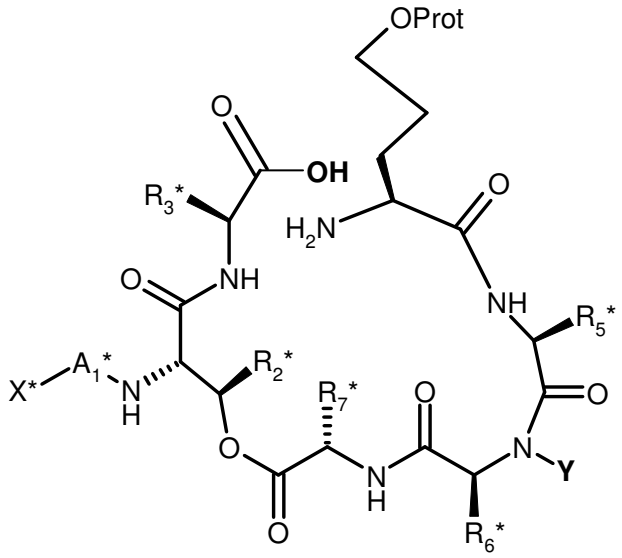
(i/b) Otra modalidad de la invención se refiere a un método o proceso anterior, el cual comprende, para la síntesis del compuesto de la fórmula II dada anteriormente, la ciclación bajo lactamización de un péptido precursor lineal, todavía no cíclico, del compuesto de la fórmula II, que lleva un grupo amino N-terminal y un grupo carboxilo C-terminal, en condiciones de reacción que permitan la formación de un enlace de amida a partir del grupo amino y el grupo carboxilo, de preferencia utilizando la química en fase de solución.

(ii/b) Una modalidad adicional de la invención se refiere al método o proceso de acuerdo con el párrafo precedente (i/b), en donde el péptido precursor lineal es de la fórmula XXV:



XXV

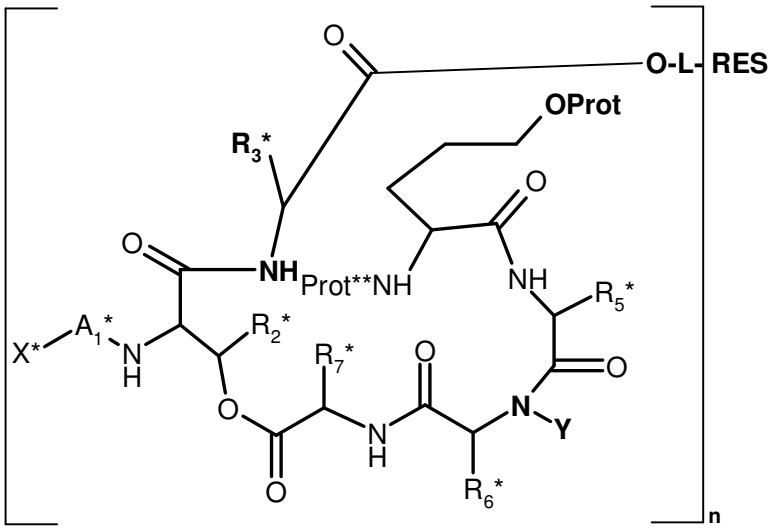
en especial XXVA:



XXVA

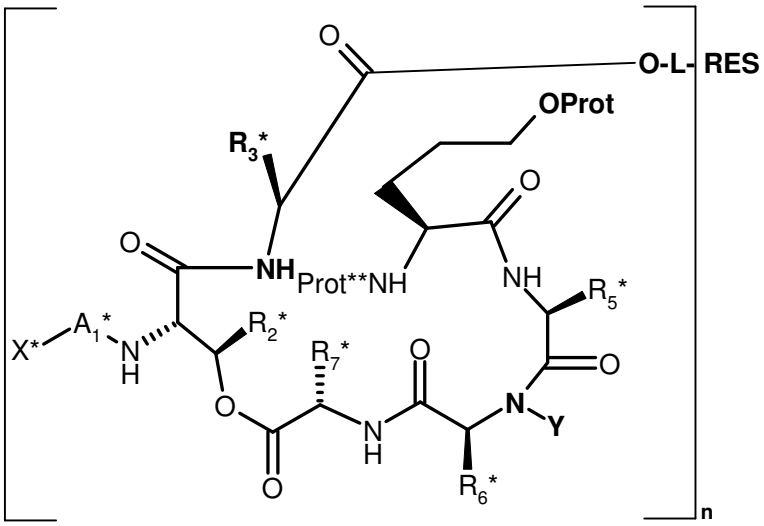
en donde X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* y Prot son como se definen para un compuesto de la fórmula II anterior.

(iii/b) Otra modalidad se refiere al método o proceso de acuerdo con el párrafo precedente (ii/b), el cual además comprende, para la síntesis del compuesto de la fórmula XXV, disociar un compuesto de la fórmula XXIV:



XXIV

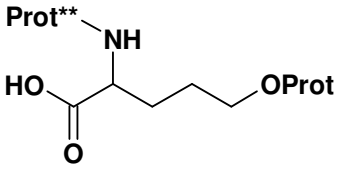
en especial XXIVA:



XXIVA

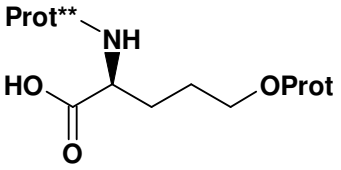
en donde X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* y Prot son como se definen para un compuesto de la fórmula II anterior, L es un enlazador disociable, RES es una resina sólida, n es un número natural, y Prot** es un grupo protector de amino que se puede remover sin la remoción paralela del grupo protector Prot y con el producto restante sobre la resina, y (antes de la disociación, en paralelo o subsiguientemente a la misma) remover el grupo protector Prot**, para proporcionar el compuesto de la fórmula XXV.

(iv/b) Una modalidad adicional de la invención se refiere al método o proceso de acuerdo con el párrafo precedente (iii/b), el cual además comprende, para la síntesis del compuesto de la fórmula XXIV, acoplar un aminoácido de la fórmula XIX:



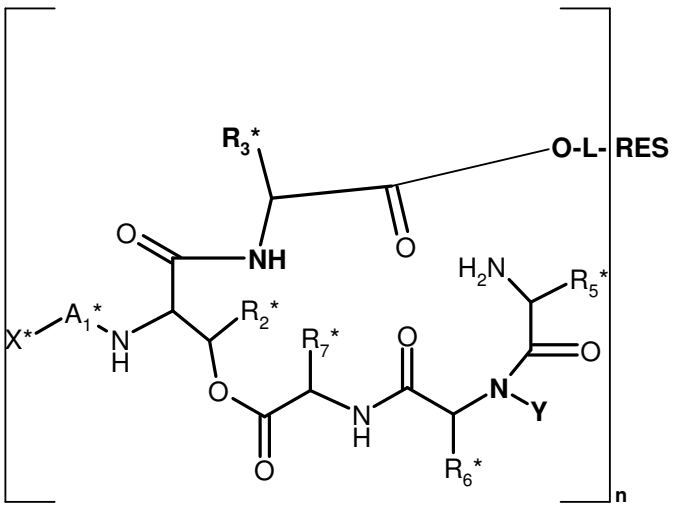
XIX,

en especial XIXA:



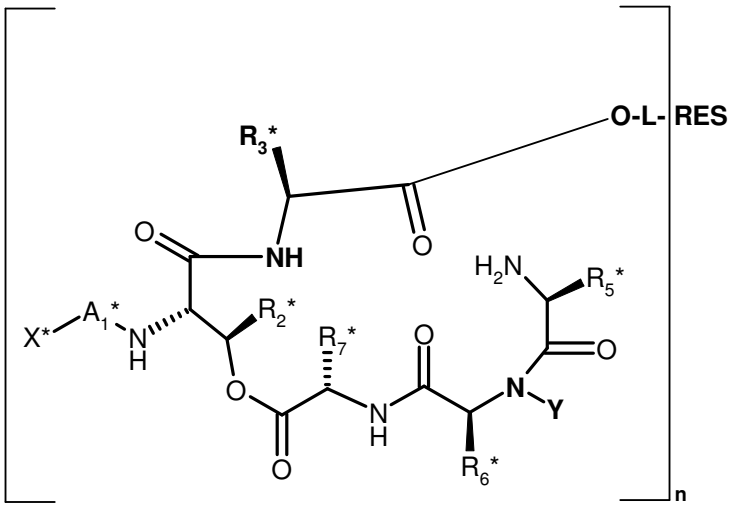
XIXA

en donde Prot es como se define para un compuesto de la fórmula II anterior, y Prot** es como se define para un compuesto de la fórmula XXIV anterior, o un derivado activado de este aminoácido, con un compuesto de la fórmula XXIII:



(XXIII)

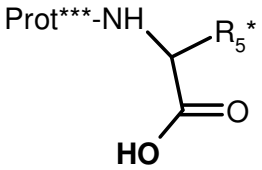
en especial XXIIIA:



XXIIIA

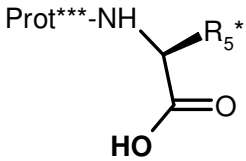
en donde X*, A_1*, R_2*, R_3*, R_5*, R_6* y R_7* son como se definen para un compuesto de la fórmula II anterior, L es un enlazador disociable, RES es una resina sólida, y n es un número natural.

(v/b) Todavía una modalidad adicional de la invención se refiere al método o proceso de acuerdo con el párrafo precedente (iv/b), el cual además comprende, para la síntesis del compuesto de la fórmula XXIII, acoplar un aminoácido de la fórmula XVII*:



XVII*,

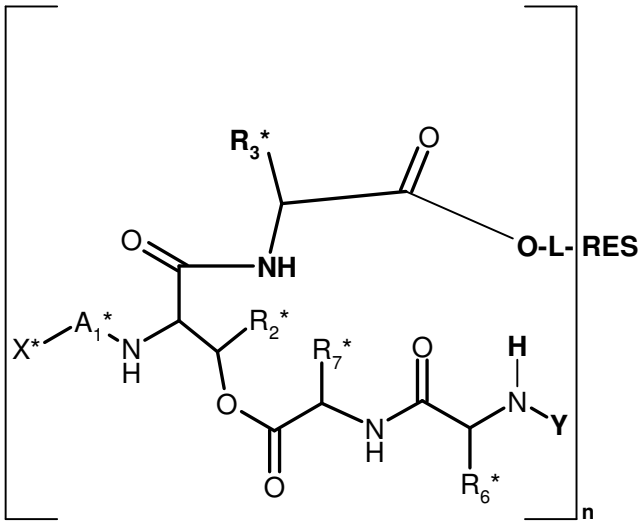
en especial XVIIA*:



XVIIA*

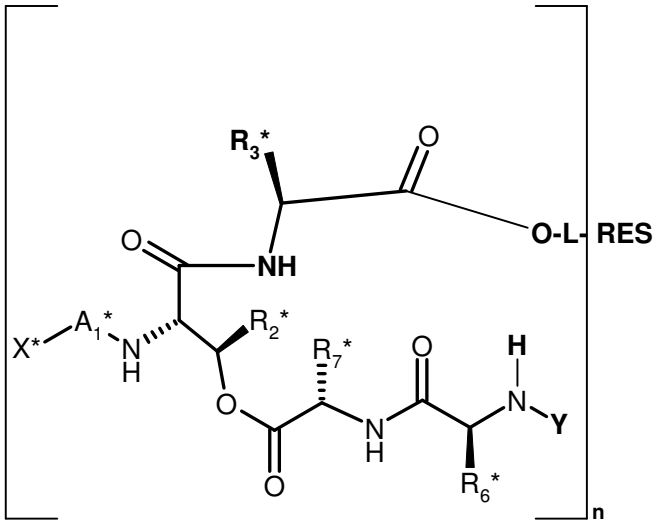
en donde R_5* es como se define para un compuesto de la

fórmula II anterior, y Prot*** es un grupo protector de amino que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina, o un derivado reactivo de este aminoácido, con un compuesto de la fórmula XXII:



XXII

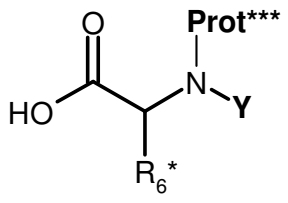
en especial XXIIA:



XXIIA

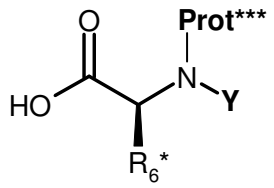
en donde X*, A_1*, R_2*, R_3*, R_6* y R_7* son como se definen para un compuesto de la fórmula II anterior, L es un enlazador disociable, RES es una resina sólida, y n es un número natural, y remover el grupo protector Prot***.

(vi/b) En todavía una modalidad adicional, la invención se refiere al método o proceso de acuerdo con el párrafo precedente (v/b), el cual además comprende, para la síntesis del compuesto de la fórmula XXII, acoplar un aminoácido de la fórmula XV*:



XV*

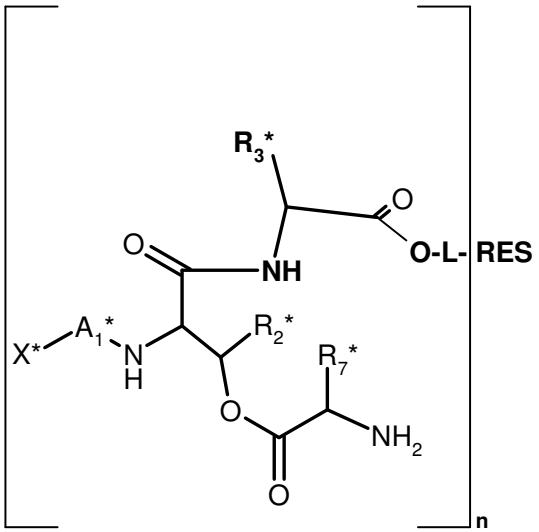
en especial XVA*:



XVA*

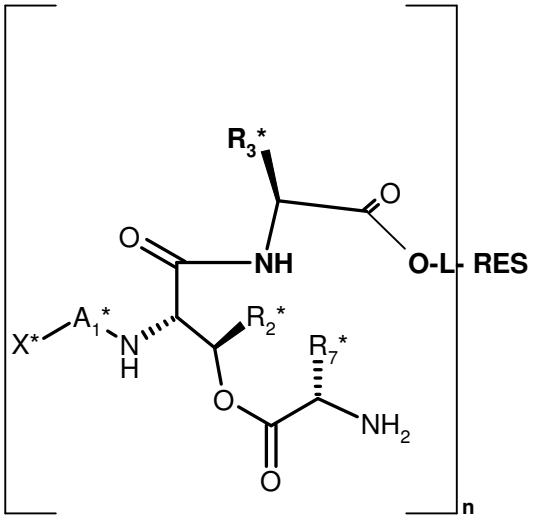
en donde R_6* e Y son como se definen para un compuesto de la fórmula II anterior, y Prot*** es un grupo protector de amino que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la

resina, o un derivado reactivo de este aminoácido, con un compuesto de la fórmula XXI:



XXI,

en especial XXIA:

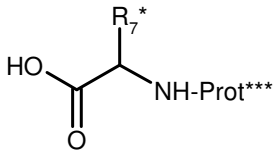


XXIA

en donde X*, A_1*, R_2*, R_3* y R_7* son como se definen

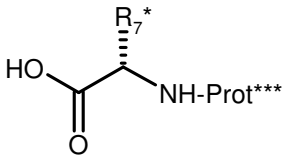
para un compuesto de la fórmula II anterior, L es un enlazador disociable, RES es una resina sólida, y n es un número natural, y remover el grupo protector Prot***.

(vii/b) Otra modalidad de la invención se refiere al método o proceso de acuerdo con el párrafo precedente (vi/b), el cual además comprende, para la síntesis de un compuesto de la fórmula XXI, hacer reaccionar un aminoácido de la fórmula XIII*:



XIII*

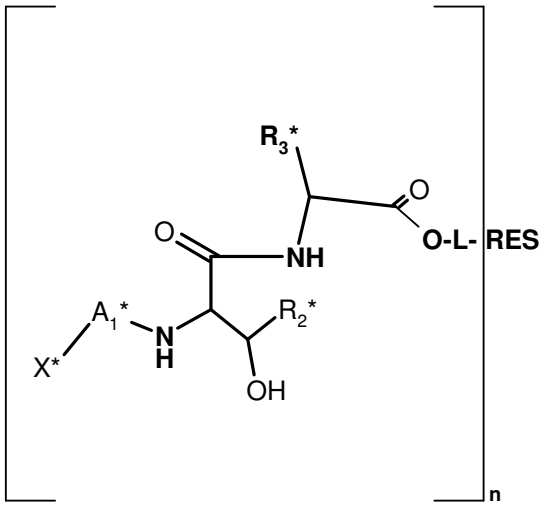
en especial XIIIA*:



XIIIA*

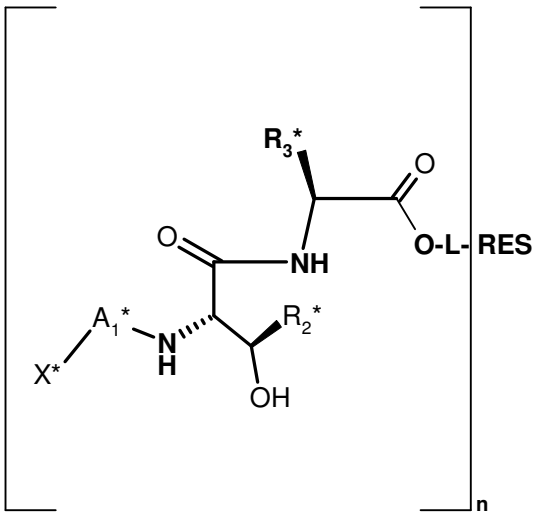
en donde Prot*** es un grupo protector de amino que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina, y R_7* es como se define para un compuesto de la fórmula II anterior, o un derivado reactivo de este aminoácido,

con el grupo hidroxilo de un compuesto de la fórmula XXVI:



XXVI

en especial XXVIA:



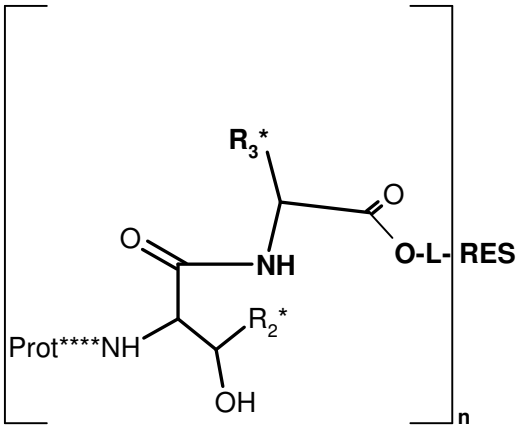
XXVIA

en donde X*, A_1*, R_2* y R_3* son como se definen para un compuesto de la fórmula II anterior, L es un enlazador disociable, RES es una resina sólida, y n es un número natural;

y remover el grupo protector Prot***.

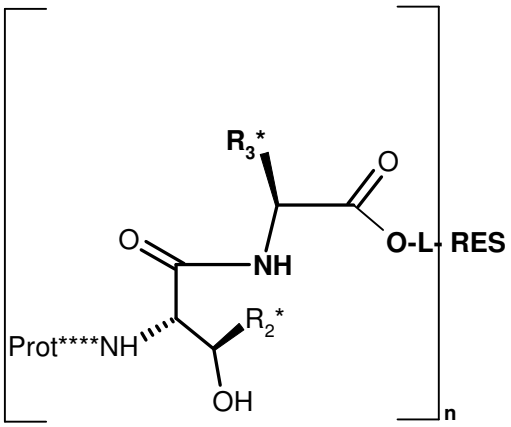
(viii/b) En una modalidad adicional, la invención se refiere al método o proceso de acuerdo con el párrafo precedente (vii/b), el cual además comprende, para la síntesis de un compuesto de la

fórmula XXVI, en especial XXVIA, acoplar un dipéptido enlazado con resina simbolizado por la fórmula XII*:



XII*

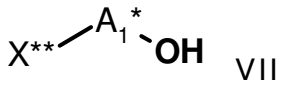
en especial XIIA*:



XIIA*

en donde Prot**** es un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes en un compuesto de la fórmula II como se define anteriormente, y con el producto restante sobre la resina, R_2* y R_3* son como se definen para un compuesto de la fórmula II anterior, L es un enlazador disociable, RES es una resina sólida, y n es un número natural, después de la remoción del grupo protector Prot****

por medio del grupo amino libre que se puede obtener de esta manera, con un ácido de la fórmula VII:

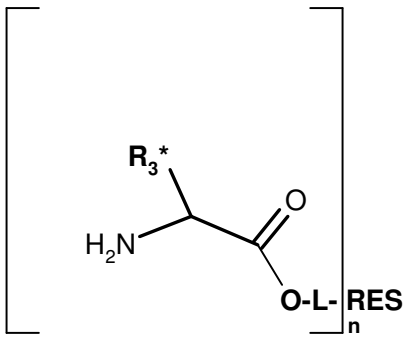


VII

en donde X** es un grupo protector de amino, o es X*, y en donde X* y A_1* son como se definen para un compuesto de la fórmula II anterior, o un derivado reactivo de dicho ácido;

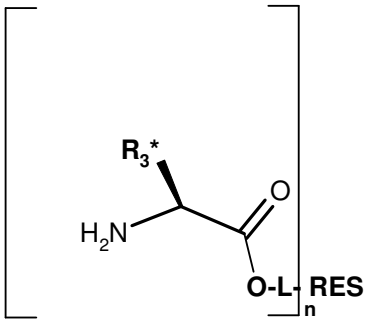
y, si X** es un grupo protector de amino, remover el grupo protector de amino X** para proporcionar el derivado de la fórmula II, en donde, en lugar de X*, está presente H, y acoplar el grupo amino resultante con un grupo acilo X* utilizando el ácido correspondiente X*-OH, en donde X* es como se define para un compuesto de la fórmula II anterior, o un derivado reactivo de dicho ácido.

(ix/b) Una modalidad todavía adicional de la invención se refiere al método o proceso de acuerdo con el párrafo precedente (viii/b), el cual además comprende, para la síntesis de un compuesto de la fórmula XII, acoplar un aminoácido enlazado con resina simbolizado por la fórmula X:



X

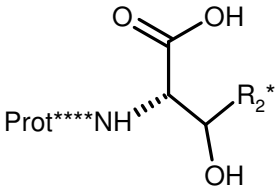
en especial XA:



XA

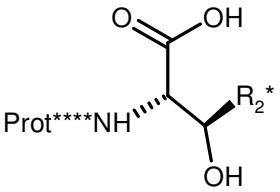
en donde R_3* es como se define para un compuesto de la fórmula II anterior, L es un enlazador disociable, RES es una resina sólida, y n es un número natural,

con un aminoácido de la fórmula XI*:



XI*,

en especial XIA*:

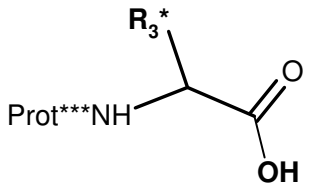


XIA*

en donde Prot**** es un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina, y R_2* es como se define para un compuesto de la fórmula II anterior, o un derivado reactivo de dicho aminoácido.

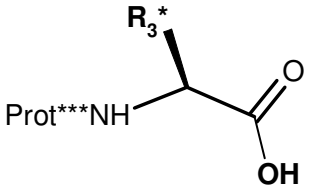
(x/b) Una modalidad adicional de la invención se refiere al

método o proceso de acuerdo con el párrafo precedente (ix/b), el cual además comprende, para obtener el aminoácido enlazado con resina de la fórmula X, acoplar un aminoácido de la fórmula IX*:



IX*

en especial IXA*:



IXA*

en donde R_3* es como se define para un compuesto de la fórmula II en la reivindicación 1, y Prot*** es un grupo protector de amino, se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina; o un derivado reactivo de dicho aminoácido de la fórmula IX, a un enlazador disociable L, el cual se enlaza a una resina sólida RES, y remover el grupo protector Prot***.

(i/c) Otra modalidad de la invención se refiere al método o proceso de acuerdo con cualquiera de los párrafos anteriores (i/a) a (x/b), en donde los símbolos A_1, R_2, R_3, R_5, R_6, R_7, X e Y, o las

fracciones no protegidas o protegidas correspondientes R_2*, R_3*, R_5*, R_6*, R_7*, X* e Y_3 se seleccionan de tal manera que, en el compuesto resultante de la fórmula I, o una sal del mismo:

A_1 es el radical bivalente de L-glutamina enlazada por medio del carbonilo de su grupo α-carboxilo con el grupo amino a la derecha de A_1 en la fórmula I, y por medio de su grupo α-amino con X, o es 2S-(2-hidroxi-3-fosfono-oxi)-propionilo;

R_2 es metilo;

R_3 es isopropilo, isobutilo (2-metil-n-propilo, dondequiera que se utilice) o bencilo, en especial isobutilo;

R_5 es butilo secundario o bencilo, en especial butilo secundario;

R_6 es 4-hidroxi-bencilo;

R_7 es isopropilo o butilo secundario (1-metil-n-propilo, dondequiera que se utilice), en especial butilo secundario;

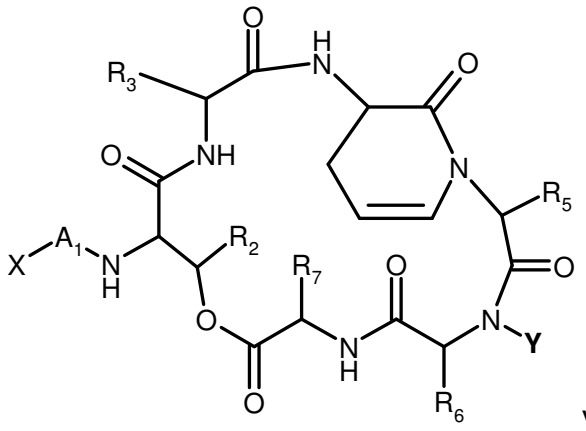
X es acetilo, o isobutirilo, o está ausente si A_1 es 2S-(2-hidroxi-3-fosfono-oxi)-propionilo;

e

Y es metilo. Este párrafo también se denomina como el párrafo a) más adelante.

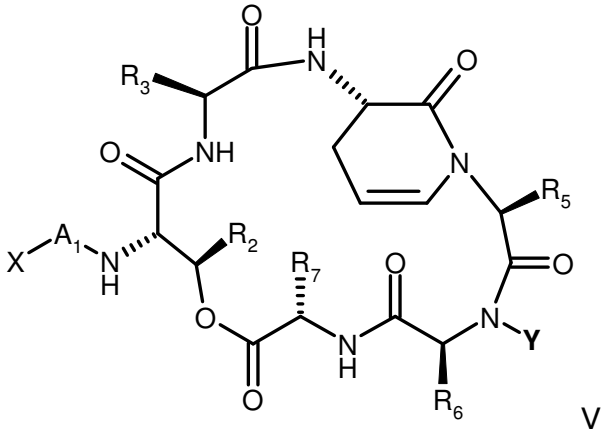
(i/d) En otra modalidad particular, la invención se refiere a un método o proceso para convertir un deshidrato de un compuesto de la fórmula I dado anteriormente, o en particular con los sustituyentes como se definen en el párrafo anterior (i/c) en el compuesto correspondiente de la fórmula I, en donde el deshidrato tiene la

fórmula V:



V,

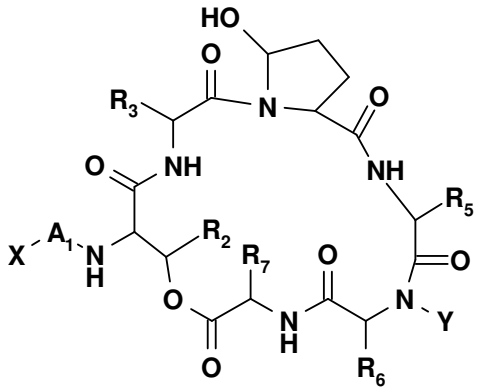
en especial VA:



VA

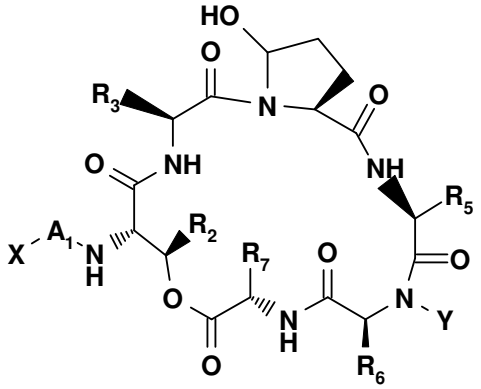
en donde Y, X, A_1, R_2, R_3, R_5, R_6 y R_7 son como se definen para un compuesto de la fórmula I anterior;

o en especial un método o proceso para cambiar el equilibrio de una mezcla de un compuesto de la fórmula I, y su deshidrato correspondiente, y/o su análogo de hemiaminal correspondiente con una estructura de cinco anillos en lugar de la estructura ahp en la fórmula I, que también se puede formar como un subproducto, y tiene la fórmula V*:



V*

en especial la fórmula VA*:



VA*,

en donde Y, X, A_1, R_2, R_3, R_5, R_6 y R_7 son como se definen para un compuesto de la fórmula I anterior, respectivamente;

en favor del compuesto de la fórmula I,

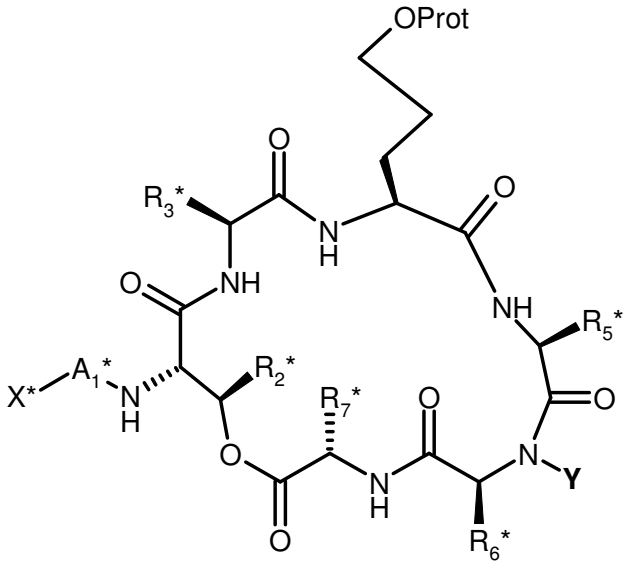
comprendiendo este método o proceso utilizar un ácido acuoso como solvente reactivo para impulsar la reacción. Este método se puede emplear independientemente (por ejemplo, también para el producto de la fermentación o biosíntesis), o en adición a los otros procesos o métodos descritos anteriormente y más adelante, para aumentar el rendimiento o para volver a convertir un compuesto de la fórmula V, en especial VA, y/o el análogo con un anillo de cinco miembros en lugar de la estructura ahp en la fórmula I, en el

compuesto correspondiente de la fórmula I.

El método descrito para la conversión del deshidrato y/o el análogo de cinco anillos (siempre con respecto al anillo de ahp deseado) en el compuesto deseado de la fórmula I, o en especial IA, por ejemplo, del deshidrato del Compuesto A a partir del Ejemplo 3 B en el Compuesto A, hace posible una síntesis directa de esta clase de compuestos. Hasta ahora, se tenía que circunvenir un tratamiento ácido como el paso final con el objeto de evitar la deshidratación del producto.

(i/e) Una modalidad adicional de la invención se refiere al método de acuerdo con el párrafo precedente (i/d), en donde el ácido es un ácido carboxílico, en especial un ácido alcanoico de 1 a 8 átomos de carbono sustituido por halógeno, más especialmente ácido trifluoro-acético o ácido tricloro-acético.

(i/f) La invención, en todavía una modalidad adicional, se refiere a un compuesto de la fórmula II:



II

en donde Prot es un grupo protector, Y es como se define para un compuesto de la fórmula I en la primera instancia anteriormente, o en particular como para la reivindicación 17, y X*, A_1*, R_2*, R_3*, R_5*, R_6*, y R_7* corresponden a X, A_1, R_2, R_3, R_5, R_6, y R_7 en la fórmula I como se definen en la reivindicación 1, o en el párrafo (ia) dado en lo anterior, respectivamente, sin embargo con la condición de que los grupos funcionales reactivos sobre estas fracciones están presentes en una forma protegida.

(i/g) En una modalidad adicional, la invención se refiere a un compuesto novedoso seleccionado a partir del grupo que consiste en los compuestos de las fórmulas II, III, IV, V, VI, VIII, X, XII, XIV, XVI, XVIII, XIX, XX, XXI, XXII, XXIII, XXIV, XXV, XXVI y XXVIII, y en especial de las fórmulas IIA, IIIA, IVA, VA, VIA, VIIIA, XA, XIIA, XIVA, XVIA, XVIIIA, XIXA, XXA, XXIA, XXIIA, XXIIIA, XXIVA, XXVA, XXVIA y XXVIIIA, todavía más especialmente al grupo que consiste en los siguientes compuestos dados en los Ejemplos: A partir del Esquema 1:, el compuesto 2, el compuesto 3, el compuesto 4, el sintón 1; a partir del Esquema 2: el compuesto 5; Fmoc-Leu-Enlazador-Resina de acuerdo con el Ejemplo 1B(2); Fmoc-Thr-Leu-Enlazador-Resina de acuerdo con el Ejemplo 1B(3); Fmoc-Gln(Trt)-Thr-Leu-Enlazador-Resina de acuerdo con el Ejemplo 1B(4); Isobutirilo-Gln(Trt)-Thr-Leu-Enlazador-Resina de acuerdo con el Ejemplo 1B(5); Isobutirilo-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Enlazador-Resina de acuerdo con el Ejemplo 1B(6); el producto del Ejemplo 1B(7) = Isobutirilo-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Enlazador-

Resina (anteriormente denominado como: Isobutirilo-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Fmoc)-Leu-Enlazador-Resina); Isobutirilo-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Enlazador-Resina (anteriormente denominado como: Isobutirilo-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Ile-Fmoc)-Leu-Enlazador-Resina) de acuerdo con el Ejemplo 1B(8); Isobutirilo-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Sintón 1-H)-Leu-Enlazador-Resina (anteriormente denominado como: Isobutirilo-Gln(Trt)-Thr(Ile-N-Me-Tyr(tBu)-Ile-Sintón 1-H)-Leu-Enlazador-Resina) de acuerdo con el Ejemplo 1B(9). Isobutirilo-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Sintón 1-H)-Leu-OH (anteriormente denominado como: Isobutirilo-Gln(Trt)-Thr(Ile-N-Me-Tyr(tBu)-Ile-Sintón 1-H)-Leu-OH) de acuerdo con el Ejemplo 1B(10), y del Esquema 3; H-Thr-Leu-Resina de acuerdo con el Ejemplo 1B(12); H-Gln(Trt)-Thr-Leu-Resina de acuerdo con el Ejemplo 1B(13); Isobutirilo-Gln(Trt)-Thr-Leu-Resina de acuerdo con el Ejemplo 1B(14); Isobutirilo-Gln(Trt)-Thr(Ile-H)-Leu-Resina de acuerdo con el Ejemplo 1B(15); Isobutirilo-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-Resina de acuerdo con el Ejemplo 1B(16); Isobutirilo-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resina de acuerdo con el Ejemplo 1B(17); Isobutirilo-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Sintón 1-H)-Leu-Resina de acuerdo con el Ejemplo 1B(18); a partir del Esquema 3, el compuesto 6 y/o 7 (éste último preferido); a partir del Esquema 4: El compuesto 8 y el isómero de hemiaminal de 5 anillos; a partir del Esquema 5, el péptido precursor 2, el compuesto 9, el compuesto 10 (siendo éste último el preferido en la presente enumeración con respecto al Esquema 5), y/o el compuesto 11;

Fmoc-Thr-Leu-Trt-Tentagel-S a partir del Ejemplo 2A(1); Fmoc-Gln(Trt)-Thr-Leu-Trt-Tentagel-S de acuerdo con el Ejemplo 2A(2); Ac-Gln(Trt)-Thr-Leu-Trt-Tentagel-S de acuerdo con el Ejemplo 2A(3); Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S de acuerdo con el Ejemplo 2A(4); Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Fmoc)-Leu-Trt-Tentagel-S (anteriormente denominado como: Ac-Gln(Trt)-Thr(Val-N-Me-Tyr(tBu)-Fmoc)-Leu-Trt-Tentagel-S) de acuerdo con el Ejemplo 2A(5); Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Fmoc)-Leu-Trt-Tentagel-S (anteriormente denominado como: Ac-Gln(Trt)-Thr(Val-N-Me-Tyr(tBu)-Phe-Fmoc)-Leu-Trt-Tentagel-S) de acuerdo con el Ejemplo 2A(6); y Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Sintón 1-H)-Leu-OH (anteriormente denominado como: Ac-Gln(Trt)-Thr(Val-N-Me-Tyr(tBu)-Phe-Sintón 1-H)-Leu-OH) (péptido precursor 2) de acuerdo con el Ejemplo 2A(7).

