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Industria y Comercio
SUPERINTENDENCIA

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Tramite 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia 0000 DIVISION DE NUEVAS CREACIONES

licación

FORMULARIO UNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

(1) SOLICITUD DE

[X]	Patente de Invención	[ ]	Patente de Modelo de Utilidad
[ ]	Capítulo I	[X]	Capítulo II

(2) SOLICITANTE (71)

Nombre: SCHERING CORPORATION

Dirección: 2000 Galloping Hill Road Kenilworth New Jersey 07033 Estados Unidos de Américs.

Nacionalidad o Domicilio: ESTADOUNIDENSE

Lugar de Constitución: New Jersey, Estados Unidos de América.

Teléfono: Fax:

E Mail:

IDENTIFICACION

C C [ ] NIT [ ]

C E [ ] Otro [ ]

Cual: _____

Número: _____

(3) REPRESENTANTE O APODERADO

Nombre: CARLOS R OLARTE

Dirección: Avda 82 No. 10-62 Piso 6

Teléfono: 634-1500 Fax: 376-2211

E Mail: patents bogota@bakernet com

IDENTIFICACION

C C [X] NIT [ ]

C E [ ] Otro [ ]

Cual: _____

Número: 79 782 747 Bta TP 74.295

(4) INVENTOR (ES) (72)

Nombre: ver documento adjunto

Dirección: ver documento adjunto

Nacionalidad o Domicilio: ver documento adjunto

(5) PCT (85)

Solicitud Internacional No: PCT/US01/12530 Fecha: Abril 17 2001

Publicación Internacional No: WO 01/81325 Fecha: Noviembre 1 2001

(6) Título (54): INHIBIDORES MACROCICLICOS DE LA SERINA PROTEASA NS-3 DEL VIRUS DE LA HEPATITIS C QUE COMPRENDEN PORCIONES ALQUIL Y ARILALANINA P2

(7) Clasificación Internacional (51): C07D 273/00 ; A61K 31/192

(8) Prioridad	(33) País de Origen	(32) Fecha	(31) Número de Solicitud
SI [X] NO [ ]	US	Abril 19 2000	60/198,204

(9) Comprobante de pago No: 51,152, 38,961 **Fecha:** 05-08-02 07-06-02

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

(10) **ANEXOS**

0002 2

- [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
- [ ] Documento de cesión del inventor al solicitante o a su causante
- [ ] Declaraciones
- [X] Copia de la solicitud internacional (Resumen descripción reivindicaciones dibujos)
- [X] Traducción de la solicitud internacional al castellano (Resumen descripción reivindicaciones dibujos)
- [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
- [ ] De ser el caso modificaciones efectuadas a la solicitud internacional
- [X] Otros Documento de Prioridad No 60/198,204

(11) **FIGURA CARATERISTICA**

(12) Solicito la concesión de la patente,

NOMBRE CARLOS R OLARTE

FIRMA

C C 79 782 747 de Btá T P 74295

Instrucciones para la presentación de los anexos ver reverso de esta página

P20020000023 3

LISTA DE INVENTORES

0003

Case No IN01155K

Attachment "A"

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Prongay, Andrew J , citizen of the United States of America, residing at 104 Willow Grove Road, Stewartsville, New Jersey 08886, U S A

Madison, Vincent S , citizen of the United States of America, residing at 12 Ronarm Drive, Mountain Lakes, New Jersey 07046, U S A

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Industria y Comercio

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Radicacion : 02083310 00003000 Folios: 588
Fecha (AMD): 2002-09-17 17:09:06
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

NIT : 800176089-2

CERTIFICACION DE EXCEDENTE 02 - 51 152
FECHA : AGOSTO 5 DE 2002

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

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No PAGO	FECHA PGO	Vr PAGO
JORGE LUIS GOMEZ	DEVOLUCION TASA			470	05/08/2002	300 000 00

C O N C E P T O

CANT	RENTISTICO	CONCEPTO		TOTAL CONCEPTO
1	50005-01-09 OTROS SERVICIOS DE PROPIEDAD I	99 OTROS SERVCIOS ADMINISTRATIVOS		300,000 00
		TOTAL :	$	300,000 00

SON: TRESCIENTOS MIL PESOS

RESPONSABLE _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No _____

ESTA CERTIFICACION DE EXCEDENTE DE PAGO SOLO PODRA SER UTILIZADA COMO PARTE DE LA CANCELACION DE UNA TARIFA VIGENTE Y NO PODRA SER SUSTITUIDA POR NINGUN RECIBO.

Carrera 13 No 27-00 Pisos 5 7 y 10
Conmutador 382 0840
Fax 350 5220
E-mail info@sic gov co
www sic gov co
Bogotá D C Colombia

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Ca. 5

NIT 900176089-2

SUPERINDUSTRIA Y COMERCIO

Radicacion : 02003318 00000000 Folios 588

Fecha (AMD): 2002-09-17 17 08.06

Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 02 - 36,961

FECHA : JUNIO 7 DE 2002

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

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No PAGO	FECHA PGO	Vr PAGO
JORGE [redacted]IS GOMEZ	CONSIGNACION	BANCO POPULAR	050-00110-6	9226066	07/06/2002	28,041,000 00

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

CANT	RENTISTICO	CONCEPTO		TOTAL CONCEPTO
1	50005-01-03 CAMBIO DE MODALIDAD Y MODIFICA	12 MARCAS Y LEMAS COMERCIALES		78,000 00
		TOTAL :	$	78,000 00

SON: SETENTA Y OCHO MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No _____

Traducción Oficial No 02-899

Efectuada el día 20 de agosto de 2002, de un documento escrito en Inglés, al cual para su identificación se le estampa el sello de María del Pilar Iglesias, Traductora e Intérprete Oficial por Resolución No 819 del 15 de abril de 1971 expedida por el Ministerio de Justicia de la Republica de Colombia

---

Traducción de las autenticaciones notariales y subsiguientes correspondientes a un Poder otorgado por **SCHERING CORPORATION** a CARLOS REINALDO OLARTE Y/O ALVARO CORREA ORDOÑEZ, documento éste que viene en español y se explica por sí solo

La firma y cargo de Edward H Mazer como Funcionario Autorizado de la compañia arriba citada fueron debidamente certificados por el Notario Publico Norma Gallina del Estado de Nueva Jersey, según consta en la Certificación Notarial, que también viene en español y que fue firmada por dicho Notario el 2 de agosto de 2002, quien tuvo a la vista la Escritura de Constitución de la Compañía reformada el 4 de abril de 1996 y el Certificado de Constitución actualizado al 16 de junio de 1997

NOTARIO ONCE DE ESTE CIRCULO PUEDE CONSTAR QUE ESTA FOTOCOPIA COINCIDE CON EL ORIGINAL QUE HE TENIDO A LA VISTA.

11 AGO 2002

BOGOTA, D.C. - COLOMBIA
JULIAN NAVARRO DE RAMIREZ
NOTARIA ONCE (11)

A

0983

APOSTILLA

(Convencion de La Haya del 5 de octubre de 1961)

1 País Estados Unidos de América

El presente documento publico

2 Fue firmado por Norma Gallina

3 Que actuó en su calidad de Notario Público del Estado de Nueva Jersey

4 Lleva el sello del mencionado Notario Publico

Se certificó

5 En Trenton, Nueva Jersey 6 El 9 de agosto de 2002

7 Por parte de John E McCormac, CPA, Tesorero de Nueva Jersey

8 Bajo el Numero A156329

9 Sello del Estado de Nueva Jersey

10 Firmado por John E McCormac

Es traducción fiel y completa efectuada por

María del Pilar Iglesias
C\C 41 339 183 de Bogotá

COMO NOTARIO ONCE DE ESTE CIRCULO DAGO CONSTAR QUE ESTA FOTOCOPIA COINCIDE CON EL ORIGINAL QUE HE TENIDO A LA VISTA.

21 AGO 2002

BOGOTA, D.C. COLOMBIA
ZULMA NAVARRO DE BAUTISTA
NOTARIA ONCE (11)

María del Pilar Iglesias
C.C. [illegible] de Bogotá
Intérprete Oficial [illegible]
16 de Abril de 1971 [illegible]

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8

APOSTILLE

(CONVENTION DE LA HAYE DU 5 OCTOBRE 1961)

1 COUNTRY UNITED STATES OF AMERICA

2 THIS PUBLIC DOCUMENT HAS BEEN SIGNED BY
NORMA GALLINA

3 ACTING IN THE CAPACITY OF
NOTARY PUBLIC OF NEW JERSEY

4. BEARS THE SEAL/STAMP OF
NORMA GALLINA, NOTARY

CERTIFIED

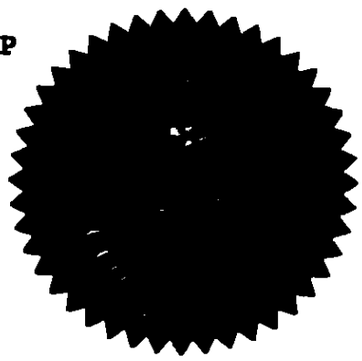
5. AT TRENTON, NEW JERSEY

6 THE 9TH DAY OF AUGUST 2002

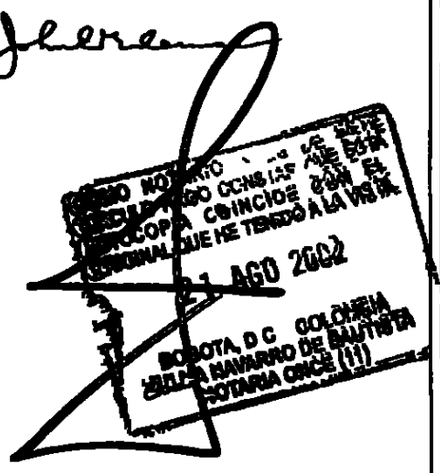
7 BY. John E McCormac, CPA, NEW JERSEY TREASURER

8 NO, A156329

9 SEAL/STAMP



10 SIGNATURE



RAIBEKC LARA RODRIGUEZ & RUEDA
MEMBERS OF THE FIRM
BAKER & McKENZIE
ATTORNEYS AT LAW

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Bogota D C COLOMBIA
Telephone (57 1) 634-1500
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NIT 860 068 840-3
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www bakernet com

POWER OF ATTORNEY

The undersigned

SCHERING CORPORATION

located in
2000 Galloping Hill Road
Kenilworth, New Jersey 07033
United States of America

declares that it hereby grants to

CARLOS REINALDO OLARTE AND/OR

ALVARO CORREA ORDOÑEZ

a full and sufficient power of attorney to apply to the proper national officers and authorities for the issue of

Patents of Invention Patents for Utility Models, Industrial Design Registrations, mergers changes of name assignments, filing and prosecution of oppositions, changes of address

to which end it empowers them to take all steps necessary before said authorities for the objects stated to file petitions' prepare descriptions make protests, declarations appeals and objections, pay imposts apply for certifications receive documents and securities, abandon applications and collect refunds. To the said mandatories sufficient power is granted hereby to receive notifications for and 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 patents, and to do everything that may be required before administrative or judicial officers of any class giving them likewise power to substitute these presents and revoke such substitutions if necessary

Done and subscribed in
Kenilworth, New Jersey, USA

On the 2nd day of August

2002

(Signature) Edward H. Mazer
Duly Authorized Officer

PODER

La abajo firmada

SCHERING CORPORATION

domiciliada en
2000 Galloping Hill Road
Kenilworth, New Jersey 07033
Estados Unidos de America

declara por el presente otorgar a

CARLOS REINALDO OLARTE Y/O

ALVARO CORREA ORDOÑEZ

poder especial amplio y bastante para recabar de las oficinas y autoridades nacionales que correspondan la obtención de
Patentes de invención Patentes de Modelo de Utilidad, Registro de Diseños Industriales fusiones, cambios de nombre, cesiones, presentación y trámite de oposiciones, cambios de dirección

a cuyo efecto los faculta para dar ante dichas autoridades, todos los pasos necesarios al objeto indicado elevar solicitudes formular descripciones protestas, declaraciones apelaciones y reclamos obtar impuestos solicitar testimonios recibir documentos y valores, desistir y percibir Se concede a los expresados mandatarios 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 las patentes se presentaren y hacer cuanto fuere necesario ante las autoridades administrativas o judiciales de cualquier orden dándoles asimismo facultad para sustituir el presente y en caso necesario revocar dichas sustituciones

Dado y suscrito en
Kenilworth, New Jersey, USA

A los 2 días del mes de AGO 2002

COMO NOTARIO ONCE DE ESTE CIRCULO HAGO CONSTAR QUE ESTA FOTOCOPIA CONCUERDA CON EL ORIGINAL QUE HE TENIDO A LA VISTA
BOGOTA D.C. COLOMBIA
JULIA HINCAPIE DE BAUTISTA
NOTARIA ONCE (11)

RAISBECK LARA RODRIGUEZ & RUEDA
MEMBERS OF THE FIRM
BAKER & McKENZIE
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FOLIO 10

NOTARIAL CERTIFICATION

On this 2nd day of August

2002 the undersigned Notary

CERTIFIES

1 That Edward H. Mazer

of legal age and domiciled in Millburn, New Jersey United States of America

set his hand on the foregoing document.

2. That the grantor is to me personally know and that he/she has legal capacity to execute the instrument.

3 That the grantor has executed the foregoing document as Duly Authorized Officer

of the judicial person

4 That the judicial person SCHERING CORPORATION

having its home office in 2000 Galloping Hill Road, Kenilworth, New Jersey 07033, USA

is duly organized has present legal existence and that the purposes for which the power of attorney is granted are within the scope of its corporate purposes

5 That the grantor has in fact the referred to authority and that his/her representation is legal

6 That I have based certifications 3 4 and 5 above on the following authentic documents for such purpose exhibited to me and which I mention specifically giving their dates and their origin or source

Certificate of Incorporation, as amended, dated April 4, 1996
Certificate of Restated Incorporation filed June 16, 1997

Notary Public

CERTIFICACION NOTARIAL

A los _____ días del mes de _____

de 2002 el suscrito Notario

DA FE

1 Que _____

mayor y domiciliado en _____

suscribió de su puño y letra el documento que antecede

2. Que conozco al otorgante y que éste tiene capacidad legal para el otorgamiento

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

de la persona jurídica

4 Que la persona jurídica _____

con sede en _____

está legalmente constituida, que tiene existencia legal actual y que el acto para el cual fue otorgado el poder está comprendido entre los que constituyen su objeto social

5. Que el otorgante tiene efectivamente la representación que ostenta y que esta es legítima

6 Que he basado las certificaciones 3 4 y 5 anteriores en los siguientes documentos auténticos que al efecto se me exhibieron y que menciono especificamente con expresión de sus fechas y de su origen o procedencia

_____

_____

_____

21 AGO. 2002

BOGOTA, D.C. COLOMBIA
RAUL GUTIERREZ DE BAUTISTA
NOTARIA CINCE (11)

NORMA GALLINA
NOTARY PUBLIC OF NEW JERSEY
My Commission Expires June 27, 2005

El Notario Publico

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14
081
11

This page is not part of

the pamphlet!

WO 01-81325

1/2

Date: 01 nov 2001

Destination Agent

Address

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(71) Applicant SCHERING CORPORATION [US/US] 2000 Galloping Hill Road Kenilworth NJ 07033-0530 (US).

(72) Inventors VENKATRAMAN, Srikanth 35 Roanoke Street, Woodbridge NJ 07095 (US). CHEN Kevin, X 44 Gill Lane, Apt 1D Iselin NJ 08830 (US). ARASAPPAN, Ashok 18 Larsen Court, Bridgewater NJ 08807 (US). NJOROGE, F., George 11 Softwood Way Warren, NJ 07059 (US) GIRIJAVALLABHAN, Viyyoor, M 10 Maplewood Drive, Parsippany NJ 07054 (US) CHAN, Tin-Yau, 26 Barlow Road Edison NJ 08817 (US). MCKITTRICK, Brian, A 67 Laurel Avenue, Bloomfield NJ 07003 (US). PRONGAY, Andrew, J. 104 Willow Grove Road, Stewartsville NJ 08886 (US). MADISON, Vincent, S. 12 Ronarm Drive, Mountain Lakes, NJ 07046 (US)

(81) Designated States *(national)* AE, AG AL, AM AT AU AZ, BA BB BG BR BY BZ, CA CH CN, CO CR, CZ, DE, DK DM DZ, EE, ES, FI, GB GD GE, HR, HU ID IL, IN IS, JP KG KR KZ, LC, LK LR LT LU LV MA MD, MG MK, MN MX MZ, NO, NZ, PL, PT RO RU SE, SG SI SK SL, TJ TM TR TT TZ, UA, UZ, VN YU ZA

(84) Designated States *(regional)* ARIPO patent (GH GM KE, LS MW MZ, SD SL SZ, TZ, UG ZW) Eurasian patent (AM AZ, BY KG KZ, MD RU TJ TM), European patent (AT BE, CH CY DE, DK ES FI FR GB GR, IE, IT LU MC NL, PT SE, TR), OAPI patent (BF BJ CF CG CI CM GA GN GW ML, MR NE, SN TD TG).

Published
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/81325 A2

(54) Title MACROCYCLIC NS3-SERINE PROTEASE INHIBITORS OF HEPATITIS C VIRUS COMPRISING ALKYL AND ARYL ALANINE P2 MOIETIES

(57) Abstract The present invention discloses novel macrocyclic compounds which have HCV protease inhibitory activity as well as methods for preparing such compounds. In another embodiment the invention discloses pharmaceutical compositions comprising such macrocycles as well as methods of using them to treat disorders associated with the HCV protease

Macrocyclic NS-3 Serine Protease Inhibitors of Hepatitis C Virus comprising Alkyl and Aryl Alanine P2 Moieties

Field of Invention

The present invention relates to novel hepatitis C virus ("HCV") protease inhibitors, pharmaceutical compositions containing one or more such inhibitors, methods of preparing such inhibitors and methods of using such inhibitors to treat hepatitis C and related disorders This invention specifically discloses novel macrocyclic compounds as inhibitors of the HCV NS3/NS4a serine protease The disclosure herein is related to that in pending patent application, Serial No _____, filed April 5, 2000

Background of the Invention

Hepatitis C virus (HCV) is a (+)-sense single-stranded RNA virus that has been implicated as the major causative agent in non-A, non-B hepatitis (NANBH), particularly in blood-associated NANBH (BB-NANBH)(see, International Patent Application Publication No WO 89/04669 and European Patent Application Publication No EP 381 216) NANBH is to be distinguished from other types of viral-induced liver disease, such as hepatitis A virus (HAV), hepatitis B virus (HBV), delta hepatitis virus (HDV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV), as well as from other forms of liver disease such as alcoholism and primary biliar cirrhosis

Recently, an HCV protease necessary for polypeptide processing and viral replication has been identified, cloned and expressed, (see, e.g., U S, Patent No 5,712,145) This approximately 3000 amino acid polyprotein contains, from the amino terminus to the carboxy terminus, a nucleocapsid protein (C), envelope proteins (E1 and E2) and several non-structural proteins (NS1, 2, 3, 4a, 5a and 5b) NS3 is an approximately 68 kda protein, encoded by approximately 1893 nucleotides of the HCV genome, and has two distinct domains (a) a serine protease domain consisting of approximately 200 of the N-terminal amino acids, and (b) an RNA-dependent ATPase domain at the C-terminus of the protein The

NS3 protease is considered a member of the chymotrypsin family because of similarities in protein sequence, overall three-dimensional structure and mechanism of catalysis Other chymotrypsin-like enzymes are elastase, factor Xa, thrombin, trypsin, plasmin, urokinase, tPA and PSA The HCV NS3 serine protease is responsible for proteolysis of the polypeptide (polyprotein) at the NS3/NS4a, NS4a/NS4b, NS4b/NS5a and NS5a/NS5b junctions and is thus responsible for generating four viral proteins during viral replication This has made the HCV NS3 serine protease an attractive target for antiviral chemotherapy

It has been determined that the NS4a protein, an approximately 6 kda polypeptide, is a co-factor for the serine protease activity of NS3 Autocleavage of the NS3/NS4a junction by the NS3/NS4a serine protease occurs intramolecularly (i.e , *cis*) while the other cleavage sites are processed intermolecularly (i.e., *trans*)

Analysis of the natural cleavage sites for HCV protease revealed the presence of cysteine at P1 and serine at P1' and that these residues are strictly conserved in the NS4a/NS4b, NS4b/NS5a and NS5a/NS5b junctions The NS3/NS4a junction contains a threonine at P1 and a serine at P1' The Cys→Thr substitution at NS3/NS4a is postulated to account for the requirement of *cis* rather than *trans* processing at this junction See, e g., Pizzi et al. (1994) Proc. Natl. Acad. Sci (USA) 91 888-892, Failla et al. (1996) Folding & Design 1:35-42 The NS3/NS4a cleavage site is also more tolerant of mutagenesis than the other sites See, e.g., Kollykhalov et al. (1994) J. Virol. 68 7525-7533 It has also been found that acidic residues in the region upstream of the cleavage site are required for efficient cleavage See, e.g., Komoda et al. (1994) J. Virol. 68 7351-7357

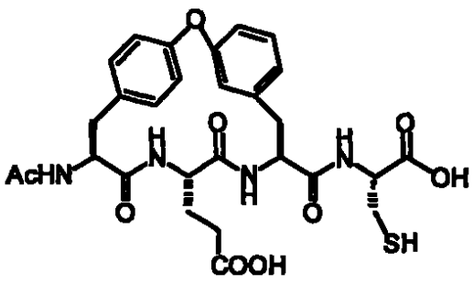
Inhibitors of HCV protease that have been reported include antioxidants (see, International Patent Application Publication No WO 98/14181), certain peptides and peptide analogs (see, International Patent Application Publication No WO 98/17679, Landro et al (1997) Biochem 36 9340-9348, Ingallinella et al (1998) Biochem 37 8906-8914, Llinàs-Brunet et al (1998) Bioorg. Med. Chem. Lett. 8 1713-1718), inhibitors based on the 70-amino acid polypeptide eglin c (Martin et al. (1998) Biochem. 37 11459-11468, inhibitors affinity selected from

human pancreatic secretory trypsin inhibitor (hPSTI-C3) and minibody repertoires (MBip) (Dimasi et al (1997) J. Virol. 71,7461-7469), cV_HE2 (a "camelized" variable domain antibody fragment) (Martin et al (1997) Protein Eng. 10 607-614), and α1-antichymotrypsin (ACT)(Elzouki et al.) (1997) J. Hepat. 27,42-28) A ribozyme designed to selectively destroy hepatitis C virus RNA has recently been disclosed (see, BioWorld Today 9(217) 4 (November 10, 1998))

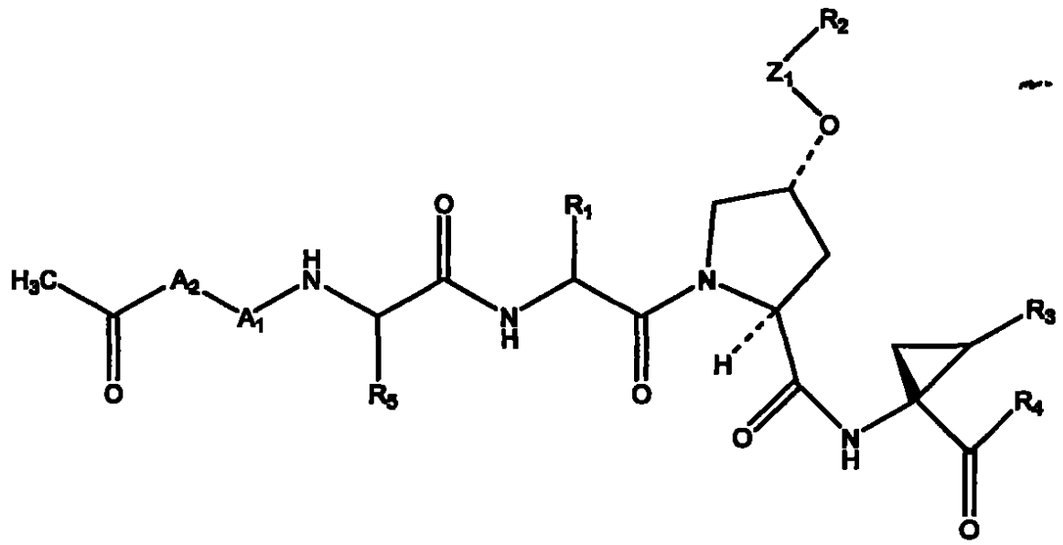
Reference is also made to the PCT Publications, No WO 98/17679, published April 30, 1998 (Vertex Pharmaceuticals Incorporated), WO 98/22496, published May 28, 1998 (F Hoffmann-La Roche AG), and WO 99/07734, published February 18, 1999 (Boehringer Ingelheim Canada Ltd)

HCV has been implicated in cirrhosis of the liver and in induction of hepatocellular carcinoma The prognosis for patients suffering from HCV infection is currently poor HCV infection is more difficult to treat than other forms of hepatitis due to the lack of immunity or remission associated with HCV infection Current data indicates a less than 50% survival rate at four years post cirrhosis diagnosis Patients diagnosed with localized resectable hepatocellular carcinoma have a five-year survival rate of 10-30%, whereas those with localized unresectable hepatocellular carcinoma have a five-year survival rate of less than 1%

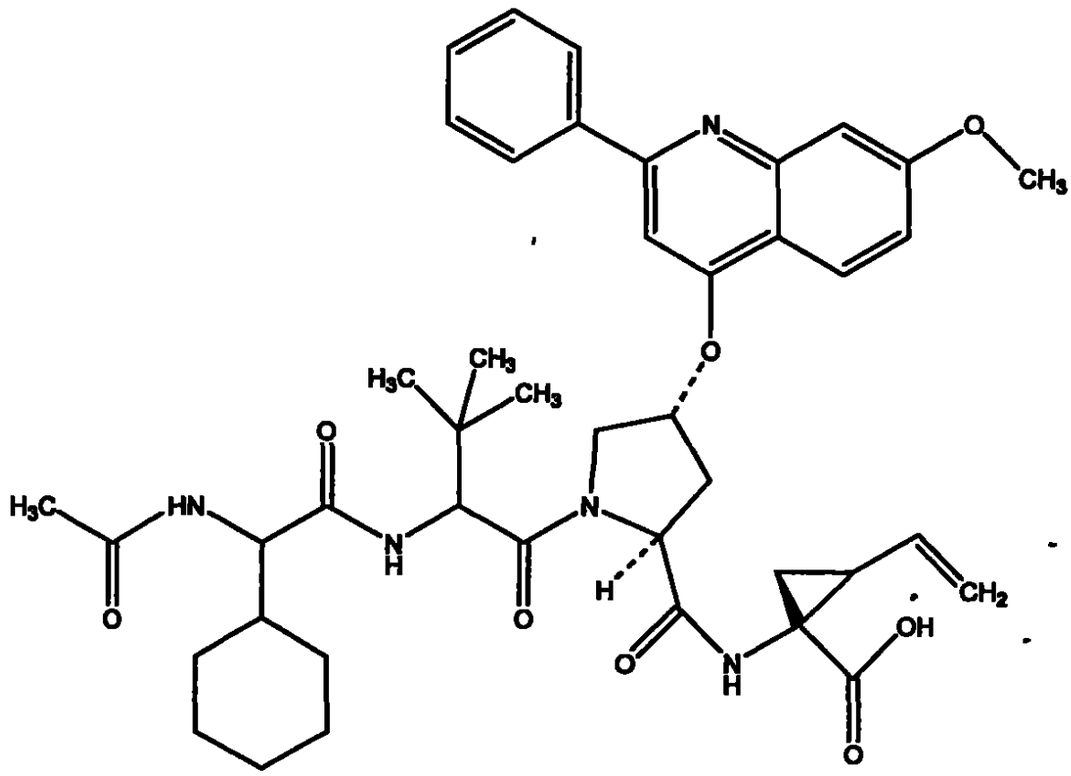
Reference is made to A Marchetti *et al*, *Synlett*, S1, 1000-1002 (1999) describing the synthesis of bicylic analogs of an inhibitor of HCV NS3 protease A compound disclosed therein has the formula



Reference is also made to WO 00/09558 (Assignee Boehringer Ingelheim Limited, Published February 24, 2000) which discloses peptide derivatives of the formula

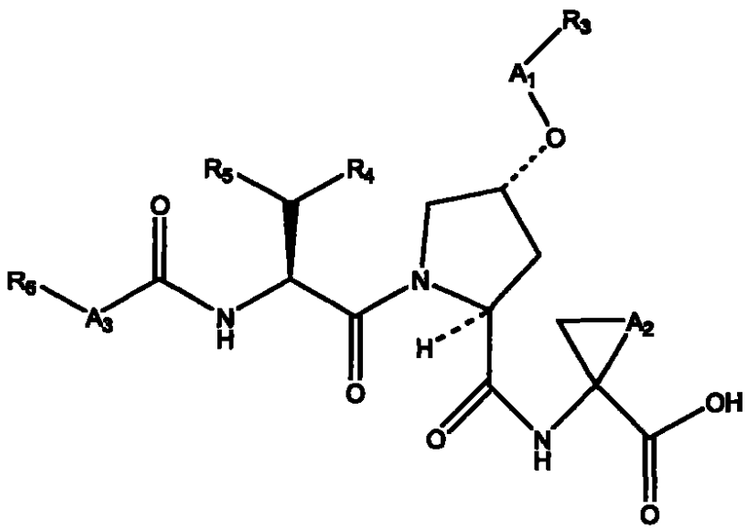


where the various elements are defined therein An illustrative compound of that series is

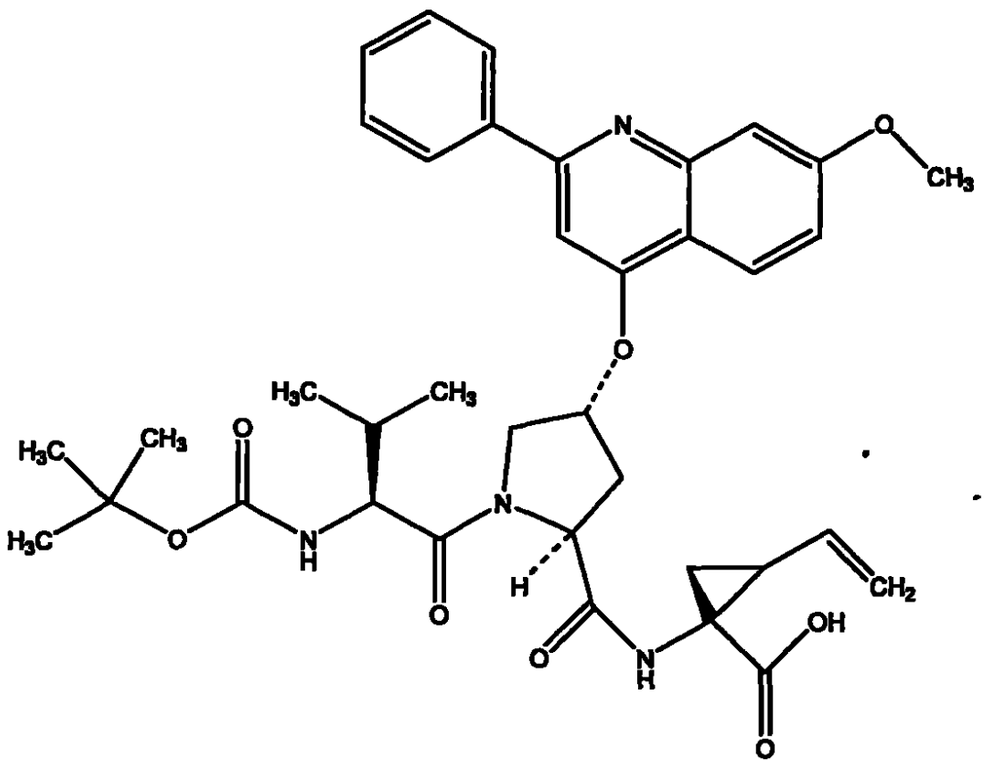


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Reference is also made to WO 00/09543 (Assignee Boehringer Ingelheim Limited, Published February 24, 2000) which discloses peptide derivatives of the formula



where the various elements are defined therein An illustrative compound of that series is



Current therapies for hepatitis C include interferon-α (INF_α) and combination therapy with ribavirin and interferon See, e g., Beremguer et al , (1998) Proc. Assoc. Am. Physicians 110(2) 98-112 These therapies suffer from a low sustained response rate and frequent side effects See, e.g., Hoofnagle et al, (1997) N. Engl. J. Med. 336 347 Currently, no vaccine is available for HCV infection

Pending patent application, Serial Number, _____, filed April 5, 2000, discloses certain macrocyclic compounds as inhibitors of the HCV protease as well as pharmaceutical compositions containing said compounds

There is a need for new treatments and therapies for HCV infection It is, therefore, an object of this invention to provide compounds useful in the treatment or prevention or amelioration of one or more symptoms of hepatitis C

It is a further object herein to provide methods of treatment or prevention or amelioration of one or more symptoms of hepatitis C

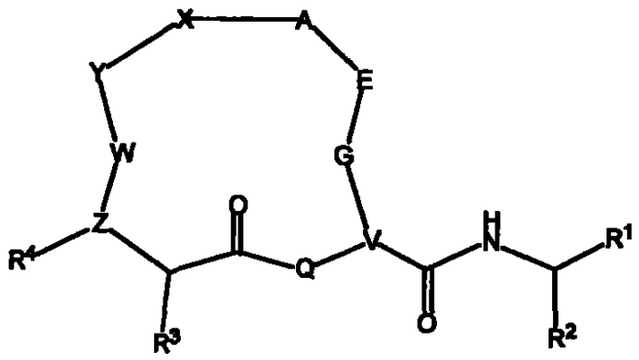
A still further object of the present invention is to provide methods for modulating the activity of serine proteases, particularly the HCV NS3/NS4a serine protease, using the compounds provided herein

Another object herein is to provide methods of modulating the processing of the HCV polypeptide using the compounds provided herein

Summary of the invention

In its many embodiments, the present invention provides a novel class of macrocyclic inhibitors of the HCV protease, pharmaceutical compositions containing one or more of the compounds, methods of preparing pharmaceutical formulations comprising one or more such compounds, and methods of treatment, prevention or amelioration or one or more of the symptoms of hepatitis C Also provided are methods of modulating the interaction of an HCV polypeptide with HCV protease Among the compounds provided herein, compounds that inhibit HCV NS3/NS4a serine protease activity are preferred The presently disclosed compounds generally contain about four or more amino acid residues and less than about twelve amino acid residues

In its principal embodiment, the present invention provides a macrocyclic compound of Formula I



Formula I

wherein

E, X and Y may be independently present or absent, and if present are independently selected from the moieties alkyl, aryl, alkyl-aryl, heteroalkyl, heteroaryl, aryl-heteroaryl, alkyl-heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, aryl ether, alkyl amino, aryl amino, alkyl-aryl amino, alkyl sulfide, alkyl-aryl sulfide, aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, aryl sulfone, alkyl-alkyl sulfoxide, alkyl-aryl sulfoxide, alkyl amide, alkyl-aryl amide, aryl amide, alkyl sulfonamide, alkyl-aryl sulfonamide, aryl sulfonamide, alkyl urea, alkyl-aryl urea, aryl urea, alkyl carbamate, alkyl-aryl carbamate, aryl carbamate, alkyl -hydrazide, alkyl-aryl hydrazide, alkyl hydroxamide, alkyl-aryl hydroxamide, alkyl sulfonyl, aryl sulfonyl, heteroalkyl sulfonyl, heteroaryl sulfonyl, alkyl carbonyl, aryl carbonyl, heteroalkyl carbonyl, heteroaryl carbonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl or a combination thereof, with the proviso that E, X and Y may optionally be additionally substituted with moieties selected from the group consisting of aromatic, alkyl, alkyl-aryl, heteroalkyl, aryl-heteroaryl, alkyl-heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, alkyl sulfide, alkyl-aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, alkyl amide, alkyl-aryl amide, alkyl sulfonamide, , alkyl amines, alkyl-aryl amines, alkyl-aryl sulfonamide, alkyl urea, alkyl-aryl urea,

alkyl carbamate, alkyl-aryl carbamate, halogen, hydroxyl amino, alkyl carbazate, aryl carbazate,

$R^1 = COR^5$ or $B(OR)_2$, wherein R^5 = H, OH, OR^8, NR^9R^{10}, CF_3, C_2F_5, C_3F_7, CF_2R^6, R^6, COR^7 wherein R^7 = H, OH, OR^8, CHR^9R^{10}, or NR^9R^{10}, wherein R^6, R^8, R^9 and R^{10} are independently selected from the group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, cycloalkyl, arylalkyl, heteroarylalkyl, $CH(R^{1'})COOR^{11}$, $CH(R^1)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})COO\ R^{11}$, $CH(R^1)CONHCH(R^{2'})CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})R'$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^{3'})COO\ R^{11}$, $CH(R^{1'})CONHCH(R^{2'})CONHCH(R^{3'})CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^{3'})CONHCH(R^4)COO\ R^{11}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^{3'})CONHCH(R^{4'})CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^{3'})CONHCH(R^4)CONHCH(R^{5'})COO\ R^{11}$, $CH(R^{1'})CONHCH(R^{2'})CONHCH(R^3)CONHCH(R^4)CONHCH(R^{5'})\ CONR^{12}R^{13}$, wherein R^1, $R^{2'}$, $R^{3'}$, $R^{4'}$, $R^{5'}$, R^{11}, R^{12}, R^{13}, and R' are independently selected from a group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, alkyl-aryl, alkyl-heteroaryl, aryl-alkyl and heteroaralkyl,

Z is selected from O, N, or CH,

W may be present or absent, and if W is present, W is selected from C=O, C=S, SO_2 or C=NR,

Q is $(NR)_p$, O, S, CH_2, CHR, CRR' or a double bond towards V,

A is O, CH_2, $(CHR)_p$, $(CHR\text{-}CHR')_p$ $(CRR')_p$, NR, S, SO_2, C=O or a bond,

G is $(CH_2)_p$, $(CHR)_p$, $(CRR')_p$, NR, O, S, SO_2, $S(O)_2NH$, C=O, or a double bond towards E or V,

V is CH, CR or N,

p is a number from 0 to 6, and

R, R', R^2, R^3 and R^4 are independently selected from the group consisting of H, C1-C10 alkyl, C2-C10 alkenyl, C3-C8 cycloalkyl, C3-C8 heterocycloalkyl, aryl, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, heteroaryl, alkyl-aryl, alkyl-

heteroaryl, (cycloalkyl)alkyl and (heterocycloalkyl)alkyl, wherein said cycloalkyl is made of three to eight carbon atoms, and zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of one to six carbon atoms,
with said alkyl, heteroalkyl, alkenyl, heteroalkenyl, aryl, heteroaryl, cycloalkyl and heterocycloalkyl moieties may be optionally substituted, with said term "substituted" referring to optional and suitable substitution with one or more moieties selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, heterocyclic, halogen, hydroxy, thio, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, sulfonamide, sulfoxide, sulfone, sulfonyl urea, hydrazide, and hydroxamate and thiourea

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs Thus, for example, the term alkyl (including the alkyl portions of alkoxy) refers to a monovalent group derived from a straight or branched chain saturated hydrocarbon by the removal of a single atom having from 1 to 8 carbon atoms, preferably from 1 to 6,

aryl – represents a carbocyclic group having from 6 to 14 carbon atoms and having at least one benzenoid ring, with all available substitutable aromatic carbon atoms of the carbocyclic group being intended as possible points of attachment. Preferred aryl groups include phenyl, 1-naphthyl, 2-naphthyl and indanyl, and especially phenyl and substituted phenyl,

aralkyl – represents a moiety containing an aryl group linked vial a lower alkyl,

alkylaryl – represents a moiety containing a lower alkyl linked via an aryl group,

cycloalkyl – represents a saturated carbocyclic ring having from 3 to 8 carbon atoms, preferably 5 or 6, optionally substituted,

heterocyclic – represents, in addition to the heteroaryl groups defined below, saturated and unsaturated cyclic organic groups having at least one O, S and/or N atom interrupting a carbocyclic ring structure that consists of one or more

rings, wherein each ring is 5-, 6- or 7-membered and may or may not have double bonds that lack delocalized pi electrons, which ring structure has from 2 to 8, preferably from 3 to 6 carbon atoms, e g , 2- or 3-pipendinyl, 2- or 3-piperazinyl, 2- or 3-morpholinyl, or 2- or 3-thiomorpholinyl,

halogen -- represents fluorine, chlorine, bromine and iodine,

heteroaryl -- represents a cyclic organic group having at least one O, S and/or N atom interrupting a carbocyclic ring structure and having a sufficient number of delocalized pi electrons to provide aromatic character, with the aromatic heterocyclic group having from 2 to 14, preferably 4 or 5 carbon atoms, e g , 2-, 3- or 4-pyridyl, 2- or 3-furyl, 2- or 3-thienyl, 2-, 4- or 5-thiazolyl, 2- or 4-imidazolyl, 2-, 4- or 5-pyrimidinyl, 2-pyrazinyl, or 3- or 4-pyndazinyl, etc Preferred heteroaryl groups are 2-, 3- and 4-pyridyl, Such heteroaryl groups may also be optionally substituted

Also included in the invention are tautomers, rotamers, enantiomers and other optical isomers of compounds of Formula I, as well as pharmaceutically acceptable salts and solvates thereof

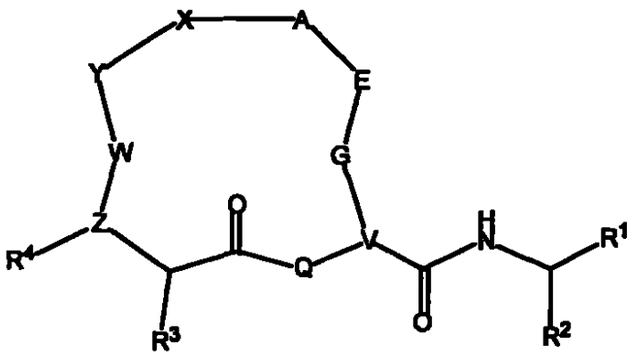
A further feature of the invention is pharmaceutical compositions containing as active ingredient a compound of Formula I (or its salt, solvate or isomers) together with a pharmaceutically acceptable carrier or excipient.

The invention also provides methods for preparing compounds of Formula I, as well as methods for treating diseases such as, for example, HCV and related disorders The methods for treating comprise administering to a patient suffering from said disease or diseases a therapeutically effective amount of a compound of Formula I, or pharmaceutical compositions comprising a compound of Formula I

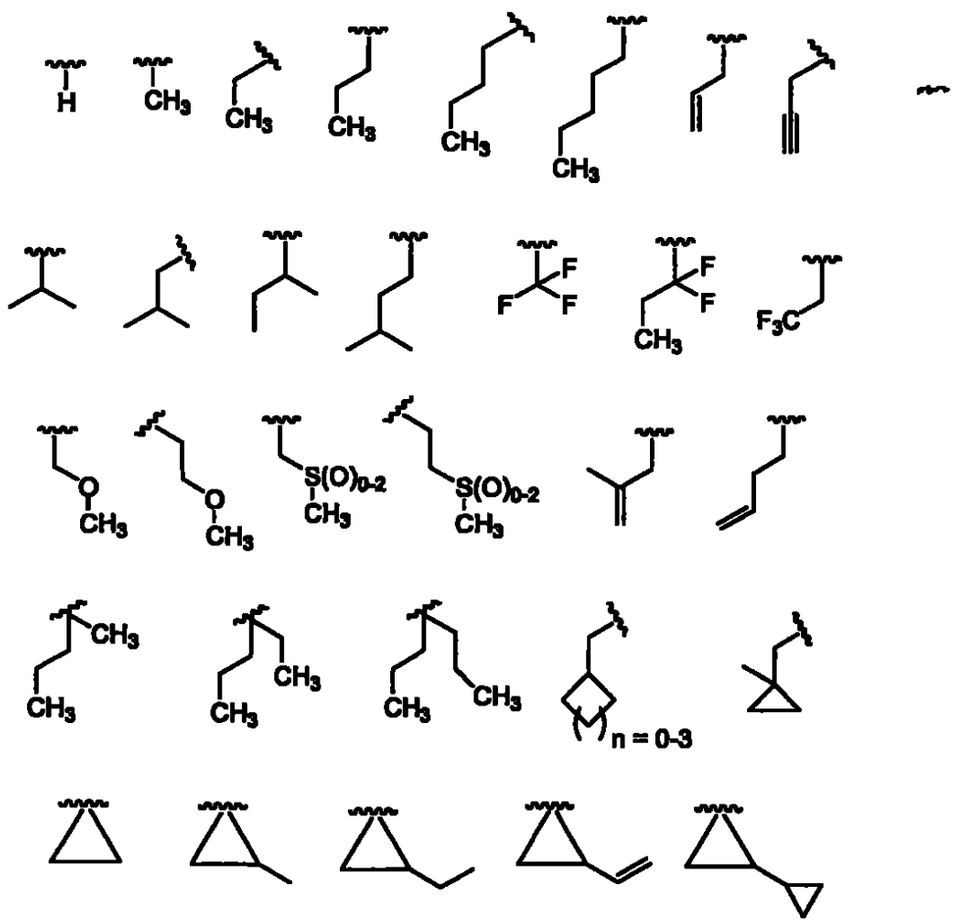
Also disclosed is the use of a compound of Formula I for the manufacture of a medicament for treating HCV and related disorders

Detailed description of preferred embodiments

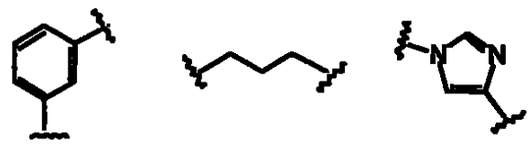
In one embodiment, the present invention discloses compounds of Formula I as inhibitors of HCV protease, especially the HCV NS3/NS4a serine protease



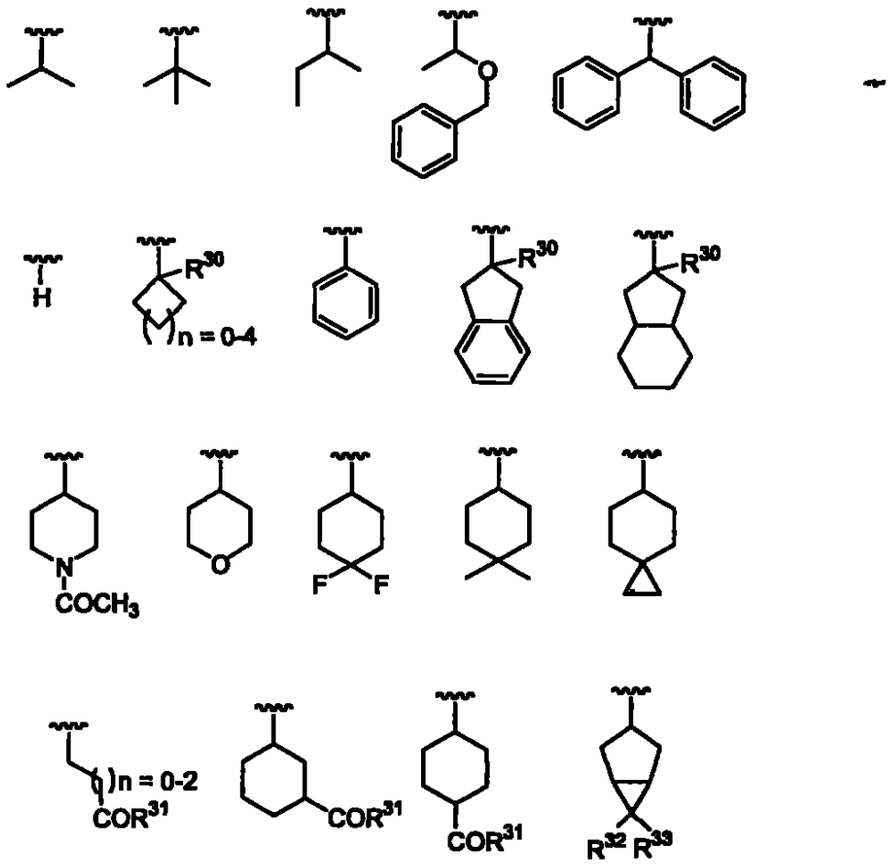
where the various moieties are defined above Some of the preferred embodiments include, but are not limited to, the following definitions of the various functionalities in the above-noted general formula I, other desired definitions for the same and additional functionalities may be found in the structures and claims of this application which are also within the contemplation of the present invention Among the preferred embodiments, R^2 in formula I may be selected from the following moieties



E may be a substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroaryl or substituted or unsubstituted cycloalkyl, with preferred representations for E being



Preferred embodiments for R^3 include the moieties

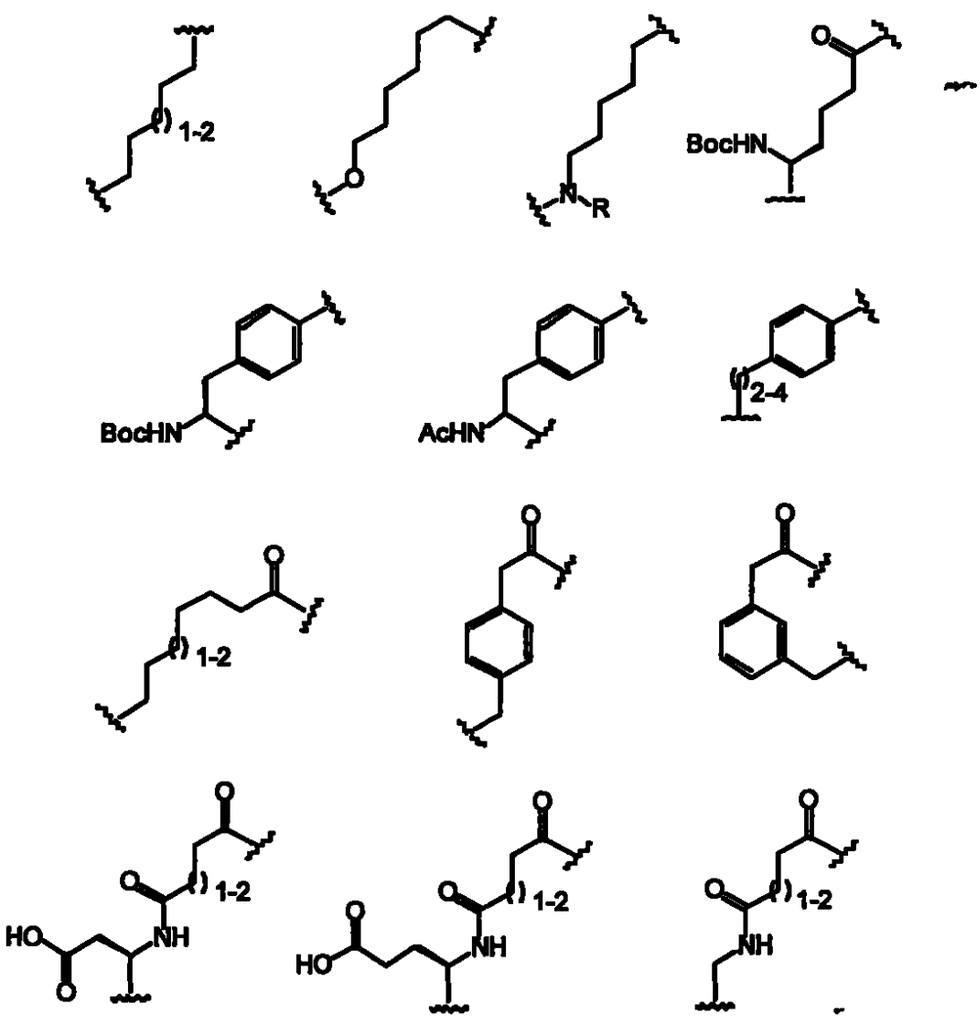


wherein R^{30} = H, CH_3 or other alkyl groups,

R^{31} = OH, O-alkyl, NH_2, N-alkyl, and

R^{32} and R^{33} may be the same or different and are selected independently from H, F, Cl, Br and CH_3

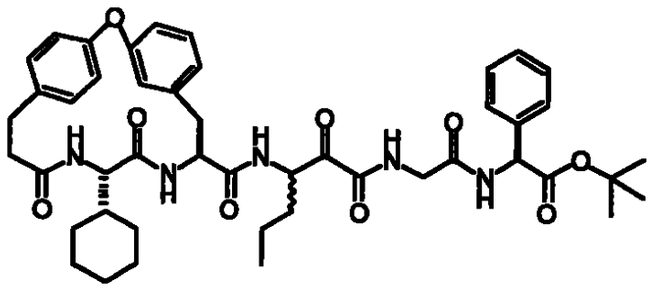
Preferred embodiments for the moiety X-Y are the following structures



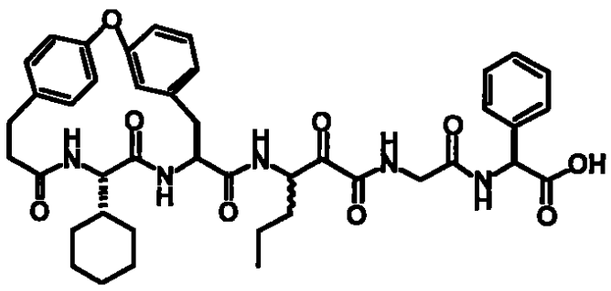
Several additional and further refinements of the above-noted various definitions for the compounds represented by Formula I are noted in the **Claims** section of this application They are also represented by the various compounds listed in the specification and claims Such refinements, definitions and limitations are to be considered as representing the entire invention of this application

Representative compounds of the invention which exhibit excellent HCV protease inhibitory activity are listed below

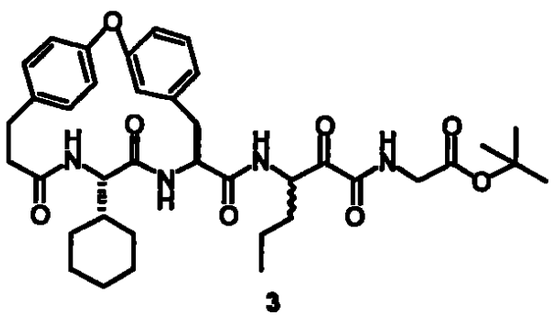
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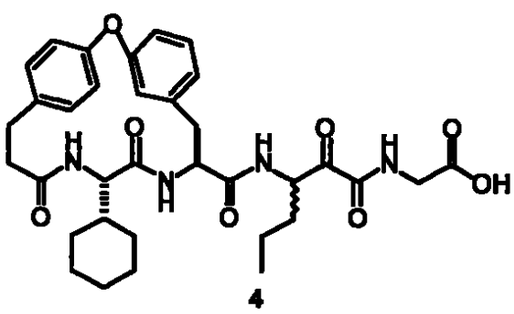
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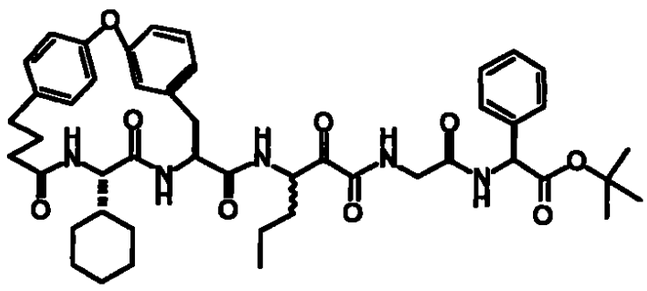
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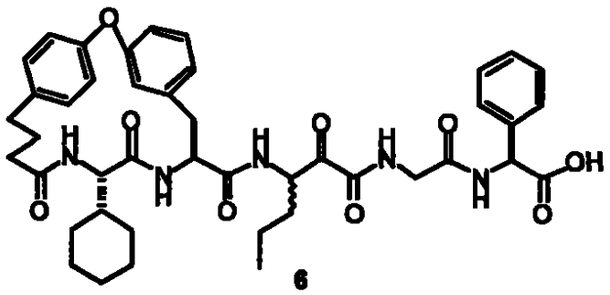
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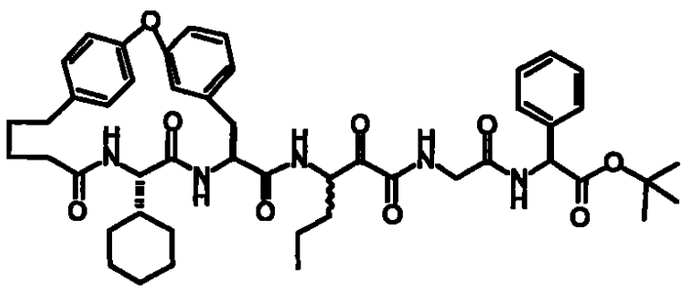
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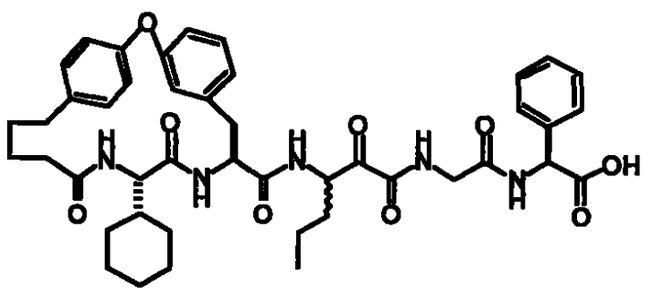
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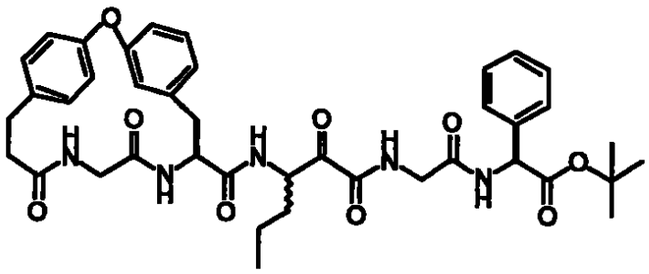
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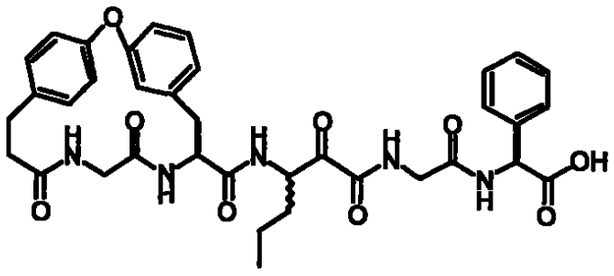
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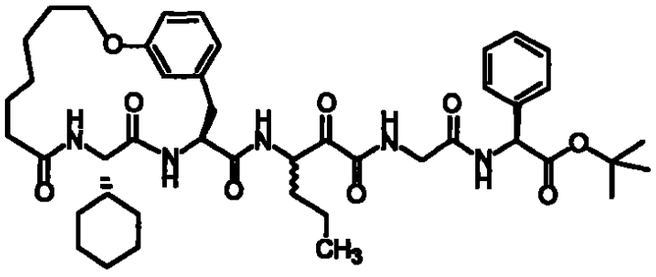
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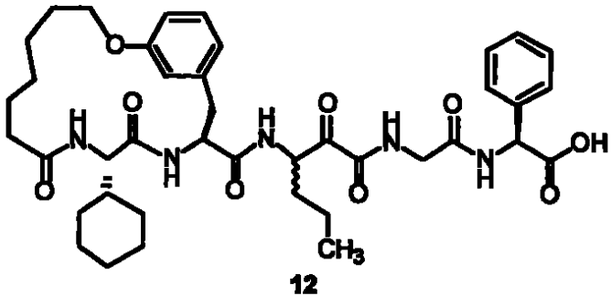
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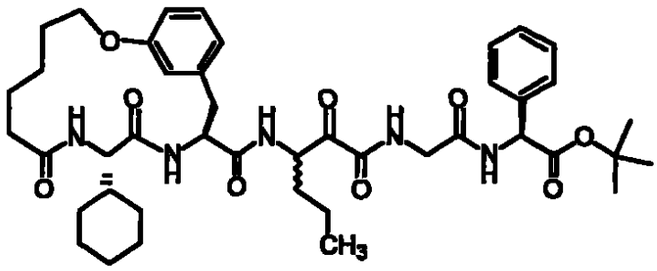
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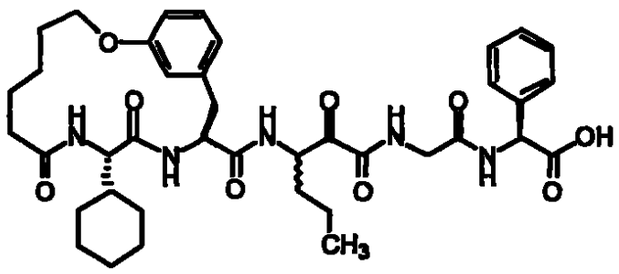
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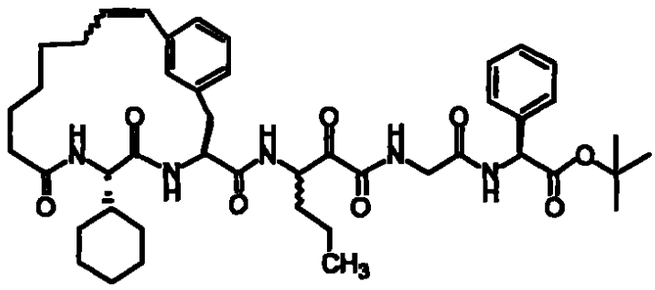
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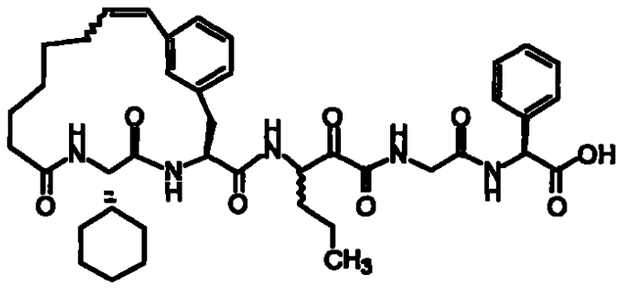
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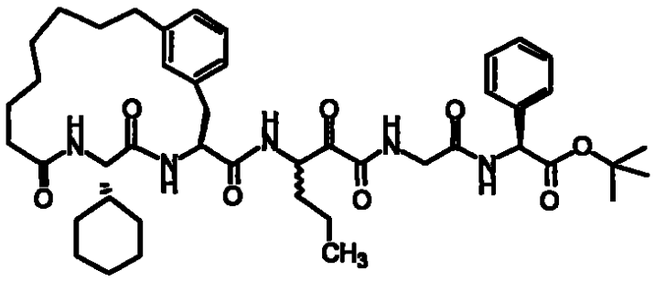


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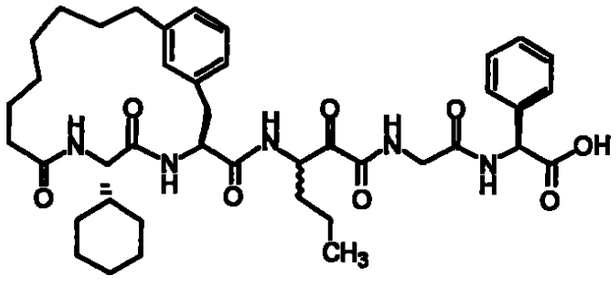


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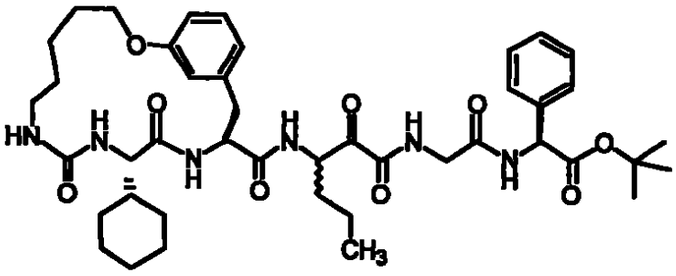
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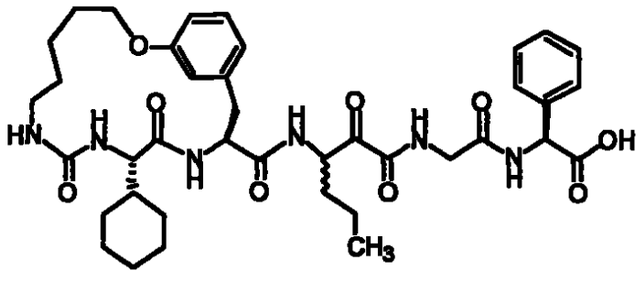
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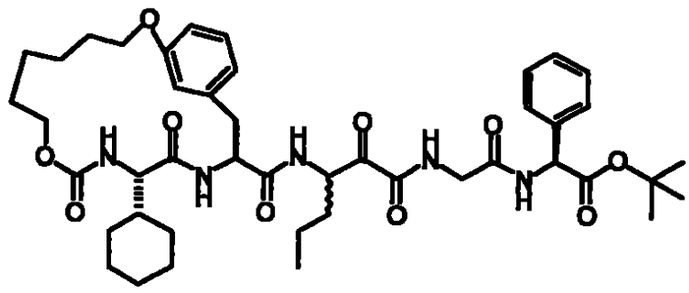
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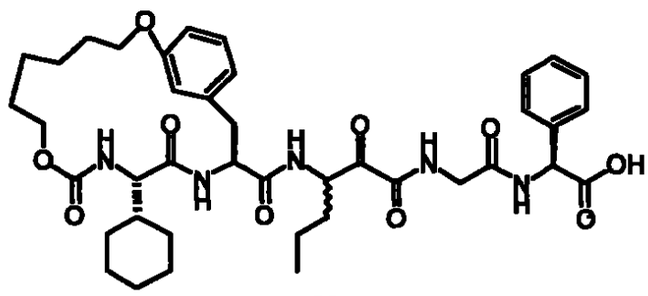
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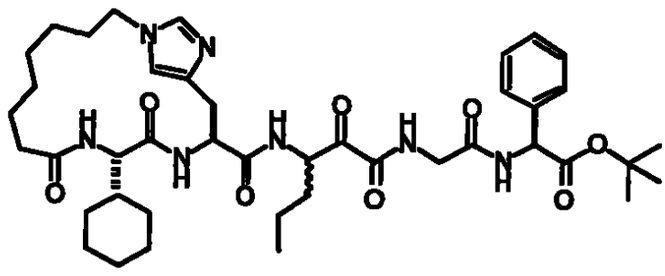
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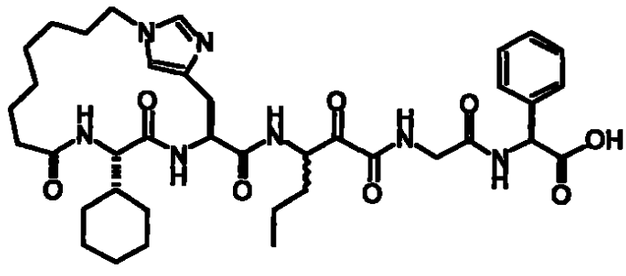
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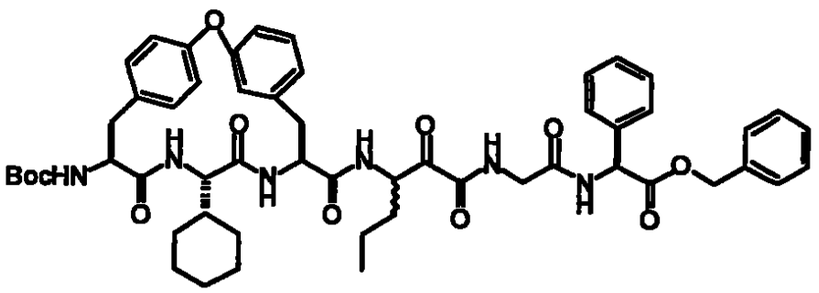
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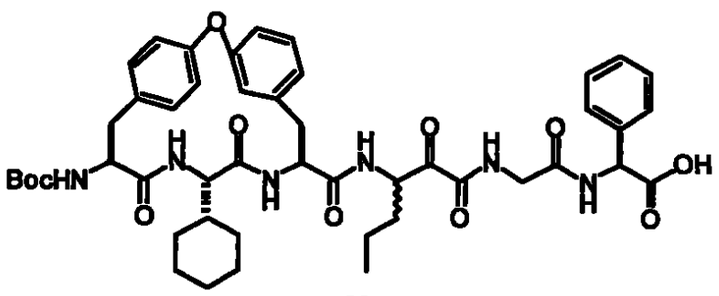
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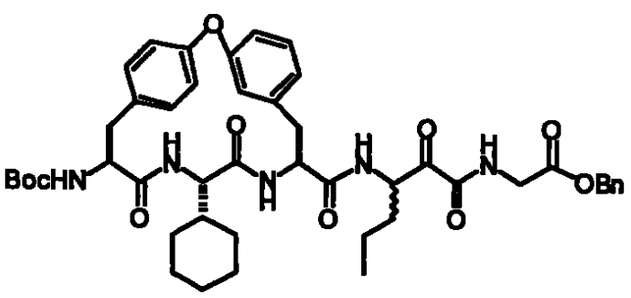
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O
O

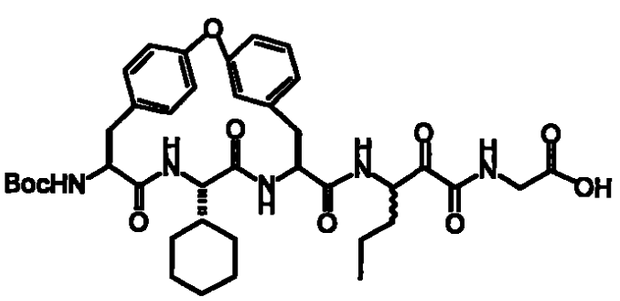
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BocHN
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N
H
OH
O

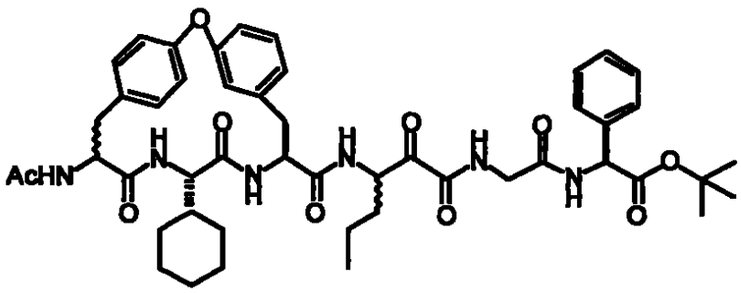
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BocHN
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H
N
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N
H
O
H
N
O
O
H
N
O
O
OBn

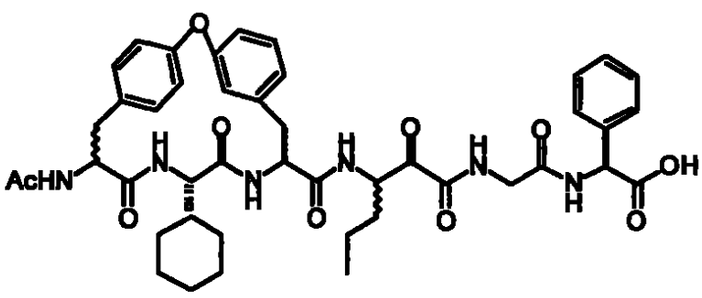
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BocHN
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O
O
OH

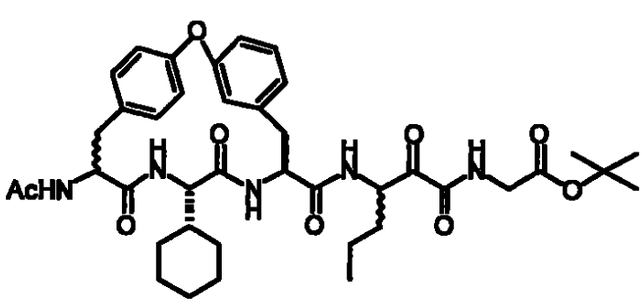
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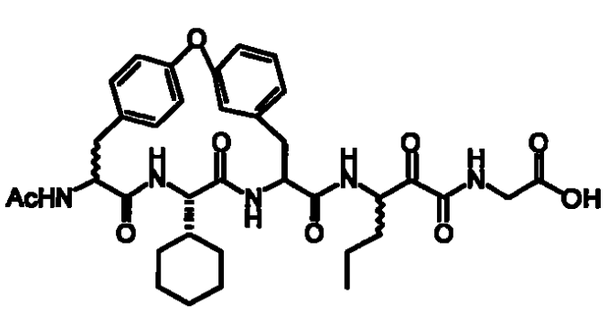
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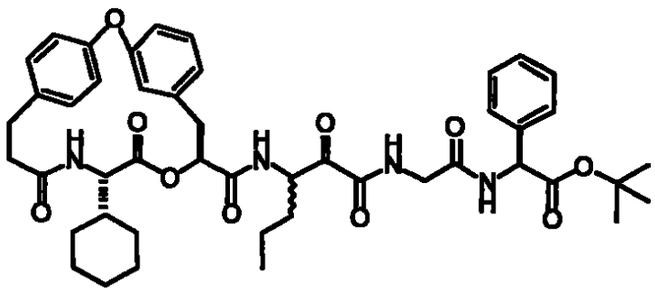
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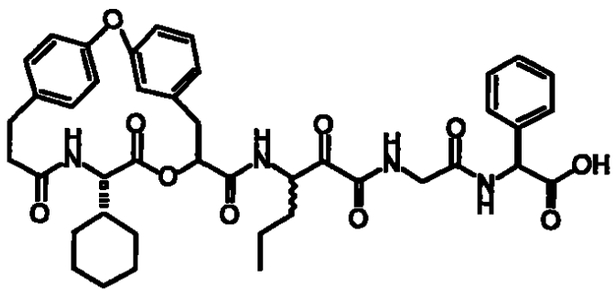
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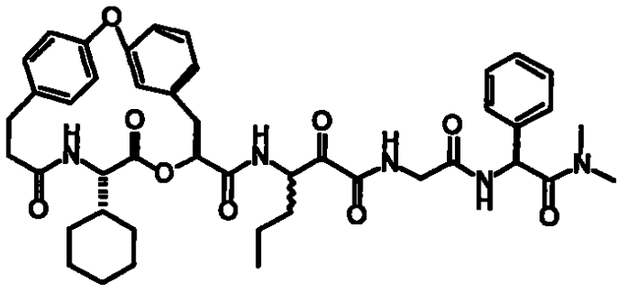
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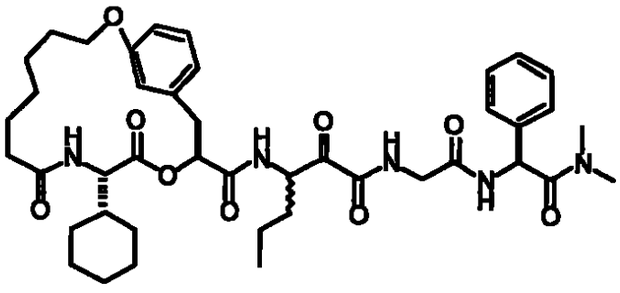
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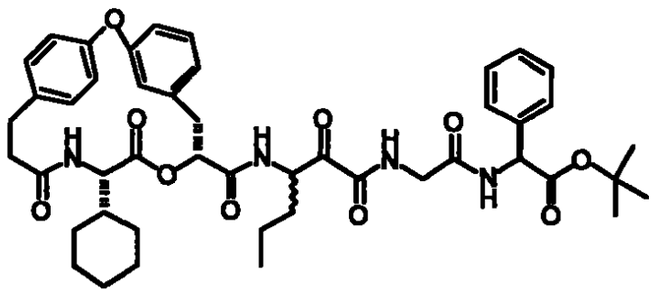
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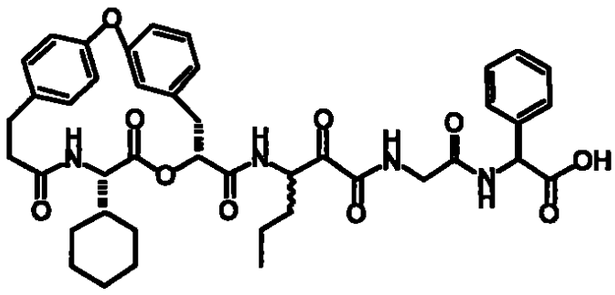
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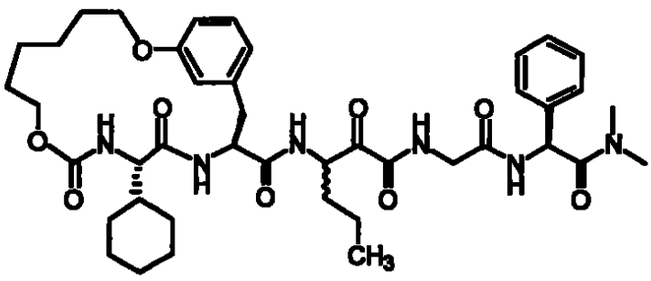
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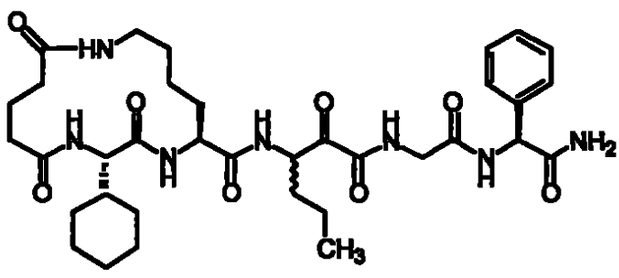
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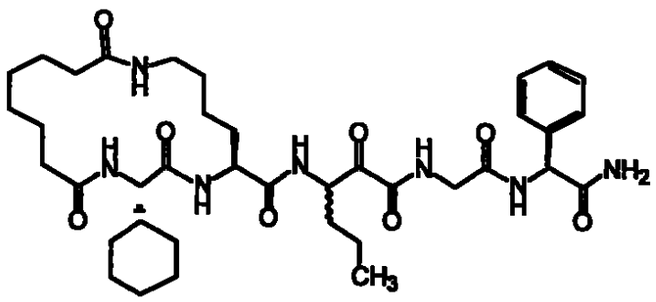
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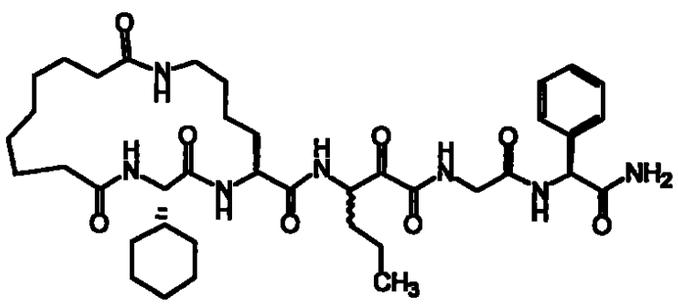
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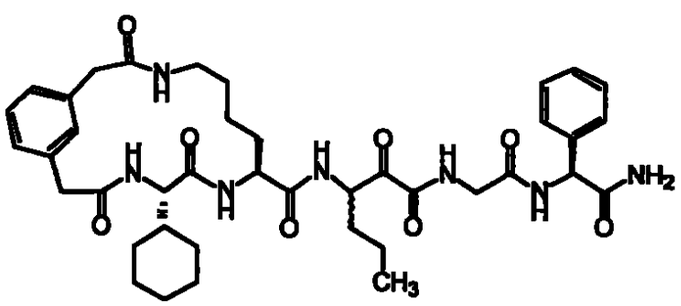
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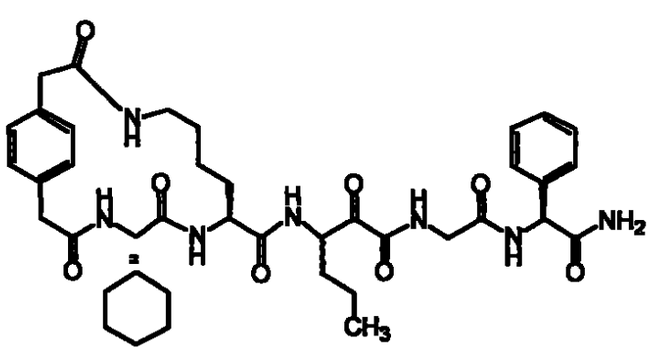
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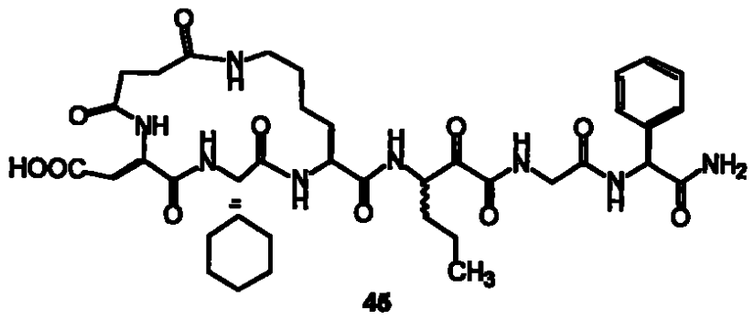
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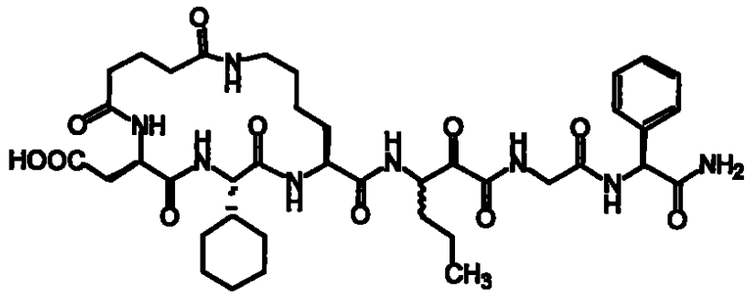
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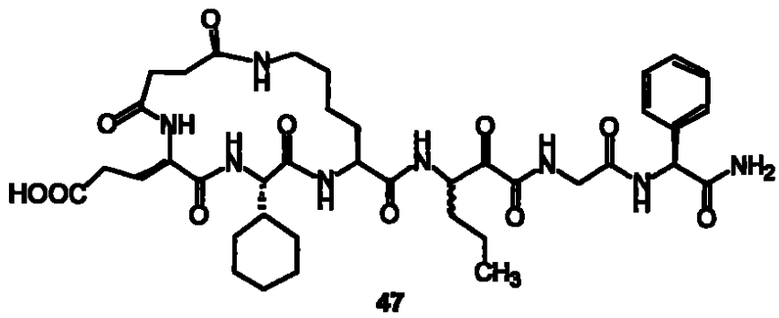
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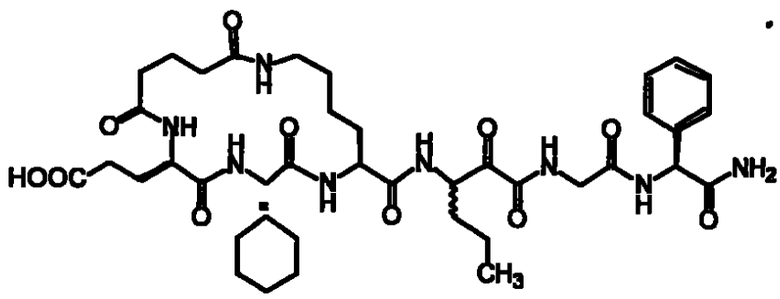
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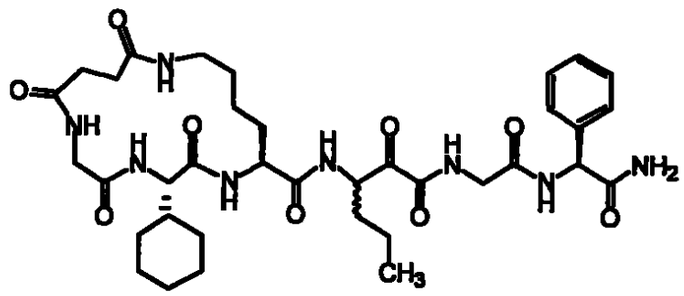
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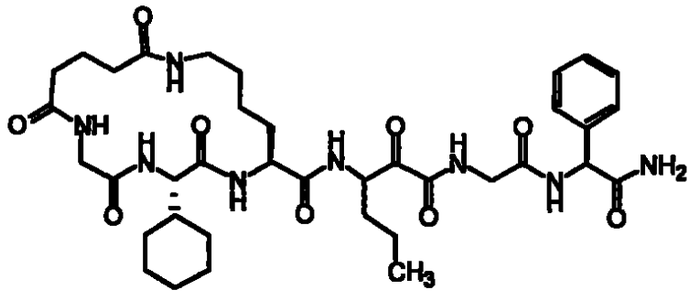
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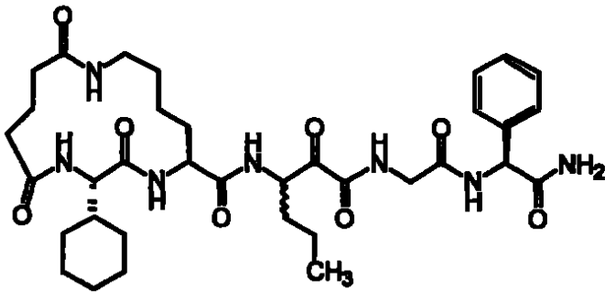
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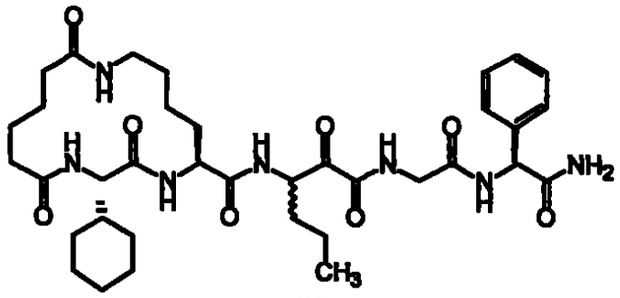
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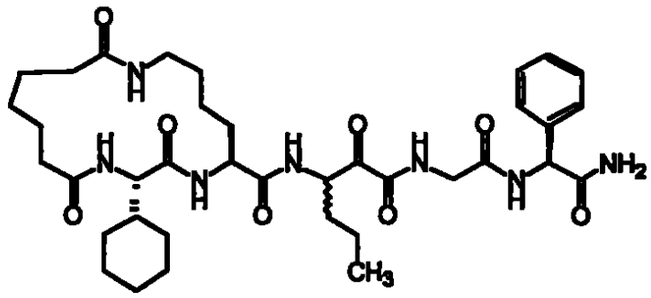
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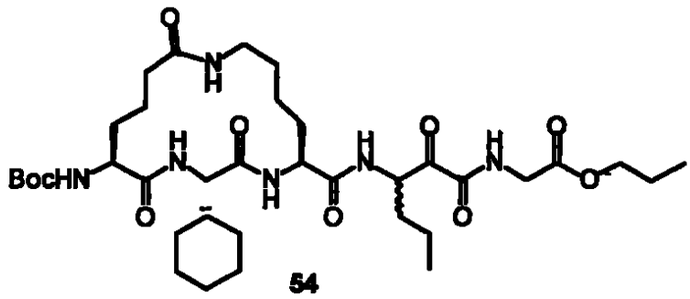
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The activity of some of the compounds are presented in **Table 1** as ranges of K_i values in nanomolar (nM) The Example numbers in **Table 1** refer to the numbers for the various structures in the EXAMPLES section found in the later parts of this application

Table 1. HCV protease continuous assay results

Example number	K_i* nM
1	B
2	A
3	B
4	B
5	B
6	A
7	B
8	A

9	B
10	B
11	B
12	A
13	B
14	B
15	B
16	A
17	B
18	A
19	B
20	A
21	B
22	A
23	B
24	B
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29	B
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31	B
32	B
33	B
34	B
35	B
36	B
37	B
38	B

39	A
40	B
41	A
42	B
43	A
44	A
45	B
46	B
47	B
48	B
49	B
50	B
51	B
52	A
53	A
54	B

HCV continous assay Ki* range

Category A= 0 001-1 0 μM, Category B= 1 1-100 μM

Some methods of synthesizing the various types of the inventive compounds are described later in this section, and also schematically described, followed by the illustrative Examples

Depending upon the structure, the compounds of the invention may form pharmaceutically acceptable salts with organic or inorganic acids, or organic or inorganic bases Examples of suitable acids for such salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic and other mineral and carboxylic acids well known to those skilled in the art For formation of salts with bases, suitable bases are, for example, NaOH, KOH, NH_4OH, tetraalkylammonium hydroxide, and the like

In another embodiment, this invention provides pharmaceutical compositions comprising the above-described inventive macrocycles as an active ingredient The pharmaceutical compositions generally additionally comprise a pharmaceutically acceptable carrier diluent, excipient or carrier (collectively referred to herein as carrier materials) Because of their HCV inhibitory activity, such pharmaceutical compositions possess utility in treating hepatitis C and related disorders

In yet another embodiment, the present invention discloses methods for preparing pharmaceutical compositions comprising the inventive macrocycle compounds as an active ingredient. In the pharmaceutical compositions and methods of the present invention, the active ingredients will typically be administered in admixture with suitable carrier materials suitably selected with respect to the intended form of administration, i e oral tablets, capsules (either solid-filled, semi-solid filled or liquid filled), powders for constitution, oral gels, elixirs, dispersible granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices For example, for oral administration in the form of tablets or capsules, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable inert carrier, such as lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid forms) and the like Moreover, when desired or needed, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated in the mixture Powders and tablets may be comprised of from about 5 to about 95 percent inventive composition Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes Among the lubricants there may be mentioned for use in these dosage forms, boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like Disintegrants include starch, methylcellulose, guar gum and the like

Sweetening and flavoring agents and preservatives may also be included where appropriate Some of the terms noted above, namely disintegrants, diluents, lubricants, binders and the like, are discussed in more detail below

Additionally, the compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one or more of the components or active ingredients to optimize the therapeutic effects, i e HCV inhibitory activity and the like Suitable dosage forms for sustained release include layered tablets containing layers of varying disintegration rates or controlled release polymeric matrices impregnated with the active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric matrices

Liquid form preparations include solutions, suspensions and emulsions As an example may be mentioned water or water-propylene glycol solutions for parenteral injections or addition of sweeteners and pacifiers for oral solutions, suspensions and emulsions Liquid form preparations may also include solutions for intranasal administration

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier such as inert compressed gas, e g nitrogen

For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides such as cocoa butter is first melted, and the active ingredient is dispersed homogeneously therein by stirring or similar mixing The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool and thereby solidify

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration Such liquid forms include solutions, suspensions and emulsions

The compounds of the invention may also be deliverable transdermally The transdermal compositions may take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose

Preferably the compound is administered orally

Preferably, the pharmaceutical preparation is in a unit dosage form In such form, the preparation is subdivided into suitably sized unit doses containing appropriate quantities of the active components, e g , an effective amount to achieve the desired purpose

The quantity of the inventive active composition in a unit dose of preparation may be generally varied or adjusted from about 1 0 milligram to about 1,000 milligrams, preferably from about 1 0 to about 950 milligrams, more preferably from about 1 0 to about 500 milligrams, and typically from about 1 to about 250 milligrams, according to the particular application The actual dosage employed may be varied depending upon the patient's age, sex, weight and severity of the condition being treated Such techniques are well known to those skilled in the art

Generally, the human oral dosage form containing the active ingredients can be administered 1 or 2 times per day The amount and frequency of the administration will be regulated according to the judgment of the attending clinician A generally recommended daily dosage regimen for oral administration may range from about 1 0 milligram to about 1,000 milligrams per day, in single or divided doses

Some useful terms are described below

Capsule - refers to a special container or enclosure made of methyl cellulose, polyvinyl alcohols, or denatured gelatins or starch for holding or containing compositions comprising the active ingredients Hard shell capsules are typically made of blends of relatively high gel strength bone and pork skin gelatins The capsule itself may contain small amounts of dyes, opaquing agents, plasticizers and preservatives

Tablet- refers to a compressed or molded solid dosage form containing the active ingredients with suitable diluents The tablet can be prepared by compression of mixtures or granulations obtained by wet granulation, dry granulation or by compaction

Oral gel- refers to the active ingredients dispersed or solubilized in a hydrophillic semi-solid matrix

Powder for constitution refers to powder blends containing the active ingredients and suitable diluents which can be suspended in water or juices

Diluent - refers to substances that usually make up the major portion of the composition or dosage form Suitable diluents include sugars such as lactose, sucrose, mannitol and sorbitol, starches derived from wheat, corn, rice and potato, and celluloses such as microcrystalline cellulose The amount of diluent in the composition can range from about 10 to about 90% by weight of the total composition, preferably from about 25 to about 75%, more preferably from about 30 to about 60% by weight, even more preferably from about 12 to about 60%

Disintegrant - refers to materials added to the composition to help it break apart (disintegrate) and release the medicaments Suitable disintegrants include starches, "cold water soluble" modified starches such as sodium carboxymethyl starch, natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar; cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose, microcrystalline celluloses and cross-linked microcrystalline celluloses such as sodium croscarmellose, alginates such as alginic acid and sodium alginate, clays such as bentonites, and effervescent mixtures The amount of disintegrant in the composition can range from about 2 to about 15% by weight of the composition, more preferably from about 4 to about 10% by weight

Binder - refers to substances that bind or "glue" powders together and make them cohesive by forming granules, thus serving as the "adhesive" in the formulation Binders add cohesive strength already available in the diluent or bulking agent Suitable binders include sugars such as sucrose, starches derived from wheat, corn rice and potato, natural gums such as acacia, gelatin and tragacanth, derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate, cellulosic materials such as methylcellulose and sodium carboxymethylcellulose and hydroxypropylmethylcellulose, polyvinylpyrrolidone, and inorganics such as magnesium aluminum silicate The amount of binder in the composition can range from about 2 to about 20% by

weight of the composition, more preferably from about 3 to about 10% by weight, even more preferably from about 3 to about 6% by weight.

Lubricant - refers to a substance added to the dosage form to enable the tablet, granules, etc after it has been compressed, to release from the mold or die by reducing friction or wear Suitable lubricants include metallic stearates such as magnesium stearate, calcium stearate or potassium stearate, stearic acid, high melting point waxes, and water soluble lubricants such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and d'l-leucine Lubricants are usually added at the very last step before compression, since they must be present on the surfaces of the granules and in between them and the parts of the tablet press The amount of lubricant in the composition can range from about 0 2 to about 5% by weight of the composition, preferably from about 0 5 to about 2%, more preferably from about 0 3 to about 1 5% by weight

Glident - material that prevents caking and improve the flow characteristics of granulations, so that flow is smooth and uniform Suitable glidents include silicon dioxide and talc The amount of glident in the composition can range from about 0 1% to about 5% by weight of the total composition, preferably from about 0 5 to about 2% by weight.

Coloring agents - excipients that provide coloration to the composition or the dosage form Such excipients can include food grade dyes and food grade dyes adsorbed onto a suitable adsorbent such as clay or aluminum oxide The amount of the coloring agent can vary from about 0 1 to about 5% by weight of the composition, preferably from about 0 1 to about 1%

Bioavailability - refers to the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed into the systemic circulation from an administered dosage form as compared to a standard or control

Conventional methods for preparing tablets are known Such methods include dry methods such as direct compression and compression of granulation produced by compaction, or wet methods or other special procedures Conventional methods for making other forms for administration such as, for example, capsules, suppositories and the like are also well known

Another embodiment of the invention discloses the use of the pharmaceutical compositions disclosed above for treatment of diseases such as, for example, hepatitis C the like The method comprises administering a therapeutically effective amount of the inventive pharmaceutical composition to a patient having such a disease or diseases and in need of such a treatment.

As stated earlier, the invention includes tautomers, enantiomers and other stereoisomers of the compounds also Thus, as one skilled in the art knows, some of the inventive compounds may exist in isomeric forms Such variations are contemplated to be within the scope of the invention

Another embodiment of the invention discloses a method of making the macrocyclic compounds disclosed herein The compounds may be prepared by several techniques known in the art Representative illustrative procedures are outlined in the following reaction schemes It is to be understood that while the following illustrative schemes describe the preparation of macrocycles predominately derived from *meta*-tyrosine or lysine at the P2 position Suitable substitution of any of both the natural and unnatural amino acids will result in the formation of the desired macrocycles based on such substitution

Abbreviations which are used in the descriptions of the schemes, preparations and the examples that follow are

THF Tetrahydrofuran

DMF *N*,*N*-Dimethylformamide

EtOAc Ethyl acetate

AcOH Acetic acid

HOOBt 3-Hydroxy-1,2,3-benzotriazin-4(3*H*)-one

EDCI 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

NMM *N*-Methylmorpholine

ADDP 1,1'-(Azodicarbobyl)dipiperidine

DEAD Diethylazodicarboxylate

MeOH Methanol

EtOH Ethanol

Et_2O Diethyl ether

Bn Benzyl

Boc tert-Butyloxycarbonyl

Cbz Benzyloxycarbonyl

Cp Cylcopentyldienyl

Ts p-toluenesulfonyl

Me Methyl

HATU O-(7-Azabenzotriazol-1-yl)-N,N N',N'-tetramethyluronium hexafluorophosphate

Chg Cyclohexylglycine

Tyr Tyrosine

G Glycerol

TG Thioglycerol

alloc allyloxycarbonyl

FMOC 9-Fluorenyl methyloxycarbonyl

Dde N-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl

tBu *tert*-butyl

equiv equivalent

rel int. relative intensity

aq aqueous

rt. room temperature

satd saturated

Hex hexane(s)

NBA Nitrobenzoic acid

PyBrOP Tris(pyrrolidino)bromophosphonium hexafluorophosphate

DMSO Dimethyl sulfoxide

TFA. Trifluoroacetic acid

HOBt. Hydroxybezotriazole

Hünigs base Diisoprpylethyl amine

BOP Benzotrizaol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate

LDA Lithium diisopropyl amide

Ph_3P Triph nyl phosphine

LAH Lithium Aluminum Hydride

DMAP 4-Dimethyl aminopyridine

DCC Dicyclohexylcarbodiimide

MCPBA meta-Chloroperbenzoic acid

BINAP 2,2'-Bis(diphenylphosphino)-1,1'-binaphtol

MeCN acetonitrile

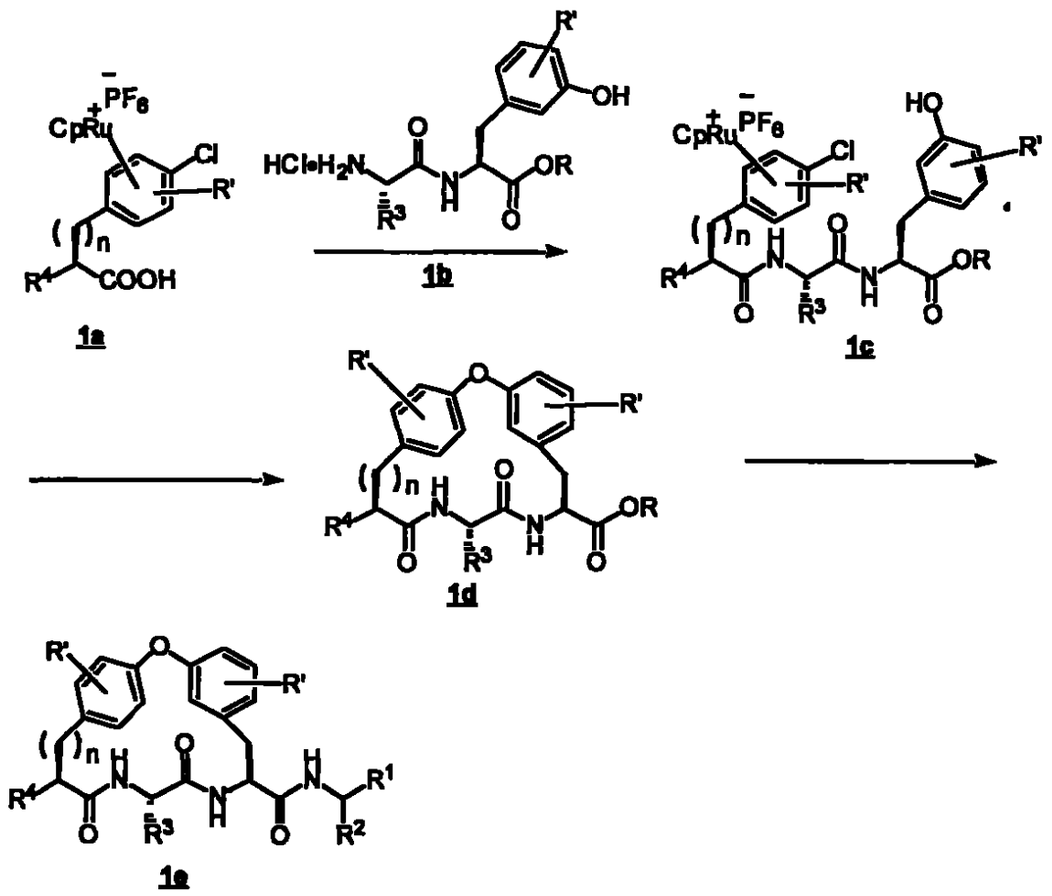
Pr Propyl

Ac Acetyl

Ph Phenyl

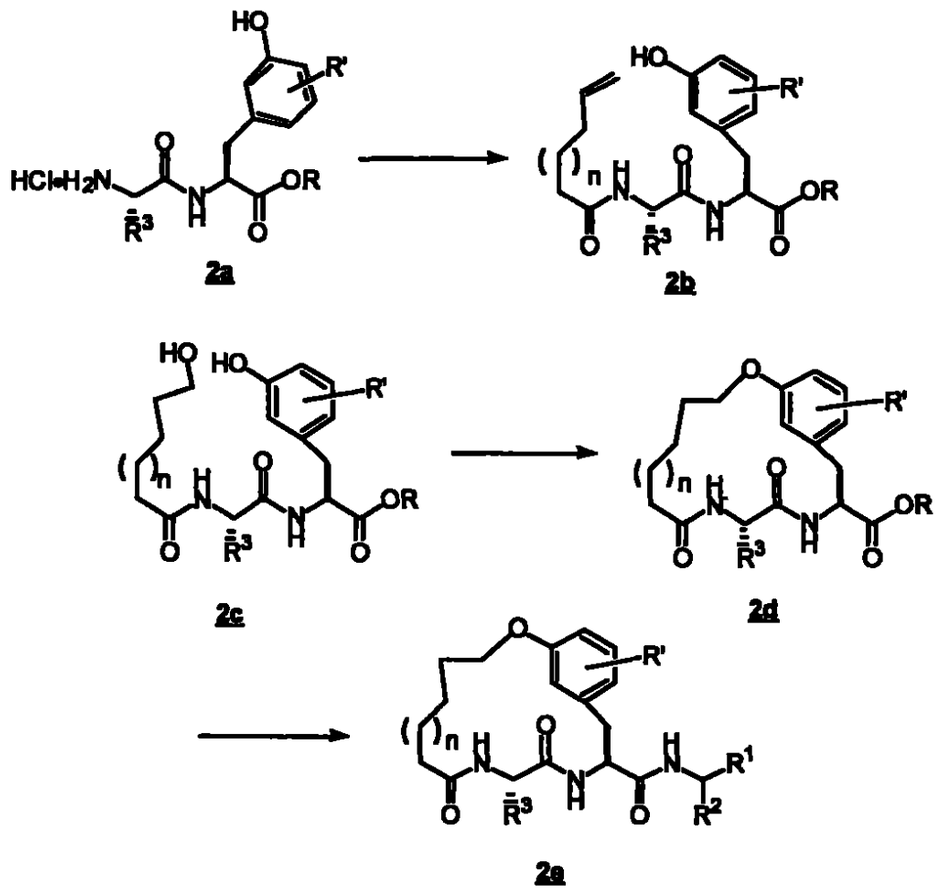
General Schemes for Synthesis of Inventive Compounds. In the following Schemes, n is a number from 1 to 6

Scheme 1



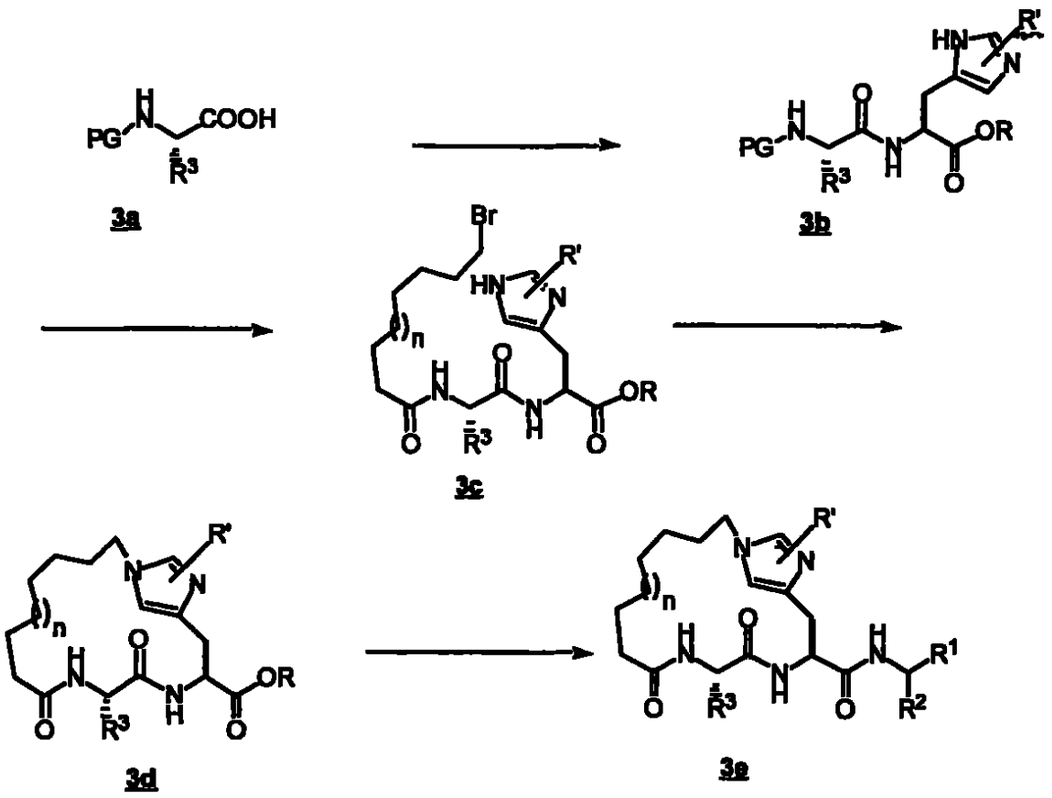
The synthesis of compounds of type **1e** wherein R^1, R^2, R^3, R' are defined above, R^4 being amide, carbamate,or hydrogen, R being alkyl, aryl or arylalkyl, began with the coupling of **1a** with the dipeptide **1b** using NMM , HOBt, and EDCI to obtain the intermediate **1c** The intermediate **1c** was treated with Cs_2CO_3 in DMF followed by photolysis to obtain compound **1d.** The macrocyclic ester **1d** was hydrolyzed and coupled with a suitable amine intermediate to generate compounds of type **1e**

Scheme 2.



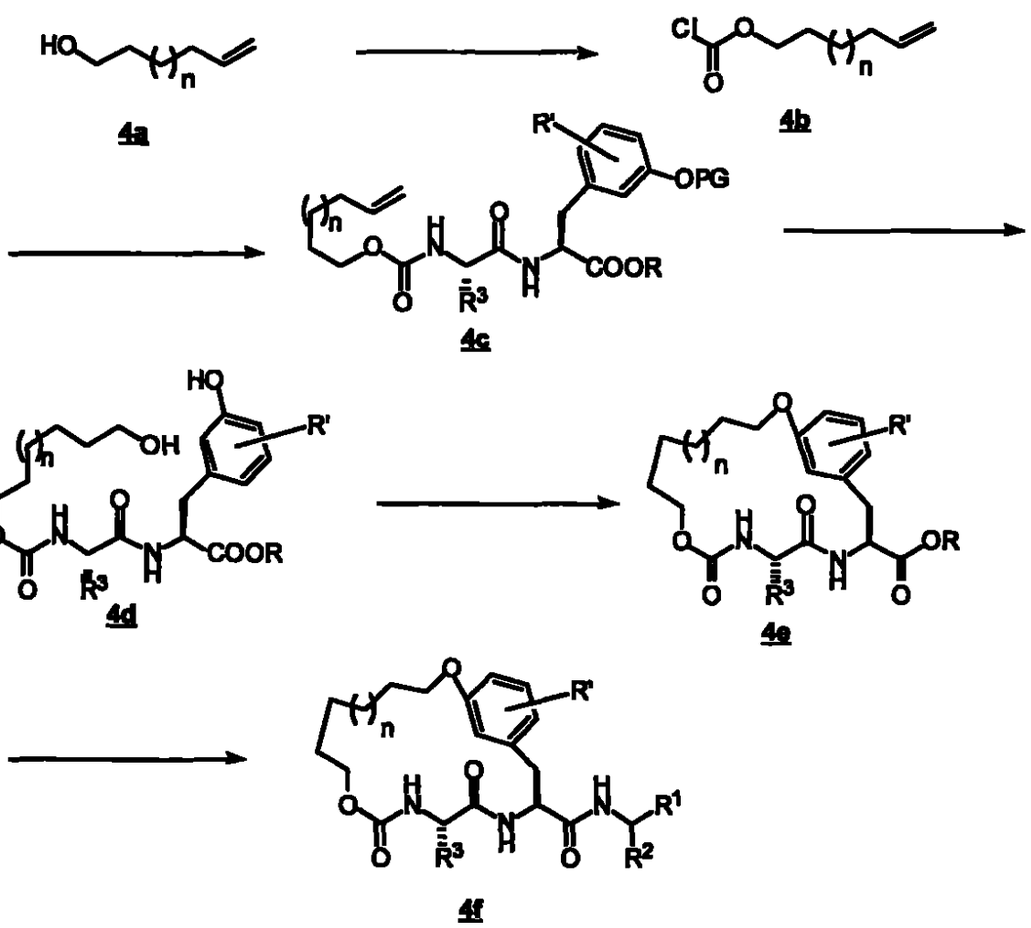
The preparation of the compound of formula **2e**, wherein R^1, R^2, R^3 and n are defined above, R' is alkyl, heteroalkyl (OR'', SR''', NR''R''' wherein R'' and R''' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position to oxygen atom, R is alkyl, aryl, or alkylaryl groups, and n is from zero to five, is outlined in **Scheme 2** The meta-tyrosine-dipeptide **2a** is coupled to an alkenyl carboxylic acid in the presence of HOOBt, EDCI HCl and NMM Hydroboration of the resulting product affords compound **2c** The macrocyclization is achieved under Mitsunobu conditions by using triphenylphosphine and ADDP (The *Mitsunobu* reaction is reviewed by D L Hughes, *Org Reactions*, 42 (1992) 335, John Wiley & Sons, New York, L Paquette, ed) After the ester is hydrolyzed to an acid with lithium hydroxide, it is coupled to an amine intermediate to afford **2e**

Scheme 3.



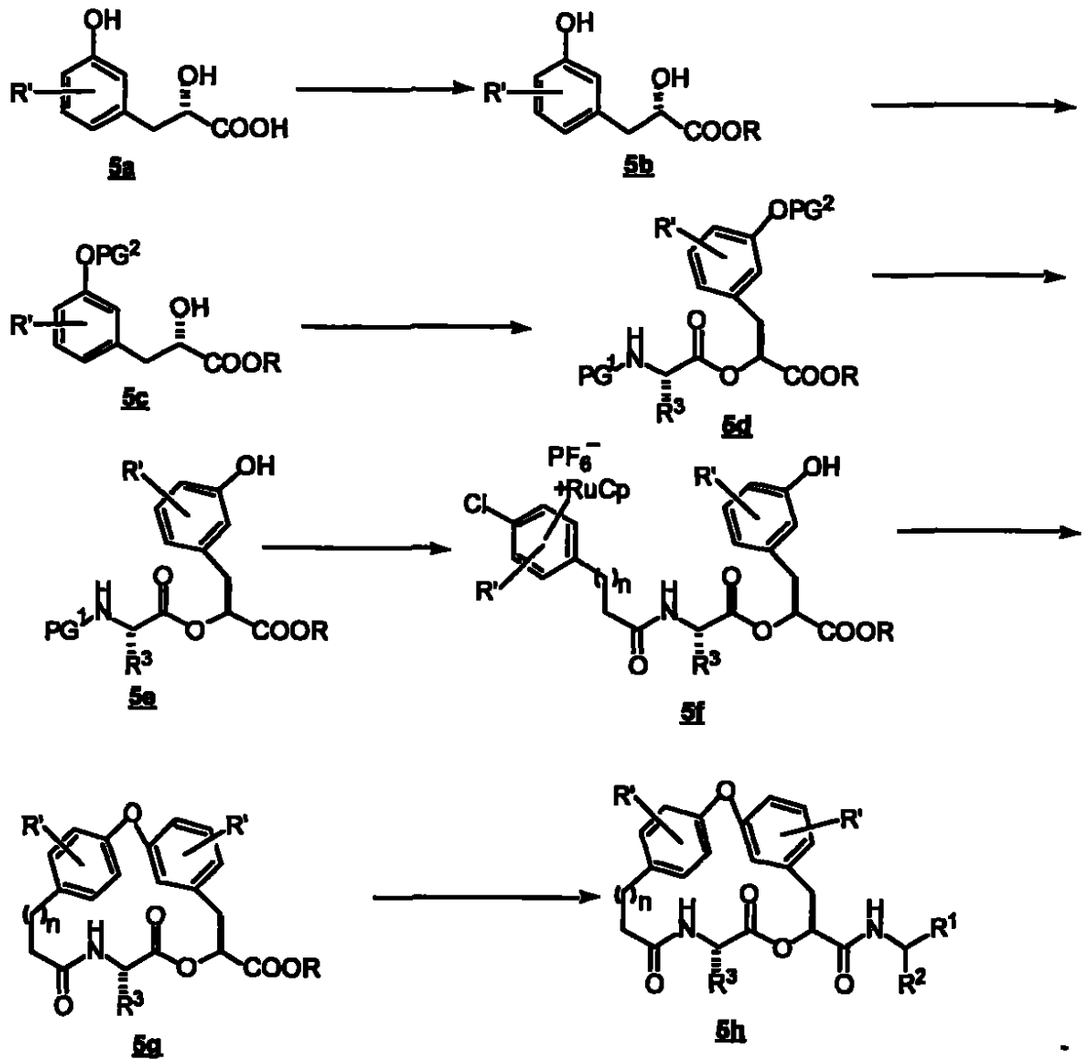
The preparation of compounds of formula 3e wherein R', R^1, R^2, R^3, R and n are defined in **Scheme 1** and PG is Cbz, Boc, or alloc is outlined in **Scheme 3** The compound 3a was coupled with substituted histidine derivative using DCC This compound 3b was deprotected and further treated with ω-bromo acids to obtain compound of the type 3c Cyclization of 3c was accomplished with NaI and Na_2CO_3 in boiling methylvinyl ketone to afford 3d The compound 3d was converted to compounds of type 3e by hydrolysis of the ester followed by coupling with the appropriate amine intermediate

Scheme 4.



The preparation of the compounds of type 4f where in R', R^1, R^2, R^3, R, n and PG are defined in **Scheme 1** was initiated from compound of the type 4a The alcohol of 4a was converted to 4b by treatment with phosgene 4b was converted to 4c by coupling with alloc protected 1b and Et_3N The alloc group of 4c was deprotected using $Pd(PPh_3)_4$ to obtain 4d which underwent cyclization under the Mitsunobu conditions to yield 4e The ester of 4e was hydrolyzed and further coupled with an amine using EDCI, HOOBt to obtain compounds of the type 4f.

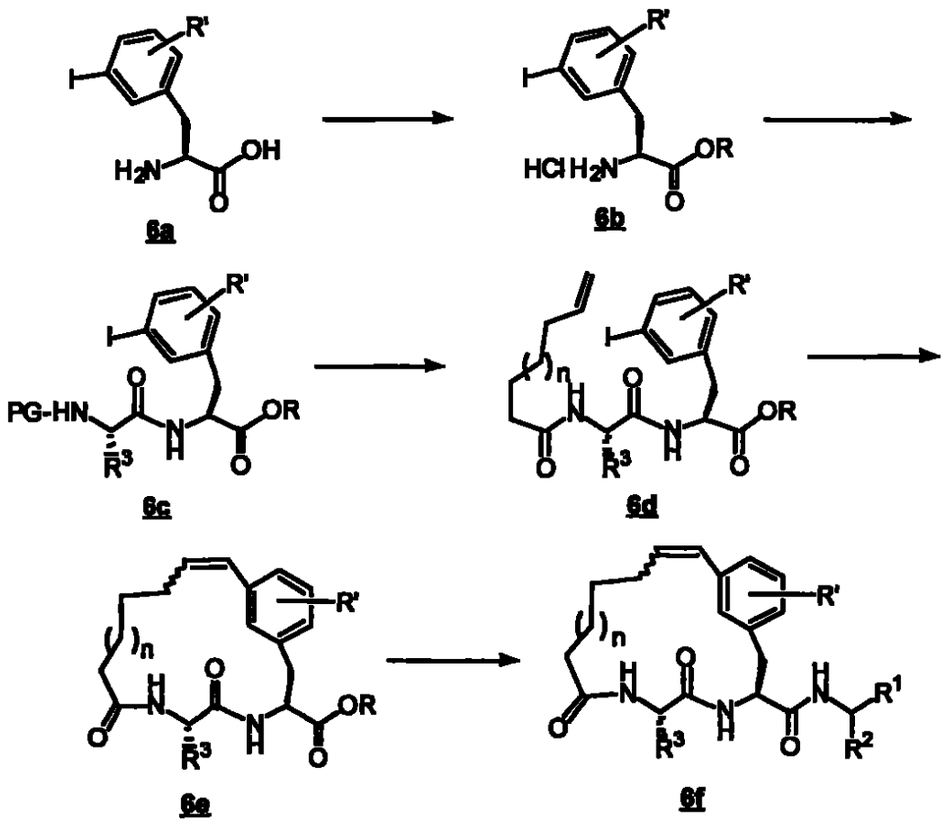
Scheme 5.



The preparation of compounds of type **5h** wherein R^1, R^2, R^3, R', PG and n are defined in **Schemes 1-3** and PG^1 being Cbz or Boc and PG^2 being alloc, began with the known compound **5a** The acid **5a** was converted to the ester by refluxing it with ROH and TsOH The phenolic oxygen of **5b** was converted to the alloc group by the treatment with alloc-chloride and triethyl amine to yield **5c.** The secondary alcohol of **5c** was converted to compounds of type **5d** by coupling with protected cyclohexyl glycine using DCC and HOBt The alloc group of compound

5d was deprotected using $Pd(Ph_3P)_4$, and dimedone 5e was deprotected and treated with EDCI, HOOBt and appropriately activated ruthenium complex, to obtain compounds of the formula of 5f The compounds of the type 5f were converted to cyclic compounds of the formula 5g by the use of Cs_2CO_3, and subsequent photolytic removal of ruthenium The ester group of 5g was hydrolyzed and coupled to an amine intermediate to obtain compounds of the type 5h

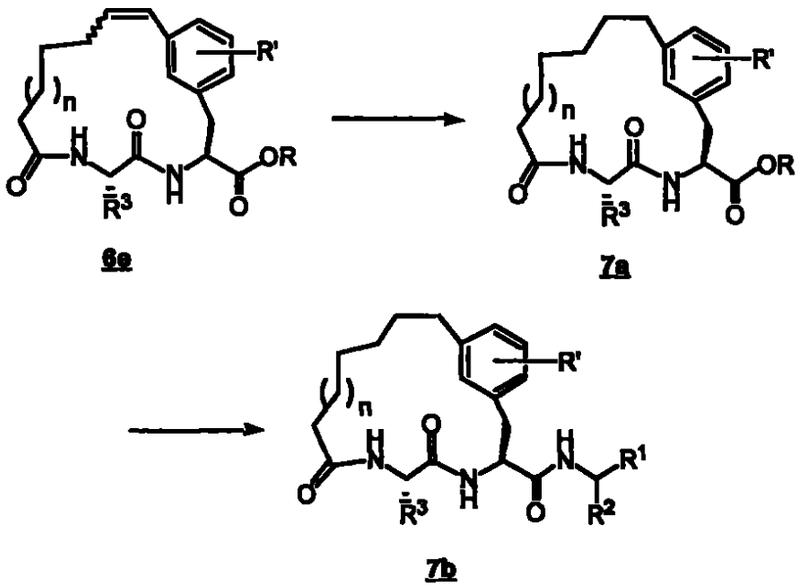
Scheme 6.



The preparation of the compound of formula 6f, wherein R^1, R^2, R^3 are defined above, R' is alkyl, heteroalkyl (OR'', SR''', NR''R''' wherein R'' and R''' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position, R is alkyl, aryl, or alkylaryl groups, PG is Cbz or Boc, and n is from zero to five, is outlined in Scheme 6 The *meta*-Iodophenylglycine 6a is converted to its ester under usual

esterification conditions (ROH, HCl) The product is then coupled to an *N*-protected amino acid in the presence of HOOBt, EDCI HCl and NMM After deprotection, the resulting amine is coupled again to a terminal alkenyl carboxylic acid to give product **6d** The intramolecular Heck reaction with a palladium catalyst provides the desired macrocyclic compound **6e** (The Heck reaction has been reviewed in detail by R F Heck, *Org React*, 27 (1989) 345-390) After the ester is hydrolyzed to an acid with lithium hydroxide, it is coupled to an amine intermediate to afford **6f**

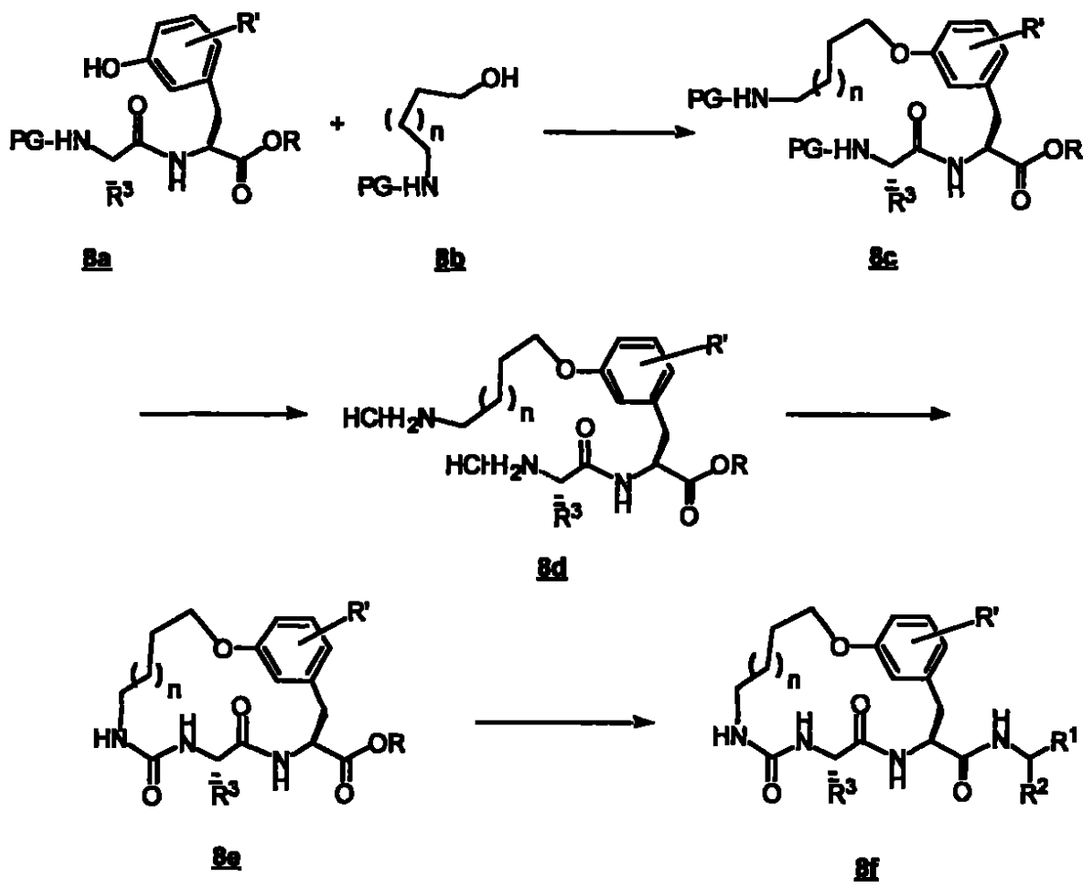
Scheme 7.



6e **7a** **7b**

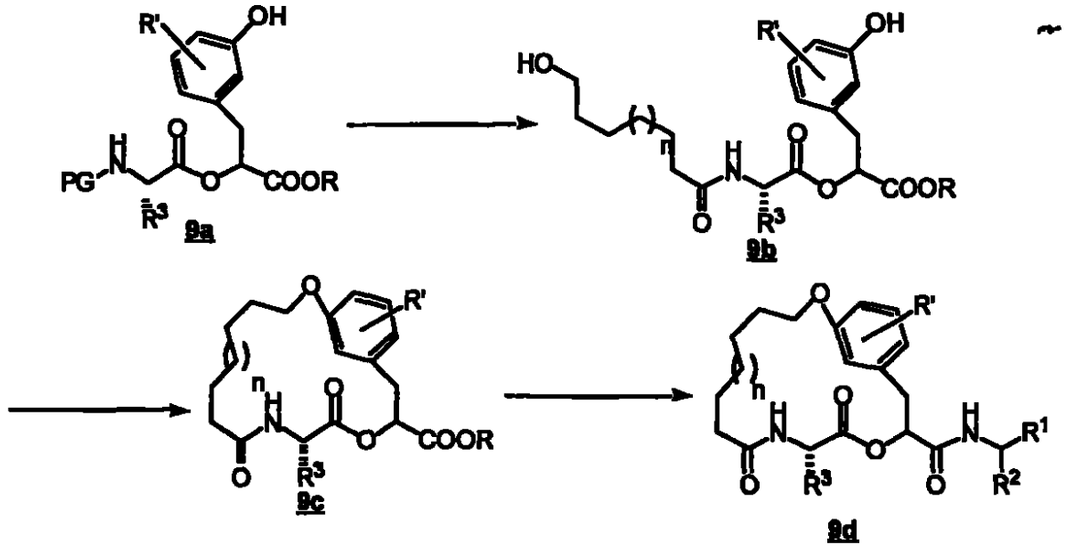
The preparation of the compound of formula **7b**, wherein R^1, R^2, R^3 are defined above, R' is alkyl, heteroalkyl (OR'', SR'', NR''R''' wherein R'' and R''' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position, R is alkyl, aryl, or alkylaryl groups, and n is from zero to five, is outlined in **Scheme 7** The hydrogenation of the double bond of **6e** gave the macrocycle **7a** Hydrolysis of the ester to acid and subsequent coupling with an amine intermediate afforded **7b**

Scheme 8.



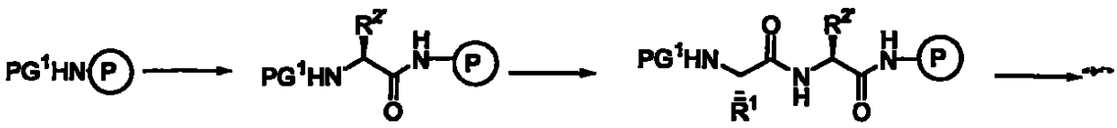
The preparation of the compound of formula **8f**, wherein R^1, R^2, R^3 are defined above, R' is alkyl, heteroalkyl (OR'', SR''', NR''R''' wherein R'' and R''' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position to oxygen atom, R is alkyl, aryl, or alkylaryl groups, PG is Boc, and n is from zero to five, is outlined in **Scheme 8** The *meta*-tyrosine-dipeptide **8a** is coupled to a terminal *N*-protected amino alcohol **8b** under Mitsunobu conditions (triphenylphosphine and ADDP) The protecting groups are removed to give diamine hydrochloride **8d**. The macrocyclization is achieved by forming the urea linkage using phosgene or carbonyl diimidazole The resulting ester is then hydrolyzed to an acid with lithium hydroxide and is subsequently coupled to an amine intermediate to afford the desired product **8f**

Scheme 9.

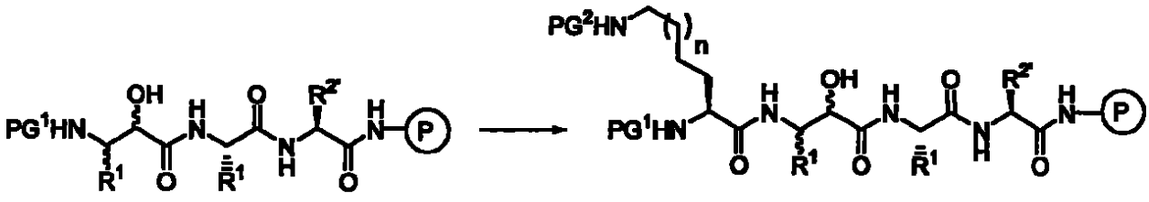


The synthesis of compounds of the type **9d** wherein the substituents R^1, R^2, R^3, R', R and PG are defined in **Scheme 1**, was initiated by the deprotection of **9a** with HCl in dioxane and coupling with ω-hydroxy acid using EDCI, HOOBt to yield compounds of the type **9b** The compounds of the type **9b** were further cyclized using Ph_3P, ADDP to generate compounds of the type **9c** The cyclic compound **9c** was deprotected and coupled with an appropriate amine intermediate to generate compound of type **9d.**

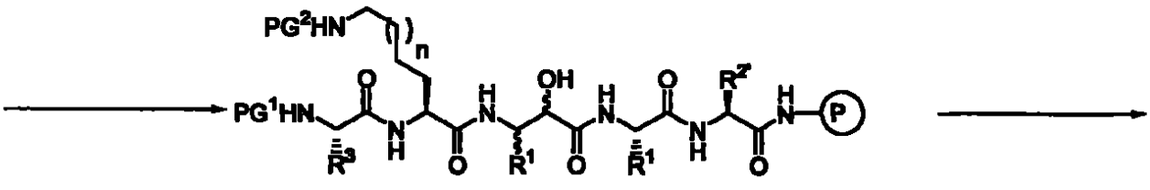
Scheme 10.



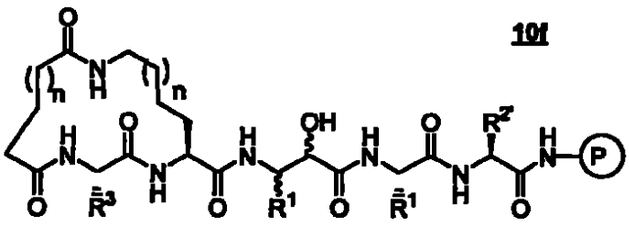
10a 10b 10c



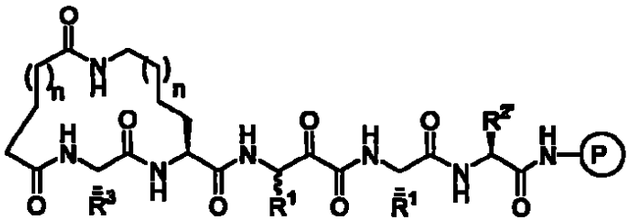
10d 10e



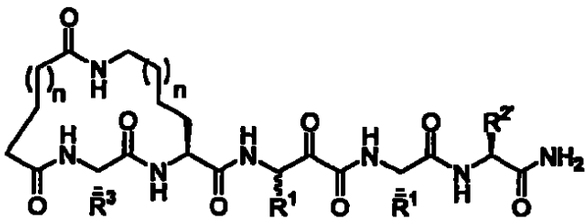
10f



10g



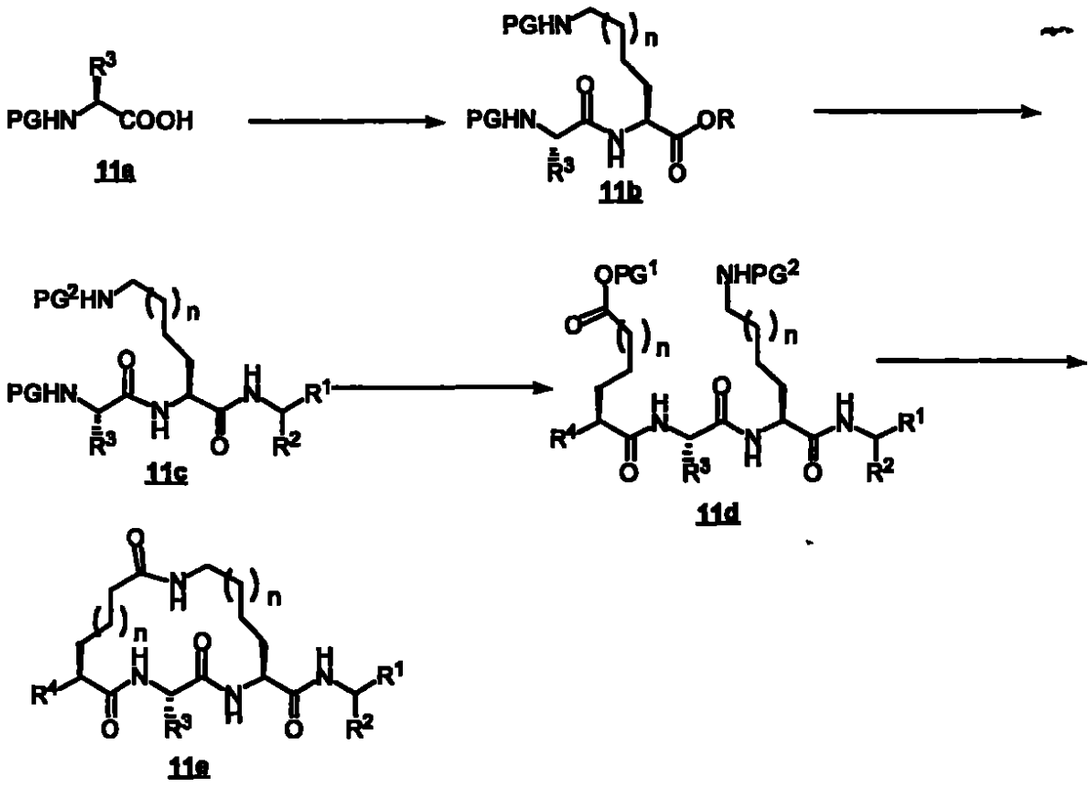
10h



10i

The synthesis of the compounds of the type 10l wherein $R^{2'}$, $R^{1'}$, R^1, R^3 are defined in **Scheme 1** PG^1 and PG^2 are defined as protecting groups namely Fmoc and Dde P is defined as a polymer support wherein the compound is immobilized, and n is a number from 1-6 The methodology used for the synthesis of molecules of type 10l is a standard solid phase peptide synthesis with Fmoc protecting group Fmoc protected Sasarin resin 10a is first deprotected by the treatment of piperidine followed by coupling with the Fmoc protected amino acid using HATU to obtain compound of type 10b. The protecting group of 10b was removed and coupled with amino acid to using HATU to obtain 10c The polymer supported 10c was deprotected by treatment with piperidine and coupled with hydroxy acid to obtain hydroxy amide of type 10d The protecting group of 10d was cleaved and coupled with protected lysine derivative using HATU to obtain compounds of type 10e The protecting group of 10e was once again deprotected and coupled with Fmoc protected amino acid to obtain compound of the type 10f The protecting groups PG^1 and PG^2 were removed and cyclized using a diacid and HATU to obtain the macrocycle 10g The compound of 10g was oxidized using Dess-Martin reagent and finally cleaved from the resin using TFA to obtain compound 10l

Scheme 11



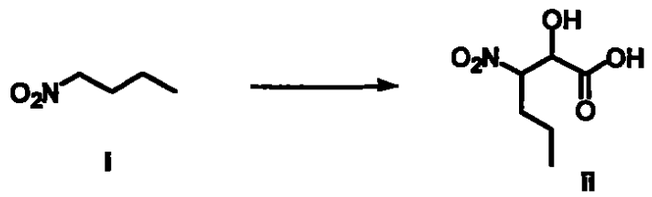
The synthesis of the compounds of type **11e** wherein R^1, R^2, R^3, R^4 and n are defined in **Scheme 1** and PG^2 is Cbz, PG^1 is Bn and PG is Boc, was initiated from protected acid **11a** **11a** was converted to compounds of type **11b** by coupling with lysine derivative using EDCI, HOOBt methodology The ester group of **11b** was hydrolyzed using LiOH H_2O followed by the coupling with an appropriate amine intermediate to obtain the compound **11c** This was further treated with HCl in dioxane and coupled with lysine intermediate using EDCI, HOOBt to form compounds of the type **11d** The compounds **11d** were deprotected and cyclized using EDCI, HOOBt to form compounds of type **11e.**

38

Preparation of Intermediates.

Intermediate A.

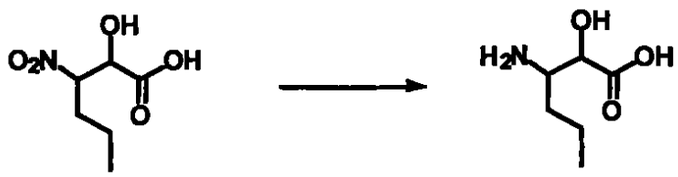
Step 1



I II

To a stirred solution of 1-nitrobutane (16 5 g, 0 16 mol) and glyoxylic acid in H_2O (28 1 g, 0 305 mol) and MeOH (122 mL) at 0°C-5°C, was added dropwise triethyl amine (93 mL, 0 667 mol) over 2 hrs The solution was warmed to room temperature , stirred overnight and concentrated to dryness to give an oil The oil was then dissolved in H_2O and acidified to pH =1 with 10% HCl, followed by extraction with EtOAc The combined organic solution was washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness to give the product II (28 1 g, 99% yield)

Step 2



II III

To a stirred solution of starting material II (240 g, 1 35 mol) in acetic acid (1 25 L) was added 10% Pd/C (37 g) The resulting solution was hydrogenated at 59 psi for 3 hrs and then at 60 psi overnight The acetic acid was then evaporated and azeotroped 3 times with toluene, then triturated with MeOH and ether The solution was then filtered and azeotroped twice with toluene to give III as an off white solid (131 g, 0 891 mol, 66%)

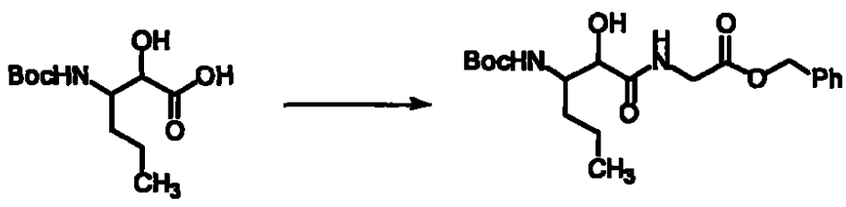
Step 3



III IV

To a stirred solution of the amino acid III (2 0 g, 013 6 mmol) in dioxane (10 mL) and H_2O (5mL) at 0°C, was added 1N NaOH solution (4 3 mL, 14 0 mmol) The resulting solution was stirred for 10 minutes, followed by addition of di-*t*-butyldicarbonate (0 110 g, 14 0 mmol) and stirred at 0 °C for 15 minutes The solution was then warmed to room temperature, stirred for 45 minutes and kept in refrigerator overnight and concentrated to dryness to give a crude material To the solution of this crude material in EtOAc (100 mL) and ice, was added $KHSO_4$ (3 36 g) and H_2O (32 mL) and stirred for 4-6 minutes The organic layer was then separated and the aqueous layer was extracted twice with EtOAc and the combined organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated to dryness to give the product IV as a clear gum (3 0 g, 89% yield)

Step 4.

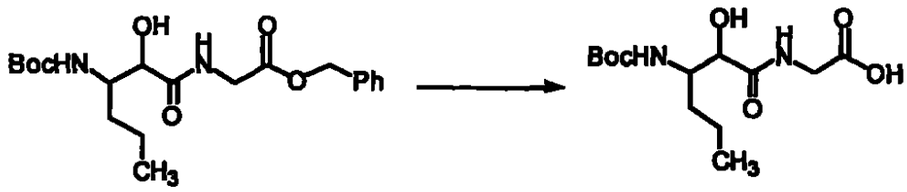


IV V

To a stirred solution of IV (3 00 g, 12 0 mmol) in DMF (15 mL) and CH_2Cl_2 (15 mL) at -20°C was added HOOBt (1 97 g, 12 0 mmol), *N*-methyl morpholine (4 0 mL, 36 0 mmol) and EDCI (2 79 g, 14 5 mmol) and stirred for 10 minutes, followed by addition of HCl H_2N-Gly-OBn (2 56 g, 13 0 mmol) The resulting solution was stirred at -20°C for 2 hrs, then kept in refrigerator overnight and

concentrated to dryness, followed by dilution with EtOAc (150 mL) The EtOAc solution was then washed twice with saturated NaHCO3, H2O, 5% H_3PO_4, brine, dried over Na_2SO_4, filtered and concentrated to dryness to give the product v (4 5 g, 94%) LRMS *m/z* MH^+= 395 1

Step 5.



v vi

The solution of starting material v (7 00 g, 17 8 mmol) in absolute ethanol (300 mL) was stirred at room temperature under a hydrogen atmosphere in the presence of Pd-C (300 mg, 10%) The reaction progress was monitored by tlc After 2h, the mixture was filtered through a celite pad and the resulting solution was concentrated *in vacuo* to give the product vi (5 40 g, quantitative) LRMS *m/z* MH^+= 305 1

Step 6.

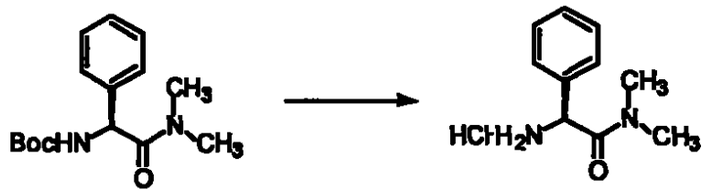


vii viii

To a solution of dimethylamine hydrochloride (1 61 g, 19 7 mmol), *N*-Boc-phenylglycine (4 50 g, 17 9 mmol), HOOBt (3 07 g, 18 8 mmol) and EDCI (4 12 g, 21 5 mmol) in anhydrous DMF (200 mL) and CH_2Cl_2 (150 mL) at -20°C was added NMM (5 90 mL, 53 7 mmol) After being stirred at this temperature for 30 min, the reaction mixture was kept in a freezer overnight (18 h) It was then allowed to warm to rt, and EtOAc (450 mL), brine (100 mL) and 5% H_3PO_4 (100 mL) were added After layers were separated, the organic solution was washed with 5% H_3PO_4 (100 mL), saturated aqueous sodium bicarbonate solution (2 X

150 mL), water (150 mL), and brine (150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford crude product viii (4 86 g) as a white solid, which was used without further purification

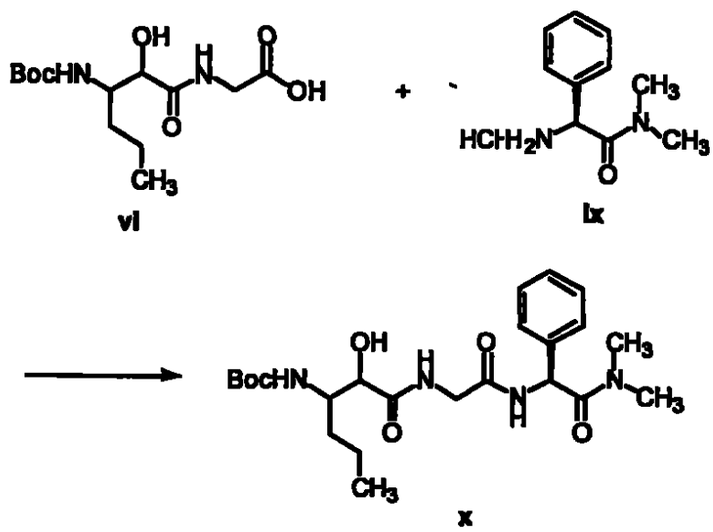
Step 7.



viii ix

The *N*-Boc-phenylglycine dimethylamide viii (4 70 g, crude) was dissolved in 4 N HCl (60 mL, 240 mmol) and the resulting solution was stirred at room temperature The progress of the reaction was monitored by TLC After 4 h, the solution was concentrated *in vacuo* to yield ix as a white solid which was used in the next reaction without further purification LRMS *m/z* MH^+= 179 0

Step 8.

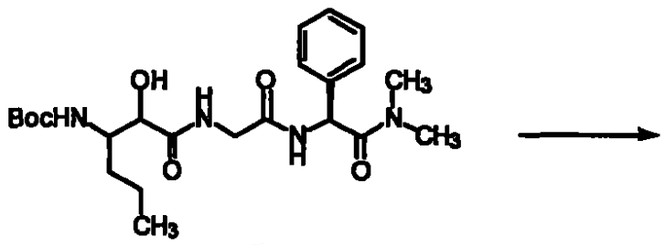


vi ix x

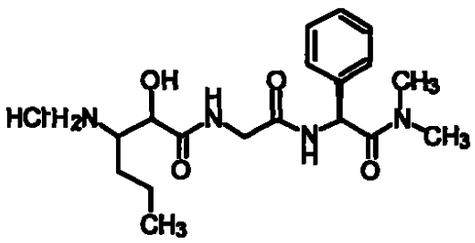
The desired compound x was prepared according to the coupling procedures described in Step 4 LRMS *m/z* MH^+= 465 1

40

Step 9.



x

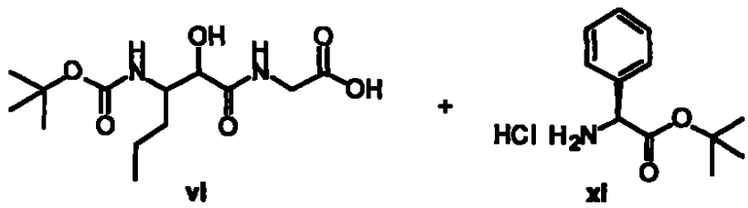


A

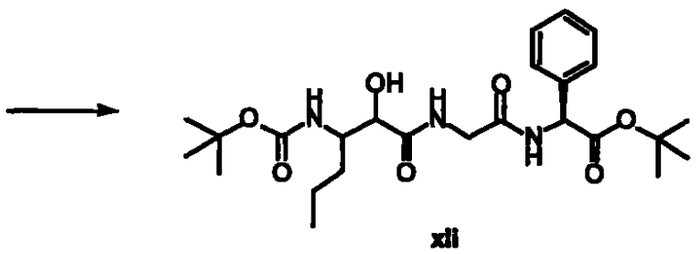
The desired intermediate A was prepared from tripeptide x according to the procedures described in Step 7 LRMS *m/z* MH^+= 365 1

Intermediate B.

Step 1.



vi xi

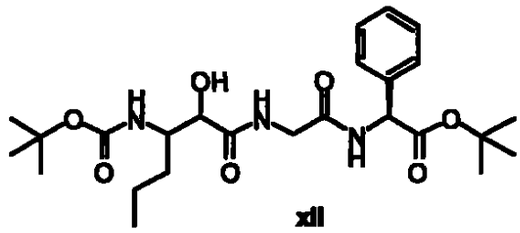


xii

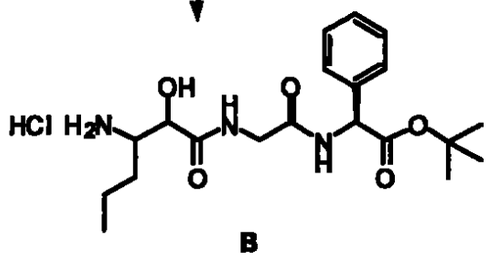
The desired product xii was obtained by the procedure described for Intermediate A, Step 8 using commercially available xi as the coupling partner The crude material was sufficiently pure for further studies A portion of the

product was purified by flash chromatography using 97/3 dichloromethane/MeOH HRMS (FAB) Calcd for $C_{25}H_{40}N_3O_7$ 494 2866 $(M+H)^+$ Found 494 2863

Step 2



xii

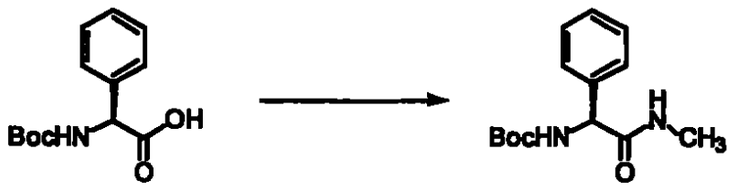


B

The desired product B was obtained by the procedure described for Intermediate A, Step 7 The crude material was used without further purification

Intermediate C.

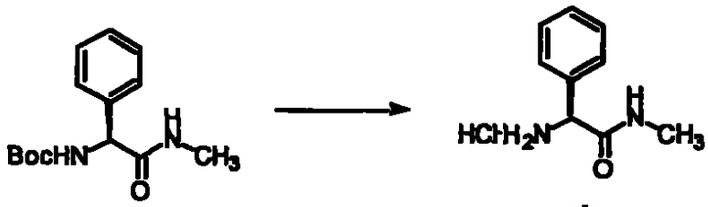
Step 1.



vii xiii

The desired compound xiii was prepared according to the coupling procedures described in Step 6 for intermediate A

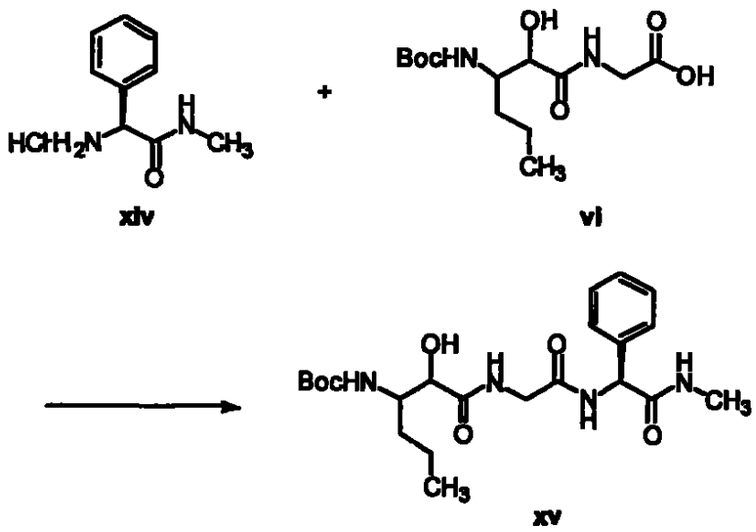
Step 2



xiii xiv

The desired compound xiv was prepared according to the procedures described in Step 7 for intermediate A

Step 3.

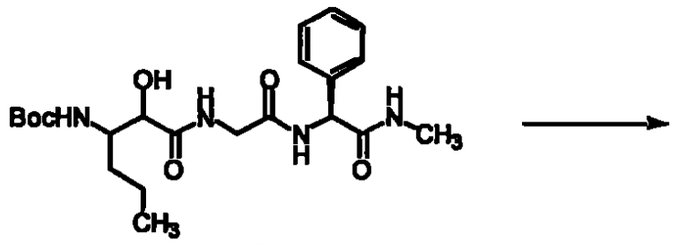


xiv vi

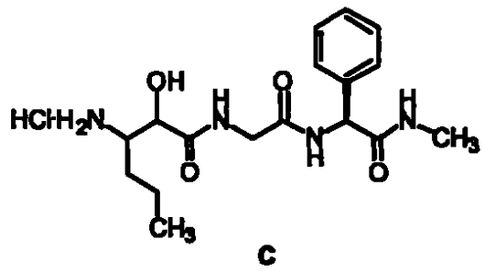
xv

The desired compound xv was prepared according to the coupling procedures described in Step 6 for intermediate A LRMS *m/z* MH^+= 451 1

Step 4.



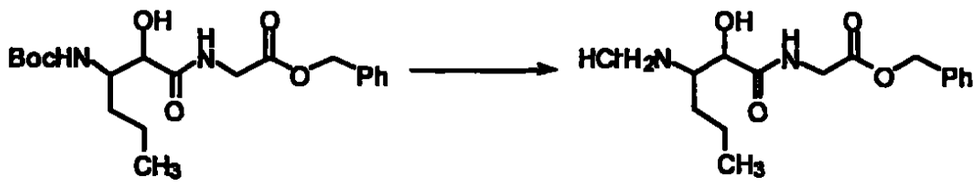
XVI



C

The desired intermediate C was prepared according to the procedures described in Step 7 for intermediate A LRMS *m/z* MH^+= 351 1 It was used without further purification

Intermediate D.



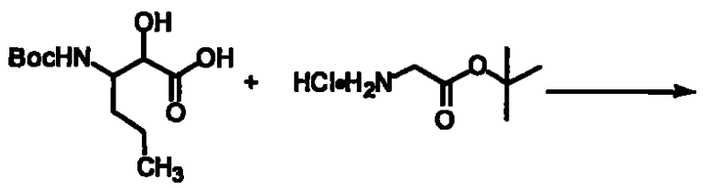
V D

The desired intermediate D was prepared from compound v according to the procedures described in Step 7 for intermediate A It was used without further purification

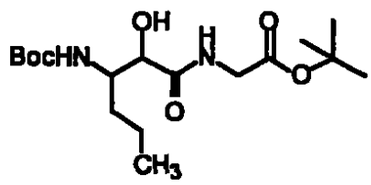
42

Intermediate E.

Step 1.



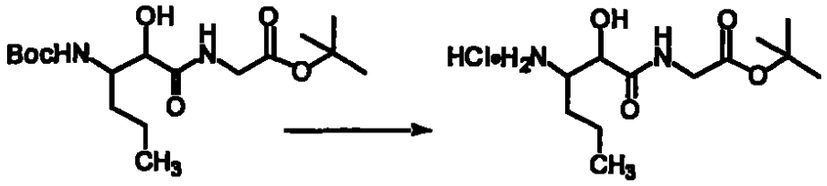
iv xvii



xviii

The desired product xviii was obtained by the procedure described for Intermediate A, Step 8 using commercially available xvii as the coupling partner The crude material was sufficiently pure for further studies

Step 2

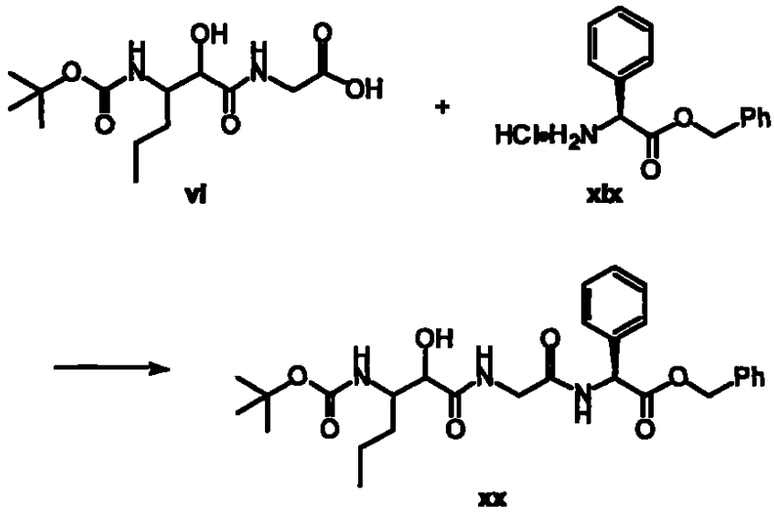


xviii E

The desired product E was obtained by the procedure described for Intermediate A, Step 7 The crude material was used without further purification

Intermediate F.

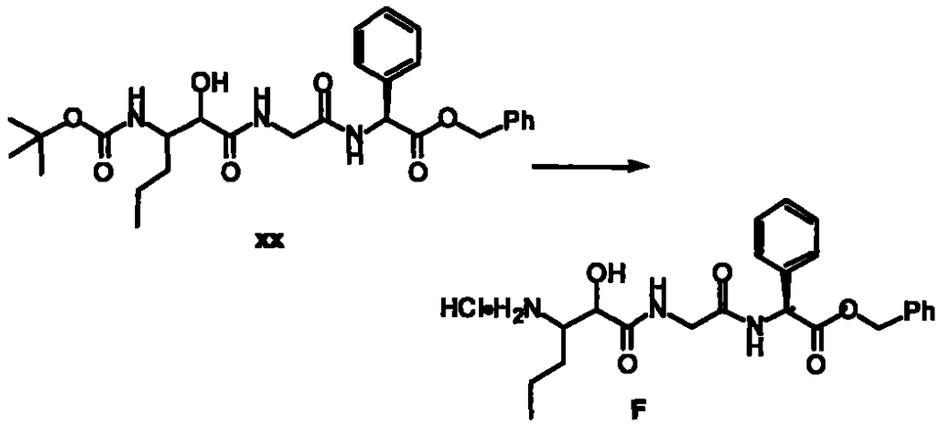
Step 1



vi xix xx

The desired product xx was obtained by the procedure described for Intermediate A, Step 4 using commercially available xix as the coupling partner The crude material was sufficiently pure for further studies

Step 2



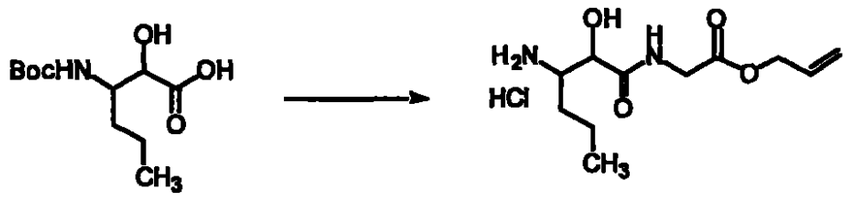
xx F

The desired product F was obtained by the procedure described for Intermediate D

43

Intermediate G.

Step 1

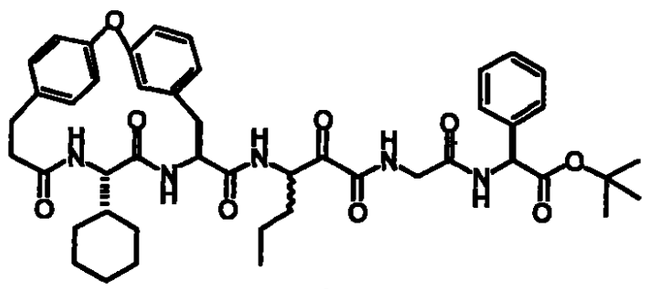


Iv G

The desired product G was obtained by the procedure described for Intermediate A, Step 4 using allylglycine as the coupling partner The crude material was sufficiently pure The crude product was treated with 4N HCl/ Dioxane and stirred at rt. for 50 min The reaction mixture was concentrated to dryness to yield intermediate G which was used without further purification

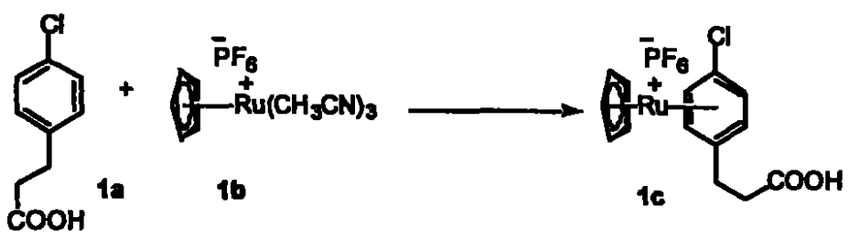
EXAMPLES

Example 1. Preparation of Compound of Formula 1.



1

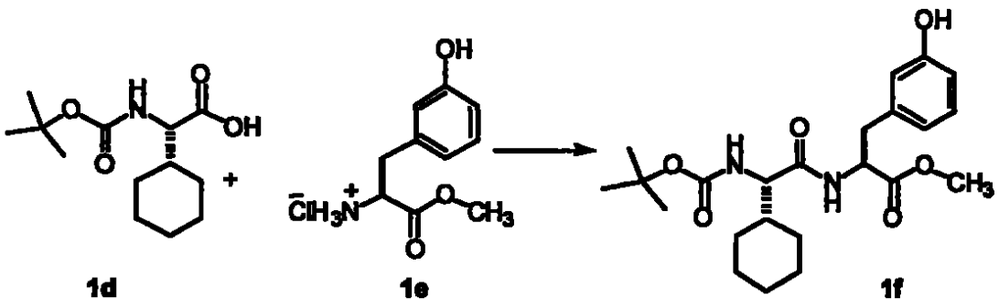
Step A.



1a 1b 1c

A solution of 4-chloropropionic acid (2 0 g, 10 8 mmol) of 1a in dichloroethane (200 mL) was treated with $CpRu(CH_3CN)_3$ PF_6 (4 7 g, 10 8 mmol, 1 0 equiv) and heated at reflux for 2 h The reaction mixture was cooled to rt when colorless crystals of the product 1c precipitated out. The crystals were filtered and washed with 1 1 mixture of Et_2O/CH_2Cl_2 and dried *in vacuo* The colorless crystals (3 3 g) were analytically pure ^1H NMR ($CD_3C(O)CD_3$, 400 MHz, ppm, δ, J) 6 77 (d, 2 H, *J* = 7 0 Hz), 6 53 (d, 2 H, *J* =7 Hz), 5 64 (s, 5 H), 2 87 (t, 2 H, *J* =7 0 Hz), 2 74 (t, 2 H, *J* =7 0Hz), MS (Electron spray, *m/z relative intensity*) 350 9 ($C_{14}H_{14}ClRu^+$, M^+, 100), CHN calcd for $C_{14}H_{14}ClF_6O_2PRu$ C=33 92% H=2 85% Cl=7 15% P=6 25% found C=34 04% H=3 04% Cl=7 09% P=5 71%

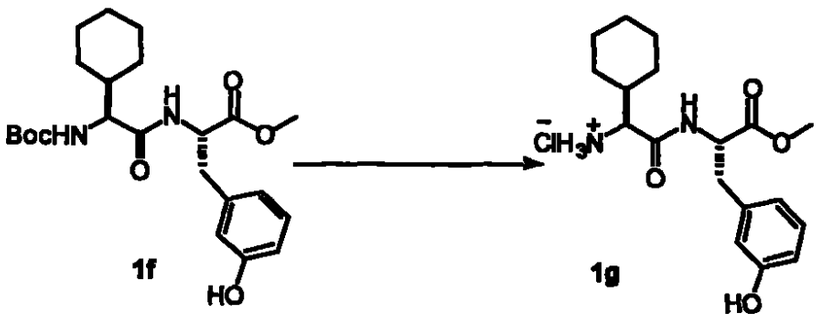
Step B



A solution of Boc-cyclohexylglycine monohydrate 1d (6 17 g, 24 00 mmol) in dry CH_2Cl_2 (50 0 mL) was treated with 4-methylmorpholine (2 64 g, 26 0 mmol, 1 1 equiv) and cooled to -10° C To this mixture was added isobutyl chloroformate (3 62 g, 3 5 mL, 1 1 equiv) and the white suspension was stirred until the bath temperature was -5 °C *meta*-Tyrosine methyl ester hydrochloride salt (6 5 g, 26 5 mmol, 1 1 equiv) was dissolved in DMF (30 mL) in a separate beaker and treated with 4-methyl morpholine (2 64 g, 26 0 mmol, 1 1 equiv) and stirred at rt. for 15 min This mixture was added to the reaction which was accompanied by evolution of CO_2 The reaction mixture was stirred at rt for 1 h and diluted with 1M aq HCl (100 mL) The aqueous layer was extracted with ethyl acetate (3x200 mL) and the combined organic layer was extracted with 1M HCl (1x100 mL), aq NaOH

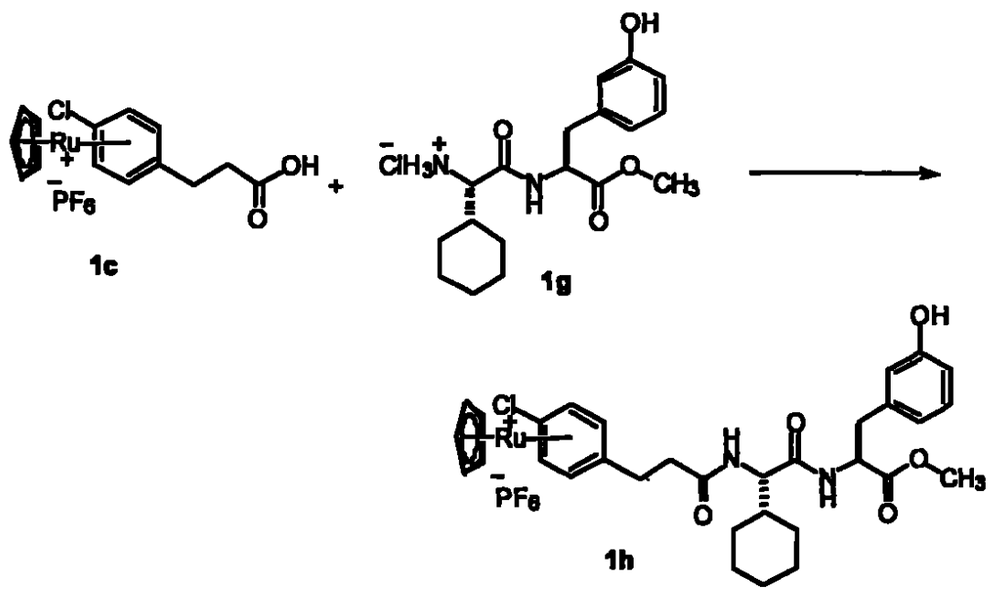
(1x100 mL), brine (1x100 mL), dried (Na_2SO_4), concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hexanes 3/7) to yield 5 3 g (53%) of coupled compound 1f as a colorless foam

Step C.



A solution of 1f (10g, 23 04 mmol) was dissolved in HCl (4M solution in dioxane, 100 mL) and stirred at rt for 2-4 h The reaction mixture was concentrated *in vacuo* and the solid was resuspended in ether It was filtered and the solid was washed with ether which was dried to give a colorless solid 1g (8 2g, 96%) ^{1}H NMR (d_4-CD_3OD, 400 MHz, δ, ppm) 7 09 (t, 1 H, *J*=8 0 Hz), 6 71-6 36 (m, 3 H), 4 69 (dd, 1H, *J*=6 0 Hz, 3 2 Hz), 3 69 (s, 3 H), 3 66 (d, 1 H, *J*=5 2 Hz), 3 15 -3 10 (dd, 1 H, *J*=5 6 Hz, 4 0 Hz), 1 87-1 69 (m, 6 H), 1 32-1 10 (m, 5 H)

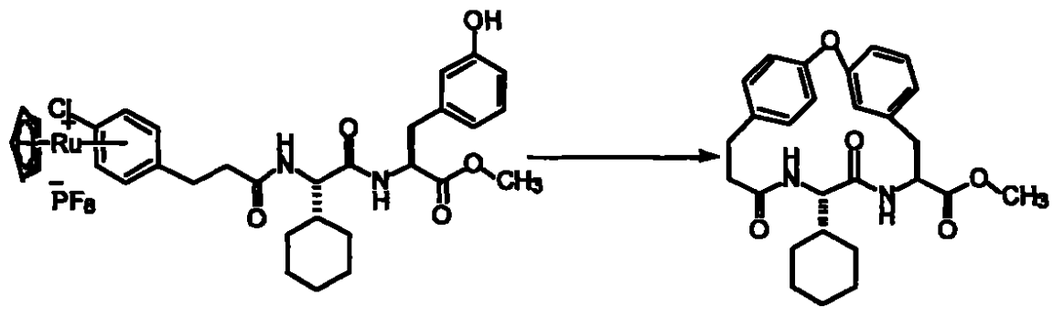
Step D.



A solution of cyclopentadiene-η^6-4-chlorophenylpropionic acid-ruthenium hexanesafluorophosphate **1c** (2 0g, 4 0 mmol) in DMF (20 mL) was treated with HOBt (810 mg, 6 0 mmol, 1 5 equiv)and Hünigs base (2 6 g, 16 0 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI•HCl (888 mg, 5 0 mmol, 1 25 equiv) The reaction mixture was stirred at 0 °C for 30 min and amine salt **1g** (1 48 g, 4 0 mmol) was added to the mixture and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with H_2O (200 mL) and extracted into CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq HCl (1x100 mL), $NaHCO_3$ (1x100 mL), brine (1x100 mL) and dried (Na_2SO_4), filtered concentrated *in vacuo* and the brown solid **1h** was used for cyclization without any further purification

0045

Step E

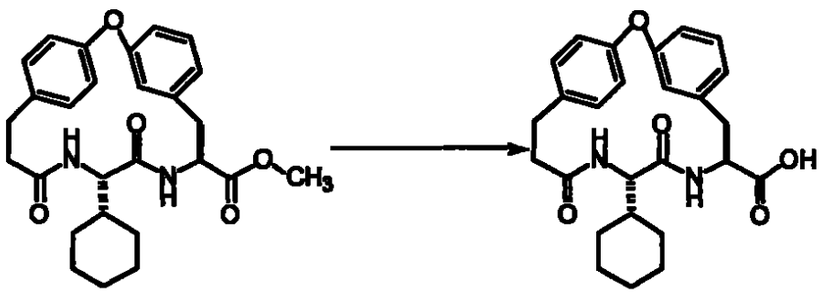


1h 1i

A solution of cyclopentadiene–η6-ruthenium-4-chlorophenylpropionic acid-cyclohexyglycine-*meta*-tyrosine-OCH_3 1h (1 47 g crude) in dry DMF (150 mL) was treated with Cs_2CO_3 (2 40 g, 7 37 mmol, 5 0 equiv) and degassed by bubbling dry N_2 into the reaction mixture The reaction mixture was stirred at rt for 16 h and the excess DMF was distilled off The residue was dissolved in H_2O (200 mL) and extracted with CH_2Cl_2 (3x100 mL) The combined organic layer was extracted with brine (100 mL), dried (Na_2SO_4), filtered concentrated *in vacuo* and the residue was used for photolytic decomplexation of ruthenium without further purification

The crude ruthenium complex was dissolved in acetonitrile (35 mL), degassed and photolysed in a Raynot (λ=350 nM) for 48 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 7 3) to yield 360 mg (52%) of a colorless solid 1i

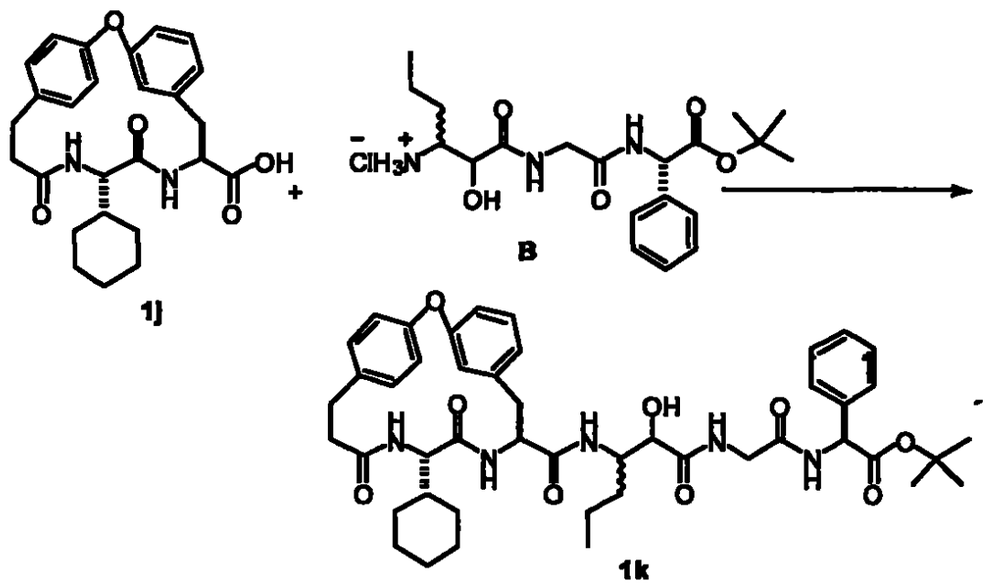
Step F.



1I 1J

A solution of biphenyl ether 1I (300 mg, 0 65 mmol) in CH_3OH (10 mL), CH_2Cl_2 (20 mL) and H_2O (5 mL) was treated with $LiOH \cdot H_2O$ (90mg, 2 2 mmol, 3 4 equiv) and stirred at rt for 2 h The reaction mixture was acidified with aq HCl (6 M) and extracted into CH_2Cl_2 (3x30 mL) The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield colorless acid 1J (200 mg, 66%)

Step G.



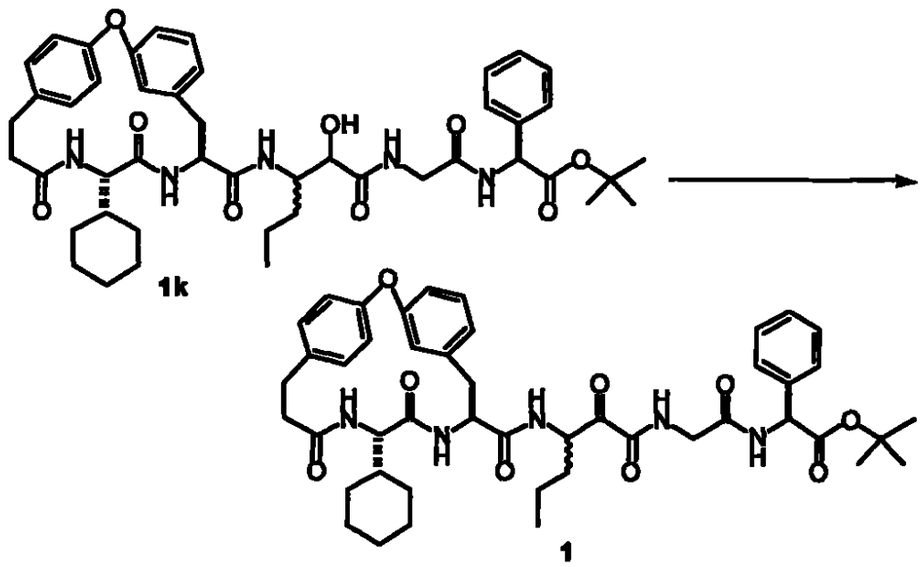
1J B

1k

A solution of acid 1j (100 mg, 0 22 mmol) in dry DMF (2 5 mL) was treated with HOOBt (45 mg, 0 33 mmol) and HUnigs base (141 mg, 1 1 mmol, 5 0 equiv)

The reaction mixture was cooled to 0° C and treated with EDCI (63 mg, 0 33 mmol, 1 5 equiv) and stirred for 20 min The reaction mixture was treated with amine B (118 mg, 0 27 mmol, 1 22 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and EtOAc (3x50 mL) The combined organic layers were extracted with aq HCl (2M), aq NaOH (2M), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 1k (79 mg) which was us d for oxidation MS (Electron spray, *m/z rel int*) 826 [$(M+1)^+$, 100], 494 (20), 94 (30)

Step H

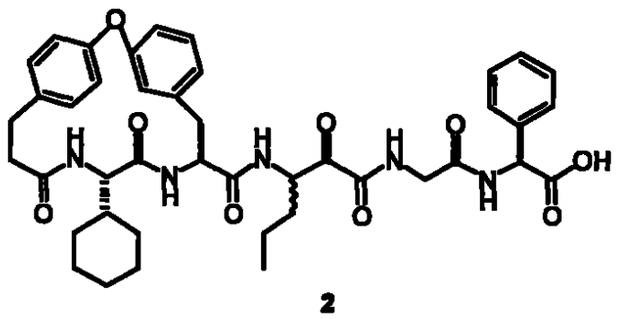


1k

1

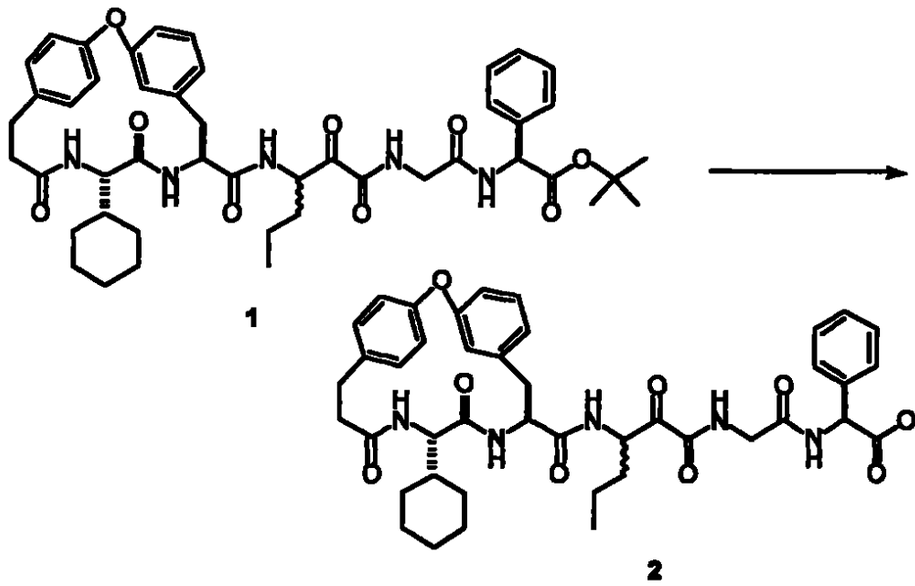
A solution of hydroxy amide 1k (130 mg, 0 16 mmol) in DMF (2 0 mL) was treated with Dess-Martin reagent (130 mg, 0 32 mmol, 2 0 equiv) The reaction mixture was stirred at rt for 2 h and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 49) to yield oxidized product 1 (55 mg, 42%) as a colorless solid MS (Electron spray, *m/z rel int*) 858 [$(M+CH_3OH+1)^+$, 100], 824 [$(M+1)^+$, 63)

Example 2 Preparation of Compound of Formula 2



2

Step A.

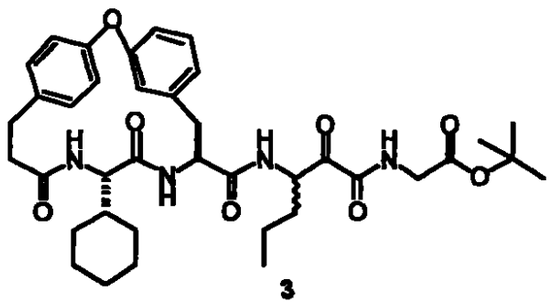


1 2

A solution of *tert*-butyl ester 1 (50 0 mg, 60 0 µmol) was treated with TFA/CH_2Cl_2 (1 1, 4 mL) and stirred at rt for 2 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1 24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes (4 0 mL) and concentrated to yield a white solid 2 (49 mg, 100%) MS (Electron spray, *m/z rel int*) 768 [$(M+1)^+$, 100)

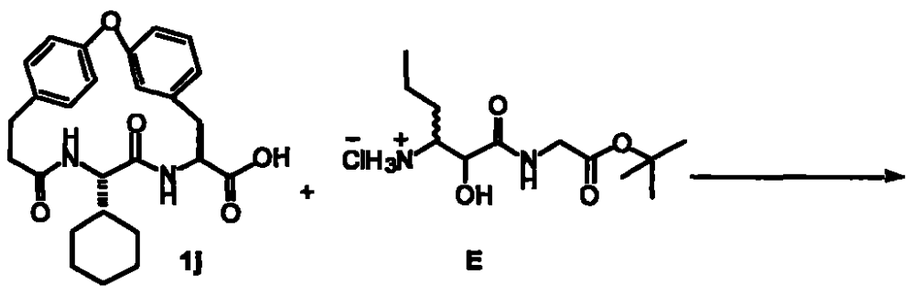
47

Example 3. Preparation of Compound of Formula 3.

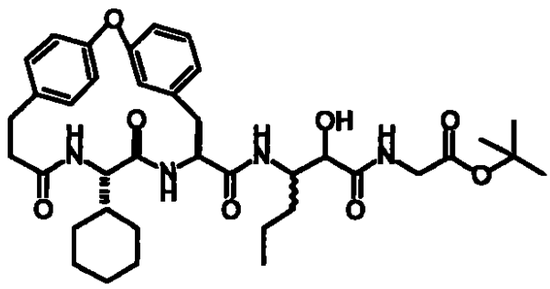


3

Step A.



1J E

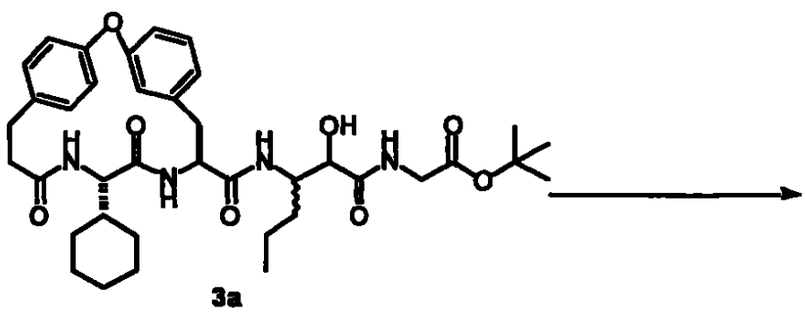


3a

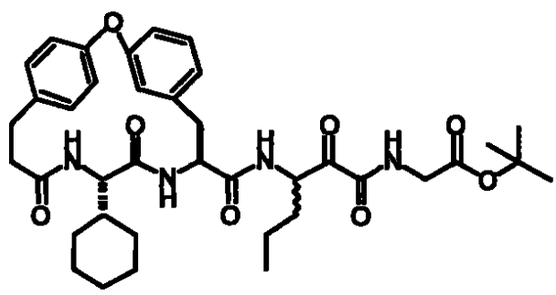
A solution of acid 1J (100 mg, 0 22 mmol) in dry DMF (2 5 mL) was treated with HOOBt (45 mg, 0 33 mmol) and Hünigs base (141 mg, 1 1 mmol, 5 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (63 mg, 0 33 mmol, 1 5 equiv) and stirred for 20 min The reaction mixture was treated with amine E (79 mg, 0 27 mmol, 1 22 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq HCl (1M, 30 mL) aq NaOH (1M, 30 mL), dried (Na_2SO_4)

filtered concentrated *in vacuo* to obtain a colorless solid **3a** (58 mg) which was used for oxidation MS (Electron spray, *m/z rel Int*) 693 [$(M+1)^+$, 100], 637 (41), 494 (55), 394 (51), 338 (13)

Step B



3a

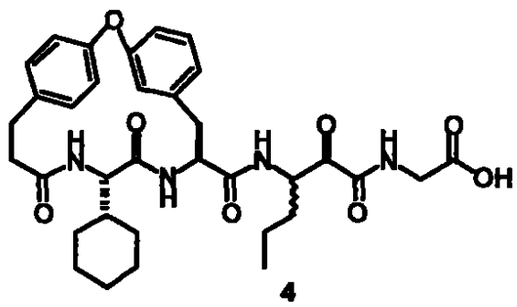


3

A solution of alcohol **3a** (95 mg, 0 14 mmol) in CH_2Cl_2 (2 0 mL) was treated with Dess-Martin reagent (116 mg, 0 28 mmol, 2 0 equiv) The reaction mixture was stirred at rt for 2 h and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 32) to yield oxidized product **3** (47 mg, 42%) as a colorless solid MS (Electron spray, *m/z-rel Int*) 691 $(M+1)^+$

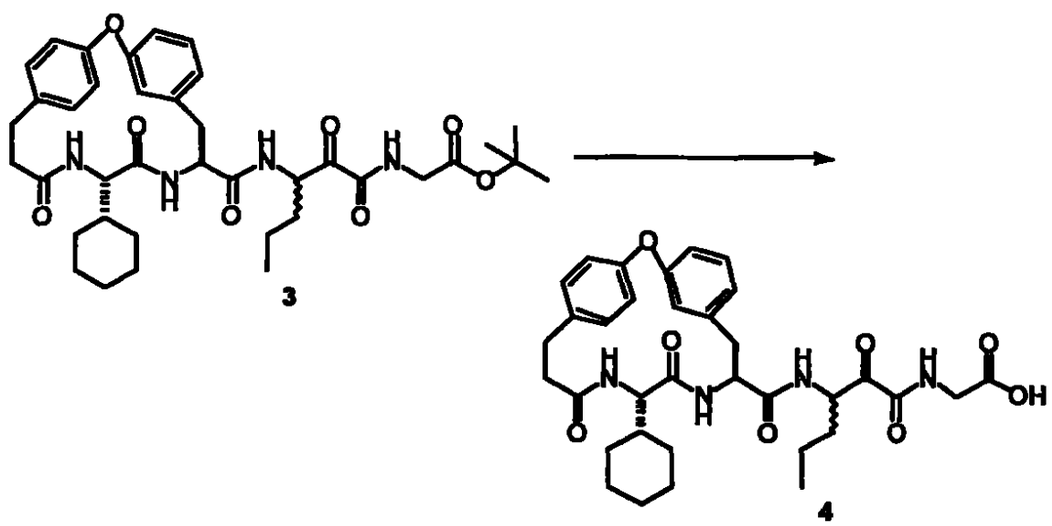
48

Example 4. Preparation of Compound of Formula 4.



4

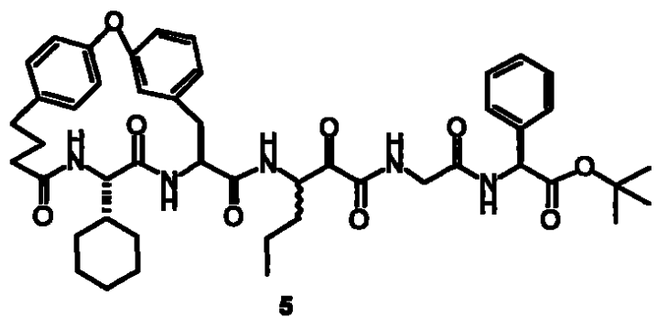
Step A



3 4

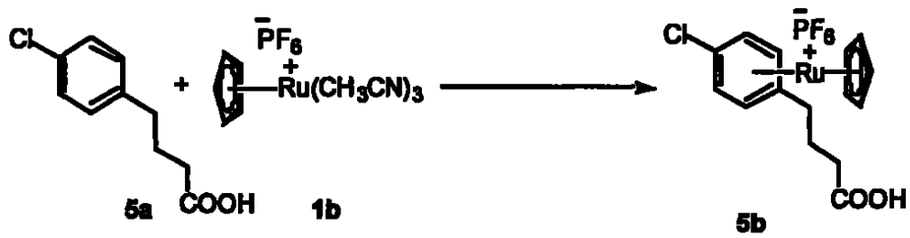
A solution of *tert*-butyl ester 3 (47 0 mg, 68 0 µmol) was treated with HCl (4M Dioxane, 5 mL) and stirred at rt for 25 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1 24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes (5 0 mL) and concentrated to yield a white solid 4 (43 mg, 100%) MS (Electron spray, *m/z rel Int*) 635 [$(M+1)^+$, 100)], 465 (62), 336 (62)

Example 5. Preparation of Compound of Formula 5.



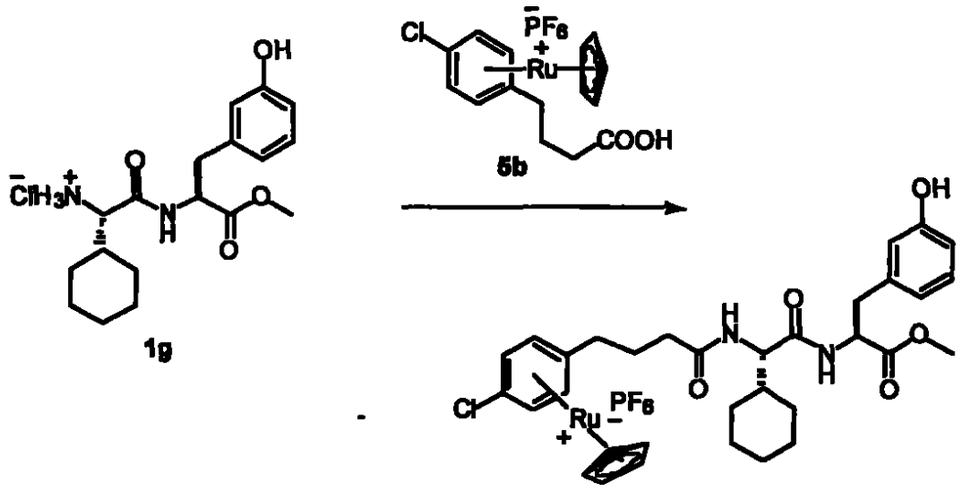
5

Step A



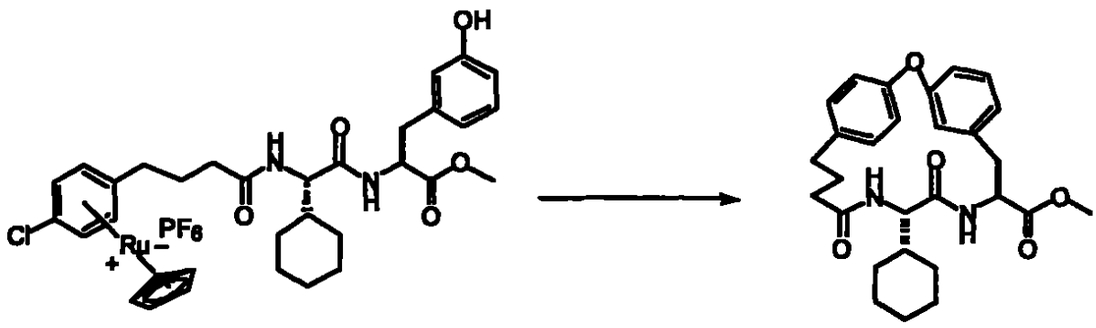
A solution of 4-chlorobutyric acid **5a** (3 0g, 15 10 mmol) in dichloroethane (200 mL) was treated with $CpRu(CH_3CN)_3PF_6$ **1b** (6 6 g, 15 10 mmol, 1 0 equiv) and heated at reflux for 2 5 h The reaction mixture was cooled to 0°C and filtered The filtrate was concentrated *in vacuo* and dissolved in CH_3CN (10 mL) and treated with a large excess of Et_2O The gum separating out was separated by decanting the ether and the residue was dissolved in CH_2Cl_2/CH_3OH (1 1, 100 mL) and concentrated *in vacuo* to obtain **5b** as a brown gum which solidifies (3 5 g, 46%)

Step B



A solution of carboxylic acid **5b** (3 12 g, 5 95 mmol) in dry DMF (20 mL) was treated with HÜnigs base (3 07 g, 24 0 mmol, 4 0 equiv, 4 4 mL) and HOBt (1 2 g, 8 93 mmol, 1 5 equiv) The reaction mixture was cooled to 0°C and the treated with EDCI (1 35 g, 7 43 mmol, 1 25 equiv) and stirred for 1h To this reaction mixture was added amine hydrochloride **1g** (2 65g, 7 14 mmol, 1.2 equiv), and the reaction mixture was stirred at rt for 12 h DMF was distilled out and the residue was diluted with water and the aqueous layer was extracted with CH_2Cl_2 The combined organic layers were extracted with aq $NaHCO_3$, aq HCl, brine, dried (Na_2SO_4), filtered, concentrated *in vacuo* and the crude product **5c** (4 3 g) was used for cyclization without further purification ^1H NMR (d_4-CD_3OD, 400 MHz, δ, ppm) 7 35 (t, 1H), 6 72-6 60 (m, 5 H), 6 33-6 20 (dd, 2 H), 5 51 (s, 5 H), 4 19 (d, 1 H), 3 68 (s, 3 H), 3 19-2 83 (m, 2 H), 2 51-2 40 (m, 2 H), 2 40-2 25 (m, 2 H), 1 99-1 59 (m, 8 H), 1 35-0 98 (m, 5 H), MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*) 695 3 ($[M-PF_6]^+$, 100), 232 (20), 171(30), HRMS calcd for $C_{34}H_{42}N_2O_5ClRu^+$ $(M-PF_6)^+$ 695 1832, found 695 1845

Step C.

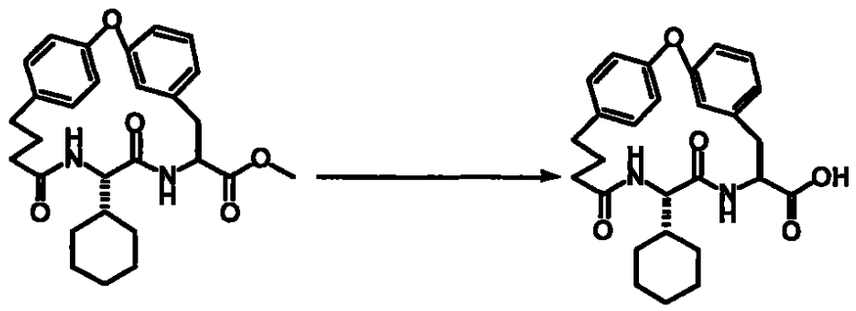


5c 5d

A solution of chloro-compound 5c (3 0 g 3 6 mmol) in dry DMF (300 mL) was degassed with dry N_2 and Cs_2CO_3 (5 2 g, 16 mmol, 4 0 equiv) and stirred at rt for 16h The solvent DMF was distilled off and the residue was diluted with water and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were dried (Na_2SO_4), filtered, concentrated *in vacuo* and dried under vacuum overnight It was used for photolytic removal of Ru without further purification MS FAB (NBA-G/TG-DMSO 695 ($[M-PF6]^+$, 100]

The cyclized compound from the previous step was dissolved in CH_3CN (35mL) and photolysed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SIO2, EtOAc/Hexanes 1 1) to yield a tan colored solid 5d (600 mg, 34%) ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm) 7 58 (d, 1H, *J*=7 6 Hz), 7 14 (t, 1 H, *J*=8 0 Hz), 6 94 (d, 2 H, *J*=8 4 Hz), 6 87 (dd, 1 H, *J*=2 4, 5 6 Hz), 6 73 (d, 1 H, *J*=7 2 Hz), 6 59 (s, 1 H), 6 57 (s, 2 H), 6 39 (d, 1 H, *J*=8 0 Hz), 4 51 (dt, 1 H, *J*=2 8, 8 0 Hz), 3 80-3 62 (m, 1 H), 3 62 (s, 3 H), 3 05-3 00 (dd, 1H, *J*=2 8, 11 6 Hz), 2 85 (dd, 1 H, *J*=8 4, 6 0 Hz), 2 76-2 72 (m, 1 H), 2 36-2 19 (m, 3 H), 2 02 (dd, 1 H, *J*=6 4, 9 2 Hz), 1 8-1 73 (m, 1 H), 1 61-1 34(m, 7H), ,1 41-0 71 (m, 7 H) MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*), 493 [$(M+1)^+$, 100], 465 (20), 232 (30), 171 (40), HRMS calcd for $C_{29}H_{37}N_2O_5$ $(M+1)^+$ 493 2702, found 493 2699

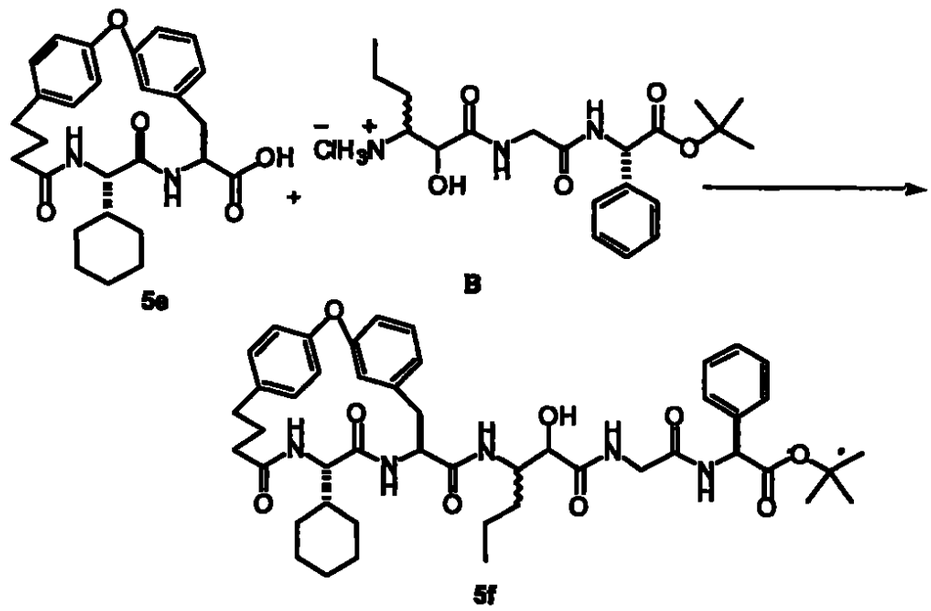
Step D



5d 5e

A solution of ether **5d** (200 mg, 0 42 mmol) in CH_3OH (5 mL), CH_2Cl_2 (10 mL) and H_2O (0 5 mL) was treated with $LiOH \cdot H_2O$ (18 mg, 0 44 mmol, 1 1 equiv) and stirred at rt for 12 h The reaction mixture was acidified with aqueous HCl (12 N, 1mL) and concentrated *in vacuo* to yield acid **5e** which was used directly for the coupling without further purification

Step E



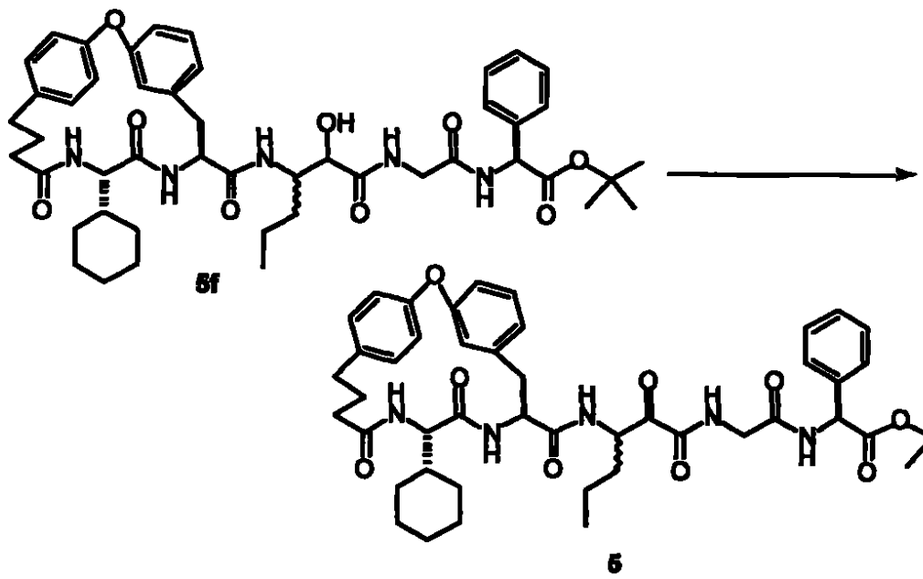
5e B

5f

A solution of acid **5e** in dry DMF (5 0 mL) was treated with HOOBt (103 mg, 0 63 mmol, 1 5 equiv), Hünigs base (216 mg, 1 68 mmol, 4 0 equiv) and amine **B** (270 mg, 0 63 mmol, 1 47 equiv) The reaction mixture was cooled to 0°

C and treated with EDCI (101 mg, 0 52 mmol, 1 25 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and EtOAc (3x50 mL) The combined organic layers were extracted with aq HCl (2M) aq NaOH (2M), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 5f (177 mg) which was used for oxidation MS (Electron spray, *m/z rel int*) 840 [$(M+1)^+$, 100], 394 (100)

Step F

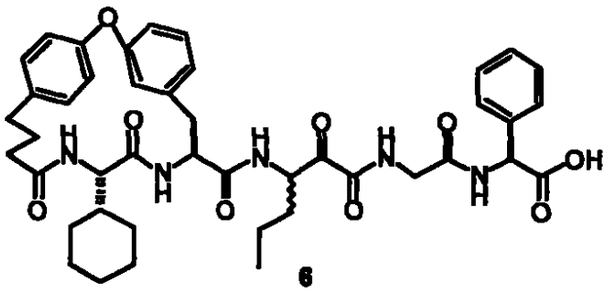


5f

5

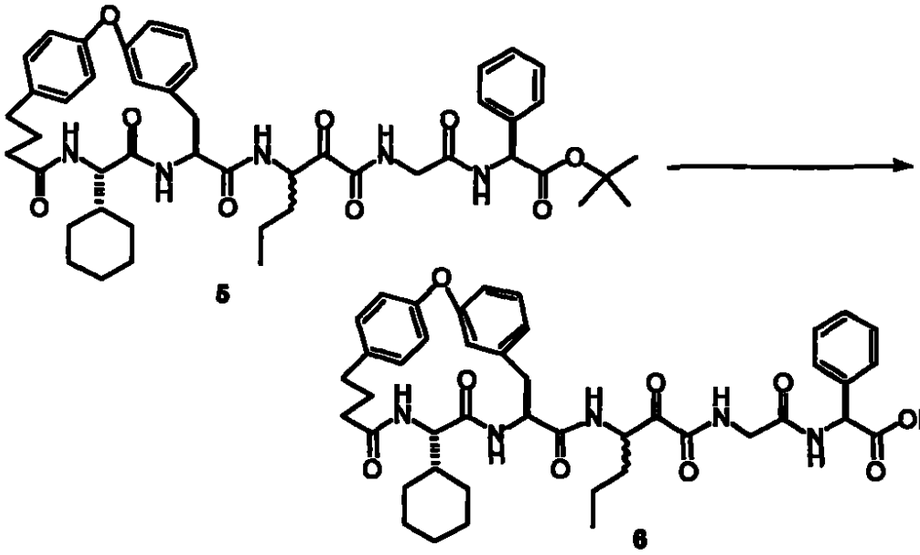
A solution of alcohol 5f (177 mg, 0 21 mmol) in CH_2Cl_2 (10 0 mL) was treated with Dess-Martin reagent (178 mg, 0 42 mmol, 2 0 equiv) The reaction mixture was stirred at rt for 3 h and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 49) to yield oxidized product 5 (23 mg, 13%) as a colorless solid MS (Electron spray *m/z rel int*) 870 [$(M+CH_3OH+1)^+$, 50], 838 [$(M+1)^+$, 100)

Example 6. Preparation of Compound of Formula 6.



