(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 648 922 B9**

(12) **CORRECTED NEW EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B3-1)
Corrections, see
Description Paragraph(s) 18, 51

(48) Corrigendum issued on:
03.04.2013 Bulletin 2013/14

(45) Mention of the grant of the patent:
01.12.2010 Bulletin 2010/48

(45) Date of publication and mention of the limitation decision:
B3-1 15.08.2012 Bulletin 2012/33

(21) Application number: **04761605.7**

(22) Date of filing: **02.08.2004**

(51) Int Cl.:
C07K 5/08 *(2006.01)* ***C07K 5/12*** *(2006.01)*

(86) International application number:
PCT/CA2004/001439

(87) International publication number:
WO 2005/012331 (10.02.2005 Gazette 2005/06)

(54) **SPATIALLY-DEFINED MACROCYCLIC COMPOUNDS USEFUL FOR DRUG DISCOVERY**

RÄUMLICH DEFINIERTE MAKROCYCLISCHE VERBINDUNGEN, DIE SICH FÜR DIE ENTDECKUNG VON ARZNEIMITTEL EIGNEN

COMPOSES MACROCYCLIQUES DEFINIS SPATIALEMENT UTILES POUR LA DECOUVERTE DE MEDICAMENTS

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

(30) Priority: **31.07.2003 US 491248 P**

(43) Date of publication of application:
26.04.2006 Bulletin 2006/17

(60) Divisional application:
10011014.7 / 2 319 859
10011035.2 / 2 316 846
10012363.7

(73) Proprietor: **Tranzyme Pharma Inc.**
Sherbrooke,
Quebec J1H 5N4 (CA)

(72) Inventors:
- **DESLONGCHAMPS, Pierre**
  Sherbrooke, Québec J1J 3M6 (CA)
- **DORY, Yves**
  Cookshire, Québec J0B 1M0 (CA)
- **PETERSON, Mark**
  Rock Forest, Québec J1N 2X1 (CA)
- **BENAKLI, Kamel**
  Montréal, Québec H3L 2J5 (CA)
- **MARSAULT, Eric**
  Sherbrooke, Québec J1J 2P6 (CA)
- **OUELLET, Luc**
  Sherbrooke, Québec J1E 1N2 (CA)
- **RAMASESHAN, Mahesh**
  Sunnyvale, CA 94087 (US)
- **VEZINA, Martin**
  Rock Forest, Québec J1N 1R4 (CA)
- **FORTIN, Daniel**
  Montréal, Québec H4P 2R2 (CA)
- **LAN, Ruoxi**
  Arlington, MA 02474 (US)
- **LI, Shigui**
  Arlington, MA 02474 (US)
- **VILLENEUVE, Gérald**
  Montréal, Québec H4L 5M3 (CA)
- **HOVEYDA, Hamid**
  Sherbrooke, Québec J1H 3J4 (CA)
- **BEAUBIEN, Sylvie**
  Sherbrooke, Québec J1L 1L5 (CA)
- **FRASER, Graeme**
  B-1330 Rixensart (BE)

(74) Representative: **Chapman, Paul Gilmour et al**
Marks & Clerk LLP
Aurora
120 Bothwell Street
Glasgow G2 7JS (GB)

EP 1 648 922 B9

(56) References cited:

WO-A-01/25257	WO-A1-96/22304
WO-A1-97/48713	WO-A1-98/46631
WO-A2-01/25257	WO-A2-02/08250
US-A- 3 997 540	US-A- 6 011 155
US-A- 6 165 985	US-B1- 6 479 460

- HARAMURA M ET AL: "DESIGN AND SYNTHESIS OF MOTILIN ANTAGONISTS DERIVED FROM THE [1-4] FRAGMENT OF PORCINE MOTILIN" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 45, no. 3, 31 January 2002 (2002-01-31), pages 670-675, XP001182553 ISSN: 0022-2623
- INGE DEPOORTERE ET AL.: "interaction of the growth hormaône-releasing peptides ghrelin and growth-hormone releasing peptide-6 with the motilin receptor in the rabbit gastric antrum" THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 305, no. 2, 11 February 2003 (2003-02-11), pages 660-667, XP002387465
- KHIAT A ET AL: "IDENTIFICATION OF THE MOTILIDE PHARMACOPHORES USING QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS" JOURNAL OF PEPTIDE RESEARCH, BLACKWELL PUBLISHING LTD., OXFORD, GB, vol. 52, no. 4, 1 October 1998 (1998-10-01), pages 321-328, XP000775977 ISSN: 1397-002X
- BEDNAREK ET AL: "Structure-Function Studies on the New Growth Hormone-Releasing Peptide, Ghrelin: Minimal Sequence of Ghrelin Necessary for Activation of Growth Hormone Secretagogue Receptor 1a" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 43, no. 23, 26 October 2000 (2000-10-26), pages 4370-4376, XP002166937 ISSN: 0022-2623
- KOGA H ET AL: "MACROLIDE-TYPE MOTILIN RECEPTOR AGONISTS: ASSESSMENT OF THE BIOLOGICAL VALUE OF THE 2'- AND 4''-HYDROXYL GROUPS OF ACID-STABLE 8,9-ANHYDROERYTHROMYCIN A 6,9-HEMIACETALS" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 4, no. 13, 1994, pages 1649-1654, XP000650032 ISSN: 0960-894X
- HARAMURA M ET AL: "DESIGN AND SYNTHESIS OF N-TERMINAL CYCLIC MOTILIN PARTIAL PEPTIDES: A NOVEL PURE MOTILIN ANTAGONIST" CHEMICAL AND PHARMACEUTICAL BULLETIN, PHARMACEUTICAL SOCIETY OF JAPAN, TOKYO, JP, vol. 49, no. 1, January 2001 (2001-01), pages 40-43, XP001182539 ISSN: 0009-2363
- TAKANASHI H ET AL: "GM-109: A NOVEL, SELECTIVE MOTILIN RECEPTOR ANTAGONIST IN THE SMOOTH MUSCLE OF THE RABBIT SMALL INTESTINE" JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, AMERICAN SOCIETY FOR PHARMACOLOGY AND, US, vol. 273, no. 2, 1995, pages 624-628, XP002913857 ISSN: 0022-3565
- ROMANOVSKIS P. ET AL.: 'Preparation of head-to-tail cyclic peptides' JOURNAL OF PEPTIDE RESEARCH vol. 52, 1998, pages 356 - 374, XP000788443
- SATO T. ET AL.: 'Medium-sized cyclophanes XV' JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS I 1973, pages 895 - 900, XP008065014
- SIANI M.A. ET AL.: 'Development and screening of a polyketide virtual library' JOURNAL OF MOLECULAR GRAPHICS AND MODELLING vol. 18, 2000, pages 497 - 511, XP004801428

Description

FIELD OF THE INVENTION

[0001] This invention relates to spatially-defined macrocyclic compounds with specific conformational control elements. It also relates to the generation of libraries of these macrocycles. These libraries are then used to select one or more macrocycle species that exhibit a specific interaction with a particular biological target.

BACKGROUND OF THE INVENTION

[0002] Among the variety of compounds that have consistently been found to possess potent and selective biological activity are natural products and peptides. Indeed, members of these classes have become useful pharmaceutical agents. Unfortunately, each type has limitations that have restricted the wider utility of these structures.

[0003] In fact, natural products often have extremely complex structures that are difficult to synthesize, particularly in the combinatorial fashion that would provide access to a greater number of analogues with which to define pharmacophoric elements and best explore modulation of the biological properties of the parent compound. Nevertheless, some efforts have been successful at constructing natural product libraries containing a modest number of analogues.

[0004] Peptides, on the other hand, have been at the forefront of the development of combinatorial chemistry due to their ease of synthesis on solid support, the reproducible and high-yielding reactions involved, and the ready availability of starting materials. Peptides being the endogenous ligands for a number of enzymes and receptors, their modification can be performed to develop even more potent agonists or inhibitors of these same receptors and enzymes. In addition, combinatorial peptide libraries have been used to find a number of previously unknown active sequences for a wide array of enzyme and receptor systems. However, peptidic compounds are plagued by the usual limitations associated with the direct use of peptides as pharmaceuticals, including rapid metabolic degradation by proteases, short pharmacokinetic half-life, difficulty in transport to site of action in tissues and organs, poor oral bioavailability and solubility, potential antigenicity, as well as high manufacturing costs.

[0005] Nevertheless, the densely functionalized and structurally diverse nature of peptides is advantageous when seeking new drug molecules. Hence, peptides are primarily used as the starting point or template for the development of new pharmaceutical leads that often results in structures that only partially resemble, if at all, the initial active peptide. In particular, the recognition potential of the amino acid side chains has resulted in attempts to incorporate these side chains into non-peptldlc rigid scaffolds that attempt to duplicate the conformational display required for optimal interaction between the molecule and the target, as well as mimic standard protein and peptide secondary structural elements. For example, sugars and aromatic rings have been exploited as rigid scaffolds containing amino acids or analogues as pendant moieties at one or more positions. Compounds and combinatorial libraries utilizing 3- and 4-substituted pyrrolidines as a central template for display of interacting functionality have been disclosed in U.S. 5,646,285 (published July 8, 1997) and U.S. 5,891,737 (published April 6, 1999).

[0006] In another approach, cyclic structures can greatly improve the pharmacological and pharmacokinetic profiles of peptides (Molecular Diversity 2000 (pub. 2002), 5, 289-304). Cyclic peptides analogues offer a number of benefits compared with the corresponding linear analogues, including restricted conformational mobility, defined topology, enhanced stability to proteolytic enzymes and modified polarity. Furthermore, cyclic peptides can enhance potency, selectivity, stability, bioavailability and membrane permeability. The stability to enzymatic degradation of the cyclic structure arises from the difficulty of such molecules to attain the extended conformation required to be recognized as a substrate for peptidases. Very large mixture libraries (10^8 members or more) of cyclic peptides have been described in WO 98/54577 (published December 3, 1998).

[0007] However, larger rings are often too flexible and can occupy too many conformations to be useful. Further, their molecular size and resulting physicochemical characteristics do not fit the typical requirements for being "drug-like." Small cyclic peptides containing the key interacting residues would provide the necessary conformational restriction, but may have other disadvantages, including synthetic difficulty, ease of dimerization, unfavorable ring strain caused by the presence of the preferred trans amide bonds, lack of stability towards metabolism and hydrolysis to release that strain and limited topological diversity.

[0008] Most attention in combinatorial chemistry has been devoted to producing diversity in terms of chemical composition. However, essentially no effort has been directed at integrating this with diversity in terms of the crucial three-dimensional structure.

[0009] The use of certain tether elements to control conformation was reported in WO 01125257. However, although those tethers were successful in restricting the conformational display of the molecule, they only were able to duplicate a portion of the spatial region accessible to a linear molecule, which can contain hundreds if not thousands of possible conformations. To better cover the available conformational space, additional tether elements that define new conformations are required. In addition, the tethers in the previous report were generally hydrophobic in nature. This effects

key properties of the macrocyclic molecules such as solubility and log P that are known to have an impact on the compound's pharmacological properties, in particular oral bioavailability. Further, variation of these physicochemical properties is often required in order to optimize the desired characteristic of a molecule as a therapeutic agent. As well, the early tethers were rather limited in their chemical functionality. Since this part of the molecule also could have interactions with a biological target in addition to its conformational control function, a greater diversity in the chemical functional groups could prove advantageous. The more chemically diverse tethers of the present invention therefore have been designed to address these limitations of the existing art and provide the following benefits:

- Access to previously inaccessible conformations
- Modification of physicochemical parameters
- Improvement of pharmacokinetic profile
- Additional interacting functionalities for modulation of biological activity

[0010] Growing evidence suggests that molecular rigidity confers favorable pharmacokinetic properties on molecules and leads to improved clinical success (J. Med. Chem. 2003, 46, 1250-1256; J. Med. Chem. 2002, 45, 2615-2623). The tethers of the present invention therefore will be extremely useful in utilizing these macrocyclic molecules in the search for new pharmaceuticals. Examples of the activity that have been exhibited by representative molecules of the invention are provided.

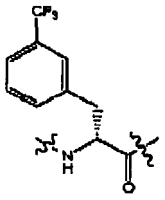
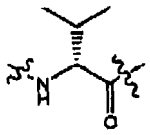
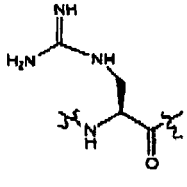
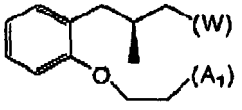
[0011] Therefore, there remains a need for specifically designed chemical entities built on a macrocyclic framework, which exploit the three-dimensional conformation changes triggered by peptidic modifications and/or by inserting specific tether-like portions, In their macrocyclic skeleton.

SUMMARY OF THE INVENTION

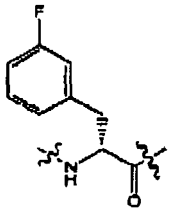
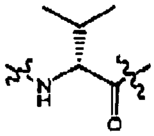
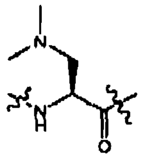
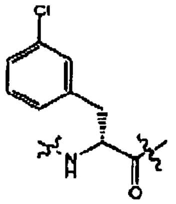
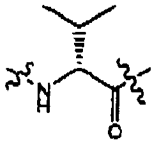
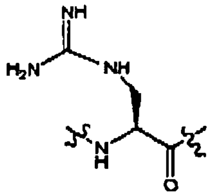
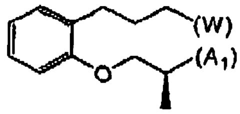
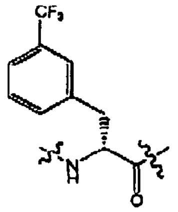
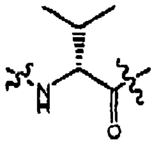
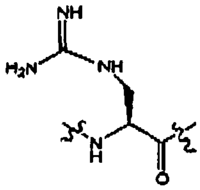
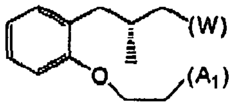
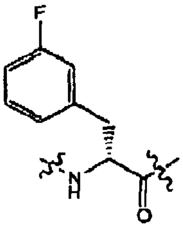
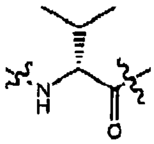
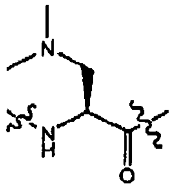
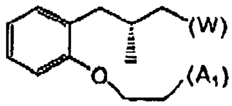
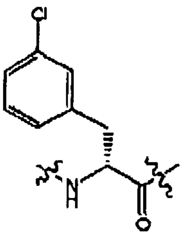
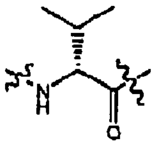
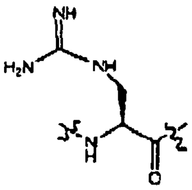
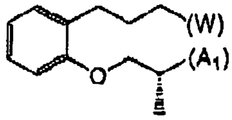
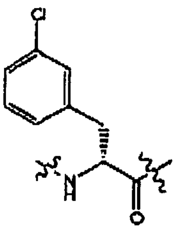
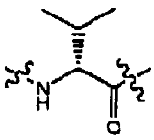
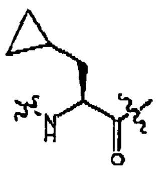
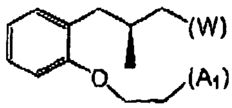
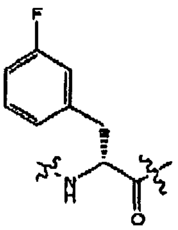
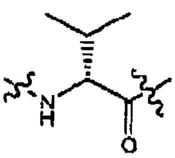
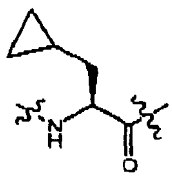
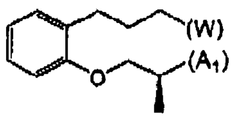
[0012] The present invention is directed towards spatially-defined macrocyclic compounds which incorporate conformational control elements in order to limit their three-dimensional structure to a small number of spatial orientations. These compounds are defined by general formula (II):

(II)

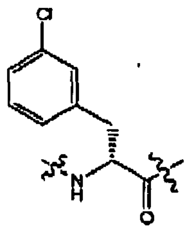
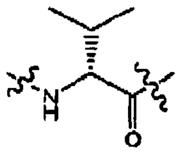
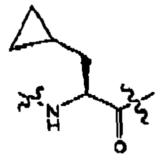
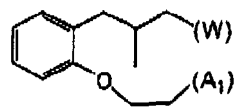
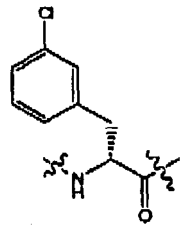
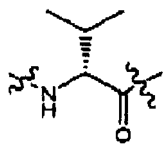
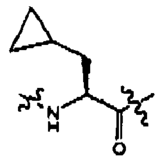
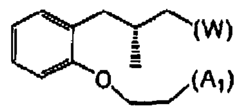
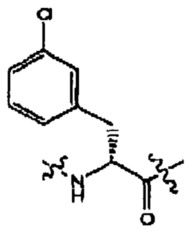
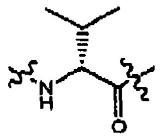
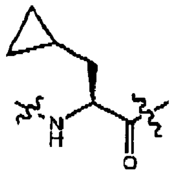
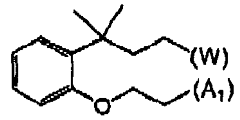
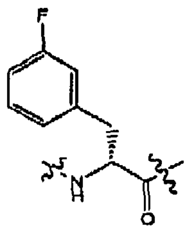
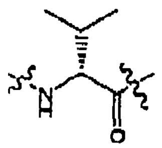
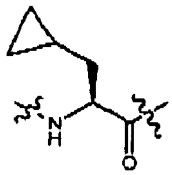
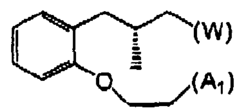
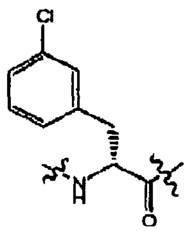
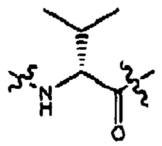
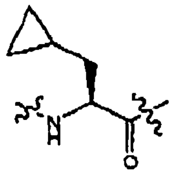
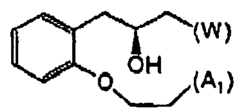
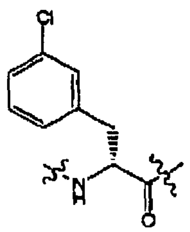
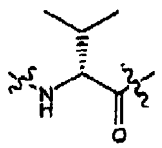
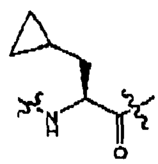
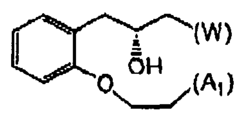
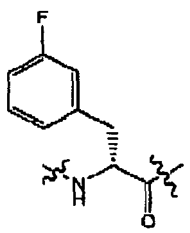
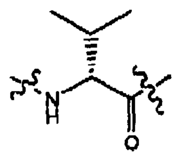
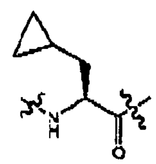
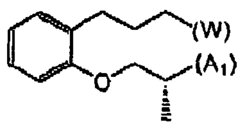
wherein W, A_1, A_2, A_3 and T are defined as below with the NH of A_1 bonded to T, the C=O of A_1 bonded to the NH of A_2, the C=O of A_2 bonded to the NH of A_3, the C=O of A_3 bonded to W, and (W) and (A_1) indicate the site of bonding of T to W and A_1, respectively:

W	A_1	A_2	A_3	T
NH				

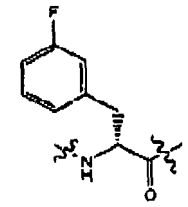
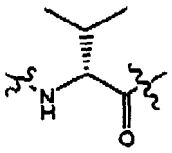
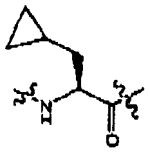
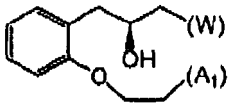
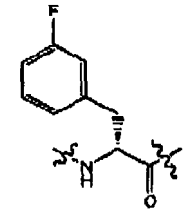
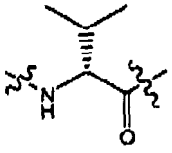
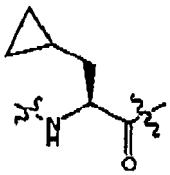
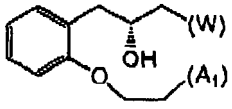
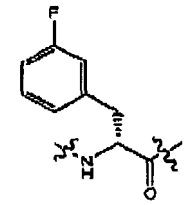
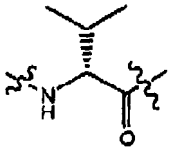
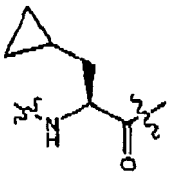
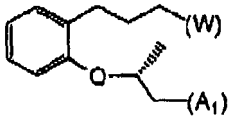
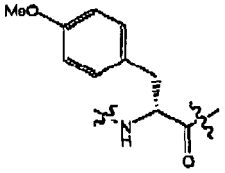
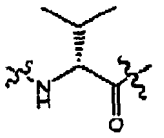
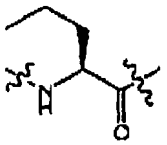
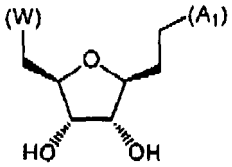
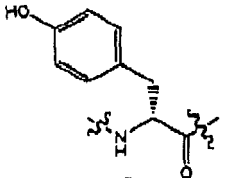
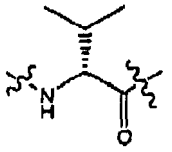
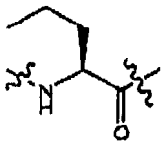
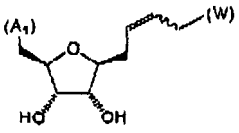
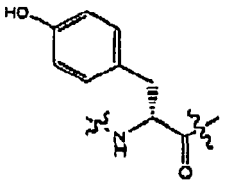
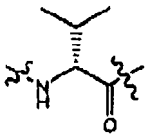
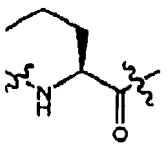
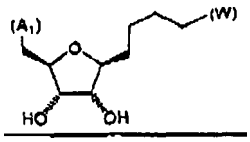
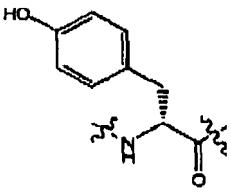
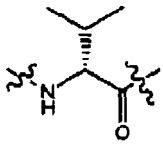
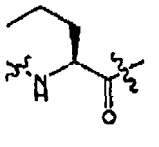
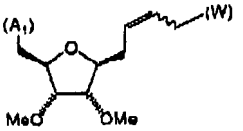
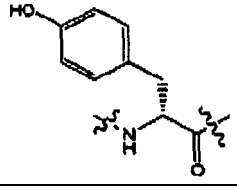
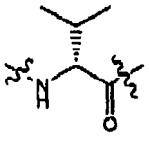
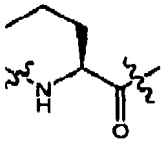
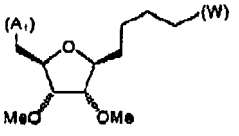
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

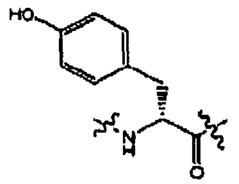
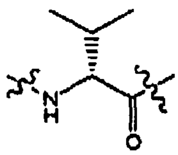
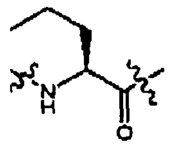
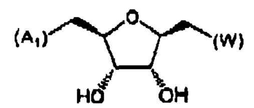
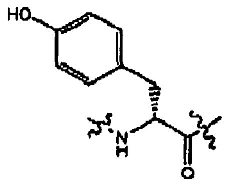
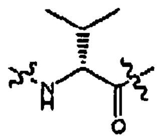
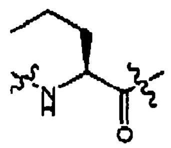
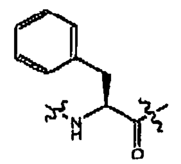
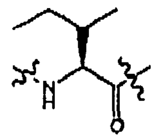
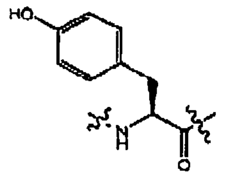
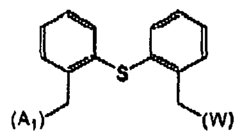
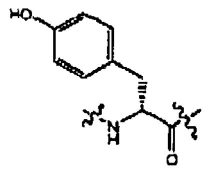
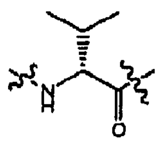
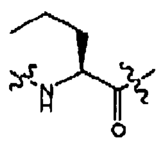
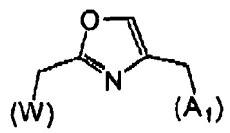
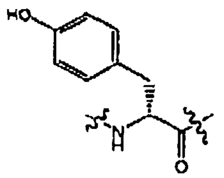
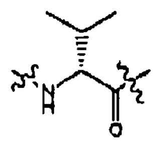
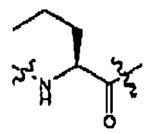
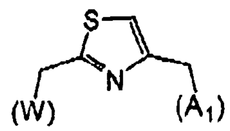
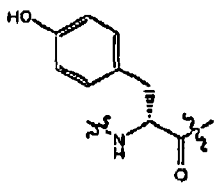
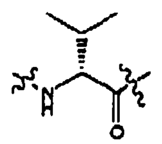
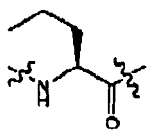
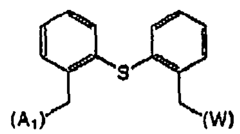
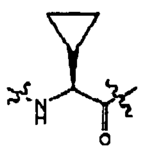
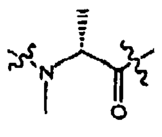
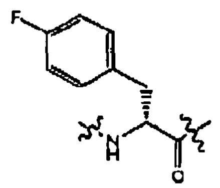
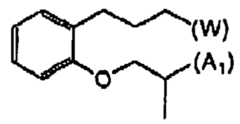
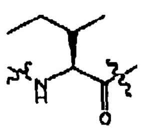
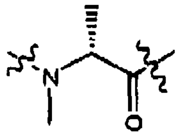
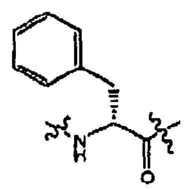
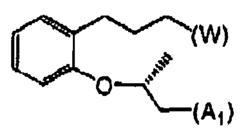
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

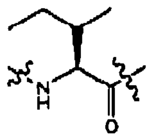
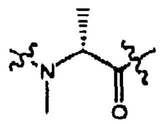
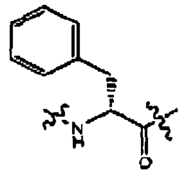
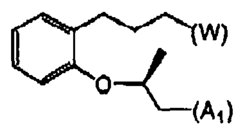
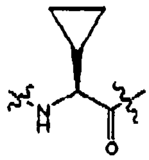
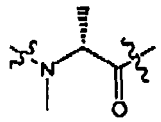
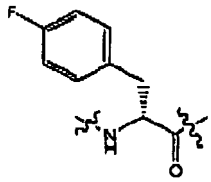
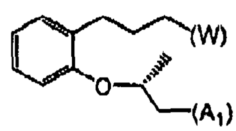
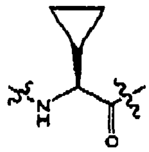
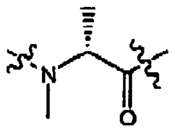
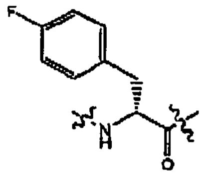
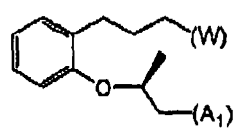
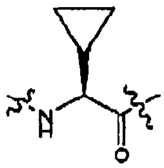
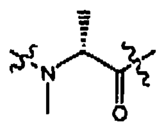
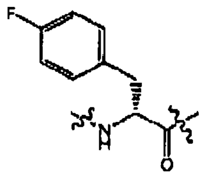
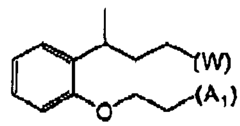
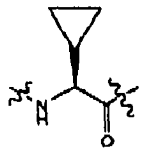
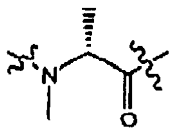
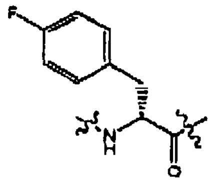
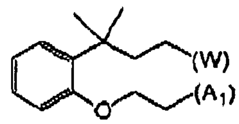
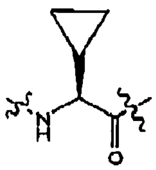
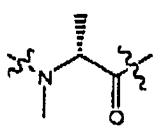
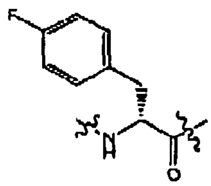
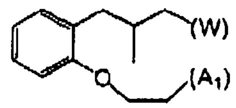
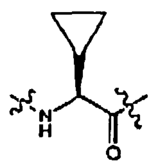
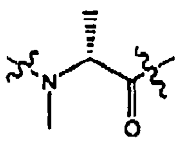
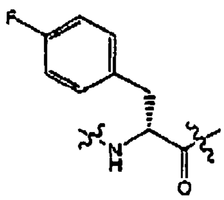
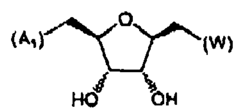
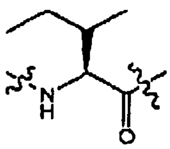
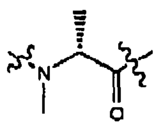
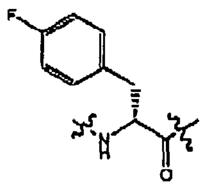
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
O				
O				
O				
O				

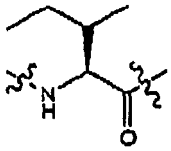
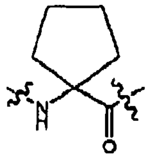
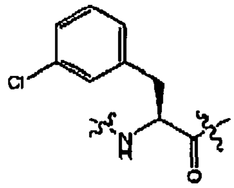
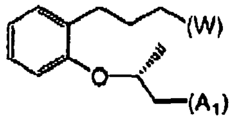
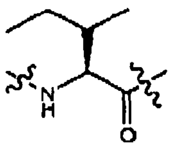
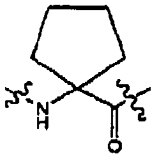
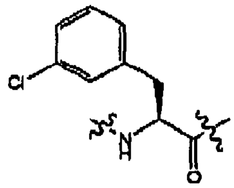
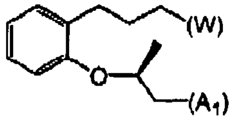
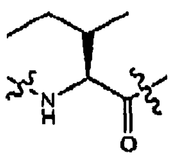
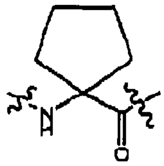
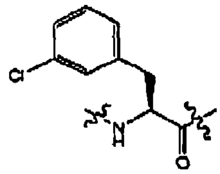
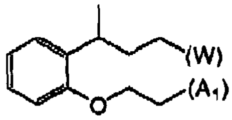
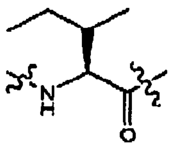
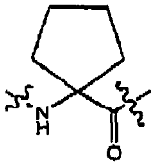
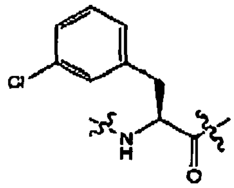
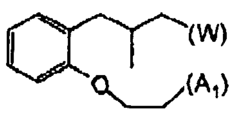
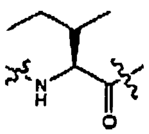
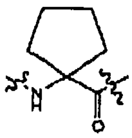
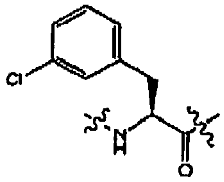
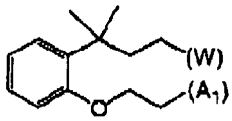
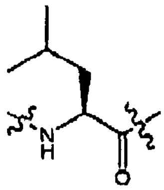
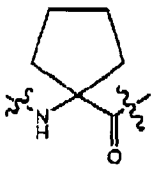
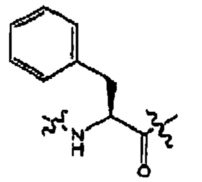
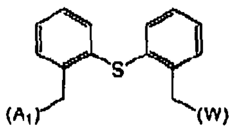
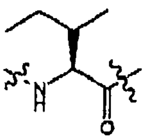
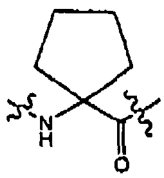
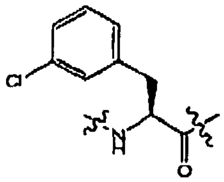
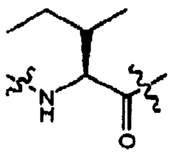
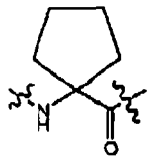
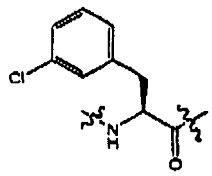
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

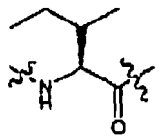
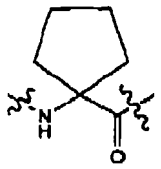
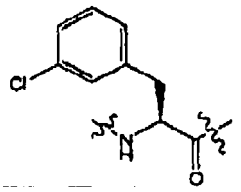
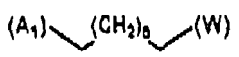
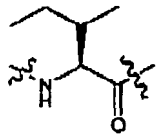
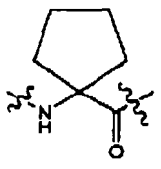
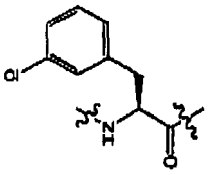
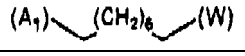
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				(A_1)—$(CH_2)_2$—(W)
NH				(A_1)—$(CH_2)_4$—(W)

(continued)

W	A_1	A_2	A_3	T
NH				
NH				

[0013] Libraries of these compounds are then used to select one or more macrocycle species that exhibit a specific interaction with a particular biological target. Such targets include enzymes and receptors. More particularly, the macrocyclic libraries of the invention serve as a readily accessible source of diverse macrocyclic compounds for use in identifying new biologically active macrocyclic compounds through pharmaceutical candidate screening assays, for use In studies defining structure/activity relationships, and/or for use in clinical investigation.

