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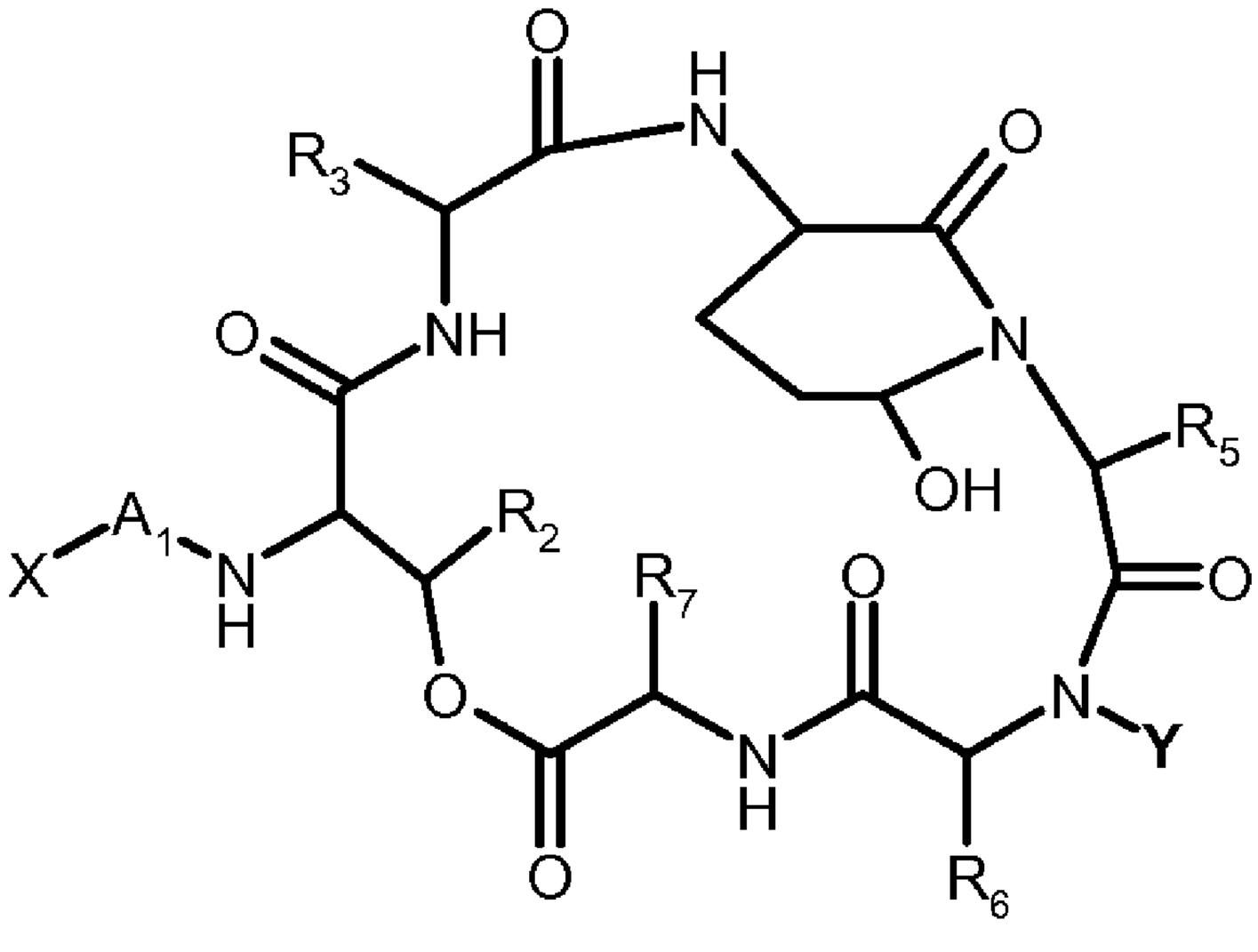
(54) Titre : PROCEDES EN PHASE DE SOLUTION POUR LA FABRICATION DE DEPSIPEPTIDES MACROCYCLIQUES, ET NOUVEAUX INTERMEDIAIRES ASSOCIES

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



(I)

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

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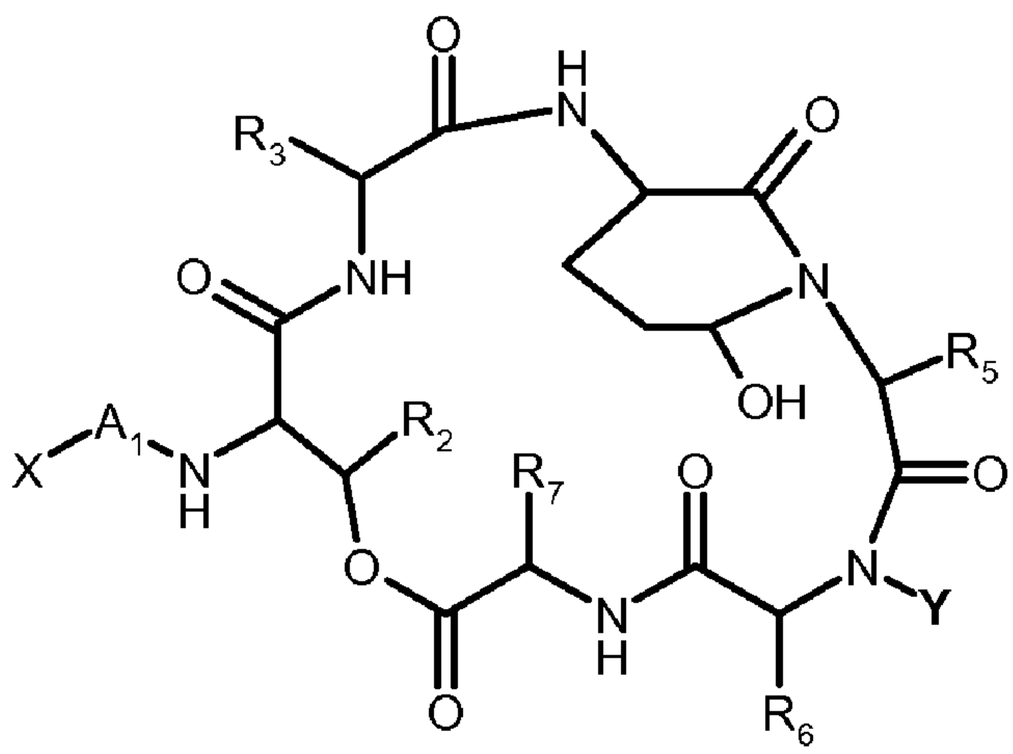
(54) Title: SOLUTION PHASE PROCESSES FOR THE MANUFACTURE OF MACROCYCLIC DEPSIPEPTIDES AND NEW INTERMEDIATES



(I)

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

WO 2014/057418 A3

Solution Phase Processes for the Manufacture of Macrocyclic Depsipeptides and New Intermediates

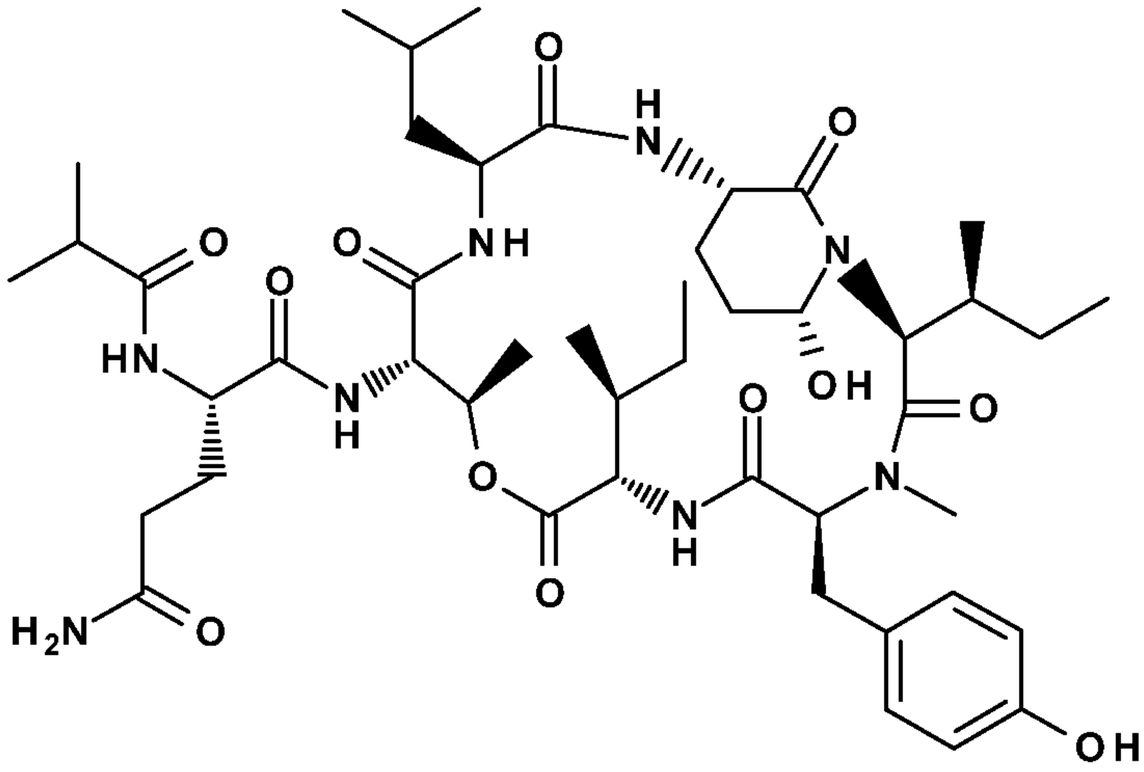
Summary of the Invention

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

Background of the invention

Numerous utilities and uses of cyclic depsipeptides are known in pharmacology. As an example, depsipeptides disclosed in WO 2009/024527 are useful for the treatment of various diseases.

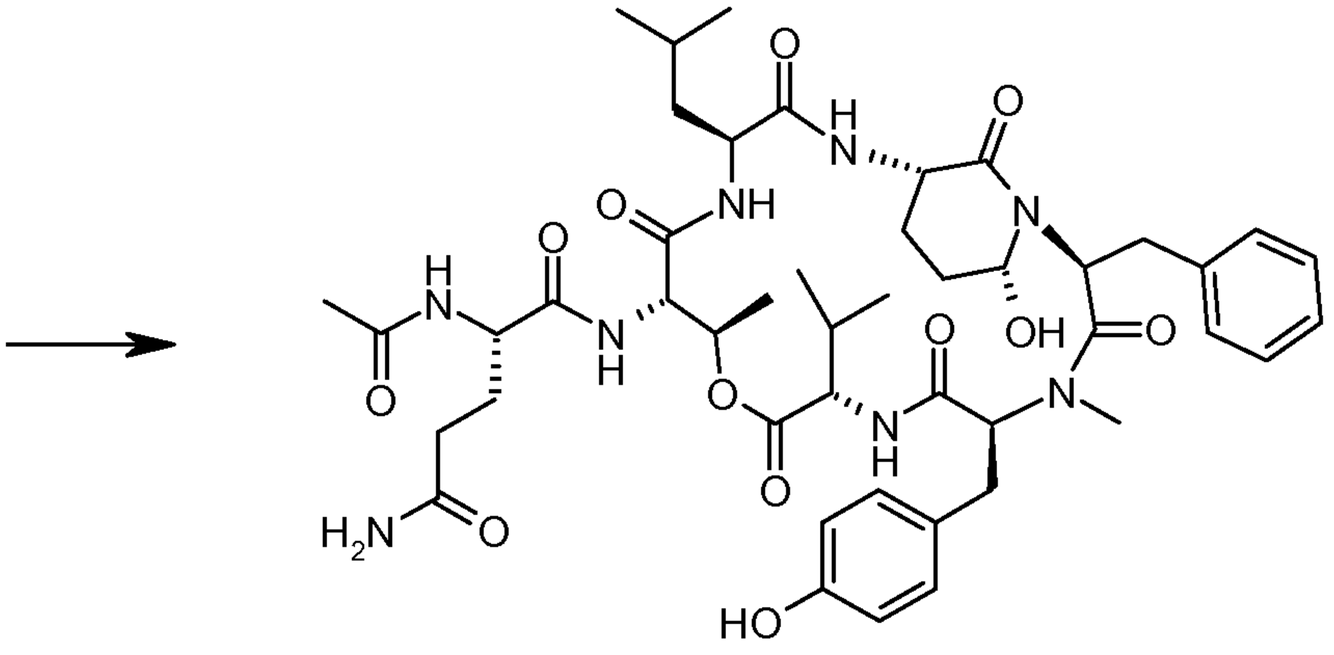
For example, the compound of the formula A



(A)

mentioned in WO 2009/024527 is useful for the treatment and/or prevention of inflammatory and/or hyperproliferative and pruritic skin diseases, for example atopic dermatitis, psoriasis, pustular psoriasis, rosacea, keloids, hypertrophic scars, acne, Netherton´s syndrome or other pruritic dermatoses such as prurigo nodularis, unspecified itch of the elderly as well as other diseases with epithelial barrier dsysfunction such as aged skin. This compound is also named “Compound A” hereinafter.

Other cyclic depsipeptides, such as BN920, formerly isolated from the cyanobacterium Nostoc, was isolated also from Microcystis. Nostopeptin (BN920) inhibited chymotrypsin with an IC50 value of 31 nM (see J. Nat. Prod. 68(9), 1324-7 (2005)). It has formula B:



Compound B (Nostopeptin BN920)

These compounds can be produced by fermentation (e.g. using *Chondromyces croactus*, myxobacteria) along with other depsipeptides comprising the so-called ahp-substructure (ahp: 3-amino-6-hydroxy-piperidin-2-one) and the corresponding dehydro-ahp substructure (dehydro-ahp: 3-amino-3,4-dihydro-1H-pyridin-2-one), also called "dehydrate" herein, respectively. Therefore, the yield of fermentation with regard to any single of these compounds is up to now rather low and cannot yet allow the manufacture of amounts sufficient on an industrial or even pilot scale.

Analogous compounds have been synthesized by solution phase synthesis, however, with poor yield and low efficiency.

The present invention therefore aims at providing a new method of manufacture allowing minimizing or removing the mentioned disadvantages.

Summary of the invention

The present invention relates to a process or method of manufacturing a depsipeptide of the formula I by pure solution phase peptide synthesis without requiring any solid phase synthesis steps.

The present invention relates to processes or methods that allow obtaining such cyclic depsipeptides with increased yield and/or in good purity, also in amounts appropriate for industrial manufacture, e.g. up to several kilograms and even tons.

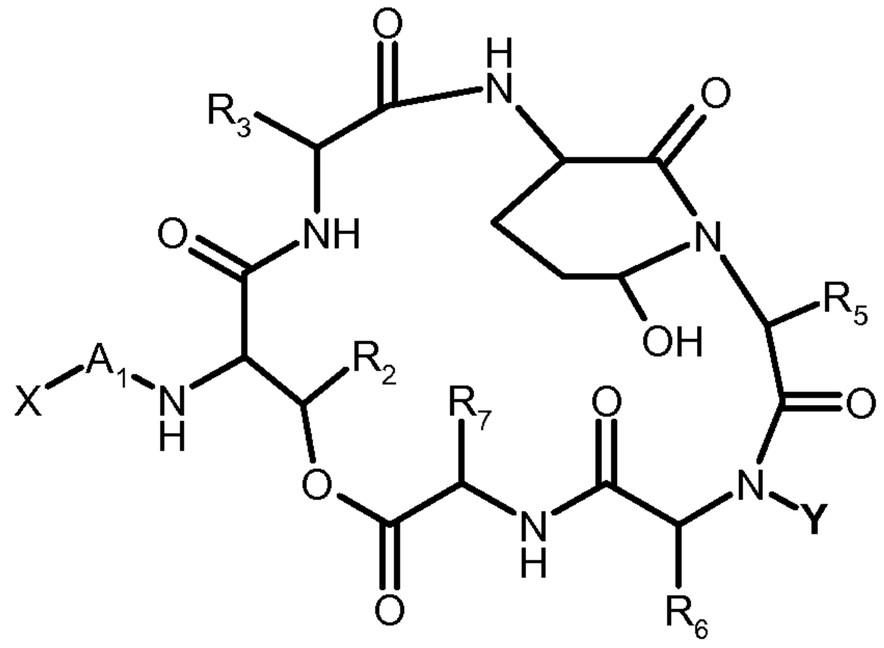
In spite of the many synthetic risks, such as racemization, tautomerization, cleavage or other side reactions during deprotection of intermediates, and the like in the synthesis of a complex molecule with many possible isomers, it has now been possible to find a manufacturing process, comprising reactions in solution and not requiring the use of any solid phase materials, that allows to produce cyclic depsipeptides of formula I in good yield and/or the required stereoisomerical purity, especially both. The synthesis of large amounts is reasonably expected to be possible based on this new way of synthesis, allowing product manufacture on an industrial scale. It is possible to reduce the amount of by-products, and even to improve yield, by converting such by-products, especially the dehydro-ahp substructure and/or an analogue of the desired ahp-comprising products with a five-ring instead of the ahp, into the desired final products. This allows to further increase yield. Especially, the synthesis allows for simple separating processes of intermediates by chromatography such as absorption chromatography, e.g. on silica gel and a reversed phase material.

The reactions can be performed in multi-purpose reactors using lower excesses of reagents (near stoichiometric or stoichiometric amounts being possible, in contrast to solid phase synthesis where usually an excess of materials to be bound on gropus on the solid phase material is required) and reactants as compared with other ways of synthesis, e.g. solid phase peptide synthesis. This, as well as the possibility to purify intermediates by the chromatographic steps indicated above, provides flexibility for the preparation and enables the scaling-up for production of multi-ton amounts of the cyclopeptides.

Especially, it has been possible to make use of an appropriate protecting group strategy.

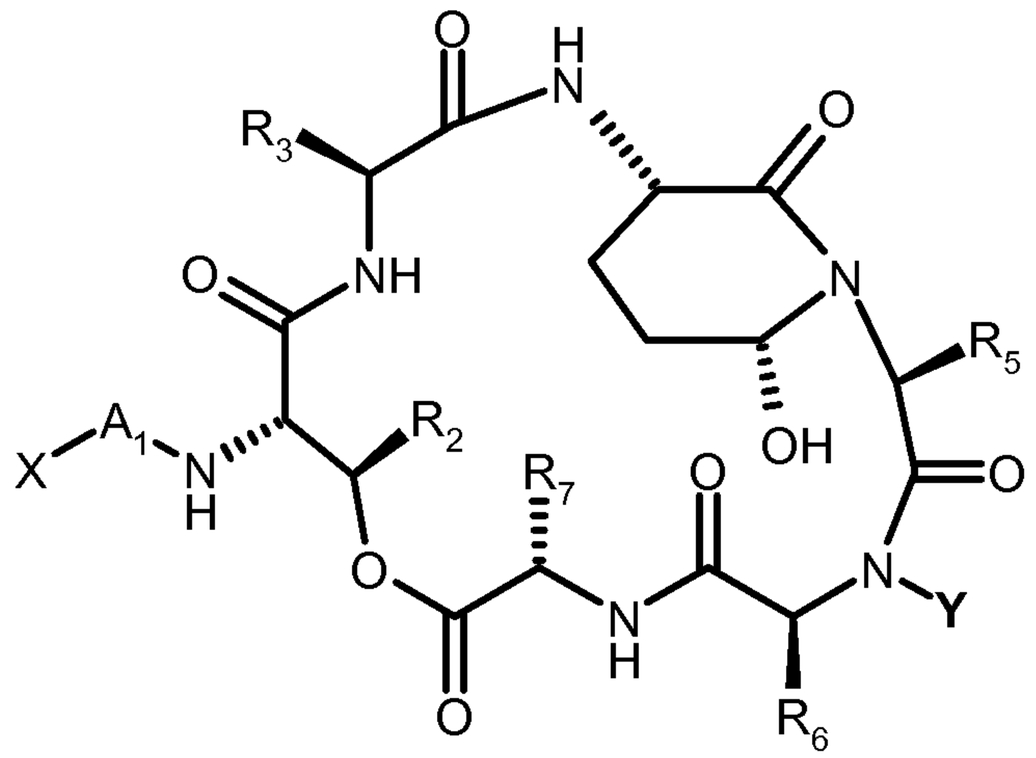
Detailed Description of the Invention

The invention thus, in a first embodiment, relates to a method or process for the preparation of a cyclic depsipeptide compound of the formula I solely by solution phase synthesis,



(I)

especially a compound of the formula IA



(IA)

wherein

A_1 is a (bivalent) moiety of an amino acid with a terminal carboxy or carbamoyl group, especially asparagine or glutamine, and is bound via a carbonyl to the rest of the molecule; or is C_{1-8}-alkanoyl or phosphorylated hydroxy-C_{1-8}-alkanoyl;