6

Step A

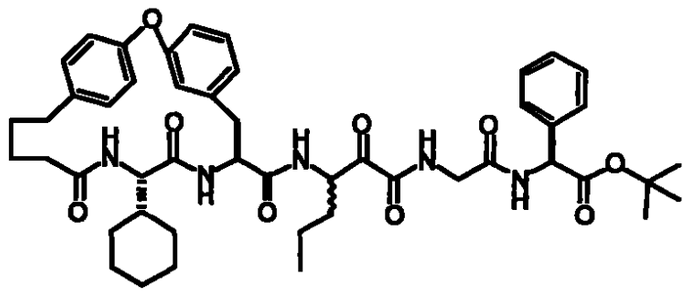


5

6

A solution of *tert*-butyl ester 5 (50 0 mg, 60 0 µmol) was treated with TFA/CH_2Cl_2 (1 1, 4 mL) and stirred at rt for 7 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1 24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes (4 0 mL) and concentrated to yield a white solid 6 (14 mg, 100%) MS (Electron spray, *m/z rel int*) 782 [$(M+1)^+$, 100)

Example 7. Preparation of Compound of Formula 7.



7

Step A



7a 7b

A solution of alcohol 7a (9 2g, 54 1 mmol) in dry CH_2Cl_2 (200 mL) was treated with DMSO (35 mL) and Et_3N (16 4g, 16 3 mmol, 23 4 mL) The reaction mixture was cooled to 0 °C and treated with Py•SO_3 (12 9 g, 81 2 mmol, 1 50 equiv) dissolved in DMSO (30 mL) The reaction mixture was stirred at 0°C for 0 5 h and rt for 6 h The reaction mixture was concentrated *in vacuo* and diluted with Et_2O (100 mL) and H_2O (200 mL) The layers were separated and the aqueous layer was extracted with Et_2O (3x100 mL) The combined organic layers were extracted with HCl (2M, 3x100 mL), brine (1x100 mL) concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hexanes 1 7) to yield aldehyde 7b which solidified to a waxy solid on standing (7 1g, 77%) CHN calcd for C_9H_9ClO C=64 11% H=5 38%, found C=64 08% H=5 30%

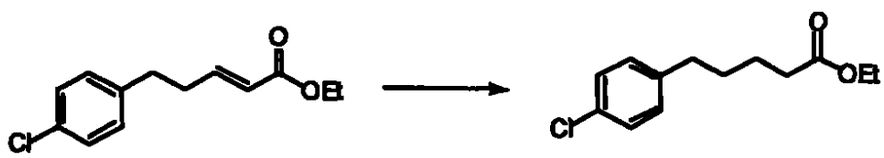
Step B



7b 7c

A solution of thiethylphosponoacetate (6 72g, 30 mmol, 1 2 equiv) in dry THF (100 mL) was treated with NaH (60% dispersion, 1 5g, 35 mmol, 1 4 equiv) at 0°C The reaction mixture was stirred at 25°C for 1 h until the H_2 evolution ceased A solution of aldehyde **7b** (4 2g, 25 0 mmol) in dry THF (5 0 mL) was added and the reaction mixture was stirred for 36 h The reaction mixture was diluted with H_2O (100 mL) and extracted with Et_2O (3x70 mL) The combined organic layer was dried (MgSO4), filtered, concentrated *in vacuo* and chromatographed to yield α,β-unsaturated ester **7c** (4 2g, 71%) which was used for reduction

Step C



7c 7d

A solution of α,β-unsaturated ester **7c** (4 2g, 8 0 mmol) in EtOAc (50 mL) was treated with Pd/C (10% w/w, 500 mg) and hydrogenated at 50 psi for 12 h The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo* to yield the reduced compound **7d** (3 9 g, 93%)

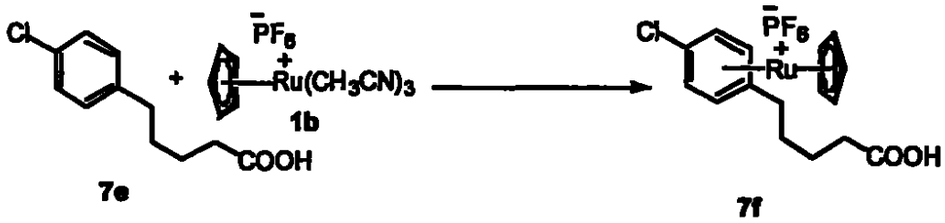
Step D



7d 7e

A solution of the ester 7d (3 9 g, 16 2 mmol) in $CH_3OH/THF/H_2O$ (1 1 0 1, 110 mL) was treated with $LiOH•H_2O$ (1 2 g, 30 mmol, 2 0 equiv) and stirred at rt for 5 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with H_2O (100 mL) and extracted into Et_2O (3x50 mL) The aqueous layer was acidified to pH ~1 (13 M HCl) and the turbid aqueous layer was extracted with Et_2O (3x100 mL) The combined organic layers were dried ($MgSO_4$) filtered concentrated *in vacuo* to yield a colorless solid 7e (3 1g, 96%) CHN calcd for $C_{11}H_{13}ClO_2$ C=62 12% H=6 16%, found C=62 27% H=6 23%

Step E

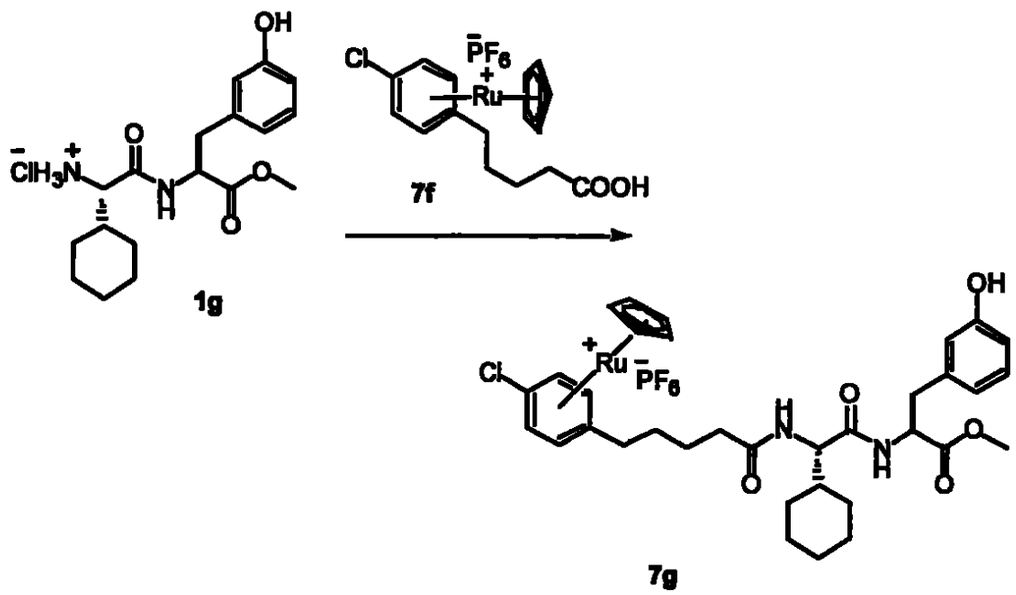


7e 7f

A solution of 4-chlorophenyl pentanoic acid 7e (3 0g, 14 15 mmol) in dichloroethane (150 mL) was treated with $CpRu(CH_3CN)_3PF_6$ 1b (6 75 g, 15 10 mmol, 1 0 equiv) and heated at reflux for 2 5 h The reaction mixture was cooled to 0° C and filtered The filtrate was concentrated *in vacuo* and dissolved in CH_3CN (20 mL) and treated with a large excess of Et_2O The gum separating out was separated by decanting the ether and the residue was dissolved in CH_2Cl_2/CH_3OH (1 1, 100 mL) and concentrated *in vacuo* to obtain 7f a brown

gum which solidifies (4 36 g, 58%) MS (Electron spray, *m/z rel int*) 379 [(M-$PF_6)^+$, 100]

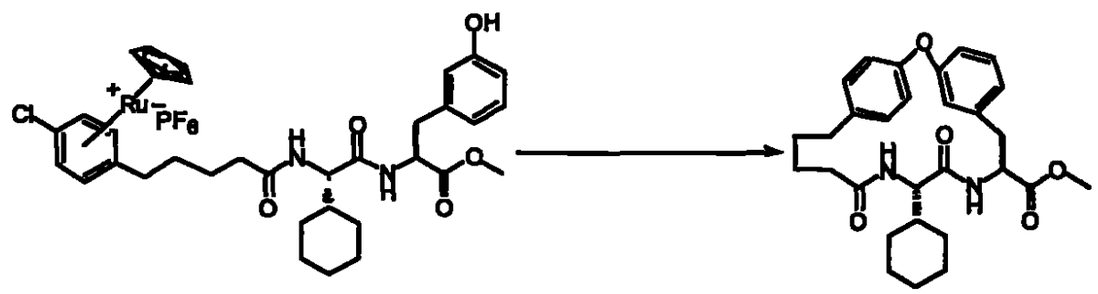
Step F



A solution of carboxylic acid 7f (3 12 g, 5 95 mmol) in dry DMF (20 mL) was treated with Hünigs base (3 07 g, 24 0 mmol, 4 0 equiv, 4 4 mL) and HOBt (1 2 g, 8 93 mmol, 1 5 equiv) The reaction mixture was cooled to 0°C and the treated with EDCI (1 35 g, 7 43 mmol, 1 25 equiv) and stirred for 1h To this reaction mixture was added amine hydrochloride 1g (2 65g, 7 14 mmol, 1 2 equiv) was added and the reaction mixture was stirred at rt for 12 h The DMF was distilled out and the residue was diluted with water and the aqueous layer was extracted with CH_2Cl_2 The combined organic layers were extracted with aq $NaHCO_3$, aq HCl, brine, dried (Na_2SO_4) filtered concentrated *in vacuo* and the crude product 7g (4 3 g) was used for further cyclization without purification ^1H NMR (d_4-CD_3OD, 400 MHz, δ, ppm) 7 35 (t, 1H), 6 72-6 60 (m, 5 H), 6 33-6 20 (dd, 2 H), 5 51 (s, 5 H), 4 19 (d, 1 H), 3 68 (s, 3 H), 3 19-2 83 (m, 2 H), 2 51-2 40 (m, 2 H), 2 40-2 25 (m, 2 H), 1 99-1 59 (m, 8 H), 1 35-0 98 (m, 5 H), MS (FAB, NBA-G/TG-

DMSO, *m/z relative intensity*) 695 3 ($[M-PF_6]^+$, 100), 232 (20), 171 (30), HRMS calcd for $C_{34}H_{42}N_2O_5ClRu^+$ (M-PF_6) 695 1832, found 695 1845

Step G



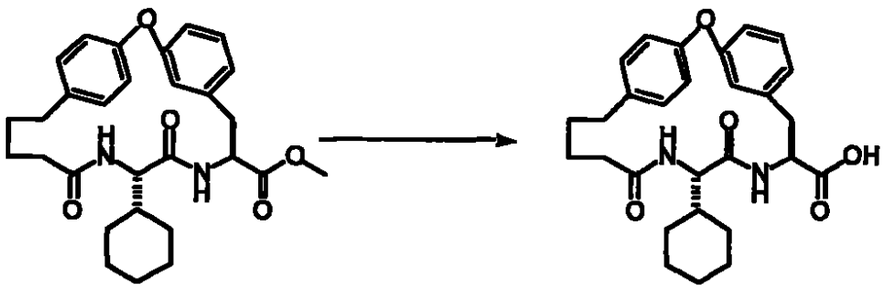
7g 7h

A solution of chloro-compound 7g (3 0 g 3 6 mmol) in dry DMF (300 mL) was degassed with dry N_2 and Cs_2CO_3 (5 2 g, 16 mmol, 4 0 equiv) and stirred at rt for 16h The solvent DMF was distilled off and the residue was diluted with water and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were dried (Na_2SO_4) filtered, concentrated *in vacuo* and dried in vacuum overnight. It was used for photolytic removal of Ru without further purification MS FAB (NBA-G/TG-DMSO 695 ($[M-PF6]^+$, 100]

The cyclized compound from the previous step was dissolved in CH_3CN (35mL) and photolysed in a Raynot (λ=350 nm) for 48 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield a tan colored solid 7h (600 mg, 34%) ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm) 7 58 (d, 1H, *J*=7 6 Hz), 7 14 (t, 1 H, *J*=8 0 Hz), 6 94 (d, 2 H, *J*=8 4 Hz), 6 87 (dd, 1 H, *J*=2 4, 5 6 Hz), 6 73 (d, 1 H, *J*=7 2 Hz), 6 59 (s, 1 H), 6 57 (s, 2 H), 6 39 (d, 1 H, *J*=8 0 Hz), 4 51 (dt, 1 H, *J*=2 8, 8 0 Hz), 3 80-3 62 (m, 1 H), 3 62 (s, 3 H), 3 05-3 00 (dd, 1H, *J*=2 8, 11 6 Hz), 2 85 (dd, 1 H, *J*=8 4, 6 0 Hz), 2 76-2 72 (m, 1 H), 2 36-2 19 (m, 3 H), 2 02 (dd, 1 H, *J*=6 4, 9 2 Hz), 1 8-1 73 (m, 1 H), 1 61-1 34(m, 7H), ,1 41-0 71 (m, 7 H) MS (FAB, NBA-G/TG-

DMSO, *m/z relative intensity*), 493 [(M+1)$^+$, 100], 465 (20), 232 (30), 171 (40), HRMS calcd for $C_{29}H_{37}N_2O_5$ (M+1)$^+$ 493 2702, found, 493 2699

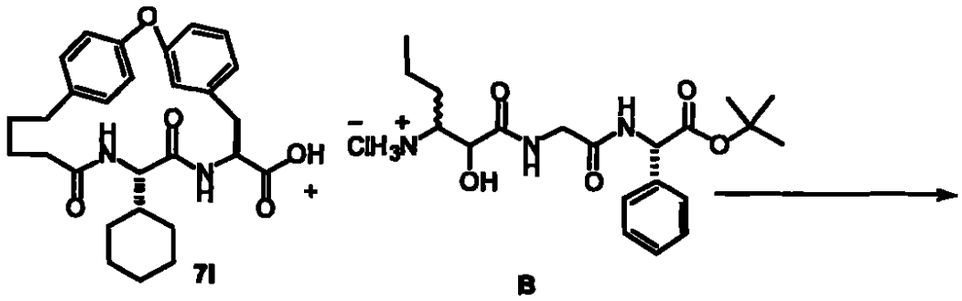
Step H



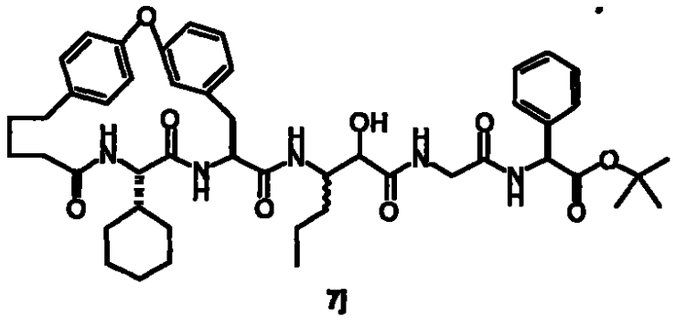
7h 7i

A solution of ether **7h** (220 mg, 0 46 mmol) in CH_3OH (3 0 mL), CH_2Cl_2 (10 mL) and H_2O (0 5 mL) was treated with $LiOH{\bullet}H_2O$ (18 mg, 0 44 mmol, 1 1 equiv) and stirred at rt for 12 h The reaction mixture was acidified with aq HCl (13 M, 1mL) and concentrated *in vacuo* to yield acid **7i** which was used directly for the coupling without further purification

Step I



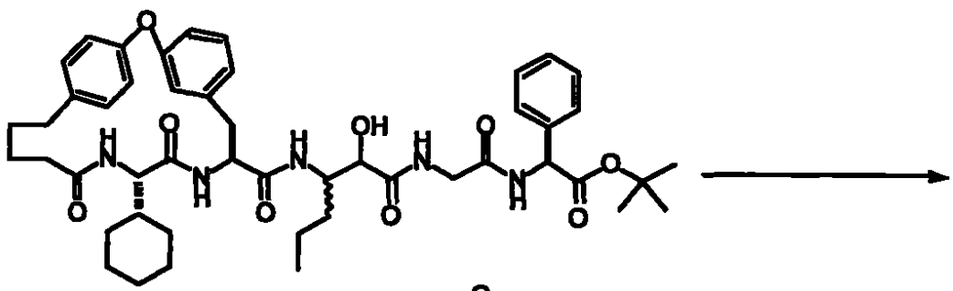
7i B



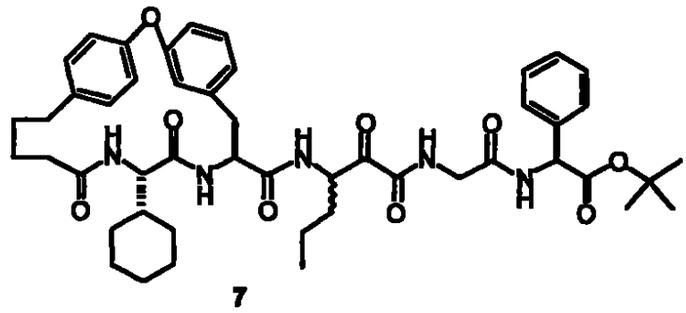
7j

A solution of acid 7I in dry DMF (3 0 mL) was treated with HOOBt (94 mg, 0 75 mmol, 1 6 equiv), Hünigs base (237 mg, 1 84 mmol, 4 0 equiv) and amine B (246 mg, 0 58 mmol, 1 47 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (110 mg, 0 58 mmol, 1 25 equiv) and stirred at 0 °C for 25 min for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The combined aqueous layers were extracted with CH_2Cl_2 (3x30 mL) The organic layers were extracted with aq HCl (1 M, 60 mL) aq NaOH (60 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 7j (230 mg) which was used for oxidation MS (Electron spray, *m/z rel Int*) 854 [$(M+1)^+$, 100], 479 (70), 327 (50), 271 1 (100)

Step J



7j



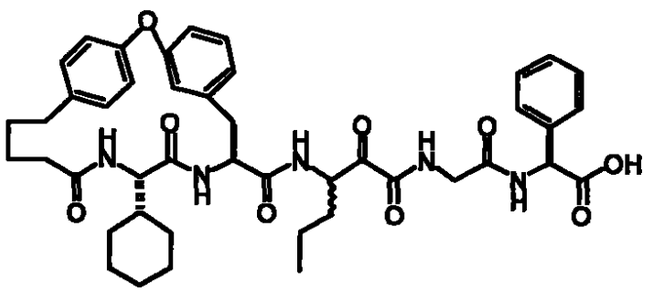
7

A solution of alcohol 7j (220 mg, 0 26 mmol) in CH_2Cl_2 (3 0 mL) was treated with Dess-Martin reagent (218 mg, 0 51 mmol, 2 0 equiv) The reaction mixture was stirred at rt and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 24) to yield oxidized product 7 (23 mg, 13%) as a colorless solid MS (FAB, *m/z, rel int*) 852 [$(M+1)^+$, 43), 796 (100), 768 (20), 461 (20), 433 (50), 405 (50), 336 (30), 294, (50)

55

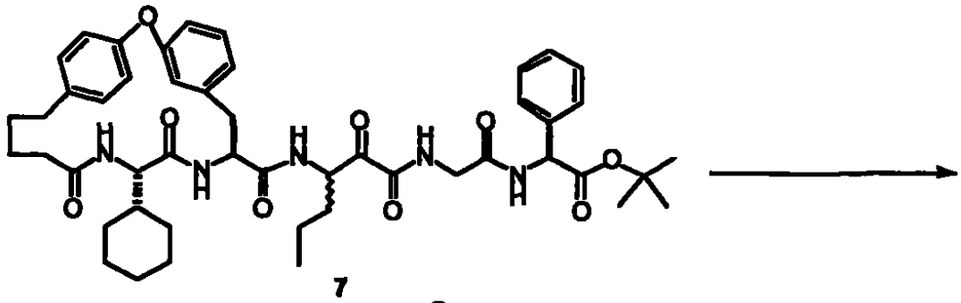
0.053

Example 8 Preparation of Compound of Formula 8.

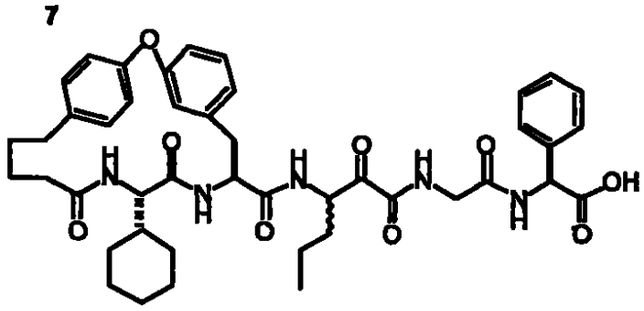


8

Step A



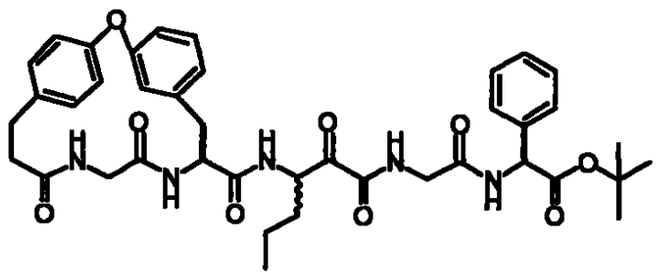
7



8

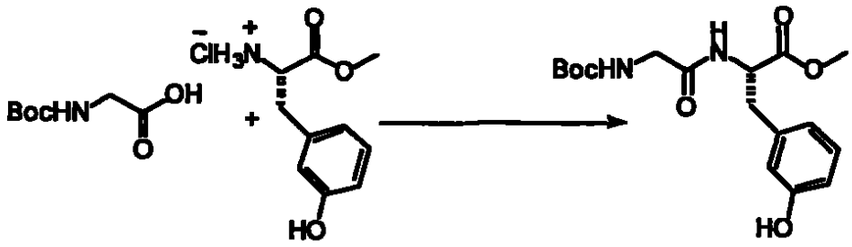
A solution of *tert*-butyl ester 7 (32 0 mg, 37 0 µmol) was treated with TFA/CH_2Cl_2 (1 1, 5 0 mL) and stirred at rt for 4 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1 24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes/CH_3OH (4 0 mL) and concentrated to yield a tan solid 8 (29 0 mg, 100%) MS (Electron spray, *m/z rel int*) 796 [$(M+1)^+$, 100)

Example 9. Preparation of Compound of Formula 9.



9

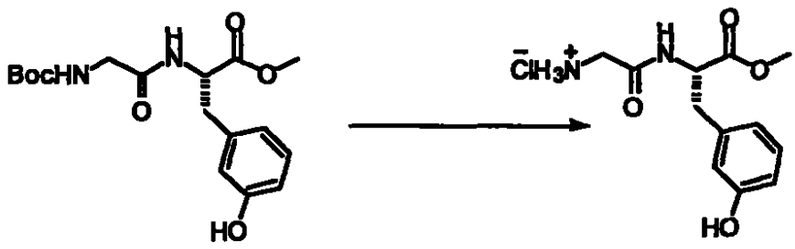
Step A



9a 1e 9b

A solution of Boc-Glycine 9a (1 75g,10 0 mmol) in dry DMF (50 mL) was treated with HOOBt (2 65 g, 15 mmol, 1 5 equiv) and EDCI (2 86g, 15 0 mmol, 1 5 equiv) The reaction mixture was treated with Hünigs base (5 16 g, 40 mmol, 4 0 equiv 7 3 mL) The reaction mixture was stirred for 1h and *meta*-tyrosine-OCH_3•HCl 1e (2 5 g, 11 5 mmol, 1 1 equiv) was added and stirred at 25 °C for 12 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with aq $NaHCO_3$ and extracted into CH_2Cl_2 The combined organic layer were concentrated and the residue purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield a colorless solid 9b (3 4g, 90%)

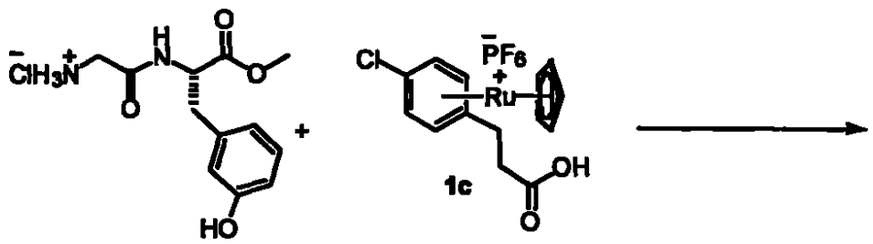
Step B



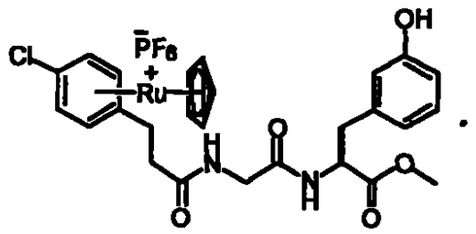
9b **9c**

A solution of **9b** (4 6g, 13 06 mmol) in HCl (4M solution in Dioxane, 50 mL) was stirred at rt. for 3h The reaction mixture was concentrated *in vacuo* and the residue was dried in high vacuum to yield **9c** as a fine powder which was used for the next step ^{1}H NMR (CD_3OD, 400 MHz, δ, ppm) 8 67 (d, 1 H, *J*=7 9 Hz),7 10-7 07 (m, 1 H), 6 68-6 64 (m, 2 H), 4 75-4 70 (m, 1 H), 3 75-3 61 (m, 2 H), 3 66 (s, 3 H), 3 10 (dd, 1 H, *J*= 5 2, 8 5 Hz), 2 90 (dd, 1 H, *J*=8 8 Hz, 5 0 Hz)

Step C



9c **1c**

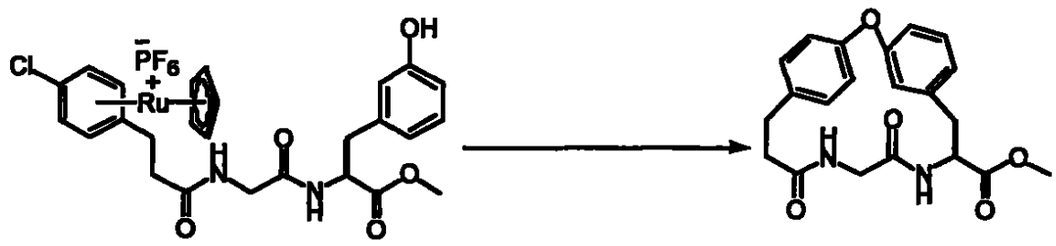


9d

A solution of [CpRu(η^6-4-chlorophenylpropionic acid)]PF_6 **1c** (3 0 g, 46 01 mmol) in dry DMF (60 mL) was treated with HOBt (1 3 g, 9 16 mmol, 1 5 equiv) and Hünigs base (3 22 g, 4 60 mL, 25 0 mmol, 4 0 equiv) The reaction mixture

was cooled to 0 ° C and treated with EDCI (1 75 g, 9 16 mmol, 1 5 equiv) The reaction mixture was stirred at 0° C for 30 min and the glycine ammonium salt.9c (1 75 g, 6 06 mmol, 1 0 equiv) was added The reaction mixture was stirred at rt for 12 and the DMF was distilled out *in vacuo* The residue was diluted with aq HCl (1M, 100 mL) and extracted into CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq $NaHCO_3$ (1x100 mL), brine (50 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* to yield a brown solid 9d (1 5 g, 34%) which was used for cyclization MS (Electron spray, *m/z relative intensity*) 585 [$(M-PF_6)^+$, 100], 459 (30), 373 (30), 198 (20)

Step D



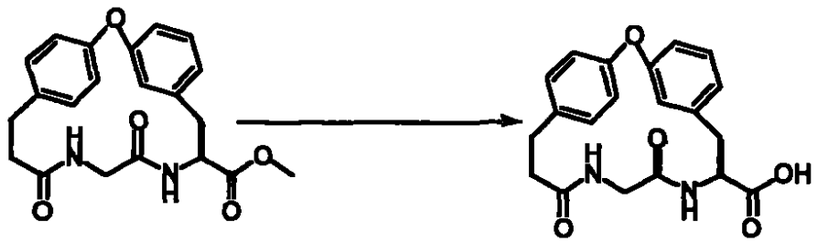
9d 9e

A solution of the ruthenium complex 9d (1 5 g 2 05 mmol) in dry DMF (100 mL) was degassed with dry N_2 at rt and Cs_2CO_3 (5 0 g, 15 mmol, 7 5 equiv) was added and stirred at rt. for 12h The solvent DMF was distilled off and the residue was diluted with water (100 mL) and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with brine (100 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* and dried in vacuum overnight. It was used for photolytic removal of Ru without further purification

The cyclized compound from the previous step was dissolved in CH_3CN (30 mL) and filtered into a quartz tube The solution was degassed and photolyzed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc) to yield a tan colored solid 9e (230 mg, 30%) ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm) 7 23-7 18 (m, 2 H), 7 09-7 01 (m, 3 H), 6 76 (dd, 1 H, *J*=2 4, 8 8 Hz), 6 66

(d, 1 H, *J*=7 6 Hz), 6 47 (d, 1 H, *J*=5 6 Hz), 6 17 (s, 1 H), 5 64 (s, 1 H), 4 69 (q, 1 H, *J*=4 4 Hz), 3 77 (s, 3 H), 3 68-3 51 (m, 2 H), 3 35 (dd, 1 H, *J*=4 0, 10 8 Hz), 3 05 (dd, 1 H, *J*=5 2, 9 2 Hz), 2 96-2 92 (m, 2 H), 2 61-2 56 (m, 1 H), 2 30-2 29 (m, 1 H), ^{13}C NMR (CDCl$_3$, 100 MHz, δ ppm) 172 3, 171 4, 168 1, 159 9, 155 4, 137 6, 136 4, 131 0, 130 0, 129 5, 123 3, 122 4, 121 0, 117 7, 117 1, 53 6, 53 0, 43 6, 39 9, 36 1, 32 3 MS (Electron spray, *m/z relative Intensity*) 383 [(M+1)$^+$, 100], 279 (20)

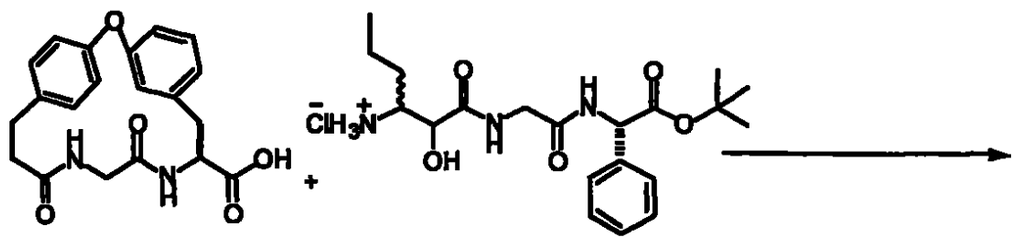
Step E



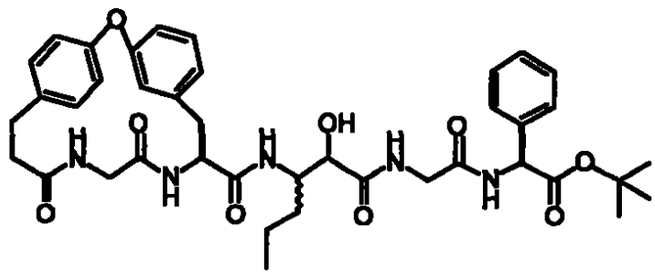
9e **9f**

A solution of cyclic compound **9e** (150 mg, 0 4 mmol) in THF (4 0 mL), H_2O (4 0 mL) was stirred at rt with LiOH•H_2O (41 0 mg, 1 0 mmol, 2 5 equiv) for 3 h The reaction mixture was acidified with conc. HCl (2 0 mL) and concentrated *in vacuo* The solid **9f** was dried *in vacuo* and used for further coupling without further purification

Step F



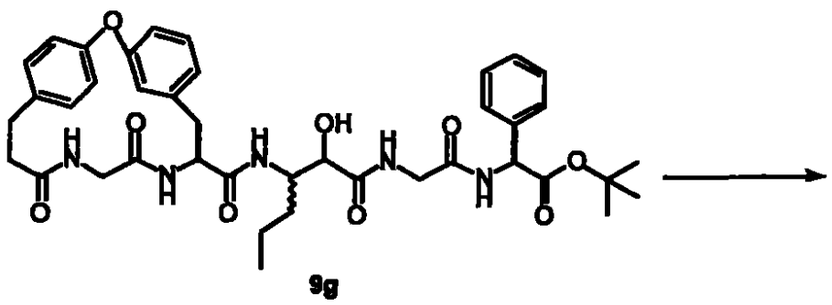
9f **B**



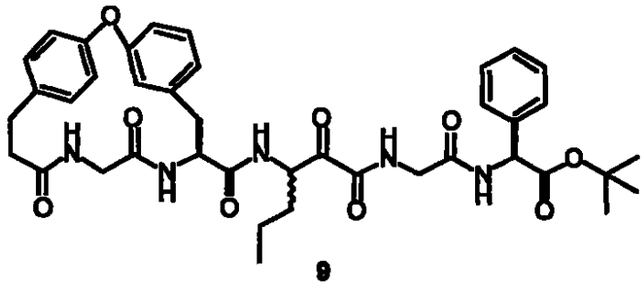
9g

A solution of the hydrolyzed acid **9f** in dry DMF (4 0 mL) and CH_2Cl_2 (4 0 mL) was treated with HOOBt (103mg, 0 58 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (206 mg, 1 60 mmol, 4 0 equiv, 295 µL) was added To this mixture was added EDCI (112 mg, 0 58 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 0 5 h and treated with the amine hydrochloride **B** (206 mg, 0 48 mmol, 1 2 equiv) The reaction mixture was stored in freezer for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with aq HCl (2M) and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq HCl (1M, 3x50 mL), aq NaOH (2M) brine (100 mL), dried (Na_2SO_4) and concentrated *in vacuo* The residue **9g** (200 mg) was oxidized without further purification

Step G



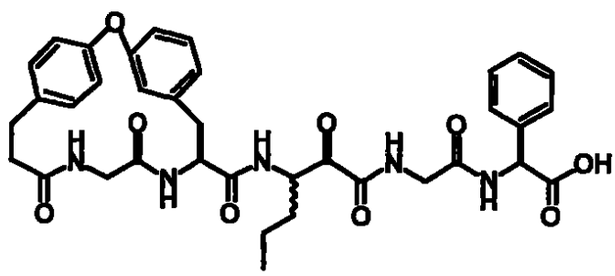
9g



9

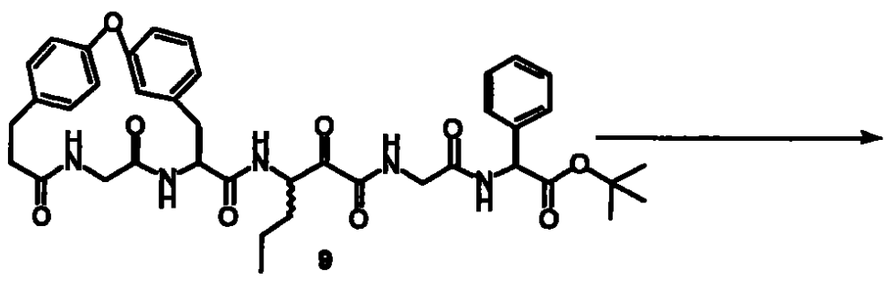
A solution of alcohol 9g (200 mg, 0 27 mmol) in CH_2Cl_2 (3 0 mL) was treated with Dess-Martin reagent (342 mg, 0 81 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 3 h and diluted with aq $NaHCO_3$ and aq $Na_2S_2O_3$ The reaction mixture was stirred at rt for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were extracted with brine (50 mL), dried (Na_2SO_4), filtered concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH (2M NH_3)/CH_2Cl_2 1 19) to yield ketoamide 9 (100 mg, 50%) of a colorless solid MS (Electron spray, *m/z relative intensity*) 742 ($[M+1]^+$, 100), 686 (80)

Example 10 Preparation of Compound of Formula 10.

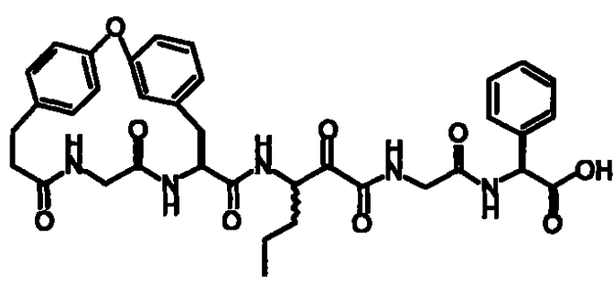


10

Step A



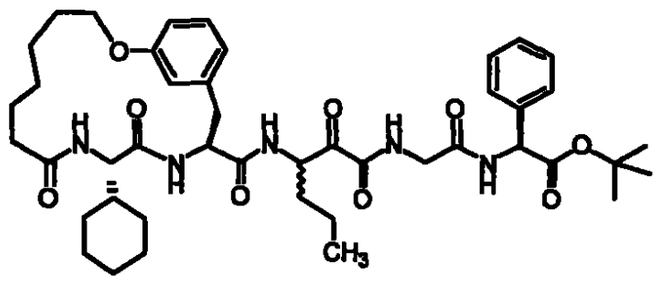
9



10

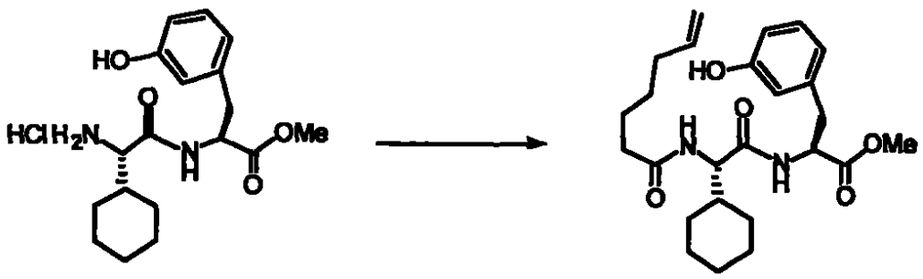
A solution of *tert*-butyl ester 9 (100 mg, 0 13 mmol) in dry CH_2Cl_2 (4 0 mL) was treated with TFA (4 0 mL) and stirred at rt for 5 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in toluene/CH_2Cl_2 and concentrated *in vacuo* several times to yield a fine colorless solid 10 MS (FAB, NBA/DMSO, *m/z relative intensity*), 686 [$(M+1)^+$, 40], 460 (20), 307 (100), 289 (60), HRMS calcd for $C_{36}H_{40}N_5O_9$ $(M+1)^+$ 686 2825, found 686.2840

Example 11 Preparation of Compound of Formula 11



11

Step A.



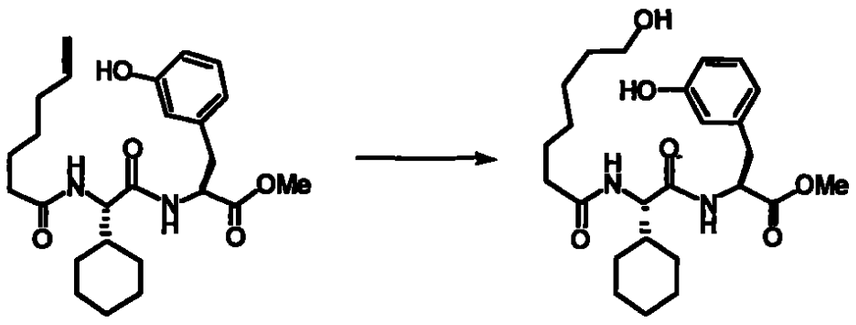
1g 11a

To a solution of amine hydrochloride 1g (1 20g, 3 23 mmol), 6-heptenoic acid (0 610 g, 4 68 mmol), HOOBt (0 765 g, 4 69 mmol) and EDCI (1 07 g, 5 58 mmol) in anhydrous DMF (50 mL) and CH_2Cl_2 (50 mL) at -20°C was added NMM (1 55 mL, 14 1 mmol) After stirring at this temperature for 30 min, the reaction mixture was kept in a freezer for 18 h It was then allowed to warm to rt EtOAc (150 mL), brine (50 mL) and 5% H_3PO_4 (50 mL) were added After separation, the organic solution was washed with 5% H_3PO_4 (80 mL), saturated aqueous sodium bicarbonate solution (2 X 80 mL), water (80 mL), and brine (80 mL), dried with magnesium sulfate, filtered and concentrated *in vacuo* Flash chromatography (2 to 5 % MeOH-CH_2Cl_2) afforded 11a (1 46 g, 3 28 mmol, quant) as a white solid

^1H NMR (400 MHz, d_6-DMSO) δ 9 25 (s, 1 H), 8 31 (d, *J* =7 2 Hz, 1 H), 7 70 (d, *J*

=9 2 Hz, 1 H), 7 05-7 01 (m, 1 H), 6 62-6 58 (m, 3 H), 5 82-5 72 (m, 1 H), 5 02-4 91 (m, 1 H), 4 43-4 38 (m, 1 H), 4 23-4 19 (m, 1 H), 3 55 (s, 3 H), 2 93-2 80 (m,2 H), 2 51-1 97 (m, 2 H), 1 66-0 86 (m, 15H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 171 9, 171 8, 171 1, 157 2, 138 6, 138 4, 129 1, 119 5, 115 8, 114 6, 113 5, 56 5, 53 5, 51 6, 36 5, 34 8, 32 8, 29 0, 28 0, 27 8, 25 8, 25 5, 24 8, HRMS, *m/z* 445 2683 (calcd for $C_{25}H_{36}N_2O_5$ 445 2702, error 4 ppm)

Step B

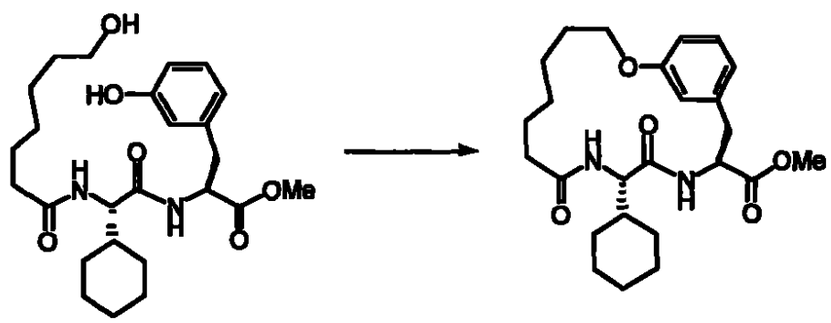


11a **11b**

To the solution of **11a** (1 46 g, 3 28 mmol) in anhydrous THF (60 mL) under nitrogen at 0°C was added borane-THF solution (12 mL, 1 0 M, 12 mmol) cautiously The resulting solution was stirred at 0°C under nitrogen for 1 h 40 min Then ethanol (4 mL) and pH 7 buffer (8 mL) were added, followed by aqueous 30 % H_2O_2 solution (7 5 mL) After stirred at 0°C for 20 min, it was warmed to rt and stirred for 2 h EtOAc (200 mL) and brine (100 mL) were added and layers were separated Aqueous solution was extracted with EtOAc (2 X 150 mL) Combined organic solution was dried with magnesium sulfate, filtrated, concentrated *in vacuo* Flash chromatography (2 to 5 % MeOH-CH_2Cl_2) afforded **11b** (1 05 g, 2 18 mmol, 68 %) as a white solid ^{1}H NMR (400 MHz, d_6-DMSO) δ 9 25 (s, 1 H), 8 30 (d, *J* =7.2 Hz, 1 H), 7 68 (d, *J* =9 2 Hz, 1 H), 7 05-7 01 (m, 1 H), 6 62-6 58 (m, 3 H), 4 43-4 18 (m, 3 H), 3 55 (s, 3 H), 3 37-3 33 (m,2 H), 2 93-2 80 (m, 2 H), 2 20-2 03 (m, 2 H), 1 66-0 87 (m, 19H), ^{13}C NMR (d_6-DMSO, 125 MHz), d 172 1, 171 8, 171 2, 157 2, 138 4, 129 1, 119 5, 115 8, 113 5, 60 7, 56 5, 53 5, 51 7,

36 5, 35 1, 32 6, 32 4, 29 0, 28 5, 28 0, 25 8, 25 6, 25 4, 25 2, HRMS, *m/z* 463 2813 (calcd for $C_{25}H_{36}N_2O_5$ 463 2808, error 1 ppm)

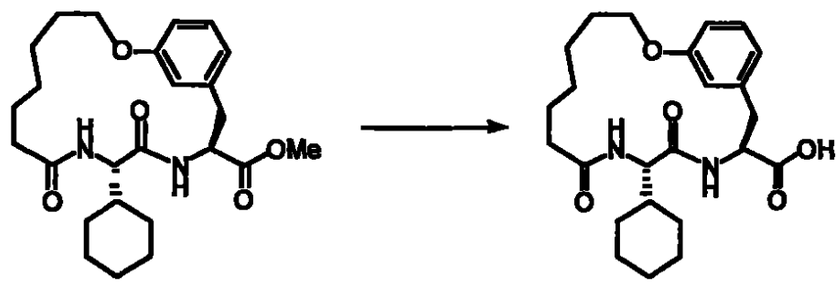
Step C.



11b 11c

To a solution of phenol alcohol **11b** (1 00 g, 2 16 mmol) and tri-*n*-butylphosphine (1 10 mL, 4 28 mmol) in anhydrous CH_2Cl_2 (100 mL) and THF (40 mL) at 0°C was added ADDP (1 08 g, 4 28 mmol) After stirring at 0°C for 1 h, the solution was warmed to rt and stirred for 3 h under nitrogen TLC indicated complete consumption of the starting material After removal of solvent *in vacuo*, the residue was partially purified by flash chromatography (0 to 3 % MeOH in CH_2Cl_2) to afford the macrocycle **11c** (650 mg, 1 46 mmol, 68 %) ^{1}H NMR (400 MHz, d_6-DMSO) δ 8 58 (d, *J* =8 3 Hz, 1 H), 7 76 (d, *J* =9 2 Hz, 1 H), 7.18-7 14 (m, 1 H), 6 76-6 65 (m, 3 H), 4 77-4 71 (m, 1 H), 4 32 (t, *J* = 8 5 Hz, 1 H), 3 97-3 93 (m, 1 H), 3 82-3 78 (m, 1 H), 3 67 (s, 3 H), 3 18-3 14 (m, 1 H), 2 98-2 92 (m, 2 H), 2 32-2 25 (m, 1 H), 2 02-2 01 (m, 1 H), 1 99-0 87 (m, 19H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 172 1, 171 6, 171 4, 160 1, 158 8, 139 0, 129 1, 121 1, 113 0, 111 9, 66 4, 56 1, 52 0, 50 1, 40 6, 34 9, 34 2, 28 7, 28 3, 26 8, 26 3, 25 9, 25 5, 25 2, 24 2, HRMS, *m/z* 445 2685 (calcd for $C_{25}H_{36}N_2O_5$ 445 2702, error 4 ppm)

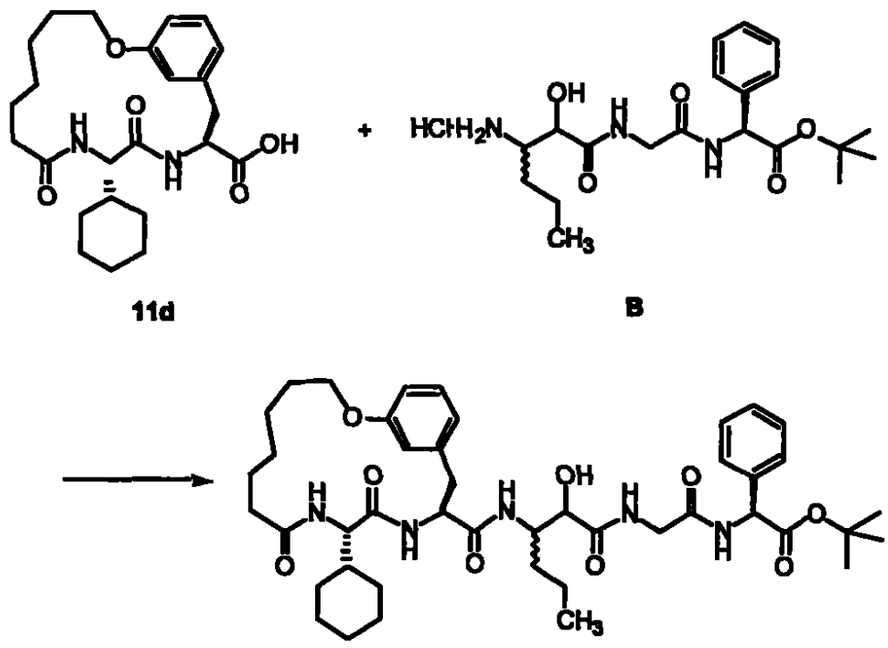
Step D



11c **11d**

Aqueous lithium hydroxide solution (70 mg, 15 mL H_2O, 2 92 mmol) was added to a solution of methyl ester **11c** (330 mg, 0 742 mmol) in THF (20 mL) and ethanol (10 mL) at rt The mixture was stirred at rt for 3 h The progress of the reaction was monitored by TLC After the solution was concentrated *in vacuo*, EtOAc (100 mL), 6 N HCl solution (10 mL) and water (50 mL) were added and the layers were separated The aqueous solution was extracted with EtOAc (2 X 80 mL) Organic solutions were combined, dried with magnesium sulfate, filtered and concentrated *in vacuo* to afford **11d** (260 mg, 0 604 mmol, 81 %) as a white solid ^1H NMR (400 MHz, d_6-DMSO) δ 8 43 (d, *J* =8 3 Hz, 1 H), 7 73 (d, *J* =9 3 Hz, 1 H), 7 17-7 13 (m, 1 H), 6 77-6 66 (m, 3 H), 4 67-4 62 (m, 1 H), 4 32-4 28 (m, 1 H), 3 98-3 93 (m, 1 H), 3 81-3 75 (m, 1 H), 3 17-3 13 (m, 1 H), 2 97-2 90 (m, 1 H), 2 32-2 26 (m, 1 H), 2 01-1 97 (m, 1 H), 1 67-0 85 (m, 19H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 173 2, 171 6, 171 3, 158 8, 139 3, 129 0, 121 1, 113 1, 111 9, 66 4, 56 1, 50 8, 35 1, 34 3, 28 8, 28 3, 26 9, 26 3, 25 9, 25 6, 25 5, 25 2, 24.2, HRMS, *m/z* 431 2564 (calcd for $C_{25}H_{36}N_2O_5$ 431 2546, error 4 ppm)

Step E

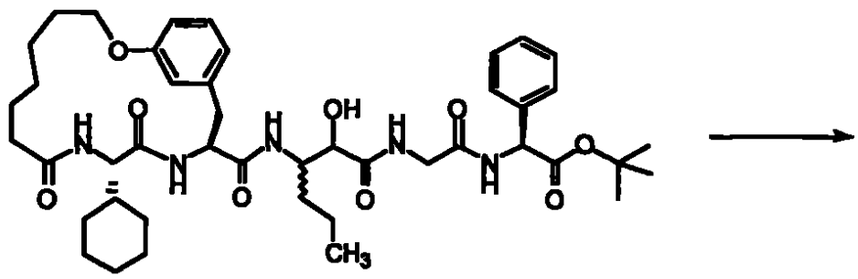


11d B

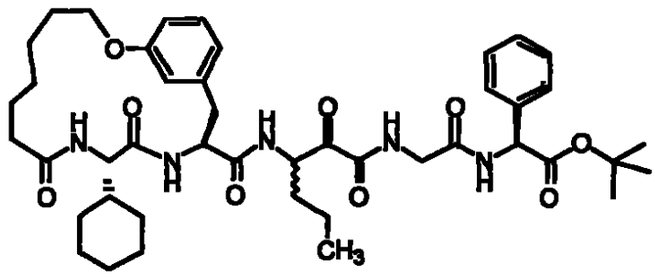
11e

To a solution of acid **11d** (0 140 g, 0 325 mmol), amine **B** (0 140 g, 0 325 mmol), HOOBt (56 mg, 0 343 mmol) and EDCI (75 mg, 0 391 mmol) in anhydrous DMF (40 mL) and CH_2Cl_2 (20 mL) at -20°C was added NMM (0 107 mL, 0 973 mmol) After stirred at this temperature for 30 min, the reaction mixture was kept in a freezer for 18 h Then EtOAc, brine and 5% H_3PO_4 were added The separated organic solution was washed, successively, with 5% H_3PO_4, saturated aqueous sodium bicarbonate solution, water, and brine, dried with magnesium sulfate, filtered and concentrated *In vacuo* Flash chromatography (2 to 5 % MeOH-CH_2Cl_2) afforded **11e** as a mixture of diastereomers (0 170 g, 0 211 mmol, 65%) as a white solid, which is used in the next reaction without further purification

Step F.



11e

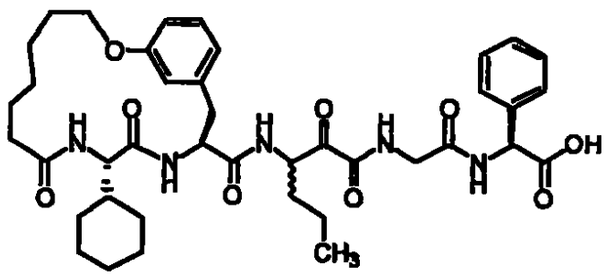


11

To the mixture of hydoxy amide 11e (0 29 g, 0 36 mmol) and Dess-Martin reagent (0 45 g, 1 06 mmol) at rt was added anhydrous CH_2Cl_2 (60 mL), DMF (3 mL) and DMSO (3 mL) The resulting solution was vigorously stirred at rt for 2 5 h More Dess-Martin reagent (300 mg, 0 71 mmol) was added and the reaction mixture was stirred for another hour Saturated aqueous sodium bicarbonate and sodium bisulfite solutions (40 mL each) were added and the mixture was vigorously stirred for 10 min before EtOAc (200 mL) and water (30 mL) were added and the layers were separated The organic solution was washed with 5% H_3PO_4 solution (2 X 100 mL) and saturated $NaHCO_3$ solution (100 mL), dried with magnesium sulfate, filtered and concentrated *in vacuo* Flash chromatography (1 to 5 % MeOH-CH_2Cl_2) afforded 11 (100 mg, 0 124 mmol, 35%) as a white solid ^1H NMR (400 MHz, d_6-DMSO) δ 8 79-8 69 (m, 2 H), 8 36-8 16 (m, 2 H), 7 72-7 68 (m, 1 H), 7 42-7 33 (m, 5 H), 7 17-7 13 (m, 1 H), 6 77-6 63 (m, 3 H), 5 30-5 27 (m, 1 H), 5 09-5 04 (m, 1 H), 4 85-4 76 (m, 1 H), 4 29-

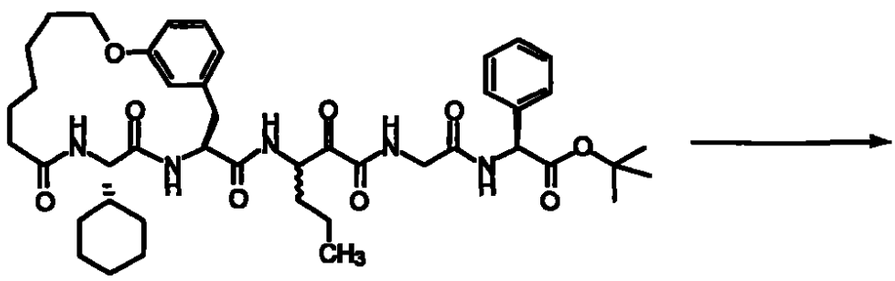
4 25 (m, 1 H), 3 98-3 74 (m, 1 H), 3 02-2 85 (m, 2 H), 2 32-2 27 (m, 1 H), 2 04-1 96 (m, 1 H), 1 72-0 81 (m, 35 H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 196 5, 196 2, 171 65, 171 61, 171 5, 171 14, 171 07, 169 4, 167 6, 160 7, 158 84, 158 79, 139 5, 139 3, 136 6, 136 5, 128 92, 128 90, 128 7, 128 6, 128 1, 127 7, 127 4, 124 9, 121 34, 121 28, 113 1, 112 9, 112 0, 111 9, 81 3, 66 34, 66 30, 56 92, 56 87, 56 3, 56 2, 53 4, 53 3, 51 5, 50 9, 41 5, 41 4, 40 8, 40 7, 36 6, 36 1, 34 4, 34 3, 31 8, 31 6, 30 4, 29 1, 28 9, 28 4, 28 3, 27 5, 26 8, 26 21, 26 17, 25 9, 25 59, 25 55, 25 0, 24 2, 18 74, 18 66, 13 5, 13 4 , HRMS, *m/z* 804 4542 (calcd for $C_{25}H_{36}N_2O_5$ 804 4548, error 1 ppm)

Example 12 Preparation of Compound of Formula 12.

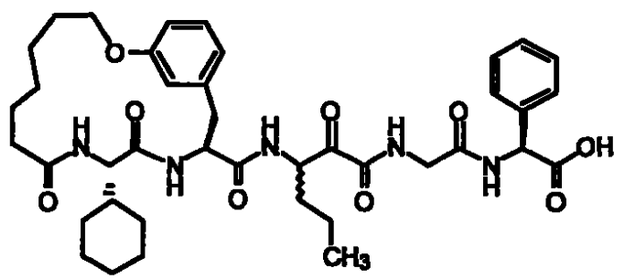


12

Step A.



11

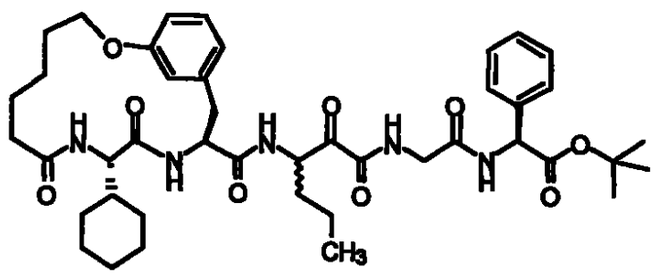


12

A solution of the *t*-Butyl ester 11 (56 8 mg, 0 0706 mmol) in trifluoroacetic acid (15 mL) and CH_2Cl_2 (15 mL) was stirred at rt for 4 h After the volatiles were removed *in vacuo*, the residue was dissolved in 50% MeOH-CH_2Cl_2 (3 mL), and concentrated to dryness *in vacuo* to afford an off-white solid 12 (50 mg, 0 0669 mmol, 95%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 8 75-8 71 (m, 2 H), 8 36-8 16 (m, 2 H), 7 72-7 69 (m, 1 H), 7 39-7 31 (m, 5 H), 7 17-7 13 (m, 1 H), 6 76-6 63 (m, 3 H), 5 37-5 35 (m, 1 H), 5 07-5 04 (m, 1 H), 4 85-4 76 (m, 1 H), 4 29-4 25 (m, 1 H), 3 97-3 74 (m, 4 H), 3 02-2 86 (m, 2 H), 2 32-2 26 (m, 1 H), 2 01-1 97 (m, 1 H), 1 70-0 82 (m, 26 H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 196 5, 196 2, 171 63, 171 59, 171 52, 171 48, 171 1, 171 06, 167 4, 160 6, 158 82, 158 78, 153.4, 139 4, 137 1, 137 0128 91, 128 88, 128 7, 128 65, 128 61, 128 5, 128 43, 128 39, 128 33, 128 32, 128 14, 128 12, 128 0, 127 7, 128 7, 127 63, 127 59, 127 5, 127 4, 126 8, 121 3, 115 9, 113 1, 112 9, 112 8, 112 0, 111 9,, 111 88, 66 33, 66 29, 56 3, 56 2, 56 17, 53 34, 53 31, 53.27, 51 1, 50 9, 41 5, 40 84, 40 77, 40 7, 40 6, 40 56, 40 53, 40 5, 38 7, 38 6, 38 56, 38 53, 36 6, 36 1, 34 4, 34 3, 31 8,

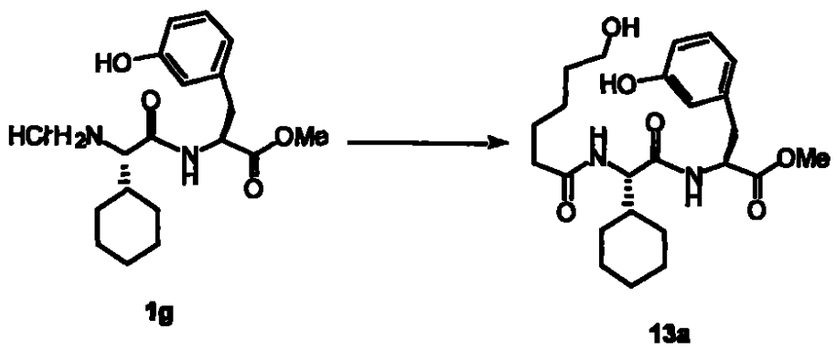
31 6, 29 4, 29 1, 29 0, e28 9, 28 4, 28 3, 28 2, 26 9, 26 8, 26 79, 26 20, 26 16, 25 88, 25 86, 25 79, 25 75, 25 71, 25 66, 25 57, 25 54, 25 4, 25 0, 24 2, 18.7, 18 6, 13 5, 13 4, HRMS, *m/z* 748 3947 (calcd for $C_{25}H_{36}N_2O_5$ 748 3922, error 3 ppm)

Example 13 Preparation of Compound of Formula 13.



13

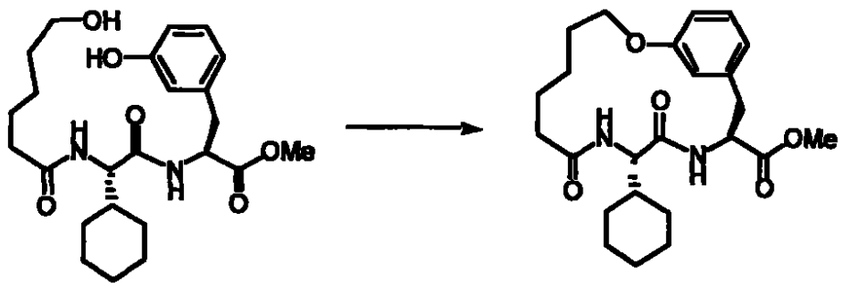
Step A



1g 13a

The desired compound **13a** was prepared according to the method of Example 11, Step A, except substituting 6-hydroxyhexanoic acid for 6-heptenoic acid (39%)

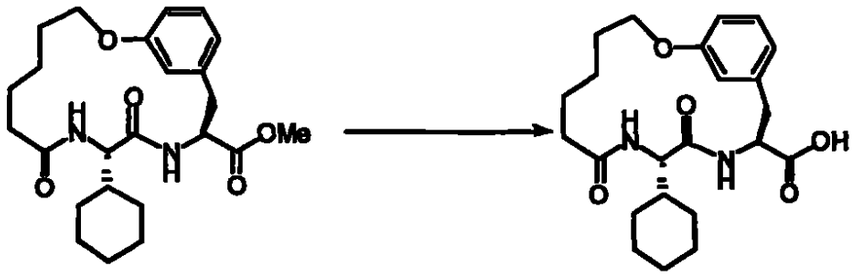
Step B.



13a 13b

The desired compound 13b was prepared from 13a according to the method of Example 11, Step C in 74% yield

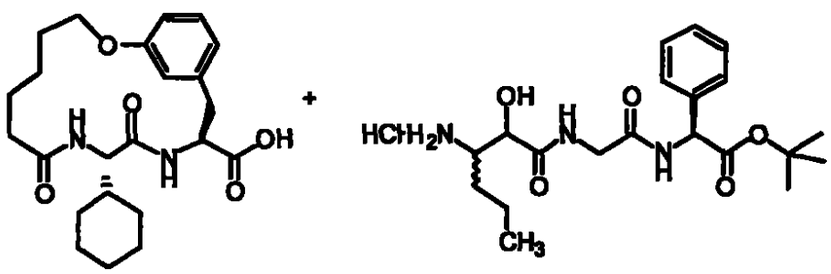
Step C



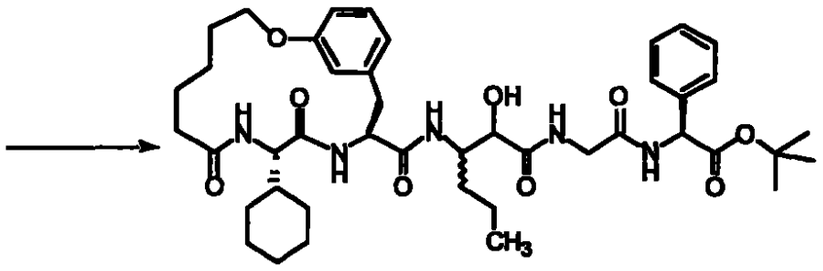
13b 13c

The desired macrocyclic acid 13c was prepared from its corresponding methyl ester 13b according to the method of Example 11, Step D in 88 % yield as a white solid

Step D.



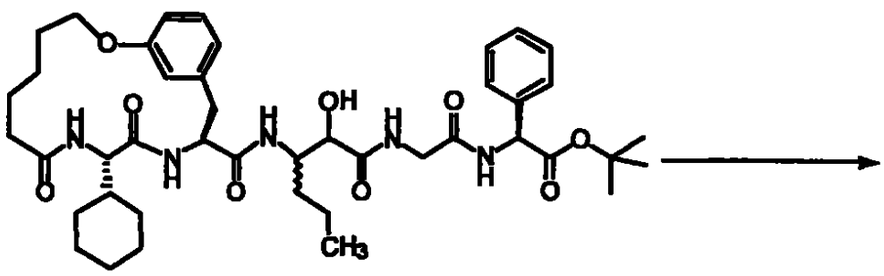
13c B



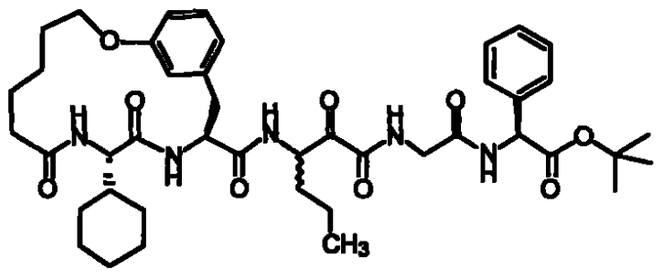
13d

The desired compound 13d was prepared from 13c and B according to the method of Example 11, Step E in 48% yield

Step E.



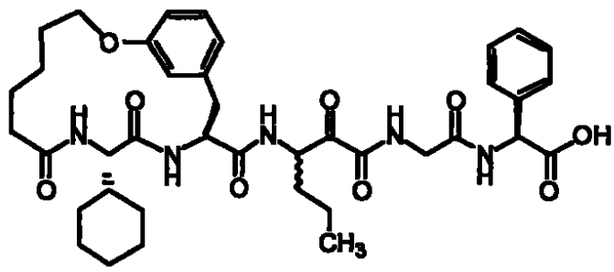
13d



13

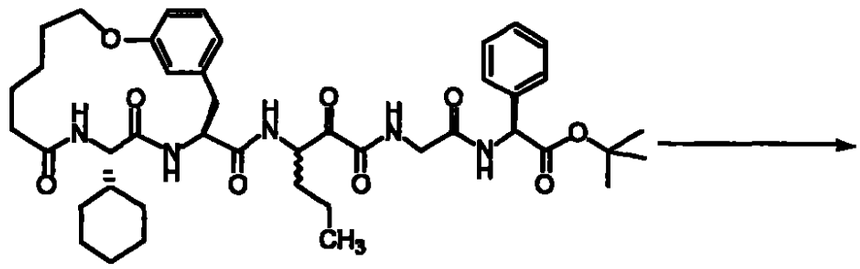
The desired compound 13 was prepared from 13d according to the method of Example 11, Step F in 70% yield

Example 14. Preparation of Compound of Formula 14.

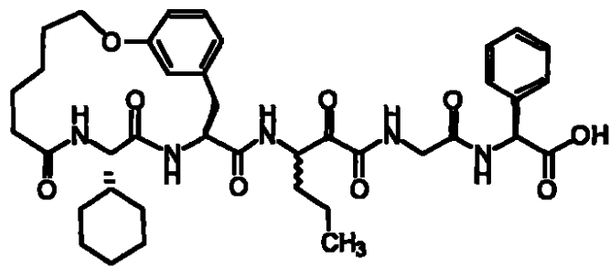


14

Step A.



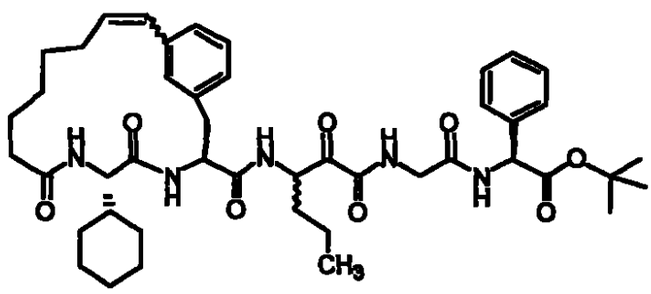
13



14

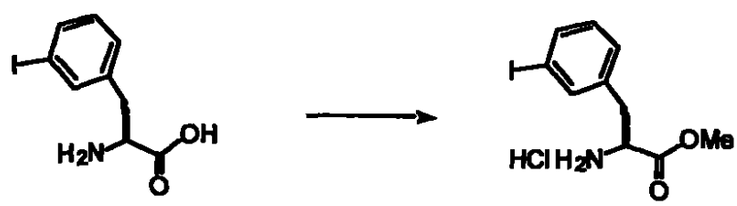
The desired compound 14 was prepared from 13 according to the method of Example 12, Step A in quantitative yield

Example 15 Preparation of Compound of Formula 15.



15

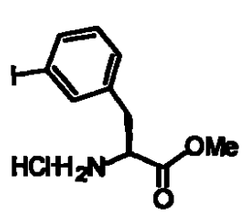
Step A



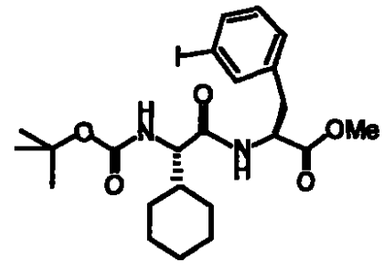
15a 15b

A solution of 3-iodo-phenylanaline **15a**, (2 50 g, 8 59 mmol) and concentrated hydrochloric acid (2 mL, 24 mmol) in methanol was heated to reflux for 18 h Removal of solvents *in vacuo* afforded a white solid **15b**, which was used in Step B without further purification

Step B



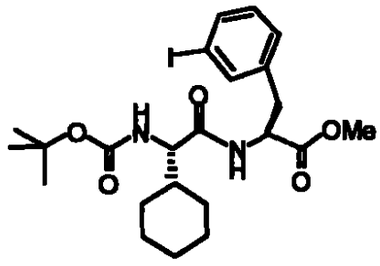
15b



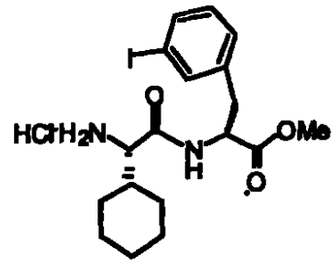
15c

The desired compound **15c** was prepared in 84% yield from **15b** according to the method of Example 11, Step A It was used in the next reaction without further purification

Step C



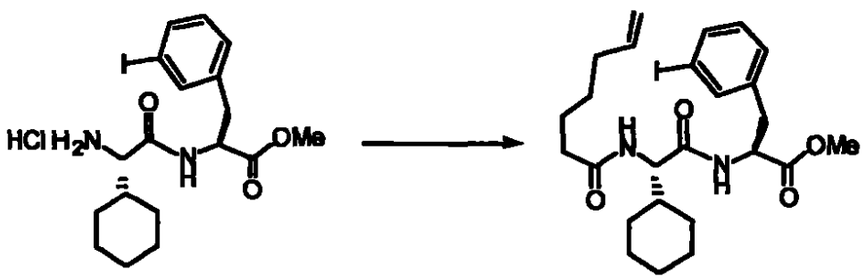
15c



15d

The desired compound **15d** was prepared from **15c** according to the method of Example 11, Step A (quantitative) It was used in the next reaction without further purification

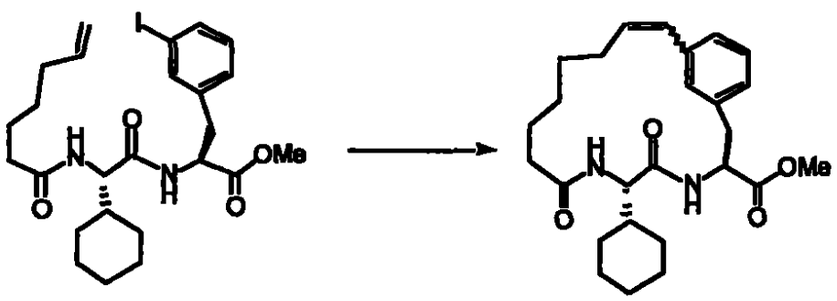
Step D.



15d 15e

The desired compound **15e** was prepared in 68% yield from **15d** according to the method of Example 11, Step A It was used in the next reaction without further purification

Step E.

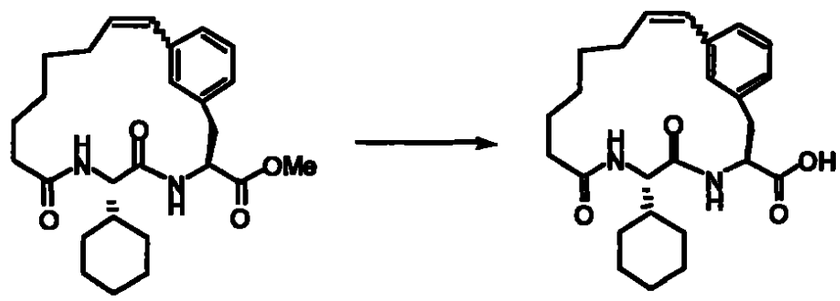


15e 15f

A solution of **15e** (1 16 g, 2 04 mmol), triethylamine (2 90 mL, 20 6 mmol) in anhydrous acetonitrile (25 mL) and DMF (20 mL) in a thick walled tube was bubbled with Argon for 5 min To this solution at rt was quickly added tetrakistriphenylphosphine palladium (0) (235 mg, 0 203 mmol) The tube was sealed with Teflon screw cap and heated to 85-90°C in an oil bath After stirring for 3 h, it was cooled to rt, opened cautiously and poured onto EtOAc (100 mL) The solution was washed with 5% H_3PO_4 (4 X 50 mL) and water (50 mL) Organic layer was dried with magnesium sulfate, filtrated, concentrated *in vacuo*

Flash chromatography (1 to 4 % MeOH-CH_2Cl_2) afforded the macrocycle **15f** (330 mg, 0 749 mmol, 37 %)

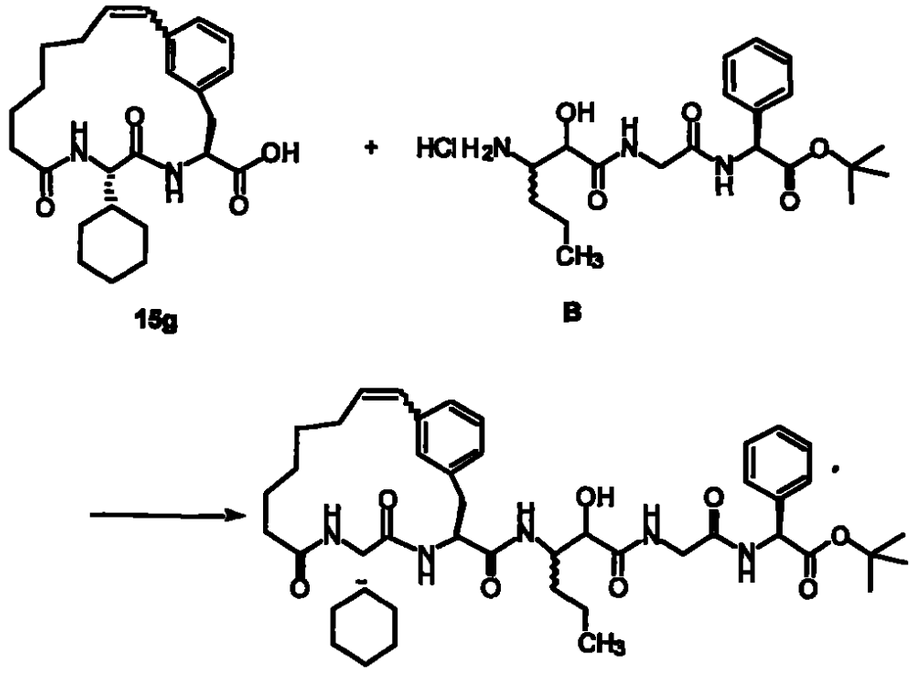
Step F



15f **15g**

The desired compound **15g** was prepared quantitatively from **15f** according to the method of Example 11, Step D It was used in the next reaction without further purification

Step G.



15g **B**

15h

23

0070 70

67

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the pamphlet!

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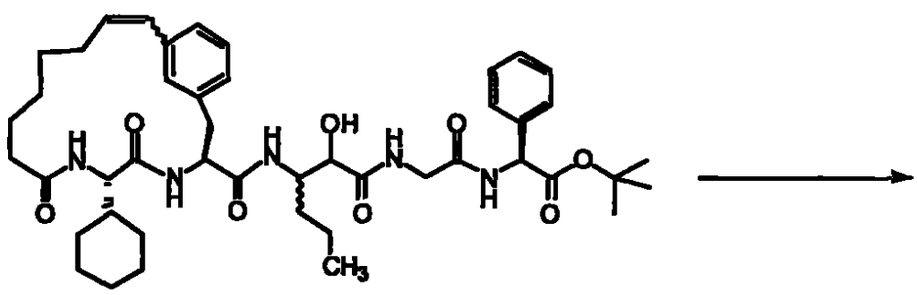
Date: 01 nov 2001

Destination Agent

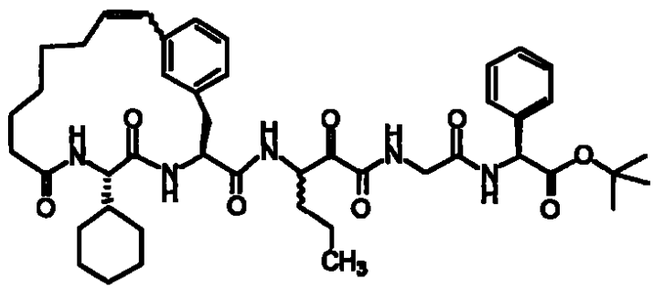
Address

The desired compound **15h** was prepared in 77 % yield from **15g** according to the method of Example 11, Step F It was used in the next reaction without further purification

Step H.



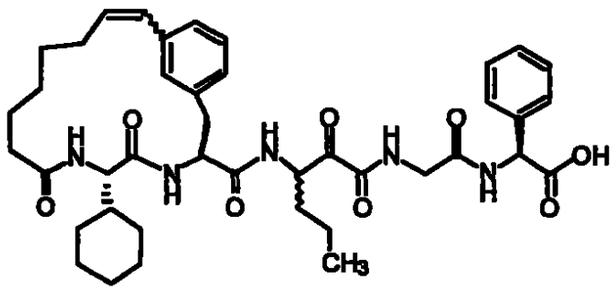
15h



15

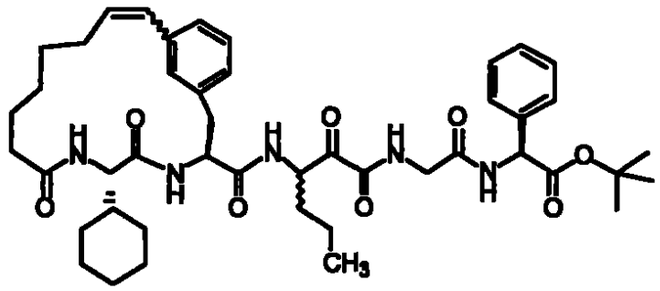
The desired compound **15** was prepared in 55 % yield from **15h** according to the method of Example 1, Step H

Example 16. Preparation of Compound of Formula 16.

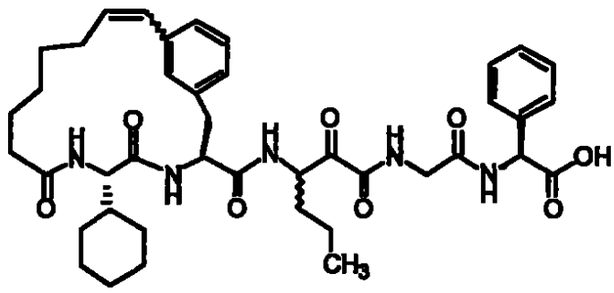


16

Step A.



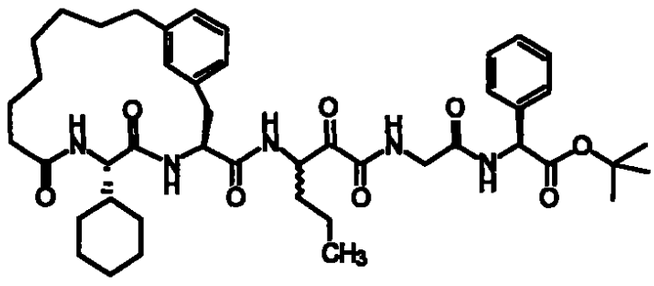
15



16

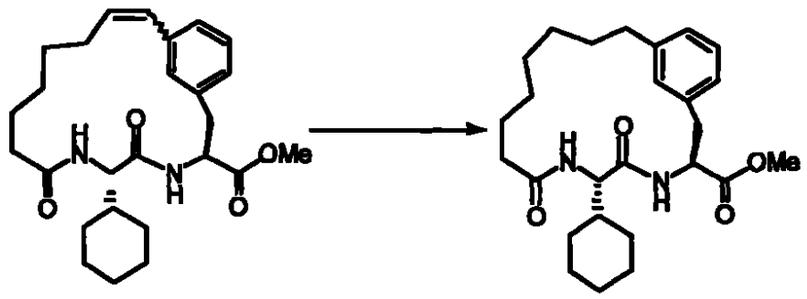
The desired compound **16** was prepared quantitatively from **15** according to the method of Example 12, Step A

Example 17. Preparation of Compound of Formula 17



17

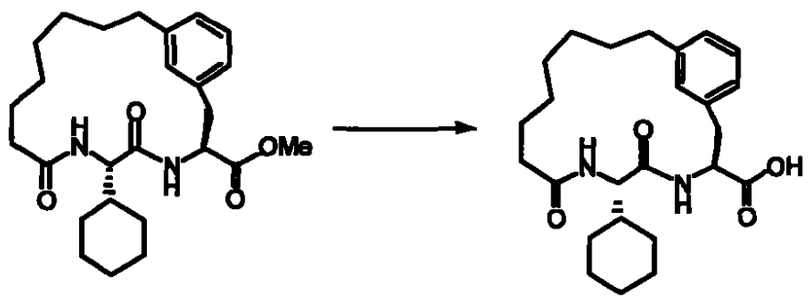
Step A.



15f 17a

To the solution of **15f** (150 mg, 0 340 mmol) in EtOH (10 mL) and EtOAc (5 mL) was added 10 % palladium on carbon (20 mg) The suspension was stirred under hydrogen for 8 h during which the reaction progress was monitored by TLC After filtration through a celite pad, solvents were removed *in vacuo* to afford the product as a white solid **17a** (150 mg, 0 339 mmol, quantitative) It was used in the next reaction without further purification

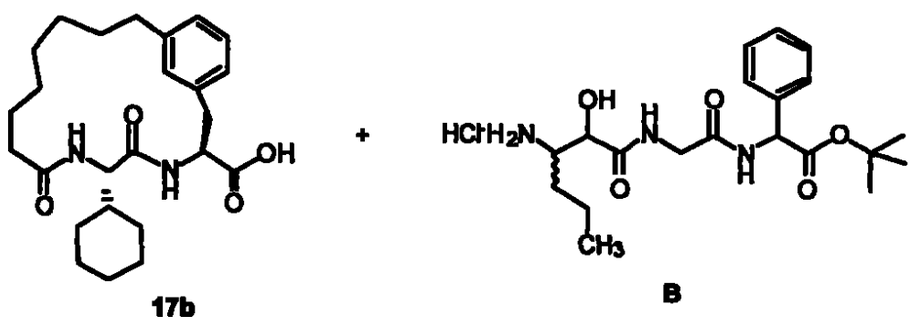
Step B.



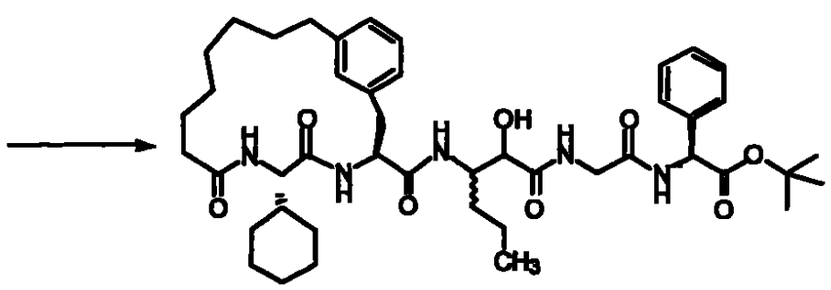
17a 17b

The desired compound **17b** was prepared from **17a** according to the method of Example 11, Step D It was used in the next reaction without further purification

Step C.



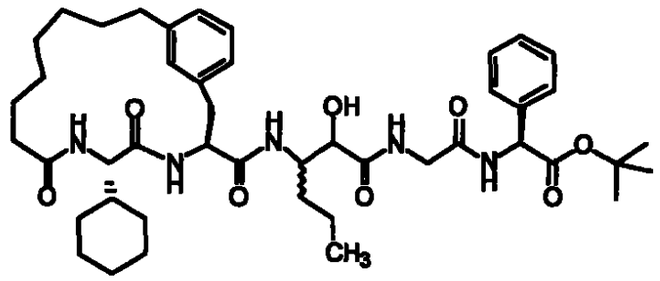
17b B



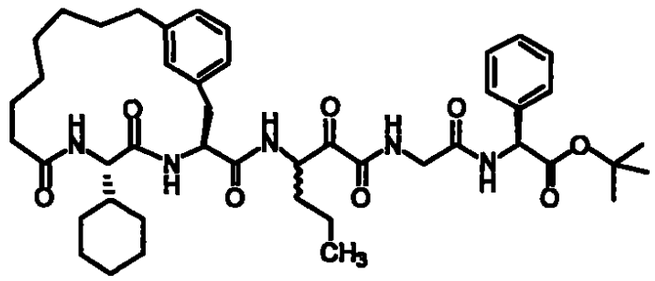
17c

The desired compound 17c was prepared in 73 % yield (Steps B and C) from 17b according to the method of Example 11, Step E It was used in the next reaction without further purification

Step D.



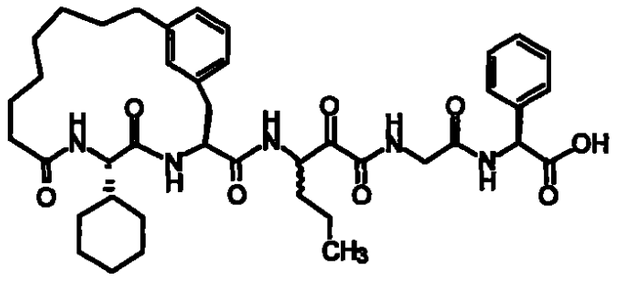
17c



17

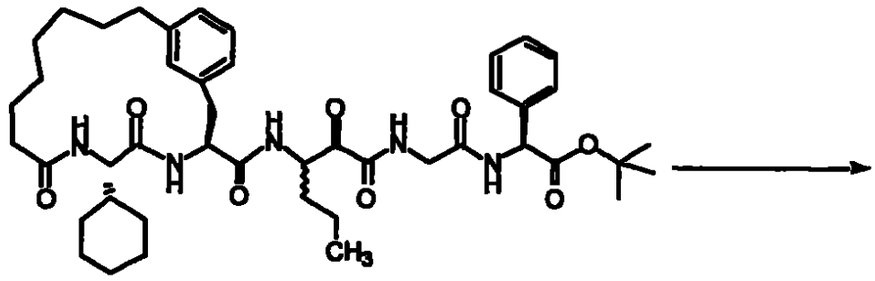
The desired compound 17 was prepared in 46 % yield from 17c according to the method of Example 11, Step F

Example 18 Preparation of Compound of Formula 18

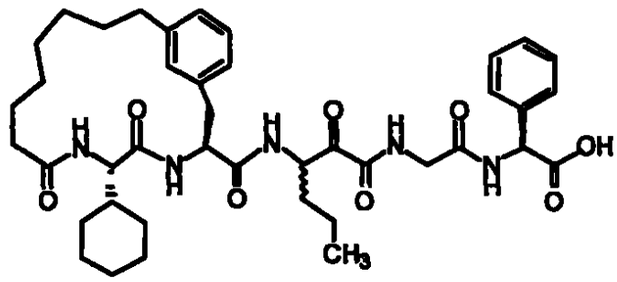


18

Step A.



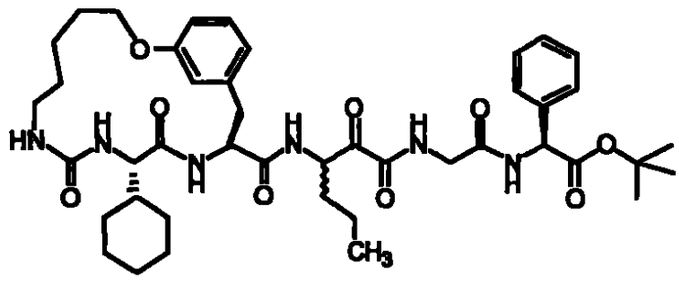
17



18

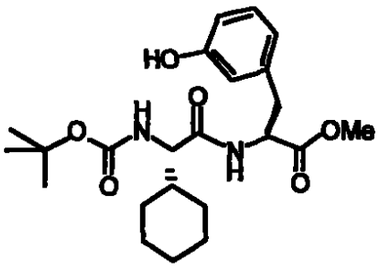
The desired compound 18 was prepared quantitatively from 17 according to the method of Example 12, Step A

Example 19. Preparation of Compound of Formula 19.

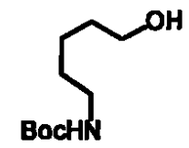


19

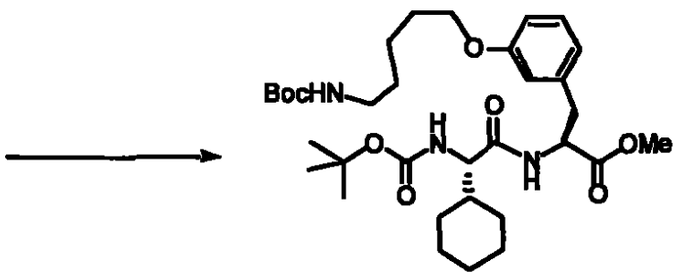
Step A.



1g



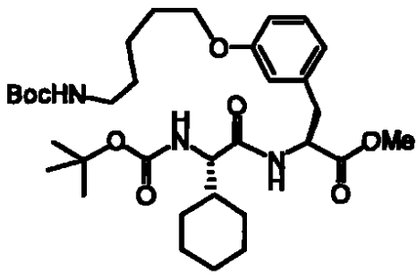
19a



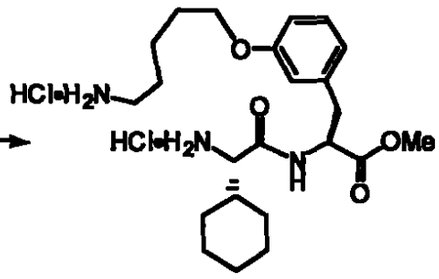
19b

The desired compound **19b** was prepared in 64 % yield from **1g** and **19a** according to the method of Example 11, Step C

Step B.



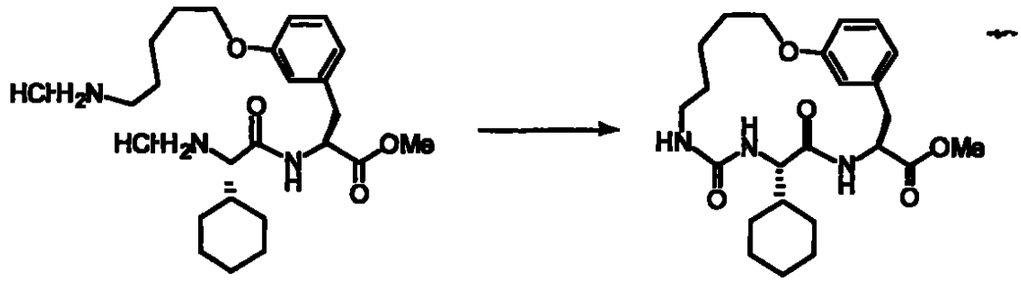
19b



19c

The desired compound **19c** was prepared from **19b** according to the method of Example 1, Step C It was used in the next reaction without further purification

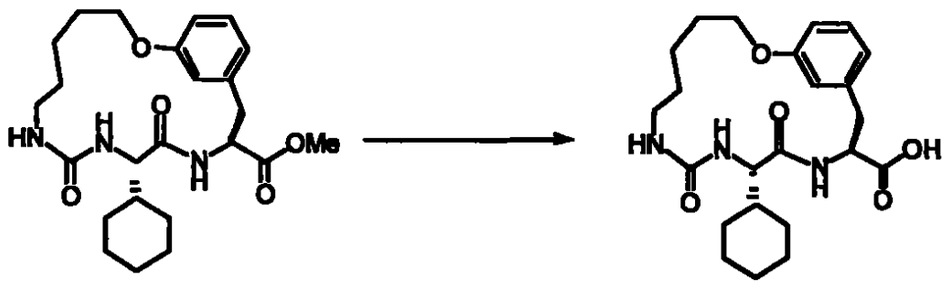
Step C.



19c 19d

To a suspension of di-amine salt **19c** (75 mg, 1 52 mmol) and carbonyl diimidazole (260 mg, 1 60 mmol) in acetonitnle (400 mL) at rt was added triethylamine (0 26 mL, 1 85 mmol) The mixture was stirred for 3 days Solvent was removed *in vacuo* The residue dissolved in EtOAc/THF (100/50 mL) and the solution was washed with 5% H_3PO_4, dried with magnesium sulfate, filtered, concentrated *in vacuo* Flash chromatography (2 to 10 % MeOH-CH_2Cl_2) afforded **19d** (290 mg, 0 651 mmol, 43 %) as a white solid

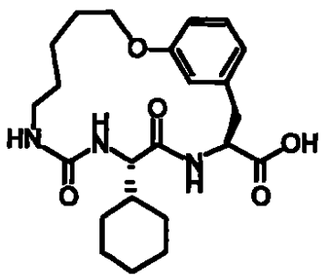
Step D.



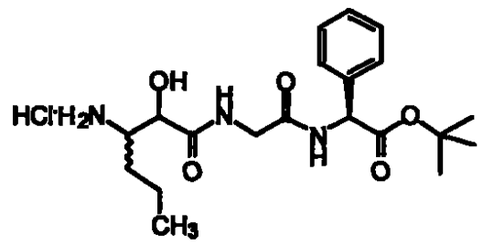
19d 19e

The desired compound **19e** was prepared in 97 % yield from **19d** according to the method of Example 11, Step D It was used in the next reaction without further purification

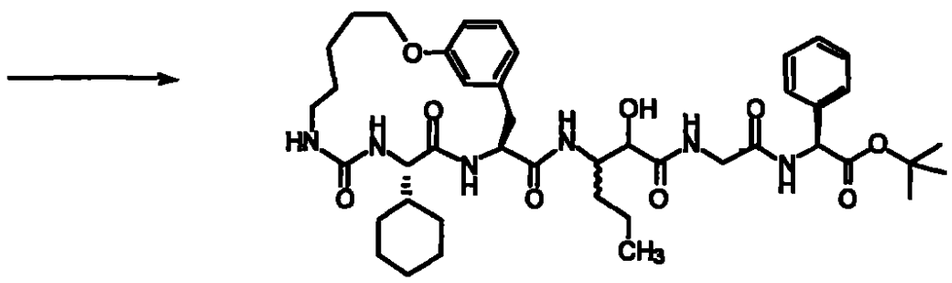
Step E



19e



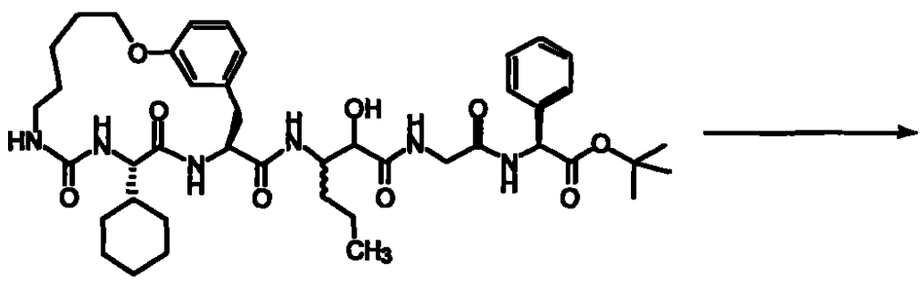
B



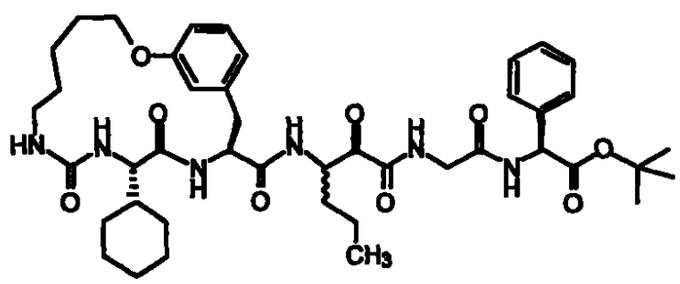
19f

The desired compound **19f** was prepared in 66 % yield from **19e** and **B** according to the method of Example 11, Step E

Step F



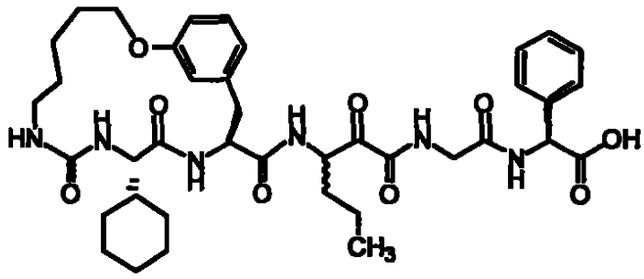
19f



19

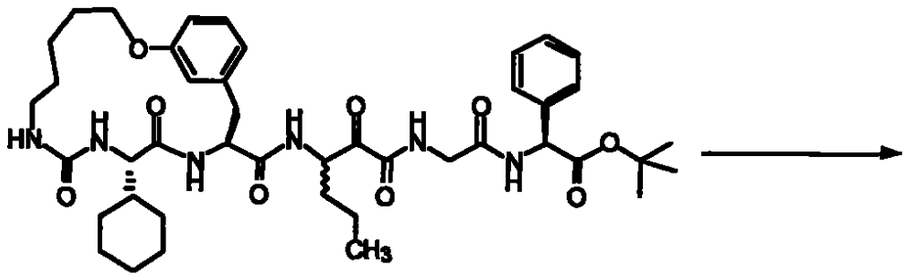
The desired compound **19** was prepared in 66 % yield from **19f** according to the method of Example 11, Step F Two products were partially separated by flash chromatography (0 to 5 % MeOH-CH_2Cl_2)

Example 20 Preparation of Compound of Formula 20.

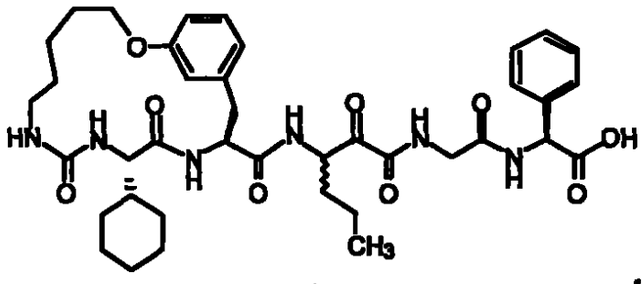


20

Step A



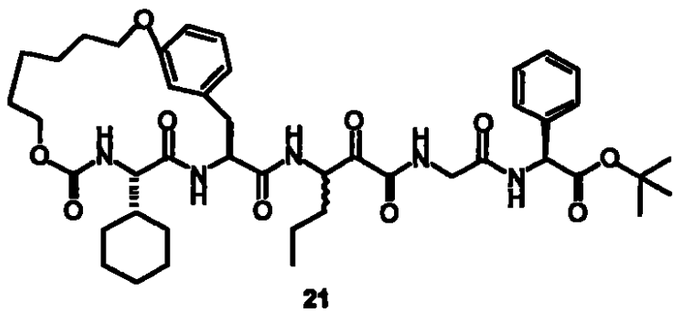
19



20

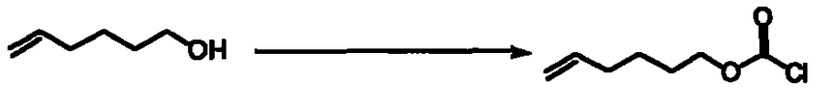
The desired compound **20** was prepared from **19** according to the method of Example 12, Step A

Example 21. Preparation of Compound of Formula 21.



21

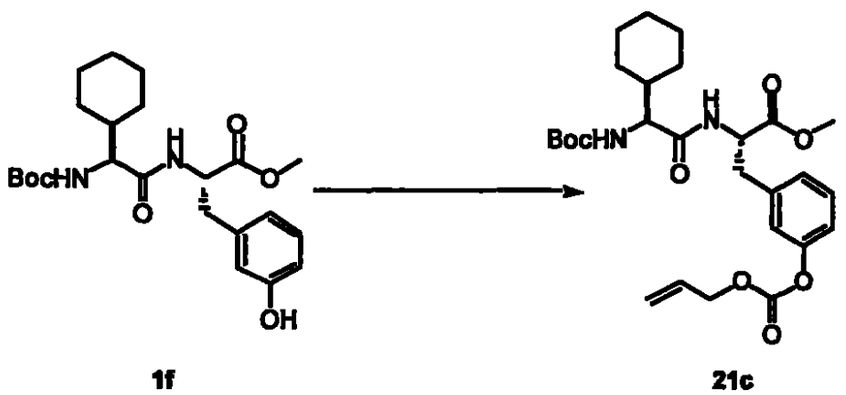
Step A



21a 21b

A solution of 5-hexene-1-ol **21a** (10g, 50 mmol) in diethylether (100 mL) was treated with triethylamine (10 1g, 100 mmol, 2 0 equiv) and cooled to 0° C A solution of phosgene in benzene (20%, 100 mL, 20g, 200 mmol, 4 0 equiv) was added dropwise and the reaction mixture was stirred at rt for 12h The triethylamine hydrochloride separating out was filtered and the filtrate was concentrated *in vacuo* The residue **21b** was used directly for further studies without purification

Step B

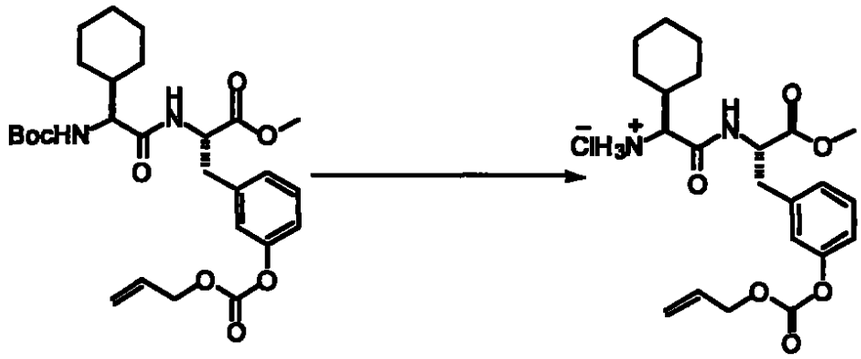


1f 21c

A solution of **1f** (8 0g, 18 43 mmol) in CH_2Cl_2 (100 mL) was treated with triethylamine (2 43 g, 24 0 mmol, 1 3 equiv) The reaction mixture was cooled to -78° C and allychloroformate (2 9g, 24 mmol, 1 3 equiv) was added dropwise The

reaction mixture was stirred at rt for 12 h and the reaction mixture was diluted with H_2O (100 mL) and aq HCl (2M, 200 mL) The aq layer was extracted with EtOAc (3x200 mL) The combined EtOAc layer were extracted with brine, dried (Na_2SO_4), filtered concentrate *in vacuo* and the residue **21c** was directly used for Boc deprotection ^1H NMR ($CHCl_3$, 300 MHz, δ, ppm) 7 29 (t, 1 H, *J*= 6 0 Hz), 7 06-6 98 (m, 3 H), 6 41 (d, 1 H, *J*=5 4 Hz), 6 05-5 95 (m, 1H), 5 42 (dd, 1H, *J*=1 2, 13 2), 5 31 (dd, 1 H, *J*=1 2, 13 2), 5 10 (d, 1 H, *J*=6 6 Hz), 4 91-4 87 (q, 1 H), 4 74 (d, 1H, *J*=4 5 Hz), 3 95-3 92 (m, 1 H), 3 70 (s, 3 H), 3 12 (d, 1 H *J*=4 2 Hz),1 81-1 51 (m, 6H), 1 43 (s, 9 H), 1 21-0 91 (m, 6 H)

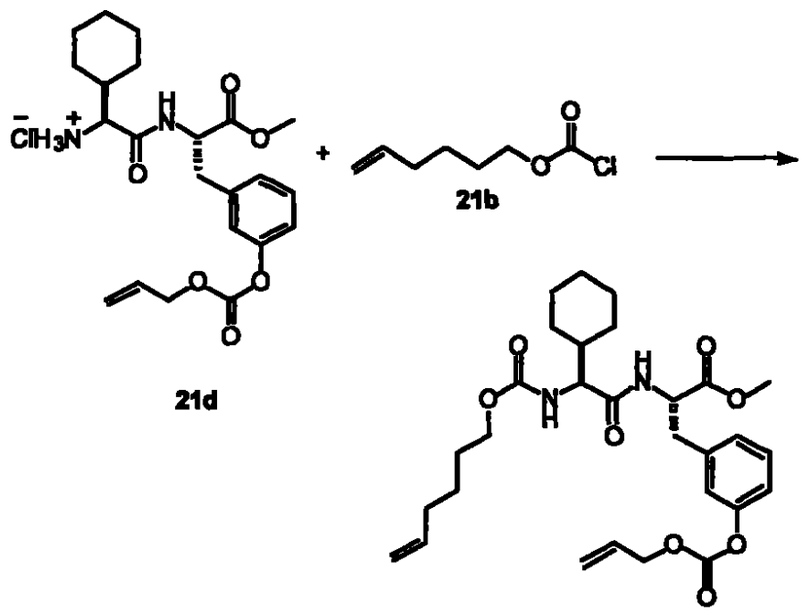
Step C



21c **21d**

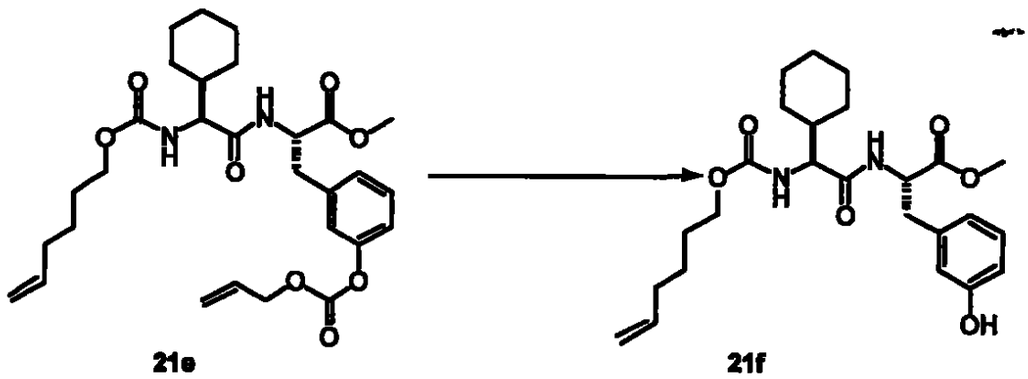
A solution of **21c** (1 5 g) in HCl (4M in dioxane, 100 mL) was stirred at rt for 3 h The disappearance of the starting material was followed by TLC and once the starting material disappeared the reaction mixture was concentrated *in vacuo* and the residue **21d** was dried in pump It was used for coupling without further purification

Step D



A solution of the aminehydrochloride **21d** (4 0g, 8 9 mmol) in CH_2Cl_2 (50 mL) was treated with triethylamine (2 73 g, 27 mmol, 3 0 equiv, 3 8 mL) and cooled to -78° C A solution of chloroformate **21b** (2 3g, 13 3 mmol, 1 5 equiv) in CH_2Cl_2 (30 mL) was added dropwise The reaction mixture was stirred overnight at rt and diluted with aq HCl (1M, 150 mL) The aqueous layer was extracted with EtOAc (3x100 mL) The combined ethyl acetate layers were extracted with H_2O (100 mL), brine (100 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 3 7) to yield **21e** as colorless solid (5g, 80%)

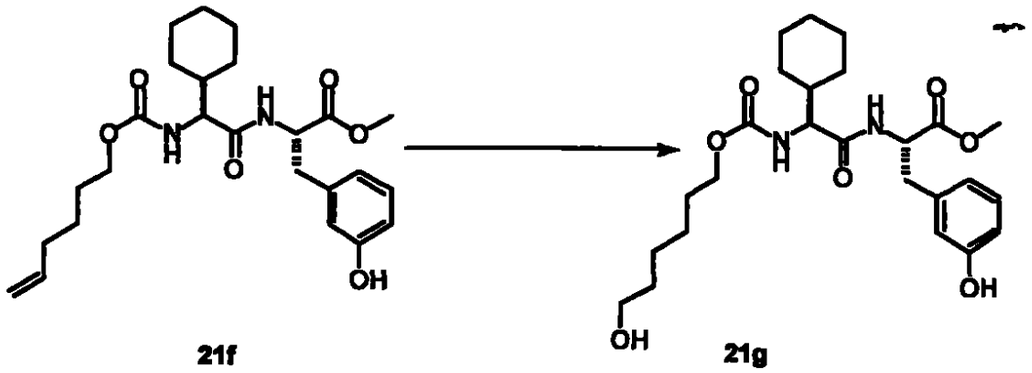
Step E



21e **21f**

A solution of alloc-protected compound **21e** (4 0 g, 7 2 mmol) in dry THF (60 0 mL) was treated with dimedione (2 01g, 14 4 mmol, 2 0 equiv), $Pd(PPh_3)_4$ (830 mg, 0 71 mmol, 10 mol%) at 0 °C and stirred at rt for 1h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes, 3 7) to yield a deprotected alcohol **21f** as a colorless solid (2 7g, 79%) ^1H NMR ($CDCl_3$, 300 MHz, δ ppm) 7 44 (bs, 1 H), 7 09 (s, 1 H, *J*=6 0 Hz), 6 75-6 72 (m, 2 H), 6 58-6 48 (m, 2 H), 5 81-5 71 (m, 1 H), 5 55 (d, 1 H, *J*=7 2 Hz, 4 98 (ddd, 1 H, *J*=1 5, 1 2, 9 Hz), 4 92 (dd, 1H, *J*=4 5, 0 9 Hz), 4 88-4 83 (m, 1 H), 4 12-3 97 (m, 1 H), 3 71 (s, 3 H), 3 09-2 98 (m, 2 H), 2 08-2 03 (m, 2 H), 1 722-1 40 (m, 10 H), 1.24-0 94(m, 5 H), ^{13}C NMR (100 MHz, δ) 171 6, 157 3, 156 6, 138 3, 136 6, 129 8, 123 5, 120 6, 117 0,114 9, 114 6, 65 7, 60 1, 53 2, 52 5, 40 4, 37 1, 33 3, 29 6, 28 6, 28 3, 26 0, 25 9, 25 1, CHN calcd for $C_{25}H_{36}N_2O_6$ C=65 20% H=7 88% N=6 08%, Found C=64 90% H=7 98% N= 6 01%

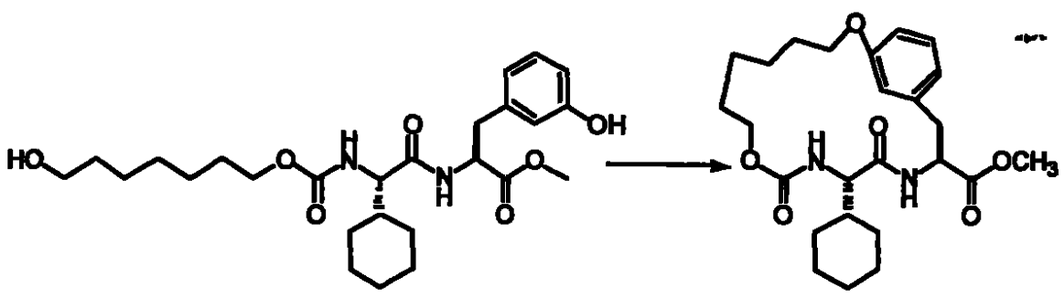
Step F



21f 21g

A solution of alkene **21f** (650 mg, 1 4 mmol) in anhydrous THF (5 2 mL) was cooled to 0°C and treated with BH_3•THF (1M soln in THF, 4 2 mL, 4 2 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 2 h and EtOH (2 0 mL) was added carefully with the evolution of hydrogen gas After the H_2 evolution was complete the reaction mixture was treated with pH 7 buffer and treated with aq H_2O_2 (30%, 5 0 mL) at 0 °C The ice bath was removed and the mixture was stirred at rt for 3-4 h The reaction mixture was extracted with EtOAc (3x100 mL) The combined organic layers were extracted with H_2O, brine, dried ($MgSO_4$), filtered, concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 3 7) to yield hydroborated product as a colorless solid **21g** (400 mg, 60%) $[\alpha]_D$ 86 4 (*c* 0 3 $CHCl_3$, 25 °C), ^1H NMR ($CDCl_3$, 400 MHz, δ) 7 26 (s, 1 H), 7 08 (t, 1 H, *J*=5 7Hz), 6 83 (d, 1 H, *J*=6 0 Hz), 6 71 (dd, 1 H, *J*=1 2, 4 5 Hz), 6 57 (bs, 1 H), 6 54 (d, 1 H, *J*=5 7 Hz), 5 68 (d, 1 H, *J*=6 9 Hz), 4 85 (dq, 1 H, *J*=4 2, 1 8 Hz), 4 05-3 97 (m, 3 H), 3 69 (s, 3 H), 3 60 (t, 2 H, *J*=4 8 Hz), 3 08-2 97 (m, 2 H), 1 77-1 53 (m, 10H), 1 42 1 25 (m, 4 H), 1 24-0 92 (m, 5 H), ^{13}C NMR ($CDCl_3$, 100 MHz, δ) 171 8, 171 8, 157 6, 156 9, 136 9, 130 0, 120 8, 117 0, 114 8, 65 7, 62 7, 60 3, 53 3, 52 7, 40 5, 37 4, 32 5, 29 7, 29 0, 28 8, 26 2, 26 0, 25 6, 25 4 MS (FAB, NBA/DMSO, *m/z, relative intensity)* 479 ($[M+1]^+$,100), 296 (40), 196 (25), 156 (25), 136 (25), 112 (20) HRMS calcd for $C_{25}H_{39}N_2O_7$ $(M+1)^+$ 479 2760, found 479 2757

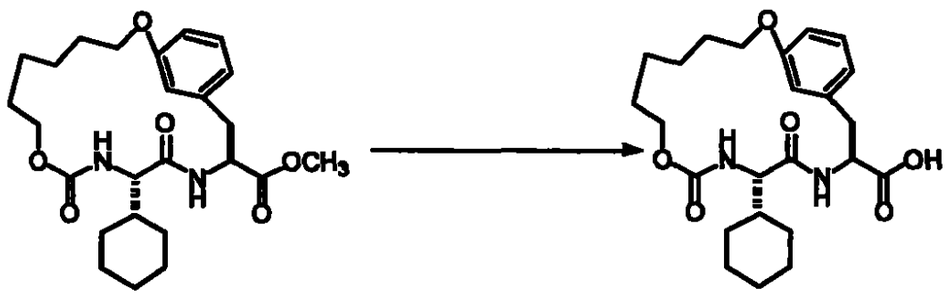
Step G



21g **21h**

A solution of PPh_3 (385 mg, 1,47 mmol, 1 75 equiv)) in CH_2Cl_2 (10 mL) was treated with the compound **21g** (400 mg, 0 84 mmol) and cooled to 0° C A solution of DEAD (220 mg, 1 26 mmol, 1 5 equiv) in CH_2Cl_2 (10 mL) was added dropwise and stirred at rt for 3 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 9) to yield cyclic product **21h** as a colorless solid (110 mg, 25%)

Step H

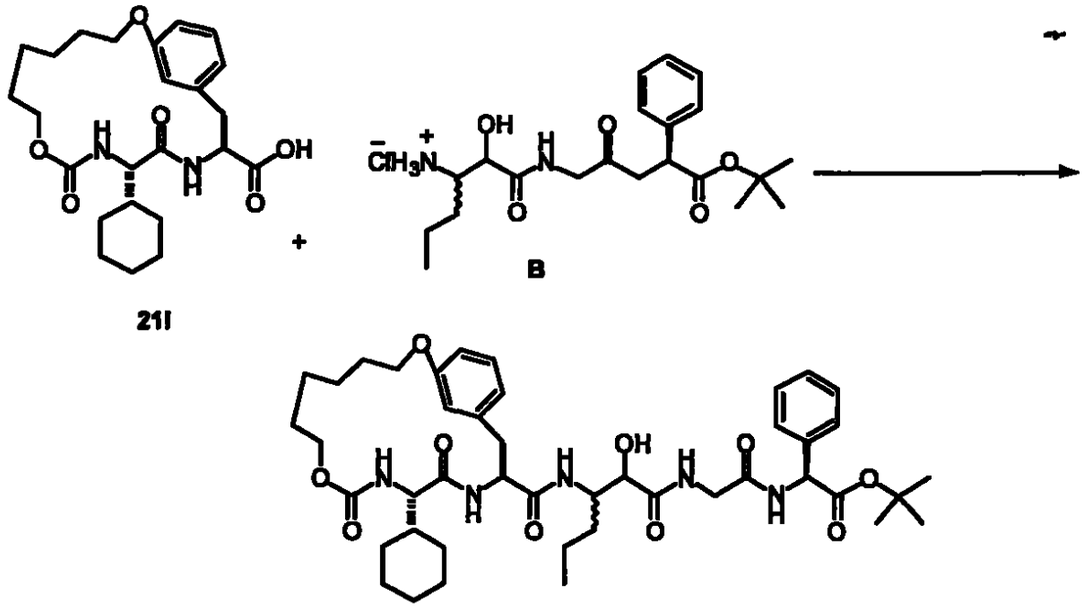


21h **21i**

A solution of cyclic carbamate **21h** (200mg, 0 44 mmol) in dioxane (30 mL), CH_3OH (20 mL) and CH_2Cl_2 (20 mL) was treated with $LiOH \cdot H_2O$ (80 mg, 2 0 mmol, 4 5 equiv) and stirred at rt for 4 h The reaction was concentrated *in vacuo* and diluted with HCl (4M soln in dioxane,10 mL) The water was removed by lyophilizer to yield the crystalline acid **21i** directly used for coupling

76

Step I

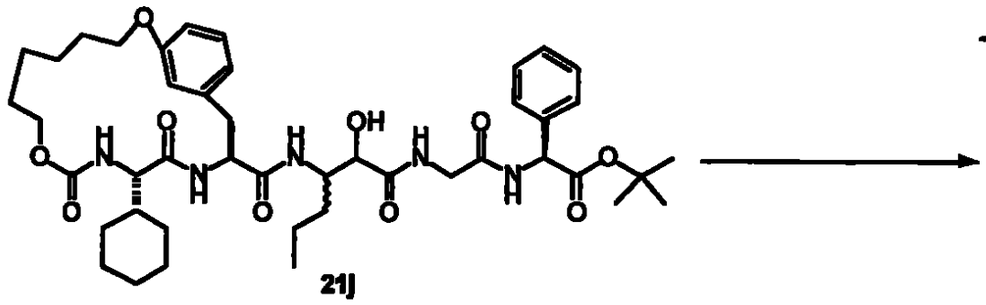


21I B

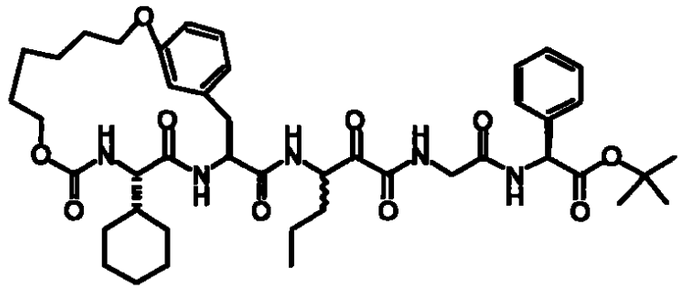
21J

A solution of the hydrolyzed acid **21I** (210 mg, 0 47 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5 0 mL) was treated with HOOBt (125 mg, 0 70 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (258 mg, 2 0 mmol, 4 0 equiv, 369 µL) was added To this mixture was added EDCI (134 mg, 0 70 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 0 5 h and treated with the amine hydrochloride **B** (253 mg, 0 58 mmol, 1 25 equiv) The reaction mixture was stored in freezer for 24 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with aq HCl (2M, 30 mL) and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq HCl (2M, 30 mL), aq NaOH (1M) brine (2x50 mL), dried ($MgSO_4$) and concentrated *in vacuo* The residue **21J** (220 mg) was oxidized without further purification

Step J



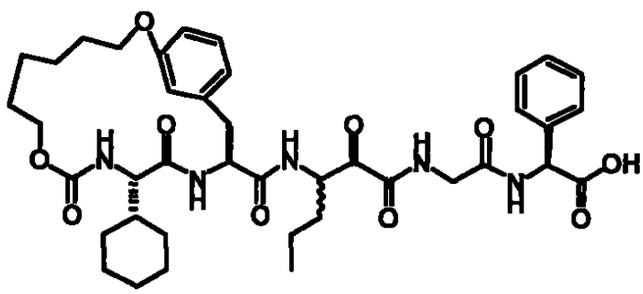
21J



21

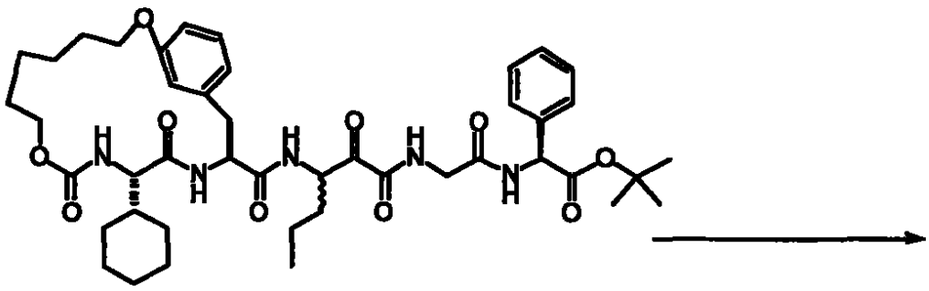
A solution of alcohol **21J** (220 mg, 0 26 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (200 mg, 0 47 mmol, 1 8 equiv) The reaction mixture was stirred at rt for 1 h and diluted with aq $NaHCO_3$ (15 mL) and aq $Na_2S_2O_3$ (15 mL) The reaction mixture was stirred at rt for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were extracted with aq Na_2CO_3, dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH (2M NH_3)/CH_2Cl_2 1 20) to yield ketoamide **21** (60 mg, 27%) of a colorless solid

Example 22: Preparation of Compound of Formula 22.

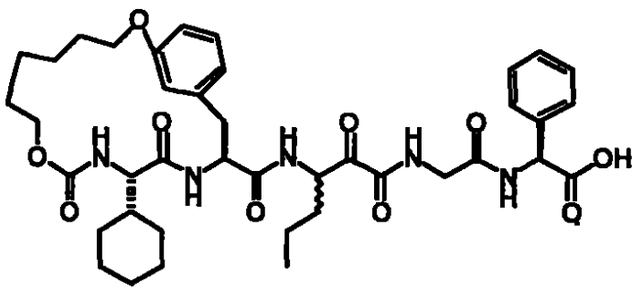


22

Step A



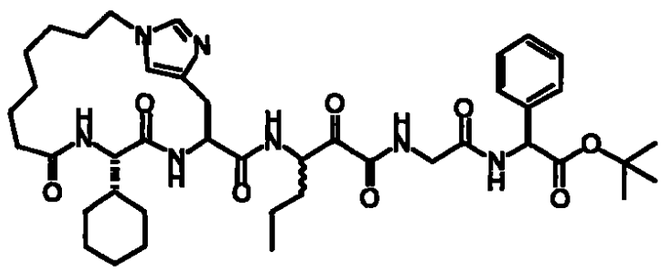
21



22

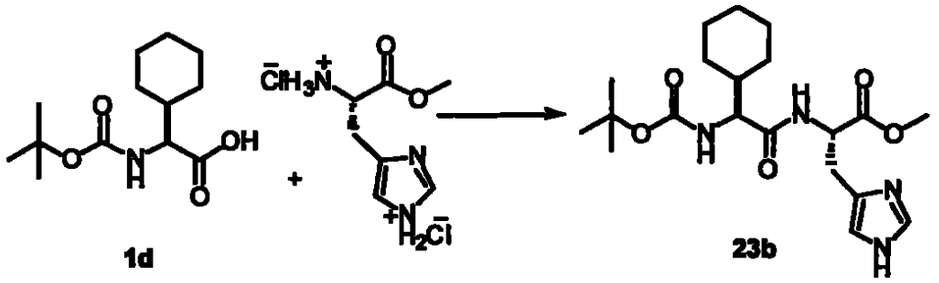
A solution of *tert*-butyl ester **21** (50 mg, 0 059 mmol) in dry CH_2Cl_2 (2 0 mL) was treated with TFA (2 0 mL) and stirred at rt. for 4 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in heptanes/CH_2Cl_2 and concentrated *in vacuo* several times to yield a fine tan solid **22** (47 mg), which was dried *in vacuo*

Example 23. Preparation of Compound of Formula 23.



23

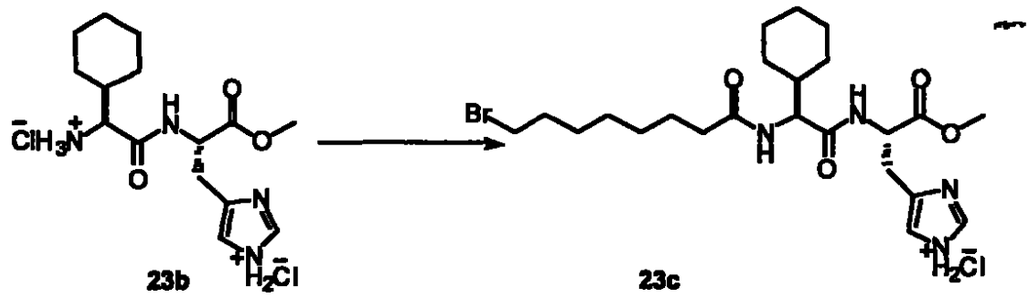
Step A



1d 23a 23b

A solution of acid **1d** (255mg, 1 0 mmol) in DMF (2 0 mL) was treated with HOBt (202 mg, 1 5 equiv) and Hünigs base (517 mg, 4 0 mmol, 4 0 equiv, 738 µL) The reaction mixture was cooled to 0° C and treated with DCC (258 mg, 1 25 mmol, 1 25 equiv) After stirring the mixture for 1h Histidine-OCH_3•2HCl **23a** (242 0 mg, 1 0 mmol) was added and stirred at rt overnight The reaction mixture was concentrated *in vacuo* and extracted in EtOAc (3x50 mL) and aq $NaHCO_3$ (50 mL) The combined organic layer was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 19) to yield dipeptide **23b** as a colorless solid (380 mg, 93%) ^1H NMR (d_6-DMSO, 400 MHz, δ, ppm) 8 17 (d, 1 H, *J*=7 2 Hz), 7 48 (s, 1 H), 6 77 (s, 1 H) 6 57 (bs, 1 H), 5 54 (d, 1H, *J*=7 6 Hz), 4 47 (q, 1 H, *J*=7 2 Hz), 3 79 (t, 1 H, *J*=8 4 Hz), 3 55 (s, 3 H), 3 36-3 20 (m,2 H), 2 94-2 82 (m, 2 H), 1 70-1 47 (bm, 6H), 1 35 (s, 9 H), 1 46-0 85 (m, 5 H) ^{13}C NMR (d_6-DMSO, 100 MHz, δ ppm) 172 5, 171 9, 157 3, 155 9, 135 4, 78 6, 59 5, 52 9, 52 3, 34 1, 29 6, 28 9, 28 6, 26 5, 26 3, 26 0, 25 2 FAB MS (NBA-G/TG-DMSO, *m/z relative intensity*) 409 [$(M+1)^+$, 100], 353 (10), 170 (20), HRMS calcd for $C_{20}H_{33}N_4O_6$ 409 2451 found 409 2466, CHN Calcd for $C_{20}H_{32}N_4O_5$ C=58 81% H=7 90%, N=13 72%, Found C=58 70% H=7.78% N=13 43%

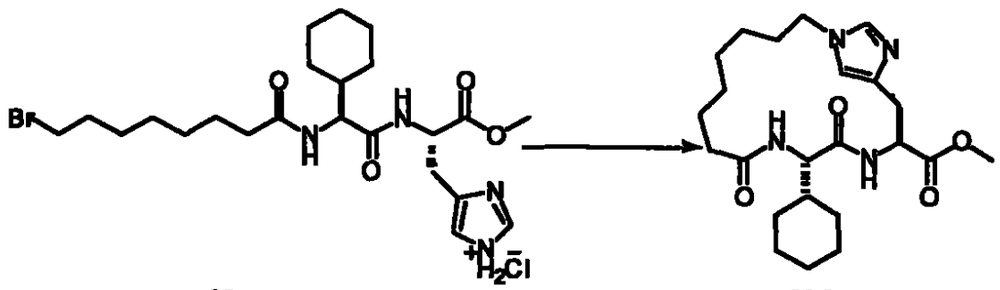
Step B.



23b 23c

A solution of ω-bromoheptenoic acid (223 mg, 1 0 mmol) in DMF (3 0 mL) was treated with deprotected amine hydrochloride **23b** (380 mg, 1 0 mmol, 1 0 equiv) and Hünigs base (387 mg, 3 0 mmol, 3 0 equiv) was added The reaction mixture was treated with PyBroP (465 mg, 1 0 mmol) and stirred at rt for 3 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2, 1 19) to yield a colorless solid (220 mg, 50 %) MS (FAB) 515 2 [$(M+1)^+$, 100], 513 2 [$(M+1)^+$, 95)], 469 (60), 433 (20), 170 (40) HRMS calcd for $C_{23}H_{38}BrN_4O_4$ 513 2076 found 513 2073

Step C

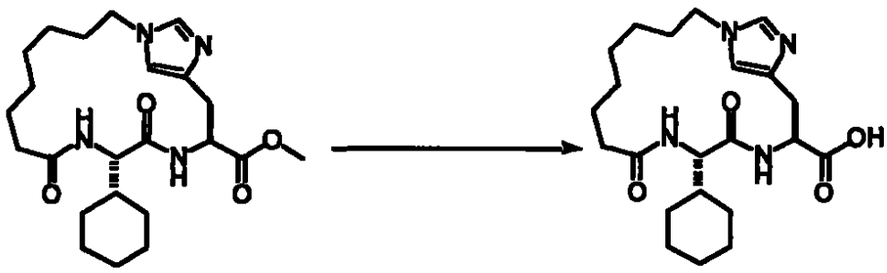


23c 23d

A solution of bromo-compound **23c** (100 mg, 0 23 mmol), in 2-butanone (4 0 mL) was treated with Na_2CO_3 (31 0 mg, 0 29 mmol, 1 25 equiv) and with LiI (50 mg, 0 37 mmol 1 3 equiv) and heated at reflux for 24 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with water The residue was extracted with CH_2Cl_2 (3x30 mL) The combined organic layer was dried(Na_2SO_4) and purified by chromatography (SiO_2, CH_3OH CH_2Cl_2 1 19) to yield the cyclized compound **23d** (25 mg, 31%), R_f 0 68 (2M NH_3 in CH_3OH CH_2Cl_2

1 19) ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm) 8 17 (d, 1 H, *J*=8 8 Hz) 7 33 (s, 1 H), 6 48 (d, 1 H, *J*=8 4 Hz), 4 90-4 85 (m, 1H), 4 26 (t, 1 H, *J*=8 0 Hz), 3 82-3 74 (m, 2 H), 3 69 (s, 3H), 3 16- 3 11 (m, 2 H) 2 91-2 84 (m, 1 H), 2 30-2 01 (m, 2 H), 1 65-1 59 (m, 11 H), 1 18-0 96 (m, 11H) ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 172 8, 172 4, 171 9, 138 2, 136 8, 57 6, 52 5, 51 7, 46 6, 41 6, 36 0, 30 9, 29 5, 28 8, 27 3, 26 7, 26 4, 26 3, 26 2, 25 2, 24 8 MS (Electron spray, *m/z relative intensity*) 433 1 [$(M+1)^+$,100], HRMS Calcd for $C_{23}H_{37}N_4O_4$ 433 2815 found 433.2822

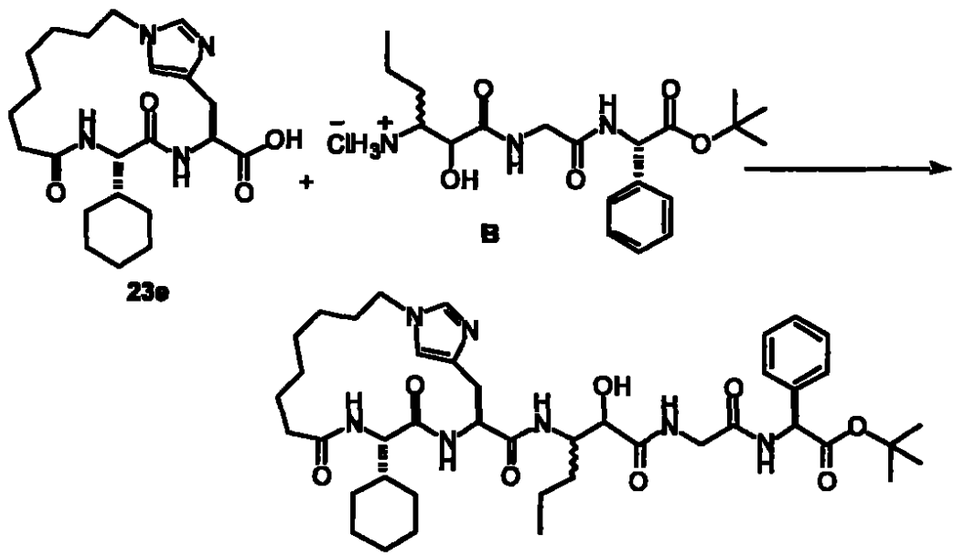
Step D



23d **23e**

A solution of methyl ester **23d** (200 mg, 0 46 mmol) in CH_3OH (5 0 mL) H_2O (0 5 mL) was treated with $LiOH{\bullet}H_2O$ (30 mg, 0 75 mmol, 1 6 equiv) The reaction mixture was stirred at rt for 15 h and concentrated *in vacuo* and dried in pump to yield hydrolyzed compound **23e** which was used for coupling directly

Step E

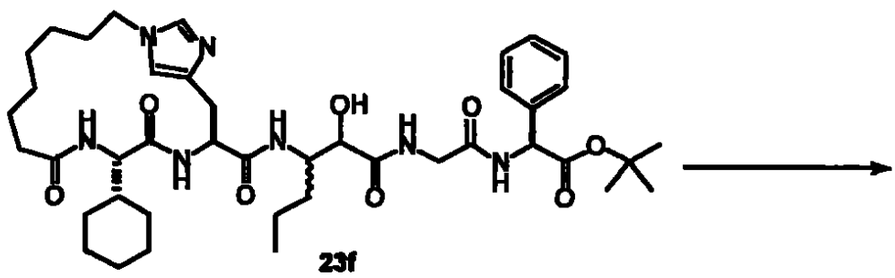


23e B

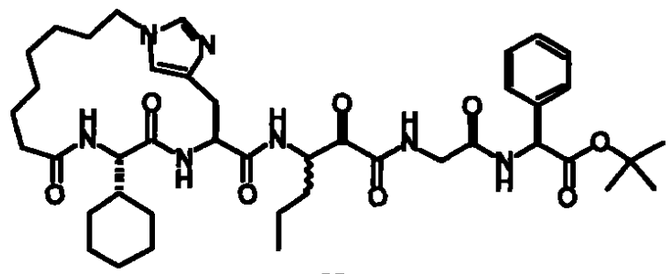
23f

A solution of the acid **23e** in CH_2Cl_2 (3 0 mL), DMF (5 0 mL) was treated with HOOBt (115 mg, 0 70 mmol, 1 50 equiv) and EDCI (113 mg, 0 60 mmol, 1 25 equiv) The reaction mixture was then treated with Et_3N (190 mg, 1 88 mmol, 271µL, 4 0 equiv) and amine hydrochloride **B** (201 mg, 0 5 mmol, 1 1 equiv) The reaction mixture was stirred at rt for 13 h and diluted with H_2O The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and the combined organic layers were extracted with aq NaOH (1M, 50 mL) and dried (Na_2SO_4) The dried organic layer was filtered and concentrated *in vacuo* to yield a colorless residue **23f** (442 mg) which was dried *in vacuo* and directly used for further oxidation MS (Electron spray, *m/z relative intensity*) 794 [$(M+1)^+$, 100]

Step F



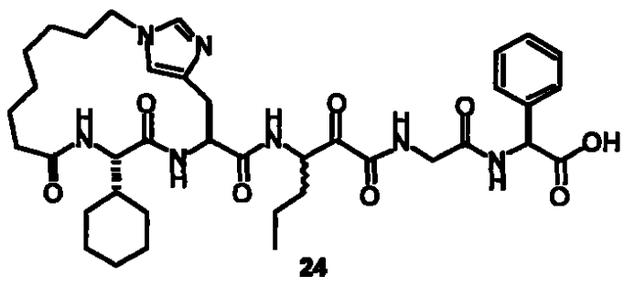
23f



23

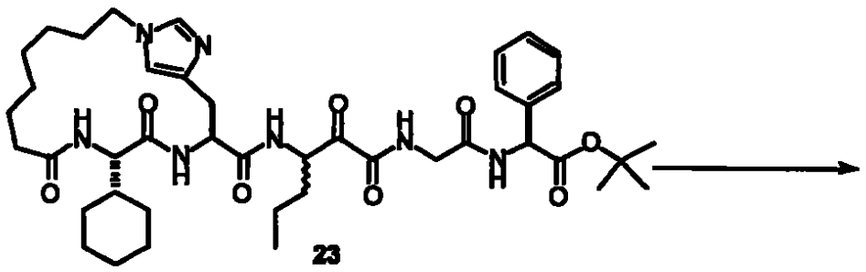
A solution of hydroxy-amide **23f** (50 mg, 0 064 mmol) in CH_2Cl_2 (3 0 mL) was treated with Dess Martin reagent (53 mg, 0 13 mmol, 2 0 equiv) and stirred at rt for 3 h The reaction mixture was diluted with aq satd $Na_2S_2O_3$ (20 mL) and stirred at rt for 15 min The aqueous layer was extracted with CH_2Cl_2 (3x30 mL) The organic layer was dried (Na_2SO_4) filtered concentrated *in vacuo* and purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2, 1 15) to yield ketoamide **23** (20mg, 40%), MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*) 824 [$(M+CH_3OH)^+$, 100], 792 [$(M+1)^+$, 60], 447 (20), HRMS calcd for $C_{42}H_{62}N_7O_8$ $(M+1)^+$ 792 4660 found 792 4659

Example 24 Preparation of Compound of Formula 24.

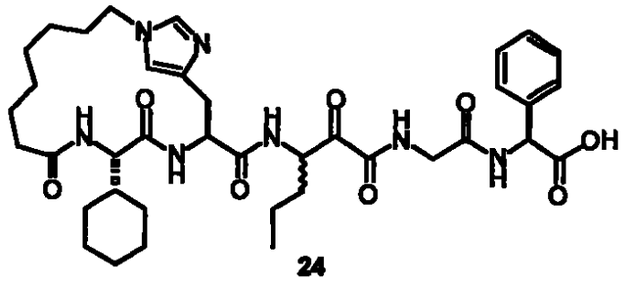


24

Step A



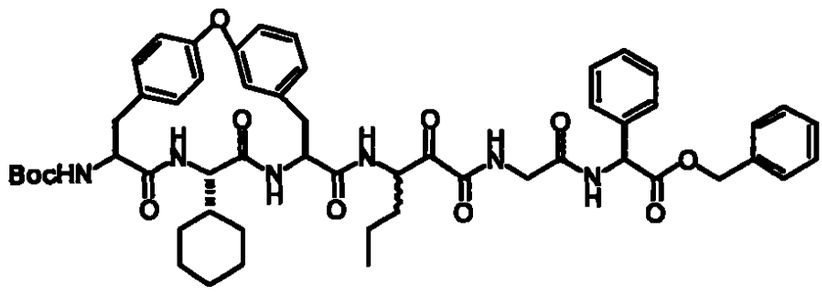
23



24

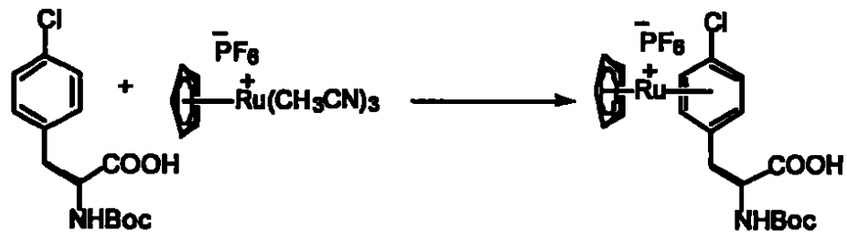
A solution of *tert*-butyl ester **23** (17 mg, 21 5 µmol) in dry CH_2Cl_2 (2 0 mL) was treated with TFA (2 0 mL) and stirred at rt. for 8 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2, 1 19) The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in CH_3OH/heptanes/CH_2Cl_2 and concentrated *in vacuo* several times to yield **24** as fine colorless solid (7 mg) MS (Electron spray, *m/z relative intensity*) 768 [$(M+CH_3OH)^+$, 100], 736 [$(M+1)^+$, 60], 46 (10)

Example 25. Preparation of Compound of Formula 25.



25

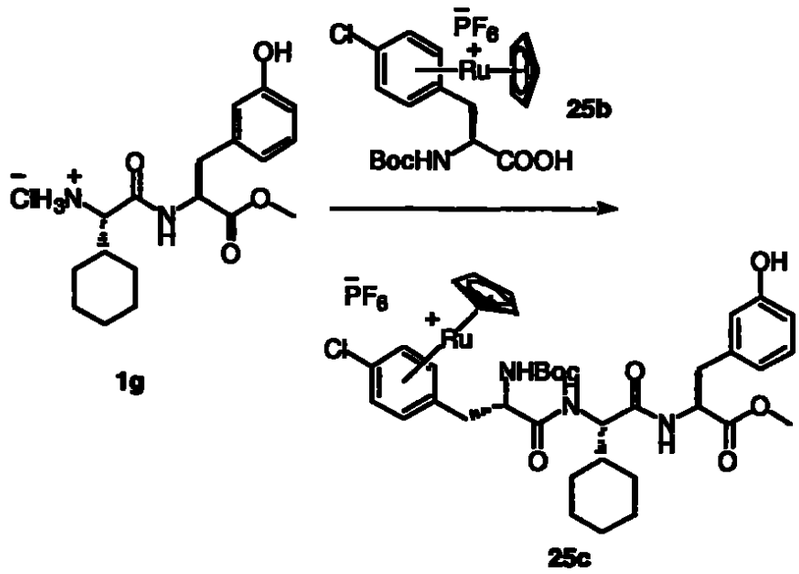
Step A



25a 1b 25b

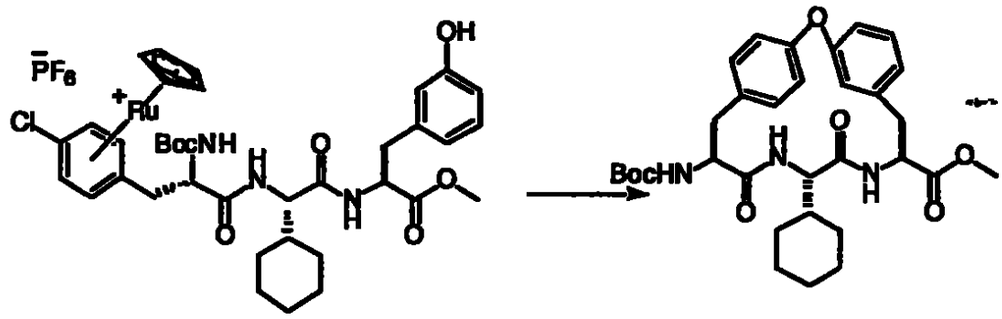
A solution of Boc-4-Chlorophenylalanine **25a** (523 mg, 1 75 mmol) in dichloroethane (37 mL) was treated with $CpRu(CH_3CN)_3$ PF_6 **1b** (760 mg, 1 75 mmol, 1 0 equiv) and heated at reflux for 2 h The reaction mixture was cooled to 0 °C and filtered The filtrate was concentrated *in vacuo* and dissolved in minimum CH_3CN and treated with a large excess of Et_2O The solid separating out was separated and dissolved in CH_2Cl_2/CH_3OH (1 1, 50 mL) and concentrated *in vacuo* to obtain **25b** as a brown foam (640 mg, 69%)

Step B



A solution of carboxylic acid **25b** (2 4 g, 3 80 mmol) in dry DMF (15 mL) was treated with Hünigs base (1 64 g, 12 64 mmol, 4 0 equiv, 2 9 mL) and HOBt (661 mg, 4 38 mmol, 1 5 equiv) The reaction mixture was cooled to 0° C and the treated with EDCI (699 mg, 3 95 mmol, 1 25 equiv) and stirred for 15 min To this reaction mixture was added amine hydrochloride **1g** (1 50 g, 4 00 mmol, 1.2 equiv) and the reaction mixture was stirred at rt for 12 h The DMF was distilled out and the residue was diluted with water (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq $NaHCO_3$ (30 mL), aq HCl (30 mL), brine, dried (Na_2SO_4) filtered concentrated *in vacuo* and the crude product **25c** (2 5 g, 69%) was used for further cyclization without purification

Step C

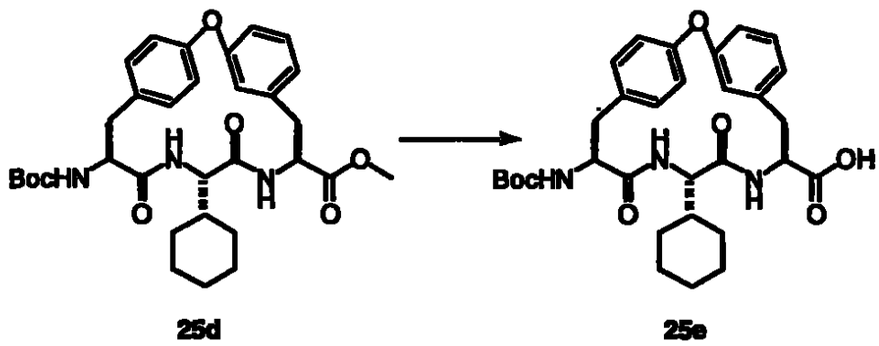


25c 25d

A solution of compound 25c (100 mg 0 11 mmol) in dry DMF (10 mL) was degassed with dry N_2 and treated with Cs_2CO_3 (170 mg, 0 5 mmol, 5 0 equiv) and stirred at rt for 12 h The solvent DMF was distilled off and the residue was diluted with water (35 mL) and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were dried (Na_2SO_4) filtered, concentrated *in vacuo* and dried in vacuum overnight It was used for photolytic removal of Ru without further purification

The cyclized compound from the previous step was dissolved in CH_3CN and photolysed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 2 1) to yield 25d as a tan colored solid (29 mg, 46 %) MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*), 580 [$(M+1)^+$, 80], 524 (100), 418 (40), 462 (30), 452 (20), 313 (60), 253 (20)

Step D



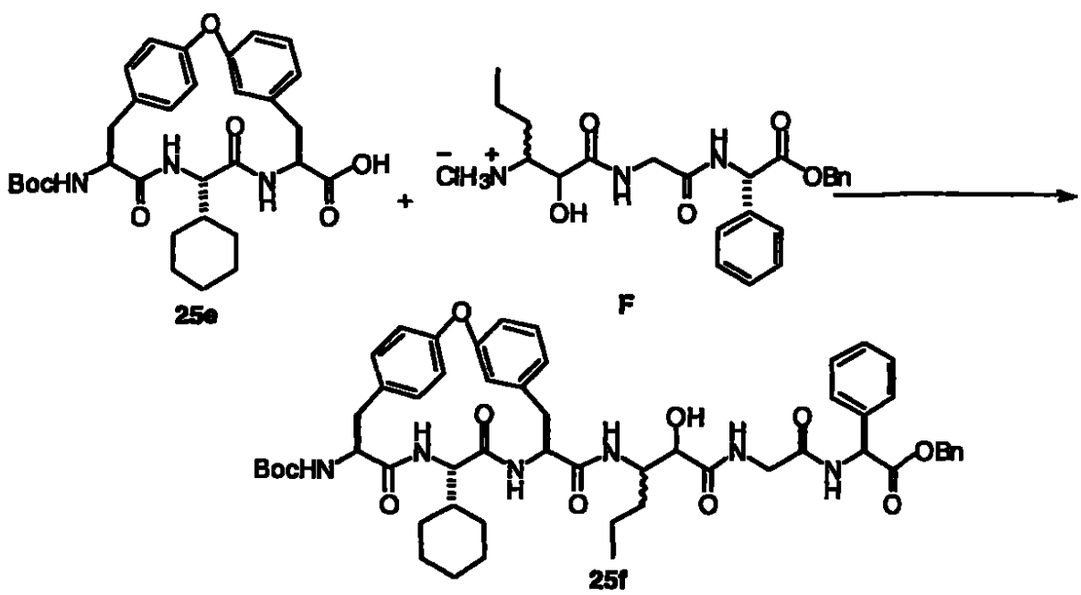
25d 25e

A solution of ester 25d (150 mg, 0.26 mmol) in THF (3 mL), CH_3OH (3.0 mL) and H_2O (3 0 mL) was treated with $LiOH \cdot H_2O$ (18 mg, 0 43 mmol, 1 65

0085 82

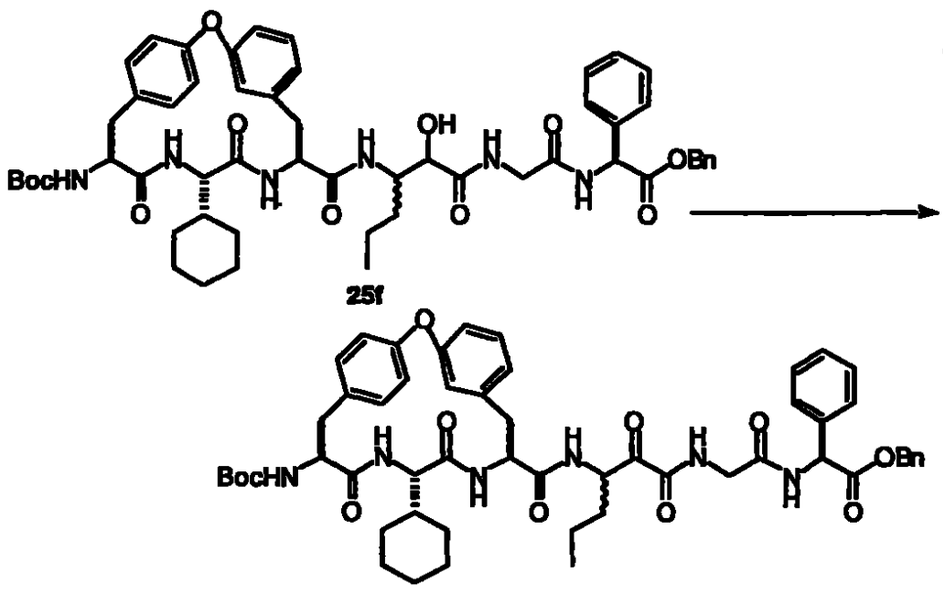
equiv) and stirred at rt for 35 min The reaction mixture was acidified with conc HCl (13 M, 1mL) and extracted in CH_2Cl_2 (3x50 mL) The combined organic layers were dried (Na_2SO_4) filtered and concentrated *in vacuo* to yield acid 25e which was used directly for the coupling without further purification

Step E



A solution of acid **25e** (150 mg, 0 27 mmol) in dry CH_2Cl_2 (2 0 mL), was treated with HOBt (62 mg, 0 40 mmol) and Hünigs base (139 mg, 1 1 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (53 mg, 0 34 mmol, 1 25 equiv) and stirred for 30 min The reaction mixture was treated with amine F (88 mg, 0 29 mmol, 1 22 equiv) and stored in the freezer for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (50 mL) The aqueous layer were extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq HCl (1M, 3x20 mL) aq NaOH (1M, 3x20 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid **25f** (138 mg) which was used for oxidation

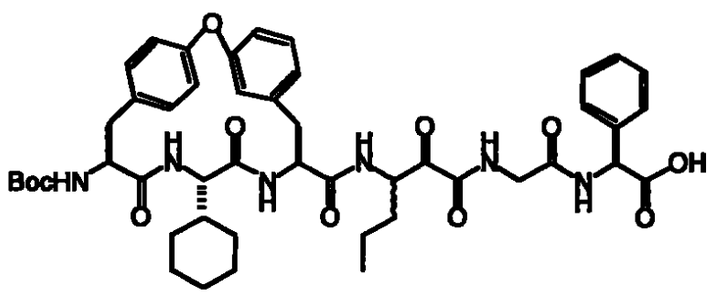
Step F



25

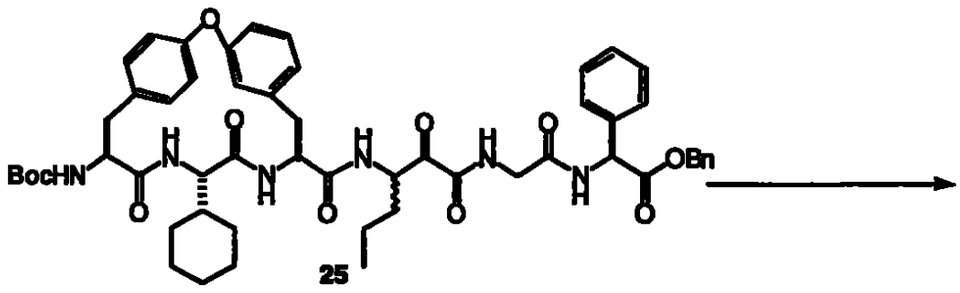
A solution of alcohol **25f** (140 mg, 0 143 mmol) in CH_2Cl_2 THF (1 1, 5 0 mL) was treated with Dess-Martin reagent (121 mg, 0.42 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 2 h and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 32) to yield oxidized product **25** (57mg, 41%) as a colorless solid

Example 26 Preparation of Compound of Formula 26.

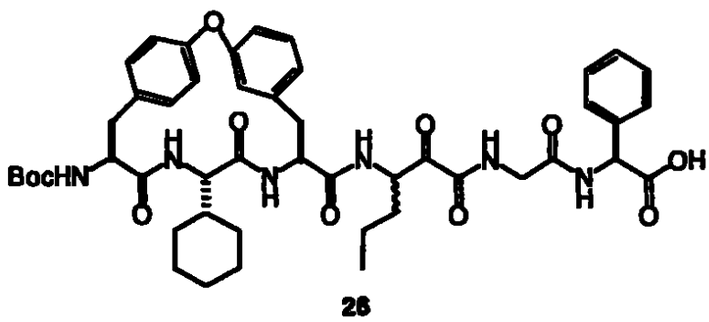


26

Step A



25

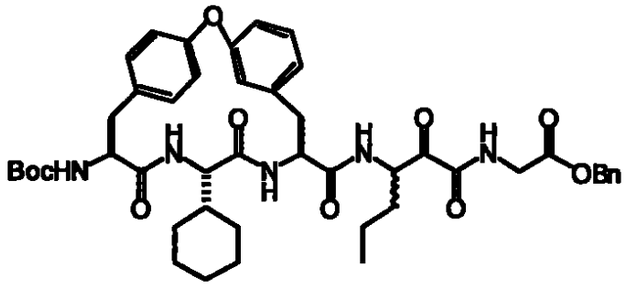


26

A solution of the benzyl ester 25 (30 mg, 38 0 μmol) in CH_3OH/THF (1 1, 4 0 mL) was treated with Pd/C (20 mg, 10%) and H_2 was bubbled through it. A drop of acetic acid was added to accelerate the reduction The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo*. The residue 26 was analyzed without further purification

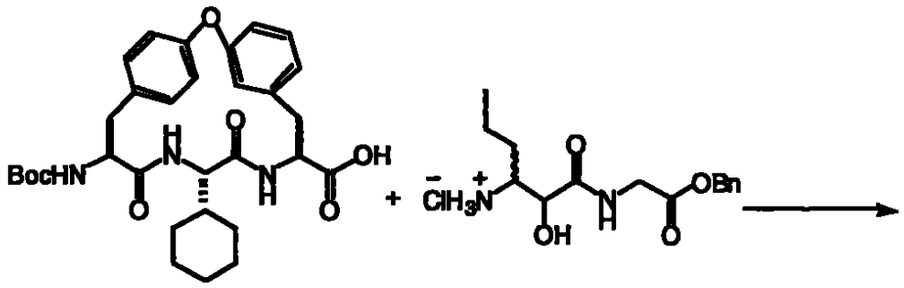
0057 84

Example 27 Preparation of Compound of Formula 27.

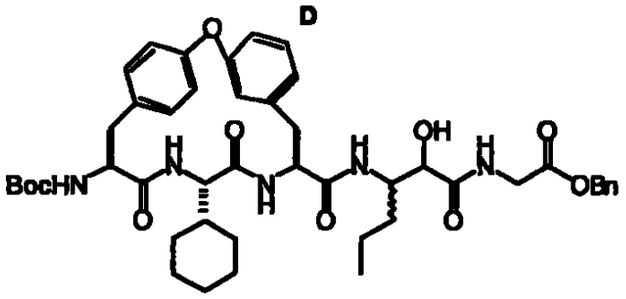


27

Step A



25e D

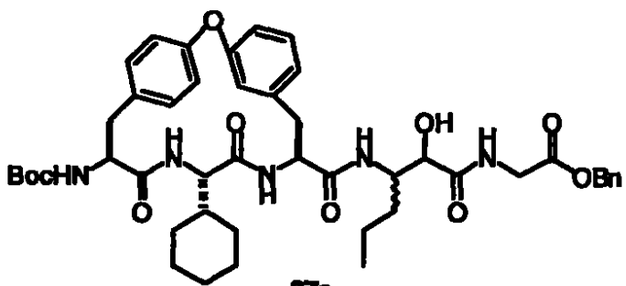


27a

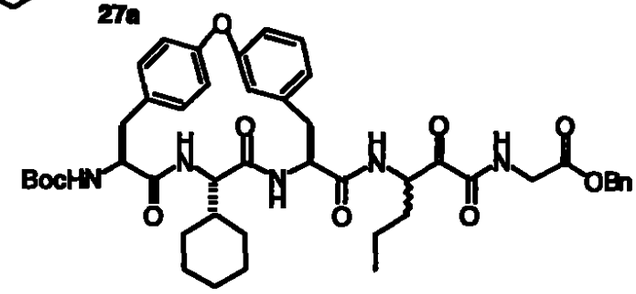
A solution of acid **25e**(100 mg, 0 17 mmol) in dry CH_2Cl_2 was treated with HOOBt (41 mg, 0 26 mmol) and Hünigs base (91 mg, 0 70 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (35 mg, 0.22 mmol, 1 25 equiv) and stirred for 30 min The reaction mixture was treated with amine D (71 mg, 0 22 mmol, 1 22 equiv) and stored in the freezer for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layers was extracted with CH_2Cl_2 (3x30 mL) The organic layers were extracted with aq HCl (1M, 30 mL) aq Na_2CO_3 (1M, 30 mL), dried (Na_2SO_4) filtered

concentrated *in vacuo* to obtain **27a** colorless solid (119 mg) which was used for oxidation MS (FAB), 842 [(M+1), 100], 765 (20), 735 (10), 657 (20), 575 (10), 492 (10), 464 (20), 446 (30) HRMS calcd for $C_{46}H_{60}N_5O_{10}$ $(M+1)^{+}$ 842 4339, found 842 4336

Step B



27a

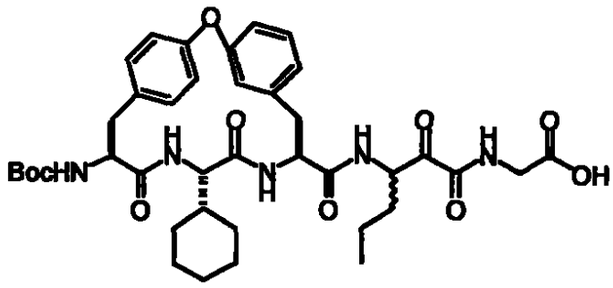


27

A solution of alcohol **27a** (120 mg, 0 143 mmol) in CH_2Cl_2 THF (1 1, 3 0 mL) was treated with Dess-Martin reagent (180 mg, 0.42 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 2 h and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 32) to yield oxidized product **27** as a colorless solid MS (FAB, NBA-G/TG-DMSO, *m/z rel int*), 840 [$(M+1)^{+}$, 50] HRMS calcd for $C_{46}H_{58}N_5O_{10}$ $(M+1)^{+}$ 840 4184, found 840 4199

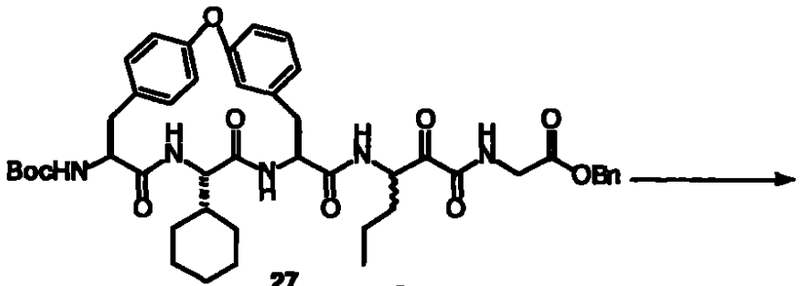
85

Example 28: Preparation of Compound of Formula 28.

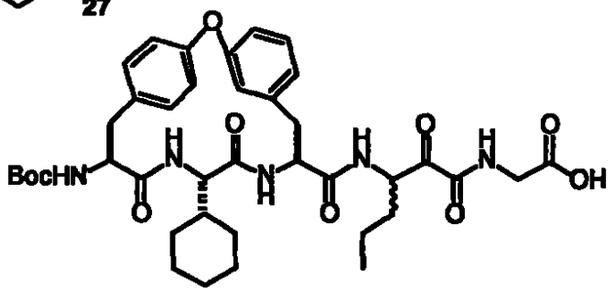


28

Step A:



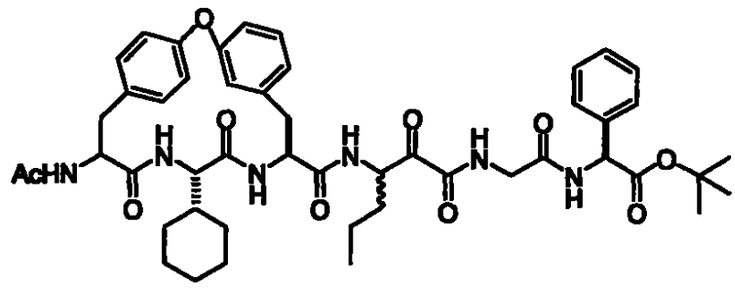
27



28

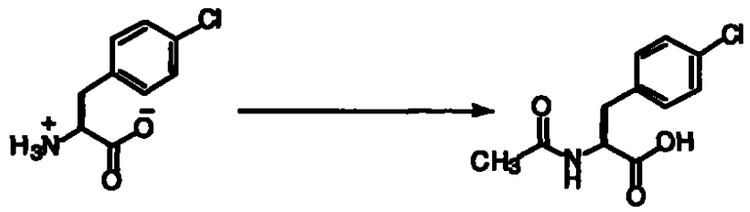
A solution of benzyl ester **27** (40 mg, 47 0 μmol) in CH_3OH/THF (1 1, 6 0 mL) was treated with Pd/C (30 mg, 10%) and H_2 was bubbled through it A drop of acetic acid was added to accelerate the reduction The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo* to yield **28**

Example 29 Preparation of Compound of Formula 29



29

Step A:

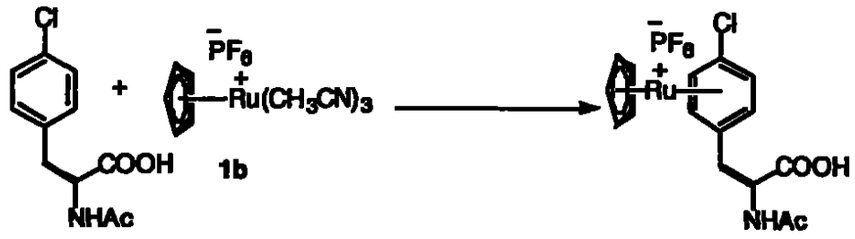


29a **29b**

A solution of 4-chlorophenylalanine **29a** (1 5g, 7 5 mmol) in THF (20 mL) and H_2O (20 mL) was treated with NaOH (900 mg, 22 5 mmol, 3 0 equiv) and cooled to 0°C, A solution of acetyl chloride (707 mg, 9 00 mmol, 1 25 mmol) in THF (10 mL) was added dropwise and the reaction mixture was stirred overnight at rt The reaction mixture was acidified with aq HCl (1M, 10 mL) and extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were dried (Na_2SO_4) filtered concentrated *in vacuo* to yield **29b** which was used in the next step without purification

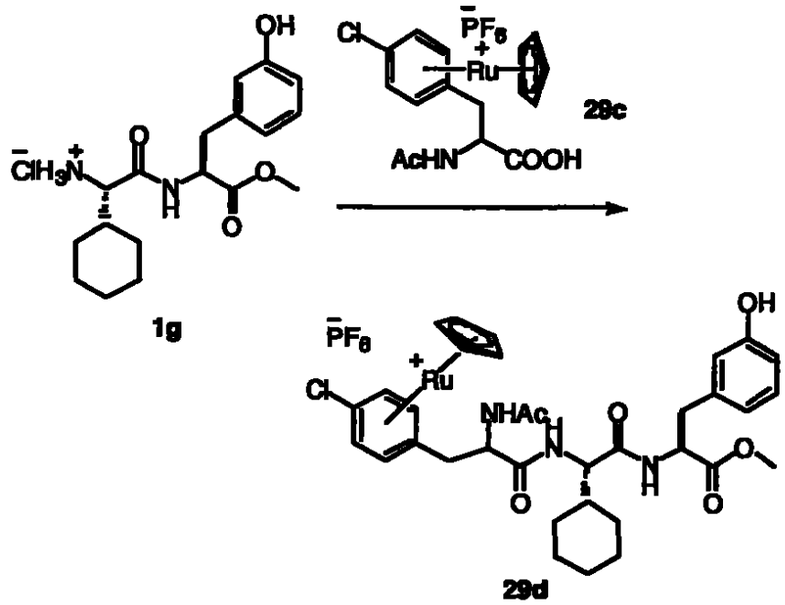
0089 86

Step B



A solution of N-acetyl-4-chlorophenylalanine **29b** (1 39 g, 5 75 mmol) in dichloroethane (118 mL) was treated with $CpRu(CH_3CN)_3$ PF_6 **1b** (2 5 g, 5 8 mmol, 1 0 equiv) and heated at reflux for 2 h The reaction mixture was cooled to 0 °C and filtered The filtrate was concentrated *in vacuo* and dissolved in CH_3CN (15 mL) and treated with Et_2O (150 mL) The gum separating out was separated by decanting the ether and the residue was dissolved in CH_2Cl_2/CH_3OH (1 1, 50 mL) and concentrated *in vacuo* to obtain **29c** as a brown foam (2.2 g, 69%) MS (Electron spray, *m/z rel int*) 408 $[(M-PF_6)^+$, 100]

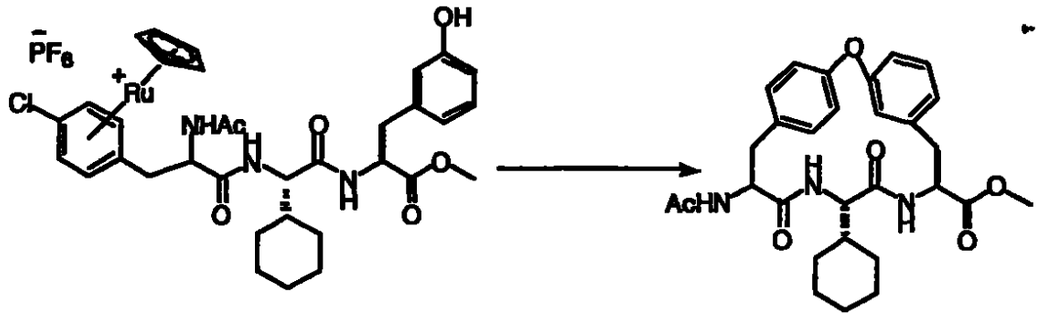
Step C



A solution of carboxylic acid **29c** (2 0 g, 4 00 mmol) in dry DMF (20 mL) was treated with HÜnigs base (2 06 g, 16 0 mmol, 4 0 equiv , 2 9 mL) and HOBt (810 mg, 6 0 mmol, 1 5 equiv) The reaction mixture was cooled to 0°C and then treated with EDCI (888 mg, 5 0 mmol, 1 25 equiv) and stirred for 0 5 h To this reaction mixture was added amine hydrochloride **1g** (1 48 g, 7 14 mmol, 1 2 equiv) and the reaction mixture was stirred at rt for 12 h The DMF was distilled out and the residue was diluted with water and the aqueous layer was extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq $NaHCO_3$ (200 mL), aq HCl (100 mL), brine, dried (Na_2SO_4) filtered concentrated *in vacuo* and the crude product **29d** (1 2 g, 38%) was used for cyclization without further purification

87

Step D

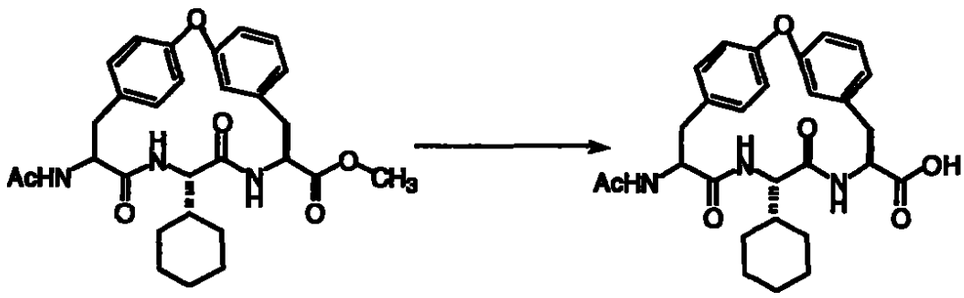


29d **29e**

A solution of chloro-compound **29d** (1 2 g 1 5 mmol) in dry DMF (120 mL) was degassed with dry N_2, treated with Cs_2CO_3 (2 4 g, 7 4 mmol, 5 0 equiv) and stirred at rt for 23 h The solvent DMF was distilled off and the residue was diluted with water (300 mL) and extracted with propionitrile (3x100 mL) The combined organic layers were dried (Na_2SO_4) filtered, concentrated *in vacuo* and dried in vacuum overnight It was used for photolytic removal of Ru without further purification

The cyclized compound from the previous step was dissolved in CH_3CN (40 mL) and photolyzed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 4 1) to yield a tan colored solid **29e** (240mg, 38 %) MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*), 522[$(M+1)^+$, 100]

Step E

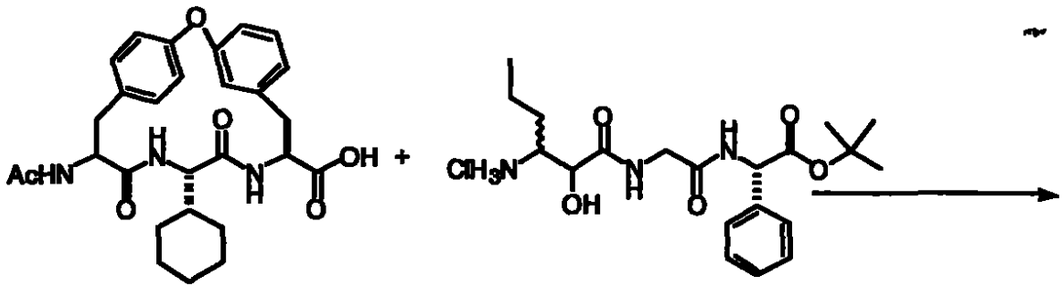


29e **29f**

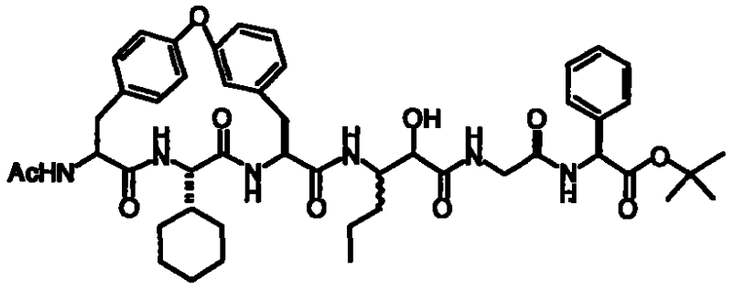
A solution of ester **29e** (200 mg, 0 42 mmol) in CH_3OH (5 mL), CH_2Cl_2 (13 mL) and H_2O (2 0 mL) was treated with LiOH H_2O (41 mg, 1 0 mmol, 2 4 equiv) and stirred at rt. for 3 h The reaction mixture was acidified with aq HCl (13 M, 1mL) and extracted in CH_2Cl_2 (3x50 mL) and EtOAc (3x50 mL) The combined organic layers were dried (Na_2SO_4) filtered and concentrated *in vacuo* to yield acid **29f** (178 mg) which was used directly for the coupling without further purification

0931 88

Step F.



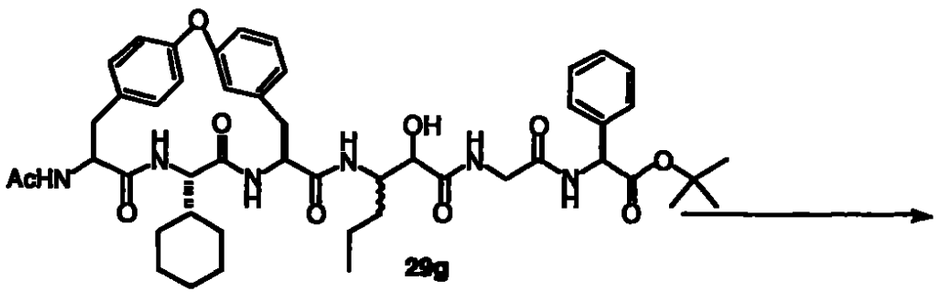
29f B



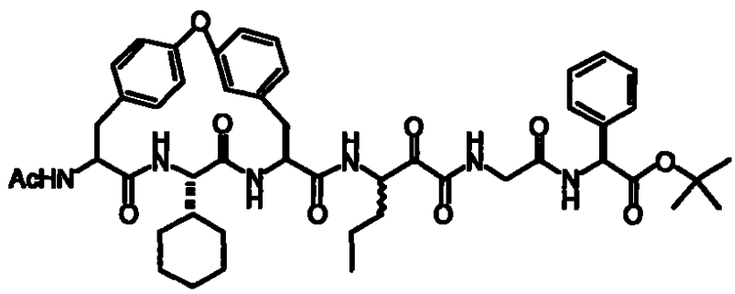
29g

A solution of acid **29f** (90 mg, 0 18 mmol) in dry DMF (1 0 mL) was treated with HOBt (45 mg, 0 33 mmol, 1 6 equiv), Hünigs base (142 mg, 1 1 mmol, 5 0 equiv) and amine **B** (118 mg, 0 28 mmol, 1 47 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (63 mg, 0 33 mmol, 1 6 equiv) and stirred at 0 °C for 20 min for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The combined aqueous layers were extracted with CH_2Cl_2 (3x30 mL) and EtOAc (3x30 mL) The organic layers were extracted with aq NaOH (2M, 30 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid **29g** (50 mg, 32%) which was used for oxidation MS (Electron spray, *m/z rel int*) 883 [$(M+1)^+$, 100], 522 (30), 394 (60)

Step G



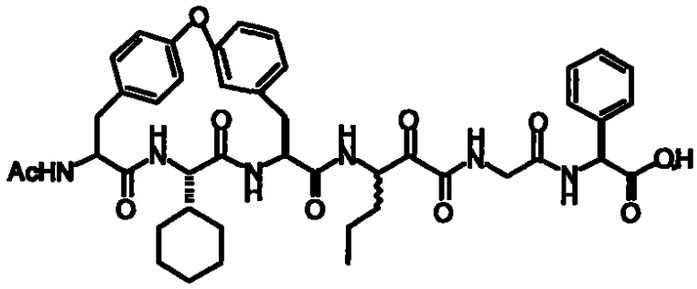
29g



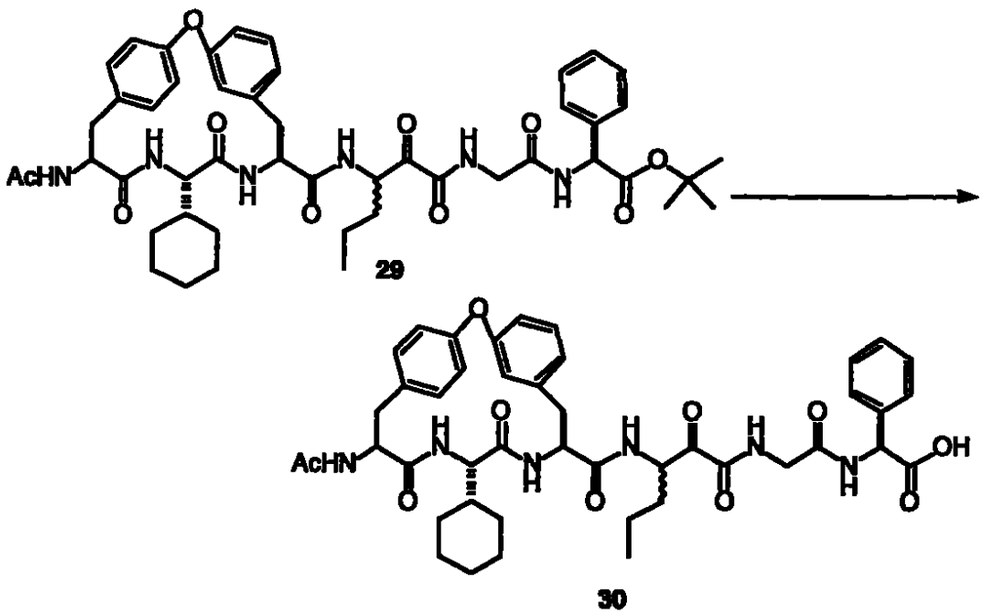
29

A suspension of alcohol **29g** (50 mg, 60 0 μmol) in CH_2Cl_2 (2 0 mL) was treated with Dess-Martin reagent (40 mg, 0 94 mmol, 2 0 equiv) The reaction mixture was stirred at rt for 3h and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 32) to yield oxidized product **29** (41 mg, 80%) as a colorless solid MS (FAB, *m/z*, *rel int*) 881 [$(M+1)^+$, 100), 825 (170), 248 (100)

Example 30 Preparation of Compound of Formula 30

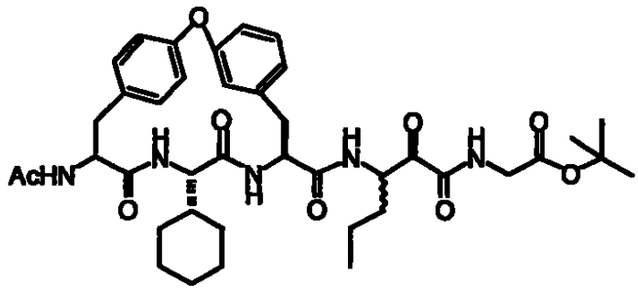


30



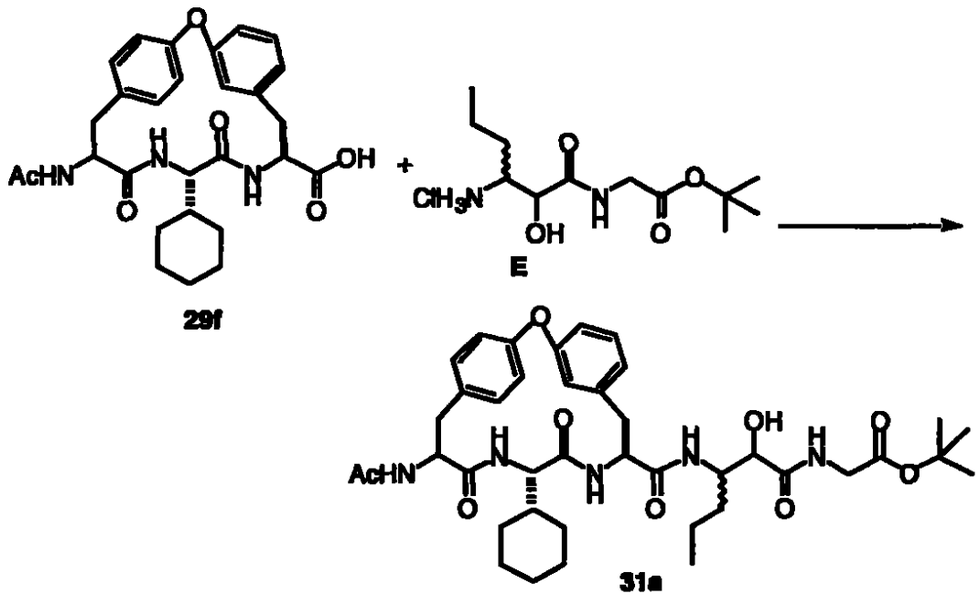
A solution of *tert*-butyl ester **29** (23 0 mg, 26 0 μmol) was treated with TFA/CH_2Cl_2 (1 1, 2 0 mL) and stirred at rt for 4 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1 24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes/CH_2Cl_2 (4 0 mL) and concentrated to yield a tan solid **30** (13 0 mg, 100%) MS (Electron spray, *m/z rel int*) 825 $[(M+1)^+$, 100)

Example 31 Preparation of Compound of Formula 31.



31

Step A



29f E

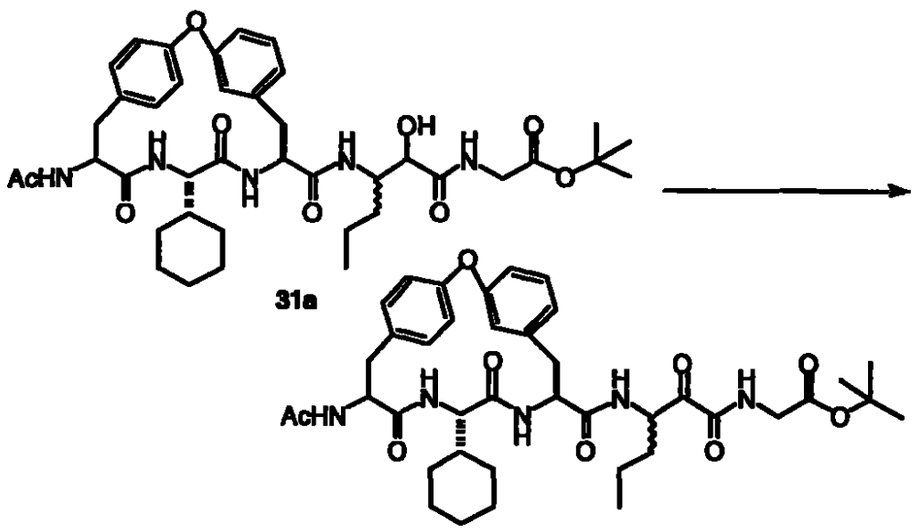
31a

A solution of acid **29f** (150 mg, 0 29 mmol) in dry DMF (4 0 mL), CH_2Cl_2 (3 0 mL) was treated with HOBt (58 mg, 0 44 mmol) and Hünigs base (149 mg, 1 1 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (82 mg, 0 44 mmol, 1 5 equiv) and stirred for 30 min The reaction mixture was treated with amine **E** (88 mg, 0 29 mmol, 1 22 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were extracted with aq HCl (1M, 3x20 mL), aq NaOH (1M, 3x20 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid **31a** (56

0093 90

mg) which was used for oxidation MS (Electron spray, *m/z rel int*) 750 [(M+1)$^+$, 20], 663 (10), 522 (10), 416 (20), 247 (30)

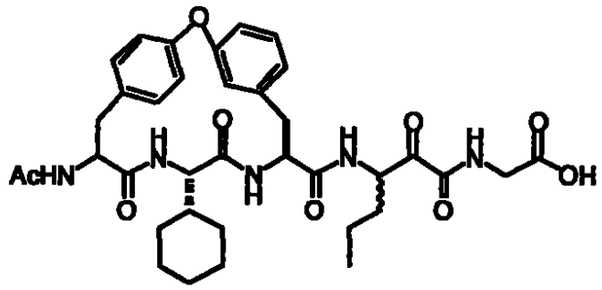
Step B



31

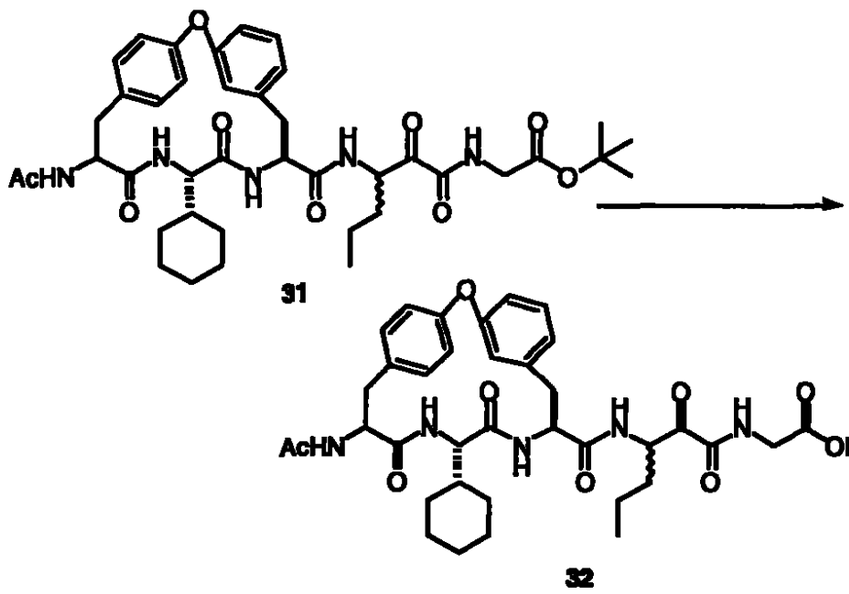
A solution of alcohol **31a** (56 mg, 75 μmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (93 mg, 0 22 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 4 h and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 19) to yield oxidized product **31** (34 mg, 60 %) as a colorless solid MS (Electron spray, *m/z rel int*) 748 [(M+1)$^+$, 35], 692 (5), 279 (100)

Example 32. Preparation of Compound of Formula 32.



32

Step A



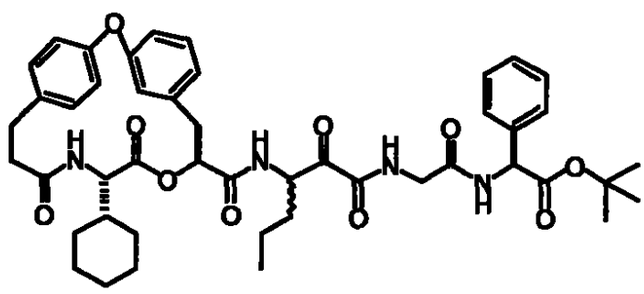
31

32

A solution of *tert*-butyl ester **31** was treated with TFA/CH_2Cl_2 (1 1, 4 0 mL) and stirred at rt for 4 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1 24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes/CH_2Cl_2 (4 0 mL) and concentrated to yield **32** as a tan solid

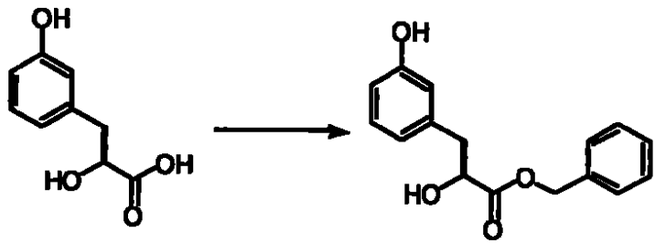
91

Example 33 Preparation of Compound of Formula 33



33

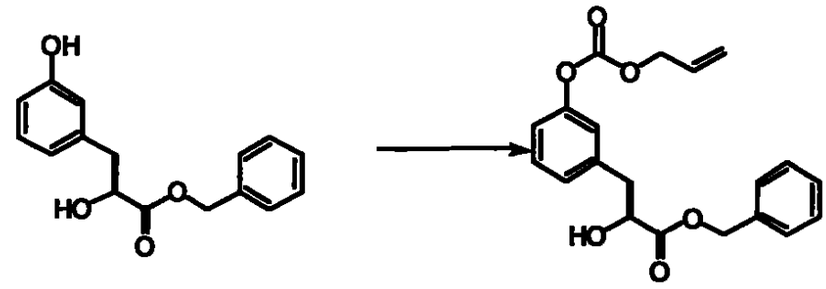
Step A



33a **33b**

A solution of acid **33a** (4 5g, 25 0 mmol) in dioxane (30 mL) and benzene (80 mL) was treated with BnOH (8 0 g, 74 mmol, 3 0 equiv) and TsOH H_2O (713 mg, 3 75 mmol, 10 mol%) The reaction mixture was heated at reflux for 5 h, when the water was separated using a Dean-Stark apparatus The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 3 7) to yield benzyl ester **33b** as a colorless oil (4 2 g, 62%), R_f 0 22 (EtOAc/Hexanes 3 7), ^{13}C NMR (CH_3OD, 75 MHz, δ) 175 1, 158 2, 139 7, 130 3, 129 5, 129 3, 121 7, 117 4, 114 6, 73 1, 67 6, 41 6, MS (FAB, G/TG-DMSO, *m/z, relative intensity*) 351 ([M+DMSO]$^+$, 70), 273 ([M+1]$^+$, 100), 255 (20), 227 (30), 181 (40), HRMS: Calcd for $C_{16}H_{17}O_4$ $(M+1)^+$ 272 1049, found 272 1054

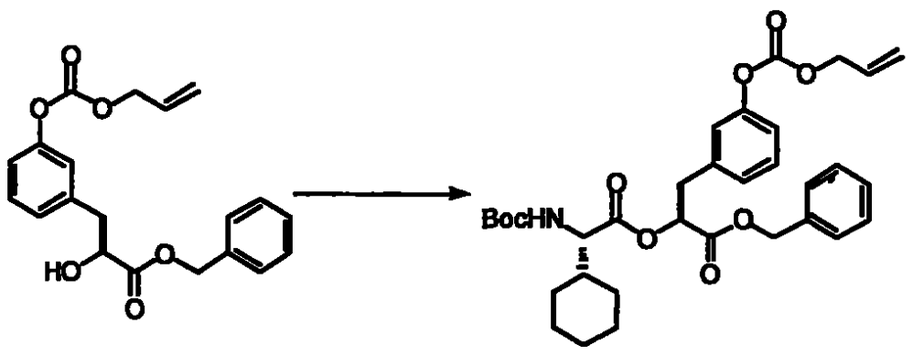
Step B



33b 33c

A solution of benzyl ester **33b** (3 8 g, 12 9 mmol) in CH_2Cl_2 (100 mL) was treated with Et_3N (1 55 g, 15 4 mmol, 2 2 mL, 1 1 equiv), cooled to -78°C (2-PrOH, dry ice) and a solution of allyl chloroformate (1 84 g, 15 36 mmol, 1 1 equiv) in CH_2Cl_2 (10 mL) was added dropwise The reaction mixture was allowed to warm up to the rt and diluted with aq HCl (1M, 100 mL) The reaction mixture was extracted with EtOAc (3x100 mL) The combined organic layer was washed with aq HCl (100 ml, 1M), brine (100 mL), dried ($MgSO_4$) concentrated *in vacuo* to yield **33c** which was used in the next step without further purification R_f - 0 43 (EtOAc/Hex 7 13), ^{13}C NMR (CH_3OD, 75 MHz, δ) 174 8, 162 5, 155 0, 152 5, 140 3, 137 1, 132 8, 130 3, 129 6, 129 5, 129 4, 123 2, 120 3, 119 4, 72 7, 70 1, 67 7, 41 2, 29 9

Step C



33c 33d

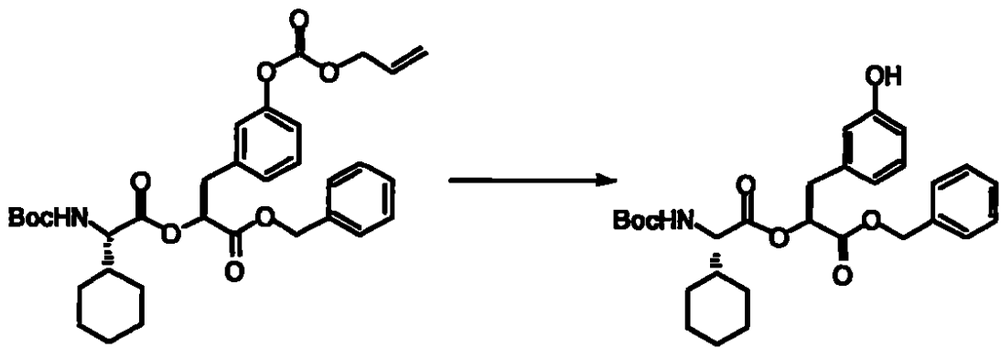
A solution of Boc-cyclohexylglycine monohydrate **1d** (6 02 g, 23 4 mmol, 2 0 equiv) was dissolved in CH_2Cl_2 and dried ($MgSO_4$) The mixture was filtered

97

0035

and the residue was further azeotropically dried with toluene The residue was dissolved in CH_2Cl_2, and treated with HOBt (4 73 g, 35 1 mmol, 2 9 equiv) EDCI (6 7 g, 35 1 mmol, 2 9 equiv) and Hünigs base (8 31 g, 64 3 mmol, 11 mL) It was stirred at rt. for 30 min and the alloc protected alcohol **33c** (4 3 g, 12 04 mmol) was added The reaction mixture was stirred at rt for 36 h and diluted with aq HCl (1M, 100 mL) and extracted with EtOAc (3x100 mL) The combined organic layers were extracted with aq NaOH (1M, 100 mL), brine (100 mL), dried, concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hex 1 4) to yield depsipeptide **33d** (7 1g 100%) R_f 0 18 (EtOAc/Hex 1 4), HRMS Calcd for $C_{28}H_{34}O_7$ $(M\text{-}Boc)^+$ 496 2335 found 496 2333

Step D.