[0014] In particular, compounds of formula (II) are disclosed as agonists or antagonists of a mammalian motillin receptor and a mammalian ghrelin receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015]

Figure (I) is a general scheme showing one approach to the solid phase synthesis of compounds of the invention. Figure (II) is a general scheme showing a second approach to the solid phase synthesis of compounds of the invention. Figures 3-17 are synthetic schemes that show routes to specific tethers (T) used for the synthesis of compounds of the invention.

DETAILED DESCRIPTION OF THE INVENTION

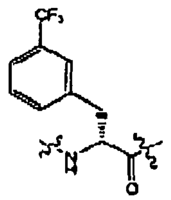
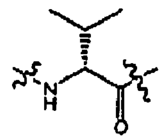
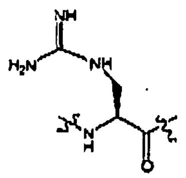
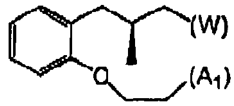
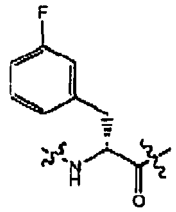
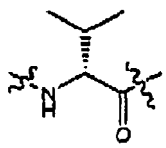
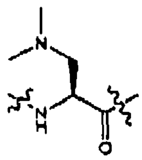
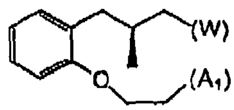
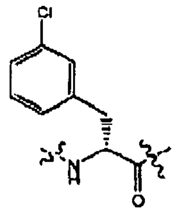
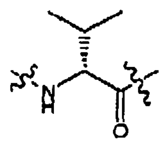
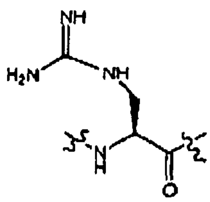
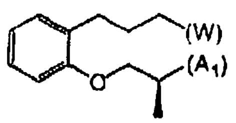
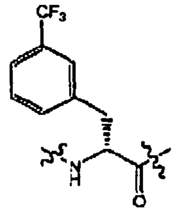
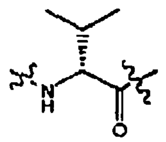
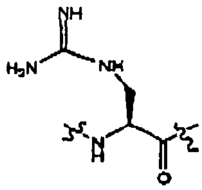
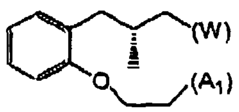
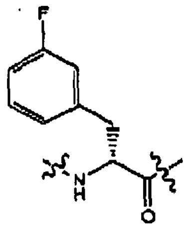
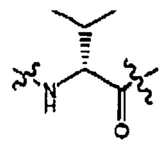
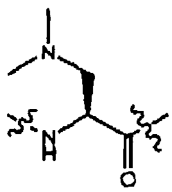
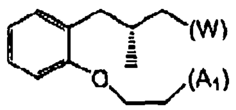
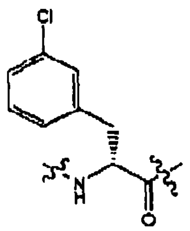
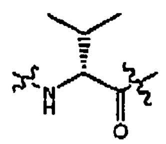
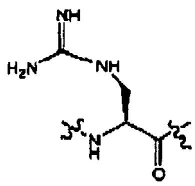
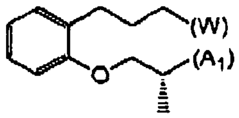
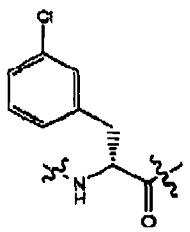
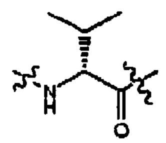
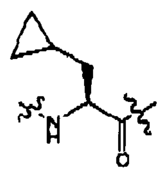
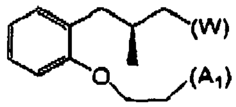
[0016] The macrocyclic compounds of the present invention incorporate a variety of tethers, thus allowing coverage of a specific section of conformational space. Furthermore, these tethers are selected on the basis of their ability to synthetically produce macrocycles in reasonable yield across a wide range of sequences. Accordingly, the compounds of the invention, which incorporate these tethers, represent a wide variety of different conformations, with some more rigid and others more flexible. In addition, some of the tethers are much more rigid in their conformation, sometimes displaying essentially only one low energy form. In these cases, improved biological results would provide excellent information on the specific, optimum bioactive conformation. Additionally, In contrast to many traditional approaches, the same synthetic routes and methods are employed in this optimization process. The ability to rapidly access such information transforms what is usually an extremely difficult and time intensive task into a much more straight forward undertaking.

[0017] As such, this invention permits the simultaneous investigation of chemical and conformational diversity within a single structural framework and therefore possesses great potential for use in increasing the speed and efficiency of research aimed at new pharmaceuticals.

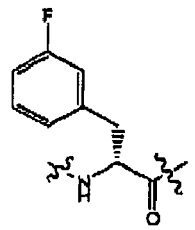
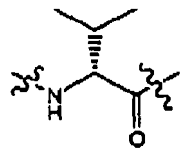
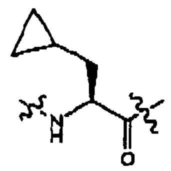
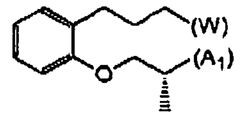
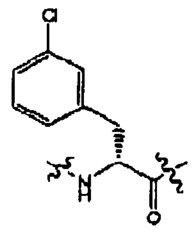
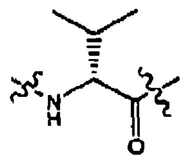
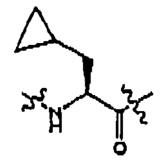
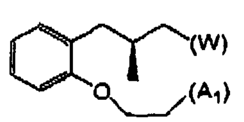
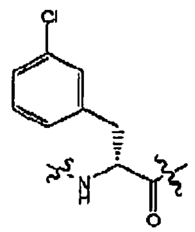
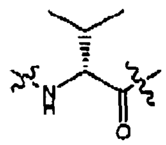
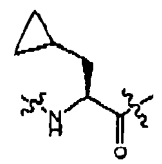
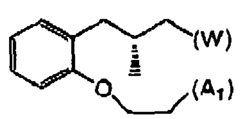
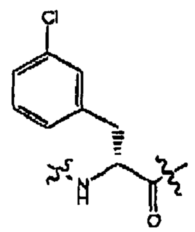
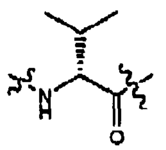
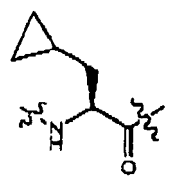
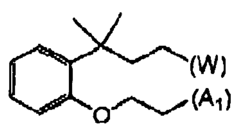
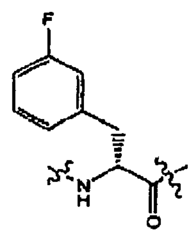
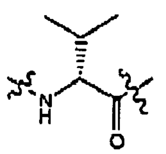
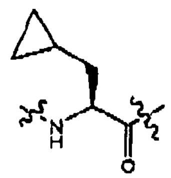
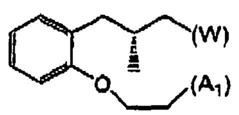
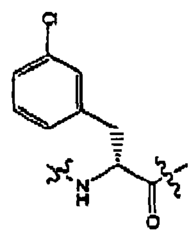
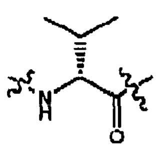
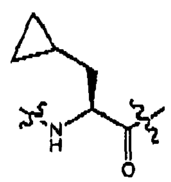
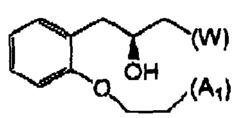
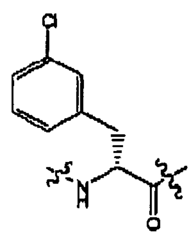
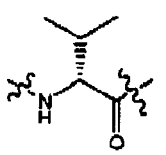
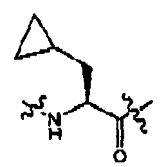
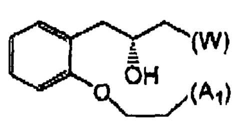
[0018] These compounds are defined by general formula (II):

(II)

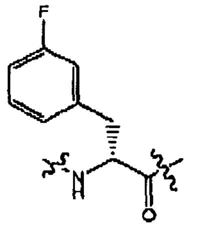
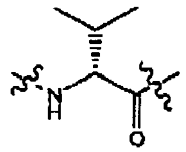
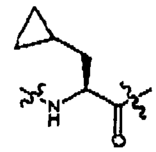
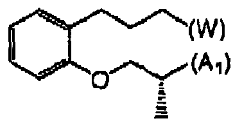
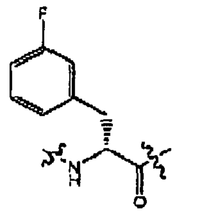
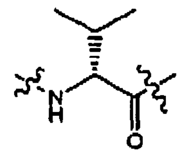
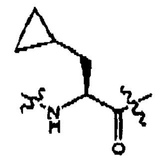
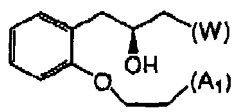
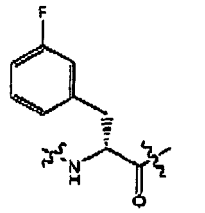
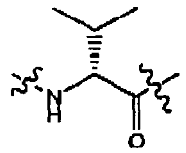
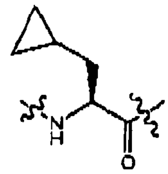
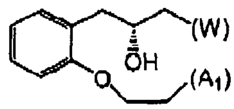
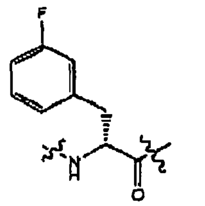
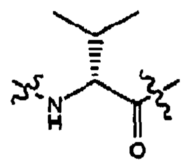
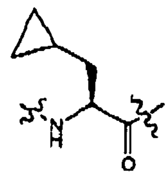
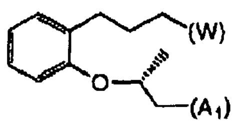
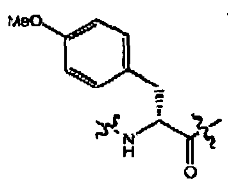
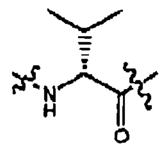
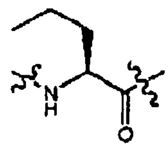
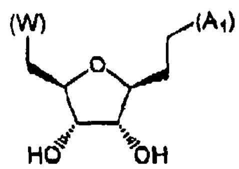
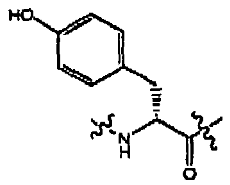
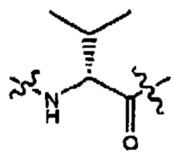
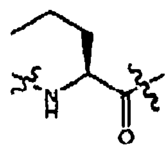
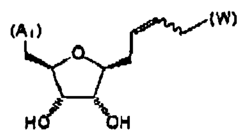
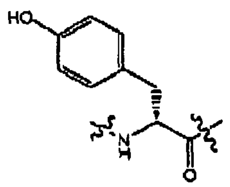
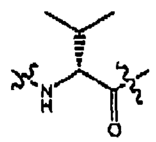
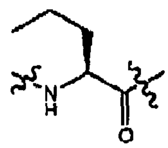
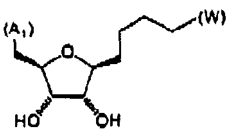
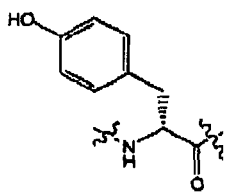
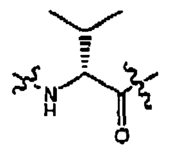
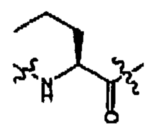
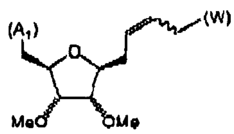
wherein W, A_1 A_2, A_3 and T are defined as below with the NH of A_1 bonded to T, the C=O of A_1 bonded to the NH of A_2, the C=O of A_2 bonded to the NH of A_3, the C=O of A_3 bonded to W, and (W) and (A_1) indicate the site of bonding of T to W and A_1, respectively:

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

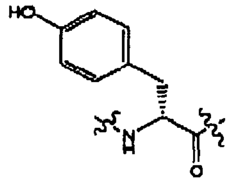
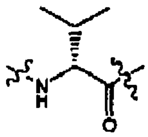
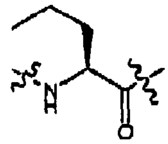
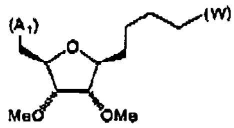
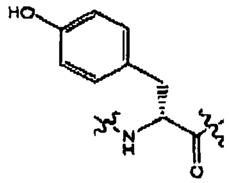
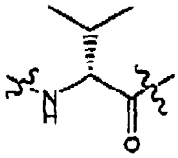
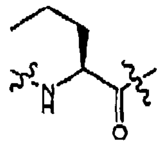
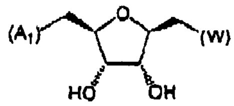
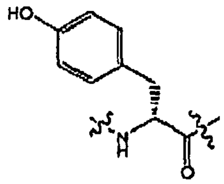
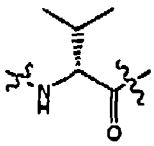
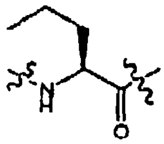
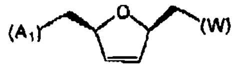
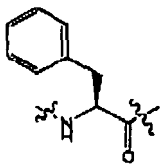
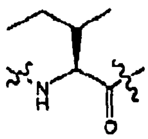
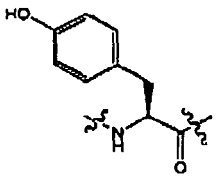
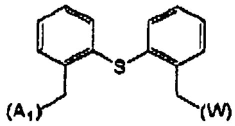
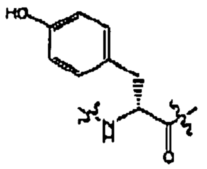
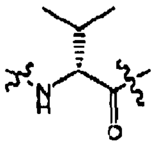
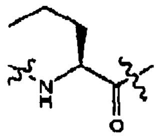
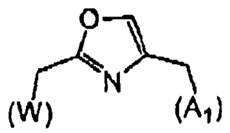
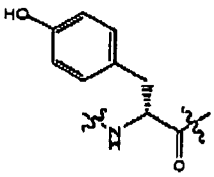
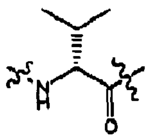
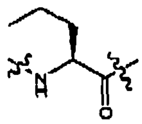
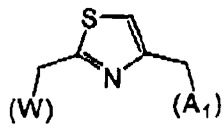
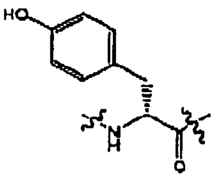
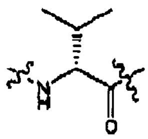
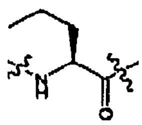
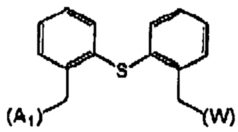
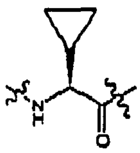
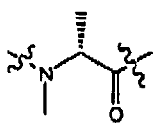
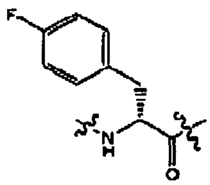
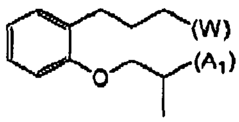
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

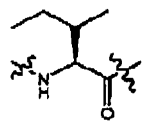
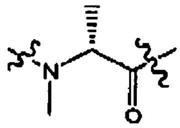
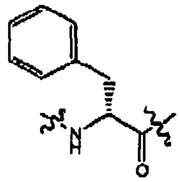
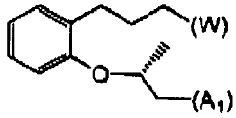
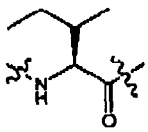
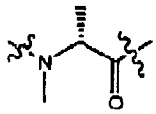
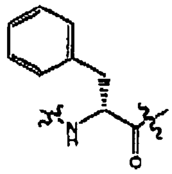
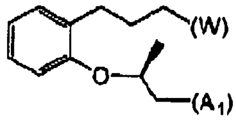
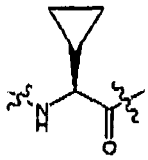
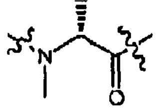
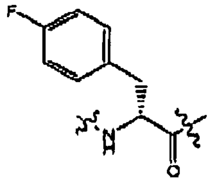
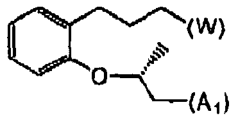
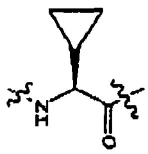
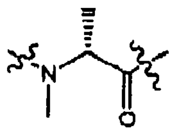
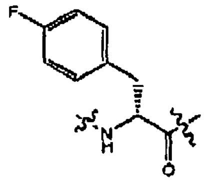
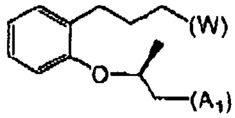
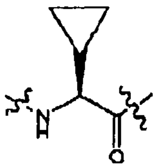
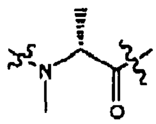
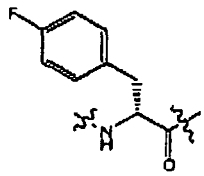
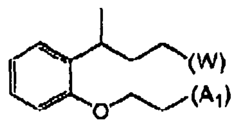
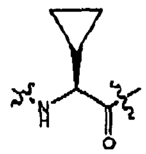
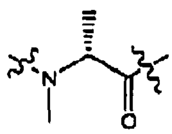
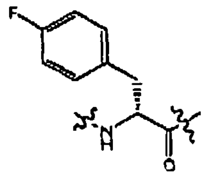
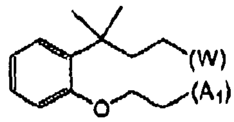
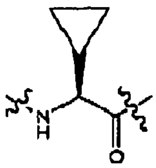
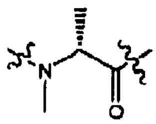
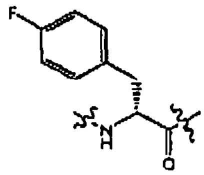
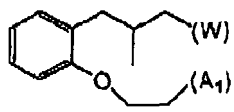
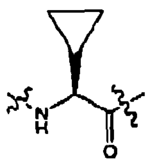
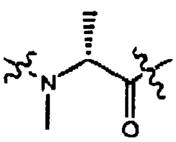
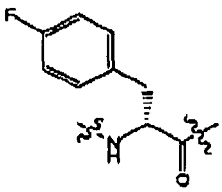
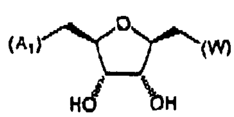
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
O				
O				
O				

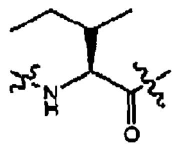
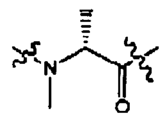
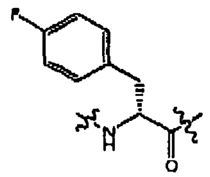
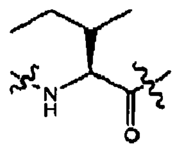
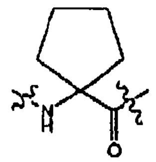
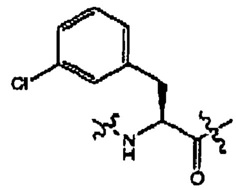
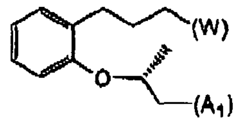
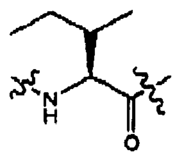
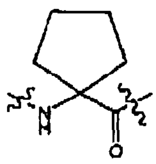
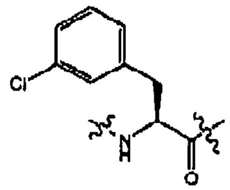
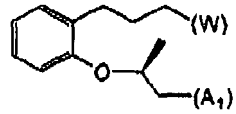
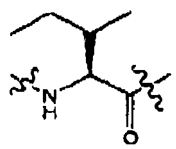
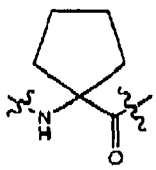
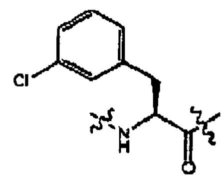
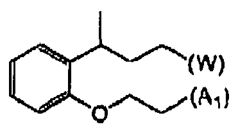
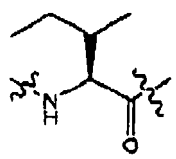
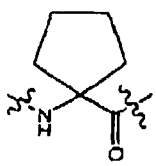
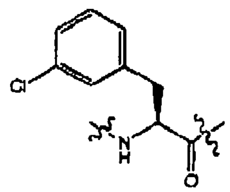
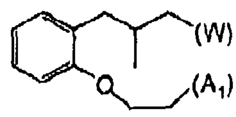
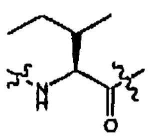
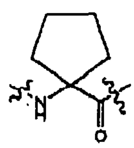
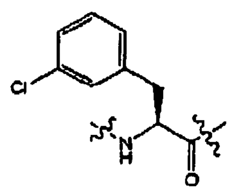
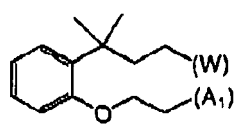
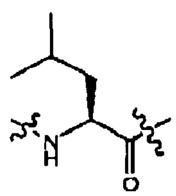
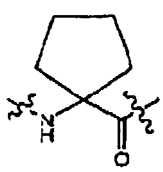
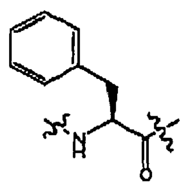
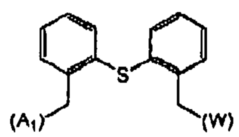
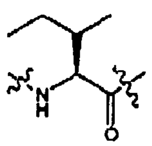
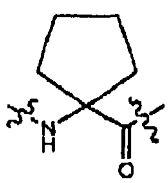
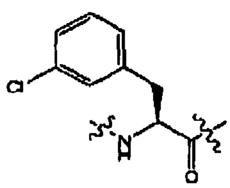
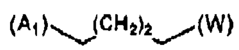
(continued)

W	A_1	A_2	A_3	T
O				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

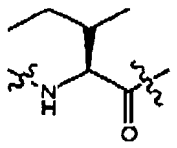
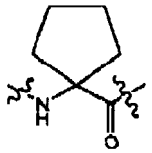
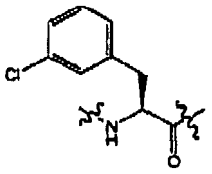
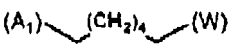
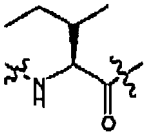
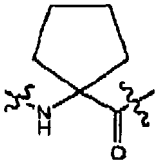
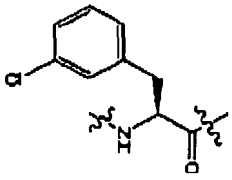
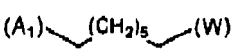
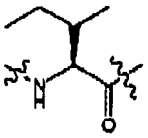
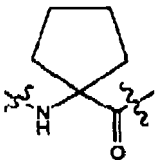
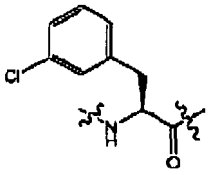
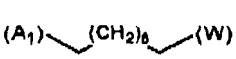
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				

[0019] The present invention has applicability to a broad range of biological targets that likewise represent diverse therapeutic indications. Active compounds initially generated could be further optimized and refined to eventually provide lead clinical candidates. A further advantage of the invention Is that these subsequent steps in the optimization process can be conducted utilizing the same basic chemical synthesis pathway, hence greatly simplifying and speeding up what is typically an extremely time-consuming phase of the overall drug discovery process.

[0020] In particular, the invention provides compounds of formula (II) which are agonists or antagonists of a mammalian motilin receptor and/or a mammalian ghrelin receptor.

Motilin, a linear 22-amino acid peptide, plays a critical regulatory role in the GI physiological system through governing of fasting gastrointestinal motor activity. As such, the peptide is periodically released from the duodenal mucosa during fasting in mammals, including humans. More precisely, motilin exerts a powerful effect on gastric motility through the contraction of gastrointestinal smooth muscle to stimulate gastric emptying, decrease intestinal transit time and initiate phase III of the migrating motor complex in the small bowel. Due to the critical and direct involvement of motilin in control of gastric motility, agents that either diminish (hypomotility) or enhance (hypermotility) the activity at the motilin receptor, are a particularly attractive area for further investigation In the search for new effective pharmaceuticals towards these indications. Macrocyclic antagonists of the motilin receptor are disclosed in U.S. Prov. Pat. Appl. Ser. No. 60/479,223. (WO 2004/111077, published December 23, 2004).

Likewise, ghrelin is a key peptide hormone involved in a number of important physiological functions including growth hormone secretion, maintenance of energy balance, appetite and gut motility. As such, antagonists of this receptor have been investigated for treatment of obesity, while ghrelin agonists have interest in treatment of a variety of diseases, including conditions caused by growth hormone deficiency, wasting syndrome, and GI disorders involving dysmotility.

Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Glu-Lys-Glu-Arg-Atn-Lys-Gla motilin (human, porcine)

Gly-Ser-Ser(Oct)-Phe-Leu-Ser-Pro-Glu-His-Gln-*arg*-*Val*-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg ghrelin (human)

EXAMPLES

Synthesis method

[0021] An assortment of synthetic strategies, involving both solution and solid phase techniques, can be used to access the macrocyclic compounds of the invention, several of which have already been disclosed in WO 01/25257.

[0022] An outline of a first approach to the solid phase synthesis of the compounds of the invention, using a thioester linker strategy is provided in Figure (I). A second approach, called ring-closing metathesis (RCM), is also generally outlined in figure (II).

In both, the construction involves four phases: first is synthesis of the building blocks, comprising mainly recognition elements for interaction at biological targets, plus the key tether moiety, primarily for control and definition of conformation. These building blocks are assembled together, typically in a sequential fashion, in a second phase employing standard chemical transformations and those described in the Standard Procedures herein. The precursors from the assembly are then cyclized in the third stage, which could involve multiple steps, to provide the macrocyclic structures. Finally, a post-cyclization processing stage involving removal of protecting groups and optional purification then provides the desired final compounds.

General Information

[0023] Reagents and solvents were of reagent quality or better and were used as obtained from various commercial suppliers unless otherwise noted. DMF, DCM, DME and THF used are of DriSolv® (EM Science, E. Merck) or synthesis grade quality except for (i) deprotection, (ii) resin capping reactions and (iii) washing. NMP used for the amino acid (AA) coupling reactions is of analytical grade. DMF was adequately degassed by placing under vacuum for a minimum of 30 min prior to use. Boc- and Fmoc-protected amino acids and side chain protected derivatives, including those of N-methyl and unnatural amino acids, were obtained from commercial suppliers or synthesized through standard methodologies known to those in the art. Ddz-amino acids were either synthesized by standard methods, or obtained commercially from Orpegen (Heidelberg, Germany) or Advanced ChemTech (Louisville, KY, USA). Bts-amino acids were synthesized by established procedures. Hydroxy acids were obtained from commercial suppliers or synthesized from the corresponding amino acids as described in the literature (Tetrahedron 1989, 45, 1639-1646; Tetrahedron 1990, 46, 6623-6632; J. Org. Chem. 1992, 57, 8239-6256.; J. Am. Chem. Soc. 1999, 121, 6197-6205). Analytical TLC was performed on precoated plates of silica gel 60F254 (0.25 mm thickness) containing a fluorescent indicator.

[0024] ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer and are referenced internally with respect to the residual proton signals of the solvent. Information about the conformation of the molecules in solution can be determined utilizing appropriate two-dimensional NMR techniques known to those skilled in the art.

[0025] HPLC analyses are performed on a Waters Alliance system 2695 running at 1 mL/min using an Xterra MS C18 column (or comparable) 4.6 x 50 mm (3.5 μm). A Waters 996 PDA provided UV data for purity assessment. An LCPackings splitter (50:40:10) allowed the flow to be separated in three parts. The first part (50%) went to a mass spectrometer (Micromass Platform II MS equipped with an APCI probe) for identity confirmation. The second part (40%) went to an evaporative light scattering detector (ELSD, Polymer Laboratories, PL-ELS-1000) for purity assessment and the last portion (10%) to a chemiluminescence nitrogen detector (CLND, Antek Model 8060) for quantitation and purity assessment. Data was captured and processed utilizing the most recent version of the Waters Millennium software package.

[0026] Preparative HPLC purifications were performed on final deprotected macrocycles using the Waters FractionLynx system, on an XTerra MS C18 column (or comparable) 19 x 100mm (5 μm). The injections were done using an At-Column-Dilution configuration with a Waters 2767 injector/collector and a Waters 515 pump running at 2 mL/min. The mass spectrometer, HPLC, and mass-directed fraction collection are controlled via MassLynx software version 3.5 with FractionLynx. Fractions (13 x 125 mm tubes) shown by MS analysis to contain the product were evaporated under reduced pressure, most typically on a centrifugal evaporator system (Genevac HT-4, ThermoSavant Discovery, SpeedVac or comparable) or, alternatively, lyophilized. Compounds were then thoroughly analyzed by LC-MS-UV-ELSD-CLND analysis for identity confirmation, purity and quantity assessment.

[0027] Automated medium pressure chromatographic purifications were performed on an Isco CombiFlash 16x system with disposable silica or C18 cartridges that permitted up to sixteen (16) samples to be run simultaneously. MS spectra were recorded on a Waters Micromass Platform II or ZQ system. HRMS spectra were recorded with a VG Micromass ZAB-ZF spectrometer. Chemical and biological information were stored and analyzed utilizing the ActivityBase database software (IDBS, Guildford, Surrey, UK).

[0028] The term "concentrated/evaporated/removed under reduced pressure" indicates evaporation utilizing a rotary evaporator under either water aspirator pressure or the stronger vacuum provided by a mechanical oil vacuum pump as appropriate for the solvent being removed. "Dry pack" indicates chromatography on silica gel that has not been pretreated with solvent, generally applied on larger scales for purifications where a large difference in R_f exists between the desired product and any impurities. "Flash chromatography" refers to the method described as such in the literature and is applied to chromatography on silica gel (230-400 mesh, EM Science) used to remove impurities some of which may be close in R_f to the desired material. Methods specific for solid phase chemistry are detailed separately.

General Methods for Solid Phase Chemistry

[0029] These methods can be equally well applied for the synthesis of single compounds or small numbers of compounds, as well as for the synthesis of libraries of compounds of the present invention.

For solid phase chemistry, the solvent choice is important not just to solubilize reactants as in solution chemistry, but

also to swell the resin. Certain solvents interact differently with the polymer matrix depending on its nature and can affect this swelling property. As an example, polystyrene (with DVB cross-links) swells best in nonpolar solvents such as DCM and toluene, while shrinking when exposed to polar solvents like alcohols. In contrast, other resins such as PEG-grafted ones like TentaGel, maintain their swelling even in polar solvents. For the reactions of the present invention, appropriate choices can be made by one skilled in the art. In general, polystyrene-DVB resins are employed with DMF and DCM common solvents. The volume of the reaction solvent required is generally 1-1.5 mL per 100 mg resin. When the term "appropriate amount of solvent" is used in the synthesis methods, it refers to this quantity. The recommended quantity of solvent roughly amounts to a 0.2 M solution of building blocks (linkers, amino acids, hydroxy acids, and tethers, used at 5 eq relative to the Initial loading of the resin). Reaction stoichiometry was determined based upon the "loading" (represents the number of active functional sites, given as mmol / g) of the starting resin.

The reaction can be conducted in any appropriate vessel, for example round bottom flask, solid phase reaction vessel equipped with a fritted filter and stopcock, or Teflon-capped jar. The vessel size should be such that there Is adequate space for the solvent, and that there is sufficient room for the resin to be effectively agitated taking into account that certain resins can swell significantly when treated with organic solvents. The solvent/resin mixture should fill about 60% of the vessel. Take note that all agitations for solid phase chemistry are best conducted with an orbital shaker (for example Forma Scientific, model 430, 160-180 rpm), except for those where scale makes use of gentle mechanical stirring more suitable, to ensure adequate mixing which is generally accepted to be important for a successful reaction.

[0030] The volume of solvent used for the resin wash is a minimum of the same volume as used for the reaction, although more is generally used to ensure complete removal of excess reagents and other soluble residual by-products. Each of the resin washes specified in the Examples should be performed for a duration of at least 5 min with agitation (unless otherwise specified) in the order listed. The number of washings Is denoted by "nx' together with the solvent or solution, where n is an integer. In the case of mixed solvent washing systems, both are listed together and denoted solvent 1/solvent 2. The ratio of the solvent mixtures DCM/MeOH and THF/MeOH used in the washing steps is (3:1) in all cases. Other mixed solvents are as listed. After washing, drying in the "standard manner" means that the resin is dried first in air (1 h), and subsequently under vacuum (oil pump usually) until full dryness is attained (minimum 30 min, to O/N).