Las siguientes definiciones (o también las definiciones ya incluidas anteriormente) pueden reemplazar a los términos más generales utilizados en las modalidades de la invención mencionadas anteriormente y más adelante, con el objeto de definir modalidades adicionales de la invención, con cualquiera de uno, dos o más, o todos los términos generales que sean reemplazables por los términos más específicos, con el objeto de definir tales modalidades de la invención:

Una fracción bivalente de un aminoácido con un grupo carboxilo o carbamoílo terminal es de preferencia un aminoácido sustituido por alfa-carbamoílo o por carboxilo de 1 a 8 átomos de

carbono, en especial la fracción bivalente de asparagina o glutamina, y se enlaza en su lado derecho en la fórmula I por medio de un carbonilo (de preferencia el carbonilo de su grupo α-carboxilo) con el resto de la molécula.

Alcanoílo de 1 a 8 átomos de carbono o hidroxi-alcanoílo de 1 a 8 átomos de carbono fosforilado (alcanoílo de 1 a 8 átomos de carbono que lleva tanto un grupo hidroxilo como un grupo fosfono (-O-P(=O)-$(OH)_2$)) A_1 es, por ejemplo, 2,3-dihidroxi-propanoílo (de preferencia en la forma S) o 2-hidroxi-3-fosfono-propanoílo (de preferencia en la forma S).

R_2 y R_2* son alquilo de 1 a 8 átomos de carbono, en especial metilo, dondequiera que se mencionen.

R_3 es la cadena lateral de un aminoácido, en especial de un aminoácido natural. De preferencia, es alquilo de 1 a 8 átomos de carbono, el cual puede ser ramificado o lineal. Muy especialmente, alquilo de 1 a 8 átomos de carbono es n-(2-metil)-propilo (isobutilo), n-(1-metil-propilo (butilo secundario) o metilo, es decir, el aminoácido que lleva la fracción es leucina, isoleucina o valina.

R_3* es la cadena lateral correspondiente en una forma protegida si está presente un grupo funcional que se tenga que impedir que participe en una reacción. De preferencia, es alquilo de 1 a 8 átomos de carbono, el cual puede ser ramificado o lineal, en especial como se define en el párrafo anterior.

Una “cadena lateral de un aminoácido” se puede seleccionar a partir de cualquier fracción, por ejemplo, una fracción orgánica

mono- o poli-cíclica, lineal, saturada, insaturada (por ejemplo, con dobles enlaces conjugados) o parcialmente saturada, por ejemplo, con hasta 20 átomos de carbono, y con 0 a 5 heteroátomos en la estructura base independientemente seleccionados a partir de N, O y S, reemplazando al número de átomos de carbono correspondiente, y puede estar sustituida por hasta tres fracciones seleccionadas a partir de amino, imino, hidroxilo, carboxilo, carbamoílo, sulfhidrilo, amidino, guanidino, O-fosfono (-O-P(=O)-$(OH)_2$). De preferencia, las cadenas laterales se seleccionan a partir de aquéllas de los 20 alfa-aminoácidos estándares de arginina, histidina, lisina, ácido aspártico, ácido glutámico, serina, treonina, asparagina, glutamina, cisteína, glicina, alanina, leucina, isoleucina, metionina, fenilalanina, triptófano, tirosina, valina y además prolina (entonces con ciclación interna, incluyendo el grupo alfa-amino).

Para los aminoácidos, se utiliza cualquiera de sus nombres o los códigos de tres letras acostumbrados en la presente divulgación, de acuerdo con la siguiente tabla:

Aminoácido	**Código de tres letras**
Alanina	Ala
Arginina	Arg
Asparagina	Asn
Ácido aspártico	Asp

Aminoácido	Código de tres letras
Asparagina o ácido aspártico	Asx
Cisteína	Cys
Ácido glutámico	Glu
Glutamina	Gln
Glutamina o ácido glutámico	Glx
Glicina	Gly
Histidina	His
isoleucina	Ile
Leucina	Leu
Lisina	Lys
Metionina	Met
Fenilalanina	Phe
Prolina	Pro
Serina	Ser
Treonina	Thr
Triptófano	Try

Aminoácido	Código de tres letras
Tirosina	Tyr
Valina	Val

R_5 es la cadena lateral de un aminoácido, de preferencia un aminoácido estándar. De preferencia, es alquilo de 1 a 8 átomos de carbono, el cual puede ser ramificado o lineal, y que está insustituido o sustituido por fenilo. Más especialmente es bencilo, n-(2-metil)-propilo, isobutilo o metilo, es decir, el aminoácido que lleva la fracción es fenilalanina, leucina, isoleucina o valina.

R_6 es la cadena lateral de un hidroxi-aminoácido, en especial de tirosina.

R_7 es la cadena lateral de un aminoácido, en especial de un aminoácido natural. De preferencia, es alquilo de 1 a 8 átomos de carbono, el cual puede ser ramificado o lineal. Más especialmente es n-(2-metil)-propilo (isobutilo), n-(1-metil)-propilo (butilo secundario) o metilo, es decir, el aminoácido que lleva la fracción es leucina, isoleucina o valina.

Alquilo de 1 a 8 átomos de carbono puede ser lineal o ramificado una o más veces; por ejemplo, puede ser n-(2-metil)-propilo, n-(1-metil)-propilo o metilo.

Todos los compuestos, en donde estén presentes grupos formadores de sales, tales como grupos básicos, por ejemplo, amino,

o imino, o grupos ácidos, por ejemplo, carboxilo o hidroxilo fenólico, se pueden utilizar en forma libre o como sales, o como mezclas de las sales y las formas libres. Por consiguiente, en siempre que se mencione un compuesto, esto incluye todas estas variantes. Por ejemplo, los grupos básicos pueden formar sales con ácidos, tales como ácidos halohídricos, por ejemplo, HCl, ácido sulfúrico, o ácidos orgánicos, tales como ácido acético, mientras que los grupos ácidos pueden formar sales con iones positivos, por ejemplo, amonio, alquil-amonio, o cationes de sales de metales alcalinos o alcalinotérreos, por ejemplo, cationes de Ca, Mg, Na, K o Li, o similares.

“O similares" o "y similares”, dondequiera que se utilicen en esta divulgación, se refieren al hecho de que la persona experta en la materia conoce otras alternativas para aquéllas mencionadas antes de esa expresión, y se pueden agregar a las expresiones específicamente mencionadas; en otras modalidades, “o similares" e "y similares” se pueden suprimir en una o más o en todas las modalidades de la invención.

Los grupos protectores Prot, Prot*, Prot**, Prot***, Prot**** y cualesquiera grupos protectores adicionales presentes sobre las fracciones A*, R_2*, R_3*, R_5*, R_6*, R_7*, X*, dondequiera que se mencionen a través de toda la presente descripción y de las reivindicaciones, se seleccionan de tal manera que permitan la protección ortogonal.

La protección ortogonal es una estrategia que permite la desprotección de múltiples grupos protectores, uno (o más pero no

todos) a la vez, donde se desee, cada uno con un conjunto dedicado de condiciones de reacción, sin afectar a los otros grupos protectores o a los enlaces con las resinas, por ejemplo, por medio de enlazadores sobre resinas de síntesis sólida. En otros términos: La estrategia utiliza diferentes clases de grupos protectores que se remueven mediante diferentes mecanismos químicos, también utilizando los enlazadores apropiados en el caso de la síntesis de péptidos en fase sólida (en donde el enlace del enlazador-resina se podrían considerar juntos como un grupo protector de carboxilo).

De preferencia, los grupos protectores se seleccionan como sigue:

El grupo protector Prot se selecciona de preferencia para resistir la remoción de cualesquiera otros grupos protectores utilizados o presentes durante la síntesis de un depsipéptido de acuerdo con la invención, por ejemplo, capaz de resistir las bases suaves (véase Prot*), pero removible con ion de fluoruro (en especial bajo condiciones anhidras), por ejemplo, $Bu_4N^+F^-$ (también si se crea *in situ*, por ejemplo, utilizando $Bu_4N^+Cl^-$ con KF • H_2O, KF con 18-corona-6, LiBr con 18-corona-6, BF_3 • dietil-éter, piridina-HF, HF en urea, $Et_3N(HF)_3$ (en donde Et es etilo) o similares, en donde el solvente se selecciona, por ejemplo, a partir del grupo que consiste en N,N-dimetil-formamida, acetonitrilo, cloroformo y tetrahidrofurano.

De preferencia, Prot es un grupo protector de éter, en especial seleccionado a partir del grupo que consiste en los grupos protectores de sililo, en donde la fracción de sililo lleva hasta tres

fracciones orgánicas enlazadas por medio de un carbono (opcionalmente por medio de un átomo de Si adicional), tal como terbutil-difenil-sililo, trimetil-sililo, tri-isopropil-sililo, terbutil-dimetil-sililo, trifenil-sililo, difenil-metil-sililo, tri-terbutil-dimetil-sililo, terbutil-metoxi-fenil-sililo, tris-(trimetil-silil)-sililo, o similares.

Prot* es un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y también sin afectar al enlace de éster formador del depsipéptido o un enlazador con una resina RES, y es estable durante los pasos de desprotección durante la síntesis del péptido precursor lineal (por ejemplo, la remoción del aliloxi-carbonilo); es de preferencia un grupo protector removible mediante complejos de trifenil-fosfina específicos en la presencia de hidruros de metales u otros reductores, por ejemplo, $(PH_3P)_4Pd$, de preferencia en combinación con hidruro de di-n-butil-estaño o hidruro de tri-n-butil-estaño, fenil-silano, borohidruro de sodio o dimedona, en un solvente apropiado, por ejemplo, tetrahidrofurano, y de preferencia no es disociable bajo condiciones que permitan la remoción de un grupo protector Prot**; por ejemplo, y Prot* se selecciona a partir del grupo que consiste en fracciones de alqu-2-eniloxilo de 3 a 8 átomos de carbono-carbonilo, por ejemplo, aliloxi-carbonilo (Alloc), 1-isopropil-aliloxi-carbonilo, 4-nitrocinamiloxi-carbonilo y 3-(3'-piridil)-prop-2-eniloxi-carbonilo.

Prot** es un grupo protector que se puede remover de la resina sin disociar otros enlaces (ninguna disociación de un aminoácido o péptido enlazado por medio del carbonilo, en especial de su grupo α-

carboxilo con el enlace por medio de un enlazador L mencionado más adelante; también sin disociar el grupo protector Prot una vez presente), en especial un grupo protector removible sin disociación de un enlace de éster (en lugar de un amida) en un depsipéptido o precursor de depsipéptido, y bajo condiciones diferentes de aquéllas para los grupos protectores Prot* y Prot, mientras que se conserva el enlace por medio del enlazador con una resina RES en donde esté presente; es de preferencia removible mediante una base suave, por ejemplo, piperidina, morfolina, diciclohexil-amina, p-dimetil-amino-piridina, di-isopropil-amina, piperazina, tris-(2-amino-etil)-amina, en un solvente apropiado, por ejemplo, N,N-dimetil-formamida, cloruro de metileno; Prot**, por ejemplo, se selecciona a partir del grupo que consiste en fluoren-9-il-metoxi-carbonilo (Fmoc); 2-(2' ó 4'-piridil)-etoxi-carbonilo y 2,2-bis-(4'-nitro-fenil)-etoxi-carbonilo.

Prot *** (un grupo protector de amino que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina), y Prot**** (un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes en un compuesto de la fórmula II como se define anteriormente y más adelante, y con el producto restante sobre la resina) son de preferencia los grupos protectores que se pueden remover como Prot** y, por ejemplo, se seleccionan a partir de aquéllos mencionados para R**, por ejemplo, fluoren-9-il-metoxi-carbonilo (Fmoc).

El método de síntesis ortogonal preferido en este caso hace

uso del método de Fmoc conocido en general para la síntesis de péptidos, utilizando síntesis de péptidos en fase sólida combinada con macrolactamización en fase de solución, y otras conversiones químicas.

De una manera alternativa, por ejemplo, el grupo protector de Boc se podría utilizar en lugar de Prot**, Prot*** y Prot**** de Fmoc.

Sin embargo, esto requerirá de diferentes grupos protectores de cadena lateral, y también el grupo hidroxilo de N-metilo-Tyr se tendrá que proteger entonces de una manera diferente para mantener la ortogonalidad de los grupos de protección.

Otros grupos protectores presentes, así como el enlace enlazador con una resina RES en donde esté presente, de preferencia no son removibles bajo condiciones bajo las cuales se puedan remover Prot* y Prot**, por ejemplo, en A*, la amida se puede N-proteger, por ejemplo, con tritilo (trifenil-metilo) (disociación, por ejemplo, con ácido trifluoro-acético (TFA); en R_6*, un hidroxilo de tirosina se puede proteger como terbutil-éter, o se puede proteger mediante terbutil-dimetil-sililo, metoxi-metilo, Boc (terbutoxi-carbonilo), o acetato de arilo (disociación con ácido trifluoro-acético (TFA)).

Los grupos protectores apropiados son conocidos en la materia, así como los métodos para su introducción y remoción. Por ejemplo, los grupos protectores, y sus métodos de introducción y remoción, se pueden seleccionar a partir de aquéllos descritos en los libros de texto convencionales, tales como “Protective Groups in

Organic Synthesis", 3ª Edición, T.W. Green y P.G.M. Wuts (Editores). J. Wiley & Sons, Inc., Nueva York, etc., 1999.

Los grupos protectores Prot, Prot*, Prot**, Prot***, Prot**** y otros grupos protectores, por consiguiente, no están limitados a aquéllos mencionados anteriormente – más bien, deben satisfacer condiciones que los hagan apropiados para la protección ortogonal, por ejemplo, como se describe anteriormente o más adelante.

Se recomienda evitar las condiciones demasiado básicas (aunque usualmente son permisibles las bases descritas para la disociación de Fmoc, tales como piperidina) para evitar la disociación del enlace (de éster) del depsipéptido.

Entre los posibles soportes sólidos para la síntesis de péptidos en fase sólida (SPPS), se pueden mencionar los siguientes:

- Soportes tipo gel sin o con espaciador: Éstos son polímeros altamente solvatados con una distribución igual de los grupos funcionales. Este tipo de soporte es el más común, e incluye:

Poliestireno: Estireno reticulado, por ejemplo, con el 1 al 2 por ciento de divinil-benceno; Poliacrilamida o polimetacrilamida: como alternativa hidrofílica al poliestireno; Polietilenglicol (PEG): El PEG-Poliestireno (PEG-PS) es más estable que el poliestireno y separa el sitio de la síntesis de la estructura base del polímero; Soportes basados en PEG: Compuestos de una red de PEG-polipropilenglicol o PEG con poliamida o poliestireno (éstos ya incluyen un espaciador, PEG);

- Soportes de tipo superficial: Los materiales desarrollados

para la funcionalización superficial, incluyendo vidrio de poros controlados, fibras de celulosa, y poliestireno altamente reticulado.

- Compuestos: Polímeros tipo gel soportados por matrices rígidas.

Usualmente estos geles llevan grupos reactivos con los que se puede enlazar un enlazador L como se menciona para diferentes precursores en lo anterior y más adelante. Por ejemplo, estos grupos incluyen grupos amino-metilo, grupos polietilenglicol con un hidroxilo terminal, y similares.

Cualquiera de estos soportes se puede utilizar en las modalidades de la presente invención.

Los soportes tipo gel se utilizan en otra modalidad especial de la invención; entre éstos, se prefieren en especial poliestireno (divinil-benceno reticulado); y resinas de poliacrilamida y poli-metacrilamida.

Entre los posibles enlazadores, se pueden utilizar todos aquéllos comúnmente conocidos y apropiados.

Los ejemplos en las posibles modalidades de la invención son el enlazador de alcohol 2-metoxi-4-benciloxi-bencílico (un Enlazador de Sasrina; Sasrina representa una resina sensible al súper-ácido, y se enlaza a los aminoácidos o péptidos por medio de OH alcohólico); la familia de enlazadores de tritilo (por ejemplo, Tritilo, 2Cl-Tritilo, que se enlazan con los aminoácidos o péptidos por medio de OH); el enlazador de 4-(2,4-dimetoxi-fenil-hidroxi-metil)-fenoxi-metilo (Rink-Ácido-Enlazador, se enlaza a los aminoácidos o péptidos por medio

de OH); o enlazadores de tris-(alcoxi)-bencil-éster (Enlazador de HAL, se enlaza a los aminoácidos o péptidos por medio de OH).

Cuando se mencionan los derivados de ácidos reactivos, en especial los aminoácidos o péptidos, por ejemplo, dipéptidos, éstos se pueden formar *in situ* o se pueden utilizar como tales.

Los derivados reactivos (o activos) utilizados como tales incluyen los haluros de acilo, por ejemplo, cloruros, fluoruros o nitro-fenil-ésteres de acilo, por ejemplo, los 2,4-dinitro-fenil-ésteres, o anhídridos de ácidos (simétricos o, por ejemplo, con ácido acético) de los grupos carboxilo de los ácidos que se vayan a hacer reaccionar.

Para la activación de aminoácidos *in situ*, se pueden aplicar los agentes de acoplamiento acostumbrados. Estos reactivos son conocidos por la persona experta en la materia, y se pueden adquirir de una manera conveniente con muchos proveedores, por ejemplo, Aldrich ChemFiles – Peptide Synthesis (Aldrich Chemical Co., Inc., Sigma-Aldrich Corporation, Milwaukee, WI, EUA) Volumen 7, Número 2, 2007 (véase http://www.sigmaaldrich.com/etc/medialib/docs/Aldrich/Brochure/al_chemfile_v7_n2.Par.0001.File.tmp/al_chemfile_v7_n2.pdf). Entre los posibles agentes de acoplamiento para la síntesis de enlaces de amida y éster, se pueden mencionar los siguientes:

Triazoles, uronio, o derivados de hexafluoro-fosfonio, por ejemplo, 1-hidroxi-benzotriazol (HOBt), 1-hidroxi-7-aza-benzotriazol (HOAt), 2-ciano-2-(hidroxi-imino)-acetato de etilo, hexafluoro-fosfato

de 2-(1H-7-azabenzotriazol-1-il)-1,1,3,3-tetrametil-uronio (metanaminio) (HATU), hexafluorofosfato de benzotriazol-1-il-oxi-tri-pirrolidino-fosfonio (PyBOP), hexafluorofosfato de benzotriazol-1-il-oxi-tris-(dimetil-amino)-fosfonio (BOP), 1-(mesitilen-2-sulfonil)-3-nitro-1,2,4-triazol (MSNT), hexafluorofosfato de 2-(1H-benzotriazol-1-il)-1,1,3,3-tetrametil-uronio (HBTU), hexafluoroborato de 2-(1H-benzotriazol-1-il)-1,1,3,3-tetrametil-uronio (TBTU), tetrafluoroborato de 2-succinimido-1,1,3,3-tetrametil-uronio (TSTU), tetrafluoroborato de 2-(5-norbornen-2,3-dicarboximido)-1,1,3,3-tetrametil-uronio (TNTU), tetrafluoroborato de O-[(ciano-(etoxi-carbonil)-metiliden)-amino]-1,1,3,3-tetrametil-uronio (TOTU), hexafluorofosfato de O-(benzotriazol-1-il)-1,3-dimetil-1,3-dimetilen-uronio (HBMDU), hexafluorofosfato de O-(benzotriazol-1-il)-1,1,3,3-bis-(tetrametilen)-uronio (HBPyU), hexafluorofosfato de O-(benzotriazol-1-il)-1,1,3,3-bis-(pentametilen)-uronio (HBPipU), 3-hidroxi-4-oxo-3,4-dihidro-1,2,3-benzotriazina (HODhbt), 1-hidroxi-7-azabenzotriazol y sus sales de uronio o fosfonio correspondientes, designadas como HAPyU y AOP, hexafluorofosfato de 1-ciano-2-etoxi-2-oxo-etiliden-amino-oxi-dimetil-amino-morfolino-carbenio (COMU), hexafluoro-fosfato de cloro-tripirrolidino-fosfonio (PyCloP), o similares;

Carbodi-imidas, por ejemplo, diciclohexil-carbodi-imida, N-(3-dimetil-amino-propil)-N'-etil-carbodi-imida, 1-terbutil-3-etil-carbodi-imida, N-ciclohexil-N'-2-morfolino-etil)-carbodi-imida o di-isopropil-carbodi-imida (en especial para la formación de éster por medio de la formación de O-acil-urea del grupo carboxílico); o

agentes formadores de éster activos, por ejemplo, 2-mercapto-benzotiazol (2-MBT),

agentes formadores de azida, por ejemplo, difenil-fosforil-azida,

anhídridos de ácidos, tales como anhídrido de ácido propan-fosfónico,

agentes de halogenación de ácidos, por ejemplo, 1-cloro-N,N,2-trimetil-1-propenil-amina, tetrafluoroborato o hexafluorofosfato de cloro-N,N,N',N'-bis-(tetrametilen)-formamidinio, hexafluorofosfato de cloro-N,N,N',N'-tetrametil-formamidinio, hexafluorofosfato de fluoro-N,N,N',N'-tetrametil-formamidinio, hexafluorofosfato de fluoro-N,N,N',N'-bis-(tetrametilen)-formamidinio,

o similares, o las mezclas de dos o más de estos agentes.

También, para el acoplamiento con éster de los compuestos de la fórmula XII o XIIA con aquéllos de la fórmula XIII o XIIIA, respectivamente, o de los compuestos de la fórmula XIII* o XIIIA* con aquéllos de la fórmula XXVI o XXVIA, respectivamente, se pueden utilizar los compuestos de carboxilo reactivos correspondientes o se pueden formar *in situ*. Aquí, se prefiere en especial MSNT como el agente de acoplamiento, debido a que éste permite el mantenimiento de una alta estereoespecificidad.

La reacción, donde sea apropiado, se puede conducir en la presencia de una base suave (por ejemplo, N-metil-morfolina, una trialquil-amina, por ejemplo, etil-di-isopropil-amina, una di-(alquil)-amino-piridina, tal como N,N-dimetil-amino-piridina, o similares,

(teniendo cuidado de que las condiciones no sean tan básicas que permitan la hidrólisis de los grupos éster, por ejemplo, el grupo éster del depsipéptido, presentes en los precursores del compuesto de la fórmula I), donde sea apropiado o se requiera, en la presencia de un solvente o mezcla de solventes apropiados, por ejemplo, una N,N-di-alquil-formamida, tal como dimetil-formamida, un hidrocarburo halogenado, por ejemplo, dicloro-metano, N-alquil-pirrolidonas, tales como N-metil-pirrolidona, nitrilos, por ejemplo, acetonitrilo, o además un hidrocarburo aromático, por ejemplo, tolueno, o las mezclas de dos o más, en donde, en el entendido de que esté presente un exceso del agente de acoplamiento, también puede haber agua presente. Las temperaturas pueden ser la temperatura ambiente o más baja o más alta, por ejemplo, en el intervalo de -20°C a 50°C.

Los aminoácidos de las fórmulas IX, IXA, XI, XIA, XIII, XIIIA, XV, XVA, XVII, XVIIA, XXVII (que se pueden obtener por ejemplo, mediante síntesis en fase de solución), XVII*, XVIIA*, XV*, XVA*, XIII*, XIIIA*, XI*, XIA*, IX* y IXA*, son conocidos o se pueden sintetizar de acuerdo con los métodos conocidos en la técnica, están comercialmente disponibles, y/o se pueden sintetizar en analogía a los métodos conocidos en la técnica.

También, los materiales de partida restantes, por ejemplo, el ácido de la fórmula XIX o VII, o el dipéptido de la fórmula XXVII o XXVIIA, son conocidos o se pueden sintetizar de acuerdo con los métodos conocidos en la técnica, están comercialmente disponibles, y/o se pueden sintetizar en analogía a métodos conocidos en la

técnica.

Por ejemplo, el sintón de la fórmula XIX se puede preparar como se describe en el Ejemplo 1 A(4) (el cual es una modalidad específica de la invención) o en analogía al mismo. La síntesis del compuesto intermediario 1 (Esquema 1) se describe en Tetrahedron 61, 1459-1480 (2005).

Las reacciones de acoplamiento para dipéptidos hacen uso de los grupos carboxílicos de los aminoácidos correspondientes en forma libre o en forma activada.

EJEMPLOS

Los siguientes ejemplos ilustran la invención sin limitar su alcance.

Abreviaturas

aq.	acuoso
Boc/BOC	terbutoxi-carbonilo
salmuera	solución de cloruro de sodio en agua (saturada a temperatura ambiente)
Bzl	bencilo
COMU	hexafluorofosfato de 1-ciano-2-etoxi-2-oxo-etiliden-amino-oxi-dimetil-amino-morfolino-carbenio
DCM	dicloro-metano
DIPEA	N,N-di-isopropil-etil-amina
DMAP	4-dimetil-amino-piridina
DMF	N,N-dimetil-formamida

Fmoc/FMOC	9-fluorenil-metoxi-carbonilo
Et	etilo
HATU	hexafluorofosfato de 2-(1H-7-aza-benzotriazol-1-il)-1,1,3,3-tetrametil-uronio (Metanaminio)
HFIP	Hexafluoro-isopropanol
HPLC	Cromatografía de líquidos de alto rendimiento
HR-MS	Espectroscopía de Masas de Alta Resolución
IPA	Acetato de isopropilo
IPC	Control En Proceso
IR	Espectroscopía infrarroja
IT	temperatura interna
Prueba de Kaiser	Prueba basada en ninhidrina para monitorear la desprotección en SPPS (véase E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, Analytical Biochemistry **1970**, *34* 595); si se menciona como OK, esto significa la desprotección de éxito
me	metilo
MS	Espectroscopía de Masas
MSNT	1-(mesitilen-2-sulfonil)-3-nitro-1,2,4-triazol

RMN	Espectroscopía de resonancia magnética nuclear
PyBOP	hexafluorofosfato de benzotriazol-1-il-oxi-tripirrolidino-fosfonio
BOP	hexafluorofosfato de benzotriazol-1-il-oxi-tris-(dimetil-amino)-fosfonio
RP	Fase inversa
RT/rt	temperatura ambiente
SPPS	síntesis de péptidos en fase sólida
TBME	terbutil-metil-éter
TFA	ácido trifluoro-acético
TEMPO	2,2,6,6-tetrametil-1-piperidiniloxilo, radical libre
RBF	matraz de fondo redondo

Para las abreviaturas de aminoácidos véase la tabla anterior.

Nombres de compuestos...

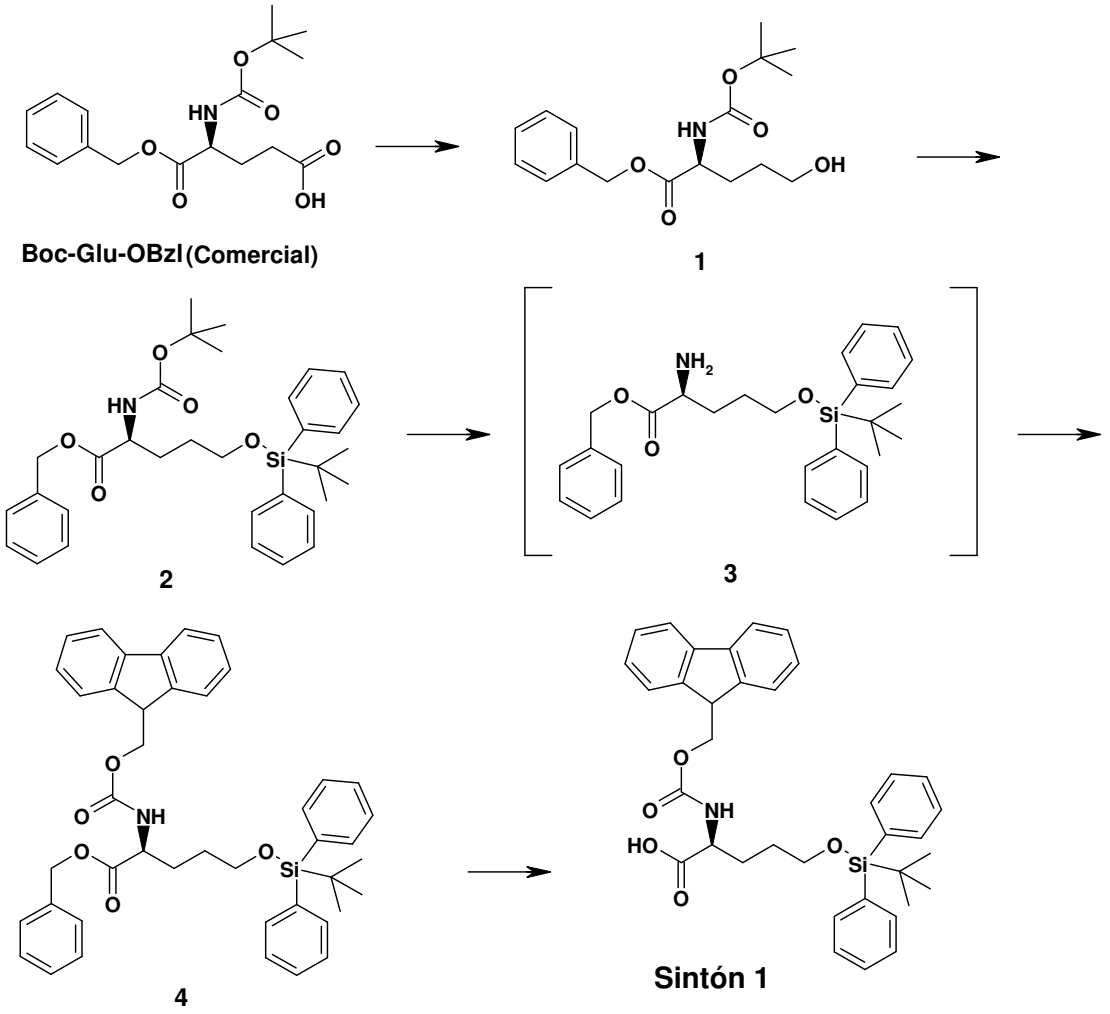
Los nombres de los oligopéptidos de cadena abierta se han derivado de acuerdo con las recomendaciones de la Comisión Conjunta sobre Nomenclatura Bioquímica (Joint Commission on Biochemical Nomenclature) (“International Union of Pure and Applied Chemistry" e "International Union of Biochemistry”) publicados en Pure & Appl. Chem. 1984, *56*, 595-624. Previamente, se utilizó la convención de nombramiento simple de péptidos para estos compuestos. Los nombres anteriores se dan entre paréntesis.

Ejemplo 1: Síntesis del compuesto A

1A Síntesis de sintón 1

(i) Alternativo 1:

Esquema de Reacción 1:



1A(1) Preparación del compuesto 1

2-((terbutoxi-carbonil)-amino)-5-hidroxipentanoato de (S)-bencilo

El compuesto 1 se preparó mediante un procedimiento similar

al procedimiento reportado en R. K. Olson, K. Ramasamy, T. Emery, *J. Org. Chem.* **1984**, *49*, 3527. BOC-Glu-OBzl (50 gramos, 148.2 milimoles) se disolvió en tetrahidrofurano (800 mililitros), y se le agregó trietil-amina (47.3 gramos, 467.4 milimoles). La solución se enfrió IT= -10°C. Se le agregó lentamente cloroformato de etilo (51.8 gramos, 98 por ciento de pureza, 467.8 milimoles), manteniendo la temperatura a IT = -10 a -15°C. La suspensión obtenida de esta manera se agitó durante una hora adicional. La IPC (HPLC) indicó la desaparición del material de partida. La mezcla de reacción se dejó calentar hasta 0°C y se le agregó agua (800 mililitros) de 0 a 5°C dentro de 25 a 30 minutos. Se hizo visible la separación de la mezcla en 2 fases. Se le agregó bromohidruro de sodio (11.8 gramos, 299.4 milimoles), en 10 porciones, bajo agitación intensiva, de 0 a 5°C. Se tuvo precaución debido a que se desprendió gas de hidrógeno durante la adición de bromohidruro de sodio. La mezcla de reacción se agitó durante 5 minutos adicionales de 0 a 5°C, y la temperatura se incrementó de 20 a 25°C dentro de 30 minutos. Se requirió precaución debido a que se continuó desprendiendo gas de hidrógeno mientras que se incrementaba la temperatura. La mezcla de reacción se agitó durante 15 minutos de 20 a 25°C antes del procesamiento.

Para el procesamiento, se agregó agua (1250 mililitros) a la mezcla de reacción, seguida por la adición de acetato de etilo (1250 mililitros). Las fases se separaron y la fase acuosa se extrajo con acetato de etilo (600 mililitros). Las capas orgánicas se combinaron y

se lavaron con salmuera (600 mililitros, 2 veces). La capa orgánica se secó sobre sulfato de magnesio, el solvente se evaporó a presión reducida y de 40 a 45°C para obtener 50.8 gramos producto crudo. Pureza de HPLC: 94 a%. El producto crudo se purificó mediante cromatografía por evaporación instantánea sobre gel de sílice con fracción de hexano/acetato de etilo (7:3 a 1:1) como la fase móvil.

Rendimiento: 40.85 gramos (85.2 por ciento). Pureza: 98 por ciento (HPLC). La MS y la RMN confirmaron la estructura propuesta.

1A(2) Preparación del compuesto 2: 2-((terbutoxi-carbonil)-amino)5-((terbutil-difenil-silil)-oxi)pentanoato de (S)-bencilo

El alcohol (el compuesto **1**) a partir del paso previo (40.3 gramos, 124.6 milimoles) se disolvió en N,N-dimetil-formamida (200 mililitros), y se le agregó imidazol (12.8 gramos, 99.5 por ciento de pureza, 186.9 milimoles). La mezcla se agitó a temperatura ambiente, hasta que se formó una solución (5 a 10 minutos). Se agregó por goteo terbutil-difenil-silil-cloruro (41.9 gramos, 98 por ciento de pureza, 149.5 milimoles), dentro de 10 minutos y se continuó la agitación durante 15 minutos adicionales a temperatura ambiente. La IPC (HPLC) indicó la desaparición del material de partida (alcohol).

Para el procesamiento, se le agregó acetato de isopropilo (400 mililitros) a la mezcla de reacción, seguido por la adición lenta de una solución acuosa saturada de medio-bicarbonato de sodio (400 mililitros). Se tuvo precaución debido a que la adición fue

exotérmica, y tuvo lugar la evolución del gas. Las fases se separaron y la fase acuosa se extrajo con acetato de isopropilo (400 mililitros). Las capas orgánicas se combinaron y se extrajeron con agua (400 mililitros). La capa orgánica se secó sobre sulfato de magnesio y el solvente se evaporó de 40 a 45°C bajo presión reducida. El residuo se secó durante la noche al vacío a 25°C para obtener 83.25 gramos el producto crudo como un aceite incoloro. El análisis de HPLC indicó la presencia de 81 a% del producto deseado y 18.6 a% de la silanola correspondiente. El producto crudo se utilizó sin mayor purificación para el siguiente paso. La RMN y la HR-MS de una muestra purificada confirmaron la estructura propuesta. HR-MS: calculada para $C_{33}H_{43}NO_5Si$: $[M+H]^+$: 562.29833; $[M+NH_4]^+$: 579.32488; $[M+Na]^+$: 584.28027. Encontrado: $[M+H]^+$: 562.29848; $[M+NH_4]^+$: 579.32489; $[M+Na]^+$: 584.27995.