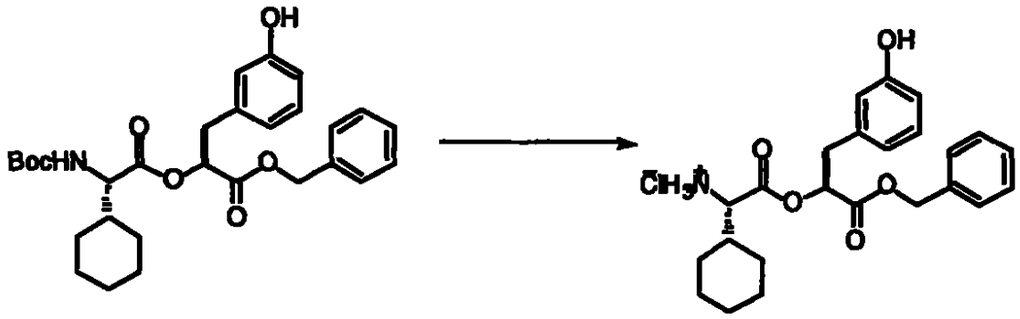


33d **33e**

A solution of alloc-protected depsipeptide **33d** (7 8 g, 13 0 mmol) in dry THF (200 mL) was treated under N_2 with dimedione (3 27 g, 23 4 mmol, 2 0 equiv) and $Pd(Ph_3P)_4$ (780 mg, 0 67 mmol, 5 mol%) The reaction mixture was stirred at rt for 1h and the disappearance of reactant was followed by TLC (EtOAc/Hex 1 4) The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 4) to yield phenol **33e** (5.2g, 78%) as a colorless foam R_f 0 52 (EtOAc/Hexanes 3 7), ^1H NMR (d_4-CD_3OD, 300 MHz, δ) 7 4-7 19 (m, 5 H), 7 15-6 99 (m, 1 H), 6 68-6 55 (m,4 H) 5 43-5 01 (m,3 H), 4 6 (bs, 2H), 4 11-4 00 (m, 1 H), 3 18-2 91 (m, 2 H), 1 80-1 55

(bs, 6H) 1 39 (s, 9 H) 1 21-0 89 (m, 6 H), ^{13}C NMR (CH_3OD, 75 MHz, δ, mixture of diastereomers) 171 6, 169 4, 169 3, 161 1, 157 1, 157 0, 137 2, 136 9, 135 4, 135 3, 129 2, 129 1, 128 2, 128 2, 128 0, 120 3, 120 1, 116 0, 115 9, 113 6, 94 8, 79 3, 73 6, 73 5, 66 7, 66 6, 58 6, 58 5, 40 0, 39 9, 36 8, 29 1, 27 7, 27 3, 25 5 MS (Electron spray, *m/z, relative intensity*) 1023 ([2M+1]$^+$, 20), 512 ([M+1]$^+$, 20), 412 ([M-Boc]$^+$, 100), 202 (40) HRMS Calcd for $C_{24}H_{30}NO_5$ (M-Boc)$^+$ 412 2123 found 412 2119

Step E

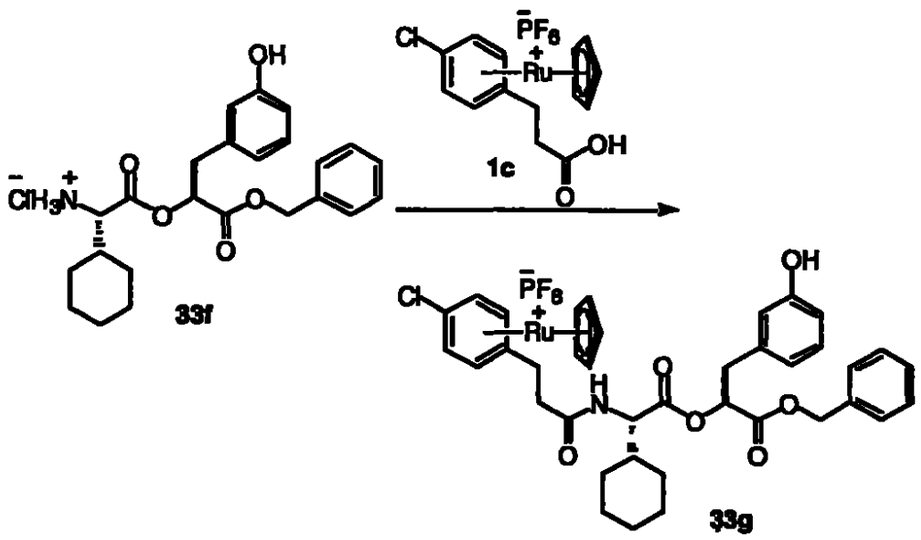


33e 33f

A solution of Boc protected amine 33e (5 2 g, 10 7 mmol) was stirred with HCl (4M, dioxane, 200 mL, 800 mmol, 80 equiv) until the starting material disappeared to the base line as indicated by TLC (EtOAc/Hex 3 7) The reaction mixture was concentrated *in vacuo* and dried in high vacuum and the residue 33f was directly used in the next step ^{1}H NMR (d_4-CD_3OD, 300 MHz, δ) 7 40-3 23 (m, 5 H), 7 07 (q, 1 H, J=13 Hz) 6 77-6 6 (m, 3 H), 5 33-5 41 (m, 1 H), 5 3-5 05 (2 AB, 2 H) 3 99-3 85 (m, 1 H) 3 35-22 (m, 2 H) 2 00-1 5 (m, 5 H), 1 50-0 80 (m, 6 H), MS (FAB, G/TG-DMSO, *m/z, relative intensity*) 412 ([M+1]$^+$, 100), HRMS Calcd for $C_{24}H_{30}NO_5$, M$^+$ 412 2123 found 412 2139

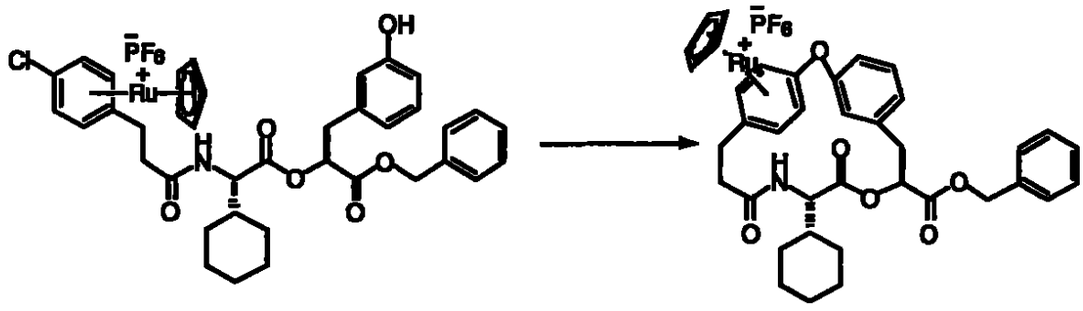
93

Step F



A solution of [CpRu(η^6- 4-chlorophenylpropionic acid)]PF_6 1c (2 0 g, 4 03 mmol) in dry DMF (20 mL) was treated with HOBt (835 mg 6 0 mmol, 1 5 equiv) and Hünigs base (2 06 g, 2 95 mL, 16 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (1 15 g, 6 0 mmol, 1 5 equiv) The reaction mixture was stirred at 0°C for 30 min and the amine hydrochloride was added 33f (1 8 g, 4 03 mmol, 1 0 equiv) in dry DMF (10 mL) The reaction mixture was stirred at rt for 12 h and the DMF was distilled out vacuo The residue was diluted with aq HCl (1M, 100 mL) and extracted into CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq $NaHCO_3$ (3x50 mL) , brine (100 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* to yield a brown solid 33g (3 5 g) which was used for cyclization, MS Electron spray, m/z, relative intensity) 743 [$(M\text{-}PF_6)^+$, 100], 304 (60), HRMS Calcd for $C_{38}H_{41}NO_6Cl^{102}Ru$ $(M\text{-}PF_6)^+$ 744 1666 found 744 1694

Step G:

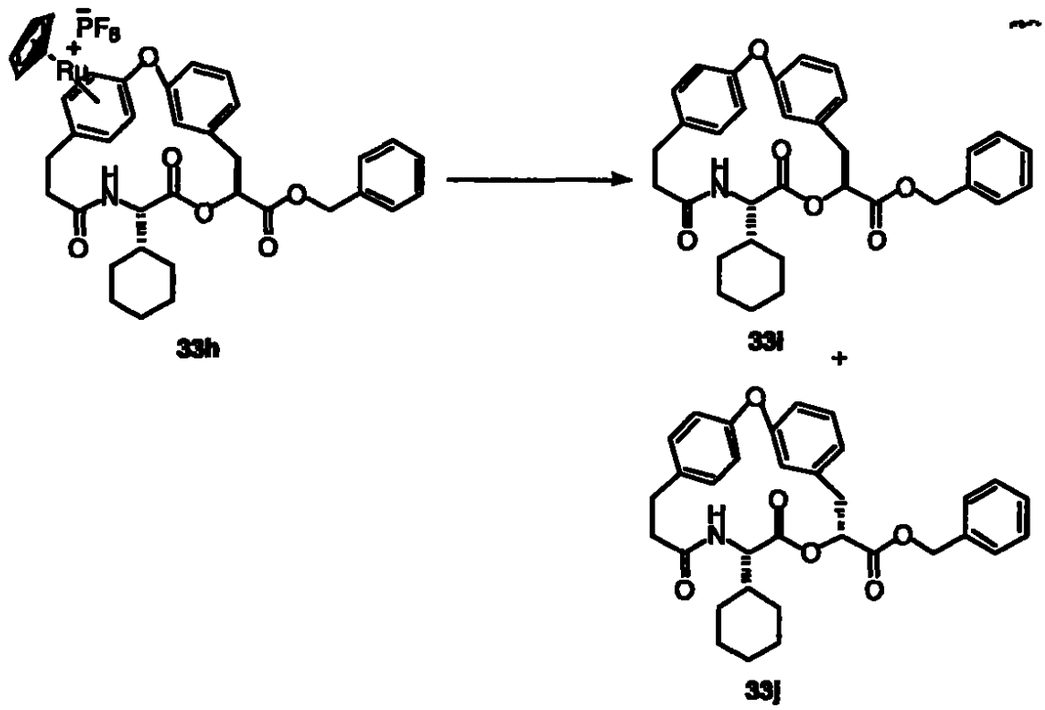


33g 33h

A solution of η^6–ruthenium complex **33g** (3 5 g, 3 93 mmol) in dry DMF (300 mL) was degassed with dry N_2 and treated with Cs_2CO_3 (6 5 g, 19 95 mmol, 5 0 equiv) and stirred at rt for 16 h The reaction mixture was concentrated *in vacuo* to remove the DMF and the residue was diluted with H_2O (100 mL) The reaction mixture was extracted with CH_2Cl_2 (3x100 mL) The combined CH_2Cl_2 layers were extracted with brine, dried (Na_2SO_4), filtered, concentrated *in vacuo* to yield **33h** which was directly used for photolysis, MS (Electron spray, m/z, relative intensity) 708 [$(M-PF_6)^+$, 100], HRMS: Calcd for $C_{38}H_{40}NO_6{}^{102}Ru$ $(M-PF_6)^+$ 708 1892 , found 708 1918

94

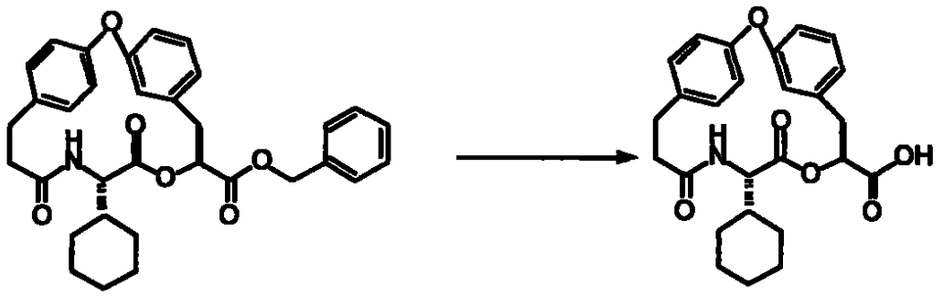
Step H



A solution of cyclized ruthenium complex **33h** (3 5g, 3 9 mmol) in CH_3CN (60 mL) was degassed and photolyzed in a quartz tube at λ=350 nm in two batches for 48 h each The reaction mixture were pooled together and purified by chromatography (SiO_2, CH_2Cl_2/Et_2O 9/1) to yield cyclic depsipeptide as a mixture of diastereomers (700 mg, 34%) The diastereomers were separated by additional chromatography (Hexanes/CH_2Cl_2/ Et_2O 6 3 1) to yield the two diastereomers **33I** (370 mg, 18%) and **33J** (216 mg 11%) as colorless solid R_f 0 28 (Hexanes EtOAc 3 2) , $[\alpha]_D$= 25 (*c* 0 15, $CHCl_3$, 20 °C) IR (neat, cm^{-1}) 3329 (w), 2960 (m) 2926 (s), 2854 (s), 1745 (s), 1680 (m), 1589 (m), 1506 (m), 1446 (m), 1365 (w), 1259 (s) 1099 (m), 1030 (s), 800 (s), 752 (m), 698 (w) 619 (w) ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 7 36-7 23 (m, 5 H), 7 18- 6 99 (m, 4 H), 6 81 (d, 1 H, *J*=7 5 Hz), 6 74 (dd, 1 H, *J*=2 7, 5 7 Hz), 6 30 (s, 1 H), 5 75 (d, 1 H, *J*=7 2 Hz), 5 61 (dd, 1 H, *J*=2 4 Hz, 5 4 Hz), 5 18, 5 14 (AB, 2 H, *J*=12 3 Hz), 4 23 (dd, 1 H, *J*=4 2 Hz, 3 3 Hz), 3 26-3 01 (m, 2 H), 2 98-2 85 (m, 2 H), 2 68-2 64 (m, 1H)

2 38-2 34 (m, 1H),1 96-1 51 (m, 6 H), 1 51-0 96 (M, 5H) ^{13}C NMR ($CDCl_3$, 75 MHz, δ, ppm) 177 3, 171 1, 168 7, 159 8, 155 3, 138 6, 135 4, 134 9, 131 2, 129 7, 129 2, 128 7, 126 6, 126 1, 123 3, 120 8, 120 8, 117 5, 114 2, 71 8, 57 5, 56 9, 41 5, 39 0, 35 7, 32 6, 31 3, 29 0, 27 6, 26 0, 25 9 FAB (NBA/DMSO, *m/e, relative intensity*) 542 [(M+1)$^+$ 100], 514 (15), 450 (5), 307 (8), 232 (5), 154 1 (17), 136 (14) HRMS Calcd for $C_{33}H_{36}NO_6$ (M+1)$^+$ 542 2543 found 542 2541 CHN Calcd for $C_{33}H_{35}NO_6$ 0 5H_2O, C 71 98% H 6 59% N 2 54%, Found C 72 56% H 7 05% N 2 63%

Step I

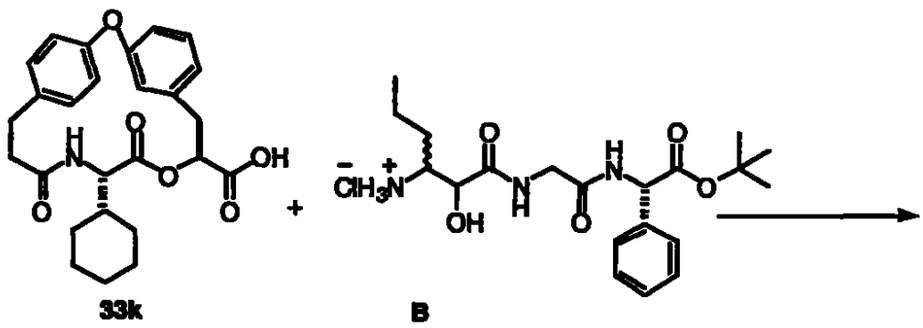


33l 33k

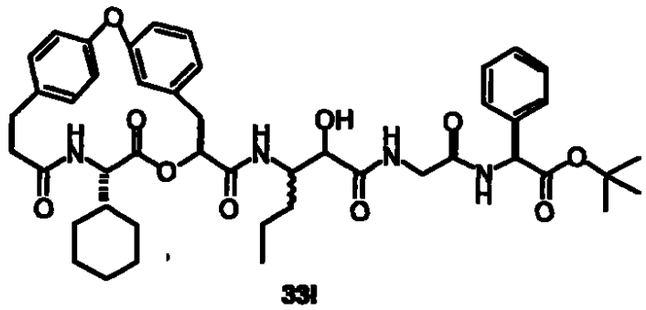
A solution of benzyl ester **33l** (360 mg, 0 66 mmol) in CH_3OH/EtOAc (1 1, 50 mL), was treated with $Pd(OH)_2$ and hydrogenated (50 psi) for 12 h The reaction mixture was filtered through a plug of celite and the cake was rinsed with CH_3OH/CH_2Cl_2 (1 1, 50 mL) The filtrate was concentrated *in vacuo* and the residue **33k** (330 mg) was used for coupling without purification R_f 0 58 (CH_3OH/CH_2Cl_2 1 19) MS (Electron spray, m/z, relative intensity) 827 2 [(M+1)$^+$, 100], 694 (20), 539 (40), 466 (10), 174 (70) HRMS Calcd for $C_{46}H_{58}N_4O_{10}$ (M+1)$^+$ 827 4231 found 827 4215

95
0898

Step J



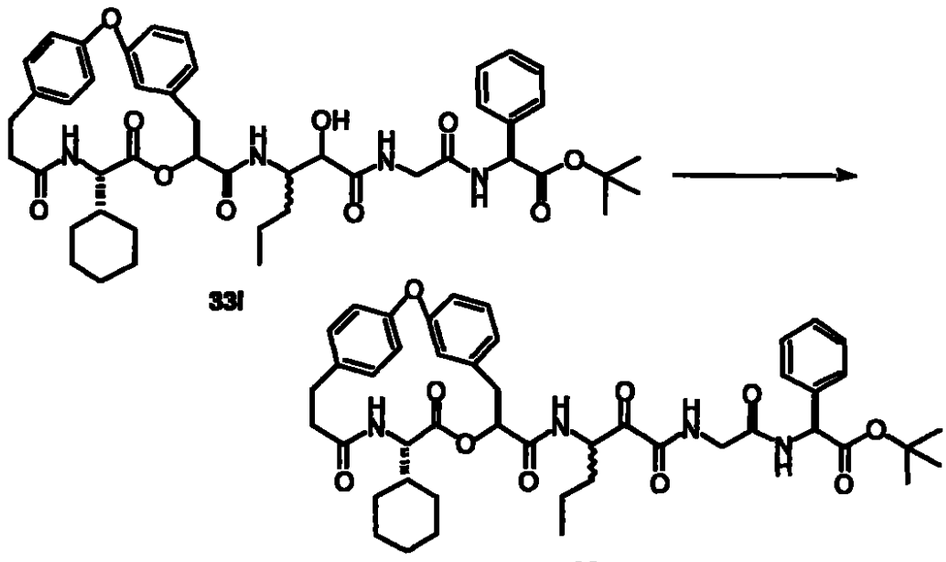
33k B



33l

A solution of acid **33k** (165 mg, 0 31 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5 0 mL) was treated with HOBt (83 mg, 0 46 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (159 mg, 1 23 mmol, 4 0 equiv, 229 µL) was added To this mixture was added EDCI (89 mg, 0 47 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 1 h and treated with the amine hydrochloride **B** (159 mg, 0 372 mmol, 1 2 equiv) The reaction mixture was stirred at rt for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with water and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer were extracted with aq HCl (1M, 3x50 mL), aq NaOH (1M, 3x50 mL) brine (100 mL) and concentrated *in vacuo* The residue **33l** was oxidized without further purification

Step K



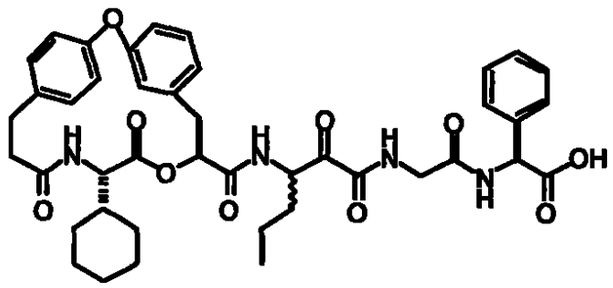
33I

33

A solution of alcohol **33I** (330 mg, 0 4 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (424 mg, 1 00 mmol, 2 5 equiv) The reaction mixture was stirred at rt for 1 h and diluted with aq $NaHCO_3$ (50 mL) and aq $Na_2S_2O_3$ (50 mL) The reaction mixture was stirred at rt for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with brine (50 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield ketoamide **33** (180 mg, 55%) of a colorless solid R_f : 0 63 (CH_3OH/CH_2Cl_2 1 19), MS (Electron spray, *m/z relative intensity*) 857 2 ($[M+CH_3OH]^+$, 40), 825 2 ($[M+1]^+$ 100)

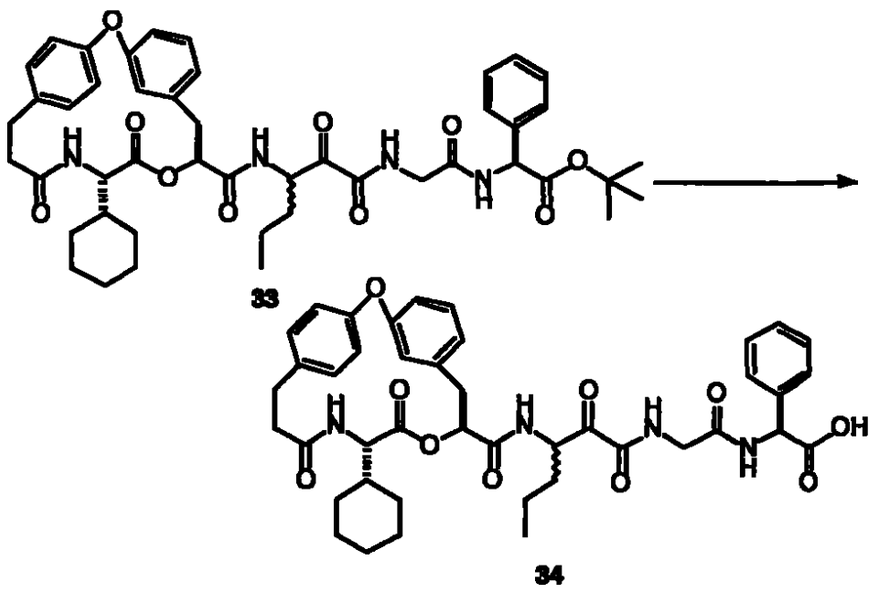
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96

Example 34 Preparation of Compound of Formula 34.



34

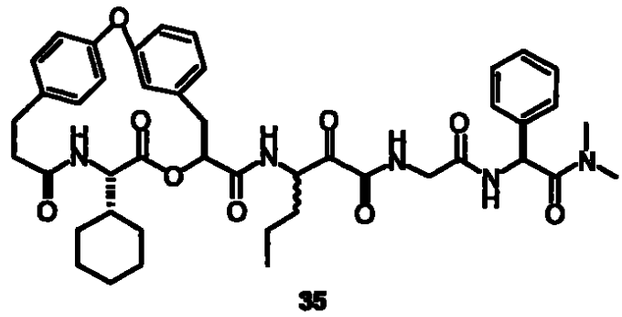
Step A



33 34

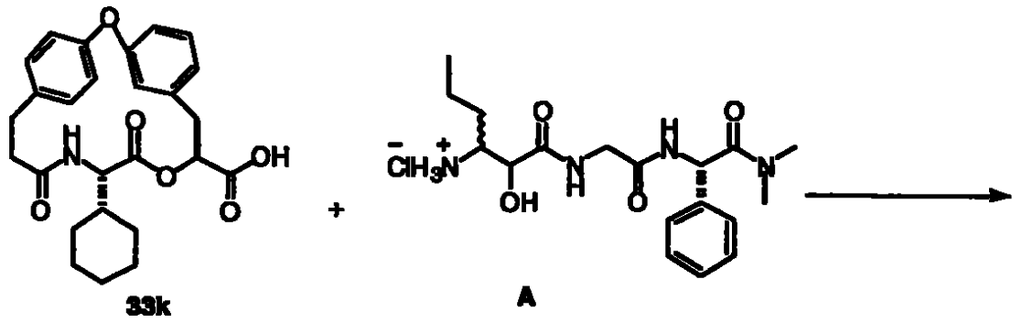
A solution of oxidized depsipeptide 33 (160 mg, 0 2 mmol) in dry CH_2Cl_2 (5 0 mL) was treated with TFA (5 0 mL) and stirred at rt for 7 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in CH_3OH/CH_2Cl_2/Hexanes (1 1 1) and concentrated *in vacuo* several times to yield a tan colored solid 34 (133 mg, 86%) which was dried *in vacuo* MS (Electron spray, *m/z relative intensity*) 769 2 $[(M+1)^+,100]$, 481 (5), 269 (25) 191 (90)

Example 35 Preparation of Compound of Formula 35.

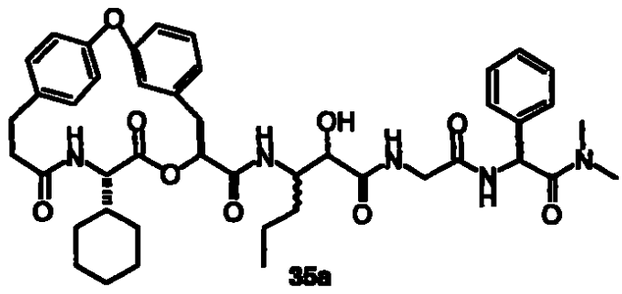


35

Step A



33k A

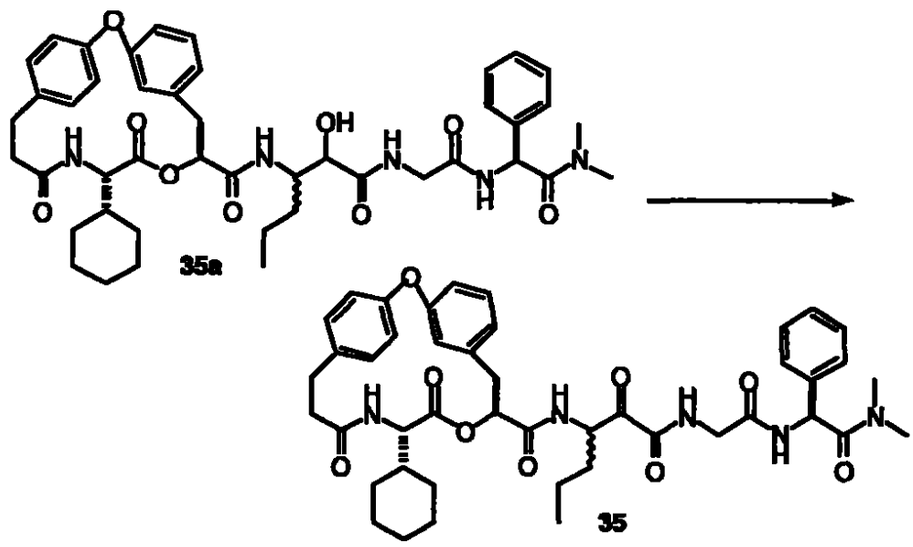


35a

A solution of acid **33k** (165 mg, 0 31 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5 0 mL) was treated with HOBt (83 mg, 0 46 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (159 mg, 1 23 mmol, 4 0 equiv , 229 µL) was added To this mixture was added EDCI (89 mg, 0 47 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 1 h and treated with the amine hydrochloride **A** (159 mg, 0 372 mmol, 1 2 equiv) The reaction mixture was stirred at rt for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with water and extracted with CH_2Cl_2 (3x50 mL) The

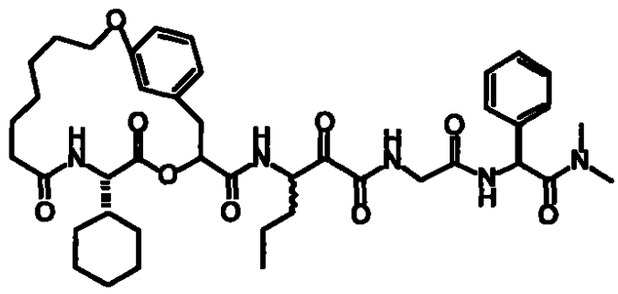
combined organic layer was extracted with aq HCl (1M, 3x50 mL), aq NaOH (1M, 3x50 mL), brine (100 mL) and concentrated *in vacuo* The residue **35a** was oxidized without further purification MS (Electron spray, *m/z relative intensity*) 798 2 [(M+1)$^+$, 30], 479 (10), 391 (20) 180 (100)

Step B.



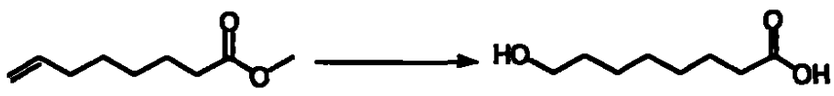
A solution of alcohol **35a** (190 mg, 0 24 mmol) in CH_2Cl_2 (10 mL) was treated with Dess-Martin reagent (423 mg 1 0 mmol, 4 0 equiv) and stirred at rt for 1h The reaction mixture was diluted with aq $NaHCO_3$ (50 mL) and extracted in CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq satd $Na_2S_2O_3$, brine (3x50 mL), dried (Na_2SO_4), filtered concentrated *in vacuo* and purified by chromatography (SiO_2, acetone/Hexanes 3 7->1 1 to yield oxidized product **35** (163 mg, 86%) as a colorless solid MS (Electron spray, *m/z relative intensity*) 796 [(M+1)$^+$, 100], 508 (20), 269 (20)

Example 36. Preparation of Compound of Formula 36



36

Step A



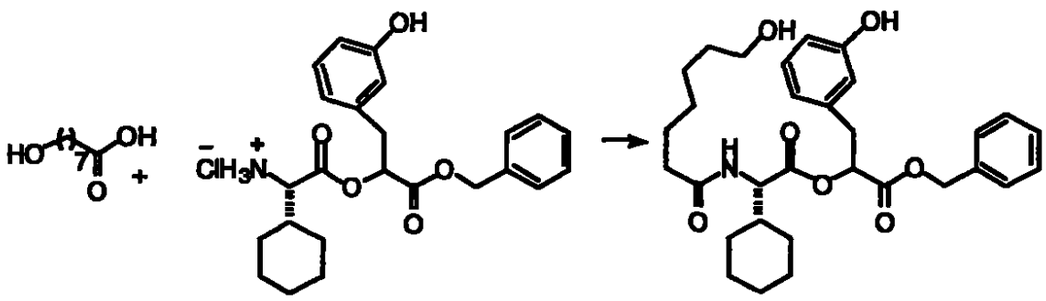
36a 36b

A solution of BH_3 THF (1M in THF, 100 mmol, 100 mL, 3 0 equiv) was added dropwise to a solution of alkene (5 0 g, 35 mmol) in THF (100 mL) at 0° C and stirred for 1h The reaction mixture was treated with ethanol (20 mL) dropwise After the evolution of hydrogen gas was complete the reaction mixture was treated with pH 7 buffer (100 mL) and H_2O_2 (30 volumes, 100 mL) The reaction mixture was stirred at rt for 4 h and quenched with aq HCl (100 mL) The aqueous layer was extracted with Et_2O (3x100 mL) The combined ether layers were extracted with aq NaOH (1M, 100 m L), brine (100 mL), dried ($MgSO_4$), filtered, concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hexanes 2/3) to yield alcohol as a colorless liquid (2 9 g, 52%)

A solution of the hydroxylated ester in THF/H_2O/CH_3OH (100 mL, 1 1 1) was treated with LiOH H_2O (2 1g, 51.2 mmol, 3 0 equiv) and stirred at rt for 3 d The reaction mixture was concentrated *in vacuo* and the aqueous layer was extracted with ether (2x40 mL) The aqueous layer was acidified to pH ~1 and extracted into EtOAc (3x50 mL) The combined organic layers were extracted with brine (100 mL), dried ($MgSO_4$) filtered concentrated *in vacuo* and the residue

36b was used as is for coupling in Step B ^{1}H NMR (300 MHz, CD_3OD, δ) 3 53 (t, 2 H, *J*=6 6 Hz), 2 72 (t, 2 H, *J*=7 2 Hz), 1 59 (t, 2 H, *J*=7.5 Hz), 1 5 (t, 2 H, *J*=7 5 Hz), 1 38-1 33 (m, 6 H)

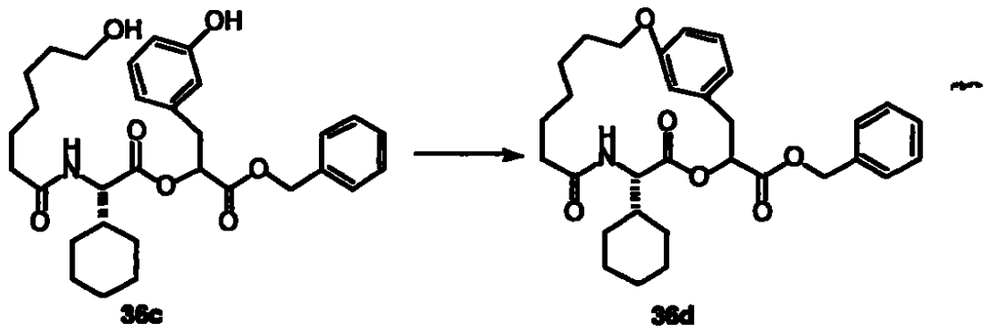
Step B



36b **33f** **36c**

A solution of ω-hydroxyl heptanoic acid **36b** (1 01g, 6 93 mmol) in CH_2Cl_2 (40 mL) was treated with Hünigs base (1 97 g, 15.24 mmol, 2 2 equiv 2 81 mL) and amine hydrochloride **33f** (3 1g, 6 93 mmol, 1 0 equiv) The reaction mixture was cooled to 0 °C and treated with PyBrOP (3 22g, 6 93 mmol, 1 0 equiv) The reaction mixture was stirred overnight at rt. and the reaction mixture was concentrated *in vacuo* The residue was purified by chromatography (EtOAc/Hexanes 1 1) to yield depsipeptide **36c** (2 5 g, 66%) as a colorless viscous oil ^{1}H NMR (CD_3OD, 400 MHz, δ, ppm) 8 07 (t, 1 H), 7 33-7 21 (m, 4 H), 7 09-7 02 (m, 1 H), 6 67-6 63 (m, 3 H), 5 25-5 06 (m, 1 H), 5 08 (q, 2 H, *J*=7 5 Hz), 4 36-4 33 (m, 1 H), 3 51 (dd, 2 H, *J*=5 4 Hz, 0 9 Hz), 3 11-2 96 (m, 2 H), 2 22-2 17 (m, 1 H), 1 99-0 90 (m, 14 H) ^{13}C NMR (CD_3OD, 75 MHz, δ, ppm, mixture of diastereomers) 172 1, 172 0, 171 8, 171 1, 170 9, 169 5, 169 3, 157 1, 157 0, 137 3, 137 0, 135 3, 135 2, 129 2, 129 1, 128 2, 128 0, 127 9, 120 3, 120 0, 116 0, 115 9, 113 6, 94 8, 73 6, 73 4, 66 8, 66 7, 60 2, 57 3, 39 6, 36 7, 28 9, 28 0, 25 6, 20 9, 19 5, 13, MS (FAB, NBA DMSO, *m/z rel int*) 562 [$(M+Na)^+$, 20], 540 [$(M+1)^+$, 100), 412 (15), 240 (50), 112 (80)

Step C:

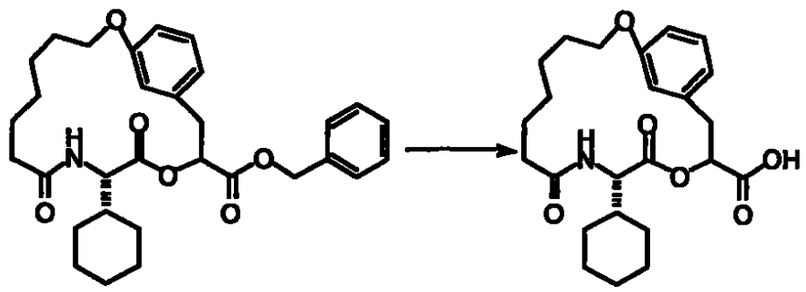


36c 36d

A solution of alcohol **36c** (2 5g, 4 63 mmol) in dry CH_2Cl_2 (50 mL) was treated with triphenylphosphine (2 67 g, 10 2 mmol, 2 2 equiv) under N_2 and cooled to 0°C The reaction mixture was treated with DEAD (1 61g, 9 26 mmol, 2 0 equiv) in CH_2Cl_2 (30 mL) The reaction mixture was warmed to rt and stirred for 2h It was concentrated *in vacuo* and purified by chromatography (Et_2O/Hex 1 3) to yield cyclic product **36d** (530 mg, 21%) as a colorless solid

MS (FAB, NBA, DMSO, *m/z rel int*), 522 [$(M+1)^+$, 100], 494 (60), 268 (20), 222(20), HRMS calcd for $C_{31}H_{40}NO_6$ ($M+1)^+$ 522 2856, Found 522 2864

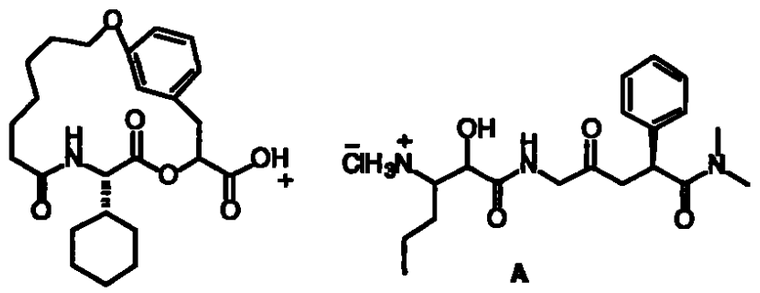
Step D



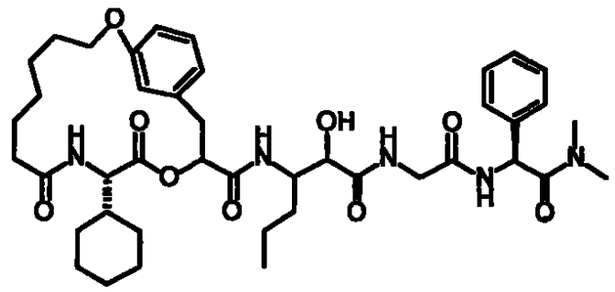
36d 36e

A solution of the benzyl ester (242 mg, 0 47 mmol) in methanol (30 mL) was treated with Pd/C (10 wt %) and hydrogenated on a Parr at 40 psi for 14h The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo* to yield a colorless solid **36e** (181 mg, 93%) which was used for coupling

Step E



36e A

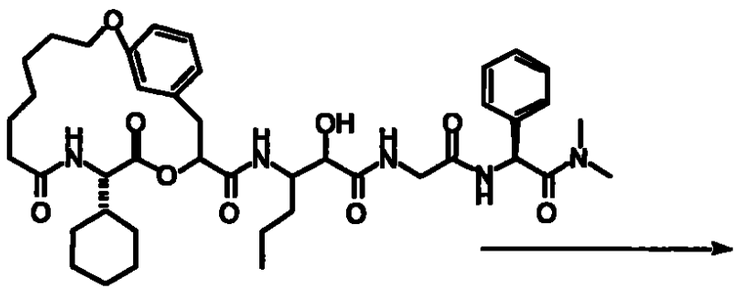


36f

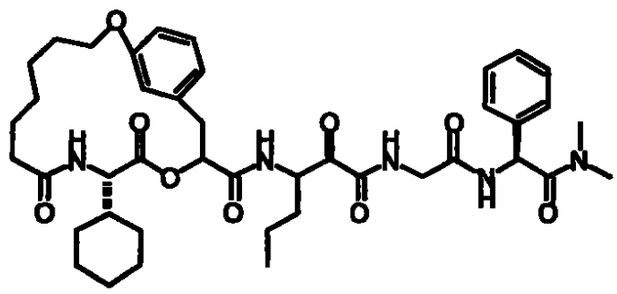
A solution of the hydrolyzed acid **36e** (167 mg, 0 39 mmol) in CH_2Cl_2 (4 0 mL) was treated with HOOBt (95 mg, 0 58 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (202 mg, 1 56 mmol, 4 0 equiv 288 µL) was added To this mixture was added EDCI (111 mg, 0.58 mmol, 1 5 equiv) and the reaction

mixture was stirred at 0° C for 0 5 h and treated with the amine hydrochloride (186 mg, 0 47 mmol, 1 20 equiv) The reaction mixture was stored in freezer for 24 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with aq HCl (2M, 30 mL) and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq HCl (2M, 30 mL), aq NaOH (1M) brine (2x50 mL), dried ($MgSO_4$) and concentrated *in vacuo* The residue **36f** (100 mg) was oxidized without further purification HRMS calcd for $C_{42}H_{60}N_5O_9$ $(M+1)^+$ 778 4391, Found 778 4399

Step F



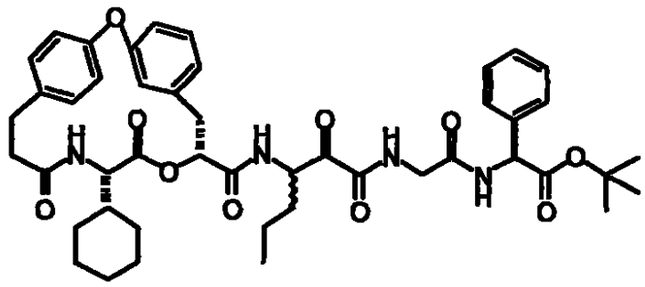
36f



36

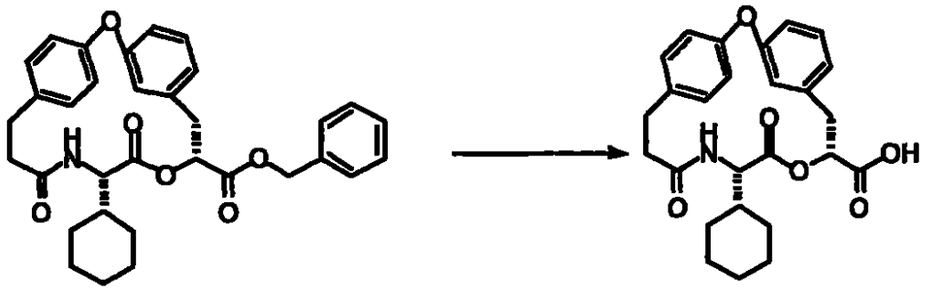
A solution of alcohol **36f** (100 mg, 0 13 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (100 mg, 0 0 23 mmol, 1 8 equiv) The reaction mixture was stirred at rt for 2 h and diluted with aq $NaHCO_3$ (15 mL) and aq $Na_2S_2O_3$ (15 mL) The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, acetone/Hexanes 3 7) to yield ketoamide **36** (61 mg, 61%) as a colorless solid, MS (FAB, NBA/DMSO, *m/z rel Int*) 776 [(M+1)+, 100], 731 (10), 598 (25), 570 (15), 485 (20), 358 (20), 247 (50)

Example 37 Preparation of Compound of Formula 37:



37

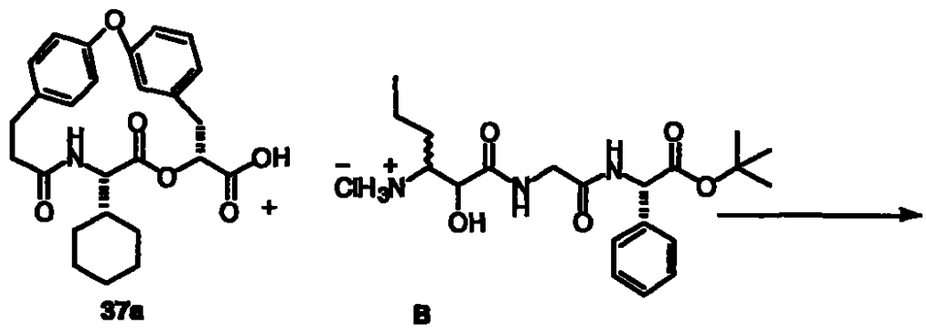
Step A



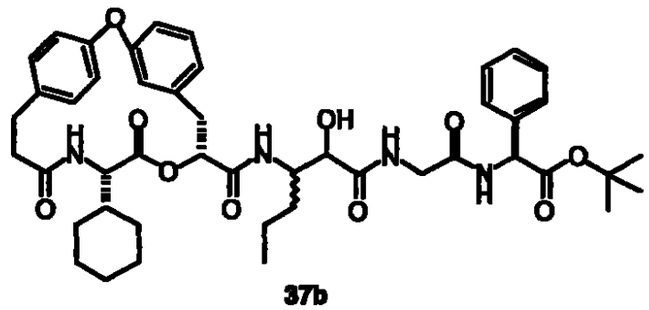
33J 37a

A solution of benzyl ester 33J (230 mg, 0 42 mmol) in CH_3OH/EtOAc (1 1, 50 mL), was treated with $Pd(OH)_2$ and hydrogenated (50 psi) for 12 h The reaction mixture was filtered through a plug of celite and the cake was rinsed with CH_3OH/CH_2Cl_2 (1 1, 50 mL) The reaction mixture was concentrated *in vacuo* and the residue **37a** (177 mg, 93%) was used for coupling without purification

Step B



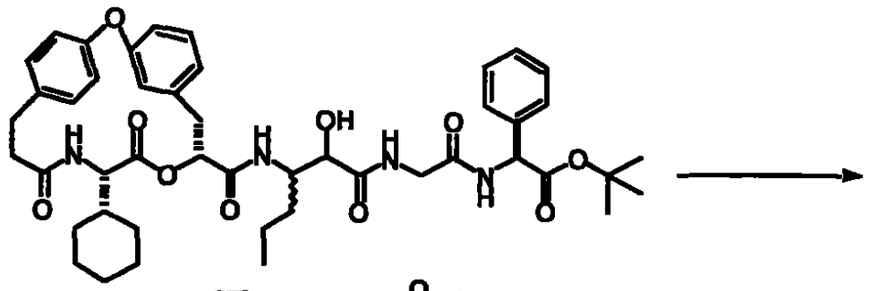
37a B



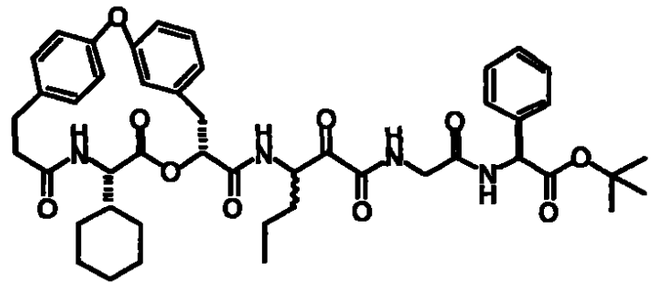
37b

A solution of acid **37a** (177 mg, 0 33 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5 0 mL) was treated with HOBt (88 mg, 0 49 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (175 mg, 1 35 mmol, 4 0 equiv, 251 µL) was added To this mixture was added EDCI (95 mg, 0 49 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 1 h and treated with the amine hydrochloride **B** (170 mg, 0 39 mmol, 1 2 equiv) The reaction mixture was stirred at rt for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with water and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq NaOH (1M, 2x50 mL), brine (100 mL) and concentrated *in vacuo* The residue **37b** (315 mg) was oxidized without further purification

Step C



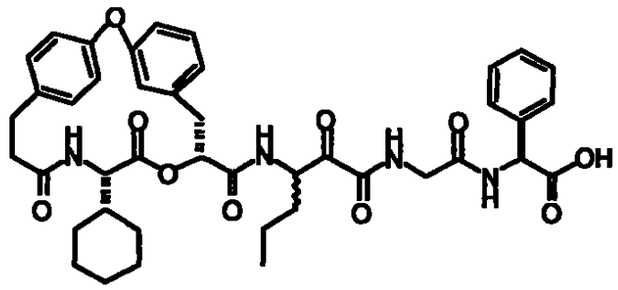
37b



37

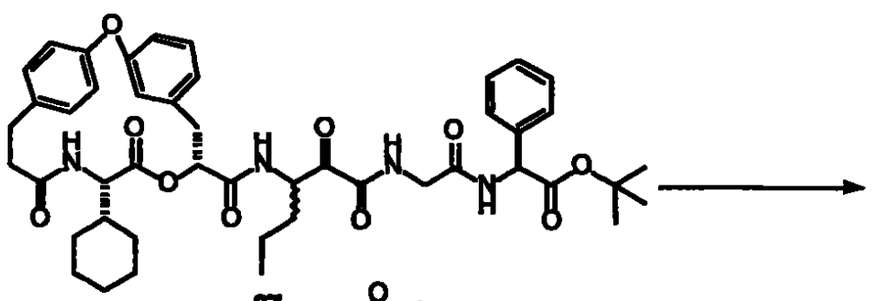
A solution of alcohol **37b** (315 mg, 0 4 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (424 mg, 1 00 mmol, 2 5 equiv) The reaction mixture was stirred at rt. for 1 h and diluted with aq $NaHCO_3$ (50 mL) and aq $Na_2S_2O_3$ (50 mL) The reaction mixture was stirred at rt. for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with brine, dried (Na_2SO_4), filtered concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield ketoamide **37** (210 mg, 66%) of a colorless solid R_f 0 63 (CH_3OH/CH_2Cl_2 1 19), MS (Electron spray, *m/z relative intensity*) 857 ($[M+CH_3OH]^+$, 33), 825 ($[M+1]^+$ 40), 191 (100)

Example 38: Preparation of Compound of Formula 38.

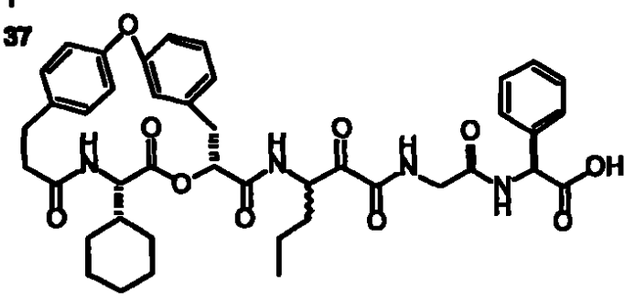


38

Step A



37

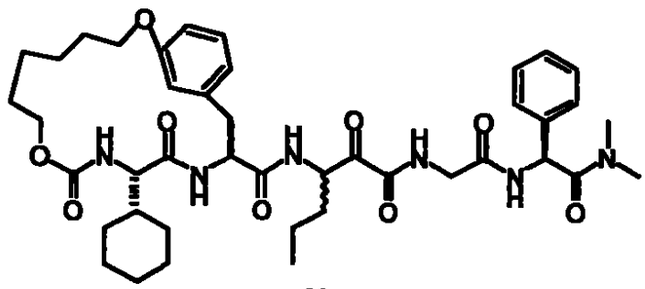


38

A solution of oxidized depsipeptide 37 (200 mg, 0 24 mmol) in dry CH_2Cl_2 (5 0 mL) was treated with TFA (5 0 mL) and stirred at rt. for 7 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in CH_3OH/CH_2Cl_2/Hexanes (1 1 1) and concentrated *in vacuo* several times to yield a tan colored solid 38 (130 mg, 87%) which was dried *in vacuo*; MS (Electron spray, *m/z relative intensity*) 769 ($[M+1]^+$, 45), 294 (45), 191 (100)

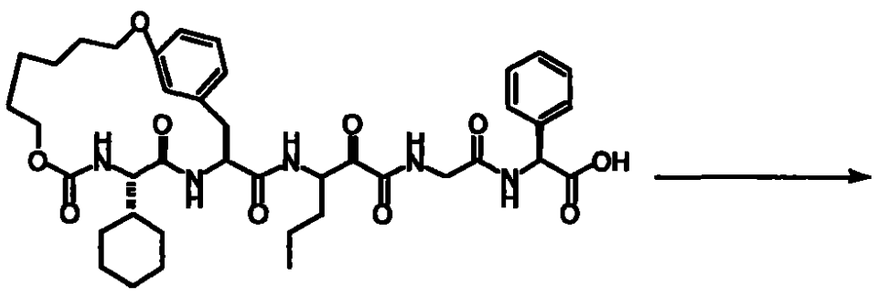
105
~~105~~
102

Example 39 Preparation of Compound of Formula 39.

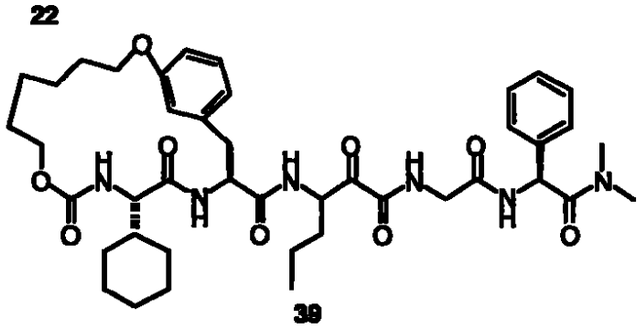


39

Step A



22

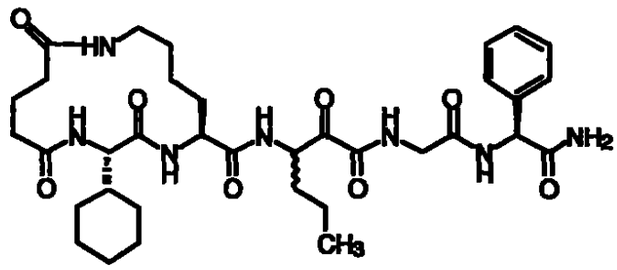


39

A solution of acid **22** (40 mg, 0 06 mmol) in CH_2Cl_2 (0 5 mL) and DMF (0 5 mL) was cooled to 0° C and treated with Me_2NH HCl (15 mg, 0 18 mmol, 3 0 equiv) and Hünigs base (31 mg, 0 24 mmol, 44 μL, 4 0 equiv) The reaction mixture was then treated with PyBrOP (55 mg, 0 12 mmol, 2 0 equiv) and stored for 12 h in the freezer The yellow reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes gradient 3 2 —>1 0) to obtain impure product which was purified once again using (acetone/Hexanes 1 6) to yield dimethyl amide **39** as a colorless solid (14 mg,

35%) MS (Electron spray, *m/z relative intensity*) 791 [(M+1)$^+$, 50], 391 (40), 276 (50), 176 (100)

Example 40 Preparation of Compound of Formula 40:

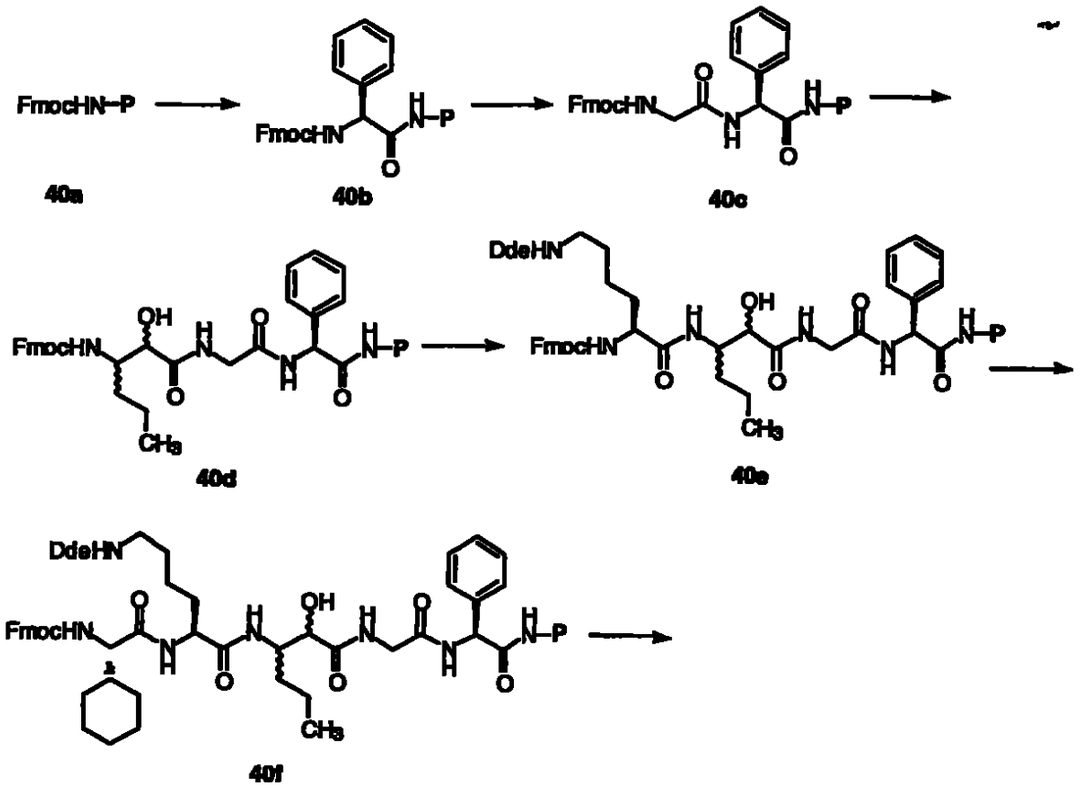


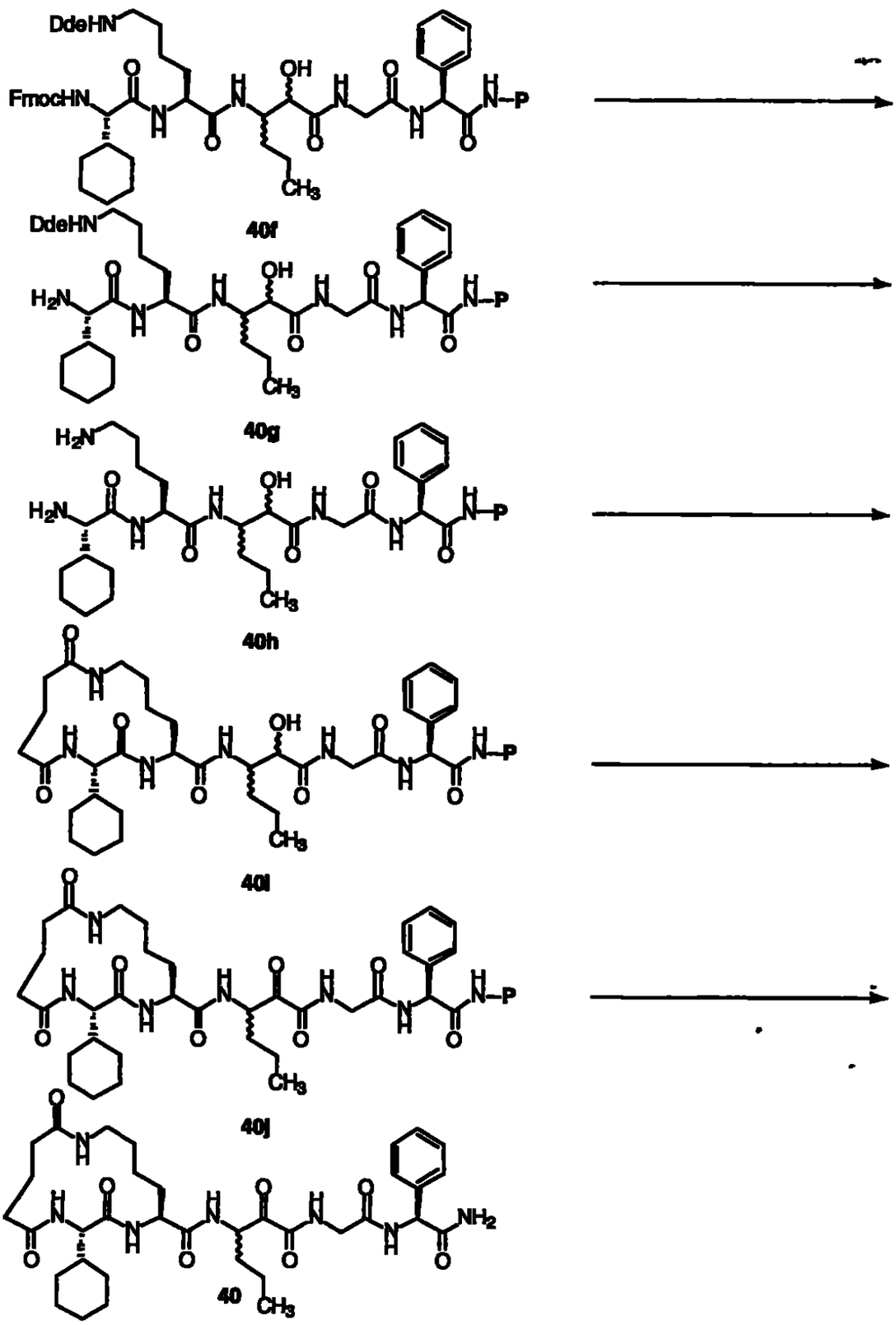
40

General procedure for solid-phase coupling reactions:

The synthesis was done in a reaction vessel which was constructed from a polypropylene syringe cartridge fitted with a polypropylene frit at the bottom The Fmoc-protected amino acids were coupled under standard solid-phase techniques Each reaction vessel was loaded with 100 mg of the starting Fmoc-Sieber resin (approximately 0 035 mmol) The resin was washed with 2 mL portions of DMF (2 times) The Fmoc protecting group was removed by treatment with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min The resin was washed with 2 mL portions of DMF (4 times) The coupling was done in DMF (2 mL), using 0 12 mmol of Fmoc-amino acid, 0 12 mmol of HATU and 0 24 mmol of DIPEA After shaking for 2 h, the reaction vessel was drained and the resin was washed with 2 mL portions of DMF (4 times) The coupling cycle was repeated with the next Fmoc-amino acid or capping group

Scheme 10

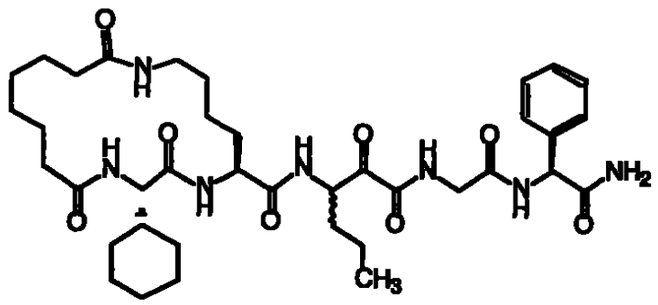

FmocHN–P
40a
FmocHN
40b
FmocHN
40c
FmocHN
40d
DdeHN
FmocHN
40e
DdeHN
FmocHN
40f


DdeHN
FmocHN
OH
N–P
CH3
40f
H2N
40g
40h
40i
40j
NH2
40

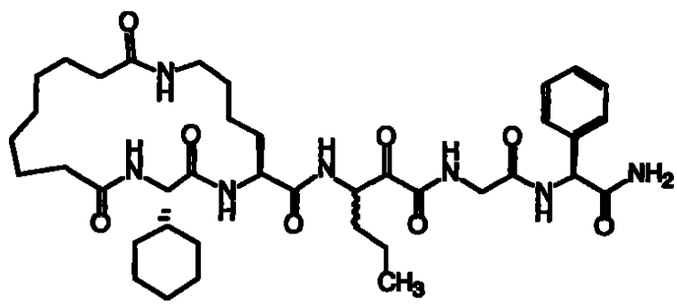
Fmoc-Sieber resin **40a** (0 035 mmol) was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-phenylglycine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0.24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound **40b** Resin-bound compound **40b** was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-glycine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0 24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound **40c** Resin-bound compound **40c** was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by N-Fmoc-propylisoserine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0 24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound **40d** Resin-bound compound **40d** was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-lysine(Dde) (0 12 mmol), HATU (0 12 mmol) and DIPEA (0.24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound **40e** Resin-bound compound **40e** was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-cyclohexylglycine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0 24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound **40f** Resin-bound compound **40f** was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min The resin was washed with 2 mL portions of DMF (4 times) to provide resin-bound compound **40g** Resin-bound compound **40g** was treated with 2 mL portions of a 2 % v/v solution of hydrazine

in DMF for 5 min (3 times) The resin was washed with 2 mL portions of DMF (4 times) to provide resin-bound compound **40h** Resin-bound compound **40h** was treated with 0 035 mmol of glutaric acid, 0 07 mmol of HATU and 0 14 mmol of DIPEA in 2 mL of DMF at room temperature for 16 h The resin was washed with 2 mL portions of DMF (4 times), THF (4 times) and DCM (4 times) to provide resin-bound compound **40i** Resin-bound compound **40i** was treated with a solution of 0 14 mmol of Dess-Martin periodinane and 0 14 mmol of t-BuOH in 2 mL of DCM at room temperature for 4 h The resin was washed with 2 mL portions of a 20 % v/v solution of IPrOH in DCM, THF, a 50 % v/v solution of THF in water (4 times), THF (4 times) and DCM (4 times) to provide resin-bound compound **40j** The resin-bound compound **40j** was treated with 4 mL of a 2 % v/v solution of TFA in DCM for 5 min The filtrate was added to 1 mL of AcOH and the solution was concentrated by vacuum centrifugation to provide compound **40** (0 0117 g, 48 % yield) MS (LCMS-Electrospray) 698 2 MH^+

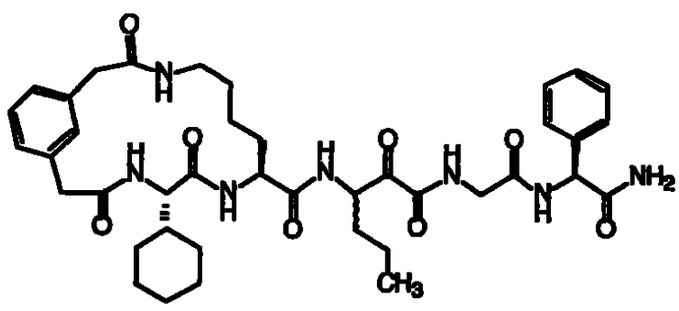
Example 41 – 53. Preparation of Compounds of Formula 41 to 53.



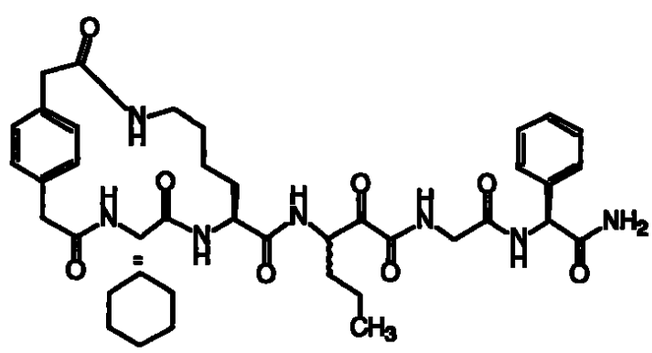
41



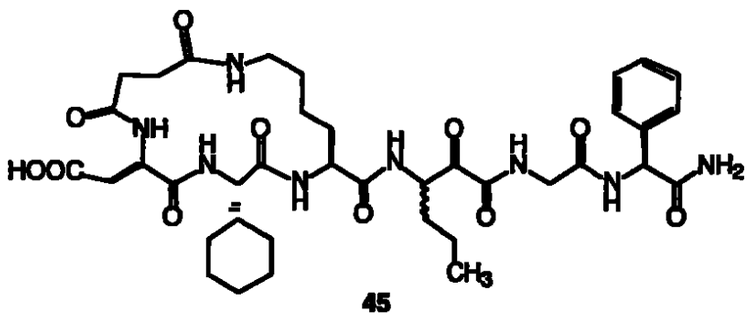
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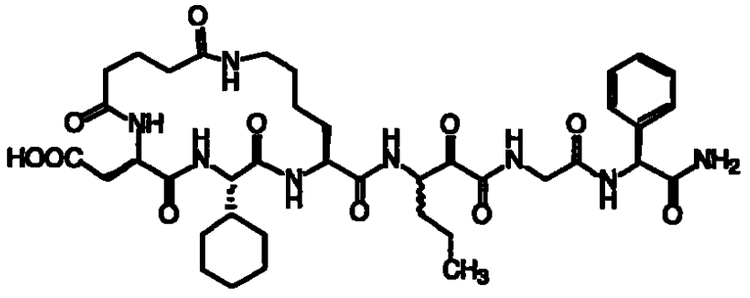
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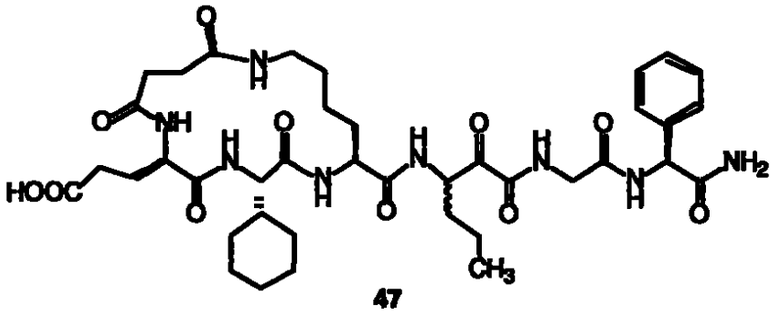
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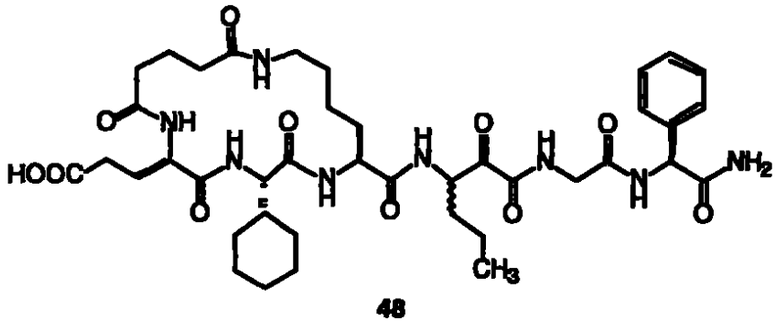
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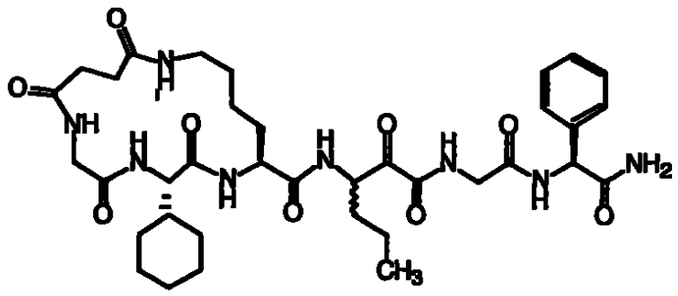
46



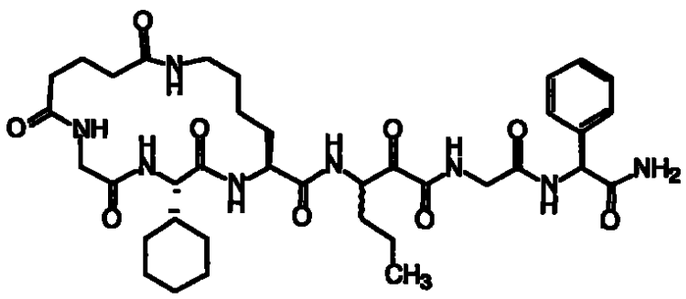
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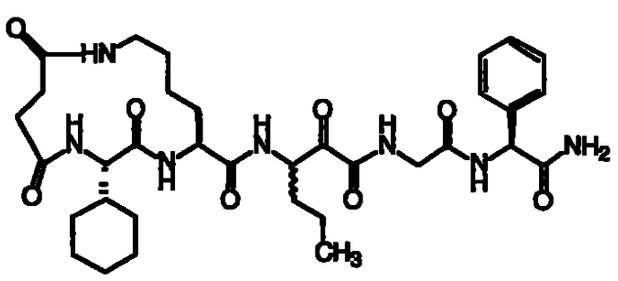
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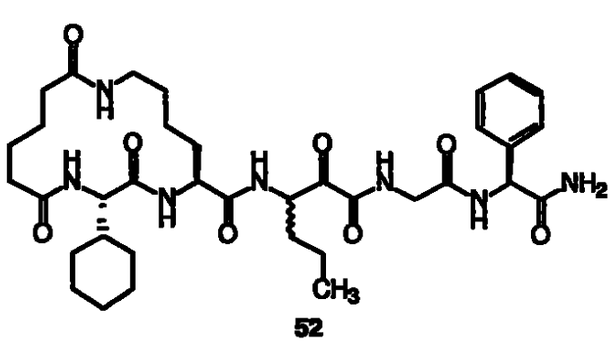
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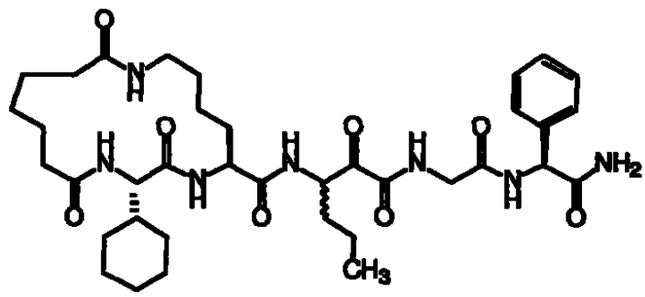
50



51



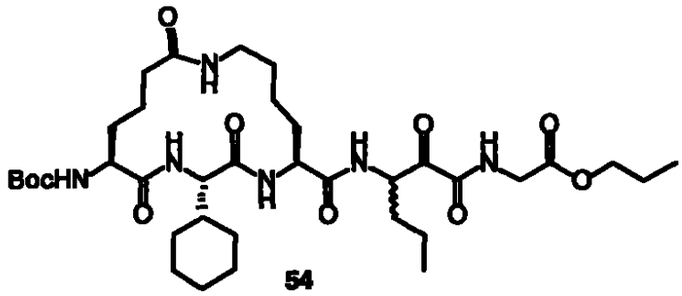
52



53

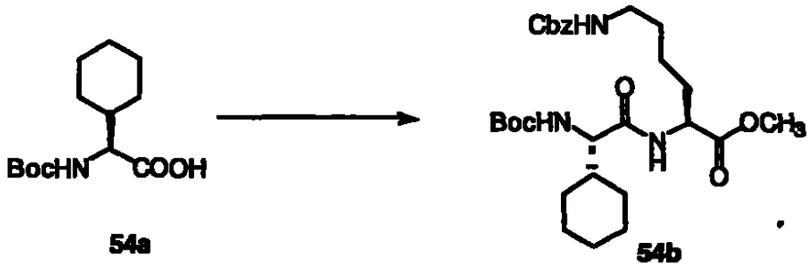
Compounds 41-53 were synthesized using solid phase methodology similar to the procedure outlined for the synthesis of Example 40

Example 54. Preparation of Compound of Formula 54.



54

Step A

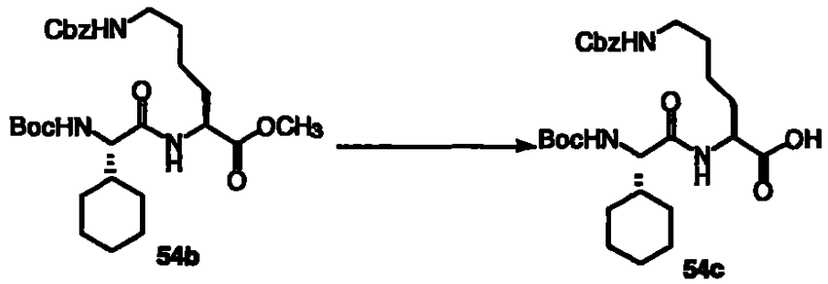


54a 54b

To a stirred solution of Boc-Cyclohexylglycine-OH (2 33 g, 9 07 mmol) in DMF (20 mL) and CH_2Cl_2 (20 mL) was added HOBT (1 48 g, 9 07 mmol), EDCI (1 91 g, 9 97 mmol) and NMM (2 99 mL, 27 2 mmol) The solution was stirred at –20°C for 10 minutes, followed by addition of H-Lys(Z)-OMe HCl and stirred for half an hour at –20°C and kept in freezer overnight The solution was then concentrated to dryness, followed by extraction with EtOAc, saturated $NaHCO_3$

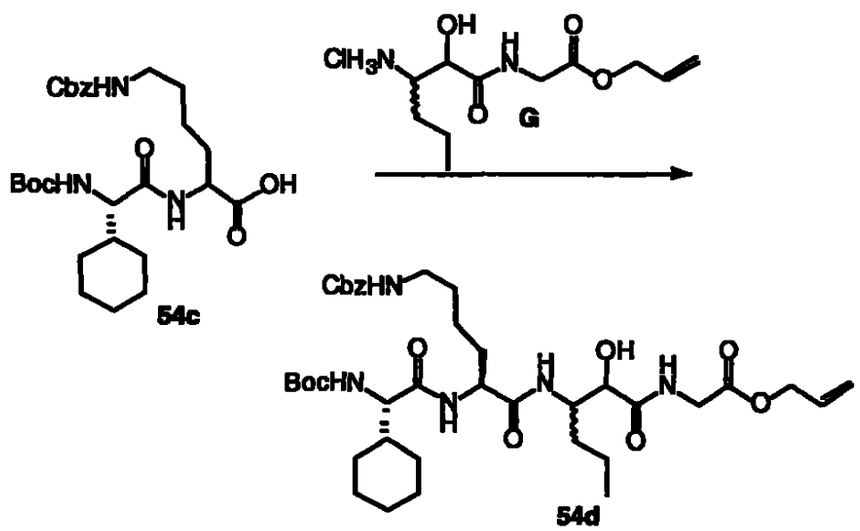
The combined organic layer was washed with H_2O, brine, dried over Na_2SO_4 and concentrated to dryness to give a white solid (4 83 g, MH^+=534 1)

Step B



To a stirred solution of **54b** (4 86 g, 8 76 mmol) in MeOH (10 mL) and H_2O (7 mL) was added LiOH (70 mg, 11.4 mmol) The white precipitate was formed and the solution was allowed to stir at room temperature overnight and then concentrated to dryness This crude material was then partitioned between CH_2Cl_2 and water The organic layer was separated and washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness to give **54c** (4 55 g, MH^+=520 1)

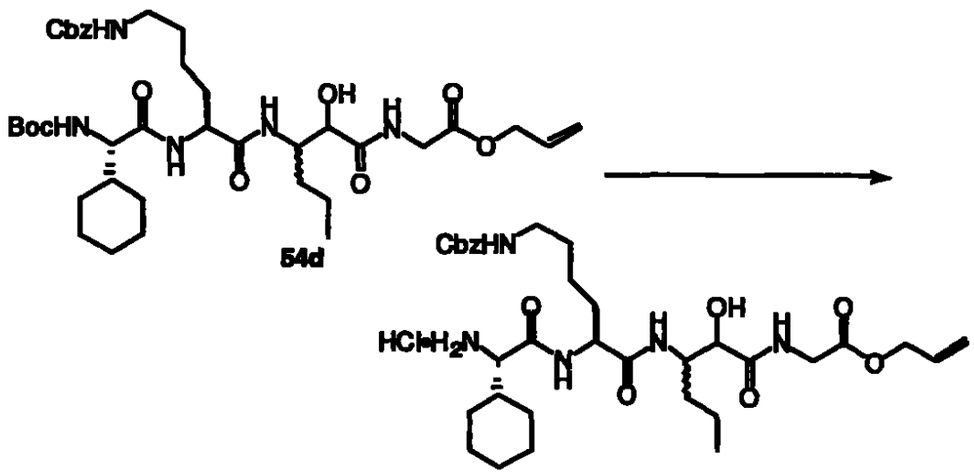
Step C



To a stirred cooling solution of **54c** (4 3 g, 8 27 mmol) in DMF (40 mL) and CH_2Cl_2 (40 mL) at –20 °C was added HOBT (1 35 g, 8 27 mmol), EDCI (1 74 g, 9 1 mmol) and NMM (2 73 mL, 8 27 mmol) The resulting solution was stirred at –20 °C for 10 minutes, followed by addition of amine **G** (2 32 g, 8 27 mmol) and

stirred at –20 °C for half an hour and kept in freezer overnight The work up procedure from step A was followed to give a **54d** (6 21 g, MH^+=746 2)

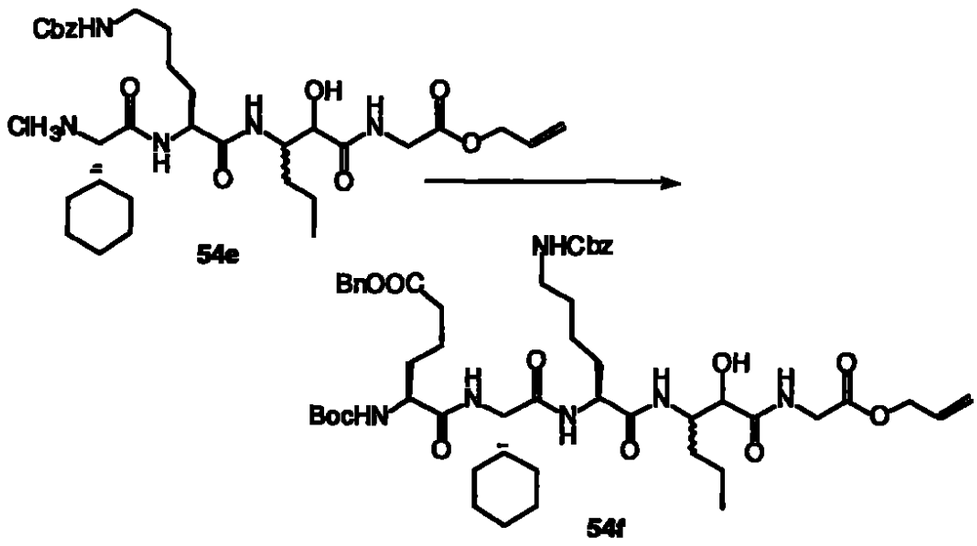
Step D.



54e

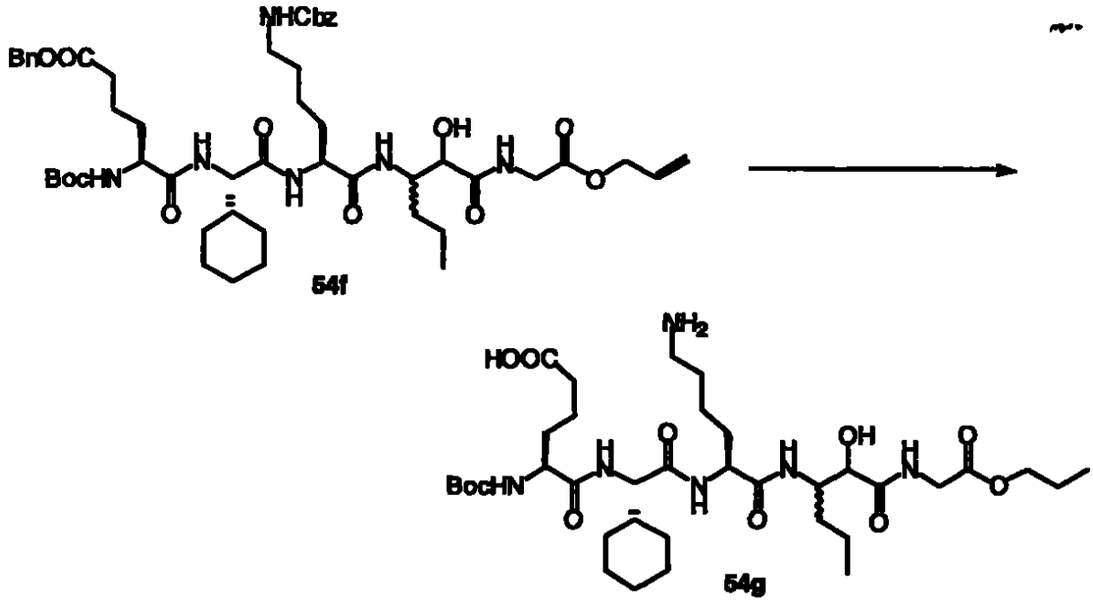
The solution of **54d** (6 16 g, 8 26 mmol) in 4N HCl/Dioxane (40 mL) was stirred at room temperature for 1 hr and concentrated to dryness to give a crude product **54e** (5 70 g, 100% yield, MH^+=646 3)

Step E



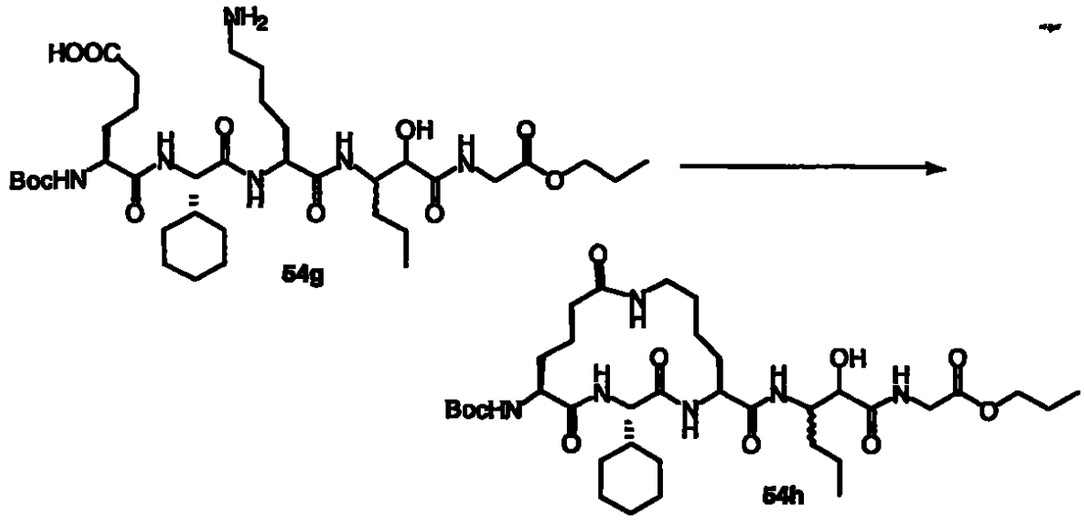
To a stirred cooling solution of Boc-Glu(OBn)-OH in DMF (25 mL) and CH_2Cl_2 (25 mL) at –20 °C, was added HOBT (1 29 g, 7 92 mmol), EDCI (1 66 g, 8 71 mmol) and NMM(2 61 mL, 23 7 mmol) The resulting solution was stirred for 10 minutes at –20°C, followed by addition **54e** (5 4 g, 7 916 mmol) and stirred for half an hour at -20°C and kept in freezer overnight Followed the work up procedure from step A to give a crude product (7 14 g, 93 5% yield)

Step F



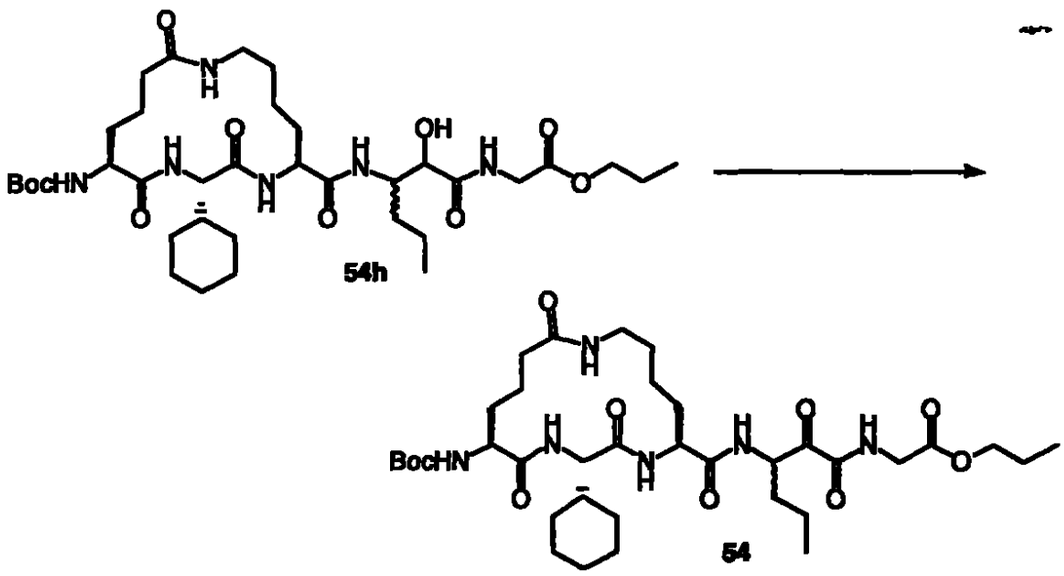
To a stirred solution of **54f** (6 9 g, 7 15 mmol) in absolute EtOH (350 mL), was added 10% Pd/C (2 8 g) in 50% H_2O (w/w) The resulting solution was purged with H_2 and stirred under H_2 balloon overnight The solution was then filtered through celite and the filtrate was washed with EtOH/CH_2Cl_2 and then concentrated to dryness to give a white solid (1 44 g) The solid was washed with 25% H_2O/ MeOH and filtered through sintered funnel, then freezed and lyophilized to give **54g** (4 12 g, 77 5% yield, MH^+=743 2)

Step G



To a stirred cooling solution of **54g** (0 5 g, 6 7 mmol) in DMF (50 mL) and CH_2Cl_2 (50 mL) at –20 °C, was added HOBT (0 219 g, 1 34 mmol), EDCI (0 271 g, 1 41 mmol) and NMM (0 296 mL, 2 69 mmol) The resulting solution was stirred at –20 °C for 25 minutes and then kept in freezer overnight The solution was concentrated to dryness, followed by extraction with EtOAc saturated $NaHCO_3$ The combined organic layer was then concentrated to dryness to give **54h** (254 mg, MH^+=725 2)

Step H



To a stirred solution of **54h** (0 2 g, 0 27 mmol) in anhydrous CH_2Cl_2 (20 mL), was added Dess-Martin periodinane (0 234 g, 0 55 mmol) The resulting solution was stirred at room temperature for 1 hr To this solution was added dropwise over half an hour the solution of H_2O (0 010 mL) in CH_2Cl_2 (20 mL) and stirred vigorously for additional 2 hrs The solution was then stirred for half an hour with 50% $Na_2S_2O_3$/50% sat $NaHCO_3$ The organic layer was separated and washed with H_2O, brine, dried over Na_2SO_4, filtered, concentrated to dryness and purified by column chromatography on silica gel, eluting with 10% MeOH/CH_2Cl_2 to yield **54** (17 mg, 62% MH^+=723 2)

Assay for HCV Protease Inhibitory Activity

Spectrophotometric Assay. Spectrophotometric assays for the HCV serine protease was performed on the inventive compounds by following the procedure described by R Zhang *et al*, *Analytical Biochemistry*, 270 (1999) 268-275, the disclosure of which is incorporated herein by reference The assay based on the proteolysis of chromogenic ester substrates is suitable for the continuous monitoring of HCV NS3 protease activity The substrates were derived from the P side of the NS5A-NS5B junction sequence (Ac-DTEDVVX(Nva), where X = A or P) whose C-terminal carboxyl groups were esterified with one of four different

chromophonc alcohols (3- or 4-nitrophenol, 7-hydroxy-4-methyl-coumann, or 4-phenylazophenol) Presented below are the synthesis, characterization and application of these novel spectrophotometric ester substrates to high throughput screening and detailed kinetic evaluation of HCV NS3 protease inhibitors

Materials and Methods.

Materials Chemical reagents for assay related buffers were obtained from Sigma Chemical Company (St. Louis, Missouri) Reagents for peptide synthesis were from Aldrich Chemicals, Novabiochem (San Diego, California), Applied Biosystems (Foster City, California) and Perseptive Biosystems (Framingham, Massachusetts) Peptides were synthesized manually or on an automated ABI model 431A synthesizer (from Applied Biosystems) UV/VIS Spectrometer model LAMBDA 12 was from Perkin Elmer (Norwalk, Connecticut) and 96-well UV plates were obtained from Corning (Corning, New York) The prewarming block was from USA Scientific (Ocala, Florida) and the 96-well plate vortexer was from Labline Instruments (Melrose Park, Illinois) A Spectramax Plus microtiter plate reader with monochrometer was obtained from Molecular Devices (Sunnyvale, California)

Enzyme Preparation. Recombinant heterodimeric HCV NS3/NS4A protease (strain 1a) was prepared by using the procedures published previously (D L. Sali *et al*, *Biochemistry*, 37 (1998) 3392-3401) Protein concentrations were determined by the Biorad dye method using recombinant HCV protease standards previously quantified by amino acid analysis Prior to assay initiation, the enzyme storage buffer (50 mM sodium phosphate pH 8 0, 300 mM NaCl, 10% glycerol, 0 05% lauryl maltoside and 10 mM DTT) was exchanged for the assay buffer (25 mM MOPS pH 6 5, 300 mM NaCl, 10% glycerol, 0 05% lauryl maltoside, 5 μM EDTA and 5 μM DTT) utilizing a Biorad Bio-Spin P-6 prepacked column

Substrate Synthesis and Purification The synthesis of the substrates was done as reported by R Zhang *et al*, (*ibid*) and was initiated by anchoring Fmoc-Nva-OH to 2-chlorotrityl chloride resin using a standard protocol (K. Barlos *et al*, *Int. J Pept Protein Res*, 37 (1991), 513-520) The peptides were subsequently

assembled, using Fmoc chemistry, either manually or on an automatic ABI model 431 peptide synthesizer The N-acetylated and fully protected peptide fragments were cleaved from the resin either by 10% acetic acid (HOAc) and 10% trifluoroethanol (TFE) in dichloromethane (DCM) for 30 min, or by 2% trifluoroacetic acid (TFA) in DCM for 10 min The combined filtrate and DCM wash was evaporated azeotropically (or repeatedly extracted by aqueous Na_2CO_3 solution) to remove the acid used in cleavage The DCM phase was dried over Na_2SO_4 and evaporated

The ester substrates were assembled using standard acid-alcohol coupling procedures (K Holmber *et al, Acta Chem Scand*, B33 (1979) 410-412) Peptide fragments were dissolved in anhydrous pyridine (30-60 mg/ml) to which 10 molar equivalents of chromophore and a catalytic amount (0 1 eq) of para-toluenesulfonic acid (pTSA) were added Dicyclohexylcarbodiimide (DCC, 3 eq) was added to initiate the coupling reactions Product formation was monitored by HPLC and found to be complete following 12-72 hour reaction at room temperature Pyridine solvent was evaporated under vacuum and further removed by azeotropic evaporation with toluene The peptide ester was deprotected with 95% TFA in DCM for two hours and extracted three times with anhydrous ethyl ether to remove excess chromophore The deprotected substrate was purified by reversed phase HPLC on a C3 or C8 column with a 30% to 60% acetonitrile gradient (using six column volumes) The overall yield following HPLC purification was approximately 20-30% The molecular mass was confirmed by electrospray ionization mass spectroscopy The substrates were stored in dry powder form under desiccation

Spectra of Substrates and Products Spectra of substrates and the corresponding chromophore products were obtained in the pH 6 5 assay buffer Extinction coefficients were determined at the optimal off-peak wavelength in 1-cm cuvettes (340 nm for 3-Np and HMC, 370 nm for PAP and 400 nm for 4-Np) using multiple dilutions The optimal off-peak wavelength was defined as that wavelength yielding the maximum fractional difference in absorbance between substrate and product (product OD - substrate OD)/substrate OD)

Protease Assay. HCV protease assays were performed at 30°C using a 200 µl reaction mix in a 96-well microtiter plate Assay buffer conditions (25 mM M PS pH 6 5, 300 mM NaCl, 10% glycerol, 0 05% lauryl maltoside, 5 µM EDTA and 5 µM DTT) were optimized for the NS3/NS4A heterodimer (D L Sali *et al*, *ibid*)) Typically, 150 µl mixtures of buffer, substrate and inhibitor were placed in wells (final concentration of DMSO • 4 % v/v) and allowed to preincubate at 30 °C for approximately 3 minutes Fifty µls of prewarmed protease (12 nM, 30°C) in assay buffer, was then used to initiate the reaction (final volume 200 µl) The plates were monitored over the length of the assay (60 minutes) for change in absorbance at the appropriate wavelength (340 nm for 3-Np and HMC, 370 nm for PAP, and 400 nm for 4-Np) using a Spectromax Plus microtiter plate reader equipped with a monochrometer (acceptable results can be obtained with plate readers that utilize cutoff filters) Proteolytic cleavage of the ester linkage between the Nva and the chromophore was monitored at the appropriate wavelength against a no enzyme blank as a control for non-enzymatic hydrolysis The evaluation of substrate kinetic parameters was performed over a 30-fold substrate concentration range (~6-200 µM) Initial velocities were determined using linear regression and kinetic constants were obtained by fitting the data to the Michaelis-Menten equation using non-linear regression analysis (Mac Curve Fit 1 1, K Raner) Turnover numbers (k_{cat}) were calculated assuming the enzyme was fully active

Evaluation of Inhibitors and Inactivators. The inhibition constants (K_i) for the competitive inhibitors Ac-D-(D-Gla)-L-I-(Cha)-C-OH (27), Ac-DTEDVVA(Nva)-OH and Ac-DTEDVVP(Nva)-OH were determined experimentally at fixed concentrations of enzyme and substrate by plotting v_o/v_i vs inhibitor concentration ($[I]_o$) according to the rearranged Michaelis-Menten equation for competitive inhibition kinetics $v_o/v_i = 1 + [I]_o/(K_i (1 + [S]_o/K_m))$, where v_o is the uninhibited initial velocity, v_i is the initial velocity in the presence of inhibitor at any given inhibitor concentration ($[I]_o$) and $[S]_o$ is the substrate concentration used Th resulting data were fitted using linear regression and the resulting slope, $1/(K_i(1+[S]_o/K_m)$, was used to calculate the K_i value

The obtained K_i values for the various macrocycles of the present invention are given in the afore-mentioned **Table 1** wherein the compounds have been arranged in the order of ranges of K_i values From these test results, it would be apparent to the skilled artisan that the compounds of the invention have excellent utility as NS3-serine protease inhibitors

Cell Bioassay Method: The cell bioassays for the HCV serine protease was performed on the inventive compounds by following the procedure described by S Agrawal *et al*, "Development and Characterization of Hepatitis C Virus Serine Protease Cell-based Trans-Cleavage Assay", *Hepatology* Supplement to Volume 30 (No 4, Part 2, October 1999), *Abstract No 615* (Proceedings of AASLD 50th Annual Meeting, Dallas, Texas, November 5-9, 1999), the disclosure of which is incorporated herein by reference The assay was performed in HeLa/Huh7 cells that were co-transfected with a plasmid that expresses a reporter protein substrate containing the NS5A/5B cleavage recognition sequence and an 1BNS4A $_{21-32}$ GS-GSNS $_{3-81}$ I17K expression vector and YFPn1 as a internal standard protein to control cytotoxicity Protease activity was measured by SDS-PAGE of total cell lysates followed by Western blot detection using a monoclonal antibody directed against the reporter substrate Quantitation of substrate cleavage was performed by scanning the immunoblot on the phosphoimager

Materials.

Plasmid DNAs

pBFP-5A/5B-GFP The reporter gene that expresses the substrate encodes a fusion protein comprised of an N' terminal blue fluorescent protein (BFP) domain and a C' terminal green fluorescent protein (GFP) domain, separated by a 25 amino acids derived from the NS5A/5B cleavage recognition sequence Both GFP and BFP are essentially homologous autofluorescent proteins that emit green or blue light, respectively, when excited by UV light of the appropriate wavelength Four amino acid substitutions in the chromophore of GFP alter the emission wavelength and convert the protein to BFP

The substrate and the resulting GFP and BFP products can be detected in cell lysates by immunologic methods using a monoclonal antibody that recognizes both proteins

The BFP-5A/5B-GFP reporter gene contains the BFP and GFP autofluorescent protein coding sequences (Quantum Biotechnologies, Inc , Montreal, Canada) separated by the NS5A/5B cleavage recognition sequence, cloned between the Nhe I and Bam HI restriction endonuclease sites of the pQBI25 cloning vector (Quantum Biotechnologies, Inc) Expression of the fusion protein is under the control of the CMV IE promoter-enhancer The bovine growth hormone p (A) sequence of the vector provides the polyadenylation signal for the mRNA The NS5A/5B cleavage sequence is SSGADTEDVVCCSMSYTWTGALVTP DNA sequencing was used to validate the clone

P1BOO2 1bNS4A21-32GS-GS NS 3-81 I17K The subtype 1b protease was cloned as an Xba1/Not1 fragment behind the CMV promoter in vector pC1neo

YFPn1: YFPn1 was purchased from CLONTECH (Palo Alto, California) Addition of third plasmid to the transfection supplies an internal standard protein to control for cytotoxicity and does not affect percentage of protease cleavage

Plasmid DNAs were maintained and propagated in DH5α cells (obtained from LifeTechnologies) in LB medium under the appropriate antibiotic selection, and purified using QIAfilter Plasmid Kits (Qiagen, Valencia, California)

Cell Culture:

HeLa cells were maintained and propagated in Eagle's Minimum Essential Media (EMEM, BioWhittaker, Walkersville, Maryland) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, and 100 u/ml penicillin-streptomycin (BioWhitaker), 2% $NaHCO_3$

Huh7 cells were maintained and propagated in Dulbecco's Modified Eagle's medium (DMEM, BioWhittaker) supplemented with 10% fetal calf serum (FCS), 100u/ml penicillin-streptomycin (BioWhitaker) and 5 ml NEAA (100x, BioWhittaker)/L

SOP Procedure

Day preceding transfection

HeLa cells were seeded in 24 well plates (Falcon 3047 plates) at a density of 6 x 10^4 cells/well and grown overnight at 37^{0}C in a 5% CO_2 incubator

Day of transfection

Plasmid DNAs were diluted to a final concentration of 0 05 μg/μl in nuclease free water (Promega, Madison, Wisconsin, cat # P119C) 0 75 μg BFP-5A/5B-GFP was combined and mixed with 0 175 μg P1B002 (0 23X) and 0 02μg of YFPn1 The DNAs were brought to a final volume of 60 μl with EMEM lacking FBS, glutamine, and antibiotics A ratio of 5 μl volumes of SuperFect Reagent (Qiagen, cat # 301305) per total μgs of DNA was added and the mixture vortexed about 10 seconds and incubated 10 min at room temperature to allow complex formation While complex formation was taking place, growth medium from cell culture plates was aspirated and cells washed 1X with 1 ml PBS without Ca^{2+}, Mg^{2+} (BioWhitaker) 350 μl EMEM (supplemented with appropriate suplements-compleat medium) was added to the tube containing the transfection complexes and the mixture pipetted up and down 2-3 times Total volume was transferred to one well of the 24 well culture plate The HeLa cells were incubated with the transfection complexes for about 3 hr at 37^{0}C and 5% CO2 The media containing the transfection complexes was removed from the cells by aspiration The cells were washed once in about 1 ml PBS, the PBS was aspirated and 495 μl of complete EMEM was added followed by 5 μl compound/well The cells were incubated 22-24 hr at 37^{0}C and 5% CO2

Preparation of Cell Lysates

The medium from each well was aspirated and washed once 1x with DPBS Cells were harvested in 100 μl of 1x Tris-SDS-BME sample buffer (OWL separation system, Portsmouth, New Hampshire, cat # ER33) and transferred to microcentrifuge tubes It was then boiled 3-5 min to lyse cells Loading was done at 10 μl/well on SDS-PAGE gel The lysates were resolved by electrophoresis on 10 cm x 10 cm 12 5% SDS-PAGE (Owl Scientific, cat # OG-0125B) run at 30 mamp in Tris-Glycine-SDS buffer (Owl Scientific) Prior to use, PVDF membrane

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(Immobilon-P, 45μm pore size, Millipore, Bedford, Massachusetts) was soaked in 100% methanol for 10 seconds and then the blot was placed in distilled water. The proteins were transferred to PVDF filter membranes (0 45μm, Millipore) at 108 mamp per gel for 90 minutes using a semi-dry electroblotter

Detection of Proteins by ECF Western Blot (Amersham Pharmacia Biotech, Little Chalfont, England), catalog #RPN 5780) The PVDF filter membranes were blocked by 5% blocking reagent (from kit) in ~10 ml PBS containing 0 05% Tween 20, pH 7 4 (Sigma Chemicals, St. Louis, Missouri, cat # 3563) for overnight at 2-4 C in refrigerator The next day, the membranes were rinsed briefly twice with TPBS containing 0 05% Tween 20 washing buffer, then washed three times each time 5 min in PBS containing 0 05% Tween 20, pH 7 4 The membranes were incubated in 12 mls of a 1 3000 dilution of anti-GFP monoclonal antibody for 30 minutes (Clontech, Palo Alto, California) in PBS containing 0 05% Tween 20, pH7.4 while at the same time 1% BSA (Albumin, bovine cat # A-2153 from Sigma) was added to reduce background The membranes were washed briefly twice with TPBS, then thrice, for 5 min each time, in TPBS washing buffer The membranes were incubated in 12 mls of a 1 600 dilution anti fluorescein-linked anti mouse Ig in TPBS for 30 minutes The membranes were washed briefly with TPBS twice, then for 5 min in TPBS washing buffer thrice For signal amplification with ECF substrate membranes were incubated in 10 ml of 1.2500 anti fluorescein alkaline phosphatase conjugate for 30 minutes The membranes were rinsed briefly with TPBS twice, then 5 min in TPBS washing buffer thrice The ECF substrate solution was prepared as per manufacturer's instructions (aliquot and freeze), membranes were incubated for 2-3 minutes, excess reagent was drained off, then were blotted with filter papers, air-dried for 9-10 minutes and then scanned

Scanning the membrane The blot was placed on the glass of phosphorimager Storm 860 The blue chemiluminiescent was set up, 200 pixcels size, 700 PMT voltage The file was opened in ImageQuant and quantitated by creating squares around the bands representing the substrate (S), the product (P) and the internal control (IC) The % cleavage of the substrate was measured as P/(S+P)x100

The inhibition in cleavage due to drug was measured compared duplicate to drug controls included on each blot. A report was created in Excel The results for some of the compounds are given below

Compound of Example 36 EC_{50} = 9μm

Compound of Example 35 EC_{50} = 20μm

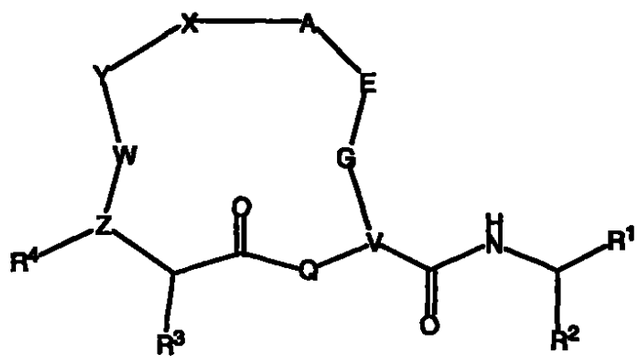
From these test results, it would be apparent to the skilled artisan that the compounds of the invention have excellent utility as NS3-serine protease inhibitors

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Claims

What is claimed is

1 A macrocyclic compound, including enantiomers, stereoisomers, rotomers and tautomers of said compound, and pharmaceutically acceptable salts or solvates of said compound having the general structure shown in Formula I



Formula I

wherein

E, X and Y may be independently present or absent, and if present are independently selected from the moieties alkyl, aryl, alkyl-aryl, heteroalkyl, heteroaryl, aryl-heteroaryl, alkyl-heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, aryl ether, alkyl amino, aryl amino, alkyl-aryl amino, alkyl sulfide, alkyl-aryl sulfide, aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, aryl sulfone, alkyl-alkyl sulfoxide, alkyl-aryl sulfoxide, alkyl amide, alkyl-aryl amide, aryl amide, alkyl sulfonamide, alkyl-aryl sulfonamide, aryl sulfonamide, alkyl urea, alkyl-aryl urea, aryl urea, alkyl carbamate, alkyl-aryl carbamate, aryl carbamate, alkyl -hydrazide, alkyl-aryl hydrazide, alkyl hydroxamide, alkyl-aryl hydroxamide, alkyl sulfonyl, aryl sulfonyl, heteroalkyl sulfonyl, heteroaryl sulfonyl, alkyl carbonyl, aryl carbonyl, heteroalkyl carbonyl, heteroaryl carbonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl or a combination thereof, with the proviso that E, X and Y may optionally be additionally substituted with moieties selected from the group consisting of aromatic, alkyl, alkyl-aryl, heteroalkyl, aryl-heteroaryl, alkyl-

heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, alkyl sulfide, alkyl-aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, alkyl amide, alkyl-aryl amide, alkyl sulfonamide, , alkyl amines, alkyl-aryl amines, alkyl-aryl sulfonamide, alkyl urea, alkyl-aryl urea, alkyl carbamate, alkyl-aryl carbamate, halogen, hydroxyl amino, alkyl carbazate, aryl carbazate,

$R^1 = COR^5$ or $B(OR)_2$, wherein R^5 = H, OH, OR^9, NR^9R^{10}, CF_3, C_2F_5, C_3F_7, CF_2R^6, R^6, COR^7 wherein R^7 = H, OH, OR^9, CHR^9R^{10}, or NR^9R^{10}, wherein R^6, R^9, R^9 and R^{10} are independently selected from the group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, cycloalkyl, arylalkyl, heteroarylalkyl, $CH(R^1)COOR^{11}$, $CH(R^1)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)R'$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONHCH(R^5)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONHCH(R^5)\ CONR^{12}R^{13}$, wherein R^1, R^2, R^3, R^4, R^5, R^{11}, R^{12}, R^{13}, and R' are independently selected from a group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, alkyl-aryl, alkyl-heteroaryl, aryl-alkyl and heteroaralkyl,

Z is selected from O, N, or CH,

W may be present or absent, and if W is present, W is selected from C=O, C=S, SO_2 or C=NR,

Q is $(NR)_p$, O, S, CH_2, CHR, CRR' or a double bond towards V,

A is O, CH_2, $(CHR)_p$, $(CHR\text{-}CHR')_p$ $(CRR')_p$, NR, S, SO_2, C=O or a bond,

G is $(CH_2)_p$, $(CHR)_p$, $(CRR')_p$, NR, O, S, SO_2, $S(O)_2NH$, C=O, or a double bond towards E or V,

V is CH, CR or N,

p is a number from 0 to 6, and

R, R', R^2, R^3 and R^4 are independently selected from the group consisting of H, C1-C10 alkyl, C2-C10 alkenyl, C3-C8 cycloalkyl, C3-C8 heterocycloalkyl,

aryl, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, heteroaryl, alkyl-aryl, alkyl-heteroaryl, (cycloalkyl)alkyl and (heterocycloalkyl)alkyl, wherein said cycloalkyl is made of three to eight carbon atoms, and zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of one to six carbon atoms,
with said alkyl, heteroalkyl, alkenyl, heteroalkenyl, aryl, heteroaryl, cycloalkyl and heterocycloalkyl moieties may be optionally substituted, with said term "substituted" referring to optional and suitable substitution with one or more moieties selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, heterocyclic, halogen, hydroxy, thio, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, sulfonamide, sulfoxide, sulfone, sulfonyl urea, hydrazide, hydroxamate and thiourea

2 The compound of claim 1, wherein $R^1 = COR^5$, and R^5 is H, OH, $COOR^9$, or $CONR^9R^{10}$

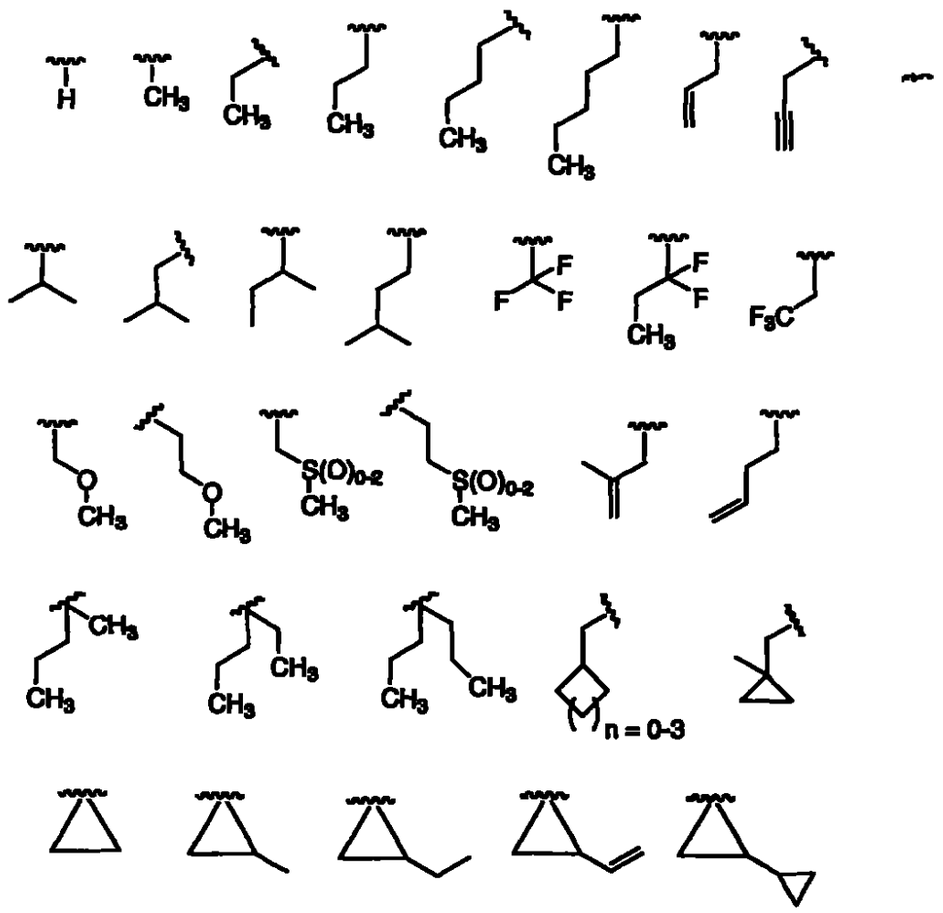
3 The compound of claim 2, wherein $R^1 = COCONR^9R^{10}$, and is R^9 is H, R^{10} is H, $CH(R^7)COOR^{11}$, $CH(R^7)$ $CONR^{12}R^{13}$, $CH(R^7)CONHCH(R^2)COOR^{11}$, $CH(R^7)CONHCH(R^2)$ $CONR^{12}R^{13}$, or $CH(R^7)CONHCH(R^2)(R')$

4 The compound of claim 3, wherein $R^{10} = CH(R^7)CONHCH(R^2)COOR^{11}$, $CH(R^7)CONHCH(R^2)$ $CONR^{12}R^{13}$, or $CH(R^7)CONHCH(R^2)(R')$, wherein R^7 is H or alkyl, and R^2 is selected from the group consisting of phenyl, substituted phenyl, hetero atom-substituted phenyl, thiophenyl, cyclohexyl, cyclopentyl, cyclopropyl, piperidyl pyridyl and 2-indanyl

5 The compound of claim 4, wherein R^7 is H

6 The compound of claim 5, wherein R^2 = phenyl, thiophenyl, cyclohexyl, 2-indanyl, cyclopentyl, pyridyl, phenyl(4-$HNSO_2NH_2$), R^{11} is H or *tert*-butyl, R^{12} and R^{13} are methyl, and R' is hydroxymethyl or tert-butoxymethyl

7 The compound of claim 1, wherein R^2 is selected from the group consisting of the following moieties



8 The compound of claim 7 wherein R^1 = COR^5, and R^5 is H, OH, $COOR^5$, or $CONR^9R^{10}$

9 The compound of claim 8 wherein V is CH

10 The compound of claim 9 wherein Q is NR or O

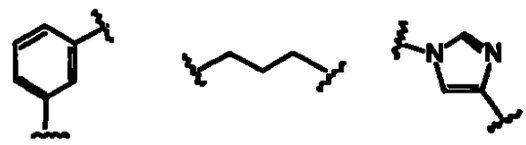
11 The compound of claim 10 wherein G is CH_2

12 The compound of claim 11 wherein A is O, NR, CH=CH or CH_2

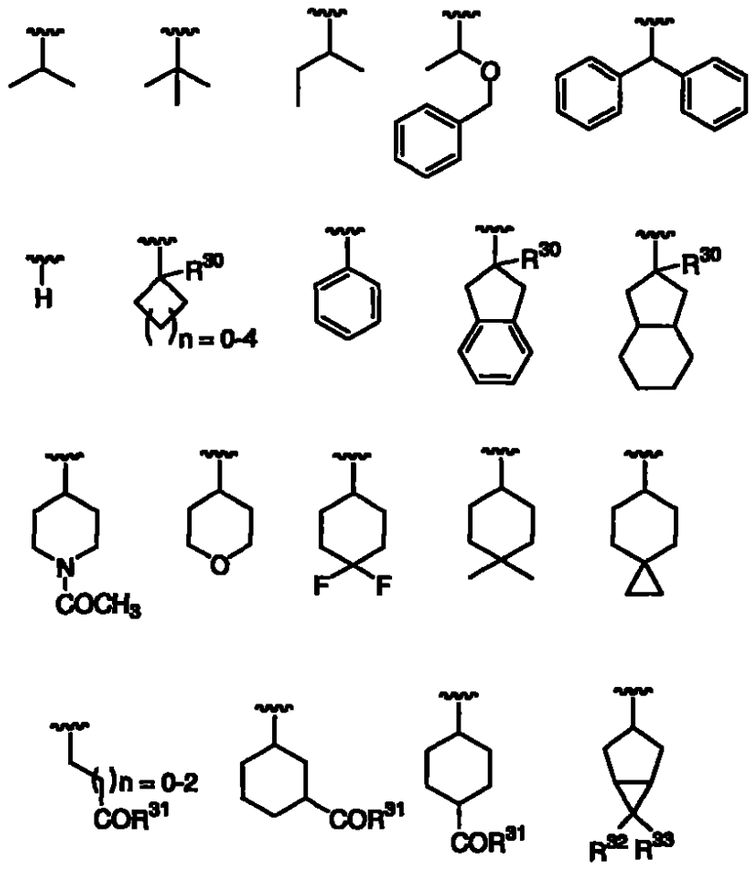
13 The compound of claim 12 wherein E is alkyl, aryl, hereroalkyl, heteroaryl, alkyl, aryl, or cycloalkyl

14 The compound of claim 13 wherein E is selected from the group consisting of the moieties

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15 The compound of claim 14 wherein R^3 is selected from the group consisting of the moieties



wherein R^{30} = H, CH_3 or other alkyl groups,

R^{31} = OH, O-alkyl, NH_2, N-alkyl, and

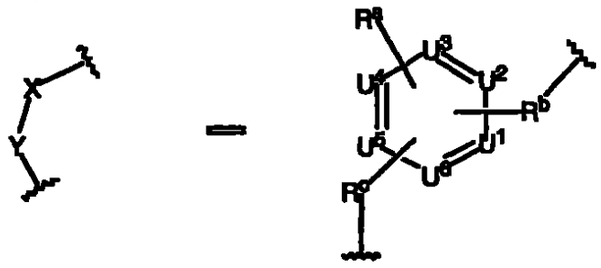
R^{32} and R^{33} may be the same or different and are selected independently from H, F, Cl, Br and CH_3

16 The compound of claim 15 wherein Z = N and R^4 = H

17 The compound of claim 16 wherein W is C=O

18 The compound of claim 17 wherein the moiety X-Y is selected from the group consisting of C1-C10 alkyl, alkyl, cycloalkyl, heteroalkyl, arylalkyl, aryl, hereroaryl and alkylaryl

19 The compound of claim 18, wherein



wherein R^b is connected directly to A and R^c is connected directly to W, and the moiety U^1, U^2, U^3, U^4, U^5 and U^6 form either a six membered carbon ring, or a five or six membered ring with one or more heteroatoms,

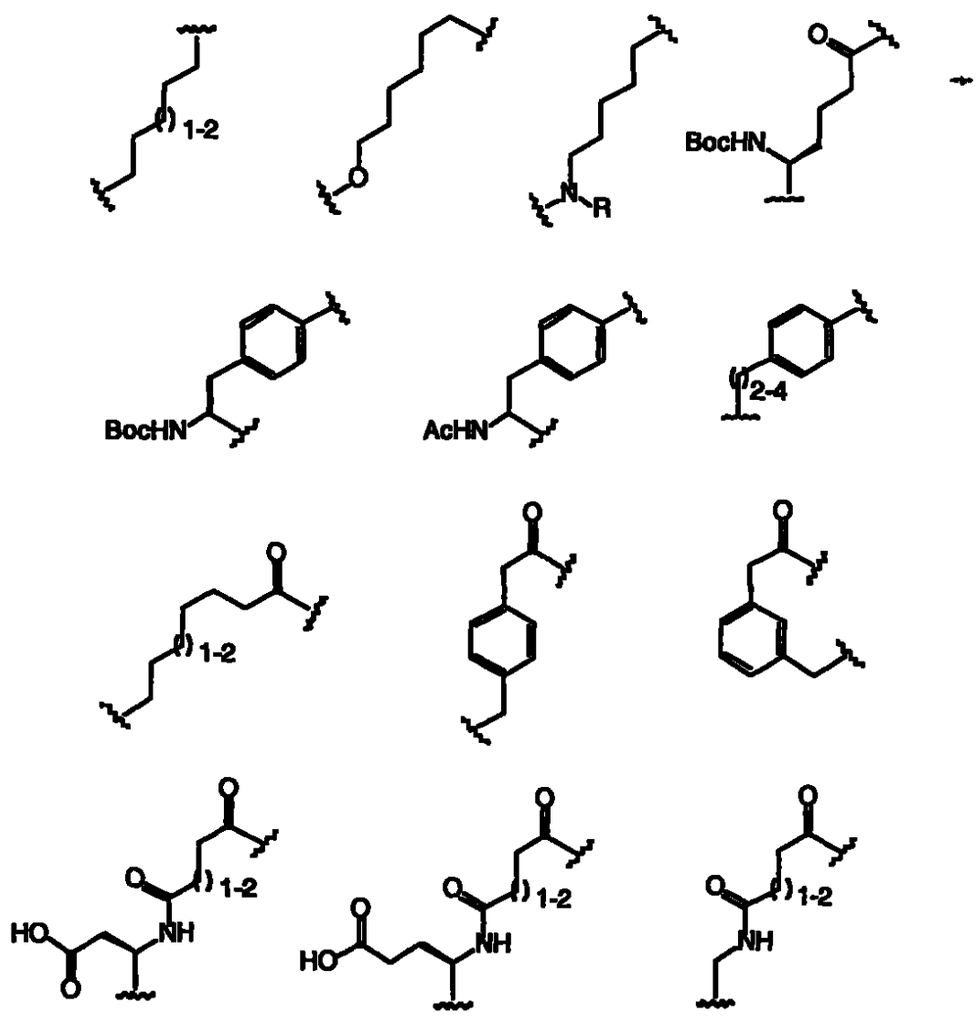
R^a = H, alkyl, alkoxy, hydroxy, alkylthio, halogen, nitro, cyano, carboxylic acid, ester, amide, amino, nitrile, or CF_3,

R^b is a bond, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, O, S, SO_2, NH, O(alkyl), S(alkyl), SO_2(alkyl) or N(alkyl), and

R^c is a bond, C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, O, S, SO_2, NH, O(alkyl), S(alkyl), SO_2(alkyl), N(alkyl) or CH_2-N(alkyl) with the CH_2 being linked to the aromatic ring

20 The compound of claim 18 wherein the moiety X-Y is selected from the group consisting of the following structures

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21 A pharmaceutical composition comprising as an active ingredient a compound of claim 1

22 The pharmaceutical composition of claim 21 for use in treating disorders associated with Hepatitis C virus

23 The pharmaceutical composition of claim 21 additionally comprising a pharmaceutically acceptable carrier

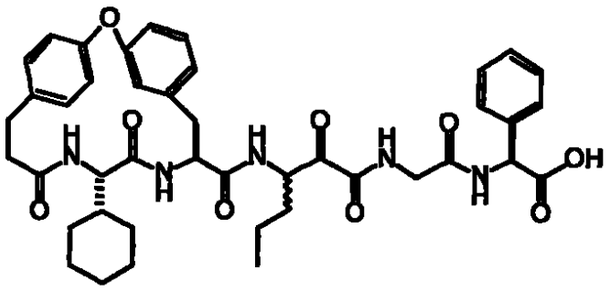
24 A method of treating disorders associated with the HCV protease, said method comprising administering to a patient in need of such treatment a pharmaceutical composition which composition comprises therapeutically effective amounts of a compound of claim 1

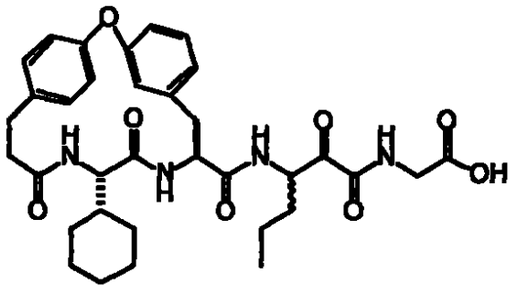
25 The use of a compound of claim 1 for the manufacture of a medicament to treat disorders associated with the HCV protease

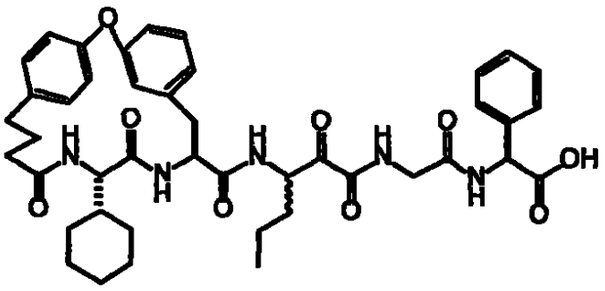
26 A method of preparing a pharmaceutical composition for treating disorders associated with the HCV protease, said method comprising bringing into intimate contact a compound of claim 1 and a pharmaceutically acceptable carrier

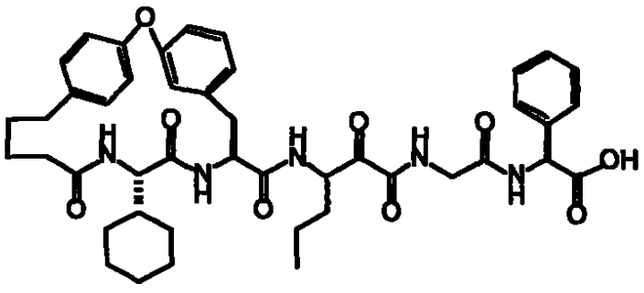
27 A compound exhibiting HCV protease inhibitory activity, including enantiomers, stereoisomers, rotamers and tautomers of said compound, and pharmaceutically acceptable salts or solvates of said compound, said compound being selected from the group of compounds with structures listed below

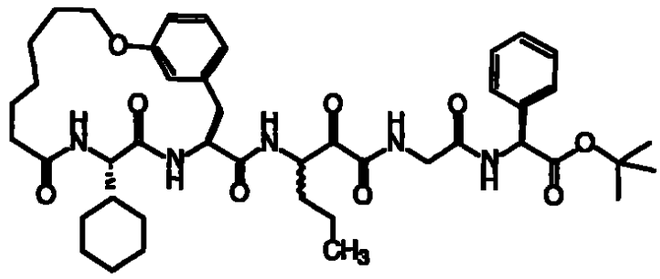
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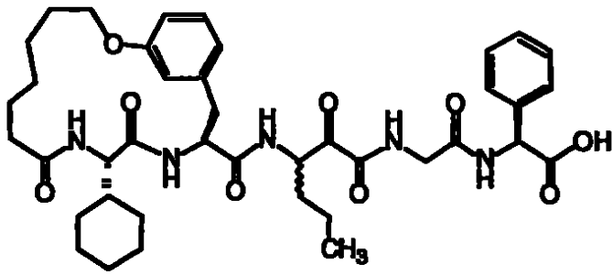


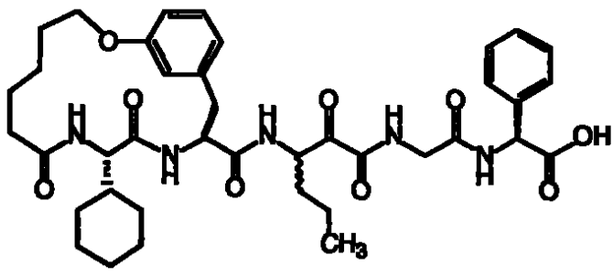


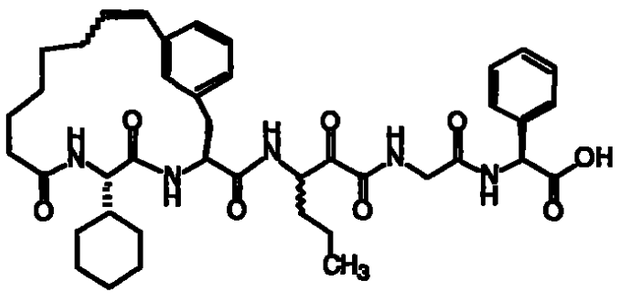


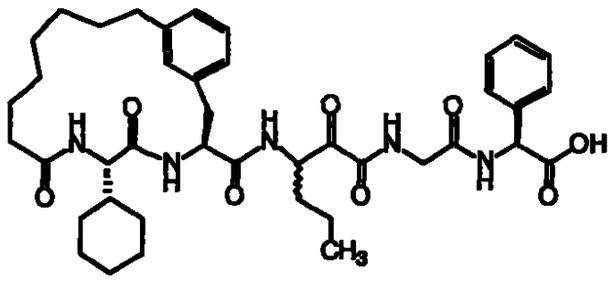


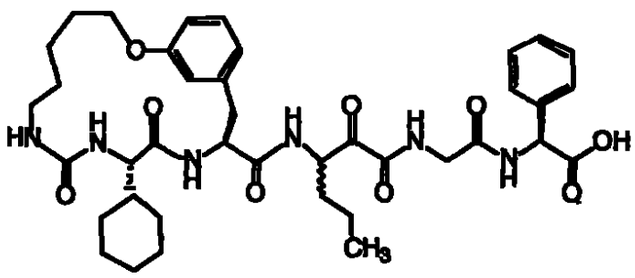


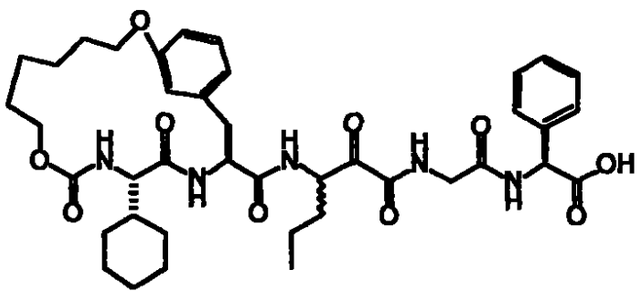


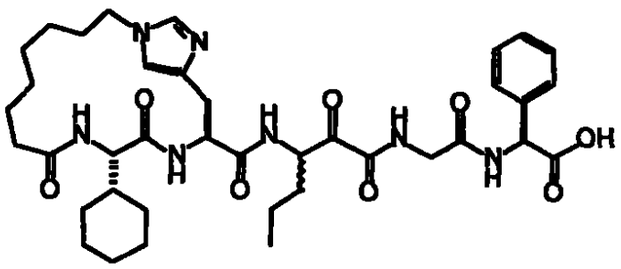


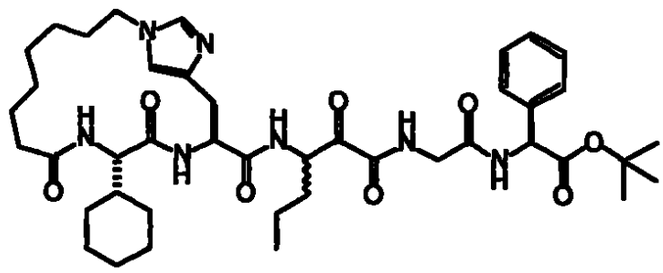


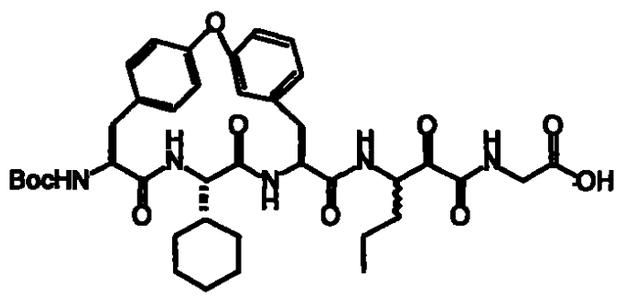


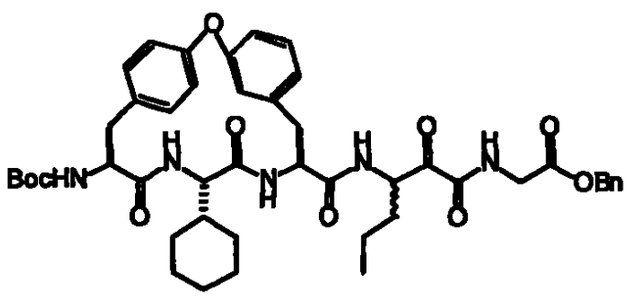


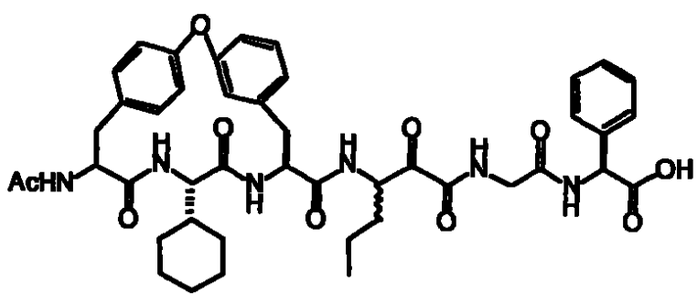


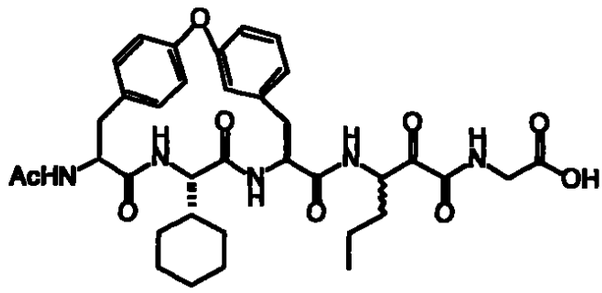


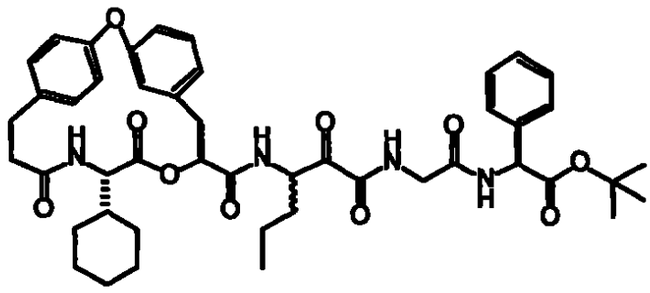


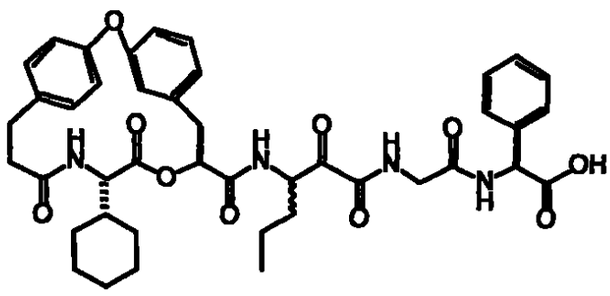

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OH

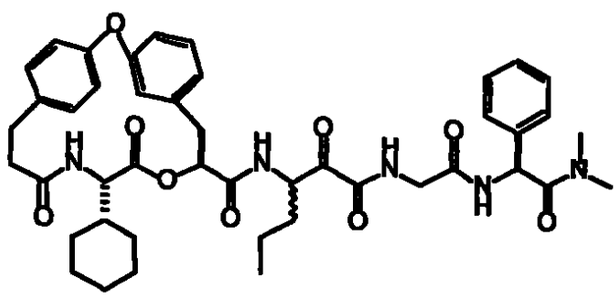

BocHN
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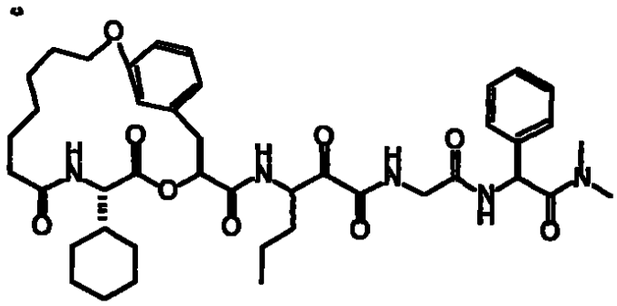

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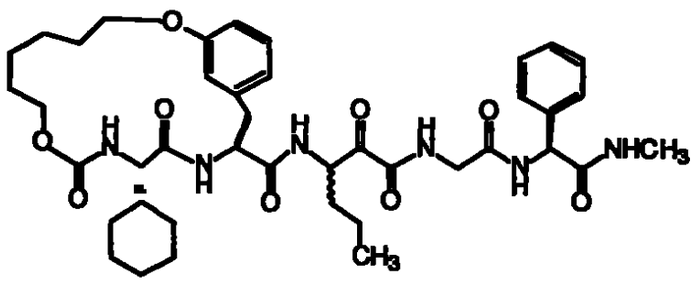

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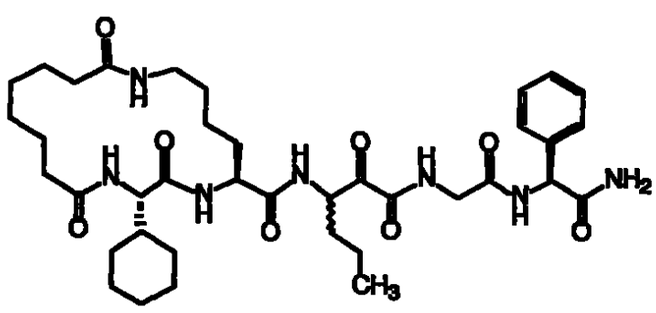


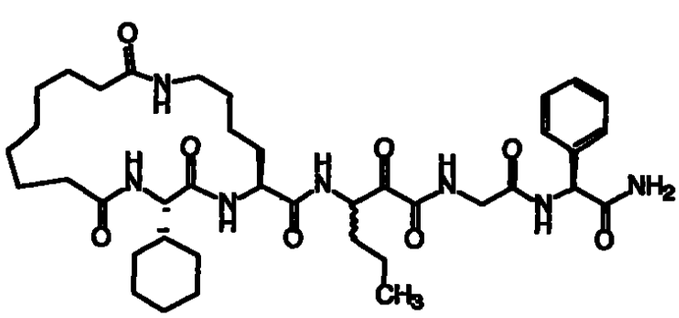

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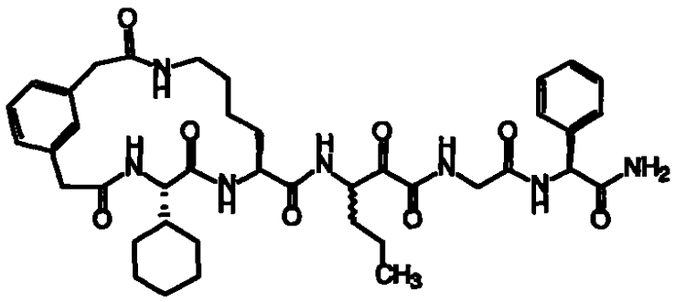


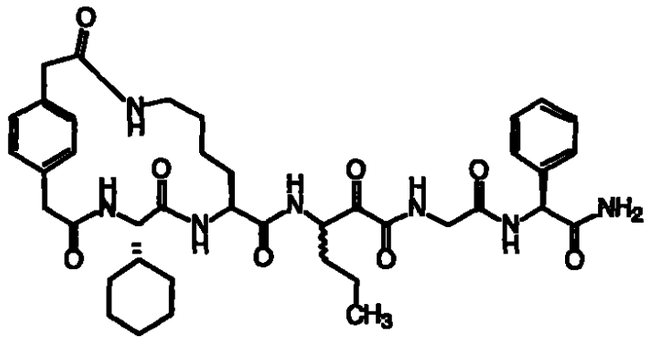


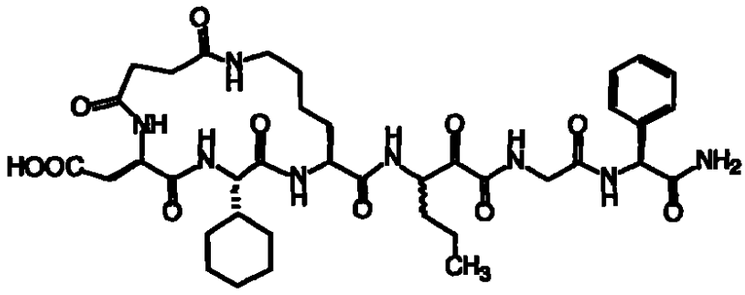

NHCH3
CH3

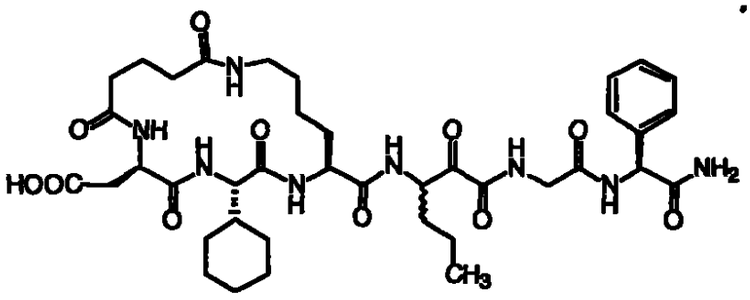

NH2
CH3

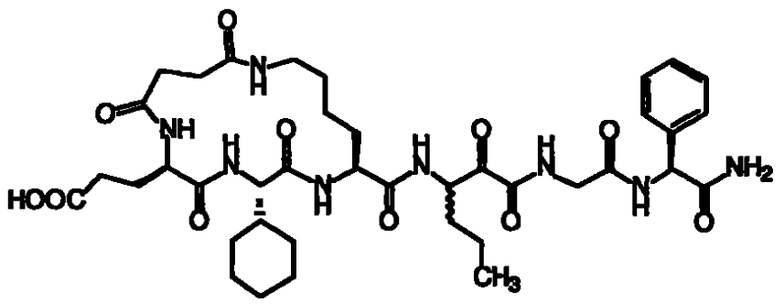

NH2
CH3

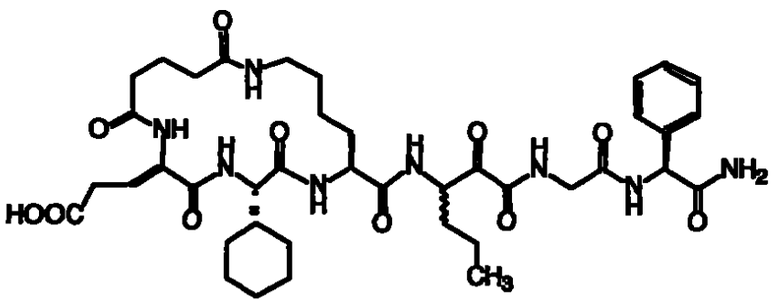


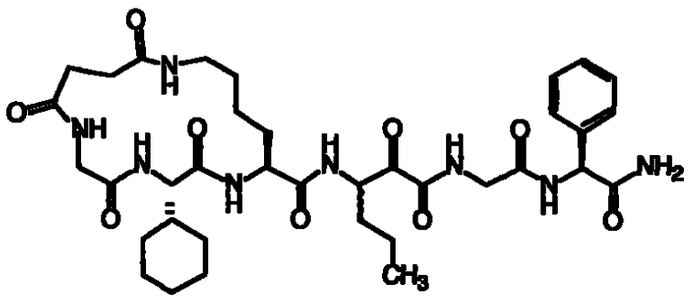


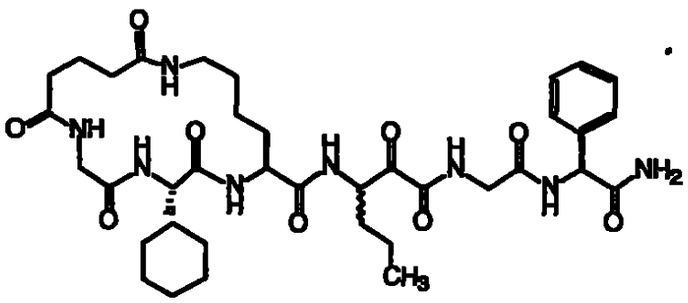












28 A pharmaceutical composition for treating disorders associated with the HCV protease, said composition comprising therapeutically effective amount of one or more compounds in claim 27 and a pharmaceutically acceptable carrier

29 The pharmaceutical composition of claim 28, additionally containing an antiviral agent

30 The pharmaceutical composition of claim 28 or claim 29, still additionally containing an interferon

31 The pharmaceutical composition of claim 30, wherein said antiviral agent is ribavirin and said interferon is α-interferon

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(84) Designated States *(regional)* ARIPO patent (GH GM KE, LS MW MZ, SD SL, SZ, TZ, UG ZW) Eurasian patent (AM AZ, BY KG KZ, MD RU TJ TM) European patent (AT BE, CH CY DE, DK, ES, FI FR GB GR, IE, IT LU MC NL, PT SE, TR), OAPI patent (BF BJ CF CG CI CM GA GN GW ML, MR NE, SN TD TG).

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1 August 2002

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(54) Title MACROCYCLIC NS3-SERINE PROTEASE INHIBITORS OF HEPATITIS C VIRUS COMPRISING ALKYL AND ARYL ALANINE P2 MOIETIES

(57) Abstract The present invention discloses novel macrocyclic compounds which have HCV protease inhibitory activity as well as methods for preparing such compounds. In another embodiment, the invention discloses pharmaceutical compositions comprising such macrocycles as well as methods of using them to treat disorders associated with the HCV protease.

WO 01/081325 A3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/12530

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K7/06 C07K14/18 C07K5/062 C07K5/065 C07K5/087
C07K5/107 A61K38/05 A61K38/07 A61K38/08 A61P31/14

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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)
IPC 7 C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 64442 A (ANGELETTI P IST RICHERCHE BIO ,MARCHETTI ANTONELLA (IT), ONTORIA J) 16 December 1999 (1999-12-16) claims, example 2C	1-28
X	MARCHETTI A ET AL "SYNTHESIS OF TWO NOVEL CYCLIC BIPHENYL ETHER ANALOGS OF AN INHIBITOR OF HCV NS3 PROTEASE" SYNLETT, THIEME VERLAG, STUTTGART, DE, no S01, June 1999 (1999-06), pages 1000-1002, XP008002749 ISSN 0936-5214 cited in the application the whole document	1-28

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

[X] Patent family members are listed in annex.

* Special categories of cited documents
A document defining the general state of the art which is not considered to be of particular relevance
E earlier document but published on or after the international filing date
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
O document referring to an oral disclosure use exhibition or other means
P document published prior to the international filing date but later than the priority date claimed
T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
& document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2002	04/06/2002

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuhr, C

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 09558 A (BOEHRINGER INGELHEIM CA LTD ,GOUDREAU NATHALIE (CA), GHIRO ELISE () 24 February 2000 (2000-02-24) cited in the application claims, examples	1-28
A	WO 99 07734 A (BAILEY MURRAY DOUGLAS ,BOEHRINGER INGELHEIM CA LTD (CA), HALMOS TE) 18 February 1999 (1999-02-18) claims, examples	1-28

International Application No. PCT/US 01 /12530

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I 2

Present claims 1-26 relate to an extremely large number of possible compounds, products and methods Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds, products and methods claimed In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds, products and methods prepared in the examples and closely related homologous compounds

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66 1(e) PCT) The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure

INTERNATI N/ 3EARCH REPORT

Information on patent family members

Inte[illegible]onal Application No
PCT/US 01/12530

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9964442 A	16-12-1999	AU 4279899 A	30-12-1999
		EP 1084137 A1	21-03-2001
		WO 9964442 A1	16-12-1999
WO 0009558 A	24-02-2000	AU 5273299 A	06-03-2000
		BG 105230 A	31-10-2001
		BR 9912943 A	08-05-2001
		WO 0009558 A1	24-02-2000
		CN 1315965 T	03-10-2001
		EP 1105422 A1	13-06-2001
		NO 20010604 A	05-02-2001
		PL 346051 A1	14-01-2002
		TR 200100438 T2	21-06-2001
WO 9907734 A	18-02-1999	AU 8846698 A	01-03-1999
		WO 9907734 A2	18-02-1999
		EP 1012180 A2	28-06-2000
		HU 0100100 A2	28-11-2001
		JP 2001512744 T	28-08-2001
		US 6143715 A	07-11-2000

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Memoria Descriptiva

de la

Patente de Invención

Sobre

"INHIBIDORES MACROCICLICOS DE LA SERINA PROTEASA NS-3 DEL VIRUS DE LA HEPATITIS C QUE COMPRENDEN PORCIONES ALQUIL Y ARILALANINA P2"

Solicitada por:

SCHERING CORPORATION, *residente en* ***2000 Galloping Hill Road, Kenilworth, Nueva Jersey 07033-0530, E U DE NORTEAMERICA***

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Inhibidores Macrocíclicos de la Serina Proteasa NS-3 del Virus de la Hepatitis C que comprenden porciones alquil y arilalanina P2

Campo de la Invención

La presente invención se relaciona con novedosos inhibidores de la proteasa del virus de la hepatitis C ("VHC"), con composiciones farmacéuticas que contienen uno o más de tales inhibidores con métodos para preparar dichos inhibidores y con métodos para usar dichos inhibidores en el tratamiento de la hepatitis C y trastornos relacionados Esta invención describe específicamente novedosos compuestos macrocíclicos como inhibidores de la serina proteasa NS3/NS4a del VHC La presente memoria descriptiva se relaciona con la de la solicitud de patente en tramite, Acta No _____, presentada el 5 de abril de 2000

Antecedentes de la Invención

El virus de la Hepatitis C (VHC) es un ARN virus de cadena simple de sentido (+) al que se ha identificado como principal agente causante en la hepatitis no A ni B (HNANB), especialmente en la NANBH asociada con la sangre (BB-NANHB) (ver Publicacion de Solicitud de Patente Internacional No WO 89/04669 y la Publicación de Solicitud de patente europea No EP 381 216) Se debe distinguir a la HNANB de otros tipos de enfermedad hepática inducida por virus, como el virus de la hepatitis A (VHA), el virus de la hepatitis B (HBV), el virus de la hepatitis delta (VHD), el citomegalovirus (CMV) y el virus de Epstein-Barr (VEB), así como de otras formas de enfermedad hepatica tal como el alcoholismo y la cirrosis biliar primaria

Recientemente se ha identificado, clonado y expresado una VHC proteasa necesaria para el procesamiento de polipéptidos y la replicacion viral (ver, por ejemplo, la patente de los Estados Unidos No 5 712 145) Esta poliproteína de aproximadamente 3000 aminoácidos contiene, del término amino al término

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carboxi, una proteina de nucleocápside (C), proteinas de envoltura (E1 y E2) y varias proteinas no estructurales (NS1, 2, 3, 4a, 5a y 5b) La NS3 es una proteina de aproximadamente 68 kDa, codificada por alrededor de 1893 nucleótidos del genoma del VHC, y tiene dos dominios diferenciados (a) un dominio de serina proteasa que consiste en aproximadamente 200 de los aminoacidos N-terminales y (b) un dominio de ATPasa dependiente del ARN en el termino C de la proteina La proteasa NS3 es considerada un miembro de la familia de la quimotripsina en virtud de las similitudes en la secuencia proteica, la estructura general tridimensional y el mecanismo de catálisis Otras enzimas tipo quimotripsina son la elastasa, el factor Xa, la trombina, tripsina, plasmina, urocinasa, tPA y PSA La serina proteasa NS3 del VHC es responsable de la proteolisis del polipéptido (poliproteina) en las uniones NS3/NS4a, NS4a/NS4b, NS4b/NS5a y NS5a/NS5b y, por consiguiente, es responsable de la generación de cuatro proteinas virales durante la replicación viral Esto ha convertido a la serina proteasa NS3 del VHC un objetivo de interés para la quimioterapia antiviral

Se ha establecido que la proteina NS4a, un polipéptido de aproximadamente 6 kDa, es un cofactor para la actividad de serina proteasa de NS3 La autoescision de la union NS3/NS4a por la serina proteasa NS3/NS4a se produce intramolecularmente (es decir, sic), en tanto que los demas sitios de escisión se procesan intermolecularmente (es decir, trans)

El análisis de los sitios de escisión naturales para la proteasa del VHC reveló la presencia de cisteina en P1 y serina en P1' y que estos residuos se conservaban estrictamente en las uniones SN4a/NS4b, NS4b/NS5a y NS5a/NS5b La unión NS3/ND4a contiene una treonina en P1 y una serina en P1' Se sugiere a sustitucion Cis→ Thr en NS3/NS4a es la razon de la necesidad del procesamiento cis, en lugar de trans, en esta union Ver, por ejemplo, Pizzi y otros (1994) Proc Natl Acad Sci (USA) 91 888-782, Failla y otros (1996)

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Folding & Design 1 35-42 El sitio de escisión NS3/NS4a) también es mas tolerante con respecto a la mutagénesis que los demas sitios Ver, por ejemplo, Kollykhalov y otros (1994) J Virol 68 7525-7533 También se ha encontrado que los residuos ácidos de la región en linea de entrada del sitio de escisión son necesarios para la escisión eficiente Ver, por ejemplo, Komoda y otros (1994) J Virol 68 7351-7357

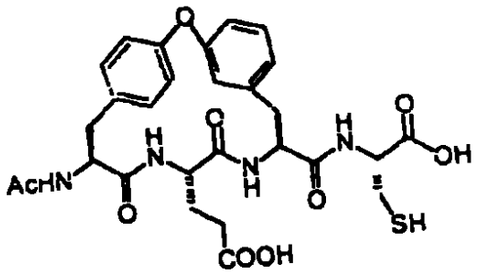
Entre los inhibidores de la proteasa del VHC que han sido dados a conocer se cuentan los antioxidantes (ver la Publicacion de solicitud de patente internacional No WO 98/14181), ciertos péptidos y análogos peptidicos (ver la Publicación de solicitud de patente internacional No WO 98/17679, Landro y otros (1997) Biochem, 36 9340-9348, Ingallinella y otros (1998) Biochem 37 8906-8914, Llinàs-Brunet y otros (1998) Bioorg Med Chem Lett 8 1713-1718), inhibidores a base del polipeptido de 70 aminoácidos eglin c (Martin y otros (1998) Biochem 37 11459-11468, inhibidores de afinidad seleccionados entre el inhibidor de la tripsina secretoria pancreática humana (hPSTI-C3) y repertorios minicorpusculares (MBip) (DIMAXI y otros (1997) (1997) J Virol 71 7461-7469), cV_HE2 (un fragmento de anticuerpo de dominio variable "camelizado") (Martin y otros (1997) Protein Eng , 10 607-614) y α1-antiquimotripsina (ACT) Elzouki y otros) (1997) J Hepat 27 42-28) Recientemente se ha descripto una ribozima diseñada para destruir selectivamente el virus ARN de la hepatitis C (ver BioWorld Today 9(217) 4 (Noviembre 10 de 1998))

Tambien se hace referencia a las publicaciones PCT No 98/17679, publicada el 30 de abril de 1998 (Vertex Pharmaceuticals Incorporated), WO 98/22496, publicada el 28 de mayo de 1998 (F Hoffmann-La Roche AG) y WO 99/07734, publicada el 17 de febrero de 1999 (Boehringer Ingelheim Canada Ltd)

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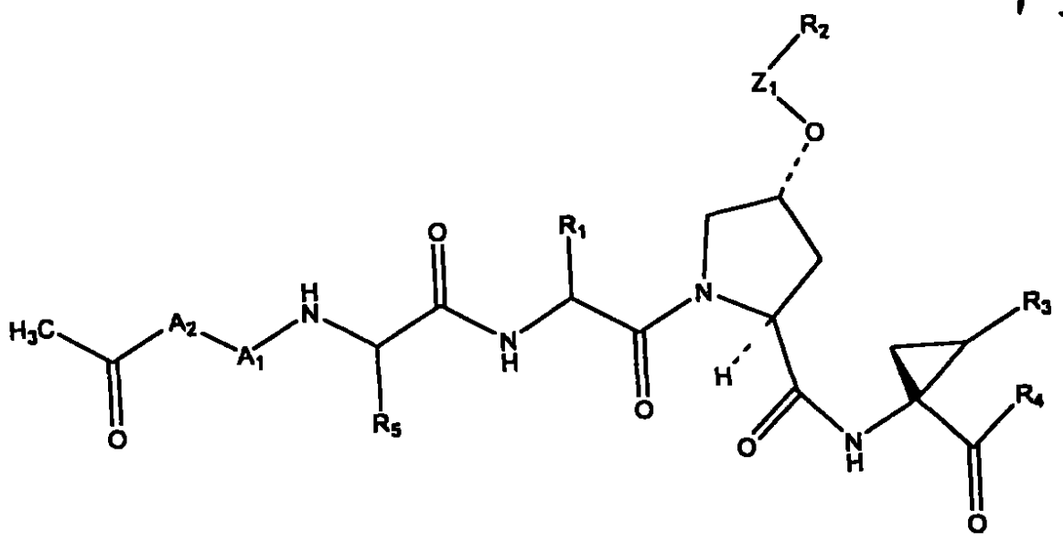
Se ha involucrado al VHC en la cirrosis hepatica y en la inducción del carcinoma hepatocelular El pronostico para pacientes que sufren una infeccion por VHC es malo en la actualidad La infección por el VHC es más dificil de trtar que otras formas de hepatitis debido a la falta de inmunidad o remisión asociada con la infeccion por VHC Los datos actuales indican una tasa de supervivencia inferior a 50 % despues de cuatro años de diagnosticarse la cirrosis Los pacientes a los que se ha diagnosticado carcinoma hepatocelular localizado operable tienen una tasa de supervivencia de 10-30 % en tanto que los que tienen carcinoma hepatocelular localizado no operable tienen una tasa de supervivencia a los cinco años inferior a 1 %

Se hace referencia a Synlett, de A Marchetti y otros, S1, 1000-1002 (1999), que describe la sintesis de análogos biciclicos de un inhibidor de la proteasa de VHC NS3 Un compuesto descripto en dicha publicación tiene la formula

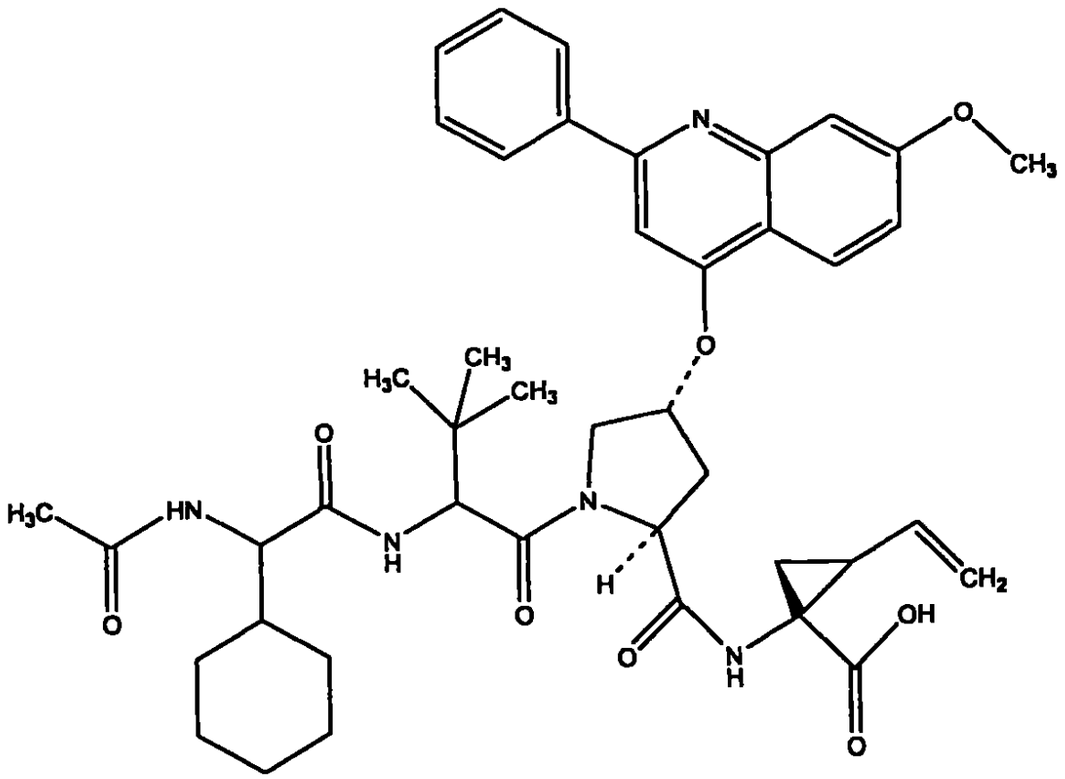


También se hace referencia a WO 00/09558 (Cesionario Boehringer Ingelheim Limited, publicada el 24 de febrero de 2000) que describe derivados peptidicos de la formula

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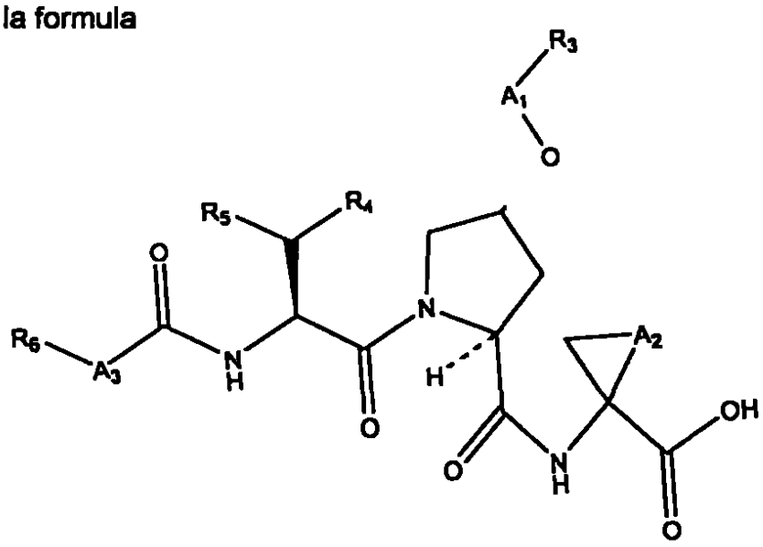


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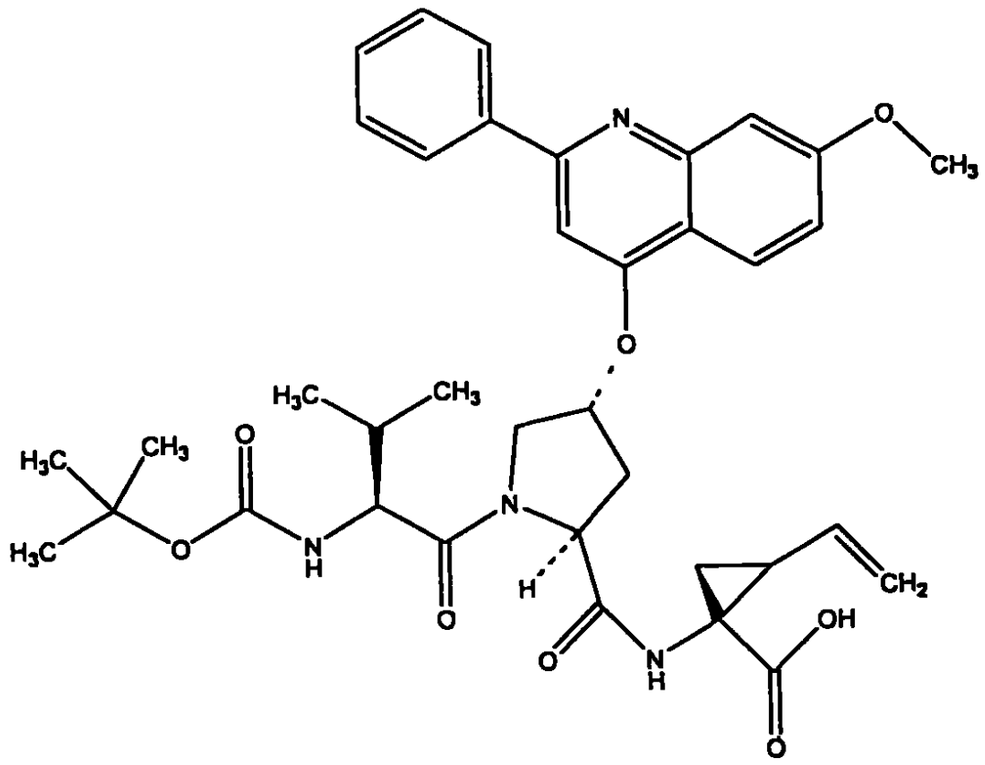


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También se hace referencia a WO 00/09543 (Cesionario Boehringer Ingelheim Limited, publicada el 24 de febrero de 2000) que describe derivados peptídicos de la formula



en la cual los diversos elementos se describen en dicho documento Un compuesto ilustrativo de dicha serie es



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Las terapias actuales para la hepatitis C incluyen interferon-α (IFN_α) y la terapia combinatoria con ribavirina e interferón Ver, por ejemplo, Beremguer y otros (1998) Proc Assoc Am Physicians 110(2) 98-112 Estas terapias padecen un bajo coeficiente de respuesta sostenida y frecuentes efectos colaterales Ver, por ejemplo, Hoofnagle y otros (1997) N Engl J Med 336 347 En la actualidad, no se dispone de una vacuna contra la infección por VHC

La solicitud de patente en trámite, Acta No _____, presentada el 5 de abril de 2000 describe ciertos compuestos macrocíclicos como inhibidores de la proteasa de VHC así como composiciones farmaceuticas que contienen dichos compuestos

Existe la necesidad de nuevos tratamientos y terapias para la infección por VHC Por lo tanto, uno de los objetivos de la presente invencion es presentar compuestos utiles en el tratamiento o prevencion o mitigación de uno o mas sintomas de la hepatitis C

Otro de los objetivos de la presente es presentar metodos de tratamiento o prevencion o mitigación de uno o mas sintomas de la hepatitis C

Un objetivo más de la presente invencion es presentar métodos para modular la actividad de las serina proteasas, especialmente la serina proteasa de VHC NS3/NS4a utilizando los compuestos presentados en la presente

Otro de los objetivos de la presente invención es presentar metodos para modular el procesamiento del polipéptido VHC empleando los compuestos provistos en la presente

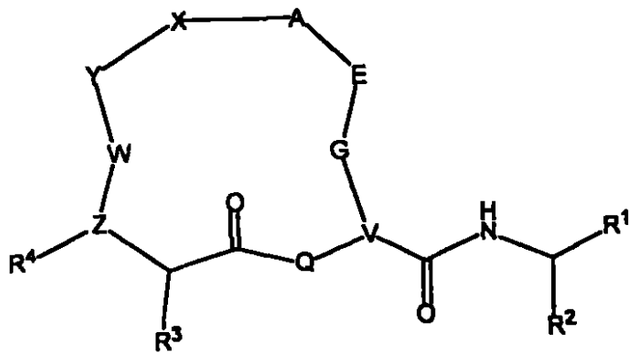
Síntesis de la Invención

En sus numerosas realizaciones, la presente invención presenta una novedosa clase de inhibidores macrocíclicos de la proteasa de VHC, composiciones farmaceuticas que contienen uno o más de dichos compuestos, métodos para la preparacion de formulaciones farmacéuticas que comprenden

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uno o más de tales compuestos y métodos de tratamiento, prevención o mitigacion de uno o más de los sintomas de la hepatitis C También se presentan métodos para modular la interaccion de un polipéptido de VHC con la proteasa de VHC Entre los compuestos presentados en la presente, se prefieren los compuestos que inhiben la actividad de serina proteasa de VHC NS3/ND4a Los compuestos descriptos en la presente contienen generalmente cuatro o más residuos aminoácidos y menos de aproximadamente doce residuos aminoácidos

En su principal realizacion, la presente invención presenta un compuesto macrociclico de la fórmula I



Formula I

en la cual

E, X e Y pueden estar independientemente presentes o ausentes y, en caso de estar presentes, son independientemente seleccionados entre las porciones alquilo, arilo, alquilarilo, heteroalquilo, heteroarilo, arilheteroarilo, alquilheteroarilo, cicloalquilo, eter alquimico, éter alquilarílico, eter arílico, alquilamino, arilamino, alquilarilamino, alquilsulfuro, alquilarilsulfuro, arilsulfuro, alquilsulfona, alquilarilsulfona, arilsulfona, alquilalquilsulfoxido, alquilarilsulfóxido, alquilamida, alquilarilamida, arilamida, alquilsulfonamida, alquilarilsulfonamida, arilsulfonamida, alquilurea, alquilarilurea, arilurea, carbamato de alquilo, carbamato de alquilarilo, carbamato de arilo, alquilhidrazida, alquilarilhidrazida,

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alquilhidroxamida, alquilarilhidroxamida, alquilsulfonilo, arilsulfonilo, heteroalquilsulfonilo, heteroarilsulfonilo, alquilcarbonilo, arilcarbonilo, heteroalquilcarbonilo, heteroarilcarbonilo, alcoxicarbonilo, ariloxicarbonilo, heteroariloxicarbonilo, alquilaminocarbonilo, arilaminocarbonilo, heteroarilaminocarbonilo o una combinación de las mismas, con la salvedad de que optativamente, E, X e Y pueden estar sustituidas además con porciones seleccionadas entre el grupo que consiste en aromático, alquilo, alquilarilo, heteroalquilo, arilheteroarilo, alquilheteroarilo, cicloalquilo, eter alquilico, éter alquilarílico, alquilsulfuro, alquilarilsulfuro, alquilsulfona, alquilarilsulfona, alquilamida, alquilarilamida, alquilsulfonamida, alquilaminas, alquilarilaminas, alquilarilsulfonamida, alquilurea, alquilarilurea, carbamato de alquilo, carbamato de alquilarilo, halógeno, hidroxilamino, carbazato de alquilo, carbazato de arilo,

R^1 = COR^5 o $B(OR)_2$, donde R^5 = H, OH, OR^8, NR^9R^{10}, CF_3, C_2F_5, C_3F_7, CF_2R^6, R^6, COR^7 donde R^7 = H, OH, OR^8, CHR^9R^{10} o NR^9R^{10}, donde R^6, R^8, R^9 y R^{10} son independientemente seleccionados entre el grupo que consiste en H, alquilo, arilo, heteroalquilo, heteroarilo, cicloalquilo, cicloalquilo, arilalquilo, heteroarilalquilo, $CH(R^1)COOR^{11}$, $CH(R^1)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})R$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^{3'})CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)CONHCH(R^4)COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)CONHCH(R^4)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^{3'})CONHCH(R^4)CONHCH(R^{5'})COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)CONHCH(R^4)CONHCH(R^{5'})CONR^{12}R^{13}$, donde R^1, $R^{2'}$, R^3, $R^{4'}$, $R^{5'}$, R^{11}, R^{12}, R^{13} y R' son independientemente seleccionados entre el grupo que consiste en H, alquilo, arilo, heteroalquilo, heteroarilo, cicloalquilo, alquilarilo, alquilheteroarilo, arilalquilo y heteroaralquilo,

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Z es seleccionado entre O, N o CH,

W puede estar presente o ausente y, en caso de estar presente, W es seleccionado entre C=O, C=S, SO_2 o C=NR,

Q es $(NR)_p$, O, S, CH_2, CHR, CRR' o un enlace doble hacia V,

A es O, CH_2, $(CHR)_p$, $(CHR\text{-}CHR')_p$, $(CRR')_p$, NR, S, SO_2, C=O o un enlace,

G es $(CH_2)_p$, $(CHR)_p$, $(CRR')_p$, NR, O, S, SO_2, $S(O)_2NH$, C=O o un enlace doble hacia E o V,

V es CH, CR o N,

p es un numero de 0 a 6 y

R, R', R^2, R^3 y R^4 son independientemente seleccionados entre el grupo que consiste en H, alquilo C1-C10, alquenilo C2-C10, cicloalquilo C3-C8, heterocicloalquilo C3-C8, arilo, alcoxi, ariloxi, alquiltio, ariltio, amino, amido, ester, ácido carboxílico, carbamato, urea, cetona, aldehido, ciano, nitro, heteroarilo, alquilarilo, alquilheteroarilo, (cicloalquil)alquilo y (heterocicloalquil)alquilo, donde dicho cicloalquilo se compone de tres a ocho átomos de carbono y de cero a seis átomos de oxigeno, nitrogeno, azufre o fósforo y dicho alquilo es de uno a seis átomos de carbono, donde dichas porciones alquilo, heteroalquilo, alquenilo, heteroalquenilo, arilo, heteroarilo cicloalquilo y heterocicloalquilo pueden estar optativamente sustituidas, y donde dicho término "sustituido" se refiere a una sustitución optativa y adecuada con una o más porciones seleccionadas entre el grupo que consiste en alquilo, alquenilo, alquinilo, arilo, aralquilo, cicloalquilo, heterocíclico, halógeno, hidroxi, tio, alcoxi, ariloxi, alquiltio, ariltio, amino, amido, éster, acido carboxílico, carbamato, urea, cetona, aldehido, ciano, nitro, sulfonamida, sulfóxido, sulfona, sulfonilurea, hidrazida e hidroxamato y tiourea

A menos que se indique lo contrario, todos los terminos tecnicos y cientificos utilizados en la presente tienen el mismo significado que los expertos en la técnica a la cual se refiere la presente invencion les dan comunmente Asi,

por ejemplo, el término alquilo (incluyendo las porciones alquilo de alcoxi) se refiere a un grupo monovalente derivado de un hidrocarburo saturado de cadena recta o ramificada por medio de la eliminación de un solo atomo, que tiene de 1 a 8 atomos de carbono, preferentemente de 1 a 6,

anilo – representa un grupo carbociclico que tiene de 6 a 14 atomos de carbono y tiene por lo menos un anillo bencenoide, pretendiéndose que todos los átomos de carbono aromaticos sustituibles libres del grupo carbociclico sean posibles puntos de union Los grupos anilo preferidos incluyen fenilo, 1-naftilo, 3-naftilo e indanilo, y especialmente fenilo y fenilo sustituido

aralquilo – representa una porción que contiene un grupo arilo ligado por medio de un alquilo inferior;

alquilarilo – representa una porcion que contiene un alquilo inferior ligado por un grupo anilo,

cicloalquilo – representa un anillo carbociclico saturado con 3 a 8 átomos de carbono, preferentemente 5 o 6, optativamente sustituido,

heterociclico – representa, además de los grupos heteroanilo definidos mas adelante, grupos organicos cíclicos saturados y no saturados con por lo menos un atomo de O, S y/o N interrumpiendo una estructura carbociclico que consiste en uno o más anillos, donde cada anillo es de 5, 6 ó 7 miembros y puede o no tener enlaces dobles que carecen de electrones pi deslocalizados, estructura de anillo que tiene de 2 a 8, preferentemente de 3 a 6 átomos de carbono, por ejemplo 2- o 3-piperidinilo, 2- o 3-piperazinilo, 2- o 3-morfolinilo o 2- o 3-tiomorfolinilo,

halogeno – representa fluor, cloro, bromo y yodo,

heteroarilo – representa un grupo organico ciclico con por lo menos un atomo de O, S y/o N que interrumpe una estructura de anillo carbociclico y tiene un numero suficiente de electrones pi deslocalizados para conferir caracter aromático, donde el grupo heterociclico aromatico tiene de 2 a 14,

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preferentemente 4 o 5 atomos de carbono, por ejemplo 2-, 3- o 4-piridilo, 2- o 3-furilo, 2- o 3-tienilo, 2-, 4- o 5-tiazolilo, 2- o 4- imidazolilo, 2-, 4- o 5-pirimidinilo, 2-pirazinilo o 3- o 4-piridazinilo, etc Los grupos heteroarilo preferidos son 2-, 3- y 4-piridilo Tales grupos heteroarilo pueden estar también optativamente sustituido

También estan incluidos en la presente invencion los tautomeros, rotámeros, enantiomeros y demas isómeros opticos de los compuestos de la fórmula I, asi como las sales farmaceuticamente aceptables y solvatos de las mismas

Otra caracteristica de la presente invención corresponde a composiciones farmacéuticas que contiene como ingrediente activo un compuesto de la fórmula I (o su sal, solvato o isomeros del mismo) junto con un vehiculo o excipiente farmacéuticamente aceptable

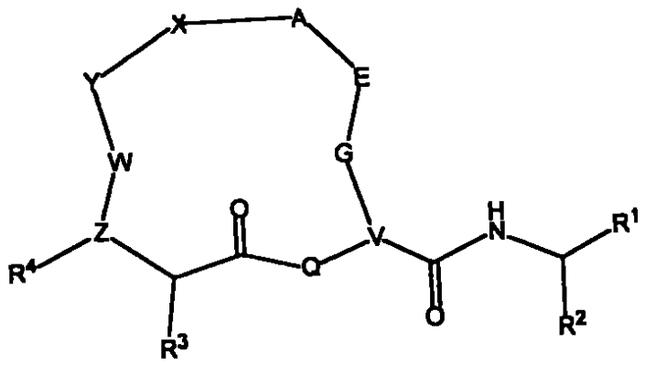
La invención presenta asimismo métodos para preparar los compuestos de la fórmula I, asi como metodos para tratar enfermedades tales como, por ejemplo, el VHC y trastornos relacionados Los metodos de tratamiento comprenden administrar a un paciente que padece dicha enfermedad o enfermedades una cantidad terapéuticamente efectiva de un compuesto de la fórmula I o composiciones farmacéuticas que comprenden un compuesto de la formula I

También se describe el uso de un compuesto de la formula I para la elaboración de un medicamento para el tratamiento del VHC y trastornos relacionados

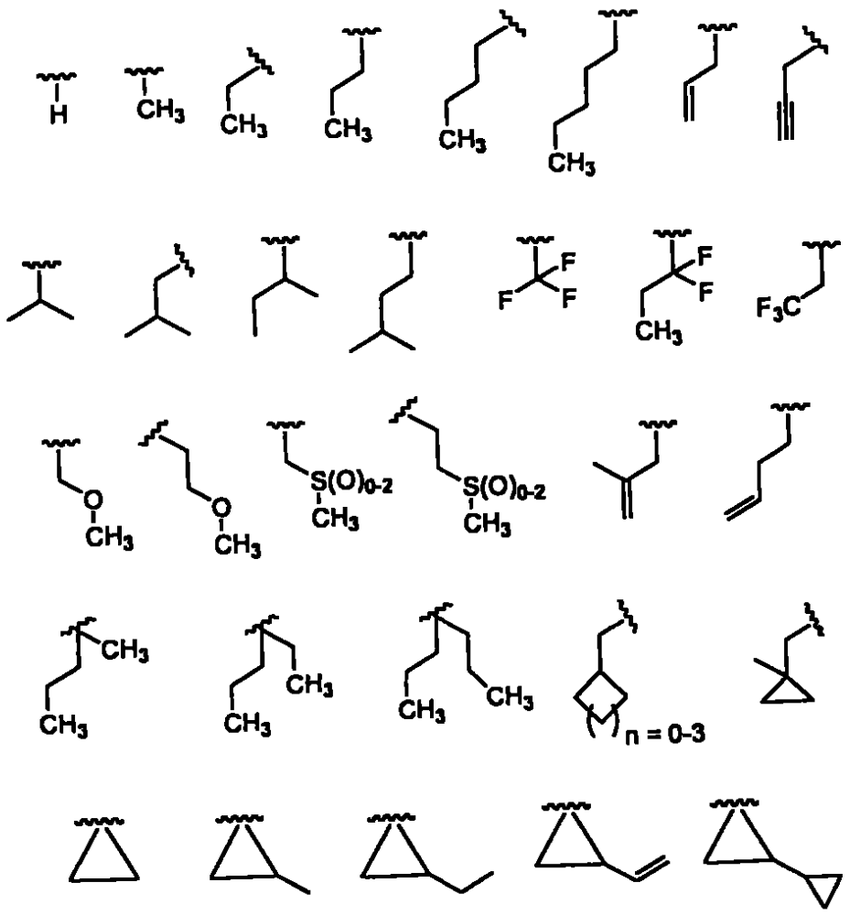
DESCRIPCIÓN DETALLADA DE LAS REALIZACIONES PREFERIDAS

En una realizacion, la presente invención describe compuestos de la formula I como inhibidores de la proteasa de VHC, especialmente la serina proteasa de VHC NS3/NS4a

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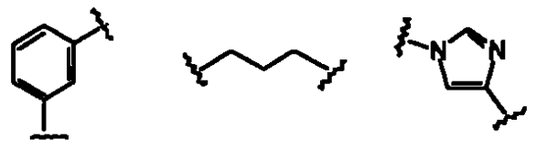


en la cual las diversas porciones son como se los definiera anteriormente Algunas de las realizaciones preferidas incluyen, aunque no se limitan a las siguientes definiciones de las diversas funcionalidades en la formula general I antes citada, se pueden encontrar otras definiciones convenientes para las mismas y otras funcionalidades en las estructuras y reivindicaciones de esta solicitud, que también estan dentro del alcance de la presente invención Entre las realizaciones preferidas, R^2 de la fórmula I puede ser seleccionado entre las siguientes porciones

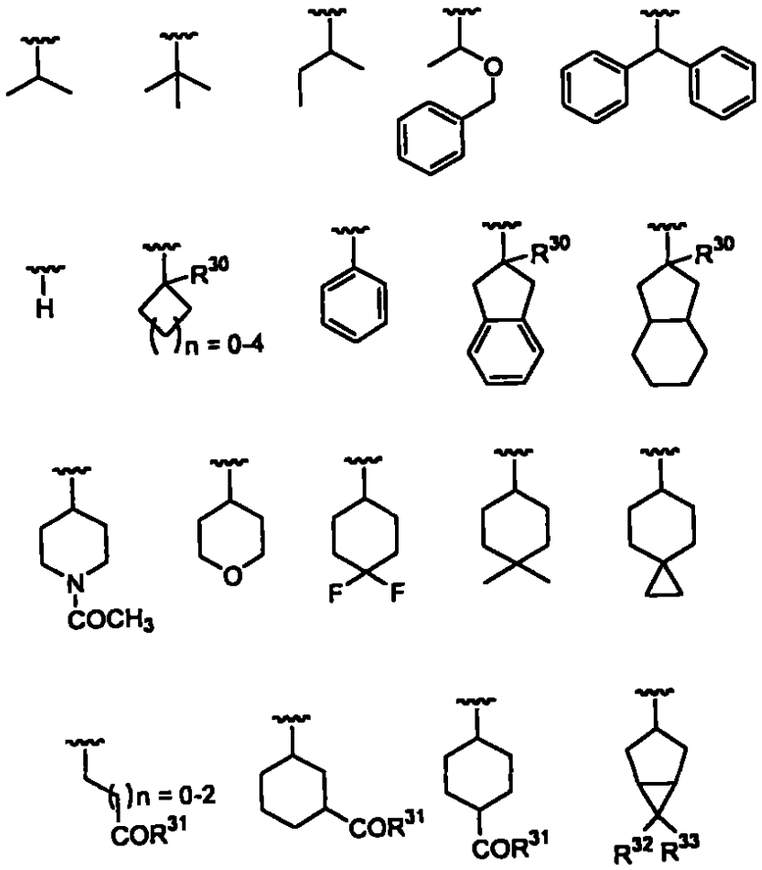


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E puede ser un alquilo sustituido o no sustituido, arilo sustituido o no sustituido o heteroalquilo sustituido o no sustituido heteroarilo sustituido o no sustituido o cicloalquilo sustituido o no sustituido, siendo las representaciones preferidas de E



Las realizaciones preferidas con respecto a R^3 incluyen las porciones



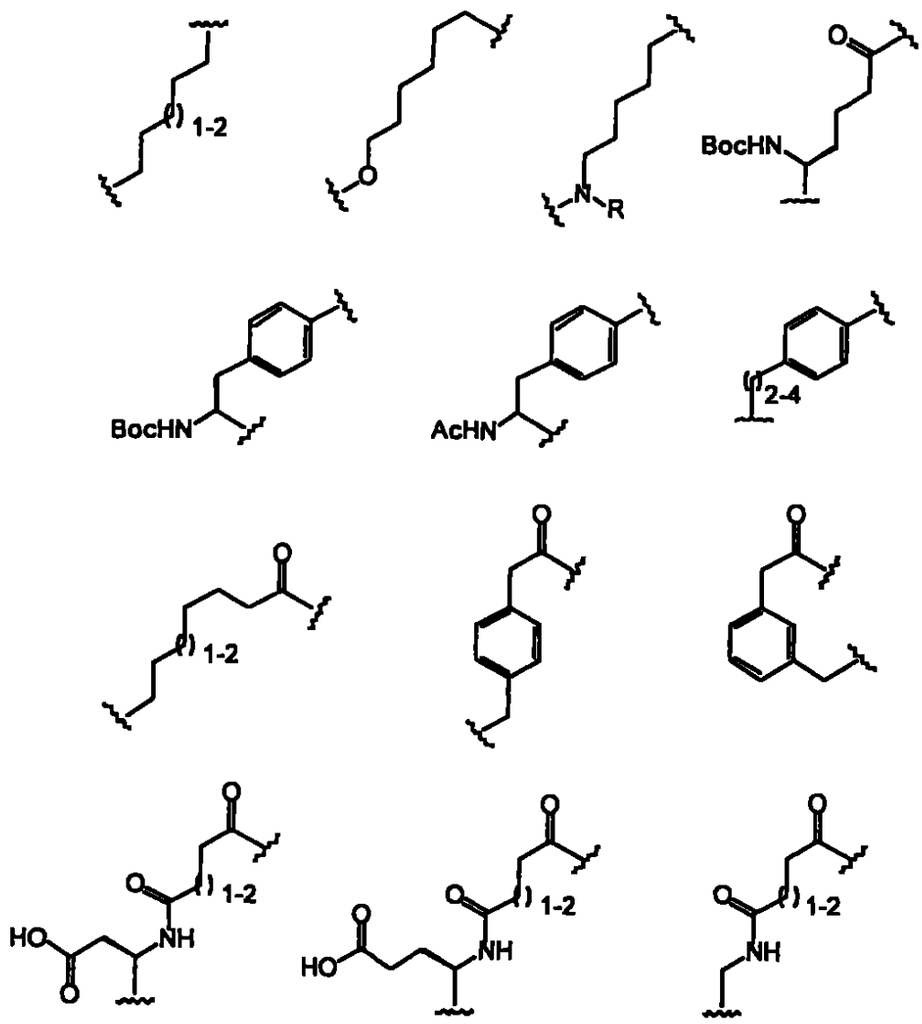
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en las cuales R^{30} = H, CH_3 u otros grupos alquilo,

R^{31} = OH, O-alquilo, NH_2, N-alquilo y

R^{32} y R^{33} pueden ser iguales o diferentes y son seleccionados independientemente entre H, f, Cl, Br y CH_3

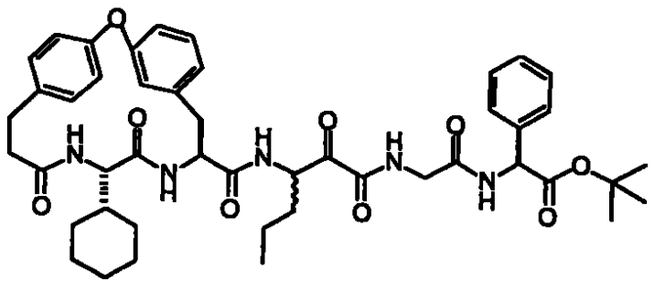
Las realizaciones preferidas para la porción X-Y son las siguientes estructuras



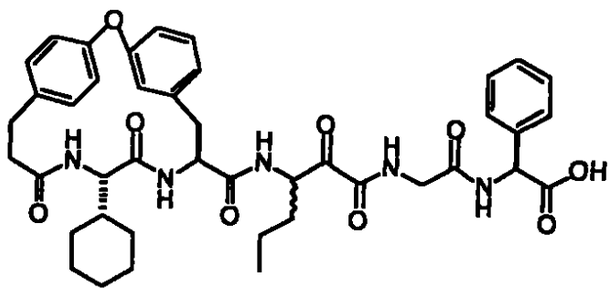
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En la seccion Reivindicaciones de la presente solicitud se consignan varias profundizaciones adicionales de las diversas definiciones antes citadas para los compuestos representados por la formula I Estas tambien están representadas por los diversos compuestos enumerados en la memoria descriptiva y las reivindicaciones Tales profundizaciones, definiciones y restricciones deben ser consideradas representativas de la totalidad de la invención presentada en esta solicitud

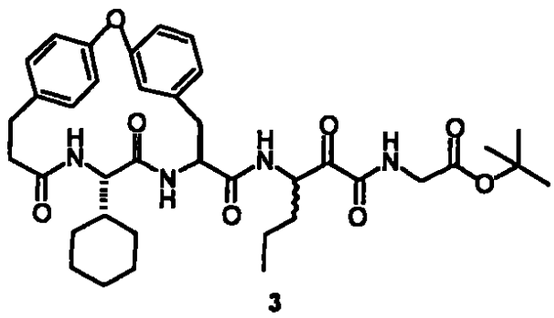
A continuación se enumeran los compuestos representativos de la presente invención, que exhiben excelente actividad inhibitoria de la proteasa de VHC



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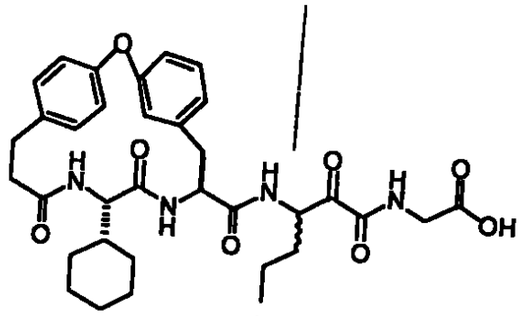


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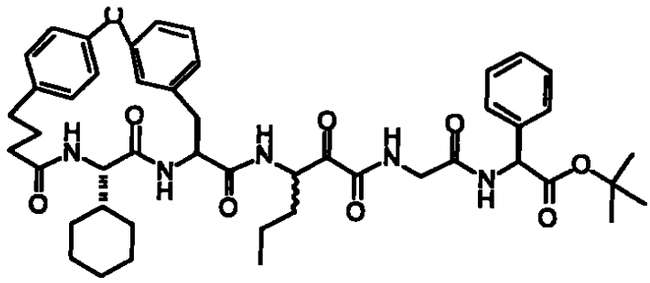


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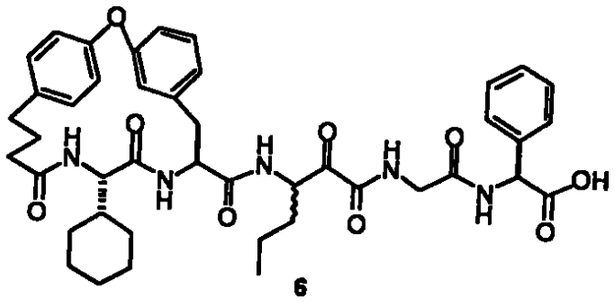
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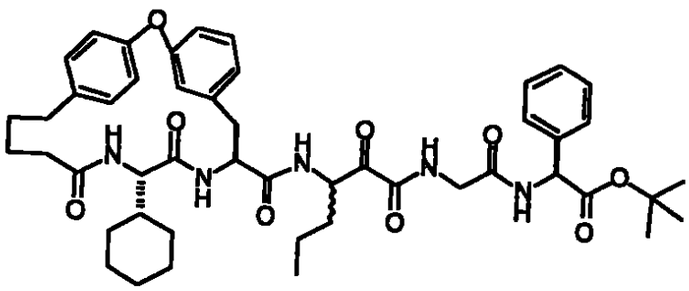
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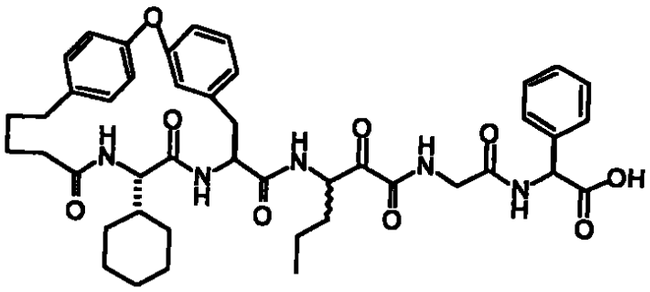
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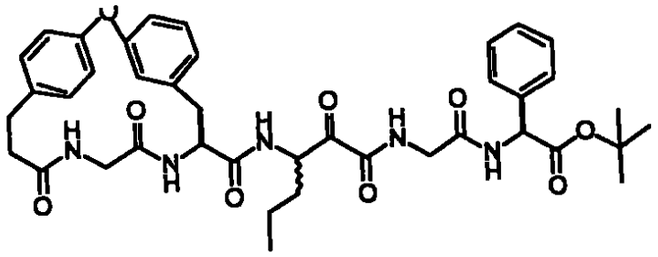


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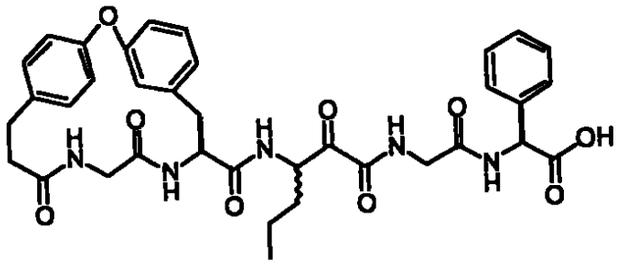


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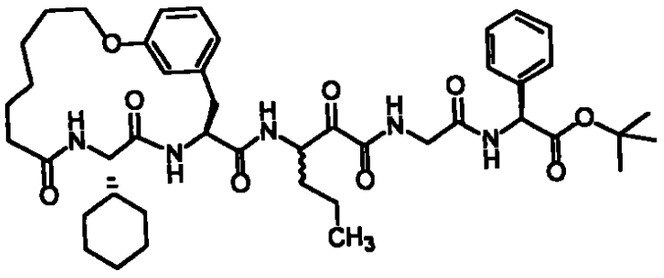
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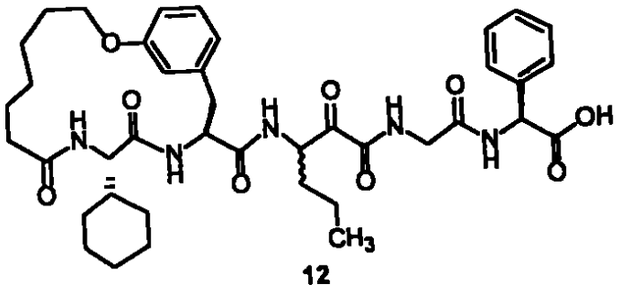
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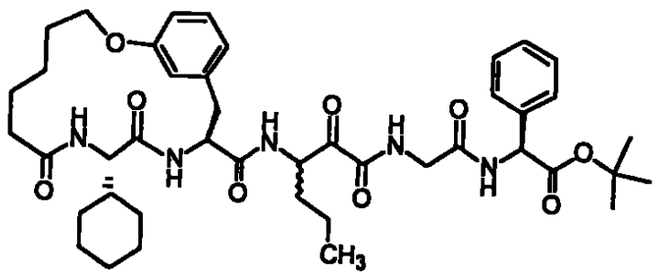


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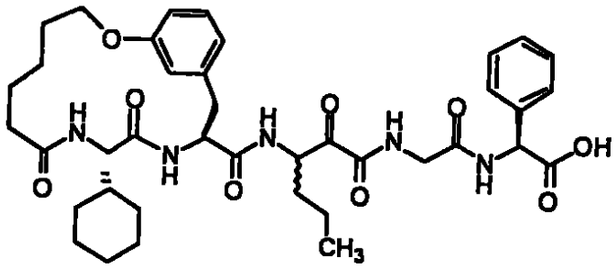


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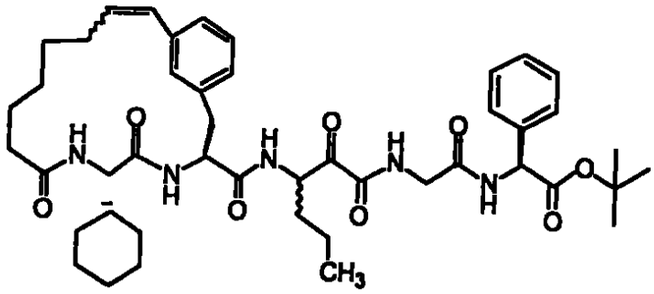
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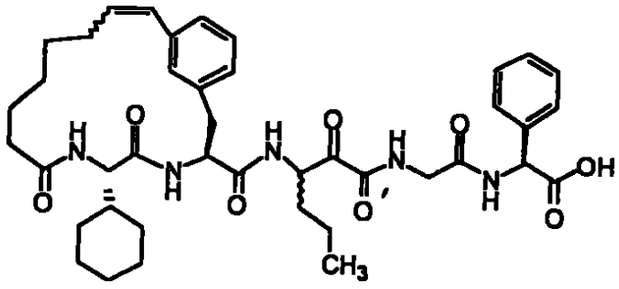
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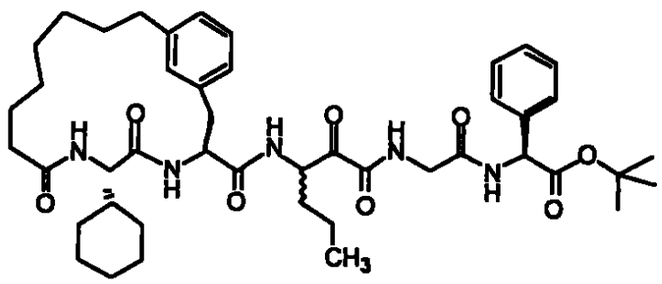


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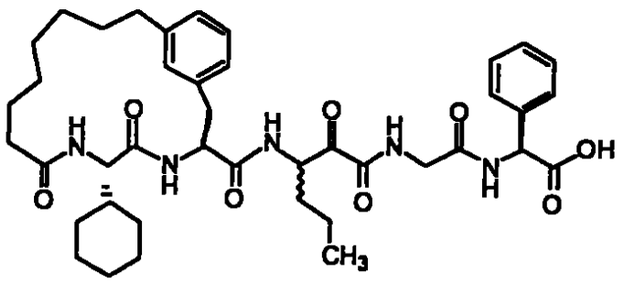


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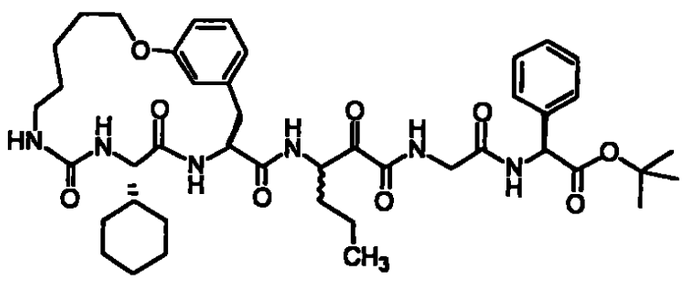
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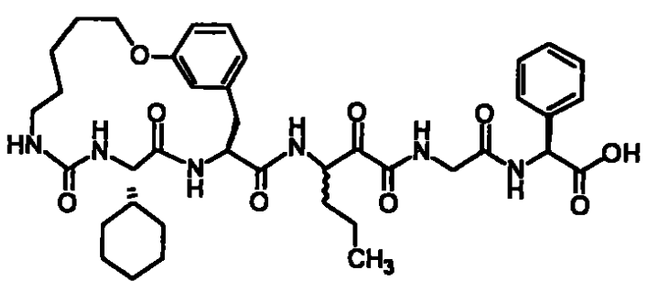
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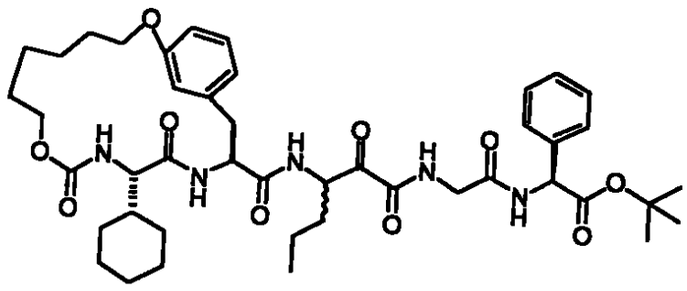


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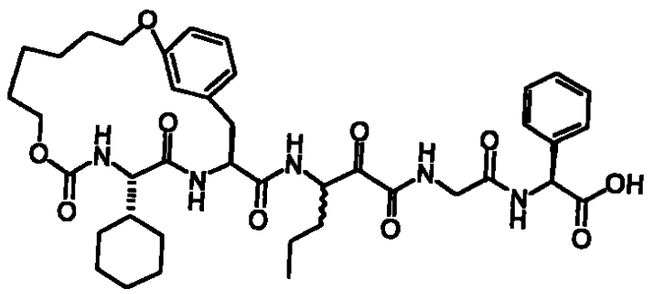


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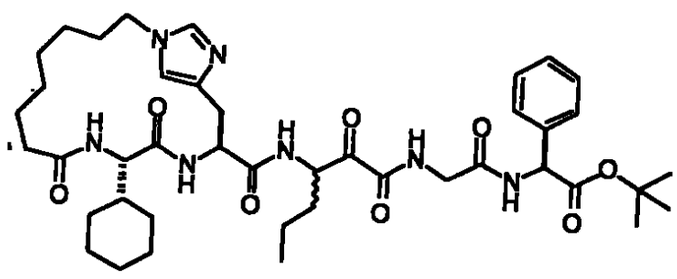
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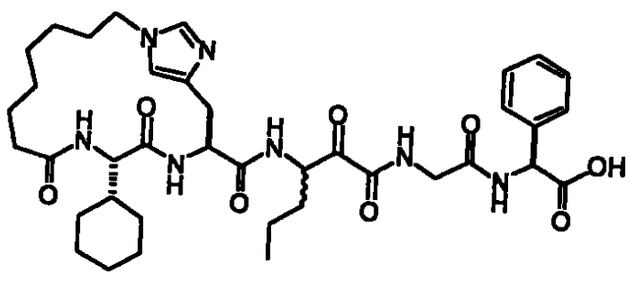
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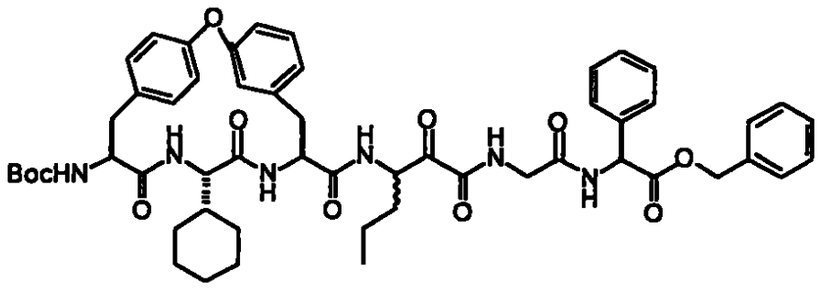