[0031] For representative examples of the new tether moieties disclosed herein, the synthetic routes presented in Figures 3-17 are employed with additional information on selected examples presented further below. Although the routes described represent a specific protection strategy, other suitable protecting groups known in the art can also be employed.

Example T12: Standard Procedure for the Synthesis of Tether T12

[0032] For an outline of this route, see Figure 3. In a 3-L flame-dried three-neck flask, a solution of (aminomethyl)phenylthiobenzyl alcohol (**12-0,** 96 g, 0.39 mol) in degassed DMF (1 L, 0.4 M) was prepared. To this was added Ddz-N_3 (0.95 eq), followed by TMG (0.39 mol, 49 mL). The reaction was stirred for 10 min, then DIPEA (68 mL, 0.39 mol) added. The mixture was heated at 50°C under N_2 until TLC indicated no Ddz-N_3 remained (48 h typically). (TLC eluent: EtOAc: Hex 50:50; detection: ninhydrin). Upon completion, to the reaction mixture was added 3 L citrate buffer and the separated aqueous layer extracted with Et_2O (3 x 1500 mL). The combined organic phase was washed sequentially with citrate buffer (2 x 200 mL), water (2 x 200 mL) and brine (2 x 200 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate evaporated under reduced pressure. A dark orange oil was obtained, which was purified by dry-pack. For this procedure, the oil was first dissolved in EtOAc:Hex:DCM:TEA (20:80:1:0.5, v/v/v/v). At this point, a little extra DCM was sometimes required to ensure complete dissolution. The solution was loaded onto the column, then the column eluted with EtOAc:Hex:DCM:Et_3N (20:80:1:0.5) until all the impurities were separated out as indicated by TLC, paying particular attention to that closest to the desired product. The elution was then continued with EtOAc:hexanes:Et_3N 30:70:0.5 (v/v/v) and finally with EtOAc:hexanes:Et_3N (50:50:0.5) to elute the desired product. After removal of the solvent from the fractions containing the product under reduced pressure, the residue was dissolved in the minimum amount of DCM, a three-fold larger volume of hexanes added, then the solvents again evaporated under reduced pressure. This treatment was repeated until an off-white foam was obtained. The latter solidified while drying under vacuum (oil pump). Alternatively, the material yielded a solid after sequential concentration with DCM (1x) and hexanes (2x). Tether **T12** was obtained as an off-white solid (85-90% yield).

Example T13: Standard Procedure for the Synthesis of Tether T13

[0033] Protected versions of tether **T13** are accessed through a route (see Figure 4) analogous to that described below in more detail for **T14,** except starting from H-Ser-OEt·HCl, in an overall yield of 14-30% for the 6 step sequence.

[0034] ^{1}H NMR ($CDCl_3$): δ 7.53 (1H, s, RR'C=C<u>H</u>-O), 6.42-6.58 (2H, m, Ph), 6.30-6.38 (1H, m, Ph), 5.40-5.50 (1H, m, NH), 4.57 (2H, s, C<u>H</u>$_2$OH), 4.40 (2H, d, C<u>H</u>$_2$NHDdz), 3.78 (6H, s, 2X(C<u>H</u>$_3$OPh)), 2.23-2.00 (1H, broad, O<u>H</u>), 1.76

(6H, s, RR'C(CH_3)$_2$).
^{13}C NMR ($CDCl_3$); δ 162, 161, 155, 149, 141, 136, 103, 99, 82, 57, 56, 39, 29.

Example T14: Standard Procedure for the Synthesis of Tether T14

[0035] See Figure 5 for an outline of the synthetic scheme.

Step T14-1: A solution of 4.4 M sodium methoxide In MeOH (1.0 mL, 4.6 mmol, 0.01 eq) in DCM (300mL) at 0°C was diluted with MeOH (35 mL). Dichloroacetonitrile (50 g, 455 mmol, 1.0 eq) was added over 45 min and the resulting mixture stirred at 0°C for 1 h. L-Cysteine ethyl ester hydrochloride (84.5 g, 455 mmol, 1.0 eq) was added and the reactionstirred O/N at rt. The reaction mixture was diluted with DCM and water. The separated aqueous phase was extracted with DCM (2x). The combined organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. The crude product obtained was acceptable for use in the next step without further purification.

Step T14-2: To a solution of the crude product from step T14-1 (455 mmol based on the theoretical yield) in DCM (500 mL) was added DIPEA (119 mL, 652.5 mmol, 1.5 eq). The resulting mixture was stirred at 50°C for 5 h, then at rt O/N. The reaction was monitored by TLC (30% EtOAc: 70% Hex; detection: UV and CMA, R_f = 0.29). Upon completion, the reaction mixture was diluted with DCM and water. The separated aqueous phase was extracted with DCM (2x). The combined organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. ^{1}H NMR was used to verify the purity and identity of the intermediate compound. The crude product obtained was acceptable for use in the next step without further purification (yield: 100%).

Step T14-3: To a solution of the crude product from step T14-2 (77 g, 375 mmol, 1.0 eq) in DMF (500 mL) was added sodium azide (122 g, 1874 mmol, 5.0 eq). The resulting mixture was mechanically stirred at 65°C O/N. The reaction was monitored by ^{1}H NMR because the starting material and product co-eluted on TLC. After completion and cooling to rt, the reaction mixture was diluted with Et_2O and an aqueous solution of saturated NH_4Cl. The separated aqueous phase was extracted with Et_2O (2x). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. ^{1}H NMR was used to verify the purity and identity of the intermediate compound. The crude product obtained was acceptable for use in the next step without further purification (yield: 93%).

Step T14-4: To a solution of the crude azide from step T14-3 (73.1 g, 345 mmol, 1.0 eq) in 95% EtOH (700 mL) was added 10% Pd/C (18.3 g, 17.3 mmol, 0.05 eq). Hydrogen gas was bubbled Into the suspension for 1h, then the resulting mixture stirred O/N with a balloon of hydrogen. The reaction was monitored by TLC (30% EtOAc: 70% Hex; detection: UV and ninhydrin.). The final product remained at the baseline and was positive to ninhydrin. If the reaction was not complete as indicated by TLC, another portion of 10% Pd/C (25% of that originally used) was added, hydrogen bubbled through the solution and the resulting suspension was stirred at rt again O/N. The reaction solution was filtered through a Celite pad and the pad rinsed thoroughly with EtOAc (until no further product was being recovered as indicated by TLC). ^{1}H NMR was used to verify the purity and identity of the intermediate compound. The crude product obtained was acceptable for use in the next step without further purification (yield: 93%).

Step T14-5: To a solution of the crude amine from step T14-4 (59.5 g, 320 mmol, 1.0 eq) in degassed (maintained on vacuum pump for 1 h) DMF (200 mL) were sequentially added Ddz-N_3 (93.3 g, 352 mmol, 1.1 eq), TMG (40.1 mL, 320 mmol, 1.0 eq) and DIPEA (55.8 mL, 320 mmol, 1.0 eq). The resulting solution was stirred at rt for 2 d. The reaction was monitored by TLC (100% EtOAc; detection: UV and ninhydrin, R_f = 0.52). Upon completion, the reaction mixture was diluted with Et_2O and an aqueous solution of citrate buffer (1 M). The separated aqueous phase was extracted with Et_2O (2x). The combined organic phase was washed with citrate buffer (1 M, 2x), water (2x), and brine (2x), then dried over $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by dry-pack (20% EtOAc: 80% Hex to 50% EtOAc: 50% Hex) to give the protected amino ester as a yellow solid. ^{1}H NMR was used to verify the identity of the intermediate compound (yield: 65%).

Step T14-6: To a solution of the protected amino ester from step T14-5 (10.5 g, 25.7 mmol, 1.0 eq) in THF (150 mL) at 0°C were added lithium borohydride (1.68 g, 77.1 mmol, 3.0 eq) and MeOH (3.1 mL, 77.1 mmol, 3.0 eq). The resulting mixture was stirred for 1 h, then identical portions of lithium borohydride and MeOH were added. The resulting mixture was stirred at rt for 3 h. The reaction was monitored by TLC (5% MeOH, 95% EtOAc; detection: UV and ninhydrin, R_f = 0.27. Note that the boronate co-eluted with the starting material, but after quenching, this spot disappeared). The reaction mixture was cooled to 0°C and water was added very slowly (100-150 mL) to quench

the reaction. On larger scales, the salts generated in the reaction were not completely soluble in the aqueous phase at this stage which complicated the extraction and led to lower yields. The resulting mixture was then stirred O/N. The aqueous phase was extracted with EtOAc (4x). The organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. The compound was purified by flash chromatography (3% MeOH, 97% EtOAc) to give tether **Ddz-T14** as a pale yellow solid (yield: 67%).
^1H NMR ($CDCl_3$, ppm): 7.53 (1H, s, RR'C=C<u>H</u>-S), 6.42-6.58 (2H, m, Ph), 6.35 (1H, t, Ph), 5.60-5.50 (1H, m, N<u>H</u>), 4.75 (2H, s, C<u>H</u>$_2$OH), 4.60 (2H, d, C<u>H</u>$_2$NHDdz), 3.78 (6H, s, 2x(C<u>H</u>$_3$OPh)), 2.70-2.50 (1H, broad, O<u>H</u>), 1.76 (6H, s, RR'C(C<u>H</u>$_3$)$_2$).
^{13}C NMR ($CDCl_3$, ppm): 170, 161, 157, 156, 149, 116, 103, 99, 82, 61, 58, 42, 29.

Example T21: Standard Procedure for the Synthesis of Tether T21

[0036] See Figure 6 for an outline of the synthetic scheme that provides the multi-step protocol for this tether containing methyl ether protection for its secondary hydroxyl groups. Alternative protection that is easier to remove, such as the acetonide, is also possible via this route.

Example T22: Standard Procedure for the Synthesis of Tether T22

[0037] An outline of the synthetic scheme that provides efficient routes to the diastereomeric forms of this tether is shown in Figure 7.

Example T24: Standard Procedure for the Synthesis of Tether T24

[0038] The synthetic approach to this tether is shown in Figure 8.

Example T24: Standard Procedure for the Synthesis of Tether T26

[0039] The synthetic scheme that provides this tether is shown in Figure 9.
MW Calc. for $C_{18}H_{25}NO_6$, 351.39; MS found $(M+H)^+$ 352

Example T33: Standard Procedure for the Synthesis of Tether T33

[0040] An outline of the synthetic scheme towards this chiral tether is shown in Figure 10. The enantiomers are accessed depending on the configuration of the starting lactic acid derivative with the (R)-isomer coming from (S)-methyl lactate and the (S)-isomer of **T33** resulting from (R)-methyl lactate
[0041] ^1H NMR ($CDCl_3$): δ 7.18-7.11 (m, 2H), 6.90 (m, 2H), 6.52 (m, 2H), 6.33(m, 1 H), 5.09 (bt, 1H), 4.52 (m, 1H), 3.77 (s, 6H), 3.08 (bq, 2H), 2.64 (bt, 2H), 1.75 (m, 8H); 1.27 (bd, 3H) ^{13}C NMR ($CDCl_3$): δ 160.8, 155.5, 149.5, 131.2, 130.6, 127.4, 121.2, 113.3, 103.2, 98,4, 80.7. 74.8, 66.5, 55,4, 40.2, 30.6, 29.3, 29.2, 27.4, 16.1

Example T38: Standard Procedure for the Synthesis of Tether T38

[0042] An outline of the synthetic scheme for racemic material is shown in Figure 11. The enatiomers are accessed through the use of the optically pure propylene oxide enantiomers. Since the center of the epoxide is inverted during the protocol, the (R)-epoxide provides **T38(S),** while the (S)-epoxide provides **T38(R).**
[0043] ^1H NMR ($CDCl_3$): δ 7.20-7.10, (m, 2H), 6.95-9.80 (m, 2H), 6.55 (bs, 2H), 6.35 (s,1 H), 5.18 (bt, 1 H), 4.12 (m, 1 H), 3.98 (m, 2H), 3.80 (s, 6H), 3.15 (bq, 2H), 2.65 (t, 2H), 1.98 (bs, 2H), 1.65 (bs, 6H), 1.25 (m, 3H).

Example T39: Standard Procedure for the Synthesis of Tether T39

[0044] See Figure 12 for an outline of the synthetic scheme for racemic product. Enantiomeric versions can be accessed via resolution methodologies or use of an asymmetric Michael addition in the third step.
[0045] ^1H NMR ($CDCl_3$): δ 7.11-7.08 (2H, m), 6.86 (1H, t), 6.76 (1H, d), 5.05 (1H, broad), 4.26-3.85 (4H, m), 3.22-3.07 (2H, m), 2.71 (1 H, broad), 1.66-1.60 (2H, m), 1.33 (9H, s), 1.17 (3H, d).
^{13}C NMR ($CDCl_3$): δ 156.1, 135.0, 127.1, 127.0, 121.4, 111.7, 69.9, 61.5, 39.8, 38.4, 28.7, 20.7.

Example T40: Standard Procedure for the Synthesis of Tether T40

[0046] An outline of the synthetic scheme for racemic material is shown in Figure 13, while Figure 14 outlines the route

to both enantiomers involving an enzymatic resolution as the key step.

[0047] ^{1}H NMR ($CDCl_3$): δ 7.11-7.08 (2H, m), 6.86 (1H, t), 6.76 (1H, d), 5.05 (1H, broad), 4.26-3.85 (4H, m), 3.22-3.07 (2H, m), 2.71 (1H, broad), 1.66-1.60 (2H, m), 1.33 (9H, s), 1.17 (3H, d).
^{13}C NMR ($CDCl_3$): δ 156.1, 135.0, 127.1, 127.0, 121.4, 111.7, 69.9, 61.5, 39.8, 38.4, 28.7, 20.7.

Example T41: Standard Procedure for the Synthesis of Tether T41

[0048] See Figure 15 for an outline of the synthetic scheme that provides an appropriately protected derivative for use in macrocycle construction via Figure 1.

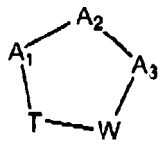
[0049] ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 3H), 1.49 (s, 3H), 1.69 (s, 3H), 1.74 (s, 3H), 1.90 (m, 2H), 2.35 (m, 1H), 3.35 (m, 2H), 3.76 (s, 6H), 3.92 (m, 2H), 4.40 (m, 2H), 5.10 (m, 1H), 6.15 (s,1 H), 6.25 (s, 2H).
^{13}C NMR ($CDCl_3$): δ 25.52 ($\underline{C}H_3$), 27.53 ($\underline{C}H_3$), 28.88 ($\underline{C}H_3$), 29.61 ($\underline{C}H_3$), 35.92 ($\underline{C}H_2$), 42.62 ($\underline{C}$H2), 55.43 (CH_3), 60.60 ($\underline{C}H_2$), 82.38 (CH), 83.33 ($\underline{C}$H), 83.68 (NH 84.96 ($\underline{C}$H), 98.26 ($\underline{C}$H), 103.23 (CH), 118.3 ($\underline{C}$q), 149.50 ($\underline{C}$q), 156.20 ($\underline{C}$q). 160,02 ($\underline{C}$q) MW Calcd. for $C_{22}H_{33}NO_8$: 439.50; MS Found: $(M+H)^+$ 440

Example T56: Standard Procedure for the Synthesis of Precursor (56.1) for Tethers T56 and T57

[0050] For some of the tether structures, specifically those arising from the ring-closing metathesis methodology (RCM, Figure 2), the tether is not added as an already assembled unit, but is constructed during the macrocyclization reaction from appropriate precursor pieces. One such example id shown in Figure 17 in which **56-1,** containing a pendant alkene moiety, will be subjected to RCM whereby the alkene will join with an alkene In another part of the substrate to form the macrocyclic ring and, hence, construct tether **T56** (or homologues). Reduction of the double bond in macrocycles containing **T56** leads to macrocycles containing **T57.** Other tethers that were constructed in this manner include **T46, T47, T49,** and **T51.**

Table 1 lists the structural features for the compounds of formula (II).
Table 2 gives the Mass Spectrum analytical data for these compounds.

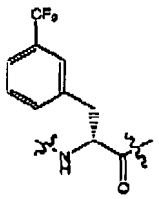
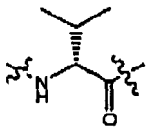
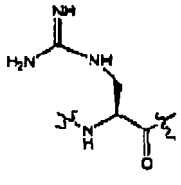
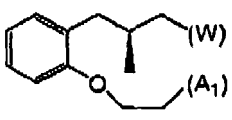
[0051] These compounds are defined by general formula (II):



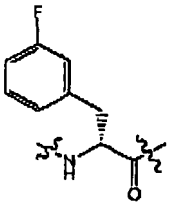
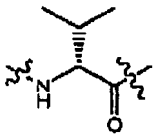
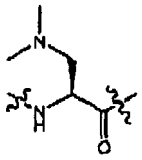
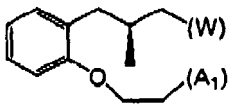
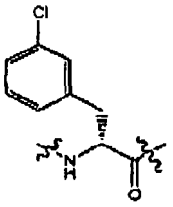
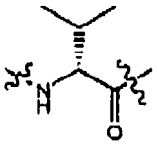
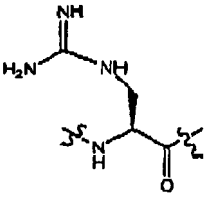
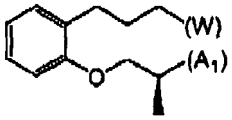
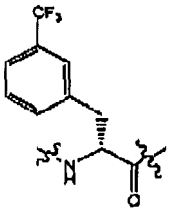
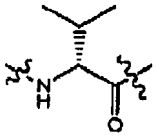
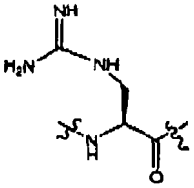
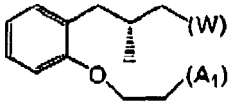
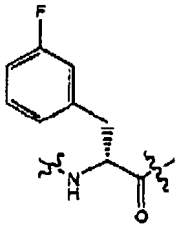
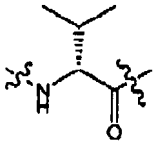
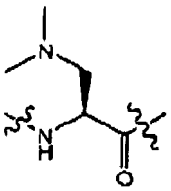
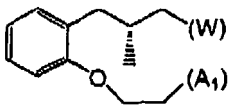
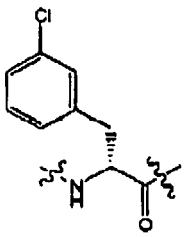
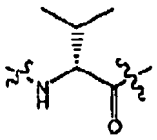
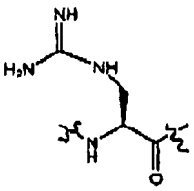
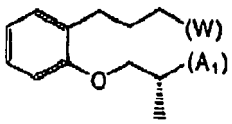
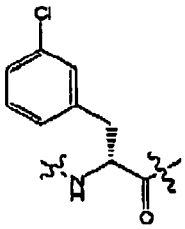
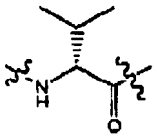
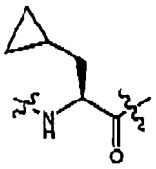
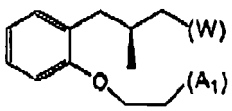
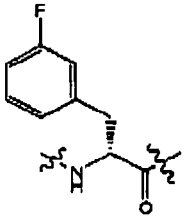
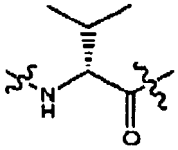
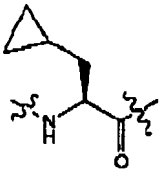
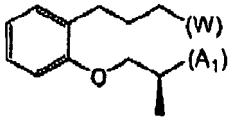
(II)

wherein W, A_1, A_2, A_3 and T are defined as below with the NH of A_1 bonded to T, the C=O of A_1 bonded to the NH of A_2, the C=O of A_2 bonded to the NH of A_3. the C=O of A_3 bonded to W, and (W) and (A_1) indicate the site of bonding of T to W and A_1, respectively:

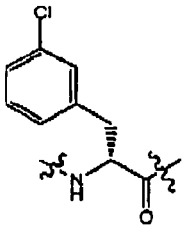
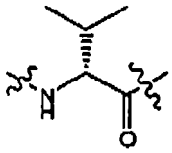
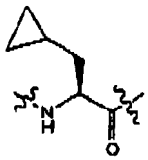
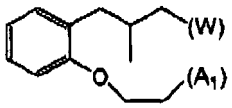
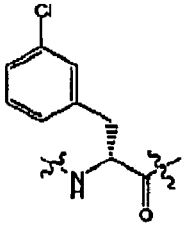
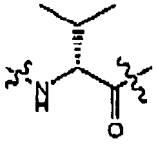
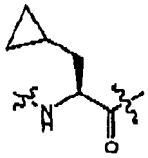
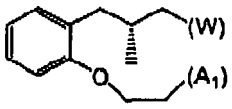
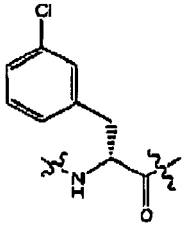
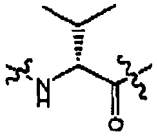
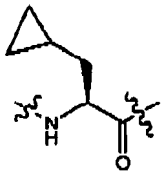
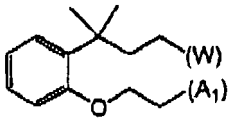
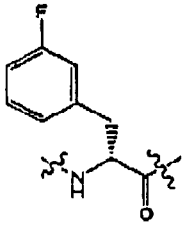
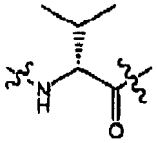
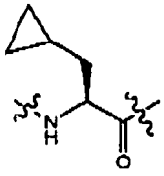
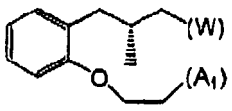
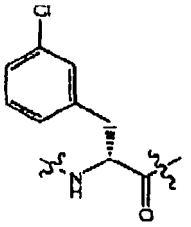
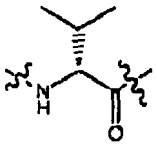
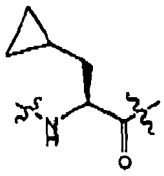
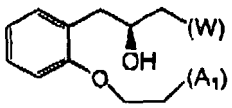
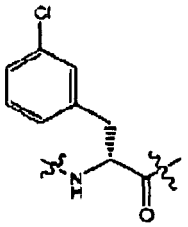
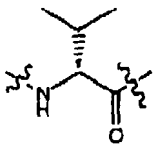
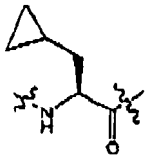
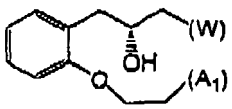
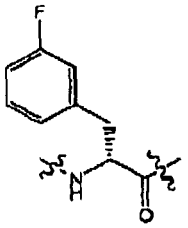
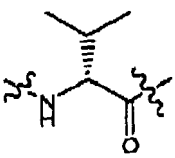
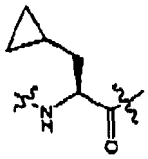
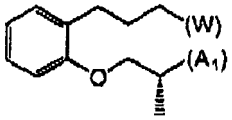
Table 1: Representative Compounds of formula (II)

Comp	W	A_1	A_2	A_3	T
201	NH				

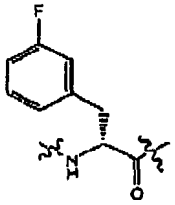
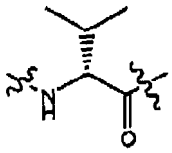
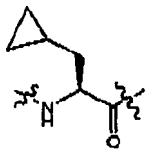
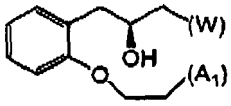
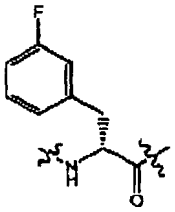
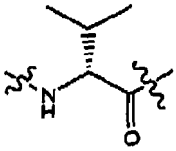
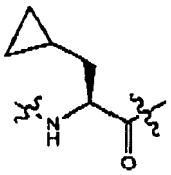
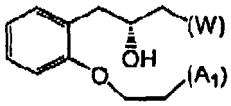
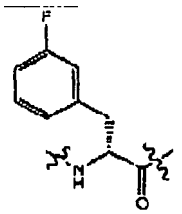
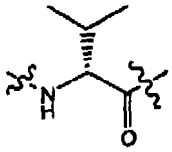
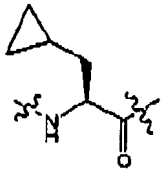
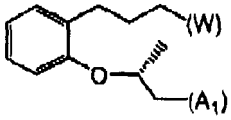
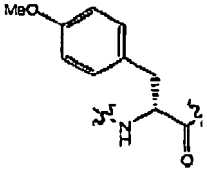
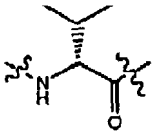
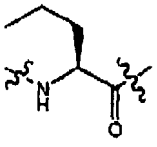
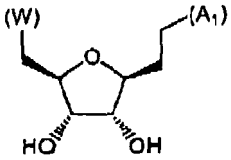
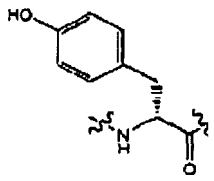
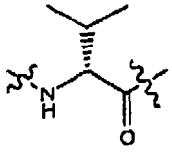
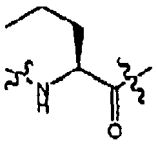
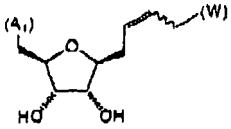
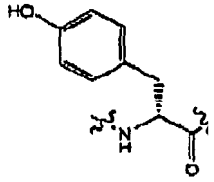
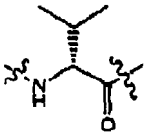
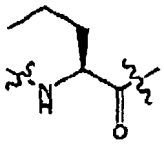
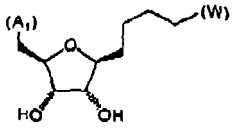
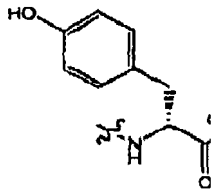
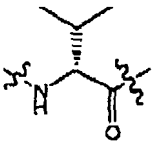
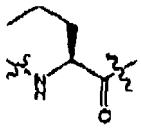
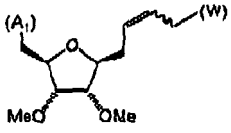
(continued)

Comp	W	A_1	A_2	A_3	T
202	NH				
203	NH				
204	NH				
205	NH				
206	NH				
207	NH				
208	NH				

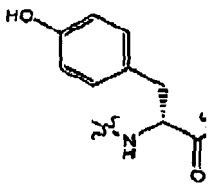
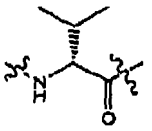
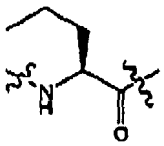
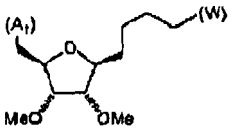
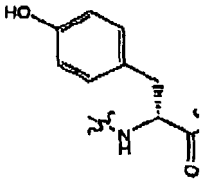
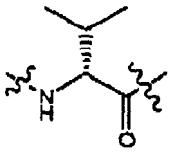
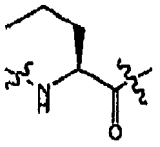
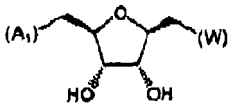
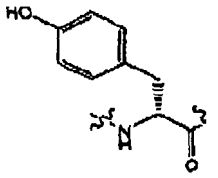
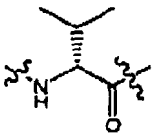
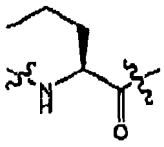
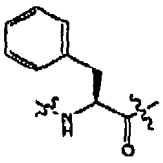
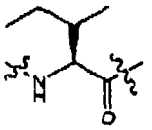
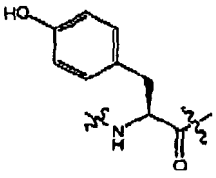
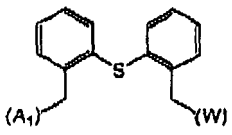
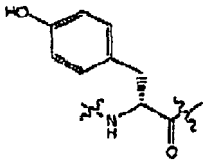
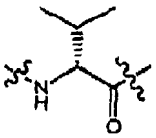
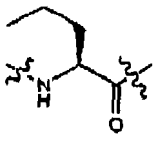
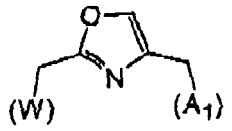
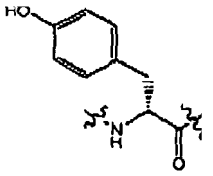
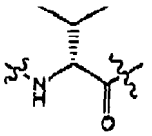
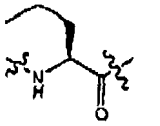
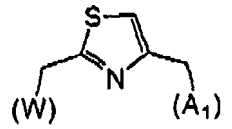
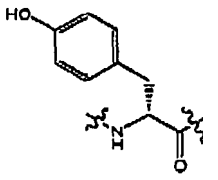
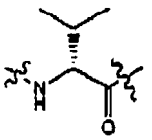
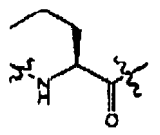
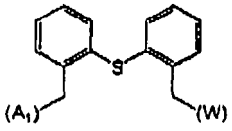
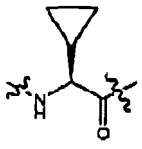
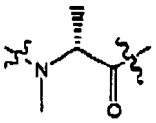
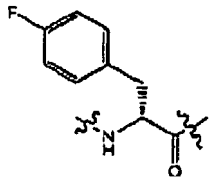
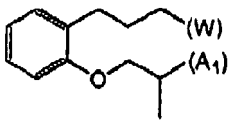
(continued)

Comp	W	A_1	A_2	A_3	T
209	NH				
211	NH				
212	NH				
214	NH				
215	NH				
216	NH				
217	NH				

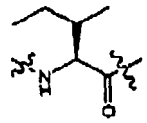
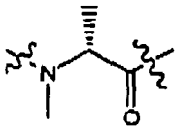
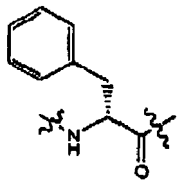
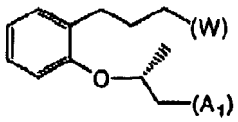
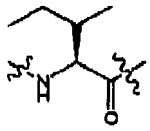
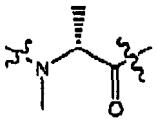
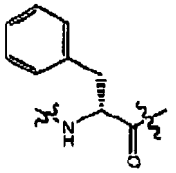
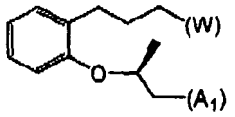
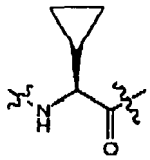
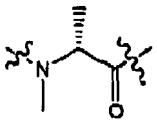
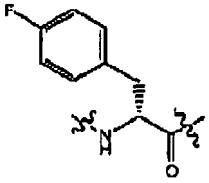
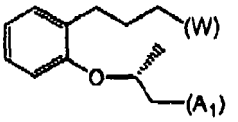
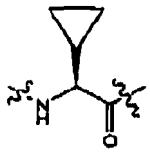
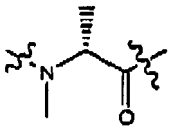
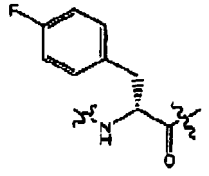
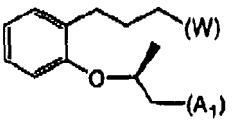
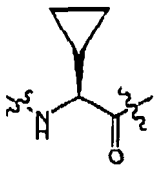
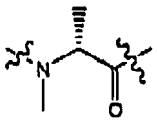
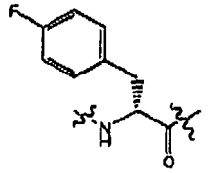
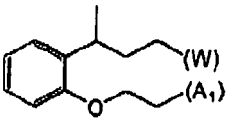
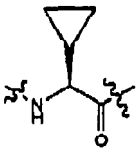
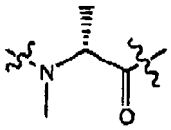
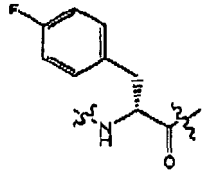
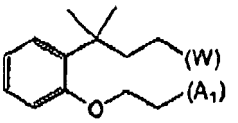
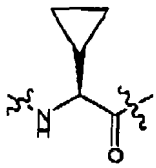
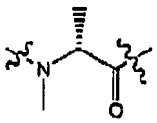
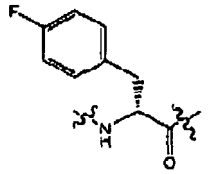
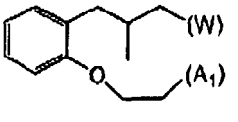
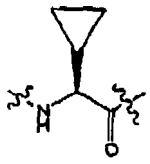
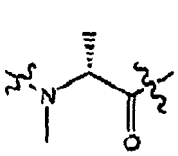
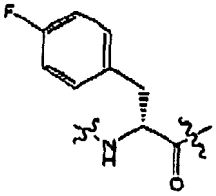
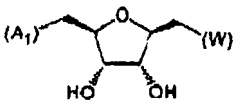
(continued)