1A(3) Preparación del compuesto 4:

2-((((9H-fluoren-9-il)-metoxi)-carbonil)-amino)-5-((terbutil-difenil-silil)-oxi)-pentanoato de (S)-bencilo

83.25 gramos el producto crudo a partir del paso previo (que corresponde a 70 gramos rendimiento teórico del compuesto **2**, 124.6 milimoles) se disolvió en dicloro-metano (650 mililitros), y se agregó por goteo ácido trifluoro-acético (358.8 gramos, 99 por ciento de pureza, 3115 milimoles) a la solución agitada intensivamente. La IPC (HPLC) después de 30 minutos indicó la disociación completa del grupo protector BOC. La mezcla de reacción (solución clara) se

transfirió en un matraz 4 de fondo redondo de 6 litros con agitador mecánico y se diluyó con 1000 mililitros de dicloro-metano. Se le agregó lentamente solución acuosa medio-saturada de carbonato de sodio (1800 mililitros) a la solución agitada intensivamente. Se tuvo precaución debido a que se observó evolución de gas fuerte durante la adición de carbonato de sodio. El pH de la fase acuosa después de completarse la adición: 9 a 10. La mezcla de reacción se agitó durante 15 minutos adicionales y las fases se separaron. La fase acuosa se extrajo con dicloro-metano (1000 mililitros), y las capas orgánicas se combinaron para obtener una solución del producto en dicloro-metano. La solución se concentró de 40 a 45°C bajo presión reducida hasta un volumen final de aproximadamente 650 mililitros. Para el siguiente paso. La HPLC indicó la presencia de 80.5 por ciento del compuesto **3** y 19.5 a% de silanola en la solución.

Para la FMOCilación, se agregó lentamente solución saturada acuosa de bicarbonato de sodio (650 mililitros) a la solución agitada intensivamente del compuesto **3**, seguido por la adición de cloruro de FMOC (36.55 gramos, 97 por ciento de pureza, 137 milimoles). Se tuvo precaución debido a que se observó evolución del gas durante la adición de FMOC-Cl. Se continuó la agitación durante 15 minutos a temperatura ambiente. Una IPC (HPLC) de la capa orgánica indicó la desaparición del Intermediario el compuesto 3 y la conversión completa en el compuesto 4. Para el procesamiento, las capas se separaron y la fase acuosa se extrajo con dicloro-metano (650 mililitros). Las capas orgánicas se combinaron y se extrajeron con

agua (650 mililitros). La capa orgánica se secó sobre sulfato de magnesio y el solvente se removió de 40 a 45°C bajo presión reducida, para obtener 112.6 gramos del producto crudo.

El producto crudo se suspendió en etanol/isopropanol/agua (89:5:6; 2400 mililitros), y la suspensión se calentó a IT 50°C para obtener una solución. La solución se enfrió 45°C, se le agregaron cristales de siembra y la temperatura se continuó hasta enfriar de 20 a 25°C dentro de 1 hora. La cristalización empezó a aproximadamente 40°C. La suspensión se agitó de 20 a 25°C durante la noche, entonces se enfrió a IT 0 a 5°C dentro de 30 minutos y se continuó la agitación durante 2 horas adicionales de 0 a 5°C. El producto se aisló mediante filtración, la torta del filtro se lavó con etanol/isopropanol/agua (89:5:6; 240 mililitros), y se secó bajo presión reducida, para obtener el compuesto **4** puro (100 a% de pureza de acuerdo con la HPLC). Rendimiento: 66 gramos (77.4 por ciento). El producto se caracterizó completamente mediante MS y RMN HR-MS: Calculada para $C_{43}H_{45}NO_5Si$: $[M+H]^+$: 684.31398; $[M+NaH_4]^+$: 701.34053; $[M+Na]^+$: 706.29592. Encontrado: $[M+H]^+$: 684.31392; $[M+NH_4]^+$: 701.34021; $[M+Na]^+$: 706.29539. El licor madre dio 30.6 gramos de una espuma, la cual comprende 30 a% de producto y 29 a% de silanola de acuerdo con la HPLC.

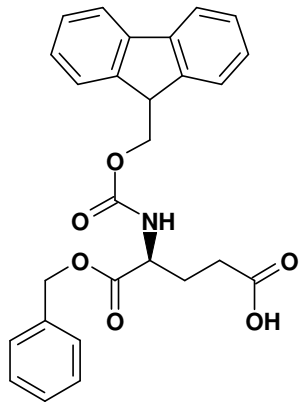
1A(4) Preparación de sintón 1 a partir del compuesto 4:

Ácido (S)-2-((((9H-Fluoren-9-il)-metoxi)-carbonil)-amino)-5-((terbutil-difenil-silil)-oxi)-pentanoico

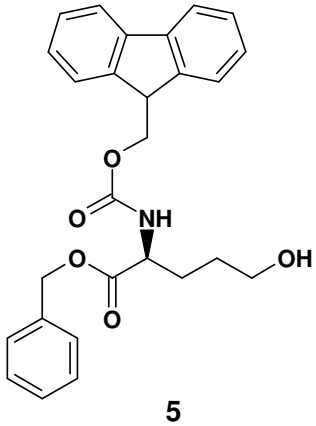
El compuesto **4** (66 gramos, 96.6 milimoles) se suspendió en etanol/alcohol isopropílico/agua (89:5:6; 3000 mililitros), y la suspensión se calentó a IT 45°C para obtener una solución. La solución se enfrió a IT 30°C. Después de hacer inerte con argón, se le agregó catalizador de paladio (10 por ciento sobre sulfato de bario; 6.6 gramos), a la solución bajo una corriente de argón. Entonces, el producto se hidrogenó bajo una presión de hidrógeno ligeramente superior a la presión atmosférica de 30 a 35°C. La hidrogenación se terminó después de 1.5 horas de acuerdo con la HPLC. La mezcla de reacción se filtró sobre un auxiliar de filtro basado en celulosa (Cellflock 40; auxiliar de filtro basado en celulosa), y auxiliar de filtro se lavó con etanol/isopropanol/agua (89:5:6; 600 mililitros). La evaporación del solvente bajo presión reducida de 45 a 50°C dio 59.58 gramos de espuma como producto crudo. El producto crudo se purificó mediante cromatografía sobre gel de sílice en 2 porciones (1 kilogramo de gel de sílice 60, 2 veces) utilizando dicloro-metano/metanol, 95:5 a 80:20 como fase móvil. Rendimiento: 52 gramos (90.6 por ciento). El producto se caracterizó totalmente mediante MS y RMN. HR-MS: Calculada para $C_{36}H_{39}NO_5Si$ $[M+H]^+$: 594.26703; $[M+NH_4]^+$: 611.29358; $[M+Na]^+$: 616.24897; Encontrado: $[M+H]^+$: 594.26743; $[M+NH_4]^+$: 611.29385; $[M+Na]^+$: 616.24900.

(ii) Alternativo 2:

Esquema de Reacción 2:



Fmoc-Glu-OBzl (Comercial)



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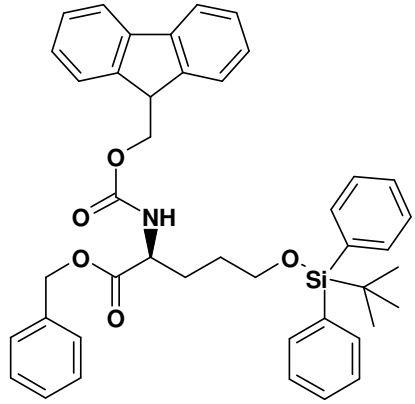
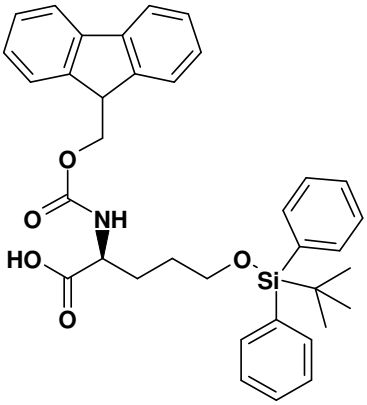


4



Sintón 1

1A(5) Preparación en un solo recipiente del compuesto 4 a partir de Fmoc-Glu-OBzl a partir del compuesto 5:

Fmoc-Glu-OBzl (5 gramos, 10.88 milimoles) se disolvió en tetrahidrofurano (80 mililitros), y se le agregó trietil-amina (3.3 gramos, 32.6 milimoles). La solución se enfrió -15°C y se le agregó cloro-formato de etilo (7.3 gramos, 67.27 milimoles) a la solución durante 30 minutos a -12°C. La suspensión obtenida de esta manera se agitó durante una hora adicional de -10 a -15°C, y la temperatura se elevó hasta 0°C. Se le agregó por goteo agua (80 mililitros) a la

mezcla de reacción, manteniendo la temperatura a 0°C. Se le agregó borohidruro de sodio (total 0.805 gramos, 21.27 milimoles), en 3 porciones (1 porción cada 10 minutos) a 0°C, y la mezcla de reacción se agitó durante 1 hora adicional a 0°C. Se tuvo precaución debido a que se desprendió gas de hidrógeno. La mezcla de reacción se diluyó con agua (100 mililitros), y se extrajo con acetato de isopropilo (150 mililitros), y las capas se separaron. La fase acuosa se extrajo otra vez con acetato de isopropilo (100 mililitros), y las capas orgánicas se combinaron. La fase orgánica combinada se lavó con solución medio-saturada de cloruro de sodio (50 mililitros, 2 veces), y la solución se concentró a presión reducida hasta un volumen final de aproximadamente 50 mililitros. La solución concentrada se filtró hasta aclararse y el residuo de filtro se lavó con acetato de isopropilo (20 mililitros). La solución del compuesto **5** obtenido de esta manera se transfirió en un matraz de fondo redondo y se le agregó imidazol (1.49 gramos, 21.89 milimoles). La mezcla se agitó durante 15 minutos a temperatura ambiente, y se le agregó terbutil-difenil-silil-cloruro (4.45 gramos, 16.19 milimoles). La mezcla de reacción se agitó durante 15 horas a temperatura ambiente. Para el procesamiento, la suspensión se diluyó con acetato de isopropilo (20 mililitros), y se extrajo con agua (50 mililitros, 3 veces). La capa orgánica se separó y el solvente se evaporó bajo presión reducida, para obtener 9.95 gramos del producto crudo. El producto crudo se purificó mediante cromatografía en columna sobre gel de sílice con isopropil-acetato/hexano (2:8) como fase móvil para obtener 6.5

gramos de un sólido, el cual se suspendió en hexano y se agitó durante 3 horas a temperatura ambiente. El precipitado se aisló mediante filtración y se secó a 50°C bajo presión reducida, para obtener 5.6 gramos del compuesto **4**. Rendimiento: 75 por ciento durante los dos pasos. HR-MS: calculada para $C_{43}H_{45}NO_5Si$: $[M+H]^+$: 684.31398; $[M+NH_4]^+$: 701.34053; $[M+Na]^+$: 706.29592. Encontrado: $[M+H]^+$: 684.31430; $[M+NH_4]^+$: 701.34073; $[M+Na]^+$: 706.29577.

1A(6) Preparación de un solo recipiente alternativa del compuesto 4 a partir de Fmoc-Glu-OBzl mediante compuesto 5 utilizando cloroformato de isopropilo en lugar de cloroformato de etilo:

Fmoc-Glu-OBzl (60 gramos, 130.579 milimoles) se disolvió en tetrahidrofurano (550 mililitros), y se le agregó trietil-amina (40.8 gramos, 403.202 milimoles). se obtuvo una solución nublada con algunos precipitados. La solución nublada/suspensión se transfirió en un embudo de goteo y se agregó a una solución previamente enfriada de cloroformato de isobutilo (54.96 gramos, 402.41 milimoles) en tetrahidrofurano (300 mililitros) en un reactor de 4.5 litros de -35 a -30°C, manteniendo esta temperatura durante la adición. Se lavaron los residuos en el embudo de goteo con tetrahidrofurano adicional (50 mililitros), y la mezcla de reacción se agitó de -35 a -30°C durante otras 2 horas. Se le agregó agua (960 mililitros) a la mezcla de reacción dentro de 45 minutos, permitiendo aumentar a la temperatura hasta 0°C. Se formó una suspensión. Se le agregó en 20 porciones borohidruro de sodio (14.4 gramos,

380.625 milimoles), dentro de 1 hora a 0°C, y la mezcla de reacción se agitó durante una hora adicional a 0°C. Se tuvo precaución debido a que se desprendió gas de hidrógeno.

La suspensión se vertió sobre metil-éter de terbutilo (600 mililitros), y el matraz de la reacción se lavó con agua (600 mililitros), la cual se le agregó al producto mezcla (2 fases). Las fases se separaron, la fase de agua se extrajo con el metil-éter de terbutilo (600 mililitros), y las fases orgánicas se combinaron. La fase orgánica se lavó con agua (600 mililitros, 2 veces), se secó sobre sulfato de magnesio anhidro (200 gramos), y el solvente se removió bajo presión reducida hasta que se logró un volumen final de 1 litro. La solución se diluyó con dimetil-formamida (600 gramos), y el solvente se evaporó bajo presión reducida, hasta que se logró un volumen final de 400 mililitros. La solución del compuesto **5** obtenido de esta manera se transfirió en un matraz de fondo redondo. Se agregó imidazol (14.4 gramos, 211.524 milimoles), a la solución de dimetil-formamida del compuesto **5** y la mezcla se agitó durante 5 minutos a temperatura ambiente. Finalmente, se le agregó por goteo TBDPS-Cl (39.6 gramos, 144.07 milimoles), durante 20 minutos de 20 a 25°C, y la mezcla de reacción se agitó durante una hora adicional a esta temperatura.

La mezcla de reacción se vertió entonces sobre acetato de etilo (1200 mililitros), y la mezcla se extrajo con agua (700 mililitros). Las capas se separaron, y la capa orgánica se lavó con agua (300 mililitros, 3 veces). La evaporación del solvente bajo presión

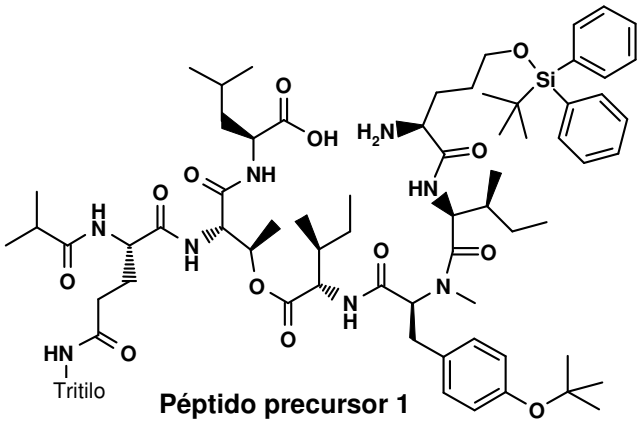
reducida dio 106 gramos del producto crudo.

El producto crudo (106 gramos) se disolvió en etanol/Isopropanol/agua (89:5:6; 1200 mililitros) de 40 a 50°C y se le agregaron los cristales de siembra (0.5 gramos el compuesto **4**). La mezcla se dejó enfriar a temperatura ambiente, y se agitó durante 17 horas a temperatura ambiente. La suspensión se enfrió a -20°C, y se agitó durante 2 horas a -20°C. El producto se aisló mediante filtración, la torta del filtro se lavó con la mezcla de solvente de etanol/isopropanol/agua (89:5:6; 200 mililitros, 3 veces), y se secó a 40°C bajo presión reducida, para obtener 66.5 gramos del compuesto **4** (74.6 por ciento de rendimiento durante los 2 pasos). La HPLC indicó >99 a% de pureza para el producto.

El producto adicional se puede aislar a partir del licor madre (34 gramos después de la evaporación del solvente), que contenga aproximadamente 30 a% del compuesto **4** de acuerdo con la HPLC.

1B Síntesis de péptido precursor 1 mediante SPPS

Péptido precursor 1: Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Sintón-1-H)-Leu-OH



Péptido precursor 1

Se ha preparado el péptido precursor 1 utilizando 2 Soportes Sólidos diferentes

Equipo:

El reactor de síntesis de fase sólida con una tela de filtro o placa de filtro de vidrio sinterizado al fondo. Un tubo colector de nitrógeno que permite drenar los contenidos del reactor mediante la tela de filtro o placa de filtro de vidrio sinterizado y válvula de fondo.

1B(1) El acoplamiento del tritil-enlazador al soporte sólido:

200 gramos de resina de amino-metil-poliestireno (reticulado con 1 por ciento de divinil-benceno, cargado de grupos amino-metilo de 1 milimol/gramo) (proveedor: Senn Chemicals AG, Dielsdorf/ Suiza) se agitaron alternativamente con varias porciones de dimetil-formamida (1600 mililitros) e isopropanol (1600 mililitros) después de dos lavados finales con dimetil-formamida, la resina se trató con una solución preparada anteriormente de ácido 4-hidroxi-difenil-metil-benzoico (91.3 gramos 300 milimoles), monohidrato de 1-hidroxi-benzotriazol (45.9 gramos, 300 milimoles), y di-isopropil-carbodi-imida (75.7 gramos, 600 milimoles) en N,N-dimetil-formamida (1600 mililitros). La mezcla de reacción se agitó durante 1.5 horas y se llevó a cabo una prueba de ninhidrina. La prueba todavía mostró grupos amino libres y, por consiguiente, se le agregó di-isopropil-carbodi-imida (7.6 gramos, 60 milimoles), y la reacción se agitó durante la noche. Una prueba de ninhidrina adicional por la mañana fue negativa y la mezcla de reacción se filtró. La resina se lavó con dimetil-formamida e isopropanol alternativamente. La resina se secó

al vacío y se redimensionó a 257 gramos de resina enlazadora seca. El material se utilizó para el siguiente paso de síntesis sin mayor análisis.

1B(2) El acoplamiento de Fmoc-Leu-OH

Preparación de Fmoc-Leu-Enlazador-Resina

Se hinchó resina enlazadora (190 gramos, 147.8 milimoles) mediante agitación en tolueno (1400 mililitros). El solvente se filtró y se remplazó por una solución de tolueno (1400 mililitros), y cloruro de acetilo (53 mililitros, 1478 milimoles). Esta mezcla se agitó durante 2 horas, se filtró, se remplazó por una mezcla idéntica, la cual se agitó durante otras 2 horas antes de la filtración. La resina clorada se lavó dos veces con tolueno y tres veces con dicloro-metano.

En un matraz de fondo redondo, se preparó una solución de Fmoc-Leu-OH (104.8 gramos, 296 milimoles), y de N-metil-morfolina (49 mililitros, 444 milimoles) en dicloro-metano (600 mililitros). Esta solución se agregó a la resina y se agitó durante la noche. Por la mañana, la solución se filtró, y la resina se lavó con dicloro-metano e isopropanol alternativamente. La resina se secó al vacío y se redimensionó a 234.7 gramos de Fmoc-Leu-Enlazador-Resina seca. Se determinó la carga con grupos Fmoc a 0.787 milimoles/gramo lo que llevó a proporcionar 185 milimoles (125 por ciento en teoría). El análisis de aminoácido en el contratista externo confirmó <0.1 por ciento de enantiómero D-Leu.

1B(3) El acoplamiento de Fmoc-Thr-OH

Preparación de Fmoc-Thr-Leu-Enlazador-Resina

Se hinchó la Fmoc-Leu-Enlazador-Resina (140 gramos, 109 milimoles) mediante agitación en dos porciones sucesivas de dimetil-formamida (1100 mililitros) durante 30 minutos cada una.

El grupo protector Fmoc se disoció por dos lavados subsiguientes de 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetilfomamida e isopropanol. Se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa confirmó la remoción satisfactoria de piperidina.

La resina se lavó con tetrahidrofurano (1200 mililitros) tres veces para preparar para el paso de acoplamiento siguiente.

En un matraz de fondo redondo se preparó una solución de Fmoc-Thr-OH (112.1 gramos, 328 milimoles), monohidrato de hidroxi-benzotriazol (51.25 gramos, 334 milimoles), y di-isopropil-carbodi-imida (51 mililitros, 655 milimoles) en tetrahidrofurano (600 mililitros).

La solución se le agregó a la resina y el pH se revisó inmediatamente (pH= 6.5). La mezcla de reacción se agitó durante 1.5 horas hasta que una muestra de ninhidrina mostró la reacción completa. La solución se filtró y la resina se lavó con dimetil-formamida e isopropanol, alternativamente. Una muestra pequeña de la resina se secó y se mandó por un análisis de aminoácido (0.13 por

ciento de D-Leu, <0.1 por ciento de D-Thr, <0.1 por ciento de L-allo-Thr, <0.1 por ciento de D-allo-Thr), la mayor parte del material se sometió al siguiente paso sin más secado.

1B(4) El acoplamiento de Fmoc-Gln(Trt)-OH

Preparación de Fmoc-Gln(Trt)-Thr-Leu-Enlazador-Resina

La Fmoc-Thr-Leu-Enlazador-Resina a partir del paso previo se hinchó mediante agitación en dos porciones subsiguientes de dimetil-formamida (1100 mililitros) durante 30 minutos cada una.

El grupo protector Fmoc se disoció por dos lavados subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetilfomamida e isopropanol. Se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa demostró la remoción satisfactoria de piperidina.

La resina se lavó con dimetil-formamida (1100 mililitros) tres veces para preparar para el paso de acoplamiento siguiente.

En un matraz de fondo redondo se preparó una solución de Fmoc-Gln(Trt)-OH (138.6 gramos, 226 milimoles), HATU (86.2 gramos, 226 milimoles), y etil-di-isopropil-amina (58.4 gramos, 452 milimoles) en N,N-dimetil-formamida (400 mililitros).

La solución se le agregó a la resina y el pH se revisó inmediatamente (pH= 10). La mezcla de reacción se agitó durante 3 horas hasta que una muestra de ninhidrina mostró la reacción

completa. La solución se filtró y la resina se lavó con dimetil-formamida e isopropanol, alternativamente.

La resina se secó al vacío y se redimensionó a 170 gramos de Fmoc-Gln(Trt)-Thr-Leu-Enlazador-Resina seca. La carga con grupos Fmoc se determinó a 0.60 milimoles/gramo indicando un rendimiento del 102 milimoles (94 por ciento en teoría durante los dos últimos pasos). El análisis de aminoácido en un contratista externo condujo a los siguientes valores: (0.13 por ciento de D-Leu, <0.1 por ciento de D-Thr, <0.1 por ciento de L-allo-Thr, <0.1 por ciento de D-allo-Thr, <0.8 por ciento de D-Gln).

1B(5) El acoplamiento del ácido isobutírico

Preparación de Isobutiril-Gln(Trt)-Thr-Leu-Enlazador-Resina

La Fmoc-Gln(Trt)-Thr-Leu-Enlazador-Resina (169 gramos, 101 milimoles) se hinchó mediante agitación en dos porciones subsiguientes de dimetil-formamida (1300 mililitros) durante 30 minutos cada una.

El grupo protector Fmoc se disoció por dos lavados subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida (1300 mililitros) durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetilfomamida e isopropanol. Se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa demostró la remoción satisfactoria de piperidina.

La resina se lavó con dimetil-formamida (1100 mililitros) tres veces para preparar el paso de acoplamiento siguiente.

En un matraz de fondo redondo se preparó una solución de ácido isobutírico (17.9 gramos, 203 milimoles), PyBOP (105.5 gramos, 203 milimoles), y etil-di-isopropil-amina (52.4 gramos, 406 milimoles) en N,N-dimetil-formamida (550 mililitros).

La solución se le agregó a la resina y el pH se revisó inmediatamente (pH= 9.5). La mezcla de reacción se agitó durante 2.5 horas hasta que una muestra de ninhidrina mostró la reacción completa. La solución se filtró y la resina se lavó con dimetil-formamida e isopropanol, alternativamente.

El lote se sometió directamente al siguiente paso sin sequedad ni análisis adicional.

1B(6) El acoplamiento de Fmoc-Ile-OH (Esterificación) Preparación de Isobutiril-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Enlazador-Resina

La Isobutiril-Gln(Trt)-Thr-Leu-Enlazador-Resina (húmeda a partir del paso anterior, 101 milimoles) se hinchó mediante agitación en tres porciones subsiguientes de dicloro-metano (1200 mililitros) durante 20 minutos cada una.

El solvente se filtró y se le agregaron MSNT (88 gramos, 297 milimoles), y Fmoc-Ile-OH (105 gramos, 297 milimoles) como sólidos. Se le agregó dicloro-metano (500 mililitros), así como una solución de imidazol de N-metilo (18.2 gramos, 223 milimoles), y etil-di-

isopropil-amina (51.2 gramos, 396 milimoles) en dicloro-metano (100 mililitros), la mezcla de reacción se agitó durante 2 horas hasta que el control en proceso de HPLC mostró la reacción completa. La solución se filtró y la resina se lavó con tres porciones de dicloro-metano, tres porciones de dimetil-formamida y tres porciones de isopropanol, subsiguientemente. La resina se secó al vacío y se redimensionó a 172 gramos de Isobutiril-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Enlazador-Resina seca. La carga Fmoc se determinó a ser 0.418 milimoles/gramo por consiguiente, indicando un rendimiento del 72 milimoles (71 por ciento durante los dos últimos pasos).

1B(7) El acoplamiento de Fmoc-N-metil-Tyr(tBu)-OH

Preparación de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Enlazador-Resina

(Anteriormente llamado: Isobutiril-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Fmoc)-Leu-Enlazador-Resina)

Isobutiril-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Enlazador-Resina (172 gramos, 72 milimoles) se hinchó mediante agitación en dos porciones subsiguientes de dimetil-formamida (1300 mililitros) durante 30 minutos cada una.

El grupo protector Fmoc se disoció por dos lavados subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida (1400 mililitros) durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetilfomamida e isopropanol. Se le agregaron

fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa probó la remoción satisfactoria de piperidina.

La resina se lavó con dimetil-formamida (1100 mililitros) tres veces para preparar el paso de acoplamiento siguiente.

Una solución de Fmoc-N-metil-Tyr(tBu)-OH (68.7 gramos, 144 milimoles), y HATU (55.1 gramos, 144 milimoles) en N,N-dimetil-formamida (700 mililitros) se preparó y, se agregó a la resina de péptido, seguida por la adición de una solución de etil-di-isopropil-amina (37.5 gramos 289 milimoles) en N,N-dimetil-formamida (100 mililitros) bajo agitación. Se revisó el pH inmediatamente después de la adición de la solución de acoplamiento y después de 1 hora de reacción dio el mismo resultado (pH 10) La solución se agitó durante 2 horas hasta que una muestra de ninhidrina mostró la reacción completa. La solución se filtró y la resina se lavó con dimetil-formamida e isopropanol, alternativamente.

El lote se sometió directamente al siguiente paso sin sequedad ni análisis adicional.

1B(8) El acoplamiento de Fmoc-Ile-OH

Preparación de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-IleFmoc)-Leu-Enlazador-Resina.

(Anteriormente llamado: Isobutiril-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Ile-Fmoc)-Leu-Enlazador-Resina)

La Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Enlazador-Resina húmeda (72 milimoles) se hinchó mediante agitación en dos

porciones subsiguientes de dimetil-formamida (1200 mililitros, y 1300 mililitros) durante 30 minutos cada una. El grupo protector Fmoc se disoció por dos lavados subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida (1400 mililitros) durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetilfomamida e isopropanol. Se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa demostró la remoción satisfactoria de piperidina.

La resina se lavó con dimetil-formamida (1100 mililitros) tres veces para preparar para el paso de acoplamiento siguiente.

En un matraz de fondo redondo se preparó una solución de Fmoc-Ile-OH (103.9 gramos, 294 milimoles) COMU (125.9 gramos, 294 milimoles), y etil-di-isopropil-amina (76 gramos, 588 milimoles) en dicloro-metano (440 mililitros), y dimetil-formamida (440 mililitros).

La solución se le agregó a la resina y la mezcla de reacción se agitó durante 20 horas. Después de este tiempo se hizo una prueba de ninhidrina, mostrando la reacción completa. La solución se filtró y la resina se lavó con dimetil-formamida e isopropanol, alternativamente.

La resina se secó al vacío y se redimensionó a 185.5 gramos de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Enlazador-Resina seca. La carga con grupos Fmoc se determinó para ser 0.40 milimoles/gramo. Por consiguiente, resultó un rendimiento

cuantitativo de 74 milimoles.

1B(9) El acoplamiento de sintón 1 y la disociación del grupo protector Fmoc final:

Preparación de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Sintón 1-H)-Leu-Enlazador-Resina

(Anteriormente llamado: Isobutiril-Gln(Trt)-Thr(Ile-N-me-Tyr-Ile-sintón-1-H)-Leu-Enlazador-Resina)

La Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Enlazador-Resina (30 gramos, 12 milimoles) se hinchó mediante agitación en dos porciones subsiguientes de dimetil-formamida (240 mililitros, y 250 mililitros) durante 30 minutos cada una.

El grupo protector Fmoc se disoció por dos lavados subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida (250 mililitros) durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetilfomamida e isopropanol. Se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa demostró la remoción satisfactoria de piperidina.

La resina se lavó con dimetil-formamida (250 mililitros) tres veces para preparar para el paso de acoplamiento siguiente.

En un matraz de fondo redondo, se preparó una solución de sintón 1 (14.6 gramos, 24.6 milimoles) PyBOP (12.85 gramos, 24.6 milimoles), y etil-di-isopropil-amina (6.4 gramos, 49.2 milimoles) en

N,N-dimetil-formamida (120 mililitros).

La solución se le agregó a la resina y la mezcla de reacción se agitó durante 3 horas. Después de este tiempo la prueba de ninhidrina confirmó la reacción completa. La solución se filtró y la resina se lavó con dimetil-formamida e isopropanol, alternativamente.

La resina de péptido resultante se hinchó mediante agitación en dos porciones subsiguientes de dimetil-formamida (250 mililitros) durante 30 minutos cada una.

El grupo protector Fmoc se disoció por dos lavados subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida (250 mililitros) durante 5 minutos y 15 minutos, respectivamente. La resina de péptido se lavó mediante varios lavados alternativos con dimetilfomamida e isopropanol.

Finalmente la resina de péptido se lavó tres veces con dicloro-metano (250 mililitros) para prepararse para la disociación del péptido.

1B(10) Disociación de péptido precursor 1 a partir del soporte sólido

Preparación de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-sintón-1-H)-Leu-OH (= Péptido precursor 1)

(Anteriormente llamado: Isobutiril-Gln(Trt)-Thr(Ile-N-me-Tyr(tBu)-Ile-sintón-1-H)-Leu-OH)

A la Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-sintón-1-H)-Leu-

Enlazador-Resina húmeda, se le agregó una mezcla del ácido acético (125 mililitros), y dicloro-metano (125 mililitros), y la suspensión se agitó durante 2 horas. La suspensión se filtró, y el filtrado se recolectó en un matraz de fondo redondo (filtrado 1). La resina se lavó dos veces con dicloro-metano (250 mililitros), y los lavados se combinaron con el filtrado 1.

La resina se trató con una porción fresca del ácido acético (125 mililitros), y dicloro-metano (125 mililitros) durante otras 2 horas. La suspensión se filtró, y el filtrado se recolectó en un matraz de fondo redondo (filtrado 2). La resina se lavó dos veces con dicloro-metano (250 mililitros), y los lavados combinado con filtrado 2.

Ácido acético (200 mililitros), y dicloro-metano (50 mililitros) se le agregaron a la resina y la suspensión se agitó durante la noche. La suspensión se filtró, y el filtrado se recolectó como el filtrado 3.

Los tres filtrados se procesaron por separado para evaluar la efectividad del método de disociación. Los filtrados se concentraron en un evaporador giratorio y se removió el ácido acético residual mediante destilación azeotrópica con tres porciones de tolueno (100 mililitros). Los residuos oleosos entonces, se secaron al alto vacío en un liofilizador. Rendimiento: filtrado 1: 12.5 gramos, filtrado 2: 2.1 gramos, filtrado 3:0.3 gramos.

El material crudo, péptido precursor 1, se purificó mediante cromatografía RP, las fracciones se concentraron en un evaporador giratorio y el concentrado se secó por congelación. Pureza: 98.8 por ciento de rendimiento: 8.5 gramos (48 por ciento para los dos últimos

pasos). El producto se caracterizó mediante, ^{1}H-RMN, ^{13}C-RMN y HR-MS. El espectro confirmó la estructura propuesta. El espectro de RMN indicó la presencia de diversas conformaciones.

HR-MS: calculada para $C_{85}H_{116}N_{8}O_{13}Si$: $[M+H]^{+}$: 1485.85039. Encontrado: $[M+H]^{+}$: 1485.84929.

Segunda Síntesis de SPPS de péptido precursor 1 utilizando una resina clorada

1B(11) Inmovilización del primer aminoácido (Fmoc-Leu-OH)

La resina de cloruro de 2-cloro-tritilo (30 gramos, L= 1.2 milimoles/gramo; Merck Novabiochem 855017) se hinchó con 240 mililitros de dicloro-metano seco durante 10 minutos y entonces el solvente se drenó, el reactor de fase sólida se mantuvo cerca bajo una corriente suave de nitrógeno. Entretanto, en un matraz de fondo redondo se mezclaron Fmoc-Leu-OH (42.6 gramos, 120.8 milimoles), y 100 mililitros de 1,4-dioxano seco. El solvente se evaporó al vacío a 45°C, entonces, se le agregó una segunda porción de 1,4 dioxano. El solvente se evaporó otra vez al vacío a 45°C hasta que se obtuvo un residuo de aceite incoloro. Entonces, a este residuo oleoso se agregaron en secuencia dicloro-metano seco (155 mililitros), y di-isopropil-etil-amina (40.4 mililitros), la solución se le agregó en una porción única a la resina pre-hinchada y, luego, 5 minutos después, se le agregó una segunda porción de di-isopropil-etil-amina (16.5 mililitros). La suspensión se agitó durante 2 horas y la reacción de medio se drenó. Con el objeto de apagar el cloruro sin reaccionar

potencial, se le agregaron 240 mililitros de una solución apagada de DCM/MeOH/DIPEA (70/15/15, volumen/volumen), y la mezcla se agitó durante 10 minutos. Se midió una carga de 0.95 milimoles/gramo.

Protocolo general para disociar Fmoc:

A la resina pre-hinchada se le agregaron 240 mililitros de una solución de 25 por ciento de piperidina en N,N-dimetil-formamida, la suspensión se agitó durante 5 minutos y entonces la mezcla de reacción se removió mediante filtración, entonces se le agregó una segunda porción de la misma solución de piperidina, la suspensión se agitó durante 15 minutos y entonces el solvente se removió mediante filtración.

Lavado de la resina con secado:

La resina se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 6 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de TBME (2 minutos, 6 veces)

La resina se secó durante la noche al vacío a 40C° para obtener 35.7 gramos de H-Leu-Resina.

1B(12): Síntesis SPPS de H-Thr-Leu-Resina

La H-Leu-Resina (30 gramos) a partir del paso previo se hinchó con 270 mililitros de dimetil-formamida durante 30 minutos, entonces el solvente se drenó.

En un matraz de fondo redondo (RBF) se mezclaron Fmoc-Thr-OH (25.0 gramos, 73.2 milimoles), BOP (40.4 gramos, 91.5 milimoles), y dimetil-formamida (250 mililitros), la mezcla se agitó durante 2 minutos y entonces se le agregó di-isopropil-etil-amina (18.9 gramos, 146.4 milimoles). La mezcla obtenida de esta manera se le agregó en una porción a la resina de péptido previamente hinchada y la mezcla de reacción se agitó durante 2.5 horas. La prueba de Kaiser fue positive y por consiguiente se hizo un segundo acoplamiento de acuerdo con las condiciones anteriormente descritas. Después de 1.0 horas la prueba de Kaiser fue negativa y la reacción se consideró que estaba completa y la reacción de medio se removió por medio de filtración.