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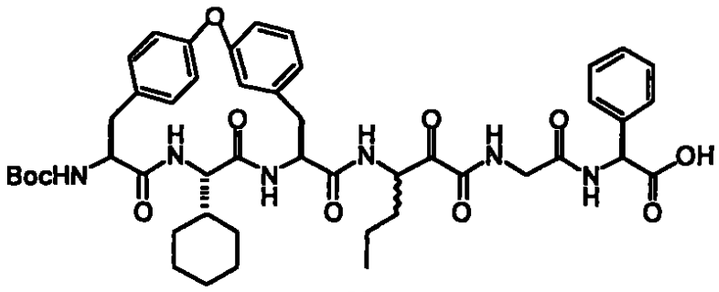


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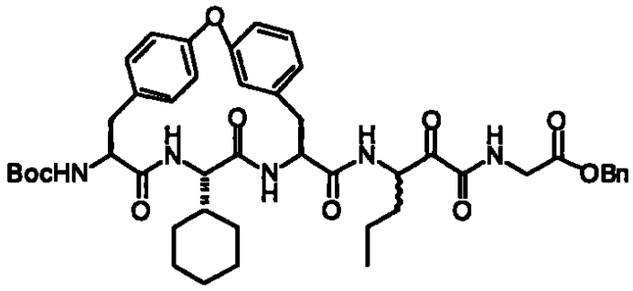
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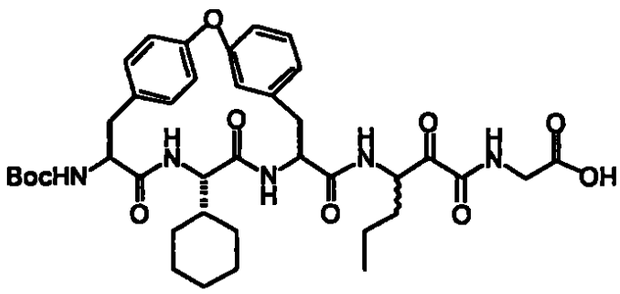
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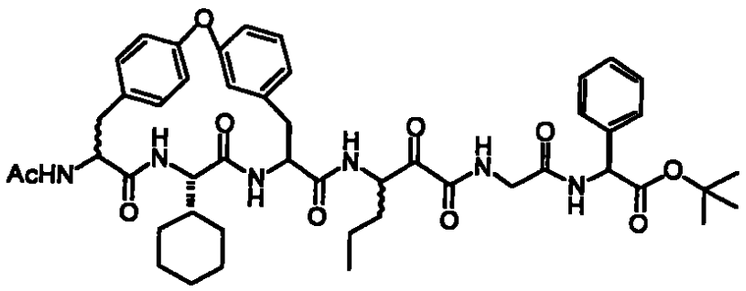


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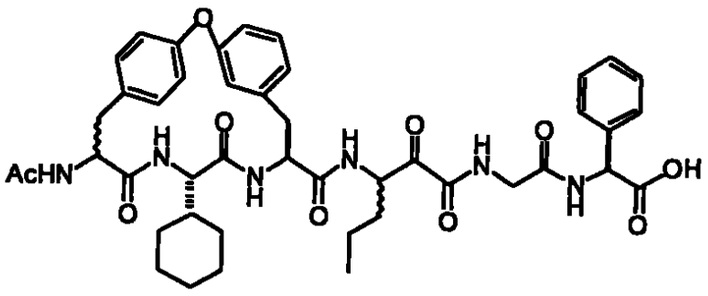


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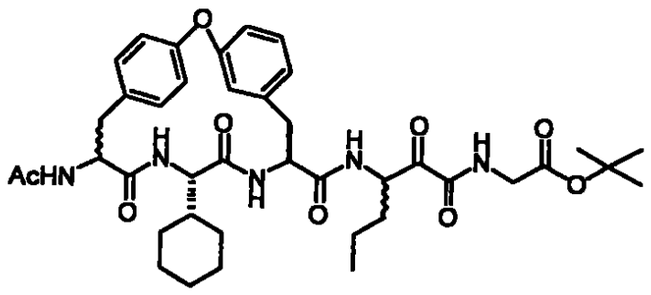
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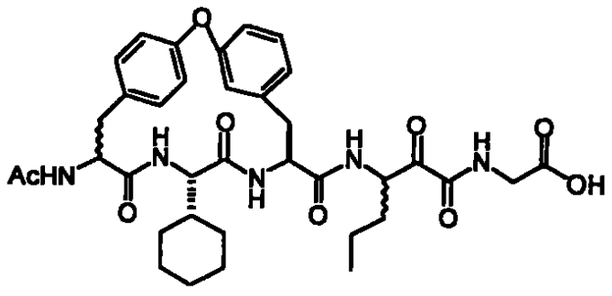
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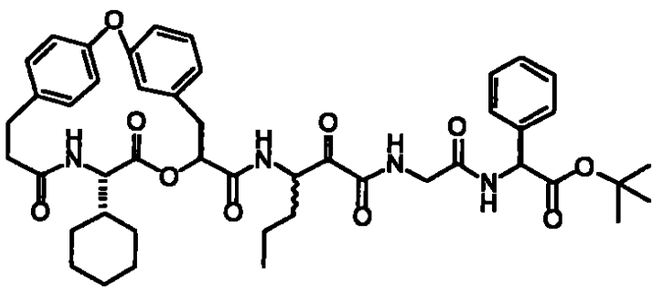


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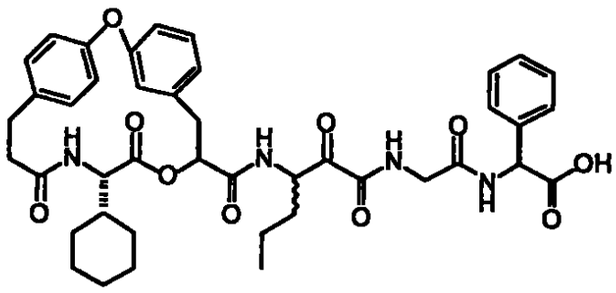


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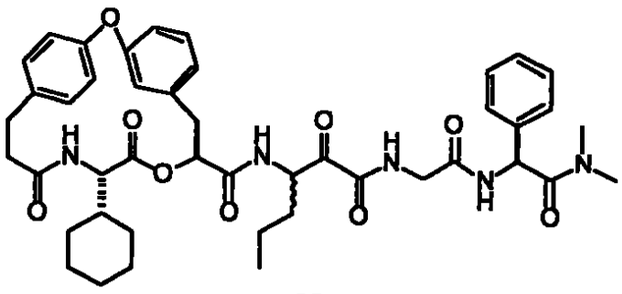
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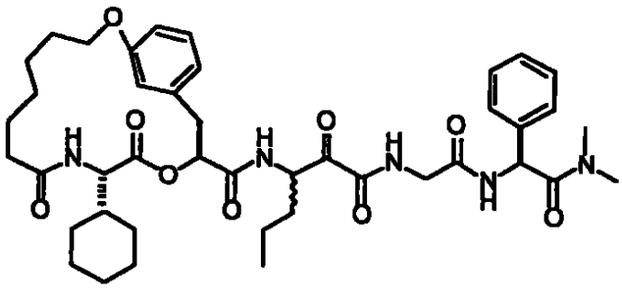
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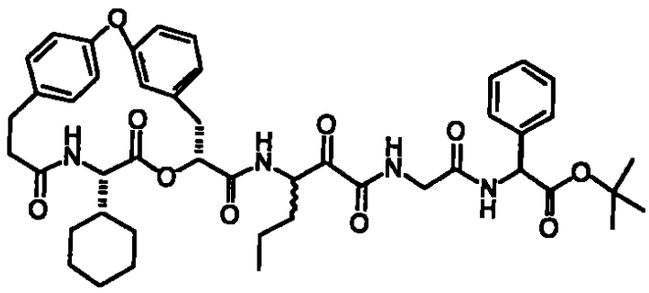
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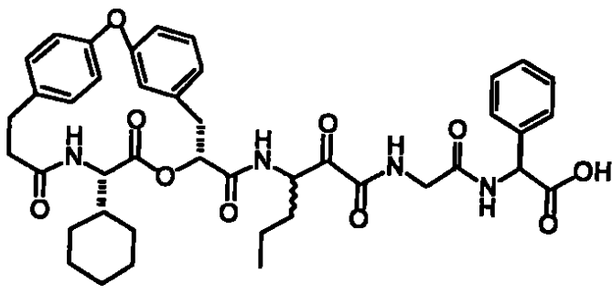
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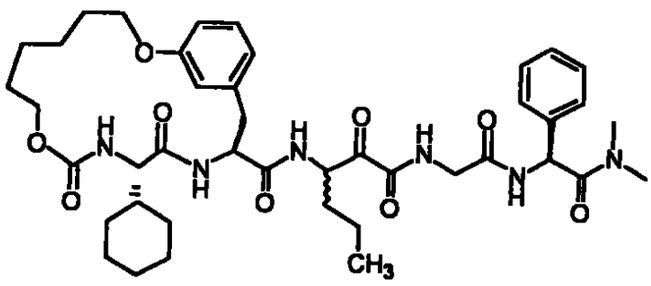
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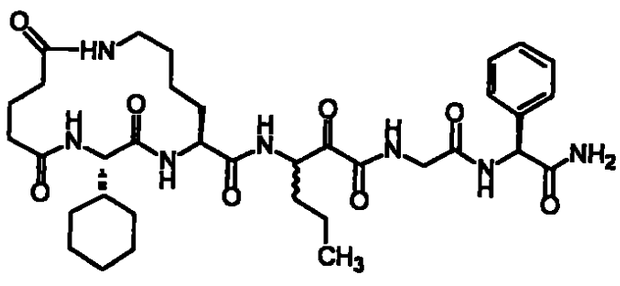
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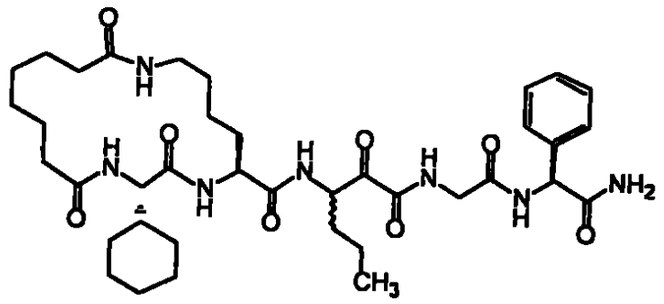
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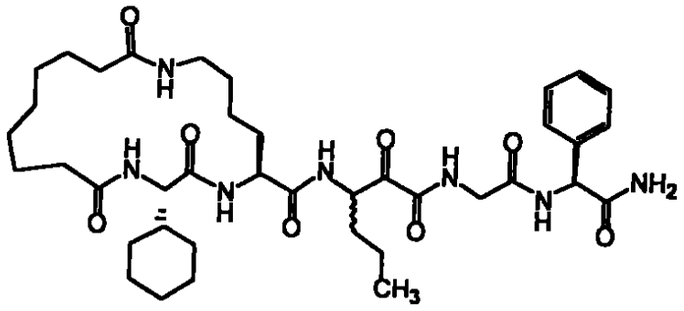
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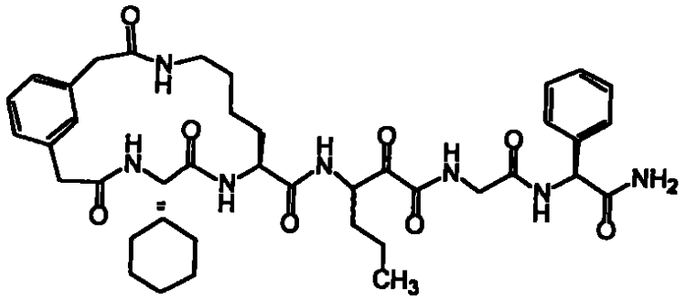
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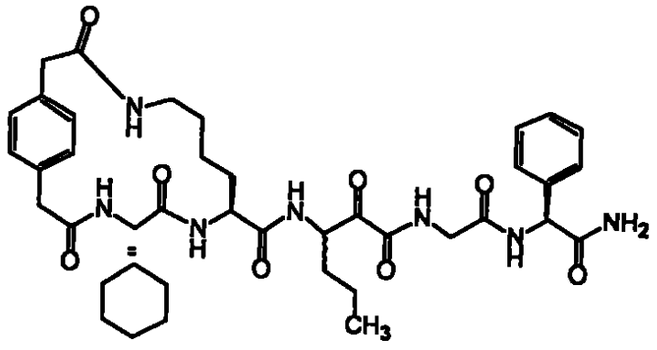
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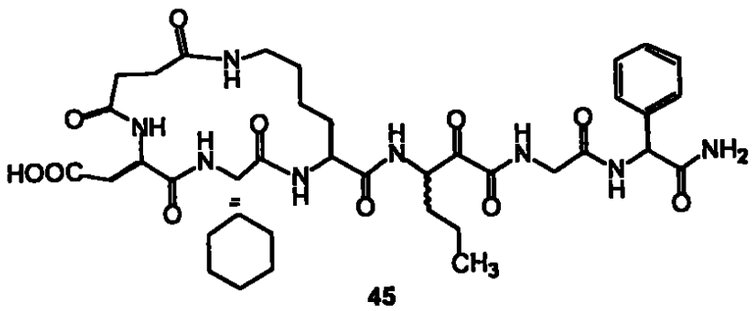


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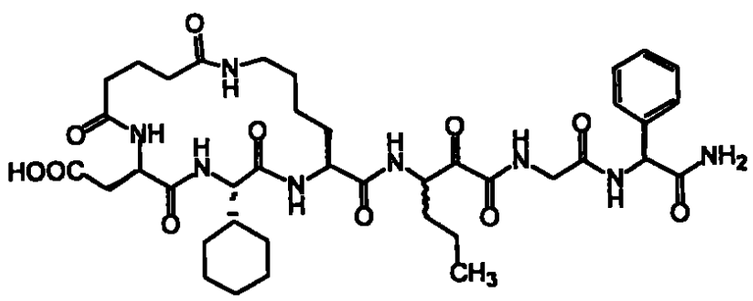


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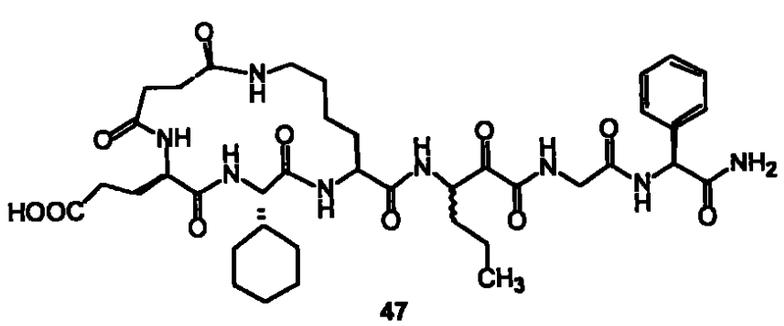
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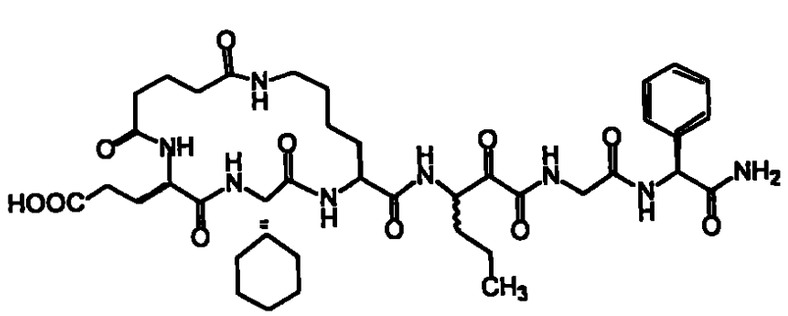
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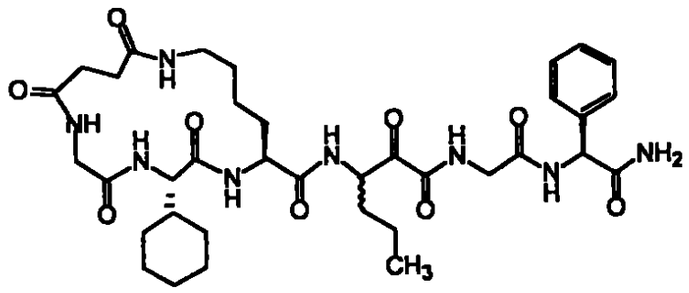


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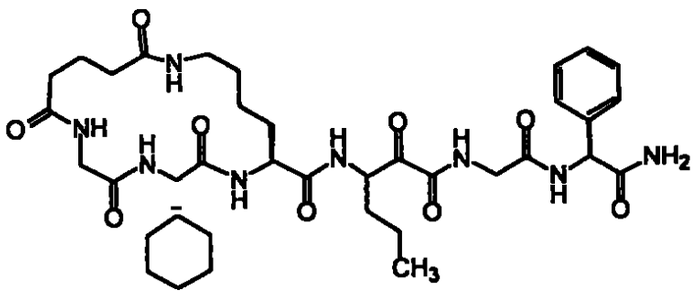


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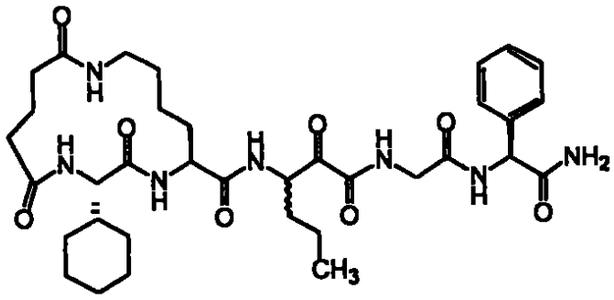
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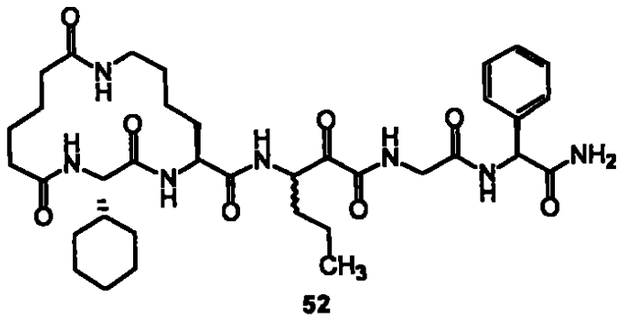
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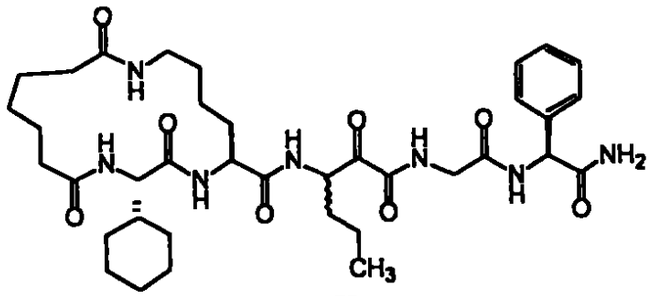


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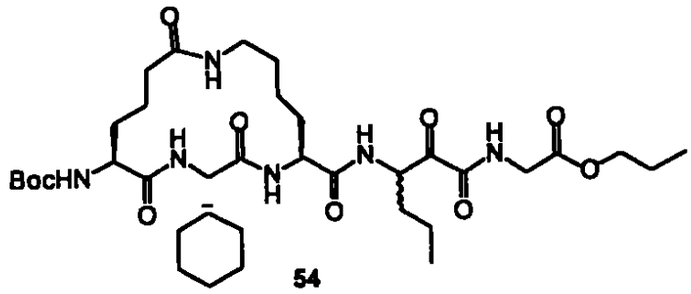


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En la tabla 1 se presenta la actividad de algunos de los compuestos como intervalos de valores de K_i en nanomoles (nM) Los numeros de Ejemplo de la Tabla 1 se refieren a los numeros para las diversas estructuras de la seccion EJEMPLOS que se encuentra en las partes subsiguientes de la presente solicitud

Tabla 1 Resultados de los ensayos continuos sobre proteasa del VHC

Ejemplo Numero	K_i* nM
1	B
2	A
3	B
4	B
5	B

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9	B
10	B
11	B
12	A
13	B
14	B
15	B
16	A
17	B
18	A
19	B
20	A
21	B
22	A
23	B
24	B
25	B
26	B
27	B
28	B
29	B
30	A
31	B
32	B
33	B
34	B
35	B
36	B
37	B
38	B

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39	A
40	B
41	A
42	B
43	A
44	A
45	B
46	B
47	B
48	B
49	B
50	B
51	B
52	A
53	A
54	B

Intervalo de K_i^* de ensayo continuo sobre VHC

Categoria A = 0,001-1,0 µM, Categoria B = 1,1-100 µM

Mas adelante en esta seccion se describen algunos métodos para sintetizar los diversos tipos de los compuestos de la invención, y tambien se los describe esquemáticamente, para proceder luego a los Ejemplos ilustrativos

Dependiendo de la estructura, los compuestos de la presente invencion pueden formar sales farmaceuticamente aceptables con ácidos orgánicos o inorganicos o con bases orgánicas o inorganicas Entre los ejemplos de ácidos adecuados para dicha formación de sales se cuentan los ácidos clorhidrico, sulfurico, fosforico, acetico, citrico, oxálico, malónico, salicilico, málico, fumarico, succínico, ascórbico, maleico, metansulfonico y otros acidos minerales y carboxilicos muy conocidos por los expertos en la técnica Para la formación de

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sales con bases, las bases adecuadas son, por ejemplo, NaOH, KOH, NH_4OH, hidróxido de tetraalquilamonio y demas

En otra realización, esta invención presenta composiciones farmacéuticas que comprenden los macrociclos antes descriptos de la invención como ingrediente activo Por lo general, las composiciones farmacéuticas comprenden además un diluyente portador, excipiente o vehiculo farmacéuticamente aceptable (a los que en la presente se denomina colectivamente como materiales portadores) A causa de su actividad inhibitoria del VHC, tales composiciones farmacéuticas son de utilidad en el tratamiento de la hepatitis C y trastornos relacionados

En otra de sus realizaciones, la presente invencion describe métodos para prepara las composiciones farmacéuticas que comprenden los compuestos macrociclicos inventivos como ingrediente activo En las composiciones farmacéuticas y los métodos de la presente invencion, los ingredientes activos se administran tipicamente mezclados con materiales portadores adecuados adecuadamente seleccionados con respecto a la forma de administracion pretendida, es decir, comprimidos, capsulas (ya sea llenadas con sólidos, llenadas con semisólidos o llenadas con liquido) orales, polvos para su constitución, geles orales, elixires, granulos dispersables, jarabes, suspensiones y demas, y de conformidad con el uso farmacéutico convencional Por ejemplo, para la administración oral en forma de comprimidos o capsulas, el componente farmacológico activo puede estar combinado con cualquier vehiculo oral inerte no tóxico farmacéuticamente aceptable, como ser lactosa, almidón, sacarosa, celulosa, estearato de magnesio, fosfato dicalcico, sulfato de calcio, talco, manitol, alcohol etílico (formas liquidas) y similares Por añadidura, cuando resulta conveniente o necesario, también se pueden incorporar a la mezcla aglutinantes, lubricantes, agentes desintegrantes y agentes colorantes adecuados Los polos y

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comprimidos pueden estar compuestos por aproximadamente 5 a 95 por ciento de la composicion de la presente invención Entre los aglutinantes adecuados se incluyen el almidon, gelatina, azucares naturales, edulcorantes de maiz, gomas naturales y sintéticas tales como acacia, alginato de sodio, carboximetilcelulosa, polietilenglicol y ceras Entre los lubricantes se pueden mencionar, para usar en estas formas de dosificación, ácido bórico, benzoato de sodio, acetato de sodio, cloruro de sodio y demás Entre los desintegrantes se cuentan el almidón, metilcelulosa, goma de guar y similares

También se pueden incluir, en caso de ser apropiado, agentes edulcorantes y saborizantes además de conservantes Más adelante se describen en forma más detallada algunos de los términos citados, como ser desintegrantes, diluyentes, lubricantes, aglutinantes y demas

Además, las composiciones de la presente invención pueden ser formuladas en forma de liberación sostenida para producir la liberación a velocidad controlada de uno o mas de los componentes o ingredientes activos, con el proposito de optimizar los efectos terapéuticos, es decir, la actividad inhibitoria del VHC y demás Las formas de dosificacion adecuadas para la liberación sostenida incluyen comprimidos estratificados que contienen capas con velocidades de desintegracion variadas o matrices poliméricas de liberación controlada impregnadas con componentes activos y moldeados en forma de comprimidos o capsulas que contienen dichas matrices poliméricas porosas impregnadas o encapsuladas

Las preparaciones en forma liquida incluyen soluciones, suspensiones y emulsiones Como ejemplos se pueden mencionar soluciones en agua o agua – propilenglicol para inyecciones parenterales o la adición de edulcorantes y suavizantes para soluciones, suspensiones y emulsiones orales Entre las

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preparaciones en forma liquida se pueden incluir además soluciones para la administracion intranasal

Las preparaciones en aerosol adecuadas par la inhalación pueden incluir soluciones y solidos en forma de polvo, que pueden estar en combinación con un vehiculo farmacéuticamente aceptable tal como un gas comprimido inerte, por ejemplo nitrógeno

Para la preparacion de supositorios, se funde en primer lugar una cera con bajo punto de fusión tal como una mezcla de gliceridos de ácidos grasos tales como la manteca de cacao y luego se dispersa el ingrediente activo de manera homogenea en la misma por agitación o similar técnica de mezclado A continuacion se vierte la mezcla homogenea fundida en moldes del tamaño conveniente, se deja enfriar y asi solidificar

Tambien se incluyen las preparaciones en forma solida destinadas a ser convertidas, poco antes de su uso, en preparaciones en forma liquida para administración oral o parenteral Tales formas liquidas incluyen soluciones, suspensiones y emulsiones

Los compuestos de la presente invencion también se pueden administrar por via transdérmica Las composiciones transdérmicas pueden tomar la forma de cremas, lociones, aerosoles y/o emulsiones y pueden estar incluidas en un parche transdérmico del tipo de matriz o de depósito, ambos convencionales en la tecnica para este fin

De preferencia, el compuesto se administra por vía oral

De preferencia, la preparación farmacéutica se presenta en forma de dosificacion unitaria En esa forma, se subdivide la preparación en dosis unitarias de tamaño adecuado que contienen cantidades apropiadas de los componentes activos, por ejemplo una cantidad efectiva para obtener el fin propuesto

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La cantidad de composicion activa de la invención en una dosis unitaria de la preparación puede ser variada o ajustada en general entre aproximadamente 1,0 miligramo y aproximadamente 1000 miligramos, preferentemente de aproximadamente 1,0 a aproximadamente 950 miligramos, mas preferentemente de aproximadamente 1,0 a aproximadamente 500 miligramos y tipicamente de aproximadamente 1 a aproximadamente 250 miligramos, de acuerdo con la aplicación especifica Se puede variar la dosis real empleada dependiendo de la edad, sexo, peso del paciente, así como de la gravedad de la afeccion en tratamiento Tales técnicas son muy conocidas por los expertos en la tecnica

Generalmente, la forma de dosificacion oral para humanos que contiene los ingredientes activos puede ser administrada 1 ó 2 veces por dia La cantidad y frecuencia de administracion se regula de acuerdo con el criterio del medico a cargo Un régimen diario de dosificacion oral generalmente recomendado puede variar entre aproximadamente 1,0 miligramo y aproximadamente 1 000 miligramos diarios, en dosis unicas o divididas

A continuación se definen algunos terminos utiles

Capsula – se refiere a un envase o envoltura especial hecho de metilcelulosa, alcoholes polivinilicos o gelatinas desnaturalizadas o almidón para sostener o contener composiciones, que comprende los ingredientes activos Las cápsulas de cáscara dura se hacen típicamente de mezclas de gelatinas de hueso y piel de cerdo con gelificacion relativamente alta La capsula en si puede contener pequeñas cantidades de tinturas, agentes opacificantes, plastificantes y conservantes

Comprimido (tableta) – se refiere a una forma de dosificacion solida moldeada o comprimida que contiene los ingredientes activos con diluyentes adecuados El comprimido se puede preparar mediante la compresion de mezclas

o granulaciones obtenidas por granulacion humeda, granulacion seca o por compactacion

Gel oral – se refiere a los ingredientes activos dispersos o solubilizados en una matriz hidrófila semisolida

Polvo para reconstitución - se refiere a mezclas de polvo que contienen los ingredientes activos y diluyentes adecuados que pueden ser suspendidos en agua o jugos

Diluyente – se refiere a sustancias que habitualmente conforman la mayor parte de la composicion o forma de dosificación Los diluyentes adecuados incluyen azucares tales como lactosa, sacarosa, manitol y sorbitol, almidones derivados del trigo, maiz, arroz y papa y celulosas tales como la celulosa microcristalina La cantidad de diluyente en la composición puede oscilar entre aproximadamente 10 y aproximadamente 90 % en peso de la composicion total, preferentemente desde aproximadamente 25 a aproximadamente 75 %, más preferentemente de aproximadamente 30 a aproximadamente 60 % en peso, aun más preferentemente de aproximadamente 12 a aproximadamente 60 %

Desintegrante – se refiere a materiales agregados a la composicion para contribuir a su desmenuzamiento (desintegración) y liberación de los medicamentos Los desintegrantes adecuados incluyen almidones, almidones modificados "solubles en agua fria" tales como el carboximetilalmidón sódico, gomas naturales y sintéticas tales como las de algarrobilla, karaya, guar, tragacanto y agar, derivados de la celulosa tales como metilcelulosa y carboximetilcelulosa sódica, celulosas microcristalinas y celulosas microcristalinas entrecruzadas tales como la croscarmelosa, alginatos tales como el ácido algínico y el alginato de sodio, arcillas como las bentonitas y mezclas efervescentes La cantidad de desintegrante incluido en la composicion puede fluctuar entre

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aproximadamente 2 y aproximadamente 15 % en peso de la composicion, mas preferentemente de aproximadamente 4 a aproximadamente 10 % en peso

Aglutinante – se refiere a sustancias que aglutinan o "pegan" los polvos entre si y los hacen cohesivos formando gránulos, actuando asi como "adhesivo" en la formulación Los aglutinantes suman a la fuerza cohesiva ya existente en el diluyente o agente reforzador Los aglutinantes adecuados incluyen azucares tales como la sacarosa, almidones derivados del trigo, maiz, arroz y papa, gomas naturales tales como acacia, gelatina y tragacanto, derivados de algas marinas tales como el ácido algínico, alginato de sodio y alginato de calcio y amonio, materiales celulosicos tales como metilcelulosa y carboximetilcelulosa sódica, asi como hidroxipropilmetilcelulosa, polivinilpirrolidona y materiales inorgánicos tales como el aluminosilicato de magnesio La cantidad de aglutinante incluida en la composición puede oscilar entre aproximadamente 2 y aproximadamente 20 % en peso de la composición, más preferentemente entre aproximadamente 3 y aproximadamente 10 % en peso, aun mas preferentemente entre aproximadamente 3 y aproximadamente 6 % en peso

Lubricante – designa una sustancia agregada a la forma de dosificación para permitir que el comprimido, los granulos, etc, sean liberados del molde o matriz, una vez comprimidos, reduciendo la friccion o el desgaste Entre los lubricantes adecuados se cuentan los estearatos metalicos tales como el estearato de magnesio, estearato de calcio o estearato de potasio, el ácido estearico, ceras con alto punto de fusión y lubricantes hidrosolubles tales como el cloruro de sodio, benzoato de sodio, acetato de sodio, oleato de sodio, glicoles de polietileno y d'l-leucina Los lubricantes se añaden por lo general en el ultimo paso antes de la compresion, puesto que deben estar presentes en la superficie de los granulos y entre éstas y las partes de la prensa para comprimidos La cantidad de lubricante incluido en la composicion puede fluctuar entre aproximadamente 0,2 y

aproximadamente 5 % en peso de la composición, preferentemente entre aproximadamente 0,5 y aproximadamente 2 %, mas preferentemente entre aproximadamente 0,3 y aproximadamente 1,5 % en peso

Deslizante – material que previene la aglomeracion y mejora las caracteristicas de flujo de las granulaciones, de manera que el flujo sea suave y uniforme Entre los deslizantes adecuados se incluyen el dioxido de silicio y el talco La cantidad de deslizante incluido en la composición puede fluctuar entre aproximadamente 0,1 % y aproximadamente 5 % en peso de la composición total, preferentemente entre aproximadamente 0,5 y aproximadamente 2 %, en peso

Agentes colorantes – excipientes que confieren coloración a la composición o a la forma de dosificacion Tales excipientes pueden incluir tinturas de grado alimentario y tinturas de grado alimentario adsorbidas en un adsorbente adecuado tal como la arcilla o el óxido de aluminio La cantidad de agente colorante puede variar entre aproximadamente 0,1 y aproximadamente 5% en peso de la composición, preferentemente entre aproximadamente 0,1 y aproximadamente 1 %

Biodisponibilidad – se refiere a la velocidad y al grado en que el ingrediente farmacologico activo o porción terapéutica es absorbido en la circulacion sistémica desde una forma de dosificación administrada en comparación con un standard o control

Los métodos convencionales de preparación de comprimidos son conocidos Tales métodos incluyen métodos en seco como la compresion directa y la compresión de granulación producida por compactación, o bien metodos humedos u otros procedimientos especiales También son conocidos los métodos convencionales para preparar otras formas de administracion tales como, por ejemplo, cápsulas, supositorios y demas

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Otra realización de la presente invención describe el uso de las composiciones farmaceuticas antes descriptas para el tratamiento de enfermedades tales como la hepatitis C y similares El metodo comprende administrar a un paciente que padece dicha enfermedad, o enfermedades, y necesita dicho tratamiento, una cantidad terapéuticamente efectiva de la composición farmaceutica de la presente invención

Como se indicara anteriormente, la invencion incluye los tautómeros, enantiomeros y tambien otros estereoisómeros de los compuestos Por consiguiente, como sabra apreciar la persona capacitada en la tecnica, algunos de los compuestos de la presente invención pueden existir en forma tautomerica Tales variaciones estan contempladas dentro del alcance de la presente invención

Otra realizacion de la presente invencion describe un método para preparar los compuestos macrociclicos descriptos en la presente Los compuestos se pueden prepara mediante varias tecnicas conocidas en la técnica Se esbozan los procedimientos ilustrativos representativos en los siguientes esquemas de reaccion Se debe tener en cuenta que, si bien los siguientes esquemas ilustrativos describen la preparacion de los macrociclos predominantemente derivados de meta-tirosina o lisina en la posicion P2, la sustitucion adecuada de cualquiera de los aminoácidos tanto naturales como no naturales dará como resultado la formación de los macrociclos deseados basados en dicha sustitución

Las siguientes son abreviaturas utilizadas en las descripciones de los esquemas, preparaciones y los ejemplos siguientes

THF, tetrahidrofurano

DMF N,N-dimetilformamida

EtOAc acetato de etilo

AcOH acido acetico

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HOOBt 3-hidroxi-1,2,3-benzotriazin-4(3H)-ona

EDCI 1-(3-dimetilaminopropil)-3-etilcarbodiimida clorhidrato

MMM N-metilmorfolina

ADDP 1,1'-(azodicarbonil)dipiperidina

DEAD dietilazodicarboxilato

MeOH Metanol

EtOH Etanol

Et_2O éter dietílico

Bn bencilo

Boc terc-butiloxicarbonilo

Cbz Benciloxicarbonilo

Cp ciclopentildienilo

Ts p-toluensulfonilo

Me Metilo

HATU = hexafluorofosfato de O-(7-azabenzotriazol-1-il)-N,N,N',N'-tetrametilamonio

Chg ciclohexilglicina

Tyr tirosina

G glicerol

TG triglicerol

alloc aliloxicarbonilo

FMOC, 9-fluorenil metiloxicarbonilo

Dde N-1-(4,4-dimetil-2,6-dioxociclohex-1-ilideno)etilo

tBu terc-butilo

equiv equivalente

int rel intensidad relativa

ac acuoso

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ta temperatura ambiente

sat saturado

Hex hexano(s)

ANB acido nitrobenzoico

PyBrOP hexafluorofosfato de tris(pirrolidino)bromfosfonio

DMSO dimetilsulfoxido

ATF acido trifluoroacético

HOBt hidroxibenzotriazol

Base de Hünigs diisopropiletilamina

BOP hexafluorofosfato de benzotriazol-1-iloxitris(dimetilamino)fosfonio

LDA diisopropilamida de litio

Ph_3P trifenilfosfina

LAH hidruro de litio y aluminio

DMAP 4-dimetilaminopirdina

DCC diciclohexilcarbodiimida

MCPBA ácido meta-cloroperbenzoico

BINAP 2,2'-bis(difenilfosfino)-1,1'-binaftol

MeCN acetonitrilo

Pr propilo

Ac acetilo

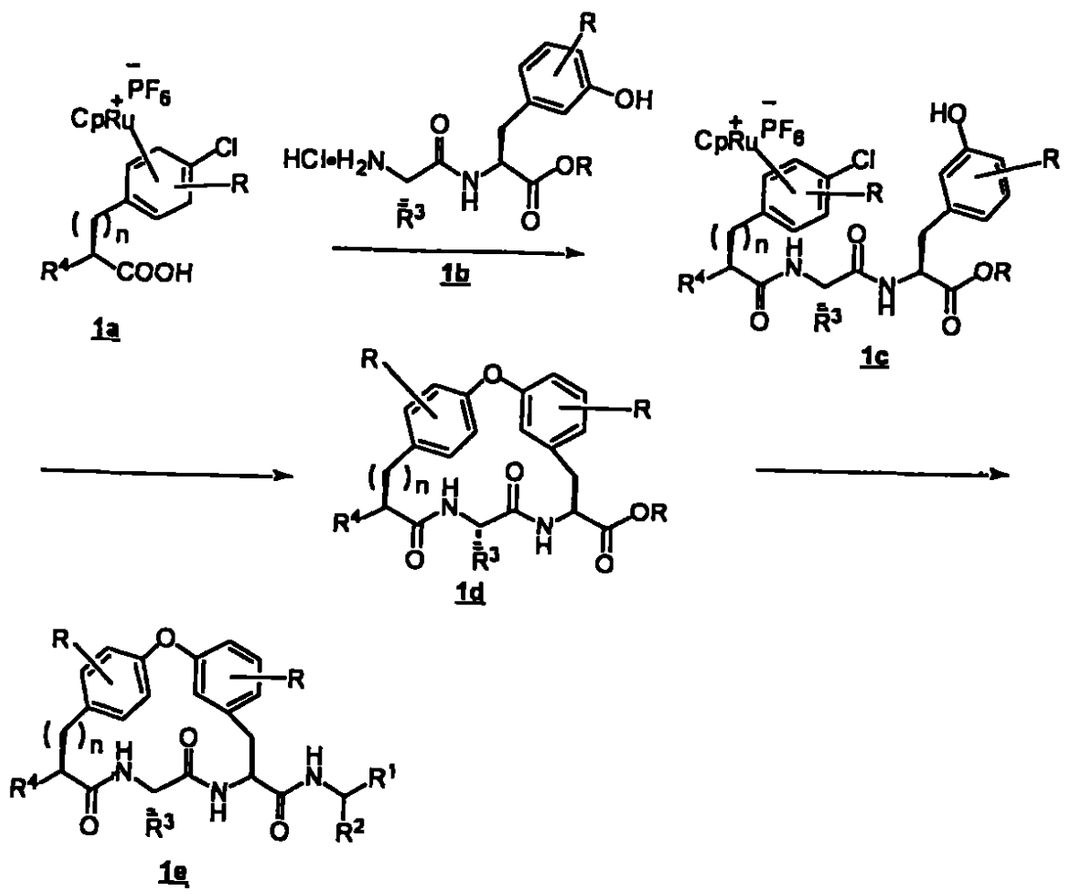
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Esquemas Generales para la Sintesis de los compuestos de la invención

En los siguientes Esquemas, n representa un numero de 1 a 6

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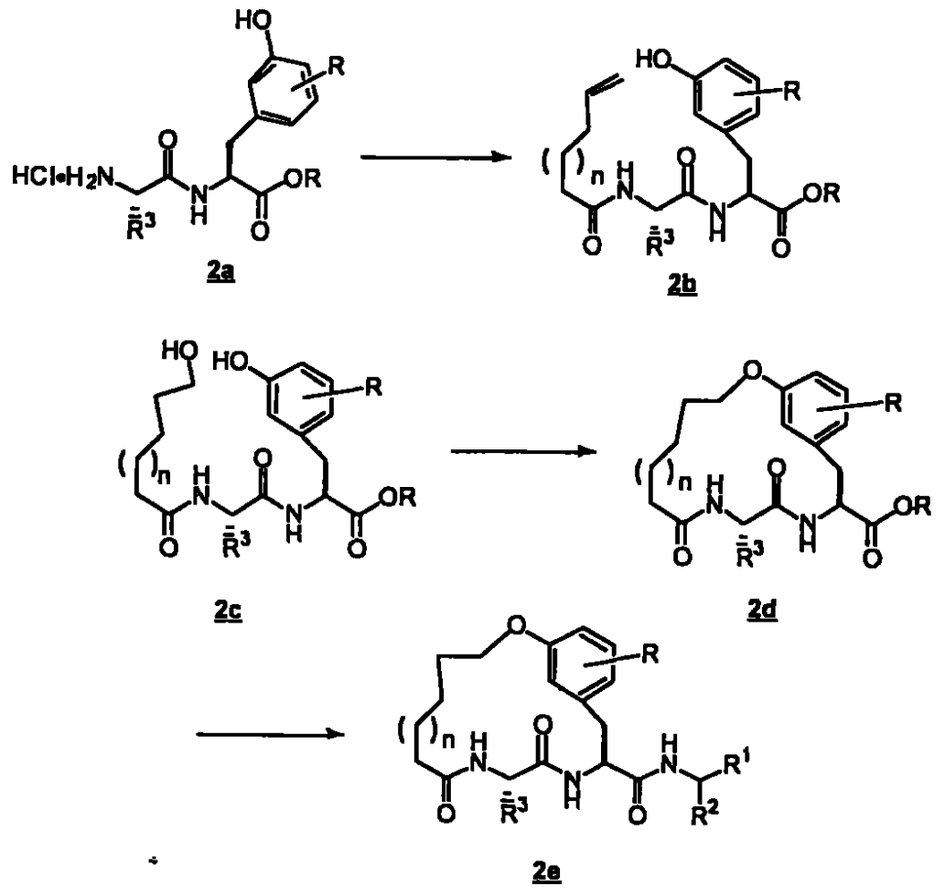
Esquema 1



1a 1b 1c 1d 1e

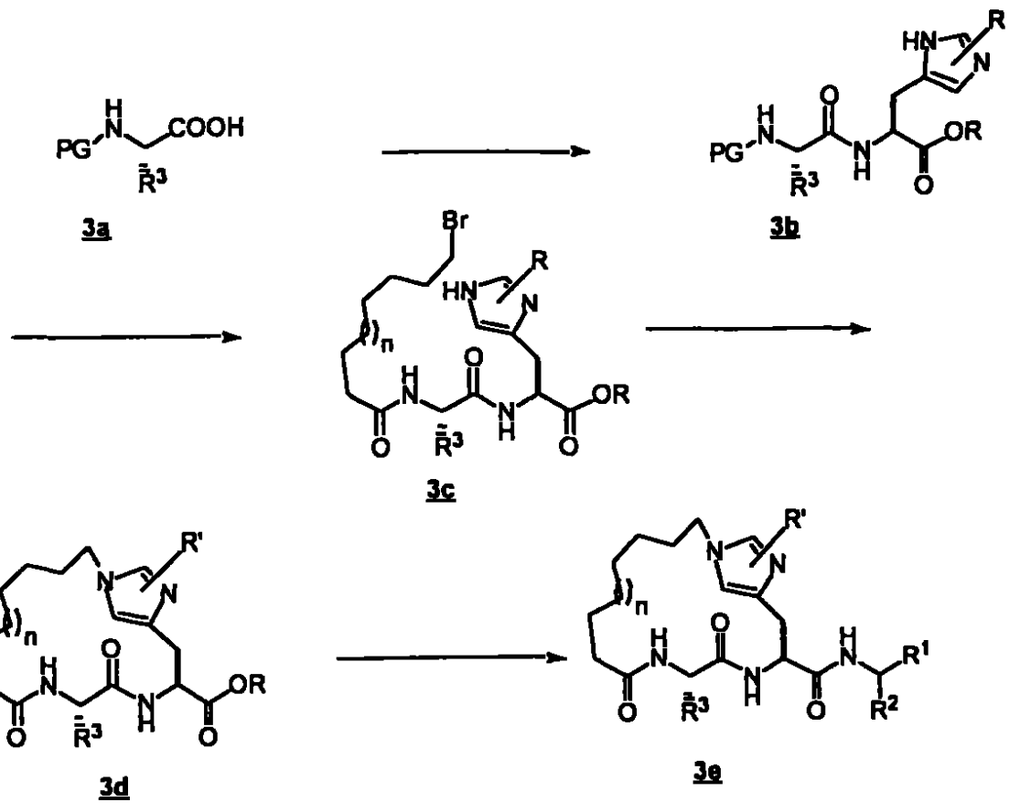
La síntesis de los compuestos del tipo 1e, en los cuales R1, R^2, R^3, R son como se los definiera anteriormente, donde R^4 es amida, carbamato o hidrogeno, R es alquilo, arilo o arilalquilo, comenzo con el acoplamiento de 1a con el dipéptido 1b mediante el uso de NMM, HOBt y EDCI para obtener el intermediario 1c Se trató al intermediario 1c con Cs_2CO_3 en DMF seguido por fotolisis para obtener el compuesto 1d Se hidrolizo el ester macrociclico 1d y se lo acopló a un intermediario aminico adecuado para generar los compuestos del tipo 1e

Esquema 2



En el Esquema 2 se resume la preparación del compuesto de la fórmula 2e, en la cual R^1, R^2, R^3 y n son como se los definiera anteriormente, R' es alquilo, heteroalquilo (OR", SR"', NR"R"' donde R" y R"' son grupos alquilo), el sustituyente halo en la posicion orto, meta o para con respecto al atomo de oxigeno, R representa grupos alquilo, arilo o alquilarilo y n es de cero a cinco Se acopla el dipeptido meta-tirosina 2a a un ácido alquenilcarboxilico en presencia de HOOBt, EDCI HCl y NMM La hidroboracion del producto resultante produce el compuesto 2c. Se obtiene la macrociclacion en condiciones de Mitsunobu utilizando trifenilfosfina y ADDP (La reaccion de Mitsunobu ha sido reseñada por D L Hughes, *Org Reactions*, 42 (1992) 335, John Wiley & Sons, Nueva York, L Paquette, edit) Una vez hidrolizado el ester a un acido con hidróxido de litio, se lo acopla a un intermediario aminico para dar 2e

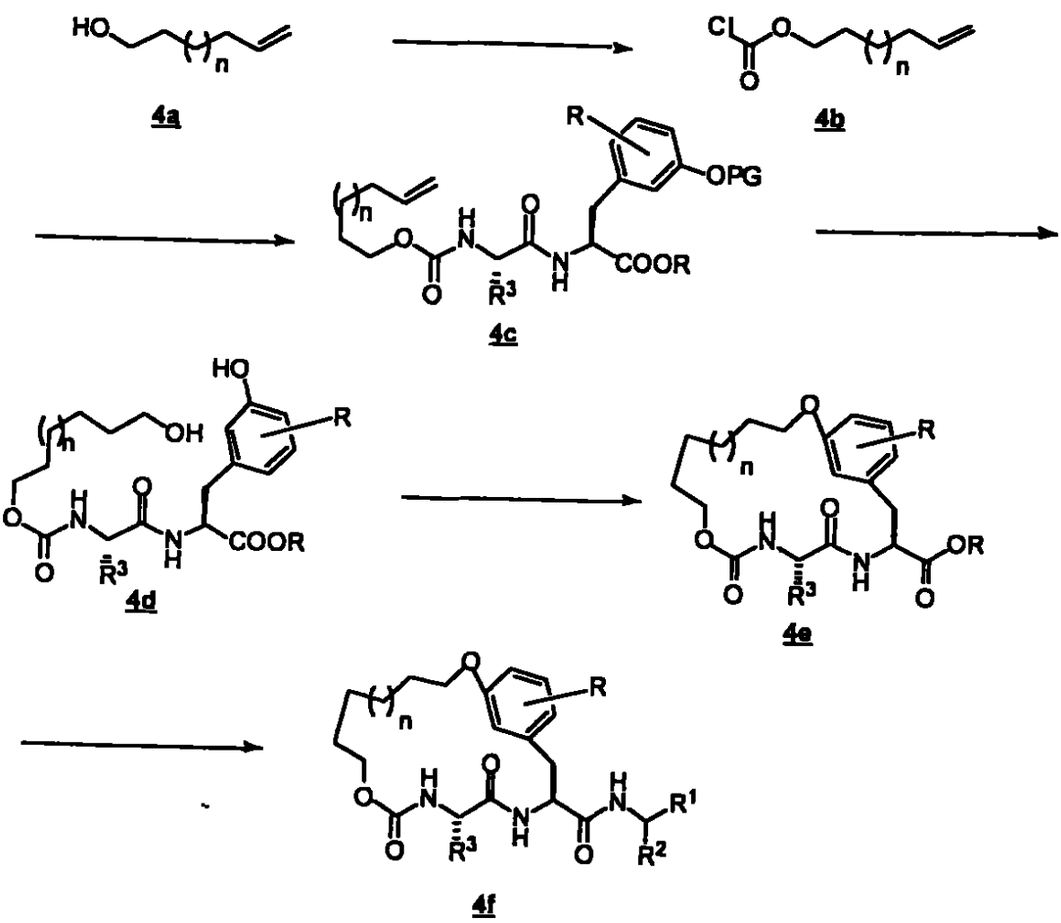
Esquema 3



En el Esquema 3 se resume la preparación del compuesto de la formula 2e, en la cual R', R^1, R^2, R^3, R y n son como se los definiera en el Esquema 1 y PG es Cbz, Boc o alloc. Se acopló al compuesto 3a con un derivado de histidina sustituido utilizando DCC Este compuesto 3b fue desprotegido y tratado tambien con ω-bromo ácidos para obtener un compuesto del tipo 3c Se logró la ciclación de 3c con NaI y Na_2CO_3 en metilvinil cetona en ebullicion para dar 3d Se convirtió el compuesto 3d a compuestos del tipo 3e por medio de la hidrolisis del éster seguida por acoplamiento con el intermediario aminico apropiado

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171

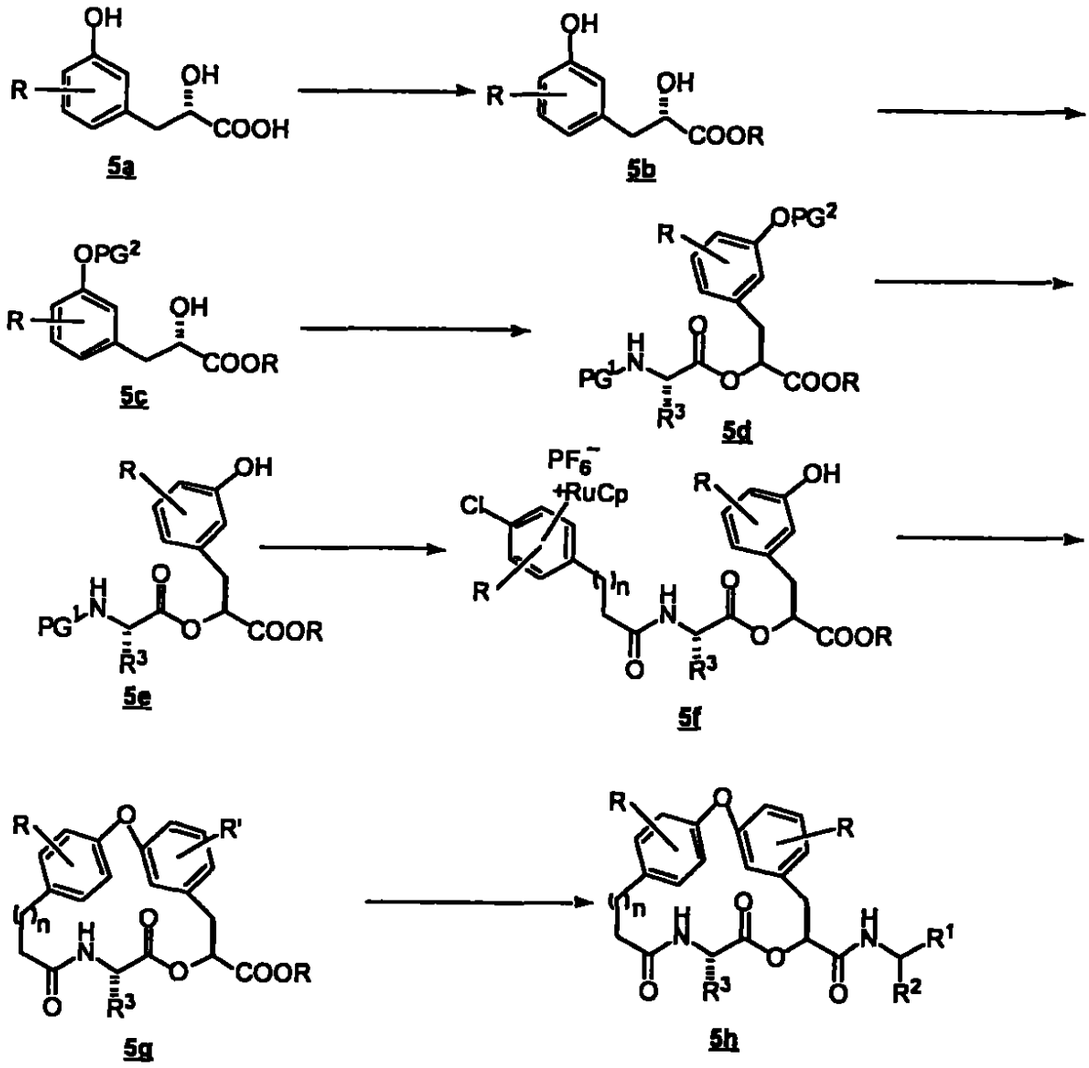
Esquema 4



Se dio comienzo a la preparacion de los compuestos del tipo 4f en los que R', R^1, R^2, R^3, R, n y PG son como se los definiera en el Esquema 1 a partir del compuesto del tipo 4a Se convirio al alcohol de 4a a 4b mediante tratamiento con fosfeno Se convirtio a 4b a 4c mediante acoplamiento con 1b protegido con alloc y Et_3N El grupo alloc de 4c fue desprotegido utilizando $Pd(PPh_3)_4$ para obtener 4d, que sufnó la ciclación en condiciones de Mitsunobu para dar 4e Se hidrolizó el ester 4e y luego se lo acoplo con una amina utilizando EDCI, HOOBt para obtener los compuestos del tipo 4f

C 176
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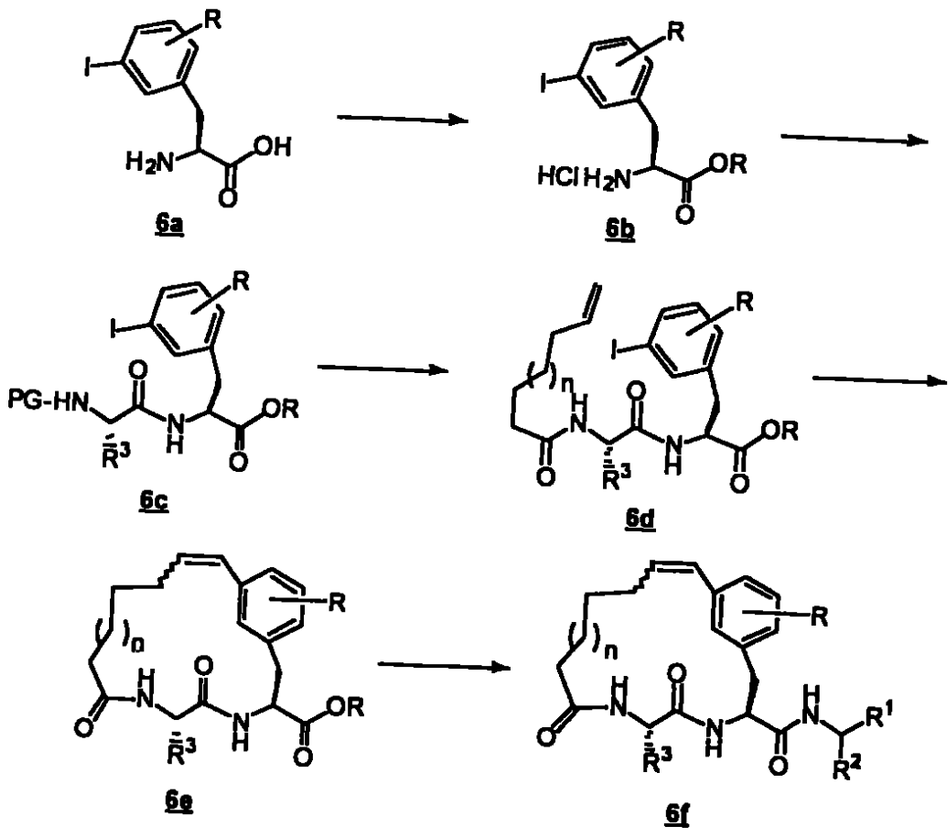
Esquema 5



Se dio comienzo a la preparación de los compuestos del tipo 5h en los que R^1, R^2, R^3, R', PG y n son como se los definiera en los Esquemas 1-3 y donde PG^1 es CBz o Boc y PG^2 es alloc, a partir del compuesto del tipo 5a Se convirtio al acido 5a al ester llevándolo a reflujo con ROH y TsOH Se convirtió al oxigeno fenólico de 5b al grupo alloc mediante tratamiento con alloc-cloruro y trietilamina para dar 5c Se convirtió al alcohol secundario de 5c a los compuestos del tipo 5d

mediante acoplamiento con ciclohexilglicina protegida utilizando Dcc y HOBt Se desprotegió al grupo alloc del compuesto 5d usando $Pd(Ph_3P)_4$y dimedona Se desprotegió a 5e y se lo trato con EDCI, HOOBt y un complejo de rutenio debidamente activado para obtener los compuestos de la fórmula 5f Los compuestos del tipo 5f fueron convertidos a compuestos cíclicos de la formula 5g mediante el uso de Cs_2CO_3 y posterior remocion fotolitica del rutenio Se hidrolizó al grupo éster de 5g y se lo acoplo a un intermediario aminico para obtener los compuestos del tipo 5h

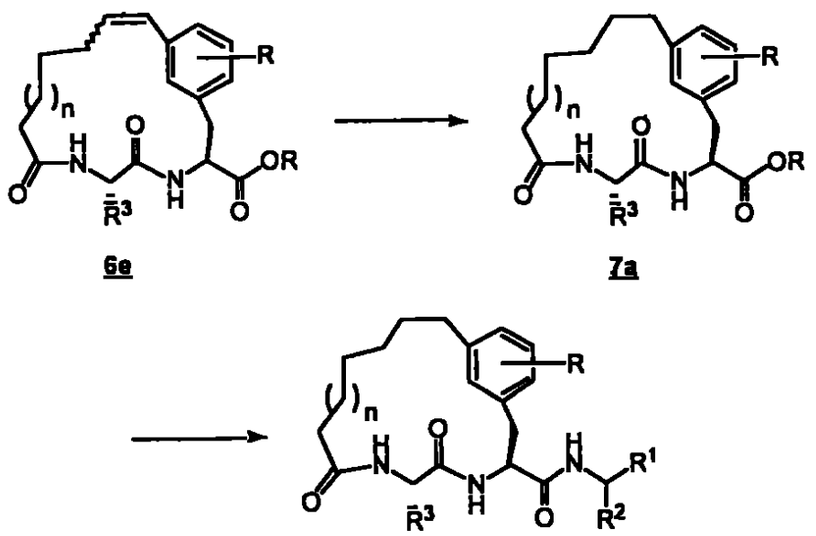
Esquema 6



~~73~~ ~~78~~
174

En el Esquema 6 se reseña la preparación del compuesto de la fórmula 6f en la cual R^1, R^2, R^3 son como se los definiera anteriormente, R' es alquilo, heteroalquilo (OR", SR", NR'R"' donde R" y R"' son grupos alquilo), el sustituyente halo en la posicion orto, meta o para, R representa grupos alquilo, arilo o alquilarilo, PG es Cbz o Boc y n es de cero a cinco Se convierte la meta-yodofenilglicina 6a a su ester en las condiciones habituales de esterificación (ROH, HCl) Luego se acopla al producto a un aminoacido N-protegido en presencia de HOOBt, EDCI-HCl y NMM Después de la desprotección, se vuelve a acoplar la amina producida a un acido alquenilcarboxilico terminal para dar el producto 6d La reaccion de Heck intramolecular con un catalizador de paladio produce el compuesto macrociclico buscado 6e (La reaccion de Heck ha sido reseñada en detalle por R F Heck, *Org, React*, 27 (1989) 345-390) Una vez hidrolizado el ester a un ácido con hidroxido de litio, se lo acopla a un intermediario aminico para producir 6f

Esquema 7



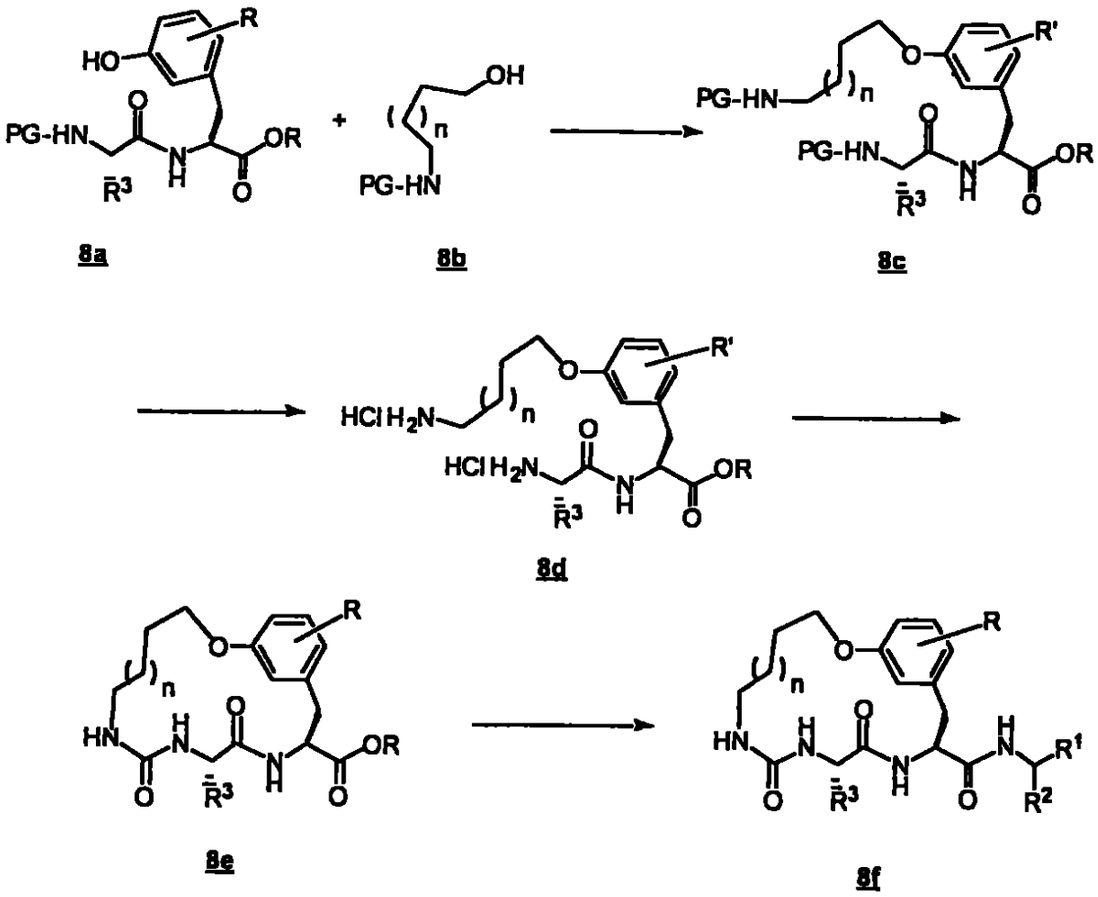
6e 7a

7b

175

En el Esquema 7 se reseña la preparación del compuesto de la fórmula 7b en la cual R^1, R^2, R^3 son como se los definiera anteriormente, R' es alquilo, heteroalquilo (OR", SR"', NR"R"' donde R" y R"' son grupos alquilo), el sustituyente halo en la posición orto, meta o para, R representa grupos alquilo, arilo o alquilarilo y n es de cero a cinco La hidrogenacion del doble enlace de 6e dio el macrociclo 7a La hidrolisis del éster a ácido y el posterior acoplamiento con un intermediario aminico produjo 7b

Esquema 8

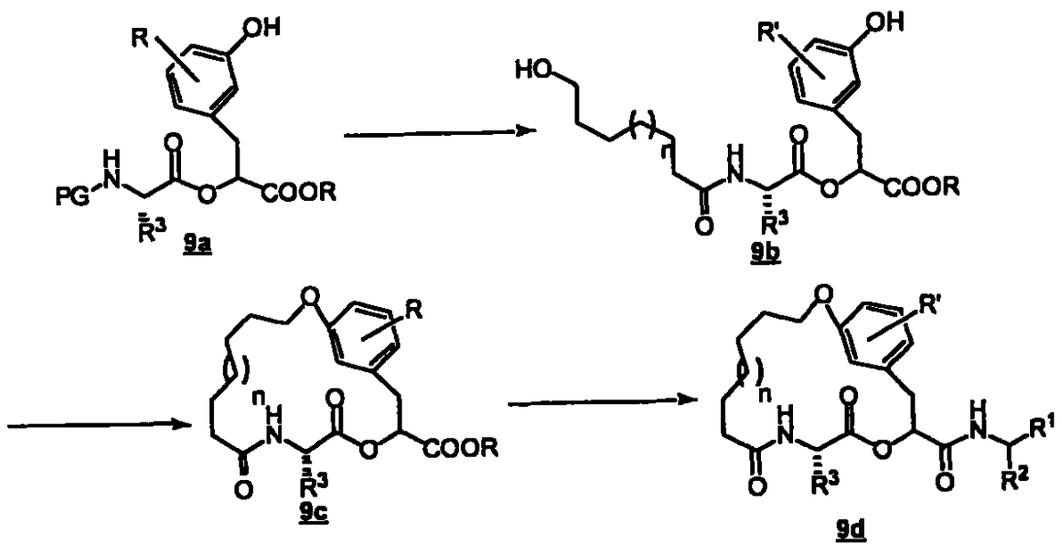


En el Esquema 8 se reseña la preparacion del compuesto de la fórmula 8f en la cual R^1, R^2, R^3 son como se los definiera anteriormente, R' es alquilo, heteroalquilo (OR", SR"', NR"R"' donde R" y R"' son grupos alquilo), el

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sustituyente halo en la posicion orto, meta o para con respecto al átomo de oxigeno, R representa grupos alquilo, arilo o alquilarilo PG es Boc y n es de cero a cinco Se acopla el meta-tirosina-dipéptido 8a a un alcohol aminico N-protegido terminal 8b en condiciones de Mitsunobu (trifenilfosfina y ADDP) Se eliminan los grupos protectores para dar el clorhidrato de diamina 8d Se obtiene la macrociclación formando el ligamiento de urea por medio del uso de fosgeno o carbonildiimidazol Luego se hidroliza el éster resultante a un ácido con hidróxido de litio y seguidamente se lo acopla a un intermediario aminico para dar el producto deseado 8f

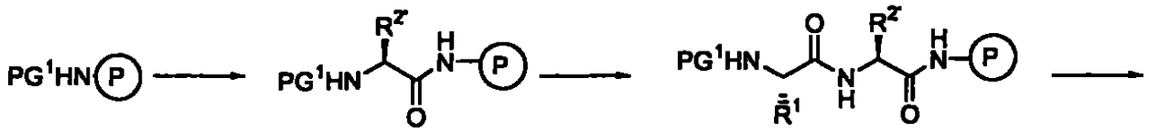
Esquema 9



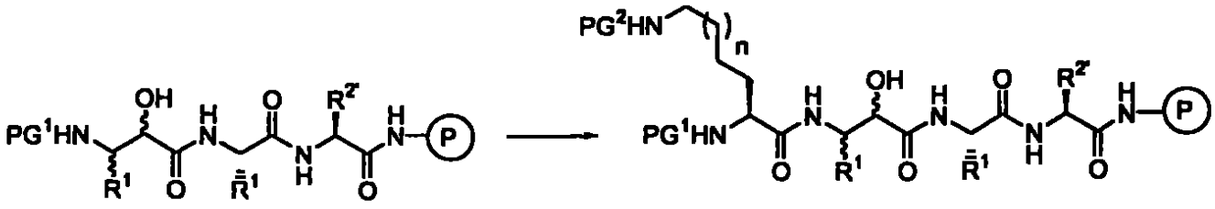
Se dio inicio a la síntesis de los compuestos del tipo 9d en los cuales los sustituyentes R^1, R^2, R^3 R' R u PG son como se los definiera en el Esquema 1, mediante la desprotección de 9a con HCl en dioxano y acoplamiento con ω-hidroxiacido utilizando EDCI, HOOBt para dar los compuestos del tipo 9b Los compuestos del tipo 9b fueron adicionalmente ciclados utilizando Ph_3P, ADDP para generar los compuestos del tipo 9c Se desprotegio al compuesto ciclico 9c y se lo acoplo con un intermediario aminico apropiado para generar el compuesto del tipo 9d

Esquema 10

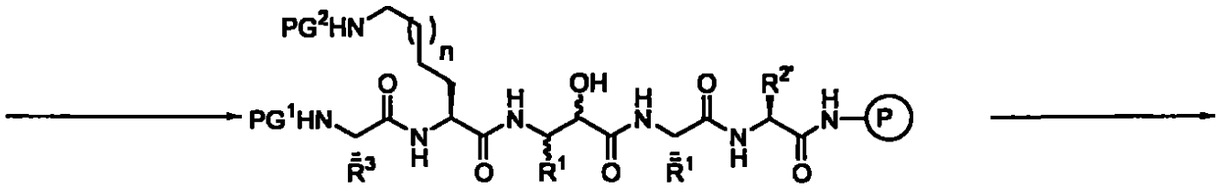
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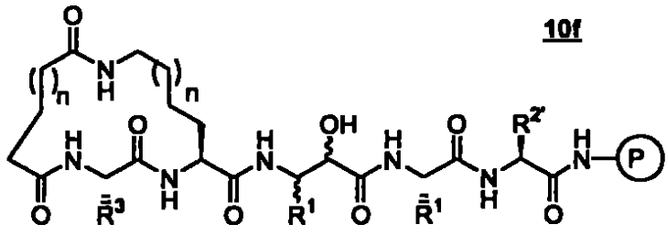
10a 10b 10c



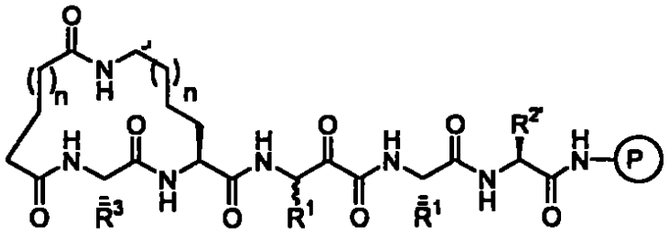
10d 10e



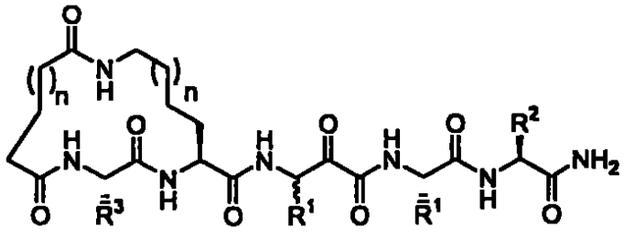
10f



10g



10h



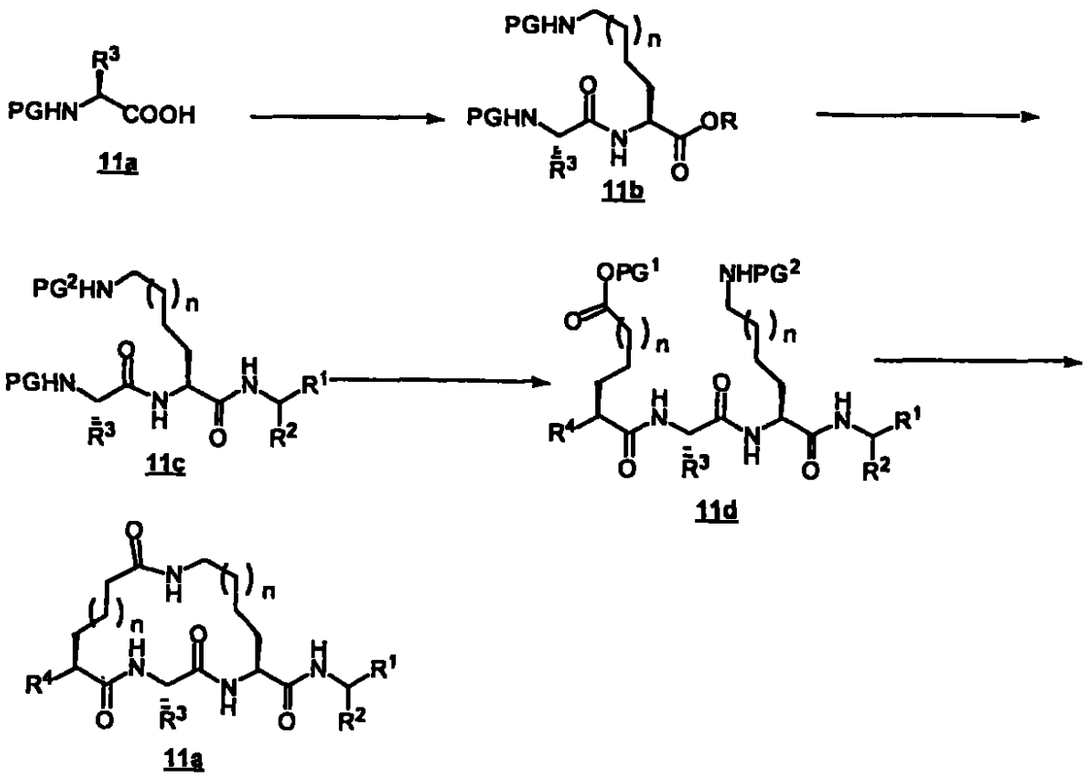
10i

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La síntesis de los compuestos del tipo 10i en los cuales R^2, R^1, R^1, R^3 se definen en el Esquema 1 PG^1 y PG^2 se definen como grupos protectors, a saber Fmoc y Dde P se define como soporte polimerico en el cual se inmoviliza el compuesto y n es un numero de 1-6 La metodologia empleada para la síntesis de las moléculas del tipo 10i es una síntesis de peptidos en fase sólida normal con un grupo protector Fmoc En primer lugar se desprotege una resina Sasarin protegida con Fmoc 10a mediante tratamiento de piperidina seguido por acoplamiento con el aminoácido protegido con Fmoc utilizando HATU para obtener el compuesto del tipo 10b Se retira el grupo protector de 10b y se acopla a este con aminoácido para usar HATU a in de obtener 10c Se desprotegio a 10c inmovilizado en un soporte polimerico mediante tratamiento con piperidina y se lo acopla con un hidroxiácido para obtener la hidroxiamida del tipo 10d Se escindió el grupo protector de 10d y se acopló a este con un derivado de lisina utilizando HATU para obtener los compuestos del tipo 10e Una vez mas de desprotegió al grupo protector de 10e y se lo acopló con un aminoacido protegido con Fmoc para obtener el compuesto del tipo 10f Se eliminaron los grupos protectores PG^1 y PG^2 y se los ciclo utilizando un diácido y HATU para obtener el macrociclo 10g Se oxido al compuesto 10g utilizando reactivo de Dess-Martin y por ultimo se lo separó de la resina utilizando ATF para obtener el compuesto 10i

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Esquema 11



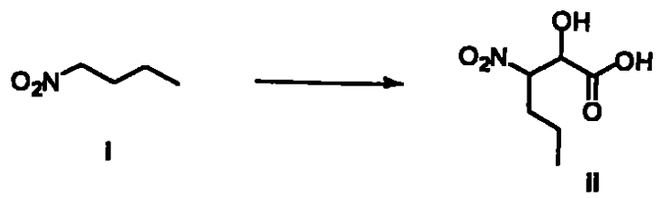
Se dio comienzo a la síntesis de los compuestos del tipo 11e en los cuales R^1, R^2, R^3, R^4 y n fueran definidos en el Esquema 1 y PG^2 es CBz, PG^1 es Bn y PG es Boc, a partir del ácido protegido 11a Se convirtió a 11a a los compuestos del tipo 11b mediante acoplamiento con un derivado de lisina utilizando metodología con EDCI, HOOBt El grupo ester de 11b fue hidrolizado empleando LiOH H_2O seguido por acoplamiento con un intermediario amínico apropiado para obtener el compuesto 11c Este fue tratado además con HCl en dioxano y acoplado con el intermediario lisina utilizando EDCI, HOOBt para formar los compuestos del tipo 11d Los compuestos del tipo 11d fueron desprotegidos y ciclados empleando EDCI, HOOBt para formar los compuestos del tipo 11e

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Preparación de los intermediarios.

Intermediario A

Paso 1



I II

A una solucion agitada de 1-nitrobutano (16,5 g, 0,16 mol) y ácido glicolico en H_2O (28,1 g, 0,305 mol) y MeOH (122 ml) a 0°C-5°C, se agregó por goteo trietilamina (93 ml, 0,667 mol) en el curso de 2 horas La solución fue calentada a temperatura ambiente, agitada durante la noche y concentrada a sequedad para dar un aceite Luego se disolvio el aceite en H_2O y se lo acidificó a pH = 1 con HCl al 10 %, seguido por extraccion con EtOAc La solución organica combinada fue lavada con salina, secada sobre Na_2SO_4, filtrada y concentrada a sequedad para dar el producto II (28,1 g, 99 % de rendimiento)

Paso 2



II III

A una solución agitada del material de partida II (240 g, 1,35 mol) en ácido acetico II (1,25 l) se agrego PD/C al 10 % (37 g) La solucion resultante fue hidrogenada a 59 psi (406 kPa) por espacio de 3 h y luego a 60 psi (413,6 kPa) durante la noche A continuación se evaporo el acido acetico y se la azeotropizo 3 veces con tolueno, luego se la trituró con MeOH o éter Seguidamente, la solución

fue filtrada y azeotropizada dos veces con tolueno para dar iii en forma de solido blanquecino (131 g, 0,891 mol, 66 %)

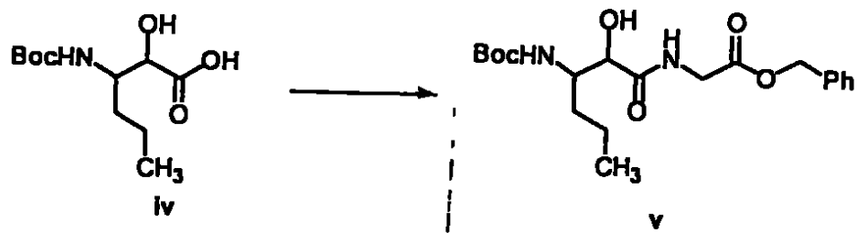
Paso 3



iii iv

A una solución agitada del aminoácido iii (2,0 g, 0,136 mmol) en dioxano (10 ml) y H_2O (5 ml) a 0°C, se agrego una solucion de NaOH 1N (4,3 ml, 14,0 mmol) La solucion resultante fue agitada por espacio de 10 minutos, tras lo cual se agregó di-t-butildicarbonato (0,110 g, 14,0 mmol) y se la agito a 0°C por un lapso de 15 minutos A continuación, la solución fue calentada a temperatura ambiente, agitada durante 45 minutos y mantenida en el refrigerador durante la noche y concentrada a sequedad para dar un material crudo A la solución de este material crudo en EtOAc (100 ml) y hielo, se agregó $KHSO_4$ (3,36 g) y H_2O (32 ml) y se la agito durante 4-6 minutos Luego se separó la capa orgánica y se extrajo la capa acuosa dos veces con EtOAc y la capa orgánica combinada fue lavada con agua, salina, secada sobre Na_2SO_4, filtrada y concentrada a sequedad para dar el producto iv en forma de goma traslucida (3,0 g, 89 % de rendimiento)

Paso 4

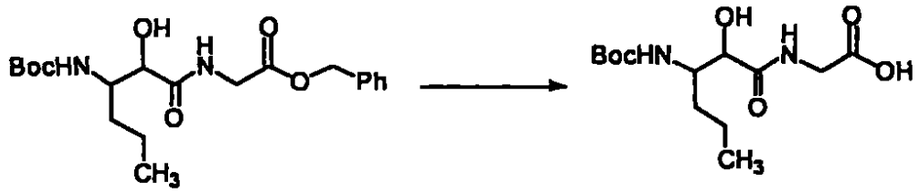


iv v

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A una solucion agitada de iv (3,00 g, 12,0 mmol) en DMF (15 ml) y CH_2Cl_2 (15 ml) a –20°C se agrego HOOBt (1,97 g, 12,0 mmol), N-metilmorfolina (4,0 ml, 36 mmol) y EDCI (2,79 g, 14,5 mmol) y se la agitó por espacio de 10 minutos, tras lo cual se agregó HCl H_2N-Gly-OBn (2,56 g, 13,0 mmol) La solución resultante fue agitada durante 2 horas, luego guardada en el refrigerador durante la noche y concentrada a sequedad, para luego diluirla con EtOAc (150 ml) Seguidamente se lavó la solución de EtOAc dos veces con $NaHCO_3$ saturado, H_2O, H_3PO_4 al 5 %, salina, se la secó sobre Na_2SO_4, se la filtro y concentro a sequedad para dar el producto v (4,5 g, 94 %) LRMS m/z MH^+ = 395,1

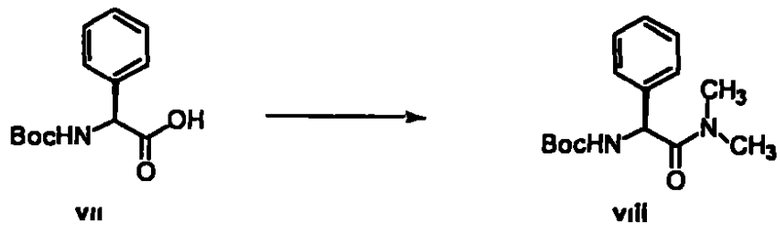
Paso 5



v vi

La solucion del material de partida v (7,00 g, 17,8 mmol) en etanol absoluto (300 ml) fue agitada a temperatura ambiente bajo atmosfera de hidrogeno en presencia de Pd-C (300 mg, 10 %) Se monitoreó el proceso de la reaccion mediante tlc Al cabo de 2 h, la mezcla fue filtrada a través de una plancha de celite y la solucion resultante fue concentrada al vacio para dar el producto vi (5,40 g, cuantitativo) LRMS m/z MH^+ = 305,1

Paso 6

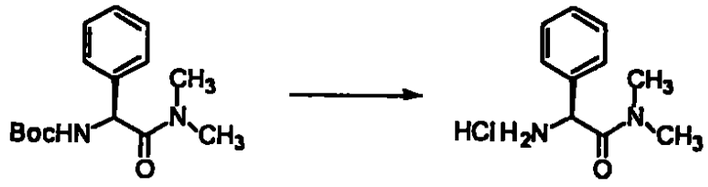


vii viii

0187 183

A una solución de clorhidrato de dimetilamina (1,61 g, 19,7 mmol), N-Boc-fenilglicina (4,50 g, 17,9 mmol), HOOBt (3,07 g, 18,8 mmol) y EDCI (4,12 g, 21,5 mmol) en DMF anhidra (200 ml) y cw (150 ml) a –20°C se agregó NMM (5,90 ml, 53,7 mmol) Despues de ser agitada a esta temperatura por espacio de 30 minutos, la mezcla de reacción fue guardada en un freezer durante la noche (18 h) Luego se la dejó entibiar a temperatura ambiente, y se agrego EtOAc (450 ml), salina (100 ml) y H_3PO_4 al 5 % (100 ml) Una vez separadas las capas, la solución orgánica fue lavada con H_3PO_4 al 5 % (100 ml), una solución acuosa saturada de bicarbonato de sodio (2 x 150 ml), agua (150 ml) y salina (150 ml), secada ($MgSO_4$), filtrada y concentrada al vacio para dar el producto crudo viii (4,86 g) en forma de sólido blanco, que fue utilizado sin más purificación

Paso 7

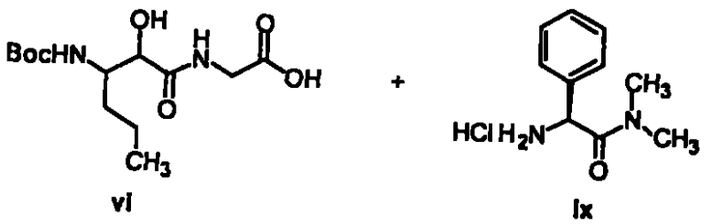


viii ix

Se disolvió la N-Boc-fenilglicina dimetilamida viii (4,70 g, cruda) en HCl 4 N (60 ml, 240 mmol) y la solucion resultante fue agitada a temperatura ambiente Se monitoreo el progreso de la reaccion por TLC Al cabo de 4 h, la solucion fue concentrada al vacio para dar ix en forma de solido blanco que fue utilizado en la siguiente reacción sin mas purificación LRMS m/z MH^+ = 179,0

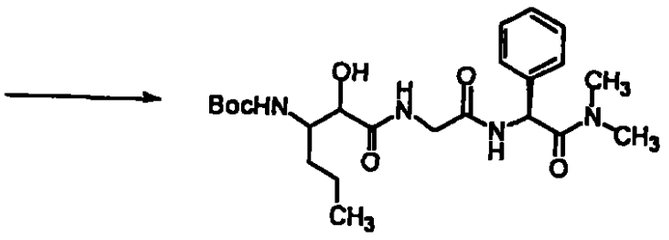
Paso 8

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vi

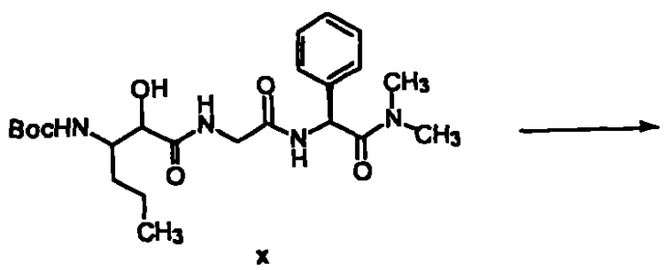
Ix



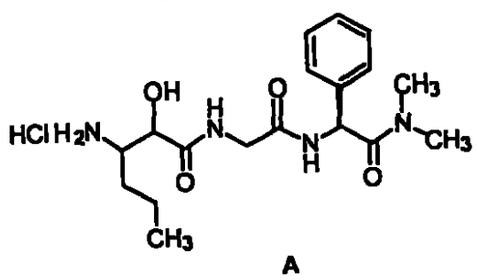
x

Se preparó el compuesto buscado x de acuerdo con los procedimientos de acoplamiento descriptos en el Paso 4 LRMS m/z MH^+ = 465,1

Paso 9



x



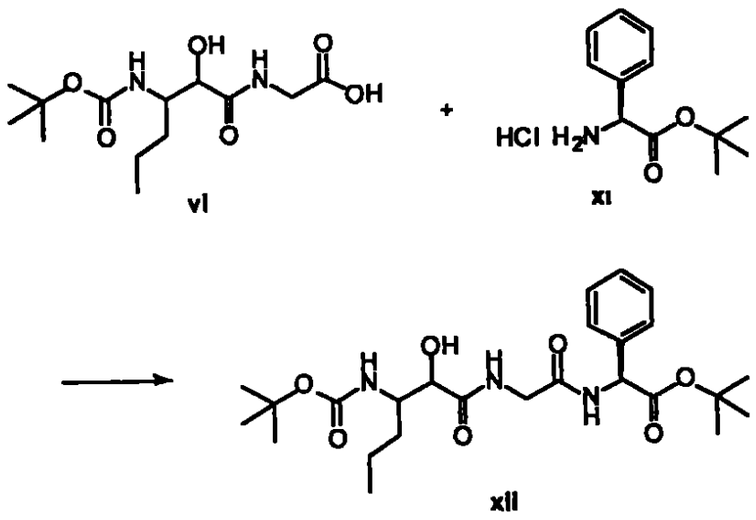
A

0189
185

Se preparo el intermediario propuesto A, a partir del tripeptido x de acuerdo con los procedimientos descriptos en el Paso 7 LRMS m/z MH^+ = 365,1

Intermediario B

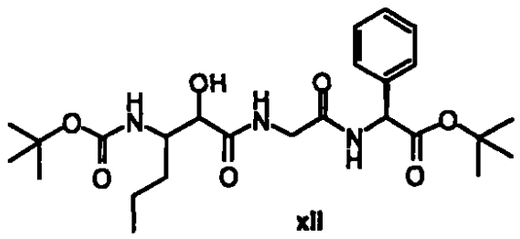
Paso 1



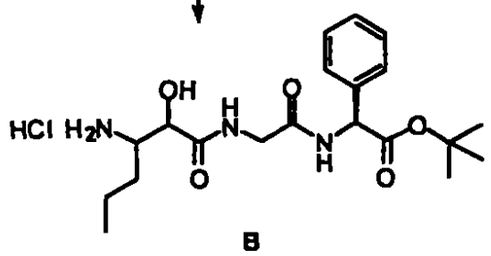
Se obtuvo el producto buscado xii mediante el procedimiento descripto para el intermediario A, paso 8 utilizando xi adquirido en el comercio como adjunto de acoplamiento El material crudo era suficientemente puro para continuar los estudios Una porción del producto fue purificada por cromatografía instantanea utilizando diclorometano/MeOH 97/3 HRMS (FAB) Calc. para $C_{25}H_{40}N_3O_7$ 494,2866 $(M+H)^+$ Encontrado 494,2863

Paso 2

01501186



xii



B

Se obtuvo el producto buscado B mediante el procedimiento descripto para el Intermediario A, Paso 7 Se utilizó el material crudo sin más purificación

Intermediario C

Paso 1



vii xiii

Se preparó el compuesto deseado xiii de acuerdo con los procedimientos de acoplamiento descriptos en el Paso 6 para el Intermediario A.

Paso 2

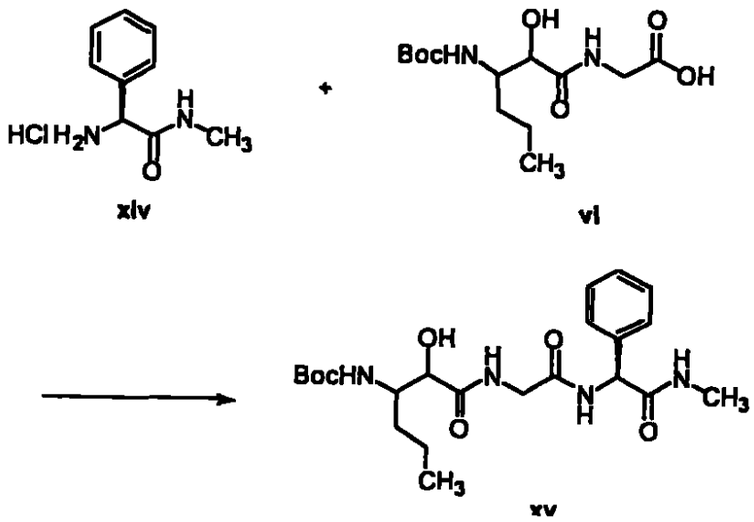


xiii xiv

0181 187

Se preparo el compuesto deseado xiv de acuerdo con los procedimientos descriptos en el Paso 7 para el intermediario A

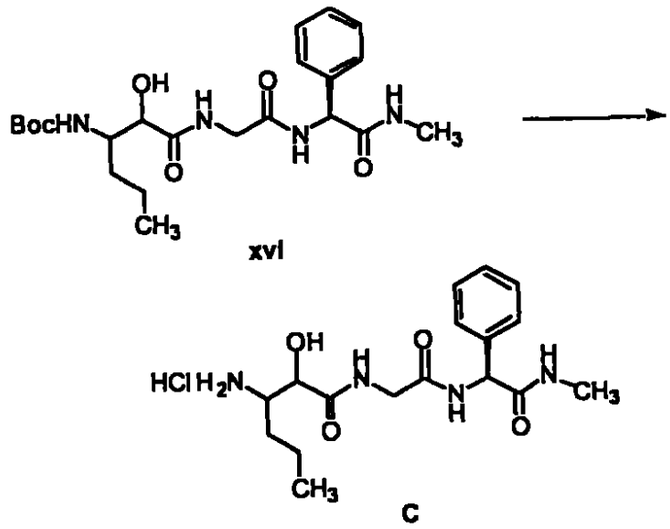
Paso 3



xiv vi xv

Se preparó el compuesto deseado xv de acuerdo con los procedimientos de acoplamiento descriptos en el Paso 6 para el intermediario A LRMS m/z MH^+ = 451,1

Paso 4

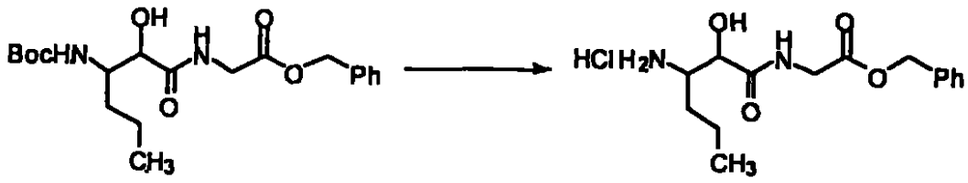


xvi C

0182 188

Se preparo el compuesto intermedio C de acuerdo con los procedimientos descriptos en el Paso 7 para el Intermediario A LRMS m/z MH^+ = 351,1 Se lo utilizo sin más purificacion

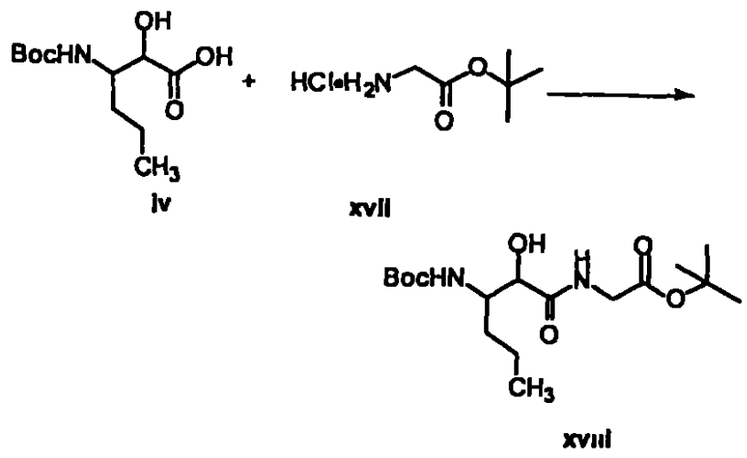
Intermediario D



Se preparo el intermediario D deseado a partir del compuesto v de acuerdo con los procedimientos descriptos en el paso 7 para el intermediario A Se lo utilizó sin más purificación

Intermediario E

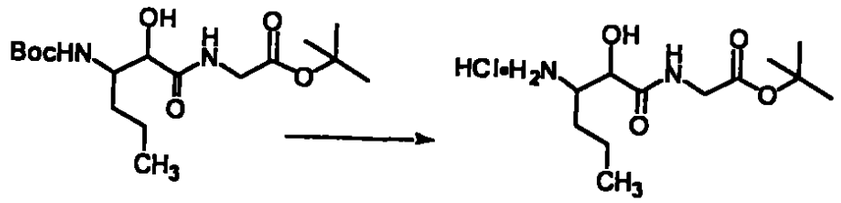
Paso 1



Se obtuvo el producto buscado xviii mediante el procedimiento descripto para el intermediario A, Paso 8, utilizando xvii adquirido en el comercio como

adjunto de acoplamiento El material crudo era suficientemente puro para continuar los estudios

Paso 2

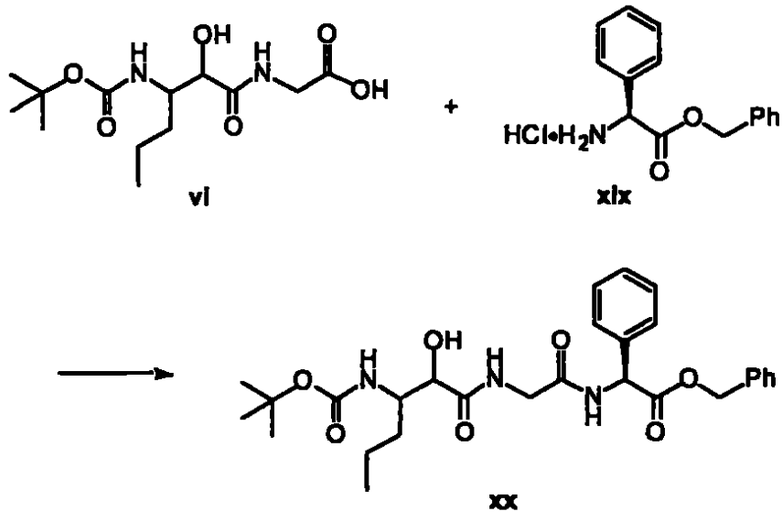


xviii E

Se obtuvo el producto deseado E mediante el procedimiento descripto para el intermediario A, Paso 7 El material crudo fue utilizado sin mas purificacion

Intermediario F

Paso 1



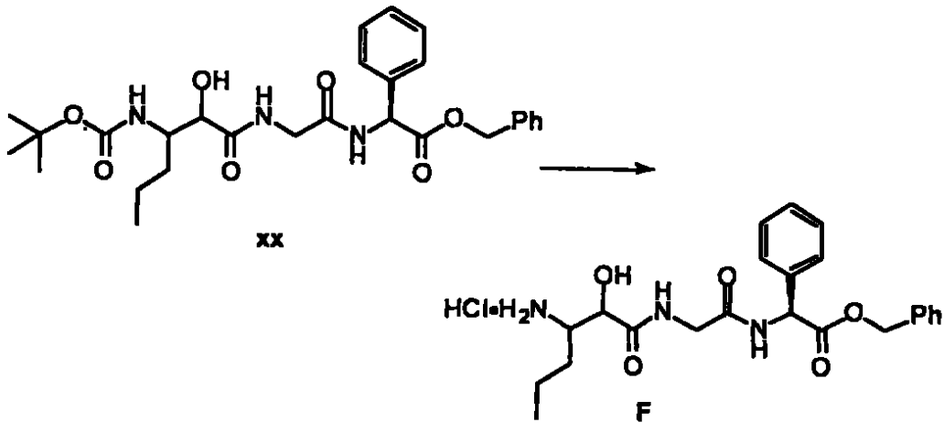
vi xix xx

Se obtuvo el producto deseado xx mediante el procedimiento descripto para el intermediario A, Paso 4, utilizando xix adquirido en el comercio como

190

adjunto de acoplamiento El material crudo era suficientemente puro para continuar los estudios

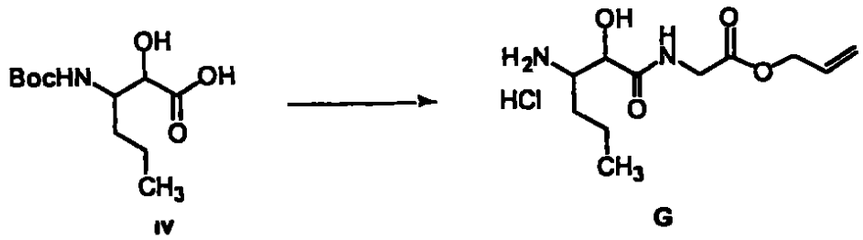
Paso 2



Se obtuvo el producto deseado F mediante el procedimiento descripto para el intermediario D

Intermediario G

Paso 1

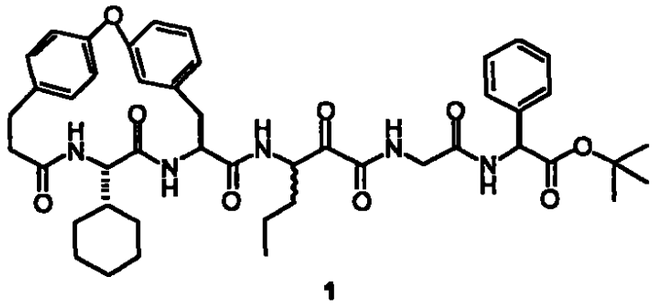


Se obtuvo el producto deseado G mediante el procedimiento descripto para el intermediario A, Paso 4, utilizando alilglicina como adjunto de acoplamiento El material crudo era suficientemente puro El producto crudo fue tratado con HCl 4N/dioxano y agitado a ta por espacio de 50 min La mezcla de reacción fue concentrada a sequedad para dar el intermediario G que fue utilizado sin mas purificacion

191

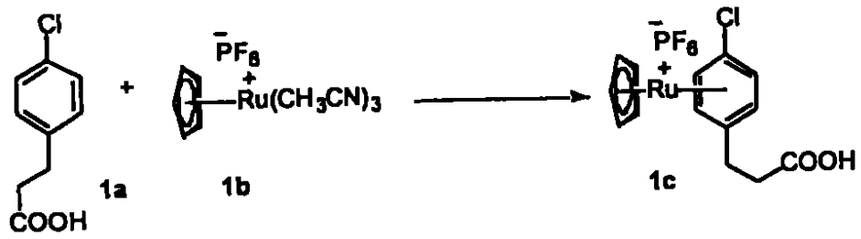
EJEMPLOS

Ejemplo 1 Preparación del Compuesto de la Fórmula 1



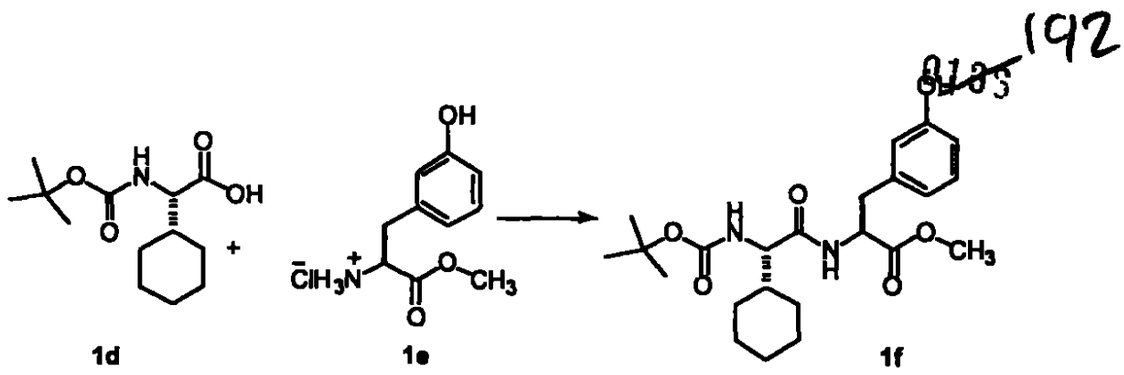
1

Paso A



Una solución de ácido 4-cloropropiónico (2,0 g, 10,8 mmol) de 1a en dicloroetano (200 ml) fue tratada con $CpRu(CH_3CN)_3$ PF_6 (4,7 g, 10,8 mmol, 10 equiv) y calentada a reflujo durante 2 h La mezcla de reaccion fue enfriada a ta , cuando precipitaron del producto 1c cristales incoloros del mismo Los cristales fueron filtrados y lavados con una mezcla 1 1 de Et_2O/CH_2Cl_2 y secados al vacio Los cristales incoloros (3,3 g) eran analiticamente puros RMN ^1H ($CD_3C(O)CD_3$, 400 MHz, ppm, δ, J) 6,77 (d, 2 H, J = 7,0 Hz), 6,53 (d, 2H, J = 7 Hz), 5,64 (s, 4H), 2,87 (t, 2H, J = 7,0 Hz), 2,74 (t, 2H, J = 7,0 Hz) EM (Electroatomizacion, intensidad relativa m/z) 350,9 ($C_{14}H_{14}ClRu^+$, M^+, 100), CHN calc para $C_{14}H_{14}ClF_6O_2PRu$ C =33,92 %, H =2,85 %, Cl =8,15 %, P =6,25 % Encontrado C =34,04 %, H =3,04 %, Cl =7,09 %, P =5,71 %

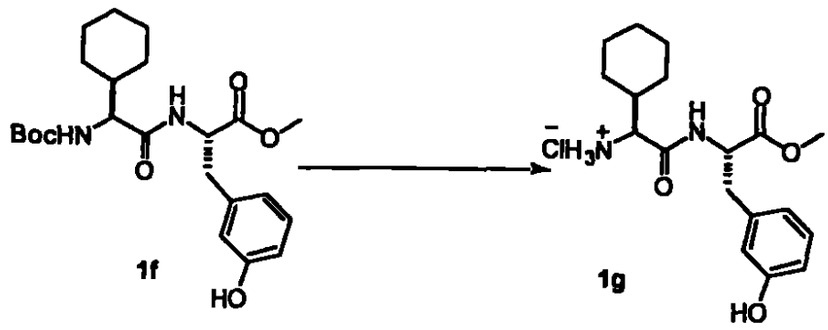
Paso B



1d 1e 1f

UNa solucion de Boc-ciclohexilglicina monohidrato 1d (6,17 g, 24,00 mmol) en CH_2Cl_2 seco (50,0 ml) fue tratada con 4-metilmorfolina (2,64 g, 26,0 mmol, 1,1 equiv) y enfriada a –10°C A esta mezcla se agregó cloroformiato de isobutilo (3,62 g, 3,5 ml, 1,1 equiv) y la suspension blanca fue agitada hasta que la temperatura del baño llego a –5°C Se disolvió la sal de clorhidrato del ester metilico de meta-tirosina (6,5 g, 26,5 mmol, 1,1 equiv) en DMF (30 ml) en un vaso separado y se la trato con 4-metilmorfolina (2,64 g, 26,0 mmol), 1,1 equiv) y se la agitó a ta por espacio de 15 min Esta mezcla fue agregada a la reaccion que estuvo acompañada por la evolucion de CO_2 La mezcla de reacción fue agitada a ta durante 1 h y diluida con HCl ac 1M (100 ml) La capa acuosa fue extraida con acetato de etilo (3x200 ml) y la capa orgánica combinada fue extraida con HCl 1M (1 x 100 ml), NaOH ac (1 x 100 ml), salina (1 x 100 ml), secada (Na_2SO_4), concentrada al vacio y purificada por cromatografia (SiO_2, EtOAc/Hexanos 3/7) para dar 5,3 g (53 %) del compuesto acoplado 1f en forma de espuma incolora

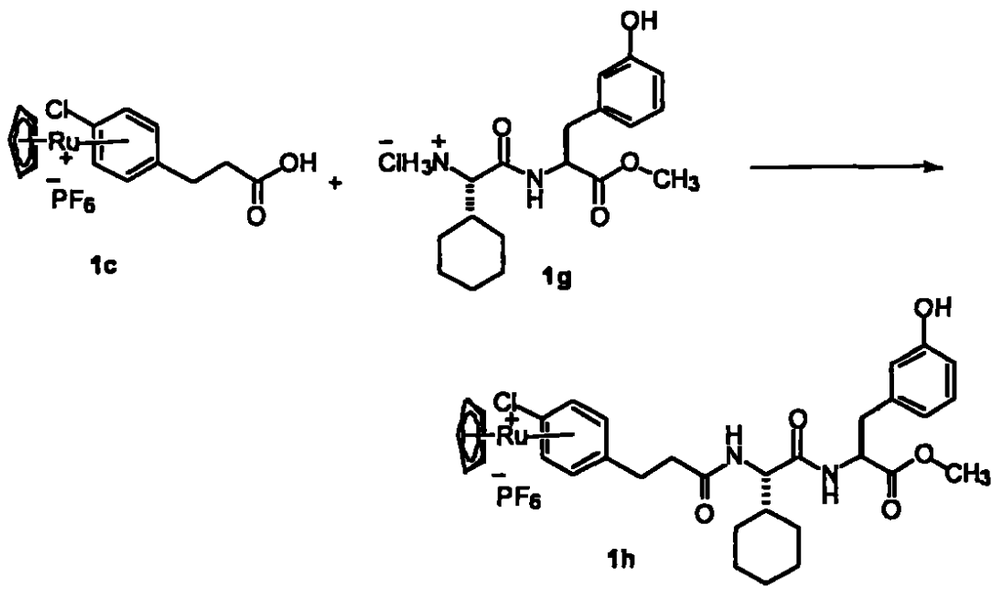
Paso C



1f 1g

Una solucion de 1f (10 g, 23,04 mmol) fue disuelta en HCl (solucion 4M en dioxano, 100 ml) y agitada a ta por espacio de 2-4 h La mezcla de reacción fue concentrada al vacio y el sólido resuspendido en eter Éste fue filtrado y el solido lavado con éter, al que se secó para dar un sólido incoloro, 1 g (8,2 g, 96 %) RMN ^1H ($CD_3C(O)CD_3$, 400 MHz, δ, ppm) 7,09 (t, 1H, J = 8,0 Hz), 6,71-6,36 (m, 3H), 4,69 (dd, 1H, J = 6,0 Hz, 3,2 Hz), 3,69 (s, 3H), 3,66 (d, 1H, J = 5,2 Hz), 3,15-3,10 (dd, 1H, J = 5,6 Hz, 4,0 Hz), 1,87 – 1,69 (m, 6H), 3,66 (d, 1H, J = 5,2 Hz), 3,15 – 3,10 (dd, 1H, J = 5,6 Hz, 4,0 Hz), 1,87 – 1,69 (m, 6H), 1,32 – 1,10 (m, 5H)

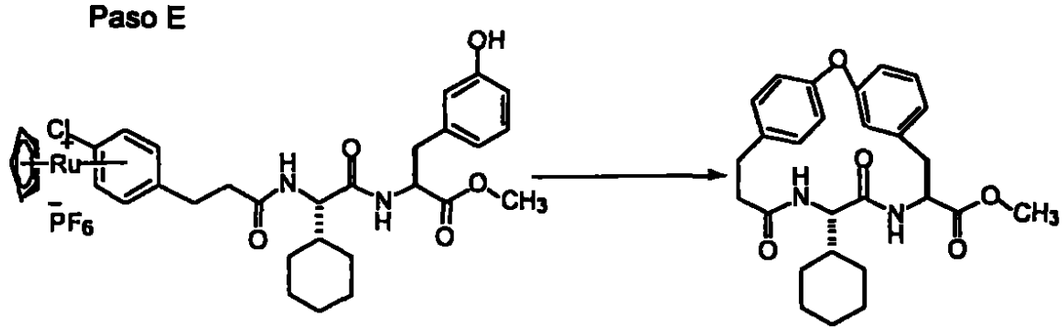
Paso D



Una solución de ácido ciclopentadien-η^6-4-clorofenilpropiónico-hexansafluorofosfato de rutenio 1c (2,0 g, 4,0 mmol) en DMF (20 ml) fue tratada con HOBt (810 mg, 6,0 mmol), 1,5 equiv) y base de Hünig (2,6 g, 16,0 mmol, 4,0 equiv) La mezcla de reacción fue enfriada a 0°C y tratada con EDCI•HCl (888 mg, 5,0 mmol, 1,25 equiv) La mezcla de reacción fue agitada a 0°C por espacio de 30 min y se agrego sal de amina 1 g (1,48 g, 4,0 mmol) a la mezcla y se la

agitó a ta durante 121 h La mezcla de reacción fue concentrada al vacio y el residuo diluido con H_2O (200 ml) y extraido con CH_2Cl_2 (3 x 100 ml) Las capas organicas combinadas fueron extraidas con HCl ac (1 x 100 ml), $NaHCO_3$ (1 x 100 ml), salina (1 x 100 ml) y secadas (Na_2SO_4), filtradas, concentradas al vacio y el sólido marron 1h fue utilizado para la ciclación sin mas purificacion

Paso E



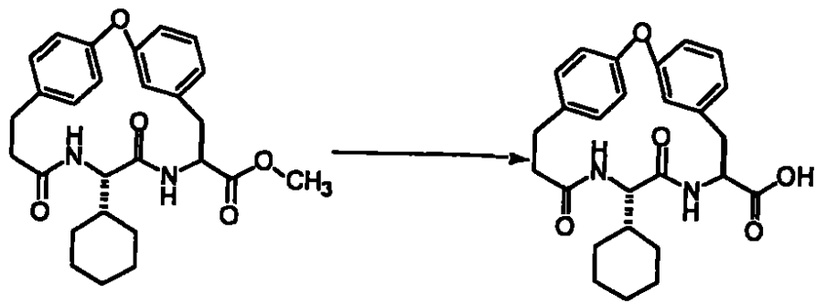
1h 1i

Una solución de acido ciclopentadien-η^6-4-clorofenilpropionico-ciclohexilglicina-meta-tirosina-OCH_3 1h (1,47 g cruda) en DMF seca (150 ml) fue tratada con Cs_2CO_3 (2,40 g, 7,37 mmol, 5,0 equiv) y desgasificada mediante el burbujeo de N_2 seco en la mezcla de reacción La mezcla de reaccion fue agitada a ta por espacio de 16 h y el exceso de DMF fue separado por destilación Se disolvio el residuo en H_2O (200 ml) y se lo extrajo con CH_2Cl_2 (3 x 100 ml) La capa orgánica combinada fue extraida con salina (100 ml), secada (Na_2SO_4), filtrada, concentrada al vacio y el residuo utilizado para la separación fotolitica del rutenio sin más purificacion

Se disolvió el complejo de rutenio crudo en acetonitriloacetonitrilo (35 ml), se lo desgasifico y fotolizó en un Raynot (λ=350 nM) por espacio de 48 h La mezcla de reacción fue concentrada al vacio y el residuo purificado por cromatografia (SiO_2, EtOAc/Hexanos 7 3) para dar 360 mg (52 %) de un sólido incoloro 1i

Paso F

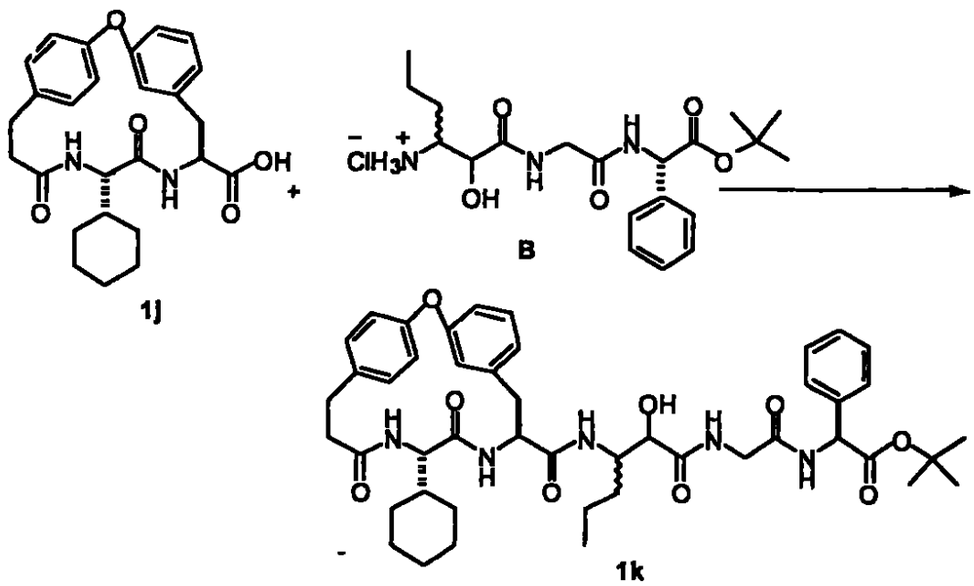
200
195



1i 1j

Una solucion de éter bifenílico 1i (300 mg, 0,65 mmol) en CH_3OH (10 ml), CH_2Cl_2 (20 ml) y H_2O (5 ml) fue tratada con $LIOH \bullet H_2O$ (90 mg, 2,2 mmol, 3,4 equiv) y agitada a temperatura ambiente durante 2 h La mezcla de reaccion fue acidificada con HCl ac (6 M) y extraida en CH_2Cl_2 (3 x 30 ml) Las capas orgánicas combinadas fueron secadas (Na_2SO_4), filtradas y concentradas al vacio para dar el ácido incoloro 1j (20 0 mg, 66 &)

Paso G



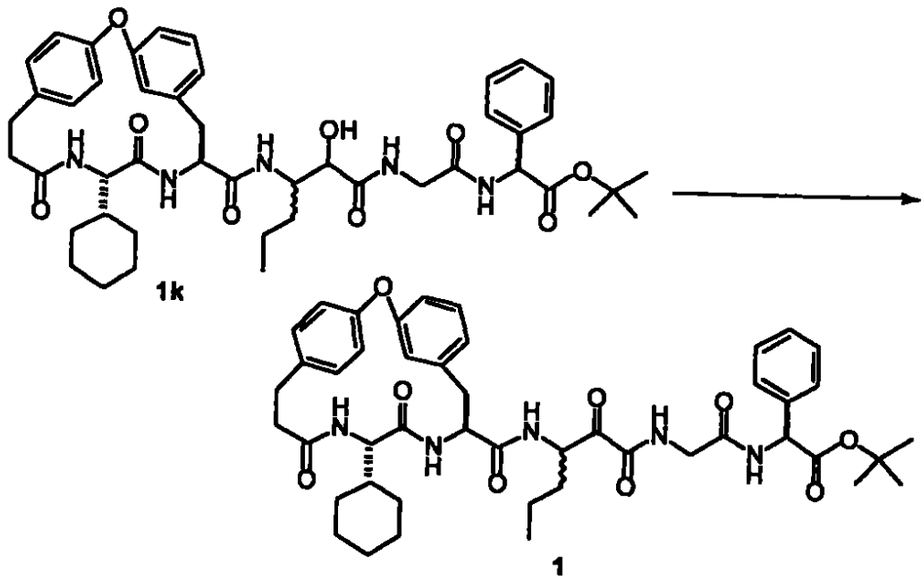
1j B 1k

Una solución del acido 1j (100 mg, 0,22 mmol) en DMF seco (2,5 ml) fue tratada con HOOBt (45 mg, 0,33 mmol) y base de Hünig (141 mg, 1,1 mmol, 5,0

201
~~0201~~ 196

equiv) La mezcla de reaccion fue enfriada a 0°C y tratada con EDCI (63 mg, 0,33 mmol, 1,5 equiv) y agitada durante 20 min La mezcla de reacción fue tratada con la amina B (118 mg, 0,27 mmol, 1,22 equiv) y agitada por espacio de 12 h a ta La mezcla de reaccion fue concentrada al vacio y diluida con H_2O (30 ml) Se extrajo la capa acuosa con CH_2Cl_2 (3 x 50 ml) y EtOAc (3 x 50 ml) Se extrajeron las capas organicas combinadas con HCl ac (2M) NaOH ac (2M), se las secó (Na_2SO_4), filtró, concentro al vacio para obtener un sólido incoloro 1k (79 mg) que fue utilizado para la oxidación EM (Electroatomización, int rel m/z) 826 [$(M+1)^+$, 100], 494 (20), 94 (40)

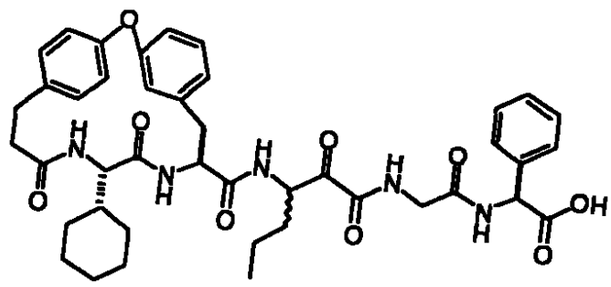
Paso H



Una solucion de la hidroxiamida 1k (130 mg, 0,16 mmol) en DMF (2,0 ml) fue tratada con reactivo de Dess-Martin (130 mg, 0,32 mmol, 2,0 equiv) La mezcla de reaccion fue agitada a ta por espacio de 2 h y la mezcla fue concentrada al vacio El residuo fue purificado por cromatografía (SiO_2,

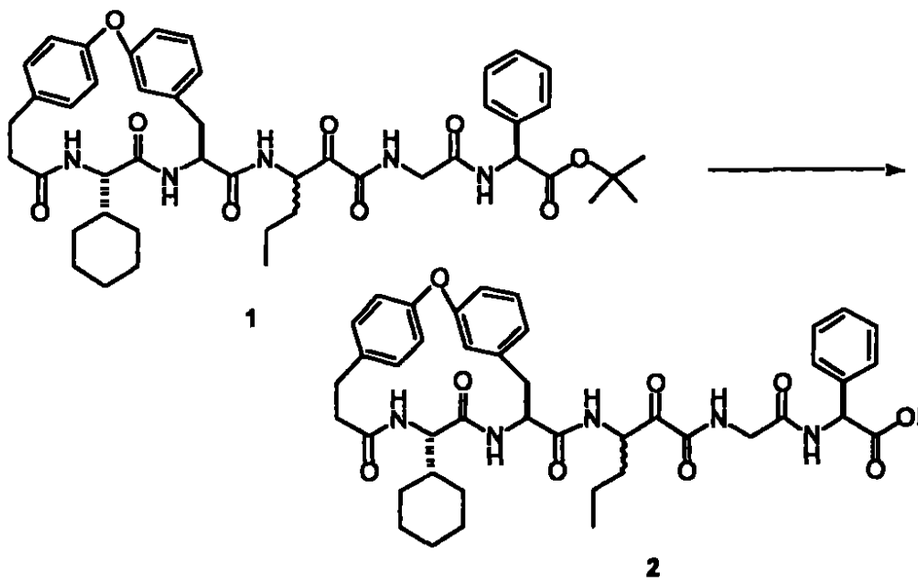
CH_3OH/CH_2Cl_2 1 49) para dar el producto oxidado 1 (55 mg, 42 %) en forma de solido incoloro EM (Electroatomizacion, int rel m/z) 858 [$(M+CH_3OH+1)^+$, 100], 824 [$(M+1)^+$, 63)

Ejemplo 2 Preparación del Compuesto de la Formula 2



2

Paso A



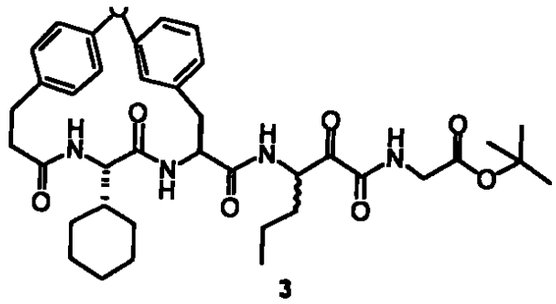
1

2

Una solución del éster terc-butílico 1 (50,0 mg, 60,0 µmol) fue tratada con ATF/CH_2Cl_2 (1 1,4 ml) y agitada a temperatura ambiente durante 2 h La desparicion del ester hasta la linea de base fue seguida por TLC (CH_3OH/CH_2Cl_2

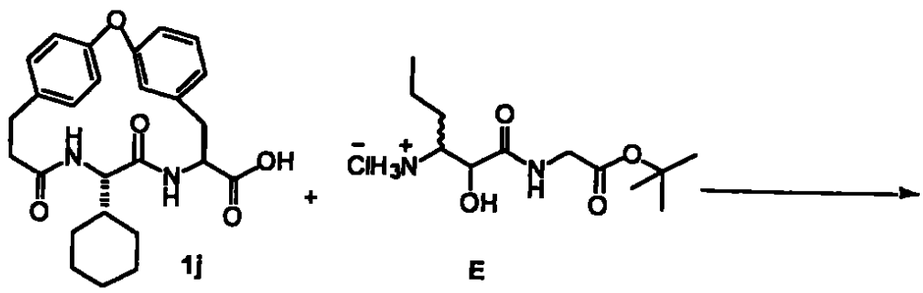
1 24) Una vez completada la desproteccion, la mezcla de reaccion fue concentrada al vacio y el residuo tratado reiteradamente con heptanos (4,0 ml) y concentrado para dar un solido blanco 2 (49 mg, 100 %) EM (Electroatomizacion, int rel m/z) 768 [(M+1)$^+$, 100)

Ejemplo 3 Preparacion del Compuesto de la Formula 3

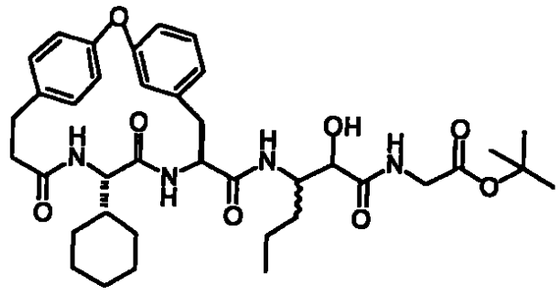


3

Paso A



1j E

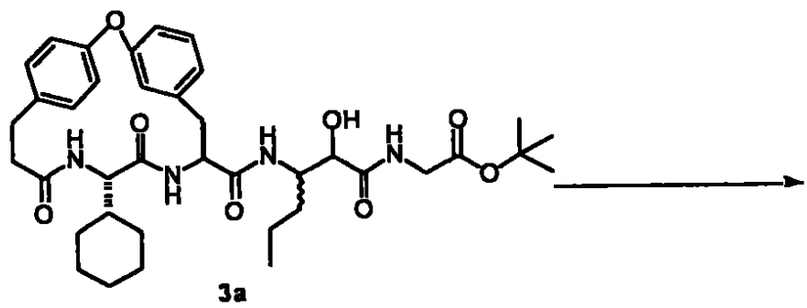


3a

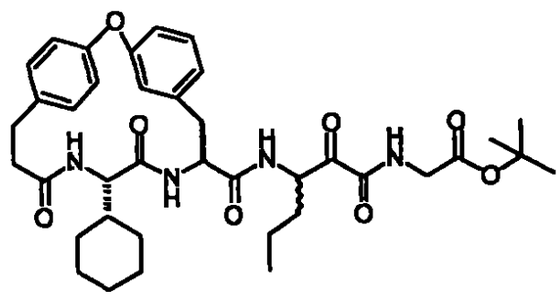
Una solución del ácido 1j (100 mg, 0,22 mmol) en DMF seco (2,5 ml) fue tratada con HOOBt (45 mg, 0,33 mmol) y base de Hünig (141 mg, 1,1 mmol, 5,0

equiv) La mezcla de reacción fue enfriada a 0°C y tratada con EDCI (63 mg, 0,33 mmol, 1,5 equiv) y agitada durante 20 min La mezcla de reacción fue tratada con la amina E (79 mg, 0,27 mmol, 1,22 equiv) y agitada por espacio de 12 h a ta La mezcla de reacción fue concentrada al vacio y diluida con H_2O (30 ml) Se extrajo la capa acuosa con CH_2Cl_2 (3 x 50 ml) Se extrajeron las capas organicas combinadas con HCl ac (1M, 30 ml), NaOH ac (1M, 30 ml), se las secó (Na_2SO_4), filtró, concentro al vacio para obtener un sólido incoloro 3a (58 mg) que fue utilizado para la oxidacion EM (Electroatomizacion, int rel m/z) 693 [$(M+1)^+$, 100], 637 (41), 494 (55), 394 (51), 338 (13)

Paso B



3a

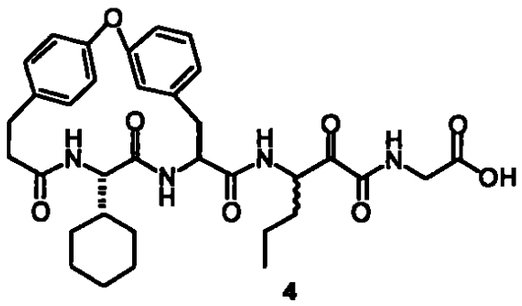


3

Una solucion del alcohol 3a (95 mg, 0,14 mmol) en CH_2Cl_2 (2,0 ml) fue tratada con reactivo de Dess-Martin (116 mg, 0,28 mmol, 2,0 equiv) La mezcla de reacción fue agitada a ta por espacio de 2 h y la mezcla fue concentrada al vacio El residuo fue purificado por cromatografía (SiO_2, CH_3OH/CH_2Cl_2 1 32) para

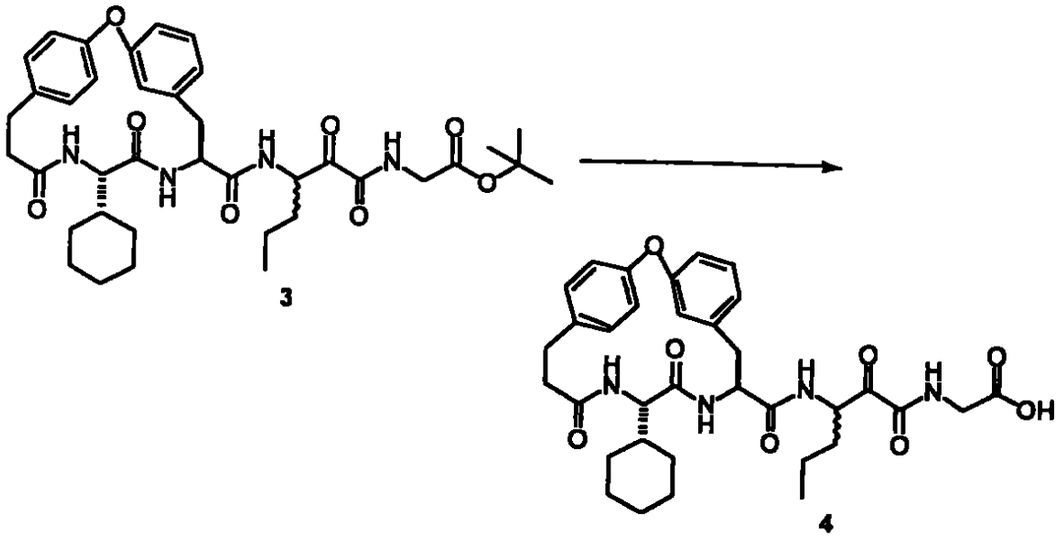
dar el producto oxidado 3 (47 mg, 42 %) en forma de sólido incoloro EM (Electroatomizacion, int rel m/z) 691 $(M+1)^+$

Ejemplo 4 Preparación del Compuesto de la Formula 4



4

Paso A



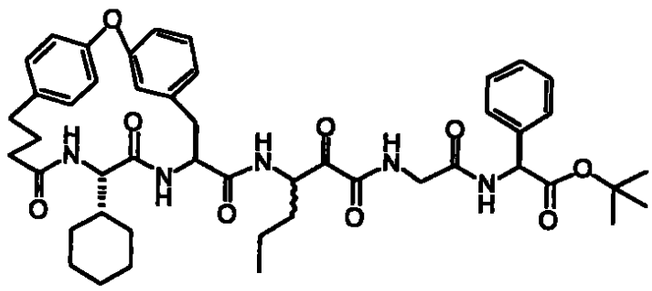
3 4

Una solucion del ester terc-butilico 3 (47,0 mg, 68,0 µmol) fue tratada con HCl (Dioxano 4M, 5 ml) y agitada a temperatura ambiente durante 25 h La desparicion del ester hasta la linea de base fue seguida por TLC (CH_3OH/CH_2Cl_2 1 24) Una vez completada la desproteccion, la mezcla de reaccion fue

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201

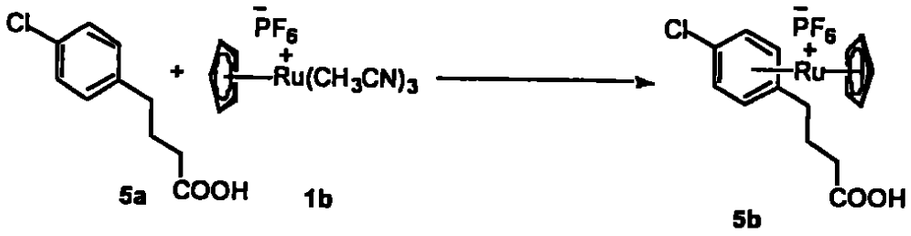
concentrada al vacio y el residuo tratado reiteradamente con heptanos (5,0 ml) y concentrado para dar un solido blanco 4 (43 mg, 100 %) EM (Electroatomizacion, int rel m/z) 635 [(M+1)$^+$, 100), 465 (62), 336 (62)

Ejemplo 5 Preparacion del Compuesto de la Formula 5



5

Paso A

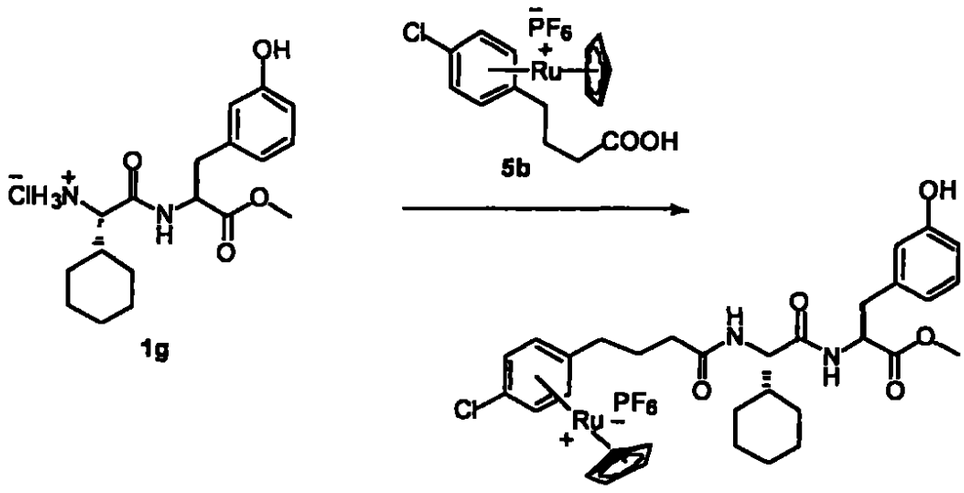


Una solucion del ácido 4-clorobutirico 5a (3,0 g, 15,10 mmol) en dicloroetano (200 ml) fue tratada con $CpRu(CH_3CN)_3PF_6$ 1b (6,6 g, 15,10 mmol, 1,0 equiv) y calentada a reflujo durante 2,5 h La mezcla de reacción fue enfriada

0285 202

a 0°C y filtrada El filtrado fue concentrado al vacio y disuelto en CH_3CN (10 ml) y tratado con un gran exceso de Et_2O La goma que se separaba fue desprendida mediante decantacion del eter y el residuo fue disuelto en CH_2Cl_2/CH_3OH (1 1, 100 ml) y concentrado al vacio para obtener 5b en forma de goma que solidifica (3,5 g, 46 %)

Paso B

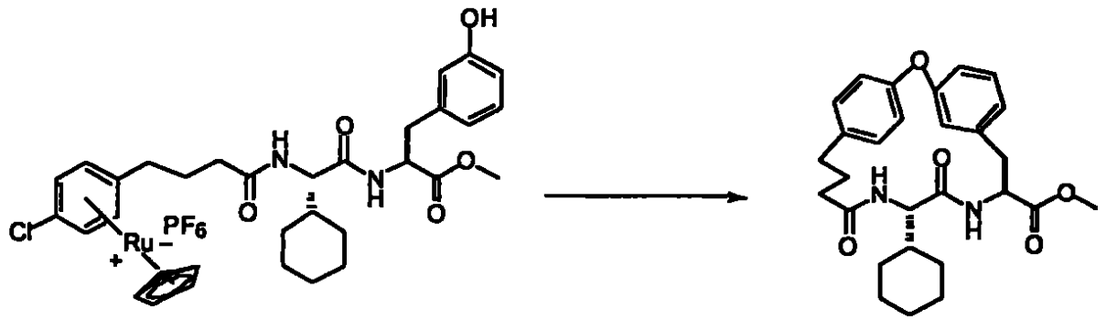


Una solución del ácido carboxílico 5b (3,12 g, 5,95 mmol) en DMF seca (20 ml) fue tratada con base de Hünig (3,07 g, 24,0 mmol, 4,0 equiv , 4,4 ml) y HOBt (1,2 g, 8,93 mmol, 1,5 equiv) La mezcla de reacción fue enfriada a 0°C y tratada con EDCI (1,35 g, 7,43 mmol, 1,25 equiv) y agitada durante 1h A esta mezcla de reacción se agregó el clorohidrato de amina 1g (2,65 g, 7,14 mmol, 1,2 equiv) y la mezcla de reacción fue agitada a ta por espacio de 12 h La DMF fue separada por destilacion y el residuo diluido con agua y la capa acuosa fue extraida con CH_2Cl_2 Se extrajeron las capas orgánicas combinadas con $NaHCO_3$ ac. HCl, salina, secadas (Na_2SO_4), filtradas, concentradas al vacio y el producto crudo 5c (4,3 g) fue utilizado para la ciclacion sin mas purificacion RMN ^1H (d_4-CD_3OD, 400 MHz, δ, ppm) 7,35 (t, 1H), 6,72 – 6,60 (m, 5H), 6,33-6,29 (dd, 2H), 5,51 (s, 5H), 4,19 (d, 1H), 3,68 (s, 3H), 3,19-2,83 (m, 2H), 2,51-2,40 (m, 2H), 2,40-2,25 (m,

203

2H), 1,99-1,59 (m, 8H), 1,35-0,98 (m, 5H), EM (FAB, NBA-G/TG-DMSO, intensidad relativa m/z) 695,3 ($[M-PF_6]^+$, 100), 232 (20), 171(30), HRMS calc para $C_{34}H_{42}N_2O_5ClRu^+$ $(M-PF_6)^+$ 695,1832, encontrado 695,1845

Paso C



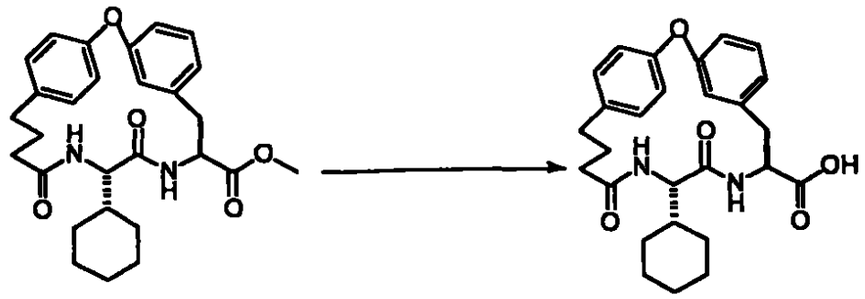
5c 5d

Una solución del compuesto cloro 5c (3,0 g, 3,6 mmol) en DMF seca (300 ml) fue desgasificada con N_2 seco y Cs_2CO_3 (5,2 g, 16 mmol, 4,0 equiv) y agitada a temperatura ambiente por espacio de 16 h La DMF solvente fue separada por destilacion y el residuo diluido con agua y extraido con CH_2Cl_2 (3x100 ml) Las capas orgánicas combinadas fueron secadas (Na_2SO_4), filtradas, concentradas), filtradas, concentradas al vacio y secadas al vacio durante la noche Se la s utilizo para la remocion fotolitica del Ru sin más purificacion EM FAB (NBA-G/TG-DMSO 695 ([M-PF6]$^+$, 100]

Se disolvió el compuesto ciclado del paso anterior en CH_3CN (35 ml) y se lo fotolizo en un Raynot (λ=350 nm) por espacio de 48 h La mezcla de reacción fue concentrada al vacio y el residuo purificado por cromatografia (SiO2, EtOAc/Hexanos 1 1) para dar un sólido de color tostado 5d (600 mg, 34 %) RMN ^1H ($CDCl_3$, 400 MHz, δ, ppm) 7,58 (d, 1H, J = 2,4, 5,6 Hz), 7,14 (t, 1H, J = 8,0 Hz), 6,94 (d, 2H, J = 8,4 Hz), 6,87 (dd, 1H, J = 2,4 Hz, 5,6 Hz), 6,73 (d, 1H, J = 7,2 Hz), 6,59 (s, 1H), 6,57 (s, 2H), 6,39 (d, 1H, J = 8,0 Hz), 4,51 (dt, 1H, J = 2,8, 8,0 Hz), 3,80 – 3,62 (m, 1H), 3,62 (s, 3H), 3,05 – 3,00 (dd, 1H, J = 2,8 Hz, 11,6 Hz), 2,85 (dd, 1H), J = 8,4, 6,0 Hz), 2,76 – 2,72 (m, 1H), 2,36-2,19 (m, 3H), 2,02 (dd, 1H, J =

6,4, 9,2 Hz), 1,8 – 1,73 (m, 1H), 1,61-1,34 (m, 7H), 1,41 –0,71 (m, 7H), EM (FAB, NBA-G/TG-DMSO, intensidad relativa m/z) 493 [(M+1)$^+$, 100] 465 (20), 232 (30), 171 (40), HRMS calc para $C_{29}H_{37}N_2O_5$ (M+1)$^+$ 493,2702, encontrado 493,2699

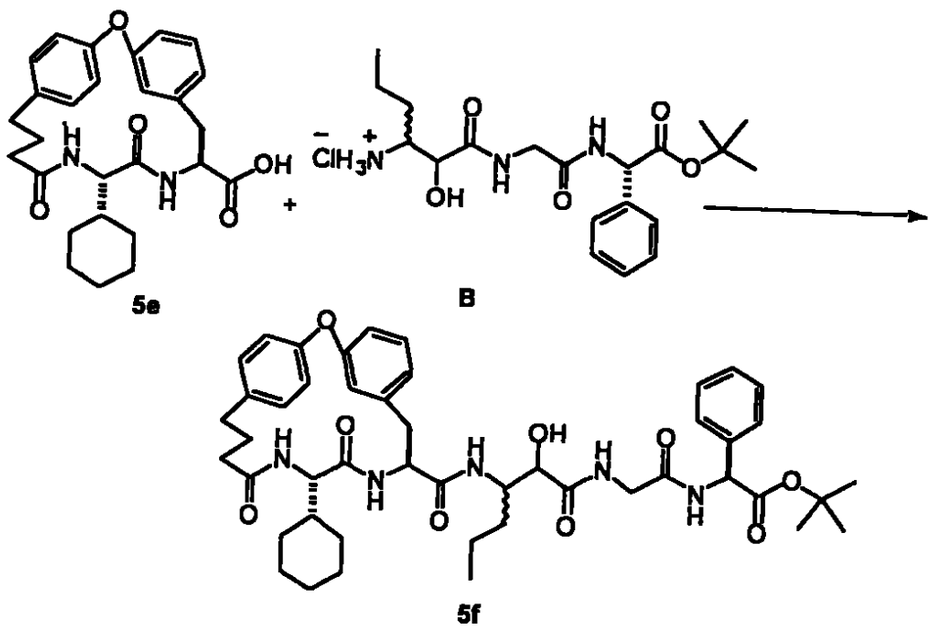
Paso D



5d 5e

Una solución del éter 5d (200 mg, 0,42 mmol) en CH_3OH (5 ml), CH_2Cl_2 (10 ml) y H_2O (0,5 ml) fue tratada con $LIOH•H_2O$ (18 mg, 0,44 mmol, 1,1 equiv) y agitada a temperatura ambiente durante 12 h La mezcla de reacción fue acidificada con HCl ac (12 M, 1 ml) y concentrada al vacío para dar el ácido 5e que fue utilizado directamente para el acoplamiento sin más purificación

Paso E



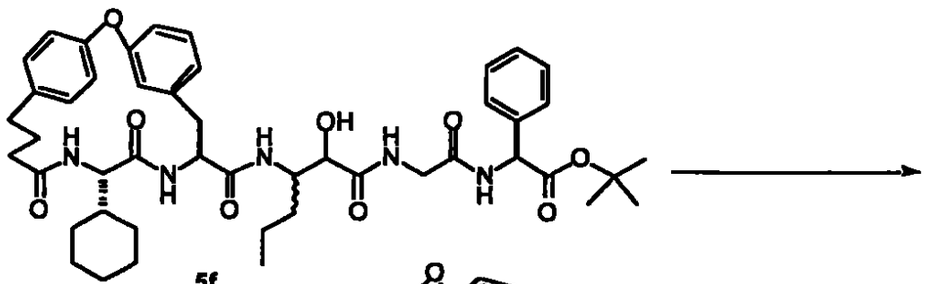
5e B

5f

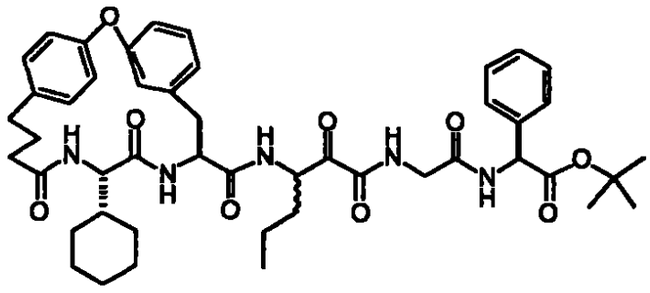
205

Una solución del acido 5e en DMF seca (5,0 ml) fue tratada con HOOBt (103 mg, 0,63 mmol, 1,5 equiv), base de Hunig (216 mg, 1,68 mmol, 4,0 equiv) y la amina B (270 mg, 0,63 mmol, 1,47 equiv) La mezcla de reaccion fue enfriada a 0°C y tratada con EDCI (101 mg, 0,52 mmol, 1,25 equiv) y agitada durante 12 h La mezcla de reaccion fue concentrada al vacio y diluida con H_2O (30 ml) Se extrajo la capa acuosa con CH_2Cl_2 (3 x 50 ml) y EtOAc (3 x 50 ml) Se extrajeron las capas orgánicas combinadas con HCl ac (2M), NaOH ac. (2M), se las seco (Na_2SO_4), filtro, concentró al vacio para obtener un sólido incoloro 5f (177 mg) que fue utilizado para la oxidacion EM (Electroatomización, int rel m/z) 840 [$(M+1)^+$, 100], 394 (100)

Paso F



5f



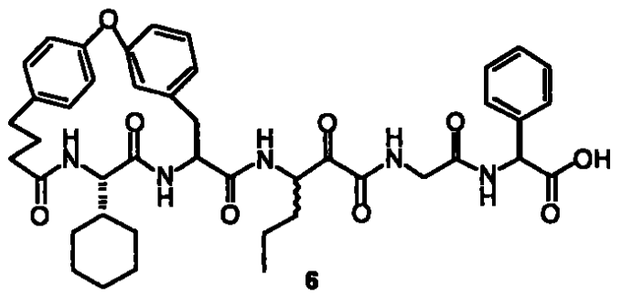
5

Una solución del alcohol 5f (177 mg, 0,21 mmol) en CH_2Cl_2 (10,0 ml) fue tratada con reactivo de Dess-Martin (178 mg, 0,42 mmol, 2,0 equiv) La mezcla de reacción fue agitada a ta por espacio de 3 h y la mezcla fue concentrada al vacio El residuo fue purificado por cromatografía (SiO_2, CH_3OH/CH_2Cl_2 1 49) para

206

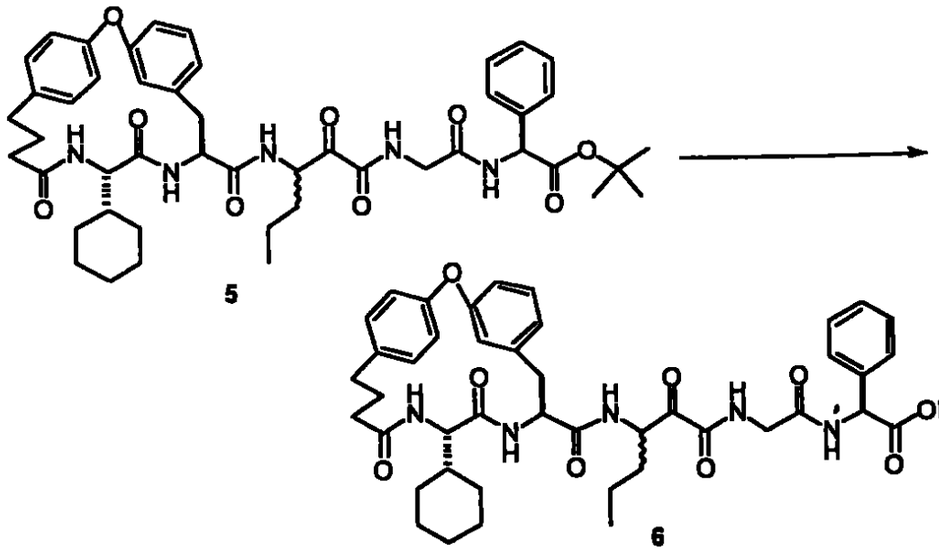
dar el producto oxidado 5 (23 mg, 13 %) en forma de solido incoloro EM (Electroatomización, int rel m/z) 870[(M+CH_3OH+1)$^+$, 40], 838 [(M+1)$^+$, 100)

Ejemplo 6 Preparación del Compuesto de la Fórmula 6



6

Paso A

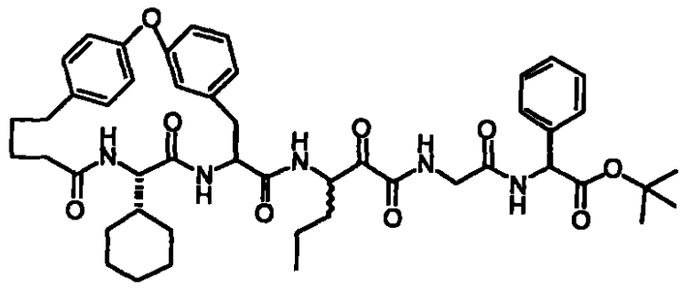


5

6

Una solución del éster terc-butílico 5 (50,0 mg, 60,0 µmol) fue tratada con ATF/CH_2Cl_2 (1 1,4 ml) y agitada a temperatura ambiente durante 7 h La desparicion del éster hasta la linea de base fue seguida por TLC (CH_3OH/CH_2Cl_2 1 24) Una vez completada la desprotección, la mezcla de reaccion fue concentrada al vacio y el residuo tratado reiteradamente con heptanos (4,0 ml) y concentrado para dar un sólido blanco 6 (14 mg, 100 %) EM (Electroatomización, int rel m/z) 782 [(M+1)$^+$, 100)

Ejemplo 7 Preparación del Compuesto de la Fórmula 7



7

Paso A



7a 7b

Una solucion del alcohol 7a (9m2 g, 54,1 mmol) en CH_2Cl_2 seco (200 ml) fue tratada con DMSO (35 ml) y Et_3N (16,4 g, 16,3 mmol, 23,4 ml) La mezcla de reacción fue enfriada a 0°C y tratada con $Py{\bullet}SO_3$ (12,9 g, 81,2 mmol, 1,50 equiv) disuelta en DMSO (30 ml) La mezcla de reaccion fue agitad a 0!C durante 0,5 h y a ta durante 6 h La mezcla de reacción fue concentrada al vacio y diluida con Et_2O (100 ml) y H_2O (200 ml) Se separaron las capas y se extrajo la capa acuosa

208

con Et_2O (3x100 ml) Se extrajeron las capas orgánicas combinadas con HCl (2M, 3x100 ml), salina (1x100 ml), se las concentro al vacio y se las purifico por cromatografia (SiO_2, EtOAc/Hexanos 1 7) para dar el aldehido 7b que solidificó tomandose un solido cereo al reposar (7,1 g, 77 %) CHN calc para C_9H_9ClO C= 64,11 % H =5,38 %, encontrado C = 64,08 % H = 5,30 %

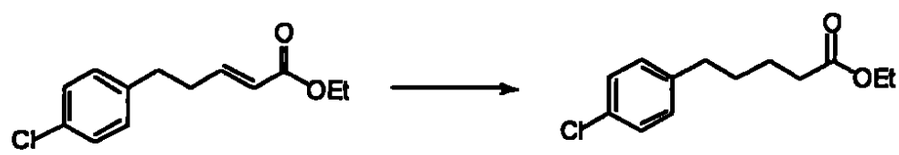
Paso B



7b 7c

Se trató una solución de trietilfosfonoacetato (6,72 g, 30 mmol, 1,2 equiv) en THF seco (100 ml) con NaH (dispersion al 60 %, 1,5 g, 35 mmol, 1,4 equiv) a 0°C La mezcla de reacción fue agitada a 25°C por espacio de 1 h hasta que la evolucion de H_2 hubo cesado Se agrego una solución del aldehido 7b (4,2 g, 25,0 mmol) en THF seco y la mezcla de reacción fue agitada durante 36 h La mezcla de reaccion fue diluida con H_2O (100 ml) y extraida con Et_2O (3x70 ml) La capa orgánica fue secada ($MgSO_4$), filtrada, concentrada al vacio y cromatografiada para dar el éster α,β-saturado 7c (4,2 g, 71 %), que fue utilizado para la reducción

Paso C



7c 7d

Se trato una solucion del ester α,β-saturado 7c (4,2 g, 8,0 mmol) en EtOAc (50 ml) con Pd/C (10 % p/p, 500 mg) y se la hidrógeno a 50 psi (34,7 kPa) por espacio de 12 h La mezcla de reacción fue filtrada a través de un cilindro de

celite y el filtrado concentrado al vacio para dar el compuesto reducido 7d (3,9 g, 93 %)

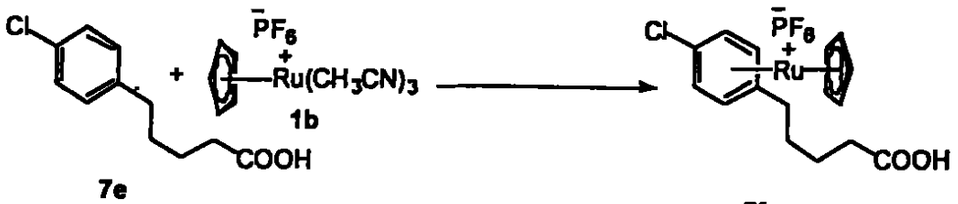
Paso D



7d 7e

Una solución del eter 7d (3,9 g, 16,2 mmol) en $CH_3OH/THF/H_2O$ (1 1 0,1, 110 ml) fue tratada con $LiOH•H_2O$ (1,2 mg, 30 mmol, 2,0 equiv) y agitada a temperatura ambiente durante 5 h La mezcla de reacción fue concentrada al vacio y el residuo diluido con H_2O (100 ml) y extraido en Et_2O (3x50 ml) La capa acuosa fue acidificada a pH ~1 (HCl 13M) y la capa acuosa turbia fue extraida con Et_2O (3x100 ml) Las capas orgánicas combinadas fueron secadas ($MgSO_4$), filtradas, concentradas al vacio para dar un sólido incoloro 7e (3,1 g, 96 &) CHN calc para calc para $C_{11}H_{13}ClO_2$ C= 62,12 % H =6,16 %, encontrado C = 62,27 % H = 6,23 %

Paso E



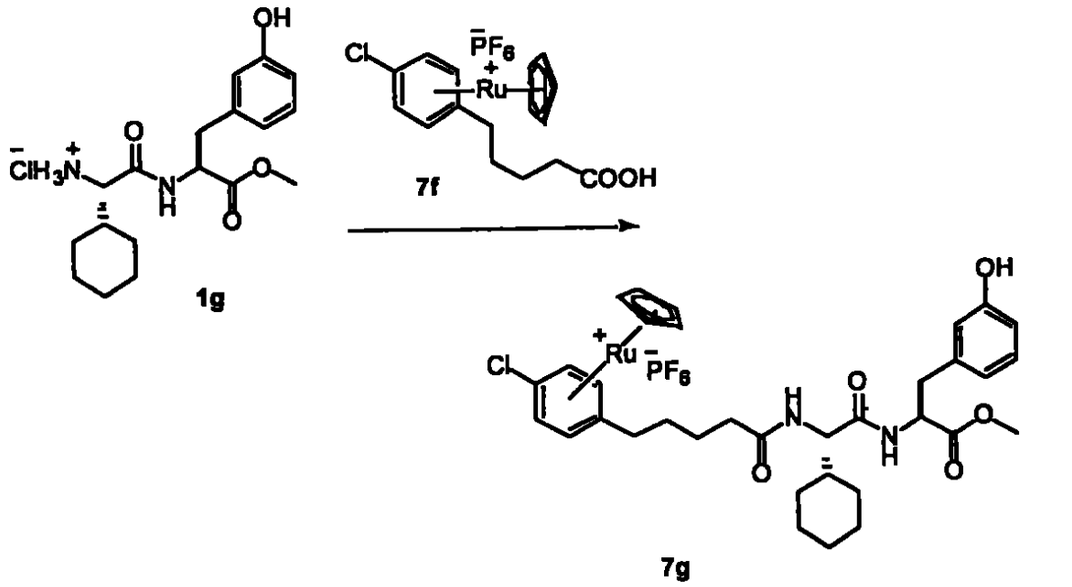
7e 7f

Una solución del ácido clorofenilpentanoico 7e (3,0 g, 14,15 mmol) en dicloroetano (150 ml) fue tratada con $CpRu(CH_3CN)_3PF_6$ 1b (6,75 g, 15,10 mmol, 1,0 equiv) y calentada a reflujo durante 2,5 h La mezcla de reacción fue enfriada a 0°C y filtrada El filtrado fue concentrado al vacio y disuelto en CH_3CN (20 ml) y tratado con un gran exceso de Et_2O La goma que se separaba fue desprendida

~~121~~ 210

mediante decantacion del eter y el residuo fue disuelto en CH_2Cl_2/CH_3OH (1 1, 100 ml) y concentrado al vacio para obtener 7f en forma de goma marron que solidifica (4,36 g, 58 %) EM EM (Electroatomización, int rel m/z) 379 $[(M\text{-}Pf_6)^+$ 100]

Paso F

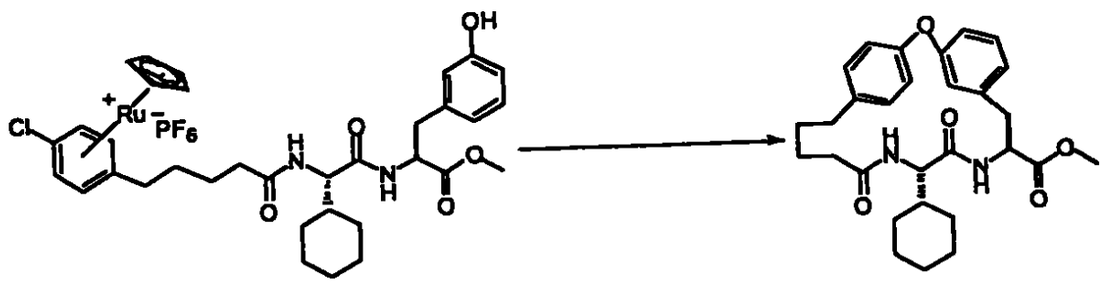


Una solucion del acido carboxilico 7f (3,12 g, 5,95 mmol) en DMF seca (20 ml) fue tratada con base de Hünig (3,07 g, 24,0 mmol, 4,0 equiv , 4,4 ml) y HOBt (1,2 g, 8,93 mmol, 1,5 equiv) La mezcla de reacción fue enfriada a 0°C y tratada con EDCI (1,35 g, 7,43 mmol, 1,25 equiv) y agitada durante 1h A esta mezcla de reacción se agrego el clorhidrato de amina 1g (2,65 g, 7,14 mmol, 1,2 equiv) y la mezcla de reacción fue agitada a ta por espacio de 12 h La DMF fue separada por destilacion y el residuo diluido con agua y la capa acuosa fue extraida con CH_2Cl_2 Se extrajeron las capas organicas combinadas con $NaHCO_3$ ac. HCl, salina, secadas (Na_2SO_4), filtradas, concentradas al vacio y el producto crudo 7g (4,3 g) fue utilizado para la ciclación posterior sin mas purificacion RMN ^1H (d_4-CD_3OD, 400 MHz, δ, ppm) 7,35 (t, 1H), 6,72 – 6,60 (m, 5H), 6,33-6,29 (dd, 2H), 5,51 (s, 5H), 4,19 (d, 1H), 3,68 (s, 3H), 3,19-2,83 (m, 2H), 2,51-2,40 (m, 2H), 2,40-

0215 211

2,25 (m, 2H), 1,99-1,59 (m, 8H), 1,35-0,98 (m, 5H), EM (FAB, NBA-G/TG-DMSO, intensidad relativa m/z) 695,3 ($[M-PF_6]^+$, 100), 232 (20), 171(30), HRMS calc para $C_{34}H_{42}N_2O_5ClRu^+$ $(M-PF_6)^+$ 695,1832, encontrado 695,1845

Paso G



7g 7h

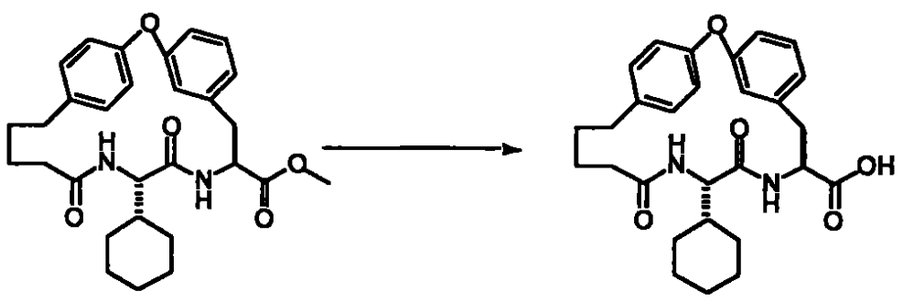
Una solución del compuesto cloro 7g (3,0 g, 3,6 mmol) en DMF seca (300 ml) fue desgasificada con N_2 seco y Cs_2CO_3 (5,2 g, 16 mmol, 4,0 equiv) y agitada a temperatura ambiente por espacio de 16 h La DMF solvente fue separada por destilación y el residuo diluido con agua y extraido con CH_2Cl_2 (3x100 ml) Las capas organicas combinadas fueron secadas (Na_2SO_4), filtradas, concentradas al vacio y secadas al vacio durante la noche Se las utilizó para la remocion fotolítica del Ru sin más purificacion EM FAB (NBA-G/TG-DMSO 695 ([M-PF6]$^+$, 100]

Se disolvio el compuesto ciclado del paso antenor en CH_3CN (35 ml) y se lo fotolizó en un Raynot (λ=350 nm) por espacio de 48 h La mezcla de reacción fue concentrada al vacio y el residuo purificado por cromatografía (SiO_2, EtOAc/Hexanos 1 1) para dar un sólido de color tostado 7h (600 mg, 34 %) RMN ^1H ($CDCl_3$, 400 MHz, δ, ppm) 7,58 (d, 1H, J = 7,6 Hz), 7,14 (t, 1H, J = 8,0 Hz), 6,94 (d, 2H, J = 8,4 Hz), 6,87 (dd, 1H, J = 2,4 Hz, 5,6 Hz), 6,73 (d, 1H, J = 7,2 Hz), 6,59 (s, 1H), 6,57 (s, 2H), 6,39 (d, 1H, J = 8,0 Hz), 4,51 (dt, 1H, J = 2,8, 8,0 Hz), 3,80 – 3,62 (m, 1H), 3,62 (s, 3H), 3,05 – 3,00 (dd, 1H, J = 2,8 Hz, 11,6 Hz), 2,85 (dd, 1H), J = 8,4, 6,0 Hz), 2,76 – 2,72 (m, 1H), 2,36-2,19 (m, 3H), 2,02 (dd, 1H, J = 6,4, 9,2 Hz), 1,8 – 1,73 (m, 1H), 1,61-1,34 (m, 7H), 1,41 –0,71 (m, 7H), EM (FAB,

0213 212

NBA-G/TG-DMSO, intensidad relativa m/z) 493 [(M+1)$^+$, 100] 465 (20), 232 (30), 171 (40), HRMS calc para $C_{29}H_{37}N_2O_5$ (M+1)$^+$ 493,2702, encontrado 493,2699

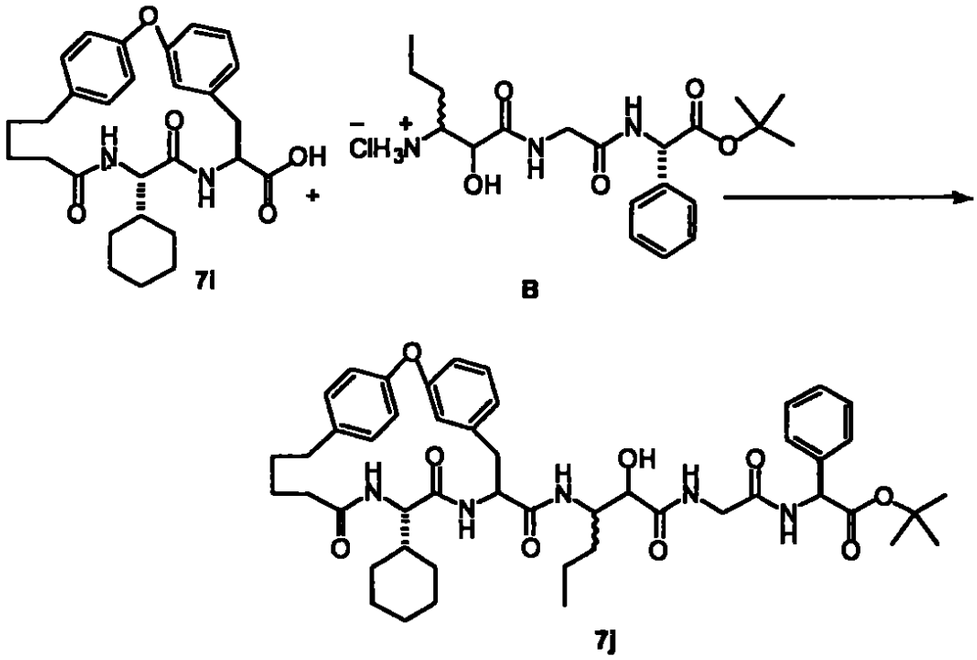
Paso H



7h 7i

Una solucion del éter 7h (220 mg, 0,46 mmol) en CH_3OH (3 ml), CH_2Cl_2 (10 ml) y H_2O (0,5 ml) fue tratada con $LIOH•H_2O$ (18 mg, 0,44 mmol, 1,1 equiv) y agitada a temperatura ambiente durante 12 h La mezcla de reacción fue acidificada con HCl ac (13 M, 1 ml) y concentrada al vacio para dar el ácido 7i que fue utilizado directamente para el acoplamiento sin mas purificación

Paso I



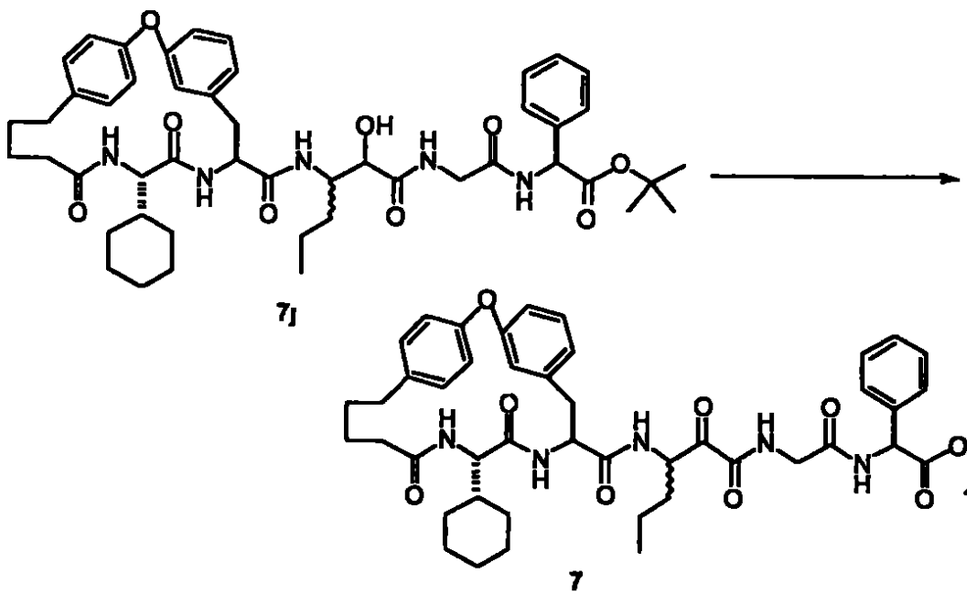
7i B

7j

0214 213

Una solución del acido 7ı en DMF seca (3,0 ml) fue tratada con HOOBt (94 mg, 0,75 mmol, 1,6 equiv), base de Hünig (237 mg, 1,84 mmol, 4,0 equiv) y la amina B (246 mg, 0,58 mmol, 1,47 equiv) La mezcla de reaccion fue enfriada a 0°C y tratada con EDCI (110 mg, 0,58 mmol, 1,25 equiv) y agitada a 0°C durante 25 min, 12 h La mezcla de reaccion fue concentrada al vacio y diluida con H_2O (30 ml) Se extrajo la capa acuosa con CH_2Cl_2 (3 x 30 ml) Se extrajeron las capas orgánicas combinadas con HCl ac (1M, 60 ml), NaOH ac (60 ml), se las seco (Na_2SO_4), filtró, concentró al vacio para obtener un solido incoloro 7ȷ (230 mg) que fue utilizado para la oxidación EM (Electroatomización, int rel m/z) 854 [(M+1)$^+$, 100], 479 (70), 327 (50), 2,71,1 (100)

Paso J



7ȷ

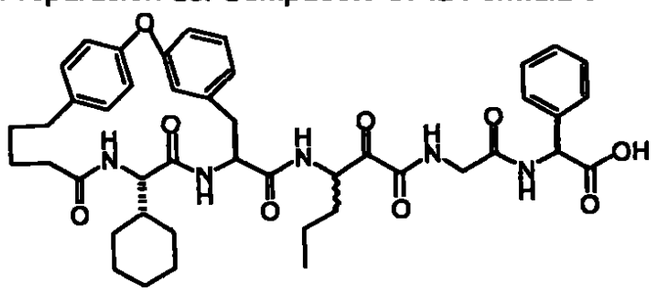
7

Una solución del alcohol 7ȷ (220 mg, 0,26 mmol) en CH_2Cl_2 (3,0 ml) fue tratada con reactivo de Dess-Martin (218 mg, 0,51 mmol, 2,0 equiv) La mezcla de reaccion fue agitada a ta y la mezcla fue concentrada al vacio El residuo fue

0218
214

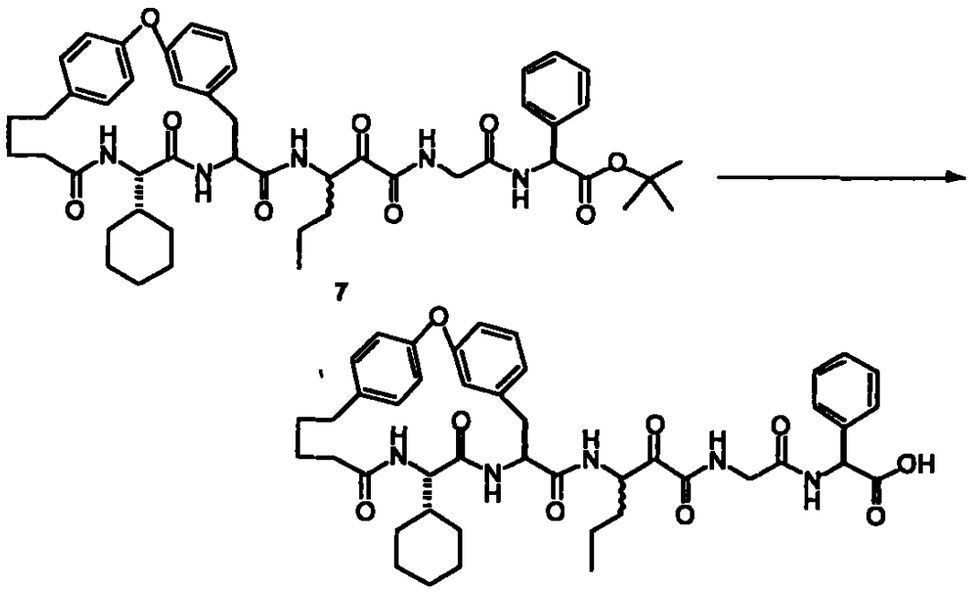
purificado por cromatografía (SiO_2, CH_3OH/CH_2Cl_2 1 24) para dar el producto oxidado 7 (23 mg, 13 %) en forma de sólido incoloro EM (Electroatomización, int rel m/z) 852 [(M+ 1)$^+$, 43), 796 (100), 768 (20), 461 (20), 433 (50), 405 (40), 336 (30), 294 (50

Ejemplo 8 Preparación del Compuesto de la Fórmula 8



8

Paso A



7

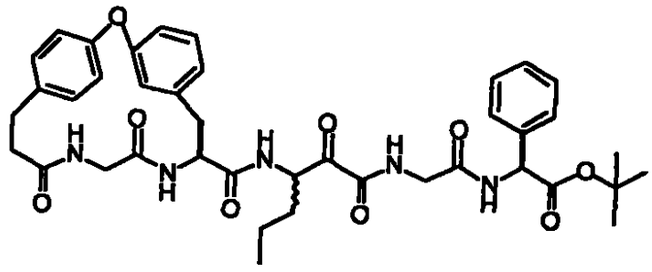
8

Una solución del ester terc-butílico 7 (32,0 mg, 37,0 µmol) fue tratada con ATF/CH_2Cl_2 (1 1, 5,0 ml) y agitada a temperatura ambiente durante 4 h La desparición del éster hasta la linea de base fue seguida por TLC (CH_3OH/CH_2Cl_2 1 24) Una vez completada la desprotección, la mezcla de reaccion fue

C37 215

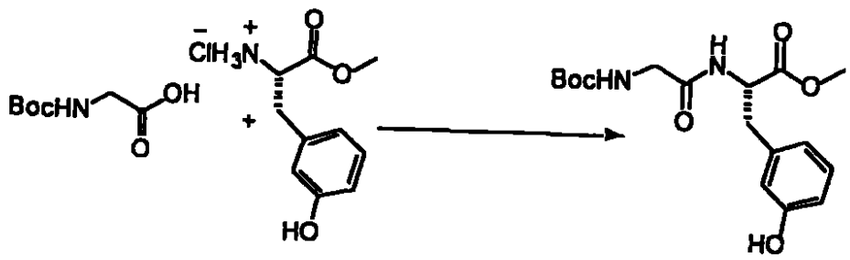
concentrada al vacio y el residuo tratado reiteradamente con heptanos/CH_3OH (4,0 ml) y concentrado para dar un sólido tostado 8 (29,0 mg, 100 %) EM (Electroatomización, int rel m/z) 796 [(M+1)$^+$, 100)

Ejemplo 9 Preparacion del Compuesto de la Fórmula 9



9

Paso A



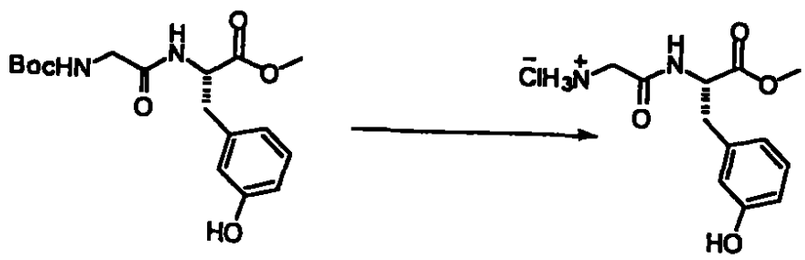
9a 1e 9b

Una solución de Boc-Glicina 9a (1,75 g, 10,0 mmol) en DMF seca (50 ml) fue tratada con HOOBt (2,65 g, 15 mmol, 1,5 equiv) y EDCI (2,86 g, 15,0 mmol, 1,5 equiv) La mezcla de reacción fue tratada con base de Hünig (5,16 g, 40 mmol, 4,0 equiv , 7,3 ml) La mezcla de reacción fue agitada durante 1 h y se agrego meta-tirosina-OCH_3•HCl 1e (2,5 g, 11,5 mmol, 1,1 equiv), agitando a 25°C durante 12 h La mezcla de reacción fue concentrada al vacio y el residuo diluido con $NaHCO_3$ y extraido en CH_2Cl_2 La capa orgánica combinada fue concentrada y el residuo purificado por cromatografía (SiO_2, EtOAc/Hexanos 1 1) para dar un sólido incoloro 9b (3,4 g, 90 %)

Paso B

216

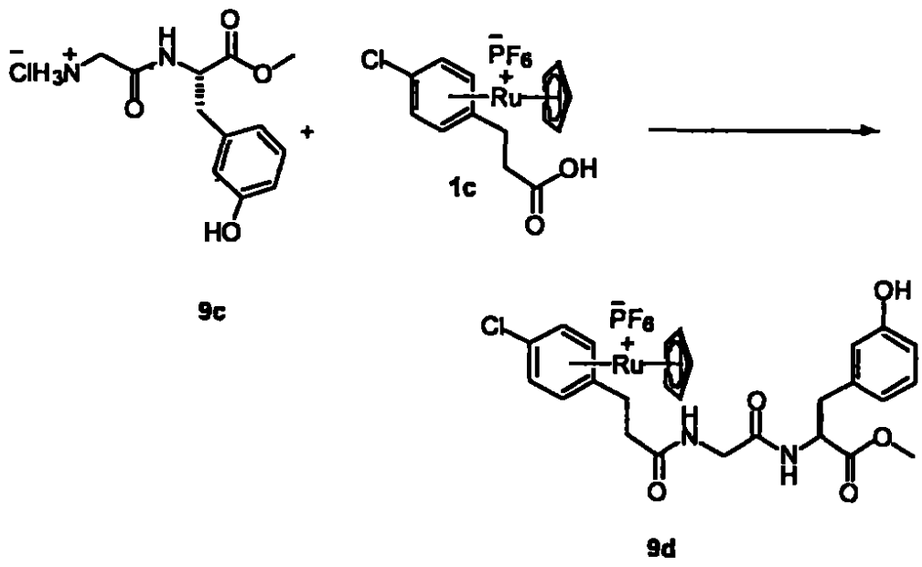
0229



9b 9c

Una solución de 9b (4,6 g, 13,06 mmol) en HCl (solución 4M en dioxano, 50 ml) fue agitada a temperatura ambiente por espacio de 3 h La mezcla de reaccion fue concentrada al vacio y el residuo secado en alto vacio para dar 9c en forma de polvo fino que fue utilizado para el siguiente paso RMN ^1H (d_4-CD_3OD, 400 MHz, δ, ppm) 8,67 (d, 1H, J = 7,9 Hz), 7,10-7,07 (m, 1H), 6,68 – 6,64 (m, 2H), 4,75-4,70 (m, 1H), 3,75-3,61 (m, 2 H), 3,66 (s, 3H), 3,10 (dd, 1H, J = 5,2, 8,5 Hz), s,90 (dd, 1H, J = 8,8, 5,0 Hz),

Paso C



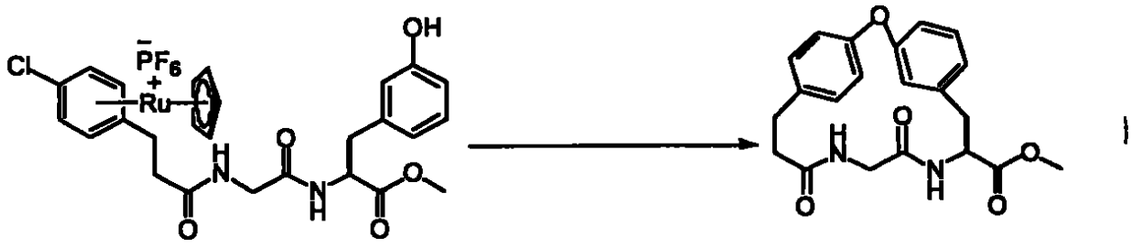
9c 1c 9d

Una solución de CpRu(ácido η^6-4-clorofenilpropiónico)-PF_6 1c (3,0 g, 46,01 mmol) en DMF seca (60 ml) fue tratada con HOBt (1,3 g, 9,16 mmol), 1,5 equiv) y

217
0221

base de Hunig (3m22 g, 4,60 mmol, 4,0 equiv) La mezcla de reaccion fue enfriada a 0°C por un lapso de 30 minutos y tratada con EDCI (1,75 g, 9,16 mmol, 1,5 equiv) La mezcla de reacción fue agitada a 0°C por espacio de 30 min y se agrego la sal de glicina-amonio 9c (1,75 g, 6,06 mmol, 1,0 equiv) la mezcla de reacción fue agitada a ta por espacio de 12 h y la DMF fue separada por destilación al vacio El residuo fue diluido con HCl ac (1M, 100 ml) y extraida en CH_2Cl_2 (3x100 ml) Las capas organicas combinadas fueron extraidas con $NaHCO_3$ ac (1 x 100 ml), salina (50 ml) y secadas (Na_2SO_4), filtradas, concentradas al vacio para dar un sólido marron 9d (1,5 g, 34 %) que fue utilizado para la ciclacion EM (Electroatomización, intensidad relativa m/z) 585 ([M-PF_6]$^+$, 100], 459 (30), 373 (30), 198 (20)

Paso D



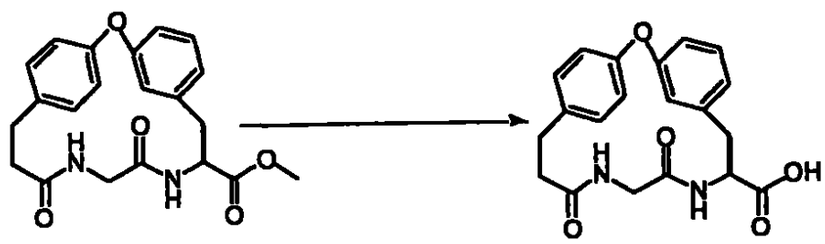
9d 9e

Una solución del compuesto rutenio 9d (1,5 g, 2,05 mmol) en DMF seca (100 ml) fue desgasificada con N_2 a ta seco y se agregó Cs_2CO_3 (5,0 g, 15 mmol, 7,5 equiv) y se agitó a temperatura ambiente por espacio de 12 h La DMF solvente fue separada por destilacion y el residuo diluido con agua (100 ml) y extraido con CH_2Cl_2 (3x100 ml) Las capas orgánicas combinadas fueron extraidas con salina (100 ml), secadas (Na_2SO_4), filtradas, concentradas al vacio y secadas al vacio durante la noche Se las utilizo para la remoción fotolitica del Ru sin más purificación

0322 218

Se disolvio el compuesto ciclado del paso anterior en CH_3CN (30 ml) y se lo filtró, introduciendolo en un tubo de cuarzo La solución fue desgasificada y fotolizada en un Raynot (λ=350 nm) por espacio de 48 h La mezcla de reacción fue concentrada al vacio y el residuo purificado por cromatografía (SiO_2, EtOAc/Hexanos 1 1) para dar un solido de color tostado 9e (230 mg, 30 %) RMN ^1H ($CDCl_3$, 400 MHz, δ, ppm) 7,23 – 7,18 (m, 2H), 7,09-7,01 (s, 3H), 6,76 (dd, 1H, J = 2,4 8,8 Hz), 6,66 (d, 1H, J = 7,6 Hz), 6,47 (d, 1H, J = 5,6 Hz), 6,17 (s, 1H), 5,64 (s, 1H), 4,69 (q, 1H, J = 4,4 Hz), 3,77 (s, 3H), 3,68-3,51 (m, 2H), 3,35 (dd, 1H, J = 4,0, 10,8 Hz), 3,05 (dd, 1H, J = 5,2, 9,2 Hz), 2,96-2,92 (m, 2H), 2,61-2,56 (m, 1H), 2,30-2,29 (m, 1H), RMN ^{13}C ($CDCl_3$ 100 MHz, δ ppm 172,3, 171,4, 168,1, 159,9, 155,4, 137,6, 136,4, 131,0, 130,0, 129,5, 123,3, 122,4, 121,0, 117,7, 117,1, 53,6, 53,0, 43,6, 39,9, 36,1, 32,3 (Electroatomización, intensidad relativa m/z) 383 [(M+1]$^+$, 100] 279 (20)

Paso E

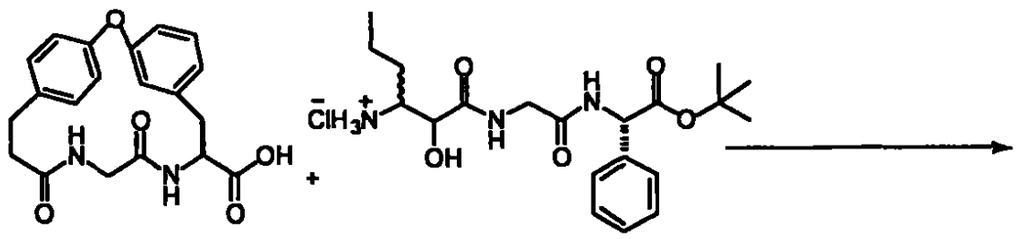


9e 9f

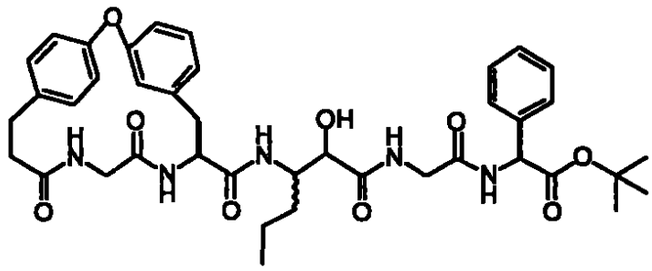
Una solución del compuesto cíclico 9e (150 mg, 0,4 mmol) en THF (4,0 ml), H_2O (4,0 ml) fue agitada a ta con $LiOH \bullet H_2O$ (41,0 mg, 1,0 mmol, 2,5 equiv) por espacio de 3 h La mezcla de reaccion fue acidificada con HCl conc. (2,0 ml) y concentrada al vacio El sólido 9f fue secado al vacio y utilizado para el posterior acoplamiento sin mas purificacion

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Paso F



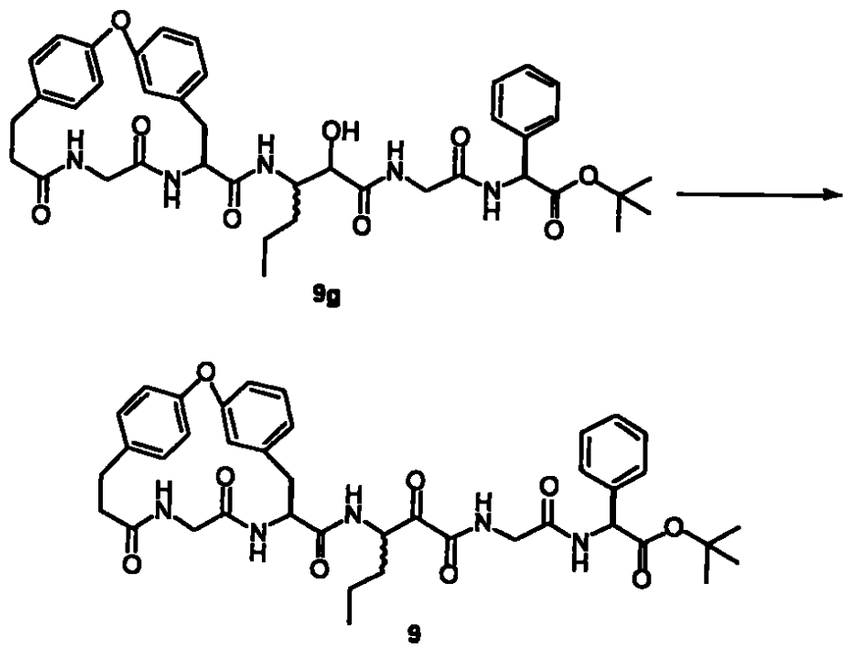
9f B



9g

Una solucion del ácido hidrolizado 9f en DMF seca (4,0 ml) y CH_2Cl_2 (4,0 ml) fue tratada con HOOBt (103 mg, 0,58 mmol, 1,5 equiv) y enfriada a 0°C y se agrego base de Hünig (206 mg, 1,60 mmol, 4,0 equiv, 295 µl) A esta mezcla se agregó EDCI (112 mg, 0,58 mmol, 1,5 equiv) y la mezcla de reacción fue agitada a 0°C durante 0,4 h y tratada con el clorhidrato de amina B (206 mg, 0,48 mmol, 1,2 equiv) La mezcla de reacción fue guardada en un freezer por espacio de 48 h y concentrada al vacio para eliminar la DMF y el CH_2Cl_2 El residuo fue diluido con HCl ac. (2M) Y extraido con CH_2Cl_2 (3x50 ml) La capa organica combinada fue extraida con HCl ac (1M, 3x50 ml), NaOH ac (2M), salina (100 ml), secada (Na_2SO_4) y concentrada al vacío El residuo 9g (200 mg) fue oxidado sin más purificación

Paso G



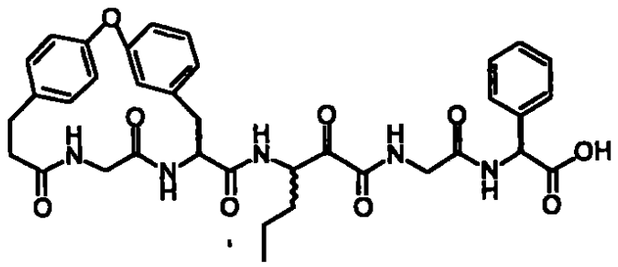
9g

9

Una solución del alcohol 9g (200 mg, 0,27 mmol) en CH_2Cl_2 (3,0 ml) fue tratada con reactivo de Dess-Martin (342 mg, 0,81 mmol, 3,0 equiv) La mezcla de reaccion fue agitada a ta durante 3 h y diluida con $NaHCO_3$ acuoso y $Na_2S_2O_3$ ac La mezcla de reaccion fue agitada a ta durante 20 min y se extrajo la mezcla de reaccion con CH_2Cl_2 (3x30 ml) Las capas orgánicas combinadas fueron extraidas con salina (50 ml), secadas (Na_2SO_4), filtradas, concentradas al vacio y el residuo fue purificado por cromatografía (SiO_2, CH_3OH (NH_3 2M)/CH_2Cl_2 1 19) para dar la cetoamida 9 (100 mg, 50 %) en forma de sólido incoloro EM (Electroatomizacion, int rel m/z) 742 [(M+ 1)$^+$, 100), 686 (80)

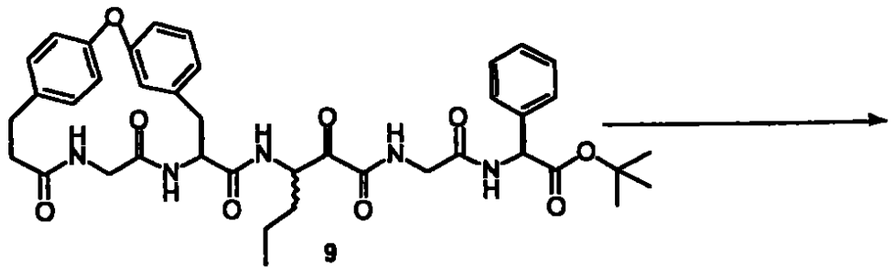
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Ejemplo 10 Preparacion del Compuesto de la Fórmula 10

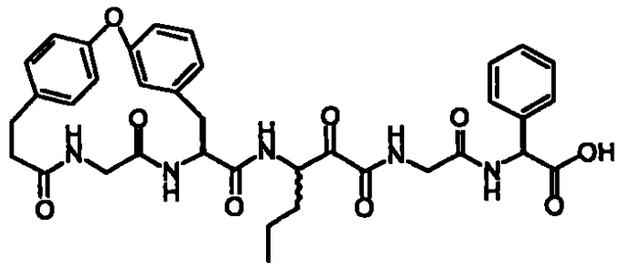


10

Paso A



9

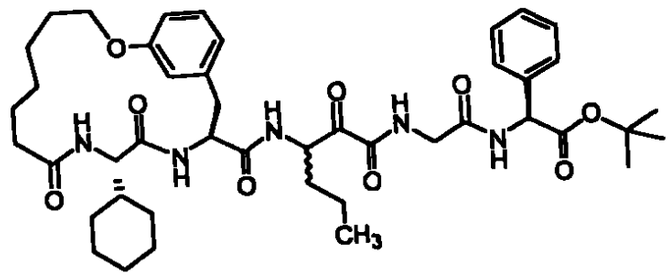


10

Una solución del éster terc-butílico 9 (100 mg, 0,3 mmol) en CH_2Cl_2 seco (4,0 ml) fue tratada con ATF (4,0 ml) y agitada a temperatura ambiente durante 5 h La mezcla de reacción fue concentrada al vacio y el residuo disuelto reiteradamente en tolueno/CH_2Cl_2 y concentrado al vacio varias veces para dar un fino sólido incoloro 10 EM (FAB, NBA/DMSO intensidad relativa m/z), 686 [(M+ 1)$^+$, 40], 460 (20), 307 (100), 289 (60), HRMS calc. para $C_{38}H_{40}N_5O_9$ (M+1)$^+$ 686,2825, encontrado 686,2840

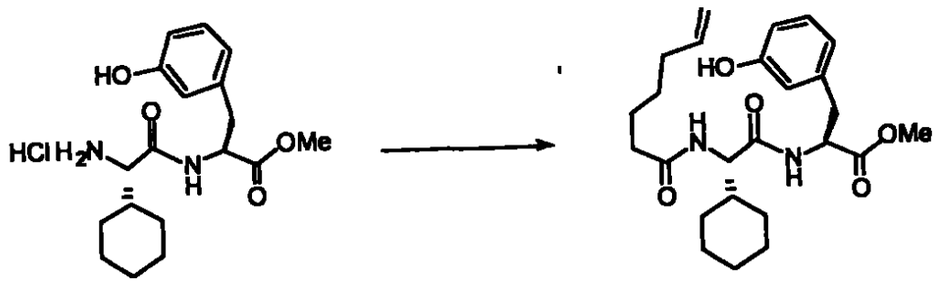
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Ejemplo 11 Preparacion del Compuesto de la Formula 11



11

Paso A

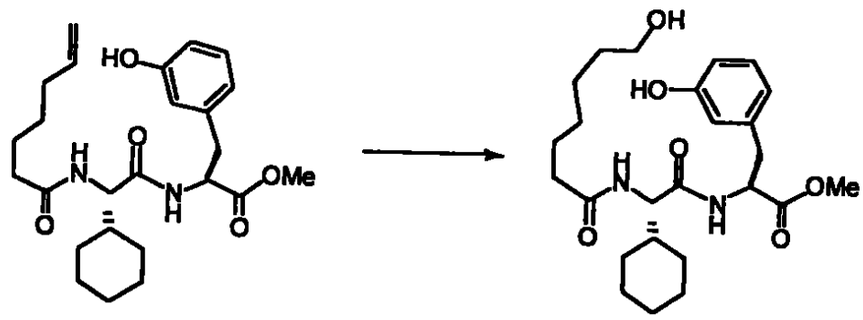


1g 11a

A una solucion del clorhidrato de amina 1g (1,20 g, 3,23 mmol), ácido 6-heptenoico (0,610 g, 4,68 mmol), HOOBt (0,765 g, 4,69 mmol) y EDCI (1,07 g, 5,58 mmol) en DMF anhidra (50 ml) y CH_2Cl_2 (50 ml) a –20°C se agregó NMM (1,55 ml, 14,1 mmol) Después de agitar a esta temperatura durante 30 min, se guardo la mezcla de reaccion en un freezer por espacio de 18 h Luego se la dejó entibiar a ta y se agregó EtOAc (150 ml), salina (50 ml) y H_3PO_4 al 5 % (50 ml) Tras la separación, se lavó la solución orgánica con H_3PO_4 al 5 % (80 ml), una solución acuosa saturada de bicarbonato de sodio (2 x 80 ml), agua (80 ml) y salina (80 ml), se la secó con sulfato de magnesio, se la filtro y concentró al vacio La cromatografía instantánea (MeOH 2 a 5 %-CH_2Cl_2) dio 11a (1,46 g, 3,28 mmol, cuant) en forma de sólido blanco (RMN ^1H (400 MHz, d_6-DMSO) δ 9,25 (s, 1H), 8,31 (d, J = 7,2 Hz, 1 H), 7,70 (d, J = 9,2 Hz, 1 H), 7,05-7,01 (m, 1 H), 6,62 –6,58 (m, 3H), 5,82-5,72 (m, 1 H), 5 ,02-4,91 (m, 1 H), 4,43-4,38 (m, 1 H), 4,23-4,19 (m,

1 H), 3,55 (s, 3 H), 2,93-2,80 (m, 2 H), 2,51-1,97 (m, 2 H), 1,66-0,86 (m, 125H), (RMN ^{13}C (d_6-DMSO, 125 MHz) δ 171,9, 171,8, 171,1, 157,2, 138,6, 138,4, 129,1, 119,5, 115,8, 114,6, 113,5, 56,5, 53,5, 51,6, 36,5, 34,8, 32,8, 29,0, 28,0, 27,8, 25,8, 25,5, 24,8, HRMS calc para $C_{25}H_{36}N_2O_5$ 445,2702, error 4 ppm)

Paso B



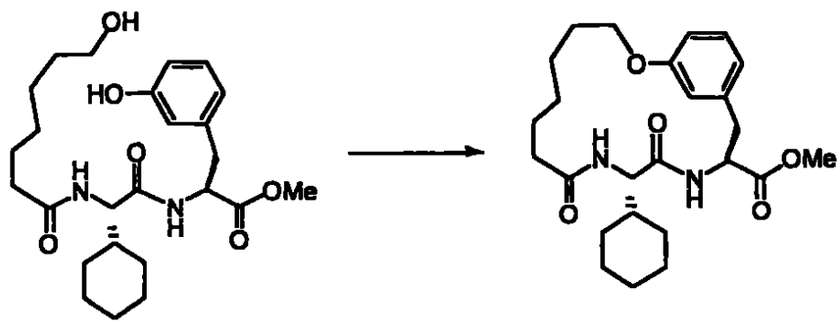
11a 11b

A una solución de 11a (1,46 g, 3,28 mmol) en THF anhido (60 ml) bajo nitrogeno a 0°C se agrego una solución de borano-THF (12 ml, 1,0 M, 12 mmol), cuidadosamente La solución resultante fue agitada a 0°C bajo nitrógeno durante 1 h 40 min Luego se agregó etanol (4 ml) y buffer pH 7 (8 ml), seguido por una solución de H_2O_2 al 30 % (7,5 ml) Después de agitar a 0°C durante 20 min, se la calentó a ta y se la agitó durante 2 h Se agrego EtOAc (200 ml) y salina (100 ml) y se separaron las capas Se extrajo la solución acuosa con EtOAc (2 x 150 ml) La solución orgánica combinada fue secada con sulfato de magnesio, filtrada y concentrada al vacio La cromatografia instantánea (MeOH 2 a 5 %-CH_2Cl_2) dio 11b (1,05 g, 2,18 mmol, 68 %) en forma de solido blanco (RMN ^1H (400 MHz, d_6-DMSO) δ 9,25 (s, 1H), 8,30 (d, J = 7,2 Hz, 1 H), 7,68 (d, J = 9,2 Hz, 1 H), 7,05-7,01 (m, 1 H), 6,62 –6,58 (m, 3H), 4,43-4,18 (m, 3 H), 3,55 (s, 3 H), 3,37-3,33 (m 2 H), 2,93-2,80 (m, 2 H), 2,20-2,03 (m, 2 H), 1,66-0,87 (m, 19H), RMN ^{13}C (d_6-DMSO, 125 MHz) d 172,1, 171,8, 171,2, 157,2, 138,4, 138,4, 129,1, 119,5, 115,8,

~~223~~ 224

113,5, 56,5, 53,5, 51,7, 36,5, 35,1, 32,6, 32,4, 29,0, 28,5, 28,0, 25,8, 25,6, 25,2, HRMS calc para $C_{25}H_{36}N_2O_5$ 463,2808, error 1 ppm)

Paso C

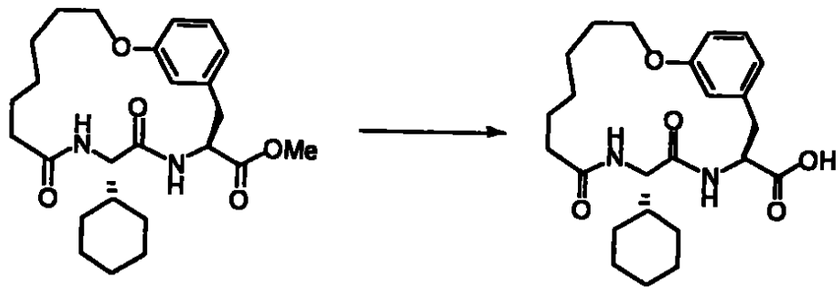


11b 11c

A una solucion del alcohol fenólico 11b (1,00 g, 2,16 mmol) y tri-n-butilfosfina (1,10 ml, 4,28 mmol) en CH_2Cl_2 anhidro (100 ml) y THF (40 ml) a 0°C se agregó ADDP (1,08 g, 4,28 mmol) Después de agitar a 0°C durante 1 h, la solución fue calentada a ta y agitada durante 3 h bajo nitrógeno La TLC indicó el consumo total del material de partida Tras la eliminación del solvente al vacio, el residuo fue parcialmente purificado por cromatografía instantánea (MeOH 0 a 3 % en CH_2Cl_2) para dar el macrociclo 11c (650 mg, 1,46 mmol, 68 %) RMN ^1H (400 MHz, d_6-DMSO) δ 8,58 (d, J = 8,3 Hz, 1 H), 7,76 (d, J = 9,2 Hz, 1 H), 7,18-7,14 (m, 1 H), 6,76 –6,65 (m, 3H), 4,77-4,71 (m, 1 H), 4,32 (t, J = 8,5 Hz, 1 H), 3,97-3,93 (m, 1 H), 3,82-378 (m, 1 H), 3,18-3,14 (m, 1 H), 2,98-2,92 (m, 2 H), 2,32-2,25 (m, 1 H) 1,99-0,87 (m, 19H), RMN ^{13}C (d_6-DMSO, 125 MHz) d 172,1, 171,6, 171,4, 160,1, 158,8, 139,0, 129,1, 121,1, 113,0, 111,9, 66,4, 56,1, 52,0, 50,1, 40,6, 34,9, 34,2, 28,7, 28,3, 26,8, 26,3, 25,9, 25,5, 25,2, 24,2,, HRMSm/z 445,2685 calc para $C_{25}H_{36}N_2O_5$ 445,2702, error 4 ppm)

225

Paso D



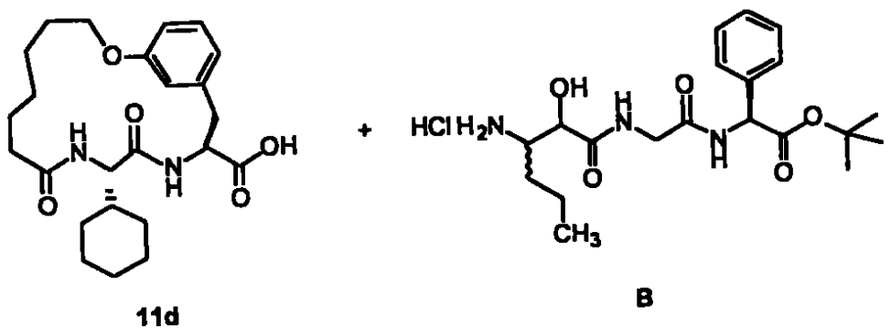
11c

11d

Se agrego una solución acuosa de hidroxido de litio (70 mg, 15 ml, H_2O, 292 mmol) a una solución del ester metilico 11c (330 mg, 0,742 mmol) en THF (20 ml) y etanol (10 ml) a ta La mezcla fue agitada a ta por espacio de 3 h Se monitoreó el progreso de la reaccion por TLC Una vez concentrada la solucion al vacio, se agregó EtOAc (100 ml), una solucion de HCl 6N (10 ml) y agua (50 ml) y se separaron las capas Se extrajo la solución acuosa con EtOAc (2 x 80 ml) Se combinaron las soluciones orgánicas, estas fueron secadas con sulfato de magnesio, filtradas y concentradas al vacio para dar 11d (260 mg, 0,604 mmol, 81 %) en forma de solido blanco RMN ^{1}H (400 MHz, d_6-DMSO) δ 8,43 (d, J = 8,3 Hz, 1 H), 7,73 (d, J = 9,3 Hz, 1 H), 7,17-7,13 (m, 1 H), 6,77 –6,66 (m, 3H), 4,67-4,62 (m, 1 H), 4,32-4,28 (m, 1 H), 3,98-3,93 (m, 1 H), 3,81-375 (m, 1 H), 3,17-3,13 (m, 1 H), 2,97-2,90 (m, 1 H), 2,32-2,26 (m, 1 H) 2,01-1,97 (m, 1 H), 1,67-0,85 (m, 19H), RMN ^{13}C (d_6-DMSO, 125 MHz) d 173,2, 171,6, 171,3, 160,1, 158,8, 139,3, 129,0, 121,1, 113,1, 111,9, 66,4, 56,1, 50,8, 35,1, 34,3, 28,8, 28,3, 26,9, 26,3, 25,9, 25,6, 25,5, 25,2, 24,2, HRMS m/z 431,2564 (calc para $C_{25}H_{36}N_2O_5$ 431,2546, error 4 ppm)

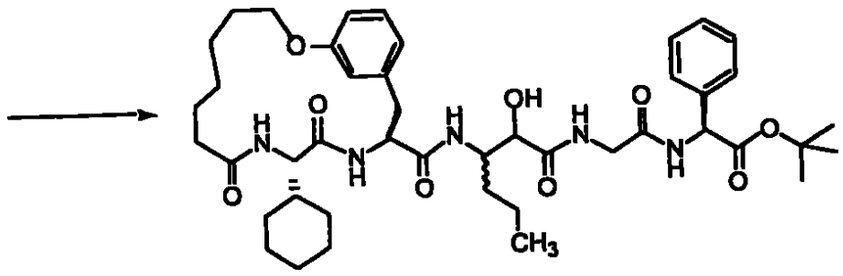
276

Paso E



11d

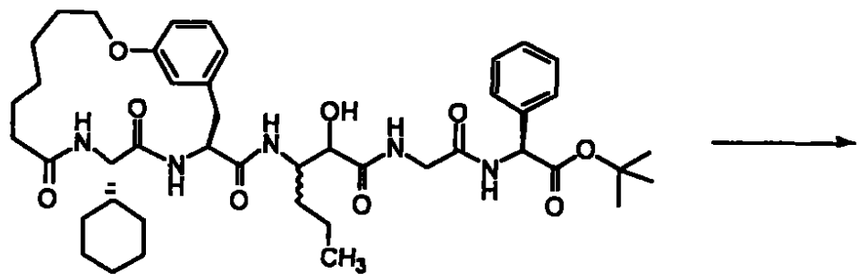
B



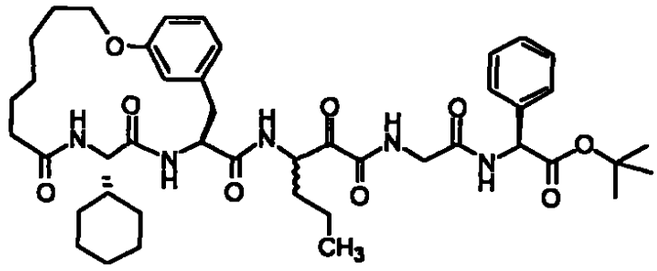
11e

A una solucion del acido 11d (0,140 g, 0,325 mmol), la amina B (0,140 g, 0,325 mmol), HOOBt (56 mg, 0,343 mmol) y EDCI (75 mg, 0,391 mmol) en DMF anhidra (40 ml) y CH_2Cl_2 (20 ml) a –20°C se agrego NMM (0,107 ml, 0,873 mmol) Despues de agitar a esta temperatura durante 30 min, se guardó la mezcla de reacción en un freezer por espacio de 18 h Luego se agregó EtOAc, salina y H_3PO_4 al 5 % Se lavo la solución organica separada sucesivamente con H_3PO_4 al 5 %, una solución acuosa saturada de bicarbonato de sodio, agua y salina, se la seco con sulfato de magnesio, se la filtro y concentró al vacio La cromatografía instantánea (MeOH 2 a 5 %-CH_2Cl_2) dio 11e como mezcla de diastereomeros (0,170 g, 0,211 mmol, 65 %) en forma de sólido blanco, que fue utilizado en la reaccion siguiente sin mas purificacion

Paso F



11e



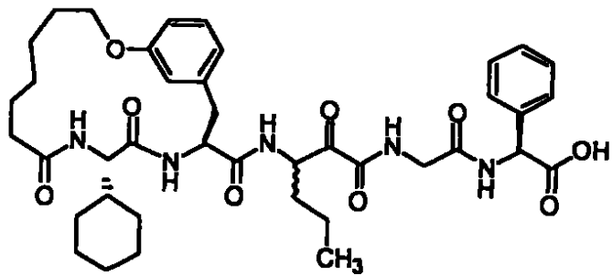
11

A la mezcla de la hidroxiamida 11e (0,29 g, 0,36 mmol) y reactivo de Dess-Martin (0,45 g, 1,06 mmol) a ta se agrego CH_2Cl_2 anhidro (60 ml), DMF (3 ml) y DMSO (3 ml) La solución resultante fue agitada vigorosamente durante 2,5 h a temperatura ambiente, se agregó más reactivo Dess-Martin (300 mg, 0,71 mmol) y se agito la mezcla de reacción durante una hora mas Se agrego una solución saturada acuosa de bicarbonato de sodio y una de bisulfito de sodio (40 ml de cada una) y se agitó vigorosamente la mezcla durante 10 min antes de la adición de EtOAc (200 ml) y agua (30 ml) y de la separación de las capas Se lavó la solución orgánica con una solución de H_3PO_4 al 5 % (2 x 100 ml) y una solucion saturada de $NaHCO_3$ (100 ml), se la secó con sulfato de magnesio, se la filtró y concentró al vacio La cromatografía instantánea (MeOH 1 a 5 %-CH_2Cl_2) dio 11 (100 mg, 0,134 mmol, 35 %) en forma de sólido RMN ^1H (400 MHz, d_6-DMSO) δ 8,79-8,69 (m, 2 H), 8,36-8,16 (m, 2 H), 7,72-7,68 (m, 1 H), 7,42-7,33 (m, 5 H),

228
0232

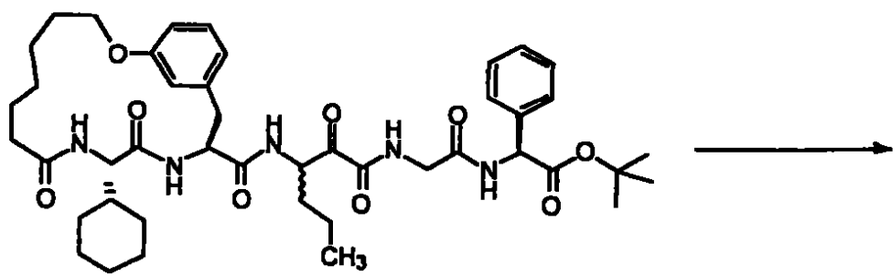
7,17-7,13 (m, 1 H), 6,77 –6,63 (m, 3H), 5,30-5,27 (m, 1 H), 5,09-5,04 (m, 1 H), 4,85-4,76 (m, 1 H), 4,29-4,25 (m, 1 H), 3,98-3,74 (m, 1 H), 3,02-2,85 (m, 2 H), 2,32-2,27 (m, 1 H) 2,04-1,96 (m, 1 H), 1,72-0,81 (m, 35 H), RMN ^{13}C (d_6-DMSO, 125 MHz) δ 196,5, 196,2, 171,5, 171,14, 171,07, 169,4, 157,6, 160,7, 158,94, 158,79, 139,5, 139,3, 136,6, 136,5, 128,92, 128,90, 128,7, 128,6, 128,1, 127,7, 127,4, 124,9, 121,34, 121,28, 113,1, 112,9, 111,9, 81,3, 66,34, 66,30, 56,92, 56,87, 56,3, 56,2, 53,4, 53,3, 51,5, 50,9, 41,5, 41,4, 40,8, 40,7, 36,6, 36,1, 25,59, 25,55, 25,0, 24,2, 18,74, 18,66, 13,5, 13,4, HRMS m/z 804,4542 (calc para $C_{25}H_{36}N_2O_5$ 804,4548, error 1 ppm)

Ejemplo 12 Preparacion del compuesto de la Fórmula 12

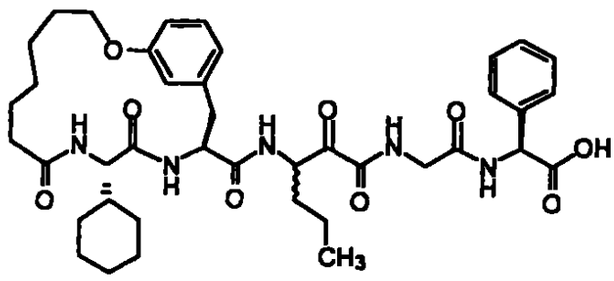


12

Paso A.