Comp	W	A_1	A_2	A_3	T
218	NH				
219	NH				
220	NH				
225	NH				
229	O				
230	O				
231	O				

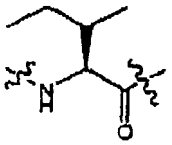
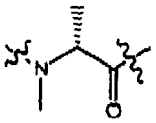
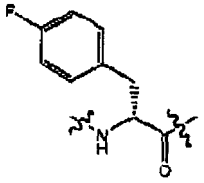
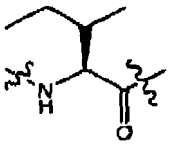
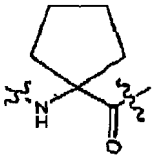
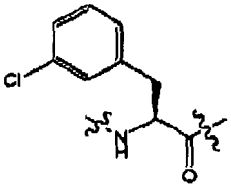
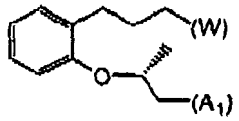
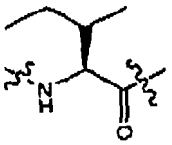
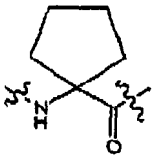
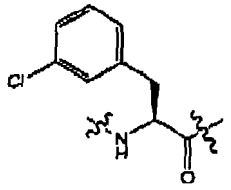
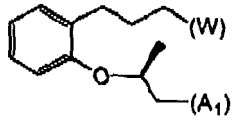
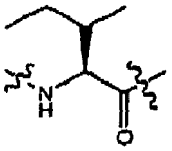
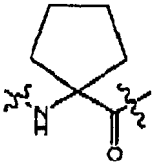
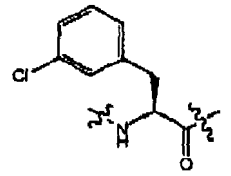
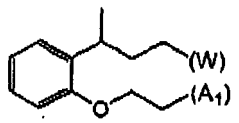
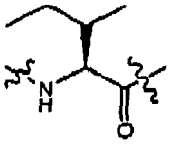
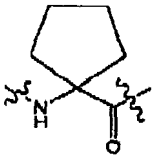
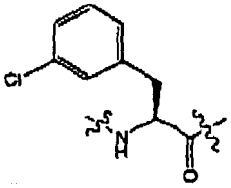
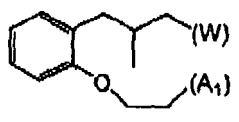
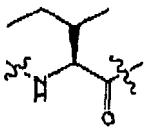
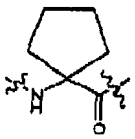
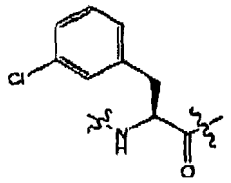
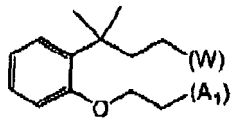
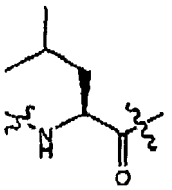
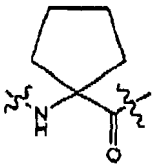
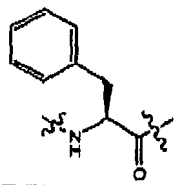
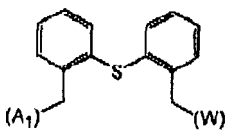
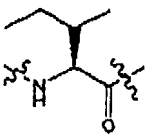
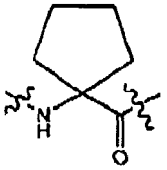
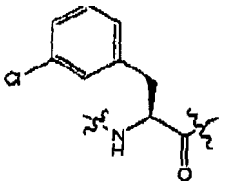
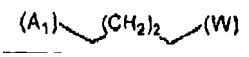
(continued)

Comp	W	A_1	A_2	A_3	T
232	O				
233	NH				
234	NH				
235	NH				
236	NH				
237	NH				
238	NH				
241	NH				

(continued)

Comp	W	A_1	A_2	A_3	T
242	NH				
243	NH				
244	NH				
245	NH				
246	NH				
247	NH				
248	NH				
249	NH				

(continued)

Comp	W	A_1	A_2	A_3	T
250	NH				
254	NH				
255	NH				
256	NH				
257	NH				
268	NH				
259	NH				
260	NH				

(continued)

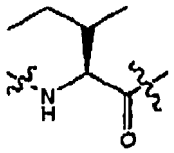
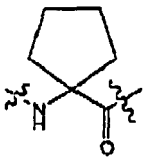
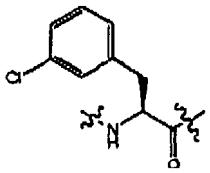
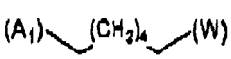
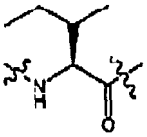
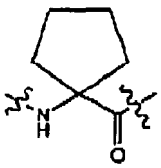
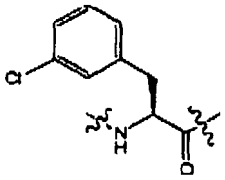
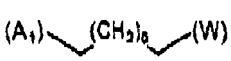
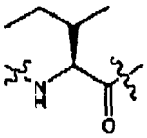
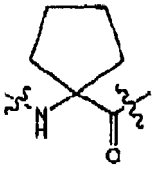
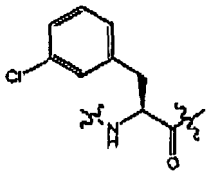
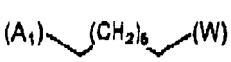
Comp	W	A_1	A_2	A_3	T
261	NH				
262	NH				
264	NH				

Table 2: Mass Spectral Analyses for Representative Compounds of formula II

Cmpd	Molecular Formula	Molecular Weight	Monoisotopic Mass	MS Found $(M+H)^+$
201	C31H42N7O4F3	633.7	633	634
202	C31H44N5O4F	569.7	569	570
203	C30H42N7O4Cl	600.2	599	600
204	C31H42N7O4F3	633.7	633	634
205	C31H44N504F	569.7	569	570
206	C30H42N7O4Cl	600.2	599	600
207	C32H43N4O4Cl	583.2	582	583
208	C32H43N4O4F	566.7	566	567
209	C32H43N404Cl	583.2	562	583
211	C32H43N4O4Cl	583.2	582	583
212	C33H45N4O4Cl	597.2	596	597
214	C32H43N4O4F	566.7	566	567
215	C31H41N4O5Cl	585.1	584	585
216	C31H41N4O5Cl	585.1	584	585
217	C32H43N4O4F	566.7	566	567
218	C31H41N4O5F	568.7	588	569
219	C31H41N4O5F	568.7	568	569
220	C32H43N4O4F	566.7	566	567
225	C27H42N4O7	534.6	534	535
229	C28H41N3O8	547.6	547	548
230	C28H43N3O8	549.7	549	550
231	C30H45N3O8	575.7	575	576

(continued)

Cmpd	Molecular Formula	Molecular Weight	Monoisotopic Mass	MS Found $(M+H)^+$
232	C30H47N3O8	577.7	577	578
233	C25H38N4O7	506.6	506	5D7
234	C25H36N4O5	472.6	472	473
235	C38H42N4O4S	650.8	650	651
236	C24H33N5O5	471.5	471	472
237	C24H33N5O4S	487.6	487	488
238	C33H40N4O4S	588.8	588	589
241	C30H39N4O4F	538.7	538	539
242	C31H44N4O4	536.7	536	537
243	C31H44N4O4	536.7	536	537
244	C30H38N4O4F	538.7	538	539
245	C30H39N4O4F	538.7	538	539
246	C30H39N4O4F	538.7	538	539
247	C31H41N4O4F	552.7	552	553
248	C30H39N4O4F	538.7	538	539
249	C24H33N4O6F	492.5	492	493
250	C26H41N4O3F	476.6	476	477
254	C33H45N4O4Cl	597.2	596	597
255	C33H45N4O4Cl	597.2	596	597
256	C33H45N4O4Cl	597.2	596	597
257	C33H45N4O4Cl	597.2	596	597
258	C34H47N4O4Cl	611.2	611	612
259	C36H42N4O3S	598.8	598	599
260	C23H35N4O3F	434.5	434	435
261	C26H39N4O3Cl	491.1	490	491
262	C27H41N4O3Cl	606.1	604	505
264	C29H45N4O3Cl	533.1	532	533

Notes

1. Molecular formulas and molecular weights (MW) are calculated automatically from the structure via ActivityBase software (IDBS, Guildford, Surrey, UK) or, for MW only, from the freeware program Molecular Weight Calculator v. 6.32

2. M+H obtained from LC-MS analysis

3. All analyses conducted on material after preparative purification

Biological Evaluation for Compounds of the Invention

[0052] The compounds of the present invention were evaluated for their ability to interact at the human motilin receptor and the human ghrelin receptor utilizing competitive radioligand binding assays as described in Method B1 and B2, respectively. Further characterization of the interaction can be performed utilizing the functional assays described in Methods B3 and B4 for the motilin and ghrelin receptors, respectively. All of these methods can be conducted, if so desired, in a high throughput manner to permit the simultaneous evaluation of many compounds.

[0053] Results for the examination of representative compounds of the present invention using Methods B1 and B2

are presented in Table 3.

Example Method B1: Competitive Radioligand Binding Assay (Motilin Receptor)

Materials:

[0054]

- Membranes were prepared from CHO cells stably transfected with the human motilin receptor and utilized at a quantity of 1.5 μg/assay point. [PerkinElmer™ SignalScreen Product #6110544]
- [^{125}I]-Motilin (PerkinElmer, #NEX-378); final concentration: 0.04-0.08 nM
- Motilin (Bachem™ #H-4385); final concentration: 1 μM
- Multiscreen Harvest plates-GF/B (Millipore™, #MAHFB1 H60)
- Deep-well polypropylene titer plate (Beckman Coulter™, #267006)
- TopSeal-A (PerkinElmer, #6005185)
- Bottom seal (Millipore, #MATAH0P00)
- MicroScint-0 (PerkinElmer, #6013611)
- Binding Buffer: 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA, 0.1% BSA

Assay Volumes:

[0055]

- 150 μL of membranes diluted in binding buffer
- 10 μL of compound diluted in binding buffer
- 10 μL of radioligand ([^{125}I]-Motilin) diluted in binding buffer

[0056] Final Test Concentrations (N=11) for Compounds:

10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005 μM.

Compound Handling:

[0057] Compounds were provided frozen on dry ice at a stock concentration of 10 mM diluted in 100% DMSO and stored at -20°C until the day of testing. On the test day, compounds were allowed to thaw at room temperature and than diluted in assay buffer according to the desired test concentrations. Under these conditions, the maximum final DMSO concentration in the assay was 0.5%.

Assay Protocol:

[0058] In deep-well plates, diluted cell membranes (1.5 μg/mL) are combined with 10 μL of either binding buffer (total binding, N=5), 1 μM motilin (non-specific binding, N=3) or the appropriate concentration of test compound. The reaction is initiated by addition of 10 μl of [^{125}I]-motilin (final conc. 0.04 - 0.06 nM) to each well. Plates are sealed with TopSeal-A, vortexed gently and incubated at room temperature for 2 hours. The reaction is arrested by filtering samples through pre-soaked (0.3% polyethyleneimine, 2 h) Multiscreen Harvest plates using a Tomtec Harvester, washed 9 times with 500 μL of cold 50 mM Tris-HCl (pH 7.4), and than plates are air-dried in a fumehood for 30 minutes. A bottom seal is applied to the plates prior to the addition of 25 μL of MicroScint-0 to each well. Plates are than sealed with TopSeal-A and counted for 30 sec per well on a TopCount Microplate Scintillation and Luminescence Counter (PerkinElmer) where results are expressed as counts per minute (cpm).

[0059] Data are analyzed by GraphPad™ Prism (GraphPad Software, San Diego, CA) using a variable slope non-linear regression analysis. K values were calculated using a K_d value of 0.16 nM for [^{125}I]-motilin (previously determined during membrane characterization).

$$D_{max} = 1 - \frac{\text{test concentration with maximal displacement} - \text{non-specific binding}}{\text{total binding} - \text{non-specific binding}} \times 100$$

where total and non-specific binding represent the cpm obtained in the absence or presence of 1 μM motilin, respectively.

Example Method B2: Competitive Radioligand Binding Assay (Ghrelin Receptor)

[0060] The competitive binding assay at the human growth hormone secretagogue receptor (hGHS-R1 a) was carried out analogously to assays described in the literature. (Bednarek MA et al. (2000), Structure-function studies on the new growth hormone-releasing peptide ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a; J Med Chem 43:4370-4376. Palucki BL et al. (2001), Spiro(indoline-3,4'-piperidine) growth hormone secretagogues as ghrelin mimetics; Bioorg Med Chem Lett 11:1955-1957.)

Materials

[0061]

- Membranes (GHS-R/HEK 293) were prepared from HEK-293 cells stably transfected with the human ghrelin receptor (hGHS-R1a). These membranes were provided by PerkinElmer BioSignal (#RBHGHSM, lot#1887) and utilized at a quantity of 0.71 μg/assay point.
- [^{125}I]-Ghrelin (PerkinElmer, #NEX-388); final concentration: 0.0070-0.0085 nM
- Ghrelin (Bachem, #H-4864); final concentration: 1 μM
- Multiscreen Harvest plates-GF/C (Millipore, #MAHFC1 H60)
- Deep-well polypropylene titer plate (Beckman Coulter, #267006)
- TopSeal-A (PerkinElmer, #6005185)
- Bottom seal (Millipore, #MATAH0P00)
- MicroScint-0 (PerkinElmer, #6013611)
- Binding Buffer: 25 mM Hepes (pH 7.4), 1 mM $CaCl_2$, 5 mM $MgCl_2$, 2.5 mM EDTA, 0.4% BSA

Assay Volumes

[0062] Competition experiments were performed in a 300 μL filtration assay format.

- 220 μL of membranes diluted in binding buffer
- 40 μL of compound diluted in binding buffer
- 40 μL of radioligand ([^{121}I]-Ghrelin) diluted in binding buffer

[0063] Final test concentrations (N = 11) for compounds of the present invention:

10, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001 μM.

Compound Handling

[0064] Compounds were provided frozen on dry ice at a stock concentration of 10 mM diluted in 100% DMSO and stored at -80°C until the day of testing. On the test day, compounds were allowed to thaw at rt overnight and then diluted in assay buffer according to the desired test concentrations. Under these conditions, the maximal final DMSO concentration in the assay was 0.1%.

Assay Protocol

[0065] In deep-well plates, 220 μL of diluted cell membranes (final concentration: 0.71 μg/well) were combined with 40 μL of either binding buffer (total binding, N = 5), 1 μM ghrelin (non-specific binding, N = 3) or the appropriate concentration of test compound (N = 2 for each test concentration). The reaction was initiated by addition of 40 μL of [^{123}I]-ghrelin (final conc. 0.0070 - 0.0085 nM) to each well. Plates were sealed with TopSeal-A, vortexed gently and incubated at rt for 30 min. The reaction was arrested by filtering samples through Multiscreen Harvest plates (pre-soaked in 0.5% polyethyleneimine) using a Tomtec Harvester, washed 9 times with 500 μL of cold 50 mM Tris-HCl (pH 7.4, 4°C), and then plates were air-dried in a fumehood for 30 min. A bottom seal was applied to the plates prior to the addition of 25 μL of MicroScint-0 to each well. Plates were than sealed with TopSeal-A and counted for 30 sec per well on a TopCount Microplate Scintillation and Luminescence Counter (PerkinElmer) using a count delay of 60 sec. Results were expressed as counts per minute (cpm).

[0066] Data were analyzed by GraphPad Prism (GraphPad Software, San Diego, CA) using a variable slope non-linear

regression analysis. K_i values were calculated using a K_d value of 0.01 nM for [^{25}I]-ghrelin (previously determined during membrane characterization).

[0067] D_{max} values were calculated using the following formula:

$$D_{max} = 1 - \frac{\text{test concentration with maximal displacement} - \text{non-specific binding}}{\text{total binding} - \text{non-specific binding}} \times 100$$

where total and non-specific binding represent the cpm obtained in the absence or presence of 1 μM ghrelin, respectively.

Example Method B3: Aequorin Functional Assay (Motilin Receptor)

Materials:

[0068]

- Membranes were prepared using AequoScreen™ (EUROSCREEN, Belgium) cell lines expressing the human motilin receptor (cell line ES-380-A; receptor accession #AF034632). This cell line is constructed by transfection of the human motilin receptor into CHO-K1 cells co-expressing $G_{\alpha 16}$ and the mitochondrially targeted Aequorin (Ref #ES-WT-A5).
- Motilin (Bachem, #H-4385)
- Assay buffer: DMEM-F12 (Dulbeccoe's Modified Eagles Medium) with 15 mM HEPES and 0.1% BSA (pH 7.0)
- Coelenterazine (Molecular Probes™, Leiden, The Netherlands)

[0069] Final Test Concentrations (N=5) for Compounds:

10, 3.16, 1, 0.316, 0.1 μM.

Compound Handling:

[0070] Compounds were provided as dry films at a quantity of approximately 1.2 μmol in preformatted 96-well plates. Compounds were dissolved in 100% DMSO at a concentration of 10 mM and stored at -20°C until further use. Daughter plates were prepared at a concentration of 500 μM in 30% DMSO with 0.1 % BSA and stored at -20°C until testing. On the test day, compounds were allowed to thaw at room temperature and than diluted in assay buffer according to the desired test concentrations. Under these conditions, the maximum final DMSO concentration in the assay was 0.6%.

Cell Preparation:

[0071] Cells are collected from culture plates with Ca^{2+} and Mg^{2+}-free phosphate buffered saline (PBS) supplemented with 5 mM EDTA, pelleted for 2 minutes at 1000 x g, resuspended in assay buffer (see above) at a density of 5 x 10^6 cells/mL and incubated overnight in the presence of 5 μM coelenterazine. After loading, cells were diluted with assay buffer to a concentration of 5 × 10^5 cells/mL.

Assay Protocol:

[0072] For agonist testing, 50 μl of the cell suspension was mixed with 50 μl of the appropriate concentration of test compound or motilin (reference agonist) in 96-well plates (duplicate samples). The emission of light resulting from receptor activation was recorded using the Functional Drug Screening System 6000 'FDSS 6000' (Hamamatsu Photonics K.K., Japan).

[0073] For antagonist testing, an approximate EC_{80} concentration of motilin (i.e. 0.5 nM; 100 μL) was injected onto the cell suspension containing the test compounds (duplicate samples) 15-30 minutes after the end of agonist testing and the consequent emission of light resulting from receptor activation was measured as described in the paragraph above.

[0074] Results are expressed as Relative Light Units (RLU). Concentration response curves were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA) by non-linear regression analysis (sigmoidal dose-response) based on the equation $E=E_{max}/X/(1+EC_{50}/C)n$ where E is the measured RLU value at a given agonist concentration (C), E_{max} is the maximal response, EC_{50} Is the concentration producing 50% stimulation and n is the slope index. For

agonist testing, results for each concentration of test compound were expressed as percent activation relative to the signal induced by motilin at a concentration equal to the EC_{80} (i.e. 0.5 nM). For antagonist testing, results for each concentration of test compound were expressed as percent inhibition relative to the signal induced by motilin at a concentration equal to the EC_{80} (i.e. 0.5 nM).

[0075] Example Method B4: Aequorin Functional Assay (Ghrelin Receptor)

Materials

[0076]

- Membranes were prepared using AequoScreen™ (EUROSCREEN, Belgium) cell lines expressing the human ghrelin receptor (cell line ES-410-A; receptor accession #60179). This cell line is constructed by transfection of the human ghrelin receptor into CHO-K1 cells co-expressing $G_{\alpha 16}$ and the mitochondrially targeted Aequorin (Ref#ES-WT-A5).
- Ghrelin (reference agonist; Bachem, #H-4864)
- Assay buffer: DMEM (Dulbecco's Modified Eagles Medium) containing 0.1% BSA (bovine serum albumin; pH 7.0.
- Coelenterazine (Molecular Probes, Leiden, The Netherlands)

[0077] Final test concentrations (N = 8) for compounds of the invention:

10, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001 μM.

Compound Handling

[0078] Stock solutions of compounds (10 mM in 100% DMSO) were provided frozen on dry ice and stored at -20°C prior to use. From the stock solution, mother solutions were made at a concentration of 500 μM by 20-fold dilution in 26% DMSO. Assay plates were then prepared by appropriate dilution in DMEM medium containing 0.1% BSA. Under these conditions, the maximal final DMSO concentration in the assay was < 0.6%.

Cell Preparation

[0079] AequoScreen™ cells were collected from culture plates with Ca^{2+} and Mg^{2+}-free phosphate buffered saline (PBS) supplemented with 5 mM EDTA, pelleted for 2 min at 1000X g, resuspended in DMEM - Ham's F12 containing 0.1% BSA at a density of 5 X 10^6 cells/mL, and Incubated overnight at rt In the presence of 5 μM coelenterazine. After loading, cells were diluted with assay buffer to a concentration of 5 x 10^5 cells/mL.

Assay Protocol

[0080] For agonist testing, 50 μL of the cell suspension was mixed with 50 nL of the appropriate concentration of test compound or ghrelin (reference agonist) in 96-well plates (duplicate samples). Ghrelin (reference agonist) was tested at several concentrations concurrently with the test compounds in orderto validate the experiment The emission of light resulting from receptor activation in response to ghrelin or test compounds was recorded using the Hamamatsu FDSS 6000 reader (Hamamatsu Photonics K.K., Japan).

Analysis and Expression of Results

[0081] Results were expressed as Relative Light Units (RLU). Concentration response curves were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA) by non-linear regression analysis (sigmoidal dose-response) based on the equation $E=E_{max}/(1+EC_{50}/C)n$ where E was the measured RLU value at a given agonist concentration (C), E_{max} was the maximal response, EC_{50} was the concentration producing 50% stimulation and n was the slope index. For agonist testing, results for each concentration of test compound are expressed as percent activation relative to the signal induced by ghrelin at a concentration equal to the EC_{50} (i.e. 3.7 nM). EC_{50}, Hill slope and $\%E_{max}$ values are reported.

Table 3: Biological Activity of Representative Compounds of formula II

Compound	Binding Affinity [K_i (μM)][1]	Receptor[2]
201	A	motilin (human)
202	A	motilin (human)

(continued)

Compound	Binding Affinity [K_i (μM)][1]	Receptor[2]
203	A	motilin (human)
204	A	motilin (human)
205	B	motilin (human)
206	B	motilin (human)
207	A	motilin (human)
208	A	motilin (human)
209	A	motilin (human)
211	A	motilin (human)
212	A	motilin (human)
214	A	motilin (human)
215	A	motilin (human)
216	A	motilin (human)
217	B	motilin (human)
218	B	motilin (human)
219	B	motilin (human)
220	B	motilin (human)
235	C	motilin (human)
236	B	motilin (human)
237	B	motilin (human)
241	A	ghrelin (human)
242	A	ghrelin (human)
243	A	ghrelin (human)
244	A	ghrelin (human)
245	A	ghrelin (human)
246	B	ghrelin (human)
247	B	ghrelin (human)
248	B	ghrelin (human)
254	A	ghrelin (human)
255	A	ghrelin (human)
256	B	ghrelin (human)
257	A	ghrelin (human)
258	B	ghrelin (human)
259	C	ghrelin (human)
260	C	ghrelin (human)
261	C	ghrelin (human)
262	B	ghrelin (human)

(continued)

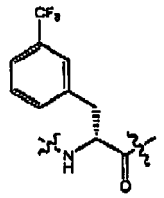
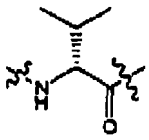
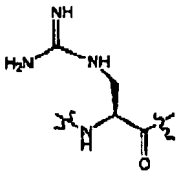
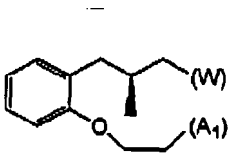
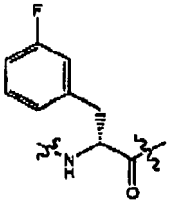
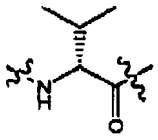
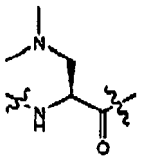
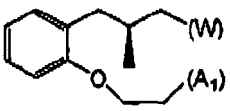
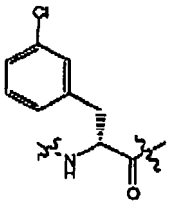
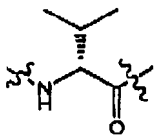
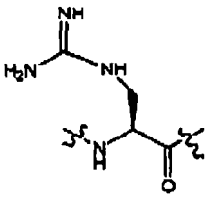
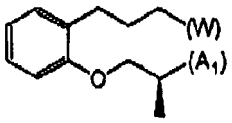
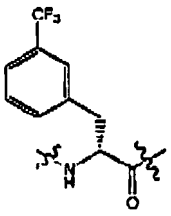
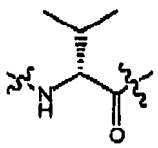
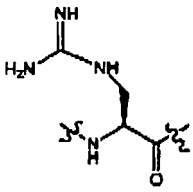
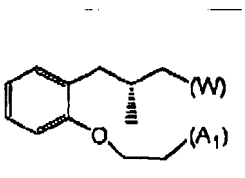
Compound	Binding Affinity [K_i (μM)][1]	Receptor[2]
264	B	ghrelin (human)
1. Activity presented indicated in the following ranges: A=0.009-0.10 μM, 8=0.1-1.0 μM, C = 1.0-10.0 μM *2. Binding conducted using the Standard Methods described in the Examples*		

Claims

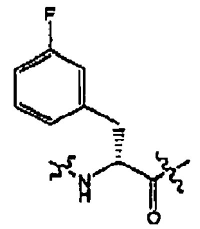
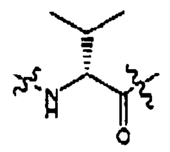
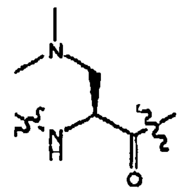
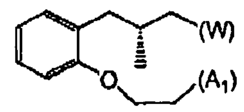
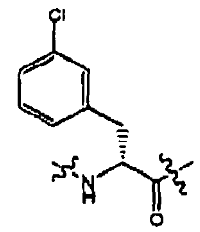
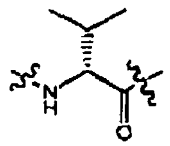
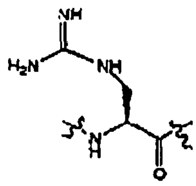
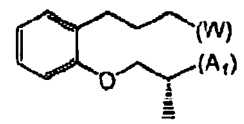
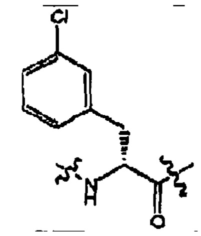
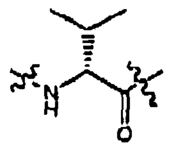
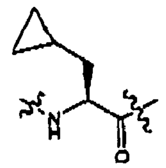
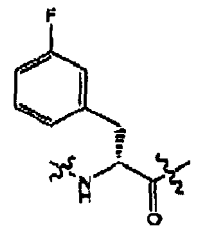
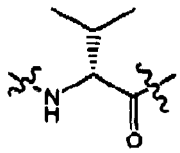
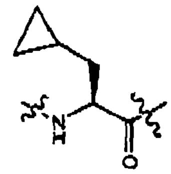
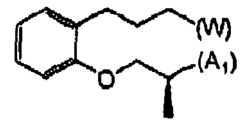
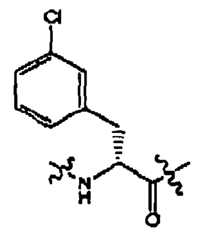
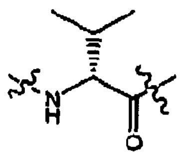
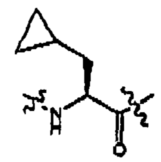
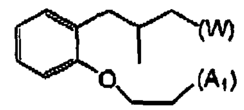
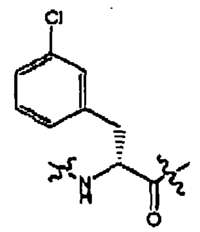
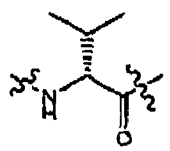
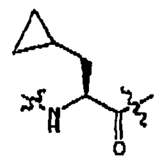
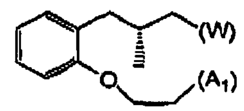
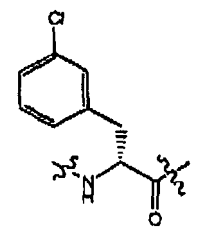
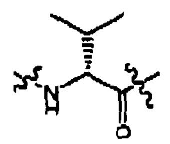
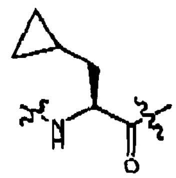
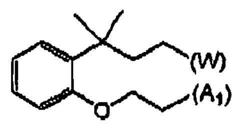
1. A compound having the structure of formula II:

(II)

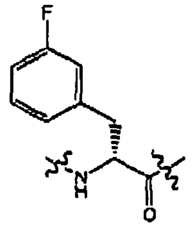
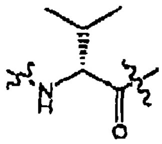
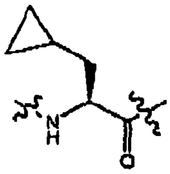
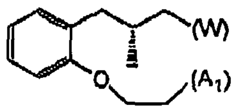
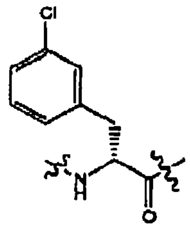
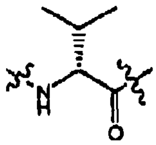
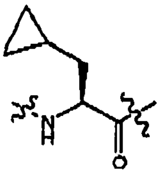
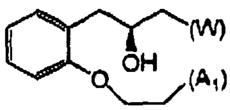
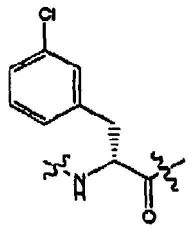
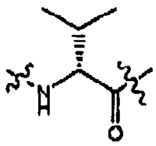
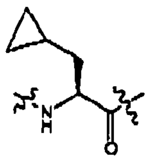
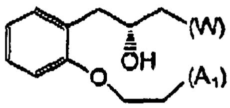
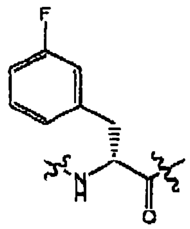
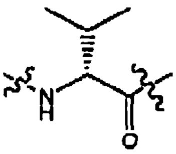
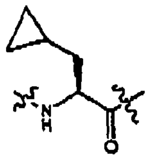
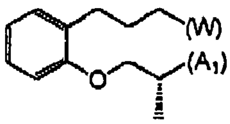
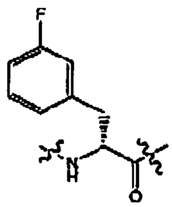
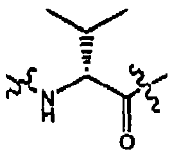
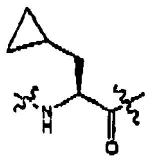
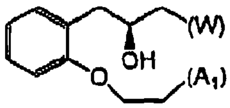
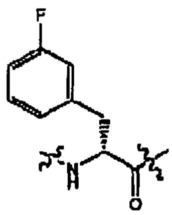
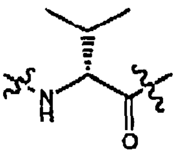
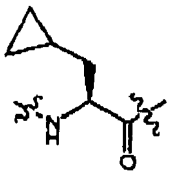
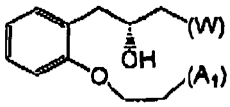
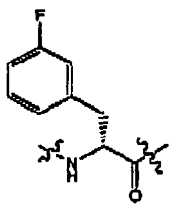
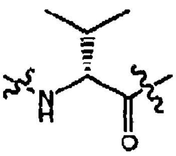
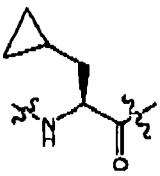
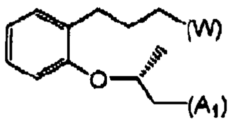
wherein W, A_1, A_2, A_3 and T are defined as below with the NH of A_1 bonded to T, the C=O of A_1 bonded to the NH of A_2, the C=O of A_2 bonded to the NH of A_3, the C=O of A_3 bonded to W, and (W) and (A_1) indicate the site of bonding of T to W and A_1, respectively:

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				

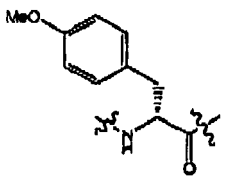
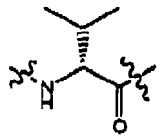
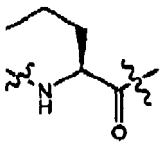
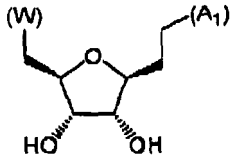
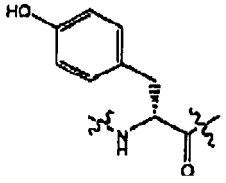
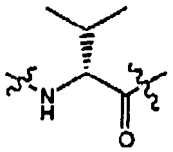
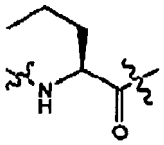
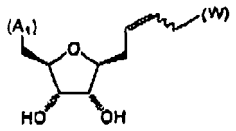
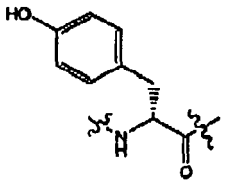
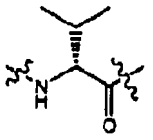
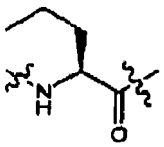
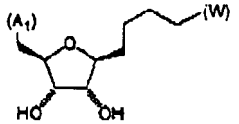
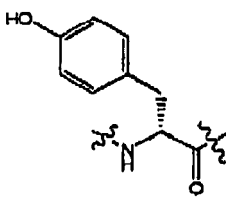
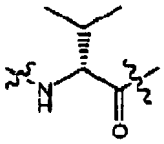
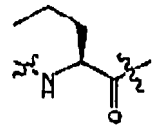
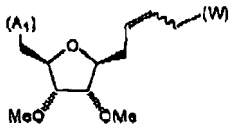
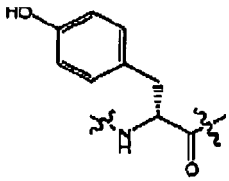
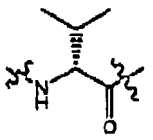
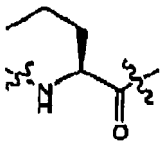
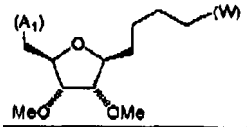
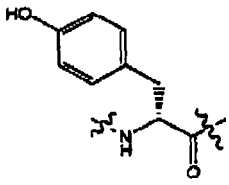
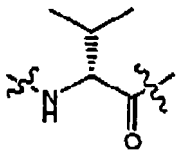
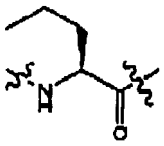
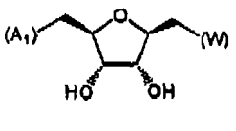
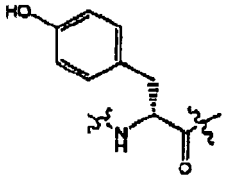
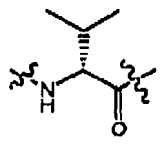
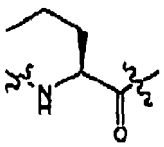
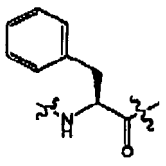
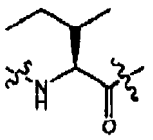
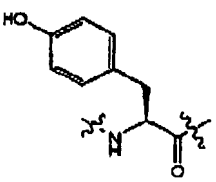
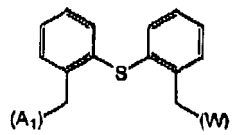
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