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

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

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

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

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

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

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

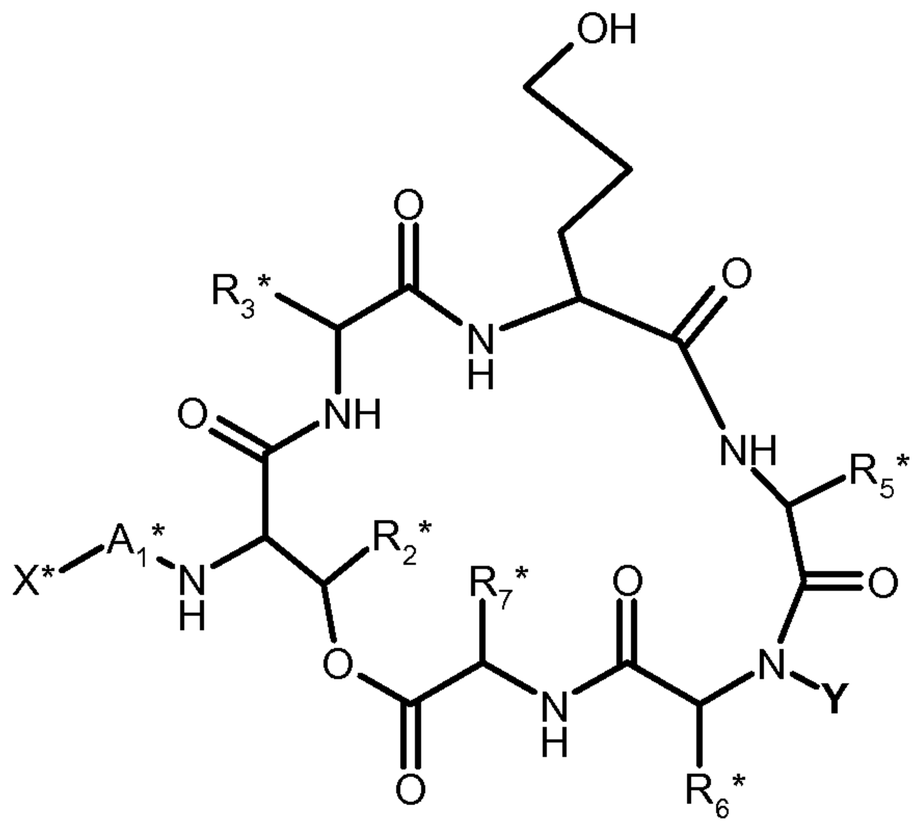
or a salt thereof,

said method comprising

either

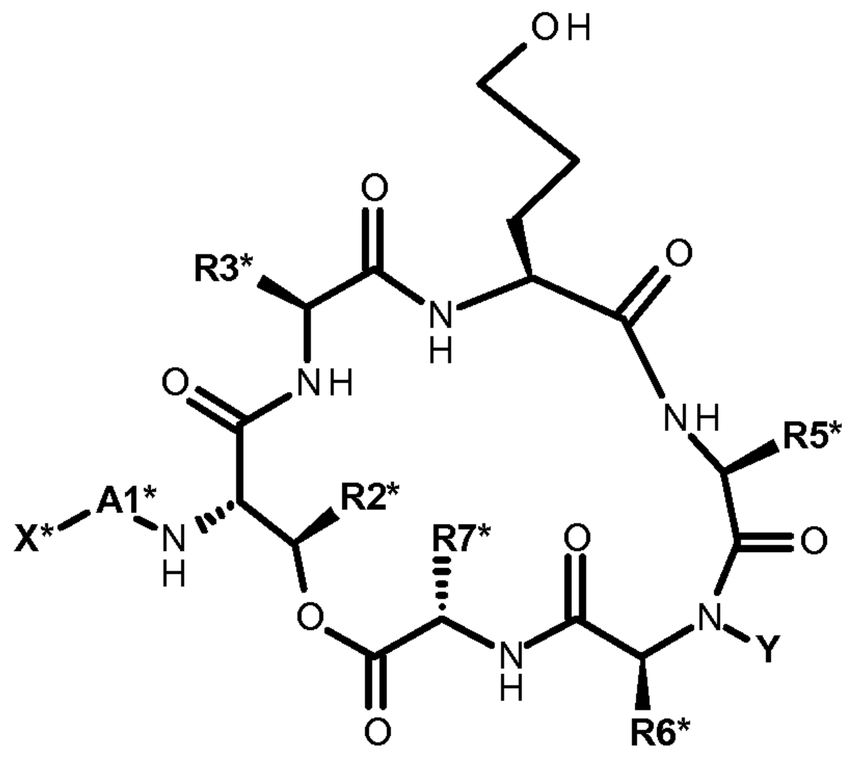
in a first variant (A)

reacting the free hydroxyl group of a compound of the formula (II),



(II)

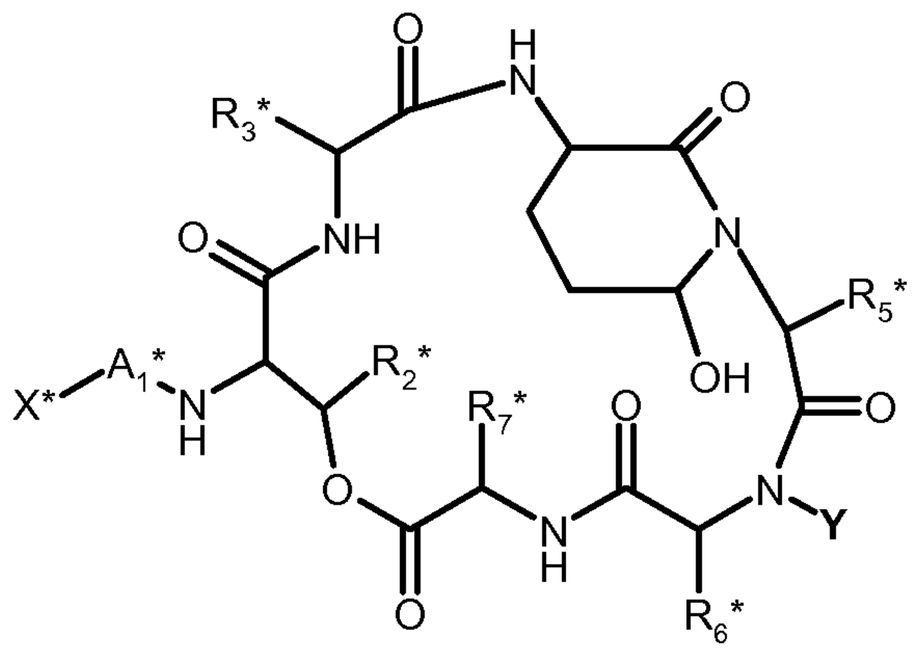
especially of the formula IIA



(IIA)

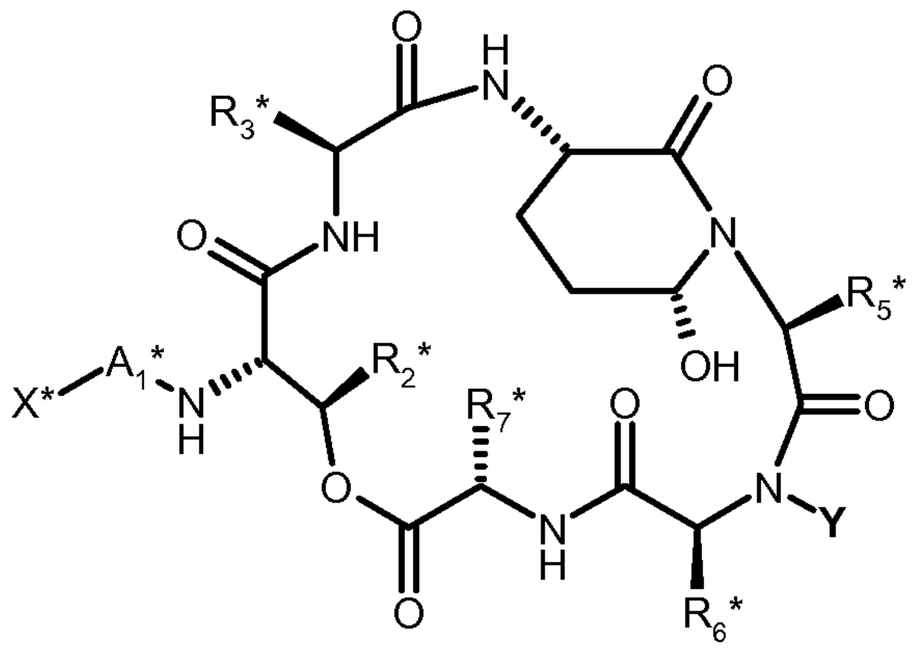
wherein Y is as defined for a compound of the formula I and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I, respectively, but with the proviso that reactive functional groups on these moieties are present in protected form at least if they could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

under oxidizing conditions to form a compound of the formula III,



(III)

especially of the formula IIIA,

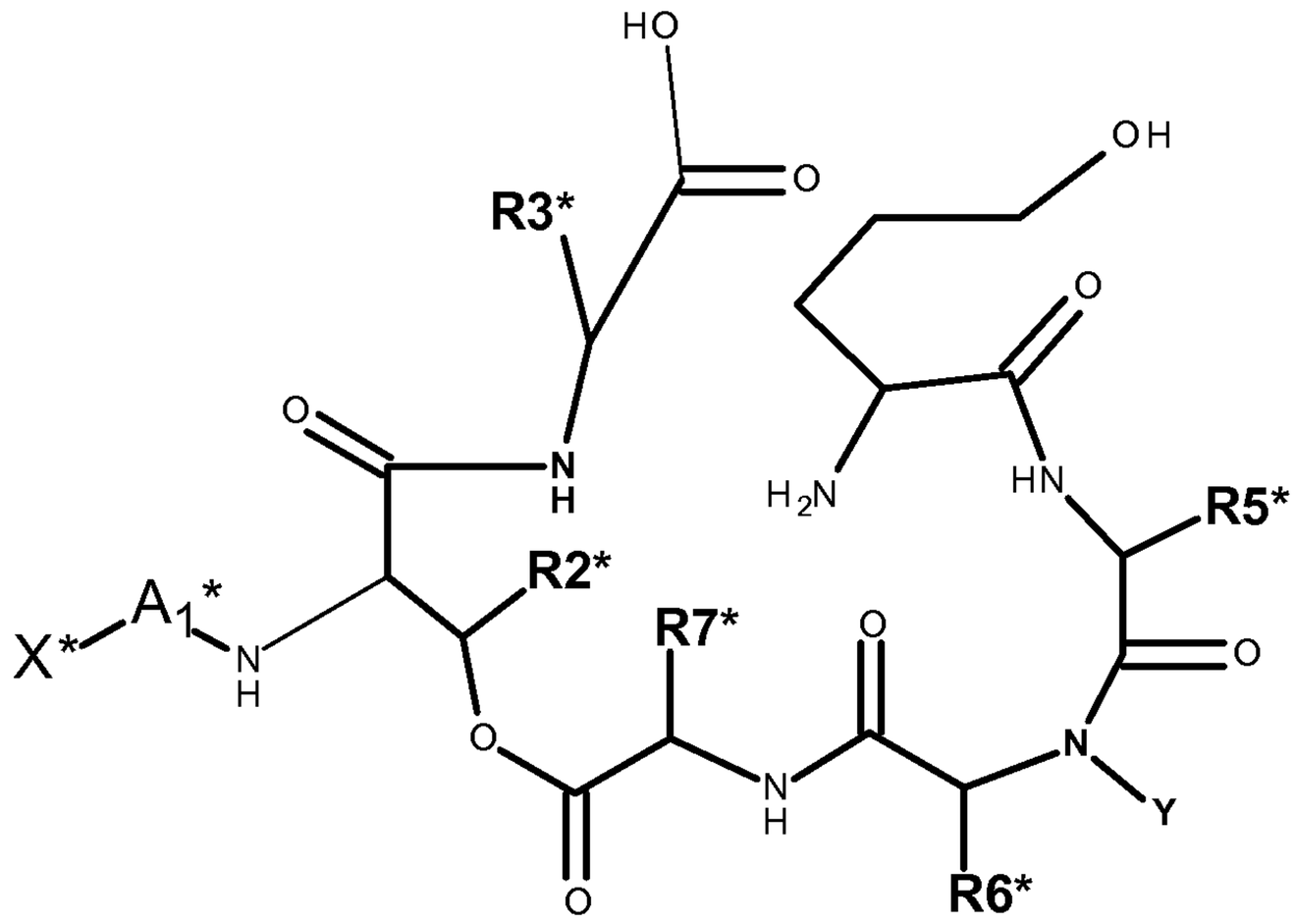


(IIIA)

and removing remaining protecting groups to yield (e.g. via the intermediates of the formula XXIII-1 (especially XXIII-1A), XXIII-2 (especially XXIII-2A) and/or XXIII-3 (especially XXIII-3A) or analogues still carrying some or all of the protecting groups in the compound of the formula II, especially IIA) a compound of the formula I, or a salt thereof,

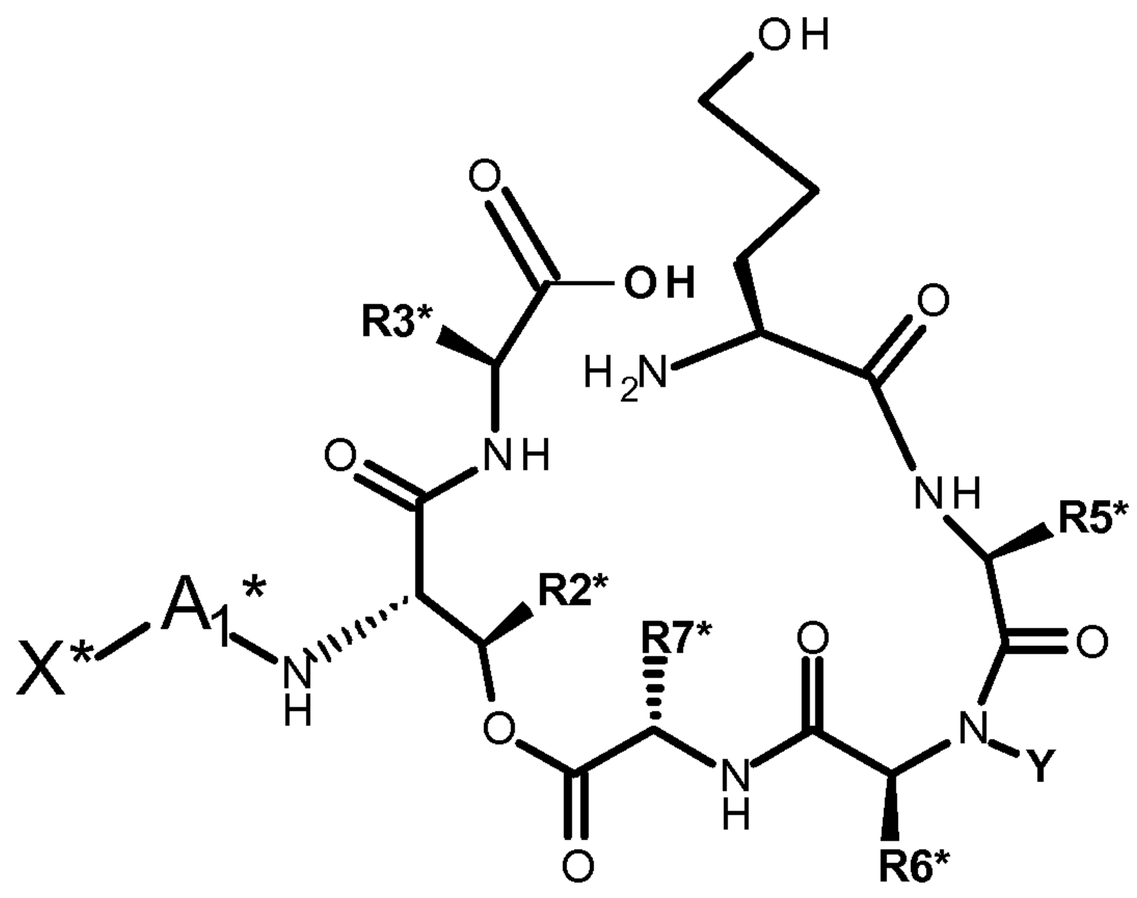
and, if desired, converting a free compound of the formula I into a salt, a salt of a compound of the formula I into a different salt of a compound of the formula I or into the free compound of the formula I and/or converting a dehydrated analogue of a compound of the formula I into the corresponding compound of the formula I;

where the compound of the formula III is (preferably) prepared by macrolactamization of a compound of the formula IV,



(IV),

especially of the formula IVA,



(IVA)

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

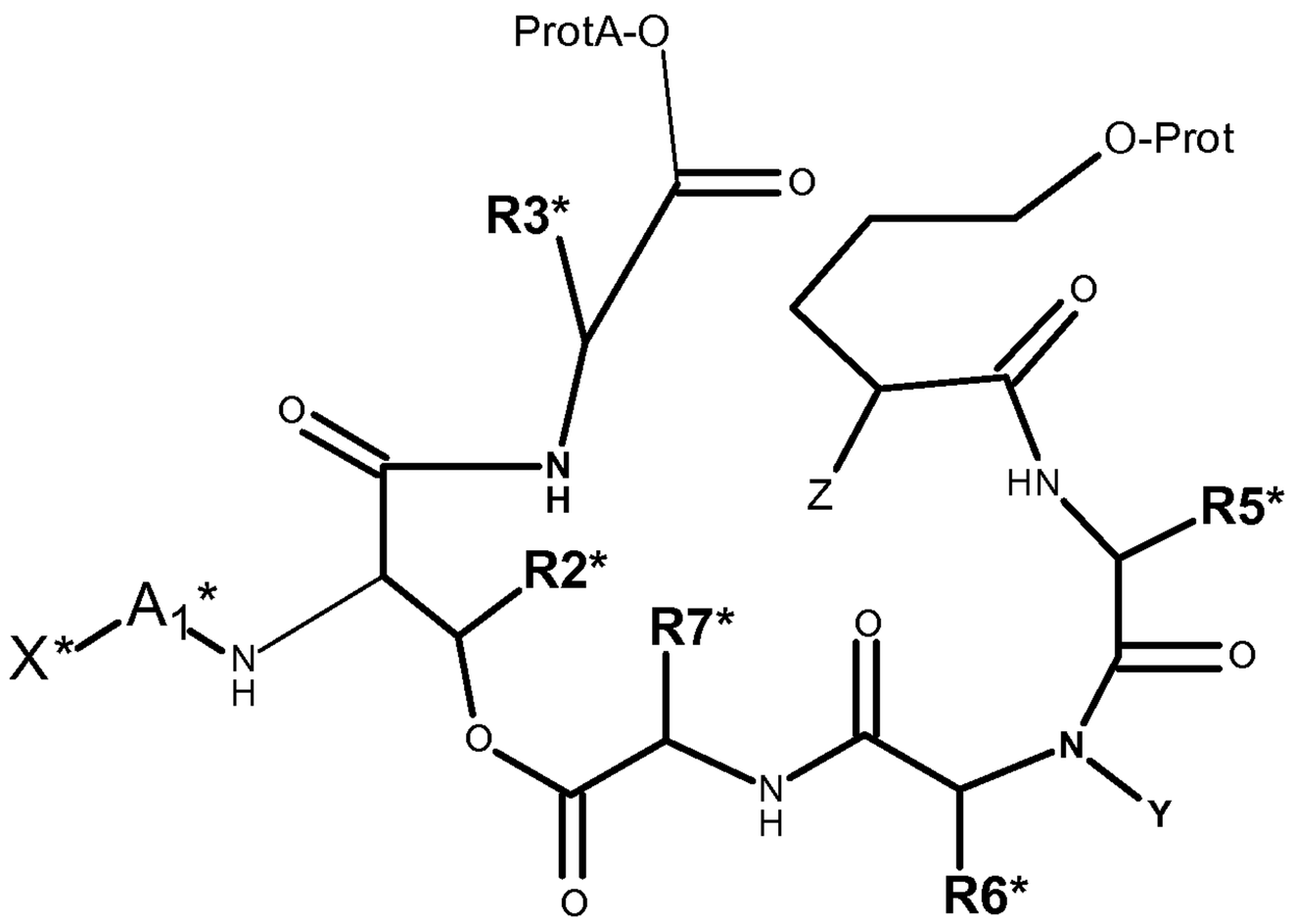
The oxidizing conditions for preparing a compound of the formula III, especially of the formula IIIA, from a compound of the formula II, especially of the formula IIA, are preferably chosen such that the hydroxyl group is oxidized directly to the aldehyde group

(and/or the hemiaminal isomers of the compound of formula XXXIII-3 given below) are formed). Suitable oxidizing conditions for the oxidation are usually using IBX in DMSO (J. Org. Chem. 1995, *60*, 7272-7276); Pyridinium dichromate or Pyridinium chlorochromate (Tetrahedron Lett. 1979, *5*, 399-402); oxalyl chloride, dimethyl sulfoxide and a tertiary amine (J. Peptide Sci. 2006, *12*, 140-146), oxoammonium salts (J. Org. Chem. 1985, *50*, 1332-1334); alkali hypochlorites catalyzed by oxoammonium salts (J Org. Chem. 1989, *54*, 2970-2972); oxoaminium salts (Tetrahedron Lett. 1988, *29*, 5671-5672), $RuCl_2(PPh_3)_3$ (Tetrahedron Lett. 1981, *22*, 1605-1608); TEMPO (1 mol%) in the presence of sodium hypochlorite (Tetrahedron Lett. 1990, *31*, 2177-2180); $NaIO_4$, TEMPO, NaBr (Tetrahedron 2006, *62*, 8928-8932); SiO_2 supported vanadium(IV)oxide and t-BuOOH (Advanced Synthesis & Catalysis 2007, *349*, 846-848). Among the possible oxidants 1-hydroxy-1,2-benziodoxol-3(1*H*)-one-1-oxide (IBX) is especially preferred. The reaction preferably takes place in an appropriate solvent, such as a cyclic ether, e.g. tetrahydrofuran or dioxin, a di-(C_1-C_8-alkyl)sulfoxide, e.g. dimethylsulfoxide, or a mixture thereof, e.g. at a temperature between 0-50°C, preferably between 20-25 °C.