11



12

Una solución del ester t-butílico 11 (56,8 mg, 0,0706 mmol) en ácido trifluoroacético (15 ml) y CH_2Cl_2 (15 ml) fue agitada a ta por espacio de 4 h Una vez eliminados todos los volátiles al vacío, se disolvió el residuo en MeOH al 50 %-CH_2Cl_2 (3 ml) y se lo concentró a sequedad al vacio para dar origen a un sólido blancuzco 12 (50 mg, 0,0669 mmol, 95 %) RMN ^1H

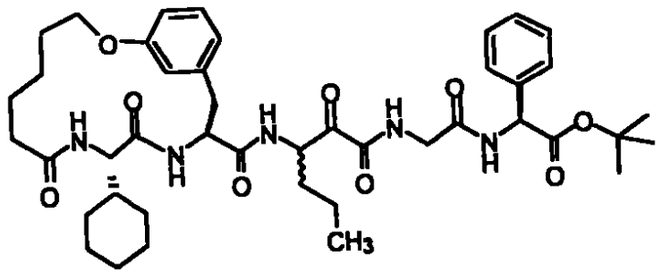
(400 MHz, d_6-DMSO) δ 8 75-8 71 (m, 2 H), 8 36-8 16 (m, 2 H), 7 72-7 69 (m, 1 H), 7 39-7 31 (m, 5 H), 7 17-7 13 (m, 1 H), 6 76-6 63 (m, 3 H), 5 37-5 35 (m, 1 H), 5 07-5 04 (m, 1 H), 4 85-4 76 (m, 1 H), 4 29-4 25 (m, 1 H), 3 97-3 74 (m, 4 H), 3 02-2 86 (m, 2 H), 2 32-2 26 (m, 1 H) 2 01-1 97 (m, 1 H), 1 70-0 82 (m, 26 H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 196 5, 196 2, 171 63, 171 59, 171 52, 171 48, 171 1, 171 06, 167 4, 160 6, 158 82, 158 78, 153 4, 139 4, 137 1, 137 0128 91, 128 88, 128 7, 128 65, 128 61, 128 5, 128 43, 128 39, 128 33, 128 32, 128 14, 128 12, 128 0, 127 7, 128 7, 127 63, 127 59, 127 5, 127 4, 126 8, 121 3 115 9, 113 1, 112 9, 112 8, 112 0, 111 9,, 111 88, 66 33, 66 29, 56 3, 56 2, 56 17, 53 34, 53 31, 53 27, 51 1, 50 9, 41 5, 40 84, 40 77, 40 7, 40 6, 40 56, 40 53, 40 5, 38 7, 38 6, 38 56, 38 53, 36 6, 36 1, 34 4, 34 3, 31 8,

230

31 6, 29 4, 29 1 29 0, e28 9, 28 4, 28 3 28 2, 26 9, 26 8, 26 79, 26 20, 26 16, 25 88, 25 86 25 79, 25 75, 25 71, 25 66, 25 57, 25 54, 25 4, 25 0, 24 2, 18 7, 18 6, 13 5, 13 4, HRMS, *m/z* 748 3947

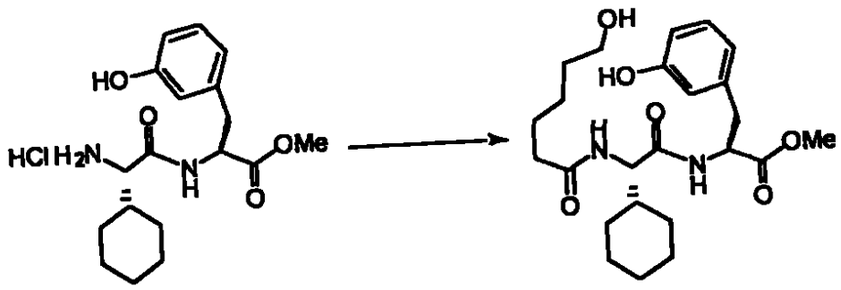
(calc para $C_{25}H_{36}N_2O_5$ 748,3922, error 3 ppm)

Ejemplo 13 Preparación del compuesto de la Fórmula 13



13

Paso A

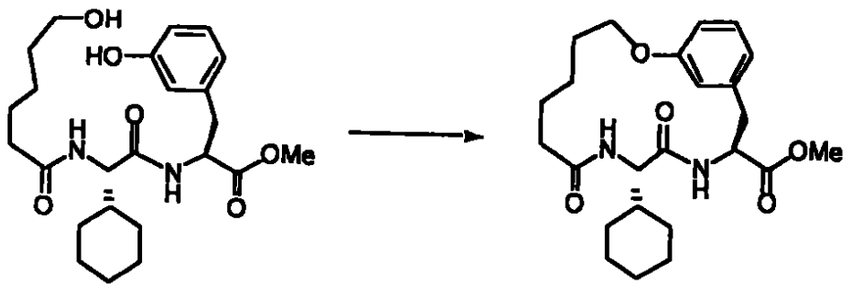


1g 13a

Se preparo el compuesto buscado 13a de acuerdo con el método del Ejemplo 11, Paso A, excepto que se sustituyo el acido 6-heptenoico por ácido 6-hidroxihexanoico (39 %)

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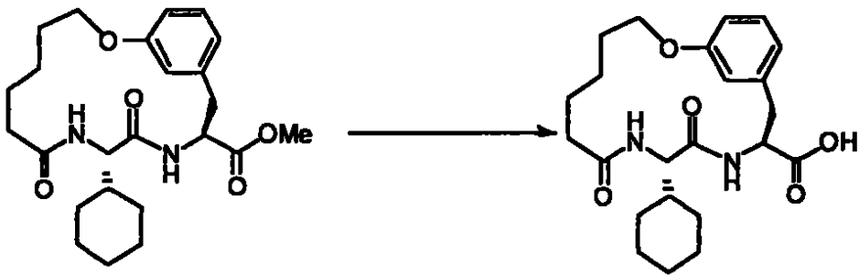
Paso B



13a 13b

Se preparo el compuesto buscado 13b de acuerdo con el método del Ejemplo 11, Paso C con 74 % de rendimiento

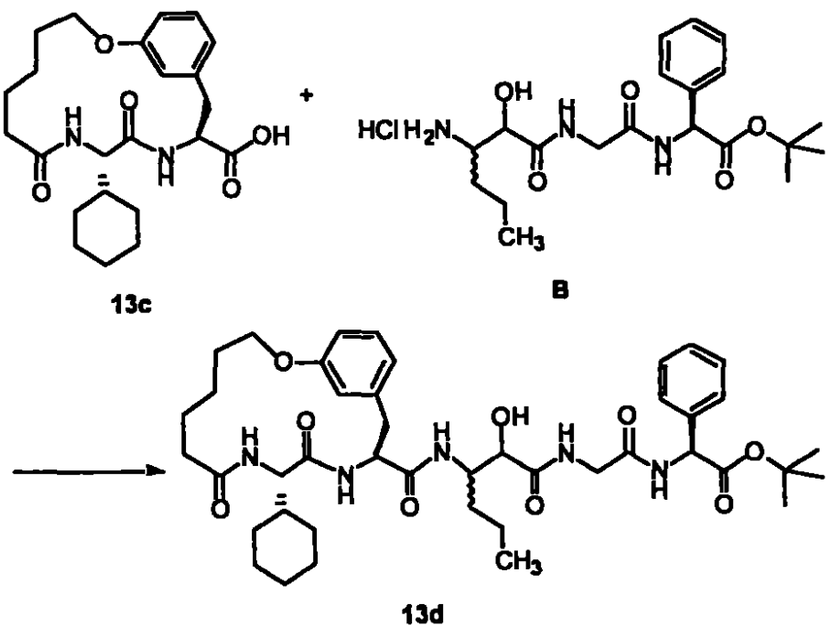
Paso C



13b 13c

Se preparo el ácido macrociclico deseado 13c a partir de su correspondiente ester metilico de acuerdo con el método del Ejemplo 11, Paso D con 88 % de rendimiento, en forma de sólido blanco

Paso D



13c

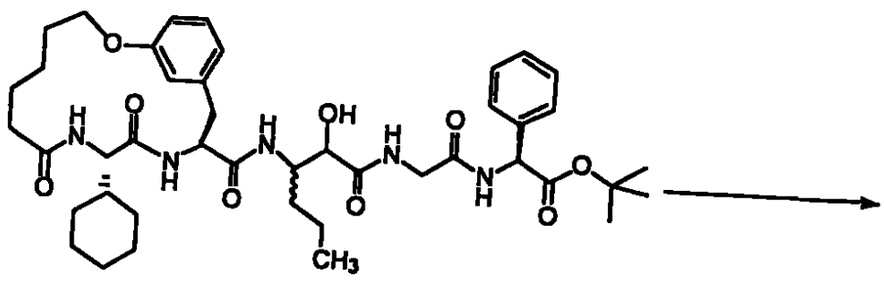
B

13d

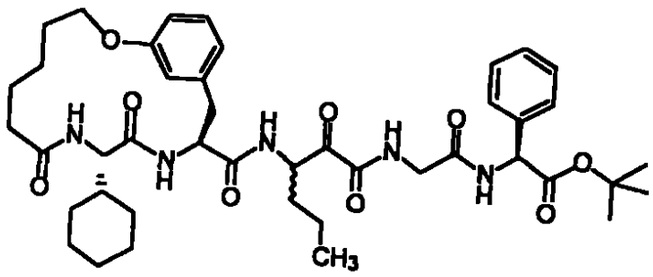
Se preparó el compuesto deseado 13d a partir de 13c y B de acuerdo con el metodo del Ejemplo 11, Paso E con 48 % de rendimiento

-AA 233

Paso E



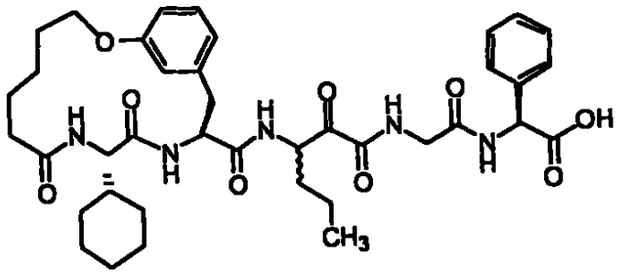
13d



13

Se preparo el compuesto deseado 13 a partir de 13d de acuerdo con el metodo del Ejemplo 11, Paso F con 70 % de rendimiento

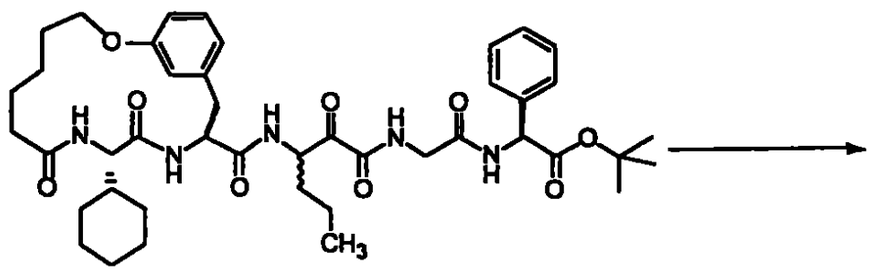
Ejemplo 14 Preparación del Compuesto de la Fórmula 14



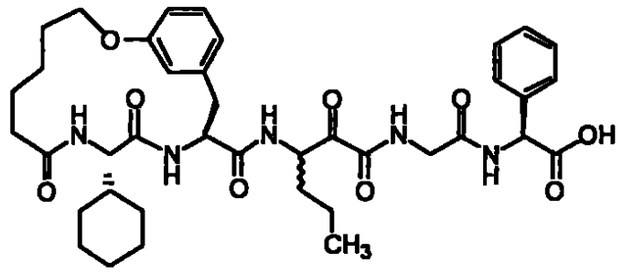
14

234

Paso A



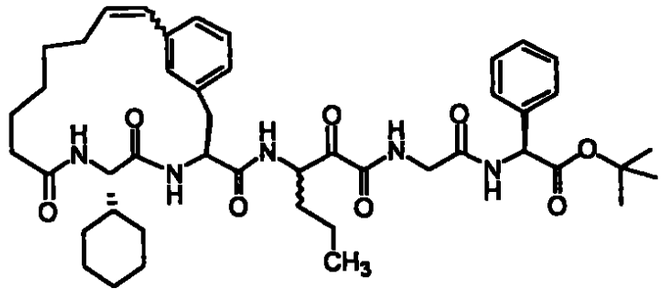
13



14

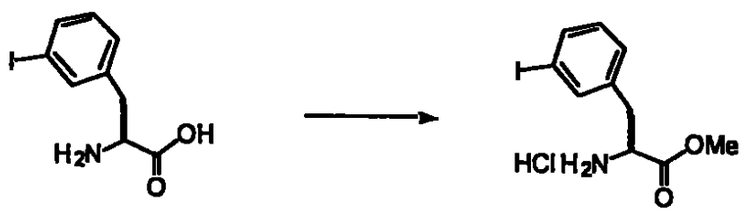
Se preparó el compuesto deseado 14 a partir de 13 de acuerdo con el metodo del Ejemplo 12, Paso A con rendimiento cuantitativo

Ejemplo 15 Preparacion del Compuesto de la Fórmula 15



15

Paso A



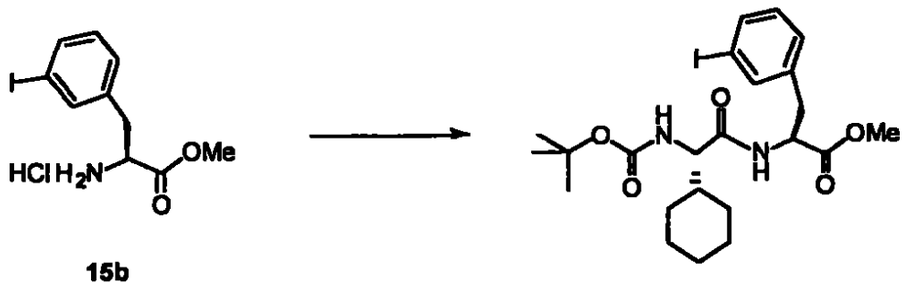
15a

15b

31 235

Una solución de 3-yodo-fenilalanina 15a (2,50 g, 8,59 mmol) y ácido clorhídrico concentrado (2 ml, 24 mmol) en metanol fue calentada a reflujo por espacio de 18 h La eliminacion de los solventes al vacio dio un solido blanco 15b, que fue utilizado en el Paso B sin más purificación

Paso B

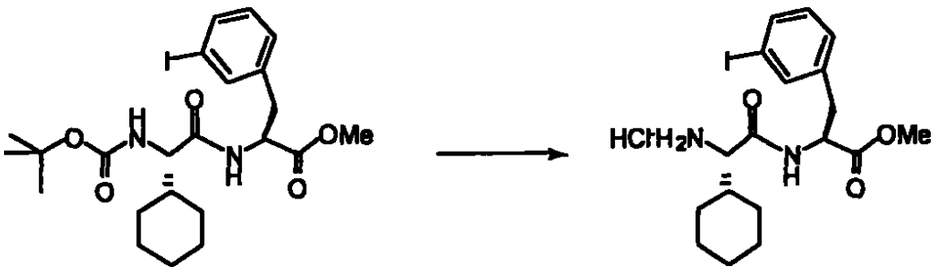


15b

15c

Se preparó el compuesto deseado 15c con 84 % de rendimiento a partir de 15b de acuerdo con el metodo del Ejemplo 11, Paso A Se lo utilizó en la siguiente reaccion sin mas purificacion

Paso C



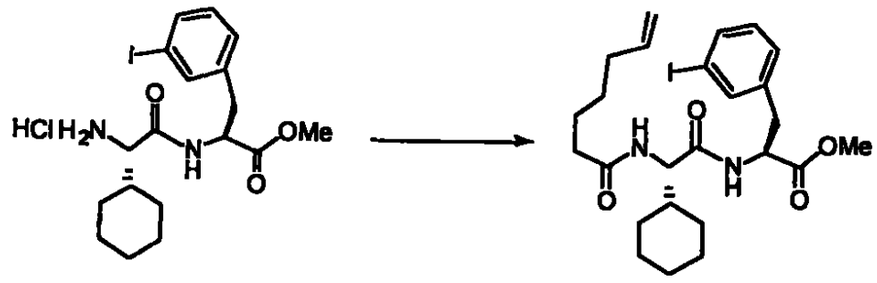
15c

15d

Se preparo el compuesto deseado 15d a partir de 15c de acuerdo con el método del Ejemplo 11, Paso A (cuantitativo) Se lo utilizo en la siguiente reacción sin más purificacion

0244 236

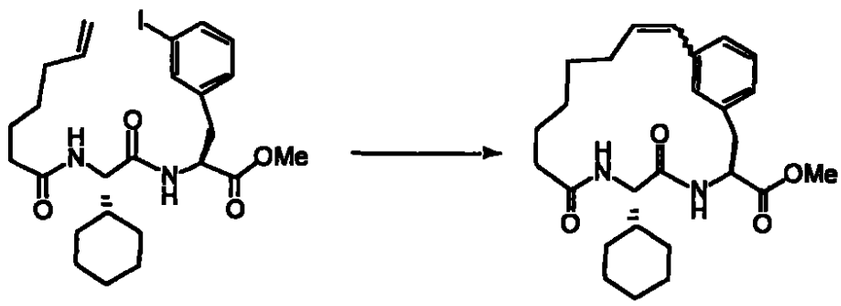
Paso D



15d 15e

Se preparo el compuesto deseado 15e con 68 % de rendimiento a partir de 15d de acuerdo con el metodo del Ejemplo 11, Paso A Se lo utilizó en la siguiente reacción sin más purificación

Paso E



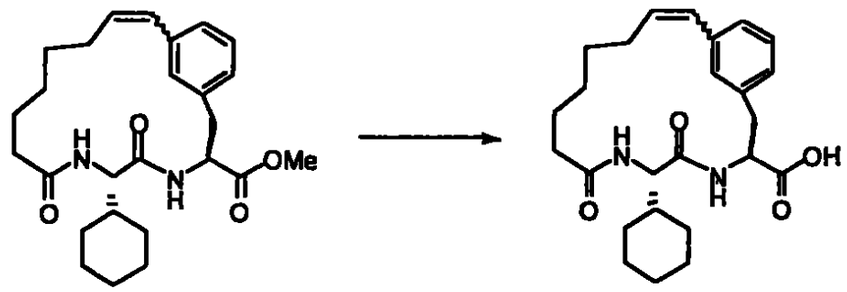
15e 15f

Una solución de 15e (1,16 g, 2,04 mmol), trietilamina (2,90 m, 20,6 mmol) en acetonitrilo anhidro (25 ml) y DMF (20 ml) en un tubo de paredes gruesas fue insuflado con argón por un lapso de 5 minutos A esta solucion a ta se agregó rápidamente tetrakis(trifenilfosfina)paladio (0) (235 mg, 0,203 mmol) El tubo fue cerrado herméticamente con una tapa a rosca de teflón y calentado a 85-90°C en un baño de aceite Después de agitar por espacio de 3 h, se lo enfrió a ta , se lo abrió con cuidado y se vertió su contenido sobre EtOAc (100 ml) La solución fue lavada con H_3PO_4 al 5 % (4 x 50 ml) y agua (50 ml) Se seco la capa organica con

0241 237

sulfato de magnesio, se la filtró, concentro al vacio La cromatografia instantánea (MeOH 1 a 4 %-CH_2Cl_2) dio el macrociclo 15f (330 mg, 0,749 mmol, 37 %)

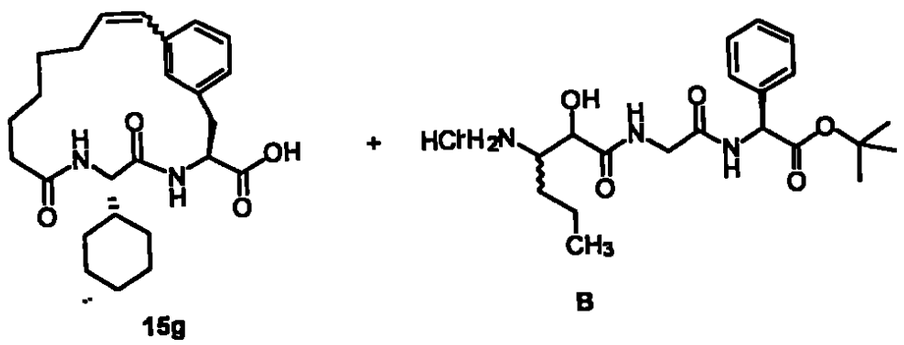
Paso F



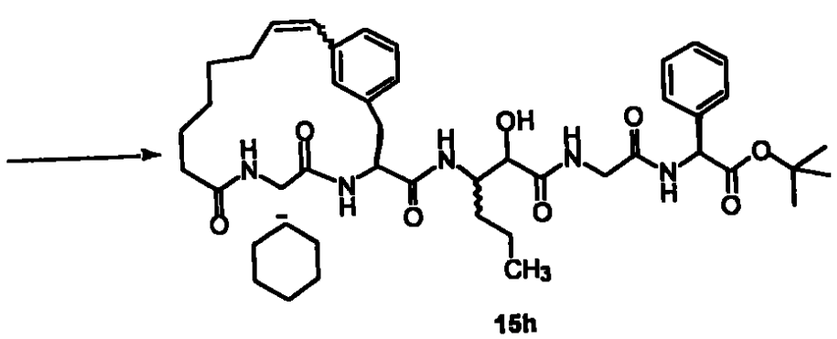
15f 15g

Se preparó el compuesto deseado 15g cuantitativamente a partir de 15f de acuerdo con el metodo del Ejemplo 11, Paso D Se lo utilizó en la siguiente reacción sin más purificación

Paso G



15g B

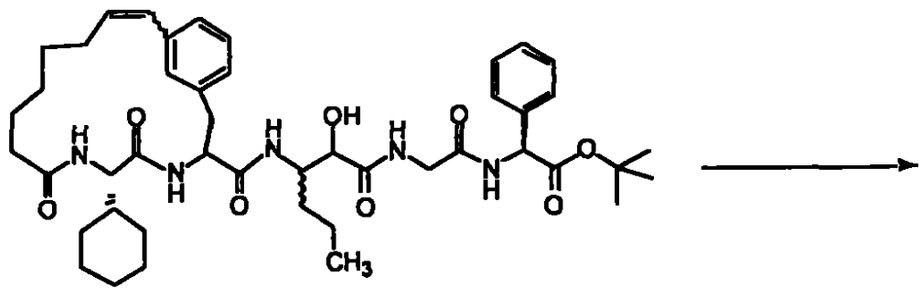


15h

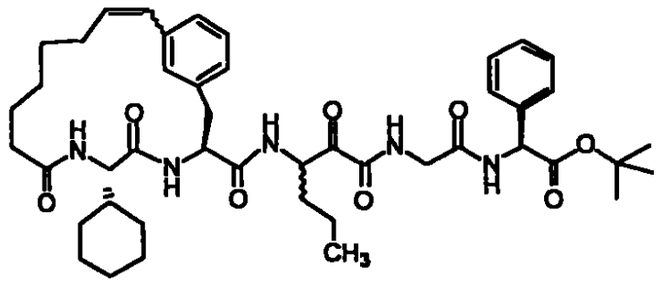
~~0242~~
238

Se preparo el compuesto deseado 15h con 78 % de rendimiento a partir de 15g de acuerdo con el metodo del Ejemplo 11, Paso F Se lo utilizo en la siguiente reaccion sin más purificación

Paso H



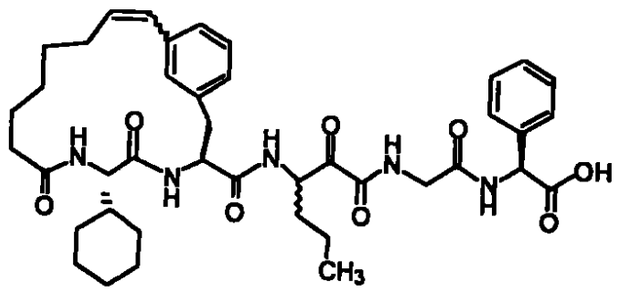
15h



15

Se preparó el compuesto deseado 15 con 55 % de rendimiento a partir de 15h de acuerdo con el metodo del Ejemplo 1, Paso H

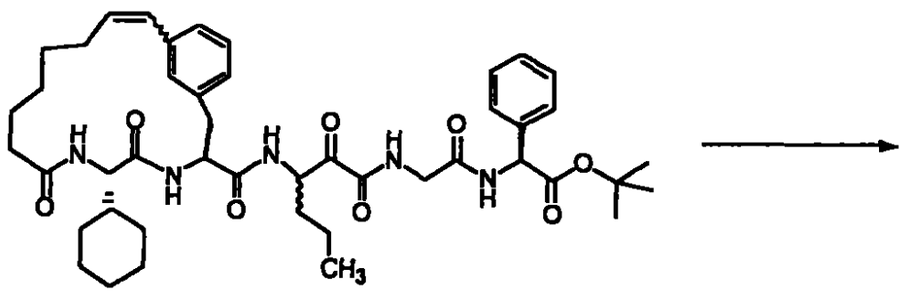
Ejemplo 16 Preparación del Compuesto de la Fórmula 16



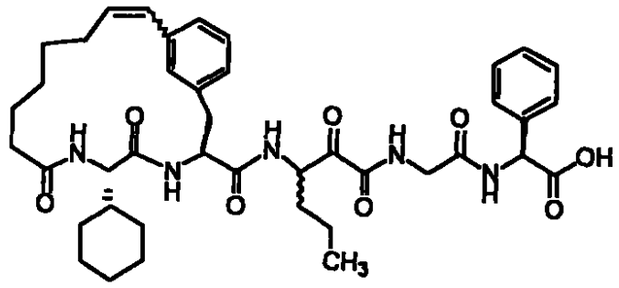
16

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Paso A



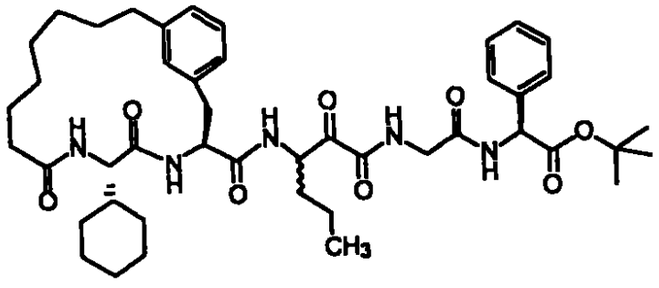
15



16

Se preparo el compuesto deseado 16 cuantitativamente a partir de 15 de acuerdo con el metodo del Ejemplo 12, Paso A

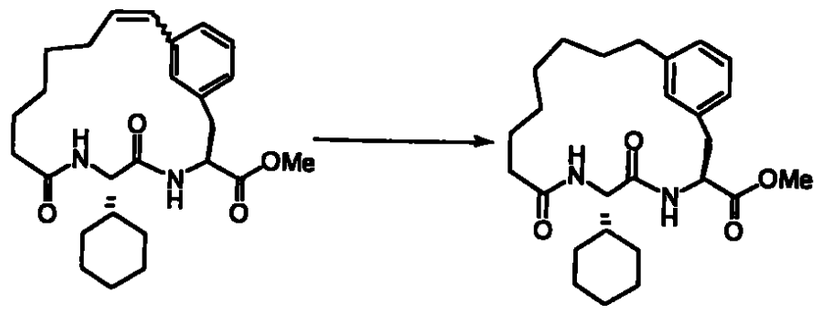
Ejemplo 17 Preparación del Compuesto de la Formula 17



17

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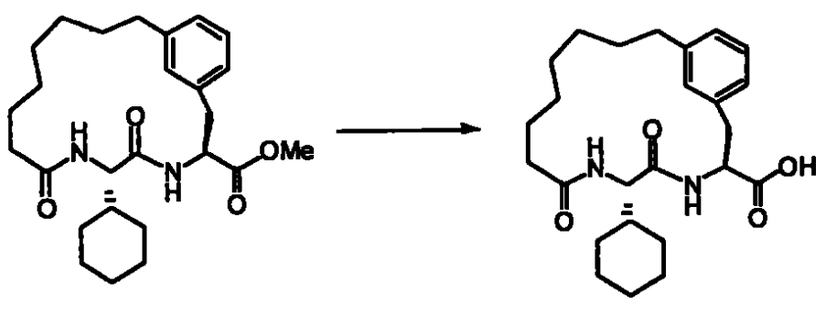
Paso A



15f 17a

A la solución de 15f (150 mg, 0,340 mmol) en EtOH (10 ml) y EtOAc (5 ml) se agregó paladio sobre carbón al 10 % (20 mg) La suspensión fue agitada bajo hidrogeno durante 8 h, lapso durante el cual se monitoreo el progreso de la reacción por TLC Después de la filtración a través de una plancha de celite, los solventes fueron eliminados al vacio para dar el producto en forma de sólido blanco 17a (150 mg, 0,339 mmol, cuantitativo) Se lo utilizo en la siguiente reacción sin mas purificación

Paso B

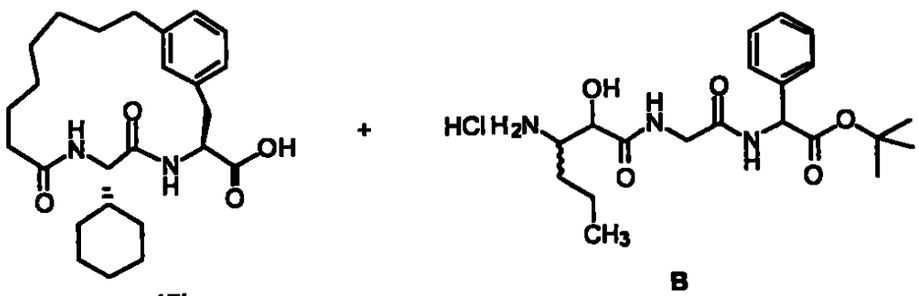


17a 17b

Se preparó el compuesto deseado 17b a partir de 17a de acuerdo con el metodo del Ejemplo 11, Paso D Se lo utilizó en la siguiente reaccion sin más purificacion

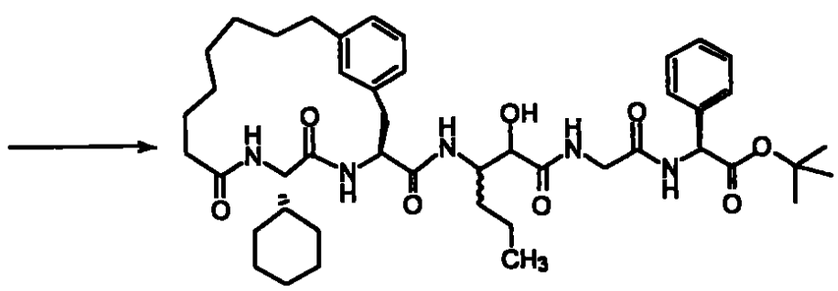
0245 241

Paso C



17b

B

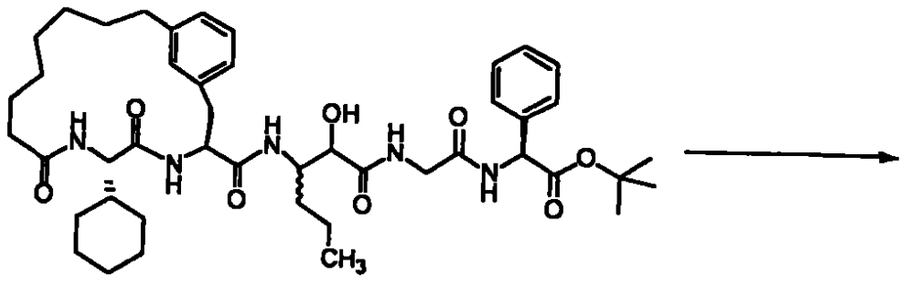


17c

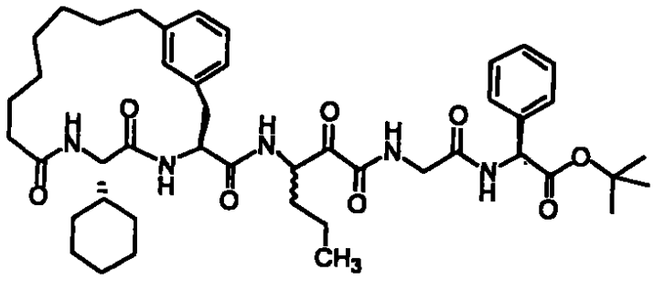
Se preparó el compuesto deseado 17c con 73 % de rendimiento (Pasos B y C) a partir de 17b de acuerdo con el metodo del Ejemplo 11, Paso E Se lo utilizo en la siguiente reaccion sin mas purificación

G7&C 242

Paso D



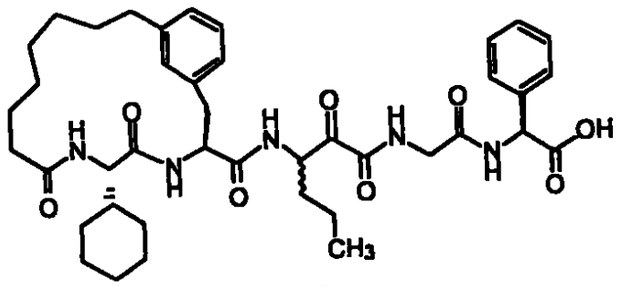
17c



17

Se preparó el compuesto deseado 17 con 46 % de rendimiento a partir de 17c de acuerdo con el método del Ejemplo 11, Paso F

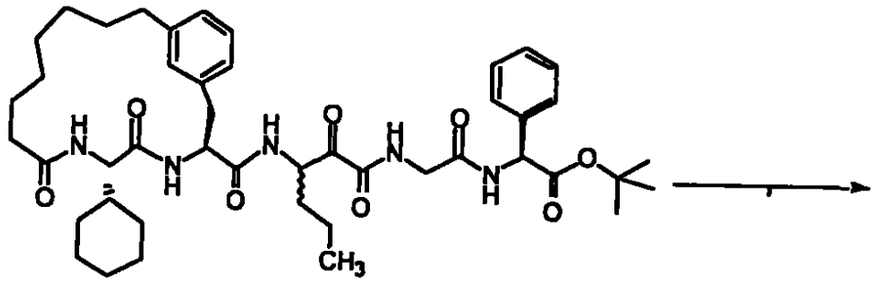
Ejemplo 18 Preparación del Compuesto de la Fórmula 18



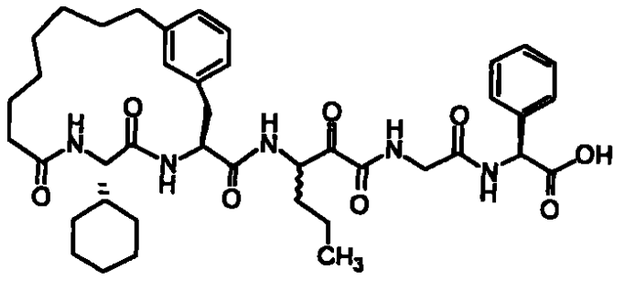
18

243

Paso A



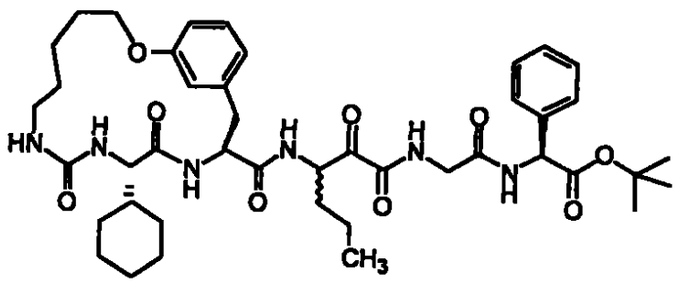
17



18

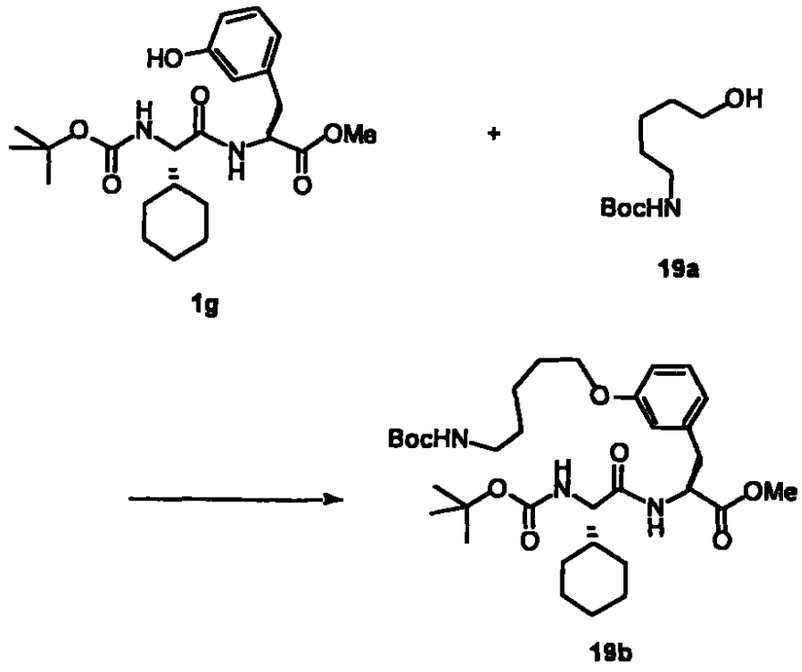
Se preparó el compuesto deseado 18 cuantitativamente a partir de 17 de acuerdo con el método del Ejemplo 12, Paso A

Ejemplo 19 Preparacion del Compuesto de la Formula 19



19

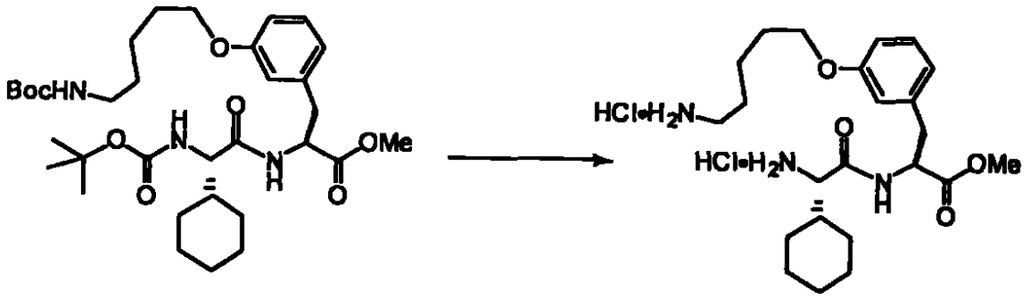
Paso A



1g 19a 19b

Se preparó el compuesto deseado 19b con 64 % de rendimiento a partir de 1g de acuerdo con el método del Ejemplo 11, Paso C

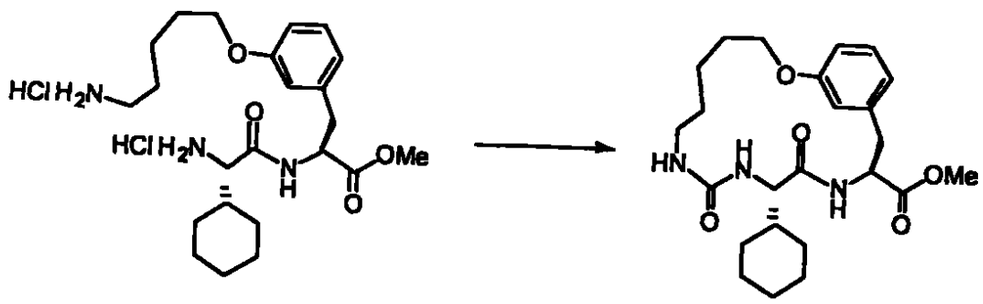
Paso B



19b 19c

Se preparó el compuesto deseado 19c a partir de 19b de acuerdo con el método del Ejemplo 1, Paso C Se lo utilizó en la siguiente reaccion sin más purificación

Paso C

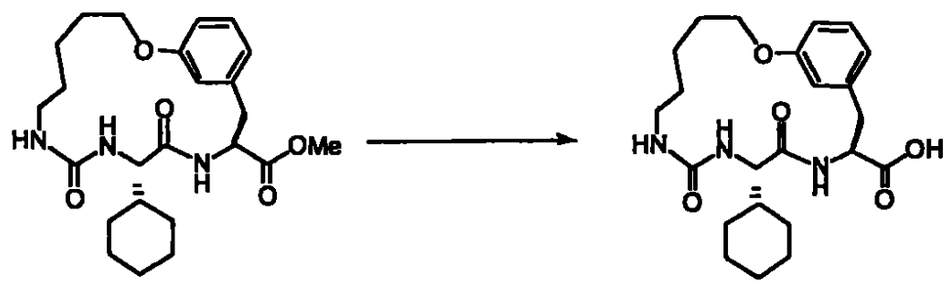


19c

19d

A una suspensión de la sal de diamina 19c (75 mg, 1,52 mmol) y carbonildiimidazol (260 mg, 1,60 mmol) en acetonitrilo (400 ml) a ta , se agrego trietilamina (0,26 ml, 1,85 mmol) La mezcla fue agitada durante un periodo de 3 dias Se eliminó el solvente al vacio Se disolvió el residuo en EtOAc/THF (100/50 ml) y se lavo la solución con H_3PO_4 al 5 %, se la secó con sulfato de magnesio, se la filtro y concentró al vacio La cromatografia instantanea (MeOH 2 a 10 %-CH_2Cl_2) dio 19d (290 mg, 0,651 mmol, 34 %) en forma de solido blanco

Paso D

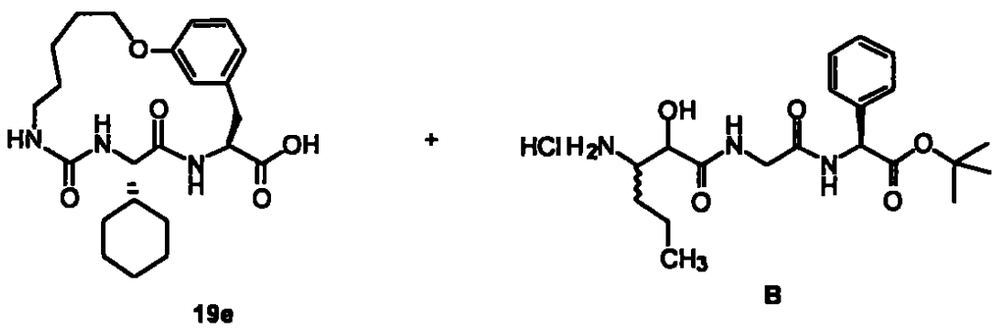


19d

19e

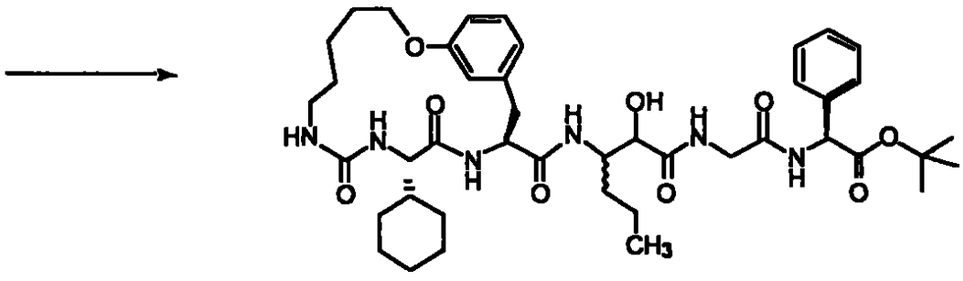
Se preparó el compuesto deseado 19e con 97 % de rendimiento a partir de 19d de acuerdo con el método del Ejemplo 11, Paso D Se lo utilizó en la siguiente reaccion sin más purificación

Paso E



19e

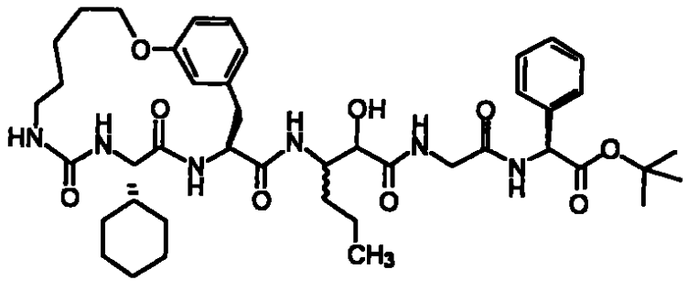
B



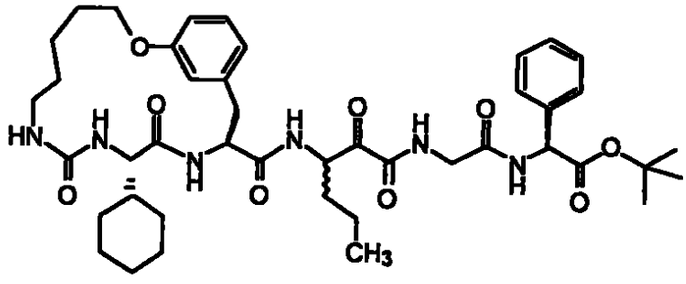
19f

Se preparo el compuesto deseado 19f con 66 % de rendimiento a partir de 19e y B de acuerdo con el método del Ejemplo 11, Paso E

Paso F



19f

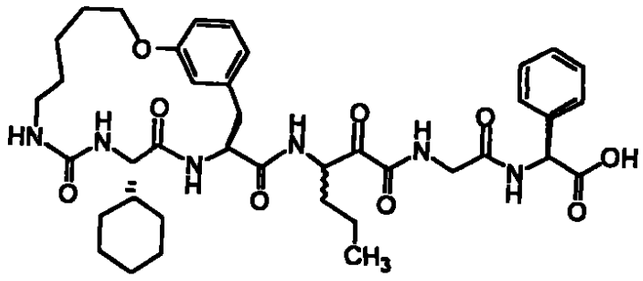


19

252
247

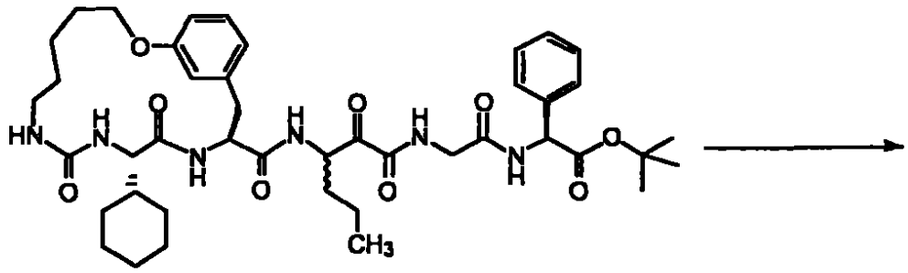
Se preparó el compuesto deseado 19 con 66 % de rendimiento a partir de 19f de acuerdo con el método del Ejemplo 11, Paso F Se separaron parcialmente dos productos por medio de cromatografía instantánea (MeOH 0 a 5 %-CH_2Cl_2)

Ejemplo 20 Preparacion del Compuesto de la Formula 20

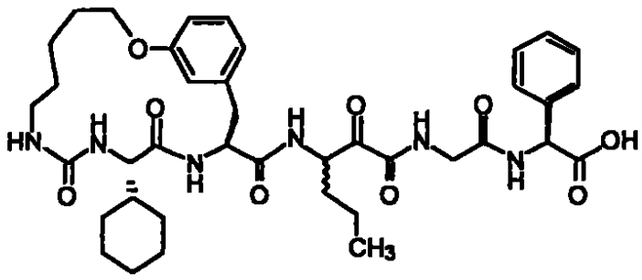


20

Paso A



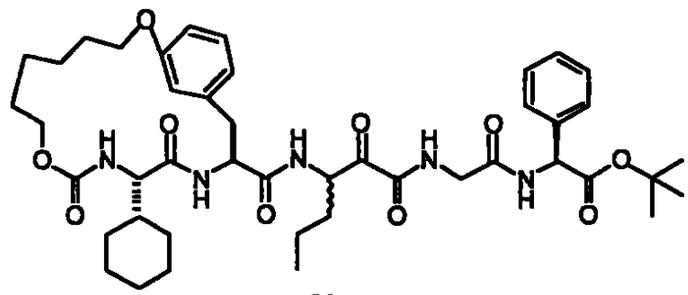
19



20

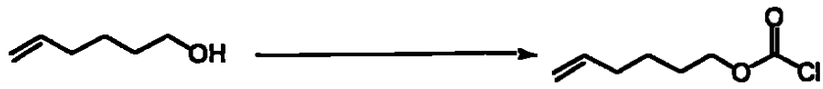
Se preparó el compuesto deseado 20 a partir de 19 de acuerdo con el método del Ejemplo 12, Paso A

Ejemplo 21 Preparacion del Compuesto de la Formula 21



21

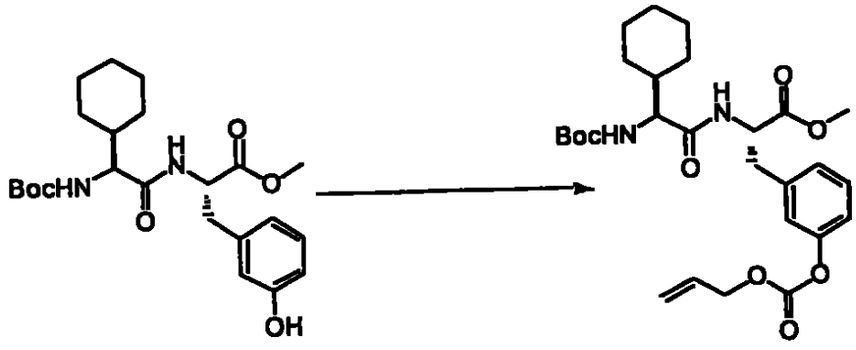
Paso A



21a 21b

Una solucion de 5-hexen-1-ol 21a (10 g, 50 mmol) en eter dietilico (100 ml) fue tratada con trietilamina (10,1 g, 100 mmol, 2,0 equiv) y enfriada a 0°C Se agregó por goteo una solucion de fosgeno en benceno (20 %, 100 ml, 20 g, 300 mmol, 4,0 equiv) y la mezcla de reacción fue agitada a temperatura ambiente por espacio de 12 h El clorhidrato de trietilamina que se separo fue eliminado por filtración y el filtrado fue concentrado al vacio El residuo 21b fue utilizado directamente para otros estudios sin purificación

Paso B



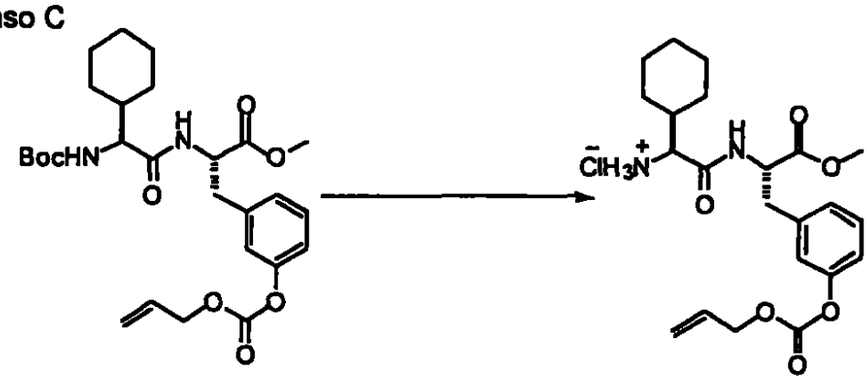
1f 21c

Una solución de 1f (8,0 g, 18,43 mmol) en CH_2Cl_2 (100 ml) fue tratada con trietilamina (2,43 g, 24,0 mmol, 1,3 equiv) La mezcla de reaccion fue enfriada a –

254
( ) 249

78°C y se agregó por goteo alilcloroformiato (2,9 g, 24 mmol, 1,3 equiv) La mezcla de reacción fue agitada a temperatura ambiente por espacio de 12 h y diluida con H_2O (100 ml) y HCl acuoso (2M, 200 ml) Se extrajo la capa acuosa con EtOAc (3x200 ml) La capa combinada de EtOAc fue extraida con salina, secada (Na_2SO_4), filtrada, concentrada al vacio y el residuo 21c fue utilizado directamente para la desproteccion del Boc RMN ^1H ($CDCl_3$, 300 MHz, δ ppm) 7,29 (t, 1 H, J = 8,0 Hz), 6,94 (d, 2 H, J = 6,0 Hz), 8,06-6,98 (m, 3 H), 6,41 (d, 1 H, J = 5,4 Hz), 6,05-5,95 (m, 1H), 5,42 (dd, 1 H, J = 1,2, 13,2), 5,31 (dd, 1 H, J) 1,2, 13,2), 5,10 (d, 1 H, J = 6,6 Hz), 4,91-4,87 (q, 1 H), 4,74 (d, 1 H, J = 4,5 Hz), 3,95-3,92 (m, 1 H), 3,70 (s, 3 H), 3,12 (d, 1 H J= 4,2Hz), 1,81-1,51 (m, 6H), 1,43 (s, 9 H), 1,21-0,91 (m, 6 H)

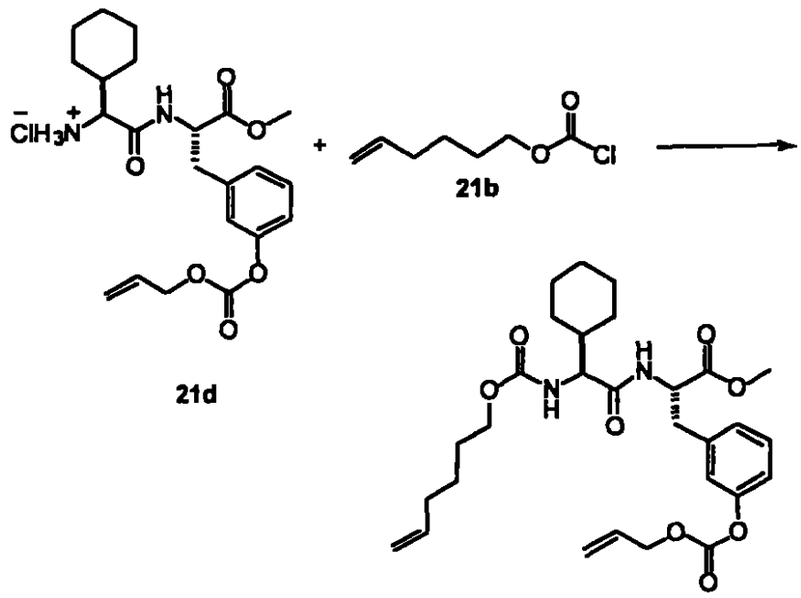
Paso C



21c 21d

Una solución de 21c (1,5 g) en HCl (solución 4M en dioxano, 100 ml) fue agitada a temperatura ambiente por espacio de 3 h La desaparicion del material de partida fue seguida por TLC y una vez desaparecido dicho material la mezcla de reacción fue concentrada al vacio y el residuo 21d fue secado en una bomba Se lo utilizó para el acoplamiento sin mas purificación

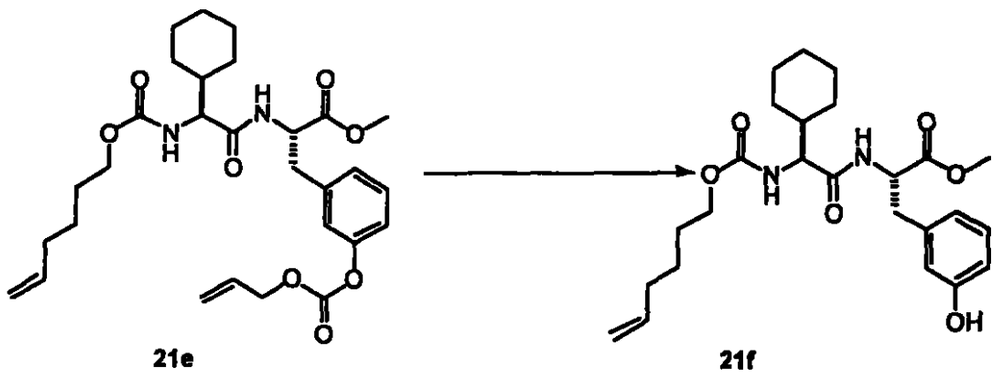
Paso D



Una solucion del clorhidrato de amina 21d (4,0 g, 8,9 mmol) en CH_2Cl_2 (50 ml) fue tratada con trietilamina (2,73 g, 27,0 mmol, 3 equiv-, 3,6 ml) y enfriada a -- 78°C Se agregó por goteo una solucion del cloroformiato 21b (2,3 g, 13,3 mmol, 1,5 equiv) en CH_2Cl_2 (30 ml) La mezcla de reacción fue agitada a temperatura ambiente durante la noche y diluida con HCl acuoso (1M, 150 ml) Se extrajo la capa acuosa con EtOAc (3x100 ml) La capa combinada de acetato de etilo fue extraida con H_2O (100 ml), salina (100 ml), secada (Na_2SO_4), filtrada, concentrada al vacio y el residuo fue purificado por cromatografía (SiO2, EtOAc/Hexanos 3 7) para dar 21e en forma de solido incoloro (5 g, 80 %)

0255 251

Paso E



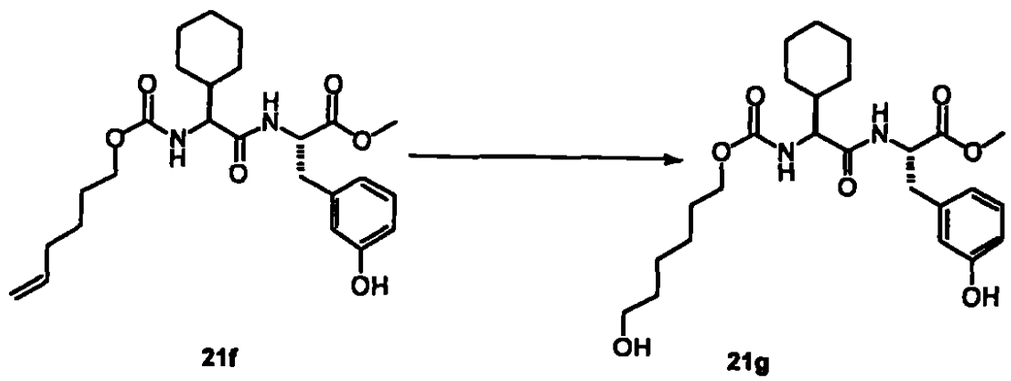
21e 21f

Una solucion del compuesto protegido con alloc 31e (4,0 g, 7,2 mmol) en THF seco (60,0 ml) fue tratada con dimediona (2,01 g, 14,4 mmol, 2,0 equiv), $Pd(PPh_3)_4$ (830 mg, 0,71 mmol, 10 % en moles) a 0°C y agitada a ta durante 1 h La mezcla de reaccion fue concentrada al vacio y el residuo purificado por cromatografía (SiO_2, EtOAc/hexanos, 3 7) para dar un alcohol desprotegido 21f en forma de sólido incoloro (2,7 g, 79 %) RMN ^1H ($CDCl_3$) δ ppm) 7,44 (bs, 1 H), 7,09 (s, 1 H, J=6,0 Hz), 6,75-6,72 (m, 2 H), 6,58-6,48 (m, 2 H), 5,81-5,71 (m, 1 H), 5,55 (d, 1 H, J=7,2 Hz, 4,98 (ddd, 1 H, J=1,5, 1,2 9 Hz), 4,92 (dd, 1H, J=4,5, 0,9 Hz), 4,88-4,83 (m, 1 H), 4,12-3,97 (m, 1 H), 3,71 (s, 3 H), 3,09-2,98 (m, 2 H), 2,08-2,03 (m, 2 H), 1,722-1,40 (m, 10 H), 1,24-0,94 (m, 5 H), RMN ^{13}C (100 MHz, δ) 171,6, 157,3, 156,6, 138,3, 136,6, 129,8, 132,5, 120,6, 117,0, 114,9, 114,6, 65,7, 60,1, 53,2, 52,5, 40,4, 37,1, 33,3, 29,6, 28,6, 28,3, 26,0, 25,9, 25,1, CHN calc para $C_{25}H_{36}N_2O_6$ C= 65,20 %, H= 7,88 %, N=6,08 %, Encontrado C= 64,90 %, H= 7,98 %, N=6,01 %,

252

0256

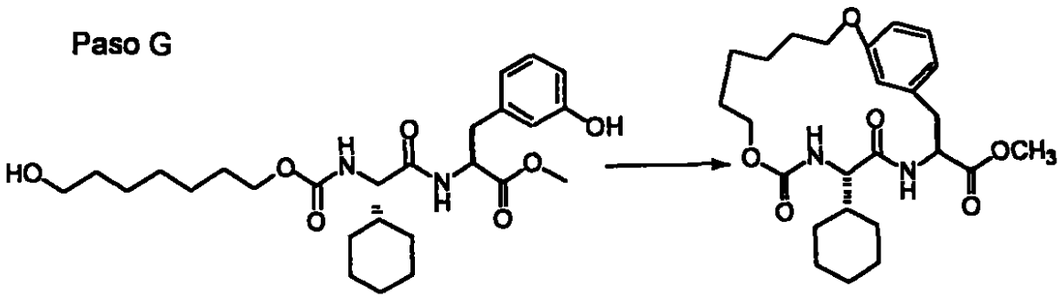
Paso F



21f 21g

Una solucion del alqueno 21f (650 mg, 1,4 mmol) en THF anhidro (5,2 ml) fue enfriada a 0°C y tratada con BH_3•THF (sol 1M en THF, 4,2 ml, 4,2 mmol, 3,0 equiv) La mezcla de reacción fue agitada a ta por espacio de 2 h y se agregó EtOH (2,0 ml) con cuidado por la evolución de gas hidrogeno Una vez completada la evolución de H_2, la mezcla de reacción fue tratada con buffer pH 7 y tratada con H_2O_2 (30 %, 5,0 ml) a 0°C Se retiro el baño helado y la mezcla fue agitada a ta durante 3-4 h La mezcla de reacción fue extraída con EtOAc (3x100 ml) Las capas orgánicas combinadas fueron extraidas con H_2O, salina, secadas ($MgSO_4$), filtradas, concentradas al vacio y el residuo fue purificado por cromatografía (SiO_2, EtOAc/hexanos, 3 7) para dar el producto hidroborado en forma de sólido incoloro 21g (400 mg, 60 %) $[\alpha]_D$ 86,4 (c 0,3 $CHCl_3$, 25°C), RMN ^1H ($CDCl_3$, 400 MHz, δ) 7,26 (s, 1 H), 7,08 (t, 1 H, J=5,7 Hz), 6,83 (d, 1 H, J=6,0 Hz), 6,71 (dd, 1 H, J=1,2, 4,5 Hz), 6,57 (bs, 1 H), 6,54 (d, 1 H, J=5,7 Hz), 5,68 (d, 1 H, J=6,9 Hz), 4,85 (dq, 1 H, J=4,2,1,8 hz), 4,05-3,97 (m, 3 H), 3,69 (s, 3 H), 3,60 (t, 2 H, J=4,8 Hz), 3,08-2,97 (m, 2 H), 1,77-1,53 (m, 10H), 1,42 1,25 (m, 4 H), 1,24-0,92 (m, 5 H), RMN ^{13}C ($CDCl_3$, 100 MHz, δ) 171,8, 181,8, 157,6, 156 9 136,9, 130,0, 120,8, 117,0, 114,8, 65,7, 62,7, 60,3, 53,3, 52,7, 40,5, 37,4, 32,5, 29,7, 29,0, 28,8, 26,2, 26,0, 25,6, 25,4, EM (FAB, NBA/DMSO, intensidad relativa m/z) 479 ($[M+1]^+$, 100), 296 (40), 196 (25), 156 (25), 136 (25), 112 (20) HRMS calc. para $C_{25}H_{39}N_2O_7$ $(M+1)^+$ 479,2760, encontrado 479,2757

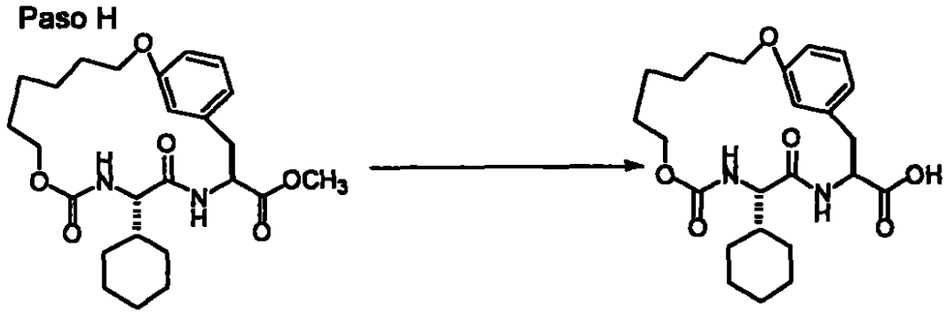
Paso G



21g 21h

Se trato una solucion de PPh_3 (385 mg, 1,47 mmol, 1,75 equiv)) en CH_2Cl_2 (10 ml) con el compuesto 21g (400 mg, 0,85 mmol) y se la enfnó a 0°C Se agregó por goteo una solucion de DEAD (220 mg, 1,26 mmol, 1,5 equiv) en CH_2Cl_2 (10 ml) y se la agitó a ta por espacio de 3 h La mezcla de reaccion fue concentrada al vacio y el residuo purificado por cromatografía (SiO_2, EtOAc/hexanos, 1 9) para dar el producto ciclico 21h en forma de sólido incoloro (110 mg, 25 %)

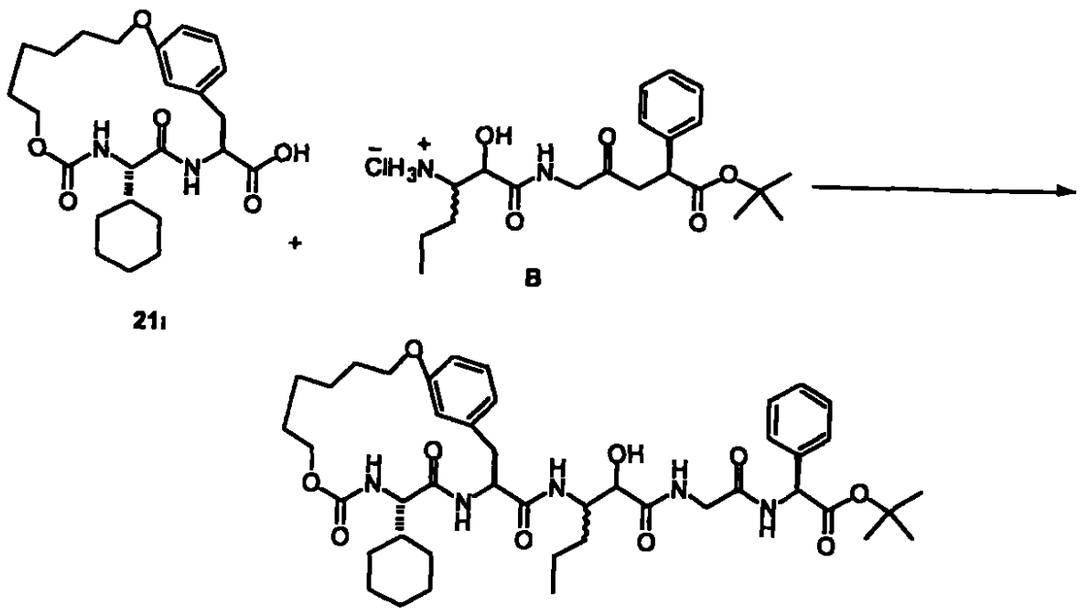
Paso H



21h 21i

Una solucion del carbamato ciclico 21h (200 mg, 0,44 mmol) en dioxano (30 ml), CH_3OH (20 ml) y CH_2Cl_2 (20 ml) fue tratada con $LiOH•H_2O$ (80 mg, 2,0 mmol, 4,5 equiv) y agitada a ta por espacio de 4 h La reacción fue concentrada al vacio y diluida con HCl (sol 4M en dioxano, 10 ml) El agua fue eliminada por liofilizacion para dar el ácido cristalino 21i que fue utilizado directamente para el acoplamiento

Paso I



21i

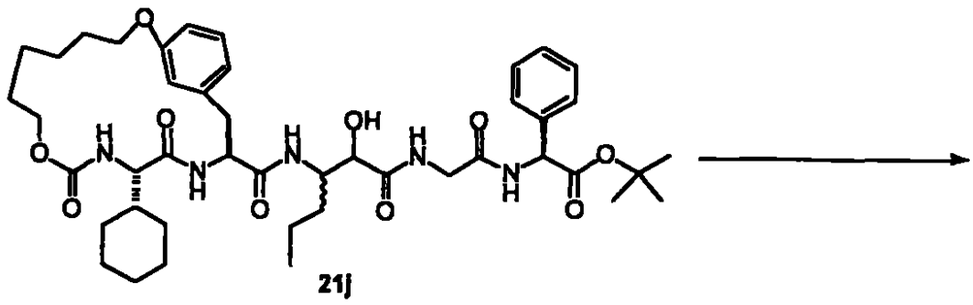
B

21j

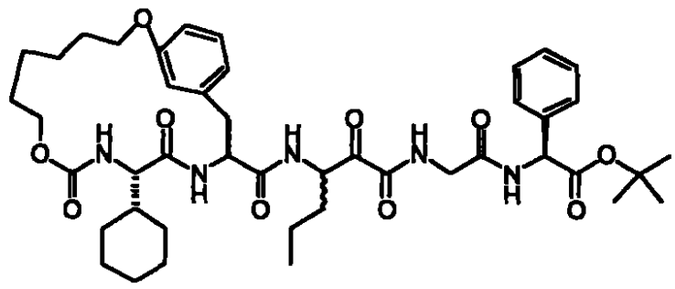
Una solución del ácido hidrolizado 21i (210 mg, 0,47 mmol) en DMF seca (5,0 ml) y CH_2Cl_2 (5,0 ml) fue tratada con HOOBt (125 mg, 0,70 mmol, 1,5 equiv) y enfriada a 0°C y se agregó base de Hünig (258 mg, 2,0 mmol, 4,0 equiv, 369 µl) A esta mezcla se agregó EDCI (134 mg, 0,70 mmol, 1,5 equiv) y la mezcla de reacción fue agitada a 0°C durante 0,5 h y tratada con el clorhidrato de amina B (253 mg, 0,58 mmol, 1,25 equiv) La mezcla de reaccion fue guardada en un freezer por espacio de 24 h y concentrada al vacio para eliminar la DMF y el CH_2Cl_2 El residuo fue diluido con HCl ac (2M, 30 ml) y extraido con CH_2Cl_2 (3x50 ml) La capa orgánica combinada fue extraida con HCl ac (2M, 30 ml), NaOH ac (1M), salina (2x50 ml), secada ($MgSO_4$) y concentrada al vacio El residuo 21j (220 mg) fue oxidado sin mas purificación

255
0259

Paso J



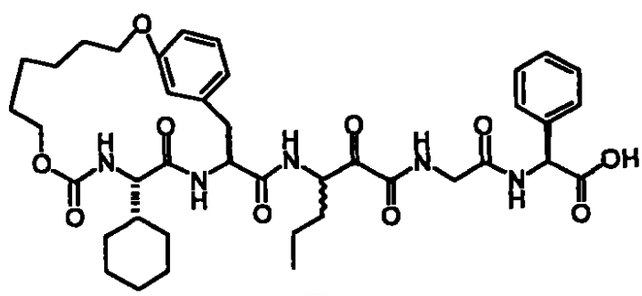
21j



21

Una solución del alcohol 21j (220 mg, 0,26 mmol) en CH_2Cl_2 (5,0 ml) fue tratada con reactivo de Dess-Martin (200 mg, 0,47 mmol, 1,8 equiv) La mezcla de reaccion fue agitada a ta durante 1 h y diluida con $NaHCO_3$ acuoso (15 ml) y $Na_2S_2O_3$ ac (15 ml) La mezcla de reaccion fue agitada a ta durante 20 min y se extrajo la mezcla de reacción con CH_2Cl_2 (3x30 ml) Las capas organicas combinadas fueron extraidas con Na_2CO_3, secadas (Na_2SO_4), filtradas, concentradas al vacio y el residuo fue purificado por cromatografía (SiO_2, CH_3OH (NH_3 2M)/CH_2Cl_2 1 20) para dar la cetoamida 21 (60 mg, 27 %) en forma de sólido incoloro

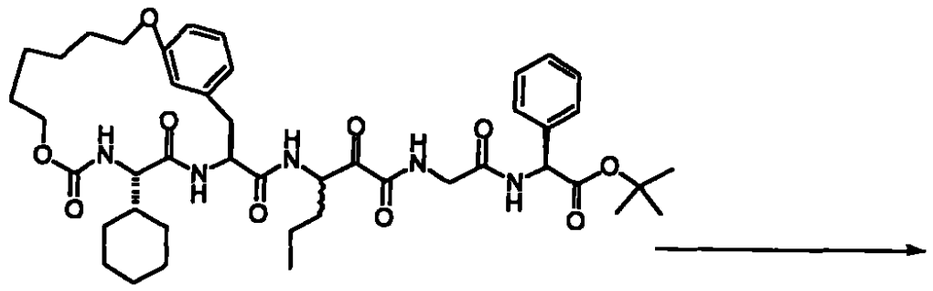
Ejemplo 22 Preparacion del Compuesto de la Fórmula 22



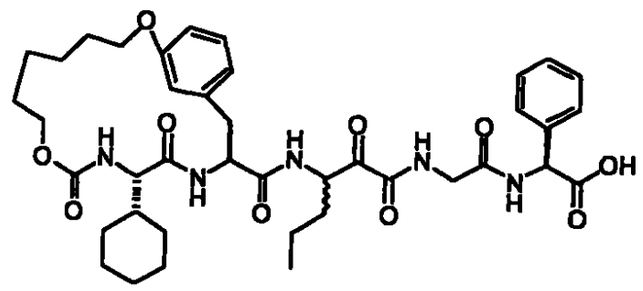
22

256

Paso A



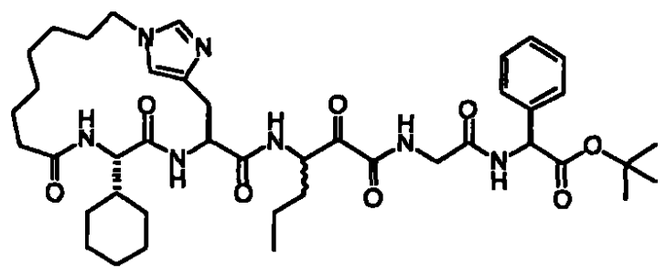
21



22

Una solucion del ester terc-butílico 21 (50 mg, 0,059 mmol) en CH_2Cl_2 seco (2,0 ml) fue tratada con ATF (2,0 ml) y agitada a ta durante 4 h La mezcla de reaccion fue concentrada al vacio y el residuo disuelto reiteradamente en heptanos/CH_2Cl_2 y concentrado al vacio varias veces para dar un fino solido tostado 22 (47 mg), que fue secado al vacio

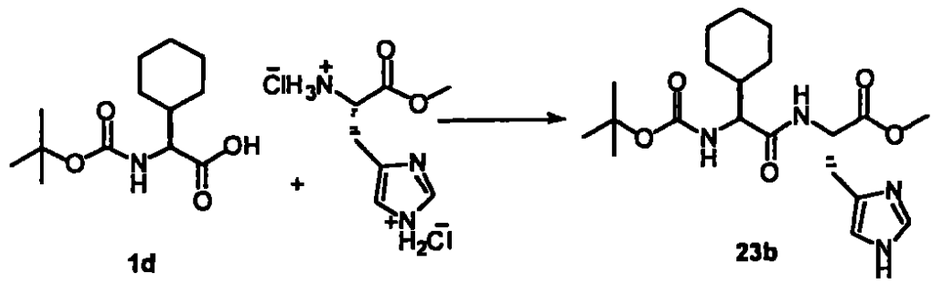
Ejemplo 23 Preparación del Compuesto de la Formula 23



23

0261 257

Paso A



1d 23a 23b

Una solución del acido 1d (255 mg, 1,0 mmol) en DMF (2,0 ml) fue tratada con HOBt (202 mg, 1,5 equiv) y base de Hunig (517 mg, 4,0 mmol, 4,0 equiv, 738 µl) La mezcla de reaccion fue enfriada a 0°C y tratada con DCC (258 mg, 1,25 mmol, 1,25 equiv) Después de agitar la mezcla durante 1 h, se agregó histidina-OCH_3•2HCl 23a (242,0 mg, 1,0 mmol) y se agitó a temperatura ambiente durante la noche La mezcla de reacción fue concentrada al vacio y extraida en EtOAc (3x50 ml) y $NaHCO_3$ ac (50 ml) La capa orgánica concentrada al vacio y el residuo purificado por cromatografia (SiO_2, CH_3OH/CH_2Cl_2 1 19) para dar el dipéptido 23b en forma de solido incoloro (380 mg, 93 %) RMN ^1H (d_6-DMSO 400 MHz, δ, ppm) 8,17 (d, 1 H, J=7,2 Hz), 7,48 (s, 1 H), 6,77 (s, 1 H), 6,57 (bs, 1 H), 5,54 (d, 1H, J=7,6 Hz), 4,47 (q, 1 H, J=7,2 Hz), 3,79 (t, 1 H, J=8,4 hz), 3,55 (s, 3 H), 3,36-3,20 (m, 2 H), 2,94-2,82 (m, 2 H), 1,70-1,47 (bm, 6H), 1,35 (s, 9 H), 1,46-0,85 (m, 5 H), RMN ^{13}C (d_6-DMSO, 100 MHz, δ ppm) 172,5, 171,9, 157,3, 155,9, 135,4, 78,6, 59,5, 52,9, 52,3, 34,1, 29,6, 28,9, 28,6, 28,6, 26,5, 26,3, 26,0, 25,2 EM FAB (NBA-G/TG-DMSO, intensidad relativa m/z) 409 [$(M+q)^+$, 100], 353 810, 170 (20), HRMS calc para $C_{20}H_{33}N_4O_8$ 409,2451, encontrado 409,2466, CHN calc para $C_{20}H_{32}N_4O_5$ C=58,81 %, H=7,90 %, N=13,72 %, Encontrado C=58,70 %, H=7,78 % N=13,43 %

0282 258

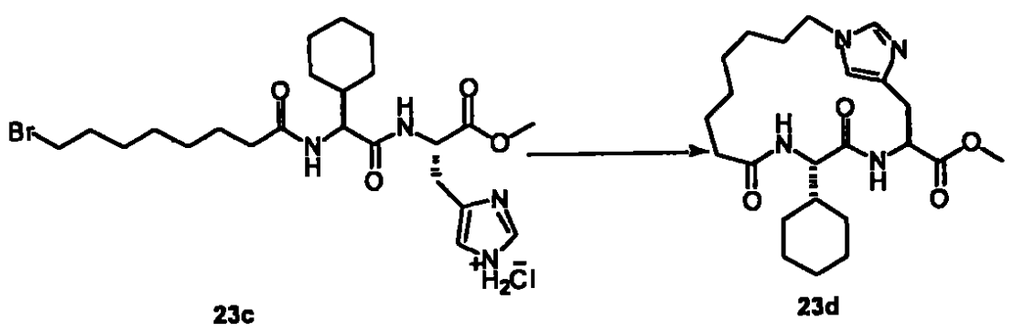
Paso B



23b 23c

Una solucion de ácido ω-bromoheptenoico (223 mg, 1,0 mmol) en DMF (3,0 ml) fue tratada con clorhidrato de amina desprotegido 23b (380 mg, 1,0 mmol, 1,0 equiv) y se agregó base de Hünig (387 mg, 3,0 mmol, 3,0 equiv) La mezcla de reaccion fue tratada con PyBroP (465 mg, 1,0 mmol) y agitada a ta durante 3 h La mezcla de reacción fue concentrada al vacio y el residuo fue purificado por cromatografía (SiO_2, CH_3OH/CH_2Cl_2, 1/19) para dar un sólido incoloro (220 mg, 50 %) EM (FAB) 415,2 [$(M+1)^+$, 100], 513,2 [$(M+1)^+$, 95)], 469 (60), 433 (20), 170 (40) HRMS calc. para $C_{23}H_{38}BrN_4O_4$ 513,2076, encontrado 513,2073

Paso C



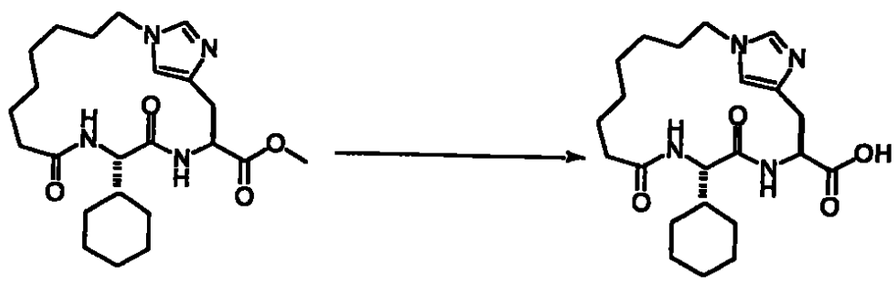
23c 23d

Una solución del compuesto bromo 23c (100 mg, 0,23 mmol) en 2-butanona (4,0 ml) fue tratada con Na_2CO_3 (31,0 mg, 0,29 mmol, 1,25 equiv) y con LiI (50 mg, 0,37 mmol 1,3 equiv) y calentada a reflujo por espacio de 24 h La mezcla de reaccion fue concentrada al vacio y el residuo diluido con agua El residuo fue extraido con CH_2Cl_2 (3x30 ml) La capa orgánica combinada fue

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secada (Na_2SO_4) y purificada por cromatografía (SiO_2, CH_3OH CH_2Cl_2 1 19) para dar el compuesto cíclado 23d (25 mg, 31 %), R_f 0,68 (NH_3 2M en CH_3OH CH_2Cl_2 1 19) RMN ^1H ($CDCl_3$, 400 MHz, δ, ppm) 8,17 (d, 1 H, J)8,8 Hz), 7,33 (s 1 H), 6,48 (d, 1 H, J=8,4 Hz) 4,90-4,85 (m, 1 H) 4 26 (t, 1 H, J=8,0 Hz), 3,82-3,74 (m, 2 H), 3,69 (s, 3 H), 3,16-3,11 (m, 2 H), 2,91-2,84 (m, 1 H), 2,30-2,01 (m, 2 H), 1,65-1,59 (m, 11 H), 1,18-0,96 (m, 11H), RMN ^{13}C ($CDCl_3$ 100 MHz, δ ppm) 172,8, 172,4, 171,9, 138,2, 136,8, 57,6, 52,5, 51,7, 46,6, 36,0, 30,9 29,5, 28,8, 27,3, 26,7, 26,4, 26,3, 26,2, 25,2, 14,8 EM (Electroatomización, intensidad relativa m/z) 433 [(M+1)$^+$, 100] HRMS calc para $C_{23}H_{37}N_4O_4$ 433,2815, encontrado 433,2822

Paso D

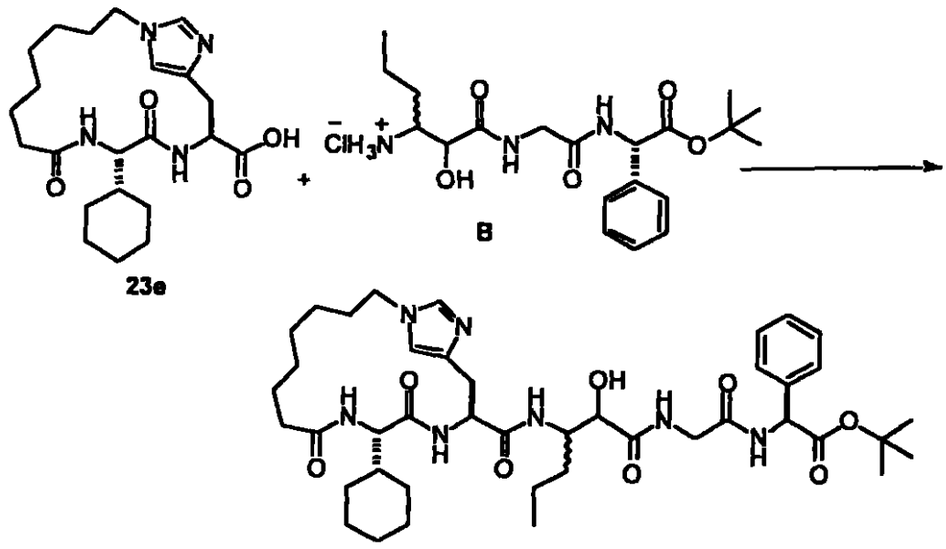


23d 23e

Una solución del ester metílico 23d (200 mg, 0,46 mmol) en CH_3OH (5,0 ml), H_2O (0,5 ml) fue tratada con $LiOH•H_2O$ (30 mg, 0,75 mmol, 1,6 equiv) La mezcla de reacción fue agitada a ta durante 15 h y concentrada al vacío y secada en bomba para dar el compuesto hidrolizado 23e que fue utilizado directamente para el acoplamiento

260

Paso E



23e

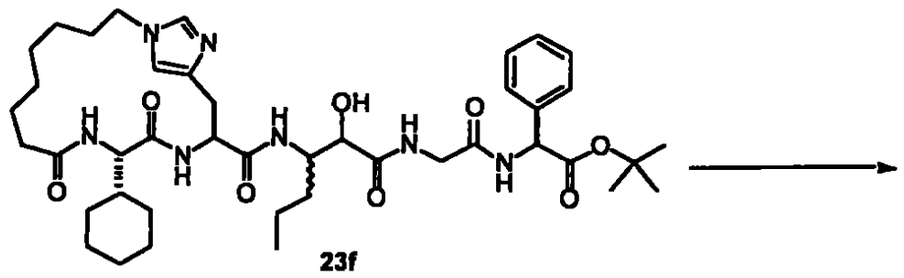
B

23f

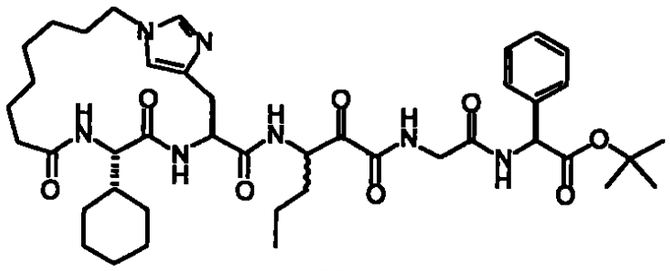
Una solución del ácido 23e en CH_2Cl_2 (3,0 ml), DMF (5,0 ml) fue tratada con HOOBt (115 mg, 0,70 mmol, 1,50 equiv) y EDCI (113 mg, 0,60 mmol, 1,25 equiv) La mezcla de reacción fue tratada con Et_3N (190 mg, 1,88 mmol, 271 µl, 4,0 equiv) y el clorhidrato de amina B (201 mg, 0,5 mmol, 1,1 equiv) La mezcla de reacción fu agitada a ta por un lapso de 13 h y diluida con H_2O La capa acuosa fue extraida con CH_2Cl_2 (3x50 ml) y las capas orgánicas combinadas fueron extraidas con NaOH ac (1M, 50 ml) y secadas (Na_2SO_4) La capa orgánica seca fue filtrada y concentrada al vacío para dar un residuo incoloro 23f (442 mg) que fue secado al vacio y utilizado directamente para la posterior oxidacion EM (Electroatomización, intensidad relativa m/z) 794 [$(M+1)^+$, 100]

761

Paso F



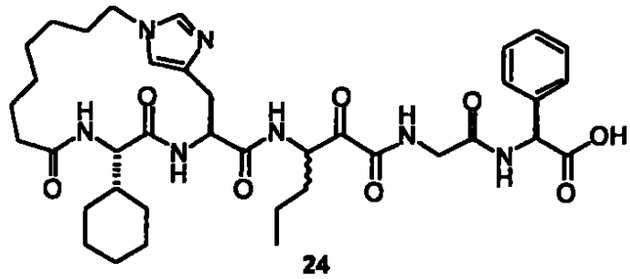
23f



23

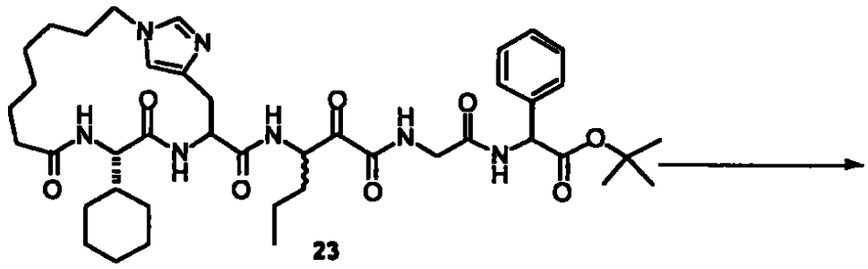
Una solución de la hidroxiamida 23f (50 mg, 0,064 mmol) en CH_2Cl_2 (3,0 ml) fue tratada con reactivo de Dess-Martin (53 mg, 0,13 mmol, 2,0 equiv) y agitada a ta durante 3 h La mezcla de reaccion fue diluida con $Na_2S_2O_3$ ac sat (20 ml) y agitada por espacio de 15 min La capa acuosa fue extraida con CH_2Cl_2 (3x30 ml) La capa orgánica fue secada (Na_2SO_4,), filtrada, concentrada al vacio y purificada por cromatografia (SiO_2, CH_3OH/CH_2Cl_2 1 15) para dar la cetoamida 23 (20 mg, 40 %) EM (FAB, NBA-G/TG-DMSO, int rel m/z) 824 [(M+ $CH_3OH)^+$, 100], 792 [$(M+1)^+$, 60], 447 (20), HRMS calc. para $C_{42}H_{62}N_7O_8$ $(M+1)^+$ 792,4660 , encontrado 792,4659

Ejemplo 24 Preparación del Compuesto de la Fórmula 24

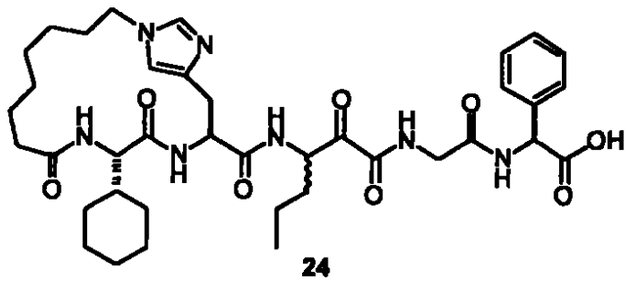


24

Paso A



23

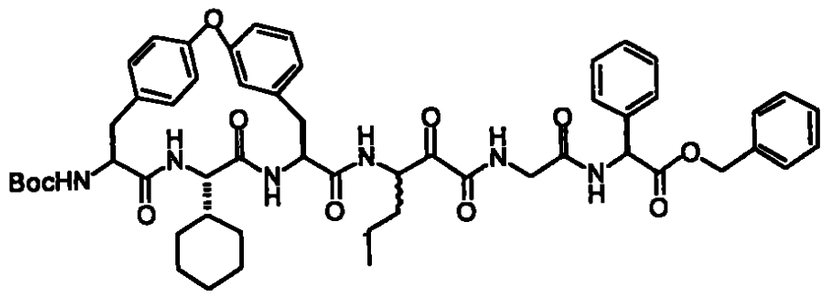


24

Una solución del ester terc-butílico 23 (17 mg, 21,5 µmol) en CH_2Cl_2 seco (2,0 ml) fue tratada con ATF (2,0 ml) y agitada a ta durante 8 h La desaparición del éster hasta la linea de base fue seguida por TLC (CH_3OH&CH_2Cl_2, 1 19) La mezcla de reaccion fue concentrada al vacio y el residuo fue repetidamente disuelto en CH_3OH/heptanos/CH_2Cl_2 y concentrado al vacio varias veces para dar 24 en forma de fino sólido incoloro (7 mg) EM (Electroatomizacion, intensidad relativa m/z) 768 [$(M+CH_3OH)^+$, 100], 736 [$(M+1)^+$, 60], 46 (10)

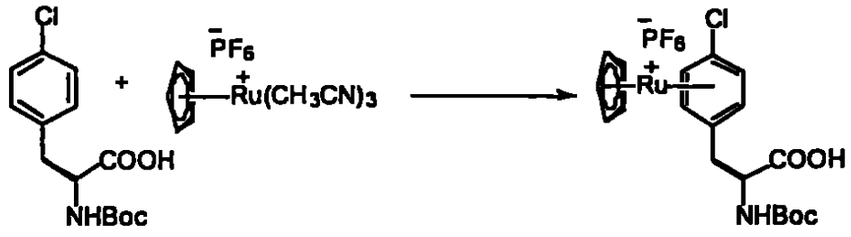
263

Ejemplo 25 Preparacion del Compuesto de la Fórmula 25



25

Paso A

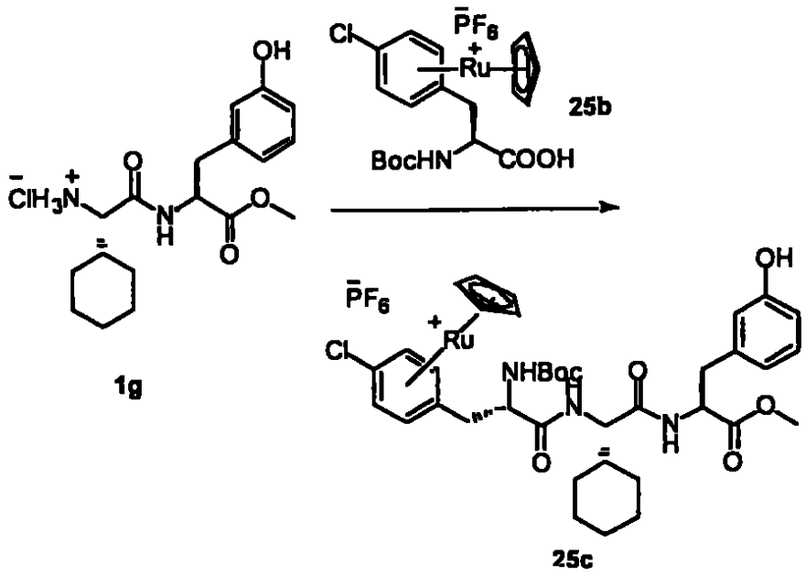


25a 1b 25b

Una solución de Boc-4-clorofenilalanina 25a (523 mg, 175 mmol) en dicloroetano (37 ml) fue tratada con $CpRu(CH_3CN)_3PF_6$ 1b (760 mg, 175 mmol, 1,0 equiv) y calentada a reflujo durante 2 h La mezcla de reacción fue enfriada a 0°C y filtrada El filtrado fue concentrado al vacio y disuelto en mínimo CH_3CN y tratado con un gran exceso de Et_2O El sólido que se separo fue desprendido y disuelto en CH_2Cl_2/CH_3OH (1 1, 50 ml) y concentrado al vacio para obtener 25b en forma de espuma marrón (640 mg, 69 %)

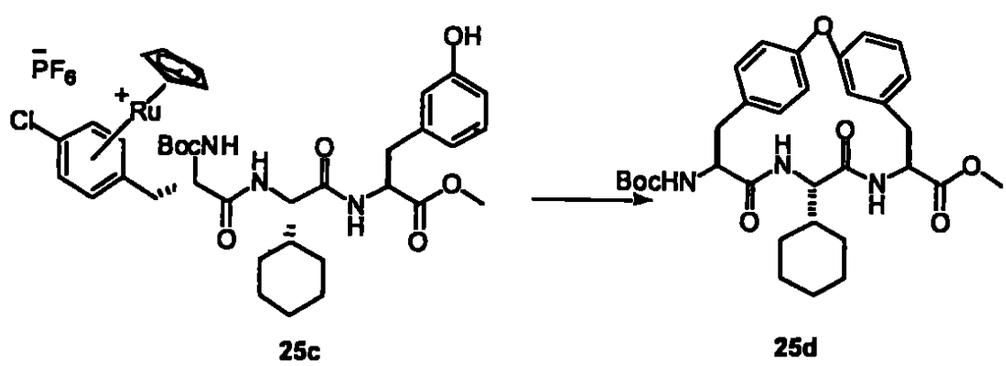
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Paso B



Una solución del ácido carboxílico 25b (2,4 g, 3,80 mmol) en DMF seca (15 ml) fue tratada con base de Hünig (1,64 g, 12,64 mmol, 4,0 equiv , 2,9 ml) y HOBt (661 mg, 4,38 mmol, 1,5 equiv) La mezcla de reacción fue enfriada a 0°C y luego tratada con EDCI (699 mg, 3,95 mmol, 1,25 equiv) y agitada durante 15 min A esta mezcla de reacción se agregó el clorohidrato de amina 1g (1,50 g, 4,00 mmol, 1,2 equiv) y la mezcla de reacción fue agitada a ta por espacio de 12 h La DMF fue separada por destilacion y el residuo diluido con agua (30 ml) y la capa acuosa fue extraida con CH_2Cl_2 (3x50 mL) Se extrajeron las capas orgánicas combinadas con $NaHCO_3$ ac. (30 ml) HCl ac (30 ml), salina, secadas (Na_2SO_4), filtradas, concentradas al vacio y el producto crudo 25c (2,5 g, 69 %)) fue utilizado para la ciclación posterior sin purificación

Paso C



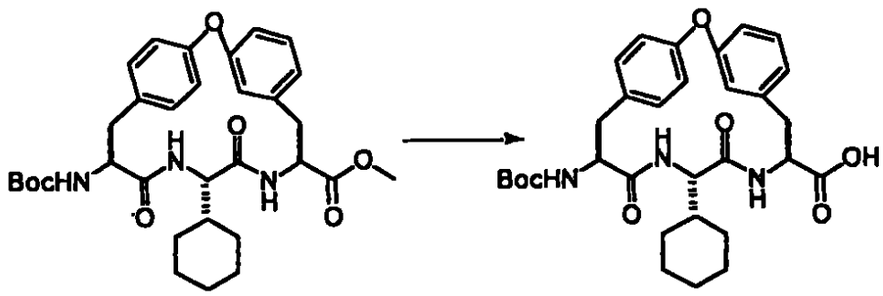
270

265

Una solución del compuesto 25c (100 mg, 0,11 mmol) en DMF seca (10 ml) fue desgasificada con N_2 seco y tratada con Cs_2CO_3 (170 mg, 0,5 mmol, 5,0 equiv) y se la agito a temperatura ambiente por espacio de 12 h La DMF solvente fue separada por destilación y el residuo fue diluido con agua (35 ml) y extraido con CH_2Cl_2 (3x100 ml) Las capas organicas combinadas fueron secadas (Na_2SO_4), filtradas, concentradas al vacio y secadas al vacio durante la noche Se las utilizo para la remoción fotolitica del Ru sin mas purificación

Se disolvió el compuesto ciclado del paso anterior en CH_3CN y se lo fotolizó en un Raynot (λ=350 nm) por espacio de 48 h La mezcla de reacción fue concentrada al vacio y el residuo purificado por cromatografía (SiO_2, EtOAc/Hexanos 2 1) para dar 25d en forma de solido de color tostado (29 mg, 46 %) EM (FAB, NBA-G/TG-DMSO, intensidad relativa m/z) 580 [$(M+1)^+$, 80] 524 (100), 418 (40), 462 (30), 452 (20), 313 (60), 253 (20)

Paso D

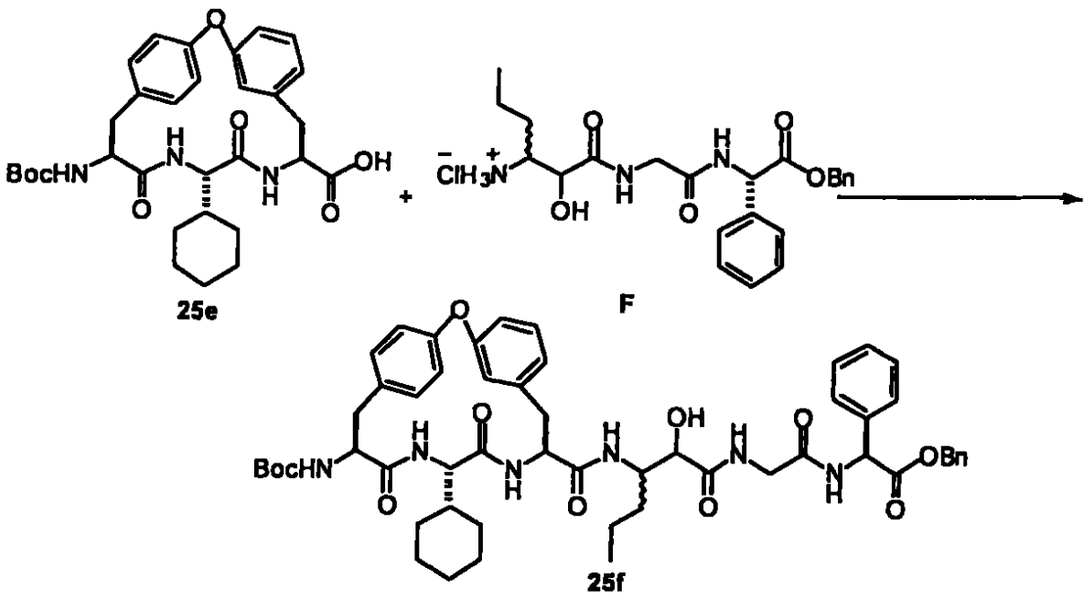


25d **25e**

Una solución del éster 25d (150 mg, 0,26 mmol) en THF (3 ml), CH_3OH (3,0 ml) y H_2O (3,0 ml) fue tratada con $LiOH \cdot H_2O$ (18 mg, 0,43 mmol, 1m65 equiv) y agitada a ta por espacio de 35 min La mezcla de reacción fue acidificada con HCl conc. (13 M, 1 ml) y extraida con CH_2Cl_2 (3x50 ml) Las capas organicas combinadas fueron secadas (Na_2SO_4), filtradas y concentradas al vacio para dar el ácido 25e que fue utilizado directamente para el acoplamiento sin más purificacion

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266

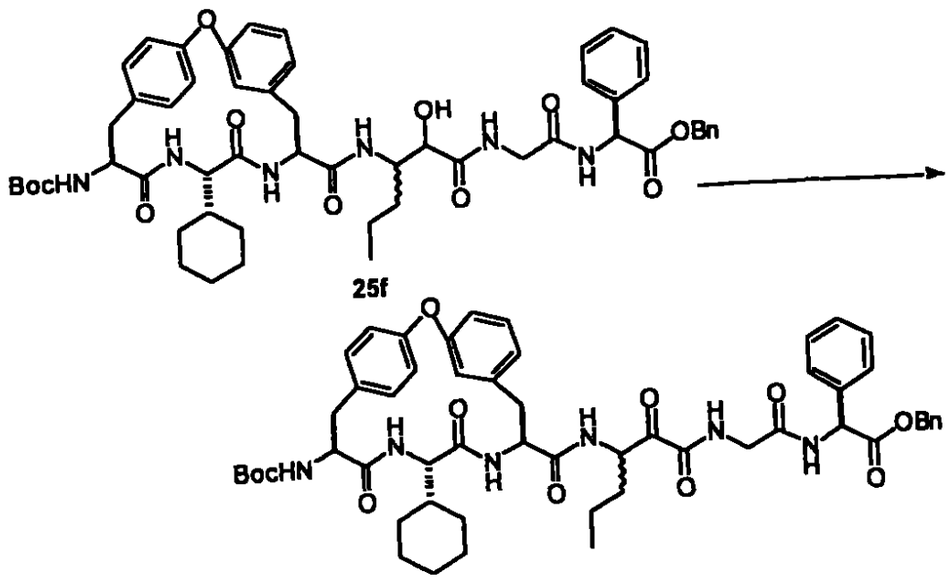
Paso E



Una solución del ácido 25e (150 mg, 0,27 mmol) en CH_2Cl_2 (2,0 ml) fue tratada con HOBt (62 mg, 0,40 mmol) y base de Hünig (139 mg, 1,1 mmol, 4,0 equiv) La mezcla de reaccion fue enfriada a 0°C y tratada con EDCI (53 mg, 0,34 mmol, 1,25 equiv) y agitada durante 30 min La mezcla de reacción fue tratada con la amina F (88 mg, 0,29 mmol, 1,22 equiv) y guardada en un freezer durante 12 h La mezcla de reacción fue concentrada al vacio y diluida cona gel de silice (50 ml) Se extrajo la capa acuosa con CH_2Cl_2 (3x50 ml) Las capas orgánicas combinadas fueron extraidas con HCl ac (1M, 3x20 ml), NaOH (1M, 3x20 ml), secadas (Na_2SO_4), filtradas, concentradas al vacio para obtener un sólido incoloro 25f (138 mg) que fue utilizado para la oxidación

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Paso F

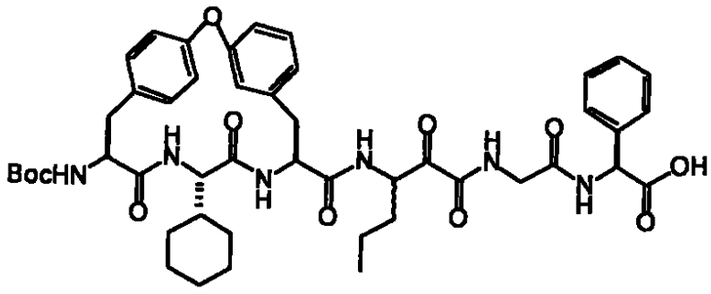


25

Una solucion del alcohol 25f (140 mg, 0,143 mmol) en CH_2Cl_2 THF (1 1, 5,0 ml) fue tratada con reactivo Dess-Martin (121 mg, 0,42 mmol, 3,0 equiv) La mezcla de reacción fue agitada a ta por espacio de 2 h y la mezcla fue concentrada al vacio El residuo fue purificado por cromatografía (SiO_2, CH_3OH/CH_2Cl_2 1 32) para dar el producto oxidado 25 (57 mg, 41 % en forma de sólido incoloro

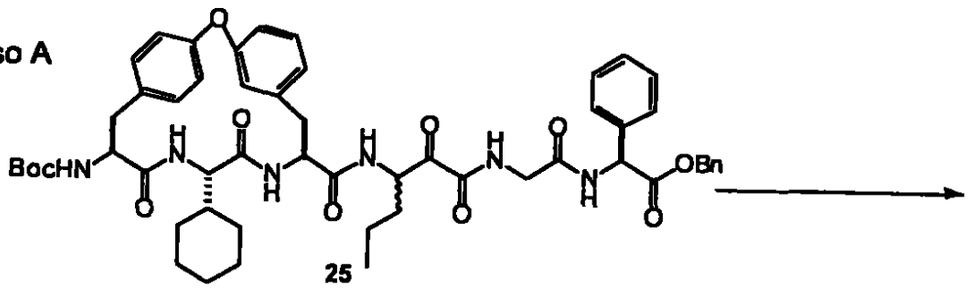
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Ejemplo 26 Preparación del Compuesto de la Fórmula 26

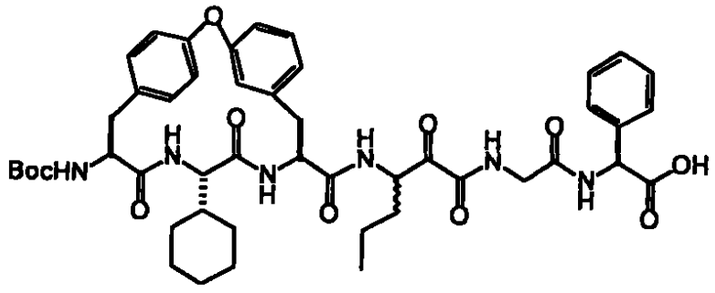


26

Paso A



25

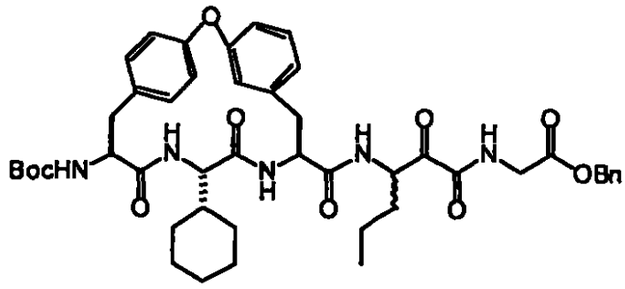


26

Una solución del ester bencílico 25 (30 mg, 38,0 µmol) en CH_3OH/THF (1 1, 4,0 ml) fue tratada con Pd/C (20 mg, 10 %) y se insufló H_2 a traves de la misma Se agregó una gota de acido acetico para acelerar la reducción La mezcla de reacción fue filtrada a través de un cilindro de celite y el filtrado fue concentrado al vacio El residuo 26 fue analizado sin más purificación

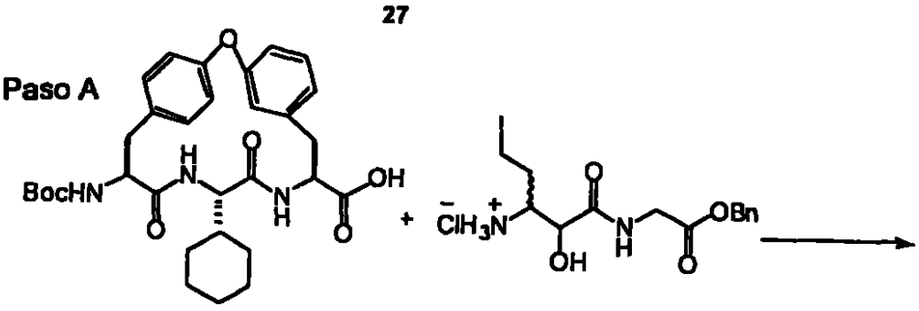
269

Ejemplo 27 Preparación del Compuesto de la Fórmula 27

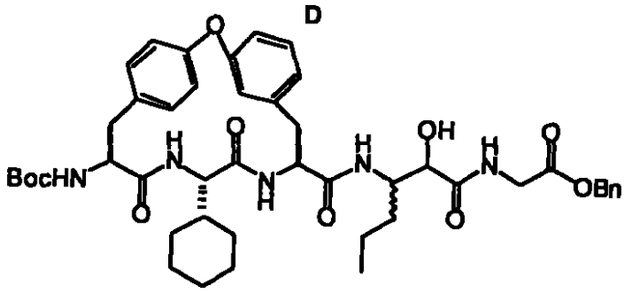


27

Paso A



25e D



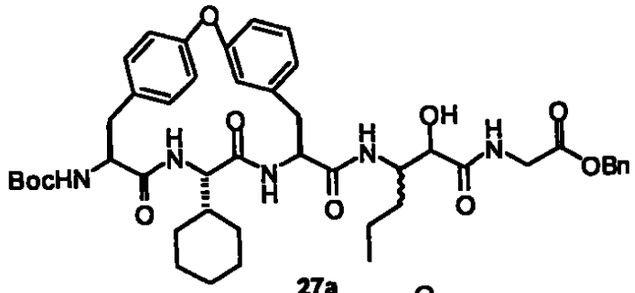
27a

Una solución del ácido 25e (100 mg, 0,17 mmol) en CH_2Cl_2 seco fue tratada con HOOBt (41 mg, 0,26 mmol) y base de Hünig (91 mg, 0,70 mmol, 4,0 equiv) La mezcla de reacción fue enfriada a 0°C y tratada con EDCI (35 mg, 0k,22 mmol, 1,25 equiv) y agitada por espacio de 30 min La mezcla de reacción fue tratada con la amina D (71 mg, 0,22 mmol, 1,22 equiv) y guardada en un freezer durante 12 h La mezcla de reacción fue concentrada al vacío y diluida con H_2O (30 ml) Las capas acuosas fueron extraidas con CH_2Cl_2 (3x30 ml) Las capas orgánicas fueron extraidas con HCl ac (1M, 30 ml), Na_2CO_3 ac (1M, 30 ml), secadas (Na_2SO_4)m filtradas, concentradas al vacio para obtener 27a en forma de solido incoloro (119 mg) que fue utilizado para la oxidacion EM (FAB), 842

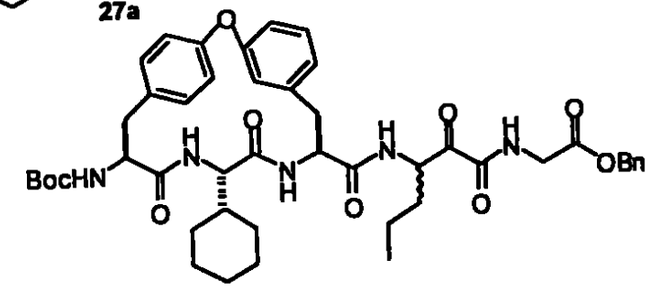
270

[(M+1)$^+$, 100] 765 (20), 735 (10), 657 (20), 575 (10), 492 (10), 464 (20), 446 (30) HRMS calc para $C_{46}H_{60}N_5O_{10}$ (M+1)$^+$ 862,4339 , encontrado 842,4336

Paso B



27a

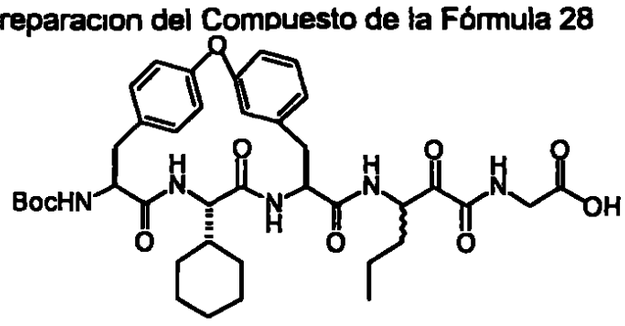


27

Una solución del alcohol 27a (120 mg, 0,143 mmol) en CH_2Cl_2 THF (1 1, 3,0 ml) fue tratada con reactivo Dess-Martin (180 mg, 0,42 mmol, 3,0 equiv) La mezcla de reaccion fue agitada a ta por espacio de 2 h y la mezcla fue concentrada al vacio El residuo fue purificado por cromatografia (SiO_2, CH_3OH/CH_2Cl_2 1 32) para dar el producto oxidado 27 en forma de solido incoloro EM (FAB, NBA-G/TG-DMSO, intensidad relativa m/z) 840 [(M+1)$^+$, 50] HRMS calc. para $C_{46}H_{58}N_5O_{10}$ (M+1)$^+$ 840,4184 , encontrado 840,4199

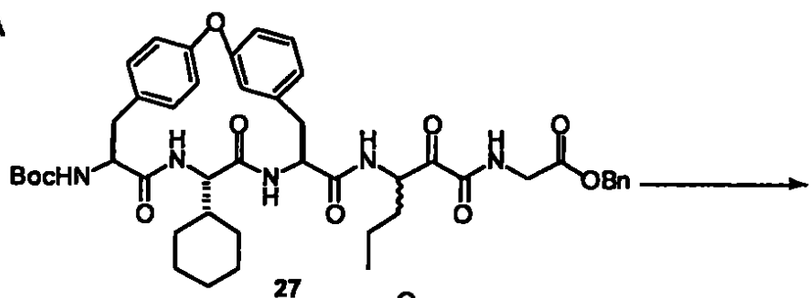
271

Ejemplo 28 Preparacion del Compuesto de la Fórmula 28

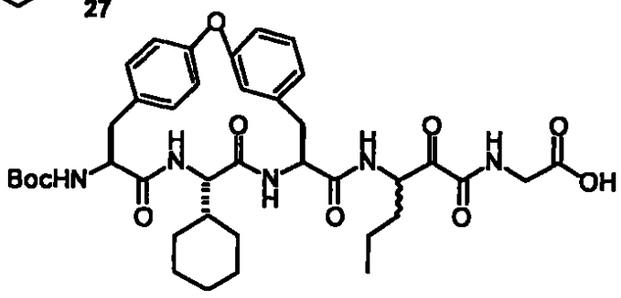


28

Paso A



27

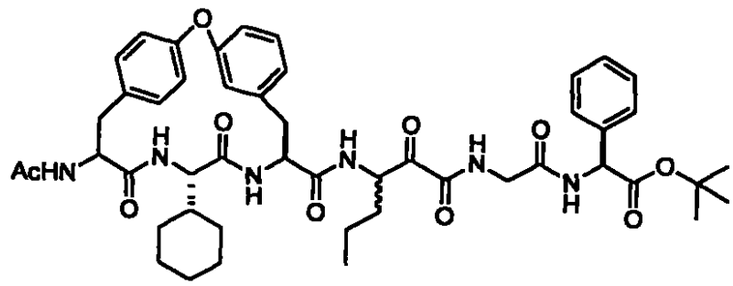


28

Una solución del ester bencílico 27 (40 mg, 47,0 µmol) en CH_3OH/THF (1 1, 6,0 ml) fue tratada con Pd/C (30 mg, 10 %) y se insufló H_2 a traves de la misma Se agregó una gota de acido acético para acelerar la reduccion La mezcla de reacción fue filtrada a traves de un cilindro de celite y el filtrado fue concentrado al vacio para dar 28

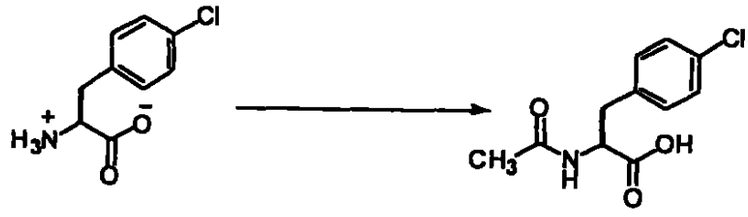
272

Ejemplo 29 Preparación del Compuesto de la Formula 29



Paso A.

29

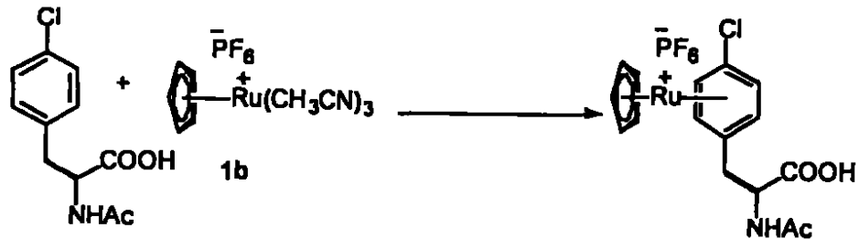


29a 29b

Una solución de 4-clorofenilalanina 29a (1,5 g, 7,5 mmol) en THF (20 ml) y H_2O (20 ml) fue tratada con NaOH (900 mg, 22,5 mmol, 3,0 equiv) y enfriada a 0°C Se agregó por goteo una solución de cloruro de acetilo (707 mg, 9,00 mmol, 1,25 mmol) en THF (10 ml) y la mezcla de reacción fue agitada durante la noche a ta La mezcla de reacción fue acidificada con HCl ac (1M, 10 ml) y extraida con CH_2Cl_2 (3x30 ml) Las capas organicas combinadas fueron secadas (Na_2SO_4), filtradas, concentradas al vacio para dar 29b, que fue utilizado en el paso siguientes sin purificacion

0277
273

Paso B

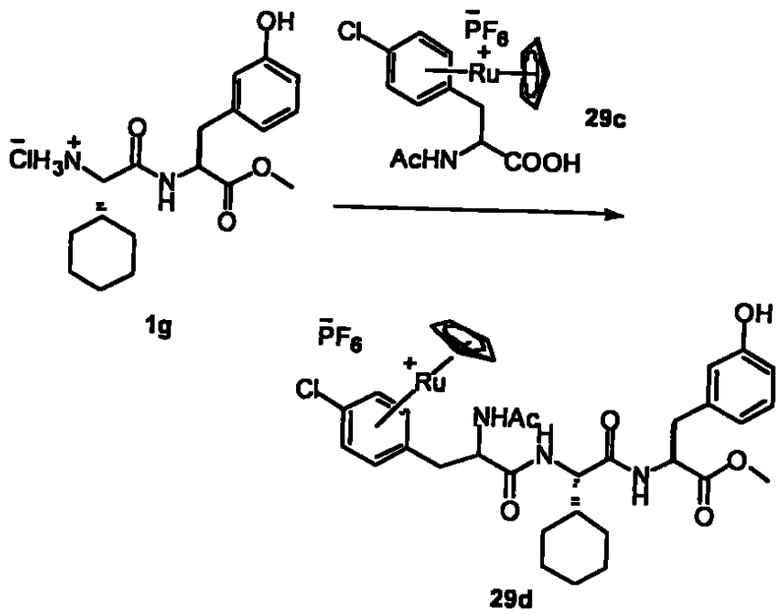


29b 29c

Una solución de N-acetil-4-clorofenilalanina 29b (1,39 g, 5,75 mmol) en dicloroetano (118 ml) fue tratada con $CpRu(CH_3CN)_3PF_6$ 1b (2,5 g, 5,8 mmol, 1,0 equiv) y calentada a reflujo durante 2 h La mezcla de reacción fue enfriada a 0°C y filtrada El filtrado fue concentrado al vacio y disuelto en CH_3CN (15 ml) y tratado con Et_2O (150 ml) La goma que se separó fue retirada por decantacion del éster y el residuo fue disuelto en CH_2Cl_2/CH_3OH (1 1, 50 ml) y concentrado al vacio para obtener 29c en forma de espuma marron (2,2 g, 69 %) EM (Electroatomización, int rel m/z) 408 [$(M-PF_6)^+$, 100]

274
278

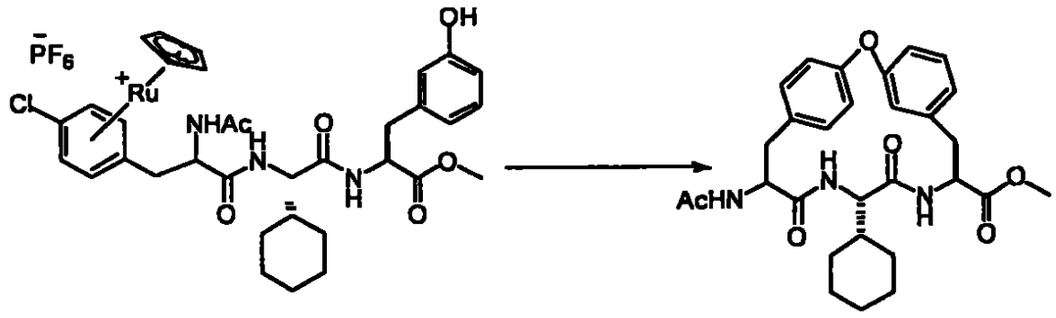
Paso C



Una solución del acido carboxilico 29c (2,0 g, 4,00 mmol) en DMF seca (20 ml) fue tratada con base de Hünig (2,06 g, 16,0 mmol, 4,0 equiv , 2,9 ml) y HOBt (810 mg, 6,0 mmol, 1,5 equiv) La mezcla de reacción fue enfriada a 0°C y luego tratada con EDCI (888 mg, 5,0 mmol, 1,25 equiv) y agitada durante 0,5 h A esta mezcla de reaccion se agregó el clorohidrato de amina 1g (1,48 g, 7,14 mmol, 1,2 equiv) y la mezcla de reacción fue agitada a ta por espacio de 12 h La DMF fue separada por destilación y el residuo diluido con agua y la capa acuosa fue extraida con CH_2Cl_2 (3x100 mL) Se extrajeron las capas orgánicas combinadas con $NaHCO_3$ ac (200 ml) HCl ac. (100 ml), salina, secadas (Na_2SO_4), filtradas, concentradas al vacio, El producto crudo 29d (1,2 g, 38 %)) fue utilizado para la ciclacion sin punficacion

275

Paso D



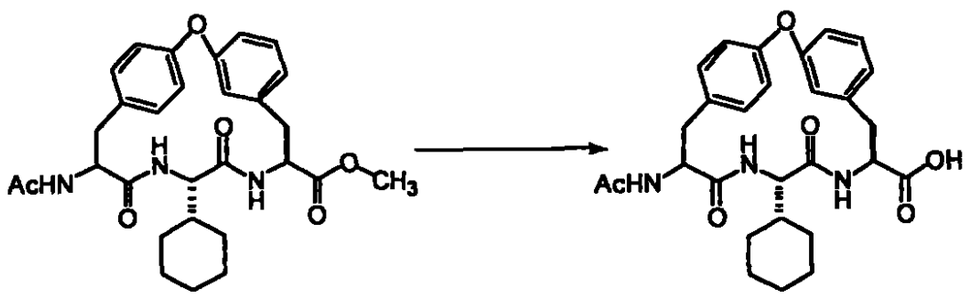
29d 29e

Una solución del compuesto cloro 29d (1,2 g, 1,5 mmol) en DMF seca (120 ml) fue desgasificada con N_2 seco, tratada con Cs_2CO_3 (2,4 g, 7,4 mmol, 5,0 equiv) y agitada a temperatura ambiente por espacio de 23 h La DMF solvente fue separada por destilación y el residuo fue diluido con agua (300 ml) y extraido con propionitrilo (3x100 ml) Las capas organicas combinadas fueron secadas (Na_2SO_4), filtradas, concentradas al vacio y secadas al vacio durante la noche Se las utilizó para la remocion fotolitica del Ru sin mas purificación

Se disolvió el compuesto ciclado del paso anterior en CH_3CN (40 ml) y se lo fotolizo en un Raynot (λ=350 nm) por espacio de 48 h La mezcla de reaccion fue concentrada al vacio y el residuo punficado por cromatografía (SiO_2, EtOAc/Hexanos 4 1) para dar un sólido de color tostado 29e (240 mg, 38 %) EM (FAB, NBA-G/TG-DMSO intensidad relativa m/z) 522 [(M+1)$^+$, 100]

276

Paso E

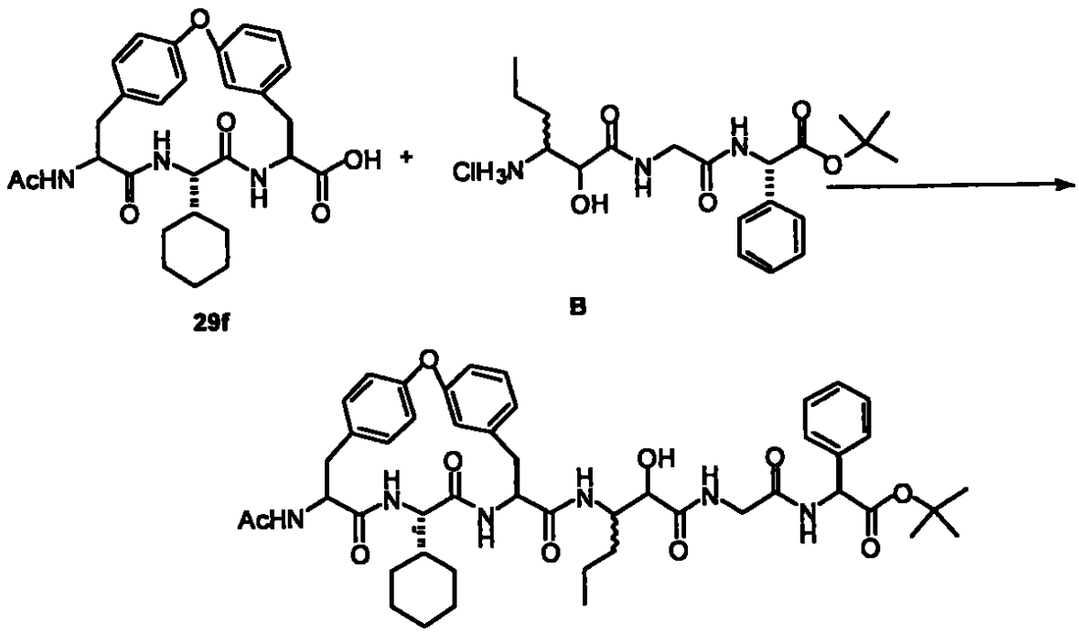


29e 29f

Una solución del éster 29e (200 mg, 0,42 mmol) en CH_3OH (5 ml), CH_2Cl_2 13 ml) y H_2O (2,0 ml) fue tratada con $LiOH \cdot H_2O$ (41 mg, 1,0 mmol, 2,4 equiv) y agitada a ta por espacio de 3 h La mezcla de reaccion fue acidificada con HCl conc (13 M, 1 ml) y extraida con CH_2Cl_2 (3x50 ml) y EtOAc (3x50 ml) Las capas orgánicas combinadas fueron secadas (Na_2SO_4), filtradas y concentradas al vacio para dar el ácido 29f (178 mg) que fue utilizado directamente para el acoplamiento sin mas purificación

277

Paso F



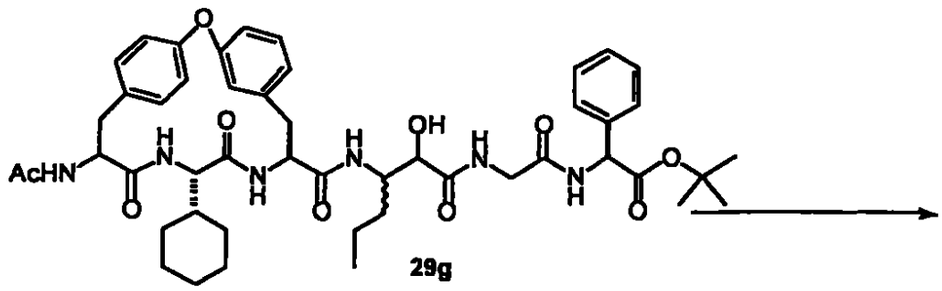
29f B

29g

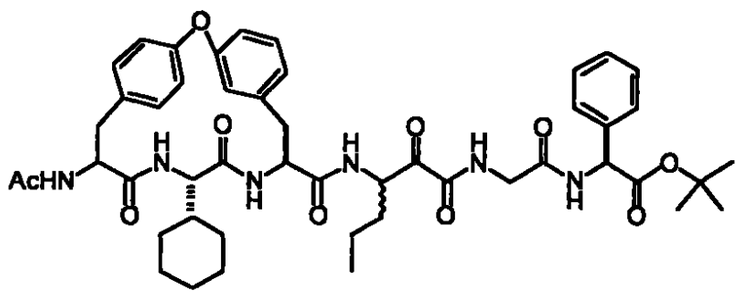
Una solucion del acido 29f (90 mg, 0,18 mmol) en DMF seca (1,0 ml) fue tratada con HOBt (45 mg, 0,33 mmol, 1,6 equiv) y base de Hünig (142 mg, 1,1 mmol, 5,0 equiv) y la amina B (118 mg, 0,28 mmol, 1,47 equiv) La mezcla de reacción fue enfriada a 0°C y tratada con EDCI (63 mg, 0,33 mmol, 1,6 equiv) y agitada a 0°C por espacio de 20 min, durante 12 h La mezcla de reaccion fue concentrada al vacio y diluida con H_2O (30 ml) Las capas orgánicas combinadas fueron extraidas con CH_2Cl_2 (3x30 ml) y EtOAc (3x30 ml) Las capas organicas combinadas fueron extraidas con NaOH ac (2M, 30 ml), secadas (Na_2SO_4), filtradas, concentradaas al vacio para obtener un solido incoloro 29g (50 mg, 32 %) que fue utilizada para la oxidacion EM (Electroatomizacion, int rel m/z) 883 [$(M+1)^+$, 100] 522 (30), 394 (60)

0282278

Paso G



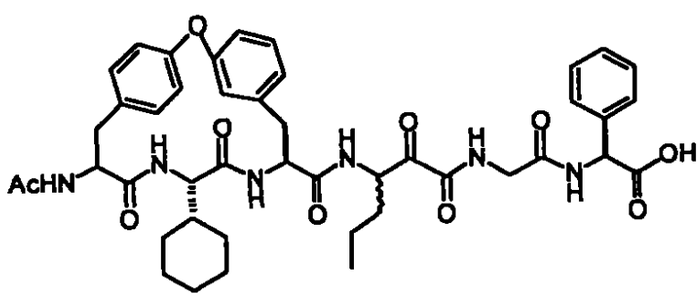
29g



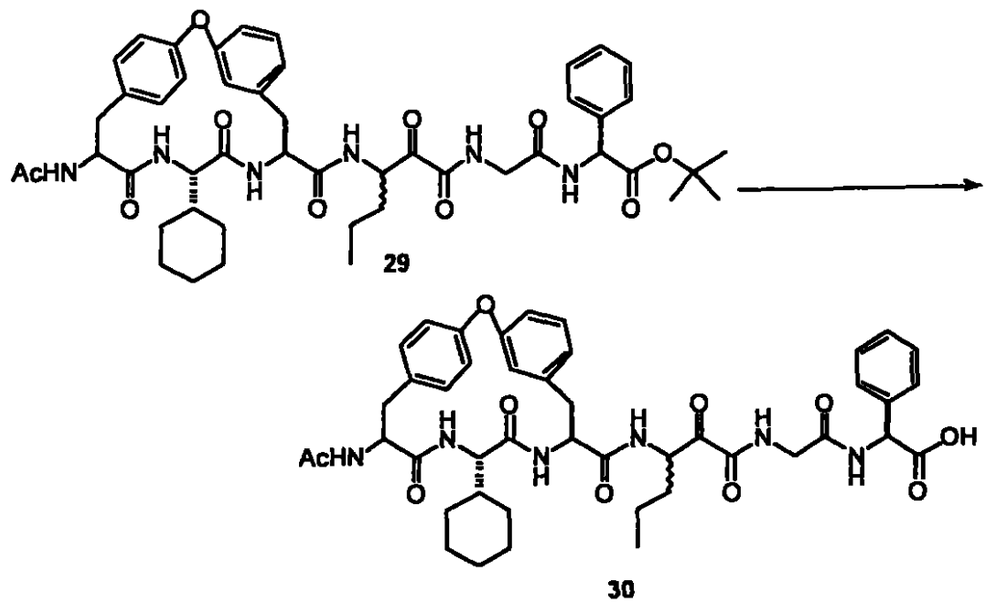
29

Una suspension del alcohol 29g (50 mg, 60,0 µmol) en CH_2Cl_2 (2,0 ml) fue tratada con reactivo Dess-Martin (40 mg, 0,94 mmol, 2,0 equiv) La mezcla de reacción fue agitada a ta por espacio de 3 h y la mezcla fue concentrada al vacio El residuo fue purificado por cromatografía (SiO_2, CH_3OH/CH_2Cl_2 1 32) para dar el producto oxidado 29 (41 mg, 80 %) en forma de sólido incoloro EM (FAB, int rel m/z) 881 [$(M+1)^+$, 100) 825 (170), 248 (100)

Ejemplo 30 Preparación del Compuesto de la formula 30



30



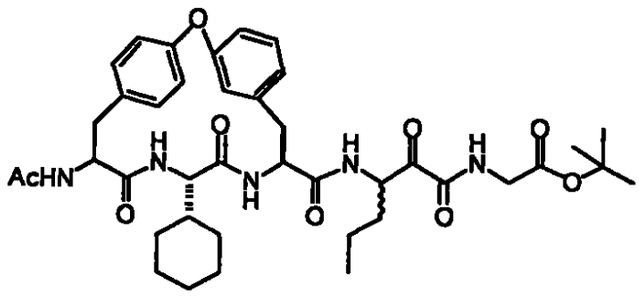
29

30

Una solucion del ester terc-butílico 29 (23,0 mg, 26,0 µmol) fue tratada con ATF/CH_2Cl_2 (1 1, 2,0 ml) y agitada a temperatura ambiente durante 4 h La desparicion del éster hasta la linea de base fue seguida por TLC (CH_3OH/CH_2Cl_2 1 24) Una vez completada la desproteccion, la mezcla de reacción fue concentrada al vacio y el residuo tratado reiteradamente con heptanos/CH_3OH (4,0 ml) y concentrado para dar un sólido tostado 30 (13,0 mg, 100 %) EM (Electroatomización, int rel m/z) 825 [$(M+1)^+$, 100)

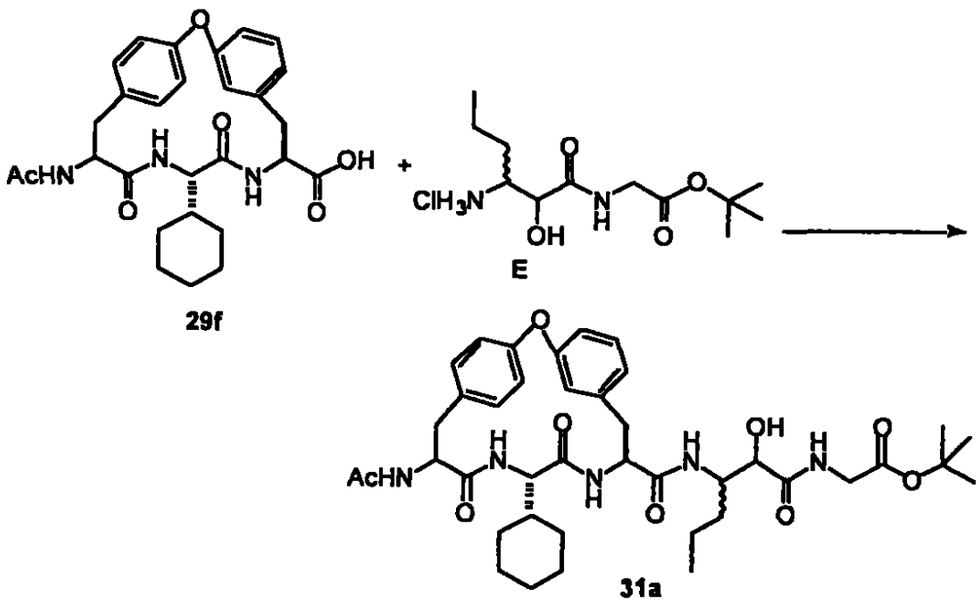
280

Ejemplo 31 Preparación del Compuesto de la fórmula 31



Paso A

31



29f

E

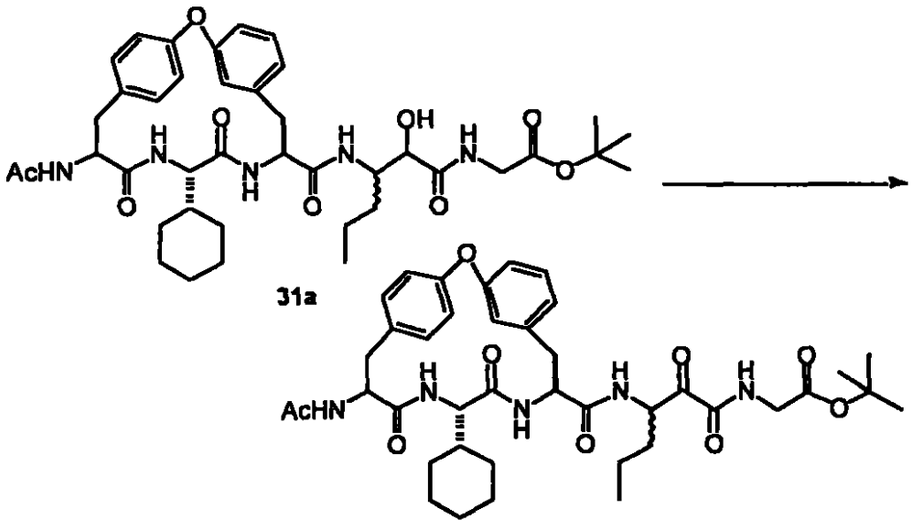
31a

Una solución del acido 29f (150 mg, 0,29 mmol) en DMF seca (4,0 ml), CH_2Cl_2 (3,0 ml) fue tratada con HOBt (58 mg, 0,44 mmol) y base de Hunig (149 mg, 1,1 mmol, 4,0 equiv) La mezcla de reaccion fue enfriada a 0°C y tratada con EDCI (82 mg, 0,44 mmol, 1,5 equiv) y agitada por espacio de 30 min La mezcla de reacción fue tratada con la amina E (88 mg, 0,29 mmol, 1,22 equiv) y agitada a temperatura ambiente por espacio de 12 h La mezcla de reaccion fue concentrada al vacio y diluida con H_2O (30 ml) La capa acuosa fue extraida con

0285
281

CH_2Cl_2 (3x30 ml) Las capas organicas combinadas fueron extraidas con HCl ac (1M, 3x20 ml), NaOH ac (1M, 3x20 ml), secadas (Na_2SO_4), filtradas, concentradaas al vacio para obtener un sólido incoloro 31a (56 mg) que fue utilizado para la oxidación EM (Electroatomización, int rel m/z) 750 [(M+1)$^+$, 20] 663 (10), 522 (10), 416 (20), 247 (30)

Paso B

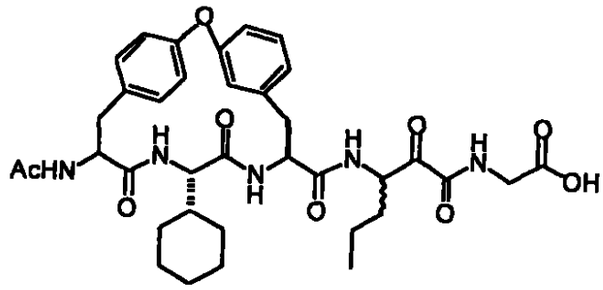


31

Una suspensión del alcohol 31a (56 mg, 75 µmol) en CH_2Cl_2 (5,0 ml) fue tratada con reactivo Dess-Martin (93 mg, 0,22 mmol, 3,0 equiv) La mezcla de reacción fue agitada a ta por espacio de 4 h y la mezcla fue concentrada al vacio El residuo fue purificado por cromatografía (SiO_2, CH_3OH/CH_2Cl_2 1 19) para dar el producto oxidado 31 (34 mg, 60 %) en forma de sólido incoloro EM (Electroatomización, int rel m/z) 748 [(M+1)$^+$, 35] 692 (5), 279 (100)

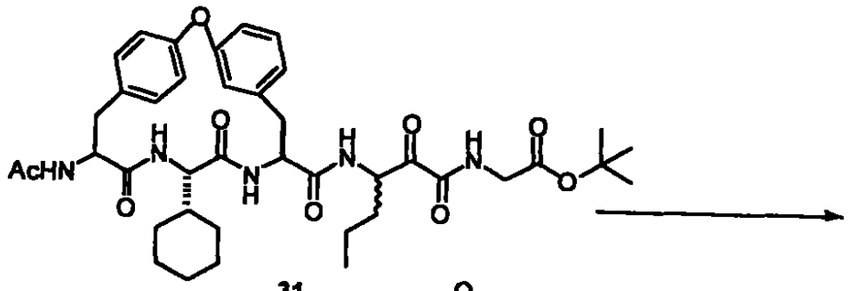
0-36 282

Ejemplo 32 Preparacion del Compuesto de la formula 32

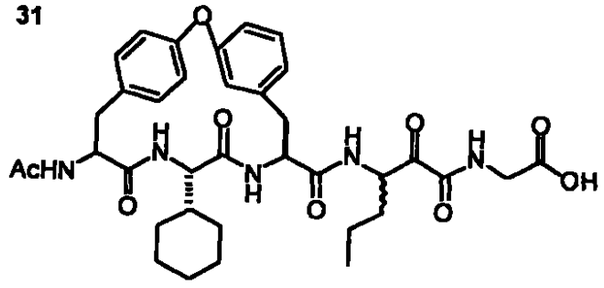


Paso A

32



31



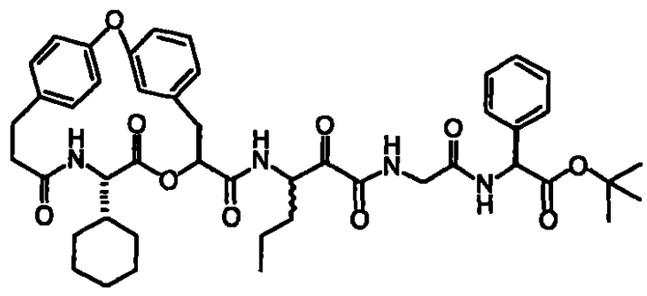
32

Una solución del éster terc-butílico 31 fue tratada con ATF/CH_2Cl_2 (1 1, 4,0 ml) y agitada a temperatura ambiente durante 4 h La desparición del éster hasta la linea de base fue seguida por TLC (CH_3OH/CH_2Cl_2 1 24) Una vez completada la desprotección, la mezcla de reacción fue concentrada al vacio y el residuo tratado reiteradamente con heptanos/CH_3OH (4,0 ml) y concentrado para dar un solido tostado 32 en forma de sólido tostado

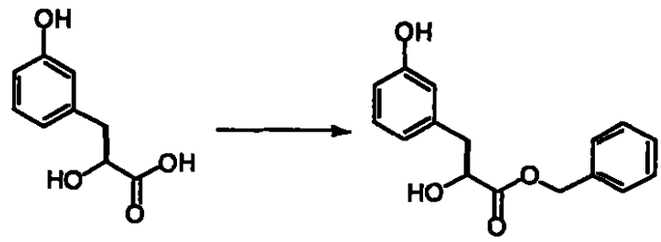
283

Ejemplo 33 Preparacion del Compuesto de la formula 33

Paso A



33



33a 33b

Una solución del acido 33a (4,5 g, 25,0 mmol) en dioxano (30 ml) y benceno (80 ml) fue tratada con BnOH (8,0 g, 74 mmol, 3,0 equiv) y TsOH H_2O (713 mg, 3,75 mmol, 10 % en moles) La mezcla de reaccion fue calentada a reflujo por espacio de 5 h, tras lo cual se separo el agua utilizando un aparato de Dean-Stark La mezcla de reaccion fue concentrada al vacio y el residuo purificado por cromatografía (SiO_2, EtOAc/Hexanos 3 7) para dar el ester bencilico 33b en forma de aceite incoloro (4,2 g, 62 %), Rf 0,22 (EtOAc/Hexanos 3 7), RMN ^{13}C (CH_3OD, 75 MHz, δ) 175,1, 158,2, 139,7, 130,3, 129,5, 129,3,

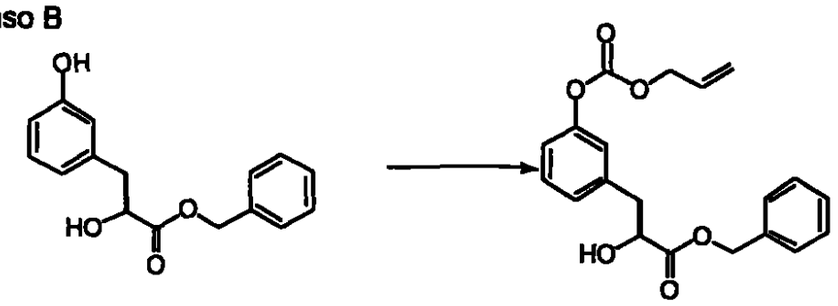
284

121,7, 117,4, 114,6, 73,1, 67,6, 41,6, EM (FAB, G/TG-DMSO, intensidad relativa)

351 (HRMS calc para $C_{16}H_{17}O_4$ $(M+1)^+$ 272,1049 , encontrado 272,1054

285

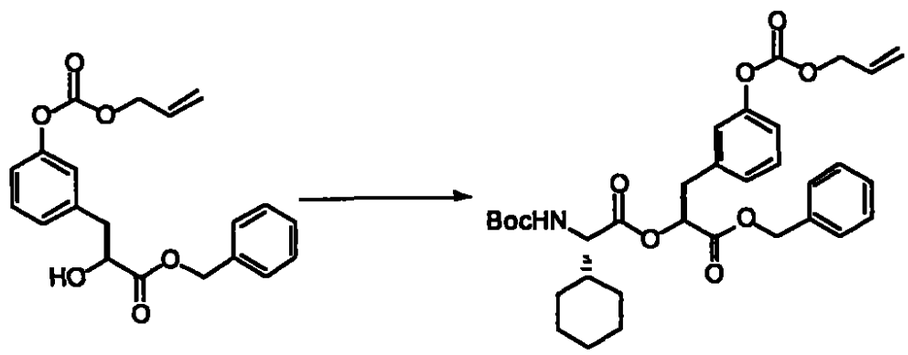
Paso B



33b 33c

Una solucion del éster bencilico 33b (3,8 g, 12,9 mmol) en CH_2Cl_2 (100 ml) fue tratada con Et_3N (1,55 g, 15,4 mmol, 1,1 equiv), enfriada a –78°C (2-PrOH, hielo seco) y se le agregó por goteo una solución de cloroformiato de alilo (1,84 g, 15,36 mmol, 1,1 equiv) en CH_2Cl_2 (10 ml) Se dejó que la mezcla de reaccion entibiara a ta y se la diluyo con HCl ac (1M, 100 ml) La mezcla de reaccion fue extraida con EtOAc (3x100 ml) La capa organica combinadas fue lavada con HCl ac. (100 ml, 1M), salina (200 ml), secada ($MgSO_4$) concenbtrada al vacio para dar 33c que fue utilizado en el siguiente paso sin más purificacion Rf 0,43 (EtOAc/Hex. 7 13), RMN ^{13}C (CH_3OD, 75 MHz, δ) 174,8, 162,5, 155,0, 152,5, 140,3, 1371, 132,8, 130,3, 129,6, 129,5, 129,4, 123,2, 120,3, 119,4, 72,7, 70,1, 67,7, 41,2, 29,9

Paso C



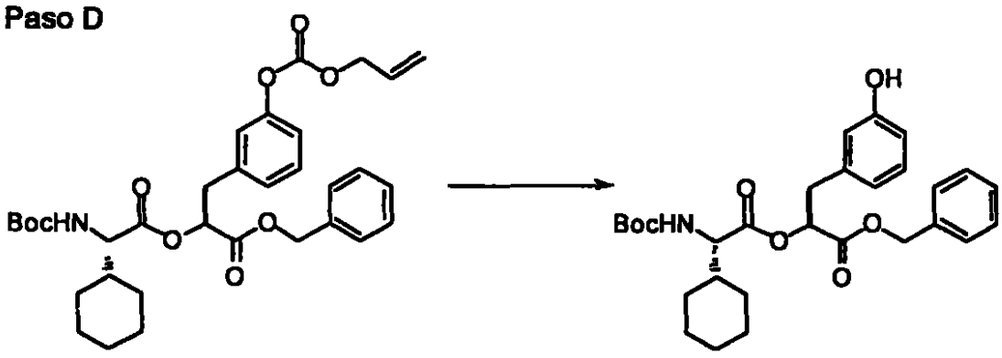
33c 33d

Una solución de Boc-ciclohexilglicina monohidrato 1d (6,02 g, 23,4 mmol, 2,0 equiv) fue disuelta en CH_2Cl_2 y secada ($MgSO_4$) Se filtró la mezcla y

0250 286

posteriormente se seco el residuo azeotrópicamente con tolueno Se disolvió el residuo en CH_2Cl_2 y se lo trató con HOBt (4,73 g, 35,1 mmol, 2,9 equiv) EDCI (6,7 g, 35,1 mmol, 2,9 equiv) y base de Hunig (8,31 g, 64,3 mmol, 11 ml) Se la agitó a ta durante 30 min y se agregó el alcohol protegido con alloc 33c (4,3 g, 12,04 mmol) La mezcla de reaccion fue agitada a ta por espacio de 36 h y diluida con HCl ac (1M, 100 ml) y extraida con EtOAc (3x100 ml) Se extrajeron las capas orgánicas combinadas con NaOH ac (1M, 100 ml), salina (100 ml), se las seco, concentro al vacio y purificó por cromatografía (SiO_2, EtOAc/Hex 1 4) para dar el depsipéptido 33d (7,1 g, 100 %) Rf 0,18 (EtOAc/Hex. 1 4), HRMS calc para $C_{28}H_{34}O_7$ $(M\text{-}Boc)^+$ 496,2335, encontrado 496,2333

Paso D



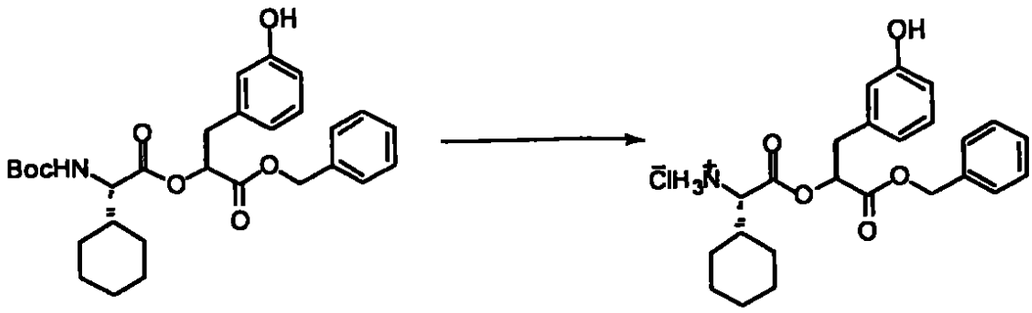
33d 33e

Una solución del depsipeptido protegido con alloc 33d (7,8 g, 13,0 mmol) en THF seco (200,0 ml) fue tratada bajo N_2 con dimediona (3m27 g, 23,4 mmol, 2,0 equiv) y $Pd(PPh_3)_4$ (780 mg, 0,67 mmol, 5 % en moles)l La mezcla de reacción fue agitada a ta durante 1 h y la desaparición del reactivo fue seguida por TLC (EtOAc/Hex 1 4) La mezcla de reacción fue concentrada al vacio y el residuo punficado por cromatografía (SiO_2, EtOAc/hexanos, 1 4) para dar el fenol 33e (5,2 g, 78 %) en forma de espuma incolora Rf 0,52 (EtOAc/Hexanos 3 7 RMN ^1H (d_4-CD_3OD, 300 MHz, δ) 7,4-719 (m, 5 H), 7,15-6,99 (m, 1 H), 6,68-6,55 (m, 4 H), 5,43-5,01 (m, 3 H), 4,6 (bs, 2 H), 4,11-4,00 (m, 1 H), 3,18-2,91 (m, 2 H), 1,80-1,55 (bs, 6 H), 1,39 (s, 9 H) 1,21-0,89 (m, 6 H), RMN ^{13}C (CD_3OD, 75 MHz, δ,

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mezcla de diastereomeros) 171,6, 169,4, 169,3, 161,1, 157,1, 157,0, 137,2, 136,9, 135,3, 129,2, 129,1, 128,2, 128,2, 128,0, 120,3, 120,1, 116,0, 115,9, 113,6, 94,8, 79,3, 73,6, 73,5, 66,7, 66,6, 58,6, 58,5, 40,0, 29,9, 26,8, 29,1, 27,7, 27,3, 25,5 EM (Electroatomización, int rel m/z) 1023 ($[2M+1]^+$, 20) 512 ($[M+a]^+$, 20), 412 ($[M-Boc]^+$, 100), 202 (40), HRMS calc para $C_{24}H_{30}NO_5$ $(M-Boc)^+$ 412,2123, Encontrado 412,2119

Paso E

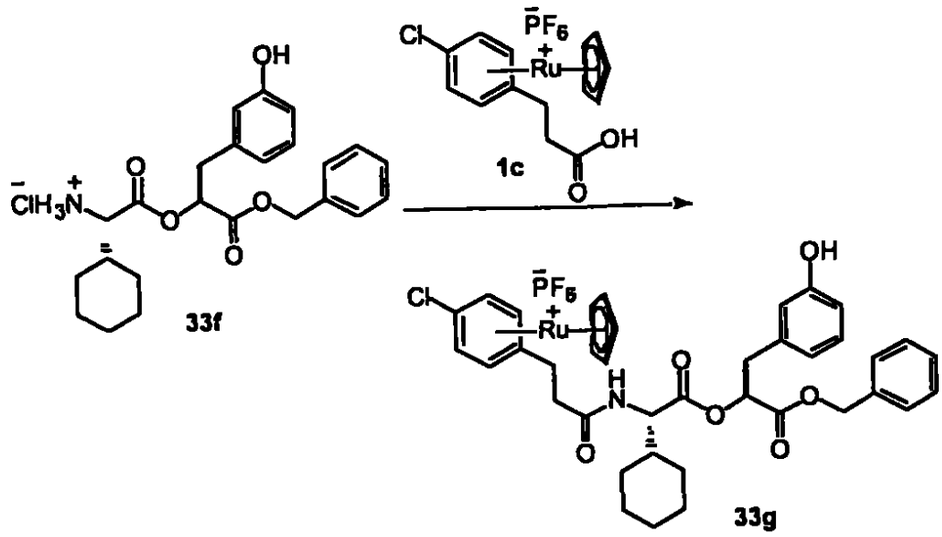


33e 33f

Una solucion de la amina protegida con Boc 33e (5,2 g, 10,7 mmol) fue agitada con HCl (4M, dioxano, 200 ml, 800 mmol, 80 equiv) hasta que el material de partida hubo desaparecido hasta la linea de base segun lo indicado por TLC (EtOAc/Hex 3 7) La mezcla de reacción fue concentrada al vacio y secada con alto vacio y el residuo 33f fue utilizado directamente en el paso siguiente RMN ^1H (d_4-CD_3OD, 300 MHz, δ) 7,40-3,23 (m, 5 H), 7,07 (q, 1 H, J=13 Hz), 6,77-6,6 (m, 3 H), 5,33-5,41 (m, 1 H), 5,3-5,05 (2 AB, 2 H), 3,99-3,85 (m, 1 H), 3,35-22 (m, 2 H), 2,00-1,5 (m, 5 H), 1,50-0,80 (m, 6 H) EM (FAB, G/TG-DMSO, m/z, intensidad relativa) 412 ($[M+1]^+$, 100), HRMS calc para $C_{24}H_{30}NO_5$ (M^+ 412,2123, Encontrado 412,2139

0432 288

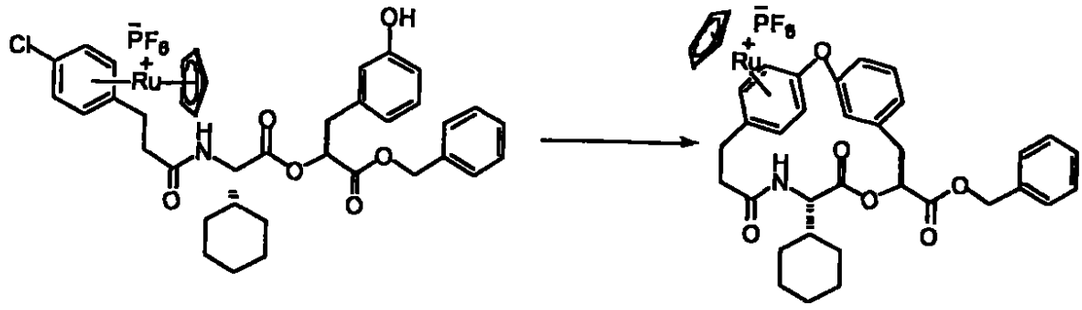
Paso F



Una solución de [CpRu(acido η^6-4-clorofenilpropionico)]PF_6 1c (2,0 g, 4,03 mmol) en DMF seca (20 ml) fue tratada con HOBt (835 mg, 6,0 mmol, 1,5 equiv) y base de Hünig (2,06 g, 2,95 ml, 16 mmol, 4,0 equiv) La mezcla de reaccion fue enfriada a 0°C y tratada con EDCI (1,15 g, 6,0 mmol, 1,5 equiv) La mezcla de reaccion fue agitada a 0°c por espacio de 30 min y se le agrego el clorhidrato de amina 33f (1,8 g, 4,03 mmol, 1,9 equiv) en DMF seca (10 ml) La mezcla de reaccion fue agitada a ta por espacio de 12 h y la DMF fue eliminada por destilación al vacio El residuo fue diluido con HCl ac (1M, 100 ml) y extraido con CH_2Cl_2 (3x100 ml) Las capas organicas combinadas fueron extraidas con $NaHCO_3$ (3x50 ml), salina (100 ml), secadas (Na_2SO_4), filt4radas, concentradas al vacio para dar un solido marrón 33g (3,5 g) que fue utilizado para la ciclación EM (Electroatomizacion, int. rel m/z) 743 [$(M-PF_6)^+$, 100] 304 (60), HRMS calc para $C_{38}H_{41}NO_6Cl^{102}Ru$ ($M PF_6)^+$ 744,1666, Encontrado 744,1694

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Paso G

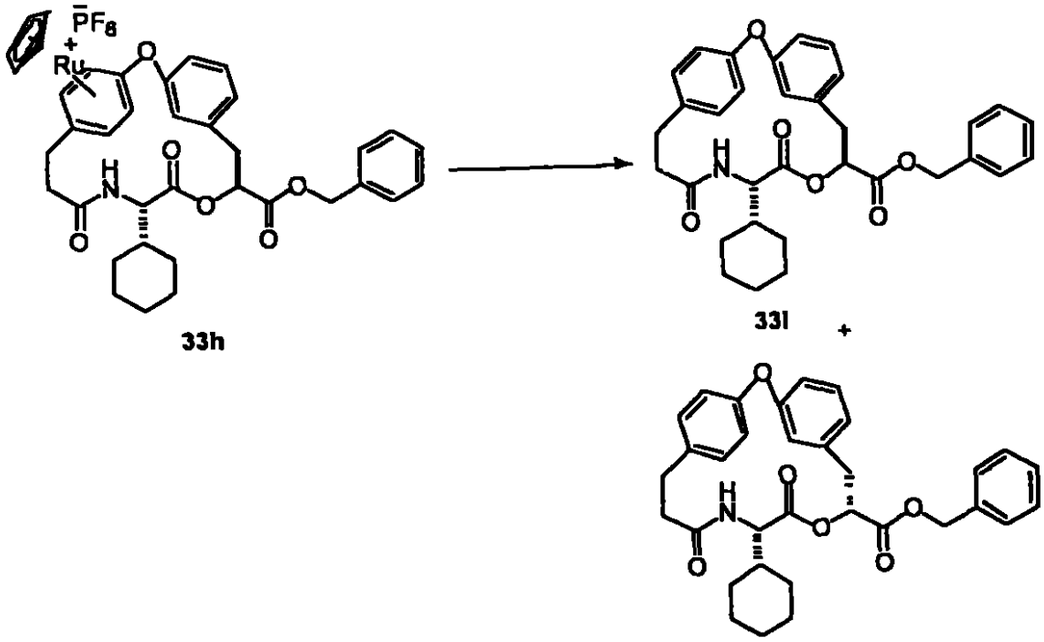


33g

33h

Una solución del complejo η^6-rutenio 33g (3,5 g, 3,93 mmol) en DMF seca (300 ml) fue desgasificada con N_2 seco, tratada con Cs_2CO_3 (6,5 g, 19,95 mmol, 5,0 equiv) y agitada a temperatura ambiente por espacio de 16 h La mezcla de reacción fue concentrada al vacio para eliminar la DMF y el residuo fue diluido con H_2O (100 ml) La mezcla de reaccion fue extraida con CH_2Cl_2 (3x100 ml) Las capas combinadas de CH_2Cl_2 fueron extraidas con salina, secadas (Na_2SO_4), filtradas, concentradas al vacio para dar 33h que fue utilizado directamente para la fotólisis EM (Electroatomizacion, intensidad relativa m/z) 708 [$(M\text{-}PF_6)^+$, 100] HRMS calc. para $C_{38}H_{40}NO_{61}O_2Ru\,(M^+PF_6)^+$ 708,1892, Encontrado 708,1918

Paso H



33h

33l +

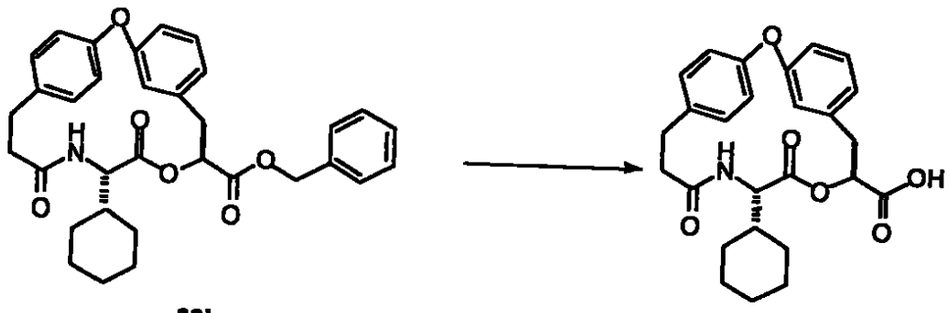
33j

Una solucion del complejo rutenio ciclado 33h (3,5 g, 3,9 mmol) en CH_3CN (60 ml) fue desgasificada y fotolizada en un tubo de cuarzo a λ=350 nm en dos lotes durante 48 horas cada uno La mezcla de reacción fue acumulada y purificada por cromatografía (SiO_2, CH_2Cl_2/Et_2O 9/1) para dar el depsipépsido cíclico en forma de mezcla de diastereómeros (700 mg, 34 %) Se separaron los diastereomeros por medio de una cromatografía adiiconal (Hexnos/CH_2Cl_2/ Et_2O 6 3 1) para dar los dos diastereómeros 33i (370 mg, 18 %) y 33j (216 mg 11 %) en forma de sólido incoloro Rf 0,28 (Hexanos EtOAc. 3 2), $[\alpha]_D$ 25 (c 0,15 $CHCl_3$, 20°C), IR (puro, cm^{-1}) 3329 (w), 2960 (m) 2926 (s), 2854 (s), 1745 (s), 1680 (m), 1589 (m), 1506 (m), 1446 (m), 1365 (w), 1259 (s) 1099 (m), 1030 (s), 800 (s), 752 (m), 6,98 (w), 619 (w) RMN ^1H ($CDCl_3$, 300 MHz, δ) 7,36-7,23 (m, 5 H), 7,18-6,99 (m 4 H), 6,81 (d, 1 H, J=7,5 Hz), 6,74 (dd, 1 H, J=2,7, 5,7 Hz), 6,30 (s, 1 H), 5,75 (d, 1 H, J=7,2 Hz), 5,61 (dd, 1 H, J=2,4 Hz, 5,4 Hz), 5,18, 5,14 (AB, 2 H, J=12,3 Hz), 4,23 (dd, 1 H, J=4,2 Hz,3,3 Hz), 326-3,01 (m, 2 H), 2,98-2,85 (m, 2 H), 2,68-2,64 (m, 1 H), 2,38-2,34 (m, 1 H), 1,96-1,51 (m, 6 H), 1,51-0,96 (M, 5H) , RMN ^{13}C

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($CDCl_3$, 75 MHz, δ ppm) 177,3, 171,1, 159,8, 155,3, 138,6, 135,4, 134,9, 131,2, 129,7, 129,2, 128,7, 126,6, 126,1, 123,3, 120,8, 120,8, 117,5, 114,2, 71,8, 57,5, 56,9, 41,5, 39,0, 35,7, 32,6, 31,3, 29,0, 27,6, 26,0, 25,9, FAB (NBA/DMSO, intensidad relativa m/e) 542 ([M+1)$^+$, 100], 514 (15), 450 (5), 307 (8), 232 (5) 154,1 (17), 136 (14) HRMS calc para $C_{33}H_{38}NO_6$ (M+1)$^+$ 542,2543, encontrado 542,2541 CHN calc para $C_{33}H_{35}NO_6$ 0,5 H_2O C 71,98 %, H 6,59 %, N 2,54 %, Encontrado C 72,56 %, H 7,05 % N 2,63 %

Paso I



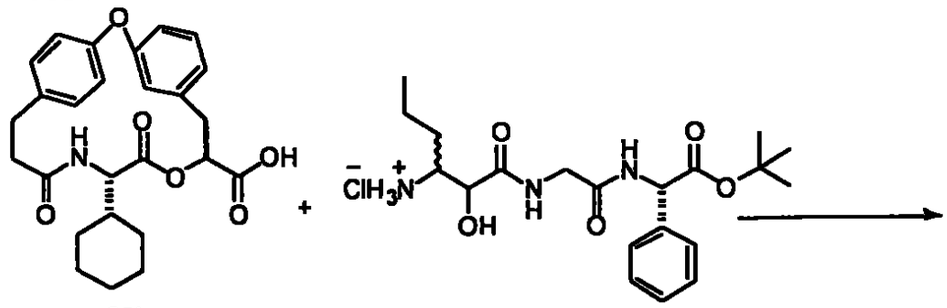
33l

33k

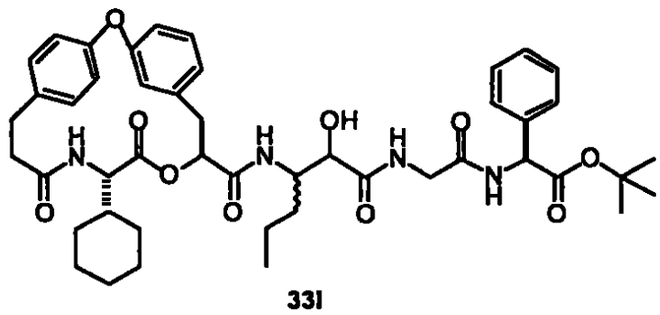
Una solución del éster bencílico 33l (360 mg, 0,66 mmol) en CH_3OH/EtOAc (1 1, 50 ml), fue tratada con $Pd(OH)_2$ e hidrogenada (50 psi, 344,7 kPa) por espacio de 12 h La mezcla de reaccion fue filtrada a través de un cilindro de celite y la torta enjuagada con CH_3OH/CH_2Cl_2 (1 1, 50 ml) El filtrado fue concentrado al vacio y el residuo 33k (330 mg,) fue utilizado para el acoplamiento sin purificación Rf 0,58 (CH_3OH/CH_2Cl_2 1 19), EM (Electroatomización, int rel m/z) 827,2 [(M+1)$^+$, 100] 694 (20), 539 (40), 466 (10), 174 (70) HRMS calc. para $C_{46}H_{58}N_4O_{10}$ (M+1)$^+$ 827,4231, Encontrado 827,4215

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Paso J



33k B

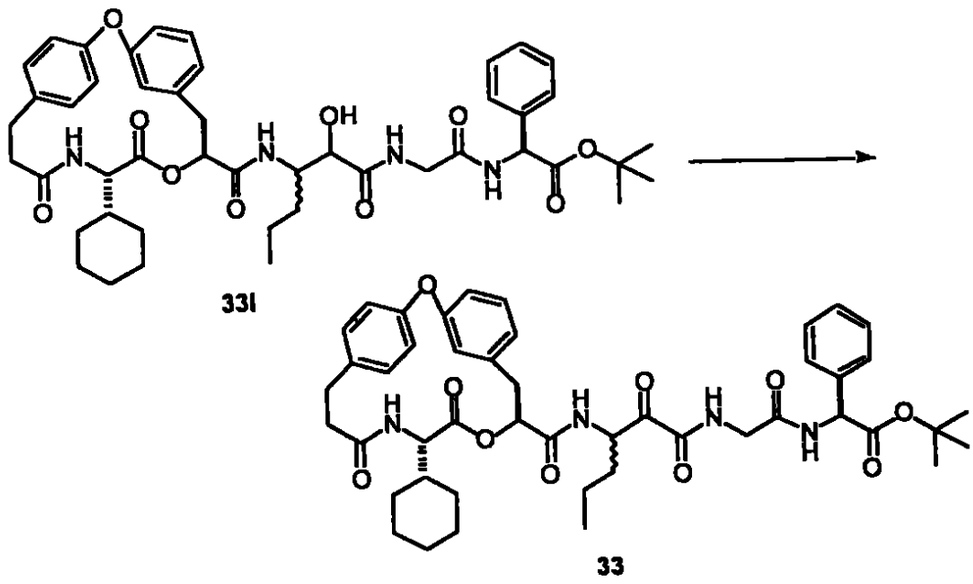


33l

Una solución del acido 33k (165 mg, 0,31 mmol) en DMF seca (5,0 ml) y CH_2Cl_2 (5,0 ml) fue tratada con HOBt (83 mg, 0,46 mmol, 1,5 equiv) y enfriada a 0°C, tras lo cual se agrego base de Hunig (159 mg, 1,23 mmol, 4,0 equiv, 229 µl) A e sta mezcla se agrego EDCI (89 mg, 0,47 mmol, 1,5 equiv) y lña mezcla de reaccion fue agitada a 0°C por espacio de 1 h y tratada con el clorhidrato de amina B (159 mg, 0,372s mmol, 1,2 equiv) La mezcla de reaccion fue agitada a temperatura ambiente por un lapso de 48 h y concentrada al vacio para eliminar la DMF y el CH_2Cl_2 El residuo fue diluido con agua y extraido con CH_2Cl_2 (3x50 ml) Las capas orgánicas combinadas fueron extraidas con HCl ac (1M, 3x50 ml), NaOH ac (1M, 3x50 ml), solución salina (100 ml) y concentradas al vacio El residuo 33l fue oxidado sin más purificacion

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293

Paso K

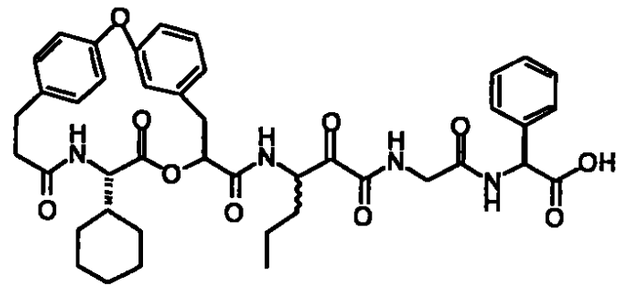


33I

33

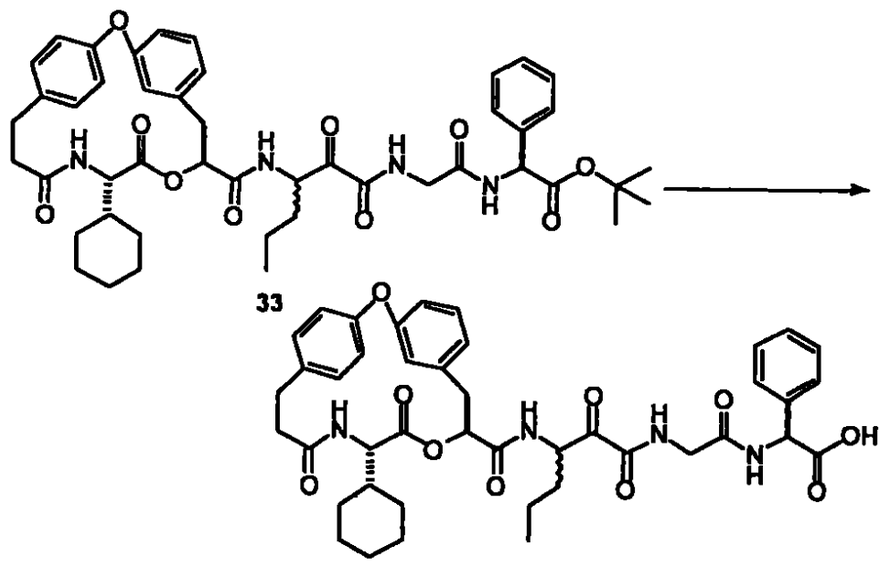
Una solución del alcohol 33I (330 mg, 0,4 mmol) en CH_2Cl_2 (5,0 ml) fue tratada con reactivo de Dess-Martin (424 mg, 1,00 mmol, 2,5 equiv) La mezcla de reacción fue agitada a ta durante 1 h y diluida con $NaHCO_3$ acuoso (15 ml) y $Na_2S_2O_3$ ac (50 ml) La mezcla de reaccion fue agitada a ta durante 20 min y se extrajo la mezcla de reacción con CH_2Cl_2 (3x50 ml) Las capas orgánicas combinadas fueron extraidas con solución salina (50 ml), secadas (Na_2SO_4), filtradas, concentradas al vacio y el residuo fue purificado por cromatografía (SiO_2, EtOAc/Hexanos 1 1) para dar la cetoamida 33 (180 mg, 55 %) en forma de solido incoloro Rf 0,63 (CH_3OH/CH_2Cl_2 1 19), EM (Electroatomizacion, int rel m/z) 857,2 ($[M+CH_3OH]^+$, 40) 825,2 ($[M+1]^+$ 100)

Ejemplo 34 Preparación del Compuesto de la fórmula 34



Paso A

34

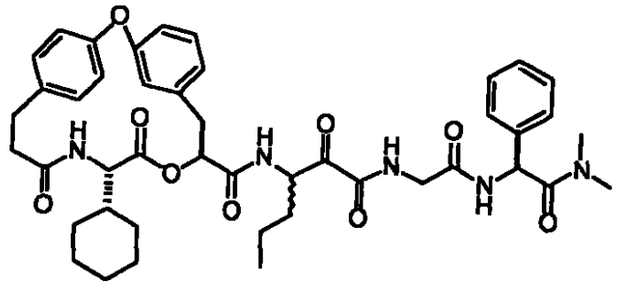


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34

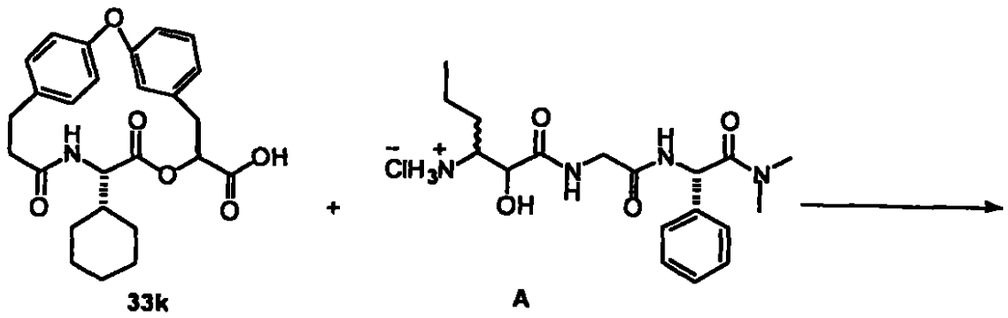
Una solución del depsipéptido oxidado 33 (160 mg, 0,2 mmol) en CH_2Cl_2 seco (5,0 ml) fue tratada con ATF (5,0 ml) y agitada a ta por espacio de 7 h La mezcla de reacción fue concentrada al vacio y el residuo disuelto reiteradamente en CH_3OH/CH_2Cl_2/ Hexanos (1 1 1) y concentrado varias veces al vacio para dar un sólido de color tostado 34 (133 mg, 86 %) que fue secado al vacio EM (Electroatomizacion, int rel m/z) 769,2 [$(M+1)^+$, 100], 481 (5), 269 (25) 191 (90)

Ejemplo 35 Preparación del Compuesto de la formula 35

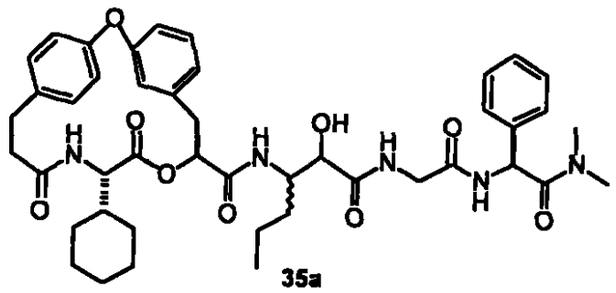


35

Paso A



33k A



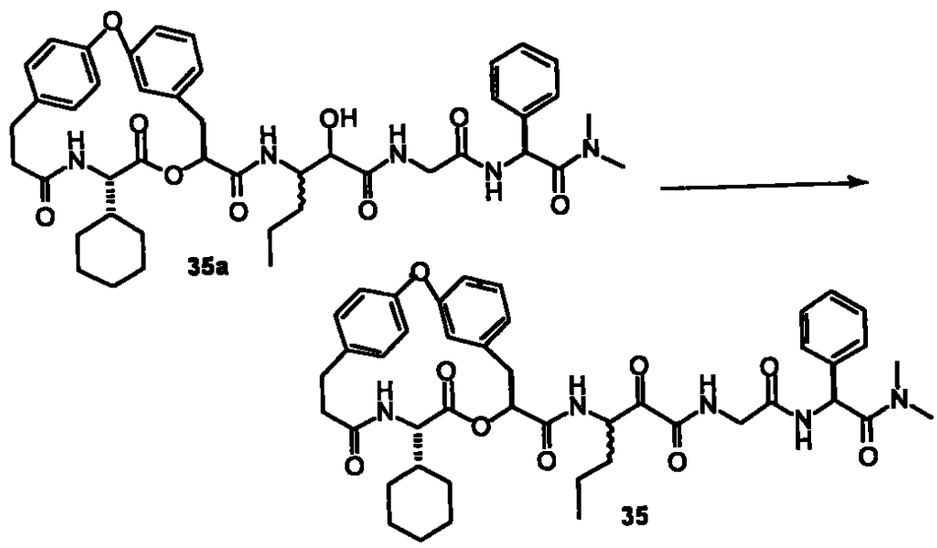
35a

Una solución del acido 33k (165 mg, 0,31 mmol) en DMF seca (5,0 ml) y CH_2Cl_2 (5,0 ml) fue tratada con HOBt (83 mg, 0,46 mmol, 1,5 equiv) y enfriada a 0°C, tras lo cual se agrego base de Hünig (159 mg, 1,23 mmol, 4,0 equiv, 229 µl) A esta mezcla se agrego EDCI (89 mg, 0,47 mmol, 1,5 equiv) y la mezcla de reacción fue agitada a 0°C por espacio de 1 h y tratada con el clorhidrato de amina A (159 mg, 0,372 mmol, 1,2 equiv) La mezcla de reaccion fue agitada a temperatura ambiente por un lapso de 48 h y concentrada al vacio para eliminar la DMF y el CH_2Cl_2 El residuo fue diluido con agua y extraido con CH_2Cl_2 (3x50 ml) Las capas orgánicas combinadas fueron extraidas con HCl ac (1M, 3x50 ml),

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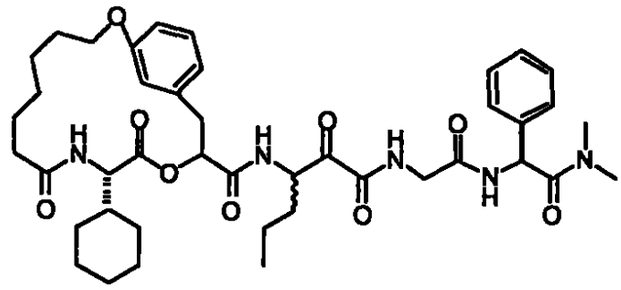
NaOH ac (1M, 3x50 ml), solución salina (100 ml) y concentradas al vacio El residuo 35a fue oxidado sin más purificacion EM (Electroatomización, int rel m/z) 798,2 [(M+1)$^+$, 30], 479 (10), 391 (20) 180 (100)

Paso B



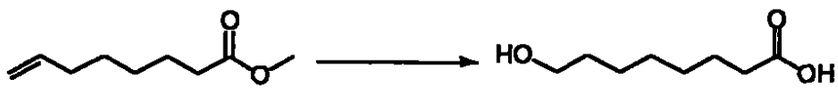
Una solucion del alcohol 35a (190 mg, 0,24 mmol) en CH_2Cl_2 (10,0 ml) fue tratada con reactivo de Dess-Martin (423 mg, 1,0 mmol, 4,0 equiv) y agitada a ta durante 1 h La mezcla de reacción fue diluida con $NaHCO_3$ acuoso (50 ml) y extraida con $Na_2S_2O_3$ ac sat , salina (3x50 ml), , secadas (Na_2SO_4), filtradas, concentradas al vacio y el residuo fue purificado por cromatografía (SiO_2, EtOAc/Hexanos 3 7 ->1 1) para dar el producto oxidado 35 (163 mg, 86 %) en forma de sólido incoloro, EM (Electroatomizacion, int. rel m/z) 796 ([M+1)$^+$ 100] 508 (20), 269 (20)

Ejemplo 36 Preparación del Compuesto de la fórmula 36



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Paso A



36a 36b

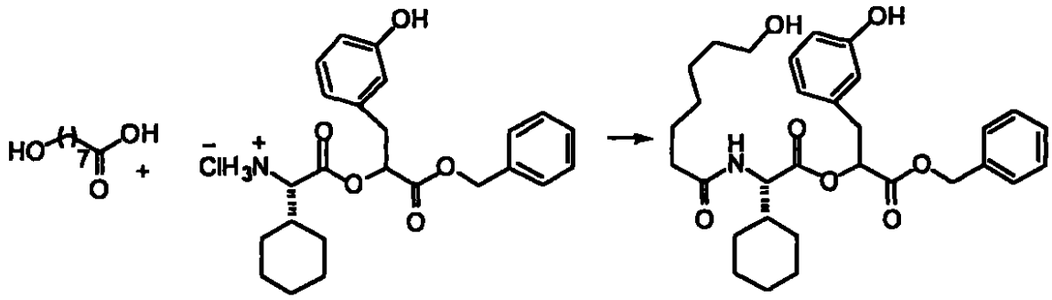
Una solucion de BH_3 THF (1 M en THF, 100 mmol, 100 ml, 3,0 equiv) fue adicionada por goteo a una solucion de alqueno (5,0 g, 35 mmol) en THF (100 ml) a 0°C y agitada por espacio de 1 h La mezcla de reacción fue tratada por goteo con etanol Una vez completada la evolucion de gas hidrógeno, la mezcla de reacción fue tratada con buffer pH 7 (100 ml) y con H_2O_2 (30 volumenes, 100 ml) La mezcla de reacción fue agitada a ta durante 4 h y apagada con HCl ac (100 ml) Se extrajo la capa acuosa con Et_2O (3x100 ml) Las capas de eter combinadas fueron extraidas con NaOH ac (1M, 100 ml), salina (100 ml), secadas ($MgSO_4$), filtradas, concentradas al vacio y purificadas por cromatografía (SiO_2, EtOAc/hexanos, 2/3) para dar un alcohol en forma de liquido incoloro 2,9 g, 52 %)

Una solución del éster hidroxilado en THF/H_2O/CH_3OH (100 ml, 1 1 1) fue tratada con LiOH H_2O (2,1 g, 51,2 mmol, 3,0 equiv) y agitada a ta por espacio de 3 d La mezcla de reaccion fue concentrada al vacio y la capa acuosa fue extraida con éter (2x40 ml) La capa acuosa fue acidificada a pH ~1 y extraida en EtOAc (3x50 ml) Las capas orgánicas combinadas fueron extraidas con solución salina

0302 303
298

(100 ml), secadas ($MgSO_4$), filtradas, concentradas al vacio y se utilizo el residuo 36b como tal para el acoplamiento en el paso B RMN ^1H (300 MHz, CD_3OD, δ) 3,53 (t, 2 H, J=6,6 Hz), 2,72 (t, 2 H, J =7,2 Hz), 1,59 (t, 2 H, J =7,5 Hz), 1,5 (t, 2 H, J =7,5 Hz), 1,38-1,33 (m, 6 H)

Paso B

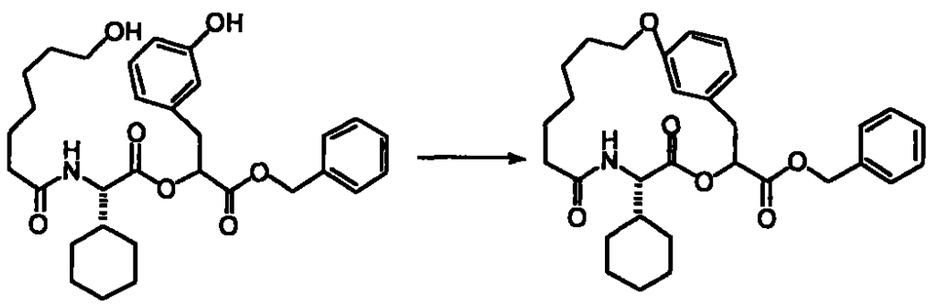


36b 33f 36c

Una solución del ácido ω-hidroxilheptanoico 36b (1 01 g, 6,93 mmol) en CH_2Cl_2 (40 ml) fue tratada con base de Hünig (1,97 g, 15,24 mmol, 2,2 equiv, 2,81 ml) y el clorhidrato de amina 33f (3,1 g, 6,93 mmol, 1,0 equiv) La mezcla de reaccion fue enfriada a 0°C y tratada con PyBrOP (3,22 g, 6,93 mmol, 1,0 equiv) La mezcla de reacción fue agitada durante la noche a temperatura ambiente y concentrada al vacio El residuo fue purificado por cromatografía ((EtOAc/Hexanos 1 1) para dar el depsipeptido 36c (2,5 g, 66 %) en forma de aceite viscoso RMN ^1H (CD_3OD, 400 MHz, δ, ppm) 8 07 (t, 1 H) 7,33-7,21 (m, 4 H), 7,09-7,02 (m, 1 H), 6,67-6,63 (m, 3 H), 5,25-5,06 (m, 1 H), 5,08 (q, 2 H, J=7,5 Hz), 4,36-4,33 (m, 1 H), 3,51 (dd, 2 H, J=5,4 Hz, 0,9 Hz), 3,11-2,96 (m, 2 H), 2,22-2,17 (m, 1 H), 1,99-0,90 (m, 14 H) RMN ^{13}C (CD_3OD, 75 MHz, δ, ppm, mezcla de diastereómeros) 172,1, 172,0, 171,8, 171,1, 170,9, 169,5, 169,3, 157,1, 157,90, 137,3, 137,0, 135,3, 135,2, 129,2, 129,1, 128,2, 128,0, 127,9, 120,3, 120,0, 116,0, 115,9, 113,6, 94,8, 73,6, 73,4, 66,8, 66,7, 60,2, 57,3, 39,6, 36,7, 28,9, 28,0, 25,6,