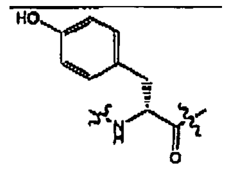
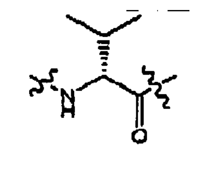
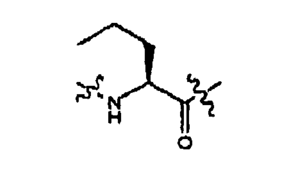
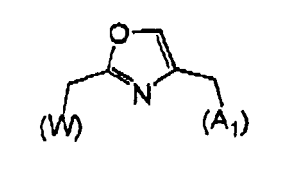
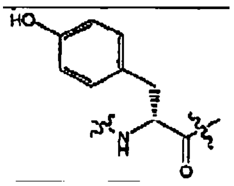
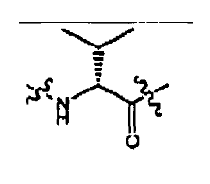
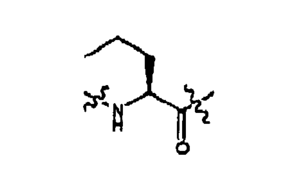
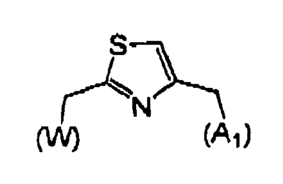
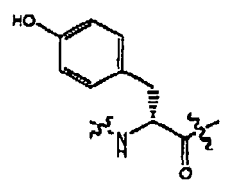
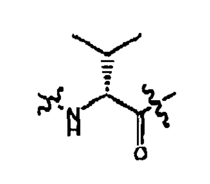
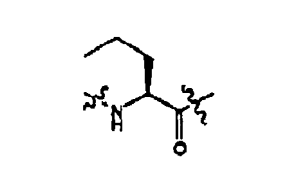
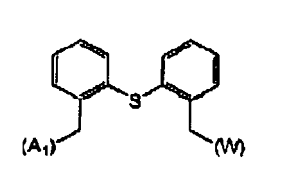
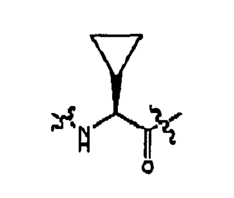
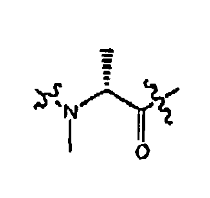
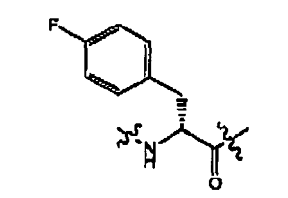
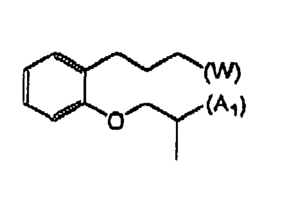
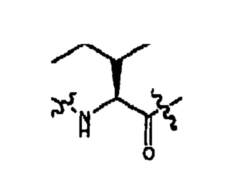
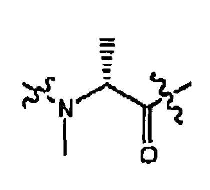
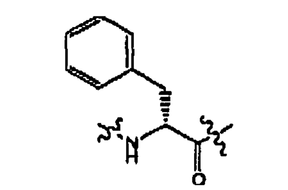
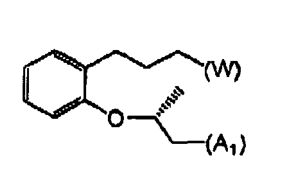
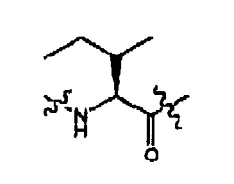
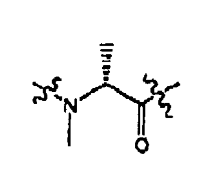
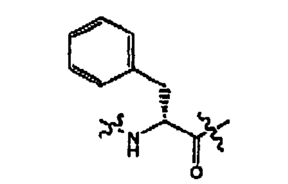
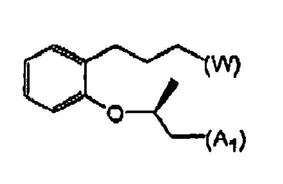
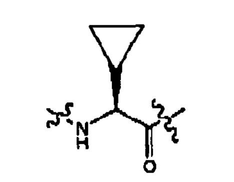
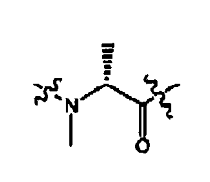
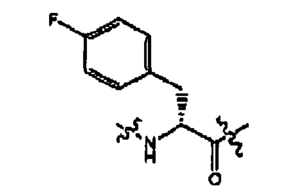
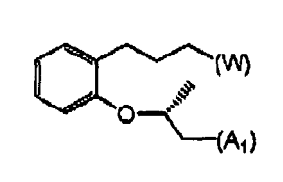
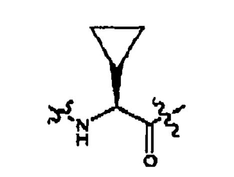
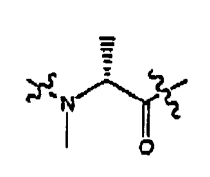
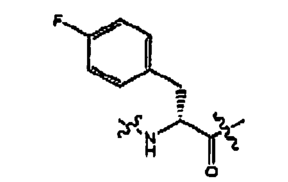
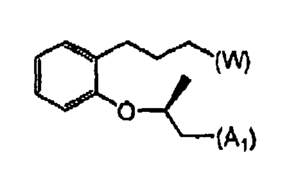
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

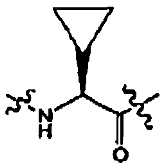
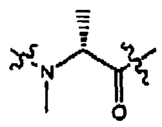
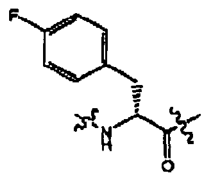
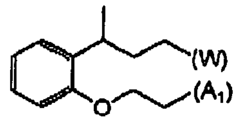
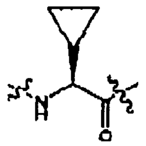
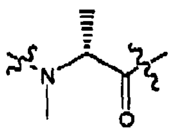
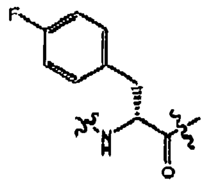
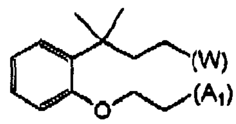
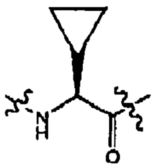
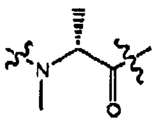
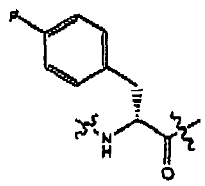
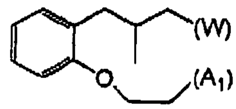
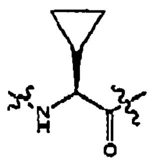
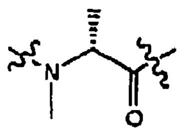
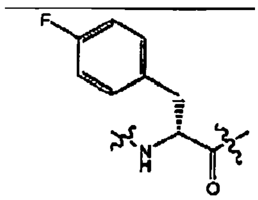
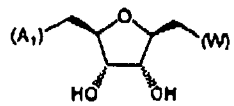
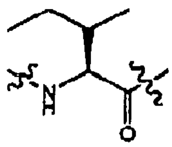
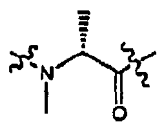
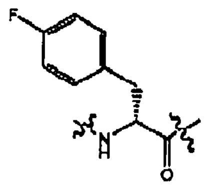
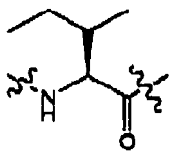
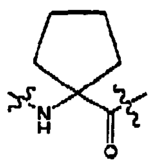
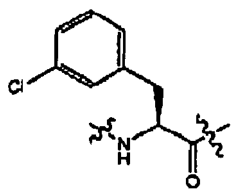
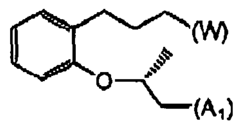
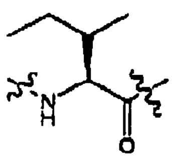
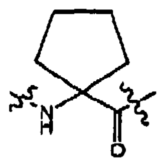
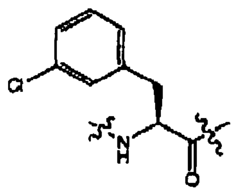
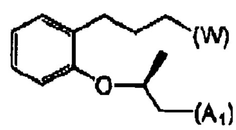
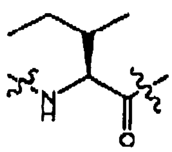
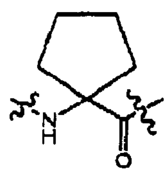
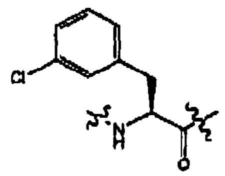
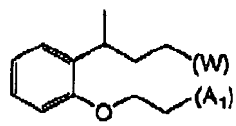
(continued)

W	A_1	A_2	A_3	T
NH				
O				
O				
O				
O				
NH				
NH				
NH				

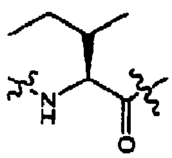
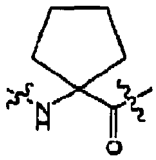
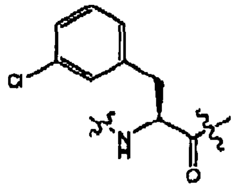
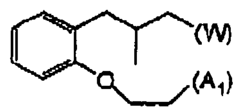
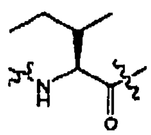
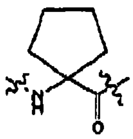
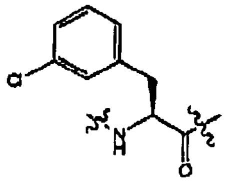
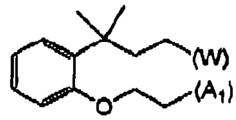
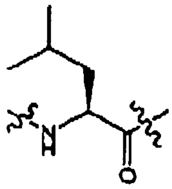
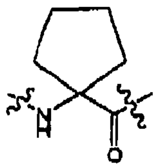
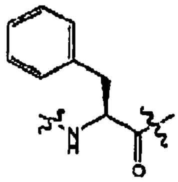
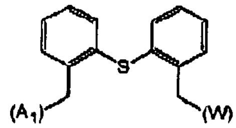
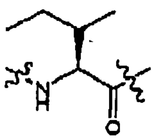
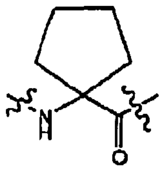
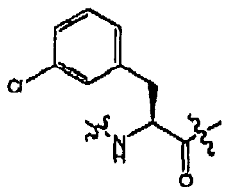
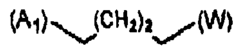
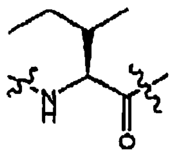
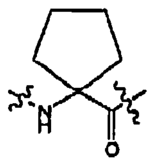
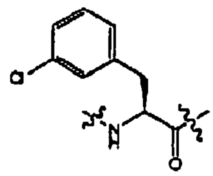
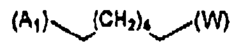
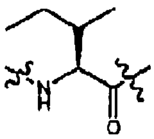
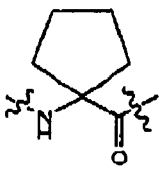
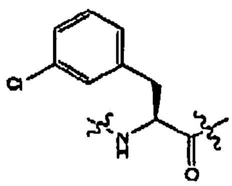
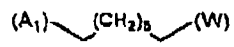
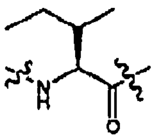
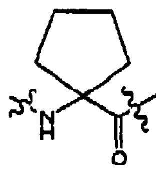
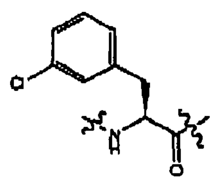
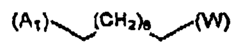
(continued)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

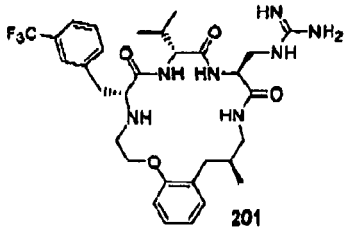
(continued)

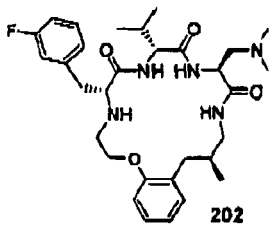
W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

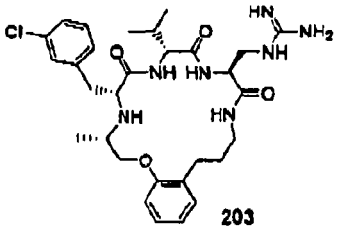
(continued)

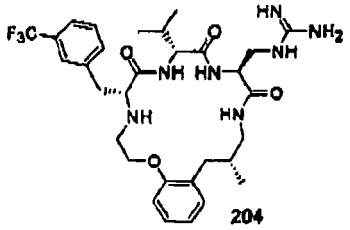
W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

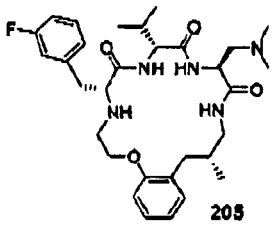
2. The compound of claim 1 having one of the following structures:

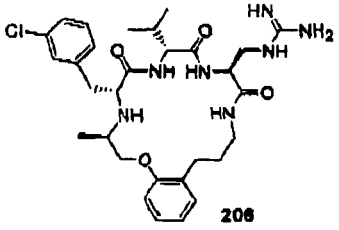

201

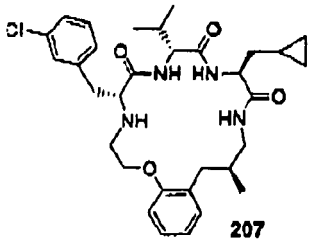

202

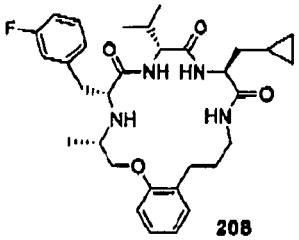

203

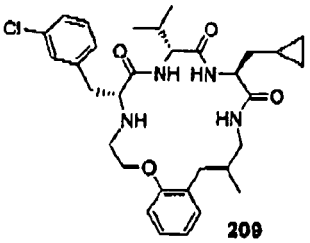

204

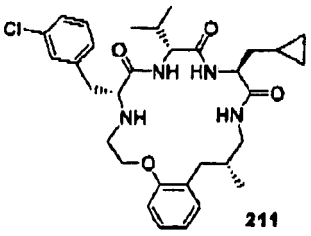

205

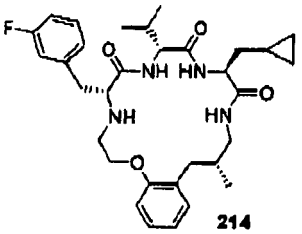

206

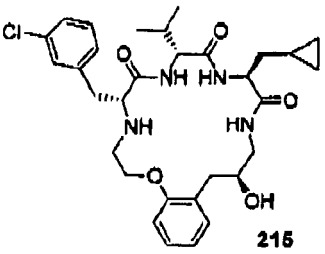

207

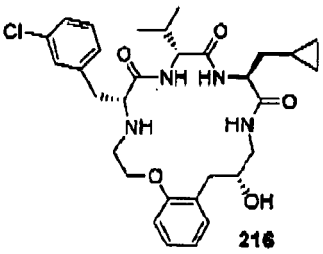

208

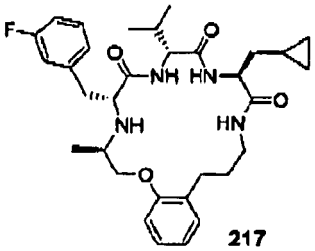

209

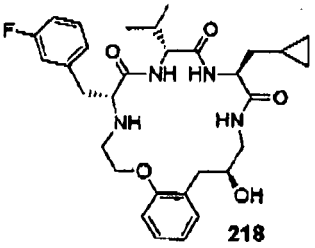

211

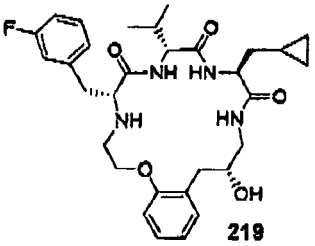

214


215

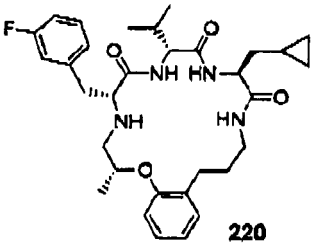

216


217

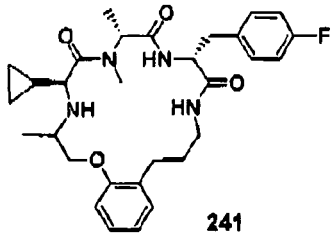

218

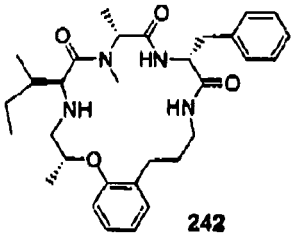

219

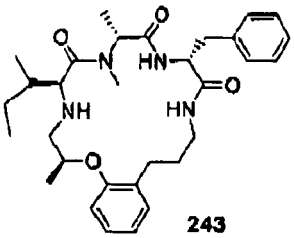
or

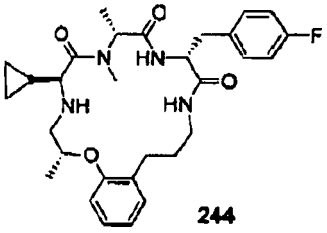

220

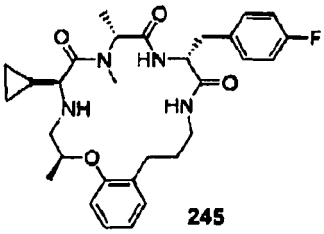
3. The compound of claim 1 having one of the following structures:

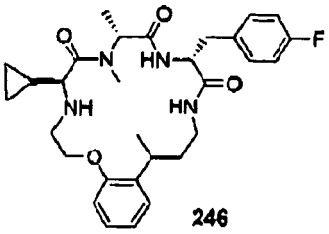

241


242

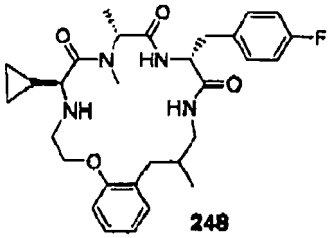

243


244

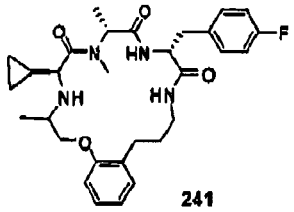

245

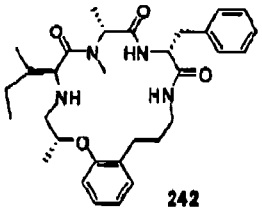

246

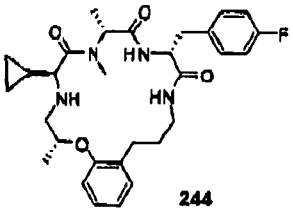
or

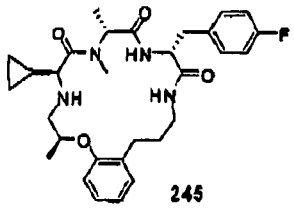

248

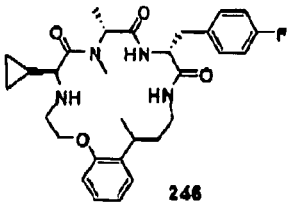
4. The compound of claim 1 having one of the following structures:

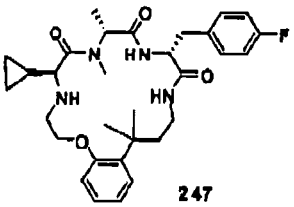

241


242


244

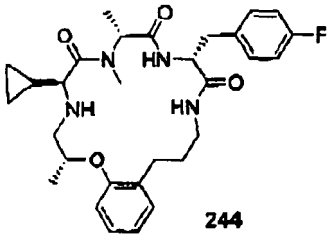

245


246


247

or

5. The compound of claim 1 having the following structure:



244

6. A pharmaceutical composition comprising a compound of any of claims 1 to 5.

7. A compound as defined in any one of claims 1 to 5 for use as a pharmaceutical.

8. Use of a compound as defined in claim 2 in the manufacture of a medicament for use in the treatment of GI disorders involving hypermotility.

9. A compound as defined in claim 2 for use in the treatment of GI disorders involving hypermotility.

10. Use of a compound as defined in any one of claims 3 to 5 in the manufacture of a medicament for use in the treatment of conditions caused by growth hormone deficiency, wasting syndrome and GI disorders involving dysmotility.

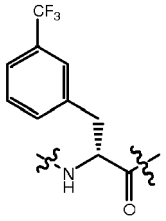
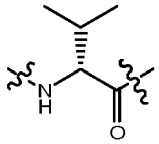
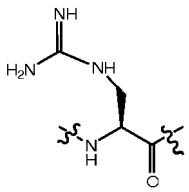
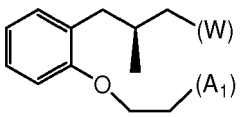
11. A compound as defined in any one of claims 3 to 5 for use in the treatment of conditions caused by growth hormone deficiency, wasting syndrome and GI disorders involving dysmotility.

Patentansprüche

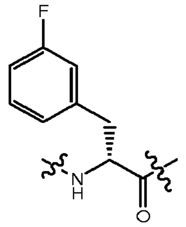
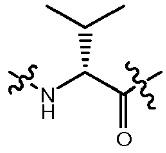
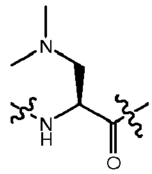
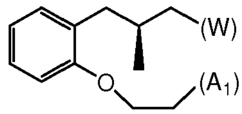
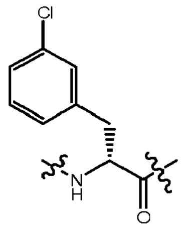
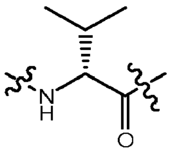
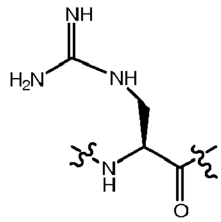
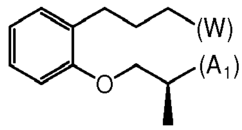
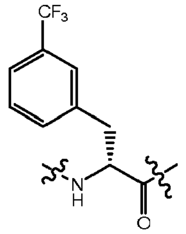
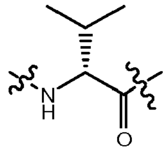
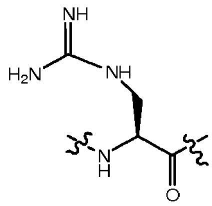
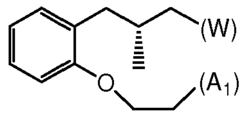
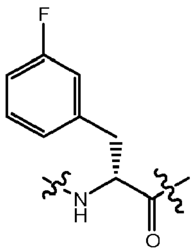
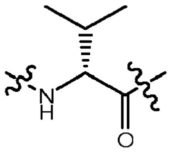
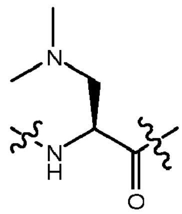
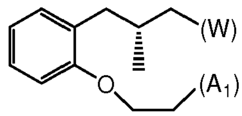
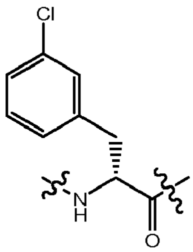
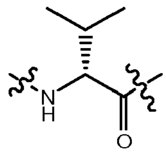
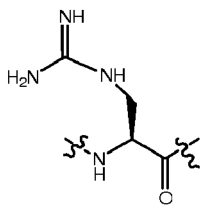
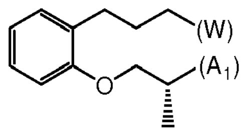
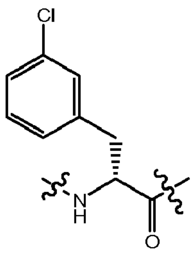
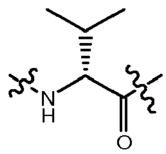
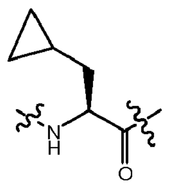
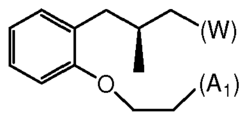
1. Verbindung mit einer Struktur gemäß Formel II:

(II)

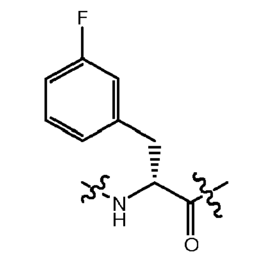
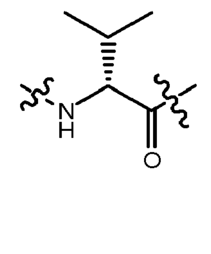
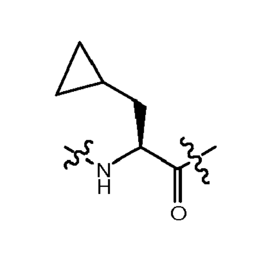
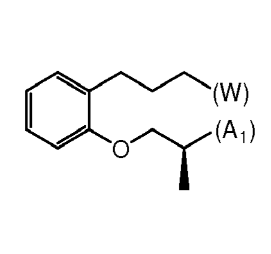
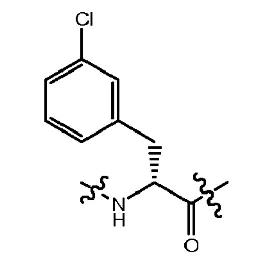
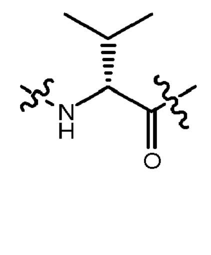
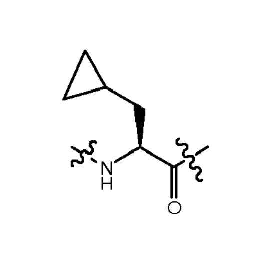
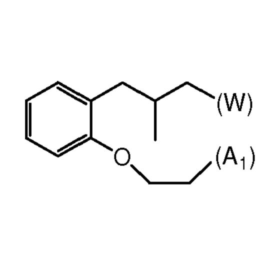
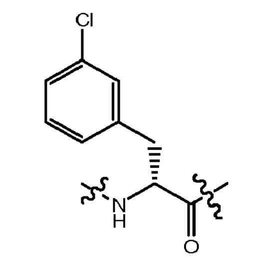
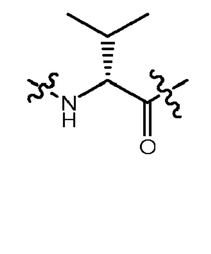
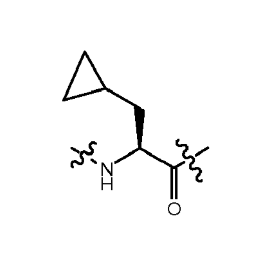
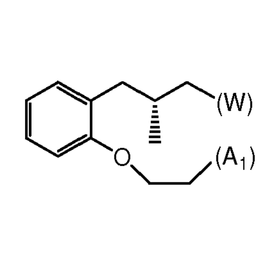
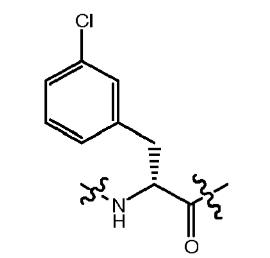
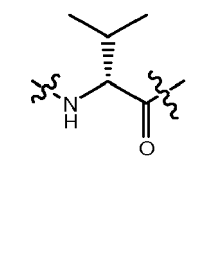
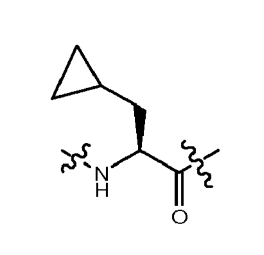
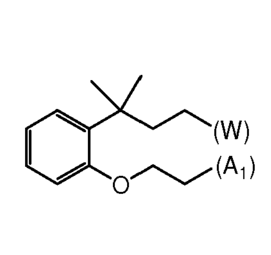
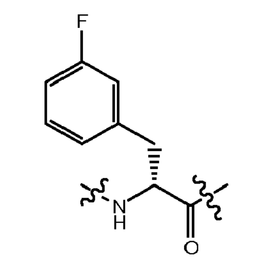
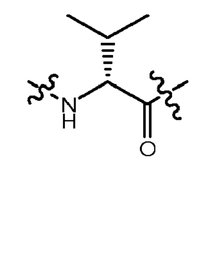
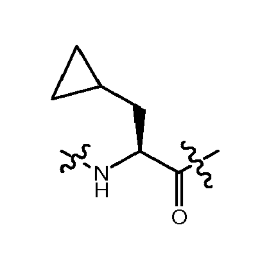
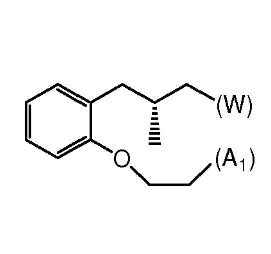
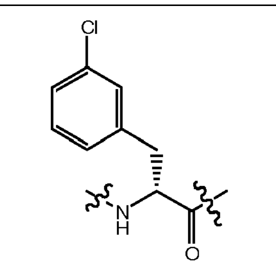
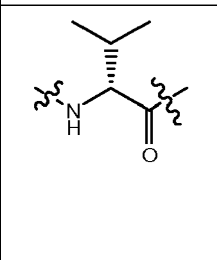
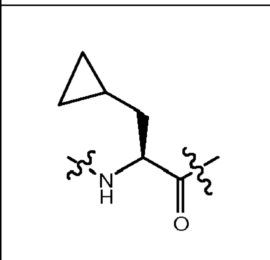
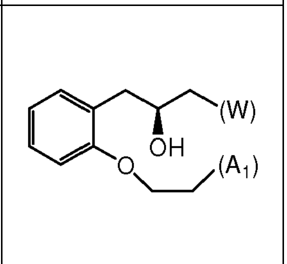
wobei W, A_1, A_2, A_3 und T wie unten definiert sind, wobei das NH von A_1 an T gebunden ist, das C=O von A_1 an das NH von A_2 gebunden ist, das C=O von A_2 an das NH von A_3 gebunden ist, das C=O von A_3 an W gebunden ist und (W) und (A_1) den Bindungsort von T an W bzw. A_1 bezeichnen:

W	A_1	A_2	A_3	T
NH				

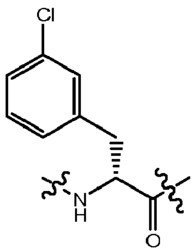
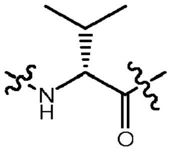
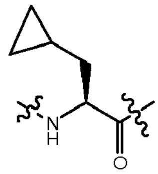
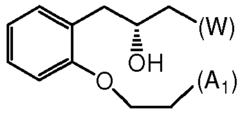
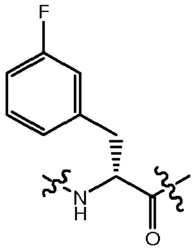
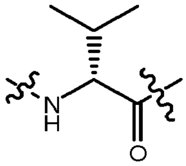
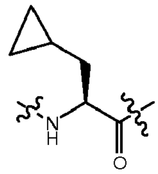
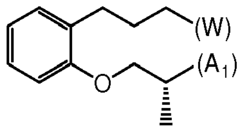
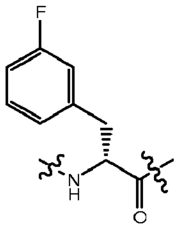
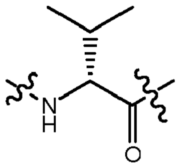
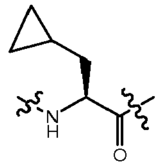
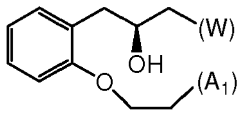
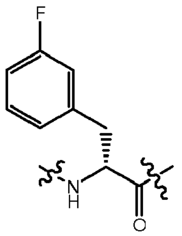
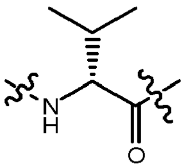
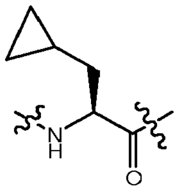
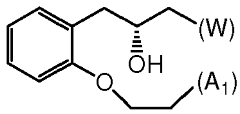
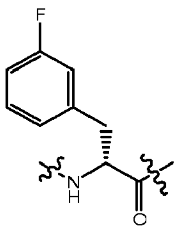
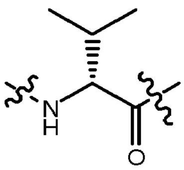
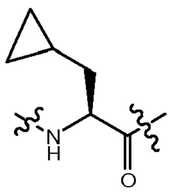
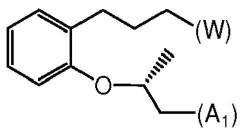
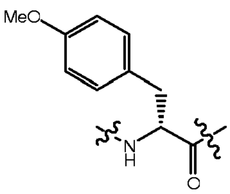
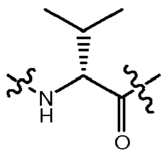
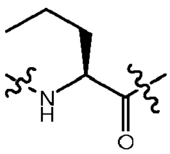
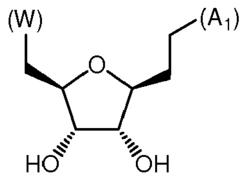
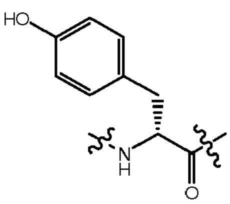
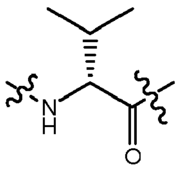
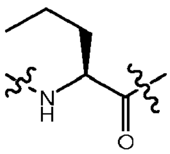
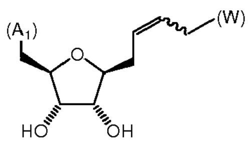
(fortgesetzt)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				