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

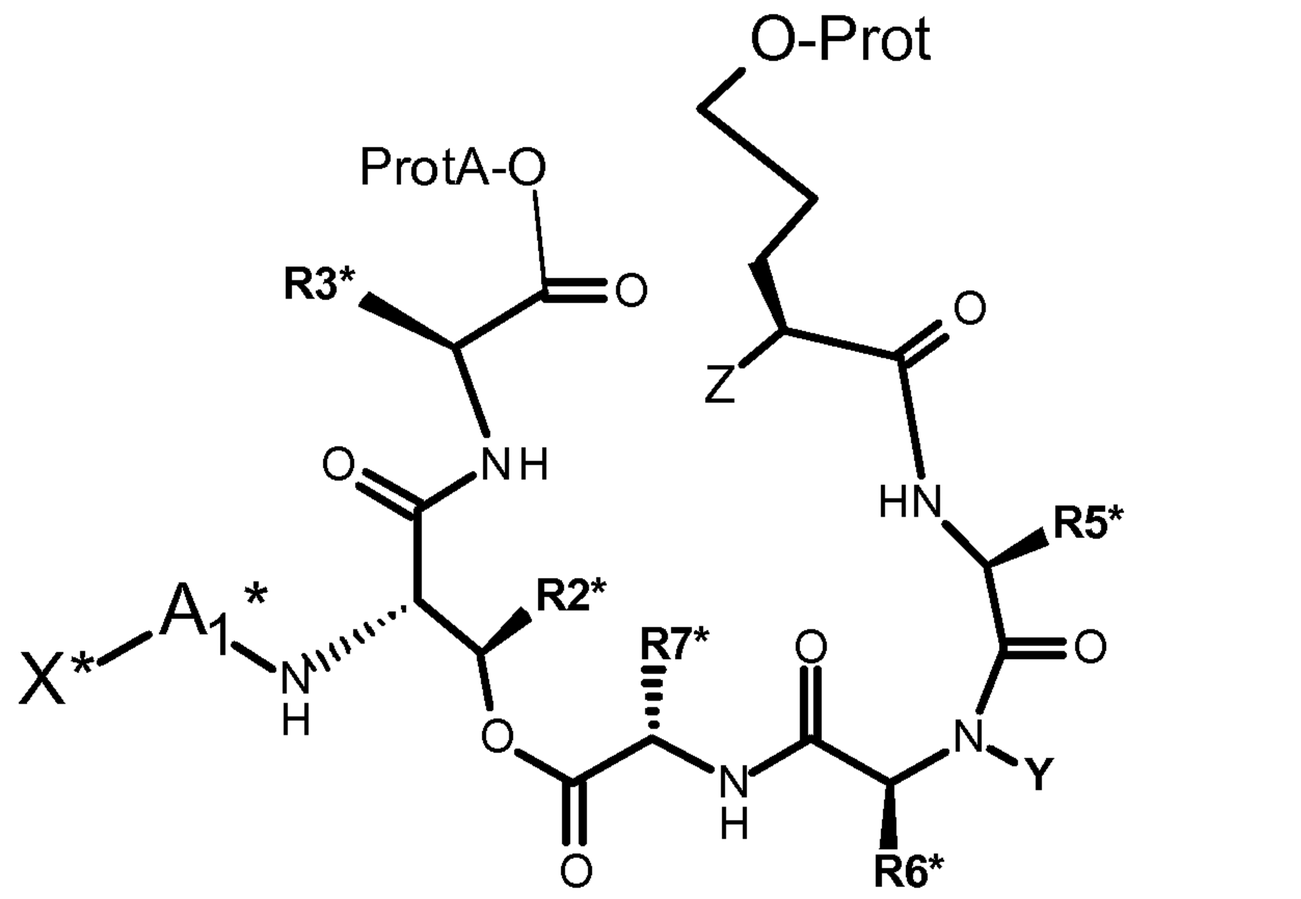
where preferably

in a further embodiment, the method or process described above, is further comprising manufacturing the compound of the formula IV in solution phase by simultaneously or sequentially removing the protecting group ProtA, the protecting group(s) of protected amino group Z and the protecting group Prot from a compound of the formula V,



(V)

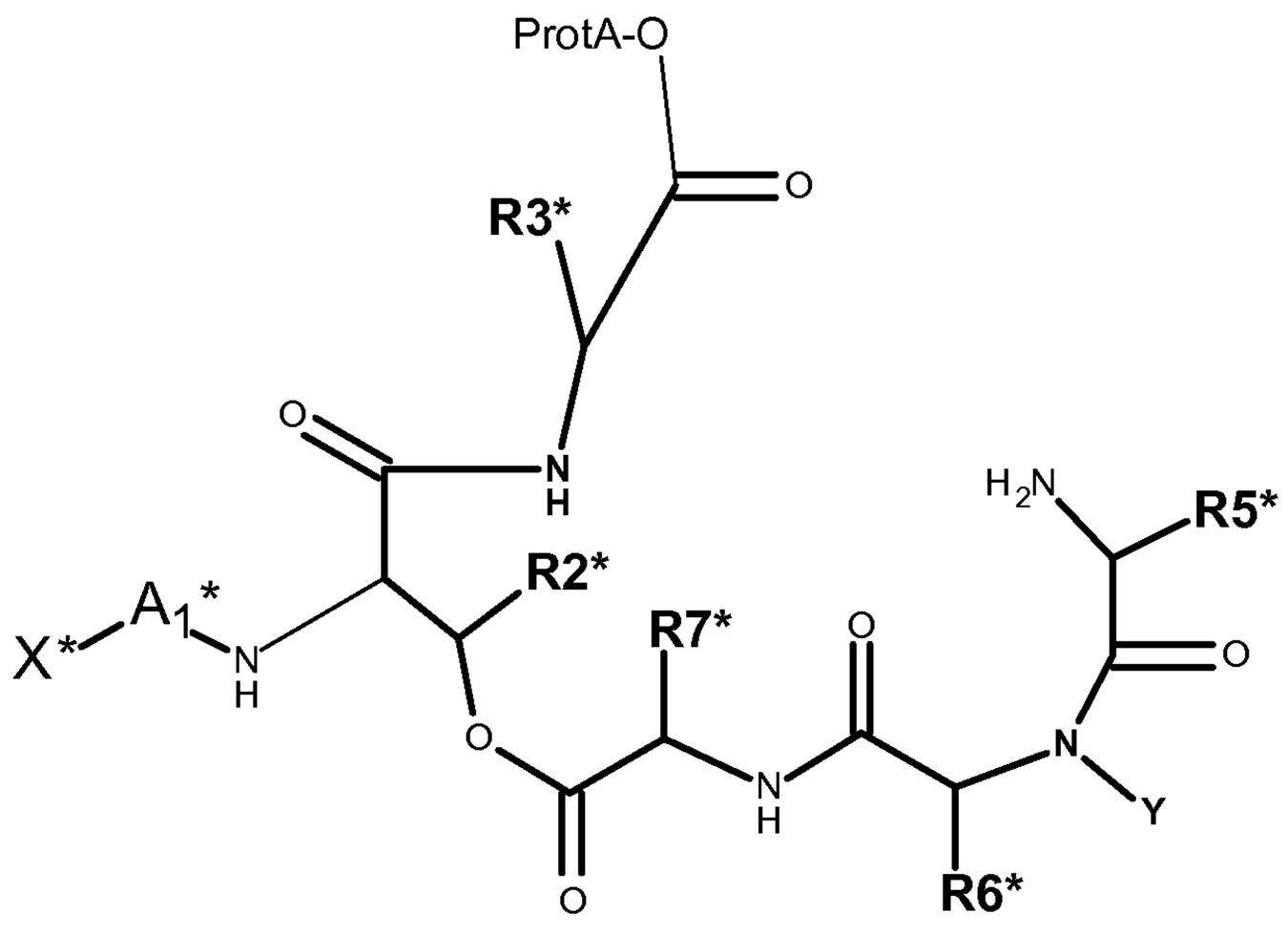
especially of the formula VA,



(VA)

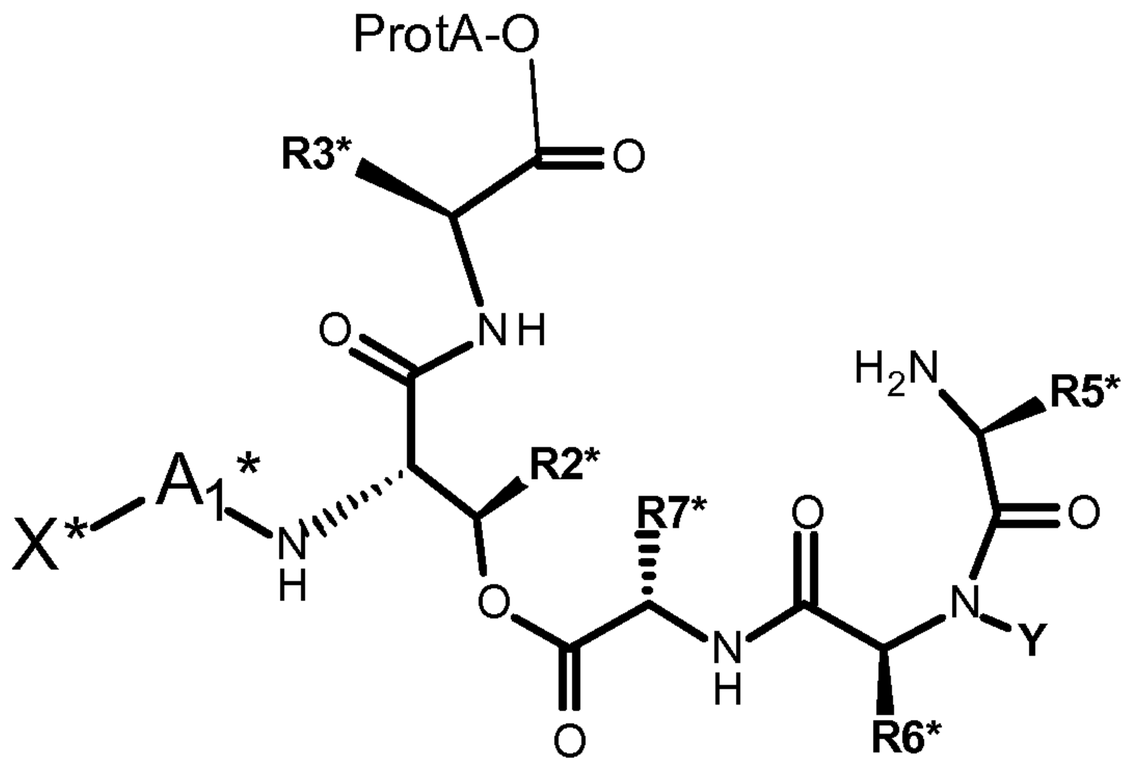
wherein ProtA is carboxy protecting group, Prot is a hydroxyl protecting group, Z is a protected amino group either of the formula NHProt** wherein Prot** is an amino protecting group that can preferably be removed under at least nearly pH neutral conditions; or Z is a protected amino group of the formula $N(Prot^*)_2$ wherein each Prot* is an amino protecting group that can be removed e.g. by catalytic hydrogenation, especially each is an arylalkyl amino protecting group; and Y, R_2^*, R_3^*, R_7^*, R_6^* and R_5^*, X^* and A_1^* are as defined for a compound of the formula II.

In yet a further embodiment, the invention relates to the method or process mentioned above, further comprising manufacturing the compound of the formula V in solution phase by coupling a compound of the formula VI,



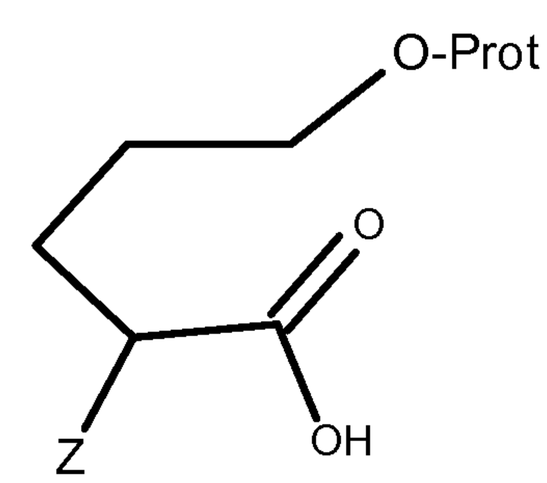
(VI),

especially of the formula VIA,



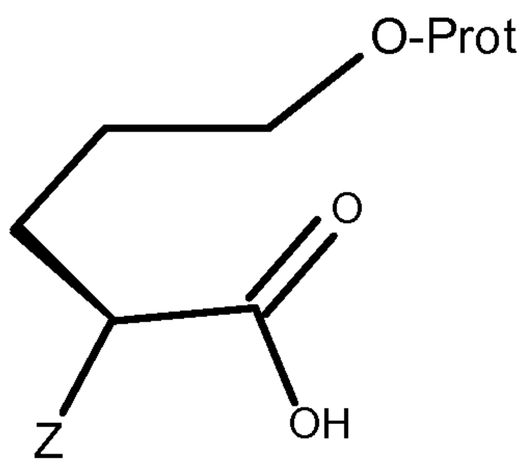
(VIA)

wherein ProtA is as defined above for a compound of the formula V and Y, R_2*, R_3*, R_7*, R_6* and R_5*, X* and A_1* are as defined above for a compound of the formula II, with an amino acid of the formula VII,



(VII)

especially of the formula VIIA,

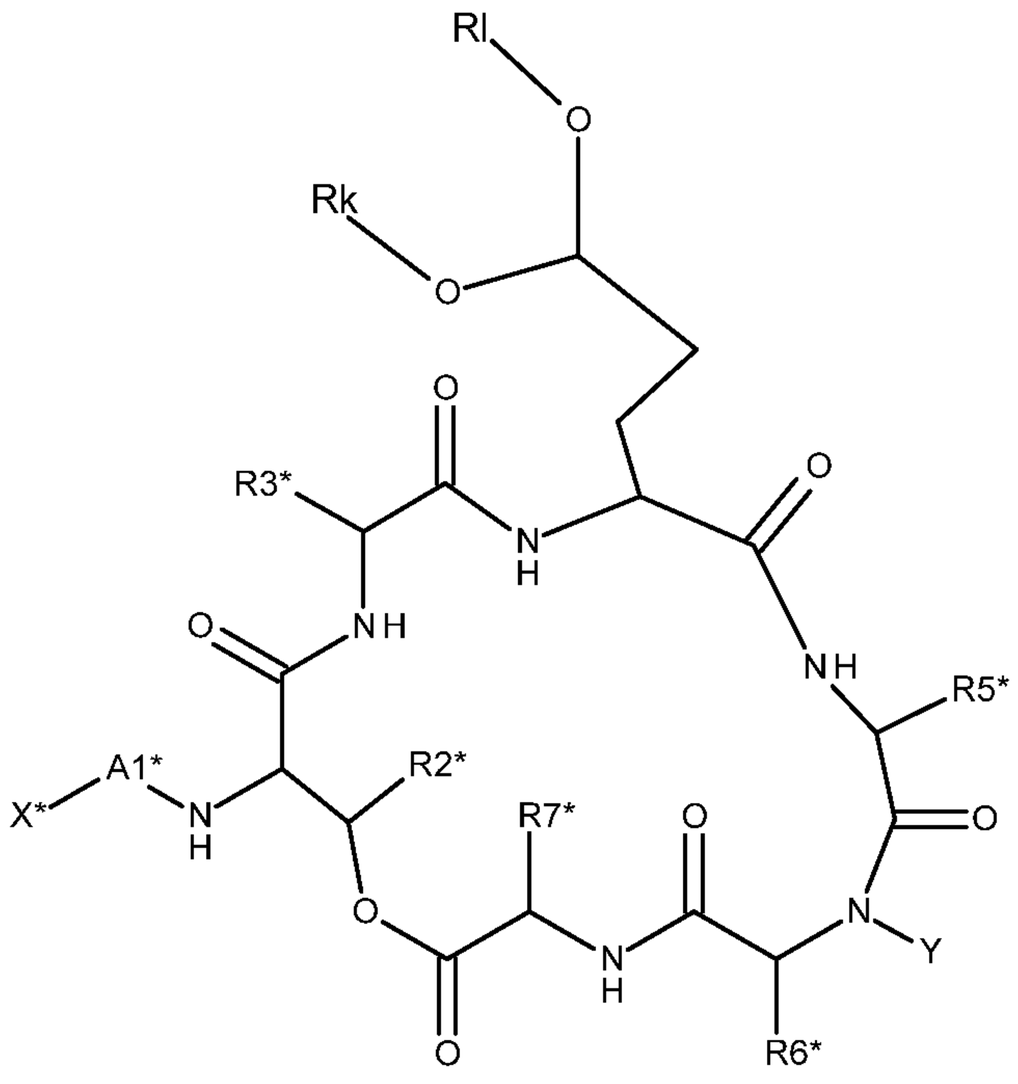


(VIIA)

wherein ProtA and Z which is preferably NHProt** are as defined above for a compound of the formula V, or an activated derivative thereof (end of variant A);

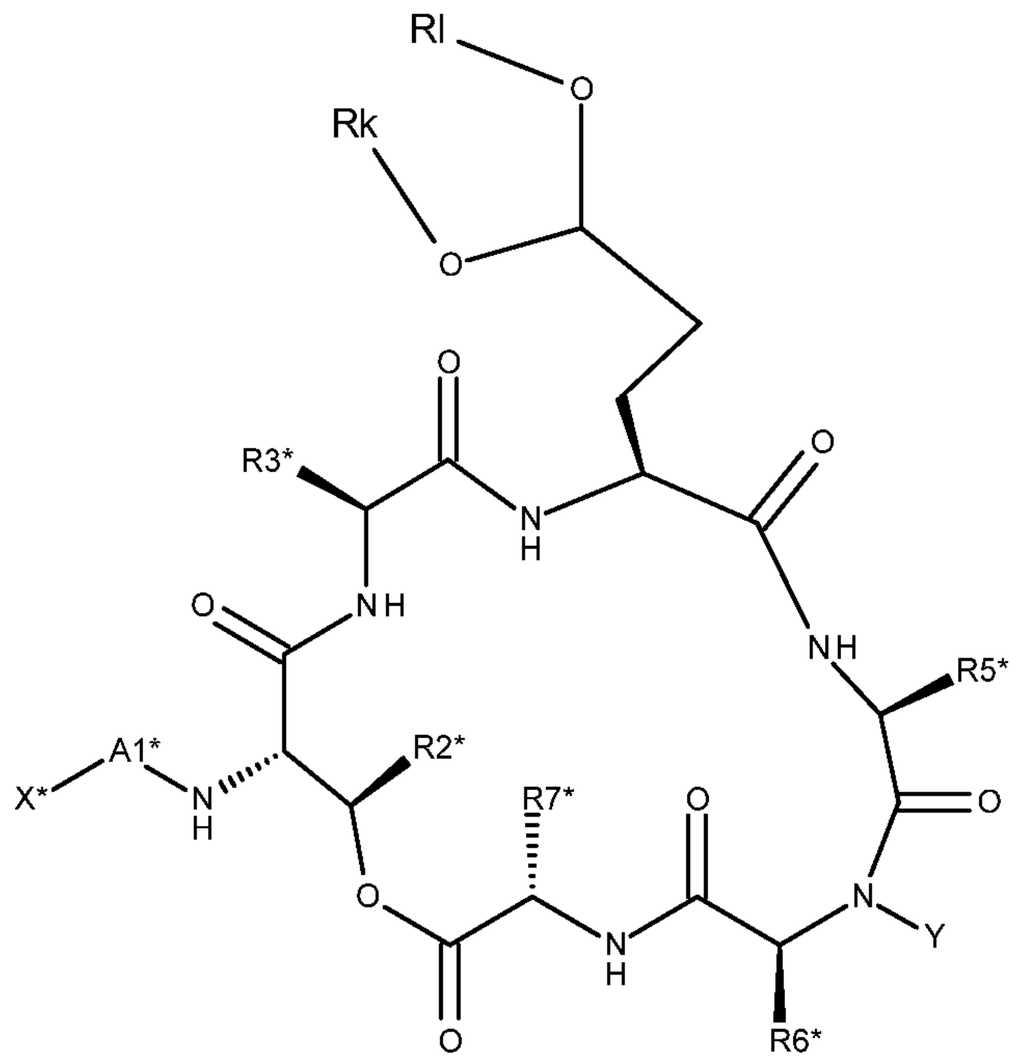
and/or (in parallel or preferably alternatively), in a variant (B),

deprotecting a compound of the formula II*



II*

especially of the formula IIA*



IIA*

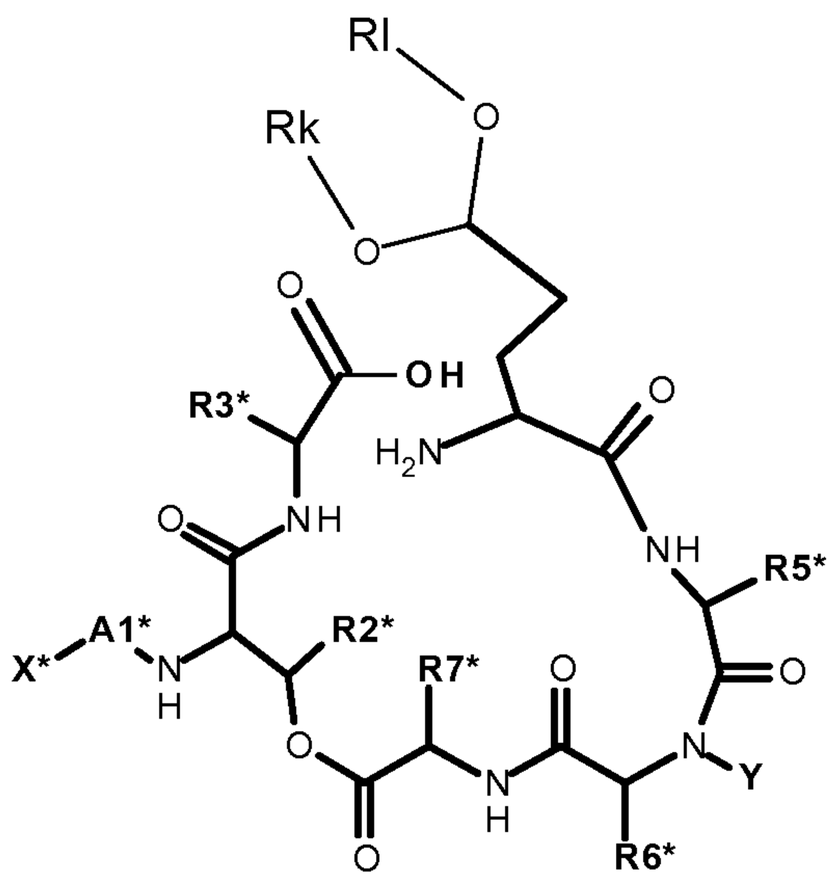
wherein the aldehyde protecting group(s) Rk and Rl are independently of each other unsubstituted or substituted alkyl or together with the two binding O atoms and the carbon atom to which the two O atoms are bound form a ring that is unsubstituted or substituted (Rk and Rl then preferably forming an unsubstituted or substituted alkylene bridge, especially unsubstituted or substituted ethylen, such as $–CH_2-CH_2-$ or $–CH_2-CH_2–CH_2-$), Y is as defined for a compound of the formula I and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I, respectively, but with the proviso that reactive functional groups on these moieties (such as amino, imino, hydroxy, carboxy, sulfhydryl, amidino, guanidino, O-phosphono $(-O-P(=O)(OH)_2)$ are present in protected form at least if they could participate in undesired side reactions, to result (e.g. via the intermediates of the formula XXIII-1 (especially XXIII-1A), XXIII-2 (especially XXIII-2A) and/or XXIII-3 (especially XXIII-3A) or analogues still carrying some or all of the protecting groups in the compound of the formula II*, especially IIA*) in a compound of the formula I, especially IA;

and, if desired, converting a free compound of the formula I, or especially IA, into a salt, a salt of a compound of the formula I into a different salt of a compound of the formula I, or especially IA, or into the free compound of the formula I, or especially IA, and/or

converting a dehydrate analogue (e.g. by-product of formula XXIII-2 or especially XXIII-2A given above) and/or five ring analogue of a compound of the formula I (e.g. by-product of formula XXIII-3 or especially XXIII-3A given above), or especially IA, into the corresponding compound of the formula I, or especially IA.