20,9, 19,5, 13, EM (FAB NBA DMSO, int rel m/z) 562, [(M+Na)$^+$, 20], 540 [(M+1)$^+$, 100), 412 (15), 240 (50), 112 (80)

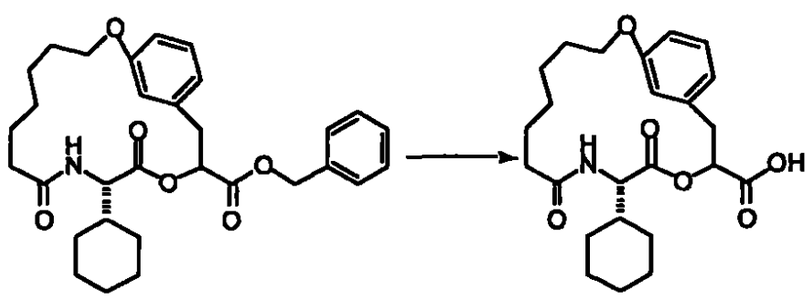
Paso C



36c 36d

Una solucion del alcohol 36c (2,5 g, 4,63 mmol) en CH_2Cl_2 seco (50 ml) fue tratada con trifenilfosfina (2,67 g, 10,2 mmol, 2,2 equiv) bajo N_2 y enfriada a 0°C La mezcla de reacción fue tratada con DEAD (1,61 g, 9,26 mmol, 2,0 equiv) en CH_2Cl_2 (30 ml) La mezcla de reaccion fue calentada a ta y agitada por espacio de 2 h Se la concentro al vacio y se la purificó por cromatografia (Et_2O/ Hex 1 3) para dar el producto cíclico 36d (530 mg, 21 %) en forma de solido incoloro EM (FAB, NBA, DMSO, int rel m/z), 522 [(M+1)$^+$, 100), 494 (60), 268 (20), 222 (20), HRMS calc para $C_{31}H_{40}NO_8$ (M+1)$^+$ 522,2856, Encontrado 522,2864

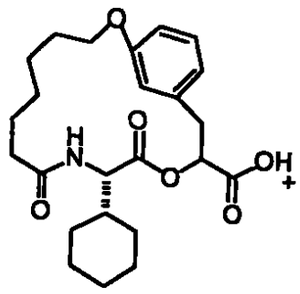
Paso D



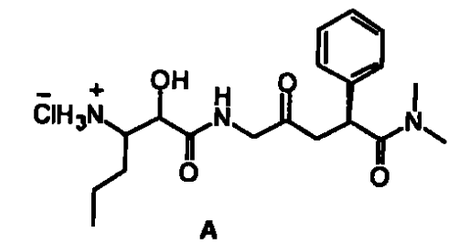
36d 36e

Una solucion del éster bencílico (242 mg, 0,47 mmol) en metanol (30 ml) fue tratada con Pd/C (10 % en peso) e hidrogenada en un Parr a 40 psi (276 kPa), durante un periodo de 14 h La mezcla de reaccion fue filtrada a través de un cilindro de celite y el filtrado fue concentrado al vacio para dar un solido incoloro 36e (181 mg, 93 %) que fue utilizado para el acoplamiento

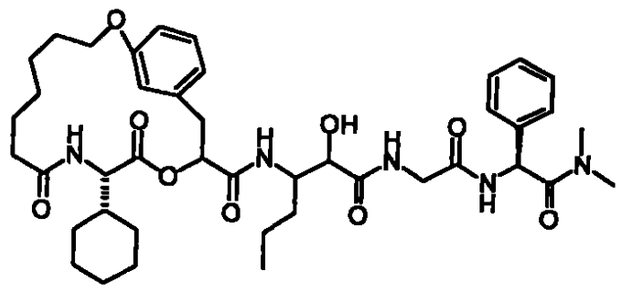
Paso E



36e



A

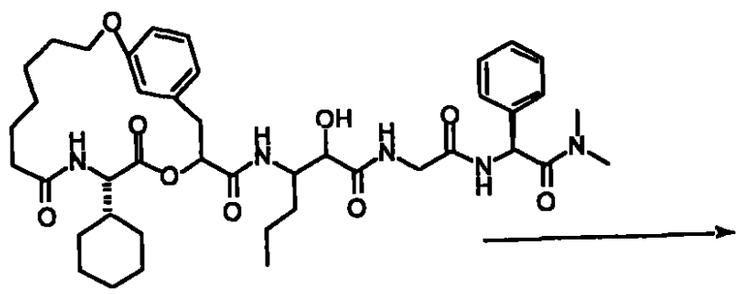


36f

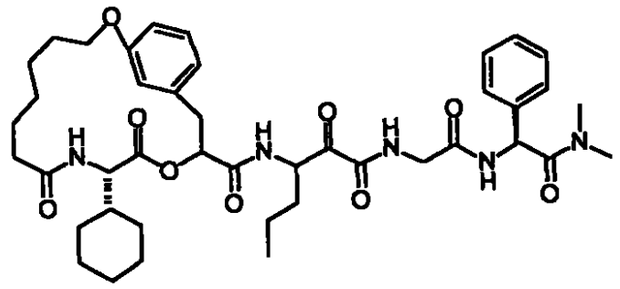
Una solución del ácido hidrolizado 36e (167 mg, 0,39 mmol) en CH_2Cl_2 (4,0 ml) fue tratada con HOOBt (95 mg, 0,58 mmol, 1,5 equiv) y enfriada a 0°C y se agregó base de Hünig (202 mg, 1,56 mmol, 4,0 equiv, 288 µl) A esta mezcla se agrego EDCI (111 mg, 0,58 mmol, 1,5 equiv) y la mezcla de reacción fue agitada a 0°C durante 0,5 h y tratada con el clorhidrato de amina (186 mg, 0,47 mmol, 1,20 equiv) La mezcla de reaccion fue guardada en un freezer por espacio de 24 h y concentrada al vacio para eliminar la DMF y el CH_2Cl_2 El residuo fue diluido con HCl ac (2M, 30 ml) y extraido con CH_2Cl_2 (3x50 ml) La capa orgánica

combinada fue extraida con HCl ac (2M, 30 ml), NaOH ac (1M), salina (2x50 ml), secada ($MgSO_4$) y concentrada al vacio El residuo 36f (100 mg) fue oxidado sin más purificación HRMS calc para $C_{42}H_{60}N_5O_9$ $(M+1)^+$ 778,4391, Encontrado 778,4399

Paso F



36f

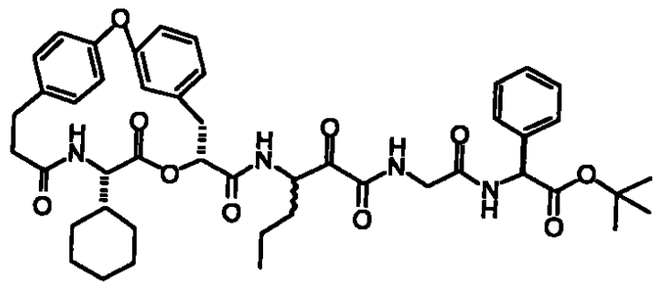


36

Una solución del alcohol 36f (100 mg, 0,13 mmol) en CH_2Cl_2 (5,0 ml) fue tratada con reactivo de Dess-Martin (100 mg, 0,0,23 mmol, 1,8 equiv) La mezcla de reacción fue agitada a ta durante 2 h y diluida con $NaHCO_3$ acuoso (15 ml) y $Na_2S_2O_3$ ac (15 ml) La mezcla de reaccion fue concentrada al vacio y el residuo fue purificado por cromatografía (SiO_2, acetona/Hexanos 3 7) para dar la cetoamida 36 (61 mg, 61 %) en forma de sólido incoloro, EM (FAB, NBA/DMSO, int rel m/z), 776 [$(M+1)^+$, 100], 731 (10), 598 (25), 570 (15), 485 (20), 358 (20), 247 (50)

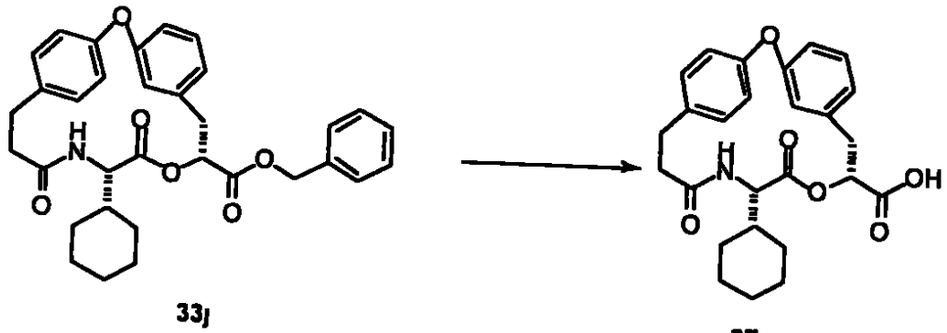
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Ejemplo 37 Preparación del Compuesto de la fórmula 37



37

Paso A

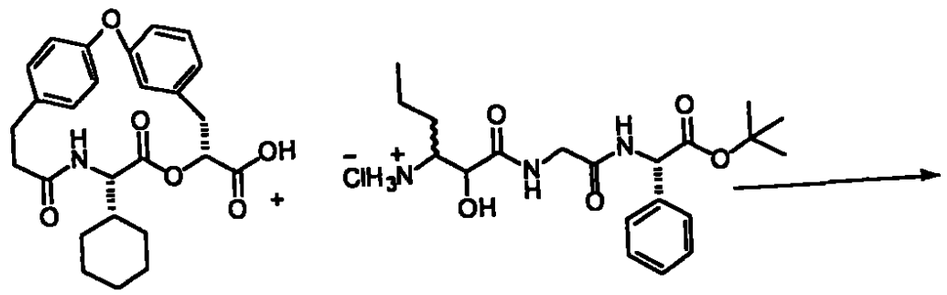


33j 37a

Una solución del ester bencílico 33i (230 mg, 0,42 mmol) en CH_3OH/EtOAc (1 1, 50 ml), fue tratada con $Pd(OH)_2$ e hidrogenada (50 psi, 344,7 kPa) por espacio de 12 h La mezcla de reacción fue filtrada a través de un cilindro de celite y la torta enjuagada con CH_3OH/CH_2Cl_2 (1 1, 50 ml) La mezcla de reaccion fue concentrada al vacio y el residuo 37a (177 mg, 93 %) fue utilizado para el acoplamiento sin purificacion

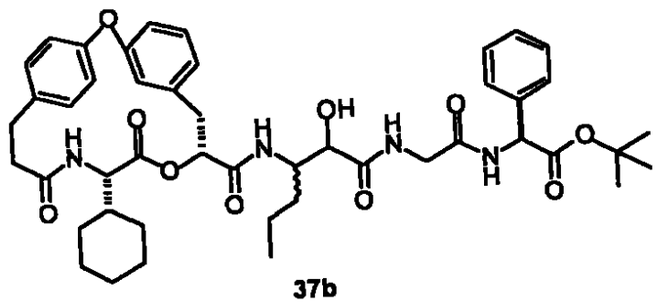
303

Paso B



37a

B

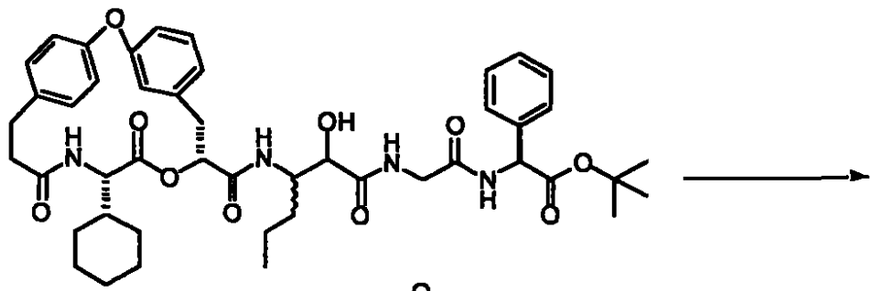


37b

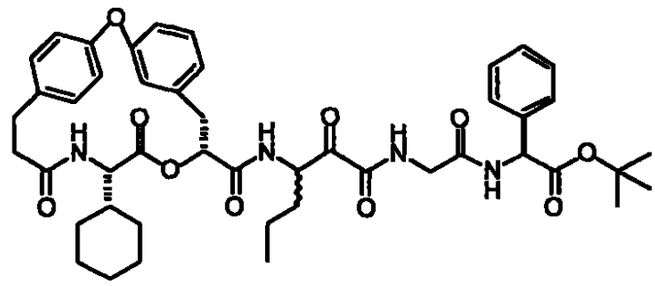
Una solucion del ácido 37a (177 mg, 0,33 mmol) en DMF seca (5,0 ml) y CH_2Cl_2 (5,0 ml) fue tratada con HOBt (88 mg, 0,49 mmol, 1,5 equiv) y enfriada a 0°C, tras lo cual se agrego base de Hünig (175 mg, 1,35 mmol, 4,0 equiv, 25 µl) A esta mezcla se agrego EDCI (95 mg, 0,49 mmol, 1,5 equiv) y la mezcla de reacción fue agitada a 0°C por espacio de 1 h y tratada con el clorhidrato de amina A (170 mg, 0,39 mmol, 1,2 equiv) La mezcla de reacción fue agitada a temperatura ambiente por un lapso de 48 h y concentrada al vacio para eliminar la DMF y el CH_2Cl_2 El residuo fue diluido con agua y extraido con CH_2Cl_2 (3x50 ml) Las capas organicas combinadas fueron extraidas con NaOH ac (1M, 2x50 ml), solucion salina (100 ml) y concentradas al vacio El residuo 37b (315 mg) fue oxidado sin más purificación

~~603~~ 304

Paso C



37b

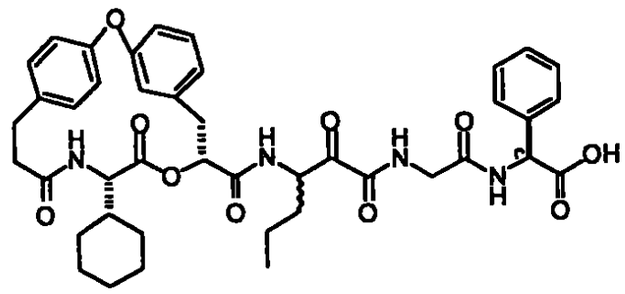


37

Una solucion del alcohol 37b (315 mg, 0,4 mmol) en CH_2Cl_2 (5,0 ml) fue tratada con reactivo de Dess-Martin (424 mg, 1,00 mmol, 2,5 equiv) La mezcla de reacción fue agitada a ta durante 1 h y diluida con $NaHCO_3$ ac (50 ml) y $Na_2S_2O_3$ ac (50 ml) La mezcla de reacción fue agitda a ta durante 20 min y extraida con CH_2Cl_2 (3x50 ml) Las capas orgánicas combinadas fuen extraidas con salina, secadas (Na_2SO_4), filtradas, concentradas al vacio y el residuo fue purificado por cromatografia (SiO_2, EtOAc/Hexanos 1 1) para dar la cetoamida 37 (210 mg, 66 %) en forma de solido incoloro, Rf 0,63 (CH_3OH/CH_2Cl_2 1 19) EM (Electroatomización, int rel m/z), 857 [$(M+CH_3OH]^+$, 33), 825 ([$M+1]^+$ 40), 91 (100)

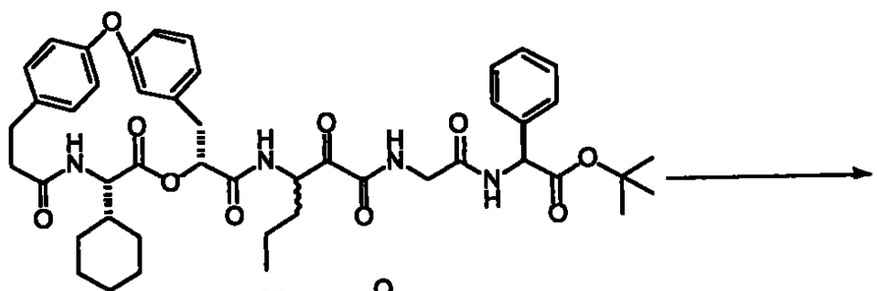
0,389 305

Ejemplo 38 Preparacion del Compuesto de la fórmula 38

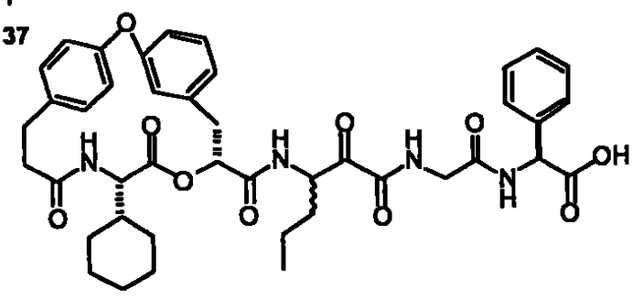


38

Paso A



37

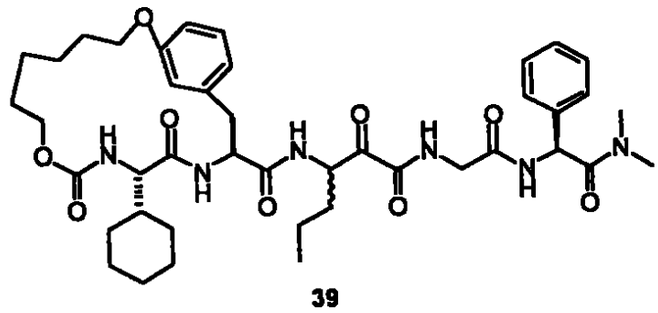


38

Una solución del depsipeptido oxidado 37 (200 mg, 0,24 mmol) en CH_2Cl_2 seco (5,0 ml) fue tratada con ATF (5,0 ml) y agitada a ta por espacio de 7 h La mezcla de reacción fue concentrada al vacio y el residuo disuelto reiteradamente en CH_3OH/CH_2Cl_2/Hexanos (1 1 1) y concentrado varias veces al vacio para dar un sólido de color tostado 38 (130 mg, 86 %) que fue secado al vacio EM (Electroatomización, int rel m/z) 769 [$(M+1)^+$, 45), 294 (45), 191 (100)

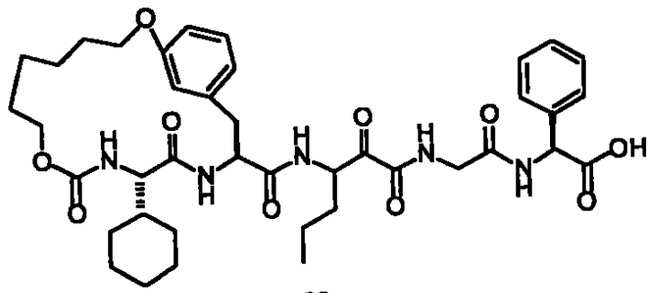
0,3,10 306

Ejemplo 39 Preparacion del Compuesto de la formula 39

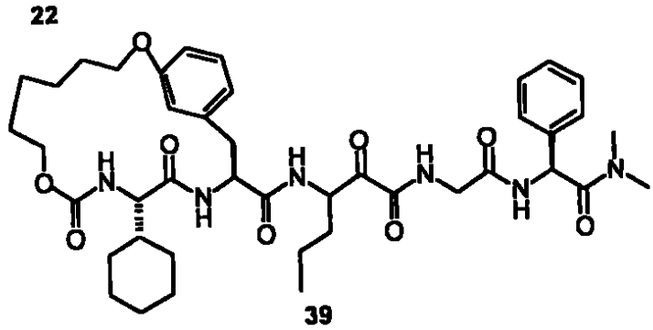


39

Paso A



22



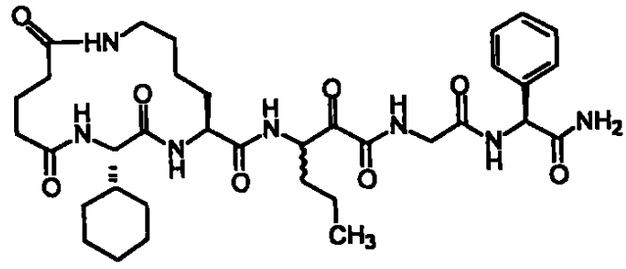
39

Una solución del ácido 22 (40 mg, 0,06 mmol) en CH_2Cl_2 (0,5 ml) y DMF (0,5 ml) fue enfriada a 0°C y tratada con Me_2NM HCl (15 mg, 0,18 mmol, 3,0 equiv) y base de Hünig (31 mg, 0,24 mmol, 44 µl, 4,0 equiv) A continuacion se trató a la mezcla de reacción con PyBrOP (55 mg, 0,12 mmol, 2,0 equiv) y se la guardo en el freezer por espacio de 12 h La mezcla de reaccion amarilla fue concentrada al vacio y el residuo fue purificado por cromatografia (SiO_2,

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EtOAc/Hexanos gradiente de 3 2 -→ 1 0) para obtener un producto impuro que fue purificado una vez mas utilizando (acetona/hexanos 1 6) para dar la dimetilamida 39 en forma de solido incoloro (14 mg, 35 %) EM (Eletroatomizacion, int rel m/z) 791, [(M+1)$^+$, 50], 391 (40), 276 (50), 176 (100)

Ejemplo 40 Preparacion del Compuesto de la formula 40



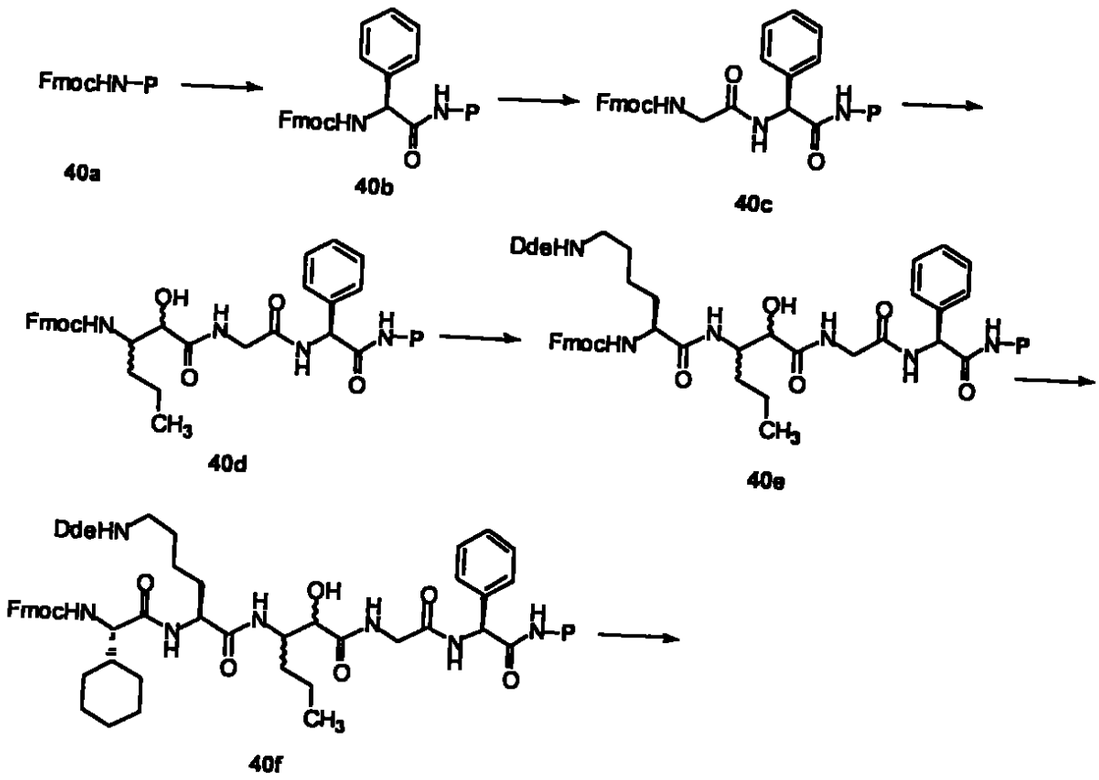
40

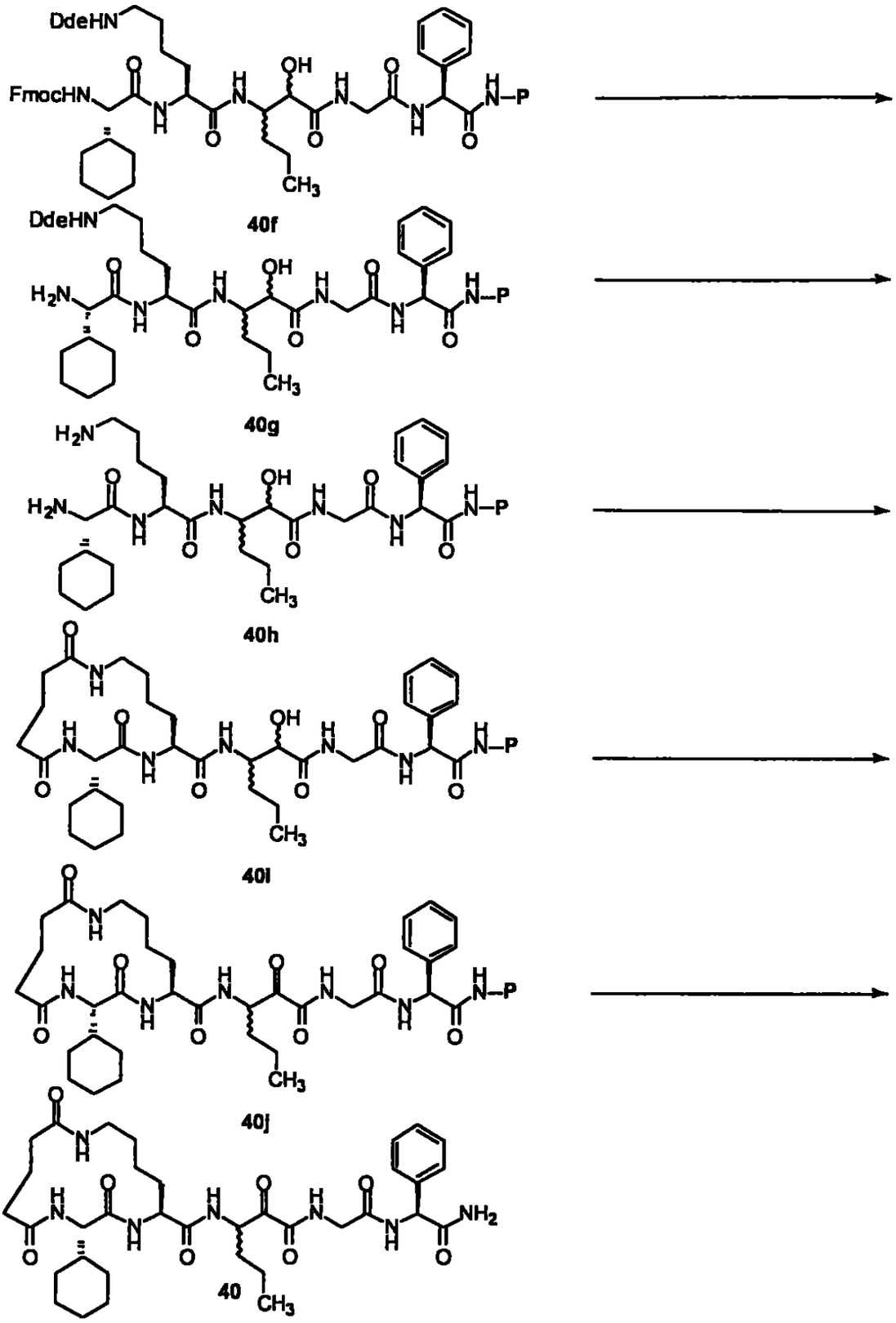
Procedimiento General para las reacciones de acoplamiento en fase solida

Se llevó a cabo la sintesis en un recipiente de reacción que fue construido con un cartucho de jeringa de polipropileno equipado con un disipador de polipropileno en el fondo Los aminoacidos protegidos con Fmoc fueron acoplados por técnicas normales de fase sólida Se cargo cada recipiente de reacción con 100 mg de la resina Fmoc-Sieber de partida (aproximadamente 0,035 mmol) Se lavó la resina con porciones de 2 ml de DMF (2 veces) El grupo protegido con Fmoc fue eliminado mediante tratamiento con 2 ml de una solución al 20 % v/v de pipendina en DMF por espacio de 20 min Se lavó la resina con porciones de 2 ml de DMF (4 veces) El acoplamiento se llevo a cabo en DMF (2 ml), utilizando 0,12 mmol de Fmoc-aminoacido, 0,12 mmol de HATU y 0,24 mmol de DIPEA. Despues de agitar durante 2 h, se dreno el recipiente de reaccion y se lavó la resina con porciones de 2 ml de DMF (4 veces) Se repitió el ciclo de acoplamiento con el siguiente Fmoc-aminoácido o grupo de casquete

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308

Esquema 10




DdeHN
FmocHN
OH
N–P
CH3
40f
DdeHN
H2N
OH
N–P
CH3
40g
H2N
H2N
OH
N–P
CH3
40h
OH
N–P
CH3
40i
N–P
CH3
40j
NH2
CH3
40

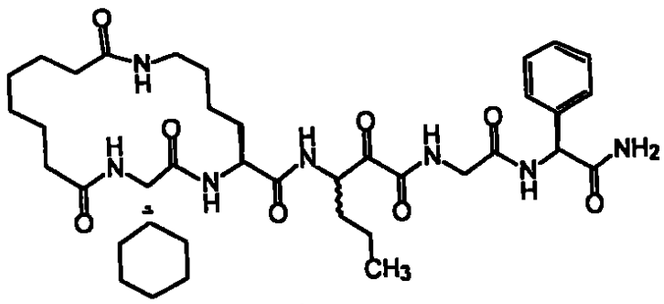
03/4
310

Se trato a la resina Sieber con Fmoc 40a (0,0365 mmol) con 2 ml de una solucionsolucion al 20 % v/v de pipendina en DMF durante 20 min, tras lo cual se lavó con porciones de 2 ml de DMF (4 veces) Se adicionó DMF (2 ml) a la resina, seguida por Fmoc-fneilglicina (0,12 mmol), HATU (0,12 mmol) y DIPEA (0,24 mmol) Despues de agitar a temperatura ambiente por espacio de 2 h, se lavo la resina con porciones de 2 ml de DMF (4 veces) para producir un compuesto unido a la resina 40b El compuesto unido a la resina 40b fue tratado con 2 ml de una solucion al 20 % v/v de pipendina en DMF durante 20 min, seguida por lavado con porciones de 2 ml de DMF (4 veces) Se adicionó DMF a la resina, seguida por Fmoc-glicina (0,12 mmol), HATU (0,12 mmol) y DIPEA (0,24 mmol) Despues de agitar a temperatura ambiente por espacio de 2 h, la resina fue lavada con porciones de 2 ml de DMF (4 veces) para producir el compuesto unido a la resina 40c Se trato al compuesto unido a la resina 40c con 2 ml de una solucion al 20 % v/v de pipendina en DMF durante 20 min, seguido por lavado con porciones de 2 ml de DMF (4 veces) Se adicionó DMF (2 ml) a la resina, seguida por N-Fmoc-propilisoserina (0,12 mmol), HATU (0,12 mmol) y DIPEA (0,24 mmol) Después de agitar a temperatura ambiente por espacio de 12 h, se lavo la resina con porciones de 2 ml de {DMF (4 veces) para producir el compuesto unido a la resina 40d Se trató al compuesto unido a la resina 40d con 2 ml de una solución al 20 % v/v de pipendina en DMF durante 20 min, seguido por lavado con porciones de 2 ml de DMF (4 veces) Se adiciono DMF (2 ml) a la resina, seguida por Fmoc-lisina(Dde) (0,12 mmol), HATU (0,12 mmol) y DIPEA (0,24 mmol) Después de agitar a temperatura ambiente por espacio de 12 h, se lavo la resina con porciones de 2 ml de DMF (4 veces) para producir el compuesto unido a la resina 40e Se trato al compuesto unido a la resina 40e con 2 ml de una solucion al 20 % v/v de pipendina en DMF durante 20 min, seguido por lavado con porciones de 2 ml de DMF (4 veces) Se adicionó DMF (2 ml) a la resina, seguida por Fmoc-

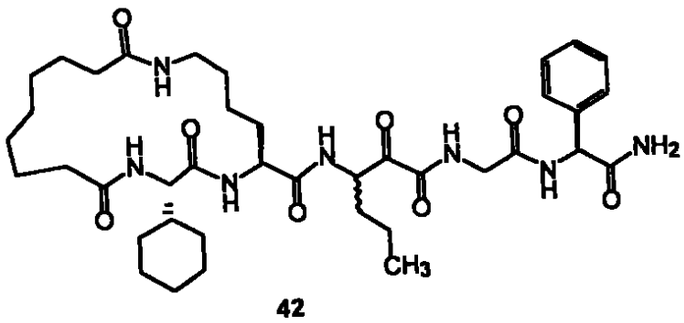
ciclohexilglicina (0,12 mmol), HATU (0,12 mmol) y DIPEA (0,24 mmol) Después de agitar a temperatura ambiente por espacio de 12 h, se lavó la resina con porciones de 2 ml de DMF (4 veces) para producir el compuesto unido a la resina 40f Se trató al compuesto unido a la resina 40f con 2 ml de una solución al 20 % v/v de piperidina en DMF durante 20 min Se lavó la resina con porciones de 2 ml de DMF (4 veces) para producir el compuesto unido a la resina 40g Se trato al compuesto unido a la resina 40g con porciones de 2 ml de una solución al 2 % v/v de hidrazina en DMF durante 5 min (3 veces) Se lavo la resina con porciones de 2 ml de DMF(4 veces) para producir el compuesto unido al a resina 40h Se trato al compuesto unido a la resina 40h con ácido glutárico 0,035 mmol, HATU 0,07 mmol y DIPEA 0,14 mmol en 2 ml de DMF a temperatura ambiente por espacio de 16 h Se lavó la resina con porciones de 2 ml de DMF (4 veces), THF (4 veces) y DCM (4 veces) para producir el compuesto unido a la resina 40i Se trato al compuesto unido a la resina 40i con una solución de periodinano de Dess-Martin 0,14 mmol y t-BuOH 0,14 mmol en 2 ml de DCM a temperatura ambiente por espacio de r h Se lavo la resina con porciones de 2 ml de una solucion al 20 % v/v de iPrOH en DCM, THF, una solución al 50 % v/v de THF en agua (4 veces), THF (4 veces) y DCM (4 veces) para producir el compuesto unido a la resina 40j El compuesto unido a la resina 40j fue tratado con 4 ml de una solucion al 2 % v/v de ATF en DCM durante 5 min Se adicionó el filtrado a 1 ml de AcOH y la solucion fue concentrada por centrifugación al vacio para dar el compuesto 40 (0,0117 g, 48 % de rendimiento) EM (LCMS-Electroatomización) 698,2 MH^+

Ejemplo 41 – 53 Preparación de los Compuestos de las Formulas 41 a 53

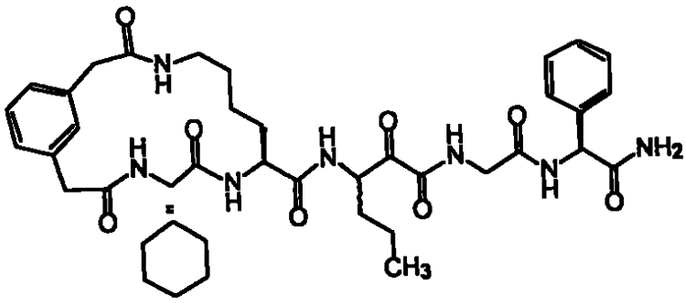
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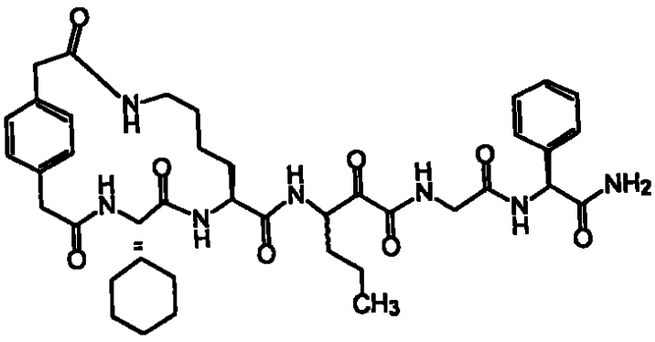
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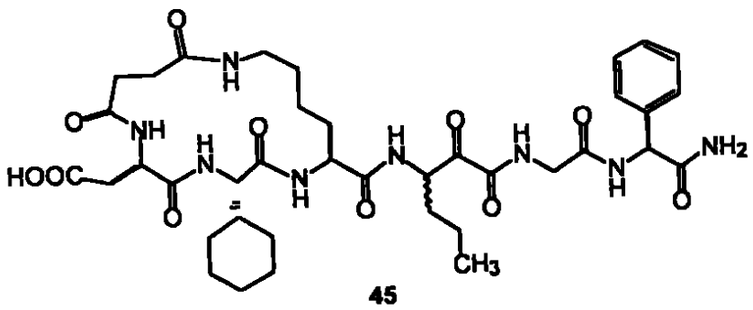


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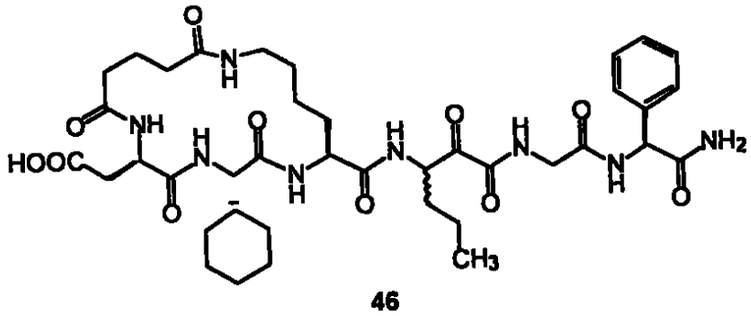


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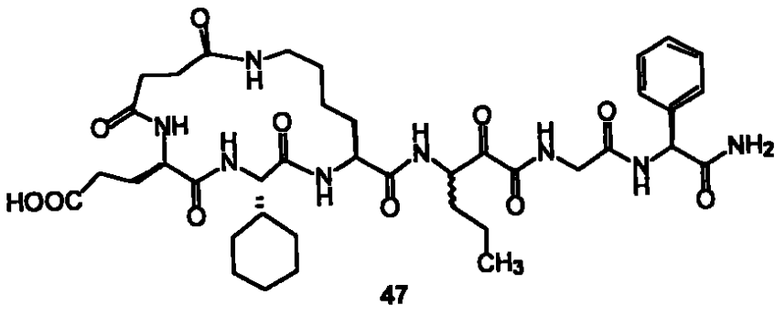
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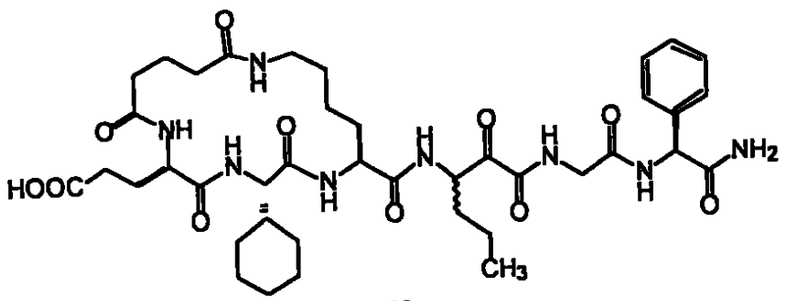
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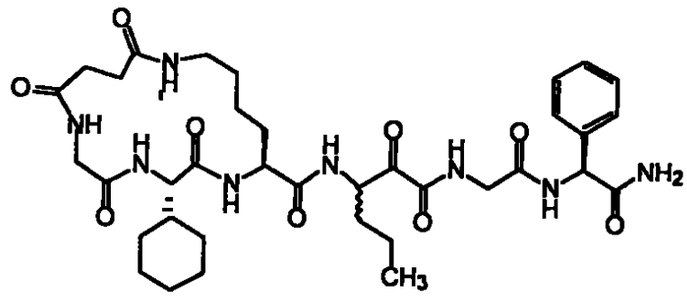


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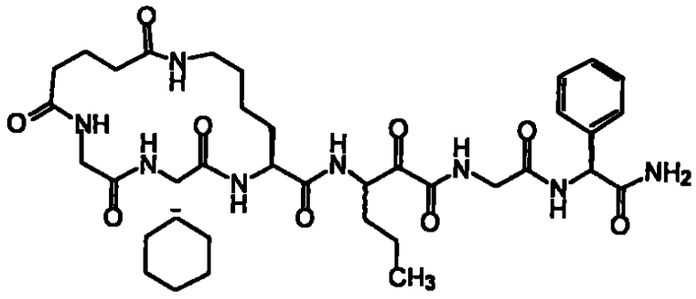


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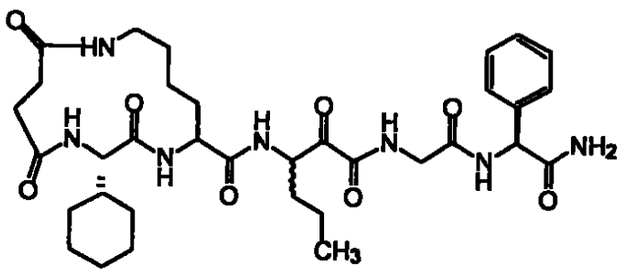
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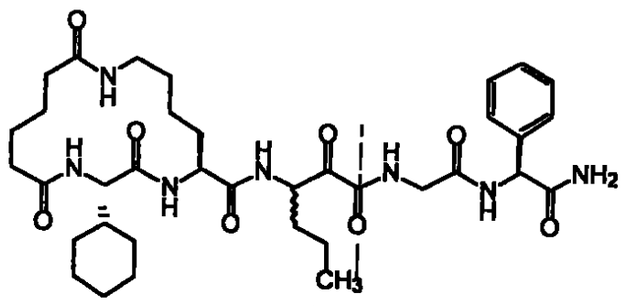
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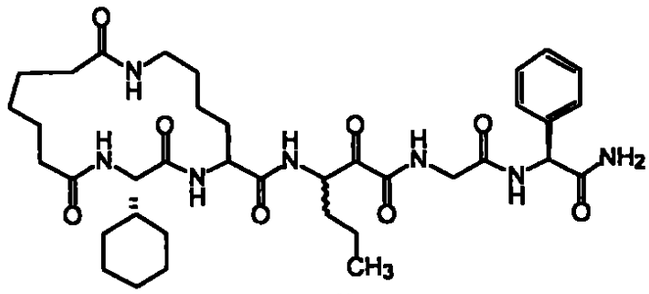


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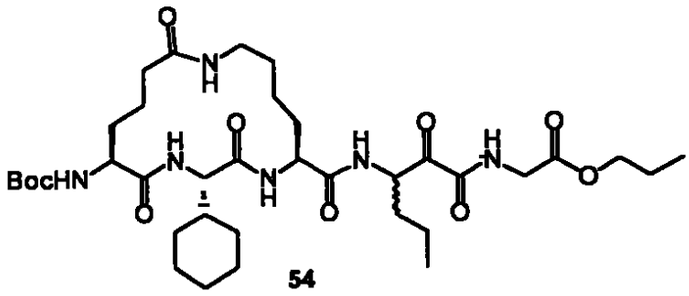
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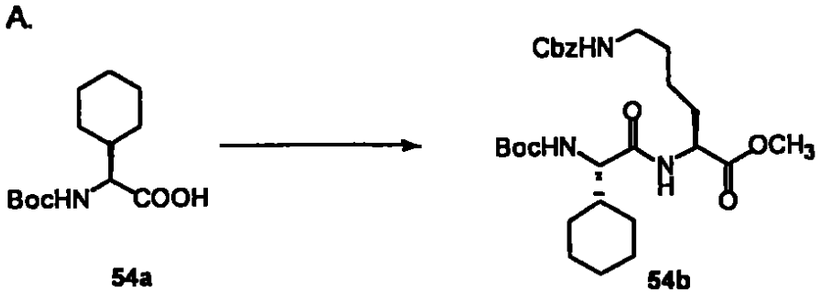
Los compuestos 41 – 53 fueron sintetizados utilizando una metodologia en fase solida similar al procedimiento reseñado para la sintesis del Ejemplo 40

Ejemplo 54 Preparacion del Compuesto de la Fórmula 54



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Paso A.



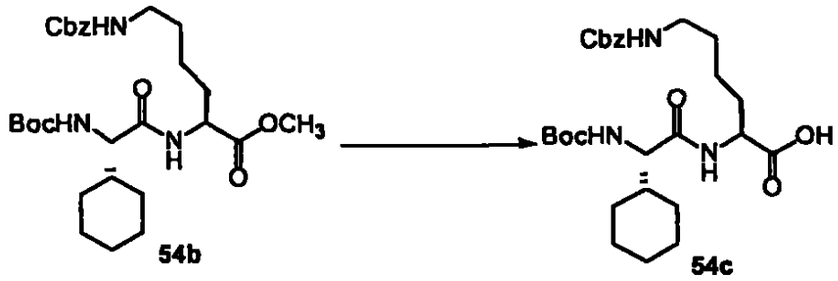
54a 54b

A una solución agitada de Boc-Ciclohexilglicina-OH (2,33 g, 9,07 mmol) en DMF (20 ml) y CH_2Cl_2 (20 ml) se adicionó HOBT (1,48 g, 9,07 mmol), EDCI (1,91 g, 9,97 mmol) y NMM (2,99 ml, 27,2 mmol La solución fue agitada a –20°C durante 10 minutos, para luego adicionar H-Lys(Z)-OMe HCl y agitar durante media hora a -20°C y mantener en el freezer durante la noche Luego se

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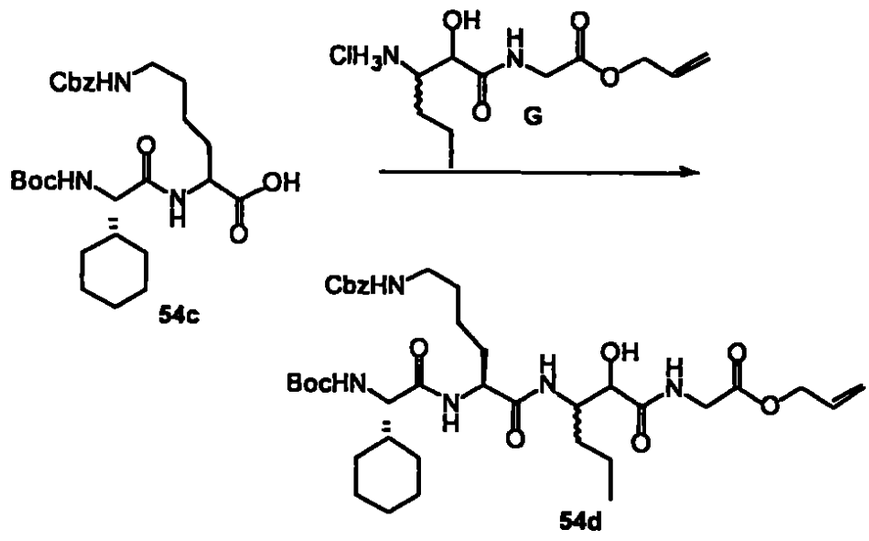
concentró la solución a sequedad, y luego se la extrajo con EtOAc, $NaHCO_3$ saturado Se lavó la capa organica combinada con H_2O, se la seco sobre Na_2SO_4, y se la concentró a sequedad para dar un solido blanco (4,83 g, MH^+ = 534,1)

Paso B



A una solución agitada de 54b (4,86 g, 8,76 mmol) en MeOH (10 ml) y H_2O (7 ml) se adiciono LiOH (70 mg, 11,4 mmol) Se formo un precipitado blanco y se dejo agitar la solución a temperatura ambiente durante la noche y luego se la concentró a sequedad Luego se repartió el material crudo entre CH_2Cl_2 y agua Se separó la capa orgánica y se la lavo con salina, se la seco sobre Na_2SO_4,, se la filtró y concentró a sequedad para dar 65c (4,55 g, MH^+ = 520,1)

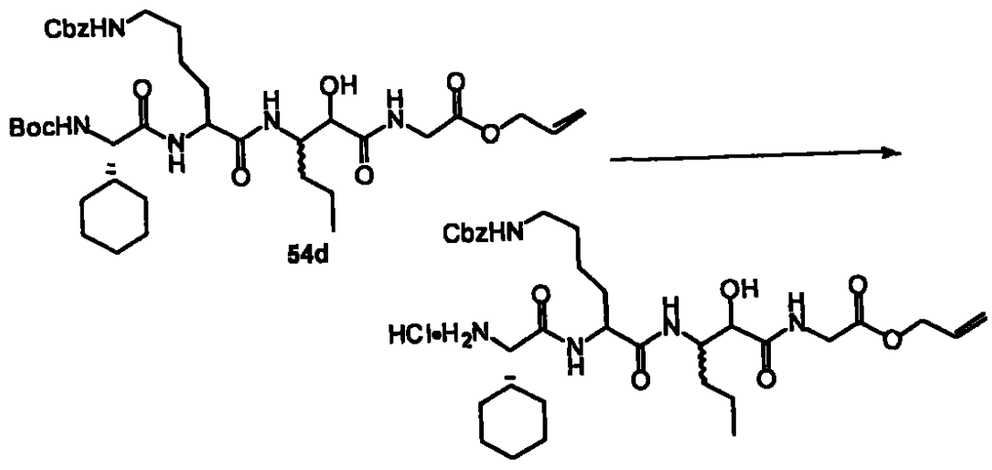
Paso C



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A una solución agitada al frio de 54c (4,3 g, 8,27 mmol) en DMf (40 ml) y CH_2Cl_2 (40 ml) a –20°C se adicionó HOBT (1,35 g, 8,27 mmol), EDCI (1,74 g, 9,1 mmol) y NMM (2,73 ml, 8,27 mmol La solucion resultante fue agitada a –20°C durante 10 minutos, para luego adicionar la amina G (2,32 g, 8,27 mmol) y agitar durante media hora a -20°C y mantener en el freezer durante la noche Se siguió el procedimiento de estimulacion del proceso del paso A para dar 54d (6,21 g, MH^+ = 746,2)

Paso D

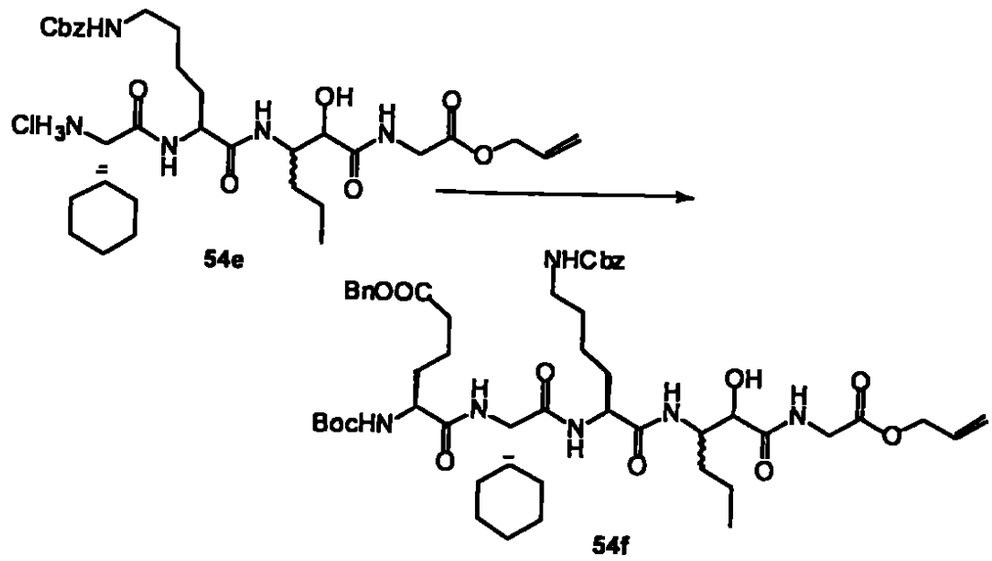


54e

Se agito la solución de 54d (6,16 g, 8,26 mmol) en HCl/Dioxano 4N (40 ml) a temperatura ambiente por espacio de 1 h y se la concentro a sequedad para dar un producto crudo 54e (5,70 g, 100 % de rendimiento, MH^+ = 646,3)

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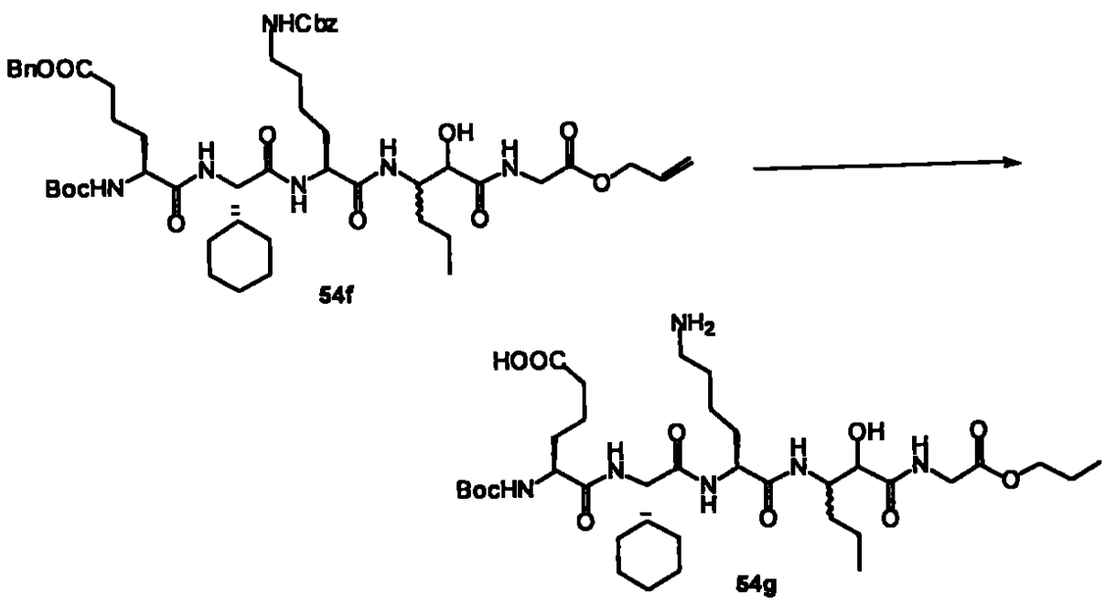
Paso E



A una solución agitada al frio de Boc-Glu(OBn)-OH en DMF (25 ml) y CH_2Cl_2 (25 ml) a –20°C se adicionó HOBT (1,29 g, 7,92 mmol), EDCI (1,66 g, 8,71 mmol) y NMM (2,61 ml, 23,7 mmol La solucion resultante fue agitada a –20°C durante 10 minutos, para luego adicionar 54e (5,4 g, 7,916 mmol) y agitar durante media hora a -20°C y mantener en el freezer durante la noche Se siguio el procedimiento de estimulación del proceso del paso A para dar un producto crudo (7,14 g, 93,5 % de rendimiento

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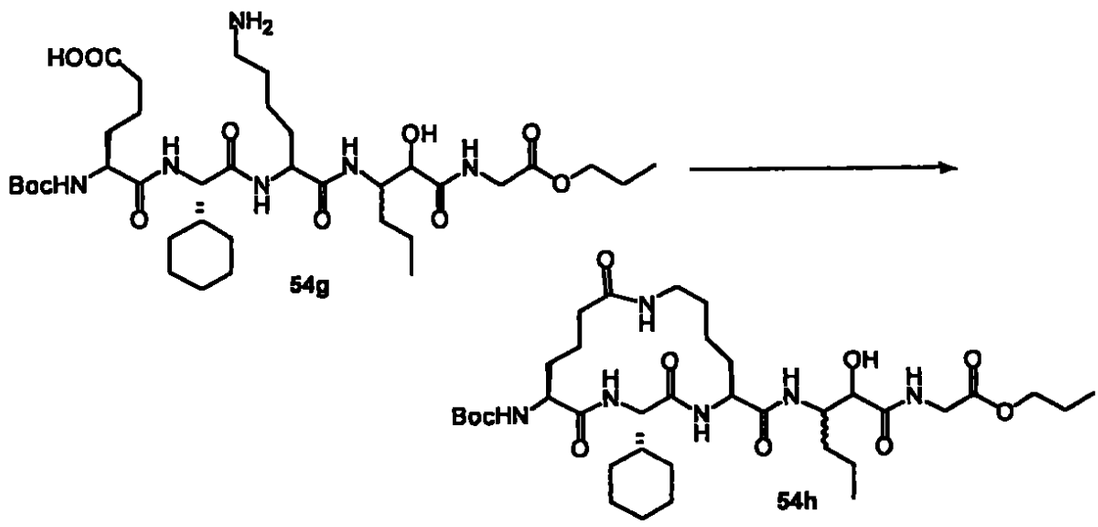
Paso F



A una solucion agitada de 54f (6,9 g, 7,15 mmol) en EtOH absoluto (350 ml) se agregó Pd/C al 10 % (2,8 g) en 50 % de H_2O (p/p) La solucion resultante fue purgada con H_2 y agitada bajo un balon de H_2 durante la noche Luego se filtró la solucion a través de celite y se lavó el filtrado con EtOH/CH_2Cl_2 y luego se lo concentró a sequedad para dar un sólido blanco (1,44 g) Se lavó el sólido con H_2O al 25 %/MeOH y se lo filtro a través de un embudo de vidrio sinterizado, luego se lo congeló y liofilizó para dar 54g (4,12 g, 77,5 % de rendimiento, MH^+ = 743,2)

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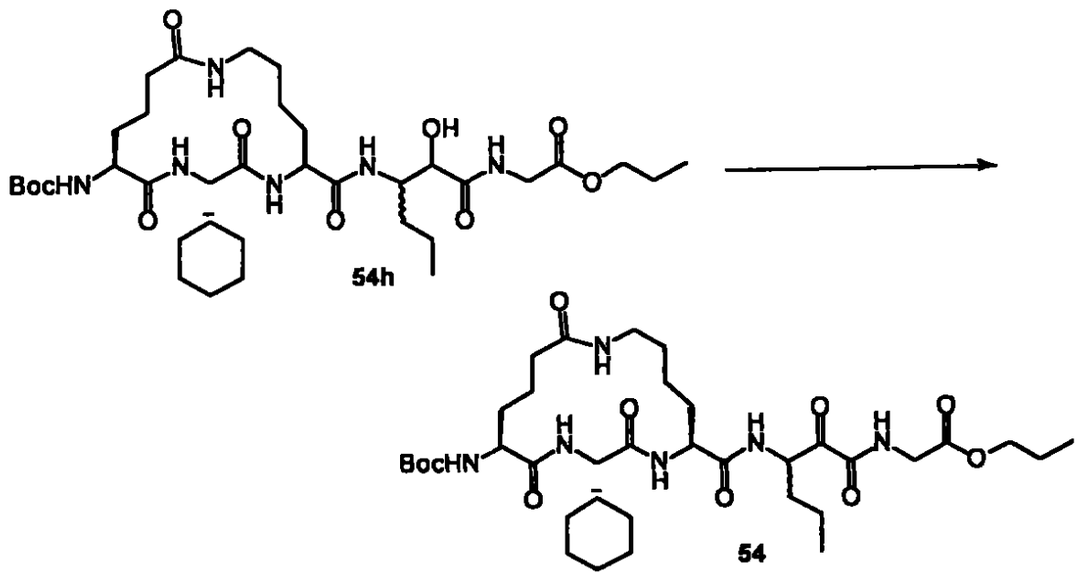
Paso G



A una solución agitada al frio de 54g (0,5 g, 6,7 mmol) en DMf (50 ml) y CH_2Cl_2 (50 ml) a –20°C se adicionó HOBT (0,219 g, 1,34 mmol), EDCI (0,271 g, 1,41 mmol) y NMM (0,296 ml, 2,69 mmol) La solución resultante fue agitada a –20°C durante 25 minutos y luego se la guardo en el freezer durante la noche La solución fue concentrada a sequedad, y luego se la extrajo con EtOAc y $NaHCO_3$ saturado A continuación se concentró a sequedad la capa orgánica combinada para dar 54h (254 mg, MH^+ = 725,2) Se siguio el procedimiento de estimulación del proceso del paso A para dar 54d)

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Paso H



A una solución agitada de 54h (0,2 g, 0,27 mmol) en CH_2Cl_2 anhidro (20 ml) se adicionó periodinano de Dess-Martin (0,234 g, 0,55 mmol) La solución resultante fue agitada a temperatura ambiente durante 1 h A esta solución se agrego por goteo, en el curso de media hora, la solución de H_2O (0,010 ml) en CH_2Cl_2 (20 ml) y se la agito vigorosamente durante 2 horas más A continuación se agitó la solucion durante media hora con 50 % de $Na_2S_2O_3$ y 50 % de $NaHCO_3$ saturado Se separó la capa orgánica, que fue lavada con H_2O, salina, secada sobre Na_2SO_4, filtrada, concentrada a sequedad y purificada por cromatografía en columna sobre gel de silice, eluyendo con MeOH al 10 %/ CH_2Cl_2 para dar 54 (17 mg, 62 % MH^+ = 723,2)

Ensayo para Determinar la Actividad Inhibitoria de la Proteasa de VHC

Análisis Espectrofotometrico Se llevaron a cabo análisis espectrofotometricos para la serina proteasa de VHC en los compuestos de la presente invención siguiendo el procedimiento descripto por R Zhang y otros, *Analytical Biochemistry*, 270 (1999) 268-275, la descripción de la cual se incorpora a la presente como referencia El analisis basado en la proteolisis de los sustratos de

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ester cromogénico es adecuado para el monitoreao continuo de la actividad de la proteasa de VHC NS3 Los sustratos fueron obtenidos del extremo P de la secuencia de empalme NS5A-NS5B (Ac-DTEDVVX(Nva), donde X = A o P), cuyos grupos carboxilo C-terminales se esterifican con uno de cuatro alcoholes cromoforicos diferentes (3- o 4-nitrofenol, 7-hidroxi-4-metil-cumarina o 4-fenilazofenol) Más adelante se presenta la sintesis, caracterizacion y aplicación de estos novedosos sustratos éster espectrofotométricos al rastreo de alto rendimiento y la evaluacion cinetica detallada de los inhibidores de la proteasa del VHC NS3

Materiales y Métodos

Materiales Los reactivos quimicos para los buffers relacionados con el ensayo fueron obtenidos de Sigma Chemical Company (St Louis, Missouri) Los reactivos para la síntesis de péptidos eran de Aldrich Chemicals, Novabiochem (San Diego, California), Applied Biosystems (Foster City, California) y Perseptive Biosystems (Framingham, Massachussets) Los péptidos fueron sintetizados a mano o en un sintetizador automático de péptidos ABI modelo 431A (de Applied Biosystems) El Espectrometro UV&VIS modelo LAMBDA 12 era de Perkin Elmer (Norwalk, Connecticut) y las placas de UV de 96 receptáculos fueron adquiridas a Corning (Corning, Nueva York) El bloque de precalentamiento era de USA Scientific (Ocala, Florida) y el generador de vórtice con placa de 96 receptáculo era de Labline Instruments (Melrose Park, Illinois) Se obtuvo un lector de placas microtituladoras Spectramax Plus con nomocrómetro de Molecular Devices (Sunnyvale, California)

Preparación de las Enzimas Se preparó proteasa de VHC NS3/NS4A heterodimerica recombinante (cepa 1a) utilizando los procedimientos publicados anteriormente (D L Sali y otros, *Biochemistry*, 37 (1998) 3392-3401) Se determinaron las concentraciones proteicas por medio del método de tincion de

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Biorad utilizando patrones de proteasa de VHC recombinante previamente cuantificada por análisis de aminoácidos Antes de dar comienzo al ensayo, se intercambió el buffer de almacenamiento de enzimas (fosfato de sodio pH 8,0 50 mM, NaCl 300 mM, glicerol al 10 %, lauril maltosido al 0,05 % y DTT 10 mM) por el buffer de ensayo (MOPS pH 6,5 25 mM, NaCl 300 mM, glicerol al 10 %, lauril maltosido al 0,05 %, EDTA 5 µM y DTT 5 µM) utilizando una columna preempaquetada Biorad Bio-Spin P-6

Sintesis y Purificacion de los Sustratos La sintesis de los sustratos se llevó a cabo de la manera dada a conocer por R Zhang y otros (Ibid) y la misma se inicio mediante el anclaje de Fmoc-Nva OH a una resina de cloruro de 2-clorotritilo utilizando un protocolo standard (K Barlos y otros, *Int J Pept Protein Res* 37 (1991), 513-520) Seguidamente, los péptidos fueron acoplados, ya sea manualmente o en un sintetizador automatico de peptidos ABI modelo 431 Los fragmentos peptidicos N-acetilados y completamente protegidos fueron escindidos de la resina con ácido acético al 10 % (HOAC) y trifluoroetanol (TFE) al 10 % en diclorometano (DCM) por espacio de 30 min, o bien con ácido trifluoroacético (ATF) al 2 % en DCM durante 10 min El filtrado combinado y el lavado con DCM fueron evaporados azeotrópicamente (o extraídos repetidamente con una solución acuosa de Na_2CO_3) para eliminar el ácido utilizado en la escisión Se secó la fase de DCM sobre Na_2SO_4, y se la evaporó

Los sustratos de ésteres fueron acoplados utilizando procedimientos de acoplamiento standard de ácido-alcohol (K Holmber y otros, *Acta Chem Scand*, B33 (1979) 410-412) Los fragmentos peptidicos fueron disueltos en piridina anhidro (30-60 mg/ml) a la cual se agregaron 10 equivalentes molares de cromóforo y una cantidad catalitica (0,1 eq) de ácido para-toluensulfónico (pTSA) Se adiciono diciclohexilcarbodiimida (DCC, 3 eq) para iniciar las reacciones de acoplamiento La formación de productos fue monitoreada por HLC y se encontro

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que se habia completado despues de 12-72 horas de reaccion a temperatura ambiente Se evaporo el solvente piridina al vacio y se la eliminó mas a fondo por evaporación azeotrópica con tolueno El éster peptidico fue desprotegido con ARF al 95 % en DCM durante dos horas y se lo extrajo tres veces con eter etilico anhidro para eliminar el exceso de cromoforo El sustrato desprotegido fue purificado por HPLC de fase inversa en una columna C3 o C8 con un gradiente de acetonitrilo de 30 % a 60 % (utilizando seis volumenes de columna) El rendimiento general después de la purificación por HPLC fue de aproximadamente 20-30 % Se confirmó la masa molecular por espectroscopia de masa por ionización por electroatomizacion Los sustratos fueron almacenados desecados en forma de polvo seco

Espectros de los Sustratos y los Productos Se obtuvieron los espectros de los sustratos y los correspondientes productos cromoforicos en el buffer de ensayo de pH 6,5 Se determinaron los coeficientes de extincion a la longitud de onda optima alejada de los picos en cubetas de 1 cm (340 nm para 3-Np y HMC, 370 nm para PAP y 400 nm para 4-Np) utilizando diluciones multiples La longitud óptima alejada de los picos se definió como la longitud de onda que da la máxima diferencia fraccionaria de absorbancia entre el sustrato y el producto (DO producto – DO del sustrato)/ DO del sustrato)

Ensayo de la Proteasa Se realizaron ensayos de proteasa de VHC a 30°C utilizando una mezcla de reacción en una placa microtituladora de 96 receptaculos Las condiciones del buffer de ensayo (MOPS 25 mM, pH 6,5, NaCl 300 mM, glicerol 10 %, lauril maltosido 0,05 %, EDTA 5 µM y DTT 5 µM) fueron optimizadas para el heterodimero NS3/NS4A (D L Sali y otros, ibidem)) Tipicamente, se colocaron 150 µl de mezclas de buffer, sustrato e inhibidor en los receptáculos (concentracion final de DMSO 4 % v/v) y se las dejó preincubar a 30°C por espacio de aproximadamente 3 minutos Luego se utilizaron cincuenta µl

de proteasa precalentada (12 nM, 30°C) en buffer de ensayo para dar comienzo a la reacción (volumen final 200 µl) Las placas fueron monitoreadas durante toda la duración del ensayo (60 minutos) para detectar los cambios de absorbancia a la longitud de onda indicada (340 nm para 3-Np y HMC, 370 nm para PAP y 400 nm para 4-Np) utilizando un lector de placas microtituladoras Spectromax Plus equipado con un monocrometro (se pueden obtener resultados aceptables con lectores de placas que utilizan filtros de corte) La escisión proteolítica del lincaje de esteres entre el Nva y el cromóforo fue monitoreada a la longitud de onda apropiada contra un testigo sin enzima como control para determinar la hidrolisis no enzimática La evaluación de los parametros cinéticos del sustrato se llevo a cabo en un rango de concentraciones de sustrato de 30 veces (~6-200 µM) Se determinaron las velocidades iniciales utilizando regresion lineal y constantes cinéticas ajustando los datos a la ecuación de Michaelis-Menten empleando un análisis de regresión no lineal (Mac Curve Fit 1 1, K Raner) Los numeros de recambio (k_{cat}) fueron calculados sobre la presunción de que la enzima era totalmente activa

Evaluación de los inhibidores e inactivadores Se determinaron las constantes de inhibicion (K_i) para los inhibidores competitivos Ac-D-(D-Gla)-L-I-(Cha)-C-OH (27), Ac-DTEDVVA(Nva)-OH y Ac-DTEDVVP(Nva)-OH experimentalmente en concentraciones fijas de enzima y sustrato representando v_o/v_i versus la concentración del inhibidor ($[I]_o$) de acuerdo con la ecuación reordenada de Mchaelis-Menten para determinar la cinética de inhibición competitiva $v_o/v_i = 1 + [I]_o/(K_i (1 + [S]_o /K_m))$, en la cual v_o es la velocidad inicial sin inhibición, v_i es la velocidad inicial en presencia de un inhibidor en cualquier concentración de inhibidor dada ($[I]_o$) y $[S]_o$ es la concentracion del sustrato utilizado Los datos asi obtenidos fueron ajustados utilizando regresion lineal y se utilizó la pendiente resultante, $1/(K_i(1+[S]_o/K_m)$ para calcular el valor K_i

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Los valores K_i obtenidos para los diversos macrociclos de la presente invención están consignados en la Tabla 1 antes mencionada, donde los compuestos han sido dispuestos en el orden de progresion de valores K_i De estos resultados de los ensayos, resultaria evidente a la persona capacitada en la tecnica que los compuestos de la presente invencion son de gran utilidad como inhibidores de la serina proteasa NS3

Método de Bioensayo Celular

Los bioensayos celulares para detectar la serina proteasa de VHC se llevaron a cabo sobre los compuestos de la invencion siguiendo el procedimiento descripto por S Agrawal y otros, "Development and Characterization of Hepatitis C Virus Serine Portease Cell-based Trans-Cleavage Assay", *Hepatology* Suplemento del Tomo 30 (No 4, Parte 2, octubre de 1999), *Abstract No* 615 (Acta de la 50ª Reunión Anual de AASLD, Dallas, Texas, 5-9 de noviembre de 1999), descripciones que se incorporan a la presente como referencia El ensayo se llevó a cabo en células HeLa/Huh7 que habian sido co-transfectadas con un plasmido que expresa un sustrato proteico reportero que contenia una secuencia de reconocimiento de segmentación NS5A/5B y un vector de expresión 1BNS4A$_{21-32}$GS-GSNS$_{3-81}$I17K y YFPn1 como proteina patrón interno para el control de la citotoxicidad Se midió la actividad de proteasa por medio de SDS-PAGE de los lisados celulares completos seguida por deteccion por transferencia Western utilizando un anticuerpo monoclonal dirigido contra el sustrato reportero La cuantificación de la segmentación de los sustratos se realizo por barrido de la inmunotransferencia en el phosphoimager

Materiales

ADN Plasmidos

pBFP-5A/5B-GFP El gen reportero que expresa el sustrato codifica una proteina de fusion compuesta por un dominio de proteina fluorescente azul N'

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terminal (BFP) y un dominio de proteina fluorescente verde C'terminal (GFP), separados por 25 aminoacidos, derivados de la secuencia de reconocimiento de segmentacion NS5A/5B La GFP y la BFP son proteinas autofluorescentes esencialmente homologas que emiten luz verde o azul, respectivamente, cuando son excitadas por luz UV de la longitud de onda apropiada Cuatro sustituciones de aminoacidos en el cromoforo de GFP alteran la longitud de onda de emisión y convierten la proteina a BFP

Se puede detectar el sustrato, asi como los productos GFP y BFP obtenidos en los lisados celulares por metodos inmunologicos empleando un anticuerpo monoclonal que reconoce a ambas proteinas

El gen reportero BFP-5A/5B-GFP contiene las secuencias codificadoras de las proteinas autofluorescentes BFP y GFP (Quantum Biotechnologies, Inc, Montreal, Canada) separadas por la secuencia de reconocimiento de segmentación NS5A/5B, clonada entre los sitios de endonucleasa de restricción Nhe I y Bam HI del vector de clonación pQBI25 (Quantum Biotechnologies, Inc) La expresion de la proteína de fusión esta bajo el control del promotor-intensificador del CMV IE La secuencia de la hormona de crecimiento bovina p (A) del vector produce la señal de poliadenilacion para el ARNm La secuencia de segmentacion NS5A/5B es SSGADTDVVCCSMSYTWTGALVTP Se utilizó la secuenciación de ADN para validar el clon

P1BOO2 1bNS4A21-32GS NS 3-81 I17K Se clono el subtipo de proteasa 1b como fragmento Xba1/Not1 detrás del promotor CMV del vector pC1neo

YFPn1 Se adquirió YFPn1 a CLONTECH (Palo Alto, California) La adición del tercer plasmido a la transfección otorga una proteina patron interno para controlar la citotoxicidad y no afecta el porcentaje de escisión de proteasa

Los ADN plásmidos se mantuvieron y propagaron en celulas DH5α (obtenidas de Life Technologies) en medio LB bajo la selección apropiada de

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antibióticos, y fueron purificados utilizando QIAfilter Plasmid Kits (Qiagen, Valencia, California)

Cultivo Celular

Las células HeLa fueron mantenidas y propagadas en Medio Esencial Mínimo de Eagle (EMEM, BioWhittaker, Walkersville, Maryland) suplementado con suero fetal bovino al 10 % (SFB), glutamina 2 mM y 100 u/ml de penicilina-estreptomicina (BioWhittaker), $NaHCO_3$ al 2 %

Las células Huh7 fueron mantenidas y propagadas en medio Eagle Modificado de Dulbecco (DMEM, BioWhittaker) suplementado con suero fetal bovino al 10 % (SFB), 100 u/ml de penicilina-estreptomicina (BioWhittaker) y NEAA 5 ml (100x, BioWhittaker)/l

Procedimiento SOP

Dia anterior a la transfección

Se sembraron celulas HeLa en placas de 24 receptaculos (placas Falcon 3047) a una densidad de 6 x 10^4 células / receptáculo y se las cultivo durante la noche a 37°C en un incubador con 5 % de CO_2

Dia de la transfeccion

Se diluyeron ADN plásmidos hasta una concentración final de 0,05 µg/µl en agua sin nucleasa (Promega, Madison, Wisconsin, # cat P119C) Se combinó 0,75 µg de BFP-4A/5B-GFP y se lo mezclo con 0,175 µg de P1B002 (0,23X) y 0,02 µg de YFPn1 Se llevo a los ADN a un volumen final de 60 µl con EMEM sin SFB, glutamina y antibióticos Se adicionó una proporción de volumenes de 5 µl de Reactivo SuperFect (Qiagen, # cat 301305) por µg totales de ADN y se sometió a la mezcla a vórtice durante aproximadamente 10 segundos y se la incubó durante 10 min a temperatura ambiente para dar lugar a la formación del complejo Mientras tenia lugar la formacion del complejo, se aspiro el medio de cultivo de las placas de cultivo celular y se lavo a las células 1X co 1 ml de PBS

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sin Ca^{2+}, Mg^{2+} (Bio Whittaker) Se agregaron 350 µl de EMEM (suplementado con el medio de suplemento-terminacion) al tubo que contenia los complejos de transfeccion y se pipeteo la mezcla con movimiento recíproco 2-3 veces Se transfino el volumen total a uno de los 24 receptáculos de una placa de cultivo Las células HeLa fueron incubados con los complejos de transfeccion por espacio de aproximadamente 3 h a 37°C y con 5 % de CO2 Se retiraron de los receptaculos los medios que contenian los complejos de transfeccion por aspiración Las células fueron lavadas una vez en alrededor de 1 ml de PBS, se aspiró el PBS y se agregaron 495 µl de EMEM completo y luego 5 µl del compuesto por receptáculo Las células fueron incubadas durante 22-24 h a 37°C y con 5 % de CO2

Preparacion de los Lisados Celulares

Se aspiró el medio de cada receptáculo y se lo lavó una vez con DPBS Las células fueron cosechadas en 100 µl de 1x buffer de muestreo Tris-SDS-BME (sistema de separacion OWL, Portsmouth, Nueva Hampshire, # cat ER33) y transferidas a tubos de microcentrifugas Luego se las hirvió durante 3-5 min para lisar las células Se realizó la carga a razón de 10 µl/receptáculo en gel SDS-PAGE Los lisados fueron resueltos por electroforesis en SDS-PAGE al 12,5 % de 10 cm x 10 cm (Owl Scientific, # cat OG-0125B) operando a 30 mamp en buffer Tris-Glicina-SDS (Owl Scientific) Antes de su uso, se empapo una membrana PVDF (Immobilon-P, tamaño de poro 45 µm, Millipore, Bedford, Massachussets) en metanol al 100 % durante 10 segundos y luego se coloco la transferencia en agua destilada Las proteinas fueron transferidas a membranas de filtro PVDF (0,45 µm, Millipore) a 108 mamp por gel por espacio de 90 minutos utilizando una membrana de electrotransferencia semiseca

Detección de Proteinas por Western Blot ECF (Amersham Pharmacia Biotech, Little Chalfont, Inglaterra), # catálogo RPN 5780) Las membranas de

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filtro PVDF fueron bloqueadas por reactivo de bloqueo al 5 % (del kit) en ~ 10 ml de PBS que contenia Tween 20, pH 7,4 (Sigma Chemicals, St Louis, Missouri, # cat 3563) durante la noche en un refrigerador a 2-4C Al dia siguiente, las membranas fueron enjuagadas brevemente dos veces con TPBS que contenia 0,05 % de buffer de lavado Tween 20, luego se las lavó tres veces durante 5 min por vez en PBS que contenia 0,05 % de Tween 20, pH 7,4 Las membranas fueron incubadas en 12 ml de una solución 1 3000 de anticuerpo monoclonal anti-GFP durante 30 minutos (Clontech, Palo Alto, California) en PBS que contenia 05 % de Tween 20, pH 7,4 en tanto que, al mismo tiempo, se agregó 1 % de BSA (Seroalbumina bovina, # cat A-2153 de Sigma) para reducir el fondo Las membranas fueron lavadas dos veces brevemente con TPBS, luego tres veces durante 5 min por vez en buffer de lavado TPBS Las membranas fueron incubadas en 12 ml de Ig anti raton anti-fluoresceina en una dilución de 1 6000 en TPBS por espacio de 30 minutos Las membranas fueron lavadas brevemente dos veces con TPBS, luego durante 5 min en buffer de lavado TPBS tres veces Para la amplificación de señal con sustrato ECF, las membranas fueron incubadas en 10 ml de conjugado anti fluoresceína fosfatasa alcalina durante 30 minutos Las membranas fueron enjuagadas brevemente dos veces con TPBS, luego tres veces durante 5 min en buffer de lavado TPBS La solución de sustrato ECF fue preparada de acuerdo con las instrucciones del fabricante (alícuotas y congelación) Las membranas fueron incubadas durante 2-3 minutos, se drenó el exceso de reactivos, luego se las escurrio con papeles de filtro, se las seco en aire durante 9-10 minutos y luego se las sometio a exploracion

Exploracion (barrido) de la membrana Se coloco el blot sobre el vidrio de un phosphoimager Storm 860 Se encendió el quimioluminiscente azul, de 200 pixeles de tamaño, un voltaje de 700 PMT Se abrio el archivo en ImageQuant y se lo cuantificó generando cuadrados alrededor de las bandas que representaban

el sustrato (S), el producto (P) y el control interno (IC) Se midió el % de escision del sustrato como P/(S+P)x100 Se midió la inhibicion de segmentacion debido al fármaco en comparación doble con controles farmacológicos incluidos en cada blot Se creo un informe en Excel A continuación se consignan los resultados para algunos de los compuestos

Compuesto del Ejemplo 36 CE_{50}= 9 µm

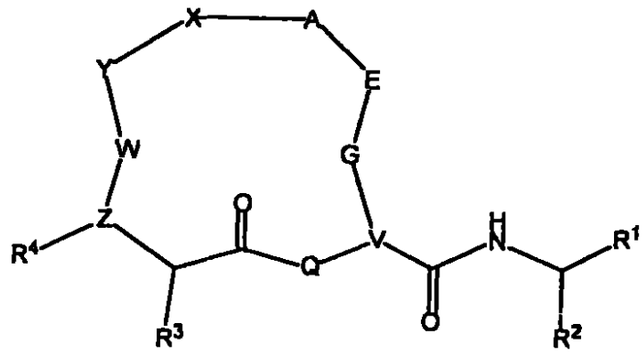
Compuesto del Ejemplo 35 CE_{50}= 20 µm

De estos resultados de los ensayos, la persona con capacitacion en la técnica consideraria evidente que los compuestos de la presente invención tienen excelente aptitud como inhibidores de la serina proteasa NS3

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REIVINDICACIONES

1 Un compuesto macrocíclico, incluyendo los enantiómeros, estereoisómeros, rotameros y tautómeros de dicho compuesto, así como las sales o solvatos farmaceuticamente aceptables de dicho compuesto, que tiene la estructura general expuesta en la Formula I



Fórmula I

en la cual

E, X e Y pueden estar independientemente presentes o ausentes y, en caso de estar presentes, son independientemente seleccionados entre las porciones alquilo, arilo, alquilarilo, heteroalquilo, heteroarilo, arilheteroarilo, alquilheteroarilo, cicloalquilo, éter alquílico, éter alquilarílico, éter arílico, alquilamino, arilamino, alquilarilamino, alquilsulfuro, alquilarilsulfuro, arilsulfuro, alquilsulfona, alquilarilsulfona, arilsulfona, alquilalquilsulfoxido, alquilarilsulfoxido, alquilamida, alquilarilamida, arilamida, alquilsulfonamida, alquilarilsulfonamida, arilsulfonamida, alquilurea, alquilarilurea, arilurea, carbamato de alquilo, carbamato de alquilarilo, carbamato de arilo, alquilhidrazida, alquilarilhidrazida, alquilhidroxamida, alquilarilhidroxamida, alquilsulfonilo, arilsulfonilo, heteroalquilsulfonilo, heteroarilsulfonilo, alquilcarbonilo, arilcarbonilo,

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heteroalquilcarbonilo, heteroarilcarbonilo, alcoxicarbonilo, ariloxicarbonilo, heteroariloxicarbonilo, alquilaminocarbonilo, arilaminocarbonilo, heteroarilaminocarbonilo o una combinación de las mismas, con la condicion de que optativamente, E, X e Y pueden estar sustituidas ademas con porciones seleccionadas entre el grupo que consiste en aromaticos, alquilo, alquilarilo, heteroalquilo, arilheteroarilo, alquilheteroarilo, cicloalquilo, eter alquilico, éter alquilarílico, alquilsulfuro, alquilarilsulfuro, alquilsulfona, alquilarilsulfona, alquilamida, alquilarilamida, alquilsulfonamida, alquilaminas, alquilarilaminas, alquilarilsulfonamida, alquilurea, alquilarilurea, carbamato de alquilo, carbamato de alquilarilo, halógeno, hidroxilamino, carbazato de alquilo, carbazato de arilo,

R^1 = COR^5 o $B(OR)_2$, donde R^5 = H, OH, OR^6, NR^9R^{10}, CF_3, C_2F_5, C_3F_7, CF_2R^6, R^6, COR^7 donde R^7 = H, OH, OR^6, CHR^9R^{10} o NR^9R^{10}, donde R^6, R^8, R^9 y R^{10} son independientemente seleccionados entre el grupo que consiste en H, alquilo, arilo, heteroalquilo, heteroarilo, cicloalquilo, cicloalquilo, arilalquilo, heteroarilalquilo, $CH(R^1)COOR^{11}$, $CH(R^1)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})R$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)CONHCH(R^4)COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)CONHCH(R^4)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^3)CONHCH(R^4)CONHCH(R^{5'})COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONHCH(R^{3'})CONHCH(R^4)CONHCH(R^{5'})CONR^{12}R^{13}$, donde R^1, $R^{2'}$, $R^{3'}$, $R^{4'}$, $R^{5'}$, R^{11}, R^{12}, R^{13} y R' son independientemente seleccionados entre el grupo que consiste en H, alquilo, arilo, heteroalquilo, heteroarilo, cicloalquilo, alquilarilo, alquilheteroarilo, arilalquilo y heteroaralquilo,

Z es seleccionado entre O, N o CH,

W puede estar presente o ausente y, en caso de estar presente, W es seleccionado entre C=O, C=S, SO_2 o C=NR,

Q es $(NR)_p$, O, S, CH_2, CHR, CRR' o un enlace doble hacia V,

A es O, CH_2, $(CHR)_p$, $(CHR\text{-}CHR')_p$, $(CRR')_p$, NR, S, SO_2, C=O o un enlace,

G es $(CH_2)_p$, $(CHR)_p$, $(CRR')_p$, NR, O, S, SO_2, $S(O)_2NH$, C=O o un enlace doble hacia E o V,

V es CH, CR o N,

p es un numero de 0 a 6 y

R, R', R^2, R^3 y R^4 son independientemente seleccionados entre el grupo que consiste en H, alquilo C1-C10, alquenilo C2-C10, cicloalquilo C3-C8, heterocicloalquilo C3-C8, arilo, alcoxi, arioxi, alquiltio, ariltio, amino, amido, éster, ácido carboxílico, carbamato, urea, cetona, aldehido, ciano, nitro, heteroarilo, alquilarilo, alquilheteroarilo, (cicloalquil)alquilo y (heterocicloalquil)alquilo, donde dicho cicloalquilo se compone de tres a ocho atomos de carbono y de cero a seis átomos de oxigeno, nitrogeno, azufre o fosforo y dicho alquilo es de uno a seis átomos de carbono, donde dichas porciones alquilo, heteroalquilo, alquenilo, heteroalquenilo, arilo, heteroarilo cicloalquilo y heterocicloalquilo pueden estar optativamente sustituidas, y donde dicho término "sustituido" se refiere a una sustitución optativa y adecuada con una o más porciones seleccionadas entre el grupo que consiste en alquilo, alquenilo, alquinilo, arilo, aralquilo, cicloalquilo, heterociclico, halógeno, hidroxi, tio, alcoxi, arioxi, alquiltio, ariltio, amino, amido, ester, acido carboxílico, carbamato, urea, cetona, aldehido, ciano, nitro, sulfonamida, sulfóxido, sulfona, sulfonilurea, hidrazida e hidroxamato y tiourea

2 El compuesto de acuerdo con la reivindicacion 1 en el cual $R^1 = COR^5$ y R^5 es H, OH, $COOR^8$ o $CONR^9R^{10}$

3 El compuesto de acuerdo con la reivindicación 2 en el cual $R^1 = COCONR^9R^{10}$ y R^9 es H, R^{10} es H, $CH(R^1)COOR^{11}$, $CH(R^1)CONR^{12}R^{13}$,

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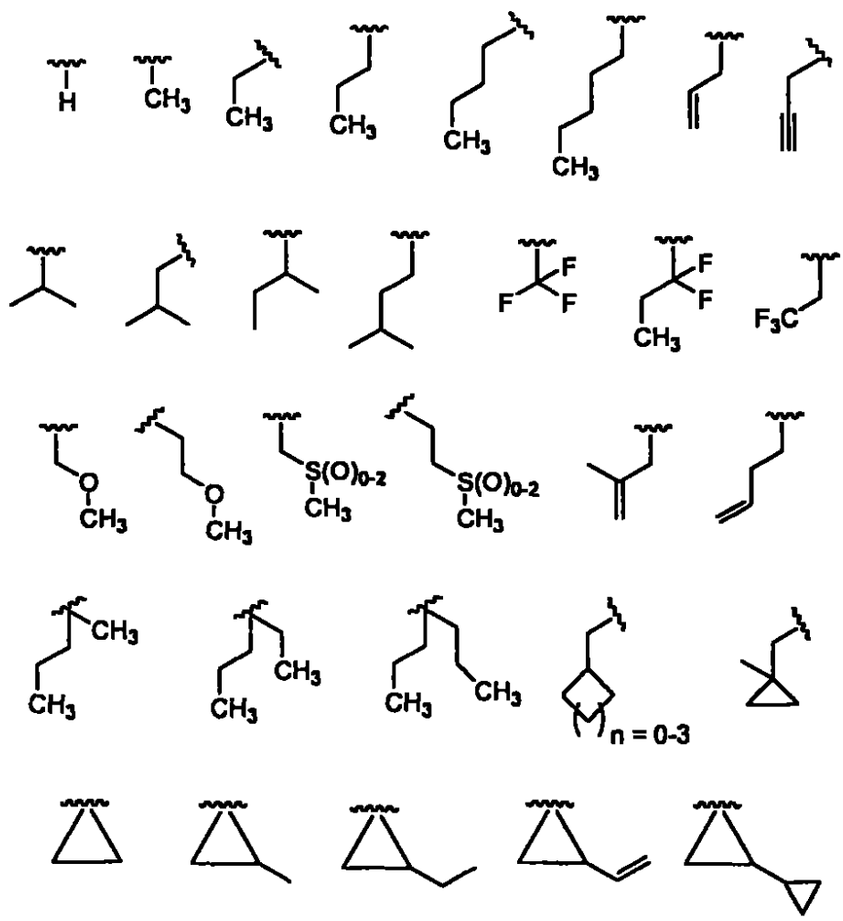
$CH(R^1)CONHCH(R^{2'})COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONR^{12}R^{13}$ o $CH(R^1)CONHCH(R^{2'})(R)$

4 El compuesto de acuerdo con la reivindicacion 3, en el cual R^{10} = $CH(R^1)CONHCH(R^{2'})COOR^{11}$, $CH(R^1)CONHCH(R^{2'})CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)(R)$ donde R' es H alquilo, y $R^{2'}$ es seleccionado entre el grupo que consiste en fenilo, fenilo sustituido, fenilo sustituido con heteroatomo, tiofenilo, ciclohexilo, ciclopentilo, ciclopropilo, piperidilo, piridilo y 2-indanilo

5 El compuesto de acuerdo con la reivindicación 4, en el cual $R^{1'}$ es H

6 El compuesto de acuerdo con la reivindicación 5, en el cual $R^{2'}$ = fenilo, tiofenilo, ciclohexilo, 2-indanilo, ciclopentilo, piridilo, fenil(4-$HNSO_2NH_2$), R^{11} es H o terc-butilo, R^{12} y R^{13} son metilo y R' es hidroximetilo o terc-butoximetilo

7 El compuesto de acuerdo con la reivindicación 1, en el cual R^2 es seleccionado entre el grupo que consiste en las siguientes porciones



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8 El compuesto de acuerdo con la reivindicación 7 en el cual $R^1 = COR^5$ y R^5 es H, OH, $COOR^8$ o $CONR^9R^{10}$

9 El compuesto de acuerdo con la reivindicación 8 en el cual V es CH

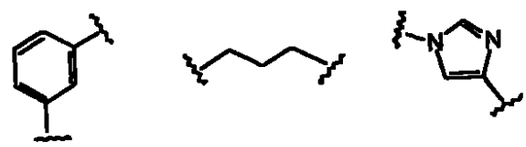
10 El compuesto de acuerdo con la reivindicación 9 en el cual Q es NR u O,

11 El compuesto de acuerdo con la reivindicación 10 en el cual G es CH_2

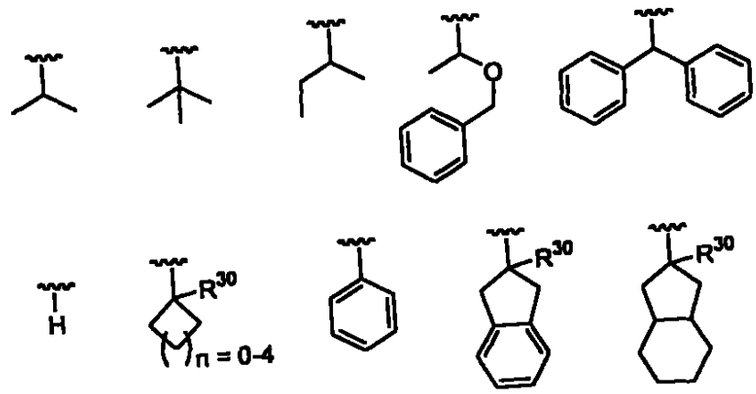
12 El compuesto de acuerdo con la reivindicacion 11 en el cual A es O NR, CH=CH o CH_2

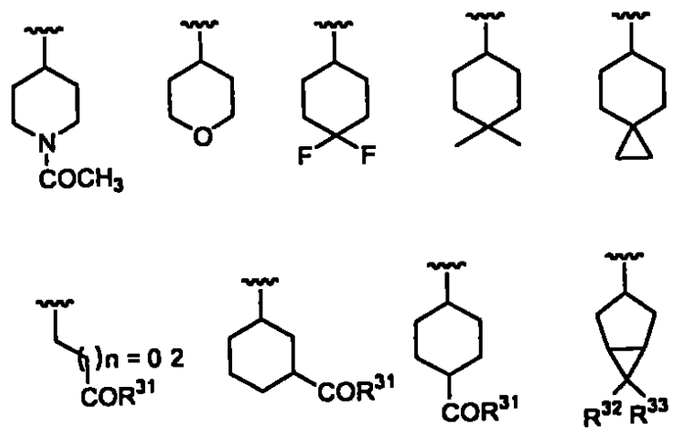
13 El compuesto de acuerdo con la reivindicación 12 en el cual E es alquilo, arilo, heteroalquilo, heteroarilo, alquilo, arilo o cicloalquilo

14 El compuesto de acuerdo con la reivindicacion 13 en el cual E es seleccionado entre el grupo que consiste en las porciones



15 El compuesto de acuerdo con la reivindicacion 13 en el cual R^3 es seleccionado entre el grupo que consiste en las porciones





en las cuales R^{30} = H, CH_3 u otros grupos alquilo,

R^{31} = OH, O-alquilo, NH_2, N-alquilo y

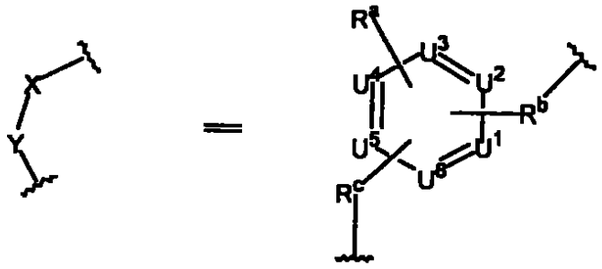
R^{32} y R^{33} pueden ser iguales o diferentes y son seleccionados independientemente entre H, F, Cl, Br y CH_3

16 El compuesto de acuerdo con la reivindicación 15 en el cual Z = N y R^4 = H

17 El compuesto de acuerdo con la reivindicacion 16 en el cual W es C=O

18 El compuesto de acuerdo con la reivindicacion 17 en el cual la porción X-U es seleccionada entre el grupo que consiste en alquilo C1-C10, alquilo, cicloalquilo, heteroalquilo, arilalquilo, arilo, heteroarilo y alquilarilo

19 El compuesto de acuerdo con la reivindicacion 18 en el cual



donde R^b está directamente conectado a A y R^c esta directamente conectado a W, y la porcion U^1, U^2, U^3, R^4, U^5 y U^6 forma un anillo de carbonos de seis miembros o un anillo de cinco o seis miembros con uno o mas heteroatomos,

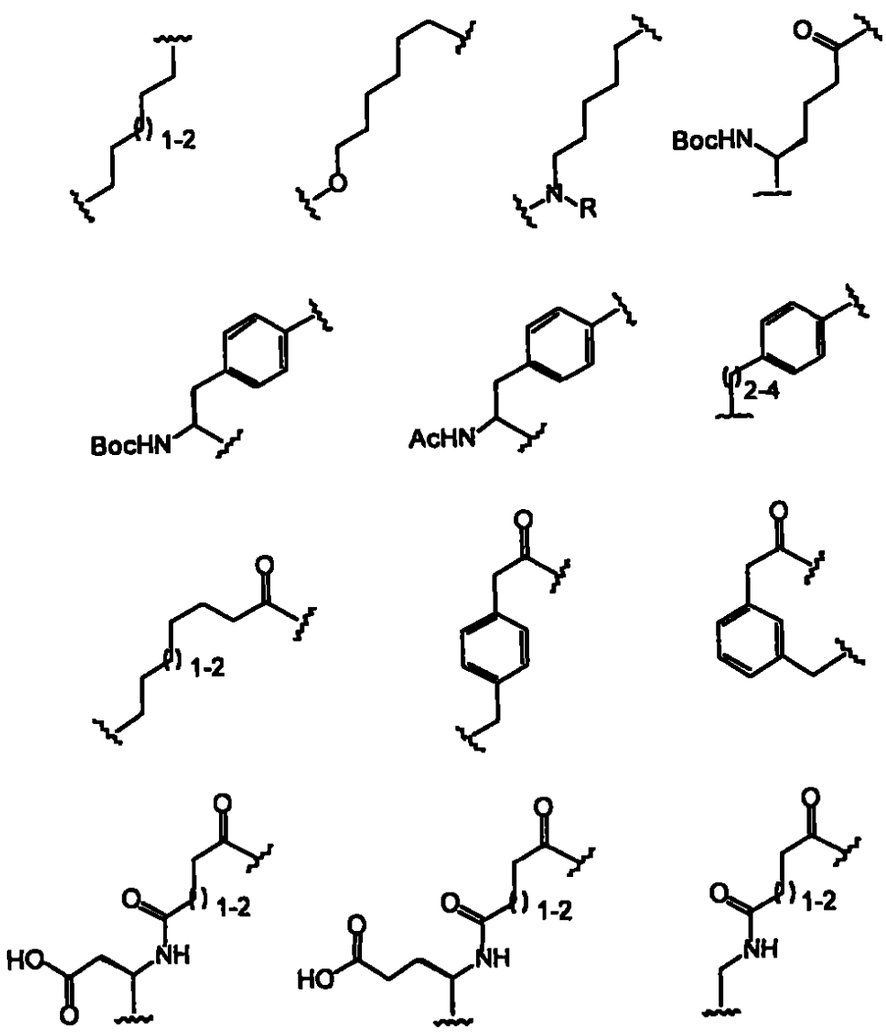
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R^a = H, alquilo, alcoxi, hidroxi, alquiltio, halogeno, nitro, ciano, acido carboxilico, ester, amida, amino, nitrilo o CF_3,

R^b es un enlace, alquilo C_1-C_6, alquenilo C_2-C_6, alquinilo C_2-C_6, O, S, SO_2 NH, O(alquilo), S(alquilo), SO_2(alquilo) o N(alquilo) y

R^c es un enlace, alquilo C_1-C_6, alquenilo C_2-C_6, alquinilo C_2-C_6, O, S, SO_2, NH, O(alquilo), S(alquilo), SO_2(alquilo), N(alquilo) o CH_2-N(alquilo), estando CH_2 ligado al anillo aromatico

20 El compuesto de acuerdo con la reivindicacion 18 en el cual la porcion X-Y es seleccionada entre el grupo que consiste en las siguientes estructuras



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21 Una composición farmacéutica que comprende, como ingrediente activo, un compuesto de acuerdo con la reivindicacion 1

22 La composicion farmaceutica de acuerdo con la reivindicación 21 para usar en el tratamiento de trastornos asociados con el virus de la Hepatitis C

23 La composicion farmaceutica de acuerdo con la reivindicacion 21 que ademas comprende un vehiculo farmacéuticamente aceptable

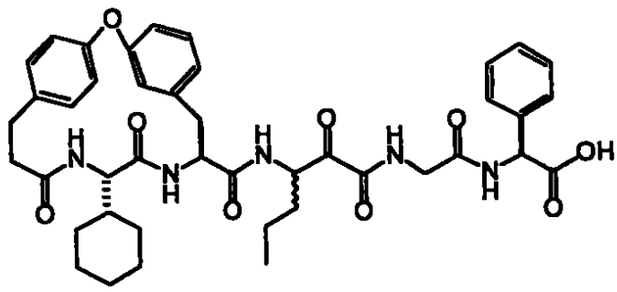
24 Un metodo para el tratamiento de trastornos asociados con la proteasa del VHC, metodo que comprende administrar a un paciente que necesita dicho tratamiento una composicion farmaceutica que comprende cantidades terapeuticamente efectivas de un compuesto de acuerdo con la reivindicación 1

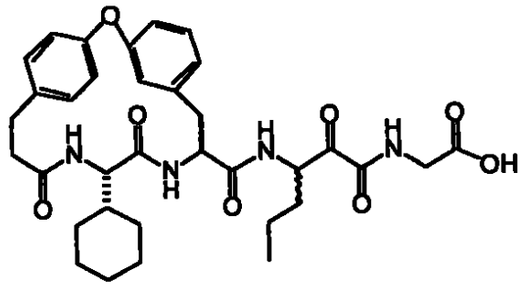
25 El uso de un compuesto de acuerdo con la reivindicación 1 para la elaboración de un medicamento para el tratamiento de trastornos asociados con la proteasa del VHC,

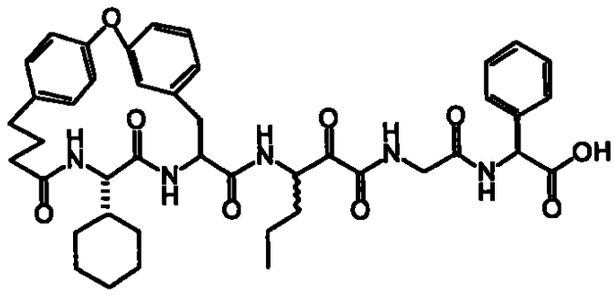
26 Un método para la preparacion de una composicion farmaceutica para el tratamiento de trastornos asociados con la proteasa del VHC, método que comprende poner en intimo contacto a un compuesto de acuerdo con la reivindicación 1 y un vehiculo farmacéuticamente aceptable

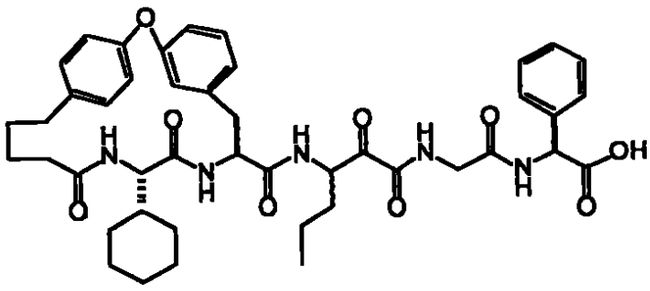
27 Un compuesto que exhibe actividad inhibitoria de la proteasa de VHC, incluyendo los enantiómeros, estereoisómeros, rotámeros y tautómeros de dicho compuesto, asi como las sales o solvatos farmaceuticamente aceptables de dicho compuesto, compuesto éste que es seleccionado entre el grupo de compuestos que tienen las estructuras enumeradas a continuación

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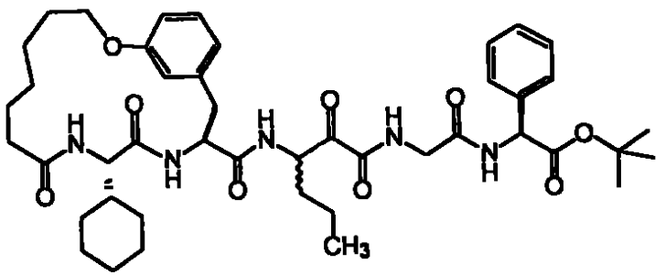


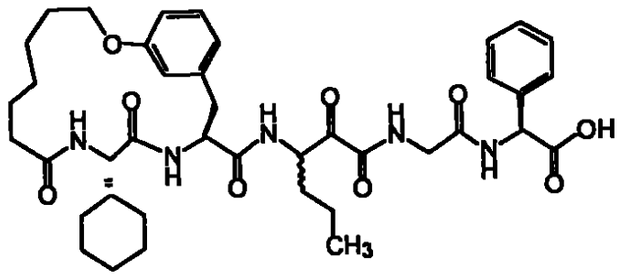


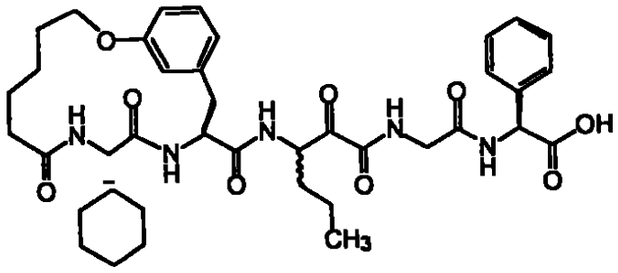


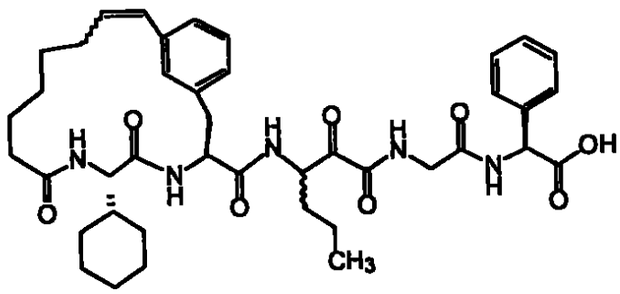


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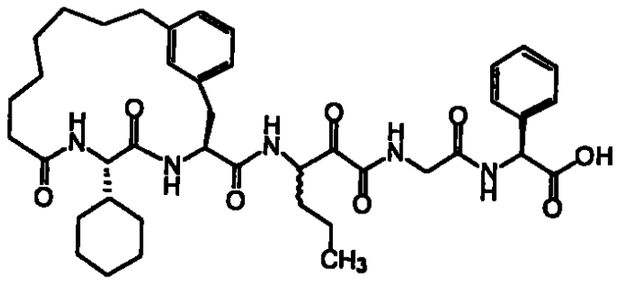


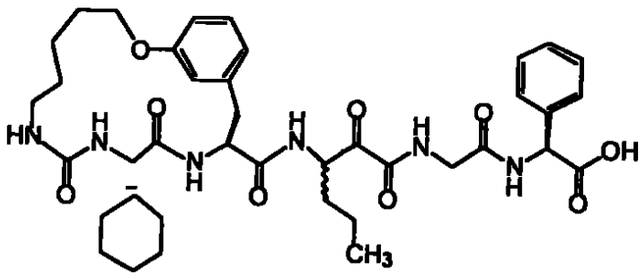


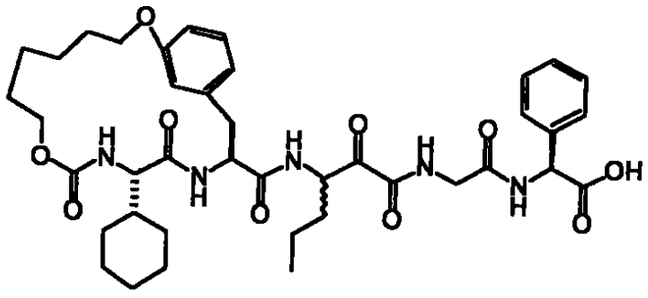


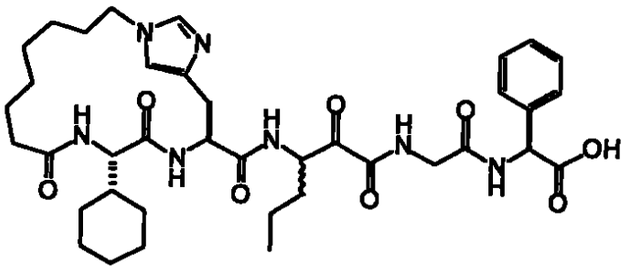


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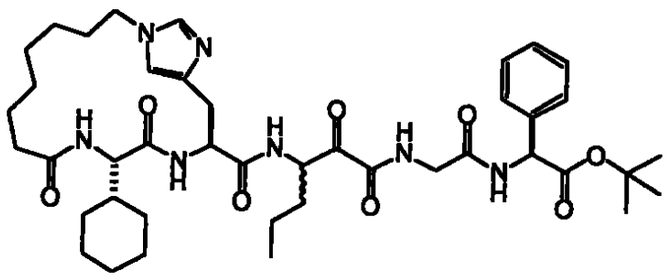


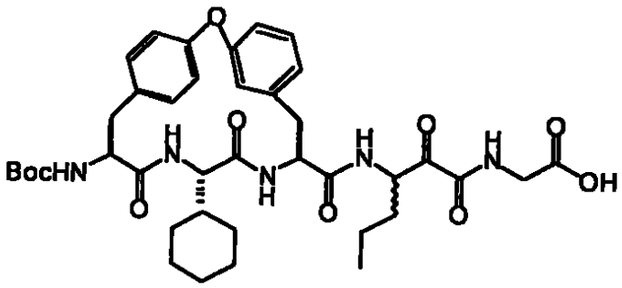


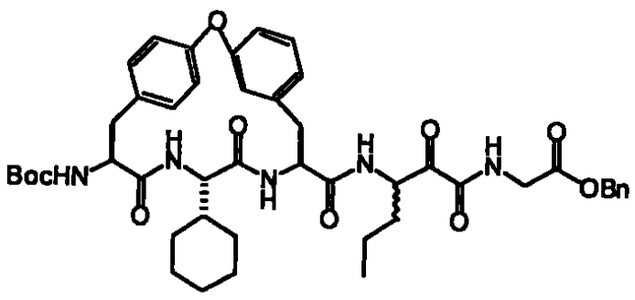


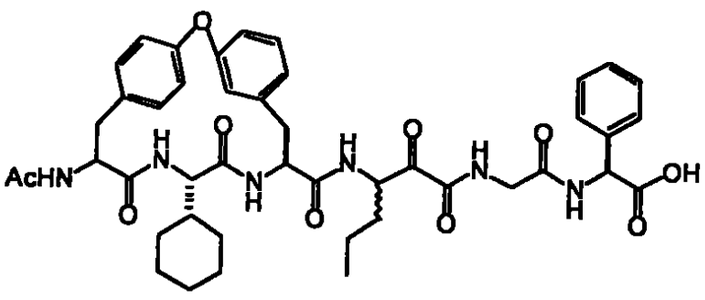


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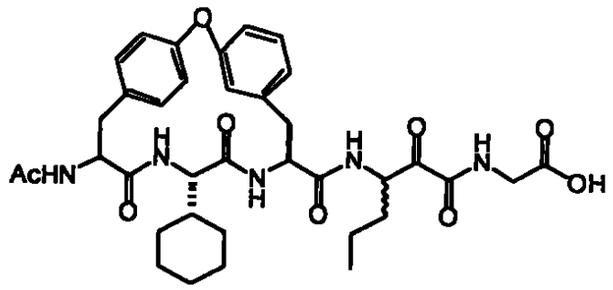


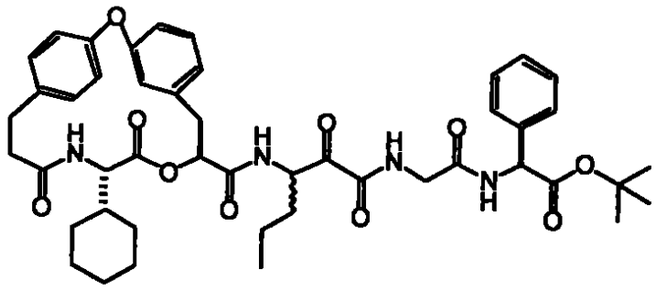


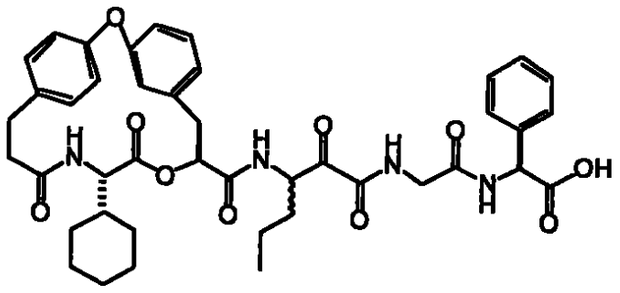


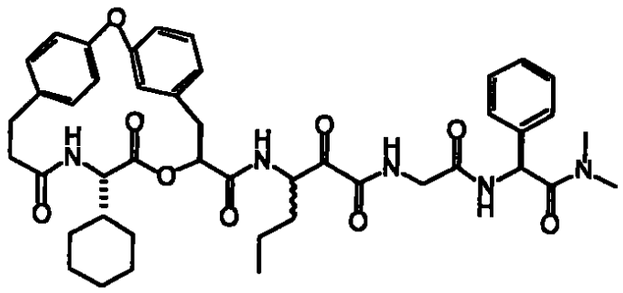


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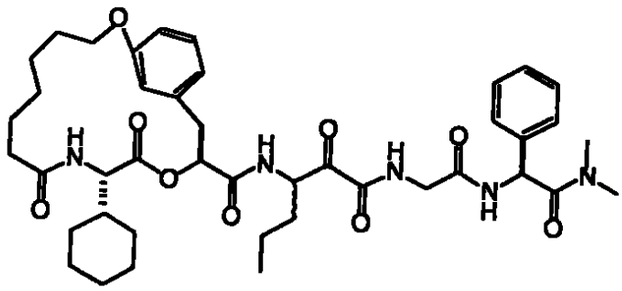


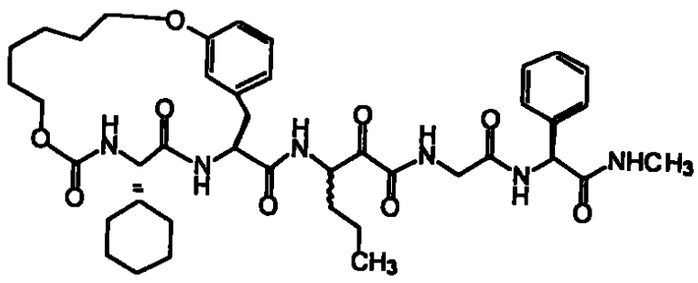


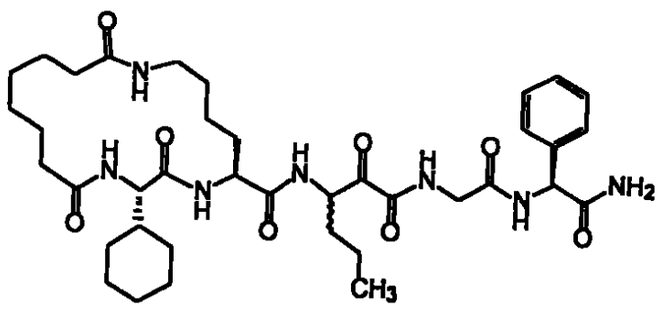


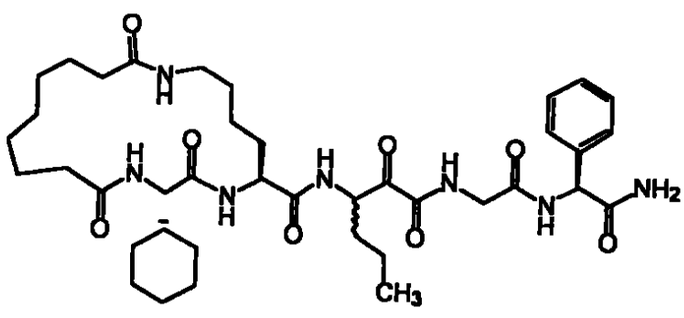


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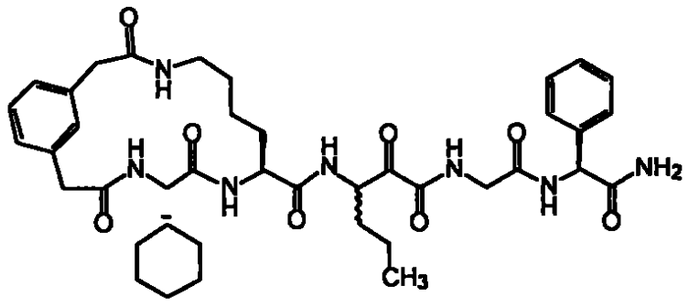


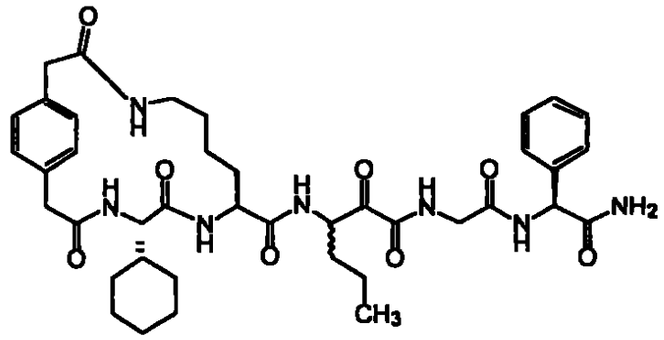


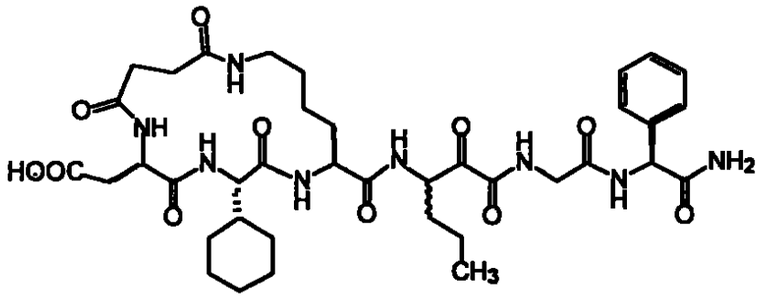


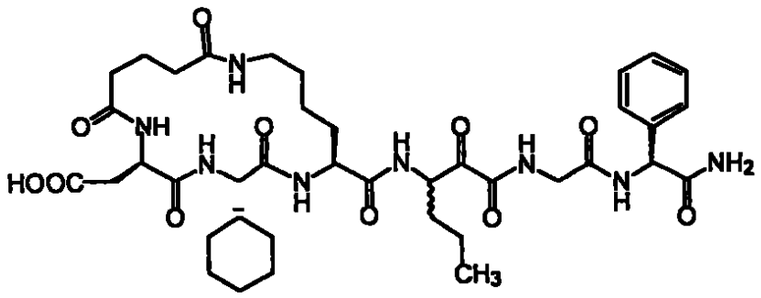


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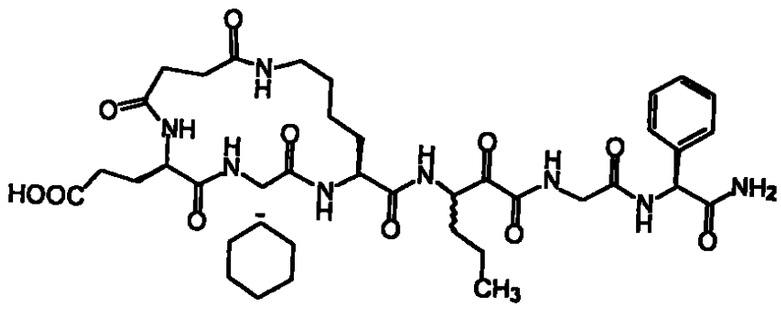


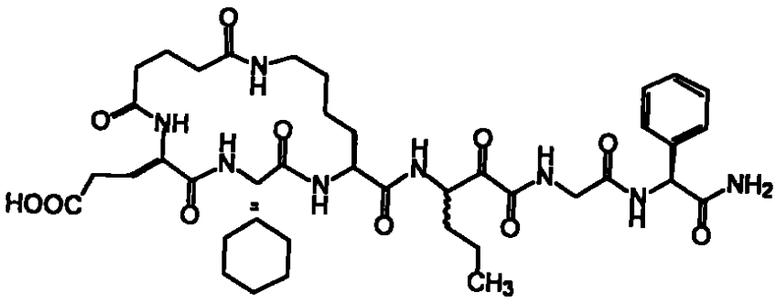


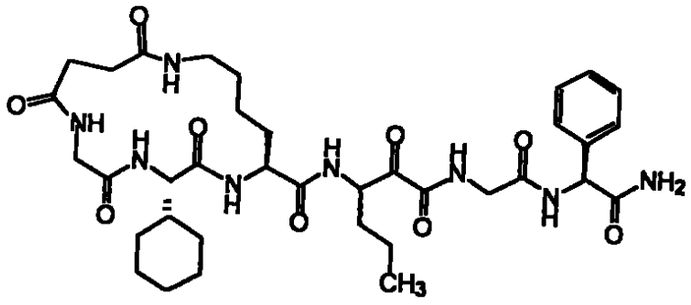


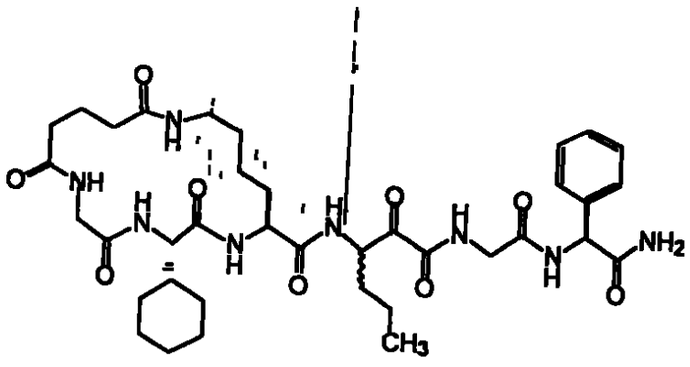


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28 Una composicion farmaceutica para el tratamiento de trastornos asociados con la proteasa del VHC, composición que comprende una cantidad terapéuticamente efectiva de uno o más compuestos de acuerdo con la reivindicacion 27 y un vehiculo farmaceuticamente aceptable

29 La composicion farmaceutica de acuerdo con la reivindicacion 28, que además contiene un agente antiviral

30 La composicion farmacéutica de acuerdo con la reivindicación 28 o la reivindicación 29, que por añadidura contiene un interferon

31 La composición farmacéutica de acuerdo con la reivindicacion 30, en el cual dicho agente antiviral es la ribavirina y dicho interferon es α-interferón

p SCHERING CORPORATION

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Resumen de la Invencion

La presente invención describe novedosos compuestos macrociclicos que tienen actividad inhibitoria de la proteasa del VHC así como metodos para la preparacion de dichos compuestos En otra realización, la invencion describe composiciones farmaceuticas que comprenden dichos macrociclicos así como metodos para su uso en el tratamiento de trastornos asociados con la proteasa de VHC

PATENT DEPARTMENT
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PATENT COOPERATION TREATY

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
KALYANARAMAN, Palaiyur S et al
SCHERING-PLOUGH CORPORATION
2000 Galloping Hill Road
Kenilworth, NJ 07033-0530
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	06/08/2002
Applicant's or agent's file reference: IN01155K	IMPORTANT NOTIFICATION

International application No	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 01/12530	17/04/2001	19/04/2000

Applicant

SCHERING CORPORATION

1 The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes if any established on the international application.

2 A copy of the report and its annexes, if any is being transmitted to the International Bureau for communication to all the elected Offices.

3 Where required by any of the elected Offices the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4 **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

For the purpose of deciding whether the claimed invention is patentable or not, the elected Offices may apply criteria additional to or different from the criteria on which the international preliminary examination report is based (see Articles 27 (5), 33 (5)). Additional criteria may include e.g. exemptions from patentability and the requirements of enabling disclosure and of clarity and support of claims.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx 523656 epmu d Fax. (+49-89) 2399-4465	DONNELLY P P Tel (+49-89) 2399 2828

Form PCT/IPEA/416 (28/06/2002) P20473

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. ATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant s or agent s file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
IN01155K		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 01/ 12530	17/04/2001	19/04/2000
International Patent Classification (IPC) or national classification and IPC		
C07D273/00		
Applicant		
SCHERING CORPORATION		

1 This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36

2 This REPORT consists of a total of 2 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70 16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

3 This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty inventive step or industrial applicability citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/10/2001	01/08/2002
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel (+49-89) 2399-0, Tx 523656 epmu d Fax (+49-89) 2399-4465	BRENNAN J Tel (+49-89) 2399 2828

Form PCT/IPEA/409 (cover sheet) (July 1998)

I Basis of the report

The basis of this international preliminary examination is the application as originally filed

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

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 66 1(e) PCT), see also international search report)

V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out

In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims, which have been the subject of an international search report, does not appear to meet the criteria mentioned in Article 33(1) PCT, i e does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claim references)

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THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 30, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111

APPLICATION NUMBER ***60/198,204***
FILING DATE ***April 19, 2000***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

E. Bornett

E. BORNETT
Certifying Officer

04-20-00 354/136

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PTO/SB/16 (8-98)
Approved for use through 01/31/2001 OMB 0651-0037
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)		
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)
SRIKANTH	VENKATRAMAN	FORDS, NEW JERSEY
KEVIN X.	CHEN	PISCATAWAY, NEW JERSEY
ASHOK	ARASAPPAN	BRIDGEWATER, NEW JERSEY
F GEORGE	NJOROGE	UNION NEW JERSEY

☒ Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

MACROCYCLIC NS-3 SERINE PROTEASE INHIBITORS OF HEPATITIS C VIRUS COMPRISING ALKYL AND ARYL ALANINE P2 MOIETIES

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☐ Customer Number → Type Customer Number here

OR

Place Customer Number Bar Code Label here

☒ Firm or Individual Name	PALAIYUR S. KALYANARAMAN				
Address	Patent Dept. K-6-1, 1990	Schering-Plough Corporation			
Address	2000 Galloping Hill Road				
City	Kenilworth	State	New Jersey	ZIP	07033-0530
Country	USA	Telephone	(908)298-5068	Fax	(908) 298-5388

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 218 ☐ Small Entity Statement

☐ Drawing(s) Number of Sheets ☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)

☐ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 19-0365

FILING FEE AMOUNT ($): $150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date 04 / 19 / 00

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PROVISIONAL APPLICATION COVER SHEET
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PROVISIONAL PATENT APPLICATION

MACROCYCLIC NS-3 SERINE PROTEASE INHIBITORS OF HEPATITIS C VIRUS COMPRISING ALKYL AND ARYL ALANINE P2 MOIETIES

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Macrocyclic NS-3 Serine Protease Inhibitors of Hepatitis C Virus comprising Alkyl and Aryl Alanine P2 Moieties

Field of Invention

The present invention relates to novel hepatitis C virus ("HCV") protease inhibitors, pharmaceutical compositions containing one or more such inhibitors, methods of preparing such inhibitors and methods of using such inhibitors to treat hepatitis C and related disorders This invention specifically discloses novel macrocyclic compounds as inhibitors of the HCV NS3/NS4a serine protease The disclosure herein is related to that in pending patent application, Serial No _____, filed April 5, 2000

Background of the Invention

Hepatitis C virus (HCV) is a (+)-sense single-stranded RNA virus that has been implicated as the major causative agent in non-A, non-B hepatitis (NANBH), particularly in blood-associated NANBH (BB-NANBH)(see, International Patent Application Publication No WO 89/04669 and European Patent Application Publication No EP 381 216) NANBH is to be distinguished from other types of viral-induced liver disease, such as hepatitis A virus (HAV), hepatitis B virus (HBV), delta hepatitis virus (HDV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV), as well as from other forms of liver disease such as alcoholism and primary biliar cirrhosis

Recently, an HCV protease necessary for polypeptide processing and viral replication has been identified, cloned and expressed, (see, e.g., U S Patent No 5,712,145) This approximately 3000 amino acid polyprotein contains, from the amino terminus to the carboxy terminus, a nucleocapsid protein (C), envelope proteins (E1 and E2) and several non-structural proteins (NS1, 2, 3, 4a, 5a and 5b) NS3 is an approximately 68 kda protein, encoded by approximately 1893 nucleotides of the HCV genome, and has two distinct domains (a) a serine protease domain consisting of approximately 200 of the N-terminal amino acids; and (b) an RNA-dependent ATPase domain at the C-terminus of the protein The

NS3 protease is considered a member of the chymotrypsin family because of similarities in protein sequence, overall three-dimensional structure and mechanism of catalysis. Other chymotrypsin-like enzymes are elastase, factor Xa, thrombin, trypsin, plasmin, urokinase, tPA and PSA The HCV NS3 serine protease is responsible for proteolysis of the polypeptide (polyprotein) at the NS3/NS4a, NS4a/NS4b, NS4b/NS5a and NS5a/NS5b junctions and is thus responsible for generating four viral proteins during viral replication This has made the HCV NS3 serine protease an attractive target for antiviral chemotherapy

It has been determined that the NS4a protein, an approximately 6 kda polypeptide, is a co-factor for the serine protease activity of NS3. Autocleavage of the NS3/NS4a junction by the NS3/NS4a serine protease occurs intramolecularly (i.e., *cis*) while the other cleavage sites are processed intermolecularly (i.e., *trans*)

Analysis of the natural cleavage sites for HCV protease revealed the presence of cysteine at P1 and serine at P1' and that these residues are strictly conserved in the NS4a/NS4b, NS4b/NS5a and NS5a/NS5b junctions. The NS3/NS4a junction contains a threonine at P1 and a serine at P1' The Cys→Thr substitution at NS3/NS4a is postulated to account for the requirement of *cis* rather than *trans* processing at this junction See, e.g., Pizzi et al. (1994) Proc. Natl. Acad. Sci (USA) 91 888-892, Failla et al. (1996) Folding & Design 1.35-42 The NS3/NS4a cleavage site is also more tolerant of mutagenesis than the other sites See, e.g., Kollykhalov et al. (1994) J. Virol. 68 7525-7533 It has also been found that acidic residues in the region upstream of the cleavage site are required for efficient cleavage See, e.g., Komoda et al. (1994) J. Virol. 68.7351-7357

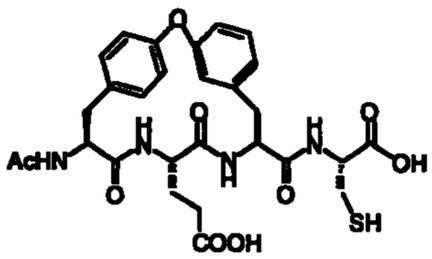
Inhibitors of HCV protease that have been reported include antioxidants (see, International Patent Application Publication No WO 98/14181), certain peptides and peptide analogs (see, International Patent Application Publication No WO 98/17679, Landro et al. (1997) Biochem. 36 9340-9348, Ingallinella et al (1998) Biochem 37 8906-8914, Llinàs-Brunet et al (1998) Bioorg. Med Chem. Lett. 8 1713-1718), inhibitors based on the 70-amino acid polypeptide eglin c (Martin et al. (1998) Biochem. 37 11459-11468, inhibitors affinity selected from

human pancreatic secretory trypsin inhibitor (hPSTI-C3) and minibody repertoires (MBip) (Dimasi et al (1997) J. Virol. 71,7461-7469), cV_HE2 (a "camelized" variable domain antibody fragment) (Martin et al (1997) Protein Eng. 10 607-614), and α1-antichymotrypsin (ACT)(Elzouki et al) (1997) J Hepat. 27,42-28) A ribozyme designed to selectively destroy hepatitis C virus RNA has recently been disclosed (see, BioWorld Today 9(217) 4 (November 10, 1998))

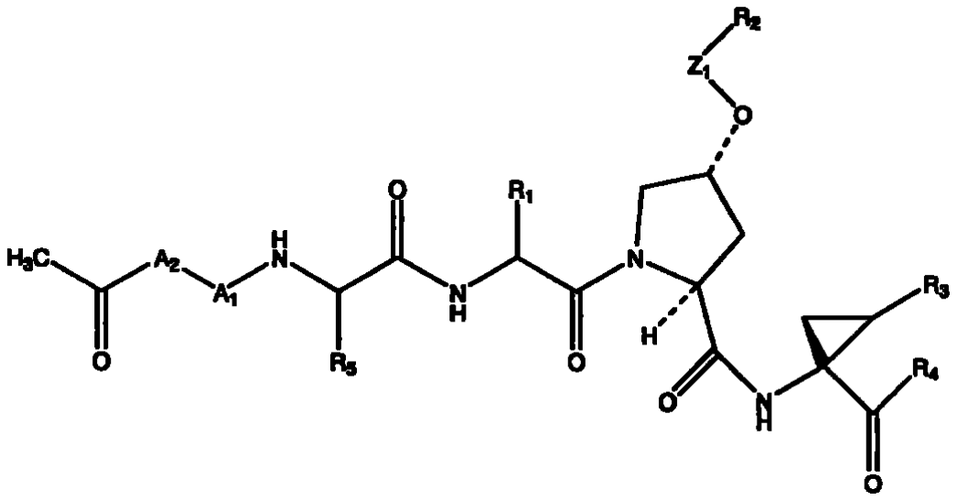
Reference is also made to the PCT Publications, No WO 98/17679, published April 30, 1998 (Vertex Pharmaceuticals Incorporated), WO 98/22496, published May 28, 1998 (F Hoffmann-La Roche AG), and WO 99/07734, published February 18, 1999 (Boehringer Ingelheim Canada Ltd)

HCV has been implicated in cirrhosis of the liver and in induction of hepatocellular carcinoma. The prognosis for patients suffering from HCV infection is currently poor HCV infection is more difficult to treat than other forms of hepatitis due to the lack of immunity or remission associated with HCV infection Current data indicates a less than 50% survival rate at four years post cirrhosis diagnosis Patients diagnosed with localized resectable hepatocellular carcinoma have a five-year survival rate of 10-30%, whereas those with localized unresectable hepatocellular carcinoma have a five-year survival rate of less than 1%

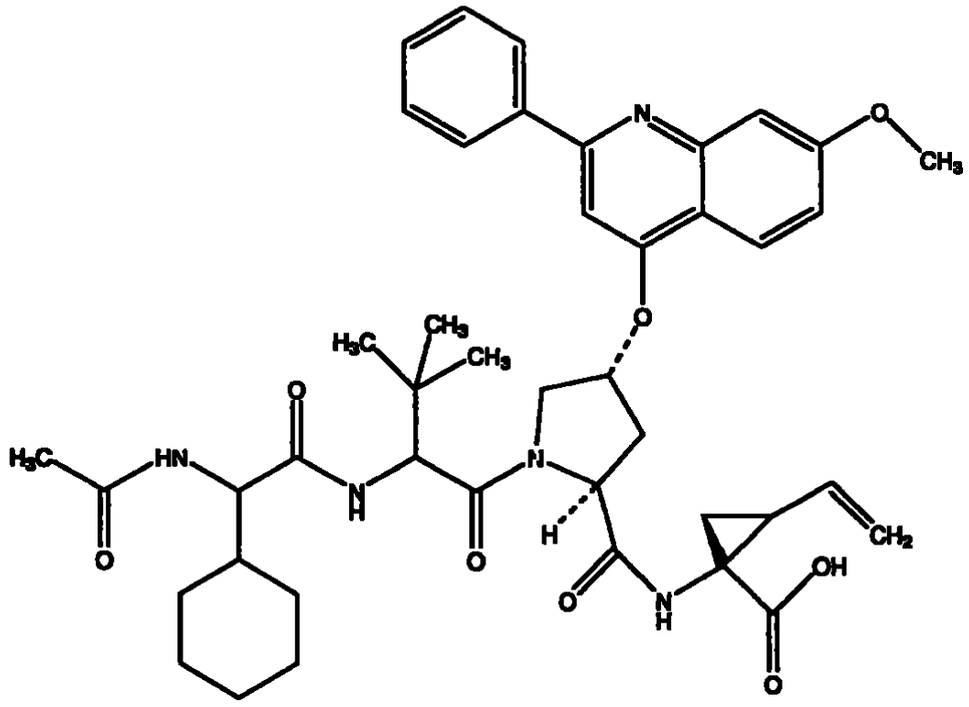
Reference is made to A Marchetti *et al*, *Synlett*, S1, 1000-1002 (1999) describing the synthesis of bicylic analogs of an inhibitor of HCV NS3 protease A compound disclosed therein has the formula.



Reference is also made to WO 00/09558 (Assignee Boehringer Ingelheim Limited, Published February 24, 2000) which discloses peptide derivatives of the formula.

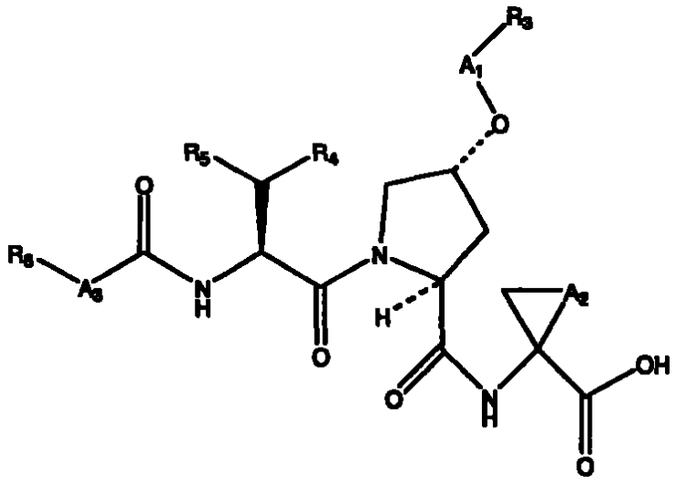


where the various elements are defined therein An illustrative compound of that series is

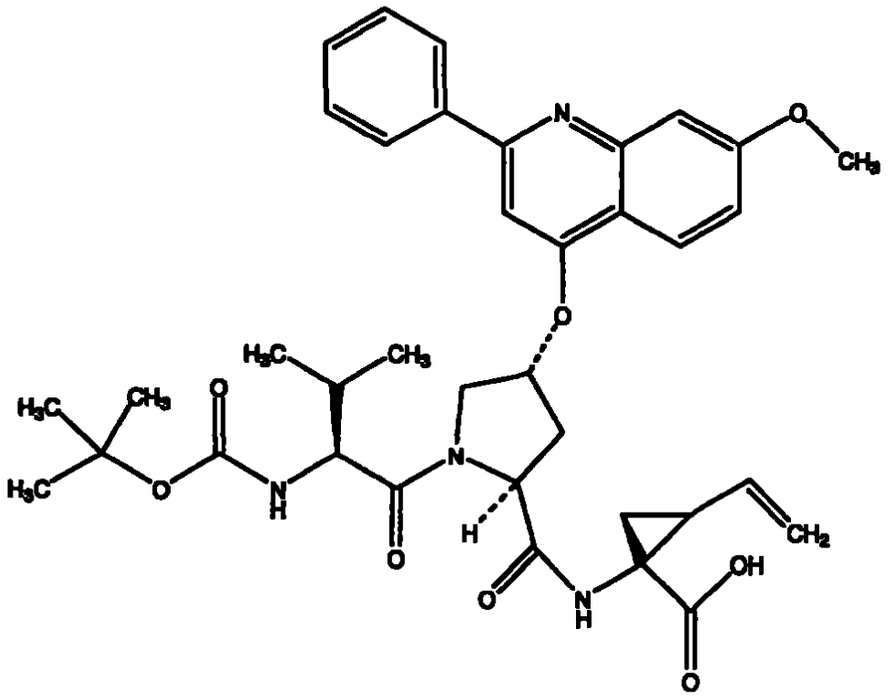


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Reference is also made to WO 00/09543 (Assignee Boehringer Ingelheim Limited, Published February 24, 2000) which discloses peptide derivatives of the formula.



where the various elements are defined therein An illustrative compound of that series is



Current therapies for hepatitis C include interferon-α (INF_α) and combination therapy with ribavirin and interferon See, e.g., Beremguer et al. (1998) Proc Assoc. Am Physicians 110(2).96-112 These therapies suffer from a low sustained response rate and frequent side effects See, e g., Hoofnagle et al. (1997) N. Engl J. Med. 336 347 Currently, no vaccine is available for HCV infection

Pending patent application, Serial Number, _____, filed April 5, 2000, discloses certain macrocyclic compounds as inhibitors of the HCV protease as well as pharmaceutical compositions containing said compounds

There is a need for new treatments and therapies for HCV infection It is, therefore, an object of this invention to provide compounds useful in the treatment or prevention or amelioration of one or more symptoms of hepatitis C

It is a further object herein to provide methods of treatment or prevention or amelioration of one or more symptoms of hepatitis C

A still further object of the present invention is to provide methods for modulating the activity of serine proteases, particularly the HCV NS3/NS4a serine protease, using the compounds provided herein

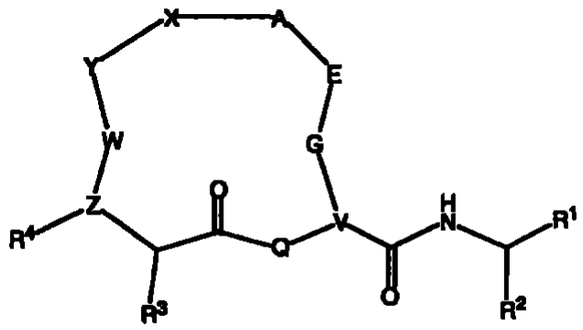
Another object herein is to provide methods of modulating the processing of the HCV polypeptide using the compounds provided herein

Summary of the Invention

In its many embodiments, the present invention provides a novel class of macrocyclic inhibitors of the HCV protease, pharmaceutical compositions containing one or more of the compounds, methods of preparing pharmaceutical formulations comprising one or more such compounds, and methods of treatment, prevention or amelioration or one or more of the symptoms of hepatitis C Also provided are methods of modulating the interaction of an HCV polypeptide with HCV protease Among the compounds provided herein, compounds that inhibit HCV NS3/NS4a serine protease activity are preferred The presently disclosed compounds generally contain about four or more amino acid residues and less than about twelve amino acid residues

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In its principal embodiment, the present invention provides a macrocyclic compound of Formula I



Formula I

wherein

E, X and Y may be independently present or absent, and if present are independently selected from the moieties alkyl, aryl, alkyl-aryl, heteroalkyl, heteroaryl, aryl-heteroaryl, alkyl-heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, aryl ether, alkyl amino, aryl amino, alkyl-aryl amino, alkyl sulfide, alkyl-aryl sulfide, aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, aryl sulfone, alkyl-alkyl sulfoxide, alkyl-aryl sulfoxide, alkyl amide, alkyl-aryl amide, aryl amide alkyl sulfonamide, alkyl-aryl sulfonamide, aryl sulfonamide, alkyl urea, alkyl-aryl urea, aryl urea, alkyl carbamate, alkyl-aryl carbamate, aryl carbamate, alkyl -hydrazide, alkyl-aryl hydrazide, alkyl hydroxamide, alkyl-aryl hydroxamide, alkyl sulfonyl, aryl sulfonyl, heteroalkyl sulfonyl, heteroaryl sulfonyl, alkyl carbonyl, aryl carbonyl, heteroalkyl carbonyl, heteroaryl carbonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl or a combination thereof, with the proviso that E, X and Y may optionally be additionally substituted with moieties selected from the group consisting of aromatic, alkyl, alkyl-aryl, heteroalkyl, aryl-heteroaryl, alkyl-heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, alkyl sulfide, alkyl-aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, alkyl amide, alkyl-aryl amide, alkyl sulfonamide, , alkyl amines, alkyl-aryl amines, alkyl-aryl sulfonamide, alkyl urea, alkyl-aryl urea,

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alkyl carbamate, alkyl-aryl carbamate, halogen, hydroxyl amino, alkyl carbazate, aryl carbazate,

$R^1 = COR^6$ or $B(OR)_2$, wherein R^6 = H, OH, OR^8, NR^9R^{10}, CF_3, C_2F_5, C_3F_7, CF_2R^8, R^8, COR^7 wherein R^7 = H, OH, OR^8, CHR^9R^{10}, or NR^9R^{10} , wherein R^6, R^8, R^9 and R^{10} are independently selected from the group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, cycloalkyl, arylalkyl, heteroarylalkyl, $CH(R^1)COOR^{11}$, $CH(R^1)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)COOR^{11}$, $CH(R^1)CONHCH(R^2)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)R'$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)COOR^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)COOR^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONHCH(R^5)COOR^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONHCH(R^5)CONR^{12}R^{13}$, wherein R^1, R^2, R^3, R^4, R^5, R^{11}, R^{12}, R^{13}, and R' are independently selected from a group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, alkyl-aryl, alkyl-heteroaryl, aryl-alkyl and heteroaralkyl,

Z is selected from O, N, or CH,

W may be present or absent, and if W is present, W is selected from C=O, C=S, SO_2 or C=NR,

Q is $(NR)_p$, O, S, CH_2, CHR, CRR' or a double bond towards V,

A is O, CH_2, $(CHR)_p$, $(CHR\text{-}CHR')_p$ $(CRR')_p$, NR, S, SO_2 , C=O or a bond,

G is $(CH_2)_p$, $(CHR)_p$, $(CRR')_p$, NR, O, S, SO_2, $S(O)_2NH$, C=O, or a double bond towards E or V,

V is CH, CR or N,

p is a number from 0 to 6, and

R, R', R^2, R^3 and R^4 are independently selected from the group consisting of H, C1-C10 alkyl, C2-C10 alkenyl, C3-C8 cycloalkyl, C3-C8 heterocycloalkyl, aryl, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, heteroaryl, alkyl-aryl, alkyl-heteroaryl, (cycloalkyl)alkyl and (heterocycloalkyl)alkyl, wherein said cycloalkyl is

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made of three to eight carbon atoms, and zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of one to six carbon atoms,
with said alkyl, heteroalkyl, alkenyl, heteroalkenyl, aryl, heteroaryl, cycloalkyl and heterocycloalkyl moieties may be optionally substituted, with said term "substituted" referring to optional and suitable substitution with one or more moieties selected from the group consisting of alkyl, alkenyl, alkynyl aryl, aralkyl, cycloalkyl, heterocyclic, halogen, hydroxy, thio, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, sulfonamide, sulfoxide, sulfone, sulfonyl urea, hydrazide, and hydroxamate and thiourea.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. Thus, for example, the term alkyl (including the alkyl portions of alkoxy) refers to a monovalent group derived from a straight or branched chain saturated hydrocarbon by the removal of a single atom having from 1 to 8 carbon atoms, preferably from 1 to 6,

aryl – represents a carbocyclic group having from 6 to 14 carbon atoms and having at least one benzenoid ring, with all available substitutable aromatic carbon atoms of the carbocyclic group being intended as possible points of attachment. Preferred aryl groups include phenyl, 1-naphthyl, 2-naphthyl and indanyl, and especially phenyl and substituted phenyl,

aralkyl -- represents a moiety containing an aryl group linked vial a lower alkyl,

alkylaryl – represents a moiety containing a lower alkyl linked via an aryl group,

cycloalkyl – represents a saturated carbocyclic ring having from 3 to 8 carbon atoms, preferably 5 or 6, optionally substituted,

heterocyclic – represents, in addition to the heteroaryl groups defined below, saturated and unsaturated cyclic organic groups having at least one O, S and/or N atom interrupting a carbocyclic ring structure that consists of one or more rings, wherein each ring is 5-, 6- or 7-membered and may or may not have double

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bonds that lack delocalized pi electrons, which ring structure has from 2 to 8, preferably from 3 to 6 carbon atoms, e g , 2- or 3-piperidinyl, 2- or 3-piperazinyl, 2- or 3-morpholinyl, or 2- or 3-thiomorpholinyl,

halogen – represents fluorine, chlorine, bromine and iodine,

heteroaryl – represents a cyclic organic group having at least one O, S and/or N atom interrupting a carbocyclic ring structure and having a sufficient number of delocalized pi electrons to provide aromatic character, with the aromatic heterocyclic group having from 2 to 14, preferably 4 or 5 carbon atoms, e g , 2- 3- or 4-pyridyl, 2- or 3-furyl, 2- or 3-thienyl, 2-, 4- or 5-thiazolyl, 2- or 4-imidazolyl, 2-, 4- or 5-pyrimidinyl, 2-pyrazinyl, or 3- or 4-pyridazinyl, etc. Preferred heteroaryl groups are 2-, 3- and 4-pyridyl, Such heteroaryl groups may also be optionally substituted

Also included in the invention are tautomers, rotamers, enantiomers and other optical isomers of compounds of Formula I, as well as pharmaceutically acceptable salts and solvates thereof

A further feature of the invention is pharmaceutical compositions containing as active ingredient a compound of Formula I (or its salt, solvate or isomers) together with a pharmaceutically acceptable carrier or excipient.

The invention also provides methods for preparing compounds of Formula I, as well as methods for treating diseases such as, for example, HCV and related disorders The methods for treating comprise administering to a patient suffering from said disease or diseases a therapeutically effective amount of a compound of Formula I, or pharmaceutical compositions comprising a compound of Formula I

Also disclosed is the use of a compound of Formula I for the manufacture of a medicament for treating HCV and related disorders

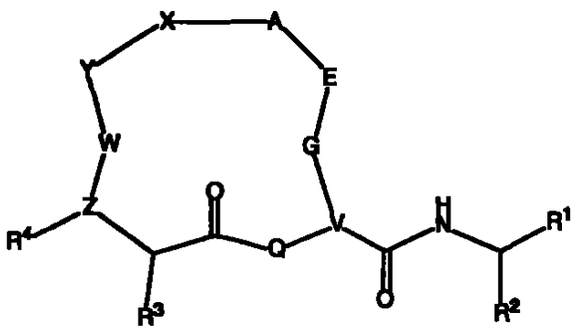
Detailed description of preferred embodiments

In one embodiment, the present invention discloses compounds of Formula I as inhibitors of HCV protease, especially the HCV NS3/NS4a serine protease

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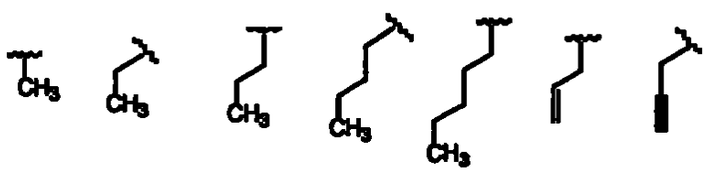


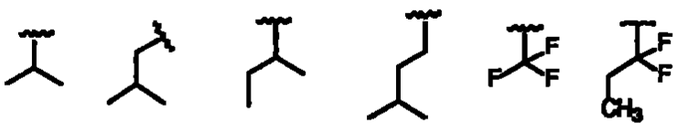
where the various moieties are defined above Some of the preferred embodiments include, but are not limited to, the following definitions of the various functionalities in the above-noted general formula I, other desired definitions for the same and additional functionalities may be found in the structures and claims of this application which are also within the contemplation of the present invention Among the preferred embodiments, R^2 in formula I may be selected from the following moieties

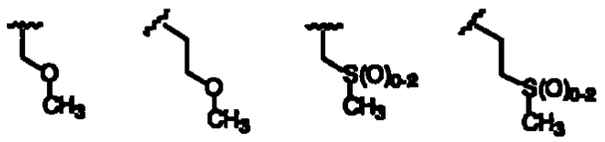
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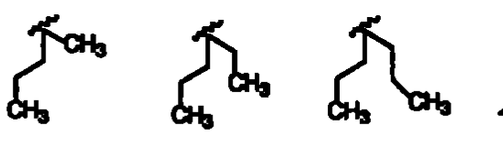
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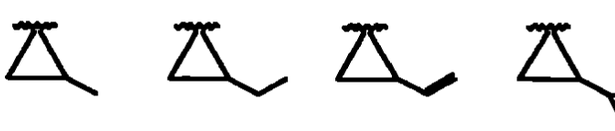
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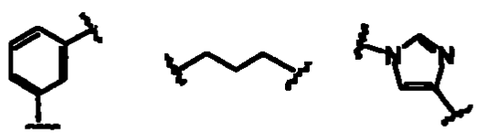






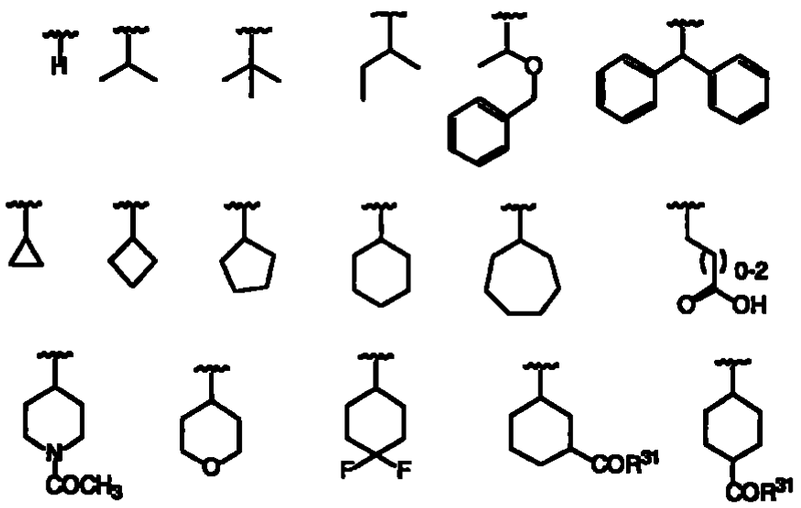


E may be a substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroaryl or substituted or unsubstituted cycloalkyl, with preferred representations for E being



Preferred embodiments for R^3 include the moieties.

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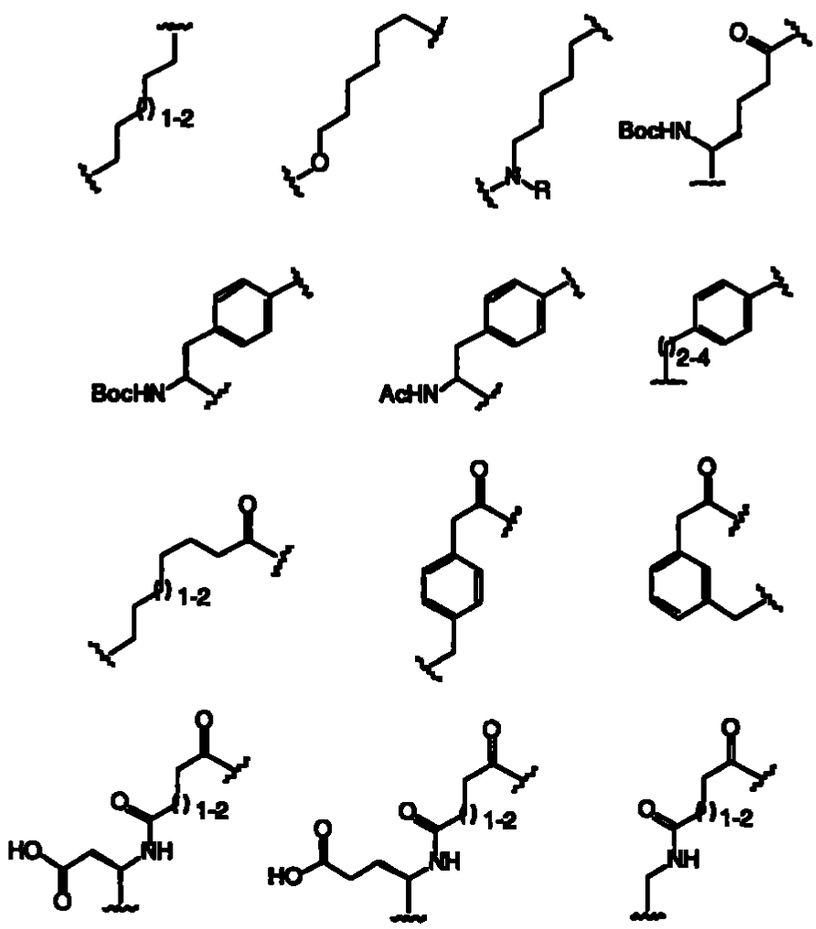


wherein R^{31} = OH, O-alkyl

Preferred embodiments for the moiety X-Y are the following structures

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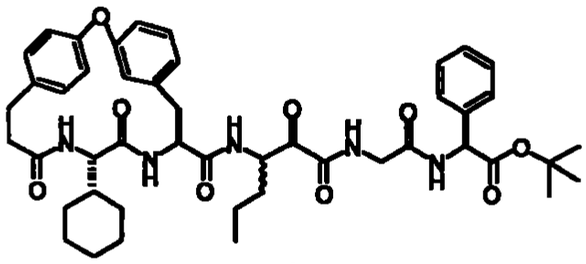
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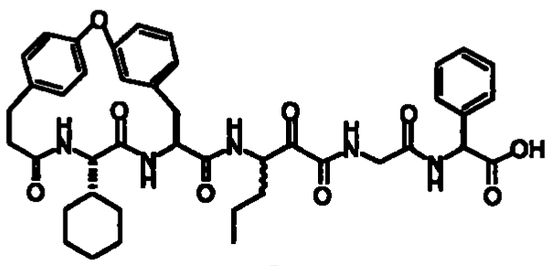
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Representative compounds of the invention which exhibit excellent HCV protease inhibitory activity are listed below:

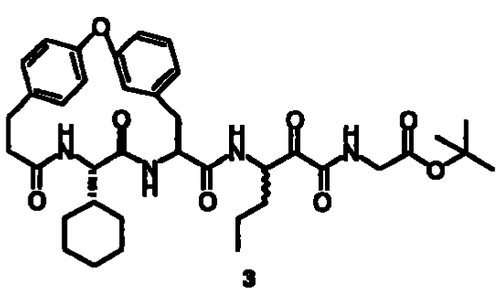
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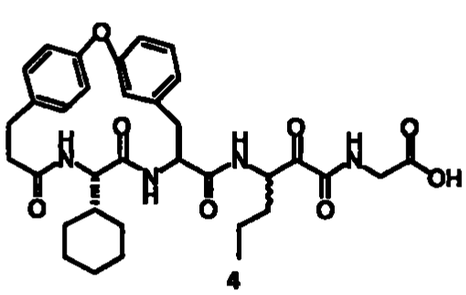
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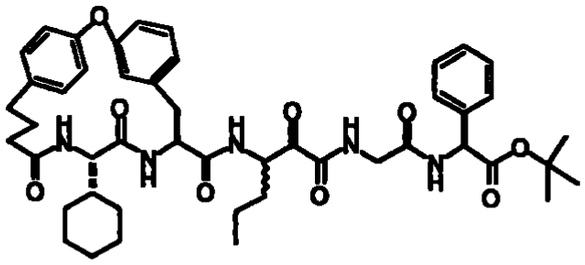


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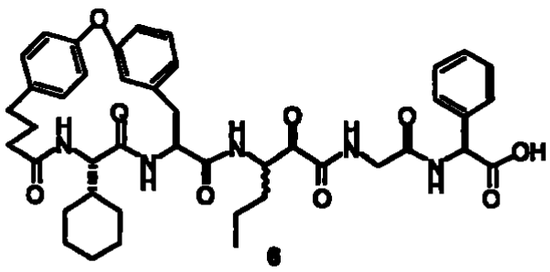


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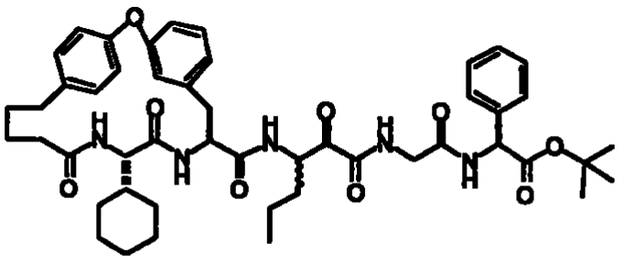
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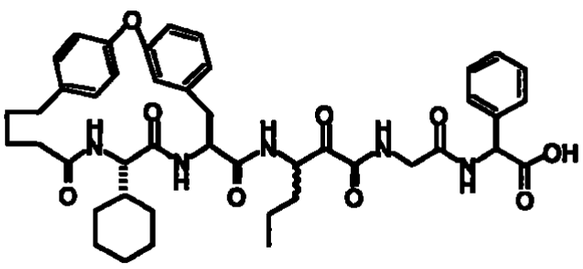
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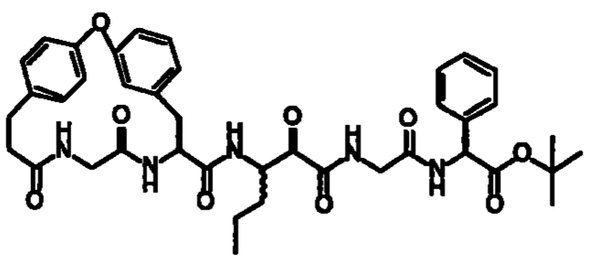
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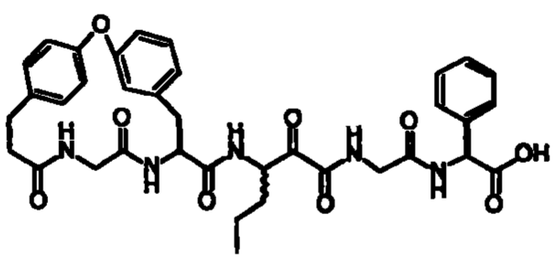
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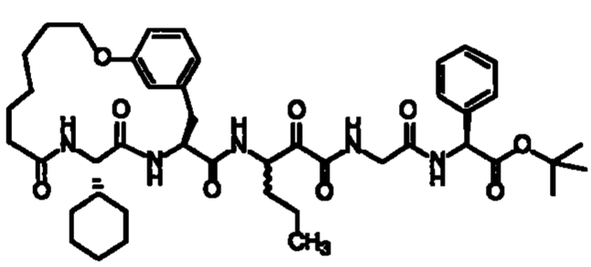
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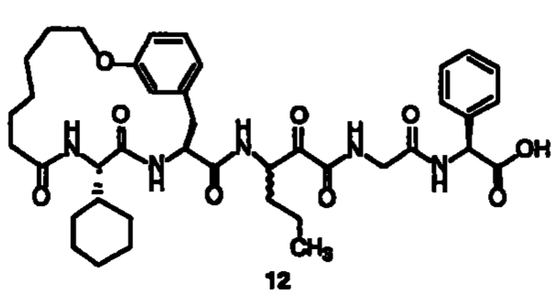
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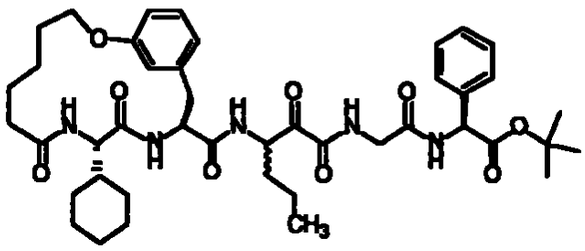
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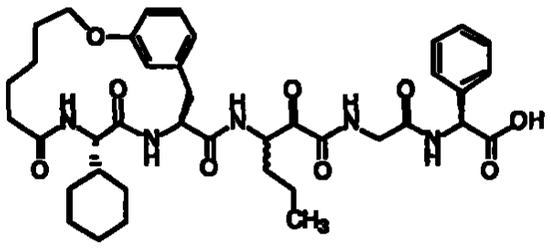
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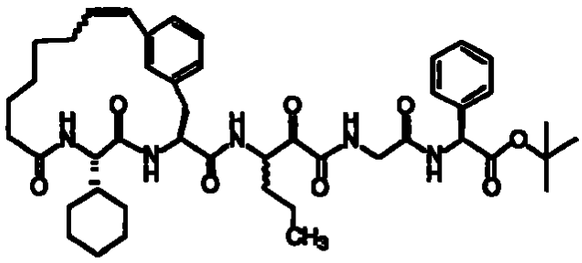
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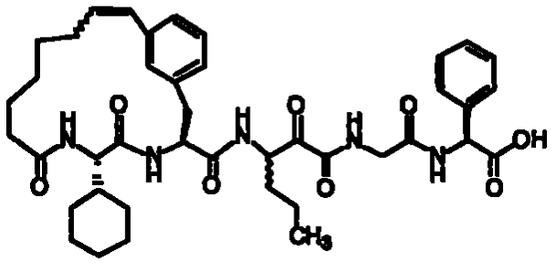
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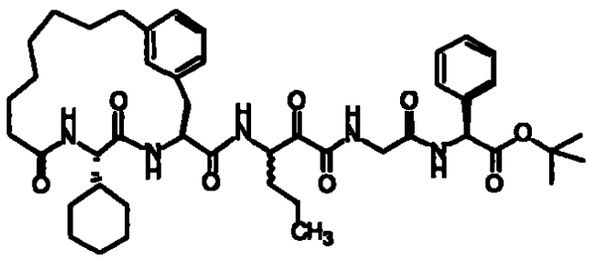


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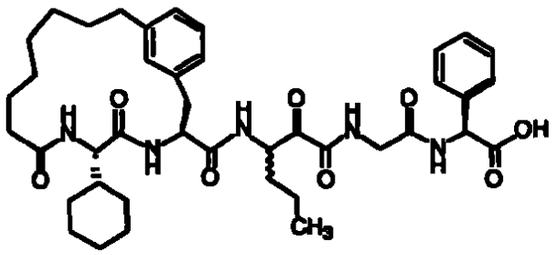
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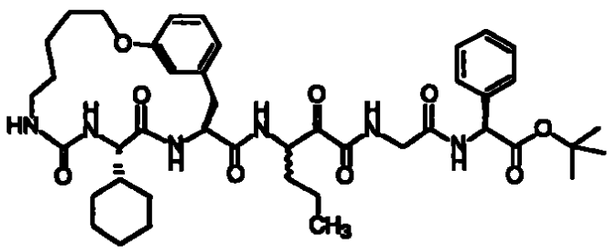
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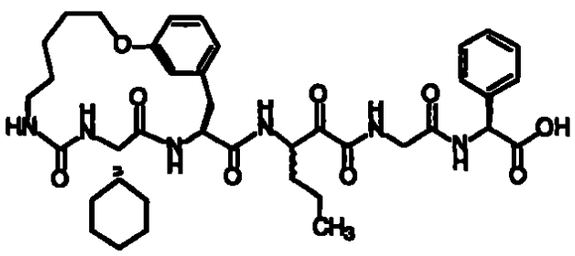
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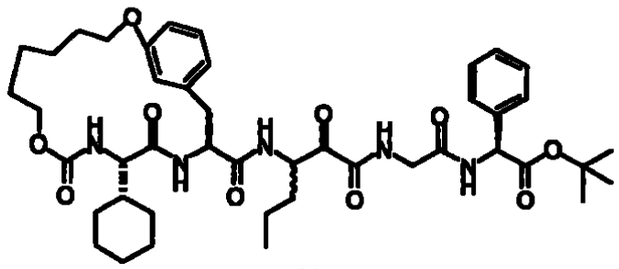
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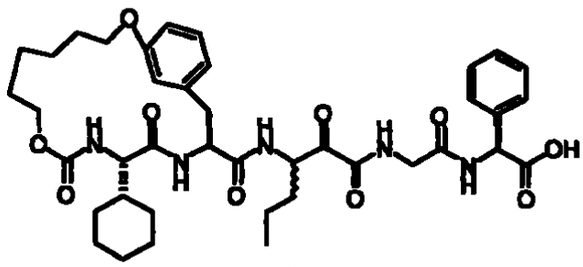
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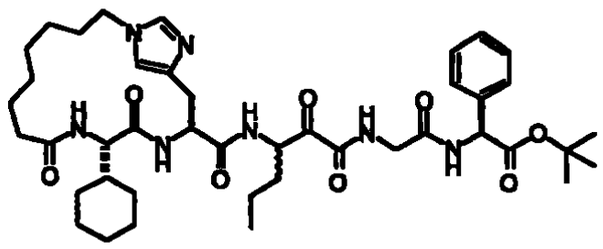
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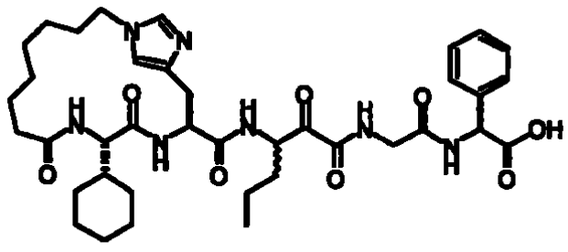
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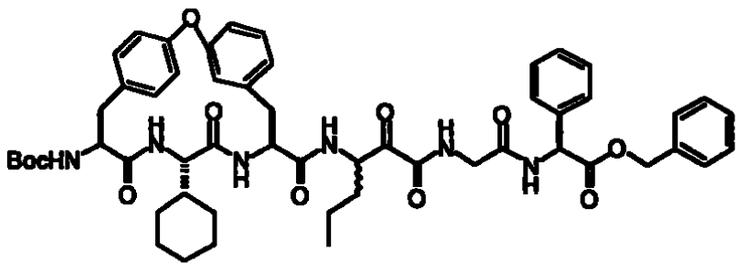


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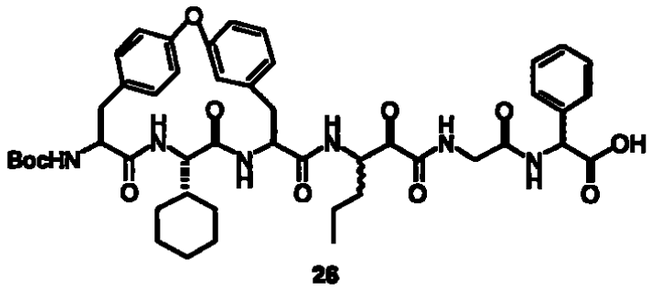


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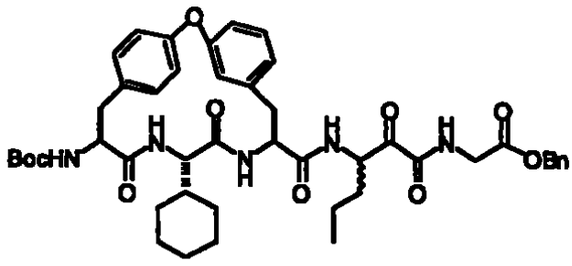
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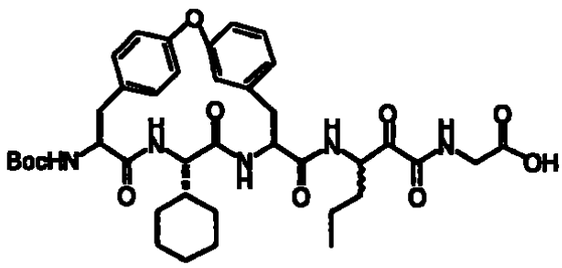
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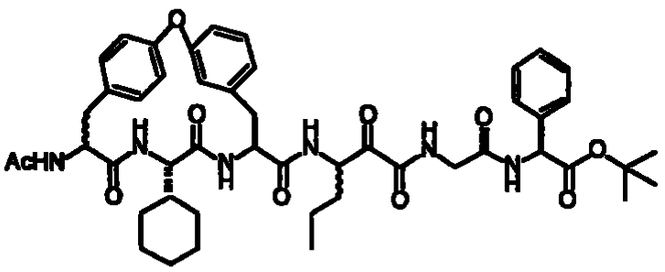
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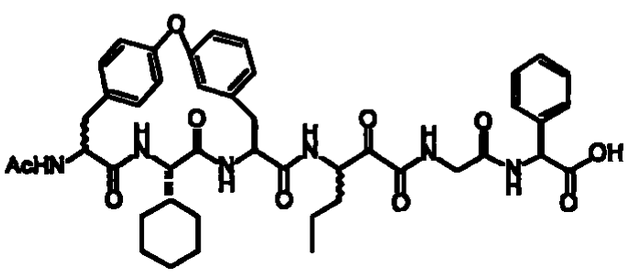
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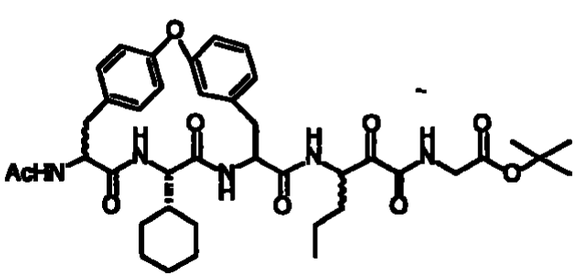
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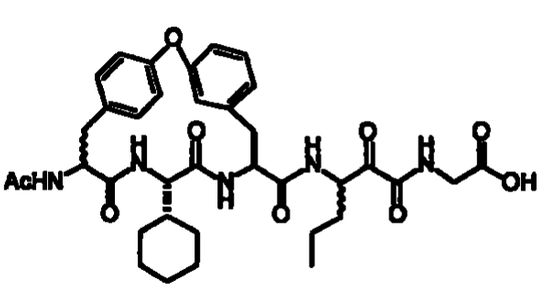
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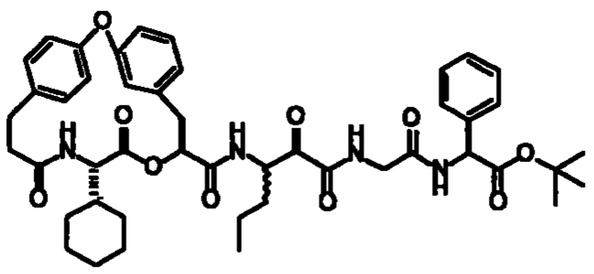


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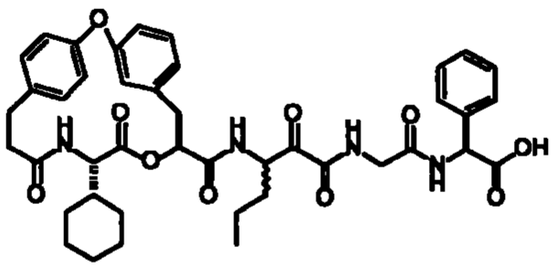
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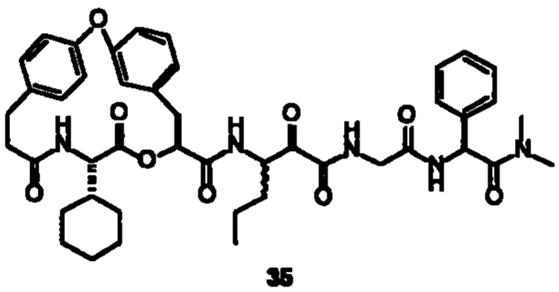
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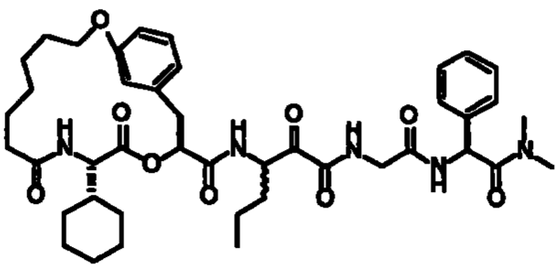
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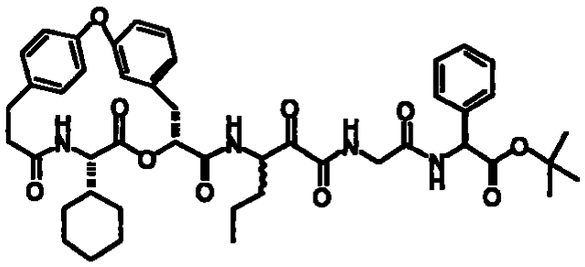


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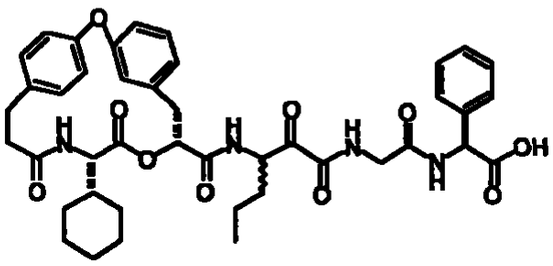


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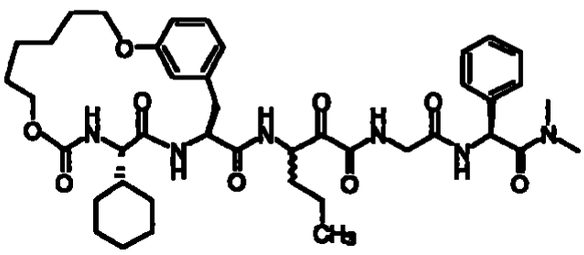
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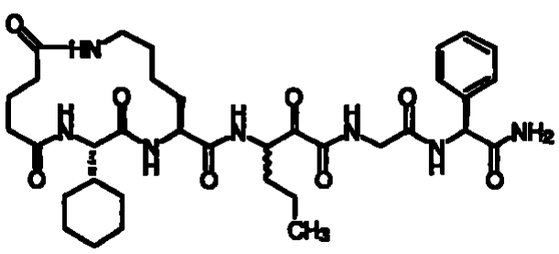
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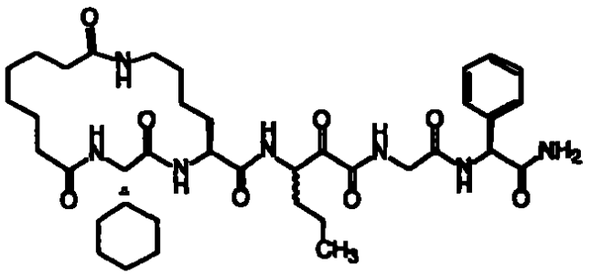


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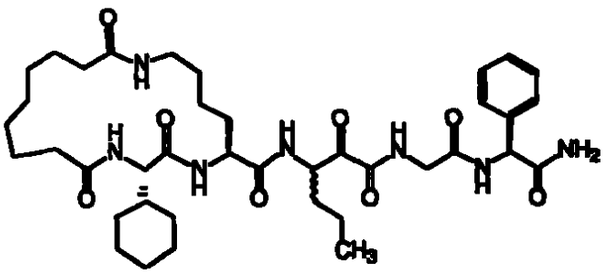


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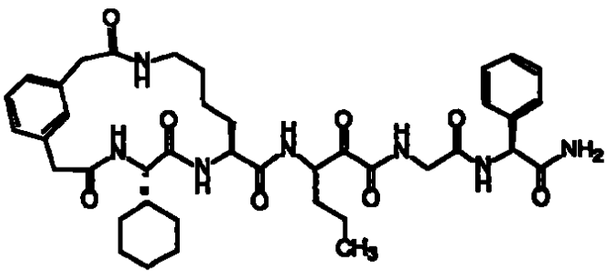
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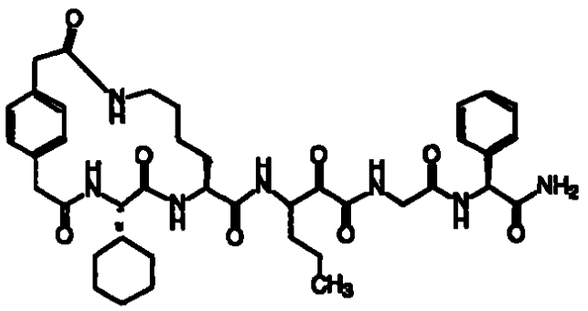
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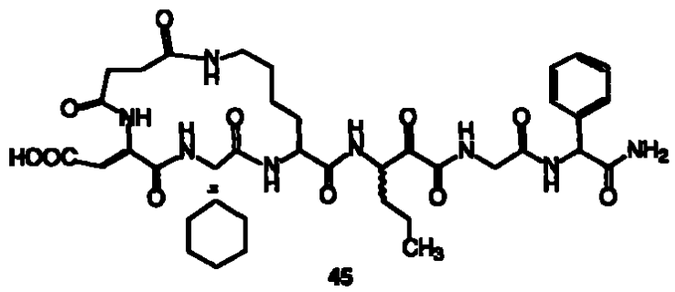
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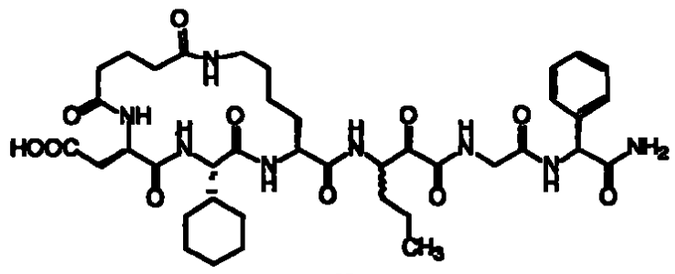
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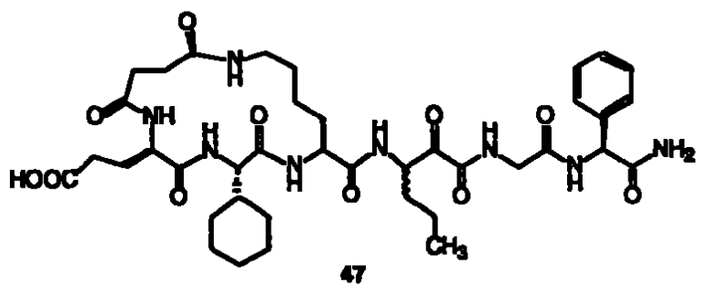
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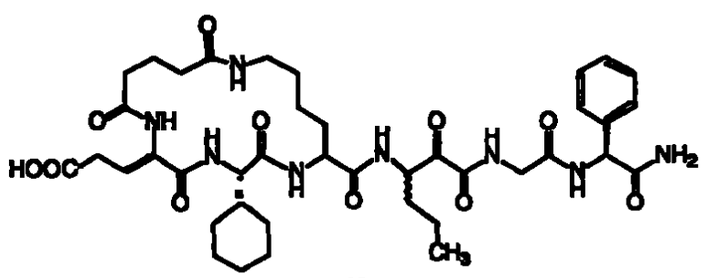
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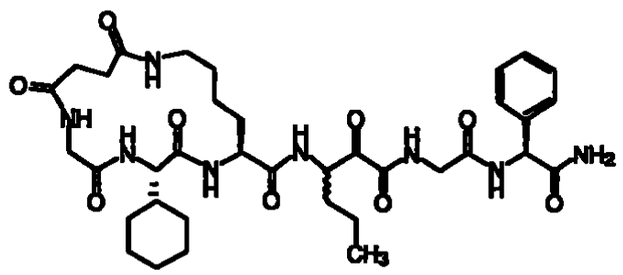
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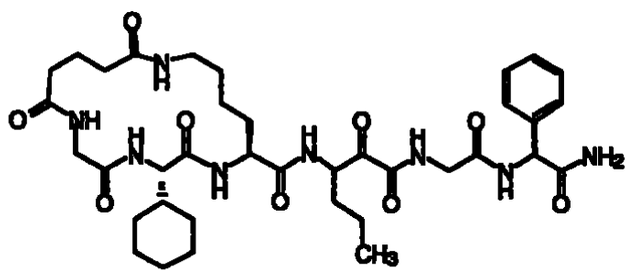
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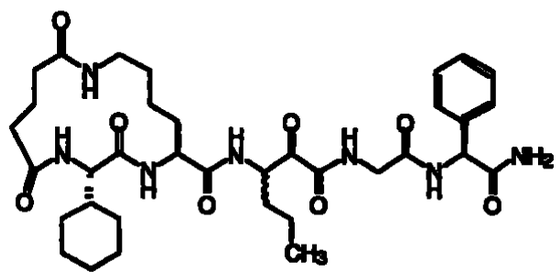
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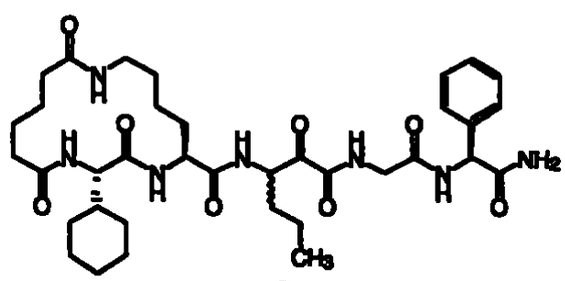
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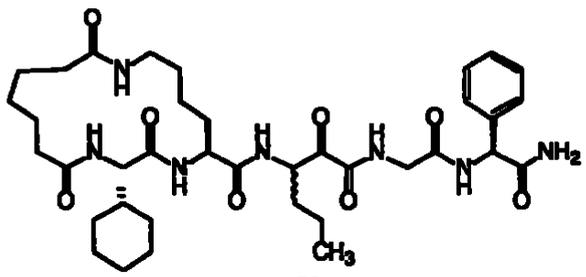
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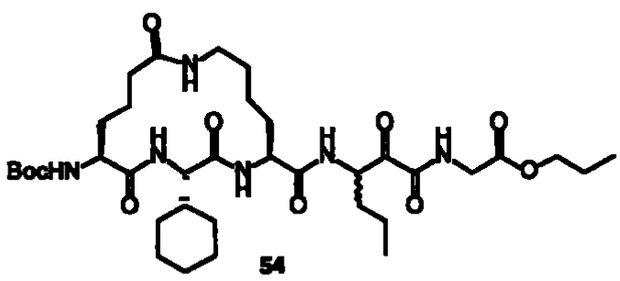
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The activity of some of the compounds are presented in Table 1 as ranges of K_i values in nanomolar (nM) The Example numbers in Table 1 refer to the numbers for the various structures in the EXAMPLES section found in the later parts of this application

Table 1. HCV protease continuous assay results

Example number	K_i* nM
1	B
2	A
3	B
4	B
5	B
6	A
7	B

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8	A
9	B
10	B
11	B
12	A
13	B
14	B
15	B
16	A
17	B
18	A
19	B
20	A
21	B
22	A
23	B
24	B
25	B
26	B
27	B
28	B
29	B
30	A
31	B
32	B
33	B
34	B
35	B
36	B

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37	B
38	B
39	A
40	B
41	A
42	B
43	A
44	A
45	B
46	B
47	B
48	B
49	B
50	B
51	B
52	A
53	A
54	B

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HCV continous assay Ki* range

Category A= 0 001-1 0 µM, Category B= 1 1-100 µM

Some methods of synthesizing the various types of the inventive compounds are described later in this section, and also schematically described, followed by the illustrative Examples

Depending upon the structure, the compounds of the invention may form pharmaceutically acceptable salts with organic or inorganic acids, or organic or inorganic bases Examples of suitable acids for such salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic and other mineral and carboxylic acids well known to those skilled in the art For formation of salts with

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bases, suitable bases are, for example, NaOH, KOH, NH_4OH, tetraalkylammonium hydroxide, and the like

In another embodiment, this invention provides pharmaceutical compositions comprising the above-described inventive macrocycles as an active ingredient The pharmaceutical compositions generally additionally comprise a pharmaceutically acceptable carrier diluent, excipient or carrier (collectively referred to herein as carrier materials) Because of their HCV inhibitory activity, such pharmaceutical compositions possess utility in treating hepatitis C and related disorders

In yet another embodiment, the present invention discloses methods for preparing pharmaceutical compositions comprising the inventive macrocycle compounds as an active ingredient In the pharmaceutical compositions and methods of the present invention, the active ingredients will typically be administered in admixture with suitable carrier materials suitably selected with respect to the intended form of administration, i e oral tablets, capsules (either solid-filled, semi-solid filled or liquid filled), powders for constitution, oral gels, elixirs, dispersible granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices For example, for oral administration in the form of tablets or capsules, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable inert carrier, such as lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid forms) and the like Moreover, when desired or needed, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated in the mixture Powders and tablets may be comprised of from about 5 to about 95 percent inventive composition. Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes Among the lubricants there may be mentioned for use in these dosage forms, boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like Disintegrants include starch, methylcellulose, guar gum and the like

Sweetening and flavoring agents and preservatives may also be included

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where appropriate Some of the terms noted above, namely disintegrants, diluents, lubricants, binders and the like, are discussed in more detail below

Additionally, the compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one or more of the components or active ingredients to optimize the therapeutic effects, i e HCV inhibitory activity and the like Suitable dosage forms for sustained release include layered tablets containing layers of varying disintegration rates or controlled release polymeric matrices impregnated with the active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric matrices

Liquid form preparations include solutions, suspensions and emulsions As an example may be mentioned water or water-propylene glycol solutions for parenteral injections or addition of sweeteners and pacifiers for oral solutions, suspensions and emulsions. Liquid form preparations may also include solutions for intranasal administration

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier such as inert compressed gas, e g nitrogen

For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides such as cocoa butter is first melted, and the active ingredient is dispersed homogeneously therein by stirring or similar mixing The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool and thereby solidify

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration Such liquid forms include solutions, suspensions and emulsions

The compounds of the invention may also be deliverable transdermally The transdermal compositions may take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose

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Preferably the compound is administered orally

Preferably, the pharmaceutical preparation is in a unit dosage form In such form, the preparation is subdivided into suitably sized unit doses containing appropriate quantities of the active components, e g , an effective amount to achieve the desired purpose

The quantity of the inventive active composition in a unit dose of preparation may be generally varied or adjusted from about 1 0 milligram to about 1,000 milligrams, preferably from about 1 0 to about 950 milligrams, more preferably from about 1 0 to about 500 milligrams, and typically from about 1 to about 250 milligrams, according to the particular application The actual dosage employed may be varied depending upon the patient's age, sex, weight and severity of the condition being treated Such techniques are well known to those skilled in the art.

Generally, the human oral dosage form containing the active ingredients can be administered 1 or 2 times per day The amount and frequency of the administration will be regulated according to the judgment of the attending clinician A generally recommended daily dosage regimen for oral administration may range from about 1 0 milligram to about 1,000 milligrams per day, in single or divided doses.

Some useful terms are described below

Capsule - refers to a special container or enclosure made of methyl cellulose, polyvinyl alcohols, or denatured gelatins or starch for holding or containing compositions comprising the active ingredients Hard shell capsules are typically made of blends of relatively high gel strength bone and pork skin gelatins The capsule itself may contain small amounts of dyes, opaquing agents, plasticizers and preservatives.

Tablet- refers to a compressed or molded solid dosage form containing the active ingredients with suitable diluents. The tablet can be prepared by compression of mixtures or granulations obtained by wet granulation, dry granulation or by compaction

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Oral gel- refers to the active ingredients dispersed or solubilized in a hydrophilic semi-solid matrix.

Powder for constitution refers to powder blends containing the active ingredients and suitable diluents which can be suspended in water or juices

Diluent - refers to substances that usually make up the major portion of the composition or dosage form Suitable diluents include sugars such as lactose, sucrose, mannitol and sorbitol, starches derived from wheat, corn, rice and potato, and celluloses such as microcrystalline cellulose The amount of diluent in the composition can range from about 10 to about 90% by weight of the total composition, preferably from about 25 to about 75%, more preferably from about 30 to about 60% by weight, even more preferably from about 12 to about 60%.

Disintegrant - refers to materials added to the composition to help it break apart (disintegrate) and release the medicaments Suitable disintegrants include starches, "cold water soluble" modified starches such as sodium carboxymethyl starch, natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar; cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose, microcrystalline celluloses and cross-linked microcrystalline celluloses such as sodium croscarmellose, alginates such as alginic acid and sodium alginate, clays such as bentonites, and effervescent mixtures The amount of disintegrant in the composition can range from about 2 to about 15% by weight of the composition, more preferably from about 4 to about 10% by weight.

Binder - refers to substances that bind or "glue" powders together and make them cohesive by forming granules, thus serving as the "adhesive" in the formulation Binders add cohesive strength already available in the diluent or bulking agent. Suitable binders include sugars such as sucrose, starches derived from wheat, corn rice and potato, natural gums such as acacia, gelatin and tragacanth, derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate, cellulosic materials such as methylcellulose and sodium carboxymethylcellulose and hydroxypropylmethylcellulose, polyvinylpyrrolidone, and inorganics such as magnesium aluminum silicate The

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amount of binder in the composition can range from about 2 to about 20% by weight of the composition, more preferably from about 3 to about 10% by weight, even more preferably from about 3 to about 6% by weight

Lubricant - refers to a substance added to the dosage form to enable the tablet, granules, etc after it has been compressed, to release from the mold or die by reducing friction or wear Suitable lubricants include metallic stearates such as magnesium stearate, calcium stearate or potassium stearate, stearic acid, high melting point waxes, and water soluble lubricants such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and d'l-leucine Lubricants are usually added at the very last step before compression, since they must be present on the surfaces of the granules and in between them and the parts of the tablet press The amount of lubricant in the composition can range from about 0.2 to about 5% by weight of the composition, preferably from about 0 5 to about 2%, more preferably from about 0.3 to about 1 5% by weight.

Glident - material that prevents caking and improve the flow characteristics of granulations, so that flow is smooth and uniform Suitable glidents include silicon dioxide and talc. The amount of glident in the composition can range from about 0 1% to about 5% by weight of the total composition, preferably from about 0 5 to about 2% by weight.

Coloring agents - excipients that provide coloration to the composition or the dosage form Such excipients can include food grade dyes and food grade dyes adsorbed onto a suitable adsorbent such as clay or aluminum oxide The amount of the coloring agent can vary from about 0 1 to about 5% by weight of the composition, preferably from about 0 1 to about 1%

Bioavailability - refers to the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed into the systemic circulation from an administered dosage form as compared to a standard or control

Conventional methods for preparing tablets are known. Such methods include dry methods such as direct compression and compression of granulation produced by compaction, or wet methods or other special procedures

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Conventional methods for making other forms for administration such as, for example, capsules, suppositories and the like are also well known

Another embodiment of the invention discloses the use of the pharmaceutical compositions disclosed above for treatment of diseases such as, for example, hepatitis C the like The method comprises administering a therapeutically effective amount of the inventive pharmaceutical composition to a patient having such a disease or diseases and in need of such a treatment

As stated earlier, the invention includes tautomers, enantiomers and other stereoisomers of the compounds also Thus, as one skilled in the art knows, some of the inventive compounds may exist in isomeric forms Such variations are contemplated to be within the scope of the invention

Another embodiment of the invention discloses a method of making the macrocyclic compounds disclosed herein The compounds may be prepared by several techniques known in the art. Representative illustrative procedures are outlined in the following reaction schemes It is to be understood that while the following illustrative schemes describe the preparation of macrocycles predominately derived from *meta*-tyrosine or lysine at the P2 position Suitable substitution of any of both the natural and unnatural amino acids will result in the formation of the desired macrocycles based on such substitution

Abbreviations which are used in the descriptions of the schemes, preparations and the examples that follow are

THF Tetrahydrofuran

DMF *N,N*-Dimethylformamide

EtOAc Ethyl acetate

AcOH Acetic acid

HOOBt: 3-Hydroxy-1,2,3-benzotriazin-4(3*H*)-one

EDCI 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

NMM *N*-Methylmorpholine

ADDP 1,1'-(Azodicarbobyl)dipiperidine

DEAD Diethylazodicarboxylate

MeOH Methanol

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EtOH Ethanol

Et_2O Diethyl ether

Bn Benzyl

Boc tert-Butyloxycarbonyl

Cbz Benzyloxycarbonyl

Cp Cylcopentyldienyl

Ts p-toluenesulfonyl

Me Methyl

HATU O-(7-Azabenzotriazol-1-yl)-N,N N',N'-tetramethyluronium hexafluorophosphate

Chg Cyclohexylglycine

Tyr Tyrosine

G Glycerol

TG Thioglycerol

alloc allyloxycarbonyl

FMOC 9-Fluorenyl methyloxycarbonyl

Dde N-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl

tBu *tert*-butyl

equiv equivalent

rel int. relative intensity

aq aqueous

rt room temperature

satd saturated

Hex hexane(s)

NBA Nitrobenzoic acid

PyBrOP Tris(pyrrolidino)bromophosphonium hexafluorophosphate

DMSO Dimethyl sulfoxide

TFA Trifluoroacetic acid

HOBt Hydroxybezotriazole

Hünigs base Diisoprpylethyl amine

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BOP Benzotrizaol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate

LDA Lithium diisopropyl amide

Ph_3P Triphenyl phosphine

LAH Lithium Aluminum Hydride

DMAP 4-Dimethyl aminopyridine

DCC Dicyclohexylcarbodiimide

MCPBA. meta-Chloroperbenzoic acid

BINAP 2,2'-Bis(diphenylphosphino)-1,1'-binaphtol

MeCN acetonitrile

Pr Propyl

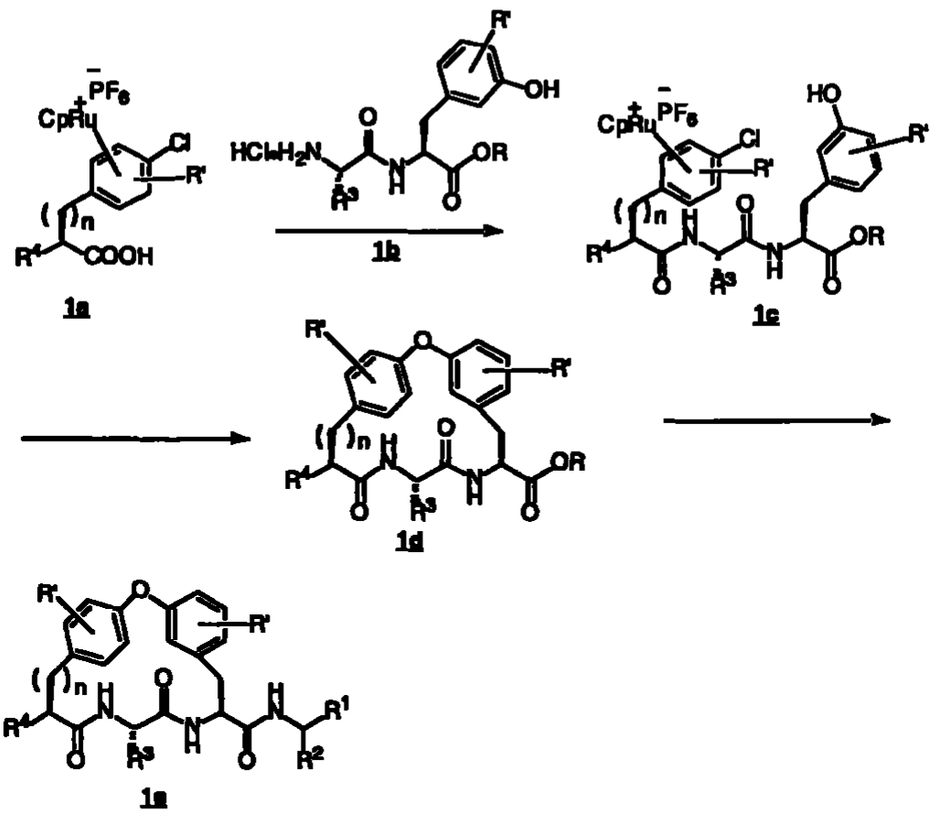
Ac. Acetyl

Ph Phenyl

General Schemes for Synthesis of Inventive Compounds. In the following Schemes, n is a number from 1 to 6

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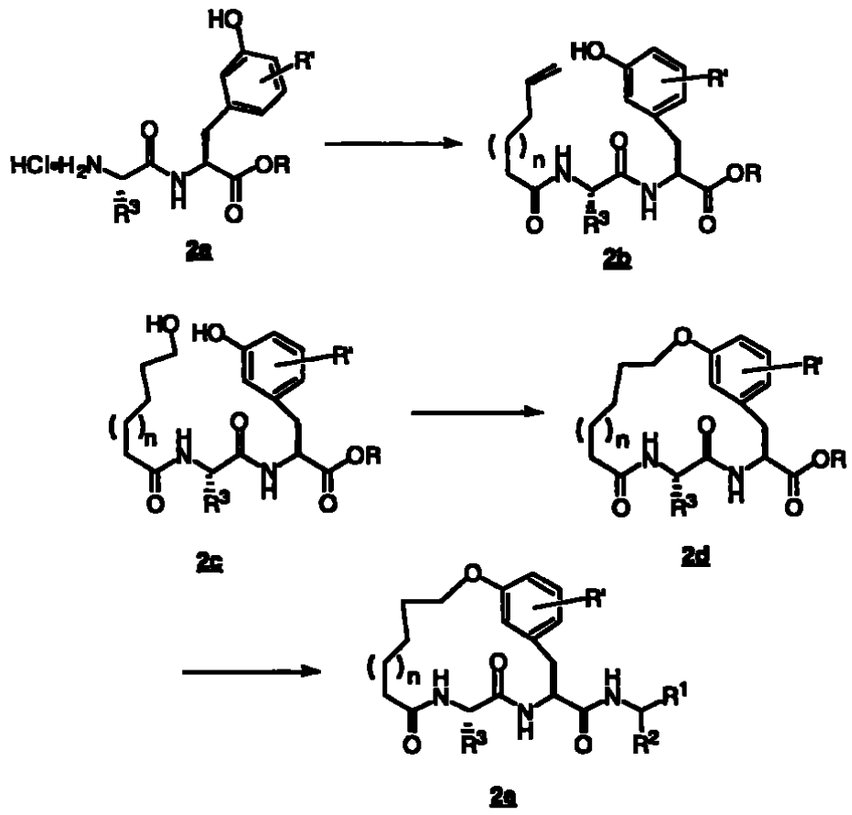
Scheme 1



The synthesis of compounds of type 1e wherein R^1, R^2, R^3, R' are defined above, R^4 being amide, carbamate, or hydrogen, R being alkyl, aryl or arylalkyl, began with the coupling of 1a with the dipeptide 1b using NMM, HOBt, and EDCI to obtain the intermediate 1c. The intermediate 1c was treated with Cs_2CO_3 in DMF followed by photolysis to obtain compound 1d. The macrocyclic ester 1d was hydrolyzed and coupled with a suitable amine intermediate to generate compounds of type 1e.