La resina se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de TBME (2 minutos, 5 veces).

La resina se secó durante la noche al vacío a 40C° proporcionando 52.2 gramos de Fmoc-Thr-Leu-Resina lista para el paso de disociar la Fmoc.

Disociación de Fmoc:

La resina (52.2 gramos) se suspendió en 272 mililitros de dimetil-formamida y se agitó durante 1.0 horas, entonces el solvente se removió mediante filtración.

El grupo protector Fmoc se disoció de la misma manera como

se describe para 1B(11), y la resina de péptido se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 6 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de TBME (2 minutos, 6 veces)

La resina se secó durante la noche al vacío a 40C° proporcionando 39.9 gramos de H-Thr-Leu-Resina

1B(13): Síntesis SPPS de H-Gln(Trt)-Thr-Leu-Resina

La H-Thr-Leu-Resina a partir del paso previo (39.9 gramos) se hinchó con 270 mililitros de dimetil-formamida durante 30 minutos entonces el solvente se drenó.

En un RBF se mezclaron Fmoc-Gln(Trt)-OH (44.7 gramos, 73.2 milimoles), BOP (40.4 gramos, 91.5 milimoles), y 250 mililitros de N,N-dimetil-formamida. La mezcla se agitó durante 2 minutos y entonces se le agregó di-isopropil-etil-amina (18.9 gramos, 146.4 milimoles). La mezcla resultante se agregó en una porción a la H-Thr-Leu-Resina previamente hinchada y la mezcla de reacción se agitó durante 2.5 horas, la prueba de Kaiser fue negativa, entonces la reacción se consideró que estaba completa y la reacción de medio se removió por medio de filtración.

La resina se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

La resina de péptido húmeda se utilizó para el paso de disociar

la Fmoc sin ninguna otra manipulación.

Disociación de Fmoc:

El grupo protector Fmoc se disoció de acuerdo con el procedimiento descrito en el Paso 1B(11). Entonces la resina se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de TBME (2 minutos, 5 veces)

La resina se secó durante la noche al vacío a 40C° proporcionando 54.4 gramos de H-Gln(Trt)-Thr-Leu-Resina.

1B(14): Síntesis SPPS de Isobutiril-Gln(Trt)-Thr-Leu-Resina

La resina de péptido a partir del paso previo (54.4 gramos) se hinchó con 270 mililitros de dimetil-formamida durante 30 minutos, entonces el solvente se drenó.

En un RBF se mezclaron ácido isobutírico (6.4 gramos, 73.2 milimoles), PyBop (38.1 gramos, 73.2 milimoles), y 230 mililitros de N,N-dimetil-formamida, la mezcla se agitó durante 2 minutos y entonces se le agregó di-isopropil-etil-amina (28.3 gramos, 219.6 milimoles. La solución se le agregó en una porción a la resina de péptido previamente hinchada y la mezcla de reacción se agitó durante 1.5 horas. La prueba de Kaiser fue negativa, entonces la reacción se consideró que estaba completa y la reacción de medio se removió por medio de filtración.

La resina se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de TBME (2 minutos, 5 veces).

La resina se secó durante la noche al vacío a 40C° proporcionando 55.8 gramos de Isobutiril-Gln(Trt)-Thr-Leu-Resina.

1B(15): Síntesis SPPS de Isobutiril-Gln(Trt)-Thr(Ile-H)-Leu-Resina

La resina de péptido que viene a partir del paso previo (55.8 gramos) se suspendió en 250 mililitros de dicloro-metano seco durante 1.0 horas. Entonces el solvente se removió mediante filtración y la resina de péptido húmeda se mantuvo bajo una corriente suave de nitrógeno y la temperatura se ajustó de 0 a 5°C.

En un RBF se mezclaron Fmoc-Ile-OH (51.7 gramos, 146.4 milimoles), y 150 mililitros de dioxano seco. El solvente se evaporó al vacío a 45°C hasta que se observó un residuo oleoso. El proceso de destilación se repitió y entonces al residuo se le agregaron 150 mililitros de DCM seco, la solución se enfrió a -10°C y se le agregó MSNT (43.3 gramos, 146.4 milimoles), la suspensión se agitó durante 3 minutos y entonces se le agregó N-metil-imidazol (14.2 gramos, 173 milimoles), la mezcla se agitó durante 2 minutos y la solución se le agregó por goteo en 10 minutos a la resina de péptido preparada anteriormente. Después de que se finalizó la adición, la suspensión se agitó bajo a nitrógeno atmósfera durante 2.0 horas

La resina se lavó como sigue:

250 mililitros DMC (2 minutos, 4 veces)

250 mililitros de dimetil-formamida (2 minutos, 3 veces)

La resina de péptido húmeda se utilizó para el paso de disociar la Fmoc sin ninguna otra manipulación.

Disociación de Fmoc:

El grupo protector Fmoc se disoció de acuerdo con el protocolo general descrito en 1B(11)

Después de que se hizo la disociación de Fmoc, la resina se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 5 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de TBME (2 minutos, 5 veces)

La resina se secó durante la noche al vacío a 40C° proporcionando 57.2 gramos de Isobutiril-Gln(Trt)-Thr(O-Ile-H)-Leu-Resina.

1B(16): Síntesis SPPS de Isobutiril-Gln(Trt)-Thr(O-Ile-Tyr(tBu)MeN)-Leu-Resina

La resina de péptido que viene a partir del paso previo (57.2 gramos) se suspendió en 250 mililitros de dimetil-formamida durante 30 minutos, para el hinchamiento, entonces el solvente se removió mediante filtración.

En un RBF se mezclaron Fmoc-NMeTyr(tBu)-OH (52.0 gramos,

109.8 milimoles), HATU (41.7 gramos, 109.8 milimoles), y 230 mililitros de dimetil-formamida y la mezcla se agitó durante 2 minutos. Entonces se le agregó di-isopropil-etil-amina (28.3 gramos, 219.6 milimoles), y la solución se agitó durante 2.0 minutos. La solución resultante se agregó entonces a la resina de péptido previamente hinchada.

La mezcla de reacción se agitó durante 1.0 hora y se llevó a cabo una prueba de Kaiser. La prueba de Kaiser fue negativa. La reacción se consideró que estaba completa y la reacción de medio se removió por medio de filtración.

La resina de péptido se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

250 mililitros de IPA (2 minutos, 1 vez)

La resina de péptido húmeda se utilizó para el paso de disociar la Fmoc sin ninguna otra manipulación.

Disociación de Fmoc:

El grupo protector Fmoc se disoció de acuerdo con el protocolo general utilizado en 1B(11).

Después de que se hizo la disociación de Fmoc, la resina se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 5 veces)

250 mililitros de IPA (2 minutos, 1 vez)

250 mililitros de dimetil-formamida (2 minutos, 5 veces)

La isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-Resina

obtenida se utilizó para el siguiente paso sin sequedad.

1B(17): Síntesis SPPS de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resina

Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-Resina a partir del paso previo se hinchó con 270 mililitros de dimetil-formamida durante 30 minutos, entonces el solvente se drenó.

En un matraz de fondo redondo se mezclaron Fmoc-Ile-OH (38.8 gramos, 109.8 milimoles), HATU (41.7 gramos, 109.8 milimoles), y dimetil-formamida (250 mililitros), la mezcla se agitó durante 2 minutos y entonces se le agregó di-isopropil-etil-amina (28.3 gramos, 219.6 milimoles). La solución se le agregó en una porción a la resina de péptido previamente hinchada y la mezcla de reacción se agitó durante 2.0 horas. La prueba de Cloranil fue positiva y entonces se llevó a cabo un segundo acoplamiento con Fmoc-Ile-OH. 3 horas después del segundo acoplamiento, la resina de péptido se aisló mediante filtración.

La resina de péptido se lavó como sigue:

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de dimetil-formamida (2 minutos, 4 veces)

La resina de péptido húmeda se utilizó para el paso de disociar la Fmoc sin ninguna otra manipulación.

Disociación de Fmoc:

El grupo protector Fmoc se removió de la misma manera como

se describe en 1B(11), y la resina de péptido se lavó como sigue.

250 mililitros de dimetil-formamida (2 minutos, 5 veces)

250 mililitros de IPA (2 minutos, 3 veces)

250 mililitros de TBME (2 minutos, 4 veces).

La resina de péptido se secó durante la noche al vacío a 40C° proporcionando 67.0 gramos de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resina.

1B(18): Síntesis SPPS de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-sintón-1-H)-Leu-Resina

La Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-Resina a partir del paso previo (33.5 gramos) se suspendió en 150 mililitros de dimetil-formamida durante 30 minutos, entonces el solvente se drenó. En un RBF se mezclaron Fmoc-sintón-1-OH (34.3 gramos, 57.8 milimoles), PyBop (30.0 gramos, 57.7 milimoles), y 113 mililitros de N,N-dimetil-formamida, la mezcla se agitó durante 2 minutos y entonces se le agregó di-isopropil-etil-amina (14.9 gramos, 115.2 milimoles). La solución se le agregó en una porción a la resina de péptido previamente hinchada y la mezcla de reacción se agitó durante 2.0 horas. La reacción de medio se removió mediante filtración.

La resina de péptido se lavó como sigue:

150 mililitros de dimetil-formamida (2 minutos, 4 veces)

150 mililitros de IPA (2 minutos, 3 veces)

150 mililitros de dimetil-formamida (2 minutos, 4 veces)

La resina de péptido húmeda se utilizó para el paso de disociar la Fmoc sin ninguna otra manipulación.

Disociación de Fmoc:

El grupo protector Fmoc se removió de la misma manera como en 1B(11), pero utilizando 150 mililitros de la solución de piperidina, y la resina de péptido se lavó como sigue.

150 mililitros de dimetil-formamida (2 minutos, 5 veces)

150 mililitros de IPA (2 minutos, 3 veces)

150 mililitros de TBME (2 minutos, 4 veces)

La resina de péptido se secó durante la noche al vacío a 40C° proporcionando 36.0 gramos de Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-sintón-1-H)-Leu-Resina.

1B(19): Síntesis de SPPS alternativa: disociación del péptido a partir del soporte sólido

La Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-sintón-1-H)-Leu-Resina a partir del paso previo (29.75 gramos) se trató con 350 mililitros de dicloro-metano seco durante 1.0 hora, el solvente se removió mediante filtración y, entonces se le agregaron 350 mililitros de una solución del 30 por ciento en volumen/volumen de HFIP en dicloro-metano. La mezcla se agitó durante 10 minutos y entonces el solvente se removió mediante filtración y se mantuvo a un lado. A la resina húmeda se le agregó una segunda porción de la misma solución de HFIP y la solución se agitó durante 10 minutos, entonces

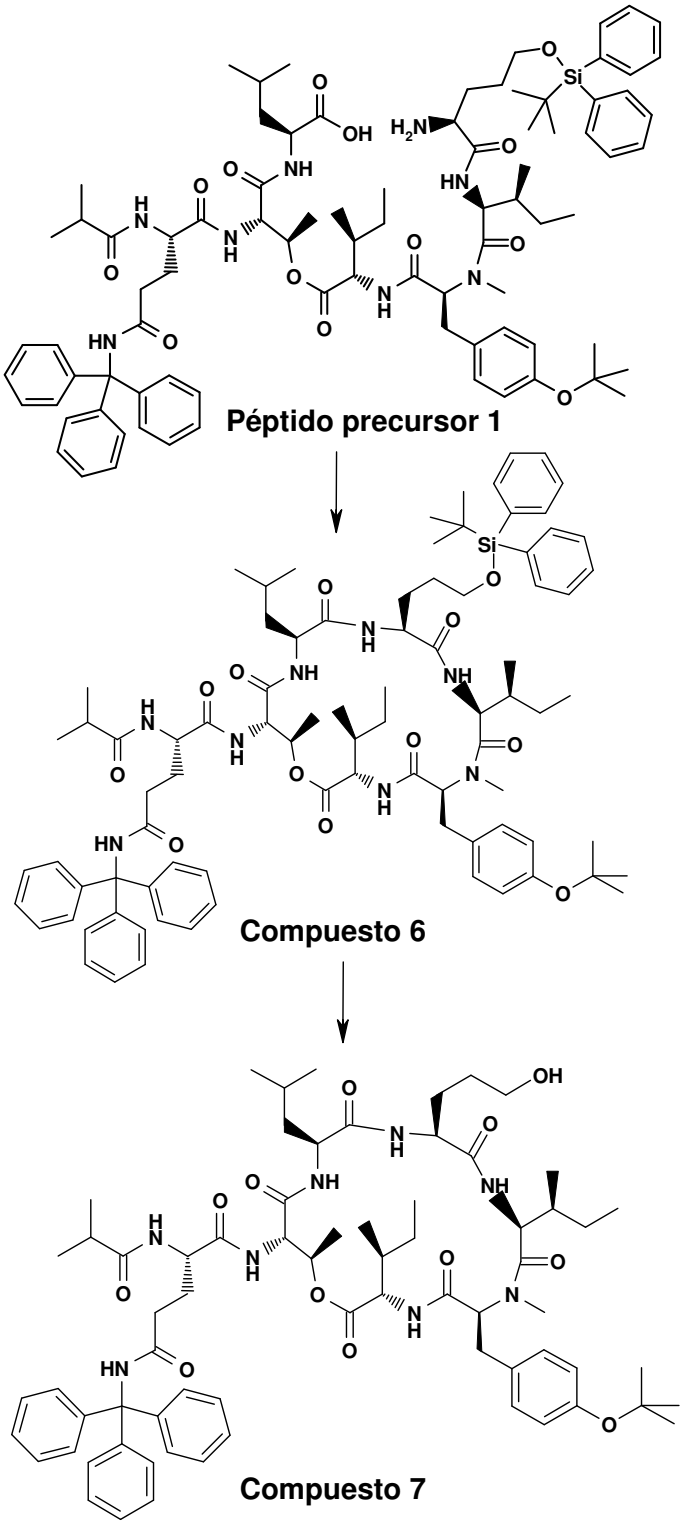
el solvente se removió mediante filtración y se agrupó con la solución previa. La resina se lavó 3 veces con dicloro-metano (350 mililitros), y los lavados se combinaron con las soluciones disociadas.

La solución combinada se concentró al vacío hasta que se observó un residuo oleoso y entonces se le agregaron 200 mililitros de tolueno, el solvente se evaporó bajo presión reducida a 45°C hasta que se obtuvo un residuo oleoso. Se le agregaron 350 mililitros de hexano y la suspensión se agitó durante 2 horas. El solvente se removió mediante filtración y la torta del filtro se lavó con hexano (50 mililitros), la torta húmeda se secó durante la noche al vacío a 35°C, para proporcionar 19.24 gramos del péptido precursor crudo **1** (= Isobutiril-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-sintón-1-H)-Leu-OH).

Parte del péptido precursor crudo 1 (6.0 gramos) se purificó mediante cromatografía RP, las fracciones de producto se concentraron en un evaporador giratorio y el concentrado se secó por congelación para obtener 2.2 gramos del péptido precursor puro 1 en una pureza del 99.3 por ciento. El producto que contiene fracciones laterales se trató de una manera similar para obtener 1.25 gramos adicionales de péptido precursor menos puro en una pureza del 71.4 por ciento. Extrapolado para la cantidad completa del péptido precursor crudo 1 (19.24 gramos), esto podría corresponder a 7.05 gramos de péptido precursor puro **1** a partir de las fracciones mayores y 4.01 gramos adicionales del péptido precursor **1** (en 71.4 por ciento de pureza) a partir de las fracciones laterales .

1C Síntesis de Fase de Solución del compuesto 8

Esquema de Reacción 3



1C(1) Síntesis del compuesto 6

(*S*)-*N*[1]-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-6-(4.terbutoxi)-bencil)-3,9-di-((*S*)-sec-butil-12-(3-((terbutil-difenil-silil)-oxi)-propil)-15-isobutil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-penta-azaciclon-onadecan-18-il)-2-isobutiramido-*N*[5]-tritil-pentan-diamida

Una solución nublada/suspensión de péptido precursor 1 (1.9 gramos, 1.28 milimoles) en acetonitrilo (120 mililitros), se le agregó durante 90 minutos a una mezcla agitada de HATU (972 miligramos, 2.56 milimoles), y DMAP (468.6 miligramos, 3.84 milimoles) en acetonitrilo (100 mililitros) a 35°C. El embudo de goteo se lavó con acetonitrilo (30 mililitros). Un IPC (HPLC) después de la adición de la suspensión indicó la ausencia del péptido precursor y la terminación de la ciclación. Para el procesamiento, el solvente se evaporó bajo presión reducida hasta un volumen final de aproximadamente 50 mililitros, y el residuo se diluyó con acetato de isopropilo (250). La fase orgánica se extrajo con agua (100 mililitros, 2 veces), y el solvente se evaporó bajo presión reducida, para obtener 2.3 gramos de producto crudo. El producto crudo se purificó mediante cromatografía por evaporación instantánea sobre gel de sílice con acetato de etilo como fase móvil para obtener 1.79 gramos (1.22 milimoles) del compuesto 6 como una espuma. Rendimiento: 95 por ciento. El producto se caracterizó mediante, ^{1}H-RMN, ^{13}C-RMN y HR-

MS. El espectro confirmó la estructura propuesta. El espectro de RMN indicó la presencia de diversas conformaciones.

HR-MS: calculada para $C_{85}H_{114}N_8O_{12}Si$: $[M+H]^+$:1467.83983; $[M+NH_4]^+$:1484.86637; $[M+Na]^+$: 1489.82177. Encontrado: $[M+H]^+$:1467.83984; $[M+NH_4]^+$:1484.86555; $[M+Na]^+$: 1489.82073.

Segundo ejemplo para la síntesis del compuesto 6 (escala de 15 gramos)

Una solución del péptido precursor **1** (15.0 gramos) en metil-éter de terbutilo (750 mililitros), se le agregó lentamente durante 1.5 horas a una solución previamente enfriada de DMAP (2.80 gramos), y HATU (5.87 gramos) en acetonitrilo (375 mililitros) a 0°C. La mezcla de reacción se agitó durante 30 minutos adicionales a temperatura ambiente. La mezcla de reacción entonces se diluyó con el metil-éter de terbutilo (750 mililitros), y se vertió sobre solución acuosa medio-saturada de NaCl (1500 mililitros). Las fases se separaron y la fase orgánica se extrajo otra vez con solución acuosa medio-saturada de NaCl (1500 mililitros). La capa orgánica se separó y el solvente se evaporó en parte bajo presión reducida hasta un volumen final de aproximadamente 175 mililitros. Esta solución se filtró sobre gel de sílice (columna con 225 gramos de gel de sílice) utilizando metil-éter de terbutilo como fase móvil. La evaporación del solvente y el secado al vacío de 40 a 45°C dio el compuesto 6 en 97.53 por ciento de pureza de acuerdo con HPLC. Rendimiento: 14.19 gramos (95.7 por ciento).

1C(2) Síntesis del compuesto 7

(*S*)-*N*^{1}-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-6-(4.terbutoxi)-bencil)-3,9-di-((*S*)-sec-butil-12-(3-(hidroxipropil)-15-isobutil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-penta-azaciclon-onadecan-18-il)-2-isobutiramido-*N*^{5}-tritil-pentan-diamida

El compuesto 6 (1.79 gramos, 1.22 milimoles) se disolvió en tetrahidrofurano (60 mililitros), y se le agregó $Et_3N(HF)_3$ (6.62 gramos, 41.1 milimoles), a temperatura ambiente. La mezcla de reacción se agitó a temperatura ambiente durante 8.5 horas, entonces se diluyó con acetato de isopropilo (200 mililitros), y la solución resultante/suspensión se le agregó lentamente a una solución saturada acuosa de $NaHCO_3$ agitada intensivamente. La fase orgánica se separó y se extrajo con agua (100 mililitros). La evaporación del solvente bajo presión reducida dio 1.86 gramos del producto crudo, el cual se purificó mediante cromatografía por evaporación instantánea sobre gel de sílice con acetato de etilo/isopropanol (95:5) como la fase móvil para obtener 1.24 gramos (1.00 milimoles) del compuesto 7. Rendimiento: 82 por ciento.

El producto se caracterizó totalmente mediante IR, RMN y MS. El espectro confirmó la estructura propuesta.

HR-MS: calculada para $C_{69}H_{96}N_8O_{12}$: $[M+H]^+$:1229.72205; $[M+NH_4]^+$: 1246.74860; $[M+Na]^+$: 1251.70399. Encontrado: $[M+H]^+$:

1229.72128; $[M+NH_4]^+$:1246.74780; $[M+Na]^+$:1251.70310.

Segundo ejemplo para la preparación del compuesto 7 (escala de 14 gramos)

El compuesto **6** (14.0 gramos, 9.537 milimoles) se disolvió en tetrahidrofurano (220 mililitros), y se le agregó metil-éter de terbutilo (116 mililitros). La solución se trató con $Et_3N(HF)_3$ (23.05 gramos) mediante la adición lenta dentro de 10 minutos. La mezcla de reacción se agitó durante 24 horas a temperatura ambiente. Para el procesamiento la mezcla de reacción se diluyó con el metil-éter de terbutilo (570 mililitros), y la mezcla se vertió sobre solución saturada medio-acuosa de $NaHCO_3$ (632 mililitros). La mezcla bifásica se agitó durante 30 minutos y las fases se separaron. La fase orgánica se extrajo con agua (280 mililitros). Ambas fases acuosas se extrajeron con el metil-éter de terbutilo (380 mililitros), y las fases orgánicas se combinaron. La fase orgánica se secó sobre $MgSO_4$ anhidro (8.0 gramos), y el solvente se evaporó en parte bajo presión reducida hasta un volumen final de aproximadamente 100 mililitros. Se le agregó tolueno (140 mililitros), y el solvente se evaporó otra vez hasta un volumen final de 80 mililitros. Esta solución se diluyó con el metil-éter de terbutilo (70 mililitros), y el producto se precipitó mediante la adición lenta de heptanos (140 mililitros) durante 30 minutos. La suspensión formada se calentó de 50 a 55°C, y se agitó durante 30 minutos a esta temperatura. La suspensión entonces se enfrió a 0°C dentro de 30 minutos, se agitó a

0°C durante 2 horas y el producto se aisló mediante filtración. El producto, un precipitado blanco, se secó al vacío, para obtener 11.08 gramos del compuesto **7**. (94.5 por ciento de rendimiento). La HPLC del producto indicó 97 a% de pureza.

1C(3) Síntesis del compuesto 8 (véase Esquema de Reacción 4 para la estructura)

(*S*)-*N*^{1}-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-6-(4.terbutoxi)-bencil)-3,9-di-((*S*)-sec-butil-15-isobutil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-12-(3-oxopropil)-1-oxa-4,7,10,13,16-penta-azaciclononadecan-18-il)-2-isobutiramido-*N*^{5}-tritil-pentan-diamida

El compuesto **7** (1.2 gramos, 0.98 milimoles) se disolvió en una mezcla de tetrahidrofurano (190 mililitros), y sulfóxido de dimetilo (62 mililitros). Se le agregó 1-óxido de 1-hidroxi-1,2-benzodioxol-3(1*H*)-ona (IBX) (2.42 gramos, 45 por ciento gramos/gramo, 3.9 milimoles), y la solución se agitó durante aproximadamente 4.5 horas, después de cuyo tiempo, la HPLC indicó la desaparición del material de partida (el compuesto 7). La mezcla de reacción se vertió entonces sobre solución de $NaHCO_3$ saturada acuosa (300 mililitros), y se extrajo con dicloro-metano (300 mililitros, 2 veces). Las capas orgánicas se combinaron y se lavaron con agua (300 mililitros, 2 veces). La evaporación del solvente bajo presión reducida dio 2.31 gramos del producto crudo como una espuma. El producto crudo se

purificó mediante cromatografía por evaporación instantánea sobre gel de sílice con acetato de etilo/isopropanol (95:5) para obtener 1.16 gramos de una mezcla del producto, la cual comprende cuando menos 2 productos con la masa deseada en LC-MS. La mezcla del producto se utilizó como tal para el siguiente paso.

HR-MS (isómero mayor): calculada para $C_{69}H_{94}N_8O_{12}$: $[M+H]^+$: 1227.70640; $[M+NH_4]^+$: 1244.73295; $[M+Na]^+$: 1249.68834 . Encontrado: $[M+H]^+$: 1227.70599; $[M+NH_4]^+$: 1244.73200; $[M+Na]^+$: 1249.68733.

HR-MS (isómero menor): calculada para $C_{69}H_{94}N_8O_{12}$: $[M+H]^+$: 1227.70640; $[M+NH_4]^+$: 1244.73295; $[M+Na]^+$: 1249.68834 . Encontrado: $[M+H]^+$: 1227.70598; $[M+NH_4]^+$: 1244.73198; $[M+Na]^+$: 1249.68751.

Segundo ejemplo para la síntesis del compuesto 8 (escala de 10 gramos)

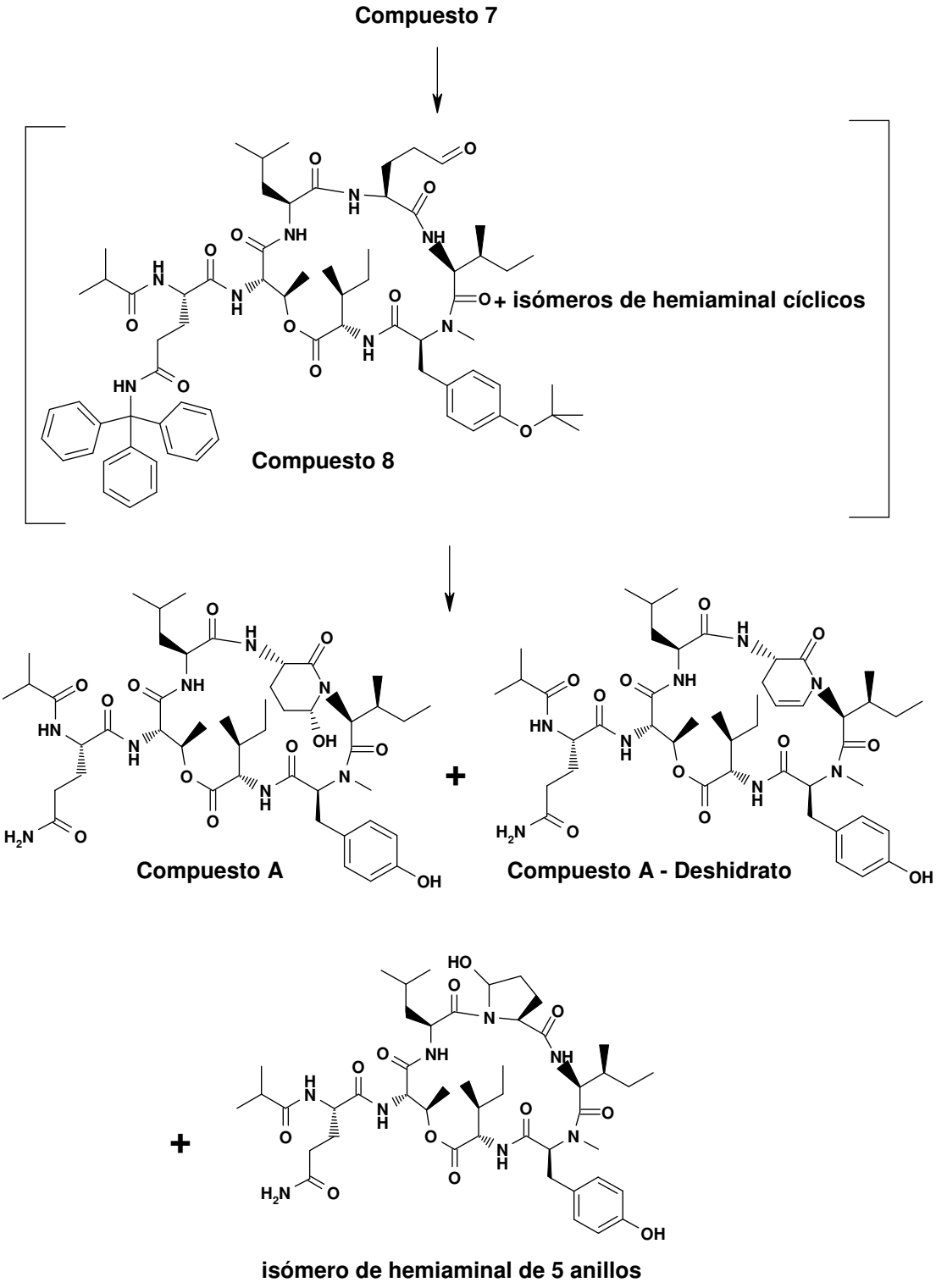
El compuesto **7** (10.0 gramos, 8.13 milimoles) se disolvió en tetrahidrofurano (127 mililitros), y sulfóxido de dimetilo (42 mililitros). A esta solución, se le agregó 1-óxido de 1-hidroxi-1,2-benzodioxol-3(1*H*)-ona (IBX) (15.18 gramos, 45 por ciento de masa/masa, 24.4 milimoles), bajo agitación intensa a temperatura ambiente. La mezcla de reacción se agitó durante 16 horas a temperatura ambiente. La mezcla de reacción se vertió entonces sobre solución acuosa de $NaHCO_3$ (500 mililitros), y se le agregó acetato de etilo (250 mililitros). La mezcla bifásica se agitó durante 30 minutos y las

capas se separaron. La fase orgánica se lavó con solución acuosa medio-saturada de NaCl (250 mililitros), y las capas se separaron. Las capas de agua se extrajeron con acetato de etilo (250 mililitros), y las fases orgánicas se combinaron. La fase orgánica combinada se secó sobre sulfato de magnesio anhidro y el solvente se evaporó en parte para obtener aproximadamente 50 gramos de solución. Esta solución se irradió sobre una columna de gel de sílice (100 gramos de gel de sílice) utilizando acetato de etilo/metanol (98:2 volumen/volumen) como la fase móvil. La solución del producto (537.4 gramos) obtenido de esta manera se trató con tolueno (135 mililitros), y la solución resultante se concentró hasta un peso final de 112 gramos mediante la evaporación parcial del solvente a 45°C bajo presión reducida. La solución resultante se enfrió a 0°C y se le agregaron heptanos (135 mililitros), durante 30 minutos. La suspensión obtenida de esta manera se agitó durante 1 hora a 0°C, el producto se aisló mediante filtración y se lavó con heptanos (30 mililitros, 2 veces). Finalmente el producto se secó al vacío a 45°C durante la noche para obtener 8.824 gramos de una mezcla del compuesto **8** y sus isómeros de hemiaminal. Rendimiento: 88.4 por ciento.

1D Síntesis del compuesto A

(S)-N^1-((2S,5S,8S,11R,12S,15S,18S,21R)-2,8-di-(S)-*sec*-butil-21-hidroxi-5-(4-hidroxi-bencil)-15-isobutil-4,11-dimetil-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-penta-azabiciclo-[16.3.1]-docosan-12-il)-2-isobutiramido-pentano-diamida

Esquema de Reacción 4:



El compuesto 8 (2.0 gramos) se disolvió en dicloro-metano (400 mililitros), y la solución se enfrió 0°C. Se le agregó ácido trifluoro-acético (115.9 gramos), a la solución agitada a 0°C, y la mezcla de reacción se agitó durante 4 horas a 0°C. Se le agregó dicloro-metano (400 mililitros), a esta temperatura, seguida por la adición de agua (20 gramos). La mezcla de reacción se dejó calentar-hasta temperatura ambiente, y se continuó la agitación durante 5 horas adicionales a temperatura ambiente. Para el procesamiento, la mezcla de reacción se vertió sobre una solución agitada de acetato de sodio (165.1 gramos) en agua (800 mililitros), y se le agregó acetato de etilo (400 mililitros) para obtener una solución. La capa superior (fase acuosa) se removió la fase orgánica inferior (fase de dicloro-metano) se lavó con agua (200 mililitros, 2 veces). Las capas de agua se extrajeron con acetato de etilo (200 mililitros), y las capas orgánicas se combinaron. El solvente se removió bajo presión reducida, para obtener el compuesto A crudo como una mezcla de isómeros de 5- y 6- anillos (véase Esquema de Reacción 5), acompañado por alcohol de tritilo y otros subproductos de la reacción.

El producto crudo se purificó mediante cromatografía RP sobre sílice Kromasil 100-10-C8 con un gradiente de acetonitrilo/agua como la fase móvil. El producto que contiene fracciones se evaporó para remover el acetonitrilo, el precipitado se disolvió en acetato de etilo y el solvente se removió bajo presión reducida, para obtener 0.895 gramos del compuesto A; rendimiento: 59.1 por ciento. El

producto se caracterizó totalmente mediante RMN y MS y el espectro del producto fue idéntico al compuesto A a partir de fermentación.

HR-MS: Calculada para $C_{46}H_{72}O_{12}N_8$: $[M+H]^+$: 929.53425; $[M+NH_4]^+$: 946.56080; $[M+Na]^+$: 951.51619. Encontrado: $[M+H]^+$: 929.53445; $[M+NH_4]^+$: 946.56129; $[M+Na]^+$: 951.51624.

^{1}H-RMN (600 MHz, d_6-DMSO) δ_H: -0.11 (3H, d, J= 6.2Hz), 0.64 (4H, m), 0.77 (3H, d, J= 6.2Hz), 0.81 (3H, t, J= 7.3Hz), 0.84 (3H, d, J= 7.0 Hz), 0.88 (3H, d, J= 6.6Hz), 1.02 (3H, d, J= 6.7Hz), 1.02 (1H, m), 1.03 (3H, d, J= 6.7Hz), 1.09 (1H, m), 1.20 (3H, d, J= 6.2Hz), 1.24 (1H, m), 1.39 (1H, m), 1.51 (1H, m), 1.75 (6H, m), 1.83 (1H. m), 1.92 (1H, m), 2.12 (2H, m), 2.47 (1H, m), 2.58 (1H, m), 2.67 (1H, m), 2.71 (3H, s), 3.16 (1H, d, J= 14.2Hz), 4.30 (1H, m), 4.34 (1H, m), 4.42 (1H, d, J= 10.6Hz), 4.45 (1H, m), 4.61 (1H, d, J= 9.2Hz), 4.71 (1H, dd, J= 9.5, 5.5Hz), 4.93 (1H, s), 5.05 (1H, dd, J= 11.4, 2.6 Hz), 5.48 (1H, m), 6.07 (1H, d, J= 2.6Hz), 6.64 (2H, d, J= 8.4Hz), 6.73 (1H, s), 6.99 (2H, d, J= 8.4Hz), 7.25 (1H, s), 7.35 (1H, d, J= 9.2Hz), 7.64 (1H, d, J= 9.5Hz), 7.73 (1H, d, J= 9.2Hz), 8.01 (1H, d, J= 7.7Hz), 8.42 (1H, d, J= 8.8Hz), 9.17 (1H, s).

^{13}C-RMN (150 MHz, d_6-DMSO) δ_C: 10.35, CH_3; 11.21 CH_3; 13.85, CH_3; 16.00, CH_3; 17.68, CH_3; 19.52, 2 x CH_3; 20.89, CH_3; 21.75, CH_2; 23.30, CH_3; 23.74, CH_2; 24.21, CH; 24.48, CH_2; 27.35, CH_2; 29.78, CH_2; 30.08, CH_3; 31.49, CH_2; 33.18, CH; 33.24, CH_2; 33.76, CH, 37.41, CH; 39.23, CH_2; 48.84, CH; 50.69, CH; 52.11, CH; 54.17 CH; 54.70, CH; 55.31, CH; 60.66, CH; 71.89, CH; 73.97, CH; 115.32, 2 x CH; 127.34, Cq; 130.37, 2 x CH; 156.27, Cq; 169.12, Cq;

169.29, Cq; 169.37, Cq; 169.79 Cq; 170.65, Cq; 172.40, Cq; 172.53, Cq; 173.87 Cq; 176.38, Cq.