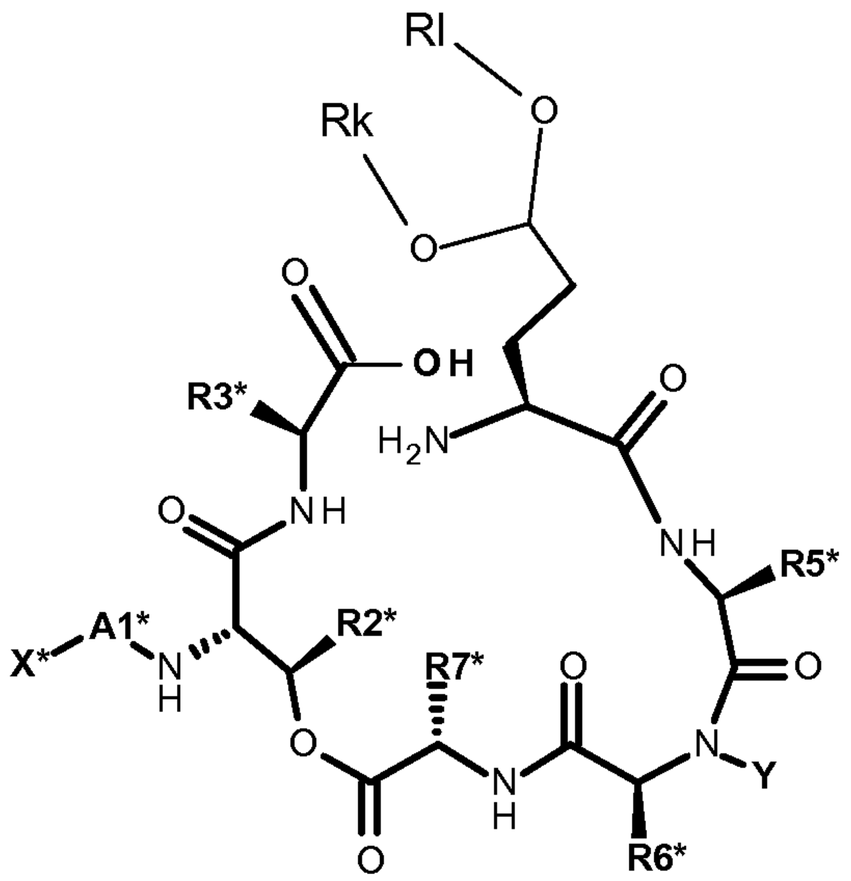
Yet a further embodiment of the invention relates to a method or process as described above, further comprising, for the synthesis of a compound of the formula II* above, especially of the formula IIA* above, cyclization under lactamization (macrolactamization) of a linear precursor peptide of the compound of the formula II* or especially of the formula IIA*, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, using Solution Phase chemistry.

In a further embodiment of the invention relates to the method or process according to the preceding paragraph, where the linear precursor peptide is of the formula IV*,



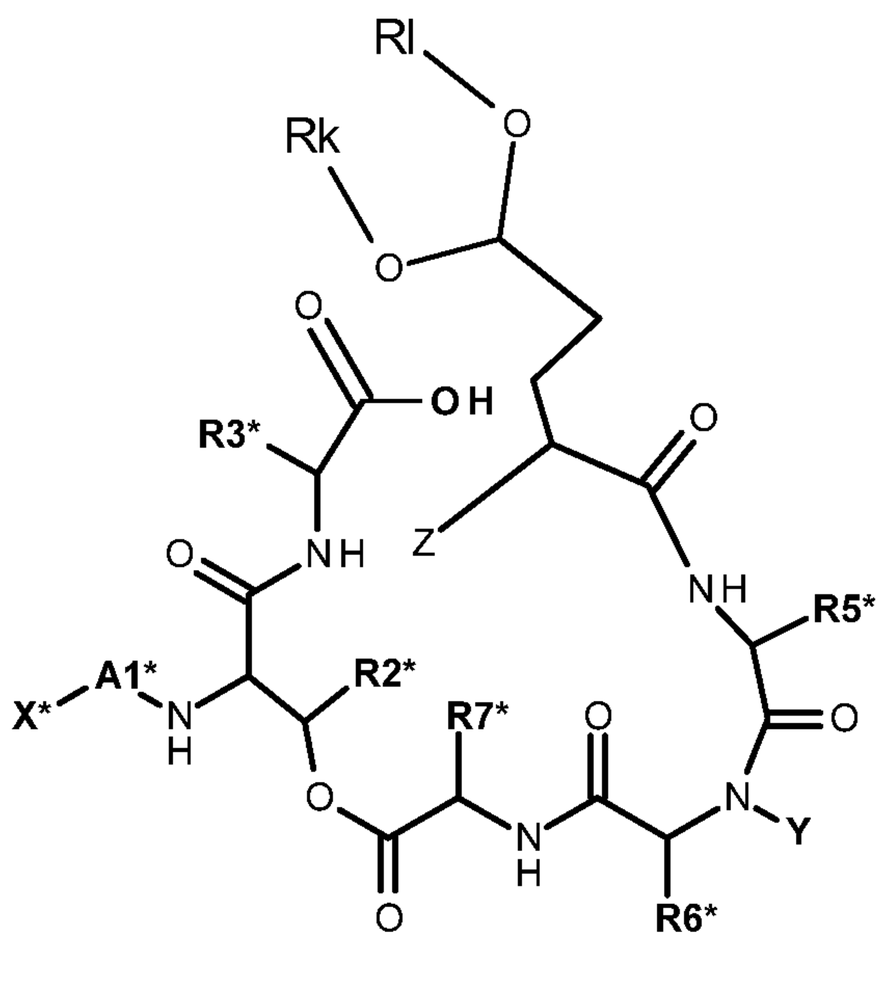
IV*

especially IVA*,



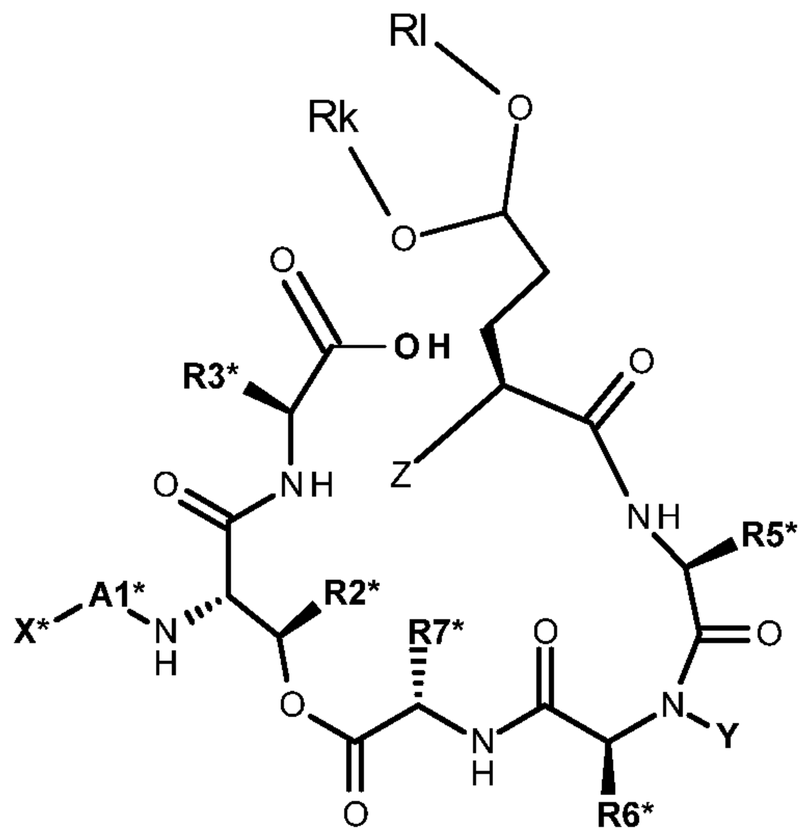
IVA*

wherein Rk, Rl, X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II* above, which can be obtained by deprotection from the corresponding compound of the formula V*,



V*

especially VA*,

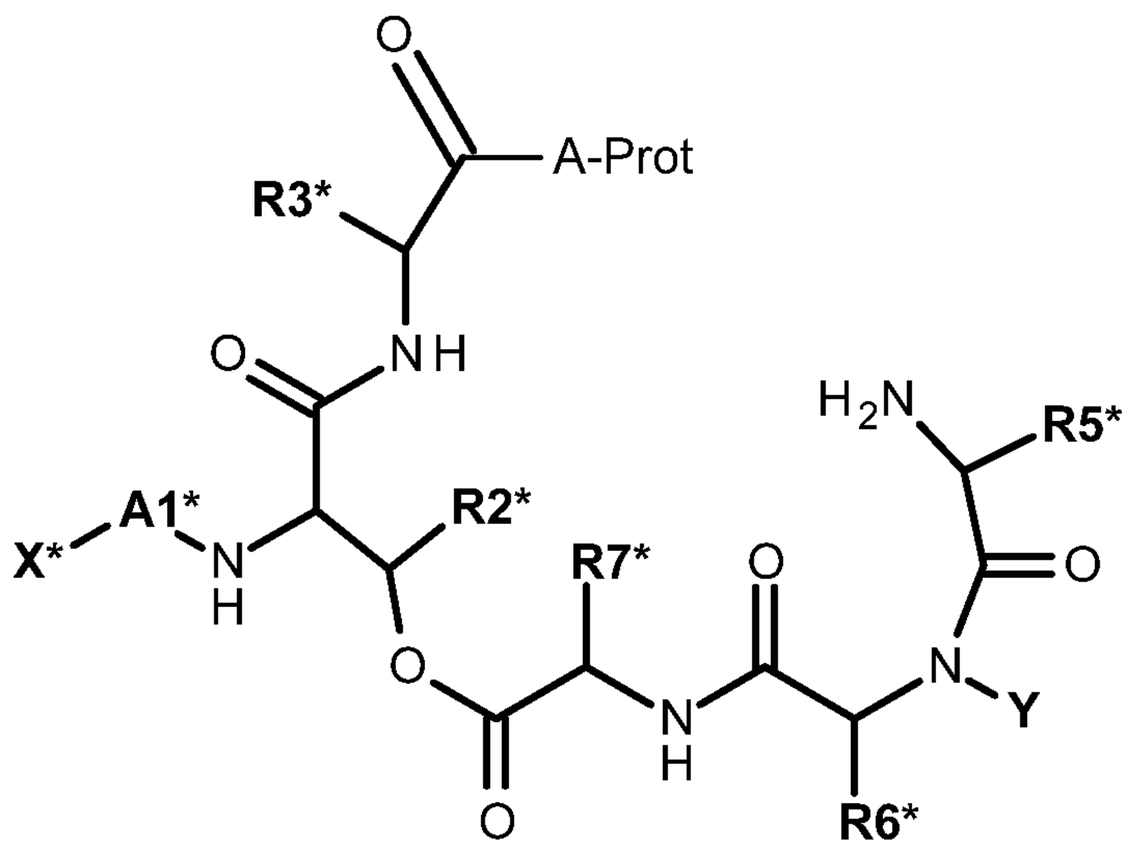


VA*

wherein Rk, Rl, X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II* above and wherein Z is as defined for a compound of the formula V above, by deprotecting the protected amino group Z.

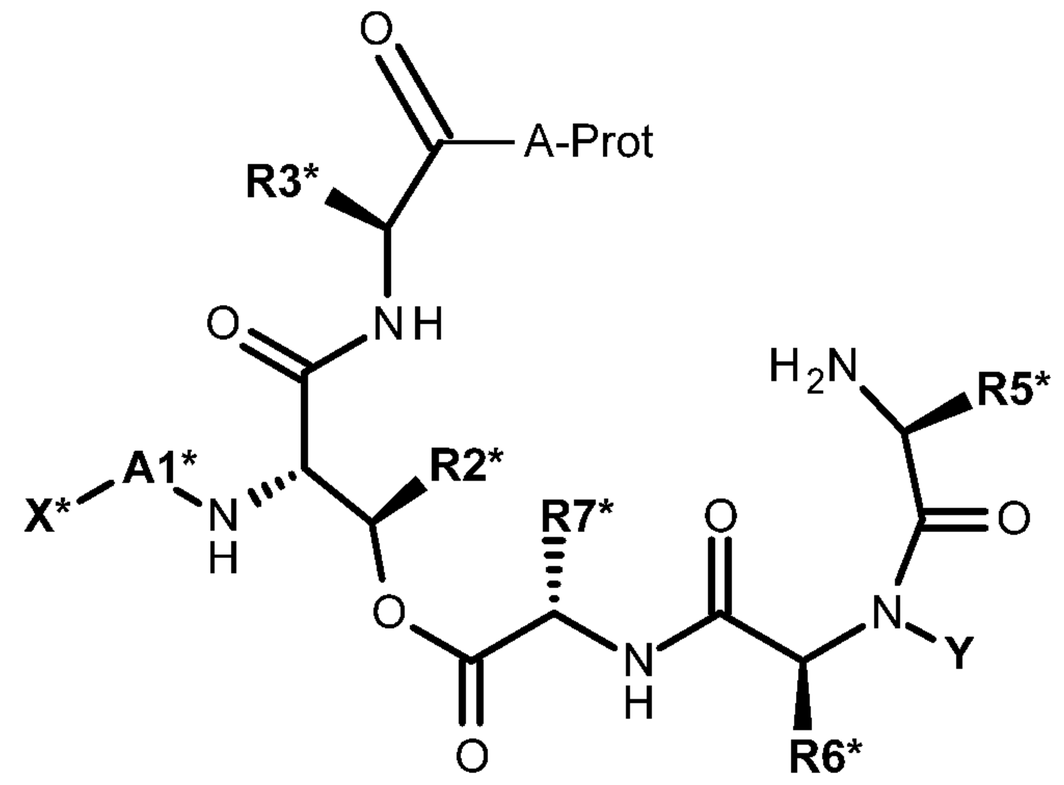
Another embodiment refers to the method or process according to the preceding paragraph, further comprising, for the synthesis of the compound of the formula V* or especially VA*,

reacting a compound of the formula VI



VI

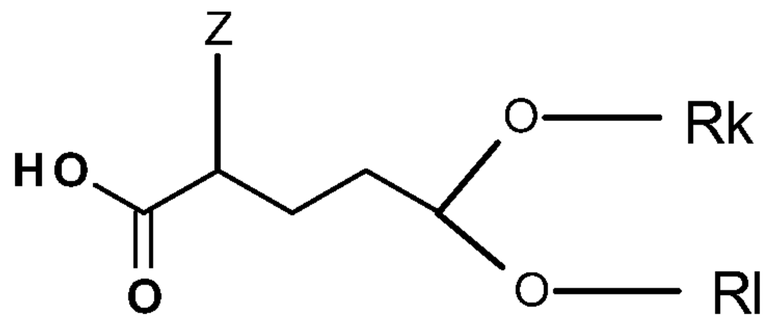
especially VIA,



VIA

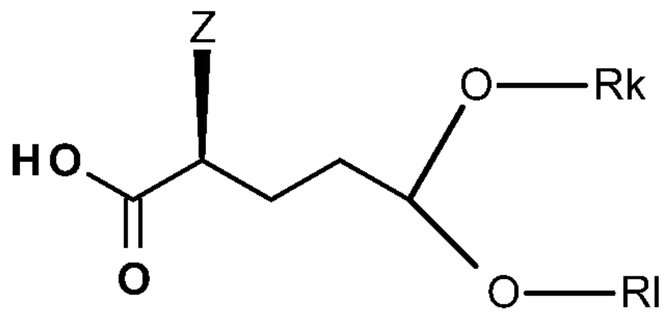
wherein Rk, Rl, X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II* above and Prot-A is a carboxy protecting group

by coupling an amino acid of the formula VII*,



VII*,

especially VIIA*,

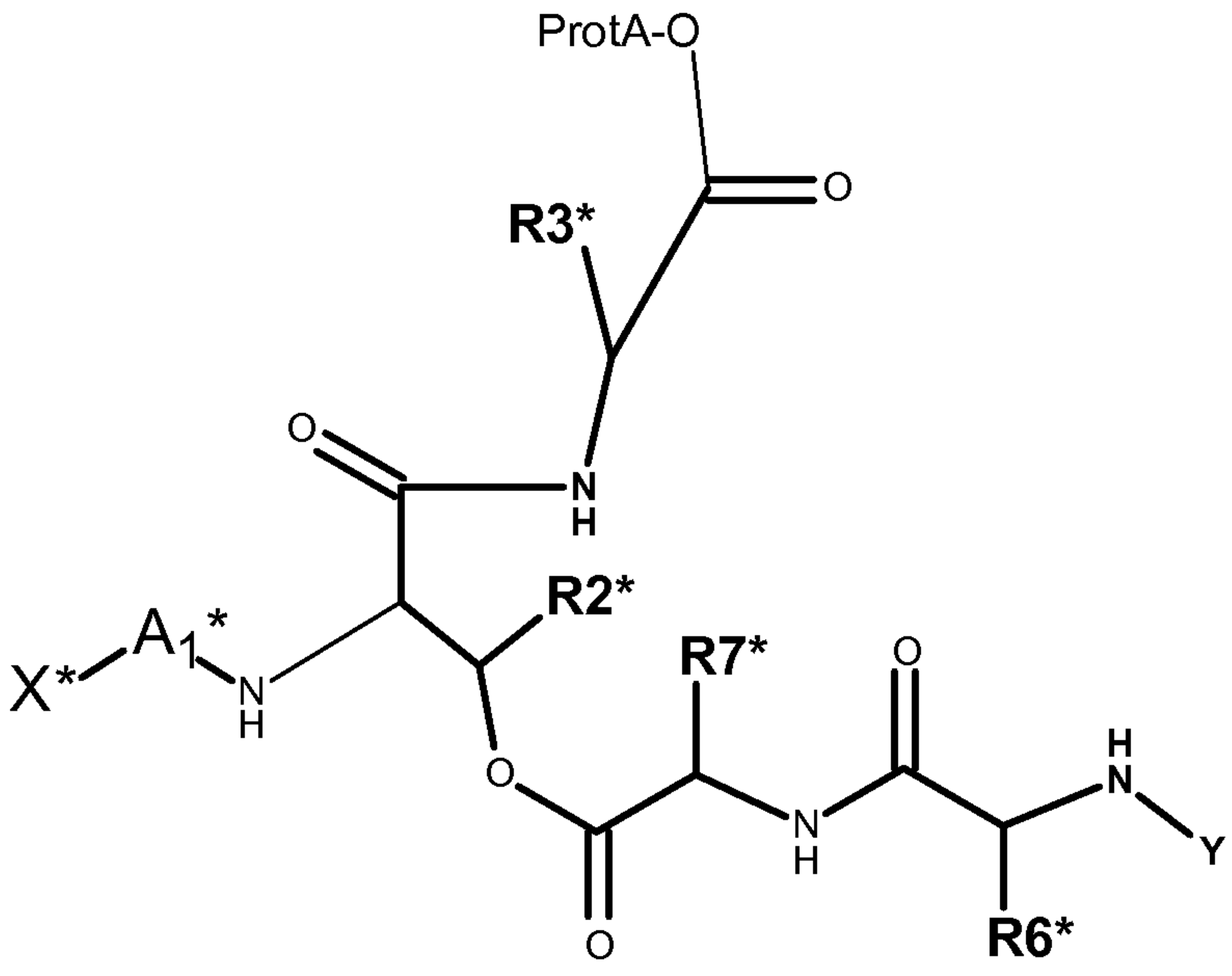


VIIA*

wherein Rk and Rl are as defined for a compound of the formula II* above and Z is as defined above for a compound of the formula V; or an activated derivative of said amino acid, to said compound of the formula VI* or VIA* **(end of variant (B)).**

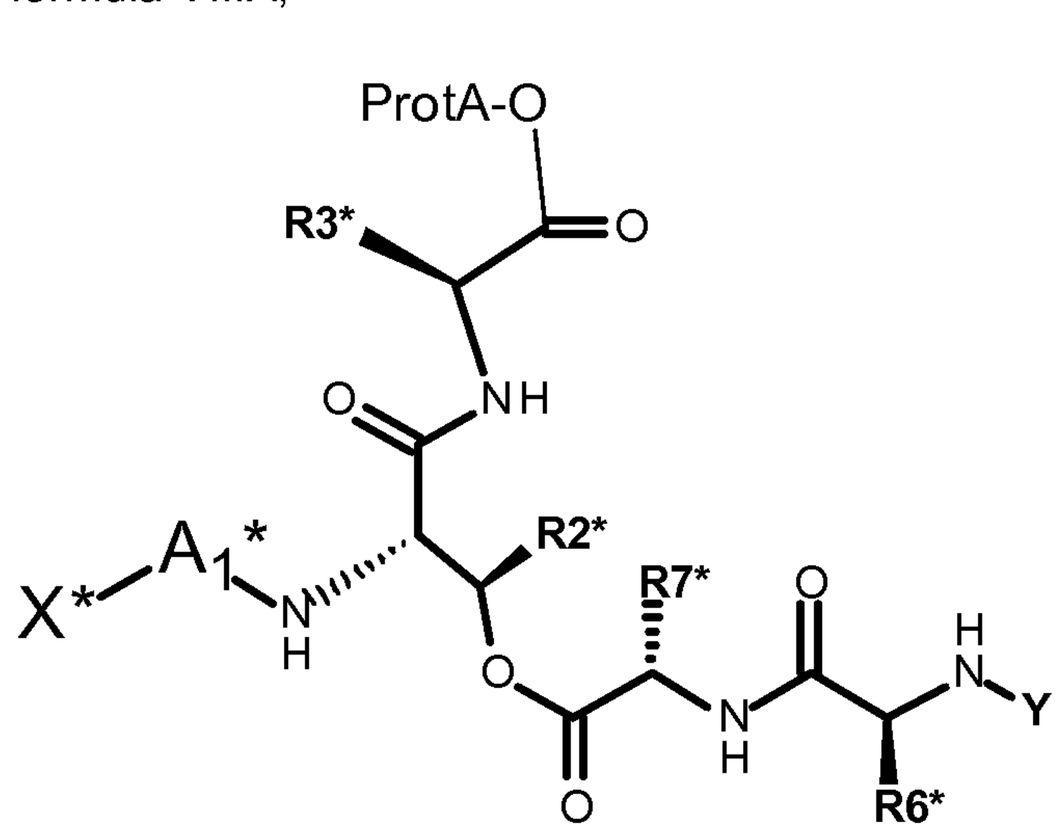
Yet a further embodiment of the invention relates to the method or process as described above (including either variant (A) or variant (B), not excluding that both are used, e.g. in

parallel), further comprising manufacturing the compound of the formula VI used in both variants in solution phase by coupling a compound of the formula VIII