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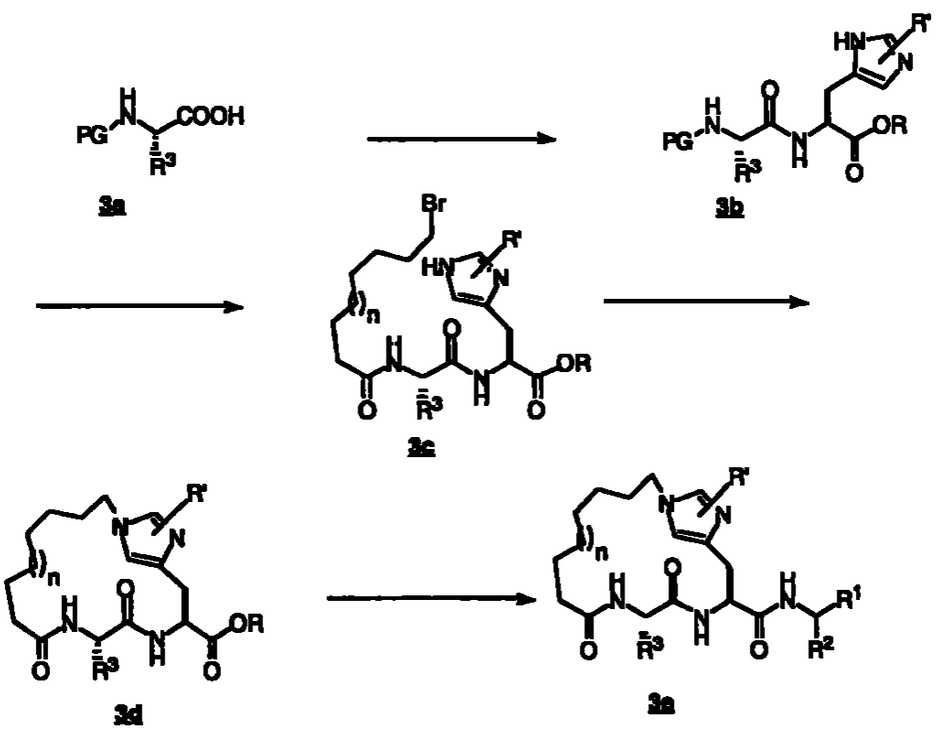
Scheme 2



The preparation of the compound of formula **2e**, wherein R^1, R^2, R^3 and n are defined above, R' is alkyl, heteroalkyl (OR", SR"', NR"R"' wherein R" and R"' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position to oxygen atom, R is alkyl, aryl, or alkylaryl groupe, and n is from zero to five, is outlined in Scheme 2 The meta-tyrosine-dipeptide **2a** is coupled to an alkenyl carboxylic acid in the presence of HOOBt, EDCI HCl and NMM Hydroboration of the resulting product affords compound **2c** The macrocyclization is achieved under Mitsunobu conditions by using triphenylphosphine and ADDP (The *Mitsunobu* reaction is reviewed by D L Hughes, *Org Reactions*, 42 (1992) 335, John Wiley & Sons, New York, L. Paquette, ed) After the ester is hydrolyzed to an acid with lithium hydroxide, it is coupled to an amine intermediate to afford **2e**

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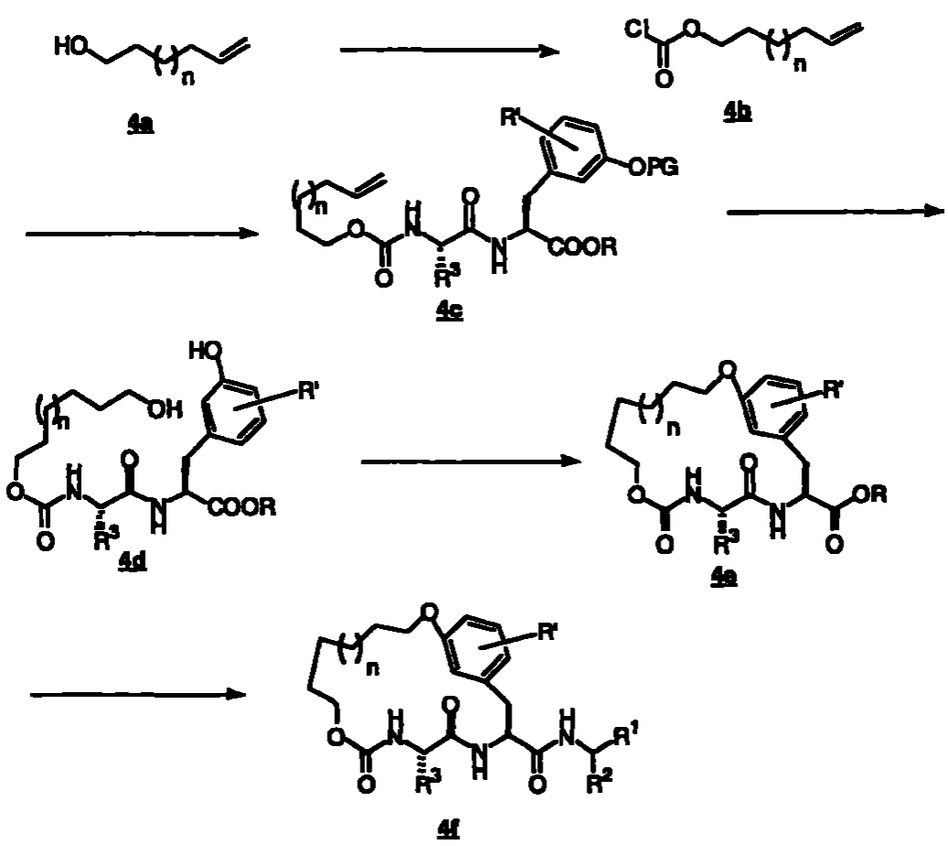
Scheme 3



The preparation of compounds of formula 3e wherein R', R^1, R^2, R^3, R and n are defined in Scheme 1 and PG is Cbz, Boc, or alloc is outlined in Scheme 3 The compound 3a was coupled with substituted histidine derivative using DCC This compound 3b was deprotected and further treated with ω-bromo acids to obtain compound of the type 3c. Cyclization of 3c was accomplished with NaI and Na_2CO_3 in boiling methylvinyl ketone to afford 3d The compound 3d was converted to compounds of type 3e by hydrolysis of the ester followed by coupling with the appropriate amine intermediate

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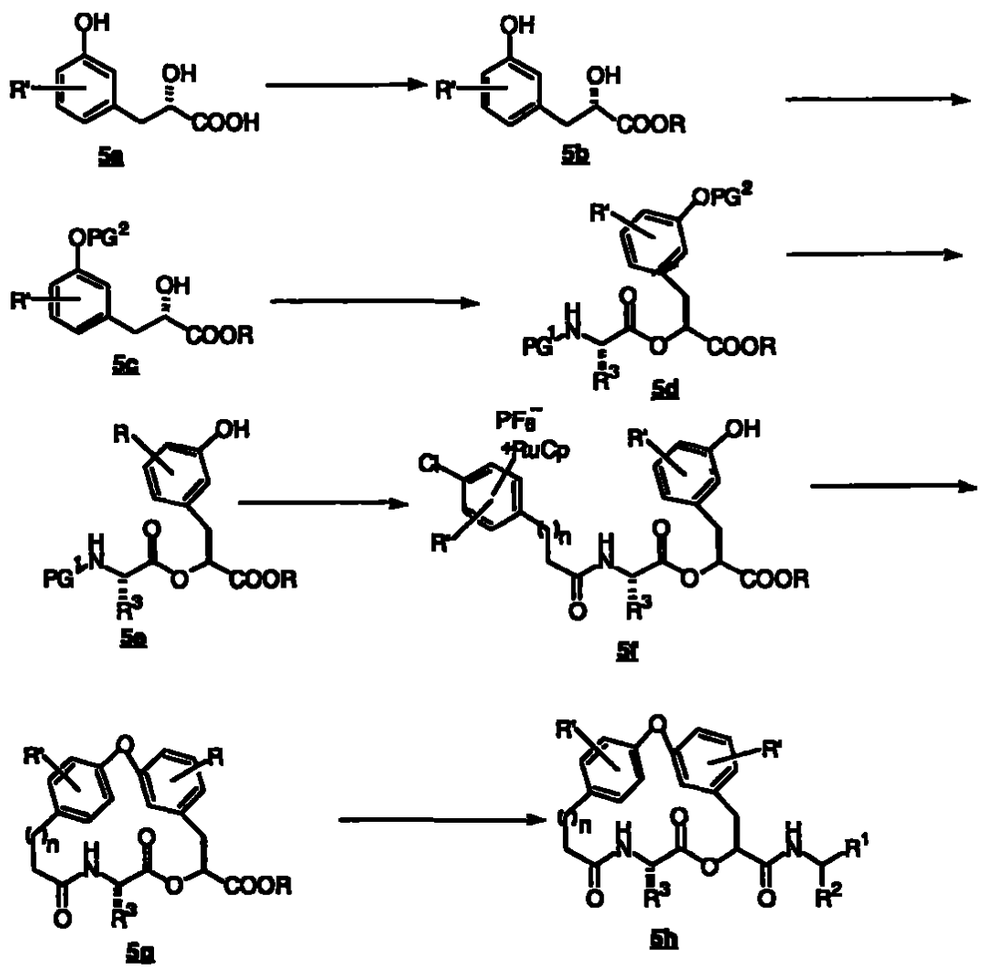
Scheme 4



The preparation of the compounds of type 4f where in R' R^1, R^2, R^3, R, n and PG are defined in **Scheme 1** was initiated from compound of the type 4a. The alcohol of 4a was converted to 4b by treatment with phosgene 4b was converted to 4c by coupling with alloc protected 1b and Et_3N The alloc group of 4c was deprotected using $Pd(PPh_3)_4$ to obtain 4d which underwent cyclization under the Mitsunobu conditions to yield 4e The ester of 4e was hydrolyzed and further coupled with an amine using EDCI, HOOBt to obtain compounds of the type 4f.

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Scheme 5.

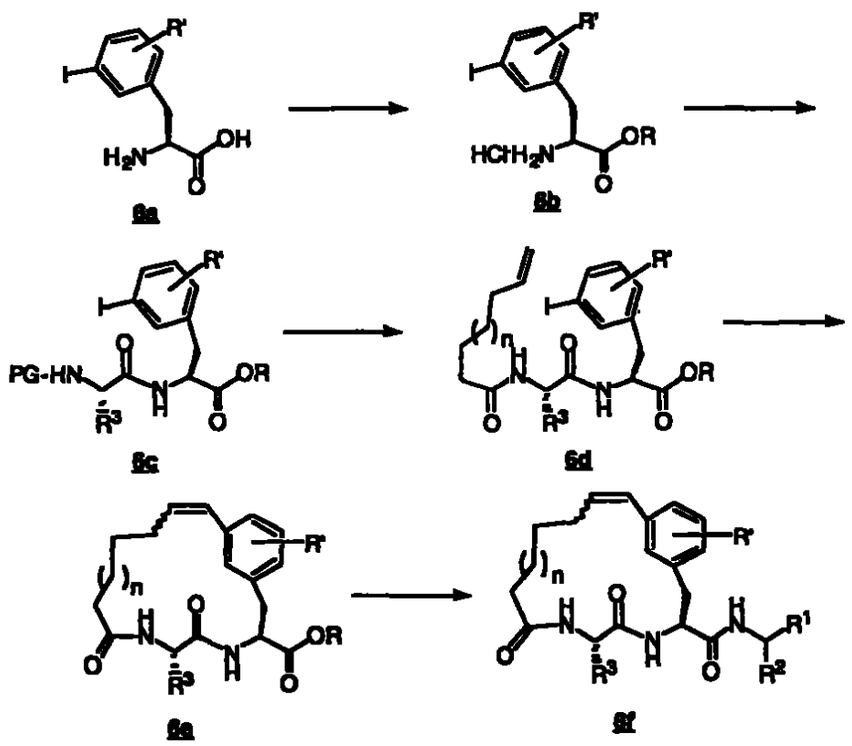


The preparation of compounds of type 5h wherein R^1, R^2, R^3, R', PG and n are defined in Schemes 1-3 and PG^1 being Cbz or Boc and PG^2 being alloc, began with the known compound 5a. The acid 5a was converted to the ester by refluxing it with ROH and TsOH. The phenolic oxygen of 5b was converted to the alloc group by the treatment with alloc-chloride and triethyl amine to yield 5c. The secondary alcohol of 5c was converted to compounds of type 5d by coupling with protected cyclohexyl glycine using DCC and HOBt. The alloc group of compound

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5d was deprotected using $Pd(Ph_3P)_4$, and dimedone 5e was deprotected and treated with EDCI, HOOBt and appropriately activated ruthenium complex to obtain compounds of the formula of 5f The compounds of the type 5f were converted to cyclic compounds of the formula 5g by the use of Cs_2CO_3, and subsequent photolytic removal of ruthenium The ester group of 5g was hydrolyzed and coupled to an amine intermediate to obtain compounds of the type 5h

Scheme 6.

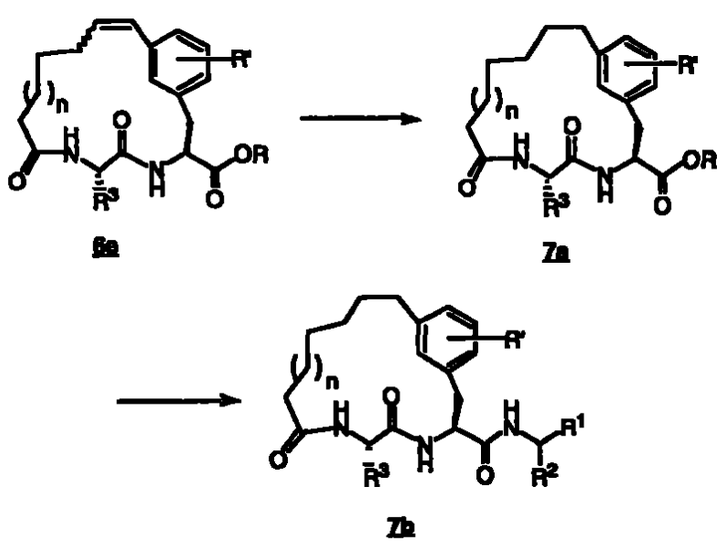


The preparation of the compound of formula 6f, wherein R^1, R^2, R^3 are defined above, R' is alkyl, heteroalkyl (OR", SR", NR"R"' wherein R" and R"' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position, R is alkyl, aryl, or alkylaryl groups, PG is Cbz or Boc, and n is from zero to five, is outlined in Scheme 6 The *meta*-Iodophenylglycine 6a is converted to its ester under usual

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esterification conditions (ROH, HCl) The product is then coupled to an *N*-protected amino acid in the presence of HOOBt, EDCI HCl and NMM After deprotection, the resulting amine is coupled again to a terminal alkenyl carboxylic acid to give product **6d**. The intramolecular Heck reaction with a palladium catalyst provides the desired macrocyclic compound **6e** (The Heck reaction has been reviewed in detail by R F Heck, *Org React*, 27 (1989) 345-390) After the ester is hydrolyzed to an acid with lithium hydroxide, it is coupled to an amine intermediate to afford **6f**.

Scheme 7.



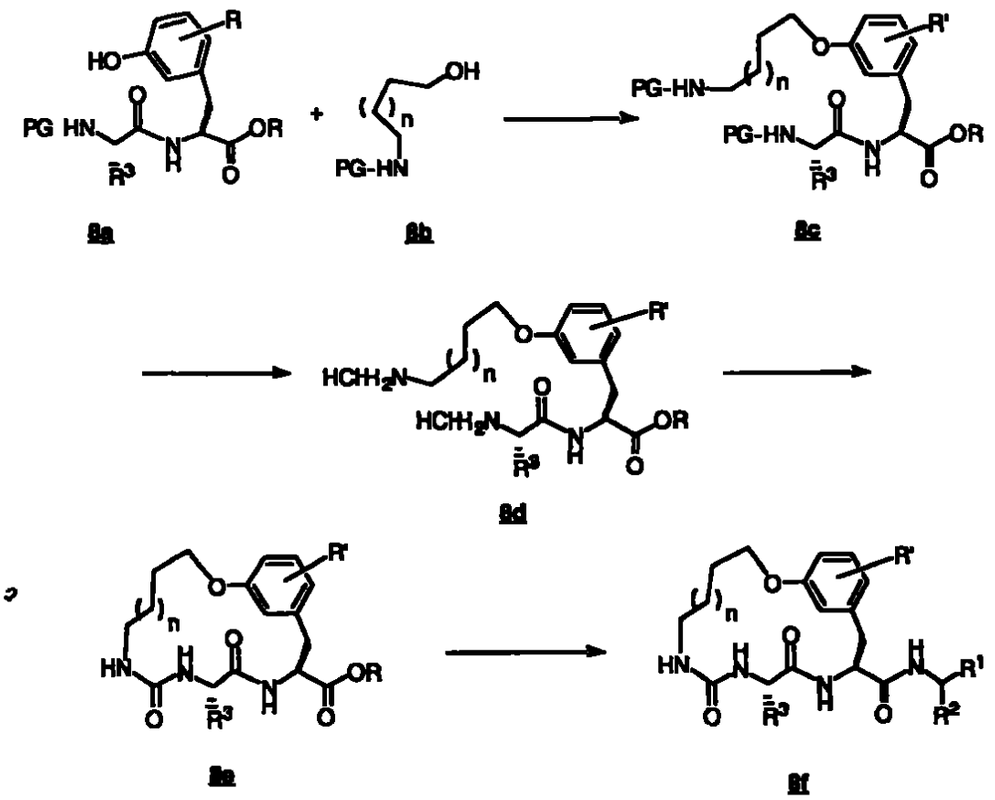
6e **7a**

7b

The preparation of the compound of formula **7b**, wherein R^1, R^2, R^3 are defined above, R' is alkyl, heteroalkyl (OR", SR", NR"R"' wherein R" and R"' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position, R is alkyl, aryl, or alkylaryl groups, and n is from zero to five, is outlined in Scheme 7 The hydrogenation of the double bond of **6e** gave the macrocycle **7a**. Hydrolysis of the ester to acid and subsequent coupling with an amine intermediate afforded **7b**

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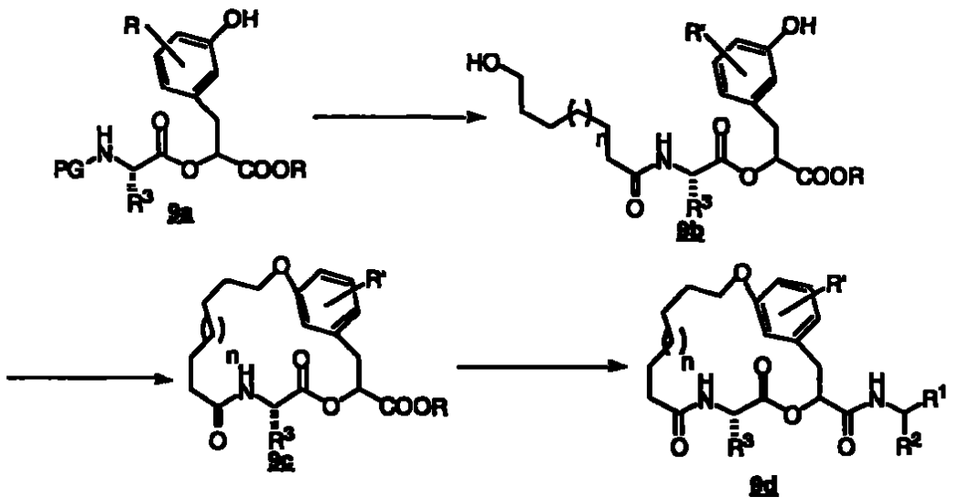
Scheme 8.



The preparation of the compound of formula 8f, wherein R^1, R^2, R^3 are defined above, R' is alkyl, heteroalkyl (OR", SR", NR"R"' wherein R" and R"' are alkyl groups), halo substituent at *ortho*, *meta*, or *para* -position to oxygen atom, R is alkyl, aryl, or alkylaryl groups, PG is Boc, and n is from zero to five, is outlined in Scheme 8 The *meta*-tyrosine-dipeptide 8a is coupled to a terminal *N*-protected amino alcohol 8b under Mitsunobu conditions (triphenylphosphine and ADDP) The protecting groups are removed to give diamine hydrochloride 8d The macrocyclization is achieved by forming the urea linkage using phosgene or carbonyl diimidazole The resulting ester is then hydrolyzed to an acid with lithium hydroxide and is subsequently coupled to an amine intermediate to afford the desired product 8f

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Scheme 9



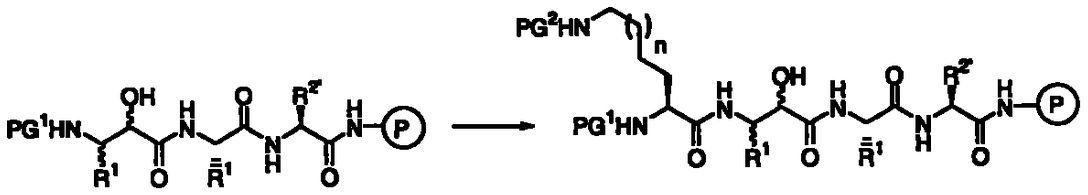
The synthesis of compounds of the type 9d wherein the substituents R^1, R^2, R^3, R', R and PG are defined in Scheme 1, was initiated by the deprotection of 9a with HCl in dioxane and coupling with ω-hydroxy acid using EDCI, HOOBt to yield compounds of the type 9b The compounds of the type 9b were further cyclized using Ph_3P, ADDP to generate compounds of the type 9c. The cyclic compound 9c was deprotected and coupled with an appropriate amine intermediate to generate compound of type 9d.

Scheme 10.

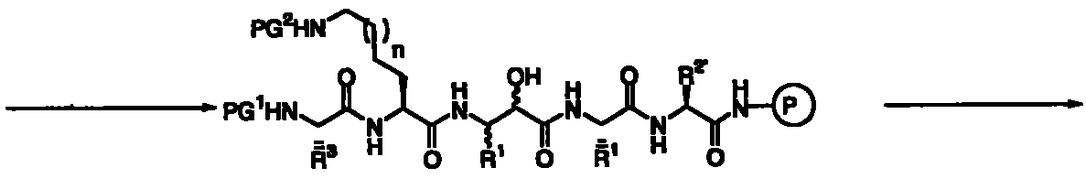
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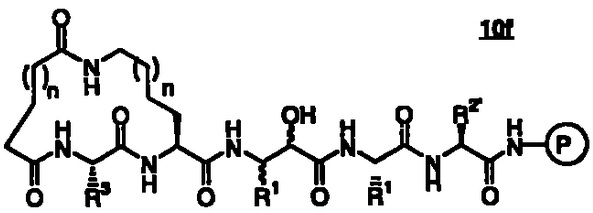
10a 10b 10c



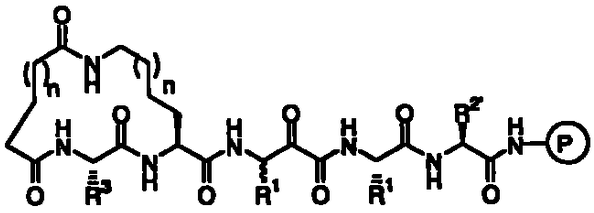
10d 10e



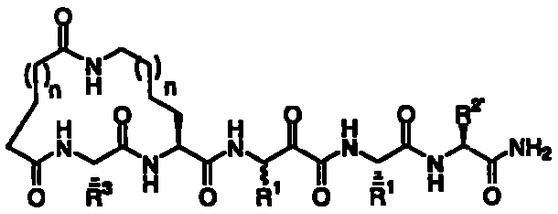
10f



10g



10h



10i

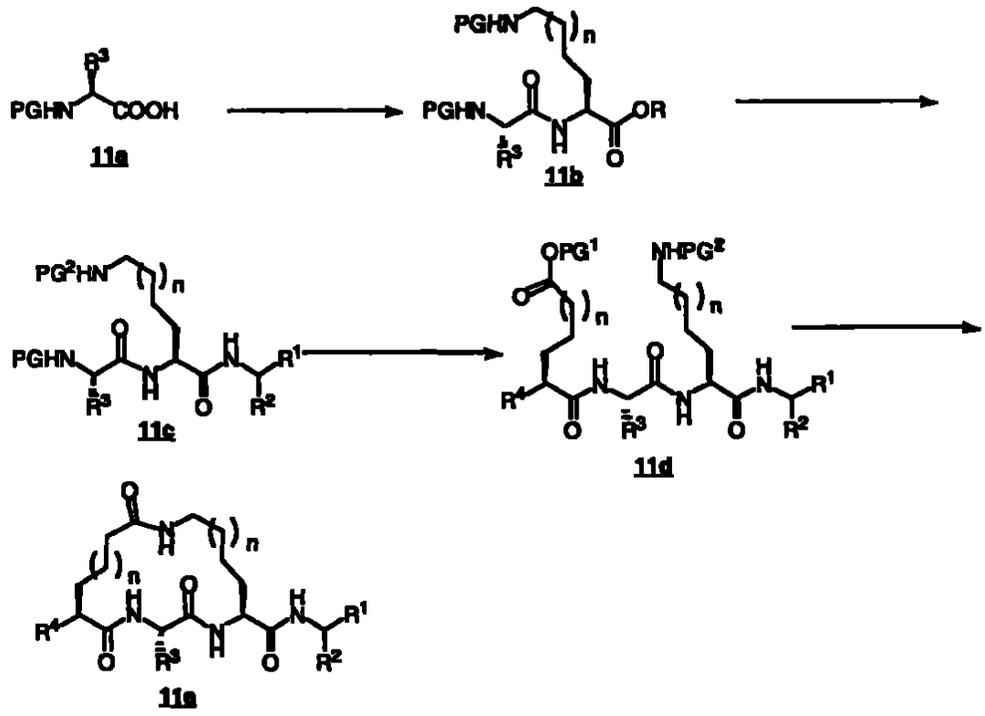
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The synthesis of the compounds of the type 10l wherein $R^{2'}$, $R^{1'}$, R^1, R^2 are defined in Scheme 1 PG^1 and PG^2 are defined as protecting groups namely Fmoc and Dde P is defined as a polymer support wherein the compound is immobilized, and n is a number from 1-6 The methodology used for the synthesis of molecules of type 10l is a standard solid phase peptide synthesis with Fmoc protecting group Fmoc protected Sasarin resin 10a is first deprotected by the treatment of piperidine followed by coupling with the Fmoc protected amino acid using HATU to obtain compound of type 10b. The protecting group of 10b was removed and coupled with amino acid to using HATU to obtain 10c The polymer supported 10c was deprotected by treatment with piperidine and coupled with hydroxy acid to obtain hydroxy amide of type 10d.The protecting group of 10d was cleaved and coupled with protected lysine derivative using HATU to obtain compounds of type 10e The protecting group of 10e was once again deprotected and coupled with Fmoc protected amino acid to obtain compound of the type 10f The protecting groups PG^1 and PG^2 were removed and cyclized using a diacid and HATU to obtain the macrocycle 10g. The compound of 10g was oxidized using Dess-Martin reagent and finally cleaved from the resin using TFA to obtain compound 10l

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Scheme 11.



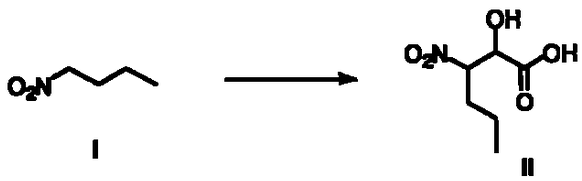
The synthesis of the compounds of type 11e wherein R^1, R^2, R^3, R^4 and n are defined in Scheme 1 and PG^2 is Cbz, PG^1 is Bn and PG is Boc, was initiated from protected acid 11a. 11a was converted to compounds of type 11b by coupling with lysine derivative using EDCI, HOOBt methodology The ester group of 11b was hydrolyzed using LiOH H_2O followed by the coupling with an appropriate amine intermediate to obtain the compound 11c This was further treated with HCl in dioxane and coupled with lysine intermediate using EDCI, HOOBt to form compounds of the type 11d. The compounds 11d were deprotected and cyclized using EDCI, HOOBt to form compounds of type 11e

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Preparation of Intermediates

Intermediate A.

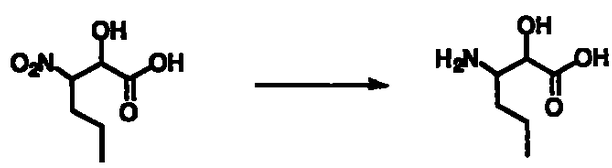
Step 1.



I II

To a stirred solution of 1-nitrobutane (16.5 g, 0 16 mol) and glyoxylic acid in H_2O (28 1 g, 0 305 mol) and MeOH (122 mL) at 0°C-5°C, was added dropwise triethyl amine (93 mL, 0 667 mol) over 2 hrs. The solution was warmed to room temperature , stirred overnight and concentrated to dryness to give an oil The oil was then dissolved in H_2O and acidified to pH =1 with 10% HCl, followed by extraction with EtOAc. The combined organic solution was washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness to give the product II (28 1 g, 99% yield)

Step 2.

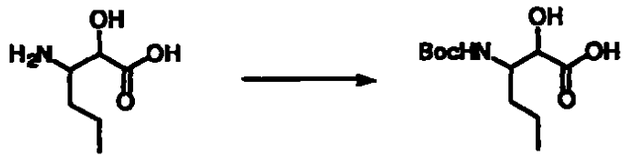


II III

To a stirred solution of starting material II (240 g, 1 35 mol) in acetic acid (1.25 L) was added 10% Pd/C (37 g) The resulting solution was hydrogenated at 59 psi for 3 hrs and then at 60 psi overnight. The acetic acid was then evaporated and azeotroped 3 times with toluene, then triturated with MeOH and ether The solution was then filtered and azeotroped twice with toluene to give III as an off white solid (131 g, 0.891 mol, 66%)

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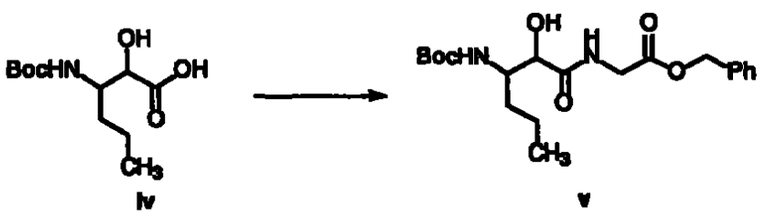
Step 3.



III IV

To a stirred solution of the amino acid III (2.0 g, 013.6 mmol) in dioxane (10 mL) and H_2O (5mL) at 0°C, was added 1N NaOH solution (4 3 mL, 14.0 mmol) The resulting solution was stirred for 10 minutes, followed by addition of di-t-butyldicarbonate (0 110 g, 14 0 mmol) and stirred at 0 °C for 15 minutes The solution was then warmed to room temperature, stirred for 45 minutes and kept in refrigerator overnight and concentrated to dryness to give a crude material To the solution of this crude material in EtOAc (100 mL) and ice, was added $KHSO_4$ (3 36 g) and H_2O (32 mL) and stirred for 4-6 minutes The organic layer was then separated and the aqueous layer was extracted twice with EtOAc and the combined organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated to dryness to give the product IV as a clear gum (3 0 g, 89% yield)

Step 4.

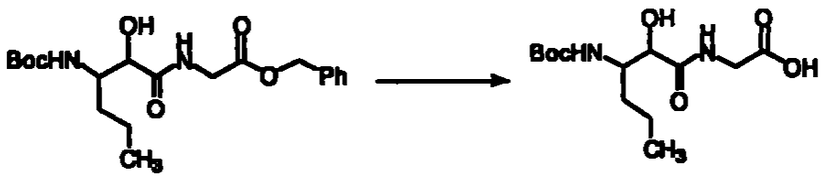


IV V

To a stirred solution of IV (3 00 g, 12.0 mmol) in DMF (15 mL) and CH_2Cl_2 (15 mL) at -20°C was added HOOBt (1 97 g, 12 0 mmol), *N*-methyl morpholine (4 0 mL, 36 0 mmol) and EDCI (2 79 g, 14 5 mmol) and stirred for 10 minutes, followed by addition of HCl H_2N-Gly-OBn (2 56 g, 13 0 mmol) The resulting solution was stirred at -20°C for 2 hrs, then kept in refrigerator overnight and

concentrated to dryness, followed by dilution with EtOAc (150 mL) The EtOAc solution was then washed twice with saturated NaHCO3, H2O, 5% H_3PO_4, brine, dried over Na_2SO_4, filtered and concentrated to dryness to give the product v (4.5 g, 94%) LRMS *m/z* MH^+= 395 1

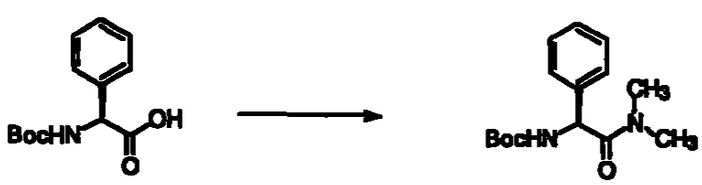
Step 5.



v vi

The solution of starting material v (7 00 g 17 8 mmol) in absolute ethanol (300 mL) was stirred at room temperature under a hydrogen atmosphere in the presence of Pd-C (300 mg, 10%) The reaction progress was monitored by tlc After 2h, the mixture was filtered through a celite pad and the resulting solution was concentrated *in vacuo* to give the product vi (5 40 g, quantitative) LRMS *m/z* MH^+= 305 1

Step 6.



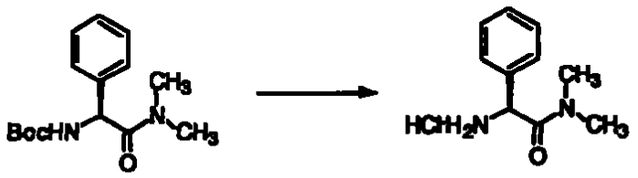
vii viii

To a solution of dimethylamine hydrochloride (1.61 g, 19 7 mmol), *N*-Boc-phenylglycine (4.50 g, 17.9 mmol), HOOBt (3 07 g, 18.8 mmol) and EDCI (4 12 g, 21 5 mmol) in anhydrous DMF (200 mL) and CH_2Cl_2 (150 mL) at -20°C was added NMM (5 90 mL, 53 7 mmol) After being stirred at this temperature for 30 min, the reaction mixture was kept in a freezer overnight (18 h) It was then allowed to warm to rt, and EtOAc (450 mL), brine (100 mL) and 5% H_3PO_4 (100 mL) were added After layers were separated, the organic solution was washed with 5% H_3PO_4 (100 mL), saturated aqueous sodium bicarbonate solution (2 X

0418 411

150 mL), water (150 mL), and brine (150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford crude product viii (4.86 g) as a white solid, which was used without further purification

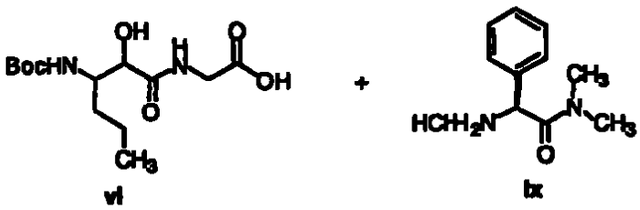
Step 7



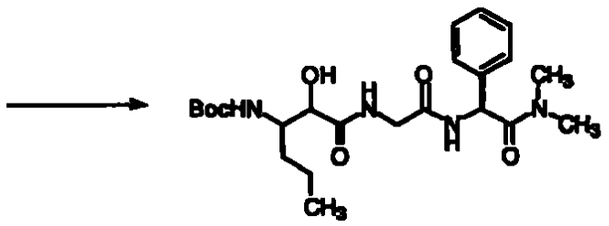
viii ix

The *N*-Boc-phenylglycine dimethylamide viii (4 70 g, crude) was dissolved in 4 N HCl (60 mL, 240 mmol) and the resulting solution was stirred at room temperature The progress of the reaction was monitored by TLC After 4 h, the solution was concentrated *in vacuo* to yield ix as a white solid which was used in the next reaction without further purification LRMS *m/z* MH^+= 179 0

Step 8.



vi ix

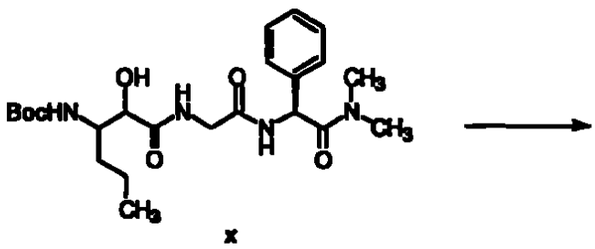


x

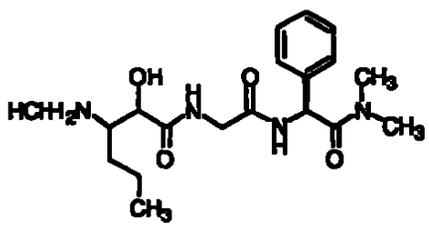
The desired compound x was prepared according to the coupling procedures described in Step 4. LRMS *m/z* MH^+= 465 1

412

Step 9.



x

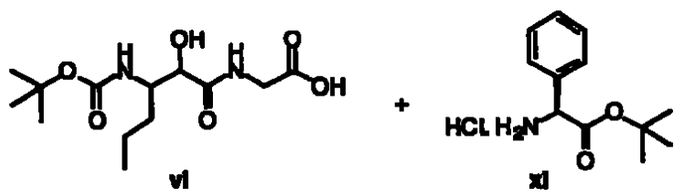


A

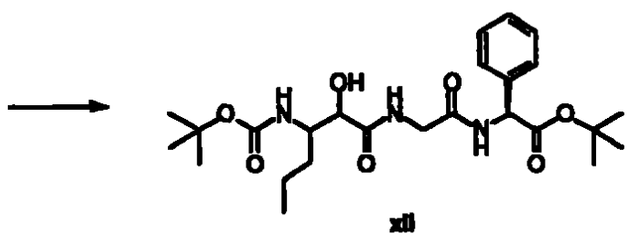
The desired intermediate A was prepared from tripeptide x according to the procedures described in Step 7 LRMS *m/z* MH^+= 365 1

Intermediate B.

Step 1.



vi xi



xii

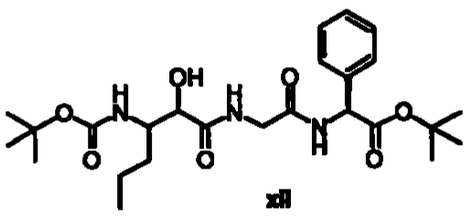
The desired product xii was obtained by the procedure described for Intermediate A, Step 8 using commercially available xi as the coupling partner The crude material was sufficiently pure for further studies A portion of the

C/2n
413

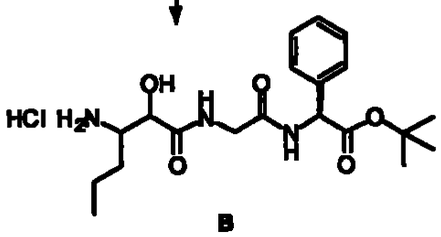
product was purified by flash chromatography using 97/3 dichloromethane/MeOH

HRMS (FAB) Calcd for $C_{25}H_{40}N_3O_7$ 494 2866 $(M+H)^+$ Found 494 2863

Step 2



xii

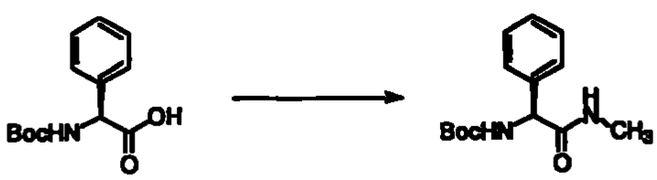


B

The desired product B was obtained by the procedure described for Intermediate A, Step 7 The crude material was used without further purification

Intermediate C.

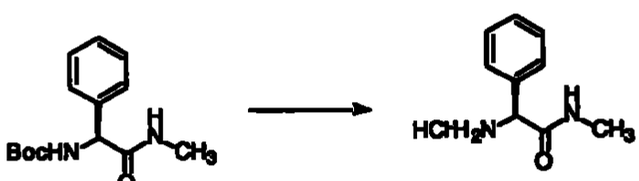
Step 1.



vii xiii

The desired compound xiii was prepared according to the coupling procedures described in Step 6 for intermediate A

Step 2.

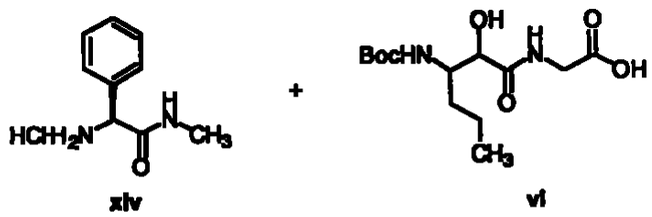


xiii xiv

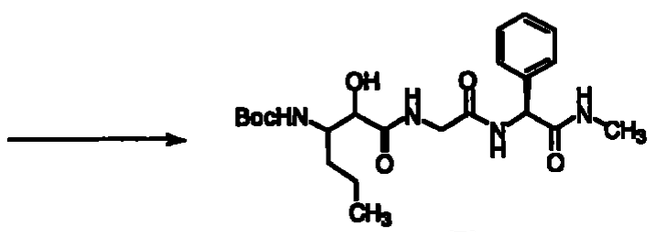
414

The desired compound xiv was prepared according to the procedures described in Step 7 for intermediate A

Step 3.



xiv vi

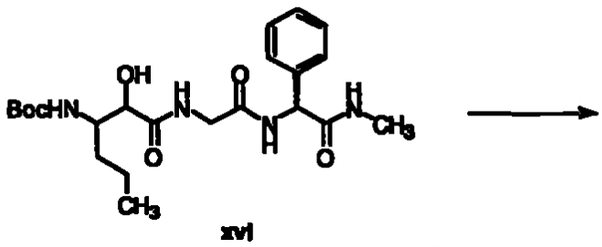


xv

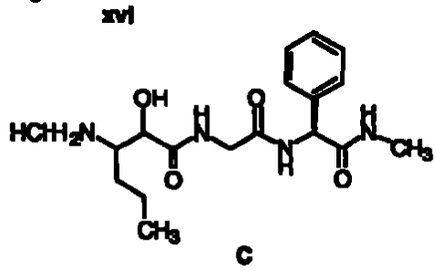
The desired compound xv was prepared according to the coupling procedures described in Step 6 for intermediate A LRMS *m/z* MH^+= 451 1

0425
415

Step 4



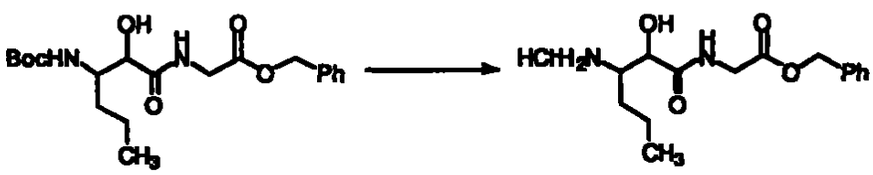
xvi



C

The desired intermediate C was prepared according to the procedures described in Step 7 for intermediate A LRMS *m/z* MH^+= 351 1 It was used without further purification

Intermediate D.



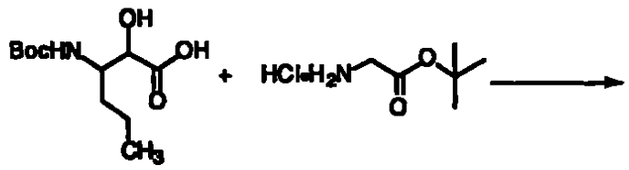
v D

The desired intermediate D was prepared from compound v according to the procedures described in Step 7 for intermediate A. It was used without further purification

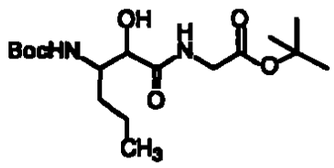
C427 416

Intermediate E.

Step 1.



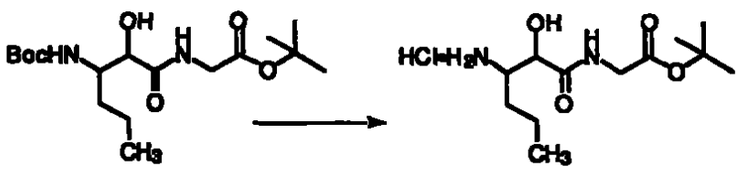
iv xvii



xviii

The desired product xviii was obtained by the procedure described for Intermediate A, Step 8 using commercially available xvii as the coupling partner The crude material was sufficiently pure for further studies

Step 2

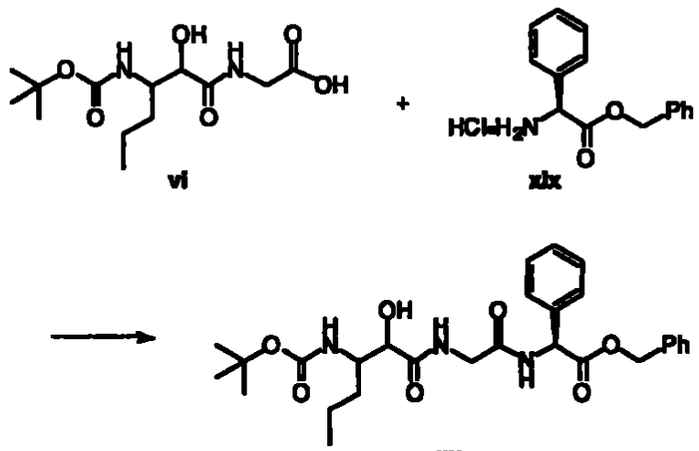


xviii E

The desired product E was obtained by the procedure described for Intermediate A, Step 7. The crude material was used without further purification

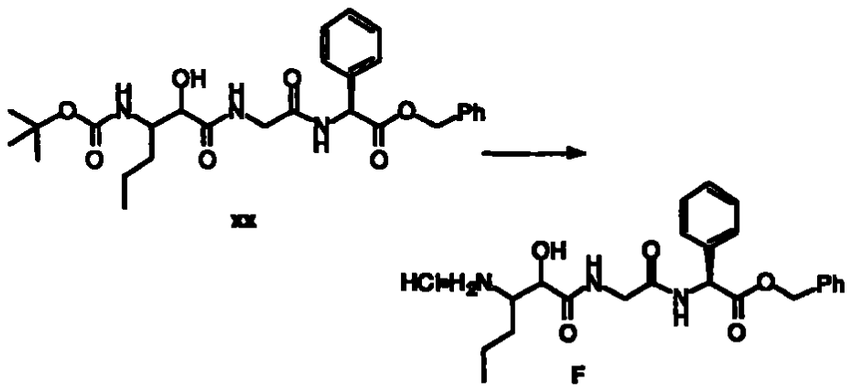
Intermediate F.

Step 1



The desired product xx was obtained by the procedure described for Intermediate A, Step 4 using commercially available xix as the coupling partner The crude material was sufficiently pure for further studies

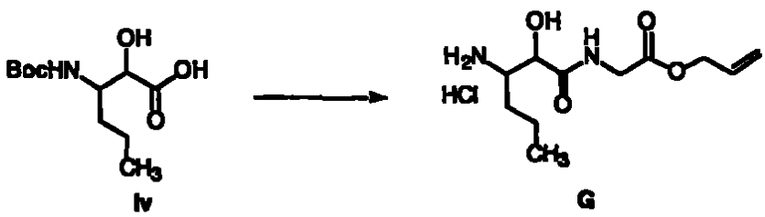
Step 2



The desired product F was obtained by the procedure described for Intermediate D

Intermediate G

Step 1

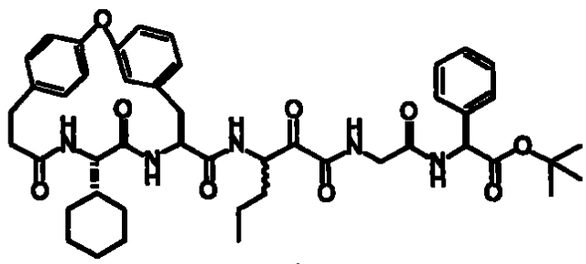


Iv G

The desired product G was obtained by the procedure described for Intermediate A, Step 4 using allylglycine as the coupling partner The crude material was sufficiently pure The crude product was treated with 4N HCl/ Dioxane and stirred at rt for 50 min The reaction mixture was concentrated to dryness to yield Intermediate G which was used without further purification

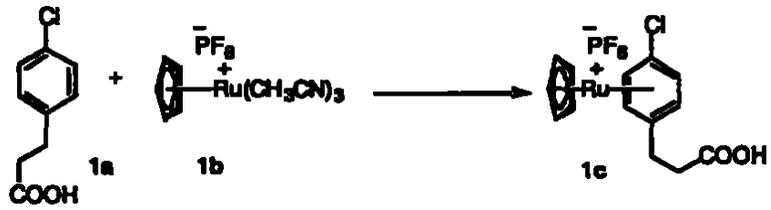
EXAMPLES

Example 1. Preparation of Compound of Formula 1.



1

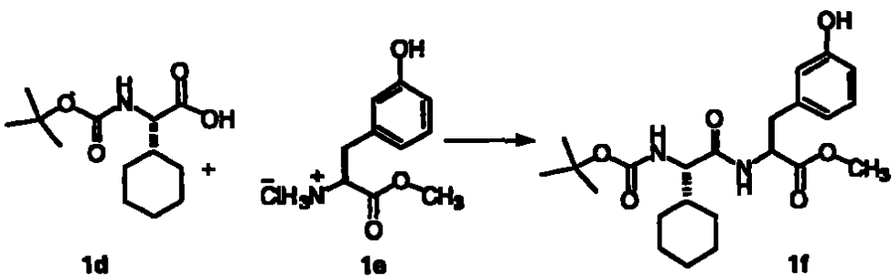
Step A.



1a 1b 1c

A solution of 4-chloropropionic acid (2 0 g, 10 8 mmol) of 1a in dichloroethane (200 mL) was treated with $CpRu(CH_3CN)_3$ PF_6 (4 7 g, 10 8 mmol, 1 0 equiv) and heated at reflux for 2 h The reaction mixture was cooled to rt when colorless crystals of the product 1c precipitated out The crystals were filtered and washed with 1 1 mixture of Et_2O/CH_2Cl_2 and dried *in vacuo*. The colorless crystals (3 3 g) were analytically pure ^1H NMR ($CD_3C(O)CD_3$, 400 MHz, ppm, δ, J) 6 77 (d, 2 H, J = 7 0 Hz), 6.53 (d, 2 H, J =7 Hz), 5 64 (s, 5 H), 2 87 (t, 2 H, J =7 0 Hz), 2 74 (t, 2 H, J =7 0Hz), MS (Electron spray, *m/z relative intensity*) 350 9 ($C_{14}H_{14}ClRu^+$, M^+, 100), CHN calcd for $C_{14}H_{14}ClF_6O_2PRu$ C=33 92% H=2 85% Cl=7 15% P=6 25% found C=34 04% H=3.04% Cl=7 09% P=5 71%

Step B.



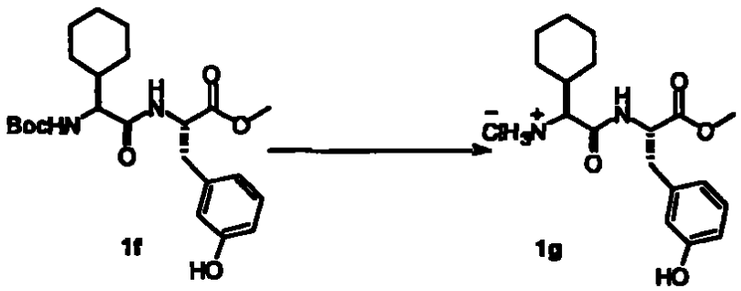
1d 1e 1f

A solution of Boc-cyclohexylglycine monohydrate 1d (6 17 g, 24 00 mmol) in dry CH_2Cl_2 (50 0 mL) was treated with 4-methylmorpholine (2.64 g, 26 0 mmol, 1 1 equiv) and cooled to -10° C To this mixture was added isobutyl chloroformate (3 62 g, 3 5 mL, 1 1 equiv) and the white suspension was stirred until the bath temperature was -5 °C *meta*-Tyrosine methyl ester hydrochloride salt (6 5 g, 26 5 mmol, 1 1 equiv) was dissolved in DMF (30 mL) in a separate beaker and treated with 4-methyl morpholine (2 64 g, 26 0 mmol, 1 1 equiv) and stirred at rt. for 15 min This mixture was added to the reaction which was accompanied by evolution of CO_2 The reaction mixture was stirred at rt for 1 h and diluted with 1M aq HCl (100 mL) The aqueous layer was extracted with ethyl acetate (3x200 mL) and the combined organic layer was extracted with 1M HCl (1x100 mL), aq NaOH

0427
420

(1x100 mL) brine (1x100 mL), dried (Na_2SO_4), concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hexanes 3/7) to yield 5 3 g (53%) of coupled compound 1f as a colorless foam

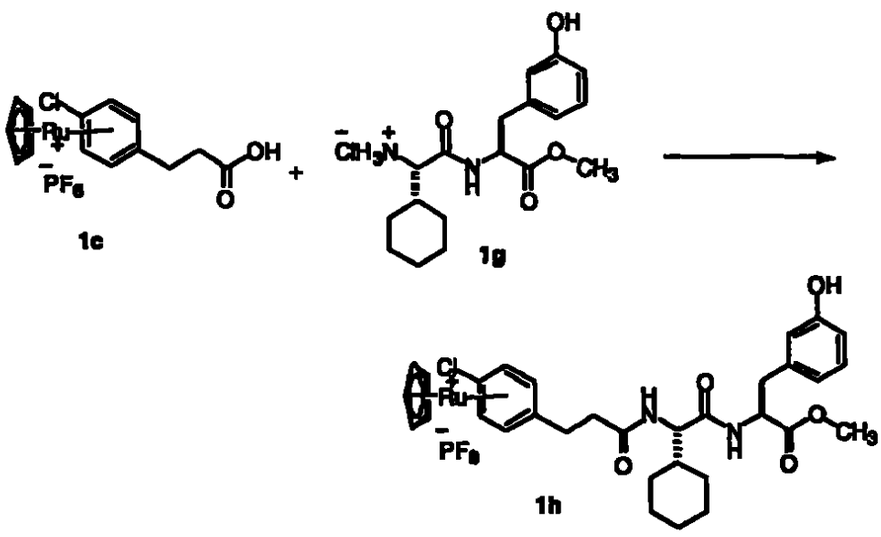
Step C.



A solution of 1f (10g, 23 04 mmol) was dissolved in HCl (4M solution in dioxane, 100 mL) and stirred at rt for 2-4 h The reaction mixture was concentrated *in vacuo* and the solid was resuspended in ether It was filtered and the solid was washed with ether which was dried to give a colorless solid 1g (6 2g, 96%) ^1H NMR (d_4-CD_3OD, 400 MHz, δ, ppm) 7 09 (t, 1 H, J=8 0 Hz), 6 71-6.36 (m, 3 H), 4 69 (dd, 1H, J=6 0 Hz, 3.2 Hz), 3 69 (s, 3 H), 3 66 (d, 1 H, J=5 2 Hz), 3 15 -3 10 (dd, 1 H, J=5 6 Hz, 4 0 Hz), 1 87-1 69 (m, 6 H), 1.32-1 10 (m, 5 H)

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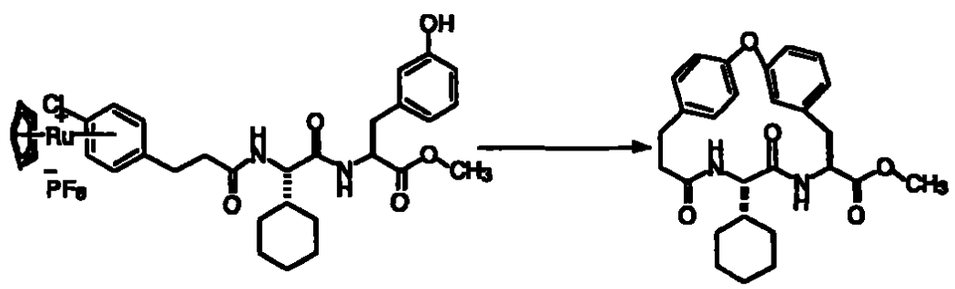
Step D.



A solution of cyclopentadiene-η^6-4-chlorophenylpropionic acid-ruthenium hexanesafluorophosphate 1c (2 0g, 4 0 mmol) in DMF (20 mL) was treated with HOBt (810 mg, 6 0 mmol, 1.5 equiv)and Hünigs base (2 6 g, 16 0 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI•HCl (888 mg, 5 0 mmol, 1 25 equiv) The reaction mixture was stirred at 0 °C for 30 min and amine salt 1g (1.48 g, 4.0 mmol) was added to the mixture and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with H_2O (200 mL) and extracted into CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq HCl (1x100 mL), $NaHCO_3$ (1x100 mL), brine (1x100 mL) and dried (Na_2SO_4), filtered concentrated *in vacuo* and the brown solid 1h was used for cyclization without any further purification

042422

Step E.



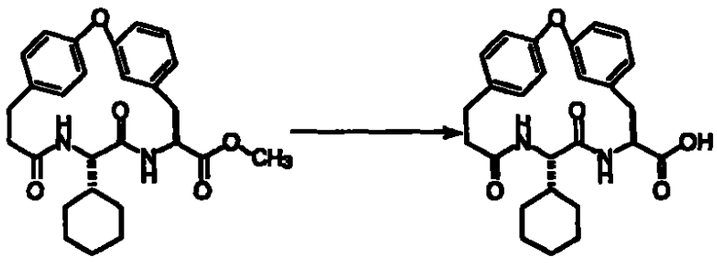
1h 1l

A solution of cyclopentadiene-η6-ruthenium-4-chlorophenylpropionic acid-cyclohexyglycine-*meta*-tyrosine-OCH_3 1h (1 47 g crude) in dry DMF (150 mL) was treated with Cs_2CO_3 (2.40 g, 7 37 mmol, 5 0 equiv) and degassed by bubbling dry N_2 into the reaction mixture The reaction mixture was stirred at rt for 16 h and the excess DMF was distilled off The residue was dissolved in H_2O (200 mL) and extracted with CH_2Cl_2 (3x100 mL) The combined organic layer was extracted with brine (100 mL), dried (Na_2SO_4), filtered concentrated *in vacuo* and the residue was used for photolytic decomplexation of ruthenium without further purification

The crude ruthenium complex was dissolved in acetonitrile (35 mL), degassed and photolysed in a Raynot (λ=350 nM) for 48 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 7:3) to yield 360 mg (52%) of a colorless solid 1l

0430
423

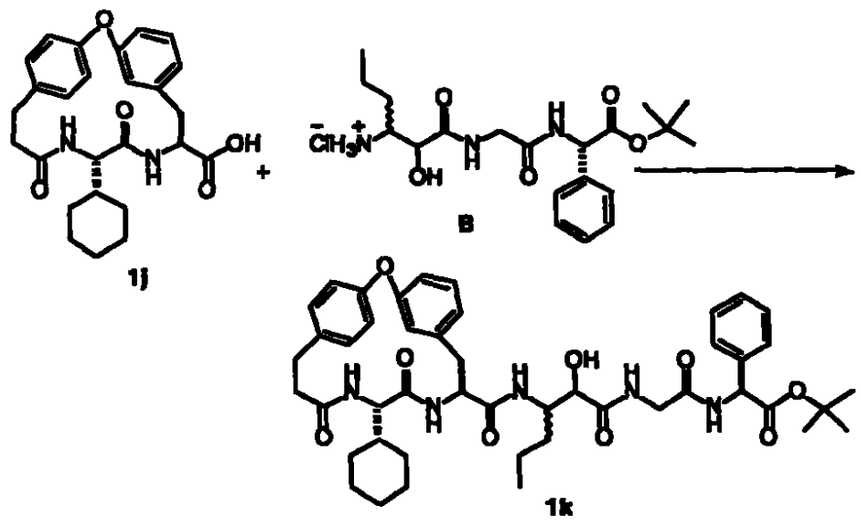
Step F.



1I 1J

A solution of biphenyl ether 1I (300 mg, 0 65 mmol) in CH_3OH (10 mL), CH_2Cl_2 (20 mL) and H_2O (5 mL) was treated with $LiOH \cdot H_2O$ (90mg, 2 2 mmol, 3.4 equiv) and stirred at rt for 2 h The reaction mixture was acidified with aq HCl (6 M) and extracted into CH_2Cl_2 (3x30 mL) The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield colorless acid 1J (200 mg, 66%)

Step G:



1J B 1k

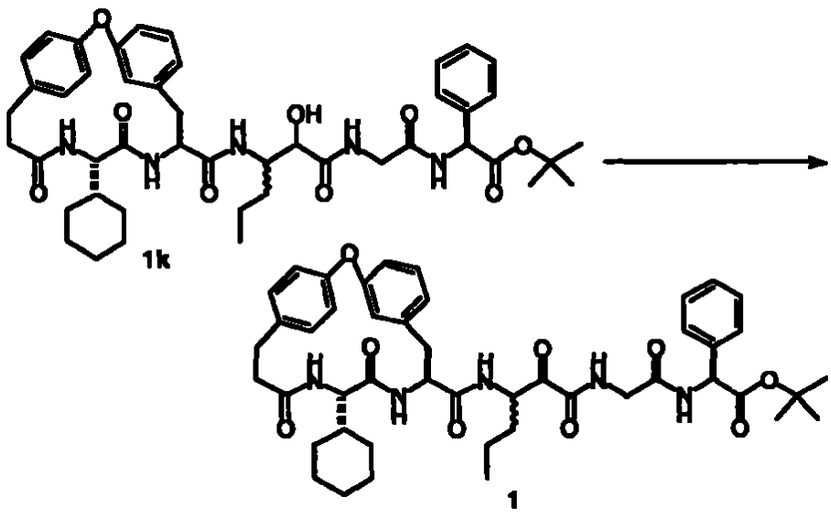
A solution of acid 1J (100 mg, 0 22 mmol) in dry DMF (2 5 mL) was treated with HOOBt (45 mg, 0 33 mmol) and Hünigs base (141 mg, 1 1 mmol, 5 0 equiv)

424

The reaction mixture was cooled to 0° C and treated with EDCI (63 mg, 0.33 mmol, 1.5 equiv) and stirred for 20 min The reaction mixture was treated with amine B (118 mg, 0 27 mmol, 1 22 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and EtOAc (3x50 mL) The combined organic layers were extracted with aq HCl (2M), aq NaOH (2M), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 1k (79 mg) which was used for oxidation MS (Electron spray, *m/z rel int*) 826 [$(M+1)^+$, 100], 494 (20), 94 (30)

0021108 1102 0105

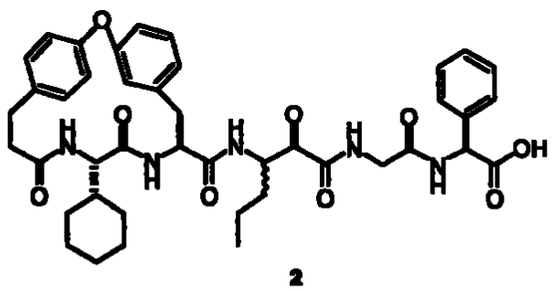
Step H.



A solution of hydroxy amide 1k (130 mg, 0 16 mmol) in DMF (2 0 mL) was treated with Dess-Martin reagent (130 mg, 0 32 mmol, 2.0 equiv) The reaction mixture was stirred at rt for 2 h and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1.49) to yield oxidized product 1 (55 mg, 42%) as a colorless solid MS (Electron spray, *m/z rel int*) 858 [$(M+CH_3OH+1)^+$, 100], 824 [$(M+1)^+$, 63)

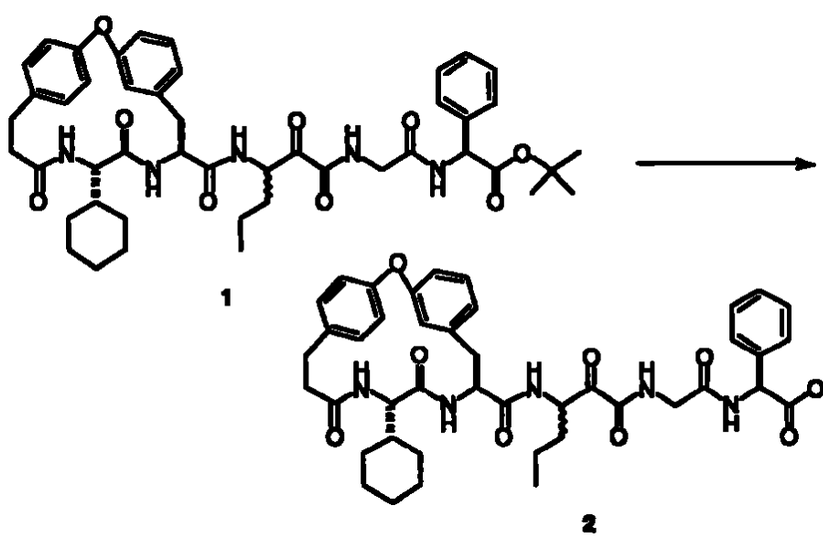
425

Example 2. Preparation of Compound of Formula 2.



2

Step A.



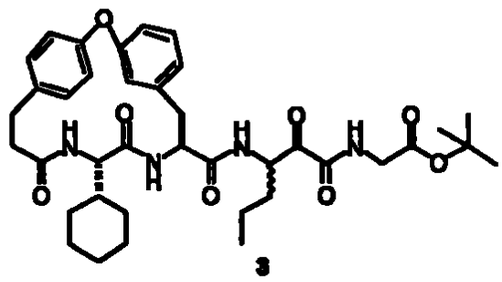
1

2

A solution of *tert*-butyl ester 1 (50 0 mg, 60 0 μmol) was treated with TFA/CH_2Cl_2 (1 1, 4 mL) and stirred at rt for 2 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1.24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes (4 0 mL) and concentrated to yield a white solid 2 (49 mg, 100%) MS (Electron spray, *m/z rel int*) 768 [$(M+1)^+$, 100)

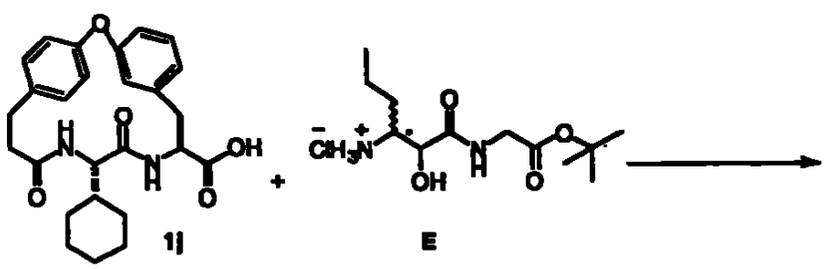
C~~03~~426

Example 3. Preparation of Compound of Formula 3.

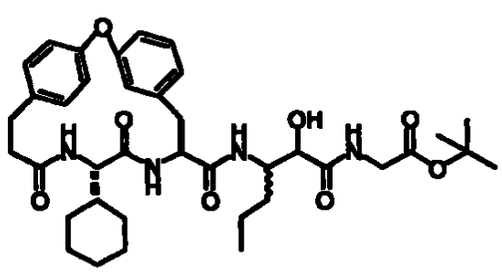


3

Step A.



1j E



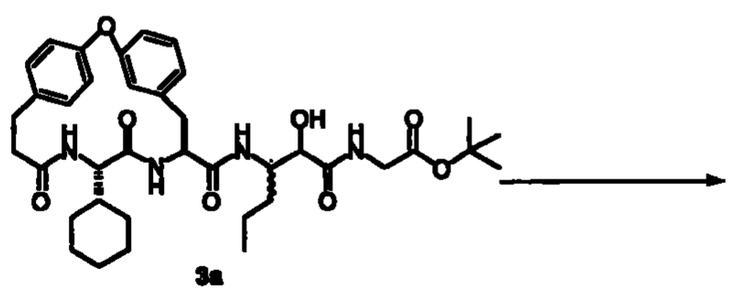
3a

A solution of acid 1j (100 mg, 0.22 mmol) in dry DMF (2.5 mL) was treated with HOOBt (45 mg, 0.33 mmol) and Hünigs base (141 mg, 1 1 mmol, 5.0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (63 mg, 0.33 mmol, 1 5 equiv) and stirred for 20 min The reaction mixture was treated with amine E (79 mg, 0.27 mmol, 1.22 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq HCl (1M, 30 mL) aq NaOH (1M, 30 mL), dried (Na_2SO_4)

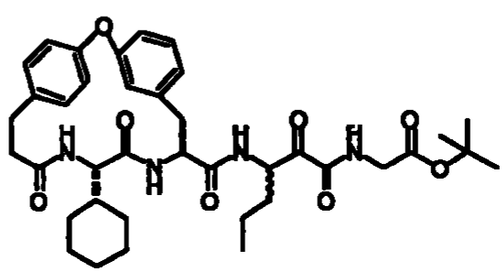
0434
427

filtered concentrated *in vacuo* to obtain a colorless solid 3a (58 mg) which was used for oxidation MS (Electron spray, *m/z rel int*) 693 [(M+1)$^+$, 100], 637 (41), 494 (55), 394 (51), 338 (13)

Step B



3a



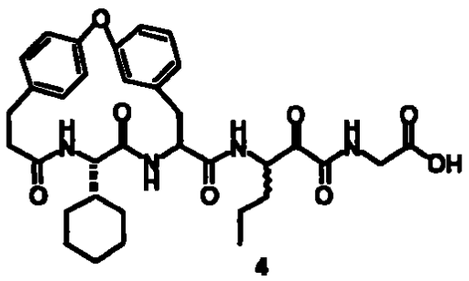
3

A solution of alcohol 3a (95 mg, 0 14 mmol) in CH_2Cl_2 (2.0 mL) was treated with Dess-Martin reagent (116 mg, 0.28 mmol, 2 0 equiv) The reaction mixture was stirred at rt for 2 h and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 32) to yield oxidized product 3 (47 mg, 42%) as a colorless solid MS (Electron spray, *m/z rel int*) 691 (M+1)$^+$

0001110 "0288019

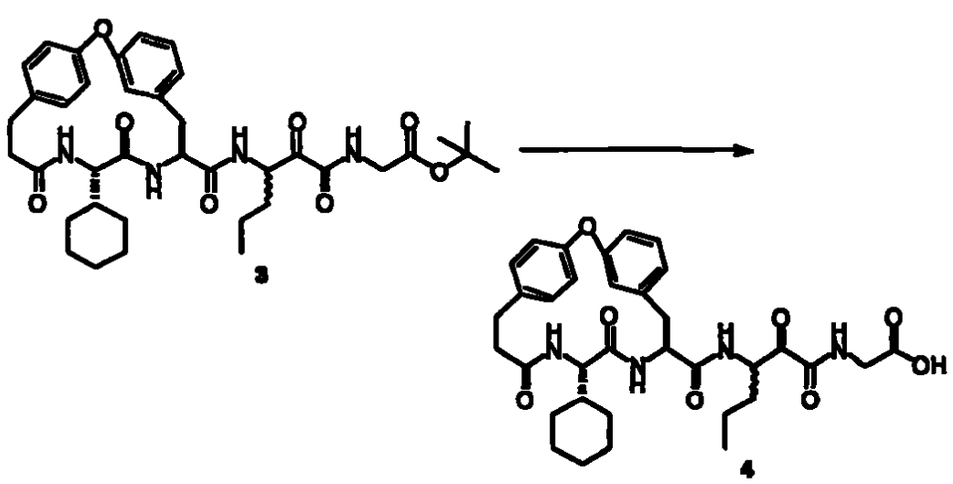
0435
428

Example 4. Preparation of Compound of Formula 4.



4

Step A.

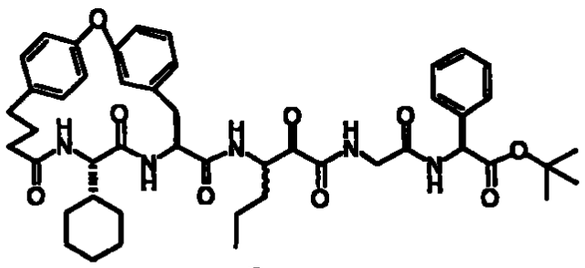


3

4

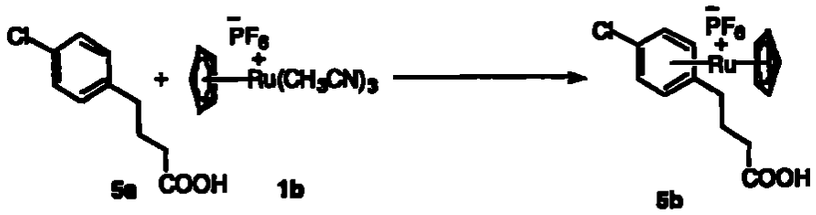
A solution of *tert*-butyl ester 3 (47.0 mg, 68.0 μmol) was treated with HCl (4M Dioxane, 5 mL) and stirred at rt for 25 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1:24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes (5 0 mL) and concentrated to yield a white solid 4 (43 mg, 100%) MS (Electron spray, *m/z rel int*) 635 [$(M+1)^+$, 100)], 465 (62), 336 (62)

Example 5. Preparation of Compound of Formula 5.



5

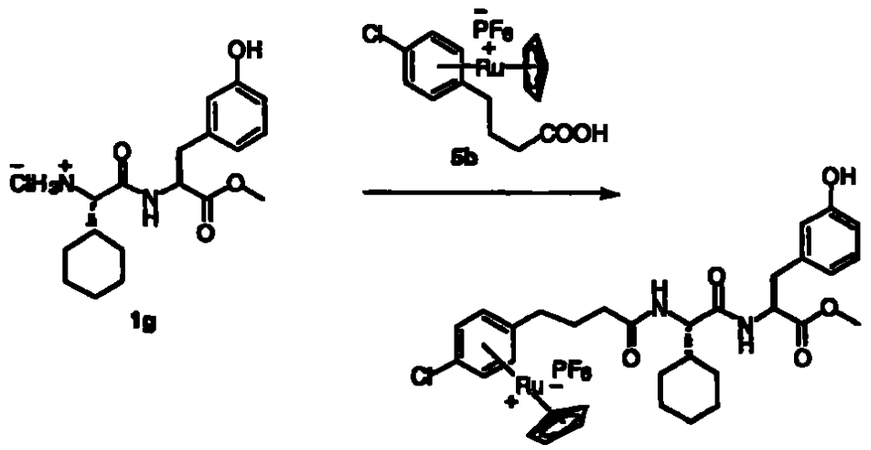
Step A



A solution of 4-chlorobutyric acid 5a (3 0g, 15 10 mmol) in dichloroethane (200 mL) was treated with $CpRu(CH_3CN)_3PF_6$ 1b (6 6 g, 15 10 mmol, 1 0 equiv) and heated at reflux for 2 5 h The reaction mixture was cooled to 0°C and filtered The filtrate was concentrated *in vacuo* and dissolved in CH_3CN (10 mL) and treated with a large excess of Et_2O The gum separating out was separated by decanting the ether and the residue was dissolved in CH_2Cl_2/CH_3OH (1 1, 100 mL) and concentrated *in vacuo* to obtain 5b as a brown gum which solidifies (3.5 g, 46%)

0/5; 430

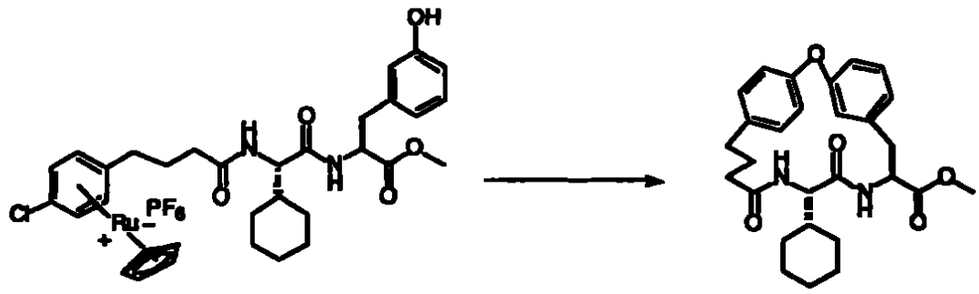
Step B



A solution of carboxylic acid 5b (3 12 g, 5 95 mmol) in dry DMF (20 mL) was treated with Hünigs base (3.07 g, 24 0 mmol, 4 0 equiv, 4.4 mL) and HOBt (1.2 g, 8 93 mmol, 1.5 equiv) The reaction mixture was cooled to 0°C and the treated with EDCI (1 35 g, 7.43 mmol, 1.25 equiv) and stirred for 1h To this reaction mixture was added amine hydrochloride 1g (2.65g, 7 14 mmol, 1.2 equiv), and the reaction mixture was stirred at rt for 12 h DMF was distilled out and the residue was diluted with water and the aqueous layer was extracted with CH_2Cl_2 The combined organic layers were extracted with aq $NaHCO_3$, aq HCl, brine, dried (Na_2SO_4), filtered, concentrated *in vacuo* and the crude product 5c (4.3 g) was used for cyclization without further purification ^{1}H NMR (d_4-CD_3OD, 400 MHz, δ, ppm) 7 35 (t, 1H), 6.72-6 60 (m, 5 H), 6 33-6.20 (dd, 2 H), 5 51 (s, 5 H), 4 19 (d, 1 H), 3 68 (s, 3 H), 3 19-2.83 (m, 2 H), 2.51-2.40 (m, 2 H), 2.40-2.25 (m, 2 H), 1 99-1.59 (m, 8 H), 1.35-0 98 (m, 5 H), MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*) 695 3 ($[M-PF_6]^+$, 100), 232 (20), 171(30), HRMS calcd for $C_{34}H_{42}N_2O_5ClRu^+$ $(M-PF_6)^+$ 695 1832, found 695 1845

431

Step C.



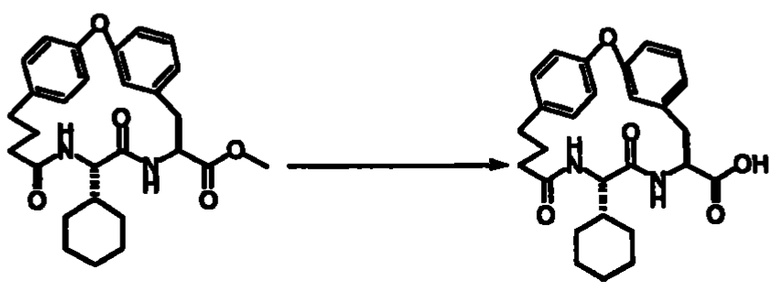
5c 5d

A solution of chloro-compound 5c (3 0 g 3 6 mmol) in dry DMF (300 mL) was degassed with dry N_2 and Cs_2CO_3 (5 2 g, 16 mmol, 4.0 equiv) and stirred at rt for 16h The solvent DMF was distilled off and the residue was diluted with water and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were dried (Na_2SO_4), filtered, concentrated *in vacuo* and dried under vacuum overnight. It was used for photolytic removal of Ru without further purification MS FAB (NBA-G/TG-DMSO 695 ([M-PF6]$^+$, 100]

The cyclized compound from the previous step was dissolved in CH_3CN (35mL) and photolysed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO2, EtOAc/Hexanes 1 1) to yield a tan colored solid 5d (600 mg, 34%) ^{1}H NMR (CDCl$_3$, 400 MHz, δ, ppm) 7.58 (d, 1H, J=7 6 Hz), 7 14 (t, 1 H, J=8.0 Hz), 6 94 (d, 2 H, J=8 4 Hz), 6.87 (dd, 1 H, J=2.4, 5 6 Hz), 6 73 (d, 1 H, J=7 2 Hz), 6.59 (s, 1 H), 6.57 (s, 2 H), 6.39 (d, 1 H, J=8 0 Hz), 4.51 (dt, 1 H, J=2.8, 8 0 Hz), 3 80-3.62 (m, 1 H), 3 62 (s, 3 H), 3 05-3 00 (dd, 1H, J=2 8, 11 6 Hz), 2 85 (dd, 1 H, J=6.4, 6 0 Hz), 2 76-2 72 (m, 1 H), 2.36-2 19 (m, 3 H), 2 02 (dd, 1 H, J=6.4, 9.2 Hz), 1.8-1 73 (m, 1 H), 1 61-1 34(m, 7H), ,1.41-0 71 (m, 7 H) MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*), 493 [(M+1)$^+$, 100], 465 (20), 232 (30), 171 (40), HRMS calcd for $C_{29}H_{37}N_2O_5$ (M+1)$^+$ 493.2702, found 493 2699

0439
432

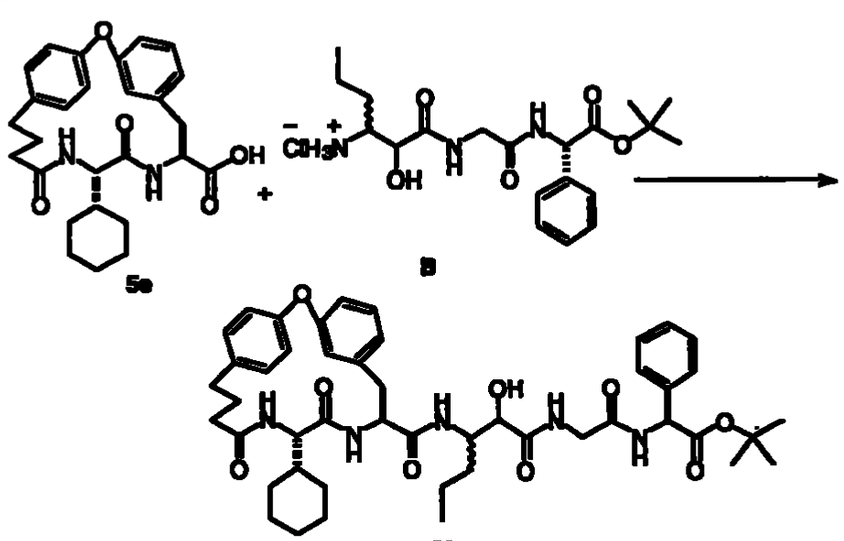
Step D



5d 5e

A solution of ether 5d (200 mg, 0.42 mmol) in CH_3OH (5 mL), CH_2Cl_2 (10 mL) and H_2O (0.5 mL) was treated with $LiOH \cdot H_2O$ (18 mg, 0.44 mmol, 1 1 equiv) and stirred at rt for 12 h The reaction mixture was acidified with aqueous HCl (12 N, 1mL) and concentrated *in vacuo* to yield acid 5e which was used directly for the coupling without further purification

Step E



5e B

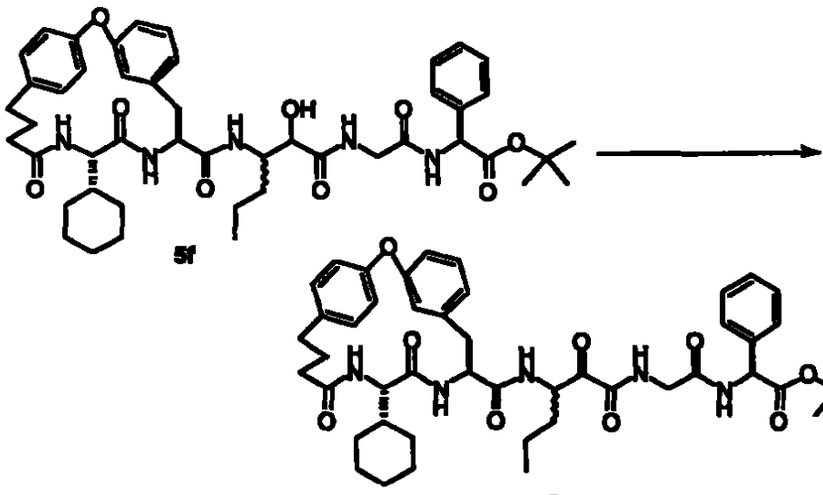
5f

A solution of acid 5e in dry DMF (5.0 mL) was treated with HOOBt (103 mg, 0 63 mmol, 1 5 equiv), Hünigs base (216 mg, 1 68 mmol, 4 0 equiv) and amine B (270 mg, 0 63 mmol, 1.47 equiv) The reaction mixture was cooled to 0°

0460433

C and treated with EDCI (101 mg, 0.52 mmol, 1.25 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x50 mL),and EtOAc (3x50 mL) The combined organic layers were extracted with aq HCl (2M) aq NaOH (2M), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 5f (177 mg) which was used for oxidation MS (Electron spray, *m/z rel int*) 840 [$(M+1)^+$, 100], 394 (100)

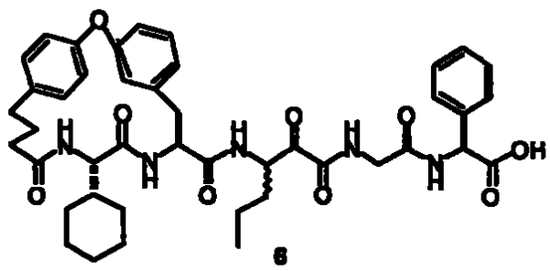
Step F



A solution of alcohol 5f (177 mg, 0.21 mmol) in CH_2Cl_2 (10 0 mL) was treated with Dess-Martin reagent (178 mg, 0.42 mmol, 2 0 equiv) The reaction mixture was stirred at rt for 3 h and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 49) to yield oxidized product 5 (23 mg, 13%) as a colorless solid MS (Electron spray, *m/z rel int*) 870 [$(M+CH_3OH+1)^+$, 50], 838 [$(M+1)^+$, 100)

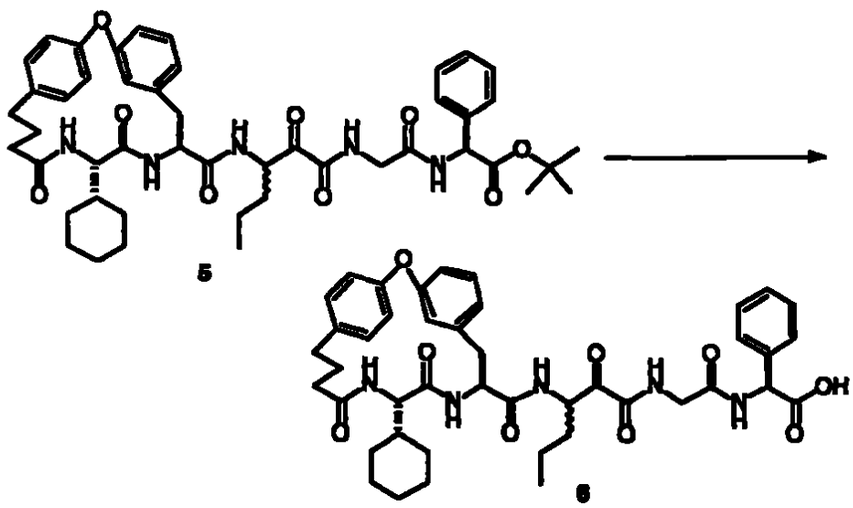
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Example 6 Preparation of Compound of Formula 6.



6

Step A



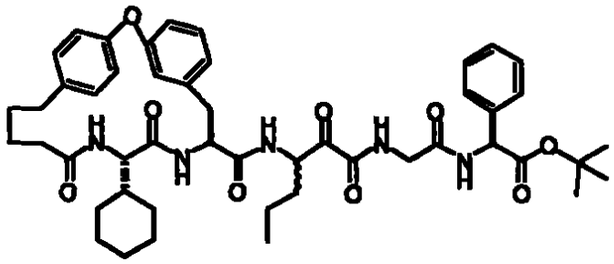
5

6

A solution of *tert*-butyl ester 5 (50.0 mg, 60.0 μmol) was treated with TFA/CH_2Cl_2 (1 1, 4 mL) and stirred at rt for 7 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1:24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes (4.0 mL) and concentrated to yield a white solid 6 (14 mg, 100%) MS (Electron spray, *m/z rel int*) 782 [(M+1)$^+$, 100)

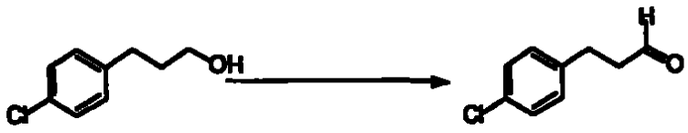
L-4492
435

Example 7 Preparation of Compound of Formula 7.



7

Step A



7a 7b

A solution of alcohol 7a (9 2g, 54 1 mmol) in dry CH_2Cl_2 (200 mL) was treated with DMSO (35 mL) and Et_3N (16 4g, 16.3 mmol, 23.4 mL) The reaction mixture was cooled to 0 °C and treated with $Py{\bullet}SO_3$ (12 9 g, 81.2 mmol, 1 50 equiv) dissolved in DMSO (30 mL) The reaction mixture was stirred at 0°C for 0 5 h and rt for 6 h. The reaction mixture was concentrated *in vacuo* and diluted with Et_2O (100 mL) and H_2O (200 mL) The layers were separated and the aqueous layer was extracted with Et_2O (3x100 mL) The combined organic layers were extracted with HCl (2M, 3x100 mL), brine (1x100 mL) concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hexanes 1 7) to yield aldehyde 7b which solidified to a waxy solid on standing (7 1g, 77%) CHN calcd for C_9H_9ClO C=64 11% H=5.38%, found C=64.08% H=5.30%

436

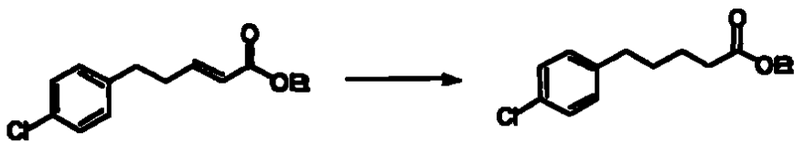
Step B



7b 7c

A solution of thiethyiphosponoacetate (6 72g, 30 mmol, 1 2 equiv) in dry THF (100 mL) was treated with NaH (60% dispersion, 1 5g, 35 mmol, 1.4 equiv) at 0°C The reaction mixture was stirred at 25°C for 1 h until the H_2 evolution ceased A solution of aldehyde 7b (4.2g, 25 0 mmol) in dry THF (5 0 mL) was added and the reaction mixture was stirred for 36 h The reaction mixture was diluted with H_2O (100 mL) and extracted with Et_2O (3x70 mL) The combined organic layer was dried (MgSO4), filtered, concentrated *in vacuo* and chromatographed to yield α,β-unsaturated ester 7c (4 2g, 71%) which was used for reduction

Step C

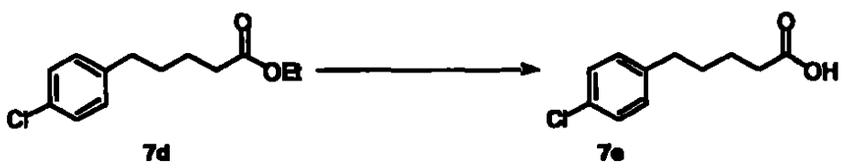


7c 7d

A solution of α,β-unsaturated ester 7c (4.2g, 8 0 mmol) in EtOAc (50 mL) was treated with Pd/C (10% w/w, 500 mg) and hydrogenated at 50 psi for 12 h The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo* to yield the reduced compound 7d (3.9 g, 93%)

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437

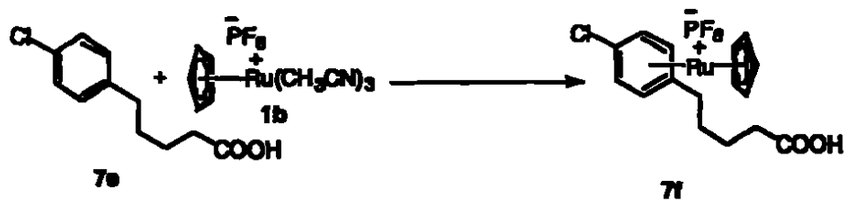
Step D



7d 7e

A solution of the ester 7d (3.9 g, 16 2 mmol) in $CH_3OH/THF/H_2O$ (1 1 0 1, 110 mL) was treated with $LiOH•H_2O$ (1 2 g, 30 mmol, 2 0 equiv) and stirred at rt for 5 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with H_2O (100 mL) and extracted into Et_2O (3x50 mL) The aqueous layer was acidified to pH ~1 (13 M HCl) and the turbid aqueous layer was extracted with Et_2O (3x100 mL) The combined organic layers were dried ($MgSO_4$) filtered concentrated *in vacuo* to yield a colorless solid 7e (3 1g, 96%) CHN calcd for $C_{11}H_{13}ClO_2$ C=62 12% H=6 16%, found C=62 27% H=6.23%

Step E

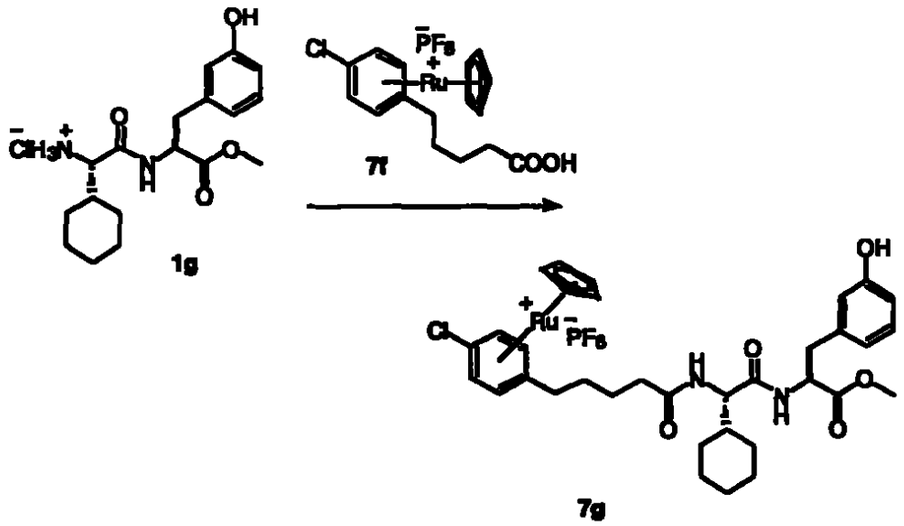


7e 7f

A solution of 4-chlorophenyl pentanoic acid 7e (3 0g, 14 15 mmol) in dichloroethane (150 mL) was treated with $CpRu(CH_3CN)_3PF_6$ 1b (6 75 g, 15 10 mmol, 1 0 equiv) and heated at reflux for 2 5 h. The reaction mixture was cooled to 0° C and filtered The filtrate was concentrated *in vacuo* and dissolved in CH_3CN (20 mL) and treated with a large excess of Et_2O The gum separating out was separated by decanting the ether and the residue was dissolved in CH_2Cl_2/CH_3OH (1 1, 100 mL) and concentrated *in vacuo* to obtain 7f a brown

gum which solidifies (4 36 g, 58%) MS (Electron spray, *m/z rel int*) 379 [(M-PF_6)$^+$, 100]

Step F



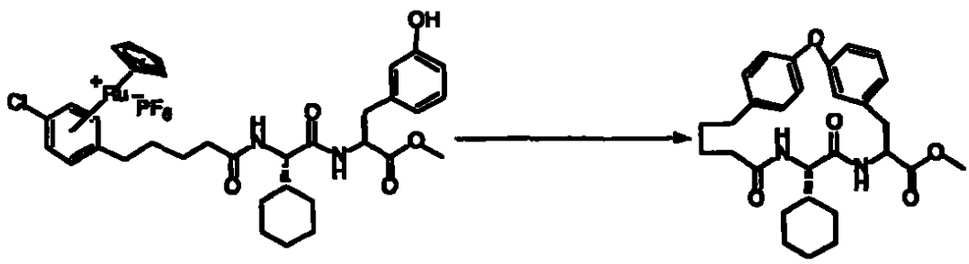
7g

A solution of carboxylic acid 7f (3.12 g, 5.95 mmol) in dry DMF (20 mL) was treated with Hünigs base (3.07 g, 24 0 mmol, 4.0 equiv, 4 4 mL) and HOBt (1 2 g, 8 93 mmol, 1 5 equiv) The reaction mixture was cooled to 0°C and the treated with EDCI (1.35 g, 7.43 mmol, 1 25 equiv) and stirred for 1h To this reaction mixture was added amine hydrochloride 1g (2.65g, 7 14 mmol, 1.2 equiv) was added and the reaction mixture was stirred at rt for 12 h The DMF was distilled out and the residue was diluted with water and the aqueous layer was extracted with CH_2Cl_2 The combined organic layers were extracted with aq $NaHCO_3$, aq HCl, brine, dried (Na_2SO_4) filtered concentrated *in vacuo* and the crude product 7g (4.3 g) was used for further cyclization without purification ^{1}H NMR (d_4-CD_3OD, 400 MHz, δ, ppm) 7.35 (t, 1H), 6.72-6 60 (m, 5 H), 6.33-6.20 (dd, 2 H), 5.51 (s, 5 H), 4 19 (d, 1 H), 3.68 (s, 3 H), 3.19-2.83 (m, 2 H), 2.51-2.40 (m, 2 H), 2 40-2 25 (m, 2 H), 1 99-1.59 (m, 8 H), 1.35-0 98 (m, 5 H), MS (FAB, NBA-G/TG-

446
0346
439

DMSO, *m/z relative intensity*) 695 3 ($[M-PF_6]^+$, 100), 232 (20), 171 (30), HRMS calcd for $C_{34}H_{42}N_2O_5ClRu^+$ (M-PF_6) 695 1832, found 695 1845

Step G



7g 7h

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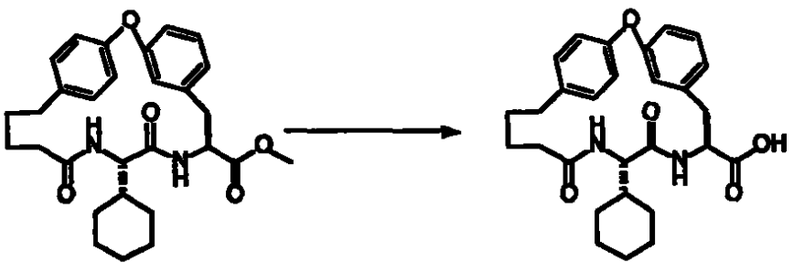
A solution of chloro-compound 7g (3 0 g 3 6 mmol) in dry DMF (300 mL) was degassed with dry N_2 and Cs_2CO_3 (5 2 g, 16 mmol, 4.0 equiv) and stirred at rt. for 16h The solvent DMF was distilled off and the residue was diluted with water and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were dried (Na_2SO_4) filtered, concentrated *in vacuo* and dried in vacuum overnight. It was used for photolytic removal of Ru without further purification. MS FAB (NBA-G/TG-DMSO 695 ([M-PF6]+, 100]

The cyclized compound from the previous step was dissolved in CH_3CN (35mL) and photolysed in a Raynot (λ=350 nm) for 48 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield a tan colored solid 7h (600 mg, 34%) ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm) 7.58 (d, 1H, *J*=7 6 Hz), 7 14 (t, 1 H, *J*=8 0 Hz), 6 94 (d, 2 H, *J*=8.4 Hz), 6 87 (dd, 1 H, *J*=2 4, 5 6 Hz), 6 73 (d, 1 H, *J*=7.2 Hz), 6.59 (s, 1 H), 6 57 (s, 2 H), 6 39 (d, 1 H, *J*=8 0 Hz), 4.51 (dt, 1 H, *J*=2.8, 8.0 Hz), 3.80-3 62 (m, 1 H), 3 62 (s, 3 H), 3 05-3 00 (dd, 1H, *J*=2.8, 11 6 Hz), 2 85 (dd, 1 H, *J*=8 4, 6 0 Hz), 2 78-2 72 (m, 1 H), 2 36-2.19 (m, 3 H), 2.02 (dd, 1 H, *J*=6.4, 9 2 Hz), 1 8-1 73 (m, 1 H), 1 61-1.34(m, 7H), ,1 41-0 71 (m, 7 H) MS (FAB, NBA-G/TG-

DMSO, *m/z relative intensity*), 493 [(M+1)$^+$, 100], 465 (20), 232 (30), 171 (40),

HRMS calcd for $C_{29}H_{37}N_2O_5$ $(M+1)^+$ 493.2702, found, 493 2699

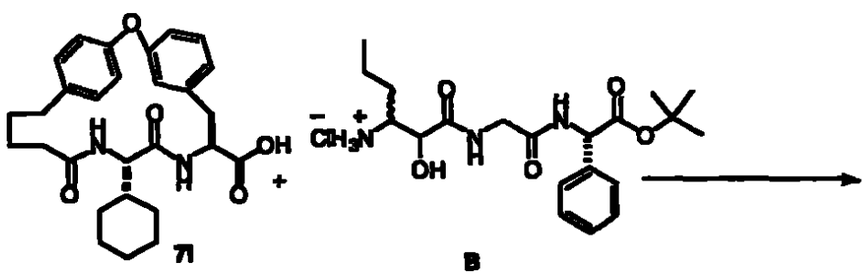
Step H



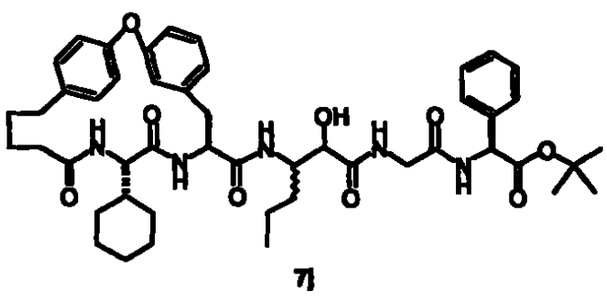
7h 7l

A solution of ether 7h (220 mg, 0.46 mmol) in CH_3OH (3 0 mL), CH_2Cl_2 (10 mL) and H_2O (0.5 mL) was treated with $LiOH•H_2O$ (18 mg, 0.44 mmol, 1 1 equiv) and stirred at rt for 12 h The reaction mixture was acidified with aq HCl (13 M, 1mL) and concentrated *in vacuo* to yield acid 7l which was used directly for the coupling without further purification.

Step I



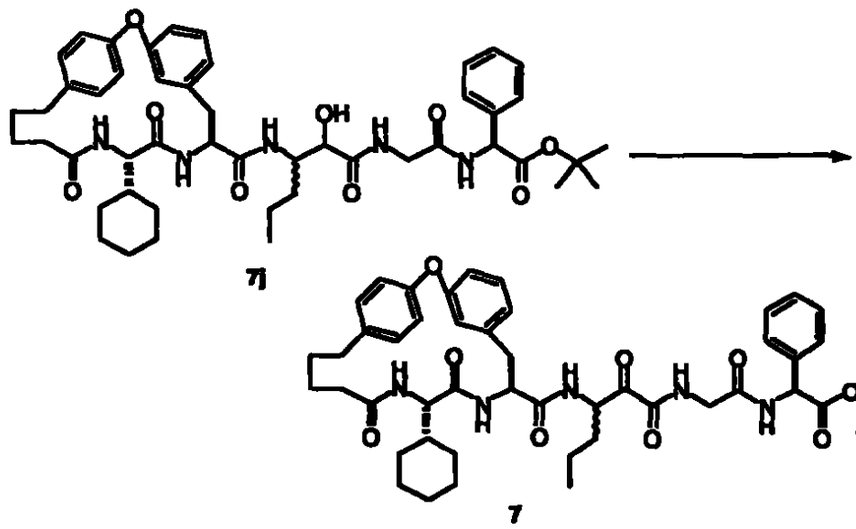
7l B



7I

A solution of acid 7I in dry DMF (3 0 mL) was treated with HOOBt (94 mg, 0 75 mmol, 1 6 equiv), Hünigs base (237 mg, 1 84 mmol, 4 0 equiv) and amine B (246 mg, 0.58 mmol, 1.47 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (110 mg, 0 58 mmol, 1 25 equiv) and stirred at 0 °C for 25 min for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The combined aqueous layers were extracted with CH_2Cl_2 (3x30 mL) The organic layers were extracted with aq HCl (1 M, 60 mL) aq NaOH (60 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 7J (230 mg) which was used for oxidation MS (Electron spray, *m/z rel int*) 854 [$(M+1)^+$, 100], 479 (70), 327 (50), 271 1 (100)

Step J

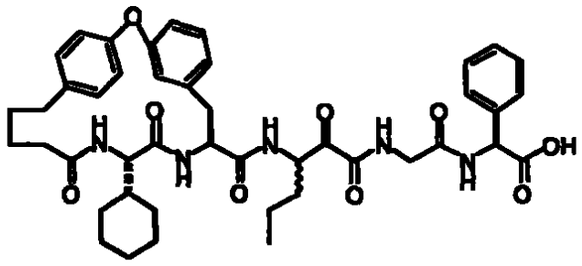


7I

7

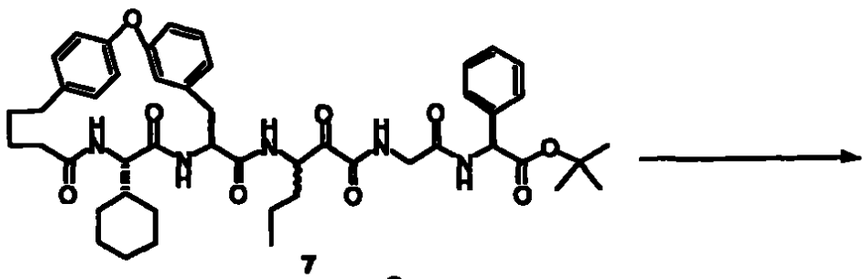
A solution of alcohol 7J (220 mg, 0 26 mmol) in CH_2Cl_2 (3.0 mL) was treated with Dess-Martin reagent (218 mg, 0 51 mmol, 2 0 equiv) The reaction mixture was stirred at rt and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 24) to yield oxidized product 7 (23 mg, 13%) as a colorless solid MS (FAB, *m/z, rel int*) 852 [$(M+1)^+$, 43), 796 (100), 768 (20), 461 (20), 433 (50), 405 (50), 336 (30), 294, (50)

Example 8 Preparation of Compound of Formula 8.

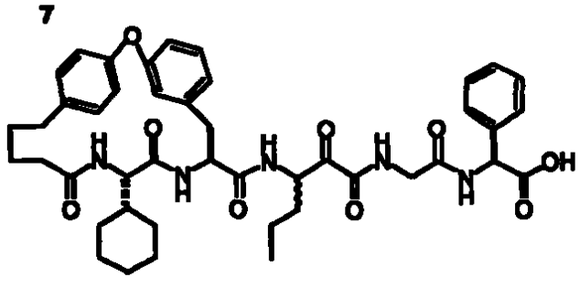


8

Step A



7

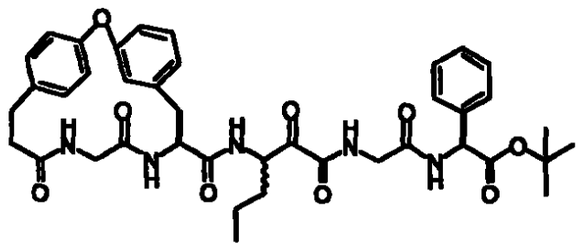


8

A solution of *tert*-butyl ester 7 (32.0 mg, 37 0 μmol) was treated with TFA/CH_2Cl_2 (1 1, 5 0 mL) and stirred at rt for 4 h. The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1:24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes/CH_3OH (4.0 mL) and concentrated to yield a tan solid 8 (29 0 mg, 100%) MS (Electron spray, *m/z rel int*) 796 [(M+1)$^+$, 100)

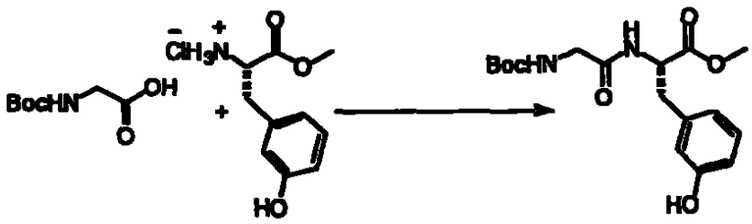
0450 450
443

Example 9. Preparation of Compound of Formula 9



9

Step A.



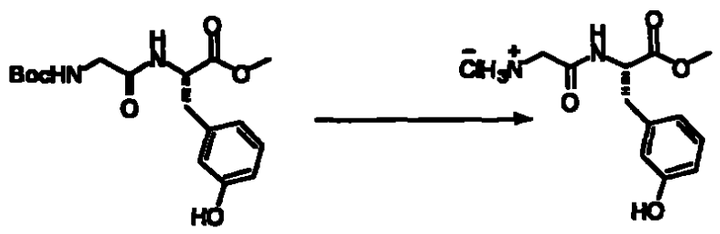
9a 1e 9b

A solution of Boc-Glycine 9a (1 75g,10 0 mmol) in dry DMF (50 mL) was treated with HOOBt (2 65 g, 15 mmol, 1 5 equiv) and EDCI (2 86g, 15 0 mmol, 1 5 equiv) The reaction mixture was treated with Hünigs base (5 16 g, 40 mmol, 4 0 equiv 7.3 mL) The reaction mixture was stirred for 1h and *meta*-tyrosine-OCH_3•HCl 1e (2 5 g, 11.5 mmol, 1 1 equiv) was added and stirred at 25 °C for 12 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with aq. $NaHCO_3$ and extracted into CH_2Cl_2 The combined organic layer were concentrated and the residue purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield a colorless solid 9b (3.4g, 90%)

0451

444

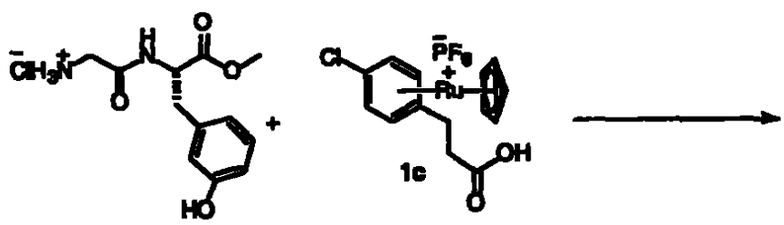
Step B.



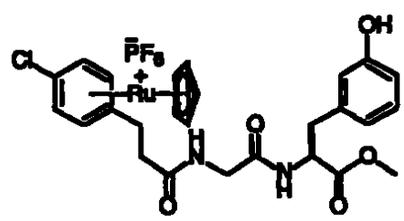
9b 9c

A solution of 9b (4 6g, 13.06 mmol) in HCl (4M solution in Dioxane, 50 mL) was stirred at rt. for 3h The reaction mixture was concentrated *in vacuo* and the residue was dried in high vacuum to yield 9c as a fine powder which was used for the next step. ^{1}H NMR (CD_3OD, 400 MHz, δ, ppm) 8.67 (d, 1 H, J=7.9 Hz),7 10-7 07 (m, 1 H), 6 68-6 64 (m, 2 H), 4.75-4 70 (m, 1 H), 3 75-3.61 (m, 2 H), 3 66 (s, 3 H), 3 10 (dd, 1 H, J= 5.2, 8.5 Hz), 2 90 (dd, 1 H, J=8.8 Hz, 5 0 Hz)

Step C



9c 1c



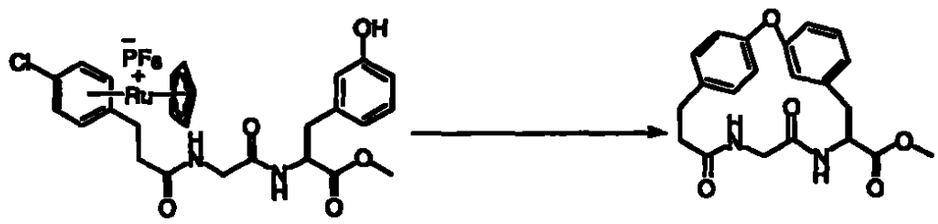
9d

A solution of [CpRu(η^6-4-chlorophenylpropionic acid)]PF_6 1c (3 0 g, 46 01 mmol) in dry DMF (60 mL) was treated with HOBt (1.3 g, 9 16 mmol, 1.5 equiv) and Hünigs base (3.22 g, 4 60 mL, 25 0 mmol, 4 0 equiv) The reaction mixture

0452
445

was cooled to 0 ° C and treated with EDCI (1 75 g, 9 16 mmol, 1 5 equiv) The reaction mixture was stirred at 0° C for 30 mn and the glycine ammonium salt 9c (1 75 g, 8.06 mmol, 1 0 equiv) was added The reaction mixture was stirred at rt for 12 and the DMF was distilled out *in vacuo* The residue was diluted with aq HCl (1M, 100 mL) and extracted into CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq $NaHCO_3$ (1x100 mL), brine (50 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* to yield a brown solid 9d (1 5 g, 34%) which was used for cyclization MS (Electron spray, *m/z relative intensity*) 585 [$(M-PF_6)^+$, 100], 459 (30), 373 (30), 198 (20)

Step D



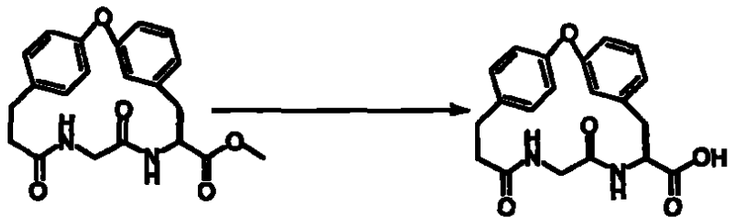
9d 9e

A solution of the ruthenium complex 9d (1.5 g 2.05 mmol) in dry DMF (100 mL) was degassed with dry N_2 at rt. and Cs_2CO_3 (5 0 g, 15 mmol, 7 5 equiv) was added and stirred at rt. for 12h The solvent DMF was distilled off and the residue was diluted with water (100 mL) and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with brine (100 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* and dried in vacuum overnight. It was used for photolytic removal of Ru without further purification

The cyclized compound from the previous step was dissolved in CH_3CN (30 mL) and filtered into a quartz tube The solution was degassed and photolyzed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc) to yield a tan colored solid 9e (230 mg, 30%) ^1H NMR ($CDCl_3$, 400 MHz,

δ, ppm) 7 23-7 18 (m, 2 H), 7 09-7 01 (m, 3 H), 6 76 (dd, 1 H, J=2.4, 8 8 Hz), 6 66 (d, 1 H, J=7 6 Hz), 6 47 (d, 1 H, J=5 6 Hz), 6.17 (s, 1 H), 5 64 (s, 1 H), 4 69 (q, 1 H, J=4 4 Hz), 3 77 (s, 3 H), 3.68-3.51 (m, 2 H), 3 35 (dd, 1 H, J=4.0, 10 8 Hz), 3 05 (dd, 1 H, J=5.2, 9 2 Hz), 2 98-2 92 (m, 2 H), 2 61-2 56 (m, 1 H), 2.30-2.29 (m, 1 H), ^{13}C NMR (CDCl$_3$, 100 MHz, δ ppm) 172.3, 171 4, 168.1, 159 9, 155.4, 137 6, 136 4, 131 0, 130 0, 129.5, 123.3, 122.4, 121 0, 117 7, 117 1, 53 6, 53 0, 43.6, 39 9, 36.1, 32.3 MS (Electron spray, *m/z relative intensity*) 383 [(M+1)$^+$, 100], 279 (20)

Step E

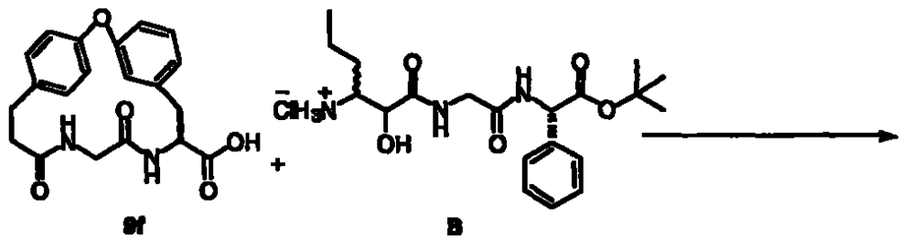


9e 9f

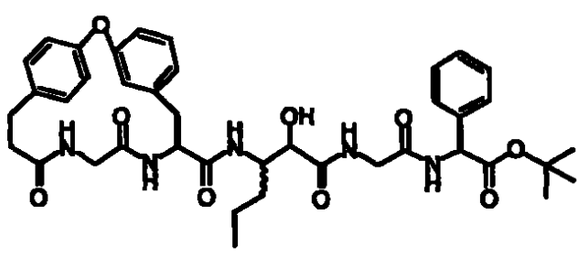
A solution of cyclic compound 9e (150 mg, 0.4 mmol) in THF (4 0 mL), H_2O (4.0 mL) was stirred at rt with LiOH•H_2O (41 0 mg, 1 0 mmol, 2.5 equiv) for 3 h The reaction mixture was acidified with conc. HCl (2 0 mL) and concentrated *in vacuo* The solid 9f was dried *in vacuo* and used for further coupling without further purification

0454
447

Step F



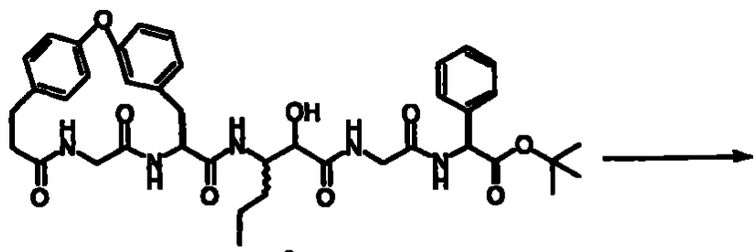
9f B



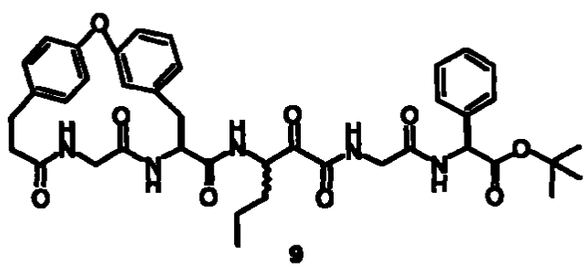
9g

A solution of the hydrolyzed acid 9f in dry DMF (4.0 mL) and CH_2Cl_2 (4.0 mL) was treated with HOOBt (103mg, 0.58 mmol, 1.5 equiv) and cooled to 0 °C and Hünigs base (206 mg, 1.60 mmol, 4.0 equiv, 295 µL) was added. To this mixture was added EDCI (112 mg, 0.58 mmol, 1.5 equiv) and the reaction mixture was stirred at 0° C for 0.5 h and treated with the amine hydrochloride B (206 mg, 0.48 mmol, 1.2 equiv.) The reaction mixture was stored in freezer for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2. The residue was diluted with aq. HCl (2M) and extracted with CH_2Cl_2 (3x50 mL). The combined organic layer was extracted with aq. HCl (1M, 3x50 mL), aq. NaOH (2M) brine (100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue 9g (200 mg) was oxidized without further purification.

Step G



9g

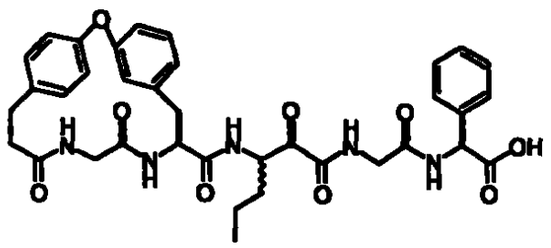


9

A solution of alcohol 9g (200 mg, 0.27 mmol) in CH_2Cl_2 (3.0 mL) was treated with Dess-Martin reagent (342 mg, 0 81 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 3 h and diluted with aq. $NaHCO_3$ and aq $Na_2S_2O_3$ The reaction mixture was stirred at rt for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were extracted with brine (50 mL), dried (Na_2SO_4), filtered concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH (2M NH_3)/CH_2Cl_2 1 19) to yield ketoamide 9 (100 mg, 50%) of a colorless solid MS (Electron spray, *m/z relative intensity*) 742 ($[M+1]^+$, 100), 686 (80)

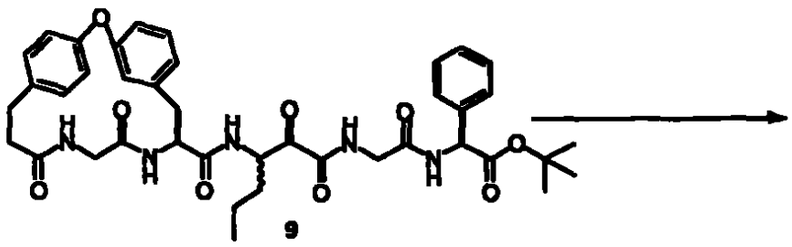
0455
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Example 10. Preparation of Compound of Formula 10:

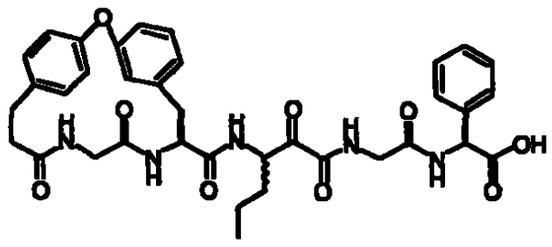


10

Step A



9

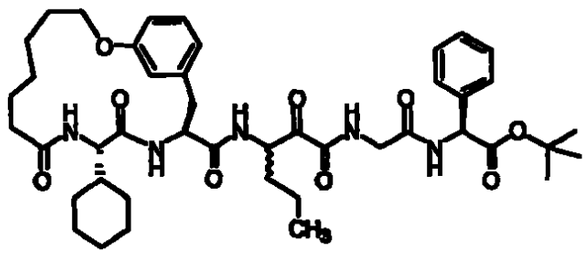


10

A solution of *tert*-butyl ester 9 (100 mg, 0 13 mmol) in dry CH_2Cl_2 (4 0 mL) was treated with TFA (4.0 mL) and stirred at rt. for 5 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in toluene/CH_2Cl_2 and concentrated *in vacuo* several times to yield a fine colorless solid 10 MS (FAB, NBA/DMSO, *m/z relative intensity*), 686 [(M+1)$^+$, 40], 460 (20), 307 (100), 289 (60), HRMS calcd for $C_{36}H_{40}N_5O_9$ (M+1)$^{+-}$ 686 2825, found 686 2840

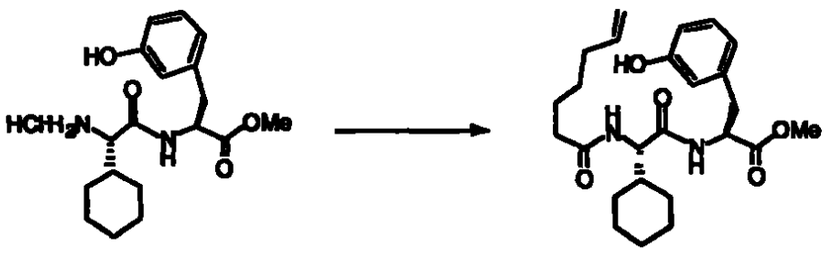
0454
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Example 11. Preparation of Compound of Formula 11.



11

Step A.



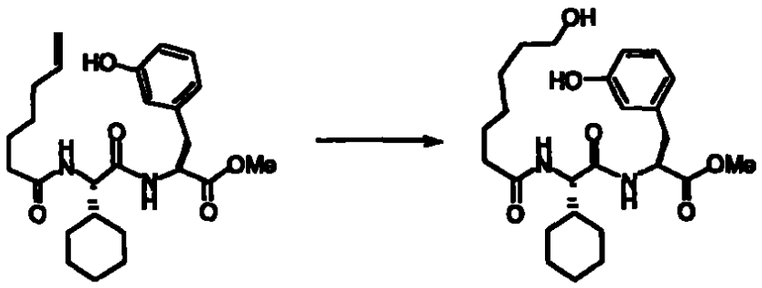
1g 11a

To a solution of amine hydrochloride 1g (1 20g, 3.23 mmol), 6-heptenoic acid (0 610 g, 4 68 mmol), HOOBt (0 765 g, 4 69 mmol) and EDCI (1 07 g, 5.58 mmol) in anhydrous DMF (50 mL) and CH_2Cl_2 (50 mL) at -20°C was added NMM (1.55 mL, 14.1 mmol) After stirring at this temperature for 30 min, the reaction mixture was kept in a freezer for 18 h It was then allowed to warm to rt. EtOAc (150 mL), brine (50 mL) and 5% H_3PO_4 (50 mL) were added After separation, the organic solution was washed with 5% H_3PO_4 (80 mL), saturated aqueous sodium bicarbonate solution (2 X 80 mL), water (80 mL), and brine (80 mL), dried with magnesium sulfate, filtered and concentrated *in vacuo*. Flash chromatography (2 to 5 % MeOH-CH_2Cl_2) afforded 11a (1.46 g, 3 28 mmol, quant.) as a white solid ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.25 (s, 1 H); 8 31 (d, J =7.2 Hz, 1 H), 7 70 (d, J

451
6158

=9 2 Hz, 1 H), 7 05-7 01 (m, 1 H), 6 62-6.58 (m, 3 H), 5 82-5 72 (m, 1 H), 5 02-4 91 (m, 1 H), 4.43-4.38 (m, 1 H), 4.23-4 19 (m, 1 H), 3.55 (s, 3 H), 2.83-2.80 (m,2 H), 2.51-1 97 (m, 2 H), 1 66-0 86 (m, 15H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 171 9, 171 8, 171 1, 157.2, 138 6, 138 4, 129 1, 119 5, 115 8, 114 6, 113.5, 56 5, 53.5, 51 6, 36.5, 34 8, 32.8, 29 0, 28 0, 27 8, 25 8, 25.5, 24 8, HRMS, *m/z* 445 2683 (calcd for $C_{25}H_{36}N_2O_5$ 445 2702, error 4 ppm)

Step B.



11a 11b

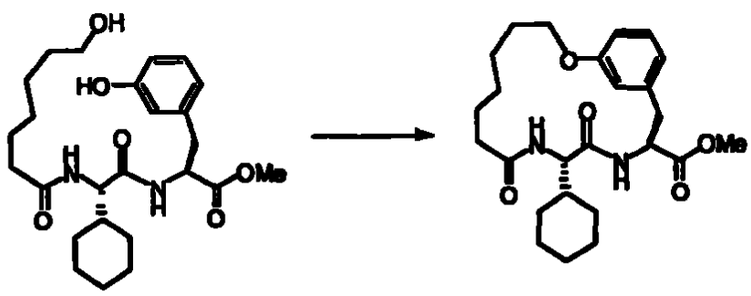
To the solution of 11a (1.46 g, 3 28 mmol) in anhydrous THF (60 mL) under nitrogen at 0°C was added borane-THF solution (12 mL, 1 0 M, 12 mmol) cautiously The resulting solution was stirred at 0°C under nitrogen for 1 h 40 min Then ethanol (4 mL) and pH 7 buffer (8 mL) were added, followed by aqueous 30 % H_2O_2 solution (7.5 mL) After stirred at 0°C for 20 min, it was warmed to rt and stirred for 2 h EtOAc (200 mL) and brine (100 mL) were added and layers were separated Aqueous solution was extracted with EtOAc (2 X 150 mL) Combined organic solution was dried with magnesium sulfate, filtrated, concentrated *in vacuo*. Flash chromatography (2 to 5 % MeOH-CH_2Cl_2) afforded 11b (1.05 g, 2 18 mmol, 68 %) as a white solid ^{1}H NMR (400 MHz, d_6-DMSO) δ 9 25 (s, 1 H), 8 30 (d, *J* =7 2 Hz, 1 H), 7 68 (d, *J* =9 2 Hz, 1 H), 7 05-7 01 (m, 1 H), 6 62-6 58 (m, 3 H), 4.43-4 18 (m, 3 H), 3 55 (s, 3 H), 3.37-3 33 (m,2 H), 2 93-2 80 (m, 2 H), 2 20-2 03 (m, 2 H), 1 66-0 87 (m, 19H), ^{13}C NMR (d_6-DMSO, 125 MHz), d 172 1,

0017820 "0110001

452
459

171.8, 171 2, 157 2, 138 4, 129 1, 119.5, 115 8, 113.5, 60 7, 56.5, 53 5, 51 7, 36.5, 35 1, 32 6, 32.4, 29 0, 28.5, 28.0, 25 8, 25 6, 25.4, 25 2, HRMS, *m/z* 463.2813 (calcd for $C_{25}H_{36}N_2O_5$ 463.2808, error 1 ppm)

Step C.

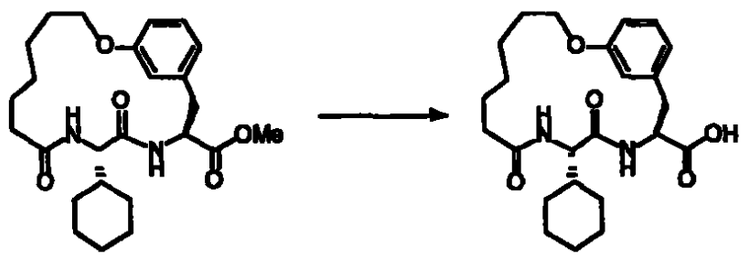


11b 11c

To a solution of phenol alcohol 11b (1 00 g, 2.16 mmol) and tri-*n*-butylphosphine (1 10 mL, 4.28 mmol) in anhydrous CH_2Cl_2 (100 mL) and THF (40 mL) at 0°C was added ADDP (1 08 g, 4 28 mmol) After stirring at 0°C for 1 h, the solution was warmed to rt and stirred for 3 h under nitrogen TLC indicated complete consumption of the starting material After removal of solvent *in vacuo*, the residue was partially purified by flash chromatography (0 to 3 % MeOH in CH_2Cl_2) to afford the macrocycle 11c (650 mg, 1.46 mmol, 68 %) ^{1}H NMR (400 MHz, d$_6$-DMSO) δ 8.58 (d, *J* =8.3 Hz, 1 H), 7 76 (d, *J* =9.2 Hz, 1 H), 7 18-7 14 (m, 1 H), 6 76-6 65 (m, 3 H), 4 77-4 71 (m, 1 H), 4 32 (t, *J* = 8.5 Hz, 1 H), 3.97-3.93 (m, 1 H), 3 82-3 78 (m, 1 H), 3 67 (s, 3 H), 3 18-3 14 (m, 1 H), 2.98-2.92 (m, 2 H), 2.32-2.25 (m, 1 H), 2.02-2.01 (m, 1 H), 1.99-0.87 (m, 19H), ^{13}C NMR (d$_6$-DMSO, 125 MHz), δ 172 1, 171 6, 171.4, 160 1, 158 8, 139 0, 129 1, 121 1, 113.0, 111 9, 66.4, 56 1, 52.0, 50 1, 40.6, 34 9, 34.2, 28 7, 28 3, 26.8, 26.3, 25 9, 25 5, 25.2, 24 2, HRMS, *m/z* 445.2685 (calcd for $C_{25}H_{38}N_2O_5$ 445.2702, error 4 ppm)

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Step D.

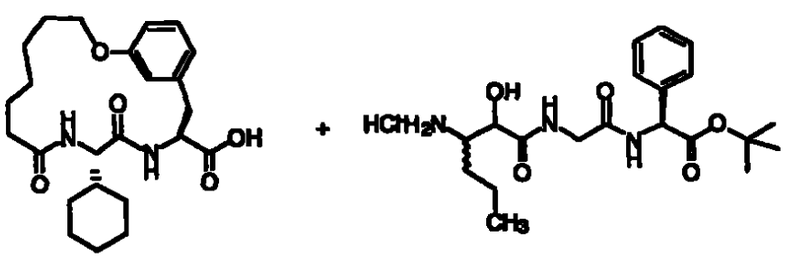


11c 11d

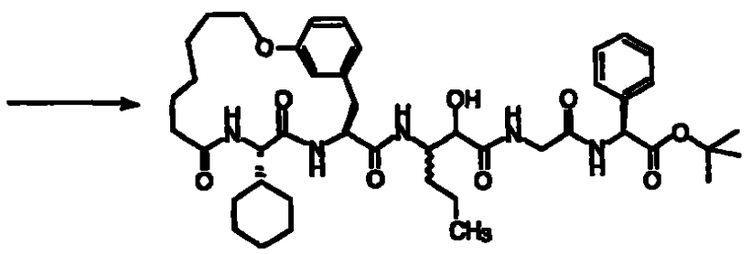
Aqueous lithium hydroxide solution (70 mg, 15 mL H_2O, 2.92 mmol) was added to a solution of methyl ester 11c (330 mg, 0 742 mmol) in THF (20 mL) and ethanol (10 mL) at rt. The mixture was stirred at rt for 3 h The progress of the reaction was monitored by TLC After the solution was concentrated *in vacuo*, EtOAc (100 mL), 6 N HCl solution (10 mL) and water (50 mL) were added and the layers were separated The aqueous solution was extracted with EtOAc (2 X 80 mL) Organic solutions were combined, dried with magnesium sulfate, filtered and concentrated *in vacuo* to afford 11d (260 mg, 0 604 mmol, 81 %) as a white solid ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.43 (d, *J* =8.3 Hz, 1 H), 7 73 (d, *J* =9.3 Hz, 1 H), 7 17-7 13 (m, 1 H), 6 77-6.66 (m, 3 H), 4 67-4 62 (m 1 H), 4.32-4.28 (m, 1 H), 3 98-3 93 (m, 1 H), 3.81-3.75 (m, 1 H), 3 17-3.13 (m, 1 H), 2.97-2.90 (m, 1 H), 2.32-2.26 (m, 1 H), 2 01-1 97 (m, 1 H), 1 67-0 85 (m, 19H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 173.2, 171 6, 171.3, 158.8, 139.3, 129 0, 121 1, 113 1, 111 9, 66.4, 56 1, 50 8, 35.1, 34.3, 28 8, 28.3, 26.9, 26.3, 25 9, 25 6, 25.5, 25 2, 24.2, HRMS, *m/z* 431 2564 (calcd for $C_{25}H_{36}N_2O_5$ 431 2548, error 4 ppm)

454
0461

Step E.



11d + B

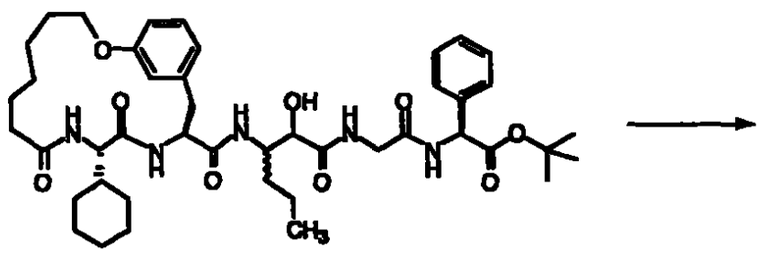


11e

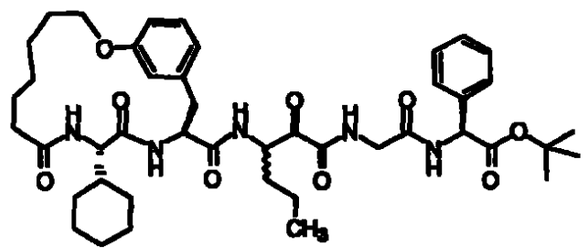
To a solution of acid 11d (0 140 g, 0.325 mmol), amine B (0 140 g, 0 325 mmol), HOOBt (56 mg, 0.343 mmol) and EDCI (75 mg, 0.391 mmol) in anhydrous DMF (40 mL) and CH_2Cl_2 (20 mL) at -20°C was added NMM (0 107 mL, 0 973 mmol) After stirred at this temperature for 30 min, the reaction mixture was kept in a freezer for 18 h Then EtOAc, brine and 5% H_3PO_4 were added The separated organic solution was washed, successively, with 5% H_3PO_4, saturated aqueous sodium bicarbonate solution, water, and brine, dried with magnesium sulfate, filtered and concentrated *in vacuo*. Flash chromatography (2 to 5 % MeOH-CH_2Cl_2) afforded 11e as a mixture of diastereomers (0 170 g, 0 211 mmol, 65%) as a white solid, which is used in the next reaction without further purification

C152 455

Step E.



11e

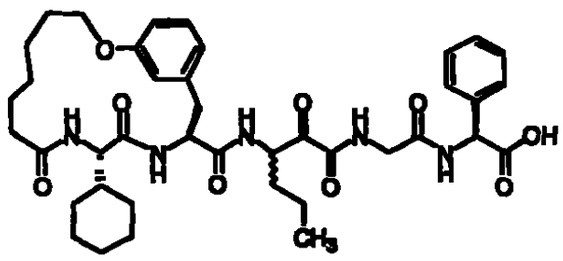


11

To the mixture of hydoxy amide 11e (0.29 g, 0.36 mmol) and Dess-Martin reagent (0.45 g, 1 06 mmol) at rt was added anhydrous CH_2Cl_2 (60 mL), DMF (3 mL) and DMSO (3 mL) The resulting solution was vigorously stirred at rt for 2.5 h More Dess-Martin reagent (300 mg, 0 71 mmol) was added and the reaction mixture was stirred for another hour Saturated aqueous sodium bicarbonate and sodium bisulfite solutions (40 mL each) were added and the mixture was vigorously stirred for 10 min before EtOAc (200 mL) and water (30 mL) were added and the layers were separated The organic solution was washed with 5% H_3PO_4 solution (2 X 100 mL) and saturated $NaHCO_3$ solution (100 mL), dried with magnesium sulfate, filtered and concentrated *in vacuo*. Flash chromatography (1 to 5 % MeOH-CH_2Cl_2) afforded 11 (100 mg, 0 124 mmol, 35%) as a white solid ^1H NMR (400 MHz, d_6-DMSO) δ 8 79-8 69 (m, 2 H), 8.36-8 16 (m, 2 H), 7 72-7 68 (m, 1 H), 7 42-7.33 (m, 5 H), 7 17-7 13 (m, 1 H), 6 77-6 63 (m, 3 H), 5.30-5.27 (m, 1 H), 5 09-5 04 (m, 1 H) 4 85-4 76 (m, 1 H), 4.29-

4.25 (m, 1 H), 3 98-3 74 (m, 1 H), 3 02-2.85 (m, 2 H), 2.32-2.27 (m, 1 H), 2 04-1 96 (m, 1 H), 1 72-0.81 (m, 35 H), ^{13}C NMR (d_6-DMSO, 125 MHz), δ 196 5, 196.2, 171 65, 171 61, 171 5, 171 14, 171.07, 169.4, 167.6, 160 7, 158.84, 158 79, 139 5, 139.3, 136.6, 136.5, 128 92, 128 90, 128 7, 128 6, 128 1, 127 7, 127 4, 124 9, 121.34, 121 28, 113 1, 112 9, 112 0, 111 9, 81.3, 68.34, 66.30, 56.92, 56 87, 56 3, 56 2, 53 4, 53.3, 51.5, 50 9, 41.5, 41.4, 40.8, 40 7, 36 6, 36 1, 34.4, 34 3, 31 8, 31 6, 30.4, 29 1, 28.9, 28.4, 28.3, 27 5, 26.8, 26.21, 26.17, 25 9, 25.59, 25.55, 25 0, 24 2, 18 74, 18.66, 13 5, 13.4 , HRMS, *m/z* 804.4542 (calcd for $C_{25}H_{36}N_2O_5$ 804.4548, error 1 ppm)

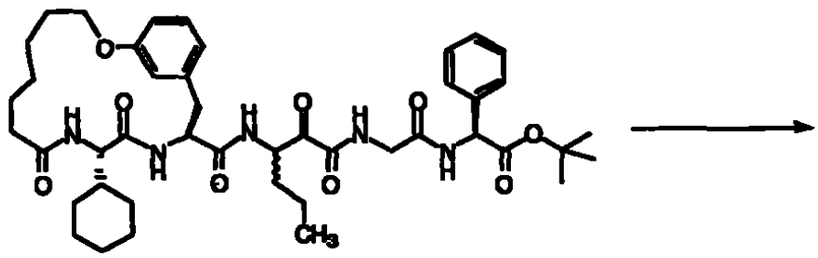
Example 12 Preparation of Compound of Formula 12:



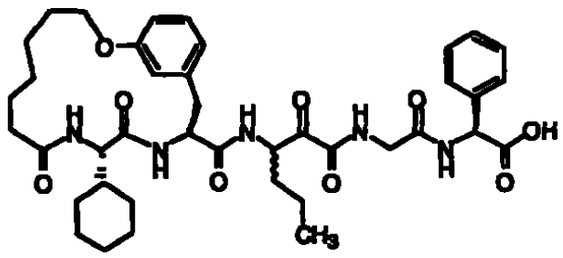
12

0184457

Step A.



11



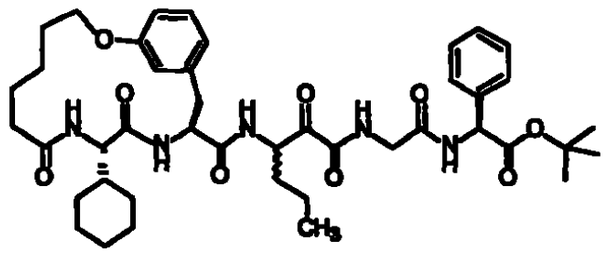
12

A solution of the *t*-Butyl ester 11 (56 8 mg, 0 0706 mmol) in trifluoroacetic acid (15 mL) and CH_2Cl_2 (15 mL) was stirred at rt for 4 h After the volatiles were removed *in vacuo*, the residue was dissolved in 50% MeOH-CH_2Cl_2 (3 mL), and concentrated to dryness *in vacuo* to afford an off-white solid 12 (50 mg, 0.0689 mmol, 95%) ^{1}H NMR (400 MHz, d$_6$-DMSO) δ 8 75-8.71 (m, 2 H), 8 36-8 16 (m, 2 H), 7 72-7 69 (m, 1 H), 7.39-7.31 (m, 5 H), 7 17-7 13 (m, 1 H), 6 76-6.63 (m, 3 H), 5 37-5.35 (m, 1 H), 5 07-5.04 (m, 1 H), 4 85-4 76 (m, 1 H), 4.29-4 25 (m, 1 H), 3 97-3.74 (m, 4 H), 3.02-2 86 (m, 2 H), 2.32-2.26 (m, 1 H), 2 01-1.97 (m, 1 H), 1 70-0 82 (m, 26 H), ^{13}C NMR (d$_6$-DMSO, 125 MHz), δ 196.5, 196.2, 171 63, 171 59, 171.52, 171.48, 171 1, 171.06, 167.4, 160 6, 158 82, 158 78, 153 4, 139 4, 137 1, 137 0128 91, 128 88, 128 7, 128.65, 128 61, 128 5, 128 43, 128 39, 128 33, 128.32, 128.14, 128.12, 128 0, 127 7, 128 7, 127 63, 127 59, 127 5, 127 4, 126 8, 121 3, 115 9, 113 1, 112 9, 112 8, 112.0, 111 9,, 111 88, 66 33, 66 29 56.3, 56 2, 56 17, 53 34, 53.31, 53.27, 51 1, 50 9 41.5, 40.84, 40 77, 40 7,

458
0465

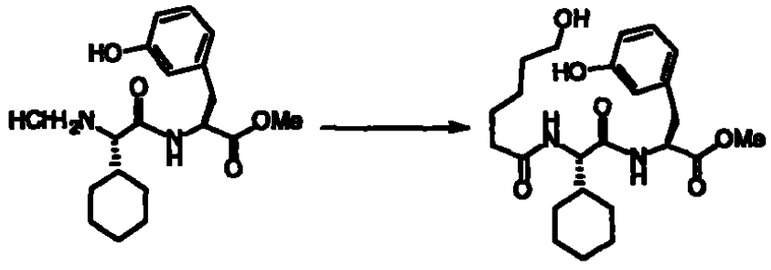
40 6, 40.56, 40.53, 40.5, 38 7, 38 6, 38 56, 38.53, 36 6, 36 1, 34.4, 34 3, 31 8, 31 6, 29 4, 29 1, 29 0, e28 9, 28.4, 28.3, 28 2, 26.9, 26 8, 26 79, 26.20, 26 16, 25 88, 25 86, 25 79, 25 75, 25 71, 25 66, 25.57, 25.54, 25.4, 25 0, 24.2, 18 7, 18 6, 13.5, 13 4, HRMS, *m/z* 748.3947 (calcd for $C_{25}H_{36}N_2O_5$. 748.3922, error 3 ppm)

Example 13. Preparation of Compound of Formula 13.



13

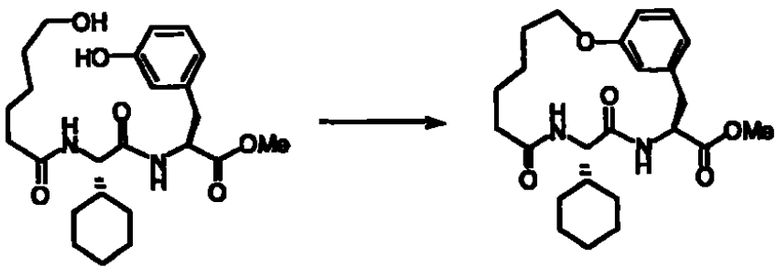
Step A.



1g 13a

The desired compound 13a was prepared according to the method of Example 11, Step A, except substituting 6-hydroxyhexanoic acid for 6-heptenoic acid (39%)

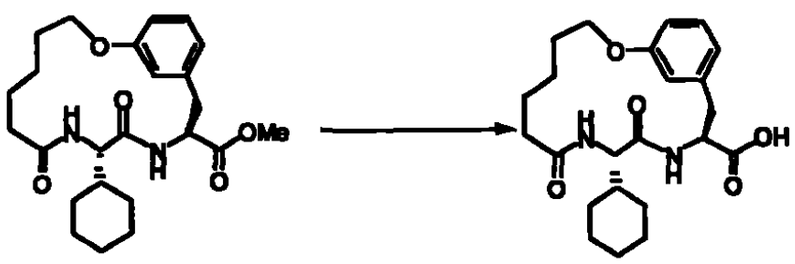
Step B.



13a 13b

The desired compound 13b was prepared from 13a according to the method of Example 11, Step C in 74% yield

Step C.

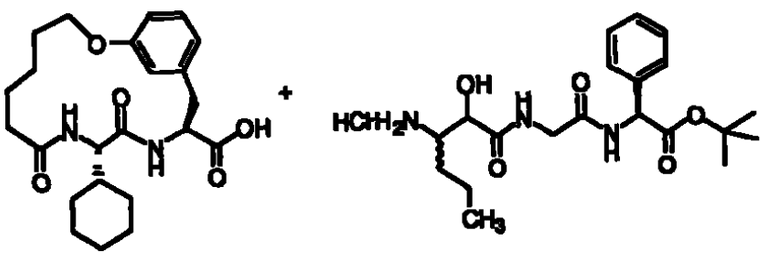


13b 13c

The desired macrocyclic acid 13c was prepared from its corresponding methyl ester 13b according to the method of Example 11, Step D in 68 % yield as a white solid

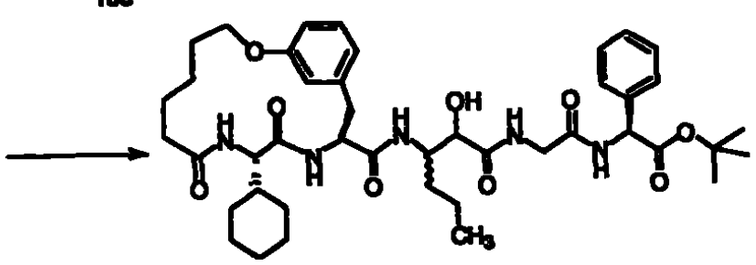
460
0167

Step D.



13c

B

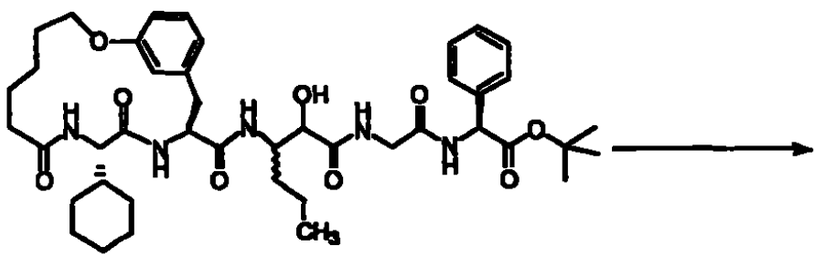


13d

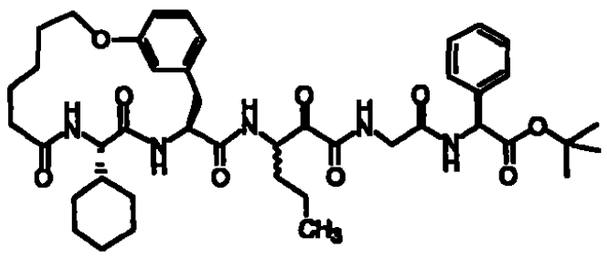
The desired compound **13d** was prepared from **13c** and **B** according to the method of Example 11, Step E in 48% yield

0463
461

Step E.



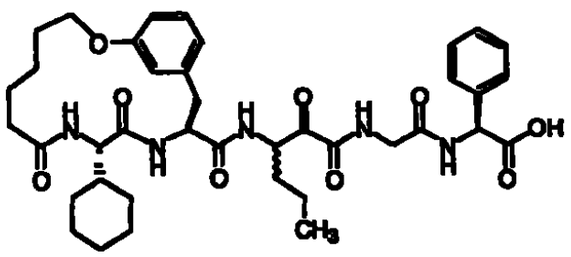
13d



13

The desired compound 13 was prepared from 13d according to the method of Example 11, Step F in 70% yield

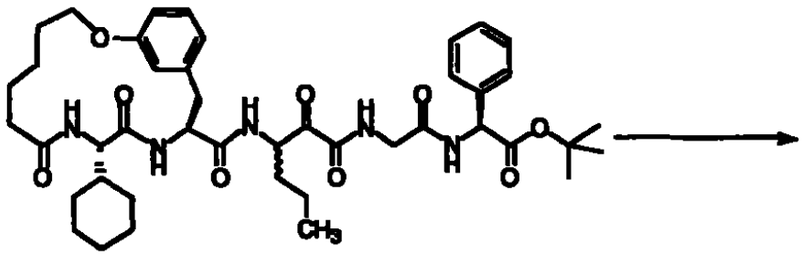
Example 14. Preparation of Compound of Formula 14.



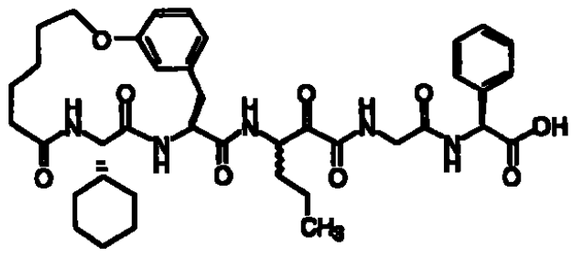
14

WO 01/96368 PCT/US01/18709

Step A.



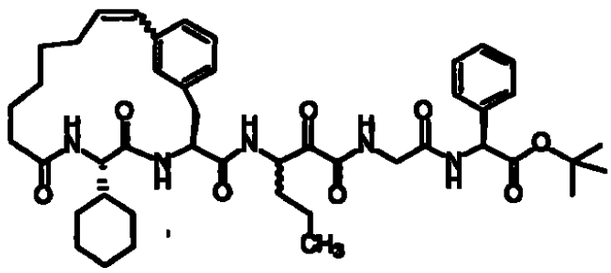
13



14

The desired compound 14 was prepared from 13 according to the method of Example 12, Step A in quantitative yield

Example 15. Preparation of Compound of Formula 15.



15

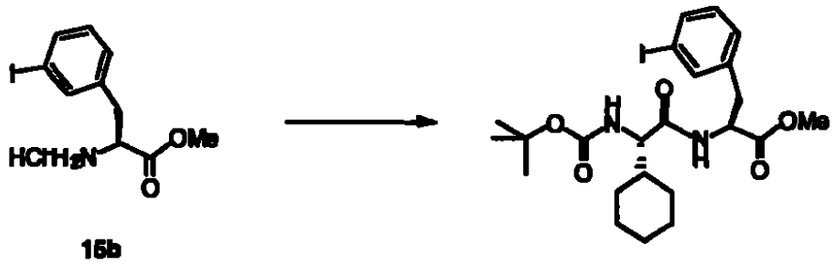
Step A.



15a 15b

A solution of 3-iodo-phenylanaline 15a, (2.50 g, 8.59 mmol) and concentrated hydrochloric acid (2 mL, 24 mmol) in methanol was heated to reflux for 18 h Removal of solvents *in vacuo* afforded a white solid 15b, which was used in Step B without further purification

Step B



15b

15c

The desired compound 15c was prepared in 84% yield from 15b according to the method of Example 11, Step A. It was used in the next reaction without further purification

Step C



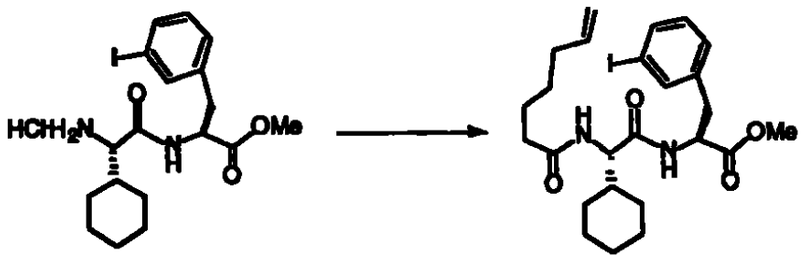
15c

15d

The desired compound 15d was prepared from 15c according to the method of Example 11, Step A (quantitative) It was used in the next reaction without further purification

Dkt 464

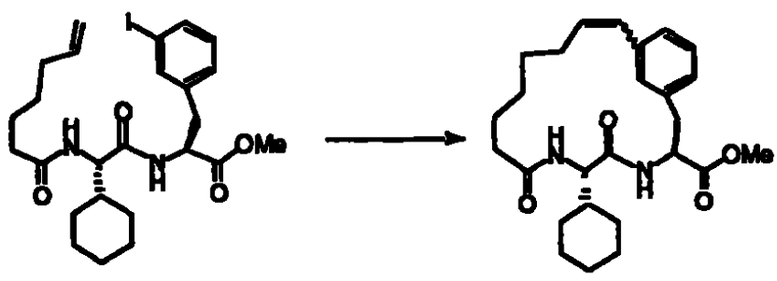
Step D.



15d 15e

The desired compound 15e was prepared in 68% yield from 15d according to the method of Example 11, Step A. It was used in the next reaction without further purification

Step E

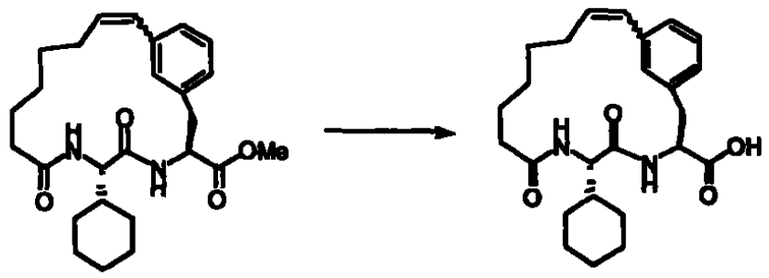


15e 15f

A solution of 15e (1 16 g, 2.04 mmol), triethylamine (2.90 mL, 20 6 mmol) in anhydrous acetonitrile (25 mL) and DMF (20 mL) in a thick walled tube was bubbled with Argon for 5 min To this solution at rt was quickly added tetrakistriphenylphosphine palladium (0) (235 mg, 0 203 mmol) The tube was sealed with Teflon screw cap and heated to 85-90°C in an oil bath After stirring for 3 h, it was cooled to rt, opened cautiously and poured onto EtOAc (100 mL) The solution was washed with 5% H_3PO_4 (4 X 50 mL) and water (50 mL) Organic layer was dried with magnesium sulfate, filtrated, concentrated *in vacuo*

Flash chromatography (1 to 4 % MeOH-CH_2Cl_2) afforded the macrocycle 15f (330 mg, 0 749 mmol, 37 %)

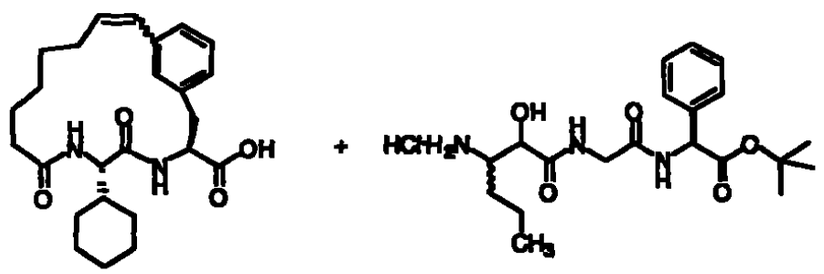
Step F



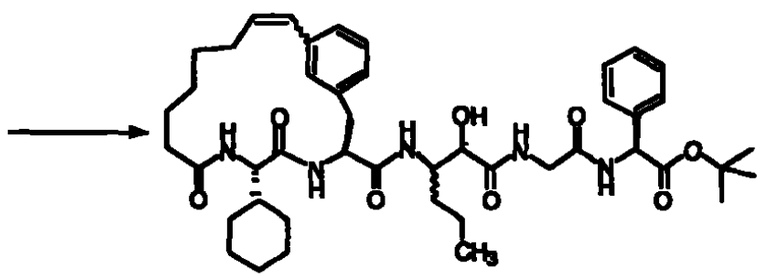
15f 15g

The desired compound 15g was prepared quantitatively from 15f according to the method of Example 11, Step D It was used in the next reaction without further purification

Step G.



15g B

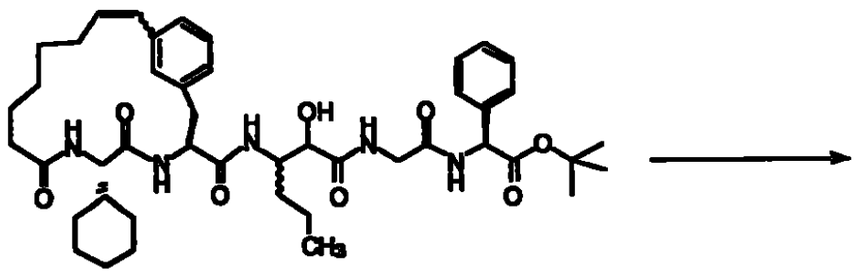


15h

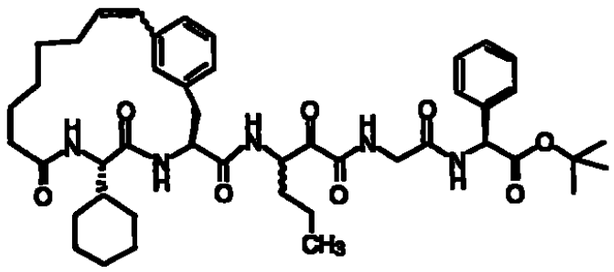
466
0473

The desired compound 15h was prepared in 77 % yield from 15g according to the method of Example 11, Step F It was used in the next reaction without further purification

Step H



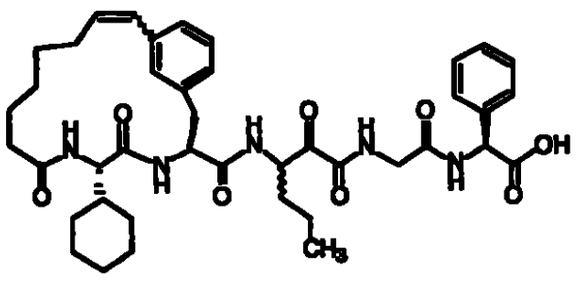
15h



15

The desired compound 15 was prepared in 55 % yield from 15h according to the method of Example 1, Step H

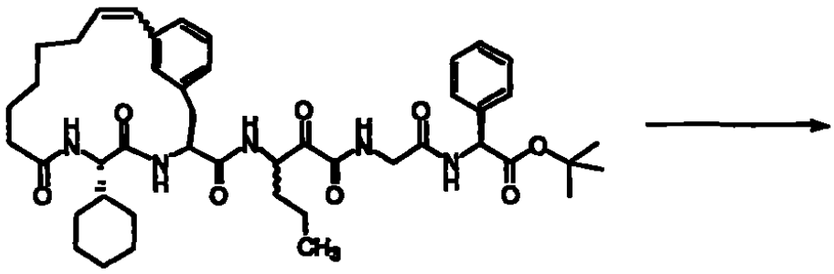
Example 16. Preparation of Compound of Formula 16.



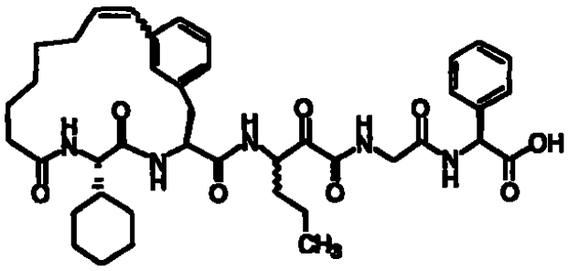
16

467

Step A.



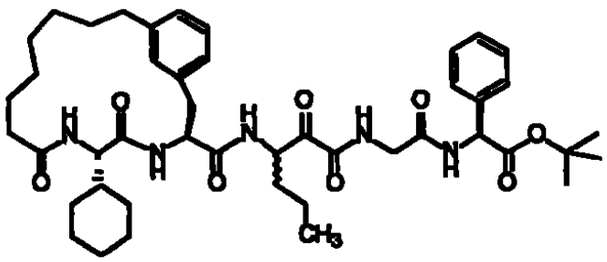
15



16

The desired compound 16 was prepared quantitatively from 15 according to the method of Example 12, Step A

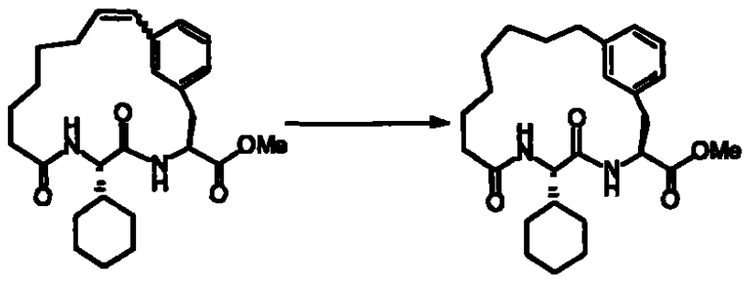
Example 17: Preparation of Compound of Formula 17.



17

468

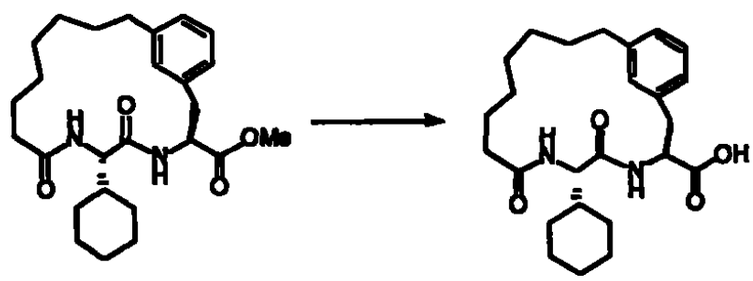
Step A.



15f 17a

To the solution of 15f (150 mg, 0.340 mmol) in EtOH (10 mL) and EtOAc (5 mL) was added 10 % palladium on carbon (20 mg) The suspension was stirred under hydrogen for 8 h during which the reaction progress was monitored by TLC After filtration through a celite pad, solvents were removed *in vacuo* to afford the product as a white solid 17a (150 mg, 0.339 mmol, quantitative) It was used in the next reaction without further purification

Step B

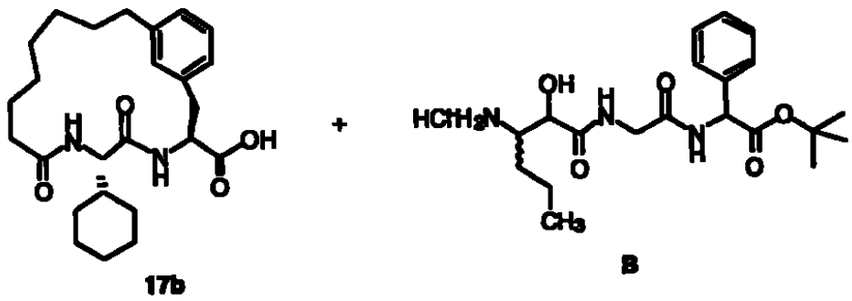


17a 17b

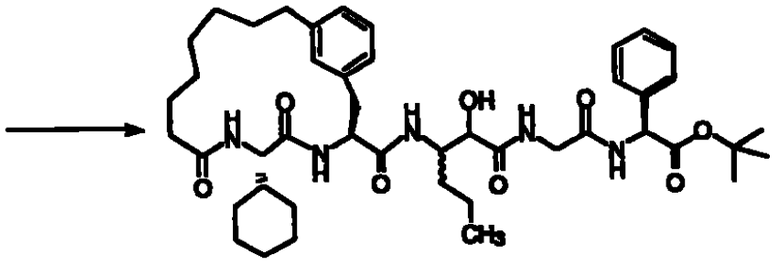
The desired compound 17b was prepared from 17a according to the method of Example 11, Step D It was used in the next reaction without further purification

469

Step C.



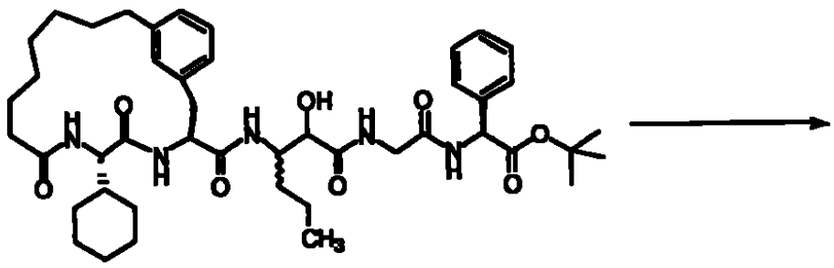
17b + B



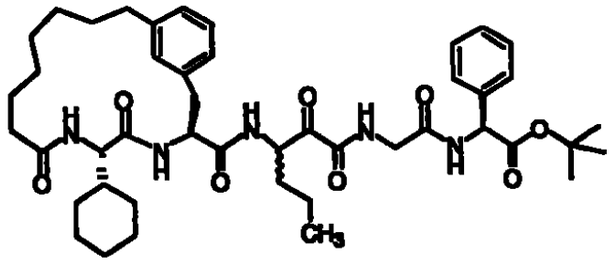
17c

The desired compound 17c was prepared in 73 % yield (Steps B and C) from 17b according to the method of Example 11, Step E It was used in the next reaction without further purification.

Step D



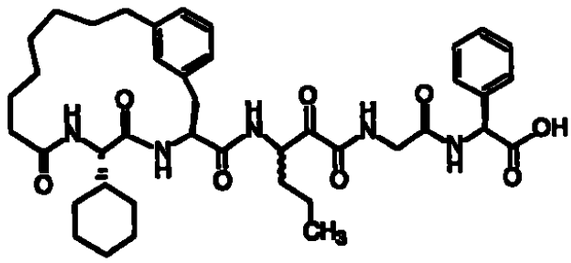
17c



17

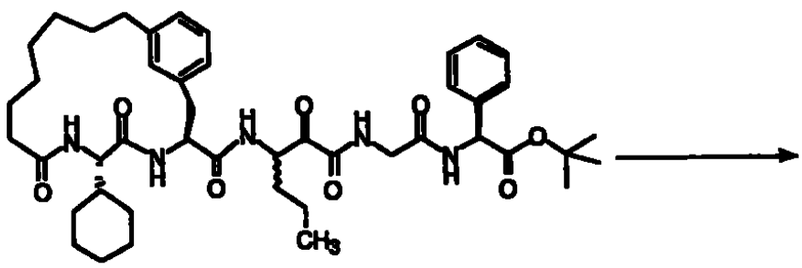
The desired compound 17 was prepared in 46 % yield from 17c according to the method of Example 11, Step F.

Example 18. Preparation of Compound of Formula 18.

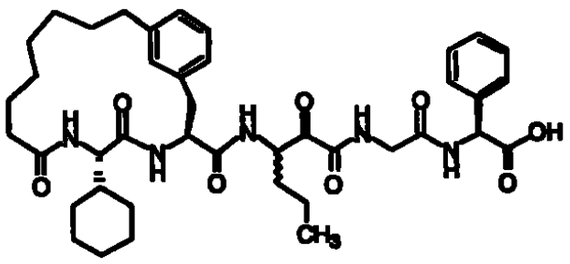


18

Step A.



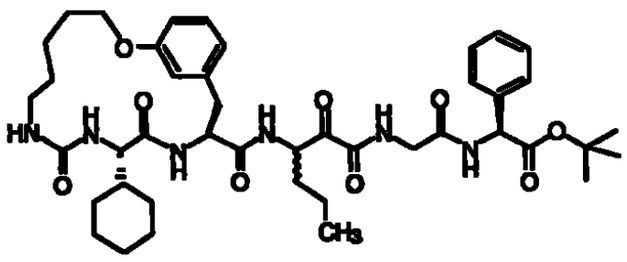
17



18

The desired compound 18 was prepared quantitatively from 17 according to the method of Example 12, Step A.

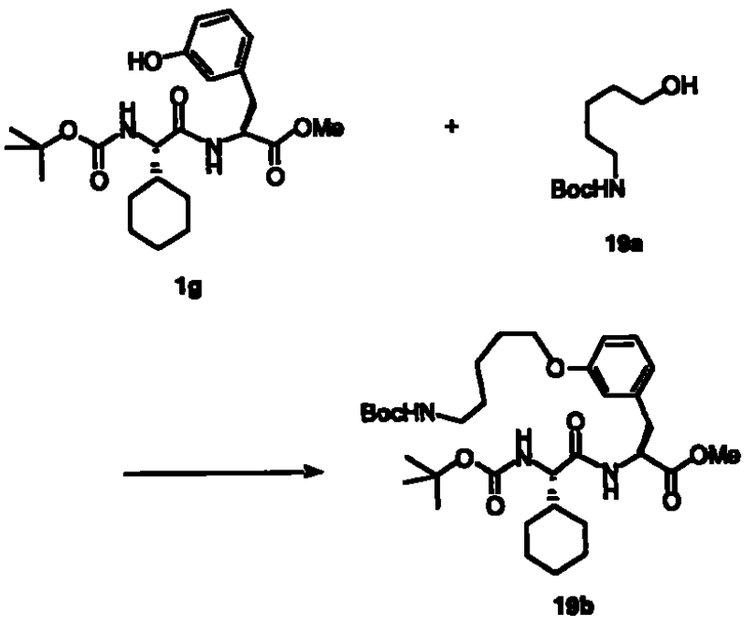
Example 19. Preparation of Compound of Formula 19.



19

09/11/02 10:01 6019

Step A.

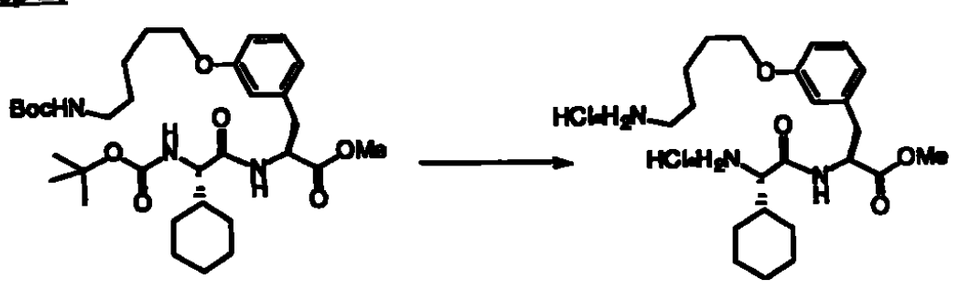


1g 19a

19b

The desired compound 19b was prepared in 64 % yield from 1g and 19a according to the method of Example 11, Step C

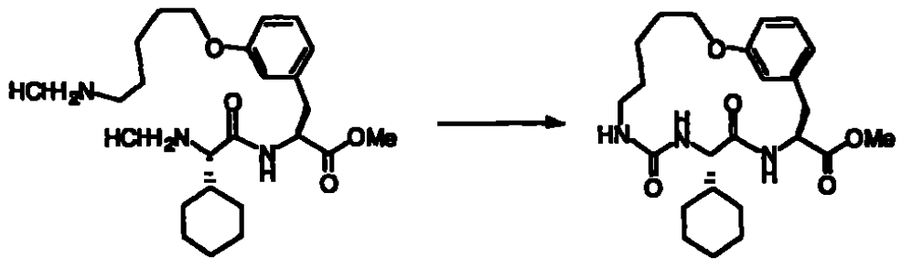
Step B.



19b 19c

The desired compound 19c was prepared from 19b according to the method of Example 1, Step C It was used in the next reaction without further purification

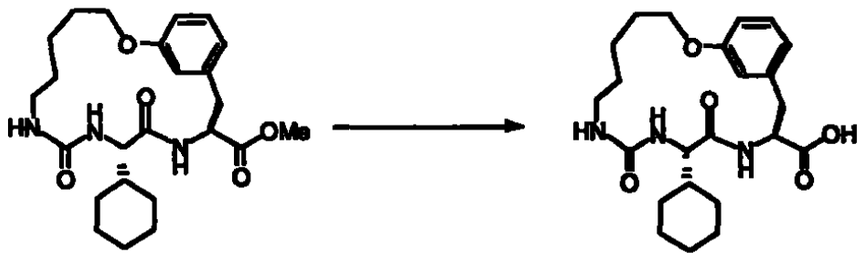
Step C



19c 19d

To a suspension of di-amine salt 19c (75 mg, 1 52 mmol) and carbonyl diimidazole (260 mg, 1.60 mmol) in acetonitrile (400 mL) at rt was added triethylamine (0.26 mL, 1 85 mmol) The mixture was stirred for 3 days Solvent was removed *in vacuo* The residue dissolved in EtOAc/THF (100/50 mL) and the solution was washed with 5% H_3PO_4, dried with magnesium sulfate, filtered, concentrated *in vacuo*. Flash chromatography (2 to 10 % MeOH-CH_2Cl_2) afforded 19d (290 mg, 0 651 mmol, 43 %) as a white solid

Step D.

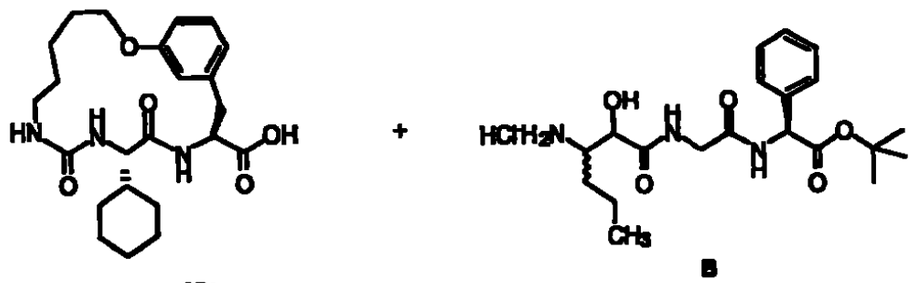


19d 19e

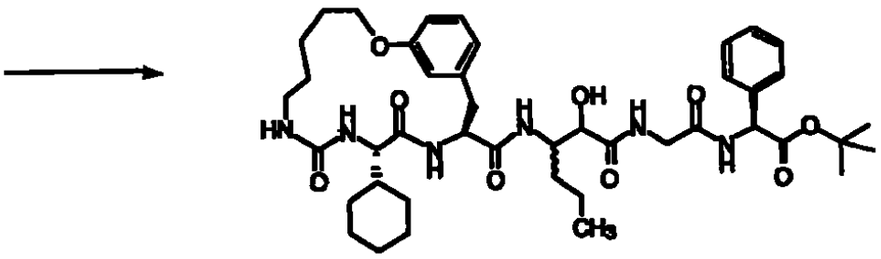
The desired compound 19e was prepared in 97 % yield from 19d according to the method of Example 11, Step D It was used in the next reaction without further purification

474

Step E.



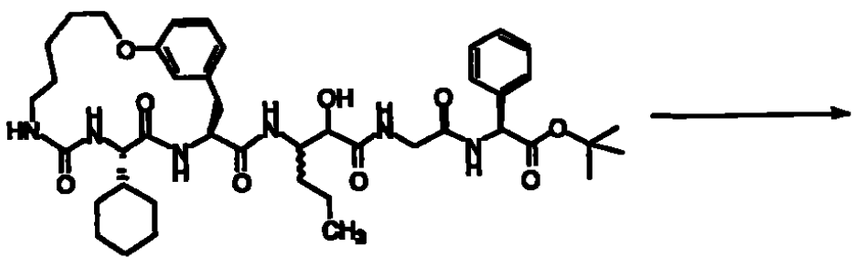
19e + B



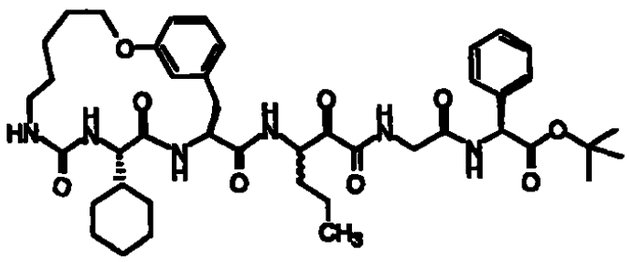
19f

The desired compound 19f was prepared in 66 % yield from 19e and B according to the method of Example 11, Step E

Step F



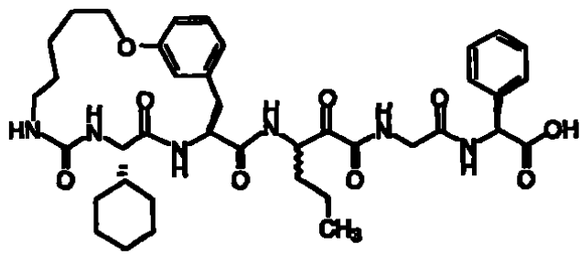
19f



18

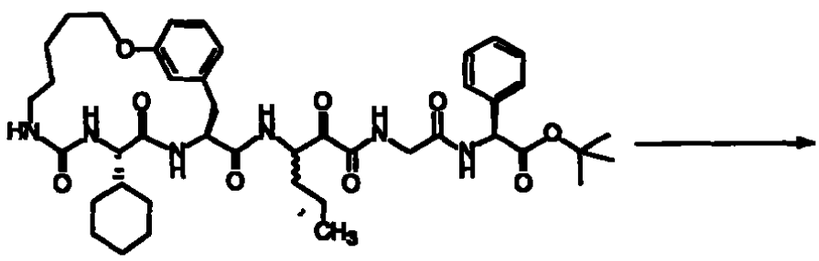
The desired compound 19 was prepared in 66 % yield from 19f according to the method of Example 11, Step F Two products were partially separated by flash chromatography (0 to 5 % MeOH-CH_2Cl_2)

Example 20. Preparation of Compound of Formula 20:

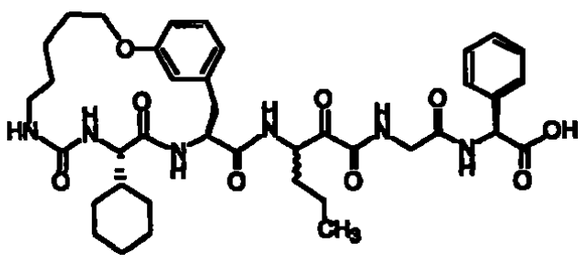


20

Step A.



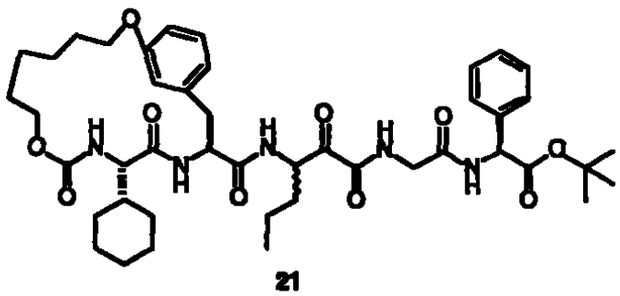
19



20

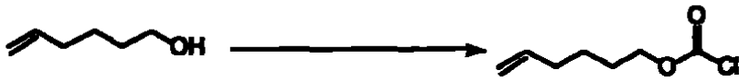
The desired compound 20 was prepared from 19 according to the method of Example 12, Step A

Example 21 Preparation of Compound of Formula 21.



21

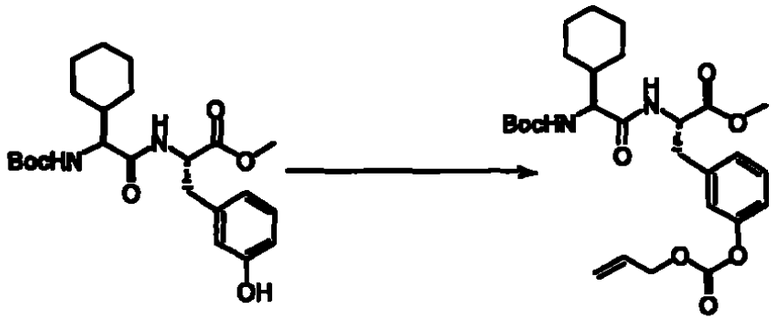
Step A



21a 21b

A solution of 5-hexene-1-ol **21a** (10g, 50 mmol) in diethylether (100 mL) was treated with triethylamine (10 1g, 100 mmol, 2 0 equiv) and cooled to 0° C A solution of phosgene in benzene (20%, 100 mL, 20g, 200 mmol, 4 0 equiv) was added dropwise and the reaction mixture was stirred at rt for 12h The triethylamine hydrochloride separating out was filtered and the filtrate was concentrated *in vacuo*. The residue **21b** was used directly for further studies without purification

Step B

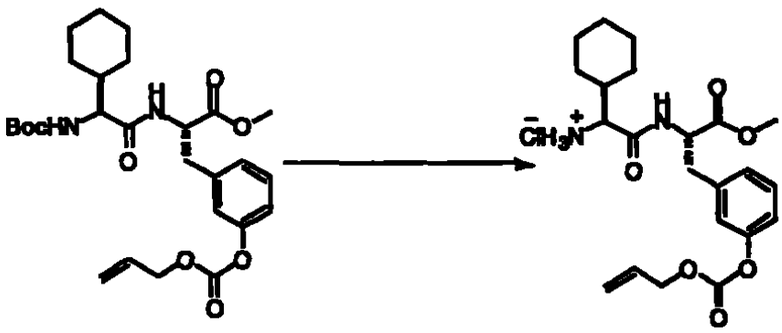


1f 21c

A solution of 1f (8 0g, 18 43 mmol) in CH_2Cl_2 (100 mL) was treated with triethylamine (2 43 g, 24.0 mmol, 1 3 equiv) The reaction mixture was cooled to -78° C and allychloroformate (2.9g, 24 mmol, 1.3 equiv) was added dropwise The

reaction mixture was stirred at rt for 12 h and the reaction mixture was diluted with H_2O (100 mL) and aq HCl (2M, 200 mL) The aq layer was extracted with EtOAc (3x200 mL) The combined EtOAc layer were extracted with brine, dried (Na_2SO_4), filtered concentrate *in vacuo* and the residue 21c was directly used for Boc deprotection ^1H NMR ($CHCl_3$, 300 MHz, δ, ppm) 7.29 (t, 1 H, J= 6.0 Hz), 7 06-6 98 (m, 3 H), 6 41 (d, 1 H, J=5 4 Hz), 6 05-5 95 (m, 1H), 5.42 (dd, 1H, J=1.2, 13 2), 5 31 (dd, 1 H, J=1 2, 13 2), 5 10 (d, 1 H, J=6 6 Hz), 4 91-4.87 (q, 1 H), 4.74 (d, 1H, J=4 5 Hz), 3 95-3 92 (m, 1 H), 3 70 (s, 3 H), 3.12 (d, 1 H J=4.2 Hz),1.81-1.51 (m, 6H), 1.43 (s, 9 H), 1.21-0 91 (m, 6 H)

Step C



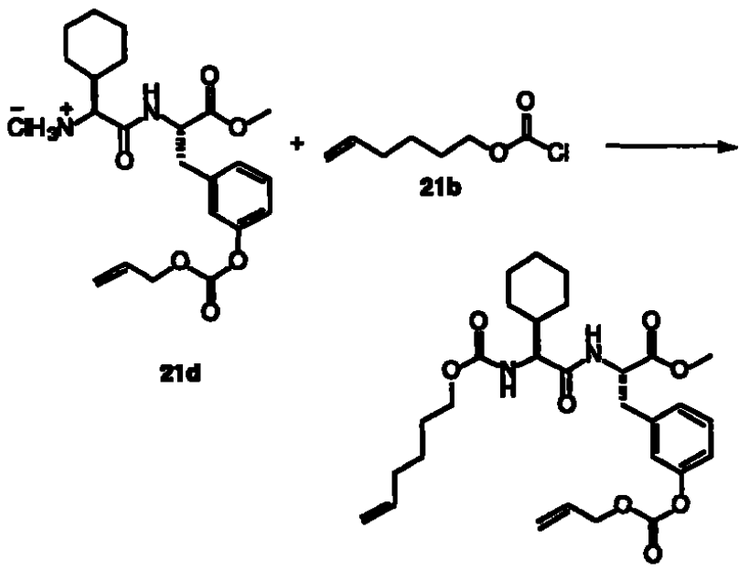
21c 21d

A solution of 21c (1 5 g) in HCl (4M in dioxane, 100 mL) was stirred at rt for 3 h The disappearance of the starting material was followed by TLC and once the starting material disappeared the reaction mixture was concentrated *in vacuo* and the residue 21d was dried in pump It was used for coupling without further purification

60/280,104 .0'11 900

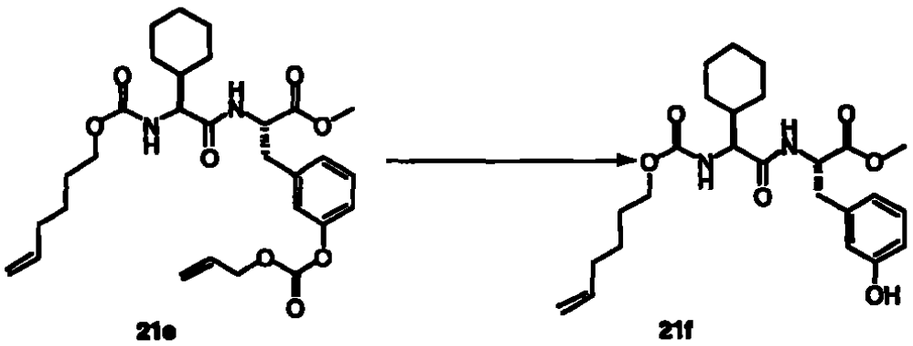
478

Step D



A solution of the aminehydrochloride 21d (4 0g, 8.9 mmol) in CH_2Cl_2 (50 mL) was treated with triethylamine (2 73 g, 27 mmol, 3 0 equiv, 3 8 mL) and cooled to -78° C A solution of chloroformate 21b (2.3g, 13 3 mmol, 1.5 equiv) in CH_2Cl_2 (30 mL) was added dropwise. The reaction mixture was stirred overnight at rt and diluted with aq HCl (1M, 150 mL) The aqueous layer was extracted with EtOAc (3x100 mL) The combined ethyl acetate layers were extracted with H_2O (100 mL), brine (100 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 3 7) to yield 21e as colorless solid (5g, 80%)

Step E

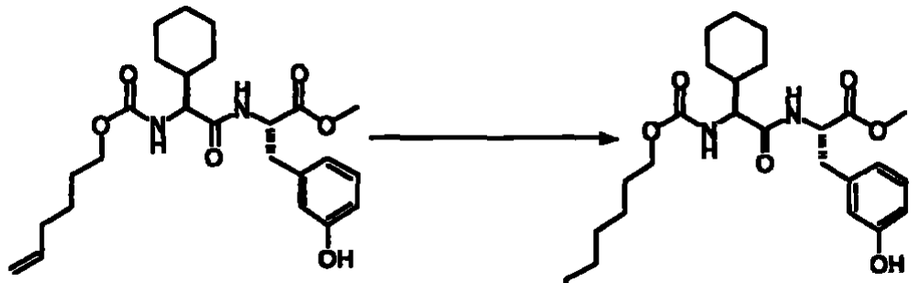


21e 21f

A solution of alloc-protected compound 21e (4 0 g, 7 2 mmol) in dry THF (60 0 mL) was treated with dimedione (2 01g, 14.4 mmol, 2 0 equiv), $Pd(PPh_3)_4$ (830 mg, 0 71 mmol, 10 mol%) at 0 °C and stirred at rt for 1h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes, 3 7) to yield a deprotected alcohol 21f as a colorless solid (2 7g, 79%) ^1H NMR ($CDCl_3$, 300 MHz, δ ppm) 7.44 (bs, 1 H), 7 09 (s, 1 H, J=6.0 Hz), 6 75-6 72 (m, 2 H), 6.58-6.48 (m, 2 H), 5.81-5 71 (m, 1 H), 5.55 (d, 1 H, J=7.2 Hz, 4.98 (ddd, 1 H, J=1 5, 1 2, 9 Hz), 4 92 (dd, 1H, J=4.5, 0 9 Hz), 4.88-4 83 (m, 1 H) 4 12-3.97 (m, 1 H), 3.71 (s, 3 H), 3.09-2.98 (m, 2 H), 2 08-2 03 (m, 2 H), 1 722-1 40 (m, 10 H), 1.24-0 94(m, 5 H), ^{13}C NMR (100 MHz, δ) 171 6, 157.3, 156 6, 138 3, 136 6, 129 8, 123 5, 120 6, 117.0,114 9, 114 6, 65 7, 60 1, 53.2, 52.5, 40.4, 37 1, 33.3, 29 6, 28 6, 28.3, 26 0, 25 9, 25 1, CHN calcd for $C_{25}H_{36}N_2O_6$ C=65 20% H=7.88% N=6.08%, Found C=64 90% H=7.98% N= 6.01%.

480

Step F

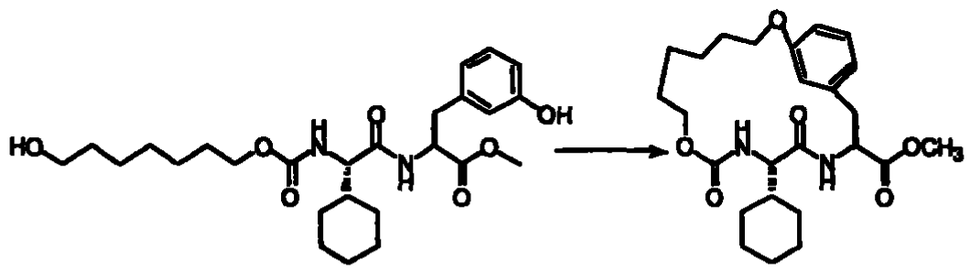


21f 21g

A solution of alkene 21f (650 mg, 1.4 mmol) in anhydrous THF (5 2 mL) was cooled to 0°C and treated with BH_3•THF (1M soln in THF, 4.2 mL, 4.2 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 2 h and EtOH (2.0 mL) was added carefully with the evolution of hydrogen gas. After the H_2 evolution was complete the reaction mixture was treated with pH 7 buffer and treated with aq H_2O_2 (30%, 5 0 mL) at 0 °C The ice bath was removed and the mixture was stirred at rt for 3-4 h The reaction mixture was extracted with EtOAc (3x100 mL) The combined organic layers were extracted with H_2O, brine, dried ($MgSO_4$), filtered, concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 3.7) to yield hydroborated product as a colorless solid 21g (400 mg, 60%) $[\alpha]_D$ 86.4 (*c* 0.3 $CHCl_3$, 25 °C), ^1H NMR ($CDCl_3$, 400 MHz, δ) 7 26 (s, 1 H), 7 08 (t, 1 H, *J*=5 7Hz), 6 83 (d, 1 H, *J*=6 0 Hz), 6.71 (dd, 1 H, *J*=1 2, 4 5 Hz), 6 57 (bs, 1 H), 6.54 (d, 1 H, *J*=5 7 Hz), 5 68 (d, 1 H, *J*=6.9 Hz), 4.85 (dq, 1 H, *J*=4.2, 1 8 Hz), 4 05-3.97 (m, 3 H), 3 69 (s, 3 H), 3 60 (t, 2 H, *J*=4.8 Hz), 3 06-2 97 (m, 2 H), 1 77-1.53 (m, 10H), 1.42 1 25 (m, 4 H), 1.24-0 92 (m, 5 H), ^{13}C NMR ($CDCl_3$, 100 MHz, δ) 171.8, 171 8, 157.8, 156.9, 136.9, 130 0, 120.8, 117 0, 114 8, 65 7, 62 7, 60.3, 53.3, 52 7, 40.5, 37.4, 32.5, 29 7, 29 0, 28 8, 26.2, 26.0, 25 6, 25 4 MS (FAB, NBA/DMSO, *m/z, relative intensity)* 479 ($[M+1]^+$,100), 296 (40), 196 (25), 156 (25), 136 (25), 112 (20) HRMS calcd for $C_{25}H_{39}N_2O_7$ $(M+1)^+$ 479 2760; found 479 2757

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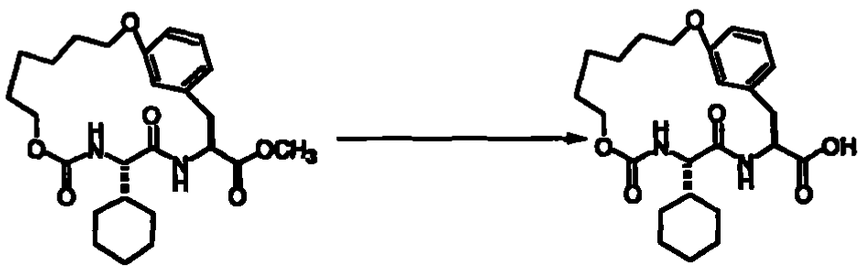
Step G



21g 21h

A solution of PPh_3 (385 mg, 1,47 mmol, 1 75 equiv)) in CH_2Cl_2 (10 mL) was treated with the compound 21g (400 mg, 0 84 mmol) and cooled to 0° C A solution of DEAD (220 mg, 1 26 mmol, 1 5 equiv) in CH_2Cl_2 (10 mL) was added dropwise and stirred at rt for 3 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 9) to yield cyclic product 21h as a colorless solid (110 mg, 25%)

Step H

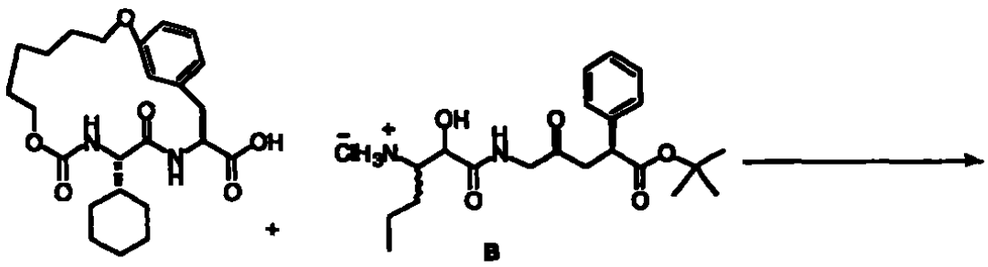


21h 21i

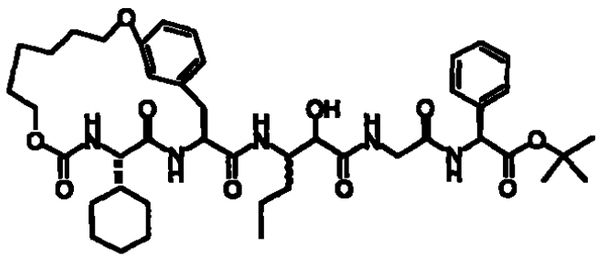
A solution of cyclic carbamate 21h (200mg, 0 44 mmol) in dioxane (30 mL), CH_3OH (20 mL) and CH_2Cl_2 (20 mL) was treated with $LiOH \cdot H_2O$ (80 mg, 2 0 mmol, 4.5 equiv) and stirred at rt for 4 h The reaction was concentrated *in vacuo* and diluted with HCl (4M soln in dioxane,10 mL) The water was removed by lyophilizer to yield the crystalline acid 21i directly used for coupling

482
0189

Step I



21I

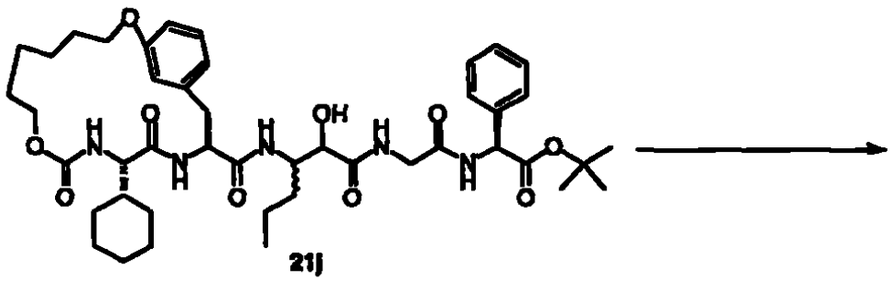


21J

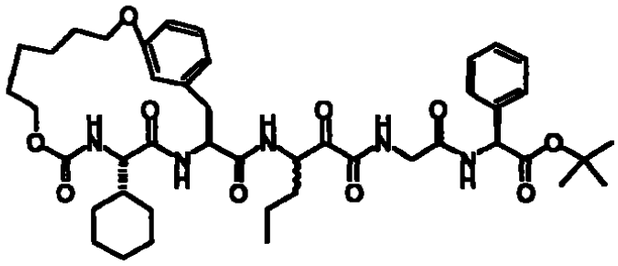
A solution of the hydrolyzed acid 21I (210 mg, 0 47 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5.0 mL) was treated with HOOBt (125 mg, 0 70 mmol, 1.5 equiv) and cooled to 0 °C and Hünigs base (258 mg, 2.0 mmol, 4.0 equiv, 369 µL) was added To this mixture was added EDCI (134 mg, 0 70 mmol, 1.5 equiv) and the reaction mixture was stirred at 0° C for 0.5 h and treated with the amine hydrochloride B (253 mg, 0.58 mmol, 1.25 equiv) The reaction mixture was stored in freezer for 24 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with aq HCl (2M, 30 mL) and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq HCl (2M, 30 mL), aq NaOH (1M) brine (2x50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue 21J (220 mg) was oxidized without further purification.