Segundo ejemplo para la síntesis del compuesto A (escala de 8.5 gramos)

El compuesto **8** (8.5 gramos, 6.924 milimoles) se disolvió en dicloro-metano (595 mililitros), y la solución se enfrió a 0°C. Se le agrego una solución del ácido trifluoro-acético (127.5 mililitros) en dicloro-metano (127.5 mililitros), a la solución fría, manteniendo la temperatura de 0 a 5°C. La mezcla de reacción se agitó durante 5.5 horas a 0°C, y se diluyó con dicloro-metano (850 mililitros), seguido por la adición de agua (42.5 mililitros). La mezcla de reacción se dejó calentar hasta la temperatura ambiente, y se agitó durante 17.5 horas a temperatura ambiente. Para el procesamiento, la mezcla de reacción se vertió sobre una mezcla acuosa bifásica agitada de acetato de sodio (169 gramos de acetato de sodio en 722 gramos de agua), y acetato de etilo (700 mililitros). Las fases se separaron y la fase orgánica se lavó nuevamente con solución acuosa de acetato de sodio (169 gramos de acetato de sodio en 722 gramos de agua). Las capas se separaron y la fase orgánica se lavó con agua (850 mililitros, 2 veces). Las fases acuosas individuales se extrajeron con acetato de etilo (700 mililitros), y las capas orgánicas se combinaron. La capa orgánica se secó sobre sulfato de magnesio anhidro y el solvente se evaporó en parte de 40 a 45°C bajo presión reducida, para obtener 187 gramos de una suspensión delgada. Se agregó heptano (187 gramos), dentro de 15 minutos y la suspensión formada

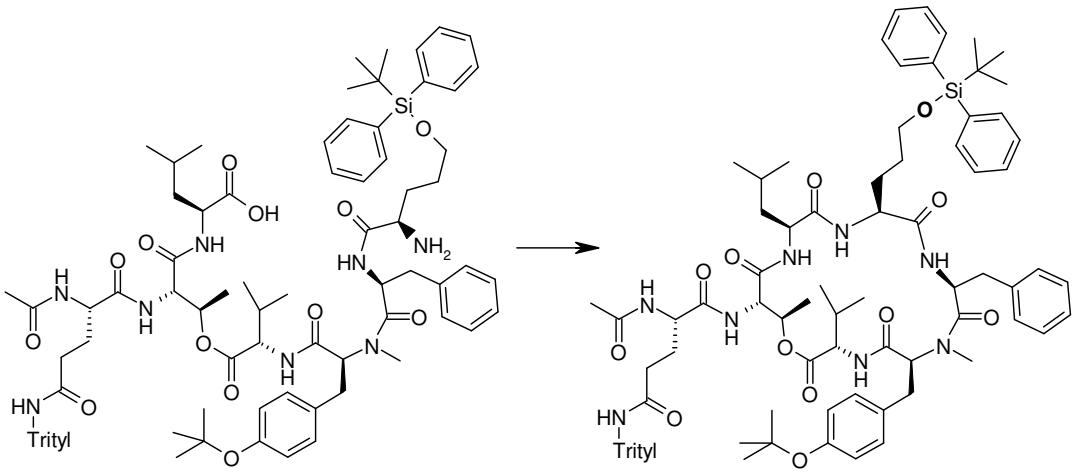
se enfrió a 0°C. La suspensión se agitó durante 1 hora a 0°C y el producto se aisló mediante filtración. El producto crudo se secó al vacío a 40°C para obtener 5.65 gramos del compuesto **A** crudo. Rendimiento crudo: 87.8 por ciento.

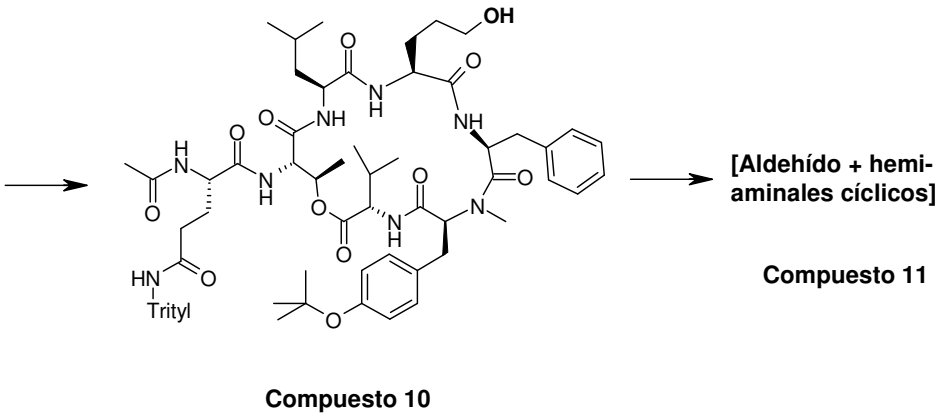
0.96 gramos del producto crudo se purificaron mediante cromatografía RP sobre sílice Kromasil 100-10-C8 con un gradiente de acetonitrilo/agua como la fase móvil. El producto que contiene las fracciones se evaporó para remover el acetonitrilo, el precipitado se disolvió en acetato de etilo y el solvente se removió en parte bajo presión reducida, para obtener 40 gramos de una solución. Se agregaron heptano (40 gramos), a la solución agitada durante 30 minutos a temperatura ambiente y la suspensión resultante se agitó a temperatura ambiente durante 2 horas adicionales. El producto se aisló mediante filtración y se lavó con acetato de etilo/heptano (1:1, 10 mililitros, 2 veces). El producto se secó al vacío de 40 a 45°C durante 16 horas para obtener 0.702 gramos del compuesto **A**. Rendimiento después de cromatografía RP y precipitación: 64 por ciento.

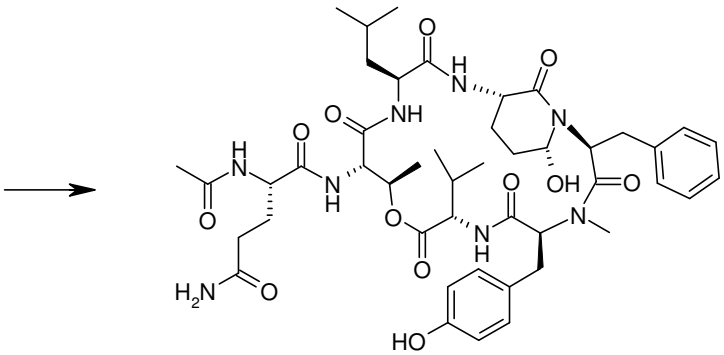
Ejemplo 2: Síntesis del compuesto B (Nostopeptina BN920)

(*S*)-2-acetamido-*N*^{1}-((1*S*,2*S*,5*S*,8*S*,11*R*,12*S*,15*S*,18*S*,21*R*)-2-bencil-21-hidroxi-5-(4-hidroxi-bencil)-15-isobutil-8-isopropil-4,11-dimetil-3,6,9,13,16,11-hexaoxo-10-oxa,1,4,7,14,17-penta-azabiciclo-[16.3.1]-docosan-12-il)-pentan-diamida

Esquema de Reacción 5:







Compuesto B (Nostopeptin BN920)

2A Síntesis de péptido precursor 2 mediante SPPS:

Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Synthon1-H)-Leu-OH

Equipo:

Sintetizador de péptido equipado con un reactor de vidrio de 250 mililitros con frita y recolector para la liberación de solvente automático, agitador y succionador de reactivos.

2A(1) Síntesis de Fmoc-Thr-Leu-Trt-Tentagel-S

La Fmoc-Leu-Trt-Tentagel-S-Resina (18.7 gramos de carga 0.37 milimoles/gramo (suministrada por Rapp Polimere GmBH, Tübingen/Alemania)) se hinchó en N,N-dimetil-formamida mediante agitación durante 30 minutos.

El grupo protector Fmoc se disoció por dos tratamientos subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. Después del lavado de resina mediante varios lavados alternativos con dimetil-formamida e isopropanol, se revisó la remoción completa de las bases en la ausencia de un color rosa después de la adición de fenolftaleína y agua al último paso de lavado.

4.7 gramos de Fmoc-Thr-OH, 5.26 gramos de HATU y 1.8 gramos de di-isopropil-etil-amina se disolvieron en 50 mililitros de N,N-dimetil-formamida. Después de 5 minutos de sacudida adicional, se le agregaron 1.8 gramos de di-isopropil-etil-amina. Después de revisar el pH (>11), la mezcla se le agregó a la resina desprotegida y se sacudió durante 2 horas. La prueba de Kaiser realizada estuvo bien y la resina se lavó mediante varios lavados alternativos con

dimetil-formamida e isopropanol. Después de secar la resina, el peso fue de 19.01 gramos. Una muestra pequeña se disoció y se revisó mediante HPLC. Se mostró un pico principal individual en una conversión satisfactoria.

2A(2) Síntesis de Fmoc-Gln(Trt)-Thr-Leu-Trt-Tentagel-S

19.01 gramos Fmoc-Thr-Leu-Trt-Tentagel-S-Resina (6.6 milimoles) se pre-hincharon en N,N-dimetil-formamida y el grupo protector Fmoc se disoció por dos tratamientos subsiguientes de 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. Después del lavado de resina mediante varios lavados alternativos con dimetil-formamida e isopropanol se revisó la remoción completa de las bases con la ausencia de un color rosa después de la adición de fenolftaleína y agua en el último paso de lavado.

8.07 gramos de Fmoc-Gln(Trt)-OH, 5.01 gramos de HATU y 3.4 gramos de di-isopropil-etil-amina se disolvieron en 50 mililitros de N,N-dimetil-formamida. Después de revisar el pH (>11), la mezcla se le agregó a la resina desprotegida y se sacudió durante 1.5 horas. La prueba de Kaiser realizada estuvo bien y la resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. La resina resultante se utilizó directamente en el siguiente paso más adelante, solo una muestra pequeña se disoció y se revisó mediante HPLC. Se mostró un pico principal individual en una conversión satisfactoria.

2A(3) Síntesis de Ac-Gln(Trt)-Thr-Leu-Trt-Tentagel-S

La resina de Fmoc-Gln(Trt)-Thr-Leu-Trt-Tentagel-S a partir de la anterior se re-hinchó en N,N-dimetil-formamida mediante sacudida en N,N-dimetil-formamida durante 10 minutos. El grupo protector Fmoc se disoció por dos tratamientos subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. Se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa demostró la remoción satisfactoria de piperidina.

0.774 gramos de ácido acético, 6.707 gramos de PyBOP y 3.33 gramos de di-isopropil-etil-amina se disolvieron en 50 mililitros de N,N-dimetil-formamida. Después de revisar el pH (>11), la mezcla se le agregó a la resina desprotegida y se sacudió durante 2.5 horas. La prueba de Kaiser realizada estuvo bien y la resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. La resina resultante se utilizó directamente en la siguiente paso más adelante, solo una muestra pequeña se disoció y se revisó mediante HPLC. Se mostró un pico principal individual en una conversión satisfactoria.

2A(4) Síntesis de Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S (Anteriormente llamado: Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S) (esterificación de cadena lateral)

La resina Ac-Gln(Trt)-Thr-Leu-Trt-Tentagel-S a partir de la anterior se re-hinchó en N,N-dimetil-formamida mediante sacudida en

N,N-dimetil-formamida durante 10 minutos.

8.7 gramos de Fmoc-Val-OH y 8.3 gramos de di-isopropil-etil-amina se disolvieron en 25 mililitros de dicloro-metano. En paralelo, 2.76 gramos de MSNT se disolvieron en otros 25 mililitros de dicloro-metano. Ambas soluciones se combinaron y después de 3 minutos de pre-activación se puso en la resina de péptido y se sacudió durante 2 horas. La resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. La resina resultante se utilizó directamente en el siguiente paso más adelante, solo una muestra pequeña se disoció y se revisó mediante HPLC. Se mostró un pico principal individual en una conversión satisfactoria.

2A(5) Síntesis de Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Fmoc)-Leu-Trt-Tentagel-S (Previamente llamado: Ac-Gln(Trt)-Thr(Val-N-me-Tyr(tBu)-Fmoc)-Leu-Trt-Tentagel-S)

La resina Ac-Gln(Trt)-Thr(Val-Fmoc)-Leu-Trt-Tentagel-S a partir de la anterior se re-hinchó en N,N-dimetil-formamida mediante sacudida en N,N-dimetil-formamida durante 10 minutos.

El grupo protector Fmoc se disoció por dos tratamientos subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa demostró la remoción satisfactoria de piperidina.

6.1 gramos de Fmoc-N-Me-Tyr(tBu)-OH, 4.8 gramos de HATU y 3.3 gramos de di-isopropil-etil-amina se disolvieron en 50 mililitros de N,N-dimetil-formamida. Después de revisar el pH (>11), la mezcla se le agregó a la resina desprotegida y se sacudió durante 2.5 horas. La prueba de Kaiser realizada estuvo bien y la resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. La resina resultante se utilizó directamente en la siguiente paso más adelante, solo una muestra pequeña se disoció y se revisó mediante HPLC. Se mostró un pico principal individual en una conversión satisfactoria.

2A(6) Síntesis de Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Fmoc)-Leu-Trt-Tentagel-S (Anteriormente llamado: Ac-Gln(Trt)-Thr(Val-N-me-Tyr(tBu)-Phe-Fmoc)-Leu-Trt-Tentagel-S)

La resina Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Fmoc)-Leu-Trt-Tentagel-S a partir de la anterior, se re-hinchó en N,N-dimetil-formamida mediante sacudida en N,N-dimetil-formamida durante 10 minutos.

El grupo protector Fmoc se disoció por dos tratamientos subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa demostró la remoción satisfactoria de piperidina.

9.66 gramos de Fmoc-Phe-OH, 9.48 gramos de HATU y 6.4 gramos de di-isopropil-etil-amina se disolvieron en 100 mililitros de N,N-dimetil-formamida. Después de revisar el pH (>11), la mezcla se le agregó a la resina desprotegida y se sacudió durante 2 horas. La prueba de Kaiser realizada estuvo bien y la resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. La resina resultante se utilizó directamente en la siguiente paso más adelante, solo una muestra pequeña se disoció y se revisó mediante HPLC. Se mostró un pico principal individual en una conversión satisfactoria.

2A(7) Síntesis de Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Synthon1-H)-Leu-OH (Anteriormente llamado: Ac-Gln(Trt)-Thr(Val-N-me-Tyr(tBu)-Phe-Synthon1-H)-Leu-OH) (= Péptido precursor 2)

La resina Ac-Gln(Trt)-Thr(Val-Tyr(tBu)Me-Phe-Fmoc)-Leu-Trt-Tentagel-S a partir de la anterior, se re-hinchó en N,N-dimetil-formamida mediante sacudida en N,N-dimetil-formamida durante 10 minutos.

El grupo protector Fmoc se disoció por dos tratamientos subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. se le agregaron fenolftaleína y agua a una muestra de la solución lavada final. La ausencia de color rosa

demostró la remoción satisfactoria de piperidina.

6.77 gramos de sintón 1, 5.9 gramos de PyBOP y 2.95 gramos de di-isopropil-etil-amina se disolvieron en 100 mililitros de N,N-dimetil-formamida. Después de revisar el pH (>11), la mezcla se le agregó a la resina desprotegida y se sacudió durante 2 horas. La prueba de Kaiser realizada estuvo bien y la resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. Después de tomar una muestra pequeña para una revisión de HPLC, el grupo protector Fmoc se disoció por dos tratamientos subsiguientes con el 20 por ciento de piperidina en N,N-dimetil-formamida durante 5 minutos y 15 minutos, respectivamente. La resina se lavó mediante varios lavados alternativos con dimetil-formamida e isopropanol. Finalmente la resina se lavó dos veces con dicloro-metano. La disociación del péptido sintetizado a partir de la resina se logró mediante sacudida en una mezcla de 80 por ciento del ácido acético en dicloro-metano durante la noche. La solución de péptido resultante se filtró y la resina se lavó dos veces con dicloro-metano. Los filtrados combinados se evaporaron y finalmente se purificaron mediante cromatografía RP utilizando un sistema de gradiente. Las fracciones recolectadas se analizaron mediante HPLC y las fracciones puras se combinaron, se evaporaron y finalmente se liofilizaron.

Rendimiento: 3.54 gramos (42 por ciento) de péptido precursor 2.

HR-MS: Calculada para $C_{85}H_{108}N_8O_{13}Si$: $[M+H]^+$ = 1477.78779;

Encontrado: $[M+H]^+$ = 1477.78691.

2B Síntesis del compuesto B

(*S*)-2-acetamido-*N*[1]-((1*S*,2*S*,5*S*,8*S*,11*R*,12*S*,15*S*,18*S*,21*R*)-2-bencil-21-hidroxi-5-(4-hidroxi-bencil)-15-isobutil-8-isopropil-4,11-dimetil-3,6,9,13,16,22-hexaoxo-10-oxa,1,4,7,14,17-penta-azabiciclo-[16.3.1]-docosan-12-il)-pentan-diamida

2B(0) Síntesis del compuesto 9

(*S*)-2-acetamido-*N*[1]-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-9-bencil-6-(4-(terbutoxi)-bencil)-12-(3-((terbutil-difenil-silil)-oxi)-propil)-15-isobutil-3-isopropil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-1-oxa, 4,7,10,13,16-penta-azaciclo-nonadecan-18-il)-*N*[5]-tritil-pentan-diamida

La lactamización de péptido precursor 2 para obtener el compuesto B se llevó a cabo bajo condiciones similares descritas para la preparación del compuesto 6. El producto, el compuesto 9, se caracterizó totalmente mediante IR, RMN y MS. El espectro confirmó la estructura propuesta.

HR-MS: Calculada para $C_{85}H_{106}N_8O_{12}Si$: $[M+H]^+$: 1459.77723; $[M+NH_4]^+$: 1476.80377. Encontrado: $[M+H]^+$: 1459.77719; $[M+NH_4]^+$: 1476.80291.

2B(1) Síntesis del compuesto 10

(*S*)-2-acetamido-*N*^{1}-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-9-bencil-6-(4-(terbutoxi)-bencil)-12-(3-(hidroxi)-propil)-15-isobutil-3-isopropil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-penta-azaciclo-nonadecan-18-il)-*N*^{5}-tritil-pentan-diamida

La reacción de des-sililación para obtener el compuesto 10 se llevó a cabo bajo condiciones similares descritas para la preparación del compuesto 7. El producto se caracterizó totalmente mediante IR, RMN y MS. El espectro confirmó la estructura propuesta.

HR-MS: Calculada para $C_{69}H_{88}N_8O_{12}$: $[M+H]^+$: 1221.65945; $[M+NH_4]^+$: 1238.6860; $[M+Na]^+$: 1243.64139. Encontrado: $[M+H]^+$: 1221.65894; $[M+NH_4]^+$: 1238.68518; {M+Na]$^+$: 1243.64001.

2B(2) Síntesis del compuesto 11 (Aldehído)

(*S*)-2-acetamido-*N*^{1}-((3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-9-bencil-6-(4-(terbutoxi)-bencil)-15-isobutil-3-isopropil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-penta-azaciclo-nonadecan-18-il)-*N*^{5}-tritil-pentan-diamida

La oxidación del compuesto 10 para obtener el compuesto 11 se llevó a cabo bajo condiciones similares descritas para la preparación del compuesto 8. El análisis de LC-MS mostró varios

picos con la masa deseada, indicando la presencia de una mezcla del aldehído, hemiaminal de 5 anillos y hemiaminal de 6 anillos. La mezcla se utilizó como tal para el siguiente paso. HR-MS (pico mayor): Calculada para $C_{69}H_{86}N_8O_{12}$: $[M+H]^+$: 1219.64380; $[M+NH_4]^+$: 1236.67035; $[M+Na]^+$: 1241.62574. Encontrado: $[M+H]^+$: 1219.64404; $[M+NH_4]^+$: 1236.67053; {$M+Na]^+$: 1241.62524.

2B(3) Síntesis del compuesto B (Nostopeptina BN920) a partir del compuesto 11

(*S*)-2-acetamido-*N*[1]-((1*S*,2*S*,5*S*,8*S*,11*R*,12S,15S,18S,21R)-2-bencil-21-hidroxi-5-(4-hidroxi-bencil)-15-isobutil-8-isopropil-4,11-dimetil-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-penta-azabiciclo-[16.3.1]-docosan-12-il)-pentan-diamida

El Compuesto(s) 11 (1.0 gramos, 0.82 milimoles) se disolvió en dicloro-metano (200 mililitros). Se le agregó ácido trifluoro-acético (57.8 gramos), de 15 a 25°C durante 10 minutos y la mezcla de reacción se agitó durante 45 horas a temperatura ambiente. La mezcla de reacción entonces se diluyó con dicloro-metano (200 mililitros), y se le agregó agua (10 mililitros). Se continuó la agitación durante 24 horas adicionales a temperatura ambiente. La mezcla de reacción se vertió sobre una solución saturada acuosa de $NaHCO_3$ (700 mililitros) dentro de 20 minutos y se le agregaron en secuencia dicloro-metano (500 mililitros), isopropanol (50 mililitros), y agua (500 mililitros). Las capas se separaron, y la capa acuosa se

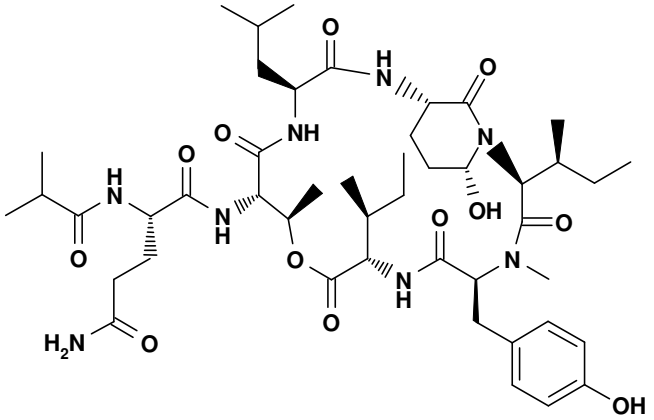
extrajo con una solución de isopropanol (50 mililitros) en dicloro-metano (500 mililitros). Las capas se volvieron a separar y la capa acuosa se extrajo varias veces con dicloro-metano (300 mililitros, 5 veces). Las capas orgánicas se combinaron y el solvente se evaporó bajo presión reducida, para obtener la mezcla del producto crudo (1.0 gramos), la cual comprende la nostopeptina deseada BN920 junto con alcohol de tritilo y otros subproductos de la reacción. El producto crudo se purificó mediante cromatografía en gel de sílice con dicloro-metano/isopropanol como la fase móvil para obtener Nostopeptina BN920 pura (212 miligramos, 97 %(A) de pureza). La recolección de las fracciones menores puras dio 402 miligramos adicionales del producto con 90 %(A) de pureza. Rendimiento a partir de todas las fracciones: 614 miligramos (81 por ciento). El producto se caracterizó totalmente mediante IR, RMN y MS. El espectro confirmó la estructura propuesta.

HR-MS: Calculada para $C_{46}H_{64}N_8O_{12}$: $[M+H]^+$: 921.47165; $[M+NH_4]^+$: 938.49820; $[M+Na]^+$: 943.45359. Encontrado: $[M+H]^+$: 921.47167; $[M+NH_4]^+$: 938.49861; $[M+Na]^+$: 943.45337.

Ejemplo 3: Cambio de equilibrio

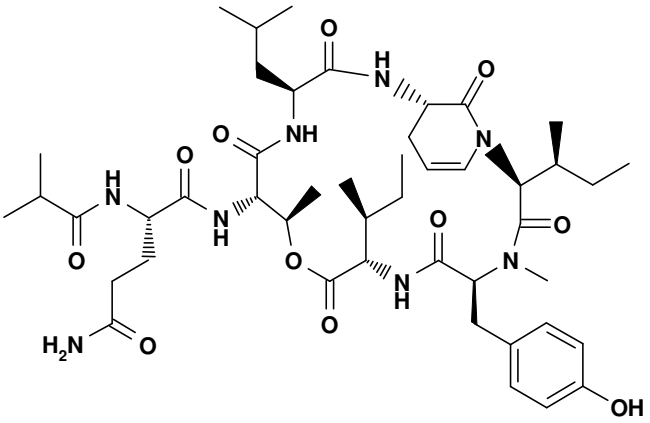
El Esquema de Reacción 4 muestra la presencia de la forma de deshidrato del compuesto A. Ahora se ha descubierto que éste se puede convertir fácilmente (de vuelta) en el compuesto A utilizando a procedimiento simple para la hidratación de la forma de deshidrato representada en el siguiente reacción esquema:

Esquema de Reacción 6:



Compuesto A

$CH_2Cl_2/TFA/H_2O$ ↑ ↓ CH_2Cl_2/TFA



Compuesto A – Deshidrato

Esto permite mejorar el rendimiento del compuesto A en cualquier tipo de síntesis (sea química como la presente divulgación o mediante el uso de fermentación como en la Publicación Internacional Número WO2009/024527).

Por ejemplo, durante disociación de los grupos protectores sensibles al ácido en un compuesto, el cual comprende la subunidad

ahp, por ejemplo, el compuesto A en el Ejemplo 1, se observa la formación de cantidades grandes del subproducto deshidratado correspondiente. Este subproducto usualmente se separa, por ejemplo, mediante cromatografía y se elimina. Esto conduce a la pérdida de producto valioso y rendimiento bajo para este paso. Por ejemplo, si el producto(s) de oxidación del compuesto 8 se somete a condiciones ácidas para disociar los grupos protectores tritil- y terbutilo (esquema 4), se forman cantidades significativas del deshidrato del Compuesto A como subproductos. Dependiendo de la concentración de ácido y las condiciones de reacción, el deshidrato del Compuesto A se podrían formar incluso como el producto mayor en esta mezcla de producto.

Por ejemplo, se observó una proporción del compuesto A/deshidrato del compuesto A (1:2) cuando trifluoro-acético/dicloro-metano del ácido (5:95 volumen/volumen) se utilizó para disociar los grupos protectores después del paso de oxidación (Ejemplo 1).

Por consiguiente se buscó por caminos para convertir el subproducto de deshidrato en el producto deseado. Ahora se ha encontrado que éste se puede lograr mediante equilibración catalizada por ácido de la mezcla del producto en la presencia de agua, bajo condiciones bien definidas. La adición de agua a la mezcla de reacción del Ejemplo 1 y la agitación subsiguiente a temperatura ambiente durante 19 horas, dio una mezcla de producto con una proporción del compuesto A/deshidrato del compuesto A de aproximadamente 96:4. Por consiguiente, la adición de agua a la

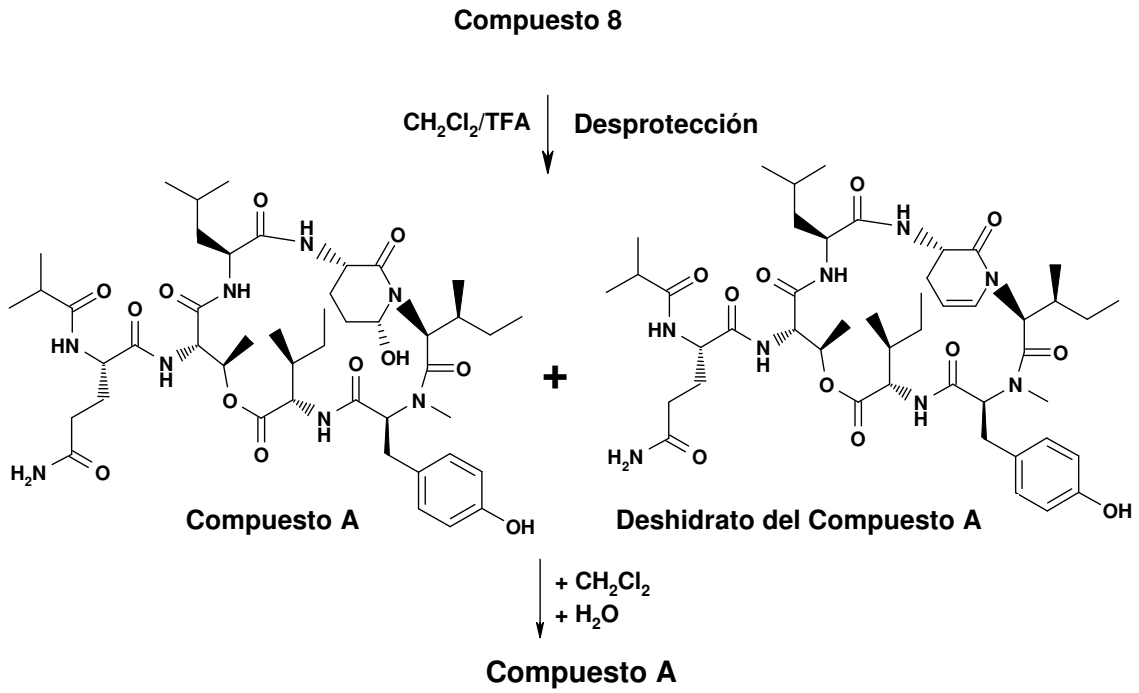
mezcla de reacción después del paso de desprotección catalizada por ácido (esquema 4) cambió la proporción del compuesto A/deshidrato del compuesto A a partir de (1:2) bajo condiciones libres de agua a (96:4) después de la adición agua y equilibración.

La formación del deshidrato del compuesto A a partir del compuesto A bajo condiciones de desprotección ácida se confirmó mediante conversión del compuesto A puro en el deshidrato del compuesto A utilizando ácido trifluoro-acético en dicloro-metano. El tratamiento del compuesto A con 33 por ciento (volumen/volumen), ácido trifluoro-acético en dicloro-metano durante 2 horas a temperatura ambiente dio una mezcla de producto del deshidrato del compuesto A/compuesto A en una proporción de 78:22 de acuerdo con la HPLC. La deshidratación se podría llevar a la conversión de >95 por ciento, cuando se le agregaron a la mezcla de reacción agentes que absorben agua, tales como tamices moleculares. Por consiguiente, la agitación del compuesto A puro en una mezcla 1:2 del ácido trifluoro-acético/DCM en la presencia de tamices moleculares dio el producto deshidratado en un rendimiento crudo cuantitativo y aproximadamente un área de 96 por ciento de pureza de HPLC (Ejemplo 3 B). Todavía hay un área aproximadamente de 4 por ciento del compuesto A presente en el producto crudo.

La conversión del deshidrato del compuesto A a partir de Ejemplo 3B en el compuesto A se demostró agitando el deshidrato del compuesto A en dicloro-metano en la presencia del ácido trifluoro-acético, y agua (Ejemplo 3C). El producto obtenido de esta

manera comprendió un área de 95.6 por ciento del compuesto A y sólo un área de 4.4 por ciento del deshidrato del compuesto A de acuerdo con la HPLC.

Esquema de Reacción 7:



Detalles experimentales para el Ejemplo 3

3A:

23 miligramos de mezcla del producto a partir de la oxidación (el compuesto 8 y aminales cíclicos derivados a partir del mismo) se disolvieron en dicloro-metano (5.7 mililitros), y se le agregó ácido trifluoro-acético (0.3 mililitros), a la solución bajo agitación intensiva. La mezcla de reacción se agitó durante 5 horas a temperatura ambiente, hasta que una IPC (HPLC) indicó una disociación completa

de los grupos protectores. El compuesto A y el deshidrato del compuesto A estuvieron presentes en la mezcla de reacción en una proporción de 1:2, junto con subproductos adicionales. La mezcla de reacción se diluyó con dicloro-metano (5.7 mililitros), y la solución agitada intensivamente se trató con agua (0.23 mililitros). La HPLC después de 19 horas de tiempo agitando a temperatura ambiente indicó una proporción de 96:4 para el compuesto A/deshidrato del compuesto A .

Para el procesamiento, la mezcla de reacción se vertió sobre una solución de acetato de sodio (1.62 gramos) en agua (23 mililitros), y se le agregó acetato de etilo (35 mililitros). Las fases se separaron, y la capa orgánica se extrajo con agua (25 mililitros, 2 veces). Las capas de agua se extrajeron con acetato de etilo (35 mililitros), y las capas orgánicas se combinaron. El solvente se evaporó a presión reducida, para obtener 22 miligramos del producto crudo como una espuma. El producto crudo se purificó mediante cromatografía por evaporación instantánea sobre gel de sílice con acetato de etilo/metanol 95:5 a 90:10 para obtener el compuesto A en un área de 96.8 por ciento de pureza, acompañado por un área de 1.8 por ciento del isómero de anillo 5.

3B:

El compuesto A (250 miligramos) se disolvió en dicloro-metano (10 mililitros), y se le agregó ácido trifluoro-acético (5 mililitros), seguido por la adición de tamices moleculares (1 gramo). La IPC (HPLC) después de 1 hora indicó una proporción de 17:83 para el

compuesto A/deshidrato del compuesto A. La mezcla de reacción se agitó para un total de 72 horas a temperatura ambiente. Para el procesamiento, se removieron los tamices moleculares mediante filtración y la solución se vertió sobre solución saturada acuosa de $NaHCO_3$. La capa acuosa se extrajo con acetato de etilo (50 mililitros). La fase orgánica se lavó con agua (20 mililitros), y el solvente se evaporó a presión reducida, y el residuo se secó al vacío, para obtener rendimiento cuantitativo (245 miligramos) del producto crudo como una espuma. El análisis de HPLC del producto crudo indicó la presencia del compuesto A/deshidrato del compuesto A a una proporción de un área aproximada de 4:96 por ciento.

HR-MS: calculada para $C_{46}H_{70}N_8O_{11}$ $[M+H]^+$= 911.52368, $[M+NH4]^+$= 928.55023, [M+Na]+= 933.50563; encontrado $[M+H]^+$= 911.52372, $[M+NH4]^+$= 928.55029, $[M+Na]^+$= 933.50538. La estructura del deshidrato del compuesto A se confirmó mediante ^{1}H-RMN.

^{1}H-RMN (600 MHz, d_6-DMSO) δ_H: 0.06 (3H, d, J= 6.6 Hz), 0.67 (3H, t, J= 7.3 Hz), 0.70 (3H, d, J= 7.0 Hz), 0.77 (3H, d, J= 6.6 Hz), 0.81 (1H, m), 0.87 (6H, m), 1.00 (3H, d, J= 7.0 Hz), 1.02 (3H, d, J= 7.0 Hz), 1.06 (1H, m), 1.16 (3H, d, J= 6.5 Hz), 1.17 (1H, m), 1.30 (1H, m), 1.41 (1H, m), 1.53 (1H, m), 1.74 (2H, m), 1.91 (2H, m), 2.01 (1H, m), 2.11 (2H, m), 2.45 (3H, m), 2.73 (3H, s), 2.74 (1H, m), 3.18 (1H, m), 4.32 (2H, m), 4.50 (1H, m), 4.54 (1H, m), 4.64 (1H, d, J= 9.5 Hz), 4.77 (1H, d, J= 11.0 Hz), 5.18 (1H, m), 5.26 (1H, m), 5.42 (1H, q, J= 6.6 Hz), 6.25 (1H, d, J= 7.3 Hz), 6.32 (1H, d, J= 7.7 Hz), 6.67

(2H, d, J= 8.4 Hz), 6.75 (1H, s), 7.04 (2H, d, J= 8.4 Hz), 7.25 (1H, s), 7.30 (1H, d, J= 8.8 Hz), 7.89 (1H, d, J= 9.2 Hz), 7.98 (1H, d, J= 8.1 Hz), 8.50 (1H, d, J= 8.4 Hz), 9.24 (1H, s).