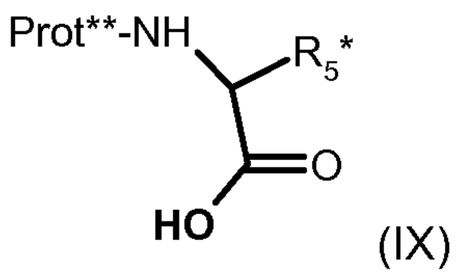
(VIII),

especially of the formula VIIIA,



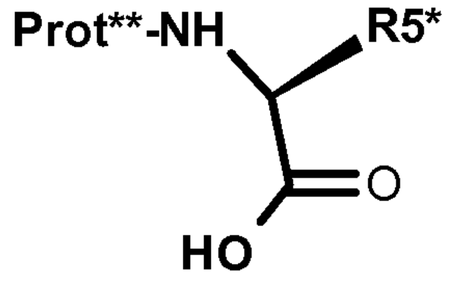
(VIIIA)

wherein ProtA is as defined above for a compound of the formula V and Y, R_2*, R_3*, R_7*, R_6* and X* and A_1* are as defined above for a compound of the formula II, with an amino acid of the formula IX,



(IX)

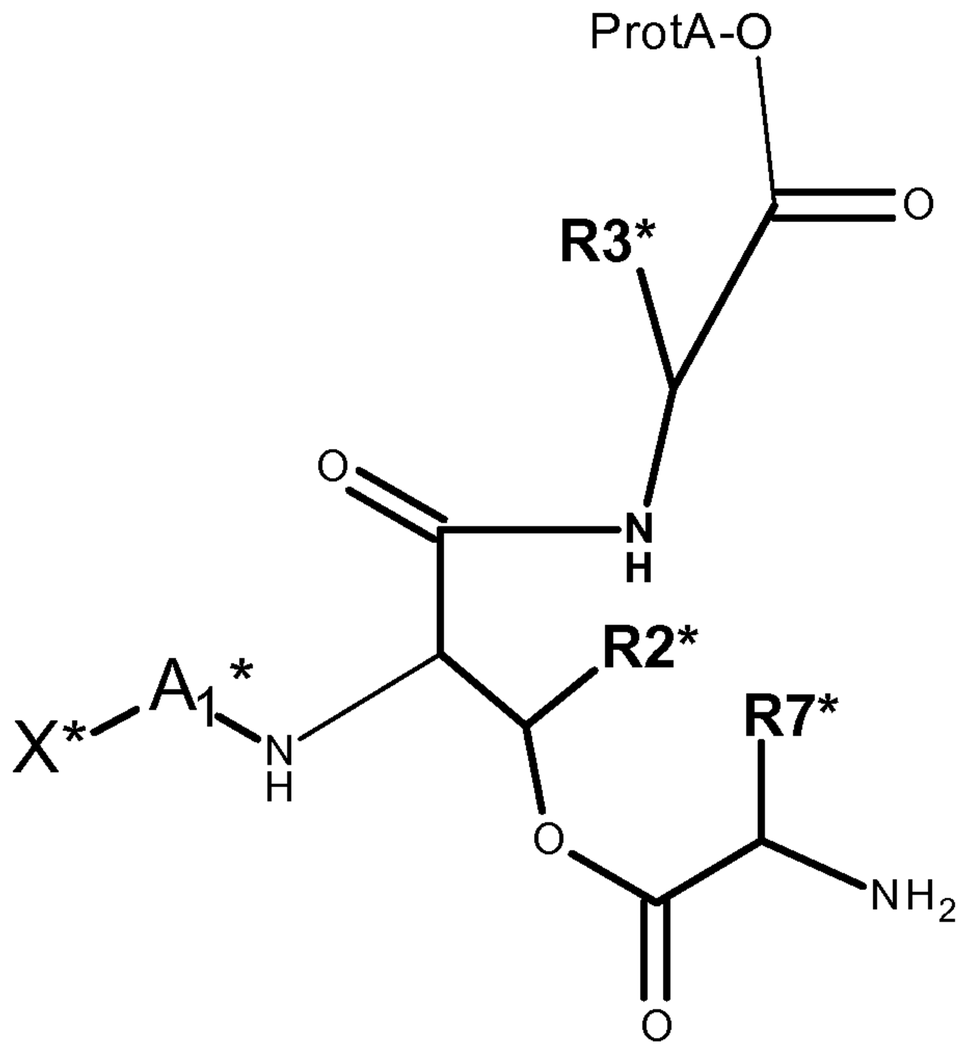
especially of the formula IXA,



(IXA)

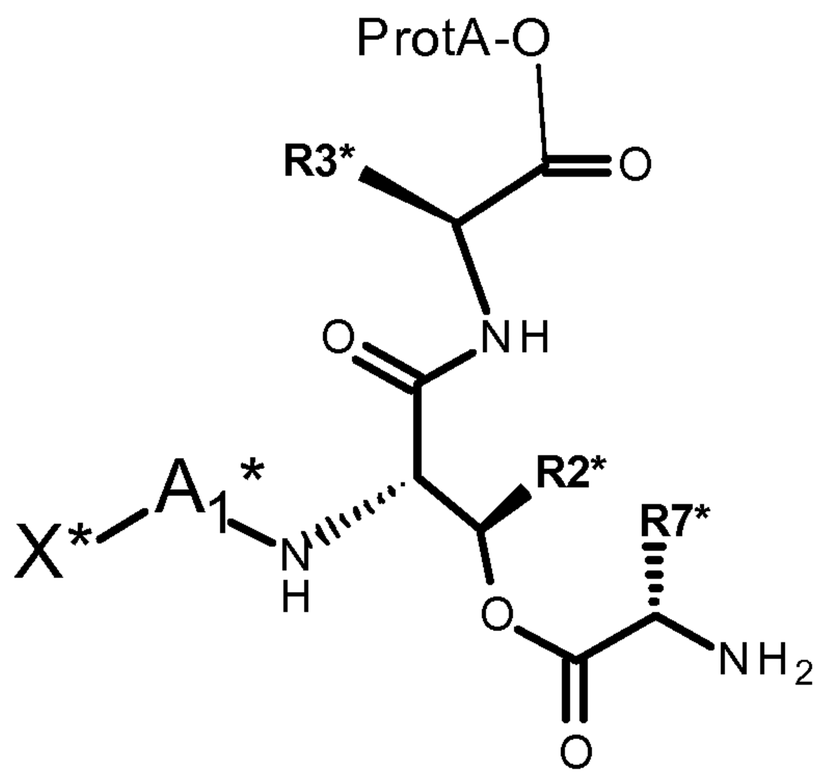
wherein Prot** is as defined for a compound of the formula V in the definition of Z and R5* is as defined above for a compound of the formula V, or a reactive derivative of said amino acid.

In another embodiment, the invention relates to the method or process mentioned above, further comprising manufacturing the compound of the formula VIII in solution phase by coupling a compound of the formula X,



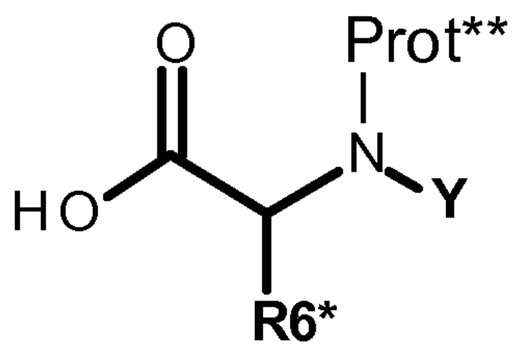
(X)

especially of the formula XA,



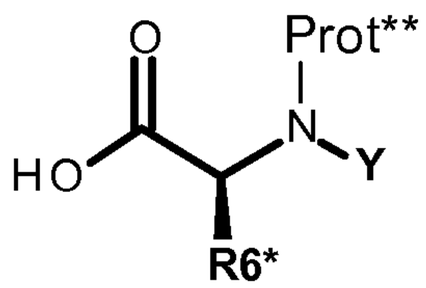
(XA)

wherein ProtA is as defined above for a compound of the formula Vand R_2*, R_3*, R_7*, X* and A_1* are as defined above for a compound of the formula II, with an amino acid of the formula XI,



(XI)

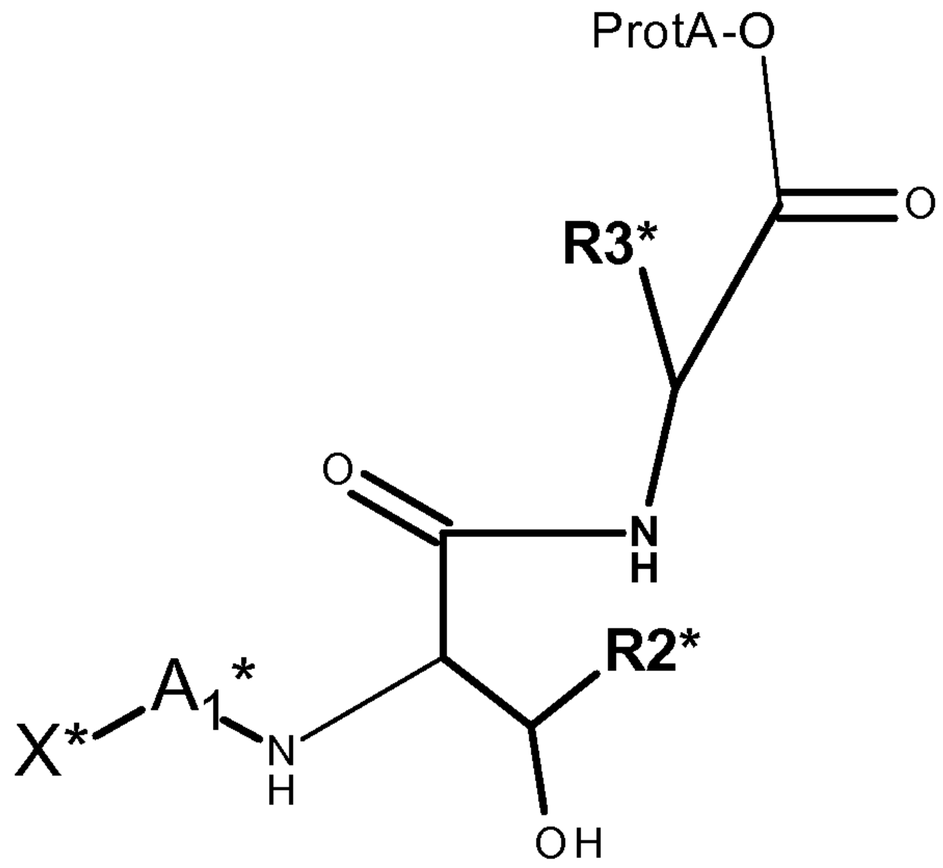
especially of the formula XIA,



(XIA)

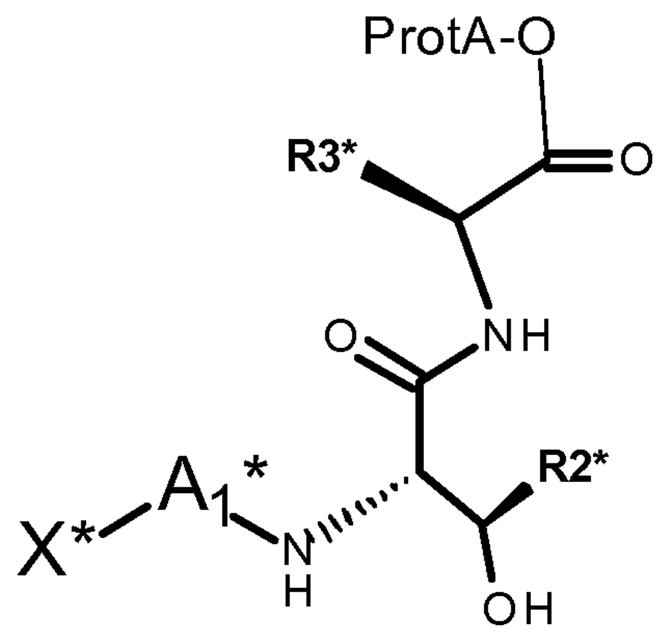
wherein Prot** and R_6* are as defined above for a compound of the formula V and Y is as defined above for a compound of the formula I, or a reactive derivative of said amino acid.

A further invention embodiment related to the above method or process, further comprising manufacturing the compound of the formula X in solution phase by esterifying a compound of the formula XII,



(XII)

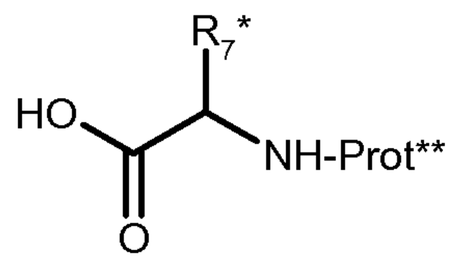
especially of the formula XIIA,



(XIIA)

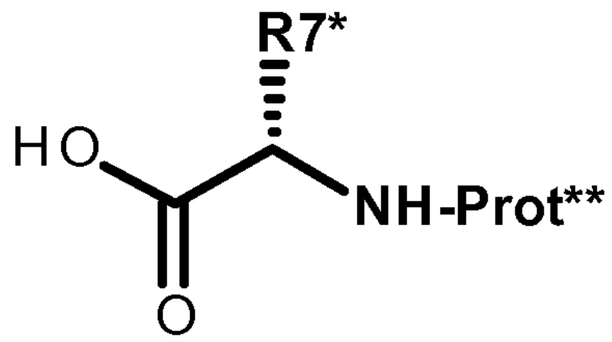
wherein ProtA is as defined above for a compound of the formula V and R_2*, R_3* X* and A_1* are as defined above for a compound of the formula II,

with an amino acid of the formula XIII



(XIII)

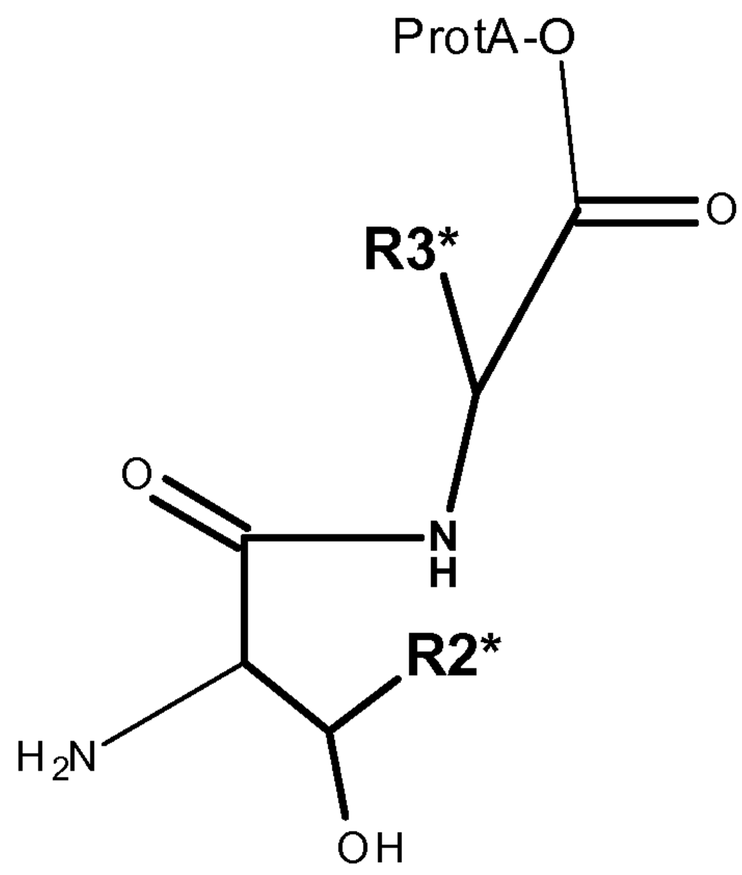
especially of the formula XIIIA,



(XIIIA)

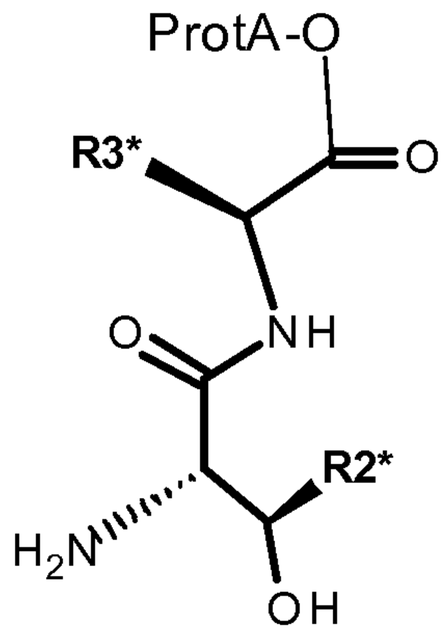
wherein Prot** and R_7* are as defined above for a compound of the formula V, or a reactive derivative of said amino acid.

In yet a further embodiment, the invention relates to the above-mentioned method or process, further comprising manufacturing the compound of the formula XII in solution phase by coupling a compound of the formula XIV,

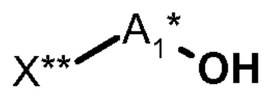


(XIV)

especially of the formula XIVA,



wherein ProtA is as defined above for a compound of the formula V and R_2* and R_3* are as defined above for a compound of the formula II, with an acid of the formula XV,

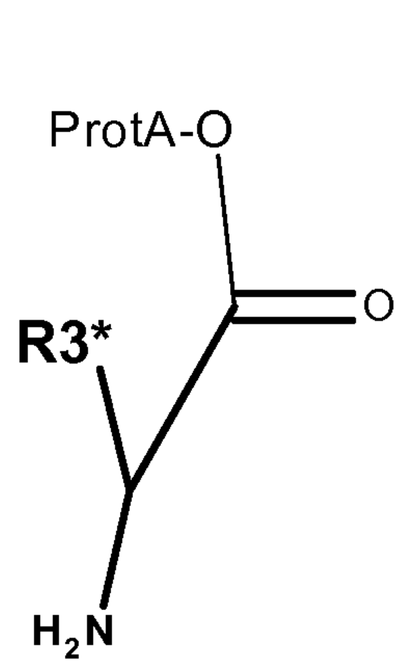


XV

or a reactive derivative thereof,

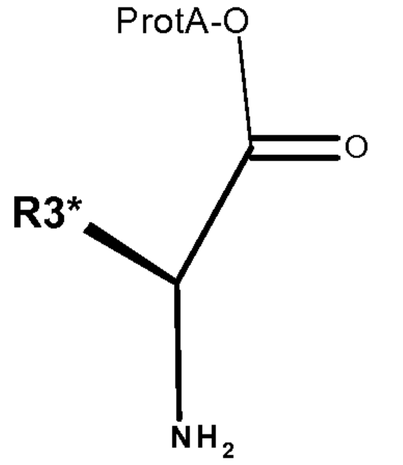
wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined above for a compound of the formula II; and, if X** is an amino protecting group, removing said amino protecting group X** to yield H instead of X* and coupling the resulting amino group with an acyl group X* using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

An embodiment of the invention also relates to the above-mentioned method or process, further comprising manufacturing the compound of the formula XV by coupling an amino acid of the formula XVI



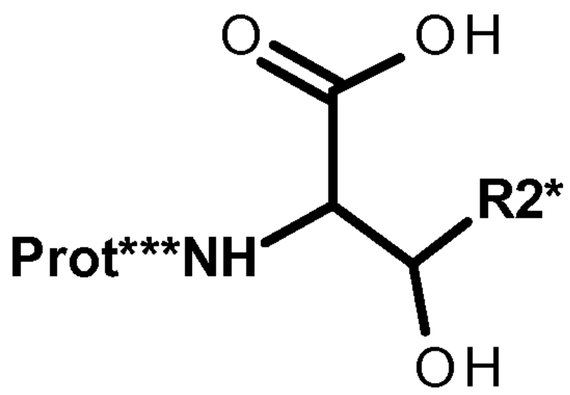
(XVI),

especially of the formula XVIA,



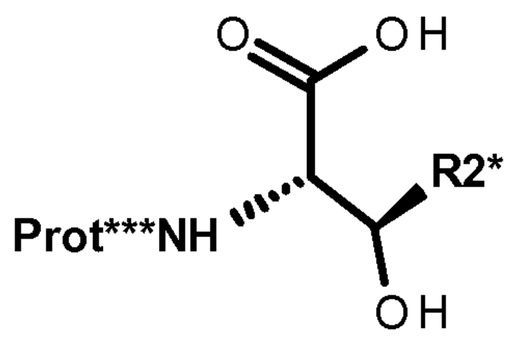
(XVIA)

wherein ProtA is as defined above for a compound of the formula V and R_3* is as defined above for a compound of the formula II, with an amino acid of the formula XVII,



(XVII)

especially of the formula XVIIA,

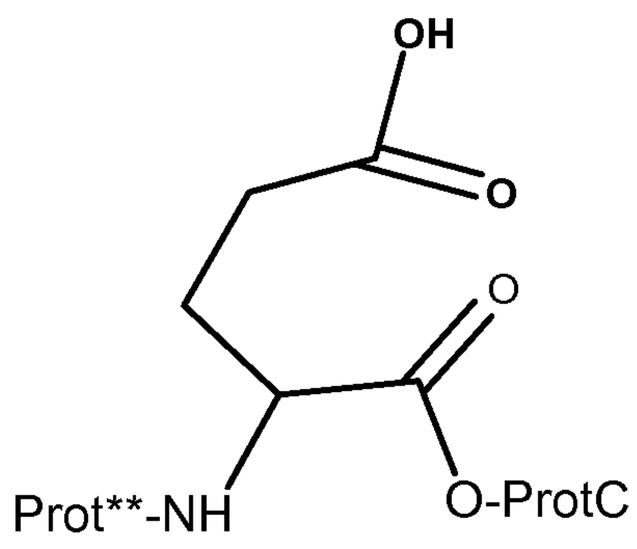


(XVIIA)

wherein Prot*** is an amino protecting group that can be cleaved off without removal of the protecting group ProtA and R_2*is as defined above for a compound of the formula II, or a reactive derivative of said amino acid,

and removing the amino protecting group Prot***.