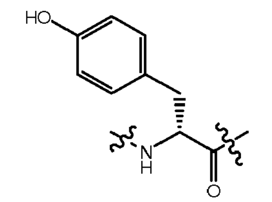
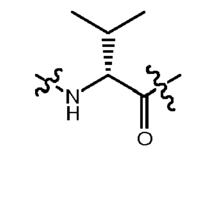
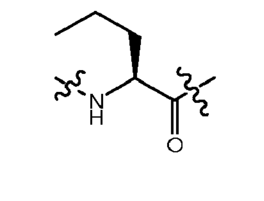
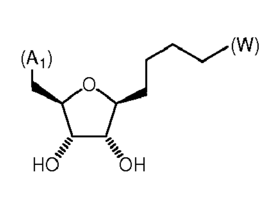
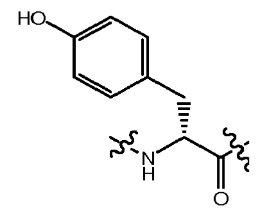
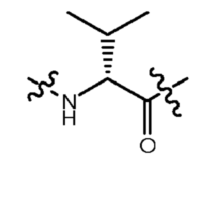
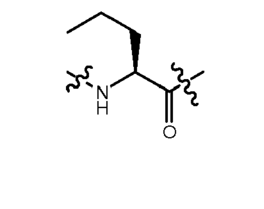
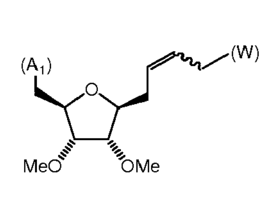
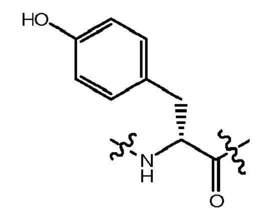
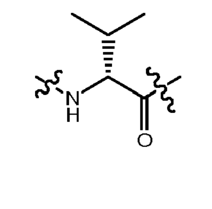
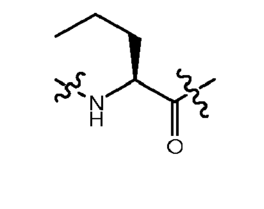
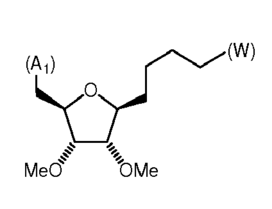
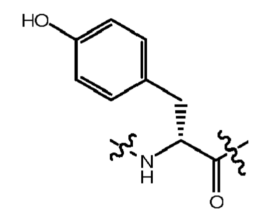
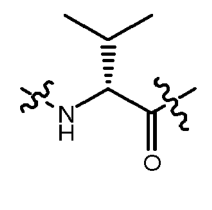
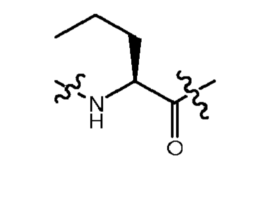
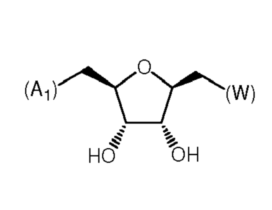
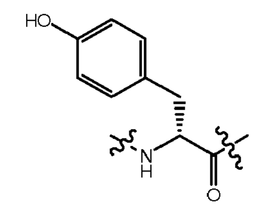
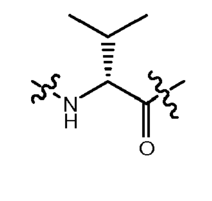
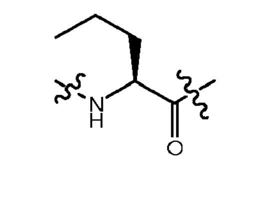
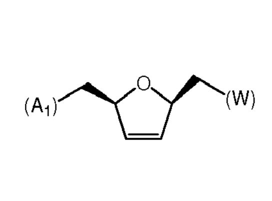
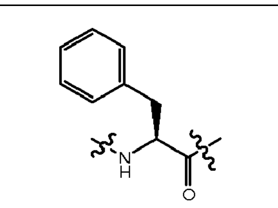
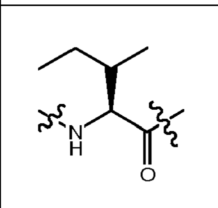
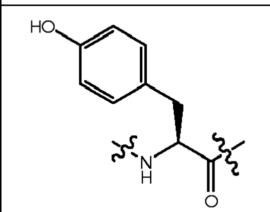
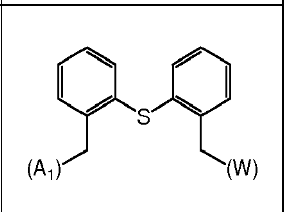
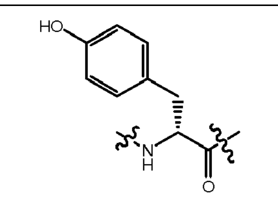
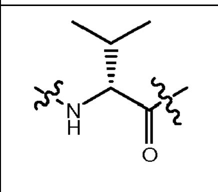
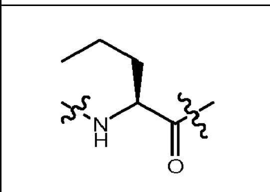
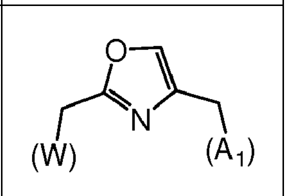
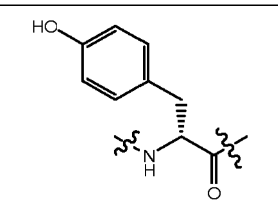
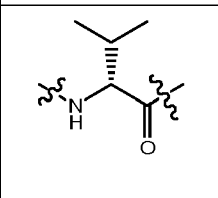
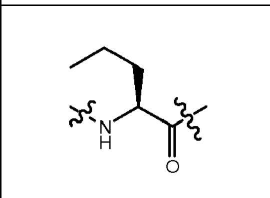
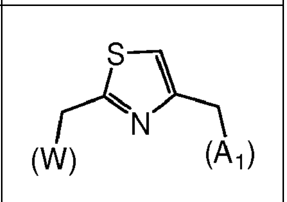
(fortgesetzt)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				

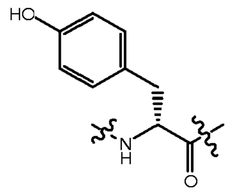
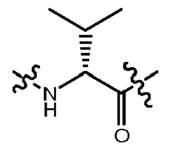
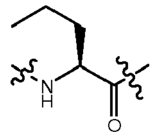
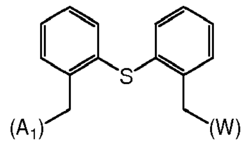
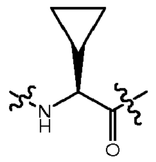
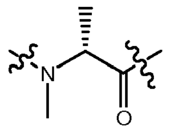
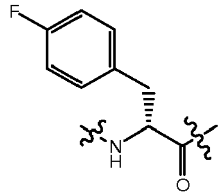
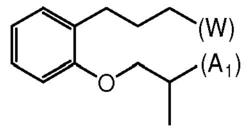
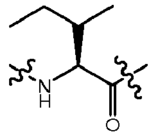
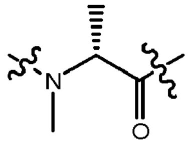
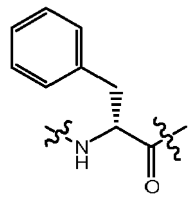
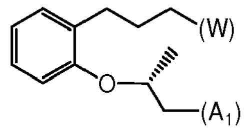
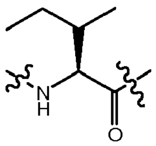
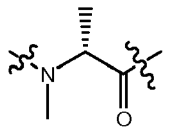
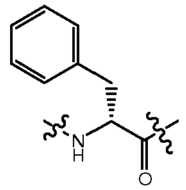
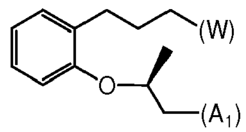
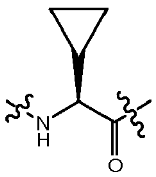
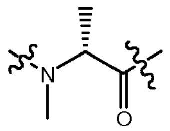
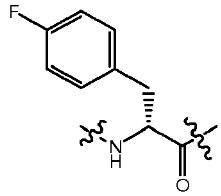
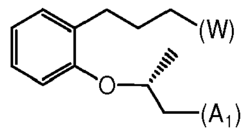
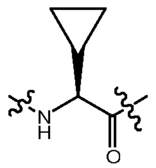
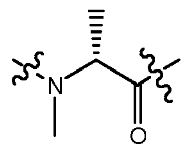
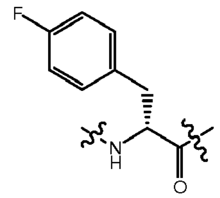
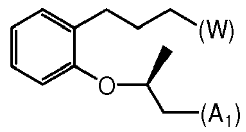
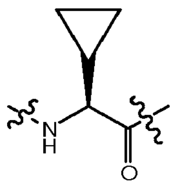
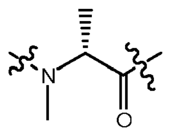
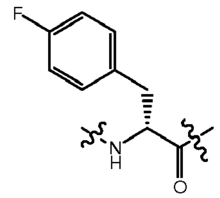
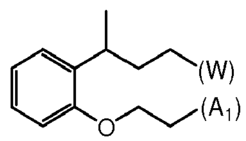
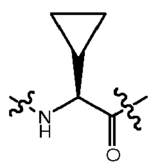
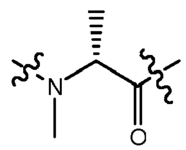
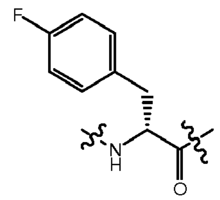
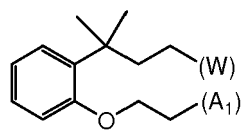
(fortgesetzt)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
O				

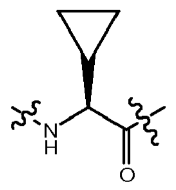
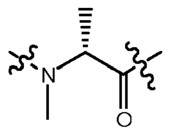
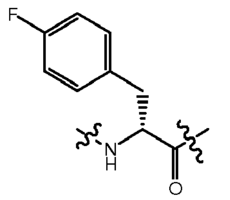
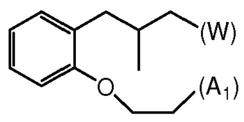
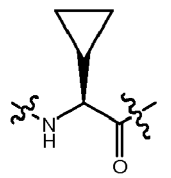
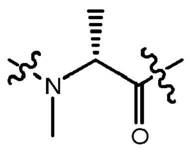
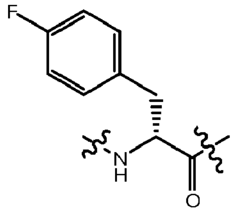
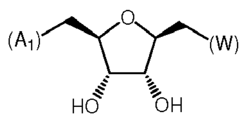
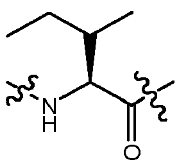
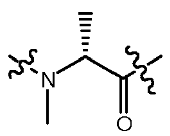
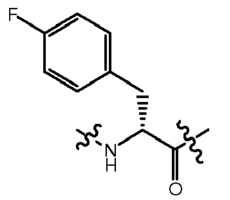
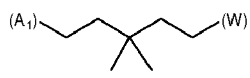
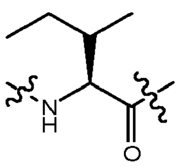
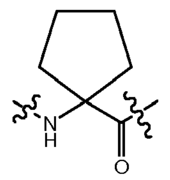
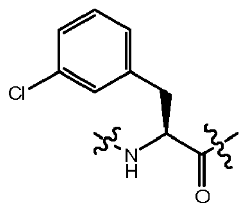
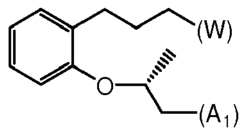
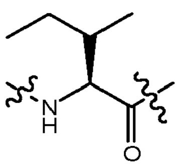
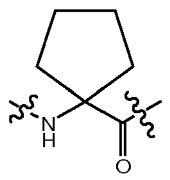
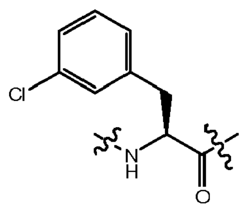
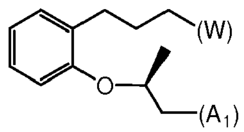
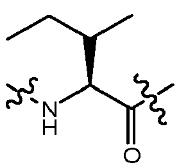
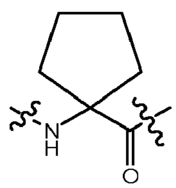
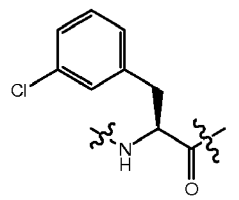
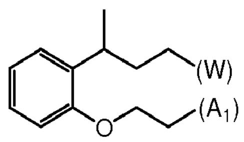
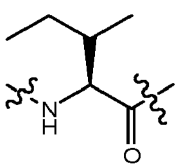
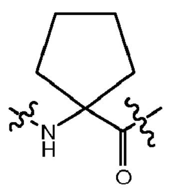
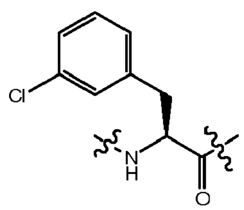
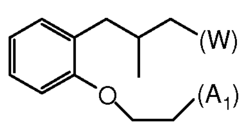
(fortgesetzt)

W	A_1	A_2	A_3	T
O				
O				
O				
NH				
NH				
NH				
NH				
NH				

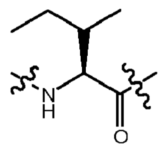
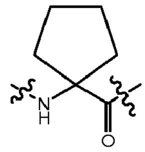
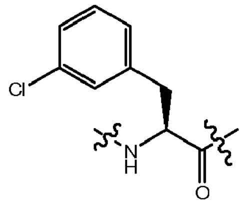
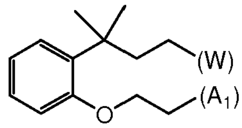
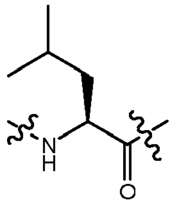
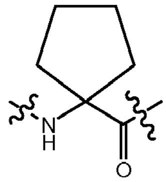
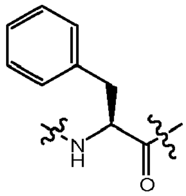
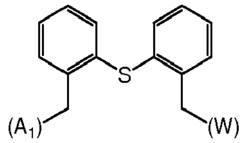
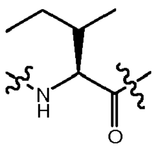
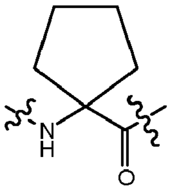
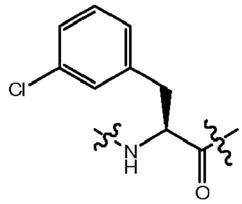
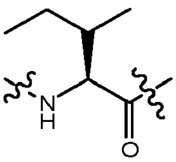
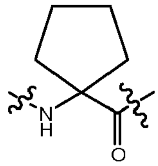
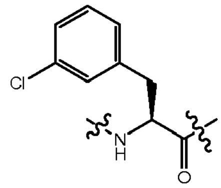
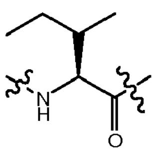
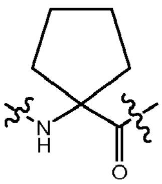
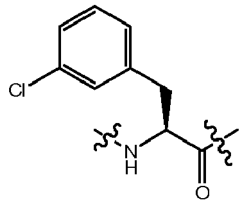
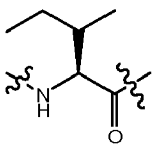
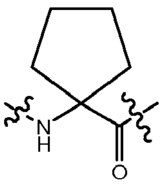
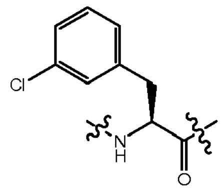
(fortgesetzt)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

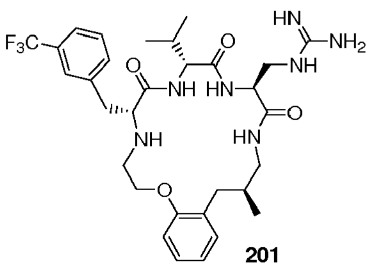
(fortgesetzt)

W	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

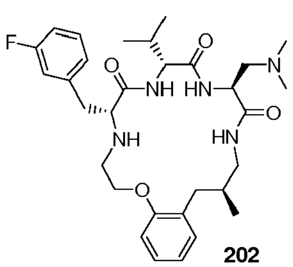
(fortgesetzt)

W	A_1	A_2	A_3	T
NH				
NH				
NH				(A_1)–$(CH_2)_2$–(W)
NH				(A_1)–$(CH_2)_4$–(W)
NH				(A_1)–$(CH_2)_5$–(W)
NH				(A_1)–$(CH_2)_6$–(W)

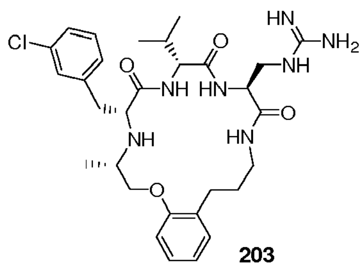
2. Verbindung nach Anspruch 1 mit einer der folgenden Strukturen:



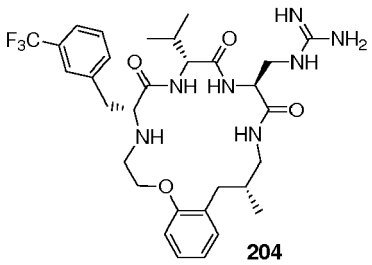
201

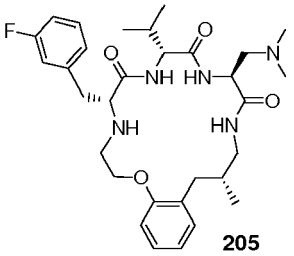


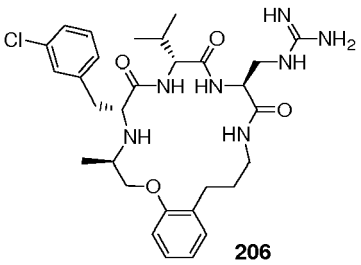
202

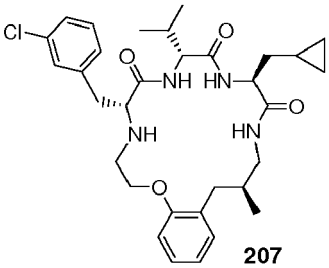


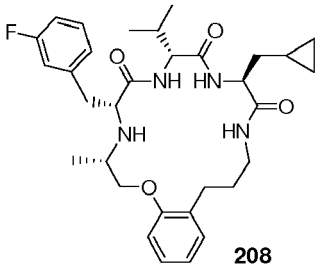
203

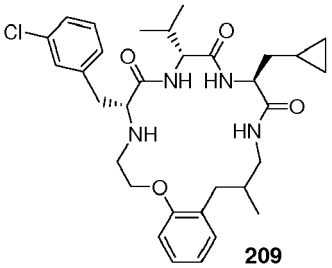

204

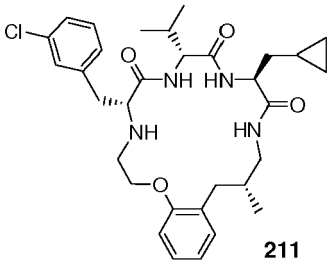

205

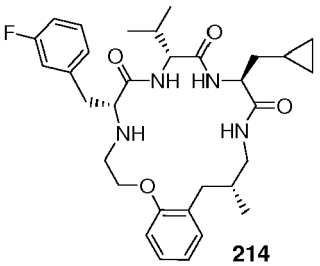

206

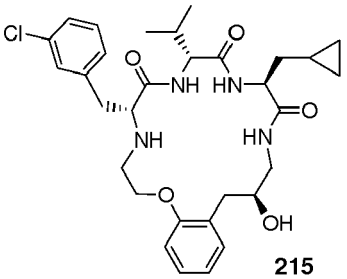

207

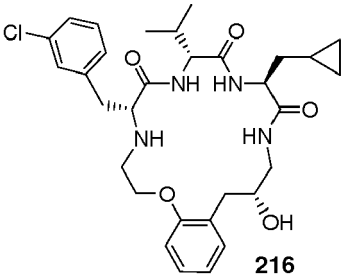

208

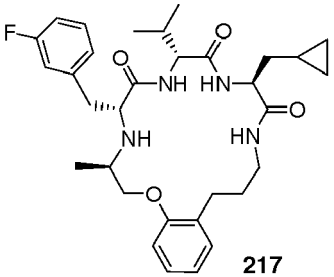

209

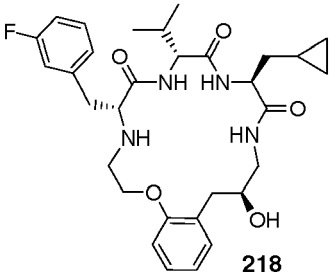

211

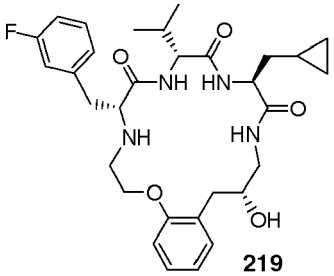

214


215

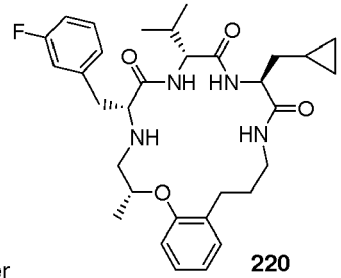

216


217

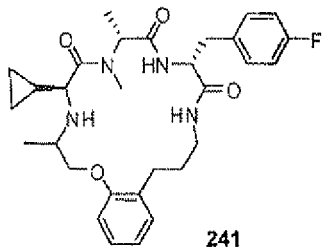

218

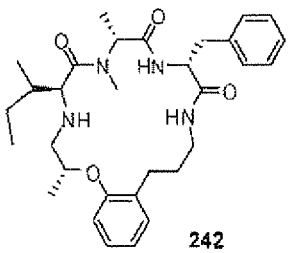

219

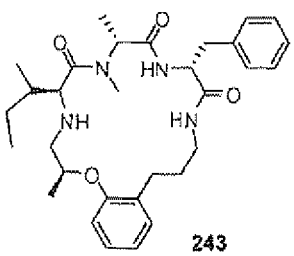
oder

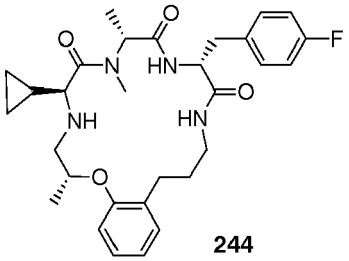

220

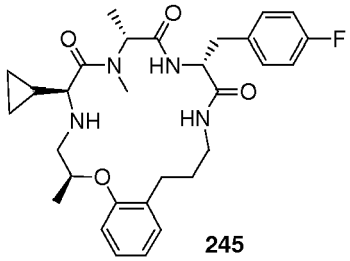
3. Verbindung nach Anspruch 1 mit einer der folgenden Strukturen:

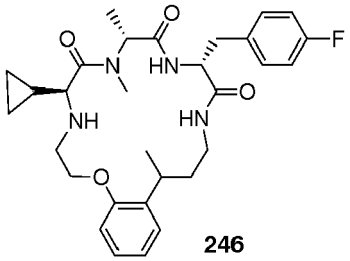

241


242

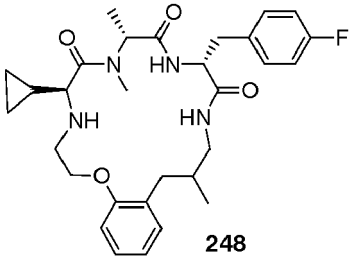

243


244

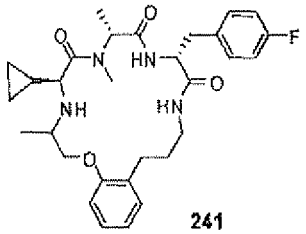

245

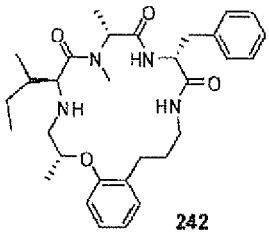

246

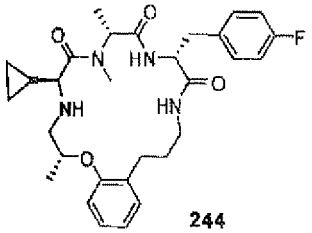
oder

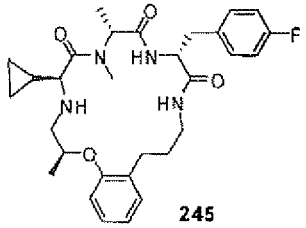

248

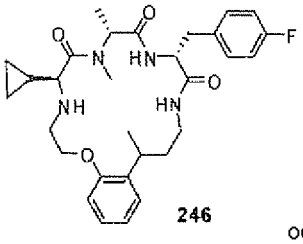
4. Verbindung nach Anspruch 1 mit einer der folgenden Strukturen:


241

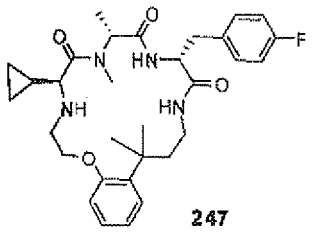

242


244

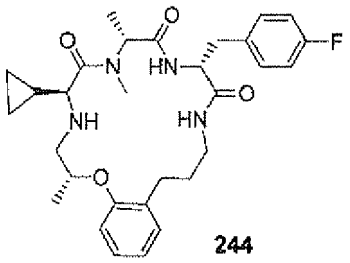

245


246

oder


247

5. Verbindung nach Anspruch 1 mit der folgenden Struktur:



244

6. Pharmazeutische Zusammensetzung, die eine Verbindung nach einem der Ansprüche 1 bis 5 aufweist.

7. Verbindung nach einem der Ansprüche 1 bis 5 zur Verwendung als Arzneimittel.

8. Verwendung einer Verbindung nach Anspruch 2 bei der Herstellung eines Medikaments zur Verwendung bei der Behandlung von Gastrointestinal-(GI-)Erkrankungen, die mit Hypermotilität verbunden sind.

9. Verbindung nach Anspruch 2 zur Verwendung bei der Behandlung von Gastrointestinalerkrankungen, die mit Hypermotilität verbunden sind.

10. Verwendung einer Verbindung nach einem der Ansprüche 3 bis 5 bei der Herstellung eines Medikaments zur Verwendung bei der Behandlung von Leiden, die durch Wachstumshormonmangel, das HIV-Kachexiesyndrom und mit Dysmotilität verbundene Gastrointestinalerkrankungen verursacht werden.

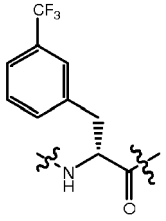
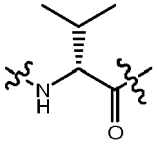
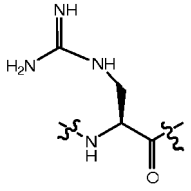
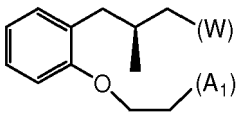
11. Verbindung nach einem der Ansprüche 3 bis 5 zur Verwendung bei der Behandlung von Leiden, die durch Wachstumshormonmangel, das HIV-Kachexiesyndrom und mit Dysmotilität verbundene Gastrointestinalerkrankungen verursacht werden.