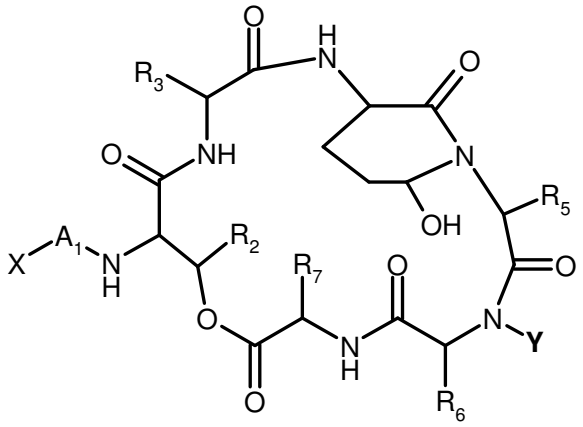
3C:

El deshidrato del compuesto A (110 miligramos) a partir de ejemplo 2 se disolvió en dicloro-metano (20 mililitros). Se le agregaron ácido trifluoro-acético (1 gramo), y agua (0.2 mililitros) y la mezcla de reacción se agitó durante 20 horas a temperatura ambiente. Para el procesamiento, la mezcla de reacción se vertió sobre acetato de etilo (50 mililitros), y la solución de acetato de etilo se extrajo con una solución saturada acuosa de $NaHCO_3$ (50 mililitros). La capa orgánica se extrajo con agua (20 mililitros), y el solvente se evaporó. El residuo se disolvió en acetato de etilo/isopropanol 9:1 (20 mililitros), y la solución se aclaró filtrada sobre un filtro de 0.45 micras. El solvente se evaporó bajo presión reducida, y el residuo se secó al vacío a 45°C para obtener 100 miligramos producto crudo. El análisis de HPLC indicó la presencia de un área aproximada de 95.6 por ciento del compuesto A y un área aproximada de 4.4 por ciento del deshidrato del compuesto A.

REIVINDICACIONES

Habiendo así especialmente descripto y determinado la naturaleza de la presente invencion y la forma como la misma ha de ser llevada a la práctica, se declara reivindicar como de propiedad y derecho exclusivo:

1. Un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:



I

en donde:

A_1 es una fracción bivalente de un aminoácido con un grupo carboxilo o carbamoílo terminal, en especial asparagina o glutamina, y se enlaza en su lado derecho en la fórmula I por medio de un carbonilo con el resto de la molécula; o es alcanoílo de 1 a 8 átomos de carbono o hidroxi-alcanoílo de 1 a 8 átomos de carbono fosforilado;

X se enlaza por medio de un N de A_1 y es acilo, o está ausente si A_1 es alcanoílo de 1 a 8 átomos de carbono o hidroxi-

alcanoílo de 1 a 8 átomos de carbono fosforilado;

R_2 es alquilo de 1 a 8 átomos de carbono, en especial metilo;

R_3 es la cadena lateral de un aminoácido, en especial de leucina, isoleucina o valina;

R_5 es la cadena lateral de un aminoácido, de preferencia de fenilalanina, leucina, isoleucina o valina;

R_6 es la cadena lateral de un hidroxi-aminoácido, en especial de tirosina;

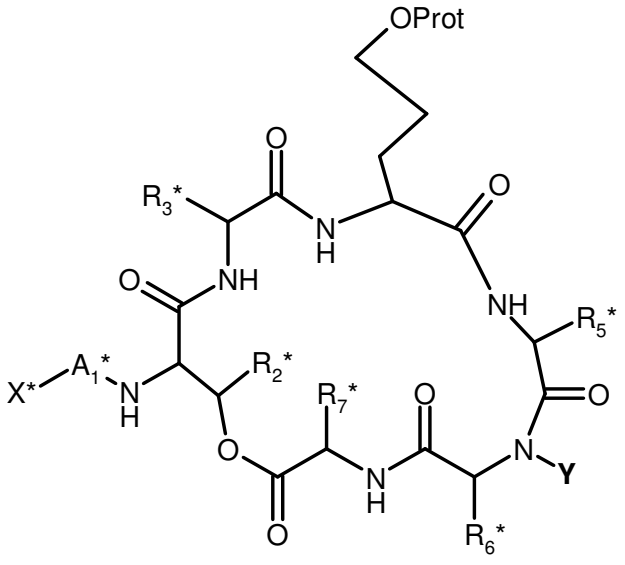
R_7 es la cadena lateral de un aminoácido, de preferencia del aminoácido leucina, isoleucina o valina; e

Y es hidrógeno o alquilo de 1 a 8 átomos de carbono;

o una sal del mismo,

comprendiendo este método:

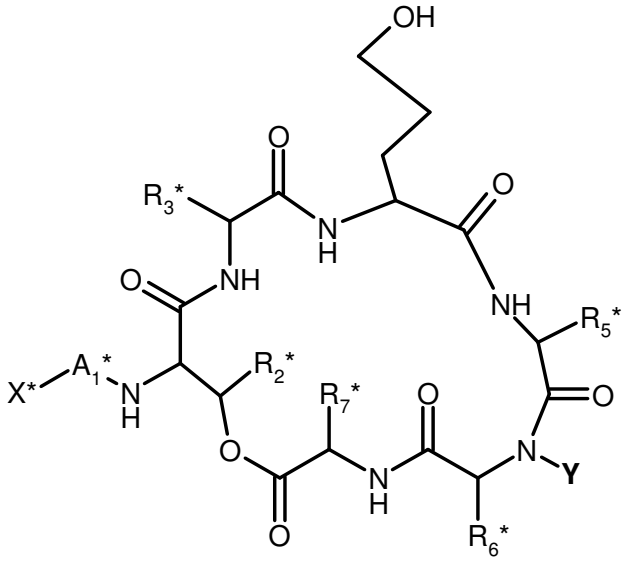
desproteger selectivamente un compuesto de la fórmula II



II

en donde Prot es un grupo protector, Y es como se define

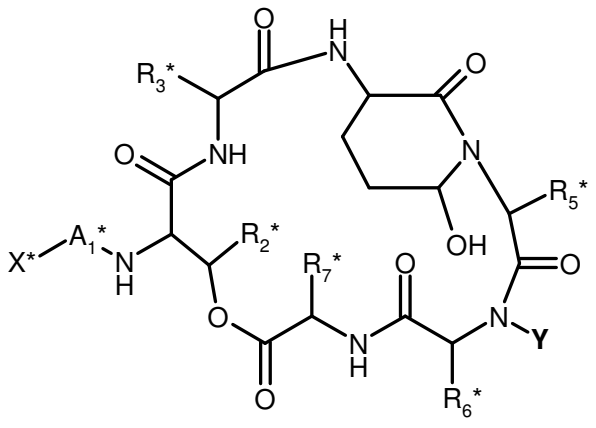
para un compuesto de la fórmula I, y X*, A_1*, R_2*, R_3*, R_5*, R_6*, y R_7* corresponden a X, A_1, R_2, R_3, R_5, R_6, y R_7 en la fórmula I, respectivamente, pero con la condición de que los grupos funcionales reactivos sobre estas fracciones están presentes en una forma protegida cuando menos si podrían participar en reacciones secundarias indeseadas, para dar como resultado un compuesto de la fórmula III:



III

en donde X*, A_1*, R_2*, R_3*, R_5*, R_6*, y R_7* tienen los significados definidos anteriormente,

hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula IV:



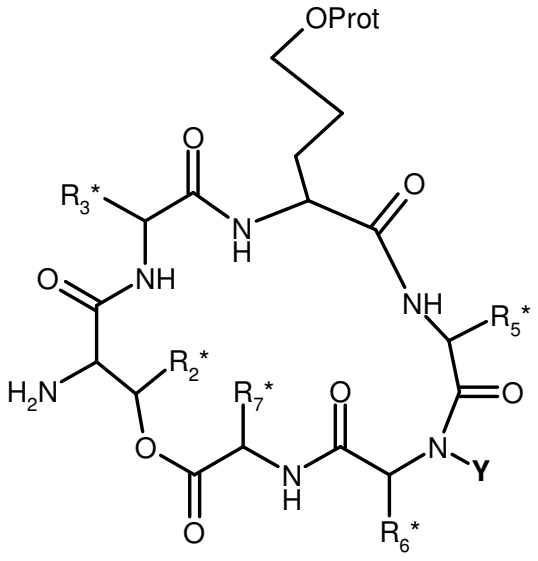
IV

y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula I, o una sal del mismo,

y, opcionalmente, convertir un compuesto libre de la fórmula I en una sal, una sal de un compuesto de la fórmula I en una sal diferente de un compuesto de la fórmula I o en el compuesto libre de la fórmula I, y/o convertir un análogo deshidratado de un compuesto de la fórmula I en el compuesto correspondiente de la fórmula I.

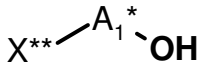
2. El método o proceso de acuerdo con la reivindicación 1, en donde el compuesto de la fórmula IV se sintetiza mediante una combinación de síntesis de péptidos en fase sólida y síntesis en fase de solución a partir de los aminoácidos de partida y precursores de cadena lateral correspondientes.

3. El método o proceso de acuerdo con cualquiera de las reivindicaciones anteriores, el cual además comprende, para la síntesis de un compuesto de la fórmula II mostrado en la reivindicación 1, hacer reaccionar un compuesto de la fórmula VI:



VI

en donde Prot es un grupo protector, Y es como se define para un compuesto de la fórmula I, y R_2*, R_3*, R_5*, R_6*, y R_7* son como se definen para un compuesto de la fórmula II en la reivindicación 1, con un ácido de la fórmula VII:



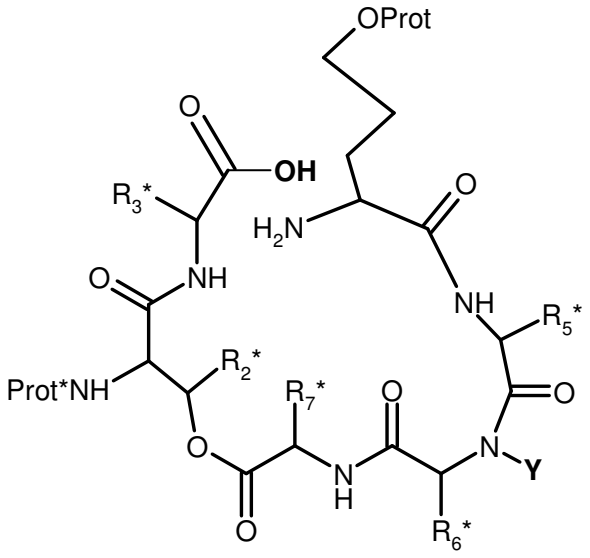
VII

o un derivado reactivo del mismo,

en donde X** es un grupo protector de amino, o es X*, y en donde X* y A_1* son como se definen para un compuesto de la fórmula II en la reivindicación 1; y, si X** es un grupo protector de amino, remover el grupo protector de amino X** para proporcionar H en lugar de X*, y acoplar el grupo amino resultante con un grupo acilo X* utilizando el ácido correspondiente X*-OH, en donde C* es como se define para un compuesto de la fórmula II en la reivindicación 1, o un derivado reactivo del mismo.

4. El método o proceso de acuerdo con la reivindicación 3, el cual además comprende la ciclación bajo lactamización de un péptido precursor lineal del compuesto de la fórmula VI, que lleva un grupo amino N-terminal y un grupo carboxilo C-terminal, en condiciones de reacción que permitan la formación de un enlace de amida a partir del grupo amino y el grupo carboxilo, de preferencia utilizando la química en fase de solución.

5. El método o proceso de acuerdo con la reivindicación 4, en donde el péptido precursor lineal es de la fórmula VIII:



VIII

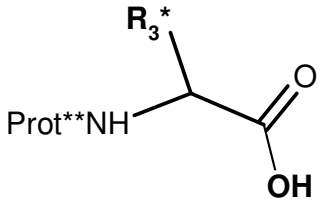
en donde Prot* es un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y es estable durante los pasos de desprotección durante la síntesis del péptido precursor lineal (por ejemplo, aliloxi-carbonilo), y R_2*, R_3*, R_5*, R_6*, y R_7* son como se definen para un compuesto de la fórmula VI en la reivindicación 4, el cual además comprende, después de la ciclación del compuesto de la fórmula VIII,

en especial VIIIA, remover el grupo protector Prot* *in situ* para proporcionar el compuesto de la fórmula VI.

6. El método o proceso de acuerdo con la reivindicación 5, en donde el péptido precursor lineal de la fórmula VIII, en especial VIIIA, se sintetiza a partir de los aminoácidos correspondientes mediante la síntesis de péptidos en fase sólida y la subsiguiente disociación a partir de la fase sólida empleada.

7. El método de acuerdo con la reivindicación 6, el cual comprende cualquiera de:

en una variante a), acoplar un aminoácido de la fórmula IX:

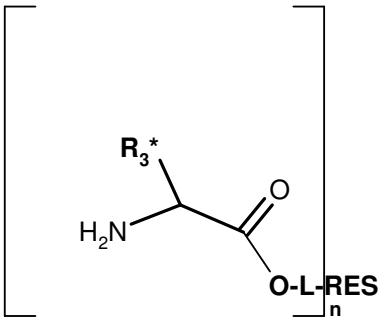


IX

en donde R_3* es como se define para un compuesto de la fórmula II en la reivindicación 1, y Prot** es un grupo protector de amino que se puede remover de la resina sin disociar otros enlaces, o un derivado reactivo de este aminoácido, por medio de un oxígeno, a un enlazador disociable L, el cual se enlaza a una resina sólida RES,

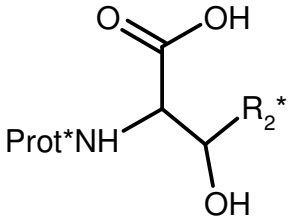
y remover el grupo protector Prot**;

acoplar el aminoácido enlazado con resina obtenido simbolizado por la fórmula X:



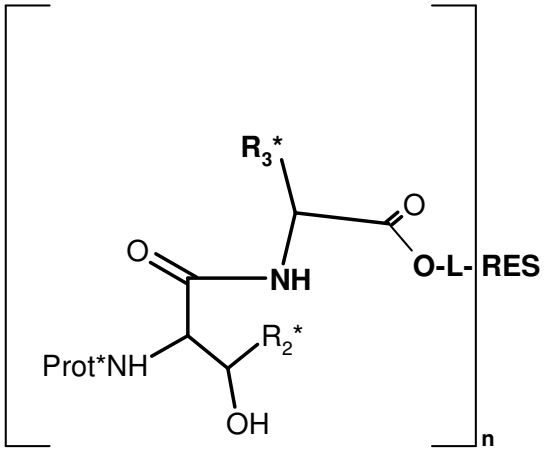
X

en donde RES y R_3* son como se definen para un compuesto de la fórmula IX, n es un número natural, y L es un enlazador disociable, con un aminoácido de la fórmula XI:



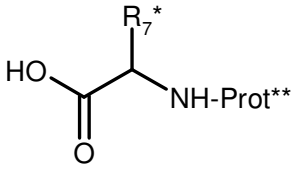
XI

en donde Prot* es como se define para un compuesto de la fórmula VIII en la reivindicación 5, y R_2* es como se define para un compuesto de la fórmula II en la reivindicación 1, o un derivado reactivo de este aminoácido, acoplar el dipéptido enlazado con resina obtenido simbolizado por la fórmula XII:



XII

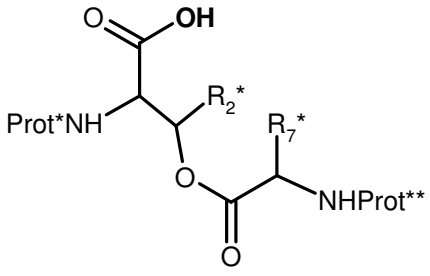
en donde Prot* es como se define para un compuesto de la fórmula VIII en la reivindicación 5, R_2* y R_3* son como se definen para un compuesto de la fórmula II en la reivindicación 1, y n, L y RES son como se definen para un compuesto de la fórmula X, por medio del grupo hidroxilo libre, con un aminoácido de la fórmula XIII:



XIII

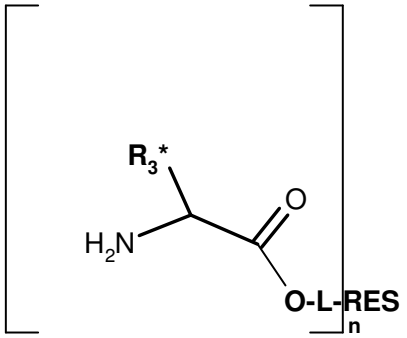
en donde Prot** es como se define para un compuesto de la fórmula IX, y R_7* es como se define para un compuesto de la fórmula II en la reivindicación 1, o un derivado reactivo de este aminoácido, y remover el grupo protector Prot**;

o, en una variante b), acoplar un dipéptido de la fórmula XXVII:



XXVII

en donde R_3* y Prot** son como se describen para un compuesto de la fórmula IX, en especial IXA, y Prot* es como se define para un compuesto de la fórmula VIII en la reivindicación 5, o un derivado reactivo de este dipéptido, con una fracción de amino-acilo, enlazada por medio de un oxígeno, a un enlazador disociable L, el cual se enlaza a una resina sólida RES, que tiene la fórmula X:

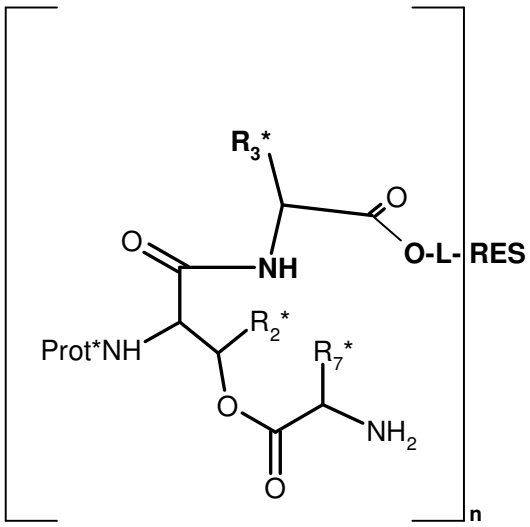


X

que se puede obtener como se describe bajo la variante a), en donde RES y R_3* son como se definen para un compuesto de la fórmula IX, y L y RES son como justamente se definieron;

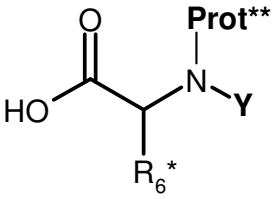
y remover el grupo protector Prot**;

y, después de las reacciones de la variante a) o de la variante b), acoplar el compuesto obtenido de la fórmula XIV:



XIV

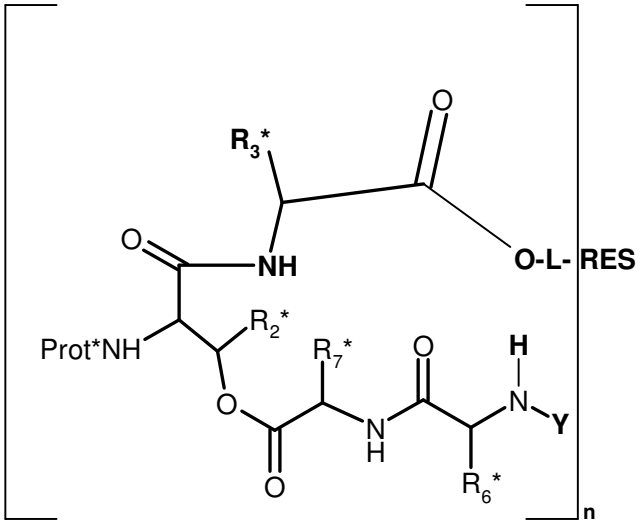
en donde R_2*, R_3* y R_7* son como se definen para un compuesto de la fórmula II en la reivindicación 1, Prot* es como se define para un compuesto de la fórmula VIII en la reivindicación 5, y n, L y RES son como se definen para un compuesto de la fórmula X, con un aminoácido de la fórmula XV:



XV

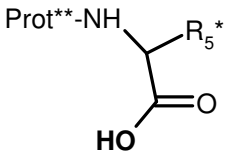
en donde R_6* e Y son como se definen para un compuesto de la fórmula II en la reivindicación 1, y Prot** es como se define para un compuesto de la fórmula IX, o un derivado reactivo de este aminoácido, y remover el grupo protector Prot**;

acoplar el compuesto obtenido de la fórmula XVI:



XVI

en donde Y, R_2*, R_3*, R_7* y R_6* son como se definen para un compuesto de la fórmula II en la reivindicación 1, Prot* es como se define para un compuesto de la fórmula VIII en la reivindicación 5, y n, L y RES son como se definen para un compuesto de la fórmula X, con un aminoácido de la fórmula XVII:

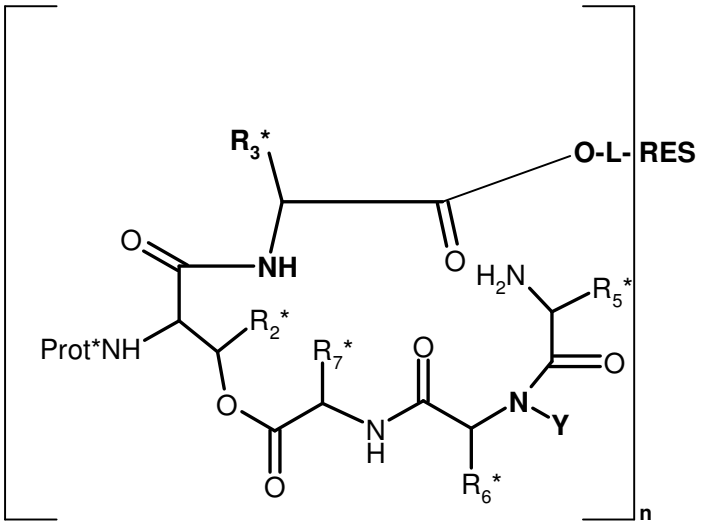


XVII

en donde R_5* es como se define para un compuesto de la fórmula II en la reivindicación 1, y Prot** es como se define para un compuesto de la fórmula IX, o un derivado reactivo de este aminoácido, y remover el grupo protector Prot**,

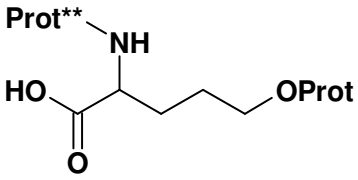
y finalmente acoplar el compuesto resultante de la

fórmula XVIII:



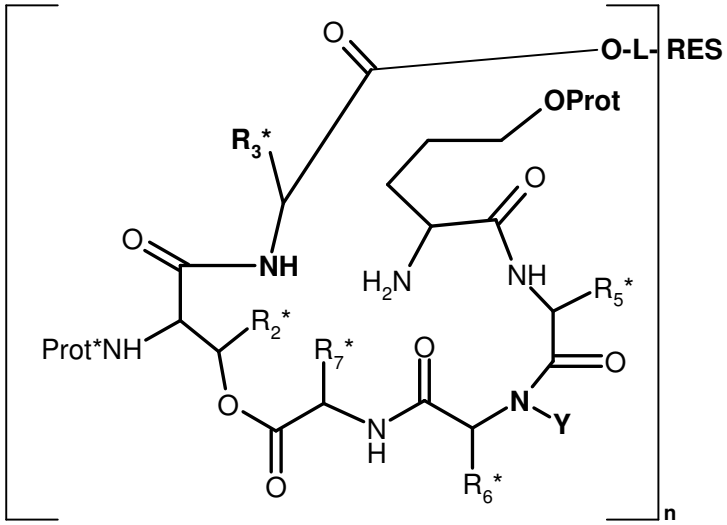
XVIII

en donde Y, R_2*, R_3*, R_7*, R_6* y R_5* son como se definen para un compuesto de la fórmula II en la reivindicación 1, Prot* es como se define para un compuesto de la fórmula VIII en la reivindicación 5, y n, L y RES son como se definen para un compuesto de la fórmula X, con un sintón de la fórmula XIX:



XIX

en donde Prot es como se define para un compuesto de la fórmula II en la reivindicación 1, y Prot** es como se define para un compuesto de la fórmula IX, o un derivado activado de dicho sintón, y remover el grupo protector Prot**, para proporcionar un compuesto de la fórmula XX:



XX

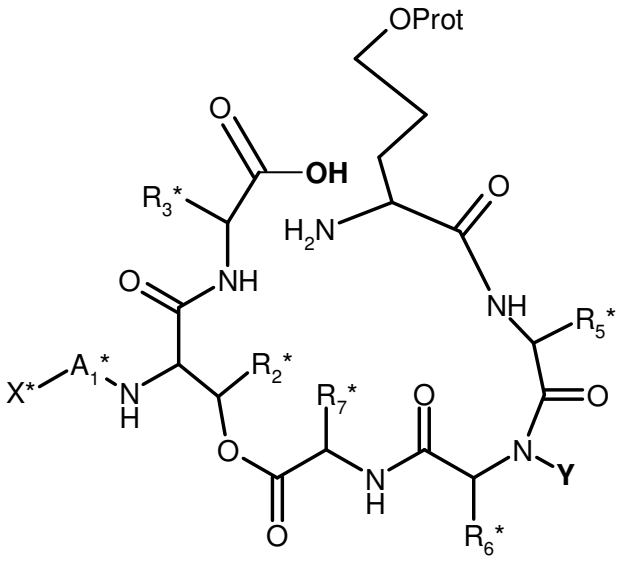
en donde Prot, Y, R_2*, R_3*, R_7*, R_6* y R_5* son como se definen para un compuesto de la fórmula II en la reivindicación 1, Prot* es como se define para un compuesto de la fórmula VIII en la reivindicación 5, y n, L y RES son como se definen para un compuesto de la fórmula X,

y disociar el péptido enlazado en fase sólida en la fórmula XX del L-RES en fase sólida, para proporcionar el compuesto correspondiente de la fórmula VIII, en especial VIIIA, como se muestra en la reivindicación 5.

8. El método o proceso de acuerdo con la reivindicación 1 o con la reivindicación 2, el cual comprende, para la síntesis del compuesto de la fórmula II de la reivindicación 1, ciclar bajo la lactamización de un péptido precursor lineal del compuesto de la fórmula II, que lleva un grupo amino N-terminal y un grupo carboxilo

C-terminal, en condiciones de reacción que permitan la formación de un enlace de amida a partir del grupo amino y el grupo carboxilo, de preferencia utilizando la química en fase de solución.

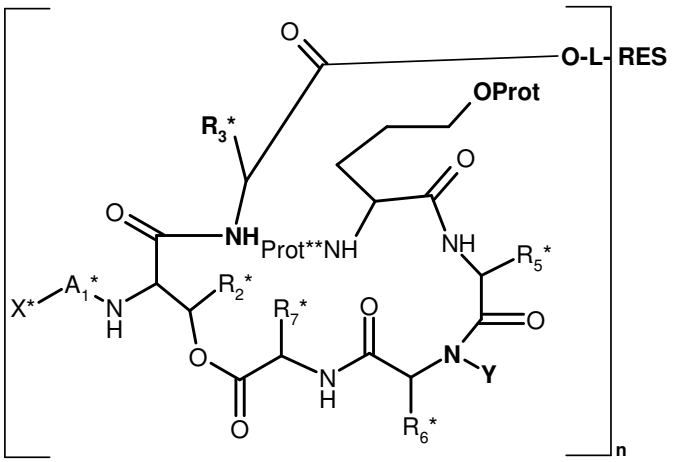
9. El método o proceso de acuerdo con la reivindicación 8, en donde el péptido precursor lineal es de la fórmula XXV:



XXV

en donde X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* y Prot son como se definen para un compuesto de la fórmula II en la reivindicación 1.

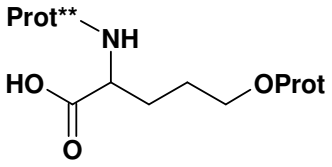
10. El método o proceso de acuerdo con la reivindicación 9, el cual además comprende, para la síntesis del compuesto de la fórmula XXV, disociar un compuesto de la fórmula XXIV:



XXIV

en donde X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* y Prot son como se definen para un compuesto de la fórmula II en la reivindicación 1, L es un enlazador disociable, RES es una resina sólida, n es un número natural, y Prot** es un grupo protector de amino que se puede remover sin la remoción paralela del grupo protector Prot y con el producto restante sobre la resina, y (antes de la disociación, en paralelo o subsiguientemente a la misma) remover el grupo protector Prot**, para proporcionar el compuesto de la fórmula XXV.

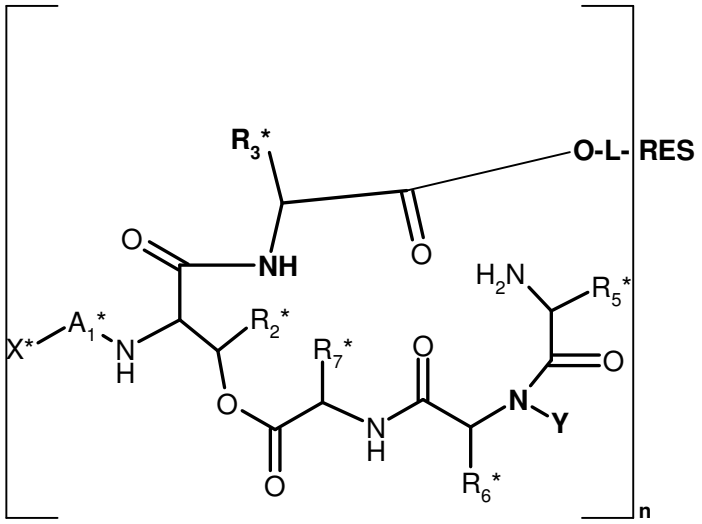
11. El método o proceso de acuerdo con la reivindicación 10, el cual además comprende, para la síntesis del compuesto de la fórmula XXIV, acoplar un aminoácido de la fórmula XIX:



XIX,

en donde Prot es como se define para un compuesto de la fórmula II en la reivindicación 1, y Prot** es como se define para un compuesto de la fórmula XXIV en la reivindicación 10, o un

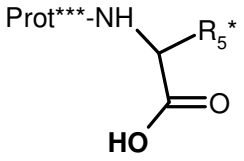
derivado activado de este aminoácido, con un compuesto de la fórmula XXIII:



XXIII

en donde X*, A_1*, R_2*, R_3*, R_5*, R_6* y R_7* son como se definen para un compuesto de la fórmula II en la reivindicación 1, L es un enlazador disociable, RES es una resina sólida, y n es un número natural.

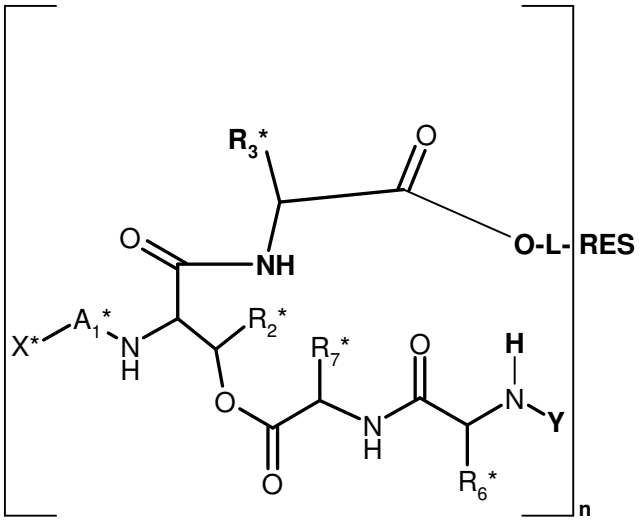
12. El método o proceso de acuerdo con la reivindicación 11, el cual además comprende, para la síntesis del compuesto de la fórmula XXIII, acoplar un aminoácido de la fórmula XVII*:



XVII*,

en donde R_5* es como se define para un compuesto de la fórmula II en la reivindicación 1, y Prot*** es un grupo protector de amino que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la

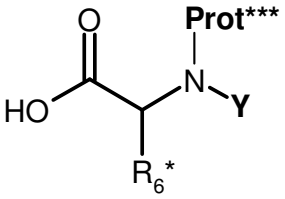
resina, o un derivado reactivo de este aminoácido, con un compuesto de la fórmula XXII:



XXII

en donde X*, A_1*, R_2*, R_3*, R_6* y R_7* son como se definen para un compuesto de la fórmula II en la reivindicación 1, L es un enlazador disociable, RES es una resina sólida, y n es un número natural, y remover el grupo protector Prot***.

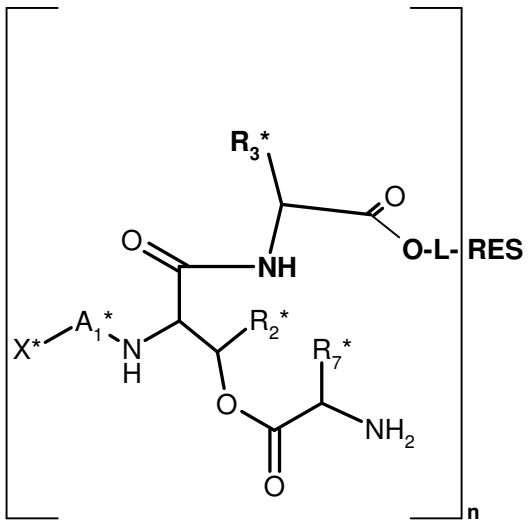
13. El método o proceso de acuerdo con la reivindicación 12, el cual además comprende, para la síntesis del compuesto de la fórmula XXII, acoplar un aminoácido de la fórmula XV*:



XV*

en donde R_6* e Y son como se definen para un compuesto de la fórmula II en la reivindicación 1, y Prot*** es un grupo protector de amino que se puede disociar selectivamente sin

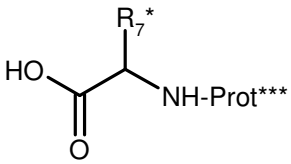
afectar a otros grupos protectores presentes, y con el producto restante sobre la resina, o un derivado reactivo de este aminoácido, con un compuesto de la fórmula XXI:



XXI,

en donde X*, A_1*, R_2*, R_3* y R_7* son como se definen para un compuesto de la fórmula II en la reivindicación 1, L es un enlazador disociable, RES es una resina sólida, y n es un número natural, y remover el grupo protector Prot***.

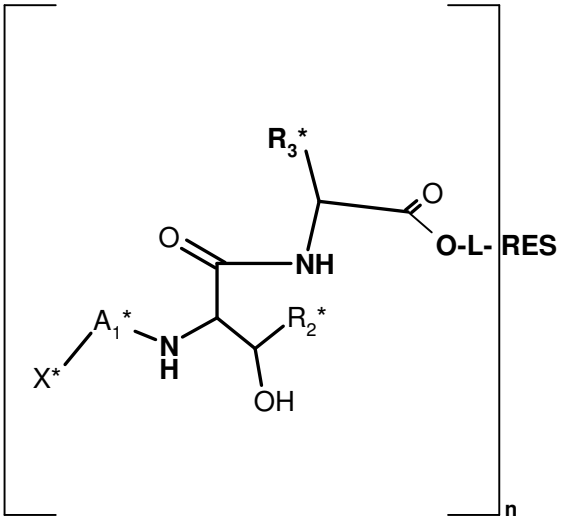
14. El método o proceso de acuerdo con la reivindicación 13, el cual además comprende, para la síntesis de un compuesto de la fórmula XXI, hacer reaccionar un aminoácido de la fórmula XIII*:



XIII*

en donde Prot*** es un grupo protector de amino que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina, y R_7* es como se define para un compuesto de la fórmula II en la reivindicación 1, o un derivado reactivo de este aminoácido,

con el grupo hidroxilo de un compuesto de la fórmula XXVI:

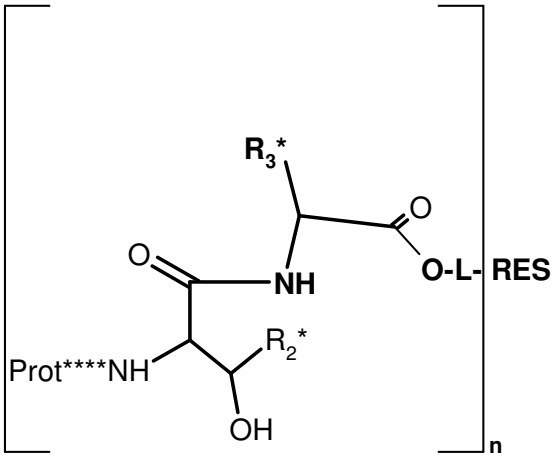


XXVI

en donde X*, A_1*, R_2* y R_3* son como se definen para un compuesto de la fórmula II en la reivindicación 1, L es un enlazador disociable, RES es una resina sólida, y n es un número natural;

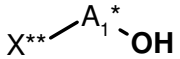
y remover el grupo protector Prot***.