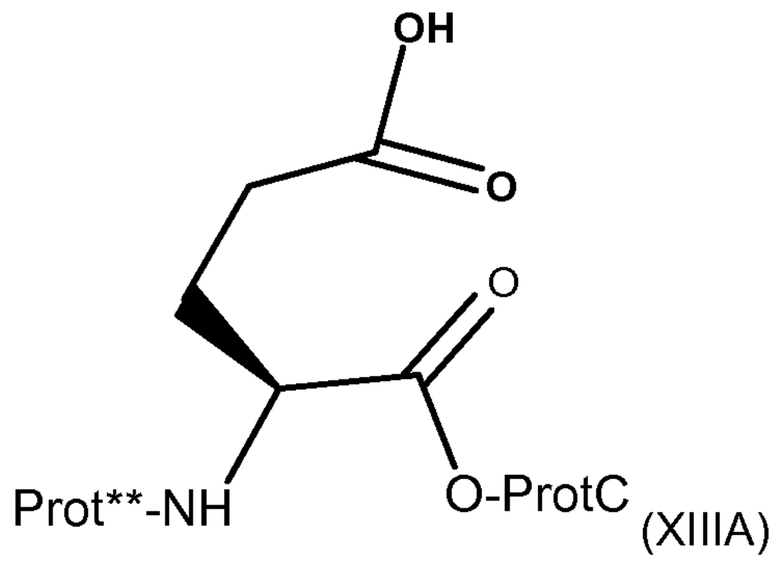
In a further embodiment, the invention relates to a method as described above, comprising manufacturing the compound of the formula VII by reducing the free carboxyl group in a compound of the formula XVIII



(XVIII)

(XVIII)

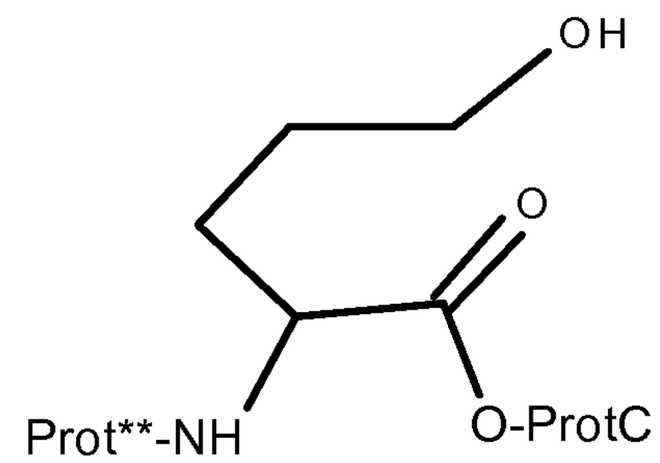
especially of the formula XVIIIA,



(XIIIA)

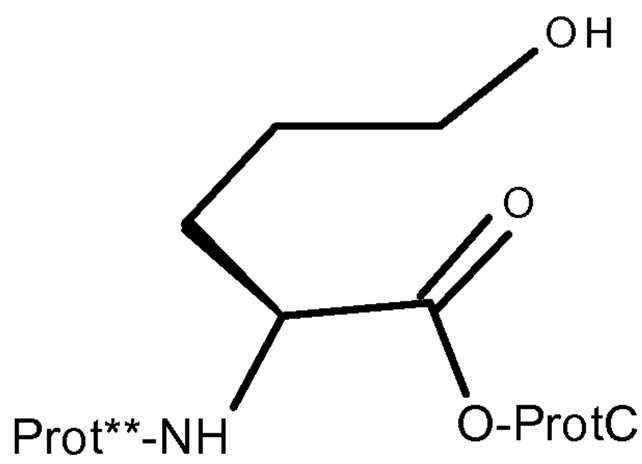
)

wherein ProtC is a carboxyl protecting group and Prot** is as defined for a compound of the formula V, to the corresponding alcohol of the formula XIX,



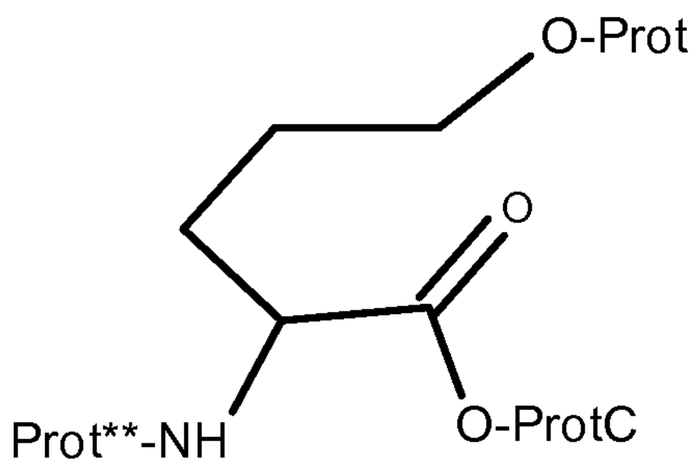
(XIX)

especially of the formula XIXA,



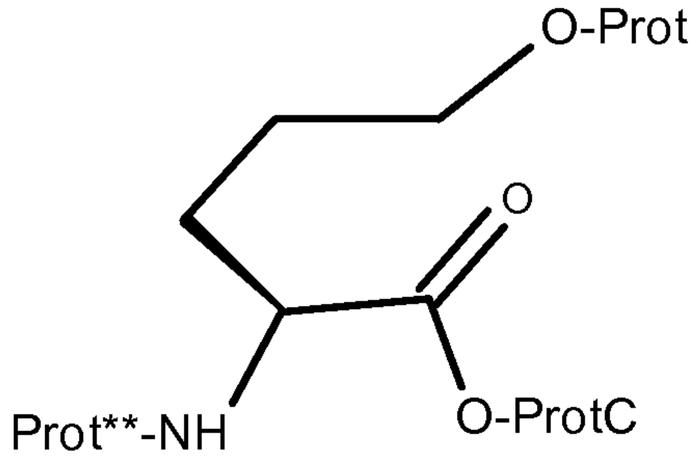
(XIXA)

wherein Prot** and ProtC are as just defined, protecting the free hydroxyl group with a reagent introducing a hydroxyl protecting group Prot to give a compound of the formula XX,



(XX)

especially of the formula XXA,

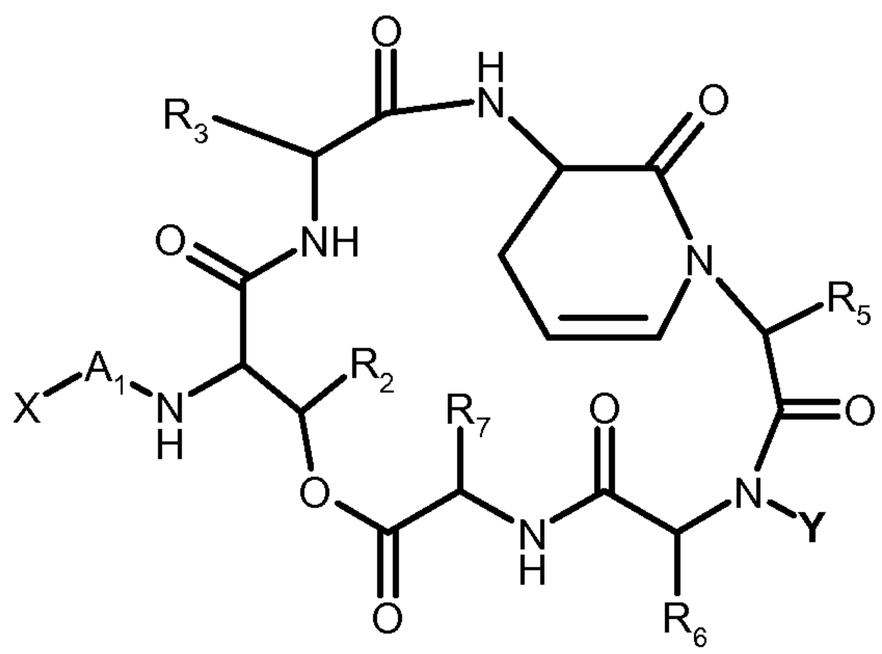


(XX)

wherein Prot**, ProtC and Prot are as just defined,

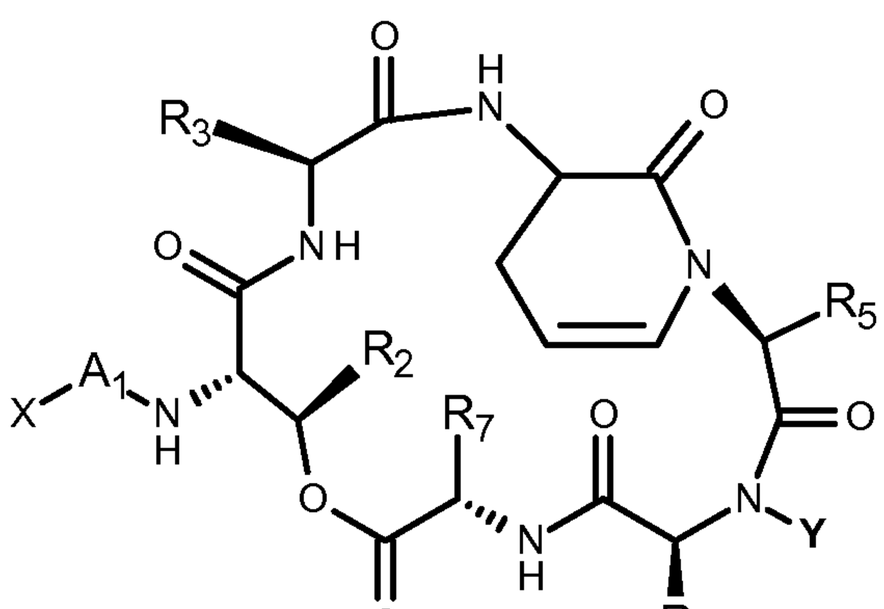
and removing the protecting group ProtC to give the compound of the formula VII.

A further embodiment of the invention relates to a process for the manufacture of a compound of the formula I, comprising converting a dehydrate of a compound of the formula I with the substituents as defined above into the corresponding compound of the formula I, where the dehydrate has the formula XXI,



(XXI),

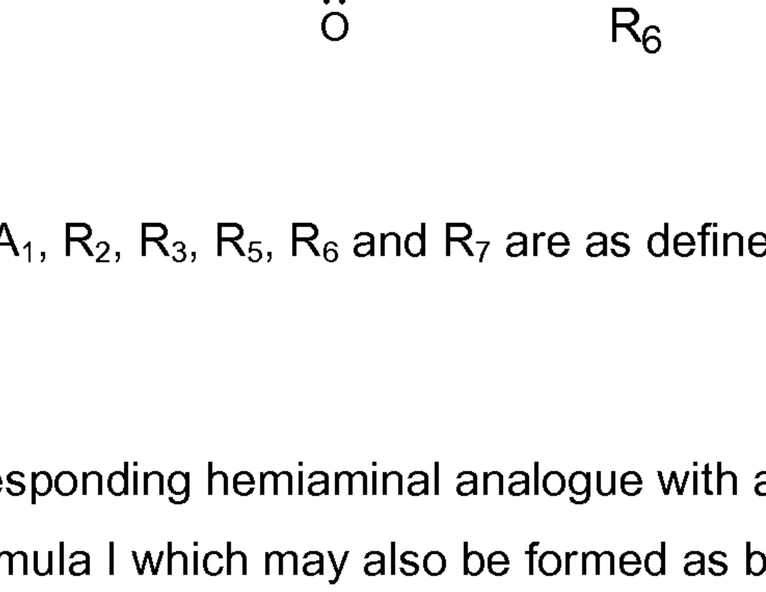
especially of the formula XXIA,



(XXIA)

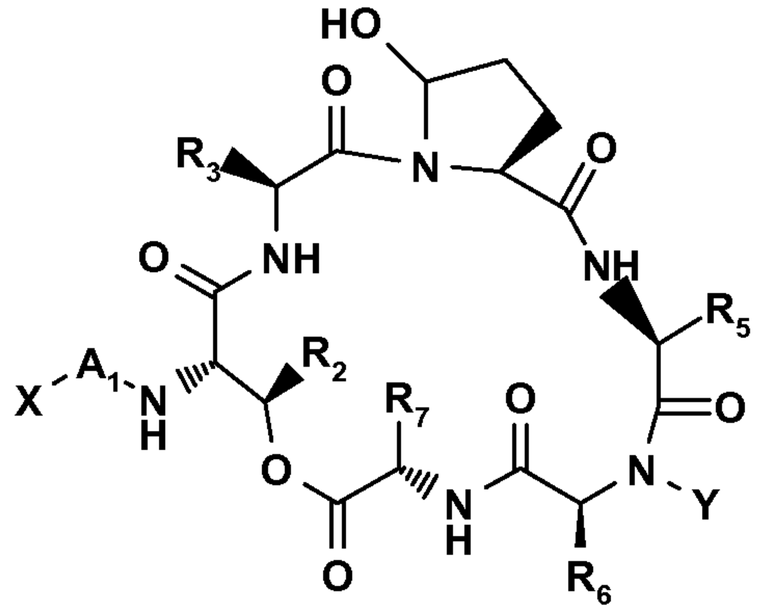
in which Y, X, A_1, R_2, R_3, R_5, R_6 and R_7 are as defined above for a compound of the formula I;

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



(XXII*)

especially the formula XXIIA*,

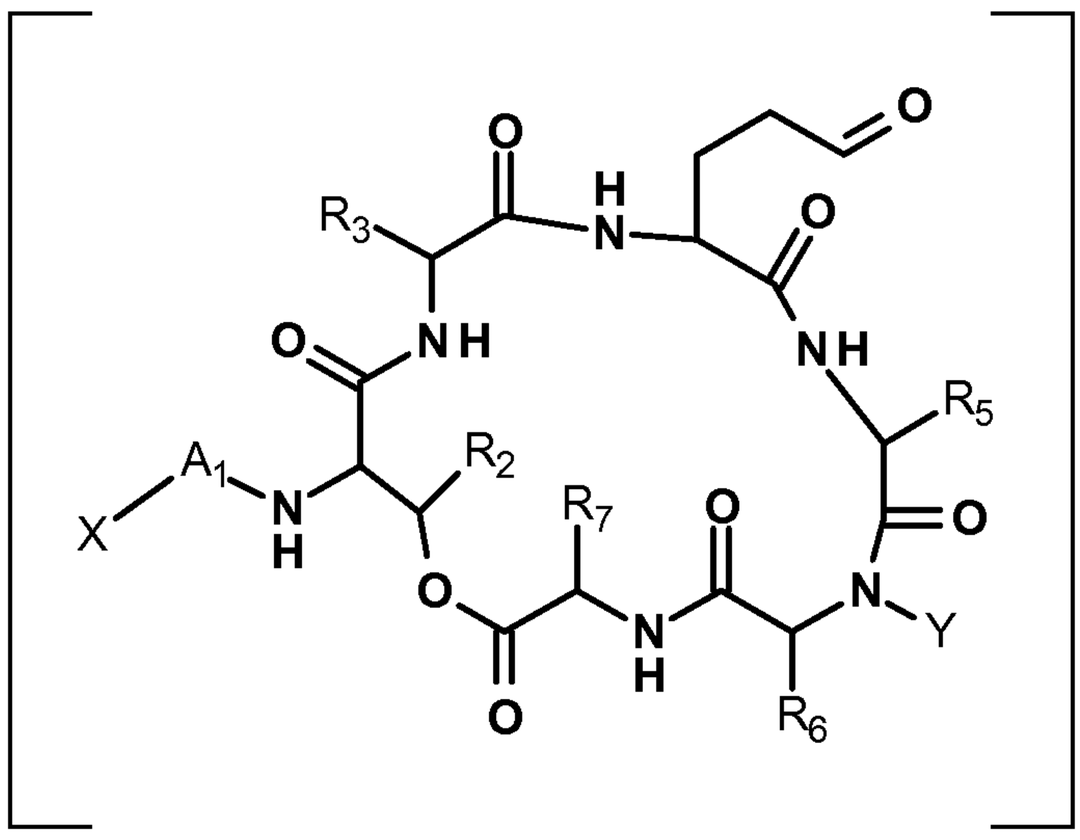


(XXIIA*),

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

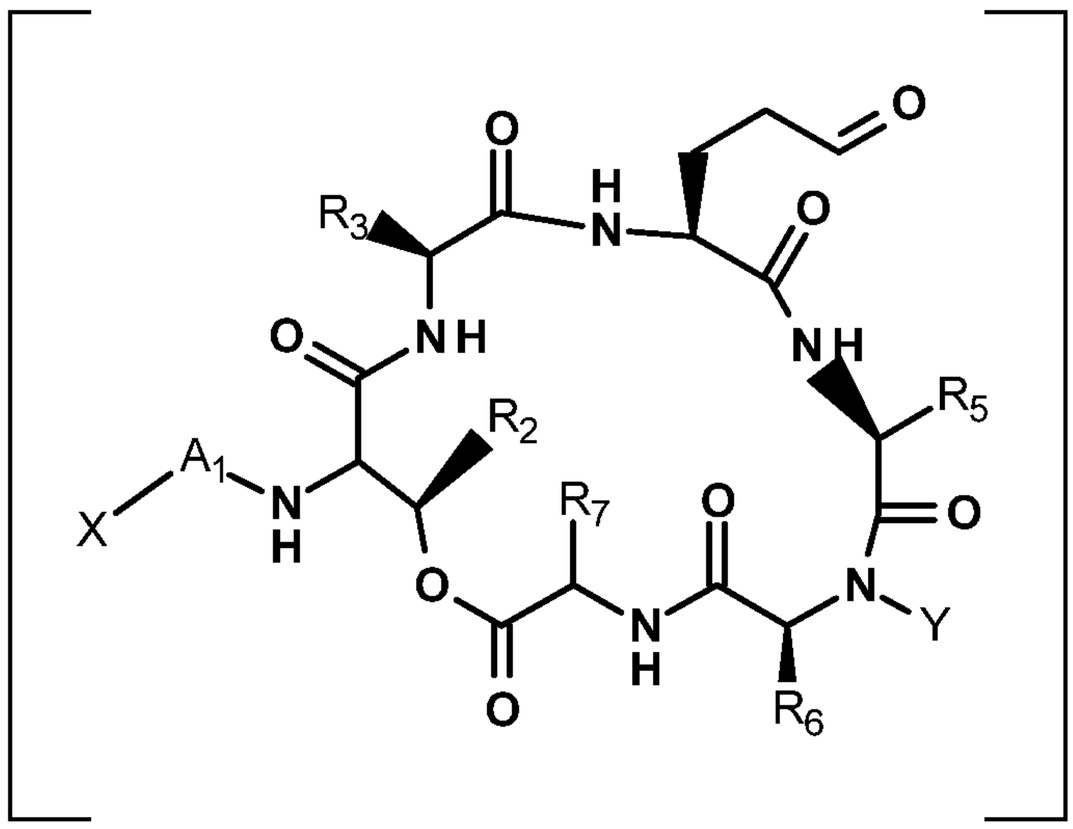
said method or process comprising using an aqueous acid as reactive solvent to drive the reaction.