Revendications

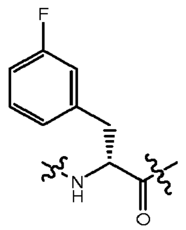
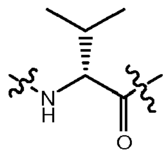
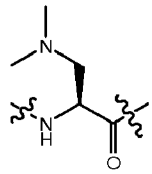
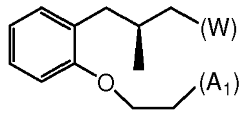
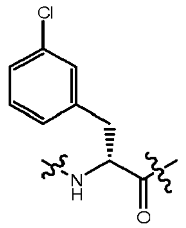
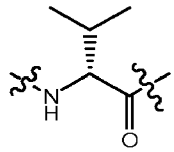
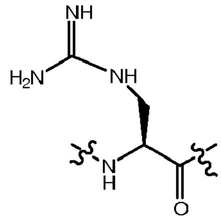
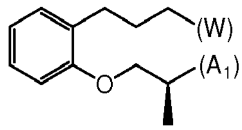
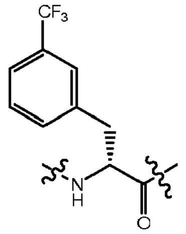
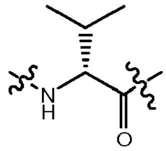
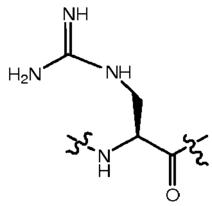
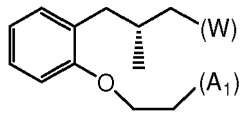
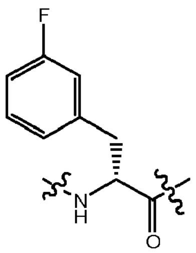
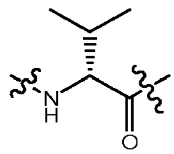
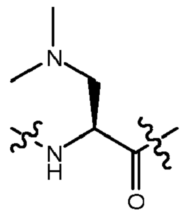
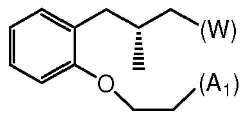
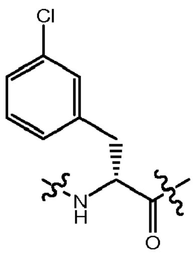
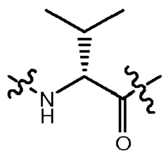
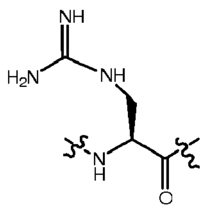
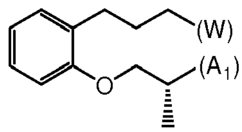
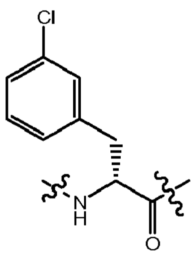
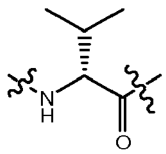
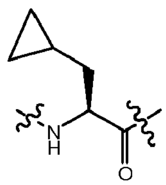
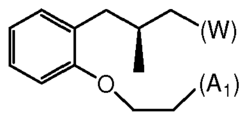
1. Composé présentant la structure de la formule II:

(II)

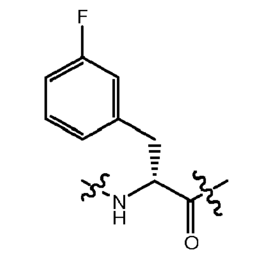
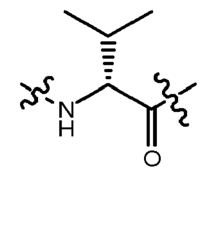
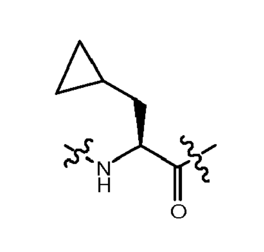
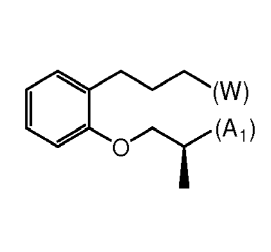
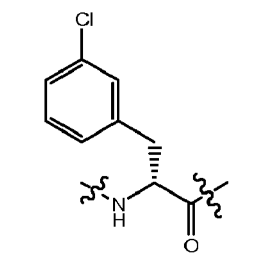
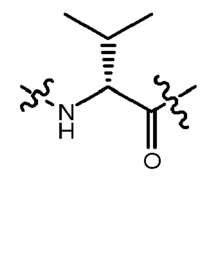
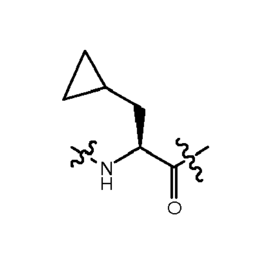
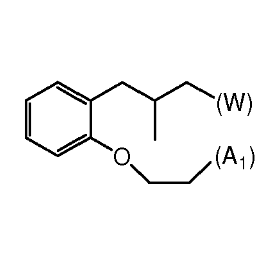
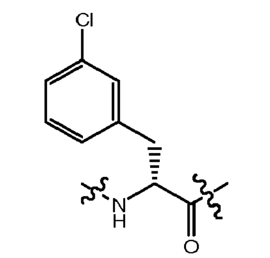
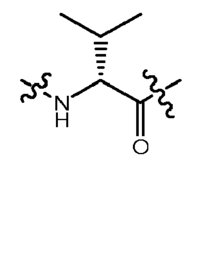
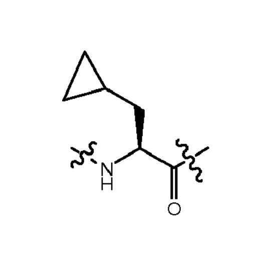
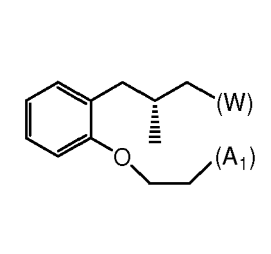
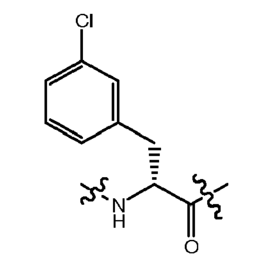
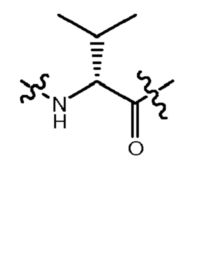
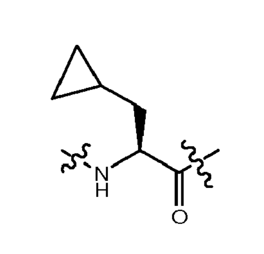
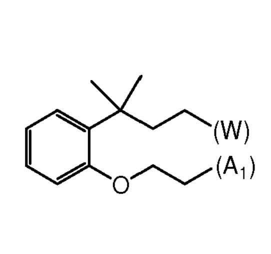
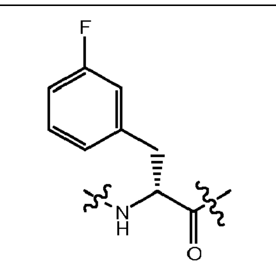
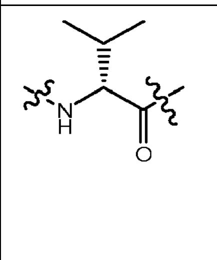
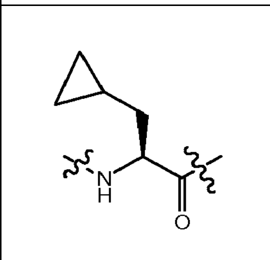
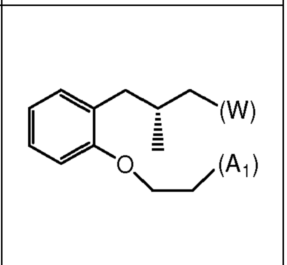
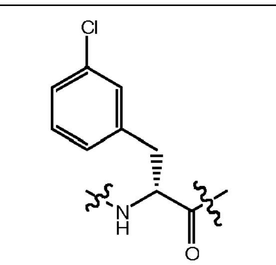
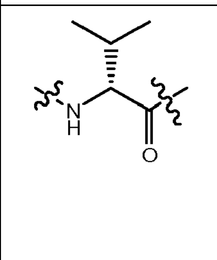
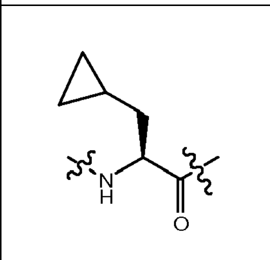
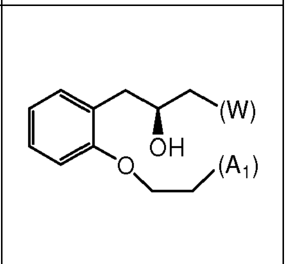
dans lequel W, A_1, A_2, A_3 et T sont définis comme ci-dessous avec le NH de A_1 lié à T, le C=O de A_1 lié au NH de A_2, le C=O de A_2 lié au NH de A_3, le C=O de A_3 lié à W, et (W) et (A_1) indiquent le site de liaison de T à W et A_1, respectivement:

w	A_1	A_2	A_3	T
NH				

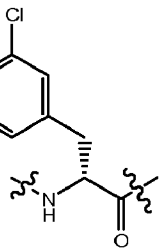
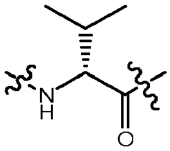
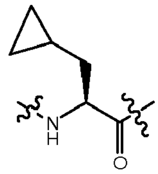
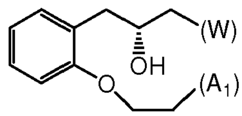
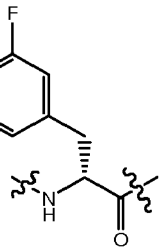
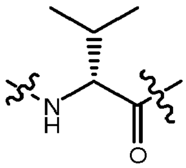
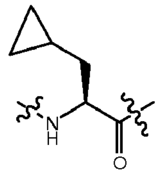
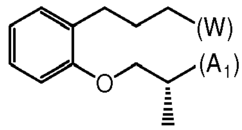
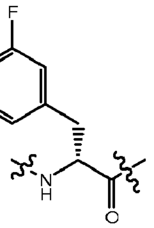
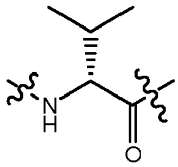
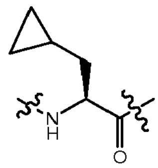
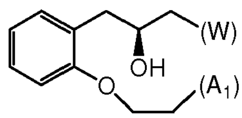
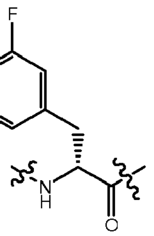
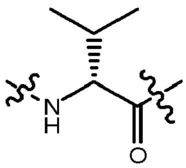
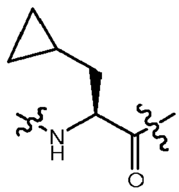
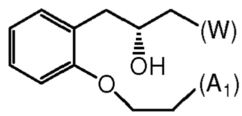
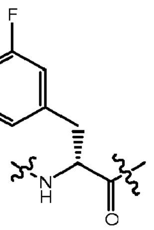
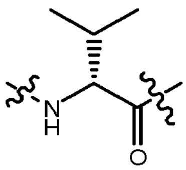
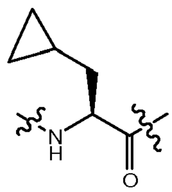
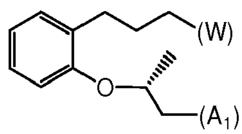
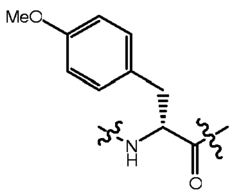
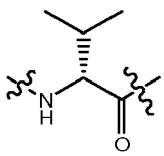
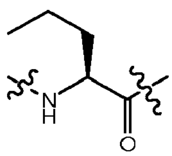
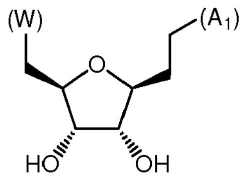
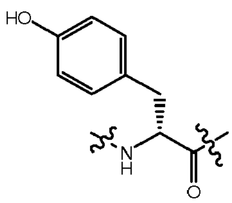
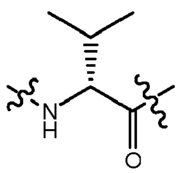
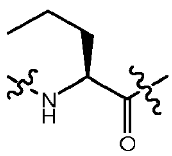
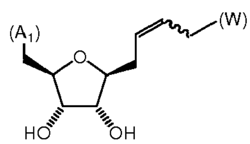
(suite)

w	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				

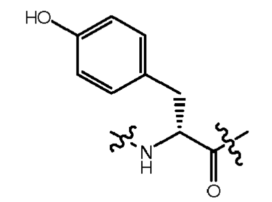
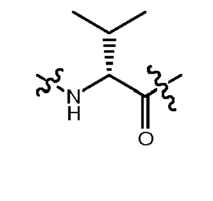
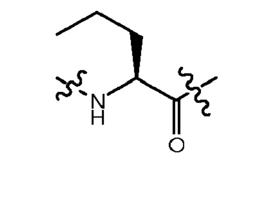
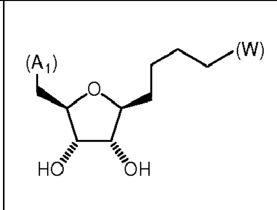
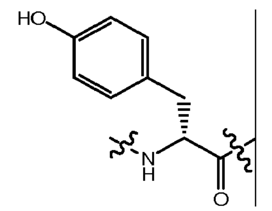
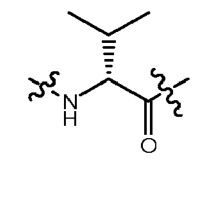
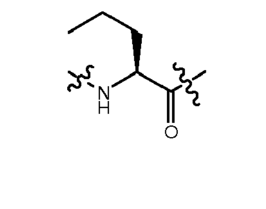
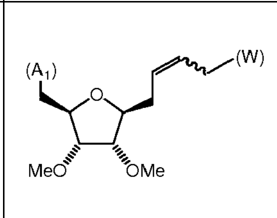
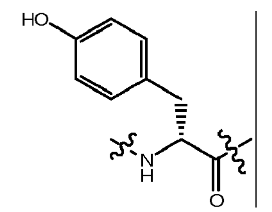
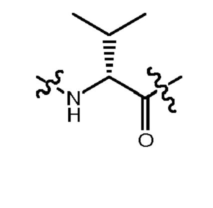
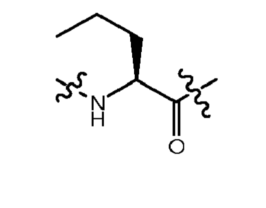
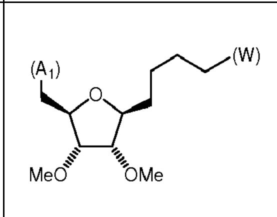
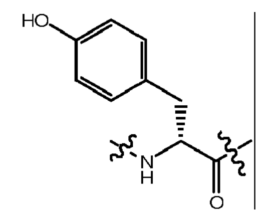
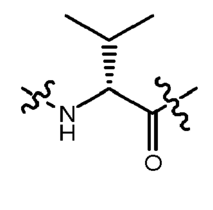
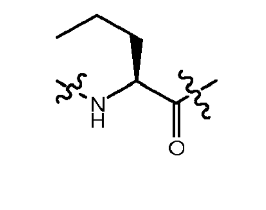
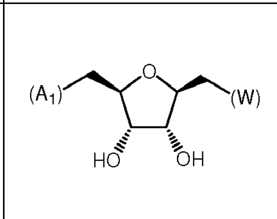
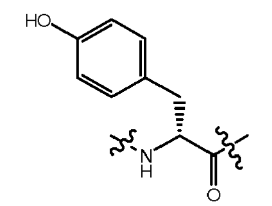
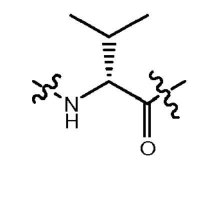
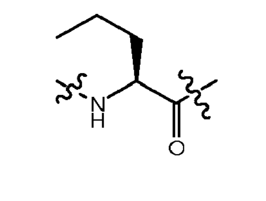
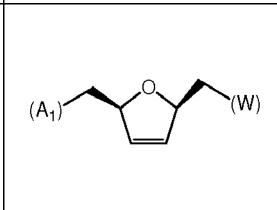
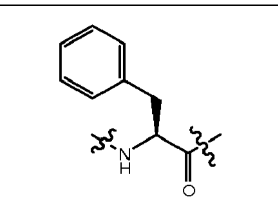
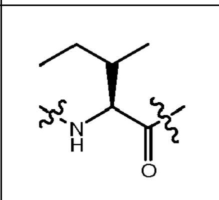
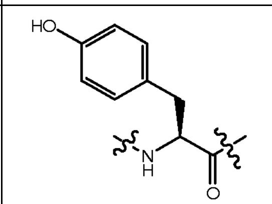
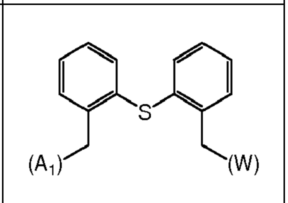
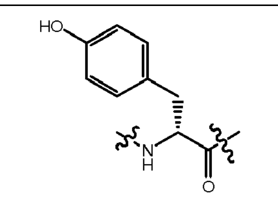
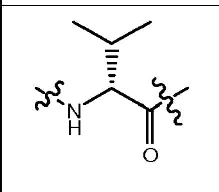
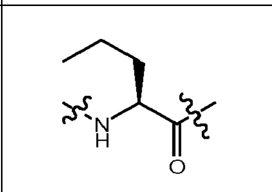
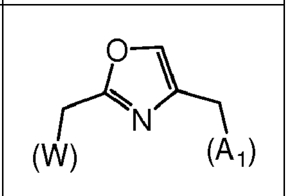
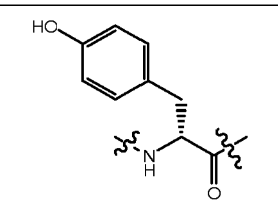
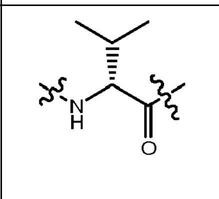
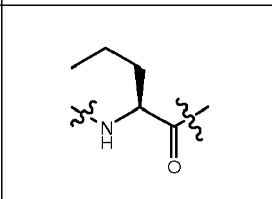
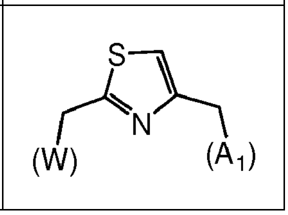
(suite)

w	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				

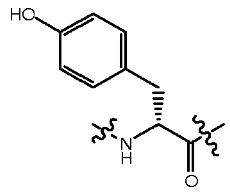
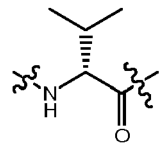
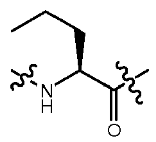
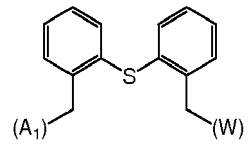
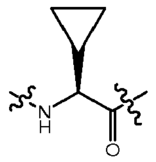
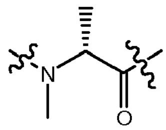
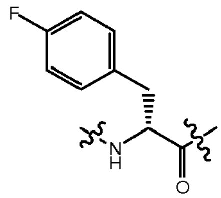
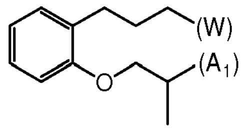
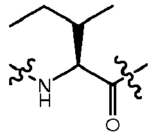
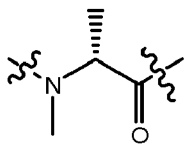
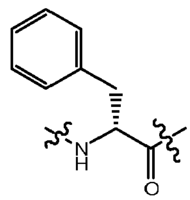
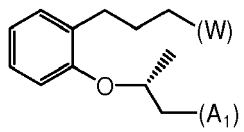
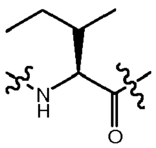
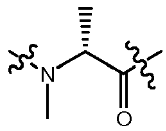
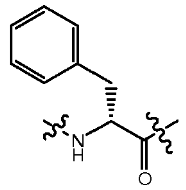
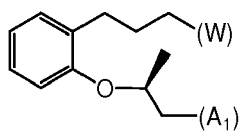
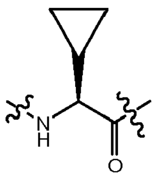
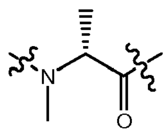
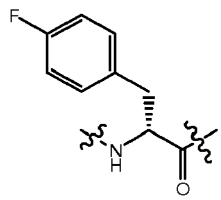
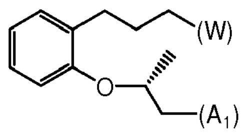
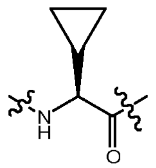
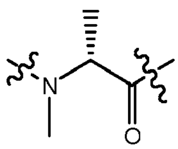
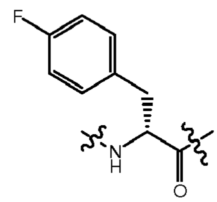
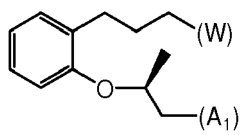
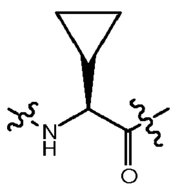
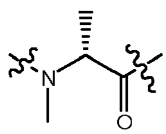
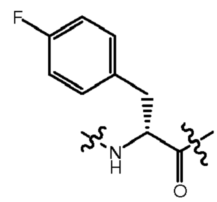
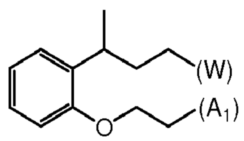
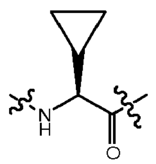
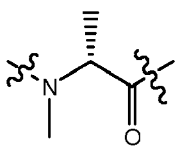
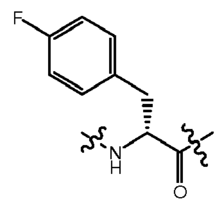
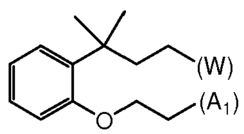
(suite)

w	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
O				

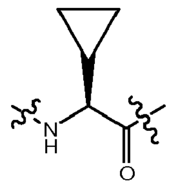
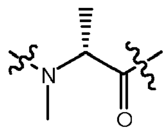
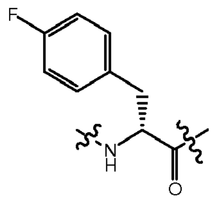
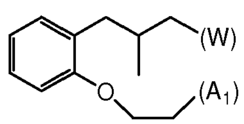
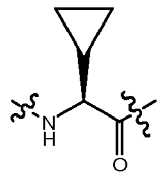
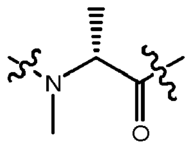
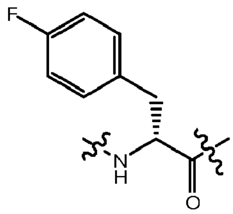
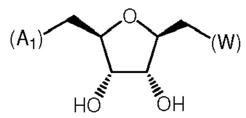
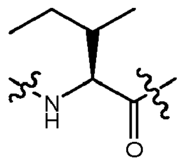
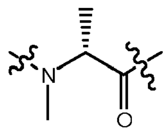
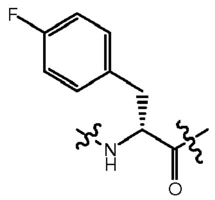
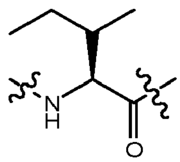
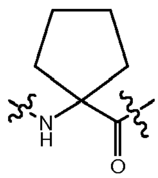
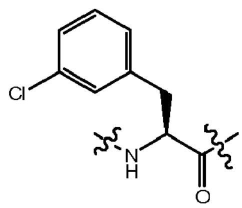
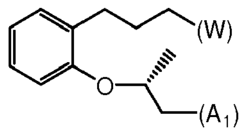
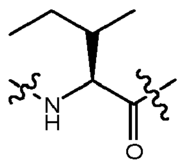
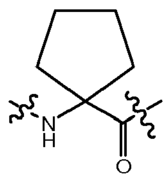
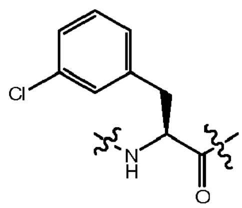
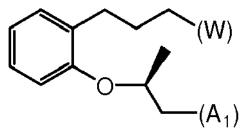
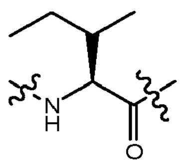
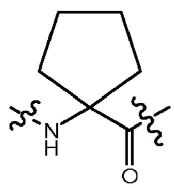
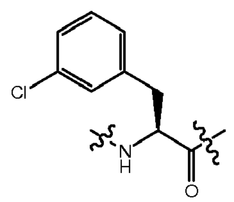
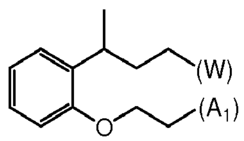
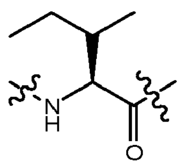
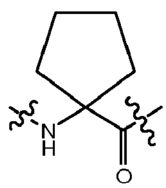
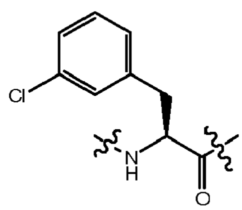
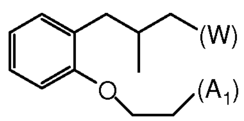
(suite)

w	A_1	A_2	A_3	T
O				
O				
O				
NH				
NH				
NH				
NH				
NH				

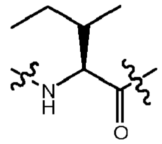
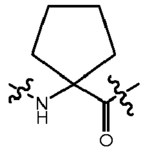
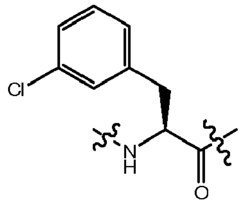
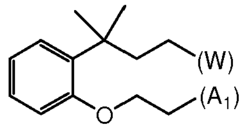
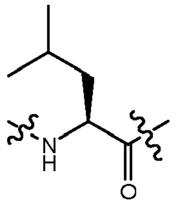
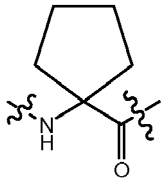
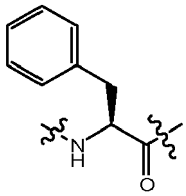
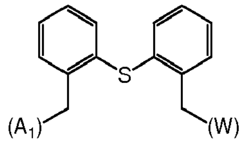
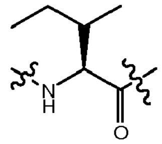
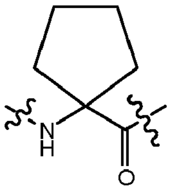
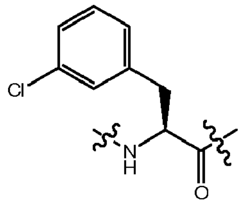
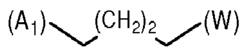
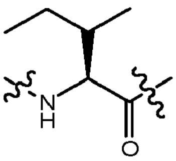
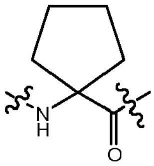
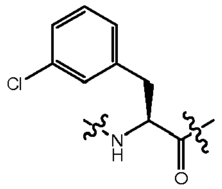
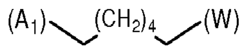
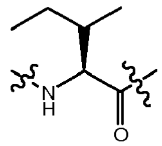
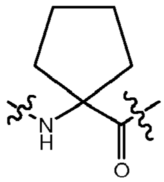
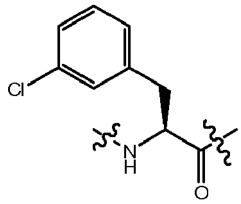
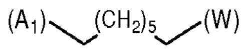
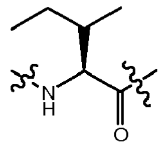
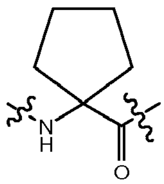
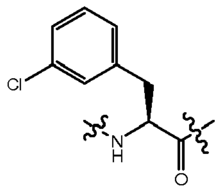
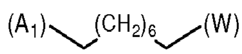
(suite)

w	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				
NH				

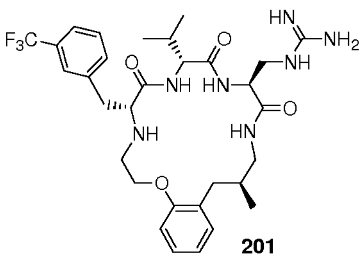
(suite)

w	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				
NH				

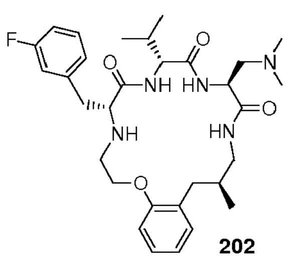
(suite)

w	A_1	A_2	A_3	T
NH				
NH				
NH				
NH				
NH				
NH				

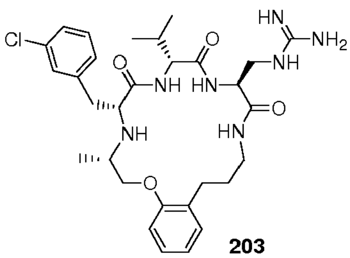
2. Composé selon la revendication 1 présentant une des structures suivantes:



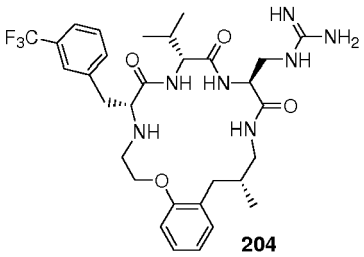
201

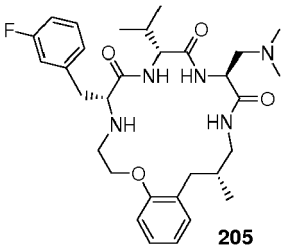


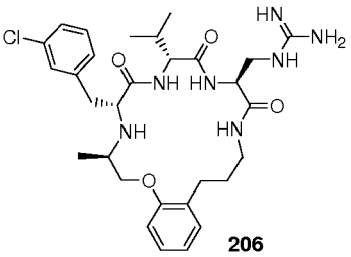
202

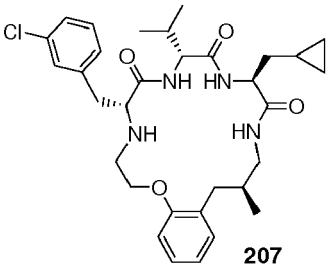


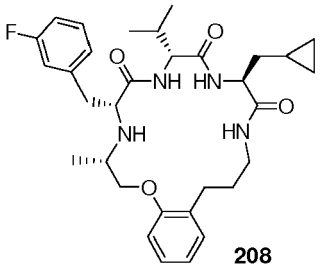
203

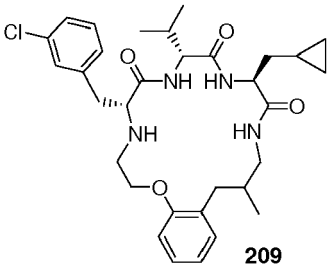

204

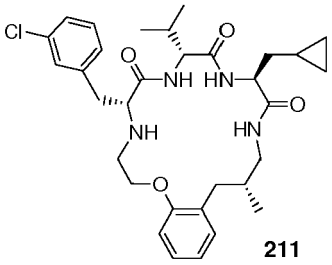

205

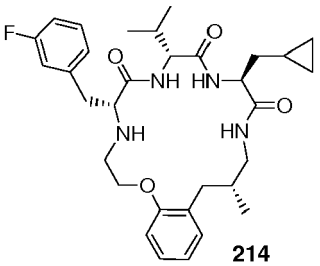

206

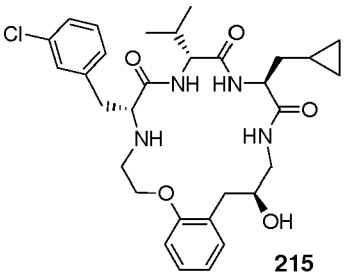

207

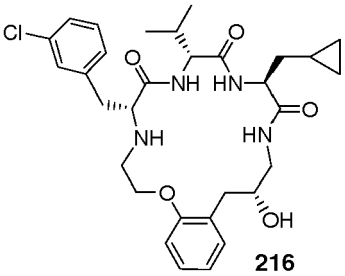

208

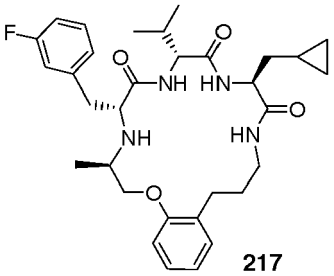

209

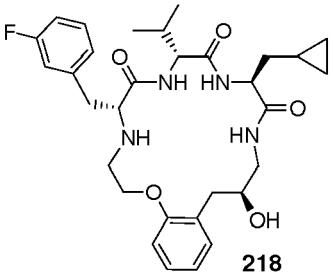

211

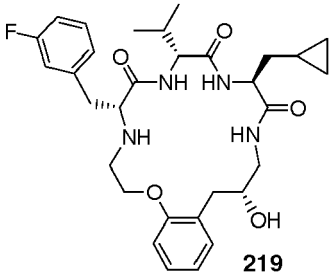

214


215

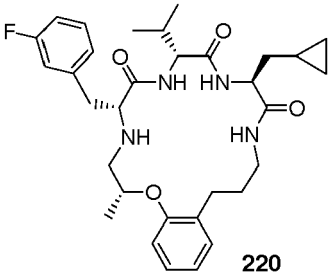

216


217

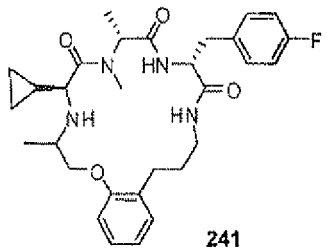

218

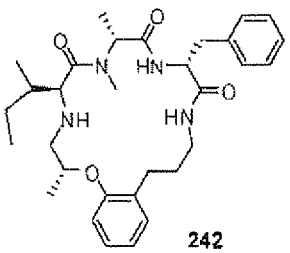

219

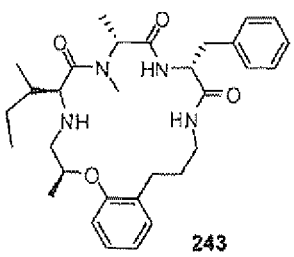
ou

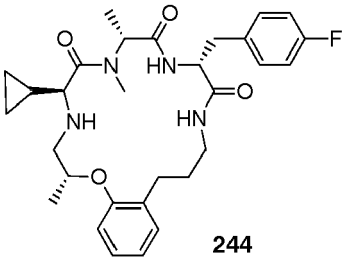

220

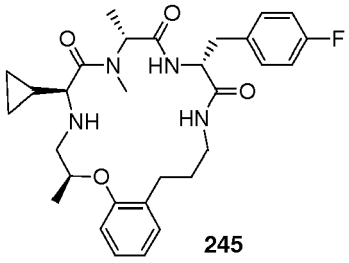
3. Composé selon la revendication 1 présentant une des structures suivantes:

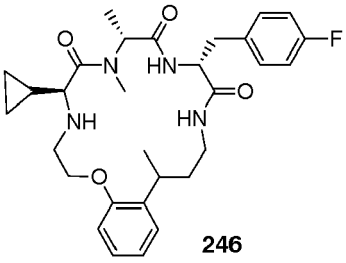

241


242

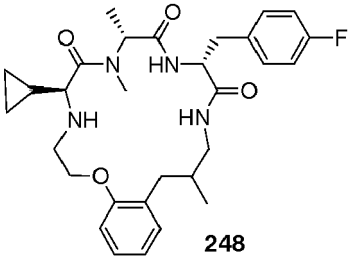

243


244

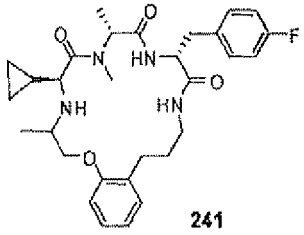

245

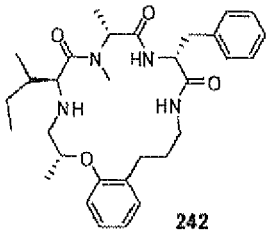

246

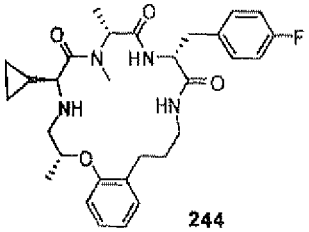
ou

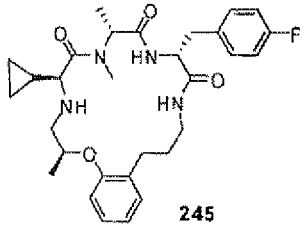

248

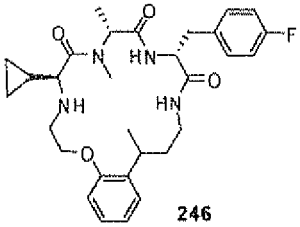
4. Composé selon la revendication 1 présentant une des structures suivantes:


241

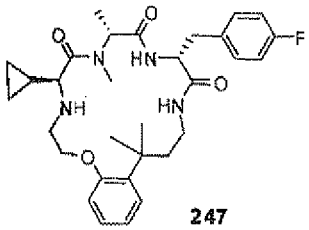

242


244

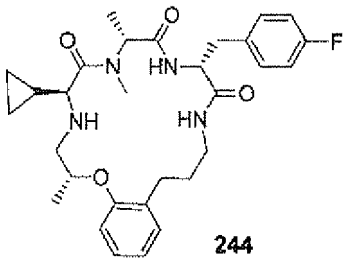

245


246

ou


247

5. Composé selon la revendication 1 présentant la structure suivante:



244

6. Composition pharmaceutique comprenant un composé selon l'une quelconque des revendications 1 à 5.

7. Composé selon l'une quelconque des revendications 1 à 5 pour une utilisation en tant que produit pharmaceutique.

8. Utilisation d'un composé selon la revendication 2 dans la fabrication d'un médicament pour une utilisation dans le traitement de troubles GI impliquant une hypermotilité.