15. El método o proceso de acuerdo con la reivindicación 14, el cual además comprende, para la síntesis de un compuesto de la fórmula XXVI, acoplar un dipéptido enlazado con resina simbolizado por la fórmula XII*:



XII*

en donde Prot**** es un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes en un compuesto de la fórmula II como se define en la reivindicación 1, y con el producto restante sobre la resina, R_2* y R_3* son como se definen para un compuesto de la fórmula II en la reivindicación 1, L es un enlazador disociable, RES es una resina sólida, y n es un número natural, después de la remoción del grupo protector Prot**** por medio del grupo amino libre obtenido de esta manera, con un ácido de la fórmula VII:



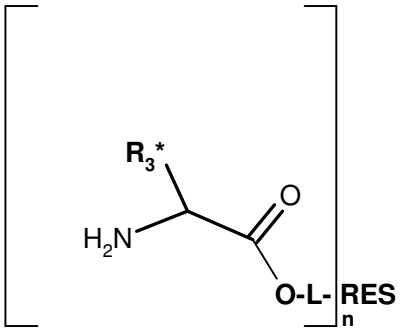
VII

en donde X** es un grupo protector de amino, o es X*, y en donde X* y A_1* son como se definen para un compuesto de la fórmula II en la reivindicación 1, o un derivado reactivo de dicho ácido;

y, si X** es un grupo protector de amino, remover el grupo protector de amino X** para proporcionar H en lugar de X*, y

acoplar el grupo amino resultante con un grupo acilo X* utilizando el ácido correspondiente X*-OH, en donde X* es como se define para un compuesto de la fórmula II en la reivindicación 1, o un derivado reactivo de dicho ácido.

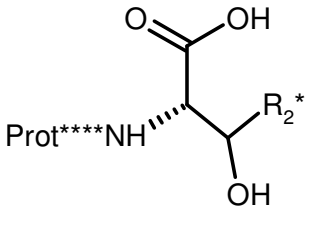
16. El método o proceso de acuerdo con la reivindicación 15, el cual además comprende, para la síntesis de un compuesto de la fórmula XII, acoplar un aminoácido enlazado con resina simbolizado por la fórmula X:



X

en donde R_3* es como se define para un compuesto de la fórmula II en la reivindicación 1, L es un enlazador disociable, RES es una resina sólida, y n es un número natural,

con un aminoácido de la fórmula XI*:

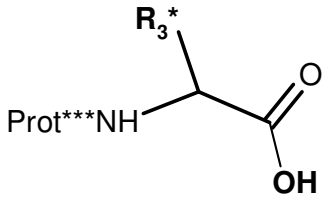


XI*,

en donde Prot**** es un grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina, y R_2* es como

se define para un compuesto de la fórmula II en la reivindicación 1, o un derivado reactivo de dicho aminoácido.

17 El método o proceso de acuerdo con la reivindicación 16, el cual además comprende, para obtener el aminoácido enlazado con resina de la fórmula X, acoplar un aminoácido de la fórmula IX*:



IX*

en donde R_3* es como se define para un compuesto de la fórmula II en la reivindicación 1, y Prot*** es un grupo protector de amino, se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y con el producto restante sobre la resina; o un derivado reactivo de dicho aminoácido de la fórmula IX, con un grupo hidroxilo enlazado por medio de un enlazador disociable L, el cual se enlaza a una resina sólida RES, y remover el grupo protector Prot***.

18. El método o proceso de acuerdo con cualquiera de las reivindicaciones 1 a 16, en donde los símbolos A_1, R_2, R_3, R_5, R_6, R_7, X e Y, o las fracciones no protegidas o protegidas correspondientes R_2*, R_3*, R_5*, R_6*, R_7*, X* e Y_3 se seleccionan de tal manera que, en el compuesto resultante de la fórmula I, o una sal del mismo,

A_1 es el radical bivalente de L-glutamina enlazada por medio del carbonilo de su grupo α-carboxilo con el grupo amino a la derecha de A_1 en la fórmula I, y por medio de su grupo α-amino con

X, o es 2S-(2-hidroxi-3-fosfono-oxi)-propionilo;

R_2 es metilo;

R_3 es isopropilo, isobutilo o bencilo, en especial isobutilo;

R_5 es butilo secundario o bencilo, en especial butilo secundario;

R_6 es 4-hidroxi-bencilo;

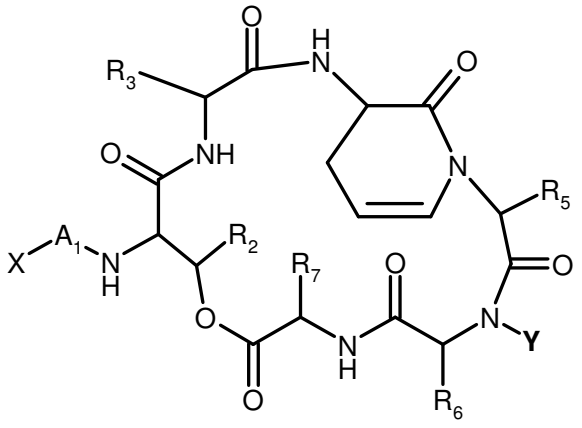
R_7 es isopropilo o butilo secundario, en especial butilo secundario;

X es acetilo, o isobutirilo, o está ausente si A_1 es 2S-(2-hidroxi-3-fosfono-oxi)-propionilo;

e

Y es metilo.

19. Un método o proceso para convertir un deshidrato de un compuesto de la fórmula I dado en la reivindicación 1, o con los sustituyentes como se definen en la reivindicación 18 en el compuesto correspondiente de la fórmula I, en donde el deshidrato tiene la fórmula V:

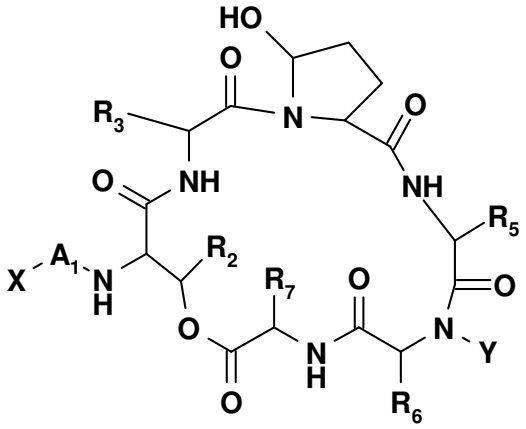


V

en donde Y, X, A_1, R_2, R_3, R_5, R_6 y R_7 son como se

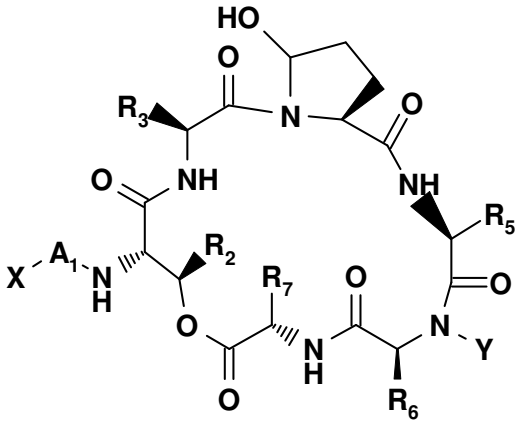
definen para un compuesto de la fórmula I en cualquiera de las reivindicaciones 1 y 17;

y/o su análogo de hemiaminal correspondiente con una estructura de cinco anillos en lugar de la estructura ahp en la fórmula I, que también se puede formar como un subproducto, y tiene la fórmula V*:



V*

en especial la fórmula VA*:



VA*,

en donde Y, X, A_1, R_2, R_3, R_5, R_6 y R_7 son como se definen para un compuesto de la fórmula I en cualquiera de las reivindicaciones 1 y 17, respectivamente;

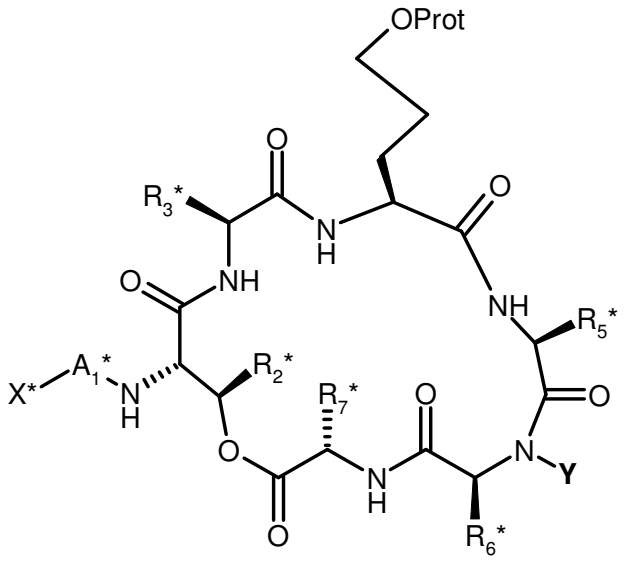
o un método o proceso para cambiar el equilibrio de una

mezcla de un compuesto de la fórmula I, y su deshidrato correspondiente y/o hemiaminal en favor del compuesto de la fórmula I,

comprendiendo este método o proceso utilizar un ácido acuoso como solvente reactivo para impulsar la reacción.

20. El método de acuerdo con la reivindicación 19, en donde el ácido es un ácido carboxílico, en especial un ácido alcanoico de 1 a 8 átomos de carbono sustituido por halógeno, más especialmente ácido trifluoro-acético o ácido tricloro-acético.

21. Un compuesto de la fórmula II:

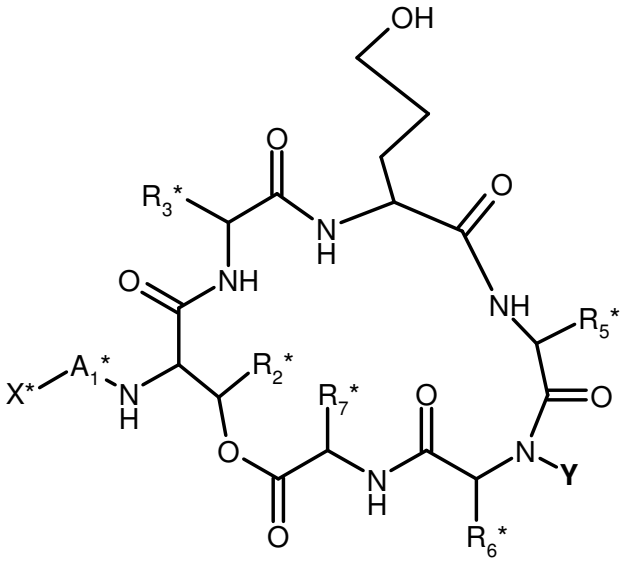


II

en donde Prot es un grupo protector, Y es como se define para un compuesto de la fórmula I en la reivindicación 1 o en la reivindicación 17, y X*, A_1*, R_2*, R_3*, R_5*, R_6*, y R_7* corresponden a X, A_1, R_2, R_3, R_5, R_6, y R_7 en la fórmula I como se define en la reivindicación 1 o en la reivindicación 17, respectivamente, sin embargo con la condición de que los grupos funcionales reactivos

sobre estas fracciones están presentes en una forma desprotegida o protegida.

22. Un compuesto de la fórmula III:



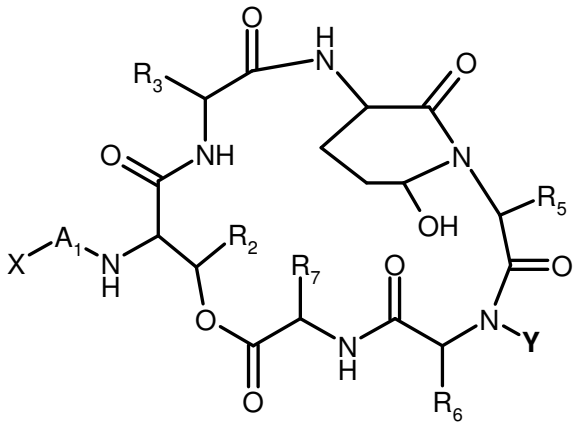
III

en donde Y es como se define para un compuesto de la fórmula I en la reivindicación 1 o en la reivindicación 17, y X*, A_1*, R_2*, R_3*, R_5*, R_6*, y R_7* corresponden a X, A_1, R_2, R_3, R_5, R_6, y R_7 en la fórmula I como se define en la reivindicación 1 o en la reivindicación 17, respectivamente, sin embargo con la condición de que los grupos funcionales reactivos diferentes del hidroxilo representado en la parte superior de la fórmula III sobre estas fracciones, están presentes en una forma desprotegida o protegida.

p.de: NOVARTIS AG

RESUMEN

La invención se refiere a un método o proceso para la elaboración química de los depsipéptidos de la fórmula I:



I

en donde los símbolos tienen el significado definido en la descripción, a nuevos intermediarios y su elaboración, así como a las modalidades de la invención relacionadas.

* * * * *

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PAT054610-WO-PCT	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/IB2012/051977	International filing date *(day/month/year)* 19.04.2012	Priority date *(day/month/year)* 20.04.2011
International Patent Classification (IPC) or national classification and IPC INV. C07K11/02		
Applicant Novartis AG		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 27 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*bis* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	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 Article 35(2) 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

Date of submission of the demand	Date of completion of this report
20.02.2013	22.08.2013
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	Authorized officer Vogt, Titus Telephone No. +49 89 2399-8477

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ 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 (under Rules 12.3(a) and 23.1(b))
   ☐ publication of the international application (under Rule 12.4(a))
   ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-90 as originally filed

   Claims, Numbers

   1-22 filed with the demand for preliminary international examination

   ☐ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:

5. ☐ 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 70.2 (e)).

6. ☐ Supplementary international search report(s) from Authority(ies) have been received and taken into account in drawing up this report (Rule 45bis.8(b) and (c)).

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees, the applicant has, within the applicable time limit:

   ☐ restricted the claims.

   ☐ paid additional fees.

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

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

   ☐ neither restricted the claims nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

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

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

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

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Item IV

Lack of unity of invention.

Art. 17(3)(a) and Rule 13 PCT require that the international application shall relate to one invention only or to a group of inventions so linked as to form a single general novel and inventive concept, which is defined by special technical features that make a contribution over the cited prior art (cf. Guidelines 10.01-10.02).

The present application is directed to the synthesis of cyclic depsipeptides having an AHP moiety.

The solution of the applicant as identified by the method of claim 1 is to oxidise a homoserine moiety to form the AHP moiety.

This solution however is not new, because it is disclosed by Yokokawa et al. (2005, 2002 and 2001). The present application is thus directed to the provision of an improved method for the synthesis of cyclic depsipeptides.

The claims appear to comprise two separate non-related solutions to this general problem, characterised by present claims 7 and 19. The solution of claim 7 focuses on an alternative method to prepare a cyclic depsipeptide intermediate by preparing a linear depsipeptide using solid phase peptide synthesis followed by cyclisation under lactamization with no relation to the presence or absence of an AHP moiety in the cyclic depsipeptide, whereas claim 19 focuses solely on the formation of the AHP moiety with no relation to the method of preparing the cyclic depsipeptide.

Hence the present claims comprise two inventions directed to two different non-related problems in the synthesis of cyclic depsipeptides containing AHP moieties.

The search report was completed for both inventions.
This international preliminary examination report will comment on both inventions.

Item V

Reasoned Statement.

<u>Subject matter of the present application.</u>

The present application is directed to the total synthesis of cyclic depsipeptides having an AHP moiety.

<u>Cited prior art documents (Rule 64(1) PCT).</u>

Reference is made to the following documents:

D1 YOKOKAWA ET AL. (2005) TETRAHEDRON 61, 1459-1480.

D2 YOKOKAWA ET AL. (2002) TETRAHEDRON LETT. 43, 8673-867.

D3 YOKOKAWA ET AL. (2001) TETRAHEDRON LETT. 42, 5903-5908.

D4 STOLZE ET AL. (2010) CHEMICAL COMM. 46, 8857-8859.

D5 STOLZE ET AL. (Jan. 2012) EUR. J. ORG. CHEM. 2012, 1616-1625.

D6 WO 2009/024527 A1.

D7 BOUREL-BONNET ET AL. (2005) J. MED. CHEM. 48, 1330-1335.

D8 STAWIKOWSKI ET AL. (2006) TETRAHEDRON LETT. 47, 8578-8590.

D5 does not form part of the prior art under Rule 64(1) PCT.

Novelty (Art. 33(2) PCT).

D1-D3 describe the complete synthesis of cyclic depsipeptides having AHP moieties, comprising fragment condensation, cyclisation under lactamisation, side chain attachment, and the formation of the AHP moiety through oxidation of homoserine in the presence of IBX in DMSO and TBAF in THF (see search report for details). The claimed subject matter is novel over D1-D3, due to the amended definition of R3.

D4 and D5 are further developments of D1-D3, wherein the synthesis of the linear precursor is performed via solid phase synthesis, and the cyclisation is performed under lactamisation (see: Scheme 3 of D4).

D6 is cited because it points away from the subject matter of present claim 19 (cf. claim 22 of D6), which seems to indicate that the AHP moiety is unstable in the presence of acidic conditions. Similar statements can be found in D1. D6 does however disclose that R3 should be the side chain of leucine (cf. D6: A3 in claim 1), which happens to be the most preferred side chain for use in variable R3 of the present application (cf. present claims 1 and 18).

D7 is cited as an example of an alternative method to D4 to prepare a cyclic depsipeptide.

D8 provides a further example for the solid phase synthesis of cyclic depsipeptides. Interestingly D8 states that:

> "*The key step in the synthesis of cyclic depsipeptides is the ring closure. Poorly chosen place for ring closure can lead to slow cyclization rates, thus facilitating side reactions such as dimerization, oligomerization, and/or*

epimerization of the C-terminal residue. Traditional methods to prepare cyclic peptides and therefore depsipeptides involve solid-phase synthesis of the selectively protected linear precursor and cyclization in solution under high dilution conditions."

To summarize: Claims 1-22 contain features which alone or in combination with the claims to which they refer do not appear to have been disclosed in the cited prior art. The subject matter of said claims therefore appears to be novel (Art. 33(2) PCT).

Inventive step (Art. 33(3) PCT).

D1 is taken as the closest prior art.

The problem to be solved with the subject matter of inventions 1 and 2 is to provide alternative methods for the synthesis of cyclic depsipeptides having an AHP moiety.

The simple statement that the linear precursor should be prepared by solid phase peptide synthesis does not require inventive skills. D4, D7 and D8 for instance already suggested and implemented that. What could be inventive is how this SPPS was implemented.

The main difference between the SPPS method of the present application and D4 is the position of the resin. In D4 the resin is attached to the side chain of an amino acid allowing on-resin cyclisation, whereas in the presently claimed method the cyclisation is performed off-resin, because the resin is attached C-terminally. D7 chose the same solution. This difference however seems to be a matter of design, preference and choice based on the presence or absence of amino acids suitable for side chain attachment of the resin and experience, rather then an inventive design resulting in an unexpected advantage. In the specification the applicant teaches that the method of cyclisation under lactamization was different from that used in D1. Specifics of this method are however not found in the claims, and moreover already appear to have been suggested by D8 (high dilution).
The difference in the R3 definition is of no influence to the method of preparation, and it is noted that the provision of novel subject matter does not require inventive skills. Moreover D6 already disclosed the presence of a Leu at the R3 position. An inventive step can therefore not be acknowledged on the basis of this feature.
Consequently an inventive step cannot be acknowledged at present for the subject matter of invention 1.

The subject matter of invention 2 is directed to modifying the equilibrium between AHP and its derivatives using an aqueous acidic solution. The claims do however not specify at which concentration TFA/water this effect occurs. Example 3C teaches that

a solution of 1g TFA in 20 ml DCM and 0.2 ml water results in the desired effect. Interestingly D1 also mentions the influence of water for the stabilisation of the AHP moiety (cf. p. 1466). D1 mentions that they suspected that the addition of water to the reaction mixture might prevent dehydration. It turned out that the addition of 0.1 M acidic phosphate buffer (pH 6) in THF was sufficient to stabilize the AHP moiety and prevent the dehydrate from forming. This statement appears to be sufficient to render the second part of the subject matter of invention 2, directed to the equilibrium shift, obvious. The first part of invention 2, concerning the conversion of AHP derivatives to AHP, does however not appear to be affected by this statement.
The presence of an inventive step for the subject matter of invention 2 can also not be acknowledged, because the expression "*aqueous acid*" appears misleading and not supported over the whole scope in view of what the applicant has actually used in example 3C to achieve the desired effect.

To summarize: the subject matter of claims 1-22 lacks an inventive step.

Industrial applicability (Art. 33(4) PCT).

The subject matter of claims 1-22 meets the requirement of industrial applicability.

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NOVARTIS

European Patent Office
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk

EPO - DG 2
22. 02. 2013
51

February 20, 2013

Demand for international preliminary examination

Applicants file reference:	**PAT054610-WO-PCT**
International application No:	**PCT/IB2012/051977**
International filing date:	**19.04.2012**

Dear Sirs,

Please find enclosed an amended set of claims (replacement pages for pages 91-111). A manuscript version of pages 91 and 108-111 is included for convenience. The amended claims form the basis for the international preliminary examination. The amendments previously submitted for this case under Rule 26 are included.

Claim Amendments (Rule 26):
Claim 18 has been re-numbered to 19. This claim now refers back to claim 18 instead of 17.
Claim 19 has been re-numbered to 19. This claim now refers back to claim 19 instead of 18.
Claim 20 has been re-numbered to 21. This claim now refers back to claim 18 instead of 17.
Claim 21 has been re-numbered to 22. This claim now refers back to claim 18 instead of 17.

Claim Amendments (Article 34):
Claim 1 has been amended so that R3 is the side chain of leucine, isoleucine or valine.
Claims 2 to 17 have not been amended.
Claim 18 has been amended so that R_3 is isobutyl.

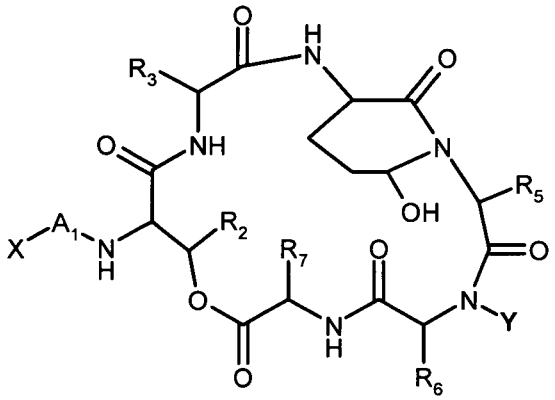
Basis for the Claim Amendments (Article 34):
Basis for the amendment to claim 1 can be found in original Claim 1, preferred embodiment.
Basis for the amendment to claim 18 can be found in original Claim 18, preferred embodiment.
Further basis can be found in the description on page 37, paragraph 6.

Yours very truly
Novartis AG

Christiane Marti
Patent Attorney I, NIBR Patents
GA No. 36671

Claims

1. A method or process for the preparation of a cyclic depsipeptide compound of the formula I,



EPO - DG 2
22. 02. 2013
51

I

wherein

A_1 is a bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group, especially asparagine or glutamine, and is bound at its right hand side in formula I via a carbonyl to the rest of the molecule; or is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

X is bound via an N of A_1 and is acyl, or is absent if A_1 is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

R_2 is C_{1-8}-alkyl, especially methyl;

R_3 is the side chain of ~~an amino acid, especially of~~ leucine, isoleucine or valine;

R_5 is the side chain of an amino acid, preferably of phenylalanine, leucine, isoleucine or valine;

R_6 is the side chain of a hydroxy amino acid, especially of tyrosine;

R_7 is the side chain of an amino acid, preferably of the amino acid leucine, isoleucine or valine; and

Y is hydrogen or C_{1-8}-alkyl;

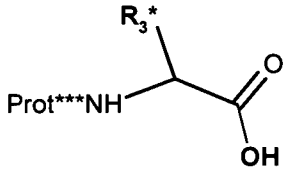
or a salt thereof,

said method comprising

selectively deprotecting a compound of the formula II

wherein Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R_2* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid.

17. The method or process according to claim 16, further comprising, for obtaining the resin bound amino acid of the formula X, coupling an amino acid of the formula IX*,



IX*

wherein R_3* is as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin; or a reactive derivative of said amino acid of the formula IX, to a hydroxy group bound via a cleavable linker L which is bound to a solid resin RES, and removing the protecting group Prot***.

18. The method or process according to any one of claims 1 to 16, where the symbols A_1, R_2, R_3, R_5, R_6, R_7, X and Y or the corresponding unprotected or protected moieties R_2*, R_3*, R_5*, R_6*, R_7*, X* and Y3 are selected so that, in the resulting compound of the formula I, or a salt thereof,

A_1 is the bivalent radical of L-glutamine bound via the carbonyl of its α-carboxyl group to the amino group at the right of A_1 in formula I and via its α-amino group to X, or is 2S-(2-hydroxy-3-phosphonooxy)-propionyl;

R_2 is methyl;

R_3 is ~~isopropyl, isobutyl or benzyl, especially~~ isobutyl;

R_5 is sec-butyl or benzyl, especially sec-butyl;

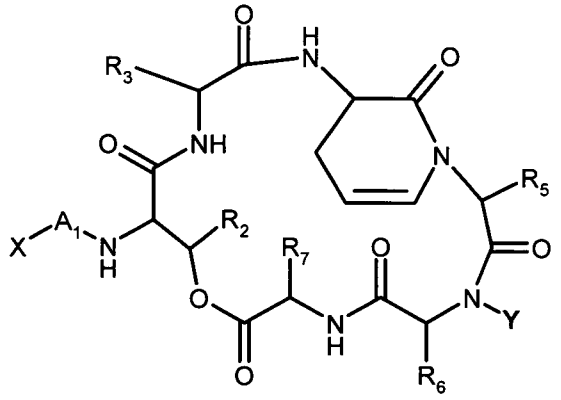
R_6 is 4-hydroxybenzyl;

R_7 is isopropyl or sec-butyl, especially sec-butyl;

X is acetyl or isobutyryl, or is absent if A_1 is 2S-(2-hydroxy-3-phosphonooxy)-propionyl and

Y is methyl.

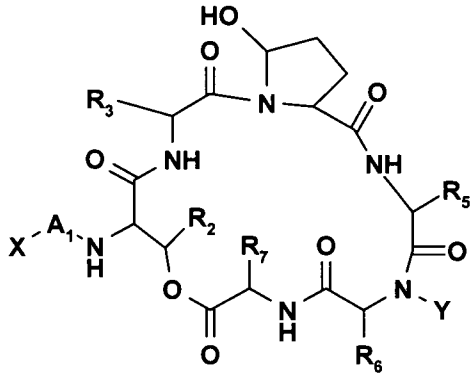
~~18~~19. A method or process for converting a dehydrate of a compound of the formula I given in claim 1 or with the substituents as defined in claim ~~17~~ 18 into the corresponding compound of the formula I, where the dehydrate has the formula V,



V,

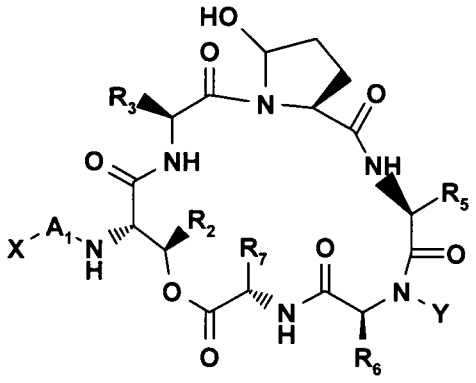
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I in any one of claims 1 and ~~17~~18;

and/or its corresponding hemiaminal analogue with a five-ring instead of the ahp structure in formula I which may also be formed as byproduct and has the formula V*,



V*

especially the formula VA*,



VA*,

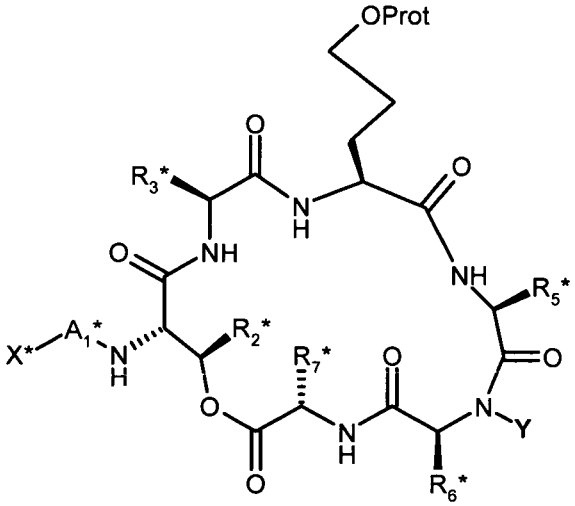
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I in any one of claims 1 and ~~17~~18, respectively;

or a method or process for shifting the equilibrium of a mixture of a compound of the formula I and its corresponding dehydrate and/or hemiaminal in favor of the compound of the formula I,

said method or process comprising using an aqueous acid as reactive solvent to drive the reaction.

~~19~~20. The method according to claim ~~18~~19, wherein the acid is a carboxylic acid, especially a halo substituted C_{1-8}alkanoic acid, more especially trifluoroacetic acid or trichloroacetic acid.

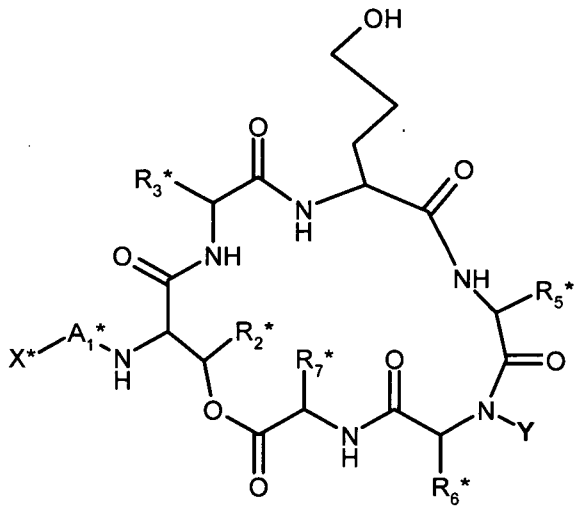
~~20~~21. A compound of the formula II,



II

wherein Prot is a protecting group, Y is as defined for a compound of the formula I in claim 1 or claim ~~17~~ 18 and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I as defined in claim 1 or in claim ~~17~~18, respectively, however with the proviso that reactive functional groups on these moieties are present in unprotected or protected form.

~~21~~22. A compound of the formula III,

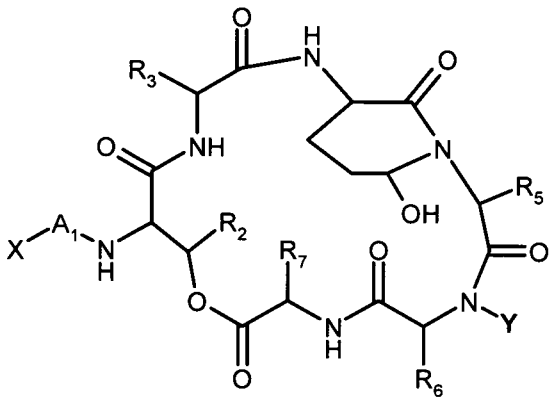


(III)

wherein Y is as defined for a compound of the formula I in claim 1 or claim ~~17~~ 18 and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I as defined in claim 1 or in claim ~~17~~18, respectively, however with the proviso that reactive functional groups other than the hydroxy represented at the top in formula III on these moieties are present in unprotected or protected form.

Claims

1. A method or process for the preparation of a cyclic depsipeptide compound of the formula I,



I

wherein

A_1 is a bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group, especially asparagine or glutamine, and is bound at its right hand side in formula I via a carbonyl to the rest of the molecule; or is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

X is bound via an N of A_1 and is acyl, or is absent if A_1 is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

R_2 is C_{1-8}-alkyl, especially methyl;

R_3 is the side chain of leucine, isoleucine or valine;

R_5 is the side chain of an amino acid, preferably of phenylalanine, leucine, isoleucine or valine;

R_6 is the side chain of a hydroxy amino acid, especially of tyrosine;

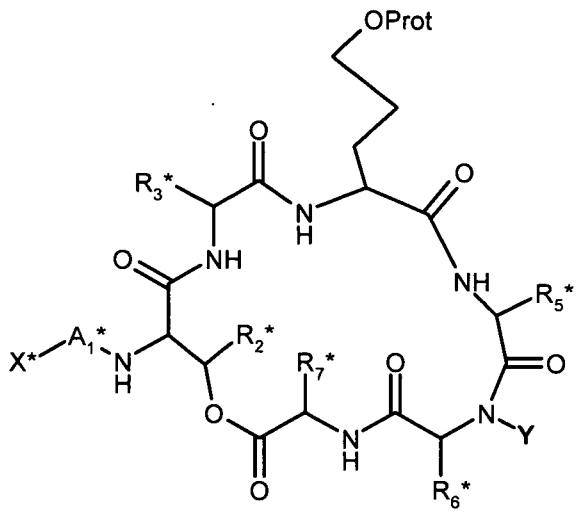
R_7 is the side chain of an amino acid, preferably of the amino acid leucine, isoleucine or valine; and

Y is hydrogen or C_{1-8}-alkyl;

or a salt thereof,

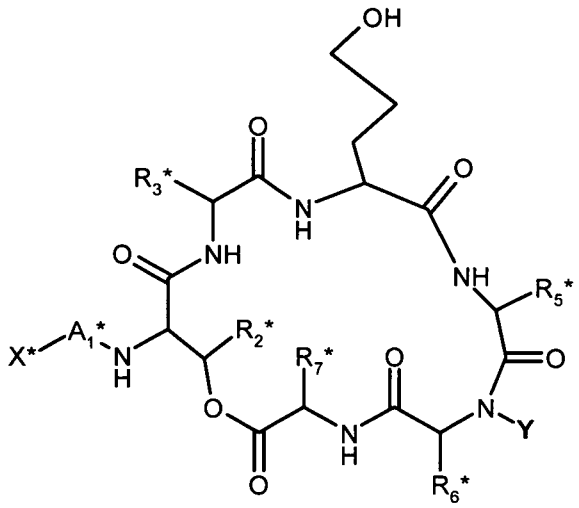
said method comprising

selectively deprotecting a compound of the formula II



II

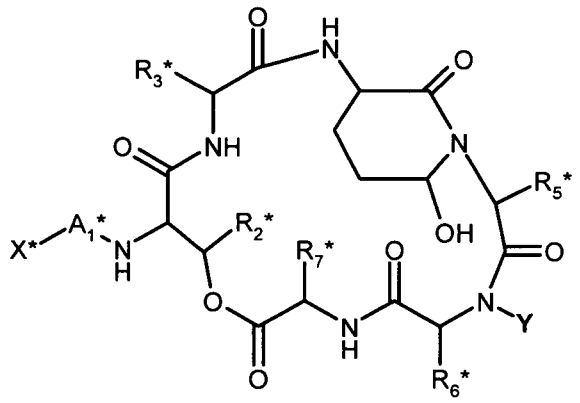
wherein Prot is a protecting group, Y is as defined for a compound of the formula I and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I, respectively, but with the proviso that reactive functional groups on these moieties are present in protected form at least if they could participate in undesired side reactions, to result in a compound of the formula III,



(III)

wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* have the meanings defined above,

reacting the free hydroxyl group under oxidizing conditions to form a compound of the formula IV



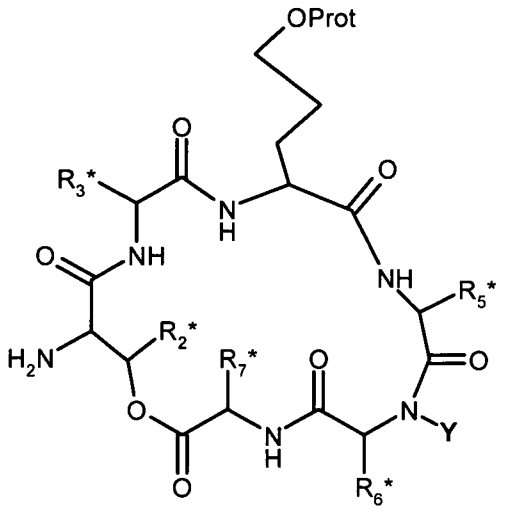
IV

and removing remaining protecting groups to yield a compound of the formula I, or a salt thereof,

and, optionally, converting a free compound of the formula I into a salt, a salt of a compound of the formula I into a different salt of a compound of the formula I or into the free compound of the formula I and/or converting a dehydrated analogue of a compound of the formula I into the corresponding compound of the formula I.