The reaction of the compounds of the formula II, especially IIA, or II*, especially IIA*, can take place e.g. via a compound mixture including one or more compounds represented by the formula XXIII-1 (aldehyde compound which is only present transiently), XXIII-2 (dehydrate) and XXIII-3 (five-ring hemi-aminal)

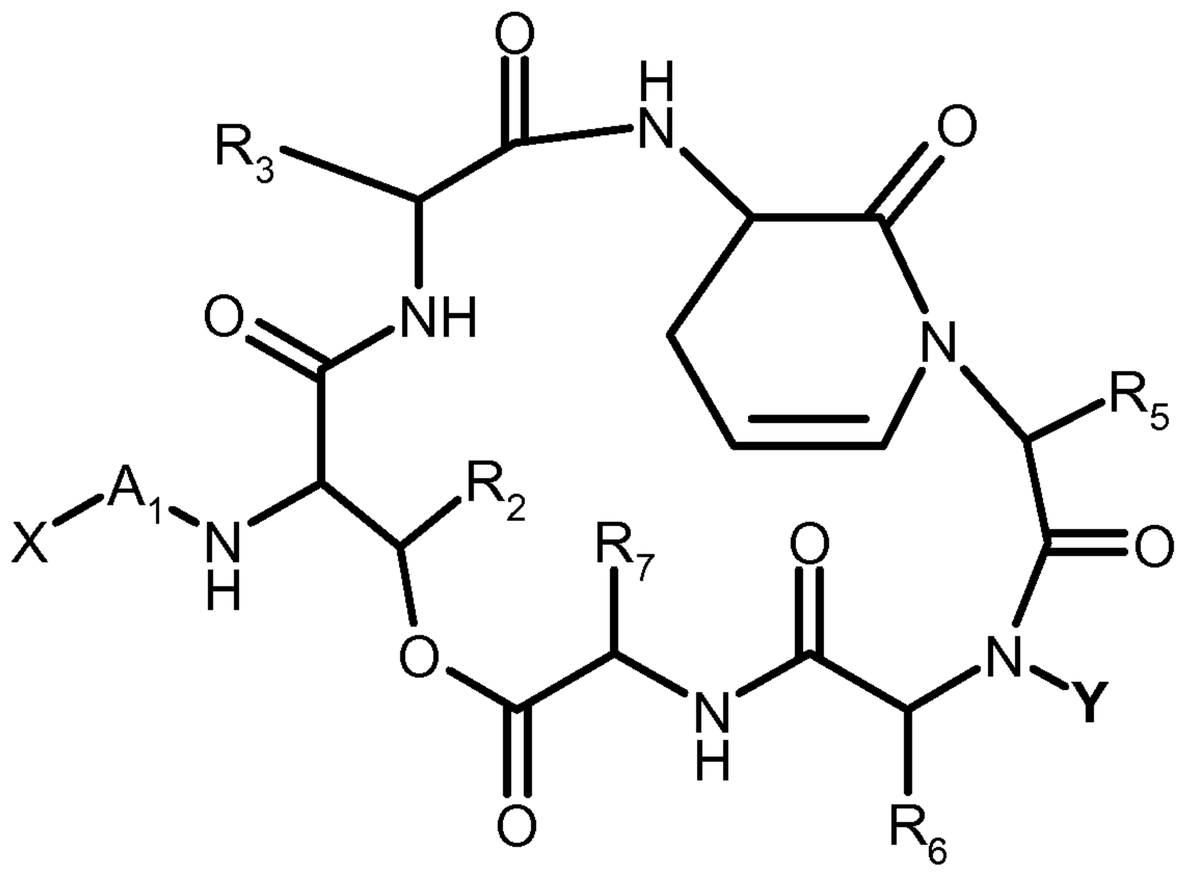


XXIII-1,

especially XXIII-1A

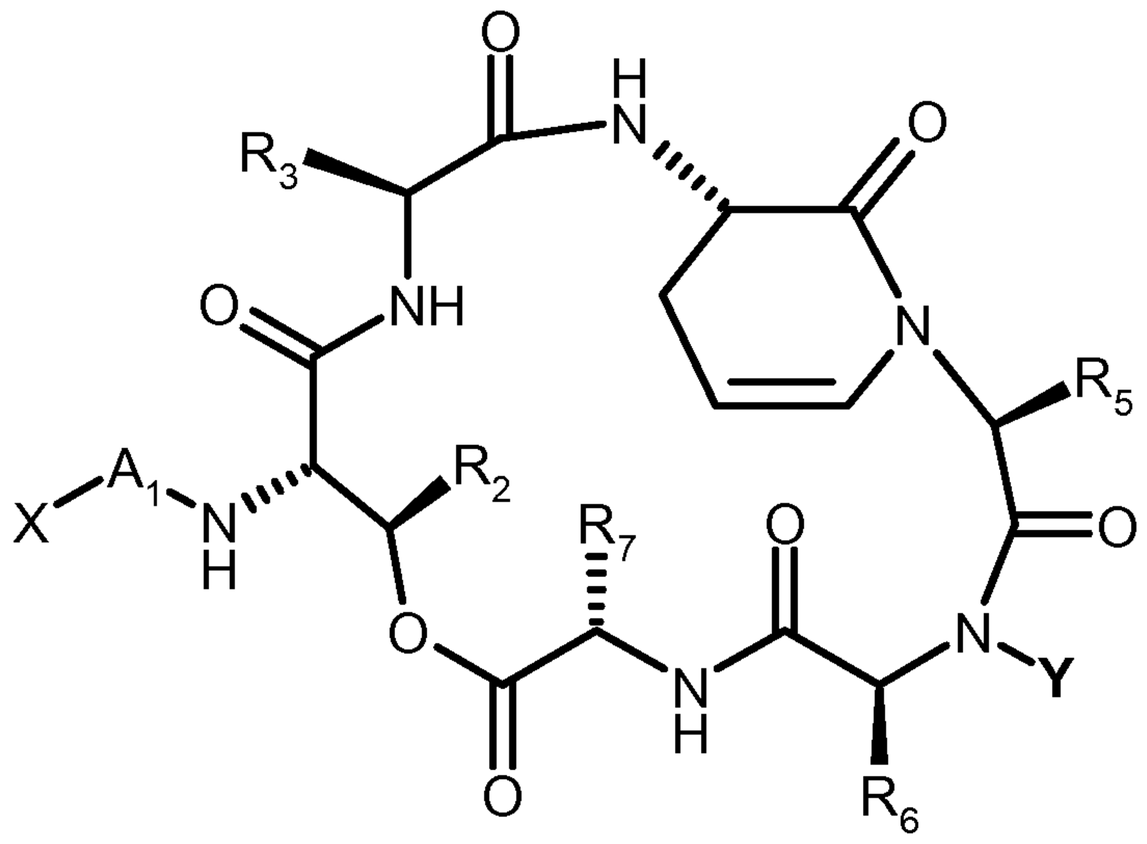


XXIII-1A;



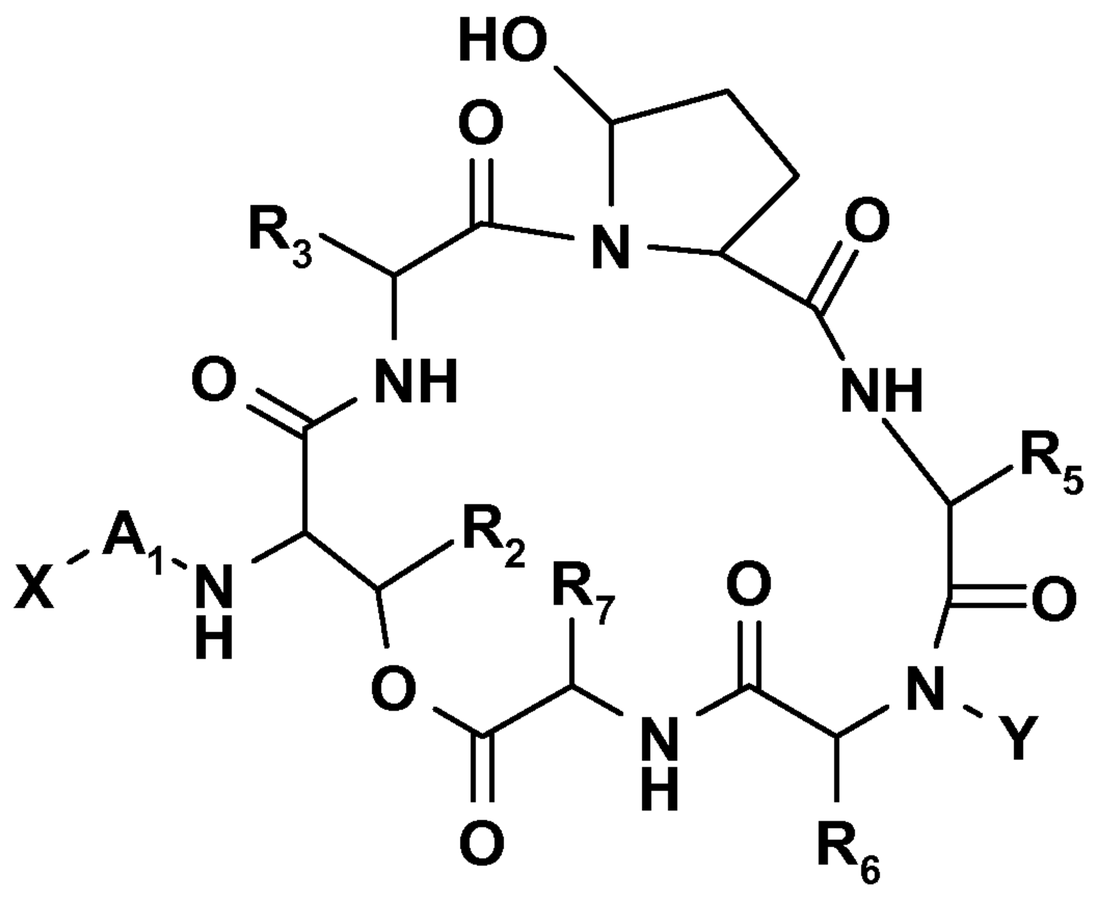
XXIII-2,

especially XXIII-2A



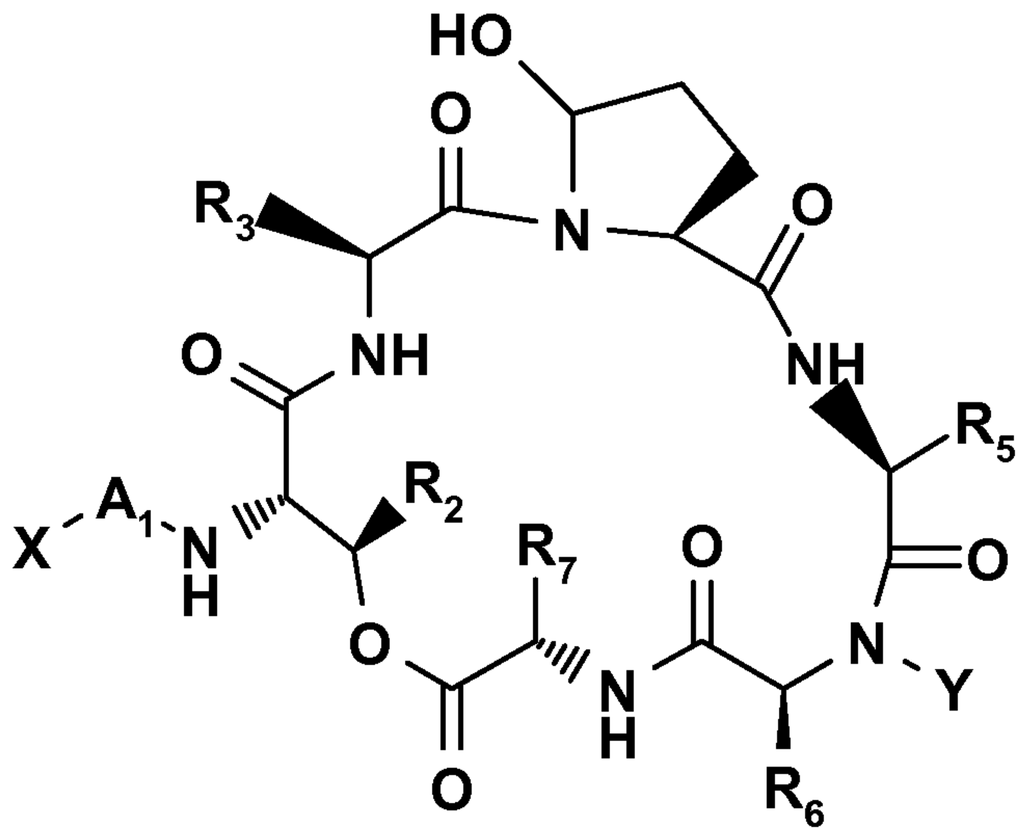
XXIII-2A;

and/or XXIII-3



XXIII-3,

especially XXIII-3A



XXIII-3A

wherein the moieties in each of the mentioned compounds XXIII-1-, -2- or -3- or the preferred variants mentioned have the following meanings:

wherein

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

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

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

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

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

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

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

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

where the compounds can also be in the form of a salt, respectively.

In a further embodiment, the invention relates to a novel compound selected from the group consisting of the following compounds given in the examples:

Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 8, Compound 9, Compound 10, Compound 12, Compound 13, Compound 14, Compound 18, Compound 21, or a salt thereof, respectively, where a salt-forming group is present.

A specific embodiment of the invention relates to the manufacture or Compound B or in particular Compound B, characterized in that in the reaction steps mentioned above and below the starting materials carrying the respective substitutents are used.

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

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

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

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

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

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

A “side chain of an amino acid” may be selected from any moiety, e.g. a mono- or poly-cyclic, linear, saturated, unsaturated (e.g. with conjugated double bonds) or partially saturated organic moiety, e.g. with up to 20 carbon atoms and 0 to 5 heteroatoms in the basis structure independently selected from N, O and S replacing the corresponding number of carbon atoms, and may be substituted by up to three moieties selected from amino, imino, hydroxy, carboxy, carbamoyl, sulfhydryl, amidino, guanidino, O-phosphono (-O-P(=O)(OH)$_2$). Preferably, the side chains are selected from those of the 20 standard alpha-amino acids arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparagine, glutamine, cysteine, glycine, alanine, leucine, isoleucine, methionine, phenylalanine, tryptophan, tyrosine, valine and further proline (then with internal cyliza-tion including the alpha-amino group).

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

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

Tyrosine	Tyr
Valine	Val

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

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

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

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

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

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

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

The protecting group Prot is preferably selected so as to be removable with fluoride ion (especially under anhydrous conditions), e.g. $Bu_4N^+F^-$ (also if created in situ, e.g. using $Bu_4N^+Cl^-$ with KF • H_2O, KF with 18-crown-6, LiBr with 18-crown-6, BF_3 • diethylether, pyridine-HF, HF in urea, $Et_3N(HF)_3$ (wherein Et is ethyl) or the like, where the solvent is e.g. selected from the group consisting of N,N-dimethylformamide, acetonitrile, chloroform and tetrahydrofurane.

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

Prot* and Prot** are a protecting group each that can be cleaved off selectively without affecting other protecting groups present. They are preferably protecting groups removable with fluoride ions (especially under anhydrous conditions), e.g. $Bu_4N^+F^-$ (also if created in situ, e.g. using $Bu_4N^+Cl^-$ with KF • H_2O, KF with 18-crown-6, LiBr with 18-crown-6, BF_3 • diethylether, pyridine-HF, HF in urea, $Et_3N(HF)_3$ (wherein Et is ethyl) or the like, where the solvent is e.g. selected from the group consisting of N,N-dimethylformamide, acetonitrile, chloroform and tetrahydrofurane, and/or removable by specific triphenylphosphin complexes in the presence of metal hydrides or other reductants, e.g. $(PH_3P)_4Pd$ or a dihalogenate thereof (e.g. $PdCl_2(PPH_3)_2$), preferably in combination with di-n-butyl tin hydride or tri-n-butyl tin hydride, phenylsilane, sodium borohydride or dimedone, in an appropriate solvent, e.g. tetrahydrofurane, and is preferably not cleavable under conditions that allow for the removal of a protecting group Prot**; for example, Prot** and Prot* is selected from the group consisting of C_3-C_8alk-2-enyloxycarbonyl moieties, e.g. allyloxycarbonyl (Alloc) (preferred; can be removed by catalytic hydrogenation, e.g. in the presence of a palladium catalyst), 1-isopropylallyloxycarbonyl, 4-nitrocinnamyloxycarbonyl and 3-(3'-pyridyl)prop-2-enyloxycarbonyl; or

fluoren-9-ylmethoxycarbonyl (Fmoc) (preferred), 2-(2' or 4'-pyridyl)ethoxycarbonyl or 2,2-bis(4'nitrophenyl)ethoxycarbonyl. Prot* is preferably an 1-aryl-alkyl group, such as benzyl, 9-fluorenylmethyl or 1-(p-methoxyphenyl)-ethyl).

Prot*** is preferably removable by addition of an acid, e.g. trifluoroacetice acid, and is e.g. tri-(1-(C_1-C_6)-alkyl)-alkoxycarbonyl, especially tert-butoxycarbonyl.

The carboxyl (-COOH) protecting group ProtA is preferably stable to deprotecting reagents used to remove protecting groups Prot** as well as Prot. The carboxy protecting group is preferably similarly removable.

The carboxyl protecting groups ProtA and ProtC are preferably removable by catalytic hydrogenation, e.g. hydrogenation in the presence of noble metal catalysts, such as Palladium or platinum, e.g. on solid carrier materials, such as carbon or insoluble salts such as barium sulfate ($BaSO_4$), in appropriate solvents, such as alcohols, e.g. methanol, ethanol, isopropylalcohol or the like, or mixtures of two or more thereof, in the absence or presence of water.

The carboxyl protecting groups ProtA and ProtC are preferably selected from the group consisting of [1-(C_1-C_8-alkyl or C_6-C_{12}-aryl)-(C_1-C_8-alkyl)]oxymethyl groups, such as methoxymethyl, benzyloxymethyl, or especially 1-aryl-alkyl groups, such as benzyl (especially preferred), 9-fluorenylmethyl or p-methoyxphenyl-ethyl.

Other protecting groups present are preferably not removable under conditions under which Prot* and Prot** can be removed, e.g. in A*, carbamoyl can be N-protected e.g. with trityl (triphenylmethyl) (cleavage with acid, e.g. with trifluoro acetic acid (TFA); in R_6* a tyrosine hydroxy can be tert-butyl protected (as tert-butoxy), or protected by tert-butyldimethylsilyl, methoxymethyl or arylacetate (cleavage with acid, e.g. TFA).

Rk and Rl are preferably aldehyde protecting groups forming an acetal, e.g. each is unsubstituted or substituted alkyl or both form, together with the two bonding O atoms and the carbon atom to which the two O atoms are bound, form a ring that is unsubsstituted or substituted.

If Rk and Rl are each independently of each other unsubstituted or substituted alkyl, this refers especially to C_1-C_7-alkyl or especially 1-aralkyl, such as 1-(C_6-C_{12}aryl)-C_1-C_7alkyl, more especially benzyl.

If Rk and Rl together with the two binding O atoms and the carbon atom to which the two O atoms are bound form a ring that is unsubstituted or substituted, Rk and Rl then preferably form an unsubstituted or substituted alkylene bridge, especially unsubstituted or substituted ethylen, such as –CH_2-CH_2-), where the substituent(s) may preferably be selected from C_1-C_7-alkyl, especially two such substituents, such as methyl, ethyl, n-propyl or isopropyl.

The aldehyde protecting group(s) Rk and Rl (which together with the binding O atoms and the carbon binding them form a protected aldehyde group (an acetal) can be removed in the presence of water by acid catalysis, especially an alpha-halo substituted alkanoic acid, such as trifluoroacetic acid or trichloroacetic acid.

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

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

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

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

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

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

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

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

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

acid anhydrides, such as propane phosphonic acid anhydride,

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

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

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

The amino acids of the formula IX, IXA, XI, XIA, XIII, XIIIA, XVI, XVIA, XVII, XVIIA, are known or they can be synthesized according to methods known in the art, they are commercially available, and/or they can be synthesized in analogy to methods known in the art.

Also the remaining starting materials, e.g. the acid of the formula XV or XVIII or XVIIIA, are known or they can be synthesized according to methods known in the art, they are

commercially available, and/or they can be synthesized in analogy to methods described in the Examples.

For example, the synthon of the formula VII can be prepared as described in Example 1 (which is a specific embodiment of the invention) or in analogy thereto, or as described in Tetrahedron 61, 1459-1480 (2005).

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

Where no specific temperature ranges are given for the reactions shown above and in the claims, the temperature is used as customary for the person skilled in the art, e.g. in the range from -20 to +50 °C, such as at about room temperature (e.g. 23 ± 2 °C).

Where no solvents are mentioned for the reactions given above and below, the solvent or solvent mixtures (including eluants for chromatography or the like) useful are selected from those customary in the art, e.g. selected from those used in the Example.

An advantage of the present process is that the starting materials shown in the steps above and below can often be used in stoichiometric or nearly stoichiometric (± 20 % deviation from the stoichiometric amount for one of the used compounds) amounts.

The intermediates can be purified or they can be used directly in subsequent steps, as appropriate and known to the person skilled in the art. The purification of the intermediates and the final product may especially make use of chromatographic methods (e.g. using silica gel and/or reverse phase materials, e.g. silica based reverse phase materials), solvent distribution methods and precipication (including crystallization) methods, or the like, and corresponding methods are known to the person skilled in the art, for example, the methods are in analogy to those described in the Example.

Example

The following Example illustrates the invention without limiting its scope. If not mentioned otherwise, reactions preferably take place at room temperature (about 23 °C).