0490
483

Step J



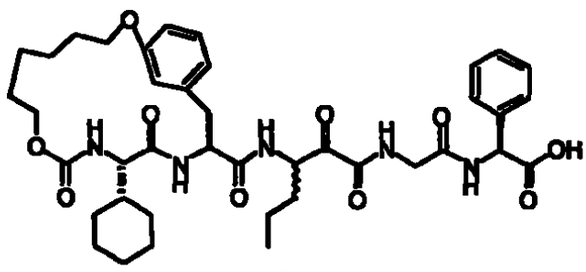
21j



21

A solution of alcohol 21j (220 mg, 0 26 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (200 mg, 0.47 mmol, 1.8 equiv) The reaction mixture was stirred at rt for 1 h and diluted with aq $NaHCO_3$ (15 mL) and aq $Na_2S_2O_3$ (15 mL) The reaction mixture was stirred at rt for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were extracted with aq Na_2CO_3, dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH (2M NH_3)/CH_2Cl_2 1 20) to yield ketoamide 21 (60 mg, 27%) of a colorless solid

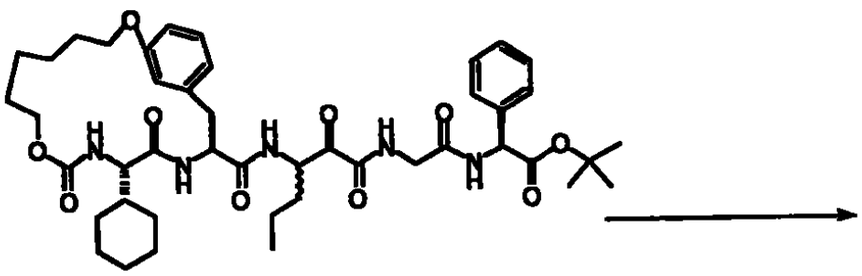
Example 22. Preparation of Compound of Formula 22.



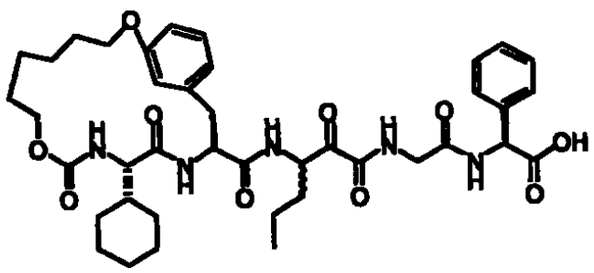
22

0/91 484

Step A



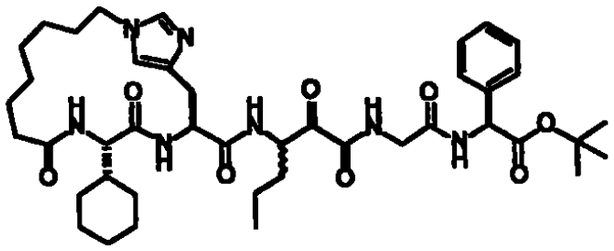
21



22

A solution of *tert*-butyl ester 21 (50 mg, 0 059 mmol) in dry CH_2Cl_2 (2 0 mL) was treated with TFA (2 0 mL) and stirred at rt. for 4 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in heptanes/CH_2Cl_2 and concentrated *in vacuo* several times to yield a fine tan solid 22 (47 mg), which was dried *in vacuo*.

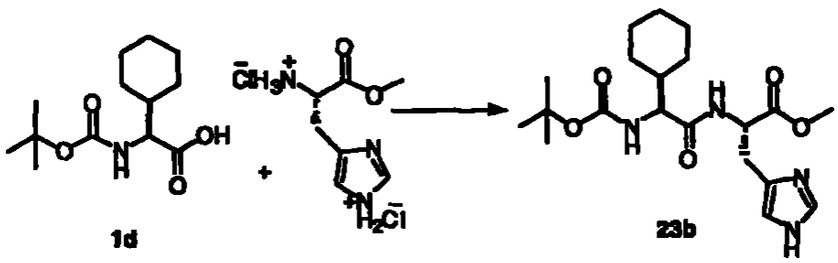
Example 23: Preparation of Compound of Formula 23.



23

60130282 .11.01300

Step A

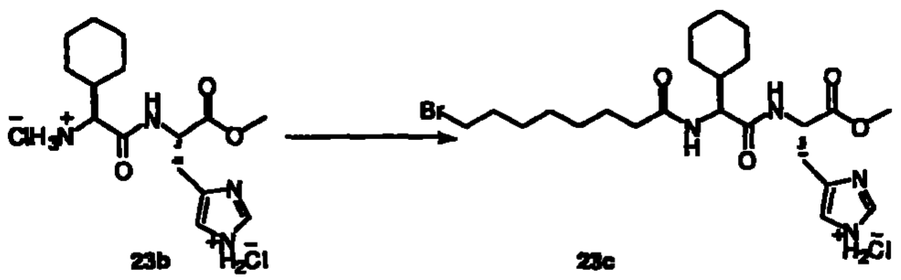


1d 23a 23b

A solution of acid 1d (255mg, 1 0 mmol) in DMF (2 0 mL) was treated with HOBt (202 mg, 1.5 equiv) and Hünigs base (517 mg, 4 0 mmol, 4 0 equiv, 738 µL) The reaction mixture was cooled to 0° C and treated with DCC (258 mg, 1.25 mmol, 1 25 equiv) After stirring the mixture for 1h Histidine-OCH_3•2HCl 23a (242.0 mg, 1 0 mmol) was added and stirred at rt overnight The reaction mixture was concentrated *in vacuo* and extracted in EtOAc (3x50 mL) and aq. $NaHCO_3$ (50 mL) The combined organic layer was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 19) to yield dipeptide 23b as a colorless solid (380 mg, 93%) ^1H NMR (d_6-DMSO, 400 MHz, δ, ppm) 8 17 (d, 1 H, *J*=7 2 Hz), 7.48 (s, 1 H), 6 77 (s, 1 H) 6 57 (bs, 1 H), 5.54 (d, 1H, *J*=7 6 Hz), 4.47 (q, 1 H, *J*=7 2 Hz), 3 79 (t, 1 H, *J*=8.4 Hz), 3 55 (s, 3 H), 3.36-3.20 (m,2 H), 2 94-2 82 (m, 2 H), 1 70-1.47 (bm, 6H), 1.35 (s, 9 H), 1 46-0 85 (m, 5 H) ^{13}C NMR (d_6-DMSO, 100 MHz, δ ppm) 172.5, 171 9, 157.3, 155.9, 135.4, 78 6, 59 5, 52.9, 52.3, 34 1, 29 6, 28.9, 28 6, 26.5, 26.3, 26 0, 25 2 FAB MS (NBA-G/TG-DMSO, *m/z relative intensity*) 409 [$(M+1)^+$, 100], 353 (10), 170 (20), HRMS calcd for $C_{20}H_{33}N_4O_6$ 409.2451 found 409.2466, CHN Calcd for $C_{20}H_{32}N_4O_5$ C=58.81% H=7 90%, N=13 72%, Found C=58 70% H=7 78% N=13.43%

~~03~~ 486

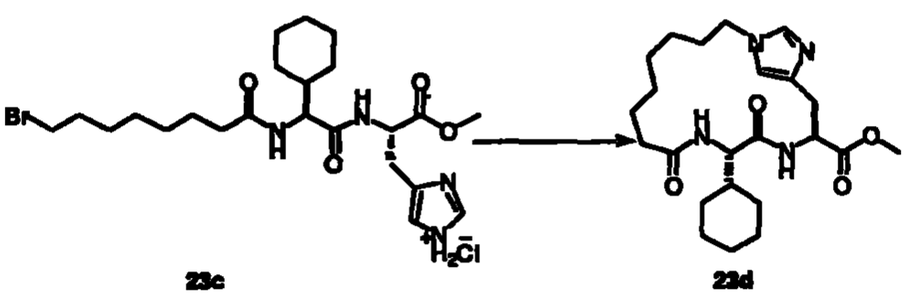
Step B



23b 23c

A solution of ω-bromoheptenoic acid (223 mg, 1 0 mmol) in DMF (3 0 mL) was treated with deprotected amine hydrochloride 23b (380 mg, 1 0 mmol, 1 0 equiv) and Hünigs base (387 mg, 3 0 mmol, 3.0 equiv) was added The reaction mixture was treated with PyBroP (465 mg, 1 0 mmol) and stirred at rt for 3 h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2, 1 19) to yield a colorless solid (220 mg, 50 %) MS (FAB) 515 2 [$(M+1)^+$, 100], 513.2 [$(M+1)^+$, 95)], 469 (60), 433 (20), 170 (40) HRMS calcd for $C_{23}H_{38}BrN_4O_4$. 513.2076 found. 513 2073

Step C:



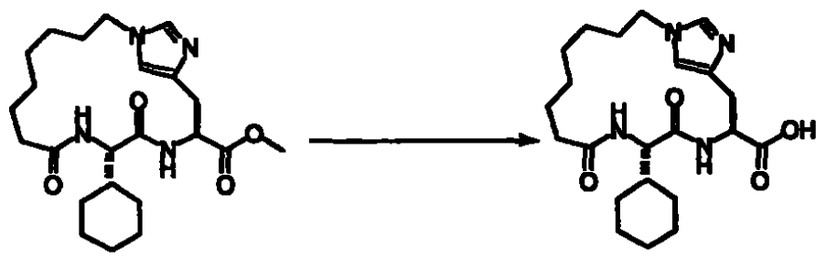
23c 23d

A solution of bromo-compound 23c (100 mg, 0 23 mmol), in 2-butanone (4 0 mL) was treated with Na_2CO_3 (31 0 mg, 0 29 mmol, 1.25 equiv) and with LiI (50 mg, 0 37 mmol 1.3 equiv) and heated at reflux for 24 h The reaction mixture was concentrated *in vacuo* and the residue was diluted with water The residue was extracted with CH_2Cl_2 (3x30 mL) The combined organic layer was dried(Na_2SO_4) and purified by chromatography (SiO_2, CH_3OH CH_2Cl_2 1 19) to yield the cyclized compound 23d (25 mg, 31%), R_f 0 68 (2M NH_3 in CH_3OH CH_2Cl_2.

494
487

1 19) ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm) 8 17 (d, 1 H, J=8 8 Hz) 7.33 (s, 1 H), 6 48 (d, 1 H, J=8.4 Hz), 4 90-4 85 (m, 1H), 4.26 (t, 1 H, J=8 0 Hz), 3 82-3 74 (m, 2 H), 3 69 (s, 3H), 3 16- 3 11 (m, 2 H) 2.91-2.84 (m, 1 H), 2.30-2.01 (m, 2 H), 1 65-1.59 (m, 11 H), 1 18-0 96 (m, 11H) ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 172.8, 172.4, 171 9, 138 2, 136 8, 57 6, 52 5, 51 7, 46 6, 41 6, 36 0, 30 9, 29 5, 28 8, 27 3, 26 7, 26.4, 26 3, 26 2, 25.2, 24.8 MS. (Electron spray, *m/z relative intensity*) 433.1 [$(M+1)^+$,100], HRMS. Calcd for $C_{23}H_{37}N_4O_4$ 433 2815 found 433 2822

Step D

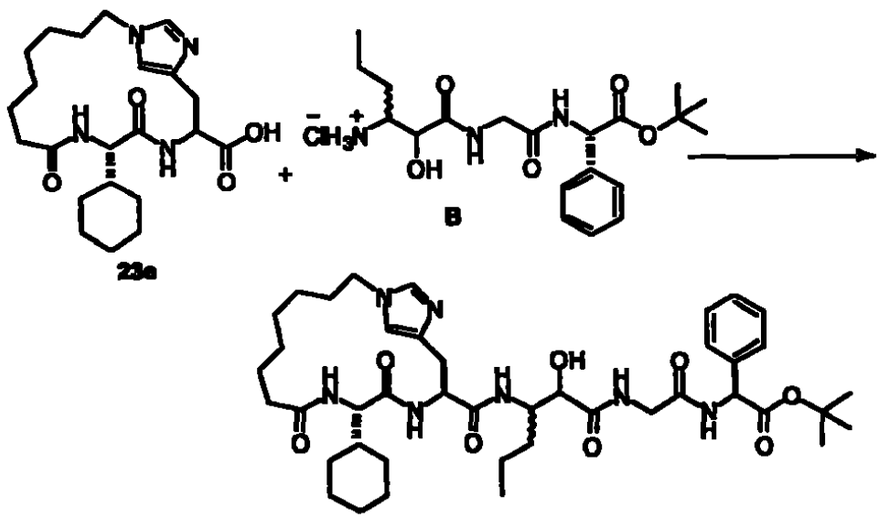


23d 23e

A solution of methyl ester **23d** (200 mg, 0 46 mmol) in CH_3OH (5 0 mL) H_2O (0.5 mL) was treated with $LiOH{\bullet}H_2O$ (30 mg, 0 75 mmol, 1 6 equiv) The reaction mixture was stirred at rt for 15 h and concentrated *in vacuo* and dried in pump to yield hydrolyzed compound **23e** which was used for coupling directly

0495
488

Step E

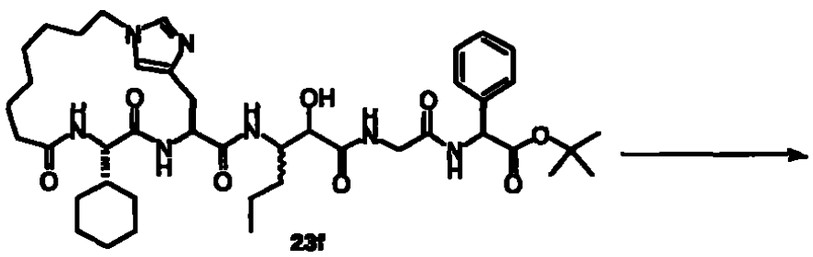


23f

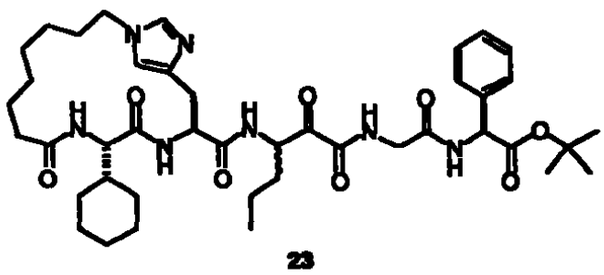
A solution of the acid **23e** in CH_2Cl_2 (3 0 mL), DMF (5 0 mL) was treated with HOOBt (115 mg, 0 70 mmol, 1.50 equiv) and EDCI (113 mg, 0 60 mmol, 1.25 equiv) The reaction mixture was then treated with Et_3N (190 mg, 1.88 mmol, 271μL, 4.0 equiv) and amine hydrochloride B (201 mg, 0.5 mmol, 1 1 equiv) The reaction mixture was stirred at rt for 13 h and diluted with H_2O The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and the combined organic layers were extracted with aq NaOH (1M, 50 mL) and dried (Na_2SO_4) The dried organic layer was filtered and concentrated *in vacuo* to yield a colorless residue **23f** (442 mg) which was dried *in vacuo* and directly used for further oxidation MS (Electron spray, *m/z relative intensity*) 794 [$(M+1)^+$, 100]

496
0496
489

Step F



23f

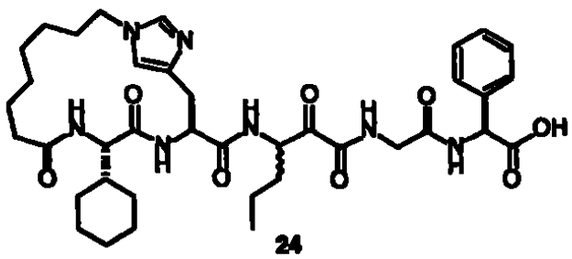


23

A solution of hydroxy-amide 23f (50 mg, 0 064 mmol) in CH_2Cl_2 (3 0 mL) was treated with Dess Martin reagent (53 mg, 0 13 mmol, 2.0 equiv) and stirred at rt for 3 h The reaction mixture was diluted with aq satd $Na_2S_2O_3$ (20 mL) and stirred at rt for 15 min The aqueous layer was extracted with CH_2Cl_2 (3x30 mL) The organic layer was dried (Na_2SO_4) filtered concentrated *in vacuo* and purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2, 1 15) to yield ketoamide 23 (20mg, 40%), MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*) 824 [$(M+CH_3OH)^+$, 100], 792 [$(M+1)^+$, 60], 447 (20), HRMS calcd for $C_{42}H_{62}N_7O_8$ $(M+1)^+$ 792.4660 found 792.4659

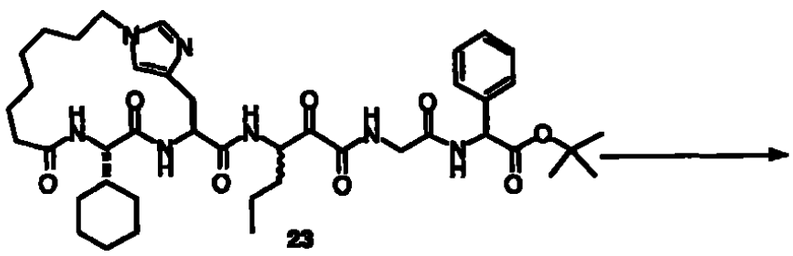
490

Example 24. Preparation of Compound of Formula 24.

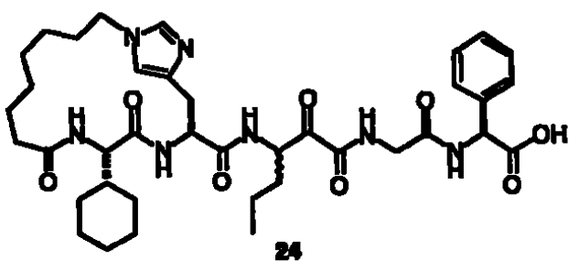


24

Step A.



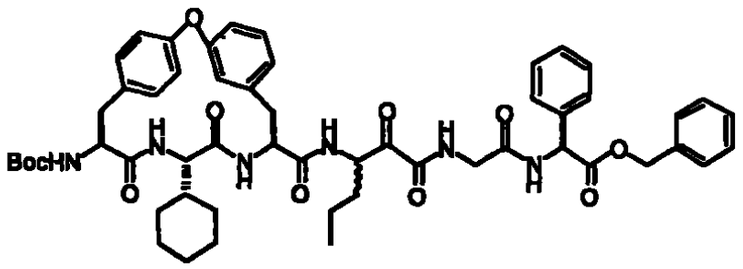
23



24

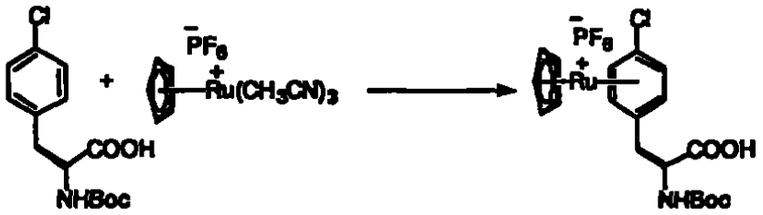
A solution of *tert*-butyl ester 23 (17 mg, 21.5 μmol) in dry CH_2Cl_2 (2 0 mL) was treated with TFA (2.0 mL) and stirred at rt. for 8 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2, 1 19) The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in CH_3OH/heptanes/CH_2Cl_2 and concentrated *in vacuo* several times to yield 24 as fine colorless solid (7 mg) MS (Electron spray, *m/z relative intensity*) 768 [$(M+CH_3OH)^+$, 100], 736 [$(M+1)^+$, 60], 48 (10)

Example 25. Preparation of Compound of Formula 25:



25

Step A

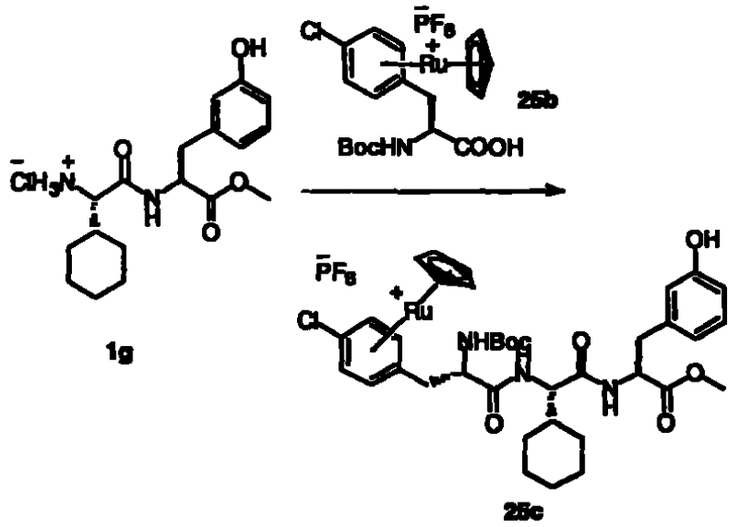


25a 1b 25b

A solution of Boc-4-Chlorophenylalanine 25a (523 mg, 1 75 mmol) in dichloroethane (37 mL) was treated with $CpRu(CH_3CN)_3$ PF_6 1b (760 mg, 1 75 mmol, 1 0 equiv) and heated at reflux for 2 h The reaction mixture was cooled to 0 °C and filtered The filtrate was concentrated *in vacuo* and dissolved in minimum CH_3CN and treated with a large excess of Et_2O The solid separating out was separated and dissolved in CH_2Cl_2/CH_3OH (1 1, 50 mL) and concentrated *in vacuo* to obtain 25b as a brown foam (640 mg, 69%)

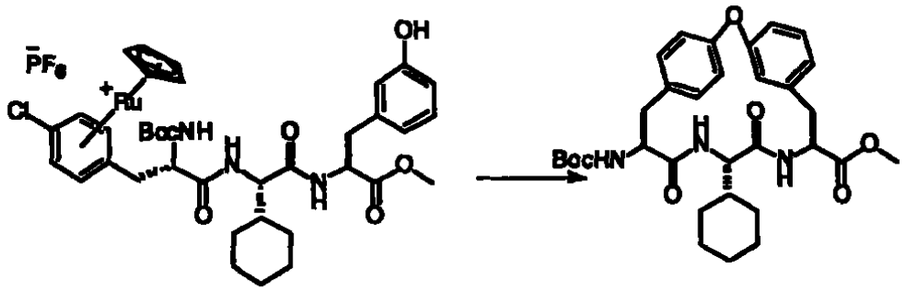
492 499

Step B



A solution of carboxylic acid **25b** (2 4 g, 3 80 mmol) in dry DMF (15 mL) was treated with Hünigs base (1 64 g, 12 64 mmol, 4 0 equiv, 2.9 mL) and HOBt (661 mg, 4.38 mmol, 1.5 equiv) The reaction mixture was cooled to 0° C and the treated with EDCI (699 mg, 3.95 mmol, 1 25 equiv) and stirred for 15 mın To this reaction mixture was added amine hydrochloride **1g** (1 50 g, 4.00 mmol, 1 2 equiv) and the reaction mixture was stirred at rt for 12 h The DMF was distilled out and the residue was diluted with water (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq $NaHCO_3$ (30 mL), aq HCl (30 mL), brine, dried (Na_2SO_4) filtered concentrated *in vacuo* and the crude product **25c** (2.5 g, 69%) was used for further cyclization without purification

Step C

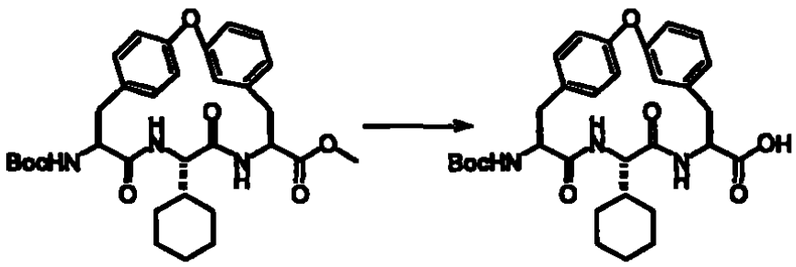


25c 25d

A solution of compound 25c (100 mg 0 11 mmol) in dry DMF (10 mL) was degassed with dry N_2 and treated with Cs_2CO_3 (170 mg, 0 5 mmol, 5 0 equiv) and stirred at rt for 12 h The solvent DMF was distilled off and the residue was diluted with water (35 mL) and extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were dried (Na_2SO_4) filtered, concentrated *in vacuo* and dried in vacuum overnight. It was used for photolytic removal of Ru without further purification

The cyclized compound from the previous step was dissolved in CH_3CN and photolysed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 2 1) to yield 25d as a tan colored solid (29 mg, 46 %) MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*), 580 [(M+1)$^+$, 80], 524 (100), 418 (40), 462 (30), 452 (20), 313 (60), 253 (20)

Step D



25d 25e

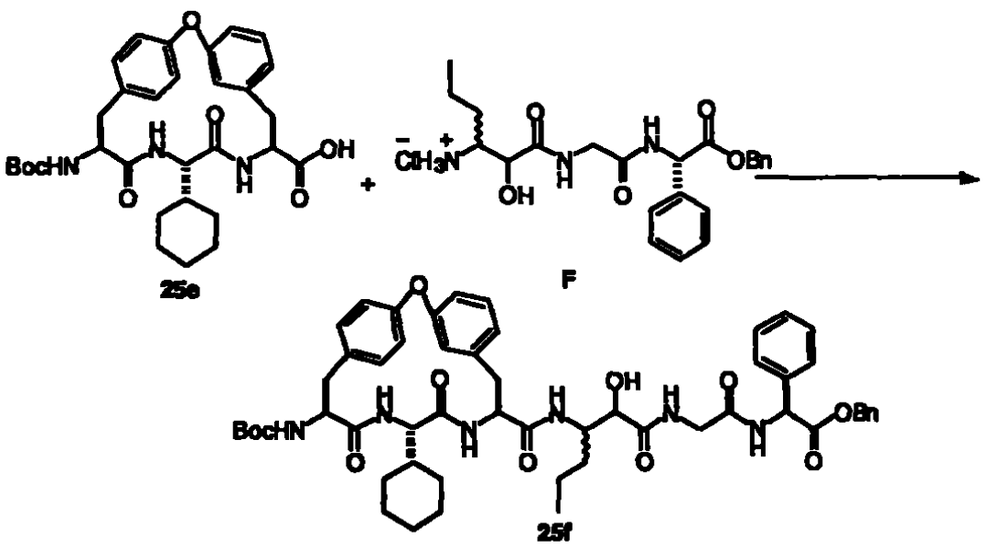
A solution of ester 25d (150 mg, 0 26 mmol) in THF (3 mL), CH_3OH (3 0 mL) and H_2O (3 0 mL) was treated with $LiOH \cdot H_2O$ (18 mg, 0.43 mmol, 1 65

507
494

equiv) and stirred at rt for 35 min The reaction mixture was acidified with conc HCl (13 M, 1mL) and extracted in CH_2Cl_2 (3x50 mL) The combined organic layers were dried (Na_2SO_4) filtered and concentrated *in vacuo* to yield acid 25e which was used directly for the coupling without further purification

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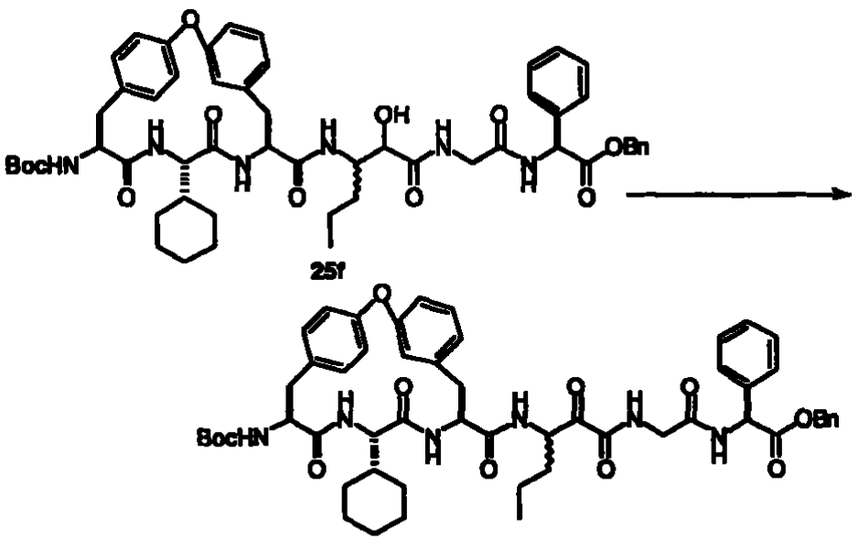
Step E



A solution of acid 25e (150 mg, 0.27 mmol) in dry CH_2Cl_2 (2 0 mL), was treated with HOBt (62 mg, 0 40 mmol) and Hünigs base (139 mg, 1 1 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (53 mg, 0 34 mmol, 1.25 equiv) and stirred for 30 min The reaction mixture was treated with amine F (88 mg, 0.29 mmol, 1 22 equiv) and stored in the freezer for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (50 mL) The aqueous layer were extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq HCl (1M, 3x20 mL) aq NaOH (1M, 3x20 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 25f (138 mg) which was used for oxidation

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496

Step F



25f

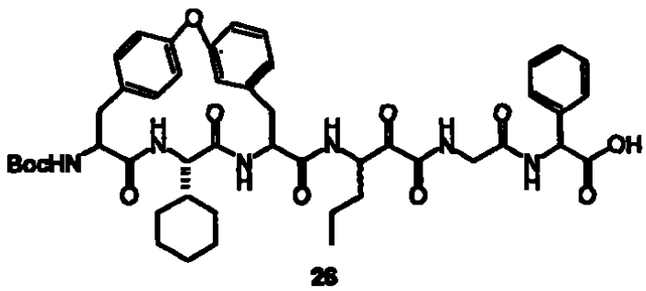
25

A solution of alcohol 25f (140 mg, 0 143 mmol) In CH_2Cl_2 THF (1 1, 5.0 mL) was treated with Dess-Martin reagent (121 mg, 0 42 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 2 h and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1:32) to yield oxidized product 25 (57mg, 41%) as a colorless solid

00710 "02317 03

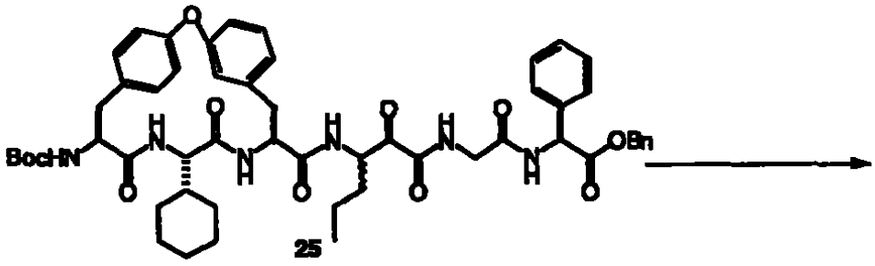
050504
497

Example 26 Preparation of Compound of Formula 26:

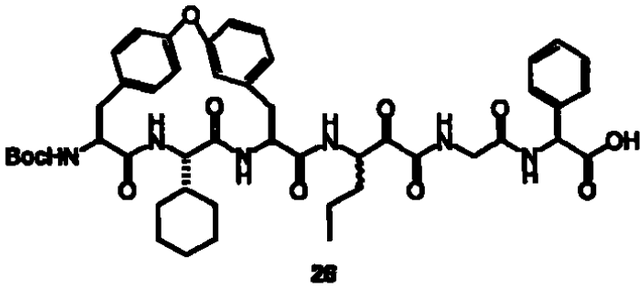


26

Step A.



25

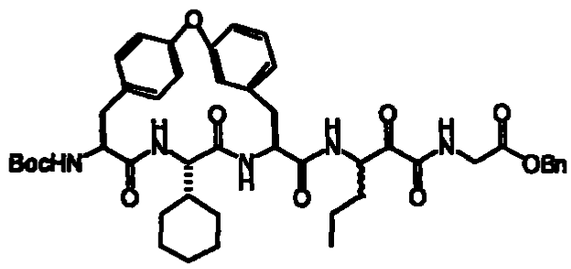


26

A solution of the benzyl ester 25 (30 mg, 38 0 μmol) in CH_3OH/THF (1 1, 4 0 mL) was treated with Pd/C (20 mg, 10%) and H_2 was bubbled through it. A drop of acetic acid was added to accelerate the reduction The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo*. The residue 26 was analyzed without further purification

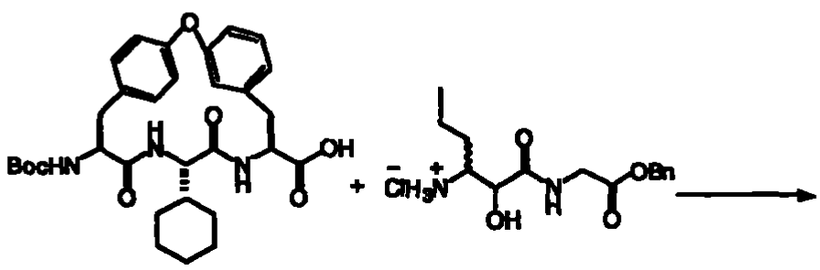
5-05
0505 498

Example 27. Preparation of Compound of Formula 27.



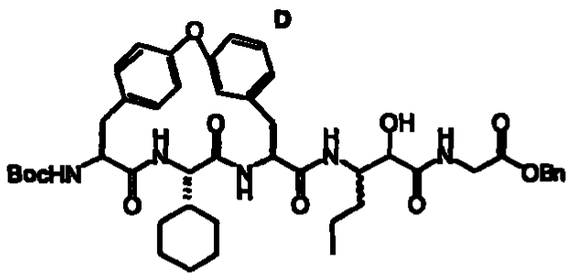
27

Step A



25e

D

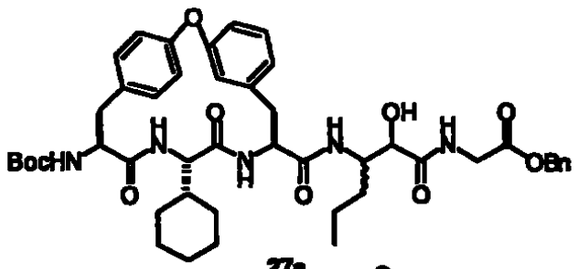


27a

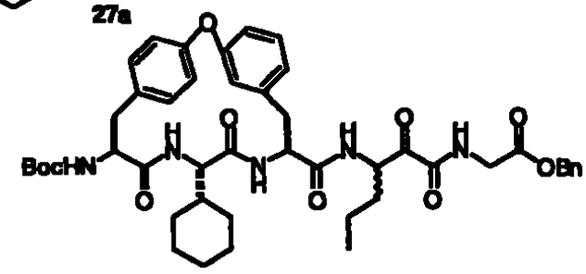
A solution of acid 25e(100 mg, 0 17 mmol) in dry CH_2Cl_2 was treated with HOOBt (41 mg, 0 26 mmol) and Hünigs base (91 mg, 0 70 mmol, 4.0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (35 mg, 0.22 mmol, 1.25 equiv) and stirred for 30 min The reaction mixture was treated with amine D (71 mg, 0 22 mmol, 1.22 equiv) and stored in the freezer for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layers was extracted with CH_2Cl_2 (3x30 mL) The organic layers were extracted with aq HCl (1M, 30 mL) aq Na_2CO_3 (1M, 30 mL), dried (Na_2SO_4) filtered

concentrated *in vacuo* to obtain **27a** colorless solid (119 mg) which was used for oxidation MS (FAB), 842 [(M+1), 100], 765 (20), 735 (10), 657 (20), 575 (10), 492 (10), 464 (20), 446 (30) HRMS calcd for $C_{48}H_{60}N_5O_{10}$ $(M+1)^+$ 842.4339, found 842 4336

Step B



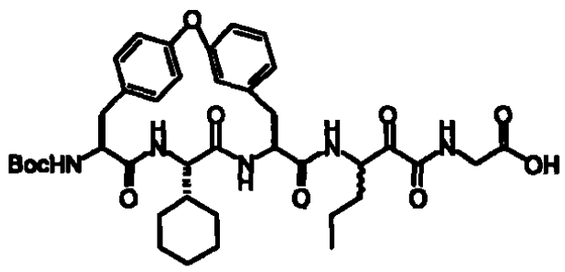
27a



27

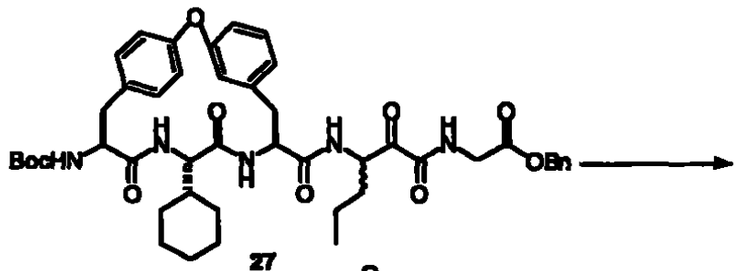
A solution of alcohol **27a** (120 mg, 0 143 mmol) in CH_2Cl_2 THF (1 1, 3 0 mL) was treated with Dess-Martin reagent (180 mg, 0.42 mmol, 3.0 equiv) The reaction mixture was stirred at rt. for 2 h and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1.32) to yield oxidized product **27** as a colorless solid MS (FAB, NBA-G/TG-DMSO, *m/z rel int*), 840 [$(M+1)^+$, 50] HRMS calcd for $C_{48}H_{58}N_5O_{10}$ $(M+1)^+$ 840.4184, found 840 4199

Example 28. Preparation of Compound of Formula 28.

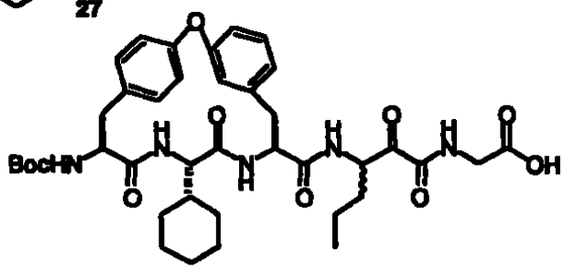


28

Step A



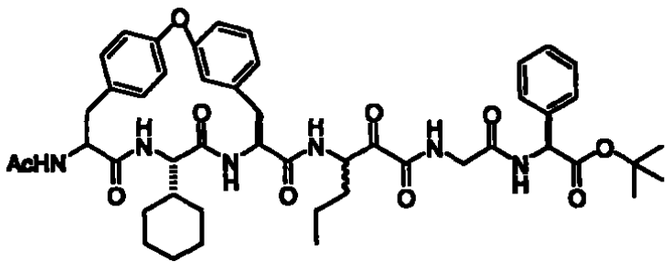
27



28

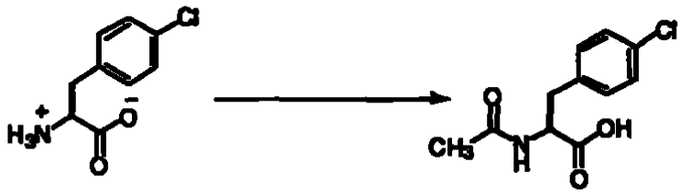
A solution of benzyl ester 27 (40 mg, 47 0 μmol) in CH_3OH/THF (1 1, 6 0 mL) was treated with Pd/C (30 mg, 10%) and H_2 was bubbled through it. A drop of acetic acid was added to accelerate the reduction The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo* to yield 28

Example 29 Preparation of Compound of Formula 29.



29

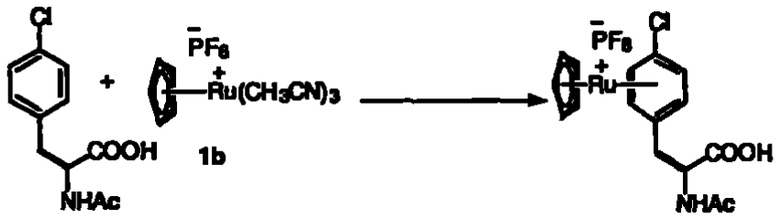
Step A.



29a 29b

A solution of 4-chlorophenylalanine 29a (1.5g, 7.5 mmol) in THF (20 mL) and H_2O (20 mL) was treated with NaOH (900 mg, 22.5 mmol, 3 0 equiv) and cooled to 0°C, A solution of acetyl chloride (707 mg, 9 00 mmol, 1.25 mmol) in THF (10 mL) was added dropwise and the reaction mixture was stirred overnight at rt. The reaction mixture was acidified with aq HCl (1M, 10 mL) and extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were dried (Na_2SO_4) filtered concentrated *in vacuo* to yield 29b which was used in the next step without purification

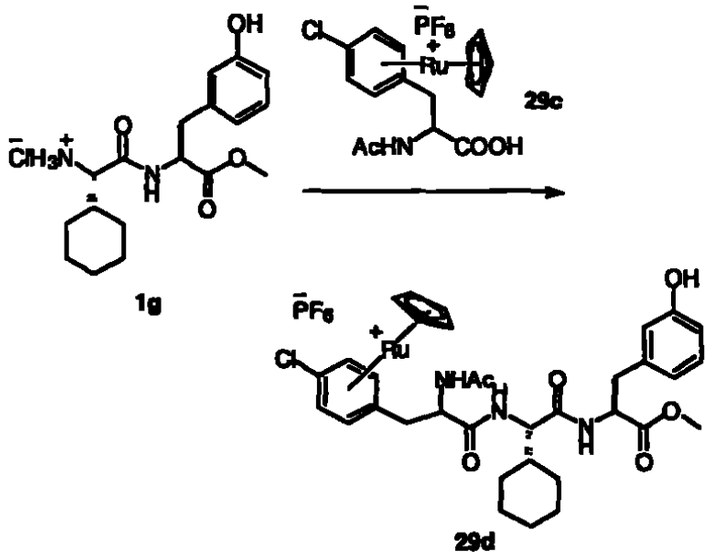
Step B



A solution of N-acetyl-4-chlorophenylalanine **29b** (1 39 g, 5 75 mmol) in dichloroethane (118 mL) was treated with $CpRu(CH_3CN)_3$ PF_6 **1b** (2 5 g, 5 8 mmol, 1 0 equiv) and heated at reflux for 2 h The reaction mixture was cooled to 0 °C and filtered The filtrate was concentrated *in vacuo* and dissolved in CH_3CN (15 mL) and treated with Et_2O (150 mL) The gum separating out was separated by decanting the ether and the residue was dissolved in CH_2Cl_2/CH_3OH (1 1, 50 mL) and concentrated *in vacuo* to obtain **29c** as a brown foam (2.2 g, 69%) MS. (Electron spray, *m/z rel int*) 408 $[(M-PF_6)^+, 100]$

0510 510 503

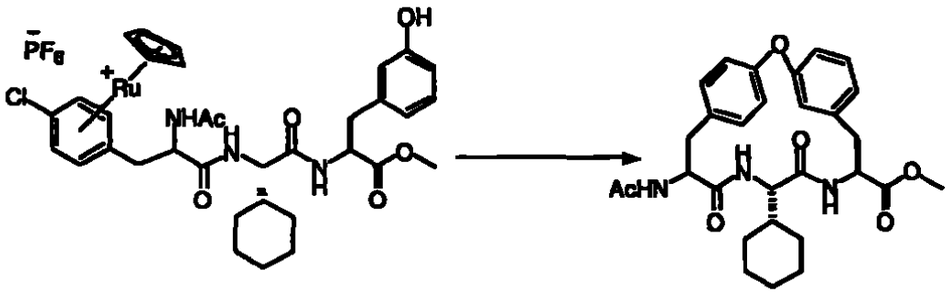
Step C



29d

A solution of carboxylic acid **29c** (2 0 g, 4 00 mmol) in dry DMF (20 mL) was treated with Hünigs base (2 06 g, 16 0 mmol, 4 0 equiv , 2.9 mL) and HOBt (810 mg, 6 0 mmol, 1.5 equiv) The reaction mixture was cooled to 0°C and then treated with EDCI (888 mg, 5 0 mmol, 1 25 equiv) and stirred for 0 5 h To this reaction mixture was added amine hydrochloride **1g** (1 48 g, 7 14 mmol, 1 2 equiv) and the reaction mixture was stirred at rt for 12 h The DMF was distilled out and the residue was diluted with water and the aqueous layer was extracted with CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq $NaHCO_3$ (200 mL), aq HCl (100 mL), brine, dried (Na_2SO_4) filtered concentrated *in vacuo* and the crude product **29d** (1 2 g, 38%) was used for cyclization without further purification

Step D



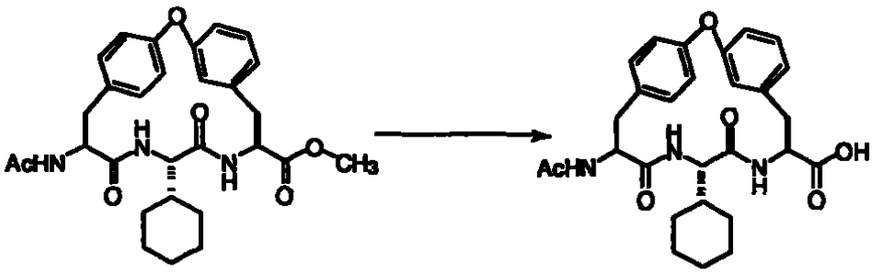
29d 29e

A solution of chloro-compound **29d** (1 2 g 1 5 mmol) in dry DMF (120 mL) was degassed with dry N_2, treated with Cs_2CO_3 (2.4 g, 7 4 mmol, 5 0 equiv) and stirred at rt for 23 h The solvent DMF was distilled off and the residue was diluted with water (300 mL) and extracted with propionitrile (3x100 mL) The combined organic layers were dried (Na_2SO_4) filtered, concentrated *in vacuo* and dried in vacuum overnight. It was used for photolytic removal of Ru without further purification

The cyclized compound from the previous step was dissolved in CH_3CN (40 mL) and photolyzed in a Raynot (λ=350 nm) for 48h The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 4 1) to yield a tan colored solid **29e** (240mg, 38 %) MS (FAB, NBA-G/TG-DMSO, *m/z relative intensity*), 522[$(M+1)^+$, 100].

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0512

Step E

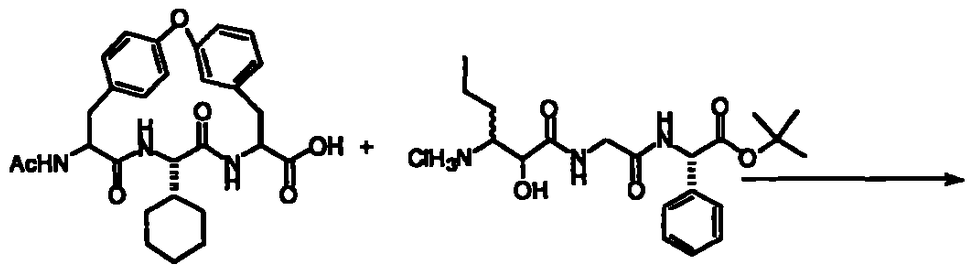


29e **29f**

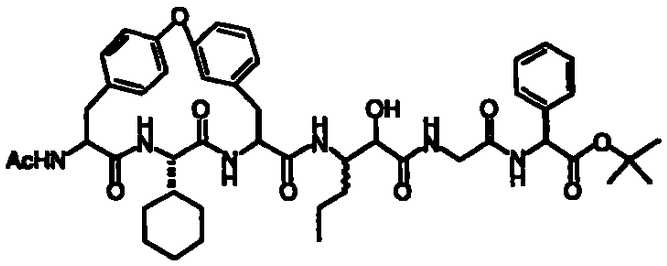
A solution of ester **29e** (200 mg, 0 42 mmol) in CH_3OH (5 mL), CH_2Cl_2 (13 mL) and H_2O (2 0 mL) was treated with $LiOH\ H_2O$ (41 mg, 1 0 mmol, 2.4 equiv) and stirred at rt for 3 h The reaction mixture was acidified with aq HCl (13 M, 1mL) and extracted in CH_2Cl_2 (3x50 mL) and EtOAc (3x50 mL) The combined organic layers were dried (Na_2SO_4) filtered and concentrated *in vacuo* to yield acid **29f** (178 mg) which was used directly for the coupling without further purification

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Step F



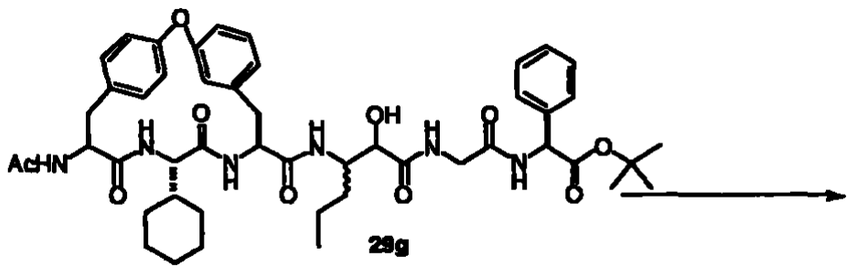
29f B



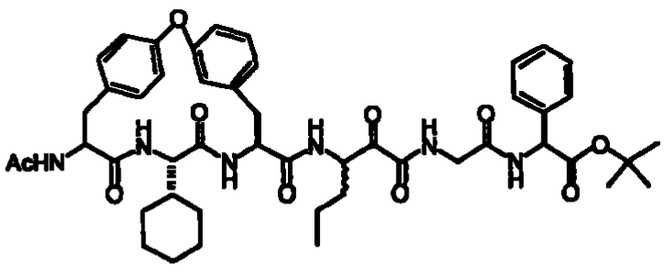
29g

A solution of acid 29f (90 mg, 0 18 mmol) in dry DMF (1 0 mL) was treated with HOBt (45 mg, 0.33 mmol, 1 6 equiv), Hünigs base (142 mg, 1 1 mmol, 5 0 equiv) and amine B (118 mg 0 28 mmol, 1 47 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (63 mg, 0 33 mmol, 1 6 equiv) and stirred at 0 °C for 20 min for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The combined aqueous layers were extracted with CH_2Cl_2 (3x30 mL) and EtOAc (3x30 mL) The organic layers were extracted with aq NaOH (2M, 30 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 29g (50 mg, 32%) which was used for oxidation MS (Electron spray, *m/z rel int*) 883 [$(M+1)^+$, 100], 522 (30), 394 (60)

Step G



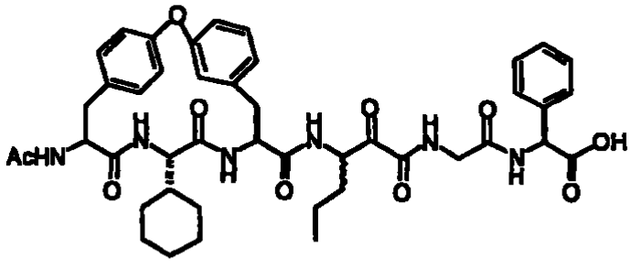
29g



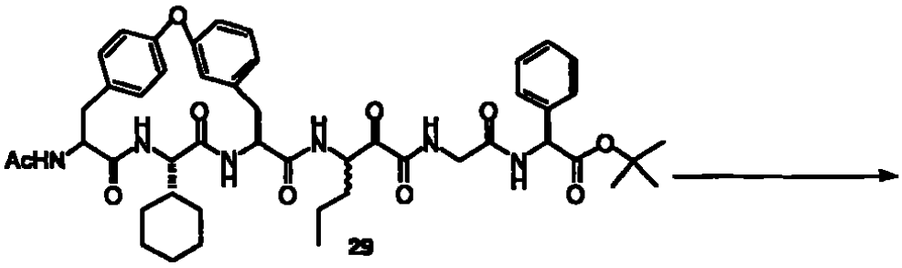
29

A suspension of alcohol **29g** (50 mg, 60 0 μmol) in CH_2Cl_2 (2 0 mL) was treated with Dess-Martin reagent (40 mg, 0 94 mmol, 2.0 equiv) The reaction mixture was stirred at rt for 3h and the mixture was concentrated *in vacuo* The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 32) to yield oxidized product **29** (41 mg, 80%) as a colorless solid MS (FAB, *m/z*, *rel int*) 881 [$(M+1)^+$, 100), 825 (170), 248 (100)

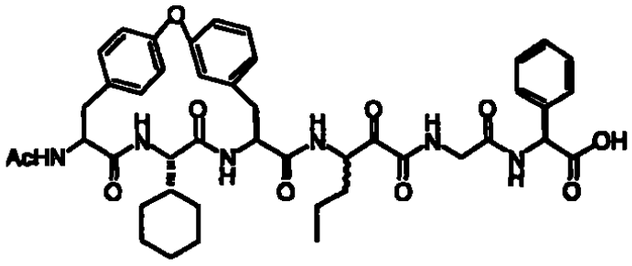
Example 30. Preparation of Compound of Formula 30.



30



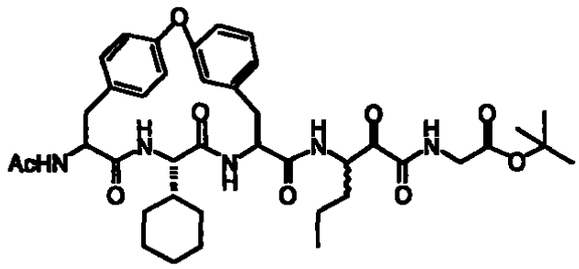
29



30

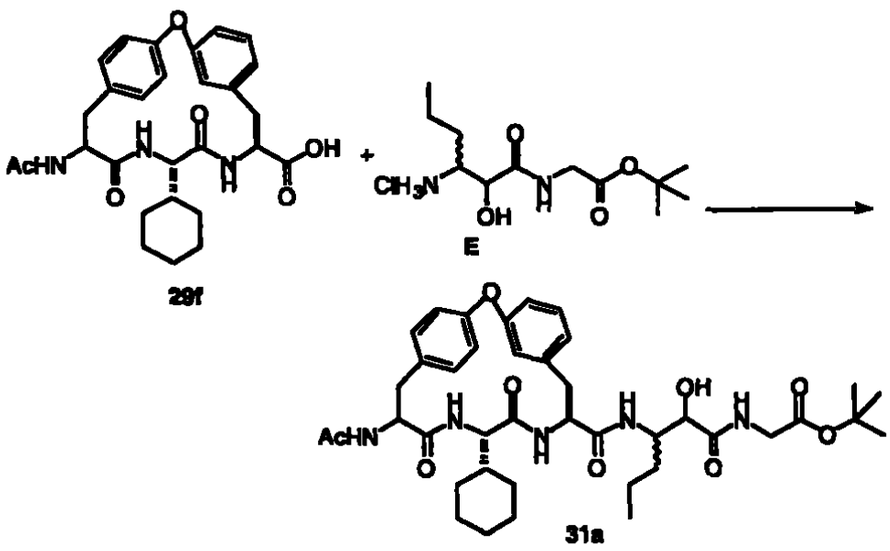
A solution of *tert*-butyl ester **29** (23 0 mg, 26 0 μmol) was treated with TFA/CH_2Cl_2 (1 1, 2 0 mL) and stirred at rt for 4 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1.24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes/CH_2Cl_2 (4 0 mL) and concentrated to yield a tan solid **30** (13 0 mg, 100%) MS (Electron spray, *m/z rel int*) 825 [$(M+1)^+$, 100)

Example 31 Preparation of Compound of Formula 31



31

Step A



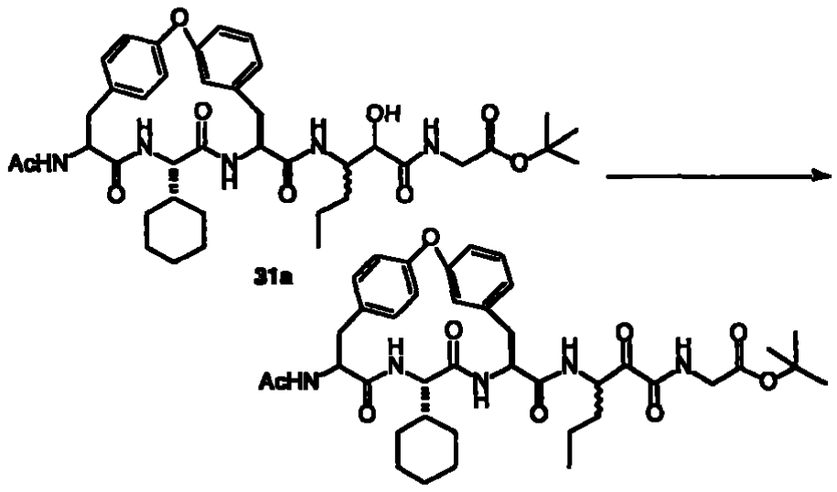
29f E

31a

A solution of acid 29f (150 mg, 0 29 mmol) in dry DMF (4 0 mL), CH_2Cl_2 (3 0 mL) was treated with HOBt (58 mg, 0 44 mmol) and Hünigs base (149 mg, 1 1 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (82 mg 0 44 mmol, 1 5 equiv) and stirred for 30 min The reaction mixture was treated with amine E (88 mg, 0 29 mmol, 1 22 equiv) and stirred at rt for 12 h The reaction mixture was concentrated *in vacuo* and diluted with H_2O (30 mL) The aqueous layer was extracted with CH_2Cl_2 (3x30 mL) The combined organic layers were extracted with aq HCl (1M, 3x20 mL), aq NaOH (1M, 3x20 mL), dried (Na_2SO_4) filtered concentrated *in vacuo* to obtain a colorless solid 31a (56

mg) which was used for oxidation MS (Electron spray, *m/z rel int*) 750 [$(M+1)^+$, 20], 663 (10), 522 (10), 416 (20), 247 (30)

Step B

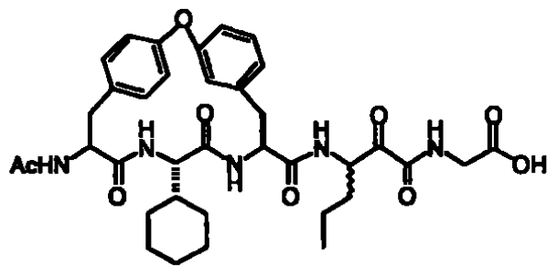


31

A solution of alcohol **31a** (56 mg 75 μmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (93 mg, 0 22 mmol, 3 0 equiv) The reaction mixture was stirred at rt for 4 h and the mixture was concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, CH_3OH/CH_2Cl_2 1 19) to yield oxidized product **31** (34 mg 60 %) as a colorless solid MS (Electron spray, *m/z rel int*) 748 [$(M+1)^+$, 35], 692 (5), 279 (100)

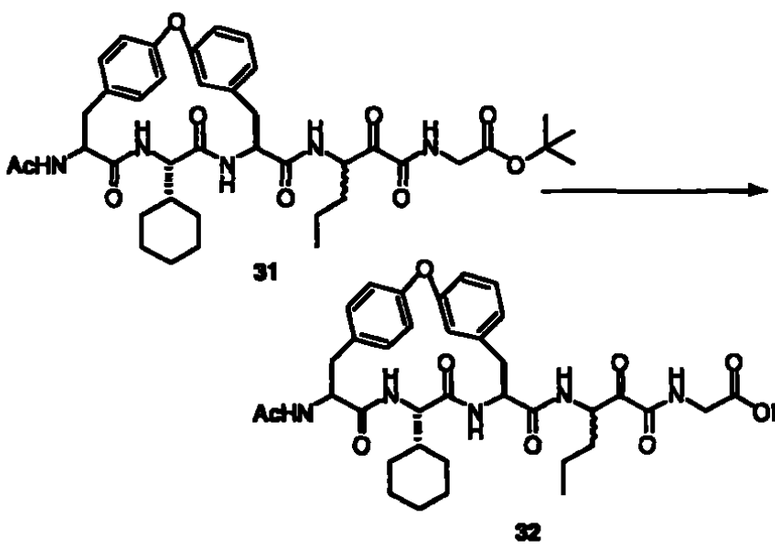
DOCID: WO2001029069

Example 32. Preparation of Compound of Formula 32



32

Step A

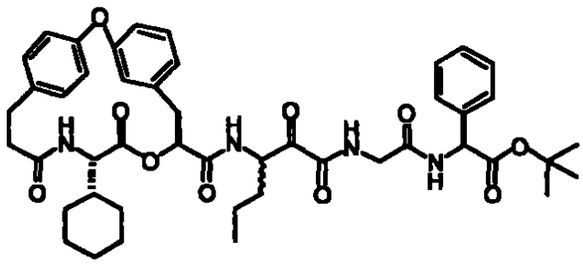


31

32

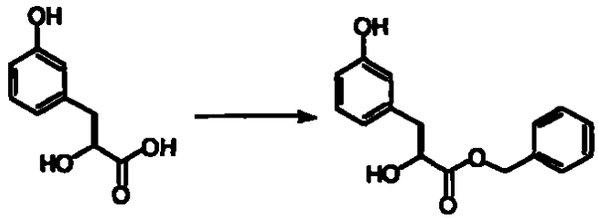
A solution of *tert*-butyl ester 31 was treated with TFA/CH_2Cl_2 (1 1, 4 0 mL) and stirred at rt. for 4 h The disappearance of the ester to the base line was followed by TLC (CH_3OH/CH_2Cl_2 1 24) After the deprotection was complete the reaction mixture was concentrated *in vacuo* and the residue was repeatedly treated with heptanes/CH_2Cl_2 (4 0 mL) and concentrated to yield 32 as a tan solid

Example 33 Preparation of Compound of Formula 33.



33

Step A.



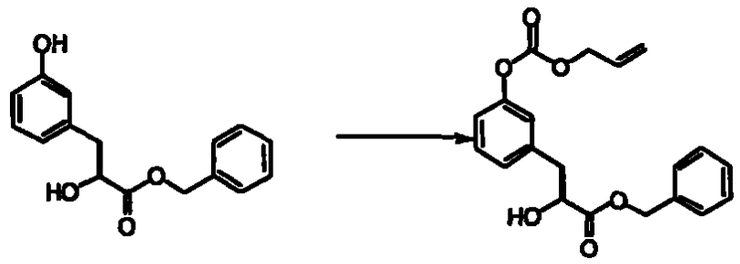
33a 33b

A solution of acid **33a** (4 5g, 25 0 mmol) in dioxane (30 mL) and benzene (80 mL) was treated with BnOH (8 0 g, 74 mmol, 3 0 equiv) and TsOH H_2O (713 mg, 3 75 mmol, 10 mol%) The reaction mixture was heated at reflux for 5 h, when the water was separated using a Dean-Stark apparatus The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 3 7) to yield benzyl ester **33b** as a colorless oil (4 2 g, 62%), R_f 0 22 (EtOAc/Hexanes 3 7), ^{13}C NMR (CH_3OD, 75 MHz, δ) 175 1, 158 2, 139 7, 130 3, 129.5, 129 3, 121 7, 117 4, 114 6, 73 1, 67 6, 41 6, MS (FAB, G/TG-DMSO, *m/z, relative intensity*) 351 ([M+DMSO] , 70), 273 ($[M+1]^+$, 100), 255 (20), 227 (30), 181 (40), HRMS Calcd for $C_{16}H_{17}O_4$ $(M+1)^+$ 272 1049, found 272 1054

0520

513

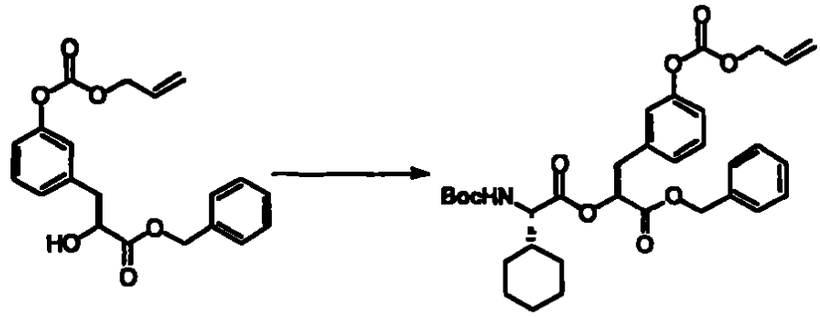
Step B



33b **33c**

A solution of benzyl ester **33b** (3 8 g, 12 9 mmol) in CH_2Cl_2 (100 mL) was treated with Et_3N (1 55 g, 15.4 mmol, 2 2 mL, 1 1 equiv), cooled to -78°C (2-PrOH, dry Ice) and a solution of allyl chloroformate (1 84 g, 15.36 mmol, 1 1 equiv) in CH_2Cl_2 (10 mL) was added dropwise The reaction mixture was allowed to warm up to the rt. and diluted with aq HCl (1M, 100 mL) The reaction mixture was extracted with EtOAc (3x100 mL) The combined organic layer was washed with aq HCl (100 ml, 1M), brine (100 mL), dried ($MgSO_4$) concentrated *in vacuo* to yield **33c** which was used in the next step without further purification R_f 0 43 (EtOAc/Hex 7 13), ^{13}C NMR (CH_3OD, 75 MHz, δ) 174 8, 162 5, 155 0, 152 5, 140 3, 137 1, 132 8, 130 3, 129 6, 129.5, 129 4, 123 2 120 3, 119 4, 72 7, 70 1, 67 7, 41 2, 29 9

Step C

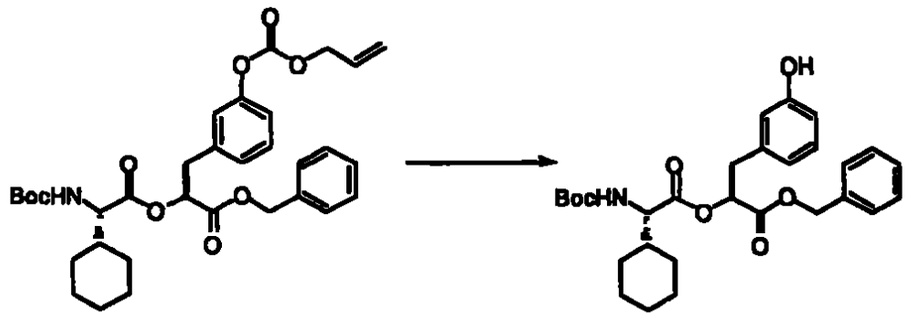


33c **33d**

A solution of Boc-cyclohexylglycine monohydrate 1d (6 02 g, 23 4 mmol, 2 0 equiv) was dissolved in CH_2Cl_2 and dried ($MgSO_4$) The mixture was filtered

and the residue was further azeotropically dried with toluene The residue was dissolved in CH_2Cl_2, and treated with HOBt (4 73 g, 35 1 mmol, 2 9 equiv) EDCI (6 7 g 35 1 mmol, 2 9 equiv) and Hünigs base (8 31 g, 64 3 mmol, 11 mL) It was stirred at rt. for 30 min and the alloc protected alcohol 33c (4 3 g, 12 04 mmol) was added The reaction mixture was stirred at rt for 36 h and diluted with aq HCl (1M, 100 mL) and extracted with EtOAc (3x100 mL) The combined organic layers were extracted with aq NaOH (1M, 100 mL), brine (100 mL), dried, concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hex 1 4) to yield depsipeptide 33d (7 1g 100%) R_f 0 18 (EtOAc/Hex 1 4), HRMS Calcd for $C_{28}H_{34}O_7$ $(M\text{-}Boc)^+$ 496 2335 found 496 2333

Step D

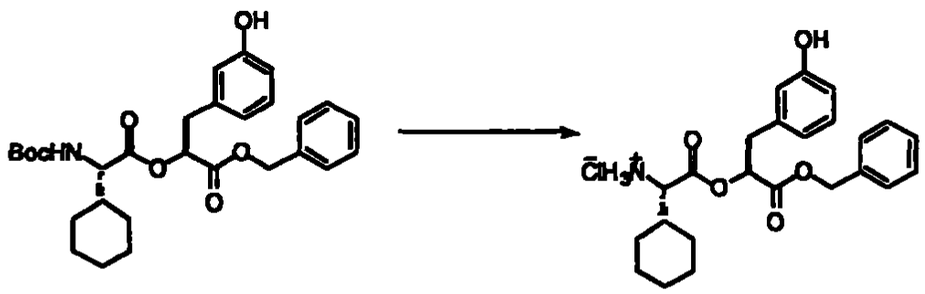


33d 33e

A solution of alloc-protected depsipeptide 33d (7 8 g, 13 0 mmol) in dry THF (200 mL) was treated under N_2 with dimedione (3 27 g, 23 4 mmol, 2 0 equiv) and $Pd(Ph_3P)_4$ (780 mg, 0 67 mmol, 5 mol%) The reaction mixture was stirred at rt for 1h and the disappearance of reactant was followed by TLC (EtOAc/Hex 1 4) The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 4) to yield phenol 33e (5 2g, 78%) as a colorless foam R_f 0 52 (EtOAc/Hexanes 3 7), ^1H NMR (d_4-CD_3OD, 300 MHz, δ) 7 4-7 19 (m 5 H), 7 15-6 99 (m, 1 H) 6 68-6 55 (m,4 H) 5 43-5 01 (m,3 H), 4 6 (bs, 2H), 4 11-4 00 (m, 1 H), 3 18-2 91 (m, 2 H), 1 80-1 55

(bs, 6H) 1 39 (s, 9 H) 1 21-0 89 (m, 6 H), ^{13}C NMR (CH_3OD, 75 MHz, δ, mixture of diastereomers) 171 6, 169 4, 169 3, 161 1, 157 1, 157 0, 137 2, 136 9, 135 4, 135 3, 129 2, 129 1, 128 2, 128 2, 128 0 120 3, 120 1, 116 0, 115 9, 113 6, 94 8 79 3, 73 6, 73 5, 66 7, 66 6, 58 6, 58 5, 40 0, 39 9, 36 8, 29 1, 27 7, 27 3, 25 5 MS (Electron spray, *m/z, relative intensity*) 1023 ($[2M+1]^+$ 20), 512 ($[M+1]^+$, 20), 412 ([M-Boc]$^+$, 100), 202 (40) HRMS Calcd for $C_{24}H_{30}NO_5$ $(M\text{-}Boc)^+$ 412 2123 found 412 2119

Step E

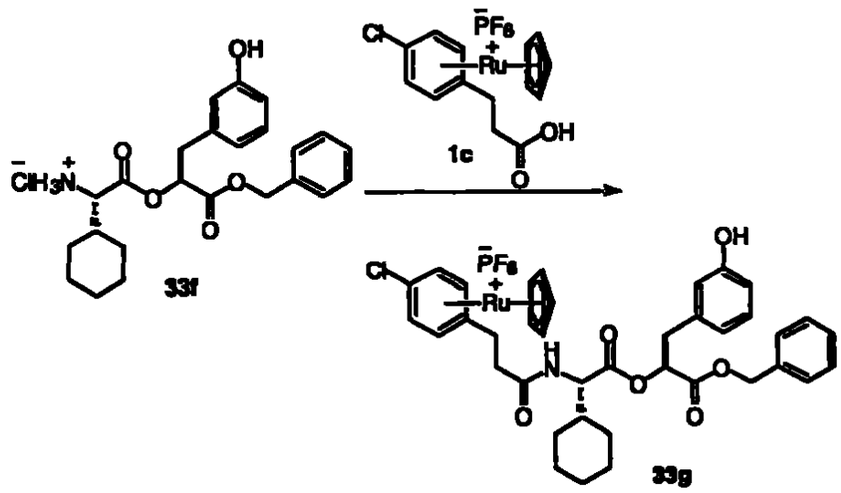


33e 33f

A solution of Boc protected amine 33e (5 2 g, 10 7 mmol) was stirred with HCl (4M, dioxane, 200 mL, 800 mmol, 80 equiv) until the starting material disappeared to the base line as indicated by TLC (EtOAc/Hex 3 7) The reaction mixture was concentrated *in vacuo* and dried in high vacuum and the residue 33f was directly used in the next step ^1H NMR (d_4-CD_3OD, 300 MHz, δ) 7.40-3 23 (m, 5 H), 7 07 (q, 1 H, J=13 Hz) 6 77-6 6 (m, 3 H), 5 33-5 41 (m, 1 H), 5 3-5 05 (2 AB, 2 H) 3 99-3 85 (m, 1 H) 3 35-22 (m, 2 H) 2 00-1 5 (m, 5 H), 1.50-0 80 (m, 6 H), MS (FAB, G/TG-DMSO, *m/z, relative intensity*) 412 ($[M+1]^+$, 100), HRMS Calcd for $C_{24}H_{30}NO_5$, M^+ 412 2123 found 412 2139

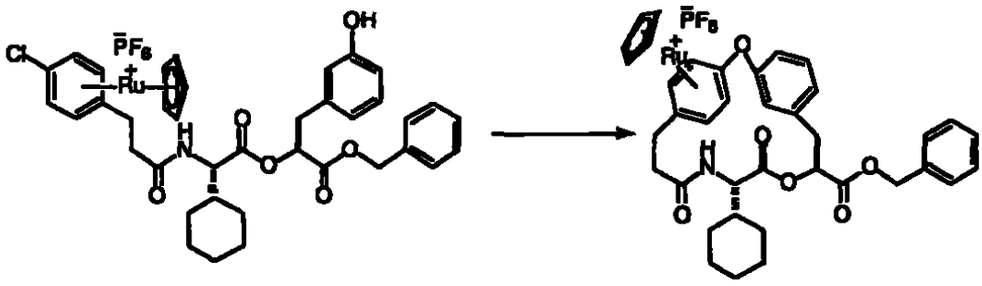
0523
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Step F



A solution of [CpRu(η^6- 4-chlorophenylpropionic acid)]PF_6 1c (2 0 g, 4 03 mmol) in dry DMF (20 mL) was treated with HOBt (835 mg 6 0 mmol, 1.5 equiv) and Hünigs base (2 06 g, 2 95 mL, 16 mmol, 4 0 equiv) The reaction mixture was cooled to 0° C and treated with EDCI (1 15 g, 6 0 mmol, 1 5 equiv) The reaction mixture was stirred at 0°C for 30 min and the amine hydrochloride was added 33f (1 8 g, 4 03 mmol, 1 0 equiv) In dry DMF (10 mL) The reaction mixture was stirred at rt for 12 h and the DMF was distilled out vacuo The residue was diluted with aq HCl (1M, 100 mL) and extracted into CH_2Cl_2 (3x100 mL) The combined organic layers were extracted with aq $NaHCO_3$ (3x50 mL) , brine (100 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* to yield a brown solid **33g** (3 5 g) which was used for cyclization, MS Electron spray, m/z, relative intensity) 743 [$(M-PF_6)^+$, 100], 304 (60), HRMS Calcd for $C_{38}H_{41}NO_8Cl^{102}Ru$ $(M-PF_6)^+$ 744 1666 found 744 1694

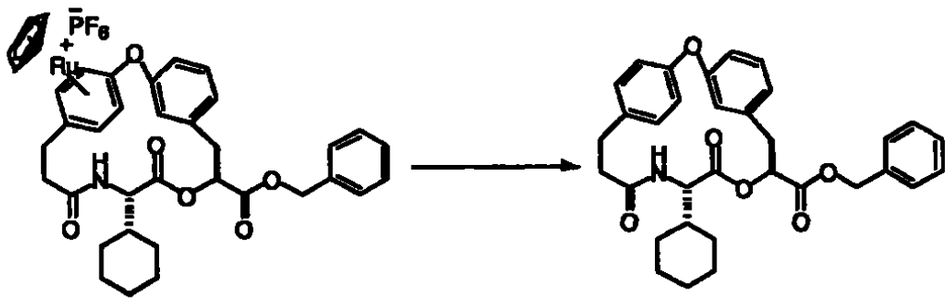
Step G



33g 33h

A solution of η^6–ruthenium complex **33g** (3 5 g, 3 93 mmol) in dry DMF (300 mL) was degassed with dry N_2 and treated with Cs_2CO_3 (6 5 g, 19 95 mmol, 5 0 equiv) and stirred at rt. for 16 h The reaction mixture was concentrated *in vacuo* to remove the DMF and the residue was diluted with H_2O (100 mL) The reaction mixture was extracted with CH_2Cl_2 (3x100 mL) The combined CH_2Cl_2 layers were extracted with brine, dried (Na_2SO_4), filtered, concentrated *in vacuo* to yield **33h** which was directly used for photolysis, MS (Electron spray, m/z, relative intensity) 708 [$(M-PF_6)^+$, 100], HRMS Calcd for $C_{38}H_{40}NO_6O_2Ru$ $(M-PF_6)^+$ 708 1892 , found 708 1918

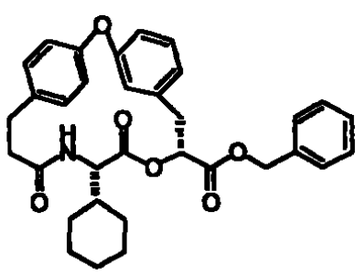
Step H



33h

33I

+

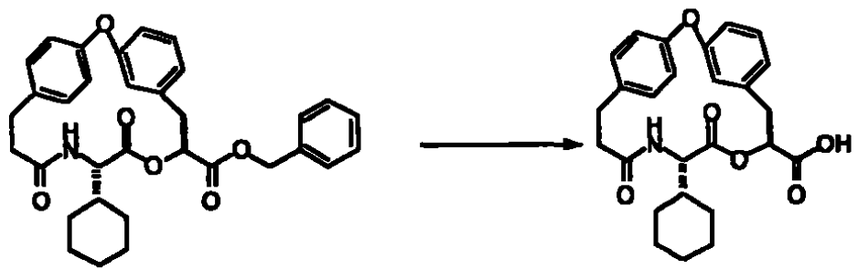


33J

A solution of cyclized ruthenium complex **33h** (3 5g, 3 9 mmol) in CH_3CN (60 mL) was degassed and photolyzed in a quartz tube at λ=350 nm in two batches for 48 h each The reaction mixture were pooled together and purified by chromatography (SiO_2, CH_2Cl_2/Et_2O 9/1) to yield cyclic depsipeptide as a mixture of diastereomers. (700 mg, 34%) The diastereomers were separated by additional chromatography (Hexanes/CH_2Cl_2/ Et_2O 6 3 1) to yield the two diastereomers **33I** (370 mg, 18%) and **33J** (216 mg 11%) as colorless solid R_f 0.28 (Hexanes EtOAc 3 2) , $[\alpha]_D$= 25 (*c* 0 15, $CHCl_3$, 20 °C) IR (neat, cm^{-1}) 3329 (w), 2960 (m) 2926 (s), 2854 (s), 1745 (s), 1680 (m), 1589 (m), 1506 (m), 1446 (m), 1385 (w), 1259 (s) 1099 (m), 1030 (s), 800 (s), 752 (m), 698 (w) 619 (w) ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 7 36-7 23 (m, 5 H), 7 18- 6 99 (m, 4 H), 6 81 (d, 1 H, *J*=7 5 Hz), 6 74 (dd, 1 H, *J*=2 7, 5 7 Hz), 6 30 (s, 1 H), 5 75 (d, 1 H, *J*=7 2 Hz), 5 61 (dd, 1 H, *J*=2 4 Hz, 5 4 Hz), 5 18, 5 14 (AB, 2 H, *J*=12 3 Hz), 4.23 (dd, 1 H, *J*=4 2 Hz, 3.3 Hz), 3 26-3 01 (m, 2 H), 2 98-2 85 (m, 2 H), 2.68-2 64 (m, 1H)

2 38-2 34 (m, 1H),1 96-1 51 (m, 6 H), 1 51-0 96 (M, 5H) ^{13}C NMR ($CDCl_3$, 75 MHz, δ, ppm) 177 3, 171 1, 168 7, 159 8, 155.3, 138 6, 135 4, 134.9, 131 2, 129 7, 129 2, 128 7, 126 6, 126 1, 123.3, 120 8, 120 8, 117 5, 114 2, 71 8, 57 5, 56 9, 41 5, 39 0, 35 7, 32 6, 31 3, 29 0, 27 6, 26 0, 25 9 FAB (NBA/DMSO, *m/e, relative intensity*) 542 [(M+1)$^+$ 100], 514 (15), 450 (5), 307 (8), 232 (5), 154 1 (17), 136 (14) HRMS Calcd for $C_{33}H_{36}NO_6$ (M+1)$^+$ 542 2543 found 542.2541 CHN Calcd for $C_{33}H_{35}NO_6.0\ 5H_2O$, C 71 98% H 6 59% N 2 54%, Found C 72 56% H 7 05% N 2 63%

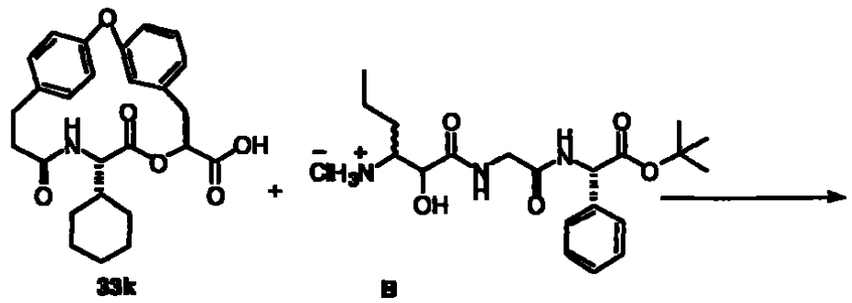
Step I



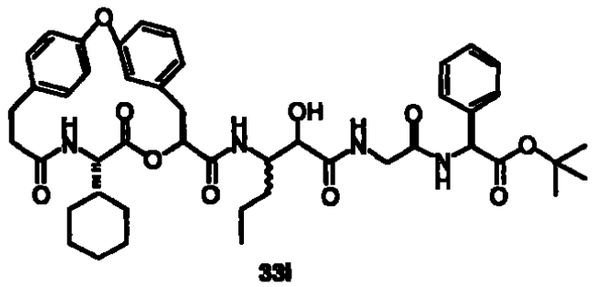
33l 33k

A solution of benzyl ester 33l (360 mg, 0 66 mmol) in CH_3OH/EtOAc (1 1, 50 mL), was treated with $Pd(OH)_2$ and hydrogenated (50 psi) for 12 h The reaction mixture was filtered through a plug of celite and the cake was rinsed with CH_3OH/CH_2Cl_2 (1 1, 50 mL) The filtrate was concentrated *in vacuo* and the residue 33k (330 mg) was used for coupling without purification R_f 0 58 (CH_3OH/CH_2Cl_2 1 19) MS (Electron spray, m/z, relative intensity) 827.2 [(M+1)$^+$, 100], 694 (20), 539 (40), 466 (10), 174 (70) HRMS Calcd for $C_{46}H_{58}N_4O_{10}$ (M+1)$^+$ 827.4231 found 827 4215

Step J



33k B

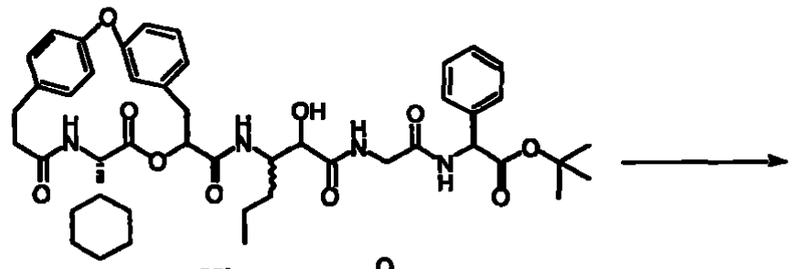


33l

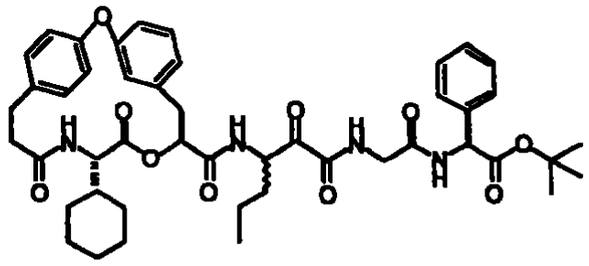
A solution of acid 33k (165 mg, 0 31 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5 0 mL) was treated with HOBt (83 mg, 0.46 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (159 mg, 1.23 mmol, 4 0 equiv , 229 µL) was added To this mixture was added EDCI (89 mg, 0 47 mmol 1 5 equiv) and the reaction mixture was stirred at 0° C for 1 h and treated with the amine hydrochloride B (159 mg, 0 372 mmol, 1 2 equiv) The reaction mixture was stirred at rt. for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with water and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer were extracted with aq HCl (1M, 3x50 mL), aq NaOH (1M, 3x50 mL) brine (100 mL) and concentrated *in vacuo* The residue 33l was oxidized without further purification

0328 521

Step K



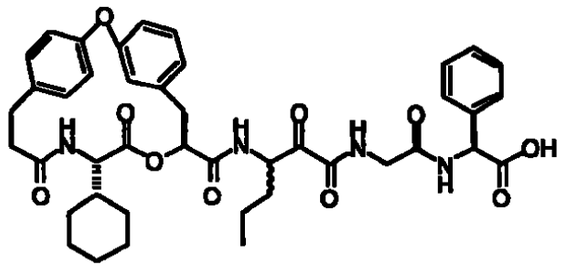
33I



33

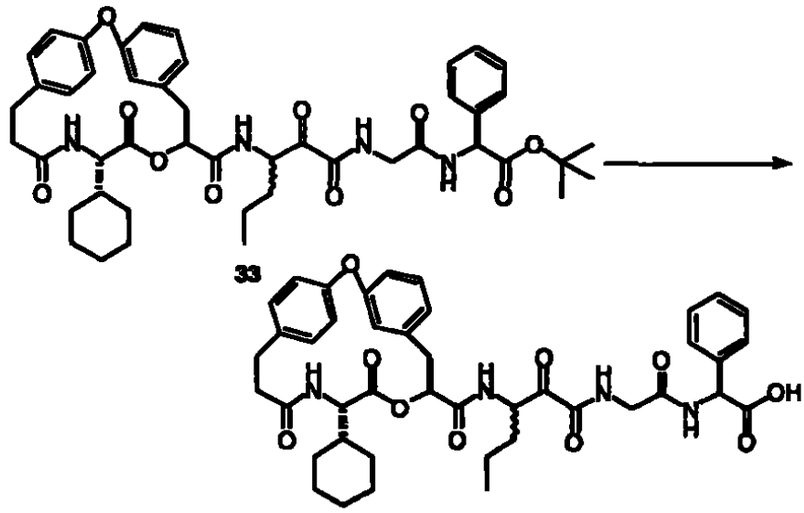
A solution of alcohol 33I (330 mg, 0 4 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (424 mg, 1 00 mmol, 2.5 equiv) The reaction mixture was stirred at rt. for 1 h and diluted with aq $NaHCO_3$ (50 mL) and aq $Na_2S_2O_3$ (50 mL) The reaction mixture was stirred at rt for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with brine (50 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield ketoamide 33 (180 mg, 55%) of a colorless solid R_f 0 63 (CH_3OH/CH_2Cl_2 1 19), MS (Electron spray, *m/z relative intensity*) 857 2 ($[M+CH_3OH]^+$, 40), 825 2 ($[M+1]^+$ 100)

Example 34 Preparation of Compound of Formula 34



34

Step A

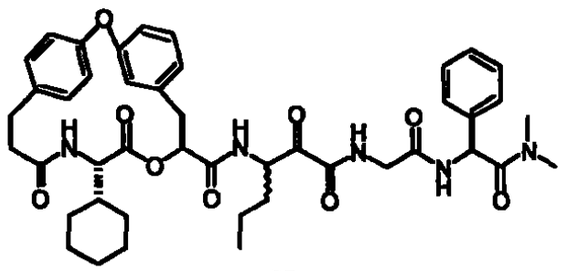


33

34

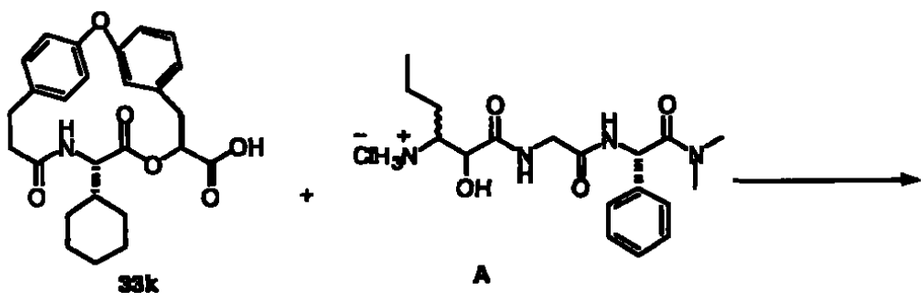
A solution of oxidized depsipeptide 33 (160 mg, 0 2 mmol) in dry CH_2Cl_2 (5 0 mL) was treated with TFA (5 0 mL) and stirred at rt. for 7 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in CH_3OH/CH_2Cl_2/Hexanes (1 1 1) and concentrated *in vacuo* several times to yield a tan colored solid 34 (133 mg, 86%) which was dried *in vacuo* MS (Electron spray *m/z relative intensity*) 769 2 $[(M+1)^+$,100], 481 (5), 269 (25) 191 (90)

Example 35 Preparation of Compound of Formula 35.



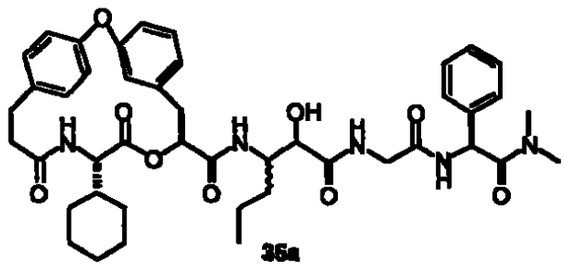
35

Step A



33k

A

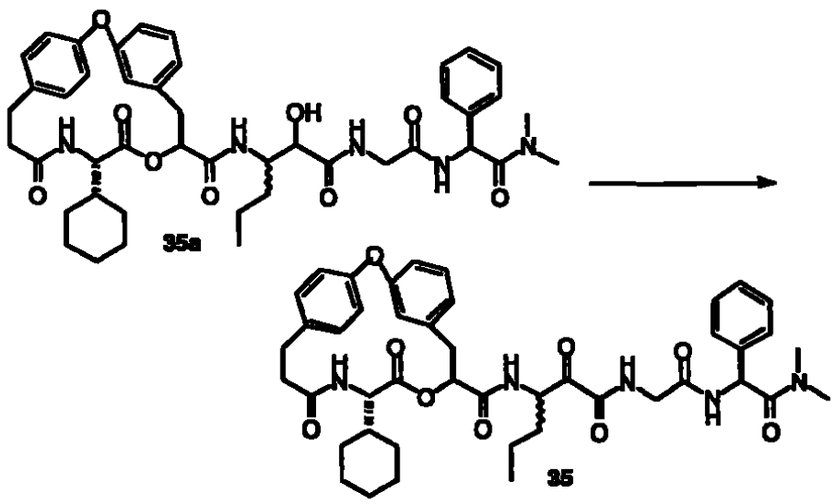


35a

A solution of acid 33k (165 mg, 0.31 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5 0 mL) was treated with HOBt (83 mg, 0 46 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (159 mg, 1 23 mmol, 4 0 equiv , 229 µL) was added To this mixture was added EDCI (89 mg, 0 47 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 1 h and treated with the amine hydrochlonde A (159 mg, 0 372 mmol, 1 2 equiv) The reaction mixture was stirred at rt for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The

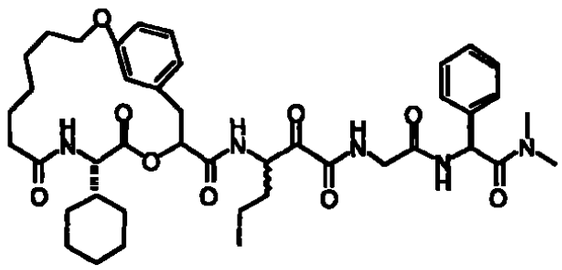
residue was diluted with water and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq HCl (1M, 3x50 mL), aq NaOH (1M, 3x50 mL), brine (100 mL) and concentrated *in vacuo*. The residue 35a was oxidized without further purification MS (Electron spray, *m/z relative intensity*) 798 2 [(M+1)$^+$, 30], 479 (10), 391 (20) 180 (100)

Step B



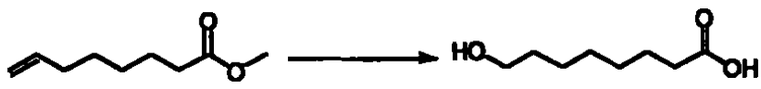
A solution of alcohol **35a** (190 mg, 0 24 mmol) in CH_2Cl_2 (10 mL) was treated with Dess-Martin reagent (423 mg 1 0 mmol, 4.0 equiv) and stirred at rt. for 1h The reaction mixture was diluted with aq $NaHCO_3$ (50 mL) and extracted in CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with aq satd $Na_2S_2O_3$, brine (3x50 mL), dried (Na_2SO_4), filtered concentrated *in vacuo* and purified by chromatography (SiO_2, acetone/Hexanes 3 7->1 1 to yield oxidized product **35** (163 mg, 86%) as a colorless solid MS (Electron spray, *m/z relative intensity*) 796 [(M+1)$^+$, 100], 508 (20), 269 (20)

Example 36. Preparation of Compound of Formula 36.



36

Step A



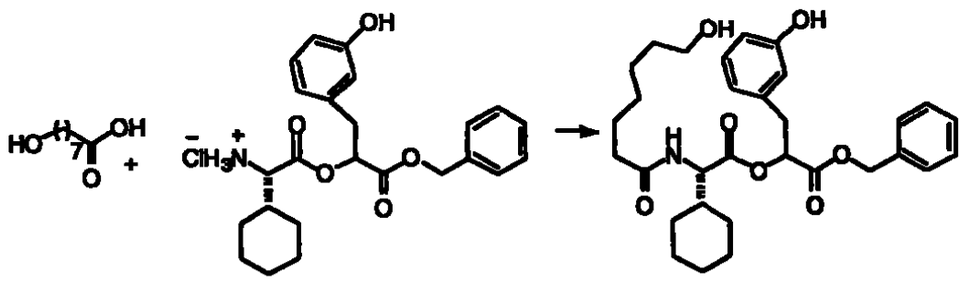
36a 36b

A solution of BH_3 THF (1M in THF, 100 mmol, 100 mL, 3 0 equiv) was added dropwise to a solution of alkene (5 0 g, 35 mmol) in THF (100 mL) at 0° C and stirred for 1h The reaction mixture was treated with ethanol (20 mL) dropwise After the evolution of hydrogen gas was complete the reaction mixture was treated with pH 7 buffer (100 mL) and H_2O_2 (30 volumes, 100 mL) The reaction mixture was stirred at rt. for 4 h and quenched with aq HCl (100 mL) The aqueous layer was extracted with Et_2O (3x100 mL) The combined ether layers were extracted with aq NaOH (1M, 100 m L), brine (100 mL), dried ($MgSO_4$), filtered, concentrated *in vacuo* and purified by chromatography (SiO_2, EtOAc/Hexanes 2/3) to yield alcohol as a colorless liquid (2 9 g, 52%)

A solution of the hydroxylated ester in THF/H_2O/CH_3OH (100 mL, 1 1 1) was treated with LiOH H_2O (2 1g, 51.2 mmol, 3 0 equiv) and stirred at rt. for 3 d The reaction mixture was concentrated *in vacuo* and the aqueous layer was extracted with ether (2x40 mL) The aqueous layer was acidified to pH ~1 and extracted into EtOAc (3x50 mL) The combined organic layers were extracted with

brine (100 mL), dried ($MgSO_4$) filtered concentrated *in vacuo* and the residue 36b was used as is for coupling in Step B ^1H NMR (300 MHz, CD_3OD, δ) 3 53 (t, 2 H, J=6 6 Hz), 2 72 (t, 2 H, J=7 2 Hz), 1 59 (t, 2 H, J=7 5 Hz), 1 5 (t, 2 H, J=7 5 Hz), 1 38-1.33 (m, 6 H)

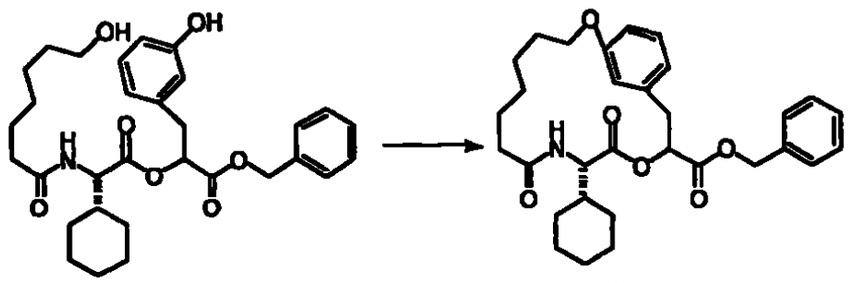
Step B



36b 33f 36c

A solution of ω-hydroxyl heptanoic acid 36b (1 01g, 6 93 mmol) in CH_2Cl_2 (40 mL) was treated with Hünigs base (1 97 g, 15.24 mmol, 2 2 equiv 2 81 mL) and amine hydrochloride 33f (3 1g, 6 93 mmol, 1 0 equiv) The reaction mixture was cooled to 0 °C and treated with PyBrOP (3 22g, 6 93 mmol, 1 0 equiv) The reaction mixture was stirred overnight at rt and the reaction mixture was concentrated *in vacuo* The residue was purified by chromatography (EtOAc/Hexanes 1 1) to yield depsipeptide 36c (2 5 g, 66%) as a colorless viscous oil ^1H NMR (CD_3OD, 400 MHz, δ, ppm) 8 07 (t, 1 H), 7 33-7.21 (m, 4 H), 7 09-7 02 (m, 1 H), 6 67-6 63 (m, 3 H), 5 25-5 08 (m, 1 H), 5 08 (q, 2 H, J=7 5 Hz), 4 36-4 33 (m, 1 H), 3 51 (dd, 2 H, J=5.4 Hz, 0 9 Hz), 3.11-2 96 (m, 2 H), 2 22-2 17 (m, 1 H), 1 99-0 90 (m, 14 H) ^{13}C NMR (CD_3OD, 75 MHz, δ, ppm, mixture of diastereomers) 172 1, 172 0, 171 8, 171 1, 170 9, 169 5, 169 3, 157 1, 157 0, 137 3, 137 0, 135 3, 135 2, 129.2, 129 1, 128 2, 128 0, 127 9, 120 3, 120 0, 116 0, 115 9, 113 6, 94 8, 73 6, 73 4, 66 8, 66 7, 60 2, 57.3, 39 6, 36 7 28 9, 28 0, 25 6, 20 9, 19 5, 13, MS (FAB, NBA DMSO, *m/z rel int*) 562 [$(M+Na)^+$, 20] 540 [$(M+1)^+$ 100), 412 (15), 240 (50), 112 (80)

Step C

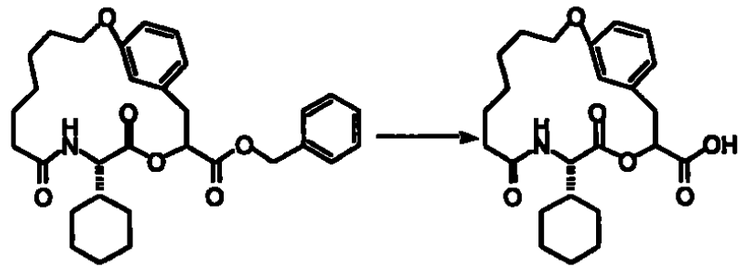


36c 36d

A solution of alcohol **36c** (2 5g, 4 63 mmol) in dry CH_2Cl_2 (50 mL) was treated with triphenylphosphine (2 67 g, 10 2 mmol, 2 2 equiv) under N_2 and cooled to 0°C The reaction mixture was treated with DEAD (1 61g, 9.26 mmol, 2 0 equiv) in CH_2Cl_2 (30 mL) The reaction mixture was warmed to rt and stirred for 2h It was concentrated *in vacuo* and purified by chromatography (Et_2O/Hex 1.3) to yield cyclic product **36d** (530 mg, 21%) as a colorless solid

MS (FAB, NBA, DMSO, *m/z rel int*), 522 [$(M+1)^+$, 100], 494 (60), 268 (20), 222(20), HRMS calcd for $C_{31}H_{40}NO_6$ $(M+1)^+$ 522.2856, Found 522 2864

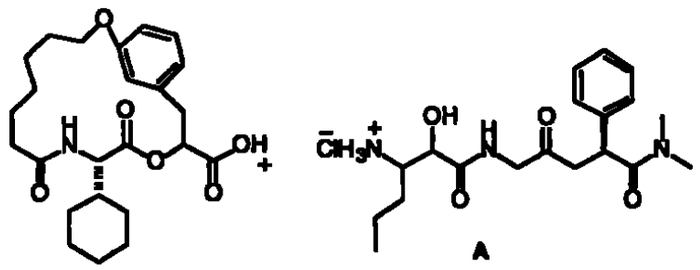
Step D



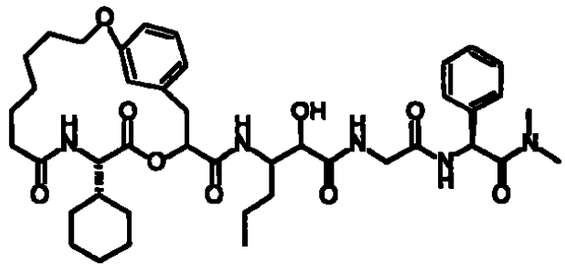
36d 36e

A solution of the benzyl ester (242 mg, 0.47 mmol) in methanol (30 mL) was treated with Pd/C (10 wt %) and hydrogenated on a Parr at 40 psi for 14h The reaction mixture was filtered through a plug of celite and the filtrate was concentrated *in vacuo* to yield a colorless solid **36e** (181 mg, 93%) which was used for coupling

Step E



36e A

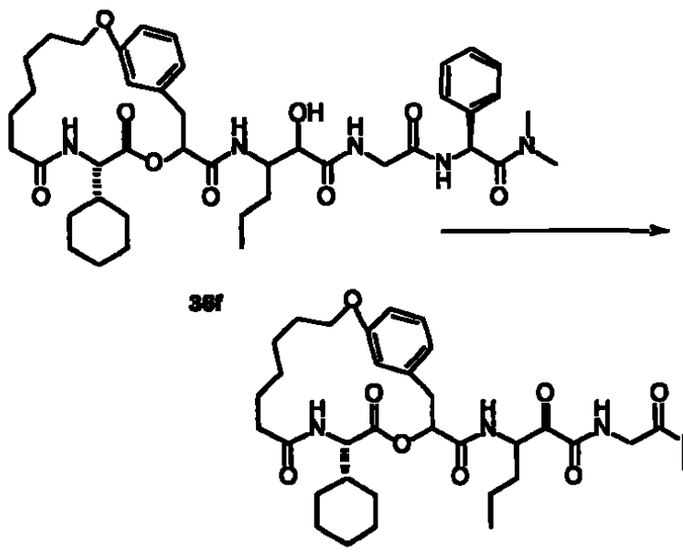


36f

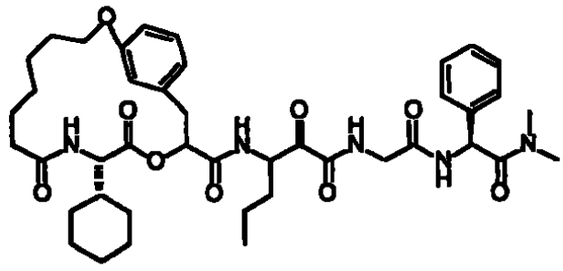
A solution of the hydrolyzed acid **36e** (167 mg, 0 39 mmol) in CH_2Cl_2 (4 0 mL) was treated with HOOBt (95 mg, 0 58 mmol, 1.5 equiv) and cooled to 0 °C and Hünigs base (202 mg, 1 56 mmol, 4 0 equiv 288 µL) was added To this

mixture was added EDCI (111 mg, 0 58 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 0 5 h and treated with the amine hydrochloride (186 mg, 0 47 mmol, 1.20 equiv) The reaction mixture was stored in freezer for 24 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with aq HCl (2M, 30 mL) and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq HCl (2M, 30 mL), aq NaOH (1M) brine (2x50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue **36f** (100 mg) was oxidized without further purification HRMS calcd for $C_{42}H_{60}N_5O_9$ $(M+1)^+$ 778 4391, Found 778.4399

Step F



36f

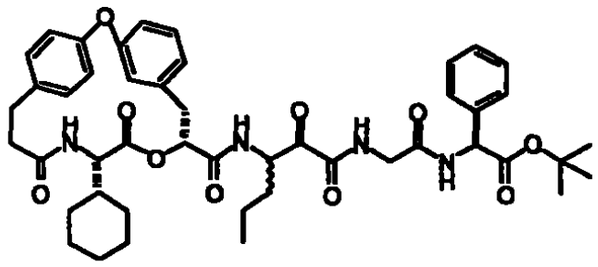


36

A solution of alcohol **36f** (100 mg, 0 13 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (100 mg, 0 0 23 mmol, 1 8 equiv) The reaction mixture was stirred at rt. for 2 h and diluted with aq $NaHCO_3$ (15 mL) and aq $Na_2S_2O_3$ (15 mL) The reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, acetone/Hexanes 3 7) to yield

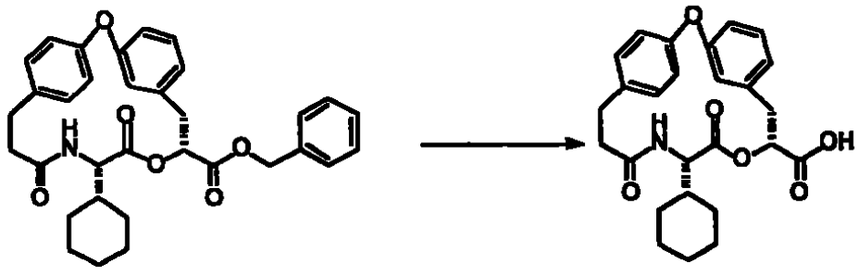
ketoamide 38 (61 mg, 61%) as a colorless solid, MS (FAB, NBA/DMSO, *m/z rel int*) 776 [(M+1)+, 100], 731 (10), 598 (25), 570 (15), 485 (20), 358 (20), 247 (50)

Example 37 Preparation of Compound of Formula 37.



37

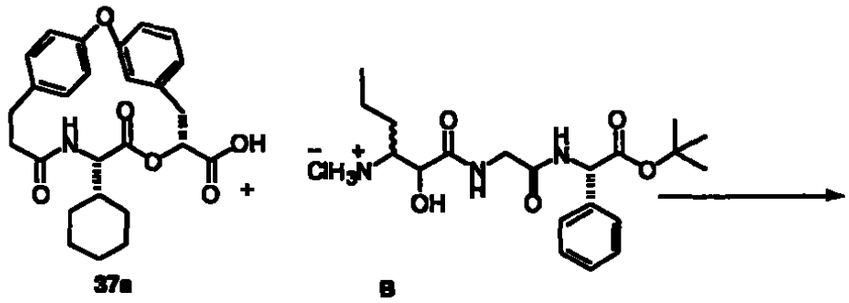
Step A



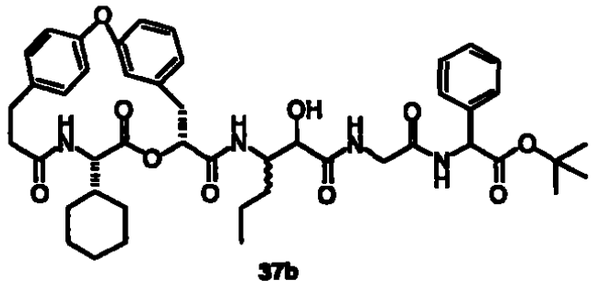
33j 37a

A solution of benzyl ester 33j (230 mg, 0 42 mmol) in CH_3OH/EtOAc (1 1, 50 mL), was treated with $Pd(OH)_2$ and hydrogenated (50 psi) for 12 h The reaction mixture was filtered through a plug of celite and the cake was rinsed with CH_3OH/CH_2Cl_2 (1 1, 50 mL) The reaction mixture was concentrated *in vacuo* and the residue 37a (177 mg, 93%) was used for coupling without purification

Step B



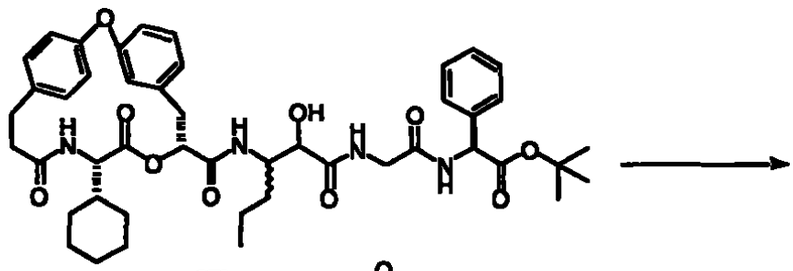
37a B



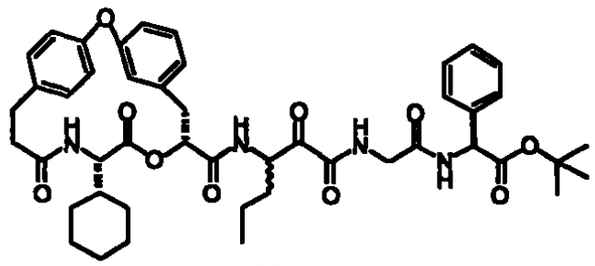
37b

A solution of acid **37a** (177 mg, 0.33 mmol) in dry DMF (5 0 mL) and CH_2Cl_2 (5 0 mL) was treated with HOBt (88 mg, 0 49 mmol, 1 5 equiv) and cooled to 0 °C and Hünigs base (175 mg, 1.35 mmol 4 0 equiv, 251 µL) was added To this mixture was added EDCI (95 mg, 0 49 mmol, 1 5 equiv) and the reaction mixture was stirred at 0° C for 1 h and treated with the amine hydrochloride **B** (170 mg, 0.39 mmol, 1 2 equiv) The reaction mixture was stirred at rt for 48 h and concentrated *in vacuo* to remove DMF and CH_2Cl_2 The residue was diluted with water and extracted with CH_2Cl_2 (3x50 mL) The combined organic layer was extracted with aq NaOH (1M, 2x50 mL), brine (100 mL) and concentrated *in vacuo*. The residue **37b** (315 mg) was oxidized without further purification

Step C



37b

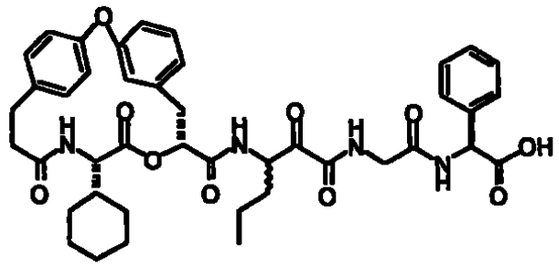


37

A solution of alcohol 37b (315 mg, 0 4 mmol) in CH_2Cl_2 (5 0 mL) was treated with Dess-Martin reagent (424 mg, 1 00 mmol, 2 5 equiv) The reaction mixture was stirred at rt. for 1 h and diluted with aq $NaHCO_3$ (50 mL) and aq $Na_2S_2O_3$ (50 mL) The reaction mixture was stirred at rt for 20 min and the reaction mixture was extracted with CH_2Cl_2 (3x50 mL) The combined organic layers were extracted with brine, dried (Na_2SO_4), filtered concentrated *in vacuo* and the residue was purified by chromatography (SiO_2, EtOAc/Hexanes 1 1) to yield ketoamide 37 (210 mg, 66%) of a colorless solid R_f 0 63 (CH_3OH/CH_2Cl_2 1 19), MS (Electron spray, *m/z relative intensity*) 857 ($[M+CH_3OH]^+$, 33), 825 ($[M+1]^+$ 40), 191 (100)

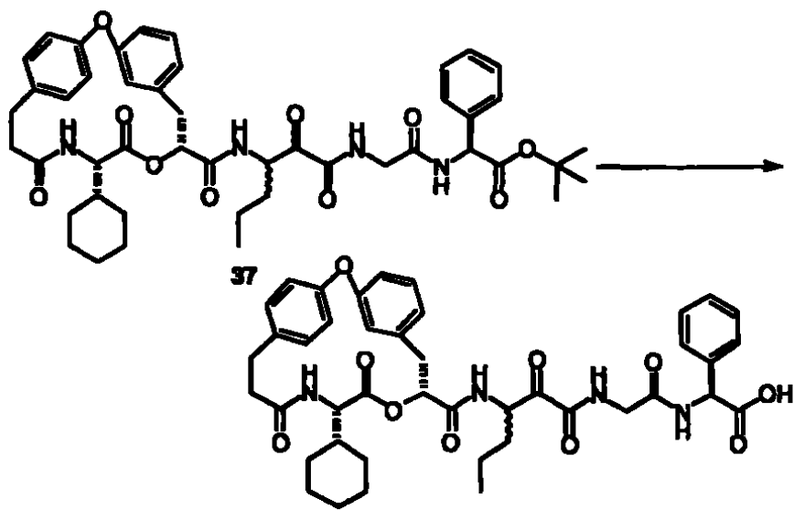
533

Example 38. Preparation of Compound of Formula 38



38

Step A

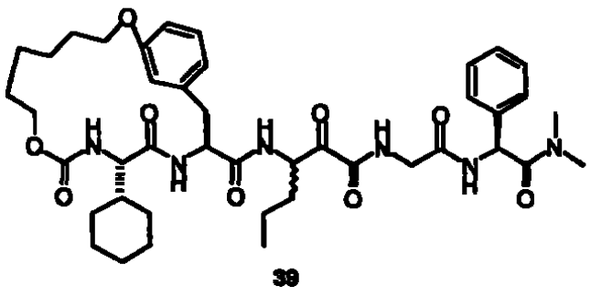


37

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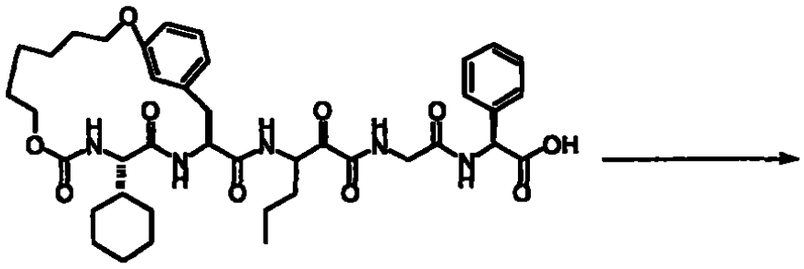
A solution of oxidized depsipeptide 37 (200 mg, 0 24 mmol) in dry CH_2Cl_2 (5 0 mL) was treated with TFA (5 0 mL) and stirred at rt. for 7 h The reaction mixture was concentrated *in vacuo* and the residue was repeatedly dissolved in CH_3OH/CH_2Cl_2/Hexanes (1 1 1) and concentrated *in vacuo* several times to yield a tan colored solid 38 (130 mg, 87%) which was dried *in vacuo*; MS (Electron spray, *m/z relative intensity*) 769 ($[M+1]^+$, 45), 294 (45), 191 (100)

Example 39 Preparation of Compound of Formula 39

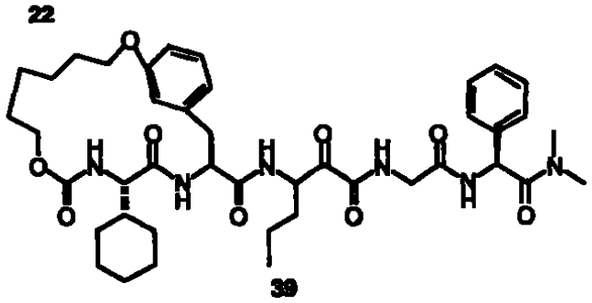


39

Step A



22

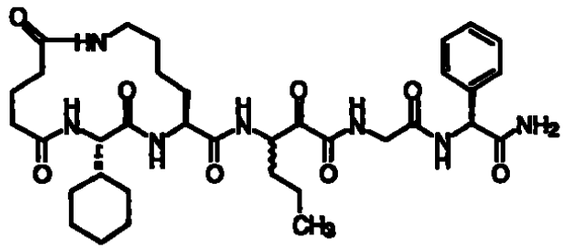


39

A solution of acid **22** (40 mg, 0 06 mmol) in CH_2Cl_2 (0.5 mL) and DMF (0 5 mL) was cooled to 0° C and treated with Me_2NH HCl (15 mg, 0 18 mmol, 3 0 equiv) and HUnigs base (31 mg, 0 24 mmol, 44 µL, 4 0 equiv) The reaction mixture was then treated with PyBrOP (55 mg, 0 12 mmol, 2 0 equiv) and stored for 12 h in the freezer The yellow reaction mixture was concentrated *in vacuo* and the residue was purified by chromatography (SiO_2 EtOAc/Hexanes gradient 3 2 —>1 0) to obtain impure product which was purified once again using (acetone/Hexanes 1 6) to yield dimethyl amide **39** as a colorless solid (14 mg,

35%) MS (Electron spray, *m/z relative intensity*) 791 [(M+1)$^+$, 50], 391 (40), 276 (50), 176 (100)

Example 40 Preparation of Compound of Formula 40

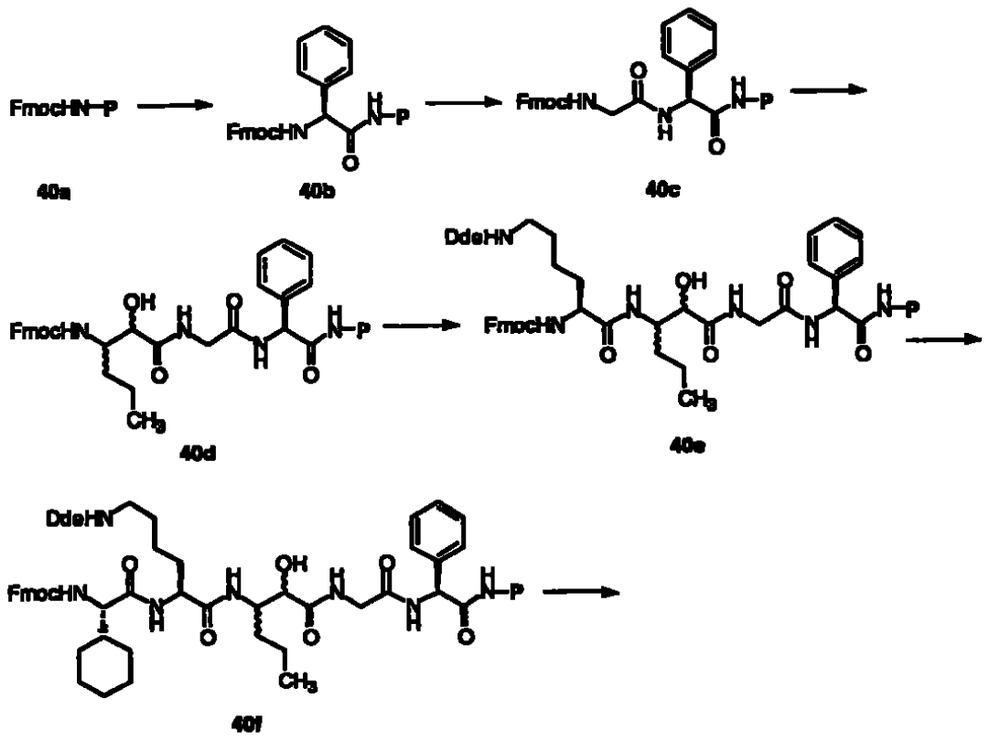


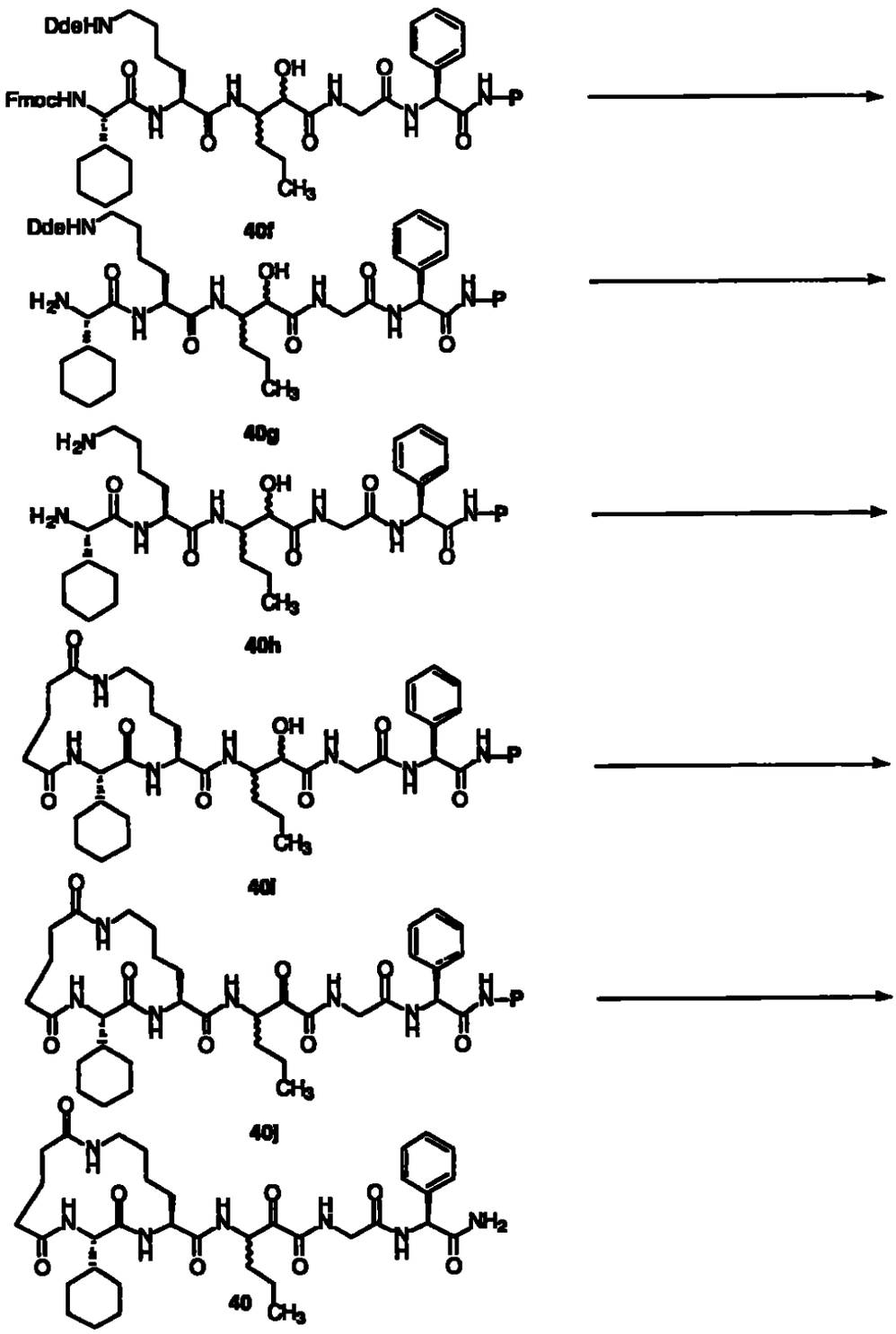
40

General procedure for solid-phase coupling reactions.

The synthesis was done in a reaction vessel which was constructed from a polypropylene syringe cartridge fitted with a polypropylene frit at the bottom The Fmoc-protected amino acids were coupled under standard solid-phase techniques Each reaction vessel was loaded with 100 mg of the starting Fmoc-Sieber resin (approximately 0 035 mmol) The resin was washed with 2 mL portions of DMF (2 times) The Fmoc protecting group was removed by treatment with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min The resin was washed with 2 mL portions of DMF (4 times) The coupling was done in DMF (2 mL), using 0 12 mmol of Fmoc-amino acid, 0 12 mmol of HATU and 0.24 mmol of DIPEA After shaking for 2 h, the reaction vessel was drained and the resin was washed with 2 mL portions of DMF (4 times) The coupling cycle was repeated with the next Fmoc-amino acid or capping group

Scheme 10





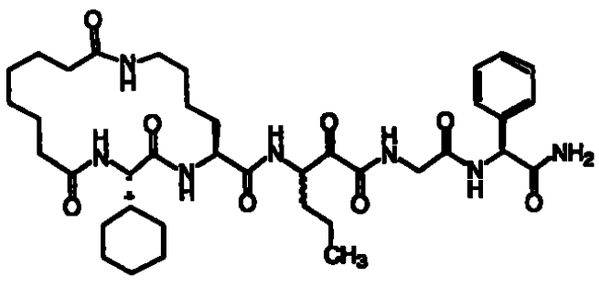
WO 98/22496 PCT/US97/21371

Fmoc-Sieber resin 40a (0 035 mmol) was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-phenylglycine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0.24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound 40b Resin-bound compound 40b was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-glycine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0 24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound 40c Resin-bound compound 40c was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by N-Fmoc-propylisoserine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0 24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound 40d Resin-bound compound 40d was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-lysine(Dde) (0 12 mmol), HATU (0 12 mmol) and DIPEA (0 24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound 40e Resin-bound compound 40e was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min followed by washing with 2 mL portions of DMF (4 times) DMF (2 mL) was added to the resin followed by Fmoc-cyclohexylglycine (0 12 mmol), HATU (0 12 mmol) and DIPEA (0 24 mmol) After shaking at room temperature for 2 h, the resin was washed with 2 mL portions of DMF (4 times) to afford resin-bound compound 40f Resin-bound compound 40f was treated with 2 mL of a 20 % v/v solution of piperidine in DMF for 20 min The resin was washed with 2 mL portions of DMF (4 times) to provide resin-bound compound 40g Resin-bound

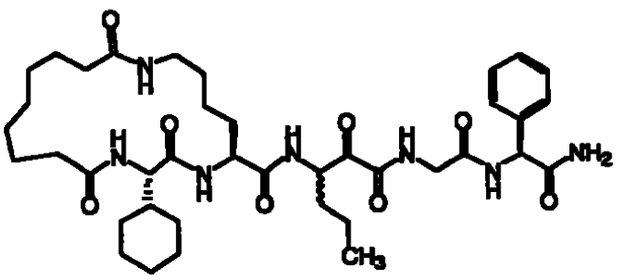
compound 40g was treated with 2 mL portions of a 2 % v/v solution of hydrazine in DMF for 5 min (3 times) The resin was washed with 2 mL portions of DMF (4 times) to provide resin-bound compound 40h Resin-bound compound 40h was treated with 0 035 mmol of glutaric acid, 0 07 mmol of HATU and 0 14 mmol of DIPEA in 2 mL of DMF at room temperature for 16 h The resin was washed with 2 mL portions of DMF (4 times), THF (4 times) and DCM (4 times) to provide resin-bound compound 40i Resin-bound compound 40i was treated with a solution of 0 14 mmol of Dess-Martin periodinane and 0 14 mmol of t-BuOH in 2 mL of DCM at room temperature for 4 h The resin was washed with 2 mL portions of a 20 % v/v solution of iPrOH in DCM THF, a 50 % v/v solution of THF in water (4 times), THF (4 times) and DCM (4 times) to provide resin-bound compound 40j The resin-bound compound 40j was treated with 4 mL of a 2 % v/v solution of TFA in DCM for 5 min The filtrate was added to 1 mL of AcOH and the solution was concentrated by vacuum centrifugation to provide compound 40 (0 0117 g, 48 % yield) MS (LCMS-Electrospray) 698.2 MH^+

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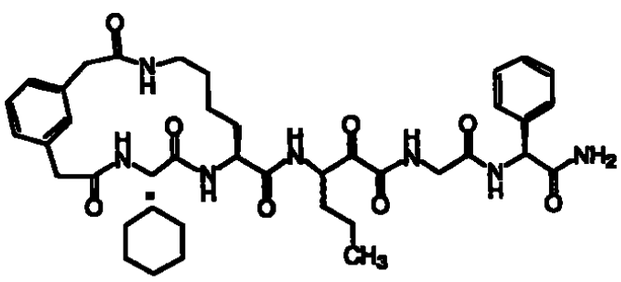
Example 41 – 53. Preparation of Compounds of Formula 41 to 53.



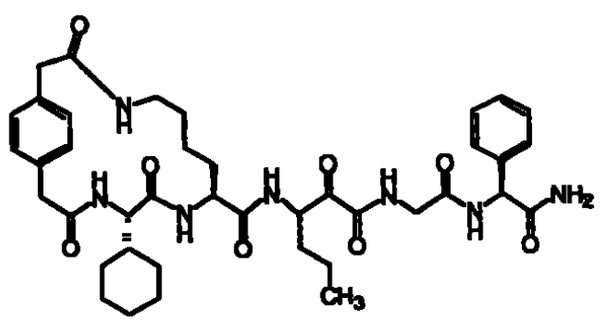
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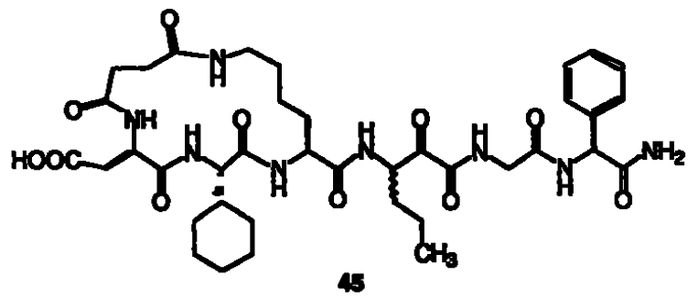
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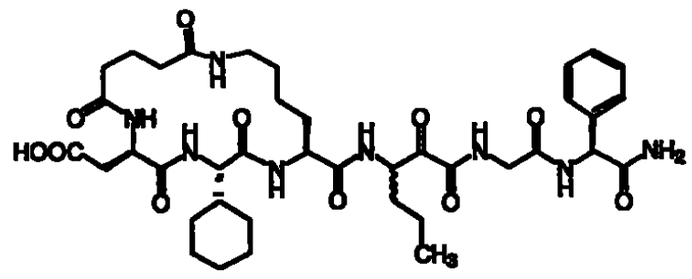
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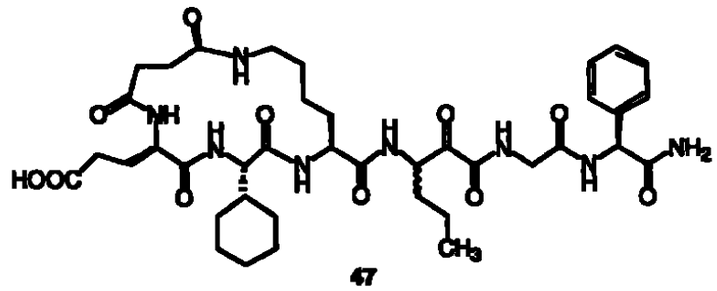
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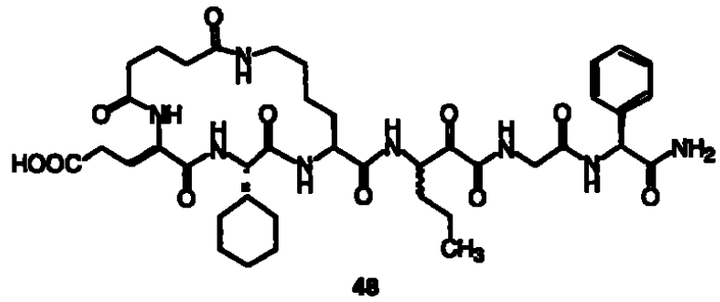
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46



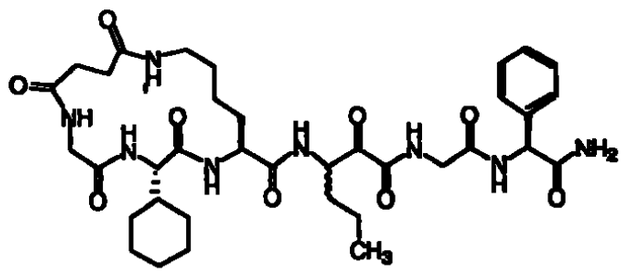
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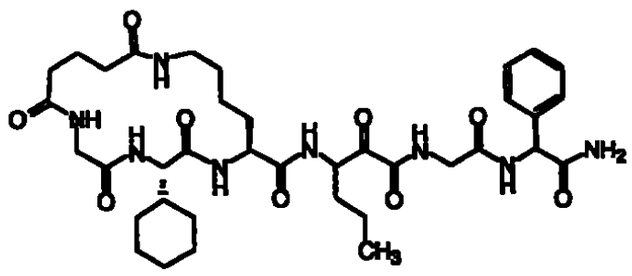
48

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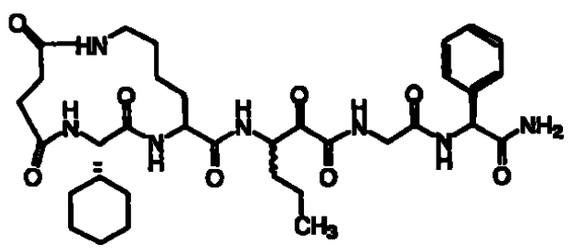
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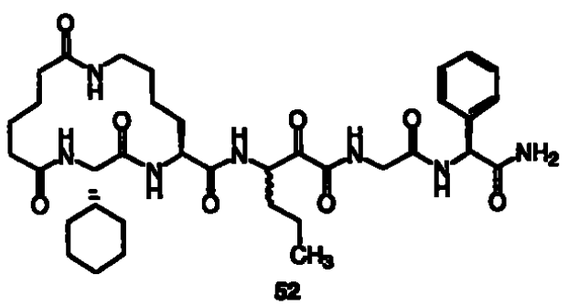
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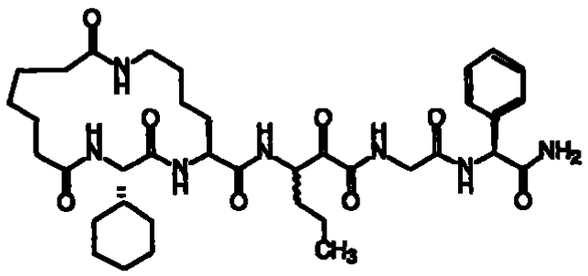


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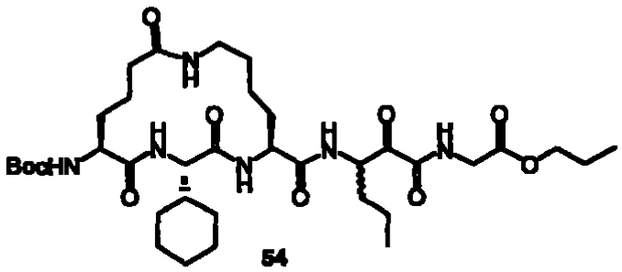
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53

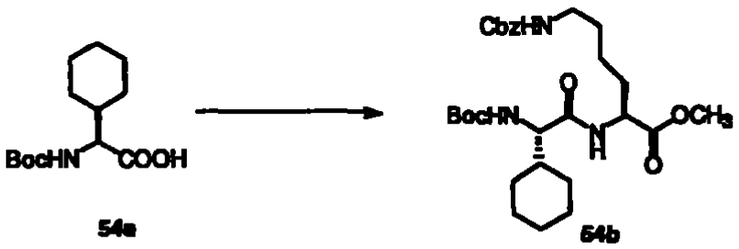
Compounds 41-53 were synthesized using solid phase methodology similar to the procedure outlined for the synthesis of Example 40

Example 54. Preparation of Compound of Formula 54.



54

Step A.

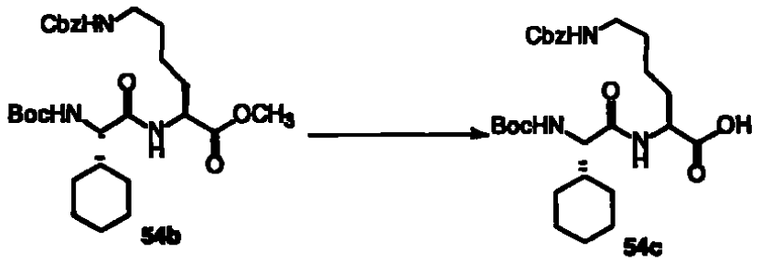


54a 54b

To a stirred solution of Boc-Cyclohexylglycine-OH (2.33 g, 9 07 mmol) in DMF (20 mL) and CH_2Cl_2 (20 mL) was added HOBT (1.48 g, 9 07 mmol), EDCI (1 91 g, 9 97 mmol) and NMM (2 99 mL, 27.2 mmol) The solution was stirred at –20°C for 10 minutes, followed by addition of H-Lys(Z)-OMe HCl and stirred for half an hour at –20°C and kept in freezer overnight. The solution was then

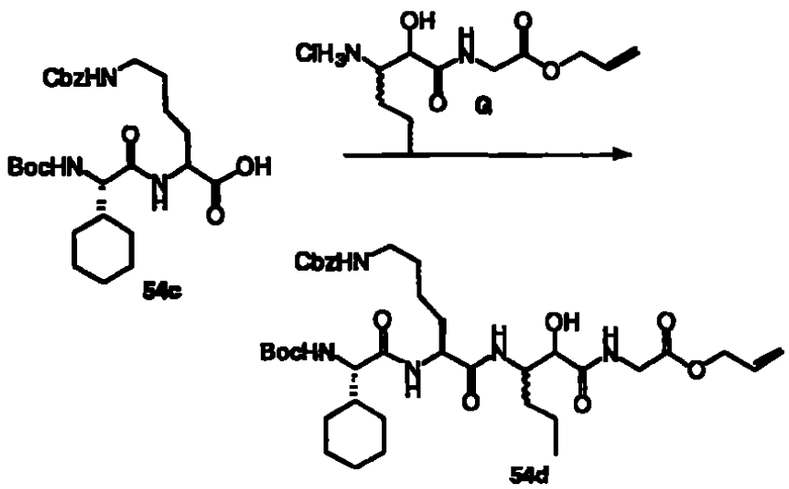
concentrated to dryness, followed by extraction with EtOAc, saturated $NaHCO_3$ The combined organic layer was washed with H_2O, brine, dried over Na_2SO_4 and concentrated to dryness to give a white solid (4 83 g, MH^+=534 1)

Step B



To a stirred solution of 54b (4 86 g, 8 76 mmol) in MeOH (10 mL) and H_2O (7 mL) was added LiOH (70 mg, 11 4 mmol) The white precipitate was formed and the solution was allowed to stir at room temperature overnight and then concentrated to dryness This crude material was then partitioned between CH_2Cl_2 and water The organic layer was separated and washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness to give 54c (4 55 g, MH^+=520 1)

Step C

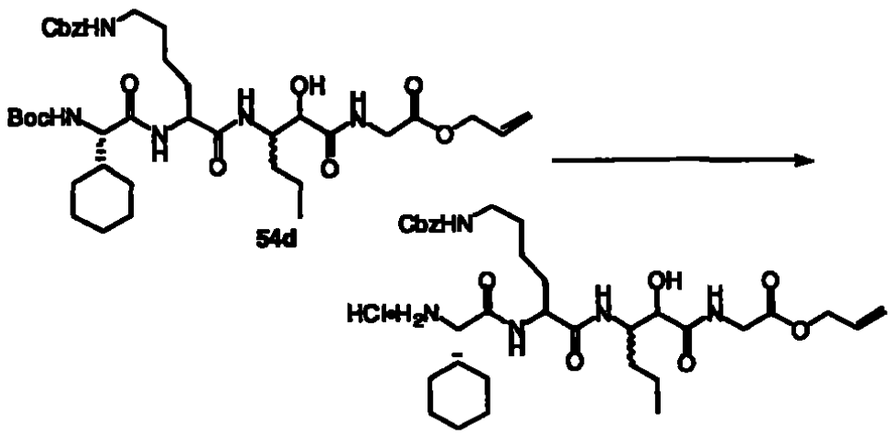


To a stirred cooling solution of 54c (4 3 g, 8 27 mmol) in DMF (40 mL) and CH_2Cl_2 (40 mL) at –20 °C was added HOBT (1.35 g, 8 27 mmol), EDCI (1 74 g,

0551
545

9 1 mmol) and NMM (2 73 mL, 8 27 mmol) The resulting solution was stirred at –20 °C for 10 minutes, followed by addition of amine G (2 32 g, 8.27 mmol) and stirred at –20 °C for half an hour and kept in freezer overnight. The work up procedure from step A was followed to give a 54d (6.21 g, MH^+=746 2)

Step D



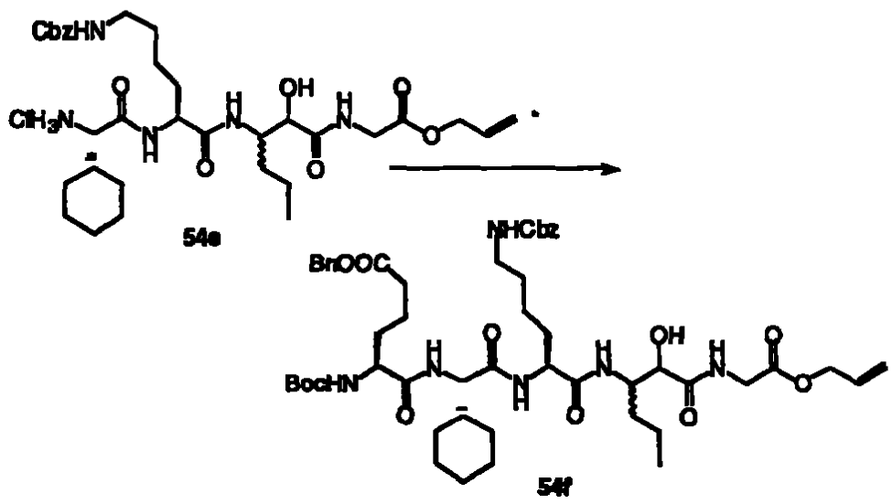
54e

The solution of 54d (6 16 g, 8 26 mmol) In 4N HCl/Dioxane (40 mL) was stirred at room temperature for 1 hr and concentrated to dryness to give a crude product 54e (5 70 g, 100% yield, MH^+=646 3)

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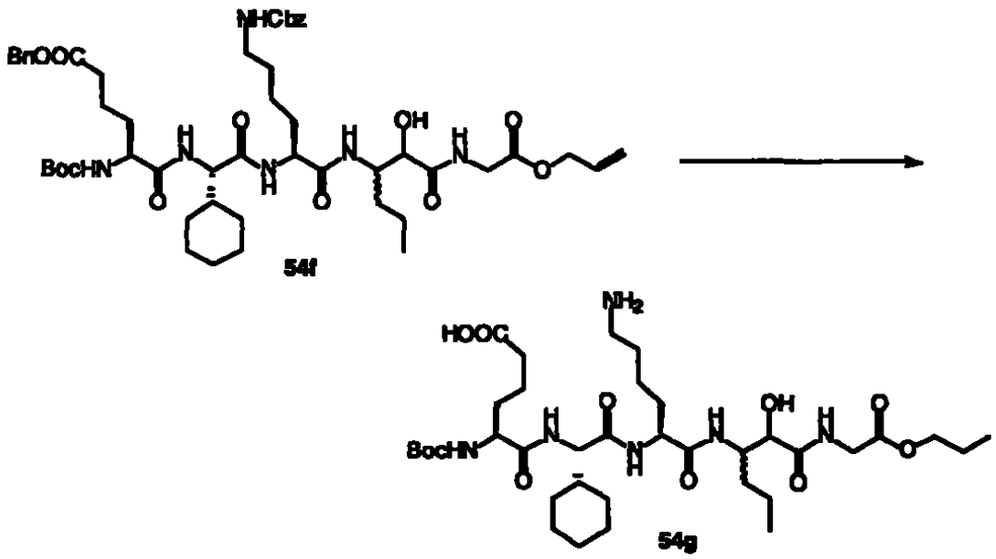
Step E.



To a stirred cooling solution of Boc-Glu(OBn)-OH in DMF (25 mL) and CH_2Cl_2 (25 mL) at –20 °C, was added HOBT (1.29 g, 7 92 mmol), EDCI (1 66 g, 8 71 mmol) and NMM(2 61 mL, 23 7 mmol) The resulting solution was stirred for 10 minutes at –20°C, followed by addition 54e (5.4 g, 7 916 mmol) and stirred for half an hour at -20°C and kept in freezer overnight. Followed the work up procedure from step A to give a crude product (7 14 g, 93 5% yield)

0553
547

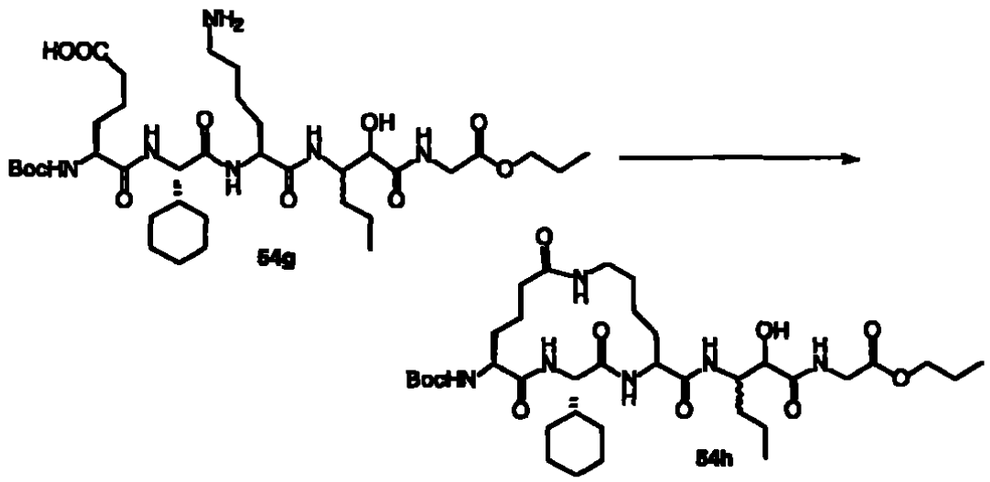
Step F



To a stirred solution of 54f (6 9 g, 7 15 mmol) in absolute EtOH (350 mL), was added 10% Pd/C (2 8 g) in 50% H_2O (w/w) The resulting solution was purged with H_2 and stirred under H_2 balloon overnight. The solution was then filtered through celite and the filtrate was washed with EtOH/CH_2Cl_2 and then concentrated to dryness to give a white solid (1.44 g) The solid was washed with 25% H_2O/ MeOH and filtered through sintered funnel, then freezed and lyophilized to give 54g (4 12 g, 77 5% yield, MH^+=743 2)

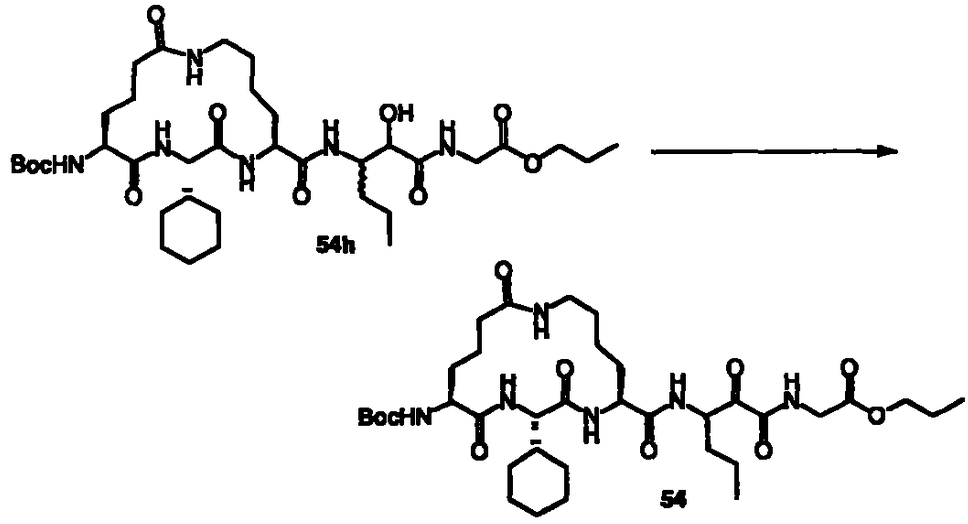
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Step G



To a stirred cooling solution of **54g** (0.5 g, 6 7 mmol) in DMF (50 mL) and CH_2Cl_2 (50 mL) at –20 °C, was added HOBT (0 219 g, 1 34 mmol), EDCI (0 271 g, 1.41 mmol) and NMM (0 296 mL, 2 69 mmol) The resulting solution was stirred at –20 °C for 25 minutes and then kept in freezer overnight. The solution was concentrated to dryness, followed by extraction with EtOAc saturated $NaHCO_3$ The combined organic layer was then concentrated to dryness to give **54h** (254 mg, MH^+=725.2)

Step H



To a stirred solution of **54h** (0 2 g, 0 27 mmol) in anhydrous CH_2Cl_2 (20 mL), was added Dess-Martin periodinane (0 234 g, 0 55 mmol) The resulting solution was stirred at room temperature for 1 hr To this solution was added dropwise over half an hour the solution of H_2O (0 010 mL) in CH_2Cl_2 (20 mL) and stirred vigorously for additional 2 hrs The solution was then stirred for half an hour with 50% $Na_2S_2O_3$/50% sat. $NaHCO_3$ The organic layer was separated and washed with H_2O, brine, dried over Na_2SO_4, filtered, concentrated to dryness and purified by column chromatography on silica gel eluting with 10% MeOH/CH_2Cl_2 to yield **54** (17 mg, 62% MH^+=723 2)

Assay for HCV Protease Inhibitory Activity.

Spectrophotometric Assay: Spectrophotometric assays for the HCV serine protease was performed on the inventive compounds by following the procedure described by R Zhang *et al*, *Analytical Biochemistry*, 270 (1999) 268-275, the disclosure of which is incorporated herein by reference The assay based on the proteolysis of chromogenic ester substrates is suitable for the continuous monitoring of HCV NS3 protease activity The substrates were derived from the P side of the NS5A-NS5B junction sequence (Ac-DTEDVVX(Nva), where X = A or

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P) whose C-terminal carboxyl groups were esterified with one of four different chromophoric alcohols (3- or 4-nitrophenol, 7-hydroxy-4-methyl-coumann, or 4-phenylazophenol) Presented below are the synthesis, characterization and application of these novel spectrophotometric ester substrates to high throughput screening and detailed kinetic evaluation of HCV NS3 protease inhibitors

Materials and Methods.

Materials Chemical reagents for assay related buffers were obtained from Sigma Chemical Company (St. Louis, Missouri) Reagents for peptide synthesis were from Aldrich Chemicals, Novabiochem (San Diego, California), Applied Biosystems (Foster City, California) and Perseptive Biosystems (Framingham, Massachusetts) Peptides were synthesized manually or on an automated ABI model 431A synthesizer (from Applied Biosystems) UV/VIS Spectrometer model LAMBDA 12 was from Perkin Elmer (Norwalk, Connecticut) and 96-well UV plates were obtained from Corning (Corning, New York) The prewarming block was from USA Scientific (Ocala, Florida) and the 96-well plate vortexer was from Labline Instruments (Melrose Park, Illinois) A Spectramax Plus microtiter plate reader with monochrometer was obtained from Molecular Devices (Sunnyvale, California)

Enzyme Preparation. Recombinant heterodimeric HCV NS3/NS4A protease (strain 1a) was prepared by using the procedures published previously (D L. Sali *et al*, *Biochemistry*, 37 (1998) 3392-3401) Protein concentrations were determined by the Biorad dye method using recombinant HCV protease standards previously quantified by amino acid analysis Prior to assay initiation, the enzyme storage buffer (50 mM sodium phosphate pH 8 0, 300 mM NaCl, 10% glycerol, 0 05% lauryl maltoside and 10 mM DTT) was exchanged for the assay buffer (25 mM MOPS pH 6 5, 300 mM NaCl, 10% glycerol, 0 05% lauryl maltoside, 5 μM EDTA and 5 μM DTT) utilizing a Biorad Bio-Spin P-6 prepacked column

Substrate Synthesis and Purification. The synthesis of the substrates was done as reported by R Zhang *et al*, (*Ibid*) and was initiated by anchoring Fmoc-Nva-

OH to 2-chlorotrityl chloride resin using a standard protocol (K Barlos *et al, Int J Pept Protein Res*, 37 (1991), 513-520) The peptides were subsequently assembled, using Fmoc chemistry, either manually or on an automatic ABI model 431 peptide synthesizer The N-acetylated and fully protected peptide fragments were cleaved from the resin either by 10% acetic acid (HOAc) and 10% trifluoroethanol (TFE) in dichloromethane (DCM) for 30 min, or by 2% trifluoroacetic acid (TFA) in DCM for 10 min The combined filtrate and DCM wash was evaporated azeotropically (or repeatedly extracted by aqueous Na_2CO_3 solution) to remove the acid used in cleavage The DCM phase was dried over Na_2SO_4 and evaporated

The ester substrates were assembled using standard acid-alcohol coupling procedures (K. Holmber *et al, Acta Chem Scand.*, B33 (1979) 410-412) Peptide fragments were dissolved in anhydrous pyridine (30-60 mg/ml) to which 10 molar equivalents of chromophore and a catalytic amount (0 1 eq) of para-toluenesulfonic acid (pTSA) were added Dicyclohexylcarbodiimide (DCC, 3 eq) was added to initiate the coupling reactions Product formation was monitored by HPLC and found to be complete following 12-72 hour reaction at room temperature Pyridine solvent was evaporated under vacuum and further removed by azeotropic evaporation with toluene The peptide ester was deprotected with 95% TFA in DCM for two hours and extracted three times with anhydrous ethyl ether to remove excess chromophore The deprotected substrate was purified by reversed phase HPLC on a C3 or C8 column with a 30% to 60% acetonitrile gradient (using six column volumes) The overall yield following HPLC purification was approximately 20-30% The molecular mass was confirmed by electrospray ionization mass spectroscopy The substrates were stored in dry powder form under desiccation

Spectra of Substrates and Products. Spectra of substrates and the corresponding chromophore products were obtained in the pH 6.5 assay buffer Extinction coefficients were determined at the optimal off-peak wavelength in 1-cm cuvettes (340 nm for 3-Np and HMC, 370 nm for PAP and 400 nm for 4-Np) using multiple

dilutions The optimal off-peak wavelength was defined as that wavelength yielding the maximum fractional difference in absorbance between substrate and product (product OD - substrate OD)/substrate OD)

Protease Assay: HCV protease assays were performed at 30°C using a 200 μl reaction mix in a 96-well microtiter plate Assay buffer conditions (25 mM MOPS pH 6 5, 300 mM NaCl, 10% glycerol, 0 05% lauryl maltoside, 5 μM EDTA and 5 μM DTT) were optimized for the NS3/NS4A heterodimer (D L Sali *et al, Ibid*)) Typically, 150 μl mixtures of buffer, substrate and inhibitor were placed in wells (final concentration of DMSO • 4 % v/v) and allowed to preincubate at 30 °C for approximately 3 minutes Fifty μls of prewarmed protease (12 nM, 30°C) in assay buffer, was then used to initiate the reaction (final volume 200 μl) The plates were monitored over the length of the assay (60 minutes) for change in absorbance at the appropriate wavelength (340 nm for 3-Np and HMC, 370 nm for PAP, and 400 nm for 4-Np) using a Spectromax Plus microtiter plate reader equipped with a monochrometer (acceptable results can be obtained with plate readers that utilize cutoff filters) Proteolytic cleavage of the ester linkage between the Nva and the chromophore was monitored at the appropriate wavelength against a no enzyme blank as a control for non-enzymatic hydrolysis The evaluation of substrate kinetic parameters was performed over a 30-fold substrate concentration range (~6-200 μM) Initial velocities were determined using linear regression and kinetic constants were obtained by fitting the data to the Michaelis-Menten equation using non-linear regression analysis (Mac Curve Fit 1 1, K Raner) Turnover numbers (k_{cat}) were calculated assuming the enzyme was fully active

Evaluation of Inhibitors and Inactivators. The inhibition constants (K_i) for the competitive inhibitors Ac-D-(D-Gla)-L-I-(Cha)-C-OH (27), Ac-DTEDVVA(Nva)-OH and Ac-DTEDVVP(Nva)-OH were determined experimentally at fixed concentrations of enzyme and substrate by plotting v_o/v_i vs inhibitor concentration ($[I]_o$) according to the rearranged Michaelis-Menten equation for competitive inhibition kinetics $v_o/v_i = 1 + [I]_o/(K_i (1 + [S]_o/K_m))$, where v_o is the uninhibited initial velocity, v_i is the initial velocity in the presence of inhibitor at any given

inhibitor concentration ($[I]_o$) and $[S]_o$ is the substrate concentration used The resulting data were fitted using linear regression and the resulting slope, $1/(K_i(1+[S]_o/K_m)$, was used to calculate the K_i value

The obtained K_i values for the various macrocycles of the present invention are given in the afore-mentioned Table 1 wherein the compounds have been arranged in the order of ranges of K_i values From these test results, it would be apparent to the skilled artisan that the compounds of the invention have excellent utility as NS3-serine protease inhibitors

Cell Bioassay Method The cell bioassays for the HCV serine protease was performed on the inventive compounds by following the procedure described by S Agrawal *et al*, "Development and Characterization of Hepatitis C Virus Serine Protease Cell-based Trans-Cleavage Assay", *Hepatology* Supplement to Volume 30 (No 4, Part 2, October 1999), *Abstract No 615* (Proceedings of AASLD 50th Annual Meeting, Dallas, Texas, November 5-9, 1999), the disclosure of which is incorporated herein by reference The assay was performed in HeLa/Huh7 cells that were co-transfected with a plasmid that expresses a reporter protein substrate containing the NS5A/5B cleavage recognition sequence and an 1BNS4A $_{21-32}$ GS-GSNS $_{3-81}$ I17K expression vector and YFPn1 as a internal standard protein to control cytotoxicity Protease activity was measured by SDS-PAGE of total cell lysates followed by Western blot detection using a monoclonal antibody directed against the reporter substrate Quantitation of substrate cleavage was performed by scanning the immunoblot on the phosphoimager

Materials.

Plasmid DNAs

pBFP-5A/5B-GFP The reporter gene that expresses the substrate encodes a fusion protein comprised of an N' terminal blue fluorescent protein (BFP) domain and a C' terminal green fluorescent protein (GFP) domain, separated by a 25 amino acids derived from the NS5A/5B cleavage recognition sequence Both GFP and BFP are essentially homologous autofluorescent proteins that emit green or blue light, respectively, when excited by UV light of the appropriate wavelength

Four amino acid substitutions in the chromophore of GFP alter the emission wavelength and convert the protein to BFP

The substrate and the resulting GFP and BFP products can be detected in cell lysates by immunologic methods using a monoclonal antibody that recognizes both proteins.

The BFP-5A/5B-GFP reporter gene contains the BFP and GFP autofluorescent protein coding sequences (Quantum Biotechnologies, Inc , Montreal, Canada) separated by the NS5A/5B cleavage recognition sequence, cloned between the Nhe I and Bam HI restriction endonuclease sites of the pQBI25 cloning vector (Quantum Biotechnologies, Inc) Expression of the fusion protein is under the control of the CMV IE promoter-enhancer The bovine growth hormone p (A) sequence of the vector provides the polyadenylation signal for the mRNA The NS5A/5B cleavage sequence is SSGADTEDVVCCSMSYTWTGALVTP DNA sequencing was used to validate the clone

P1BOO2. 1bNS4A21-32GS-GS NS 3-81 I17K. The subtype 1b protease was cloned as an Xba1/Not1 fragment behind the CMV promoter in vector pC1neo

YFPn1 YFPn1 was purchased from CLONTECH (Palo Alto, California) Addition of third plasmid to the transfection supplies an internal standard protein to control for cytotoxicity and does not affect percentage of protease cleavage

Plasmid DNAs were maintained and propagated in DH5α cells (obtained from LifeTechnologies) in LB medium under the appropriate antibiotic selection, and purified using QIAfilter Plasmid Kits (Qiagen, Valencia, California)

Cell Culture

HeLa cells were maintained and propagated in Eagle's Minimum Essential Media (EMEM, BioWhittaker, Walkersville, Maryland) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, and 100 u/ml penicillin-streptomycin (BioWhitaker), 2% $NaHCO_3$.

Huh7 cells were maintained and propagated in Dulbecco's Modified Eagle's medium (DMEM, BioWhittaker) supplemented with 10% fetal calf serum (FCS),

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100u/ml penicillin-streptomycin (BioWhitaker) and 5 ml NEAA (100x, BioWhittaker)/L

SOP Procedure

Day preceding transfection

HeLa cells were seeded in 24 well plates (Falcon 3047 plates) at a density of 6 x 10^4 cells/well and grown overnight at 37^{0}C in a 5% CO_2 incubator

Day of transfection

Plasmid DNAs were diluted to a final concentration of 0 05 μg/μl in nuclease free water (Promega, Madison, Wisconsin, cat # P119C) 0 75 μg BFP-5A/5B-GFP was combined and mixed with 0 175 μg P1B002 (0 23X) and 0 02μg of YFPn1 The DNAs were brought to a final volume of 60 μl with EMEM lacking FBS, glutamine, and antibiotics A ratio of 5 μl volumes of SuperFect Reagent (Qiagen, cat # 301305) per total μgs of DNA was added and the mixture vortexed about 10 seconds and incubated 10 min at room temperature to allow complex formation While complex formation was taking place, growth medium from cell culture plates was aspirated and cells washed 1X with 1 ml PBS without Ca^{2+}, Mg^{2+} (BioWhitaker) 350 μl EMEM (supplemented with appropriate suplements-compleat medium) was added to the tube containing the transfection complexes and the mixture pipetted up and down 2-3 times Total volume was transferred to one well of the 24 well culture plate The HeLa cells were incubated with the transfection complexes for about 3 hr at 37^{0}C and 5% CO2 The media containing the transfection complexes was removed from the cells by aspiration The cells were washed once in about 1 ml PBS, the PBS was aspirated and 495 μl of complete EMEM was added followed by 5 μl compound/well The cells were incubated 22-24 hr at 37^{0}C and 5% CO2

Preparation of Cell Lysates

The medium from each well was aspirated and washed once 1x with DPBS Cells were harvested in 100 μl of 1x Tris-SDS-BME sample buffer (OWL separation system, Portsmouth, New Hampshire, cat # ER33) and transferred to microcentrifuge tubes It was then boiled 3-5 min to lyse cells Loading was done

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at 10 μl/well on SDS-PAGE gel The lysates were resolved by electrophoresis on 10 cm x 10 cm 12 5% SDS-PAGE (Owl Scientific, cat # OG-0125B) run at 30 mamp in Tris-Glycine-SDS buffer (Owl Scientific) Prior to use, PVDF membrane (Immobilon-P, 45μm pore size, Millipore, Bedford, Massachusetts) was soaked in 100% methanol for 10 seconds and then the blot was placed in distilled water The proteins were transferred to PVDF filter membranes (0 45μm, Millipore) at 108 mamp per gel for 90 minutes using a semi-dry electroblotter

Detection of Proteins by ECF Western Blot (Amersham Pharmacia Biotech, Little Chalfont, England), catalog #RPN 5780) The PVDF filter membranes were blocked by 5% blocking reagent (from kit) in ~10 ml PBS containing 0 05% Tween 20, pH 7 4 (Sigma Chemicals, St. Louis, Missouri, cat # 3563) for overnight at 2-4 C in refrigerator The next day, the membranes were rinsed briefly twice with TPBS containing 0 05% Tween 20 washing buffer, then washed three times each time 5 min in PBS containing 0 05% Tween 20, pH 7 4 The membranes were incubated in 12 mls of a 1 3000 dilution of anti-GFP monoclonal antibody for 30 minutes (Clontech, Palo Alto, California) in PBS containing 0 05% Tween 20, pH7 4 while at the same time 1% BSA (Albumin, bovine cat # A-2153 from Sigma) was added to reduce background The membranes were washed briefly twice with TPBS, then thrice, for 5 min each time, in TPBS washing buffer The membranes were incubated in 12 mls of a 1 600 dilution anti fluorescein-linked anti mouse Ig in TPBS for 30 minutes The membranes were washed briefly with TPBS twice, then for 5 min in TPBS washing buffer thrice For signal amplification with ECF substrate membranes were incubated in 10 ml of 1.2500 anti fluorescein alkaline phosphatase conjugate for 30 minutes The membranes were rinsed briefly with TPBS twice, then 5 min in TPBS washing buffer thrice The ECF substrate solution was prepared as per manufacturer's instructions (aliquot and freeze), membranes were incubated for 2-3 minutes, excess reagent was drained off, then were blotted with filter papers, air-dried for 9-10 minutes and then scanned

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Scanning the membrane. The blot was placed on the glass of phosphoimager Storm 860 The blue chemiluminiescent was set up, 200 pixcels size, 700 PMT voltage The file was opened in ImageQuant and quantitated by creating squares around the bands representing the substrate (S), the product (P) and the internal control (IC) The % cleavage of the substrate was measured as P/(S+P)x100 The inhibition in cleavage due to drug was measured compared duplicate to drug controls included on each blot A report was created in Excel The results for some of the compounds are given below

Compound of Example 36 EC_{50} = 9μm

Compound of Example 35 EC_{50} = 20μm

From these test results, it would be apparent to the skilled artisan that the compounds of the invention have excellent utility as NS3-serine protease inhibitors

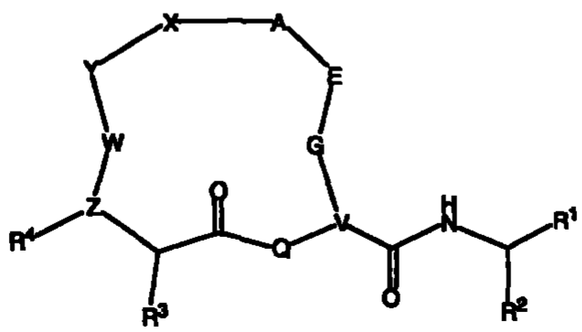
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Claims

What is claimed is.

1 A macrocyclic compound, including enantiomers, stereoisomers, rotomers and tautomers of said compound, and pharmaceutically acceptable salts or solvates of said compound having the general structure shown in Formula I



Formula I

wherein

E, X and Y may be independently present or absent, and if present are independently selected from the moieties alkyl, aryl, alkyl-aryl, heteroalkyl, heteroaryl, aryl-heteroaryl, alkyl-heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, aryl ether, alkyl amino, aryl amino, alkyl-aryl amino, alkyl sulfide, alkyl-aryl sulfide, aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, aryl sulfone, alkyl-alkyl sulfoxide, alkyl-aryl sulfoxide, alkyl amide, alkyl-aryl amide, aryl amide, alkyl sulfonamide, alkyl-aryl sulfonamide, aryl sulfonamide, alkyl urea, alkyl-aryl urea, aryl urea, alkyl carbamate, alkyl-aryl carbamate, aryl carbamate, alkyl -hydrazide, alkyl-aryl hydrazide, alkyl hydroxamide, alkyl-aryl hydroxamide, alkyl sulfonyl, aryl sulfonyl, heteroalkyl sulfonyl, heteroaryl sulfonyl, alkyl carbonyl, aryl carbonyl, heteroalkyl carbonyl, heteroaryl carbonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl or a combination thereof, with the proviso that E, X and Y may optionally be additionally substituted with moieties selected from the group consisting of aromatic, alkyl, alkyl-aryl, heteroalkyl, aryl-heteroaryl, alkyl-

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heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, alkyl sulfide, alkyl-aryl sulfide, alkyl sulfone, alkyl-aryl sulfone, alkyl amide, alkyl-aryl amide, alkyl sulfonamide, , alkyl amines, alkyl-aryl amines, alkyl-aryl sulfonamide, alkyl urea, alkyl-aryl urea, alkyl carbamate, alkyl-aryl carbamate, halogen, hydroxyl amino, alkyl carbazate, aryl carbazate,

R' = COR^5 or $B(OR)_2$, wherein R^5 = H, OH, OR^9, NR^9R^{10}, CF_3, C_2F_5, C_3F_7, CF_2R^6, R^6, COR^7 wherein R^7 = H, OH, OR^8, CHR^9R^{10}, or NR^9R^{10} , wherein R^6, R^8, R^9 and R^{10} are independently selected from the group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, cycloalkyl, arylalkyl, heteroarylalkyl, $CH(R^1)COOR^{11}$, $CH(R^1)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)R'$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONHCH(R^5)COO\ R^{11}$, $CH(R^1)CONHCH(R^2)CONHCH(R^3)CONHCH(R^4)CONHCH(R^5)\ CONR^{12}R^{13}$, wherein R', R^2, R^3, R^4, R^5, R^{11}, R^{12}, R^{13}, and R' are independently selected from a group consisting of H, alkyl, aryl, heteroalkyl, heteroaryl, cycloalkyl, alkyl-aryl, alkyl-heteroaryl, aryl-alkyl and heteroaralkyl,

Z is selected from O, N, or CH,

W may be present or absent, and if W is present, W is selected from C=O, C=S, SO_2 or C=NR,

Q is $(NR)_p$, O, S, CH_2, CHR, CRR' or a double bond towards V,

A is O, CH_2, $(CHR)_p$, $(CHR\text{-}CHR')_p$ $(CRR')_p$, NR, S, SO_2 , C=O or a bond,

G is $(CH_2)_p$, $(CHR)_p$, $(CRR')_p$, NR, O, S, SO_2, $S(O)_2NH$, C=O, or a double bond towards E or V,

V is CH, CR or N,

p is a number from 0 to 6, and

R, R', R^2, R^3 and R^4 are independently selected from the group consisting of H, C1-C10 alkyl, C2-C10 alkenyl, C3-C8 cycloalkyl, C3-C8 heterocycloalkyl,

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aryl, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, heteroaryl, alkyl-aryl, alkyl-heteroaryl, (cycloalkyl)alkyl and (heterocycloalkyl)alkyl, wherein said cycloalkyl is made of three to eight carbon atoms, and zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of one to six carbon atoms,
with said alkyl, heteroalkyl, alkenyl, heteroalkenyl, aryl, heteroaryl, cycloalkyl and heterocycloalkyl moieties may be optionally substituted, with said term "substituted" referring to optional and suitable substitution with one or more moieties selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, heterocyclic, halogen, hydroxy, thio, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, ester, carboxylic acid, carbamate, urea, ketone, aldehyde, cyano, nitro, sulfonamide, sulfoxide, sulfone, sulfonyl urea, hydrazide, hydroxamate and thiourea.

2 The compound of claim 1, wherein $R^1 = COR^8$, and R^8 is H, OH, $COOR^9$, or $CONR^9R^{10}$

3 The compound of claim 2, wherein $R^1 = COCONR^9R^{10}$, and is R^9 is H, R^{10} is H, $CH(R^1)COOR^{11}$, $CH(R^1)$ $CONR^{12}R^{13}$, $CH(R^1)CONHCH(R^6)COOR^{11}$, $CH(R^1)CONHCH(R^6)$ $CONR^{12}R^{13}$, or $CH(R^1)CONHCH(R^6)(R')$

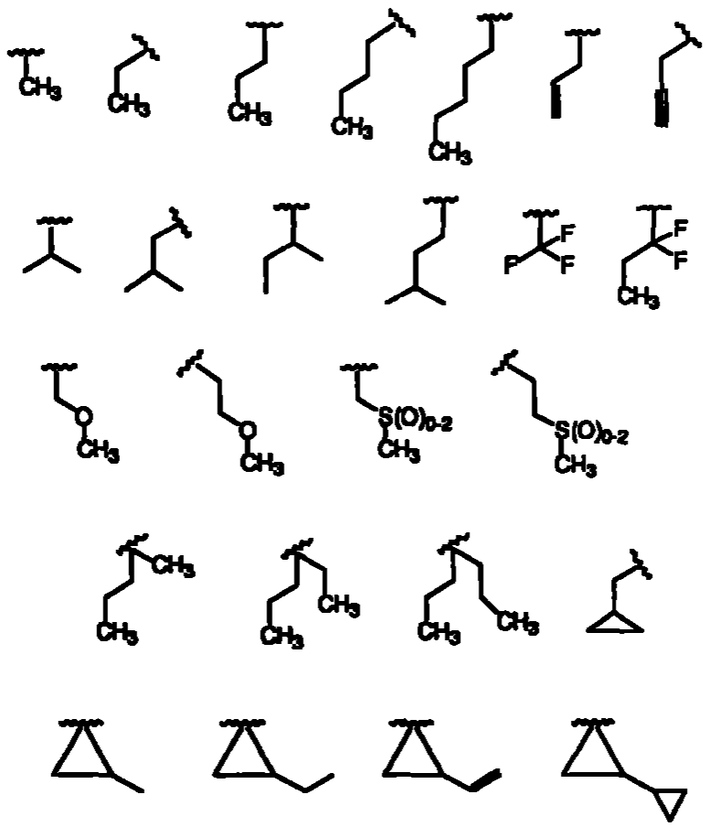
4 The compound of claim 3, wherein $R^{10} = CH(R^1)CONHCH(R^6)COOR^{11}$, $CH(R^1)CONHCH(R^6)$ $CONR^{12}R^{13}$, or $CH(R^1)CONHCH(R^6)(R')$, wherein R^1 is H or alkyl, and R^6 is selected from the group consisting of phenyl, substituted phenyl, hetero atom-substituted phenyl, thiophenyl, cyclohexyl, cyclopentyl, cyclopropyl, piperidyl and pyridyl

5 The compound of claim 4, wherein R^1 is H

6 The compound of claim 5, wherein R^6 = phenyl, thiophenyl, cyclohexyl, cyclopentyl, pyridyl, or phenyl(4-$HNSO_2NH_2$), R^{11} is H, *tert*-butyl, or CH_2-phenyl, R^{12} and R^{13} are independent combinations of H, methyl, and R' is hydroxymethyl

7 The compound of claim 1, wherein R^8 is selected from the group consisting of the following moieties

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8 The compound of claim 7 wherein $R^1 = COR^9$, and R^2 is H, OH, $COOR^9$, or $CONR^9R^{10}$

9 The compound of claim 8 wherein V is CH

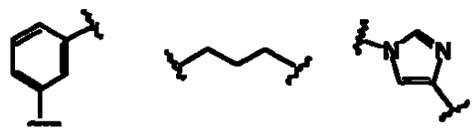
10 The compound of claim 9 wherein Q is NR or O

11 The compound of claim 10 wherein G is CH_2

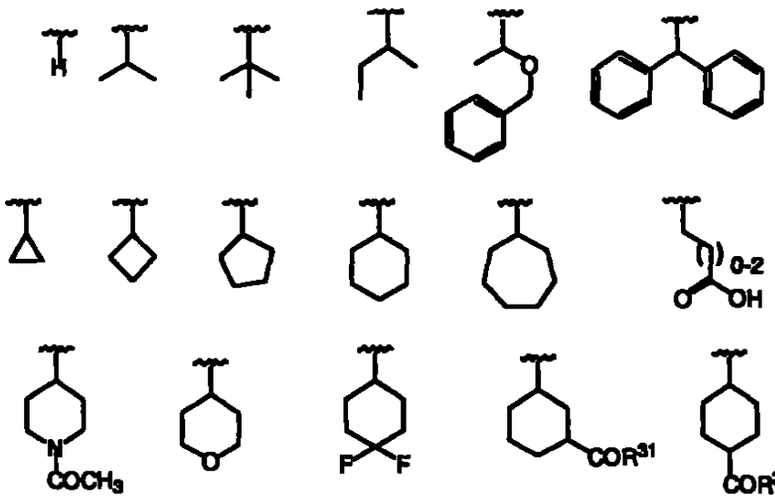
12 The compound of claim 11 wherein A is O, NR, CH=CH or CH_2.

13 The compound of claim 12 wherein E is alkyl, aryl, hereroalkyl, heteroaryl, alkyl, aryl, or cycloalkyl

14 The compound of claim 13 wherein E is selected from the group consisting of the moieties



15 The compound of claim 14 wherein R^3 is selected from the group consisting of the moieties



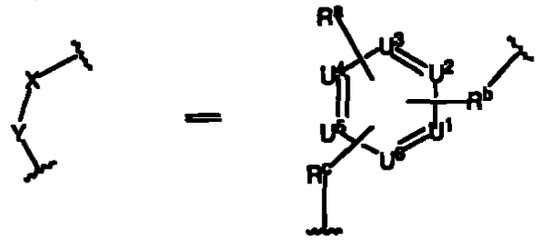
wherein R^{31} = OH or O-alkyl

16 The compound of claim 15 wherein Z = N and R^4 = H

17 The compound of claim 16 wherein W is C=O

18 The compound of claim 17 wherein the moiety X-Y is selected from the group consisting of C1-C10 alkyl, alkyl, cycloalkyl, heteroalkyl, arylalkyl, aryl, hereroaryl and alkylaryl

19 The compound of claim 18, wherein



wherein R^b is connected directly to A and R^c is connected directly to W, and the moiety U^1, U^2, U^3, U^4, U^5 and U^6 form either a six membered carbon ring, or a five or six membered ring with one or more heteroatoms,

R^a = H, alkyl, alkoxy, hydroxy alkylthio, halogen, nitro, cyano, carboxylic acid, ester, amide, amino, nitrile, or CF_3,

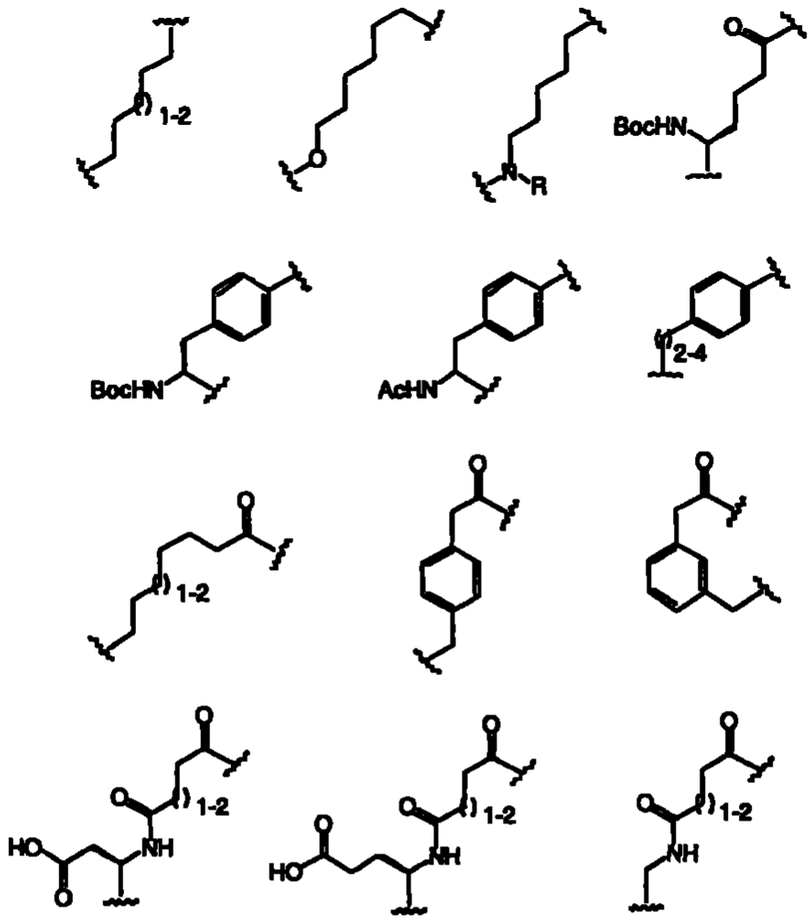
R^b is a bond, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, O, S, SO_2, NH, O(alkyl), S(alkyl), SO_2(alkyl) or N(alkyl), and

R^c is a bond, C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, O, S, NH, O(alkyl), S(alkyl), or N(alkyl)

20 The compound of claim 18 wherein the moiety X-Y is selected from the group consisting of the following structures

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21 A pharmaceutical composition comprising as an active ingredient a compound of claim 1

22 The pharmaceutical composition of claim 21 for use in treating disorders associated with Hepatitis C virus

23 The pharmaceutical composition of claim 21 additionally comprising a pharmaceutically acceptable carrier

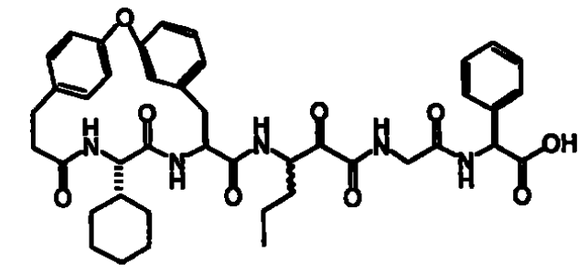
24 A method of treating disorders associated with the HCV protease, said method comprising administering to a patient in need of such treatment a pharmaceutical composition which composition comprises therapeutically effective amounts of a compound of claim 1

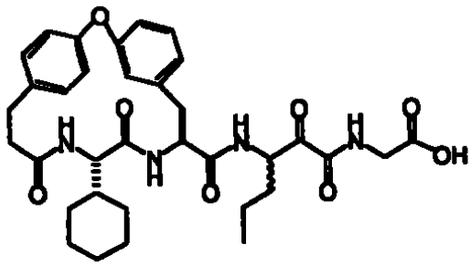
25 The use of a compound of claim 1 for the manufacture of a medicament to treat disorders associated with the HCV protease

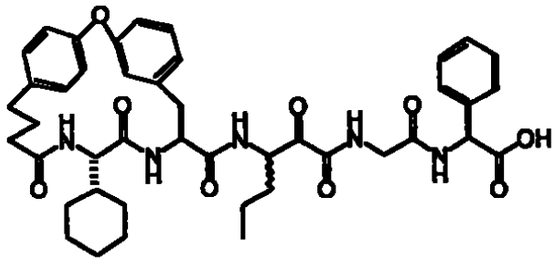
26 A method of preparing a pharmaceutical composition for treating disorders associated with the HCV protease, said method comprising bringing into intimate contact a compound of claim 1 and a pharmaceutically acceptable carrier

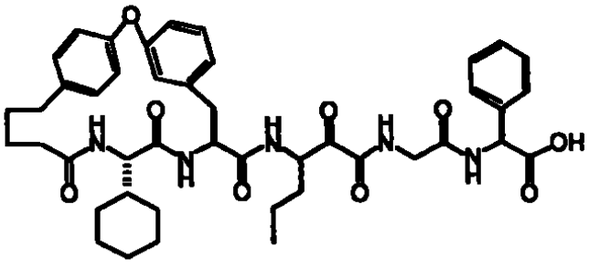
27 A compound exhibiting HCV protease inhibitory activity, including enantiomers, stereoisomers, rotamers and tautomers of said compound, and pharmaceutically acceptable salts or solvates of said compound, said compound being selected from the group of compounds with structures listed below

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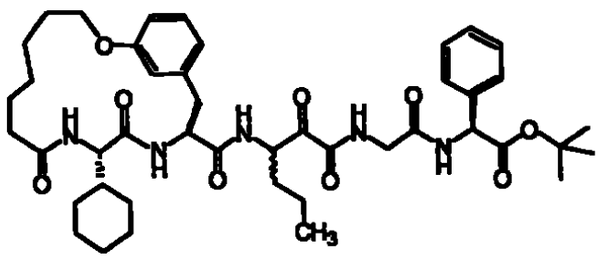


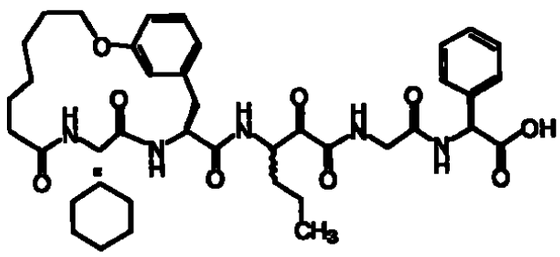


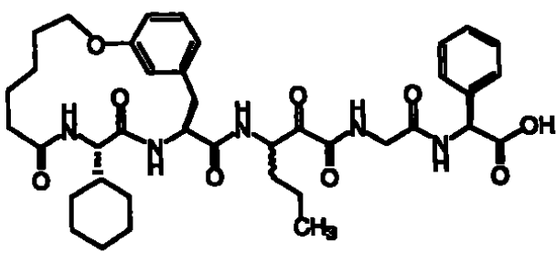


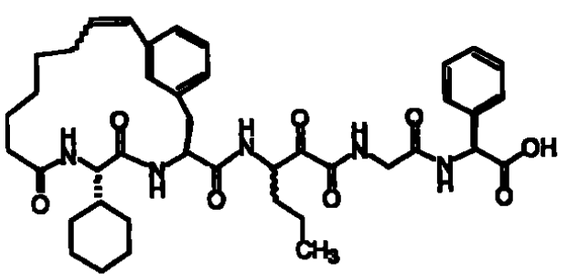
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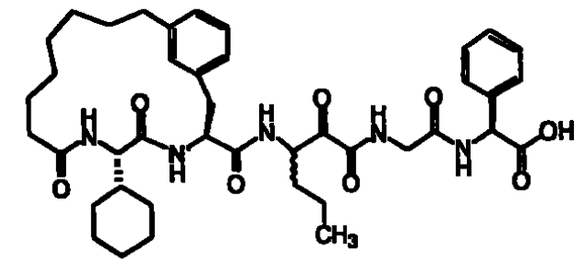


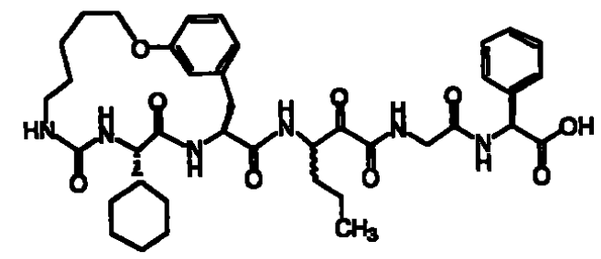


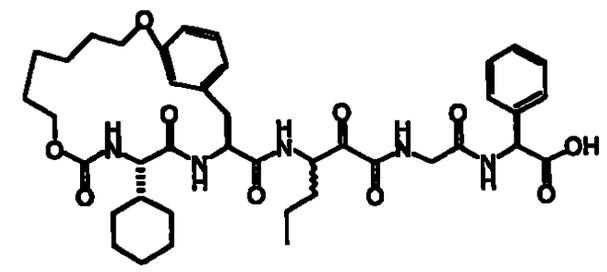


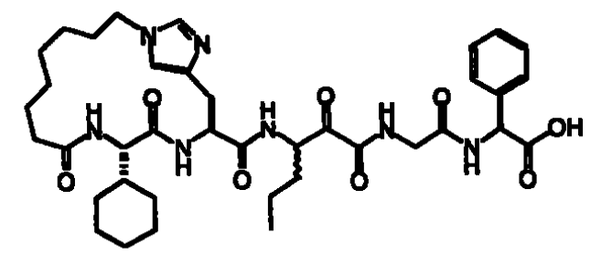
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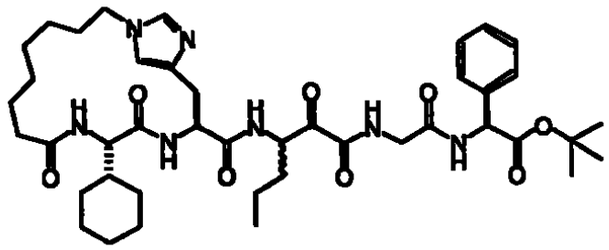


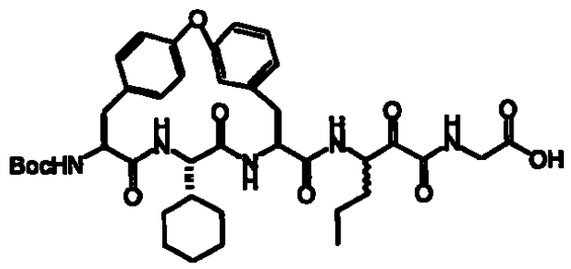


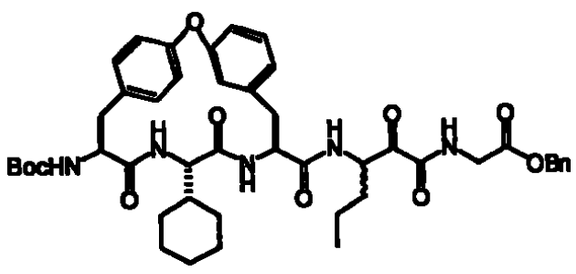


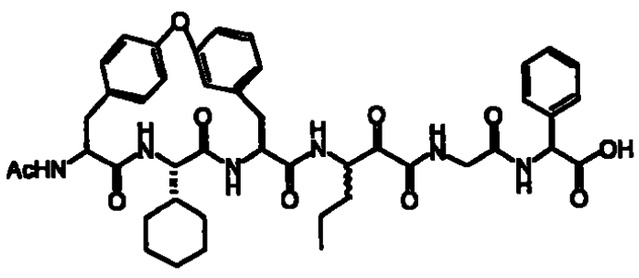


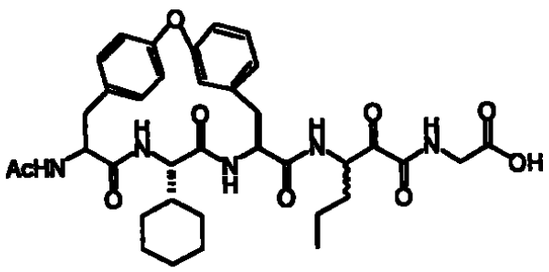
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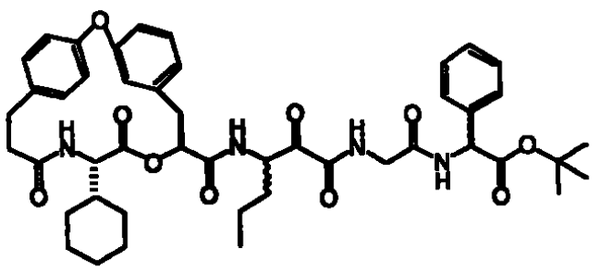


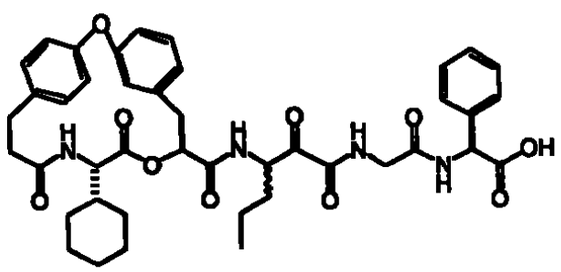

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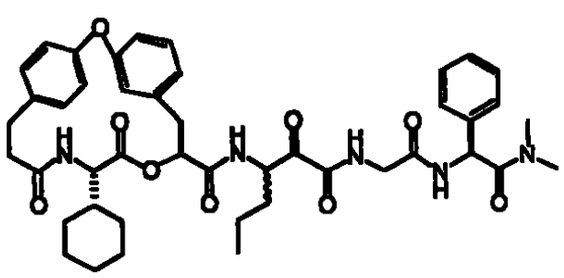

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OBn


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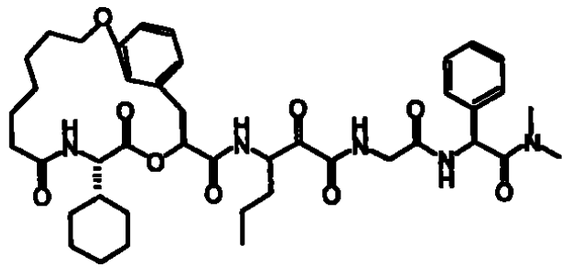


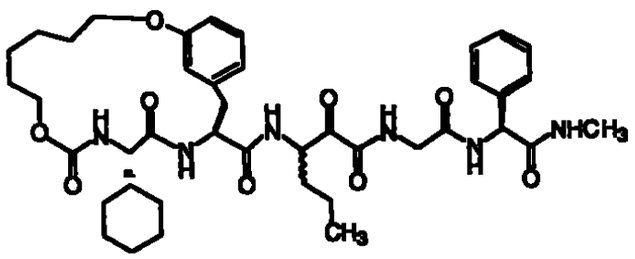


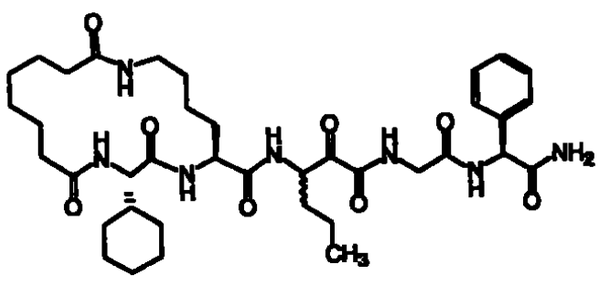


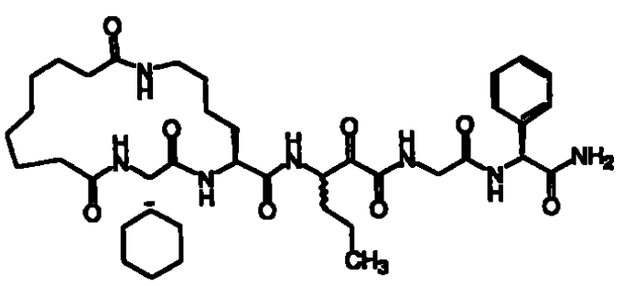


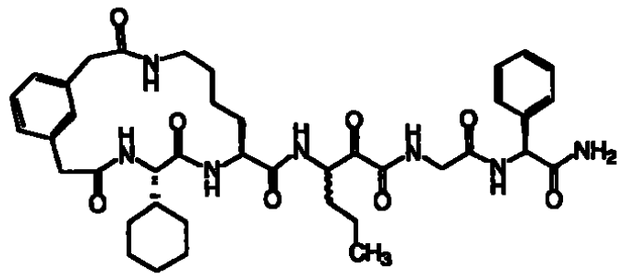
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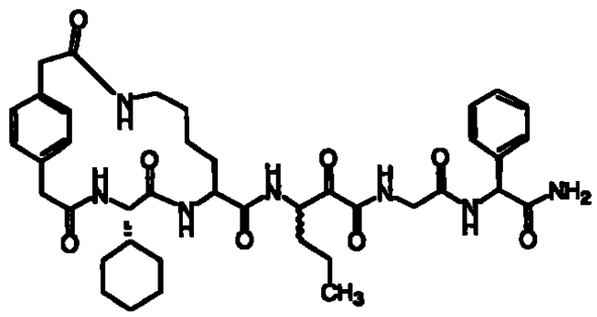


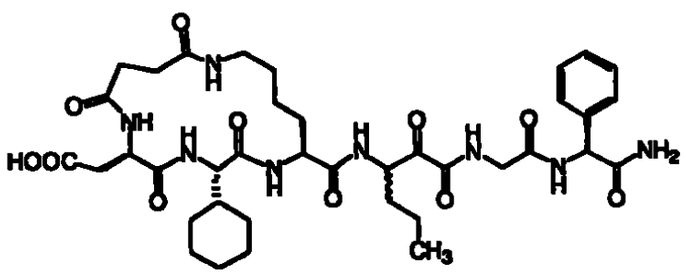

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CH3

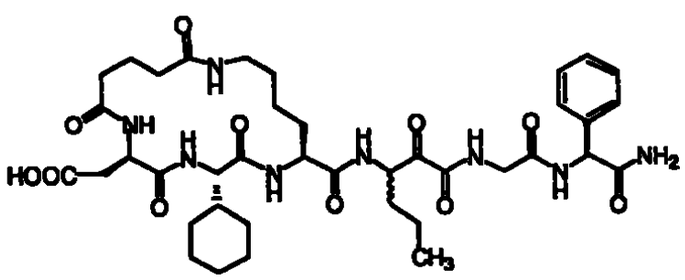

NH2
CH3


NH2
CH3

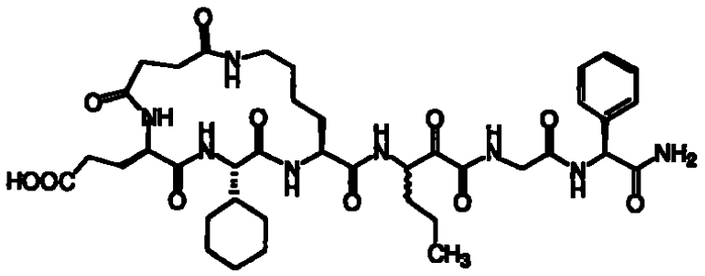


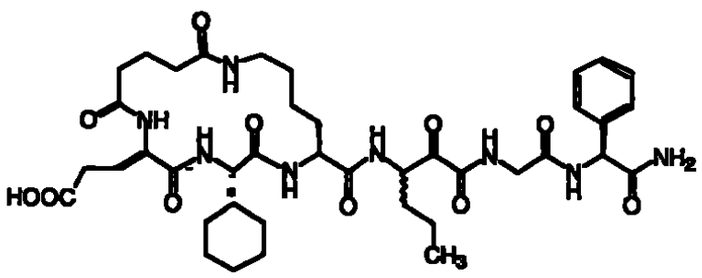


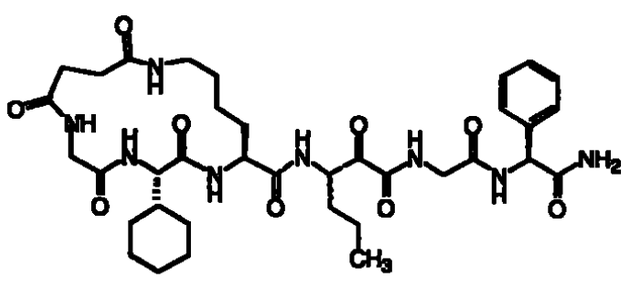


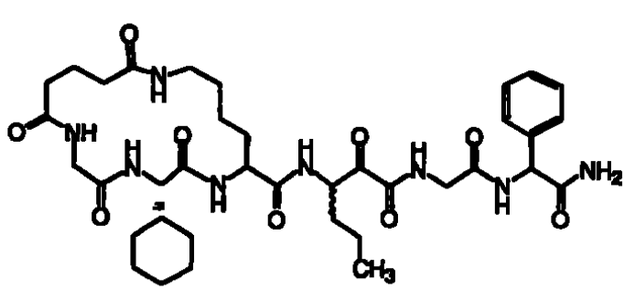


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28 A pharmaceutical composition for treating disorders associated with the HCV protease, said composition comprising therapeutically effective amount of one or more compounds in claim 27 and a pharmaceutically acceptable carrier

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Abstract of the Invention

The present invention discloses novel macrocyclic compounds which have HCV protease inhibitory activity as well as methods for preparing such compounds In another embodiment, the invention discloses pharmaceutical compositions comprising such macrocycles as well as methods of using them to treat disorders associated with the HCV protease

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Attorney Docket No IN01155P

DECLARATION AND POWER OF ATTORNEY FOR PROVISIONAL PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MACROCYCLIC NS-3 SERINE PROTEASE INHIBITORS OF HEPATITIS C VIRUS COMPRISING ALKYL AND ARYL ALANINE P2 MOIETIES

the specification of which is attached hereto

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 C F R §1 56(a)

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith (List name and registration number)

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09078.1001

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POST OFFICE ADDRESS	POST OFFICE ADDRESS 12 RONARM DRIVE	CITY MOUNTAIN LAKES	STATE & ZIP CODE/COUNTRY NEW JERSEY 07046

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

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Date	Date	Date

Signature of Fourth Inventor	Signature of Fifth Inventor	Signature of Sixth Inventor
F GEORGE NJOROGE	VIYYOOR MOOPIL GIRIJAVALLABHAN	ASHIT K GANGULY
Date	Date	Date

Signature of SeventhInventor	Signature of Eighth Inventor	Signature of Ninth Inventor
TIN YAU CHAN	BRIAN ALEXANDER MC KITTRICK	NANHUA HUGH YAO
Date	Date	Date

Signature of Tenth Inventor	Signature of Eleventh Inventor
ANDREW JOSEPH PRONGAY	VINCENT STEWART MADISON
Date	Date

09/710,020

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Industria y Comercio

SUPERINTENDENCIA

REQUISITOS NECESARIOS PARA PRESENTACIÓN Y AGILIZACIÓN DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCIÓN [ ] **MODELO DE UTILIDAD**	[ ]	[ ]
• Indicación de que se solicita la concesión de 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	[ ]	[ ]
COMPLETA [ ] INCOMPLETA	[ ]	[ ]
DISEÑO INDUSTRIAL [ ]		
• Indicación de que se solicita el registro de Diseño Industrial.	[ ]	[ ]
• Datos de identificación del solicitante o de la persona que presenta la solicitud	[ ]	[ ]
• Representación gráfica y foto gráfica del Diseño Industrial o muestra del material que incorpora el diseño	[ ]	[ ]
• Comprobante de pago de las tasas establecidas	[ ]	[ ]
COMPLETA [ ] INCOMPLETA	[ ]	[ ]
ESQUEMA DE TRAZADO [ ]		
• Indicación de que se solicita el registro de un Esquema de trazado	[ ]	[ ]
• Datos de identificación del solicitante o de la persona que presenta la solicitud	[ ]	[ ]
• Representación gráfica del esquema de trazado	[ ]	[ ]
• Comprobante de pago de las tasas establecidas	[ ]	[ ]
COMPLETA [ ] INCOMPLETA	[ ]	[ ]

Firma Responsable

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