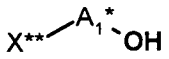
2. The method or process according to claim 1, wherein the compound of the formula IV is synthesized by a combination of Solid Phase Peptide Synthesis and Solution Phase synthesis from the corresponding starting amino acids and side chain precursors.

3. The method or process according to any one of the preceding claims, further comprising, for the synthesis of a compound of the formula II shown in claim 1, reacting a compound of the formula VI,



VI

wherein Prot is a protecting group, Y is as defined for a compound of the formula I and R_2*, R_3*, R_5*, R_6*, and R_7* are as defined for a compound of the formula II in claim 1, with an acid of the formula VII



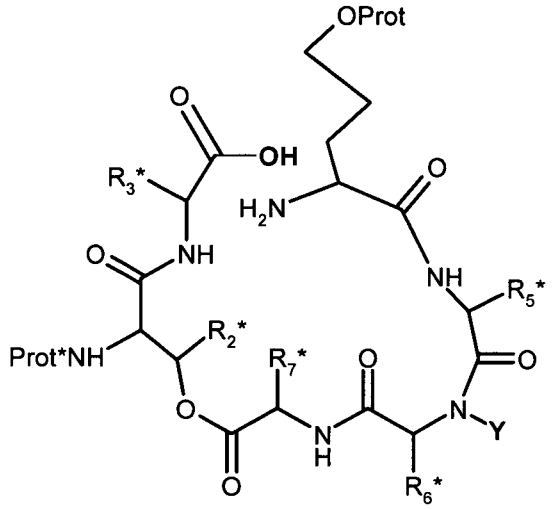
VII

or a reactive derivative thereof,

wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined for a compound of the formula II in claim 1; and, if X** is an amino protecting group, removing said amino protecting group X** to yield H instead of X* and coupling the resulting amino group with an acyl group X* using the corresponding acid X*-OH wherein C* is as defined for a compound of the formula II in claim 1, or a reactive derivative thereof.

4. The method or process according to claim 3, further comprising cyclization under lactamization of a linear precursor peptide of the compound of the formula VI, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, preferably using Solution Phase chemistry.

5. The method or process according to claim 4, where the linear precursor peptide is of the formula VIII,



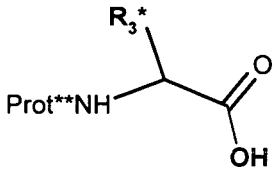
VIII,

wherein Prot* is a protecting group that can be cleaved off selectively without affecting other protecting groups present and is stable during deprotection steps during synthesis of the linear precursor peptide (e.g. allyloxycarbonyl) and R_2*, R_3*, R_5*, R_6*, and R_7* are as defined for a compound of the formula VI in claim 4, further comprising, after cyclisation of the compound of the formula VIII, especially VIIIA, removing the protecting group Prot* *in situ* to yield the compound of the formula VI.

6. The method or process according to claim 5, where the linear precursor peptide of the formula VIII, especially VIIIA, is synthesized from the corresponding amino acids by solid phase peptide synthesis and subsequent cleaving-off from the employed solid phase.

7. The method according to claim 6, comprising either

in a variant a), coupling an amino acid of the formula IX,

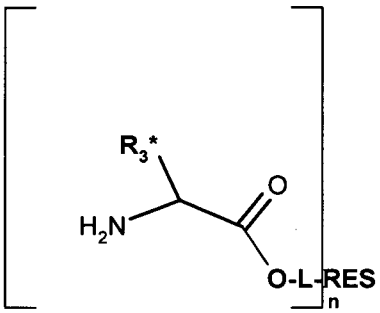


IX

wherein R_3* is as defined for a compound of the formula II in claim 1 and Prot** is an amino protecting group that can be removed on the resin without cleaving other bonds, or a reactive derivative of said amino acid, via an oxygen to a cleavable linker L which is bound to a solid resin RES,

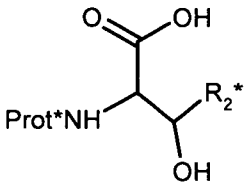
and removing the protecting group Prot**;

coupling the obtained resin bound amino acid symbolized by the formula X,



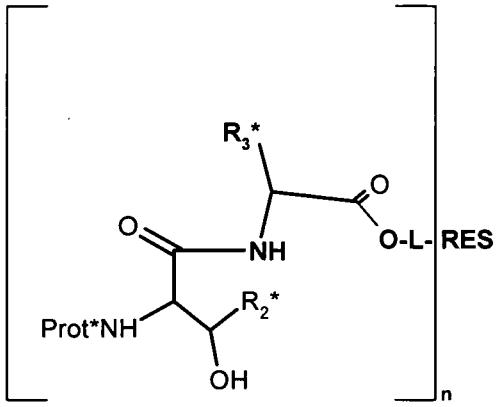
X

in which RES and R_3* are as defined for a compound of the formula IX, n is a natural number and L is a cleavable linker, with an amino acid of the formula XI,



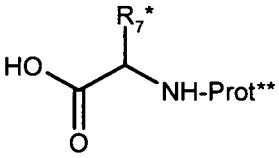
XI

wherein Prot* is as defined for a compound of the formula VIII in claim 5 and R_2* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid, coupling the obtained resin bound dipeptide symbolized by the formula XII,



XII

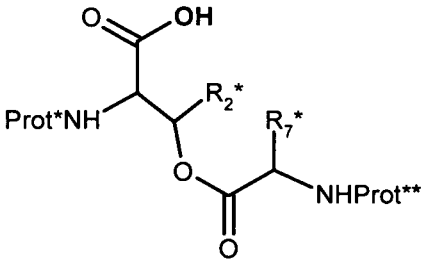
in which Prot* is as defined for a compound of the formula VIII in claim 5, R_2* and R_3* are as defined for a compound of the formula II in claim 1, and n, L and RES are as defined for a compound of the formula X, via the free hydroxy group with an amino acid of the formula XIII,



XIII

wherein Prot** is as defined for a compound of the formula IX and R_7* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid, and removing the protecting group Prot**;

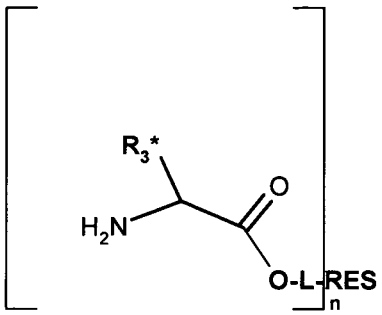
or, in a variant b), coupling a dipeptide of the formula XXVII,



XXVII

wherein R_3* and Prot** are as described for a compound of the formula IX, especially IXA, and Prot* is as defined for a compound of the formula VIII in claim 5, or a reactive

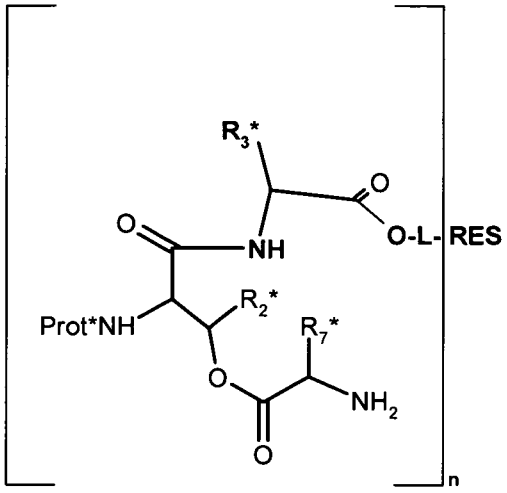
derivative of said dipeptide, to an amino acyl moiety, bound via an oxygen to a cleavable linker L which is bound to a solid resin RES, having the formula X,



X

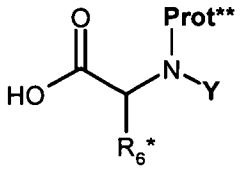
that can be obtained as described under variant a), in which RES, and R_3* are as defined for a compound of the formula IX and L and RES are as just defined; and removing the protecting group Prot**;

and, after the reactions of variant a) or of variant b), coupling the obtained compound of the formula XIV,



XIV

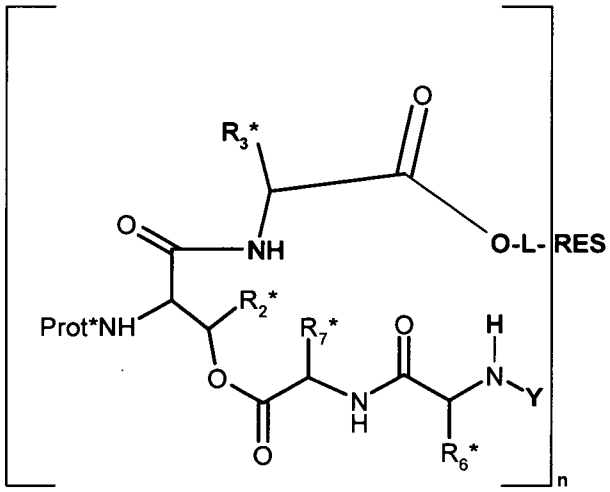
wherein R_2*, R_3* and R_7* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X, with an amino acid of the formula XV,



XV

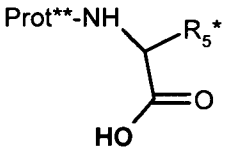
in which R_6* and Y are as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula IX, or a reactive derivative of said amino acid, and removing the protecting group Prot**;

coupling the obtained compound of the formula XVI



XVI

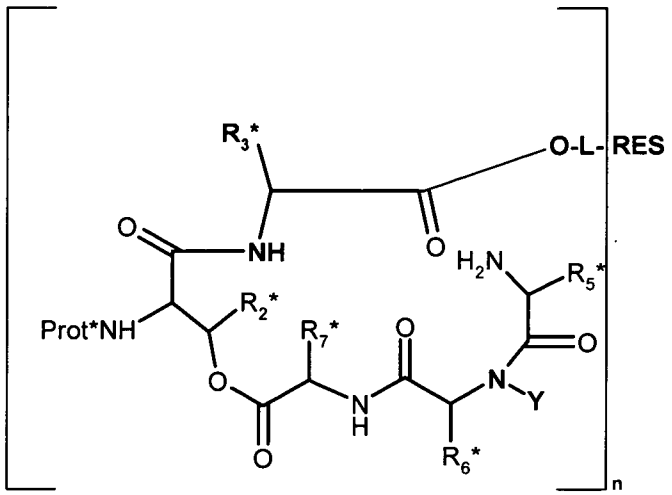
wherein Y, R_2*, R_3*, R_7* and R_6* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X, with an amino acid of the formula XVII,



XVII

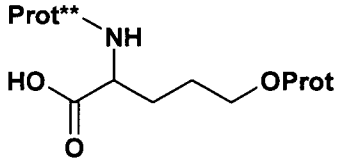
wherein R_5* is as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula IX, or a reactive derivative of said amino acid, and removing the protecting group Prot**,

and finally coupling the resulting compound of the formula XVIII,



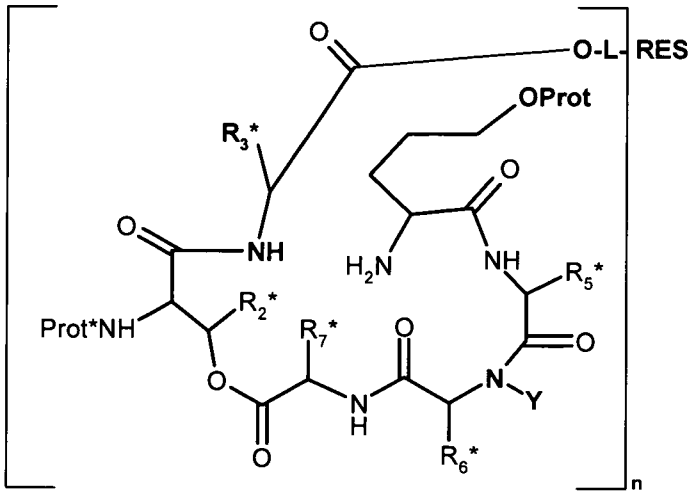
XVIII

wherein Y, R_2*, R_3*, R_7*, R_6* and R_5* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X, to a synthon of the formula XIX,



XIX

wherein Prot is as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula IX, or an activated derivative of said synthon, and removing the protecting group Prot** to yield a compound of the formula XX,



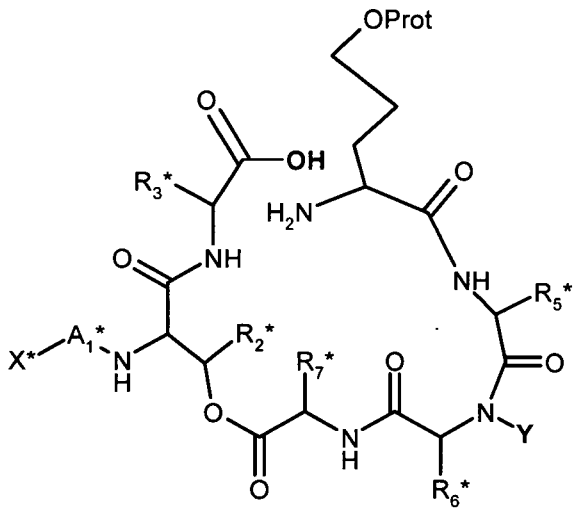
XX

wherein Prot, Y, R_2*, R_3*, R_7*, R_6* and R_5* are as defined for a compound of the formula II in claim 1, Prot* is as defined for a compound of the formula VIII in claim 5 and n, L and RES are as defined for a compound of the formula X,

and cleaving the solid phase bound peptide in formula XX off the solid phase L-RES to yield the corresponding compound of the formula VIII, especially VIIIA, as shown in claim 5.

8. The method or process according to claim 1 or claim 2, comprising, for the synthesis of the compound of the formula II of claim 1, cyclizing under lactamization of a linear precursor peptide of the compound of the formula II, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, preferably using Solution Phase chemistry.

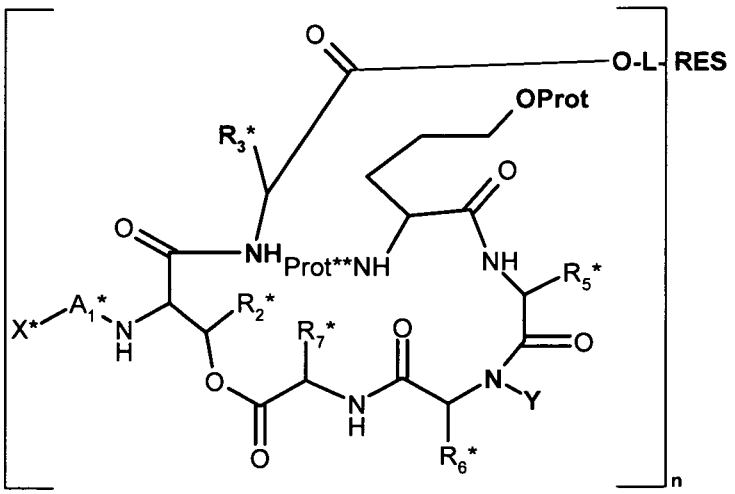
9. The method or process according to claim 8, where the linear precursor peptide is of the formula XXV,



XXV

wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* and Prot are as defined for a compound of the formula II in claim 1.

10. The method or process according to claim 9, further comprising, for the synthesis of the compound of the formula XXV, cleaving a compound of the formula XXIV,

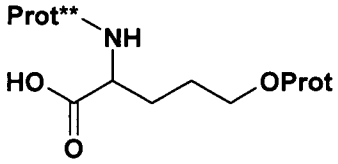


XXIV

wherein X*, A_1*, R_2*, R_3*, R_5*, R_6*, R_7* and Prot are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, n is a natural number and Prot** is an amino protecting group that can be removed without parallel removal of the protecting group Prot and with the product remaining on the resin, and (before the

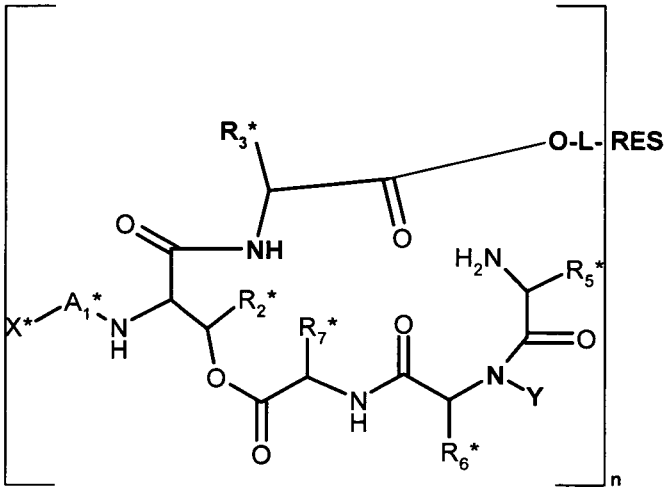
cleavage, in parallel or subsequently to it) removing the protecting group Prot** to yield the compound of the formula XXV.

11. The method or process according to claim 10, further comprising, for the synthesis of the compound of the formula XXIV, coupling an amino acid of the formula XIX,



XIX,

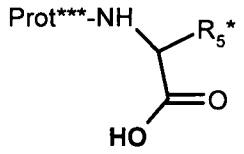
wherein Prot is as defined for a compound of the formula II in claim 1 and Prot** is as defined for a compound of the formula XXIV in claim 10, or an activated derivative of said amino acid, with a compound of the formula XXIII,



(XXIII)

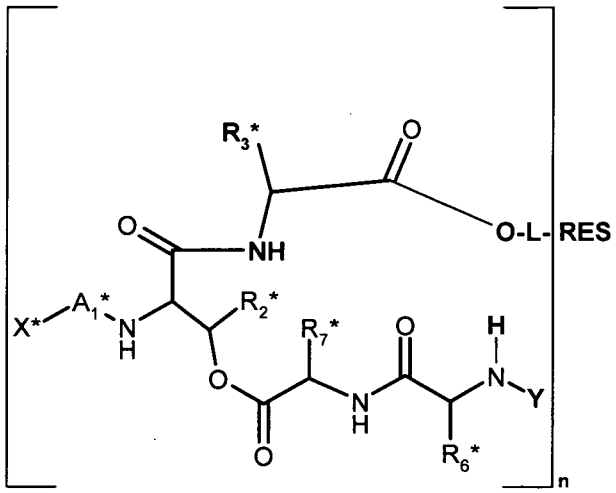
wherein X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number.

12. The method or process according to claim 11, further comprising, for the synthesis of the compound of the formula XXIII, coupling an amino acid of the formula XVII*



XVII*,

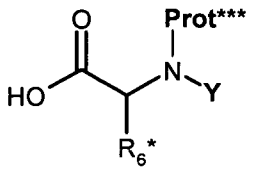
wherein R_5* is as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula XXII,



XXII

wherein X*, A_1*, R_2*, R_3*, R_6* and R_7* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number, and removing the protecting group Prot***.

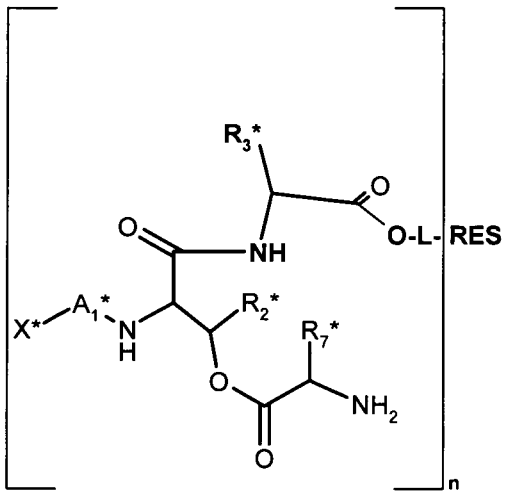
13. The method or process according to claim 12, further comprising, for the synthesis of the compound of the formula XXII, coupling an amino acid of the formula XV*,



XV*

in which R_6* and Y are as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group that can be cleaved off selectively without affecting other

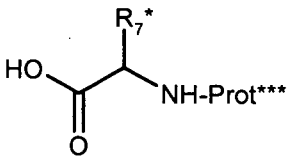
protecting groups present and with the product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula XXI,



XXI,

wherein X*, A_1*, R_2*, R_3* and R_7* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number, and removing the protecting group Prot***.

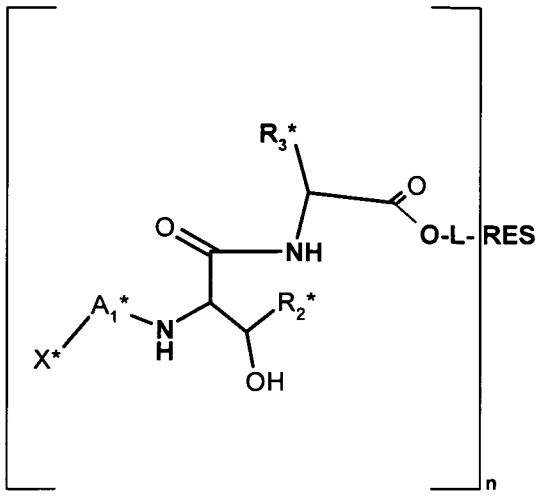
14. The method or process according to claim 13, further comprising, for the synthesis of a compound of the formula XXI, reacting an amino acid of the formula XIII*,



XIII*

wherein Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R_7* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid,

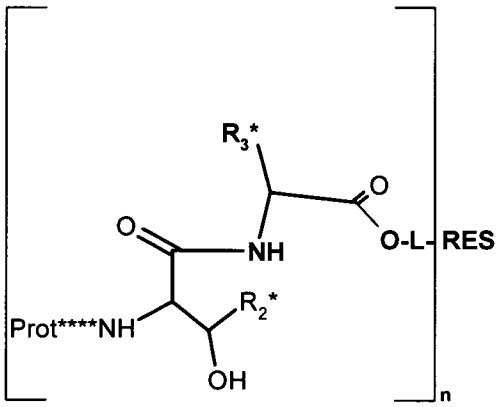
with the hydroxyl group of a compound of the formula XXVI,



XXVI

wherein X*, A_1*, R_2* and R_3* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number;
and removing the protecting group Prot***.

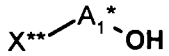
15. The method or process according to claim 14, further comprising, for the synthesis of a compound of the formula XXVI, coupling a resin bound dipeptide symbolized by the formula XII*,



XII*

in which Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present in a compound of the formula II as defined in claim 1 and with the product remaining on the resin, R_2* and R_3* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n

is a natural number, after removal of the protecting group Prot**** via the thus obtained free amino group, with an acid of the formula VII

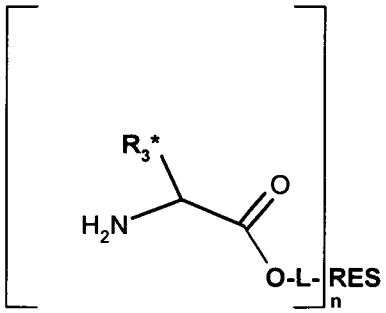


VII

wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined for a compound of the formula II in claim 1, or a reactive derivative of said acid;

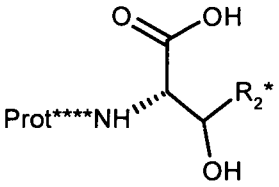
and, if X** is an amino protecting group, removing said amino protecting group X** to yield H instead of X* and coupling the resulting amino group with an acyl group X* using the corresponding acid X*-OH wherein X* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said acid.

16. The method or process according to claim 15, further comprising, for the synthesis of a compound of the formula XII, coupling a resin bound amino acid symbolized by the formula X,



X

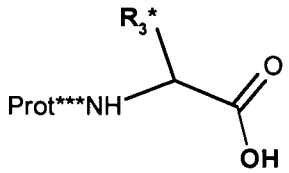
wherein R_3* is as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number,
with an amino acid of the formula XI*,



XI*,

wherein Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R_2* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid.

17. The method or process according to claim 16, further comprising, for obtaining the resin bound amino acid of the formula X, coupling an amino acid of the formula IX*,



IX*

wherein R_3* is as defined for a compound of the formula II in claim 1 and Prot*** is an amino protecting group can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin; or a reactive derivative of said amino acid of the formula IX, to a hydroxy group bound via a cleavable linker L which is bound to a solid resin RES, and removing the protecting group Prot***.

18. The method or process according to any one of claims 1 to 16, where the symbols A_1, R_2, R_3, R_5, R_6, R_7, X and Y or the corresponding unprotected or protected moieties R_2*, R_3*; R_5*, R_6*, R_7*, X* and Y3 are selected so that, in the resulting compound of the formula I, or a salt thereof,

A_1 is the bivalent radical of L-glutamine bound via the carbonyl of its α-carboxyl group to the amino group at the right of A_1 in formula I and via its α-amino group to X, or is 2S-(2-hydroxy-3-phosphonooxy)-propionyl;

R_2 is methyl;

R_3 is isobutyl;

R_5 is sec-butyl or benzyl, especially sec-butyl;

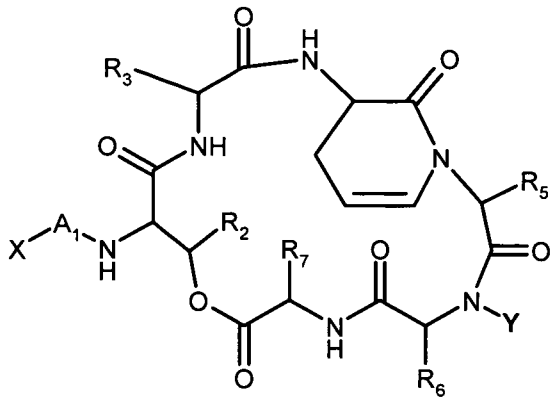
R_6 is 4-hydroxybenzyl;

R_7 is isopropyl or sec-butyl, especially sec-butyl;

X is acetyl or isobutyryl, or is absent if A_1 is 2S-(2-hydroxy-3-phosphonooxy)-propionyl and

Y is methyl.

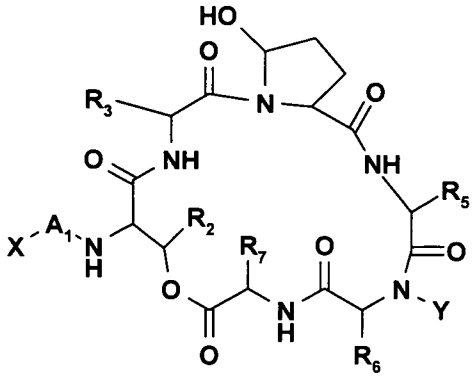
19. A method or process for converting a dehydrate of a compound of the formula I given in claim 1 or with the substituents as defined in claim 18 into the corresponding compound of the formula I, where the dehydrate has the formula V,



V,

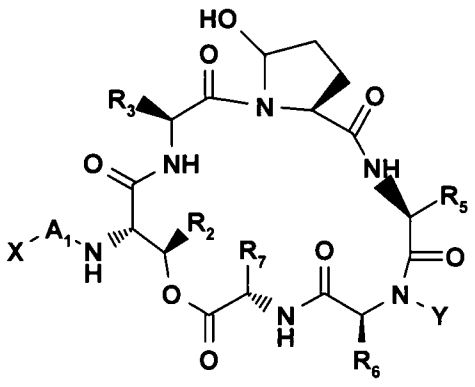
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I in any one of claims 1 and 18;

and/or its corresponding hemiaminal analogue with a five-ring instead of the ahp structure in formula I which may also be formed as byproduct and has the formula V*,



V*

especially the formula VA*,



VA*,

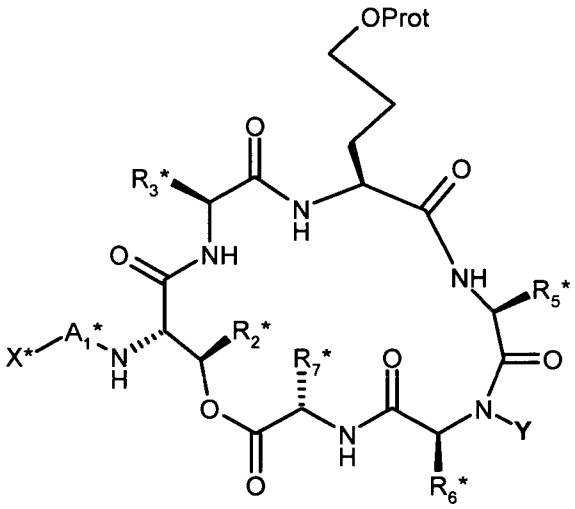
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined for a compound of the formula I in any one of claims 1 and 18, respectively;

or a method or process for shifting the equilibrium of a mixture of a compound of the formula I and its corresponding dehydrate and/or hemiaminal in favor of the compound of the formula I,

said method or process comprising using an aqueous acid as reactive solvent to drive the reaction.

20. The method according to claim 19, wherein the acid is a carboxylic acid, especially a halo substituted C_{1-8}alkanoic acid, more especially trifluoroacetic acid or trichloroacetic acid.

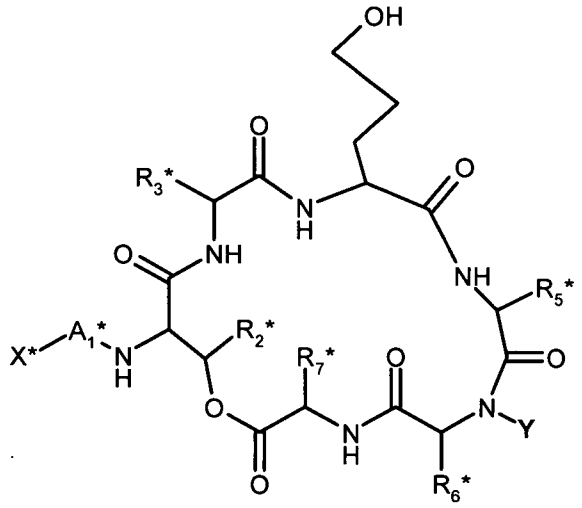
21. A compound of the formula II,



II

wherein Prot is a protecting group, Y is as defined for a compound of the formula I in claim 1 or claim 18 and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I as defined in claim 1 or in claim 18, respectively, however with the proviso that reactive functional groups on these moieties are present in unprotected or protected form.

22. A compound of the formula III,



(III)

wherein Y is as defined for a compound of the formula I in claim 1 or claim 18 and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I as defined in claim 1 or in claim 18, respectively, however with the proviso that reactive functional groups other than the hydroxy represented at the top in formula III on these moieties are present in unprotected or protected form.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 54610-WO-PCT	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/IB2012/051977	International filing date *(day/month/year)* 19/04/2012	(Earliest) Priority Date *(day/month/year)* 20/04/2011
Applicant NOVARTIS AG		

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 7 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. [ ] 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, 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/IB2012/051977

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/051977

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K11/02
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BEILSTEIN Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YOKOKAWA F ET AL: "Synthetic studies of the cyclic depsipeptides bearing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit. Total synthesis of the proposed structure of micropeptin T-20", TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 61, no. 6, 7 February 2005 (2005-02-07), pages 1459-1480, XP027860763, ISSN: 0040-4020 [retrieved on 2005-02-07] Scheme: 1, 6, 9, 10,11,12; 2. Synthetic strategy;compounds 2,32-34,44,45,46,51 ----- -/--	1-21

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2012	07/08/2012

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

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YOKOKAWA F ET AL: "Total synthesis of somamide A, an Ahp (3-amino-6-hydroxy-2-piperidone)-containin g cyclic depsipeptide", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 43, no. 48, 25 November 2002 (2002-11-25), pages 8673-8677, XP004390459, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(02)02178-0 Scheme 5compounds 1,4, 24,25 -----	1-21
X	YOKOKAWA F ET AL: "Synthetic studies of micropeptin T-20, a novel 3-amino-6-hydroxy-2-piperidone (Ahp)-containing cyclic depsipeptide", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 42, no. 34, 20 August 2001 (2001-08-20), pages 5903-5908, XP004295953, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(01)01136-4 Schemes;compounds 15,16,20,21,28 -----	1-21
A	SARA C. STOLZE ET AL: "Solid phase total synthesis of the 3-amino-6-hydroxy-2-piperidone (Ahp) cyclodepsipeptide and protease inhibitor Symplocamide A", CHEMICAL COMMUNICATIONS, vol. 46, no. 46, 1 January 2010 (2010-01-01), page 8857, XP55033621, ISSN: 1359-7345, DOI: 10.1039/c0cc02889d the whole document -----	1-21
A,P	SARA C. STOLZE ET AL: "Development of a Solid-Phase Approach to the Natural Product Class of Ahp-Containing Cyclodepsipeptides", EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, vol. 2012, no. 8, 23 January 2012 (2012-01-23), pages 1616-1625, XP55033623, ISSN: 1434-193X, DOI: 10.1002/ejoc.201101757 the whole document -----	1-21

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/051977

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/024527 A1 (NOVARTIS AG [CH]; KRASTEL PHILIPP [DE]; LIECHTY BRIGITTA-MARIA [CH]; M) 26 February 2009 (2009-02-26)	18
A	claims	1-17, 19-21

Y	LINE BOUREL-BONNET ET AL: "Solid-Phase Total Synthesis of Kahalalide A and Related Analogues", JOURNAL OF MEDICINAL CHEMISTRY, vol. 48, no. 5, 1 March 2005 (2005-03-01), pages 1330-1335, XP55033736, ISSN: 0022-2623, DOI: 10.1021/jm049841x Scheme 1	1-17

Y	STAWIKOWSKI M ET AL: "A novel strategy for the solid-phase synthesis of cyclic lipodepsipeptides", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 47, no. 48, 27 November 2006 (2006-11-27), pages 8587-8590, XP025003510, ISSN: 0040-4039, DOI: 10.1016/J.TETLET.2006.09.116 [retrieved on 2006-11-27] Scheme 1; page 8578, left-hand column, paragraph 3.	1-17

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2012/051977

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009024527 A1	26-02-2009	AR 068343 A1	11-11-2009
		AU 2008290567 A1	26-02-2009
		CA 2696570 A1	26-02-2009
		CO 6260099 A2	22-03-2011
		CR 11251 A	08-03-2010
		EA 201000329 A1	30-08-2010
		EC SP109968 A	31-03-2010
		EP 2183273 A1	12-05-2010
		JP 2010536731 A	02-12-2010
		KR 20100044893 A	30-04-2010
		MA 31701 B1	01-09-2010
		NZ 583284 A	27-04-2012
		PE 10072009 A1	14-08-2009
		SV 2010003485 A	23-03-2011
		TW 200922613 A	01-06-2009
		US 2009156472 A1	18-06-2009
		WO 2009024527 A1	26-02-2009
		ZA 201000776 A	27-10-2010

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-17, 20, 21

   Synthesis of an AHP containing cyclic depsipeptide.

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2. claim: 18

   A method of converting a dehydrate or hemiaminal of the compound of formula I, or modiffying the equilibrium of a reaction comprising using an aqueous acid as reactive solvent.