9. Composé selon la revendication 2 pour une utilisation dans le traitement de troubles GI impliquant une hypermotilité.

10. Utilisation d'un composé selon l'une quelconque des revendications 3 à 5 dans la fabrication d'un médicament pour une utilisation dans le traitement d'états entraînés par une déficience en hormone de croissance, un syndrome de cachexie et des troubles GI impliquant une dysmotilité.

11. Composé selon l'une quelconque des revendications 3 à 5 pour une utilisation dans le traitement d'états entraînés par une déficience en hormone de croissance, un syndrome de cachexie et des troubles GI impliquant une dysmotilité.

Figure (I)

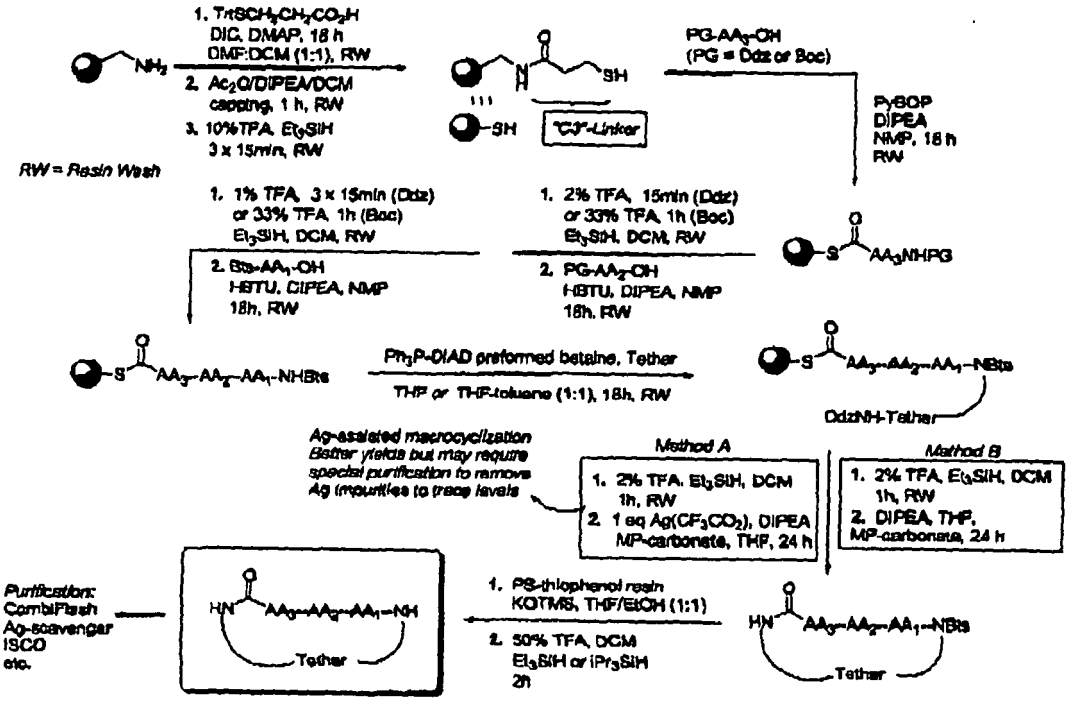

1. TrtSCH2CH2CO2H
DIC, DMAP, 18 h
DMF:DCM (1:1), RW
2. Ac2O/DIPEA/DCM
capping, 1 h, RW
3. 10%TFA, Et3SiH
3 x 15min, RW
RW = Resin Wash
"C3"-Linker
PG-AA3-OH
(PG = Ddz or Boc)
PyBOP
DIPEA
NMP, 18 h
RW
1. 1% TFA, 3 x 15min (Ddz)
or 33% TFA, 1h (Boc)
Et3SiH, DCM, RW
2. Bts-AA1-OH
HBTU, DIPEA, NMP
18h, RW
1. 2% TFA, 15min (Ddz)
or 33% TFA, 1h (Boc)
Et3SiH, DCM, RW
2. PG-AA2-OH
HBTU, DIPEA, NMP
18h, RW
Ph3P-DIAD preformed betaine, Tether
THF or THF-toluene (1:1), 18h, RW
DdzNH-Tether
Ag-assisted macrocyclization
Better yields but may require
special purification to remove
Ag impurities to trace levels
Method A
1. 2% TFA, Et3SiH, DCM
1h, RW
2. 1 eq Ag(CF3CO2), DIPEA
MP-carbonate, THF, 24 h
Method B
1. 2% TFA, Et3SiH, DCM
1h, RW
2. DIPEA, THF,
MP-carbonate, 24 h
Purification:
CombiFlash
Ag-scavenger
ISCO
etc.
Tether
1. PS-thiophenol resin
KOTMS, THF/EtOH (1:1)
2. 50% TFA, DCM
Et3SiH or iPr3SiH
2h
Tether

Figure (2)

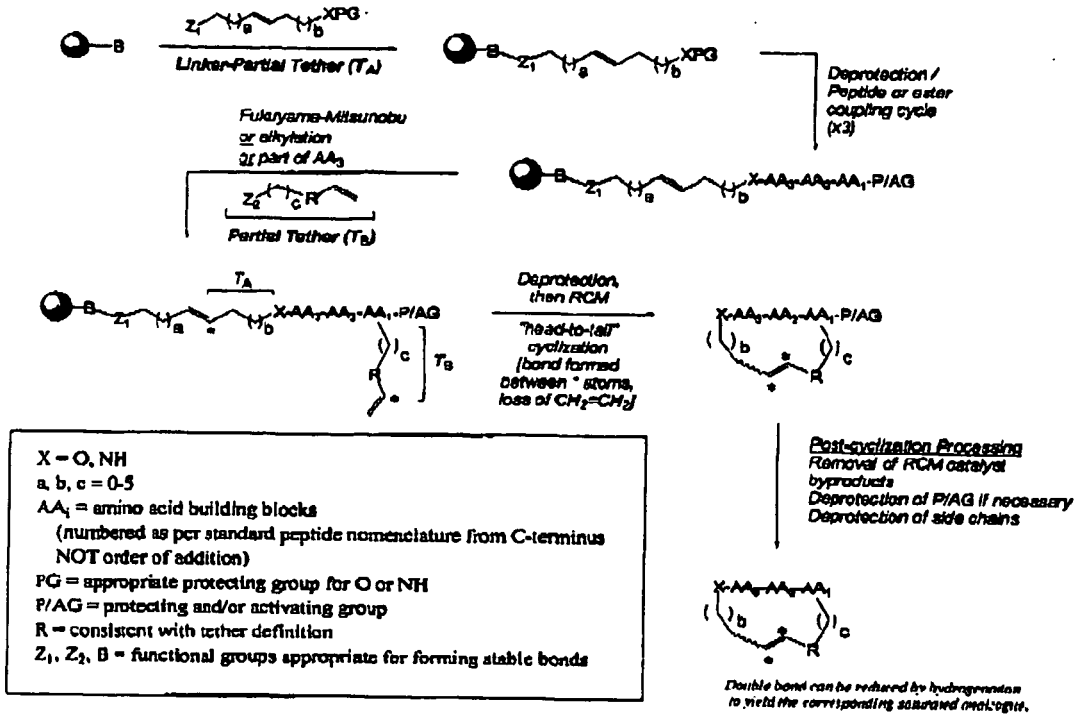

Linker-Partial Tether (T_A)
Deprotection / Peptide or ester coupling cycle (x3)
Fukuyama-Mitsunobu or alkylation of part of AA_3
Partial Tether (T_B)
Deprotection, then RCM
"head-to-tail" cyclization (bond formed between * atoms, loss of $CH_2=CH_2$)
Post-cyclization Processing
Removal of RCM catalyst byproducts
Deprotection of P/AG if necessary
Deprotection of side chains
X = O, NH
a, b, c = 0-5
AA_i = amino acid building blocks (numbered as per standard peptide nomenclature from C-terminus NOT order of addition)
PG = appropriate protecting group for O or NH
P/AG = protecting and/or activating group
R = consistent with tether definition
Z_1, Z_2, B = functional groups appropriate for forming stable bonds
Double bond can be reduced by hydrogenation to yield the corresponding saturated analogue.

Figure 3

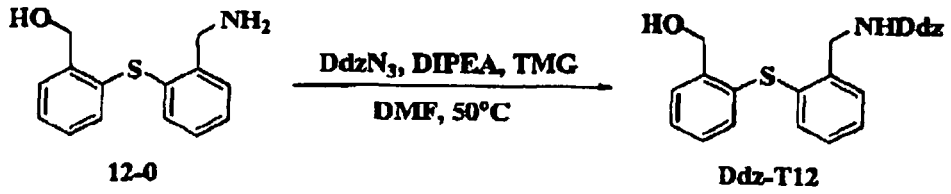


Figure 4

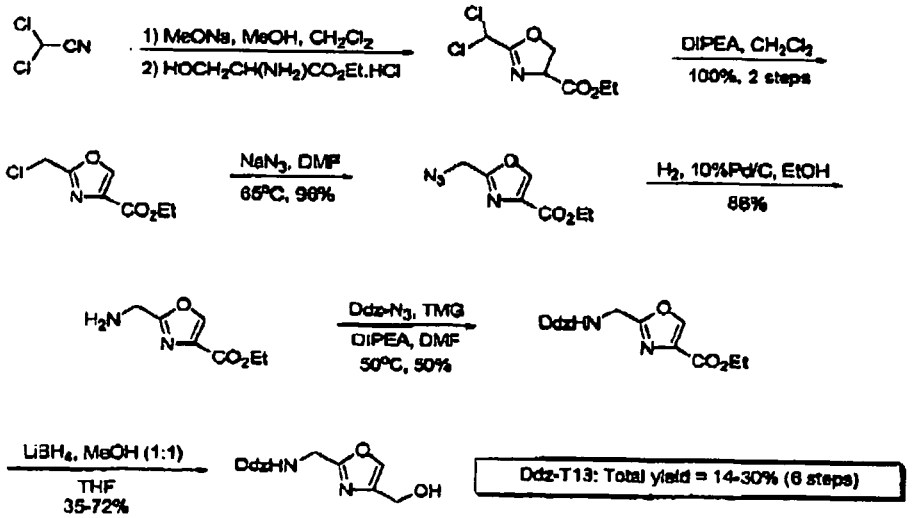


Figure 5

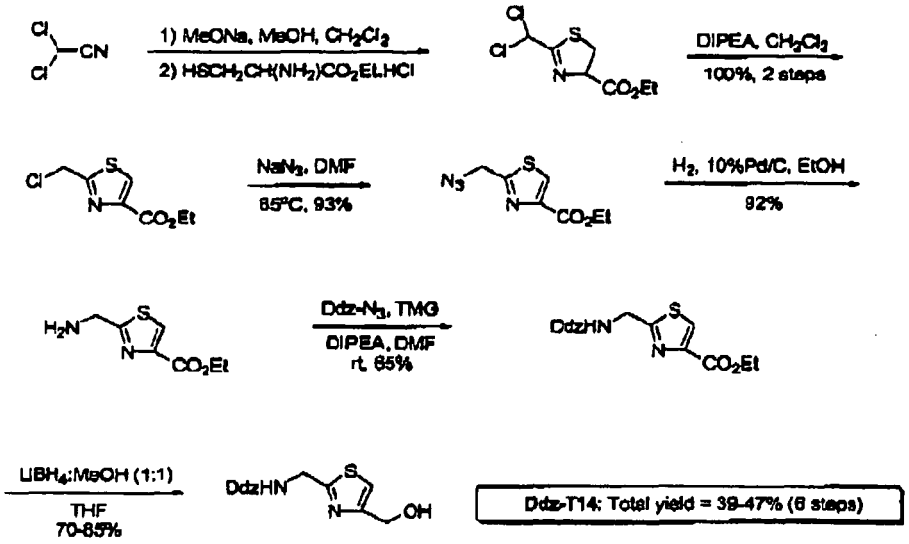
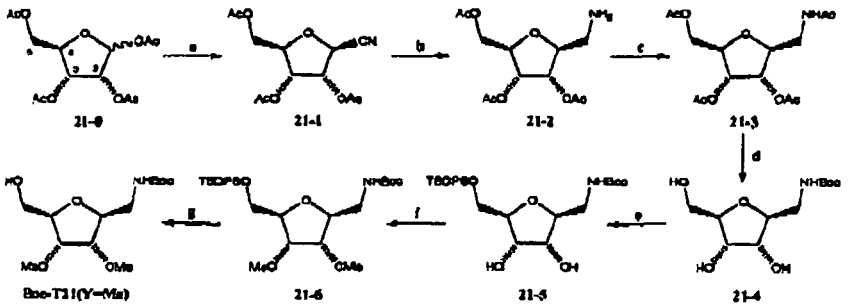
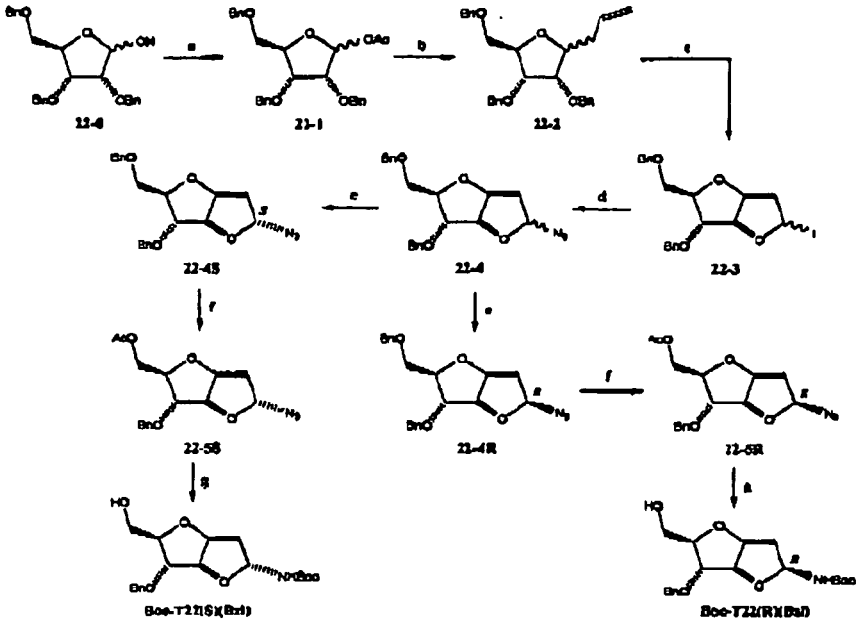


Figure 6



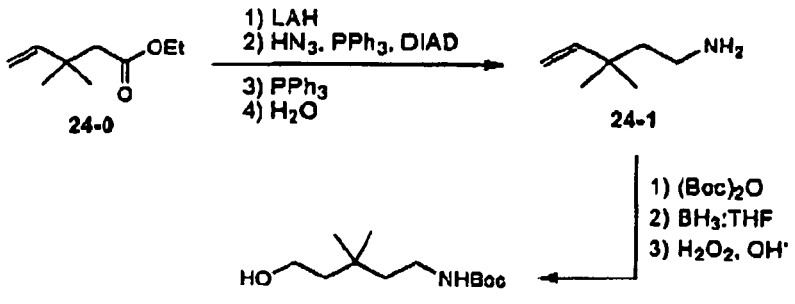
Reagents: a) TMSCN, $BF_3{\cdot}Et_2O$, 85%; b) $NaBH_3(O_2CCF_3)$, [S22] THF, 28°C, 80%; c) pyr, Ac_2O, 90%; d) 1. Boc_2O, DMAP, THF, 88%; 2. NaOMe, MeOH, 95%; e) TBDPSCl, pyr, DMAP, 70%; f) MeI, Ag_2O, DMF-CH_3CN, 50°C, 90%; g) TBAF, THF, 90%.

Figure 7



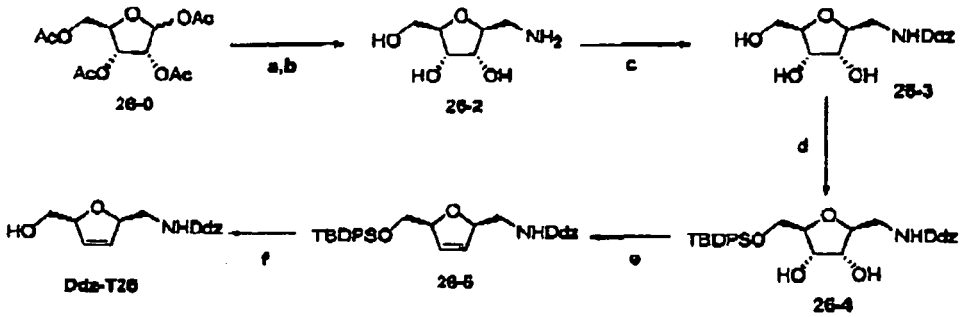
Reagents: a) Ac_2O/pyr , b) CH_2=CH-CH_2TMS, $BF_3{\cdot}Et_2O$, (85%); c) NIS, THF, 6 d, 80% (from α-anomer only, β-anomer used for T56 and T57); d) NaN_3, DMF, 70°C, 85%; e) flash chromatography separation of diastereomers; f) TFA/Ac_2O, 75%; g) 1. MeONa, MeOH, 90%; 2. PPh_3, 40°C, THF-H_2O, then Boc_2O, $NaHCO_3$, 90%.

Figure 8



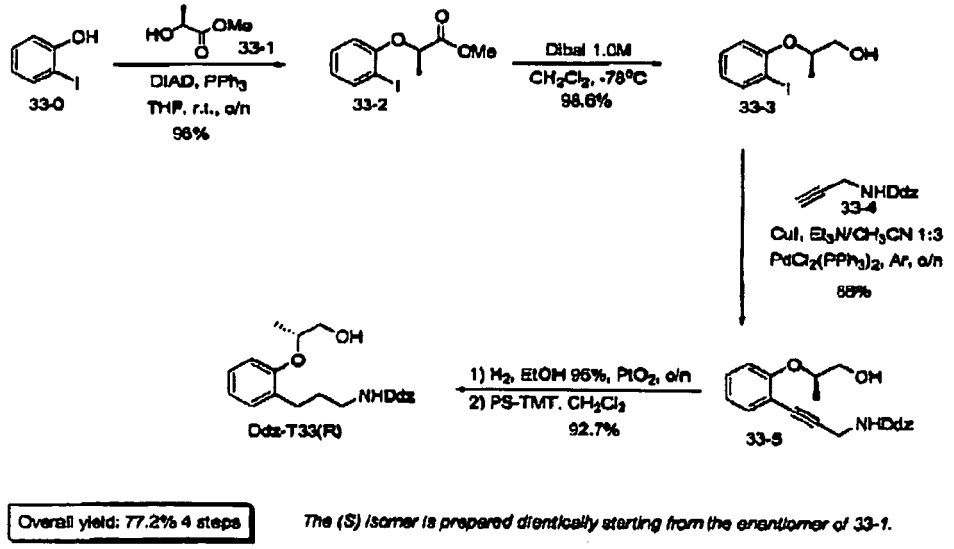
T24
(37% overall)

Figure 9



a-TMSCN, $BF_3.OEt_2$, DCM, 67%; b-LAH, THF; c-Ddz-OPh, TEA, DMF, 50° C, 60%; d-TBDPSCl, DMAP, Py/DCM, 90%; e- I_2, Im, PPh_3, $C_6H_5CH_3$/THF, reflux, 80%; f- TBAF, THF, 90%.

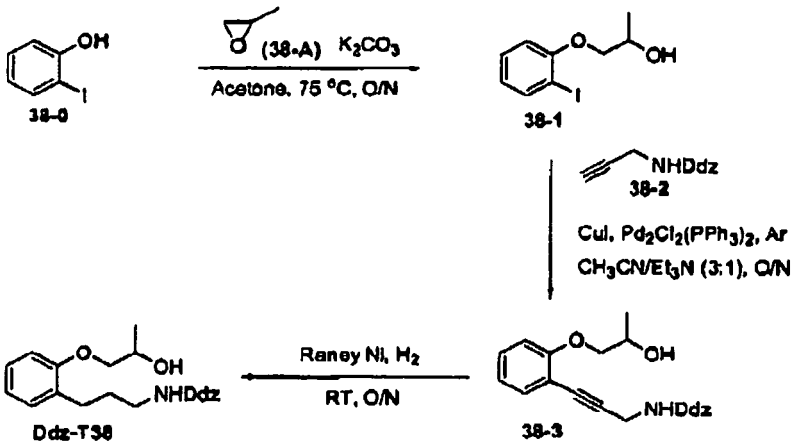
Figure 10



Overall yield: 77.2% 4 steps

The (S) isomer is prepared dientically starting from the enantiomer of 33-1.

Figure 11



Chiral versions of this tether are obtained starting from the enantiomers of 38-A.

Figure 12

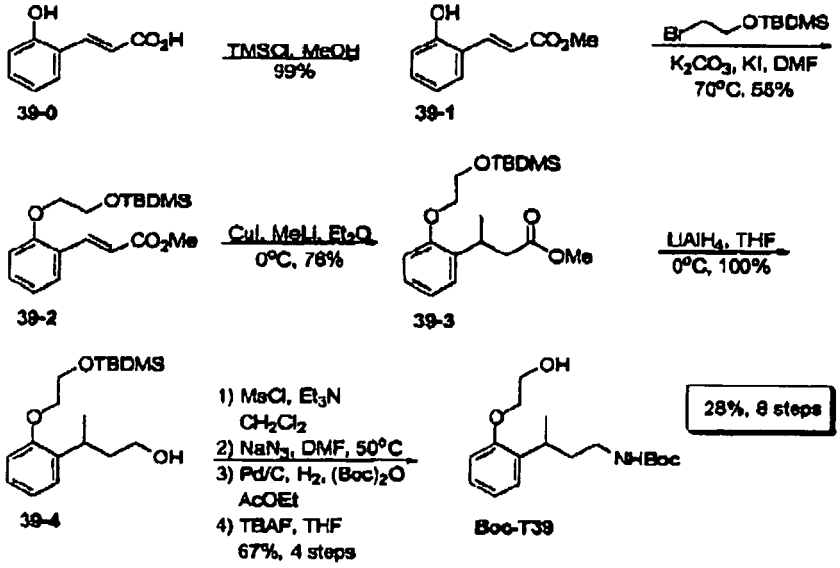


Figure 13

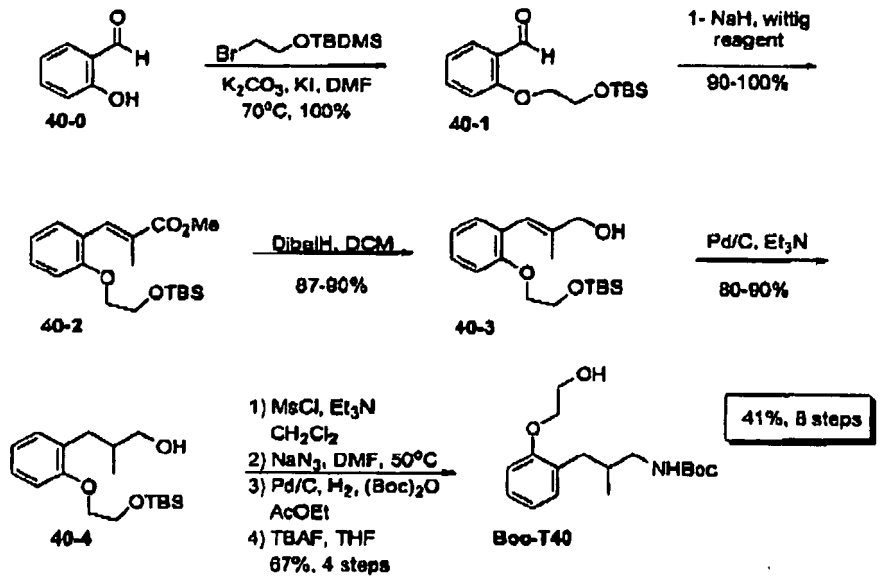

40-0
Br OTBDMS
K2CO3, KI, DMF
70°C, 100%
40-1
1- NaH, wittig reagent
90-100%
CO2Me
OTBS
40-2
DibalH, DCM
87-90%
OH
OTBS
40-3
Pd/C, Et3N
80-90%
OH
OTBS
40-4
1) MsCl, Et3N
CH2Cl2
2) NaN3, DMF, 50°C
3) Pd/C, H2, (Boc)2O
AcOEt
4) TBAF, THF
67%, 4 steps
NHBoc
Boc-T40
41%, 8 steps

Figure 14

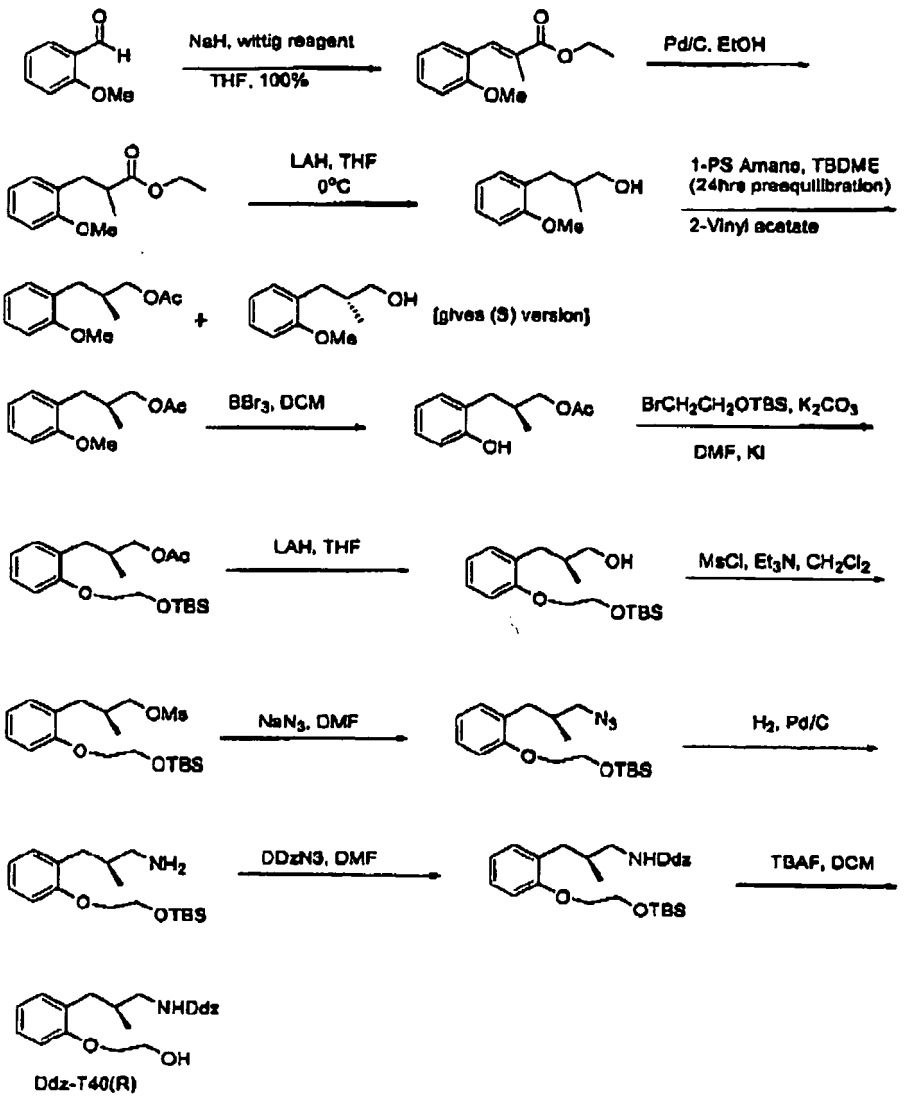

NaH, wittig reagent
THF, 100%
Pd/C, EtOH
LAH, THF
0°C
1-PS Amano, TBDME
(24hrs preequilibration)
2-Vinyl acetate
(gives (S) version)
BBr3, DCM
BrCH2CH2OTBS, K2CO3
DMF, KI
LAH, THF
MsCl, Et3N, CH2Cl2
NaN3, DMF
H2, Pd/C
DDzN3, DMF
TBAF, DCM
Ddz-T40(R)

Figure 15

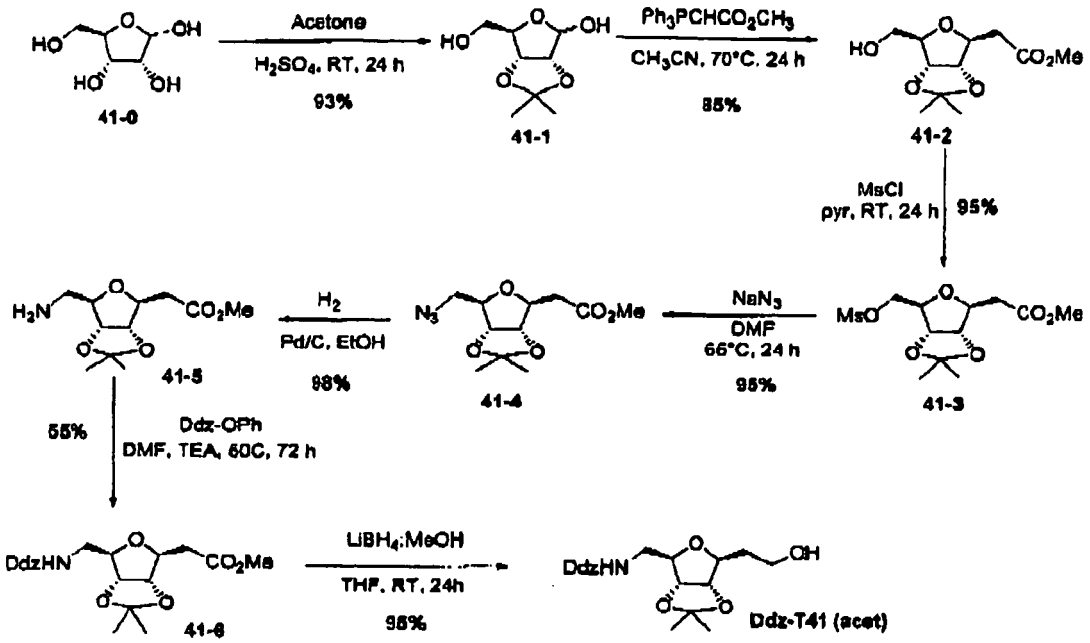

Acetone
H2SO4, RT, 24 h
93%
Ph3PCHCO2CH3
CH3CN, 70°C, 24 h
85%
MsCl
pyr, RT, 24 h
95%
NaN3
DMF
66°C, 24 h
95%
H2
Pd/C, EtOH
98%
55%
Ddz-OPh
DMF, TEA, 60C, 72 h
LiBH4:MeOH
THF, RT, 24h
95%
41-0
41-1
41-2
41-3
41-4
41-5
41-6
Ddz-T41 (acet)

Figure 16

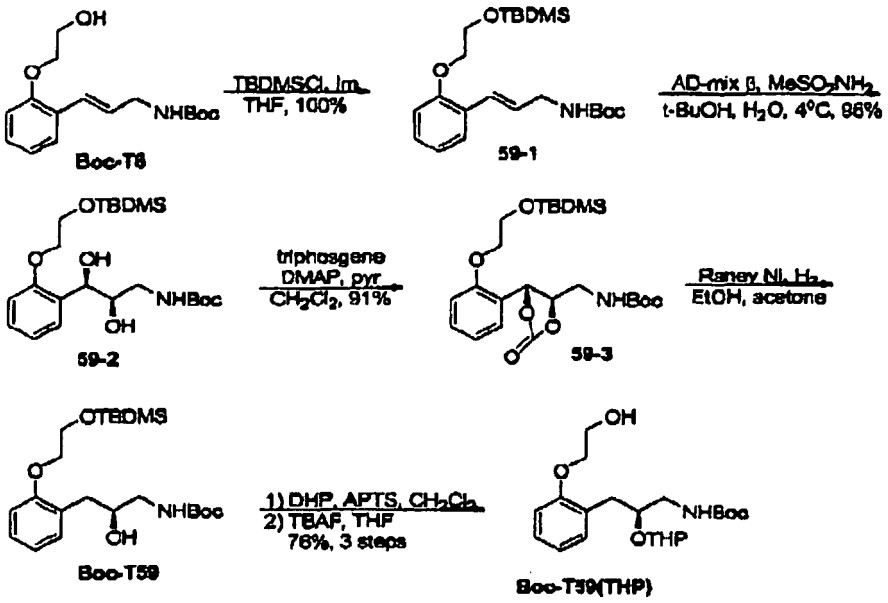
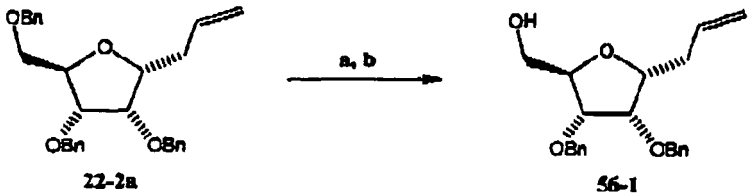


Figure 17



Reagents: a) TFA/ Ac_2O, 75%; b) MeONa, MeOH, 90%

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5646285 A **[0005]**
- US 5891737 A **[0005]**
- WO 9854577 A **[0006]**
- WO 01125257 A **[0009]**
- US 60479223 B **[0020]**
- WO 2004111077 A **[0020]**
- WO 0125257 A **[0021]**

Non-patent literature cited in the description

- *Molecular Diversity,* 2000, vol. 5, 289-304 **[0006]**
- *J. Med. Chem.,* 2003, vol. 46, 1250-1256 **[0010]**
- *J. Med. Chem.,* 2002, vol. 45, 2615-2623 **[0010]**
- *Tetrahedron,* 1989, vol. 45, 1639-1646 **[0023]**
- *Tetrahedron,* 1990, vol. 46, 6623-6632 **[0023]**
- *J. Org. Chem.,* 1992, vol. 57, 8239-6256 **[0023]**
- *J. Am. Chem. Soc.,* 1999, vol. 121, 6197-6205 **[0023]**
- **PALUCKI BL et al.** *J Med Chem,* 2001, vol. 43, 4370-4376 **[0060]**
- *Bioorg Med Chem Lett,* vol. 11, 1955-1957 **[0060]**