Abbreviations

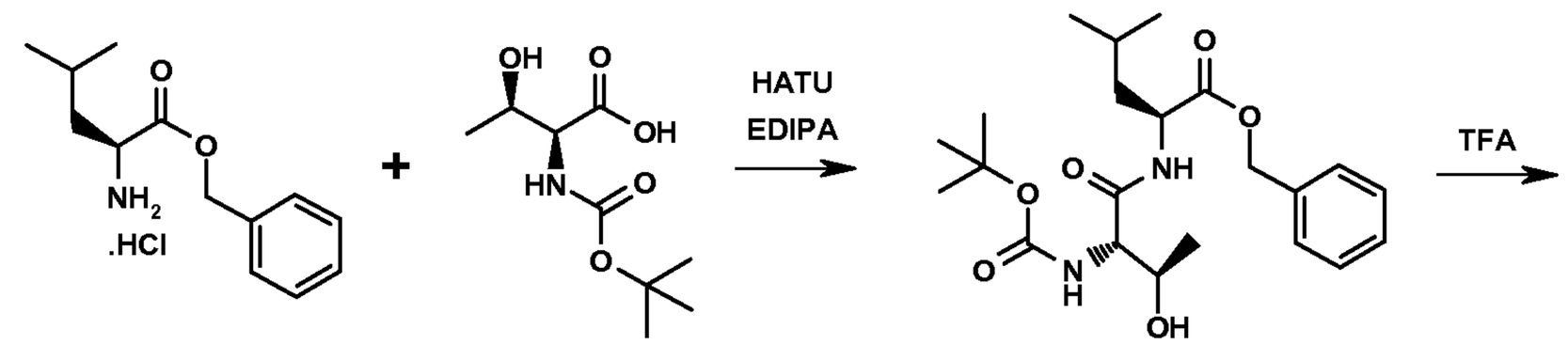
aq.	Aqueous
Alloc	Allyloxycarbonyl
Boc/BOC	tert-Butoxycarbonyl
brine	sodium chloride solution in water (saturated at RT)
Bu	butyl
Bzl or Bz.	benzyl
COMU	1-cyano-2-ethoxy-2-oxoethylideneaminooxy-dimethylamino-morpholino-carbenium hexafluorophosphate
DCM	dichloromethane
DIPEA	N,N-diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
EDC	1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide
EDIPA	Ethyldiisopropylamine
Et	ethyl
Fmoc/FMOC	9-fluorenymethoxycarbonyl
HATU	2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyluronium hexafluorophosphate Methanaminium
HPLC	High Performance Liquid Chromatography
HR-MS	High Resolution Mass Spectroscopy
IBX	1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide
IPC	In-Process Control
IR	Infrared Spectroscopy
IT	internal temperature
Kaiser test	Ninhydrin-based test to monitor deprotection in SPPS (see E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, Analytical Biochemistry 34 595 (1970)); if mentioned to be OK, this means successful deprotection.
Me, Me	methyl
MS	Mass Spectroscopy
MSNT	1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole

NMR	Nuclear Magnetic Resonance Spetroscopy
PPH_3	triphenylphosphine
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
RP	Reversed Phase
RT/rt	room temperature
TBAF	tetrabutylammoniumfluoride
TBDPS	tert-butyl-diphenyl-silyl
TFA	trifluoroacetic acid
Trityl	triphenylmethyl

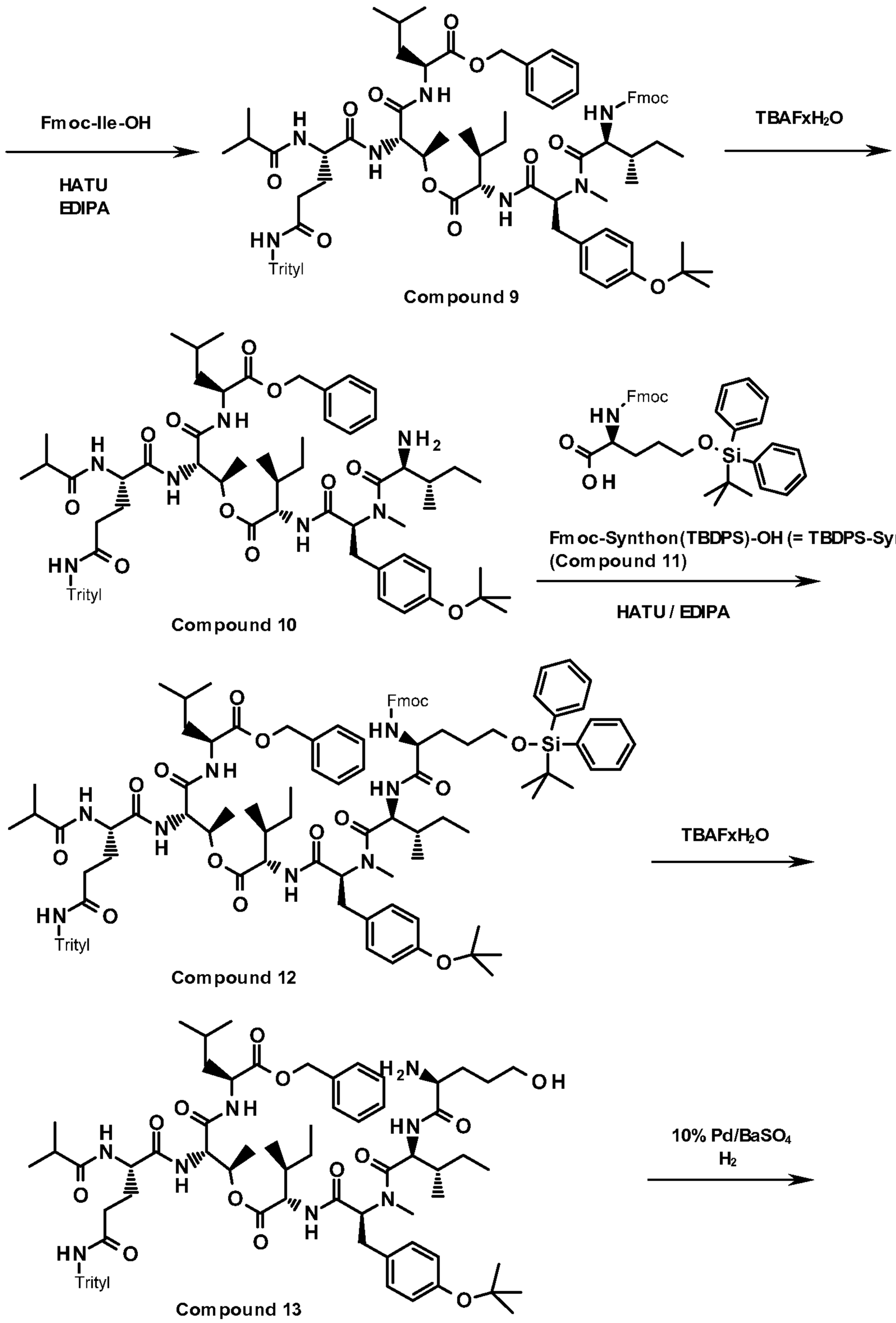
The following Example illustrates the invention without affecting its scope, though it described a preferred variant:

In the following, the manufacture of Compound A according to the invention is first described in terms of reaction schemes and short descriptions. Details are then added in an Experimental part.

Scheme 1:


NH2
.HCl
OH
HN
Trityl
HATU
EDIPA
TFA
Leucine-benzyl ester hydrochloride
N-Boc-Threonine
Compound 1
Compound 2
Compound 3
Compound 4
Fmoc-Ile-OH
EDC.HCl
DMAP
TBAFxH2O
fmoc
Fmoc-Tyr(tBu)Me-OH
Compound 5
Compound 6
Compound 7
Compound 8

Scheme 1 continued:



Compound 9

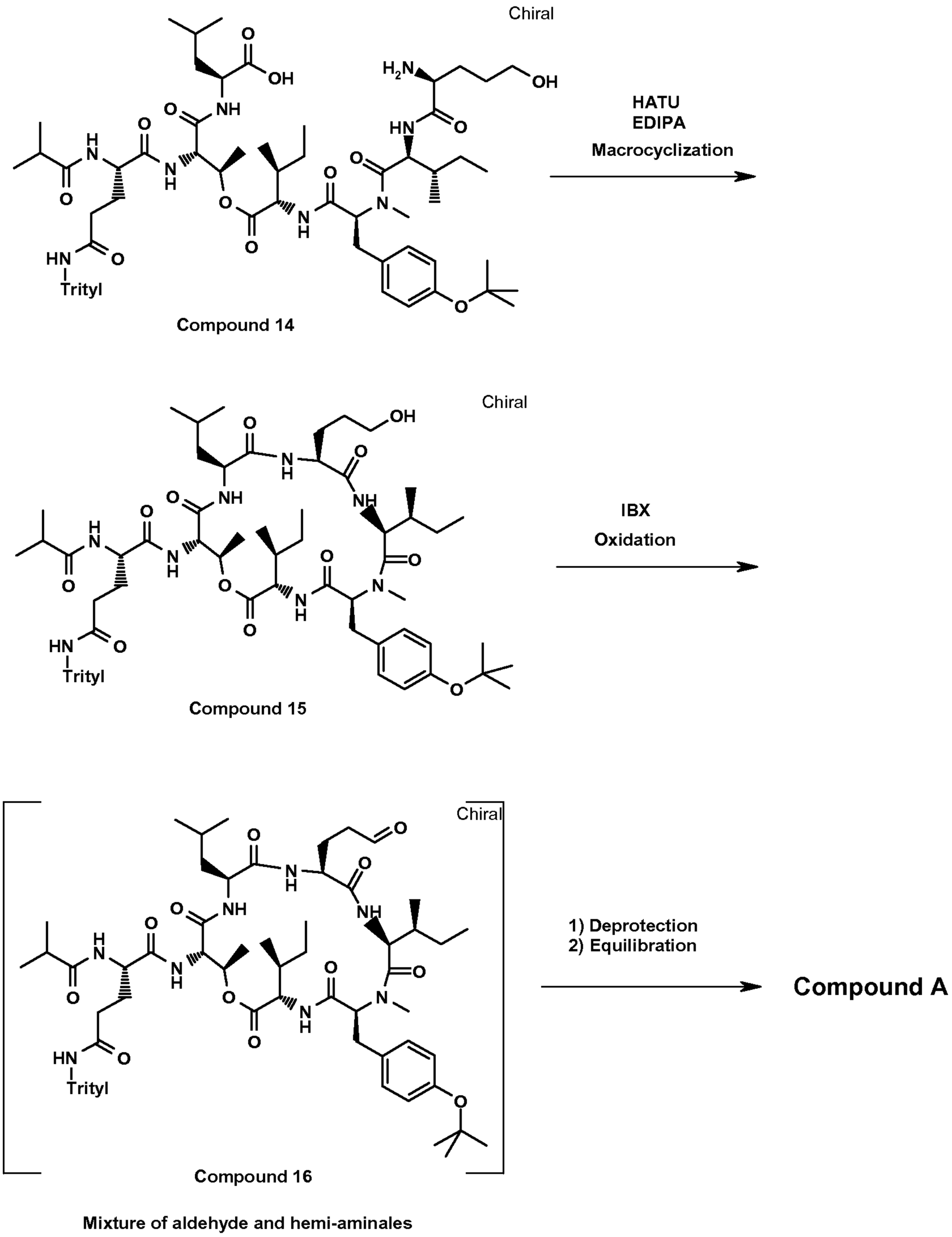
Compound 10

Compound 11

Compound 12

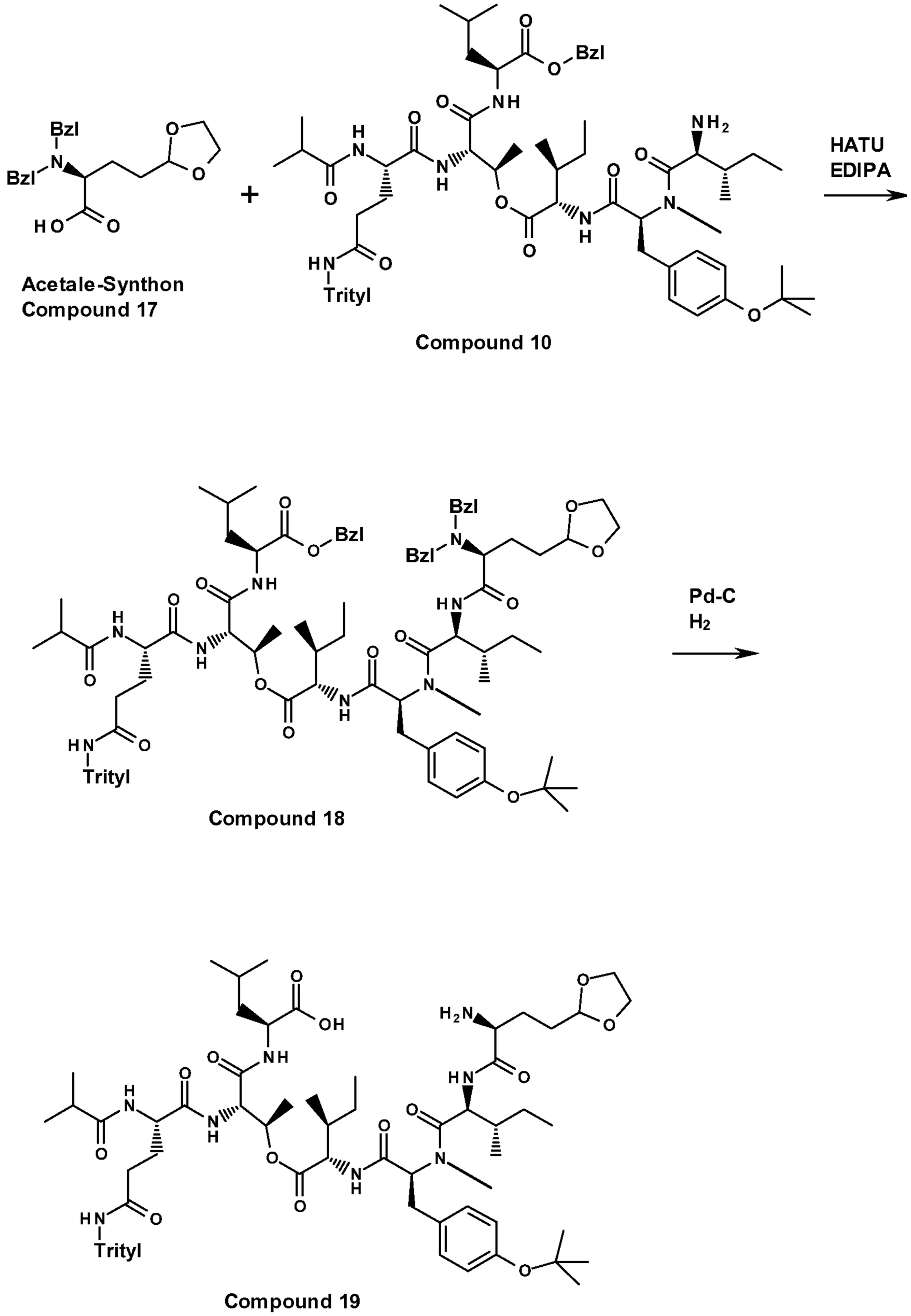
Compound 13

Scheme 1 (continued)

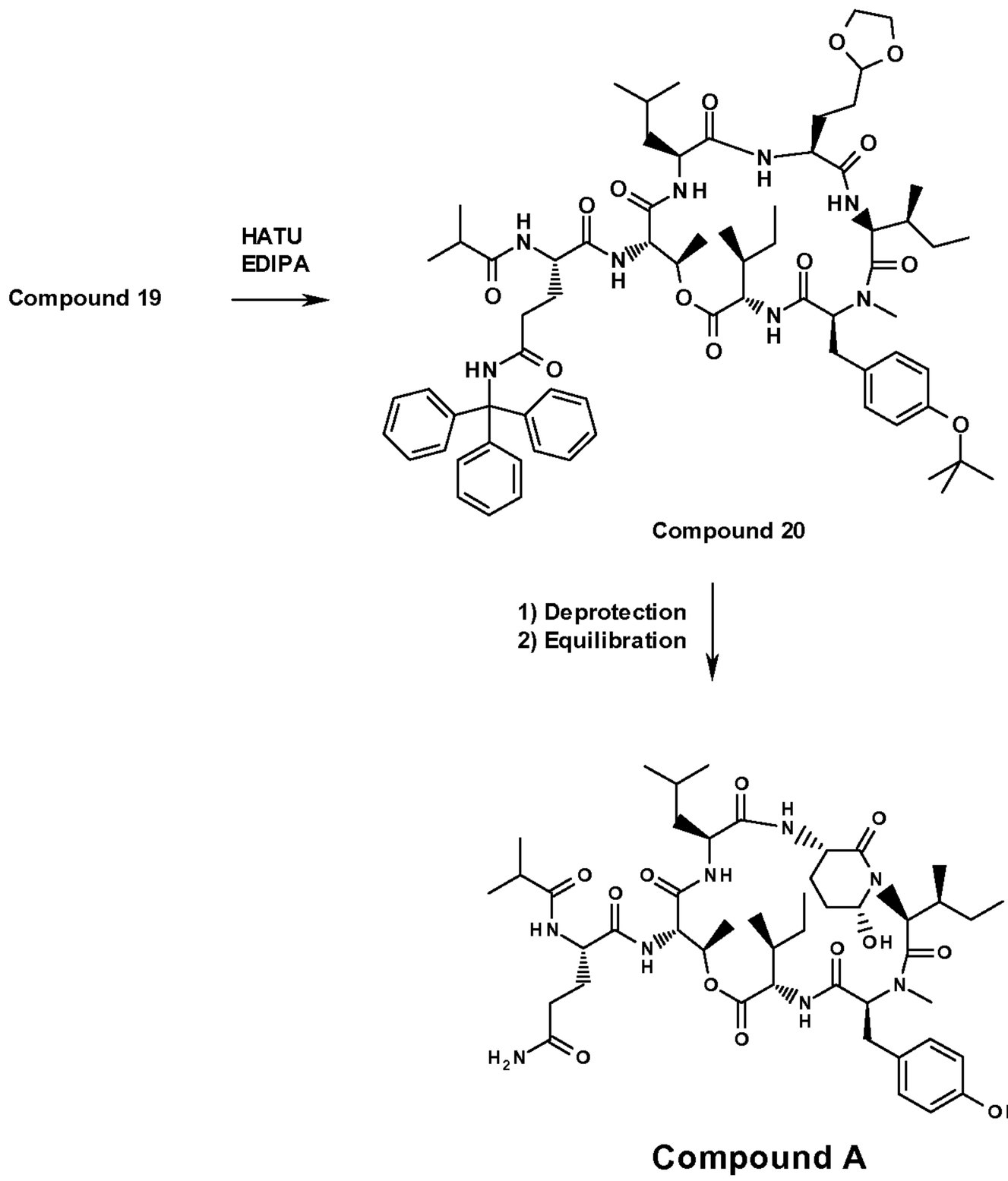


Alternatively, instead of using Synthon Compound 11 above, an Acetale-synthon (Compound 17 in the following scheme) can be used:

Scheme 2:


Bzl
Bzl
HO
HATU
EDIPA
Acetale-Synthon
Compound 17
Trityl
Compound 10
Pd-C
H2
Trityl
Compound 18
Trityl
Compound 19

Scheme 2 (continued)



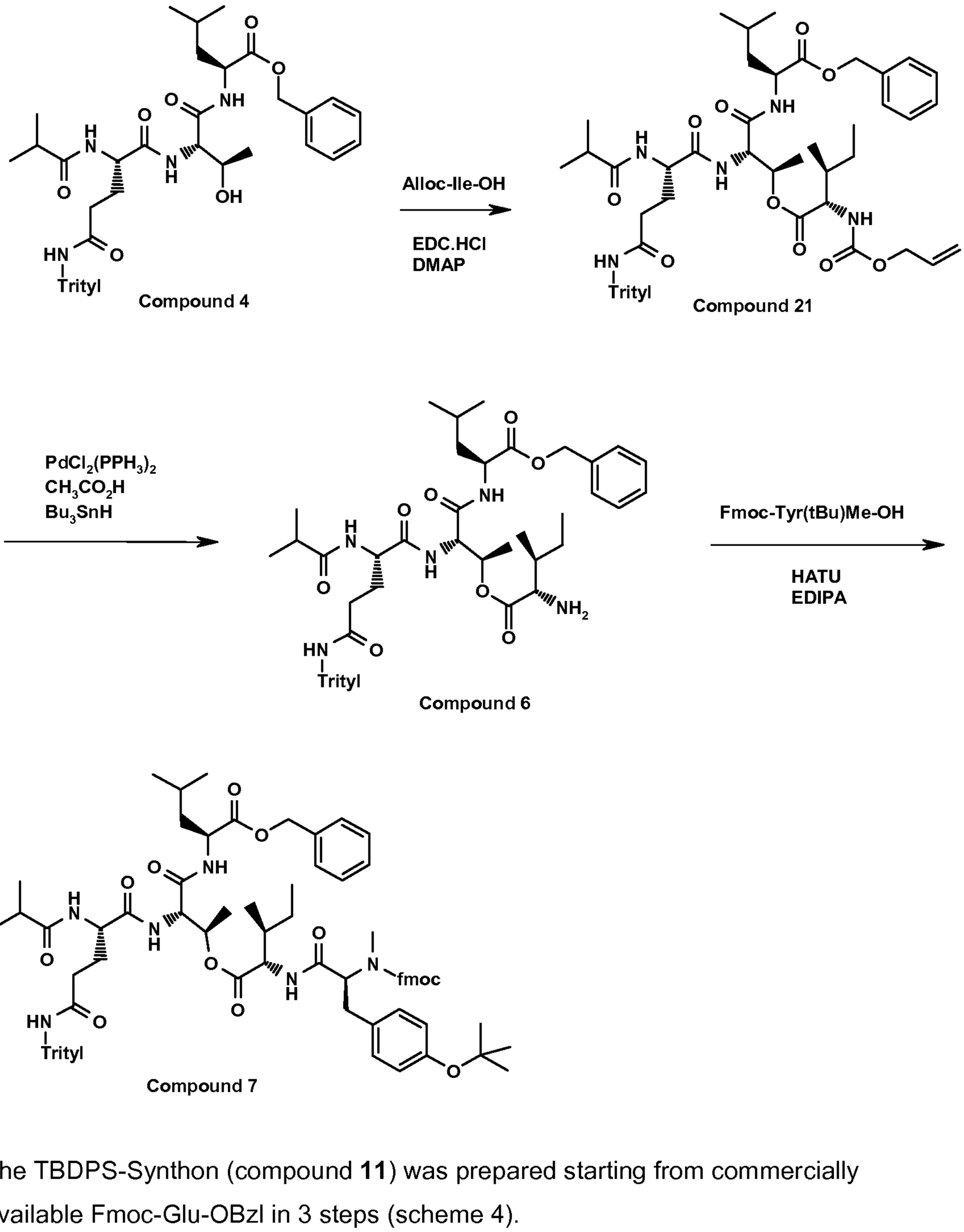
The protected aldehyde synthon (Acetale-synthon, compound **17**) was coupled with compound **10** (scheme 2) using standard coupling conditions (HATU/EDIPA) to obtain compound **18** in 91% yield. Hydrogenation of compound **18** with palladium on charcoal removed all benzyl protecting groups in one step and gave compound **19** in 94% crude yield. Macrolactamization of the crude compound **19** using HATU as activation reagent and DMAP as tertiary base gave the macrocyclic compound **20** in 64% yield and ca. 98 a% purity after purification by flash chromatography on silica gel (scheme 3). Finally, de-protection/equilibration of compound 20 and purification of the product by silica gel chromatography delivered the desired Compound A in 75% yield and 97 a% purity. The product was characterized by NMR, HR-MS and IR. The spectra confirmed the structure and were identical to the

spectra of the natural product and the product from the previous solution synthesis using the TBDPS-Synthon (Compound 11).

It is noteworthy to mention that the reactions described here were not optimized. For example, the macrocyclization was slightly improved using compound **19** from SPPS and the product, compound **20**, was obtained in 81% yield. Therefore, there is a high potential for improvements in this approach.

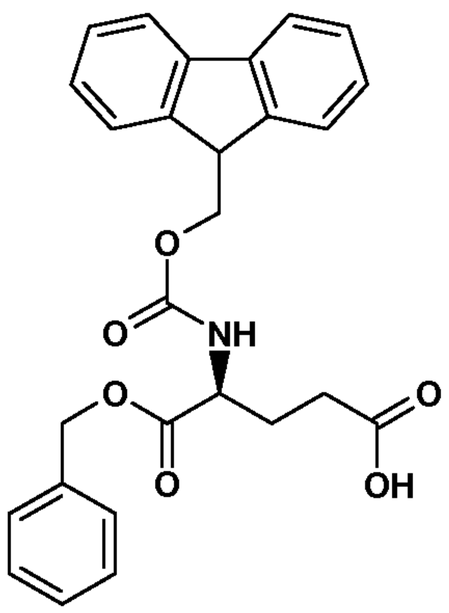
Although the Fmoc-protecting group proved to be efficient for the solution phase synthesis of Compound A, other protecting groups might also be used as appropriate. For example, compound **4** was esterified with Alloc-Ile-OH to obtain compound **21** (scheme 3). Palladium catalysed cleavage of the alloc-protecting group and subsequent coupling with Fmoc-N-methyl-tyrosine gave compound **7** in 85% yield over 2 steps.

Scheme 3:

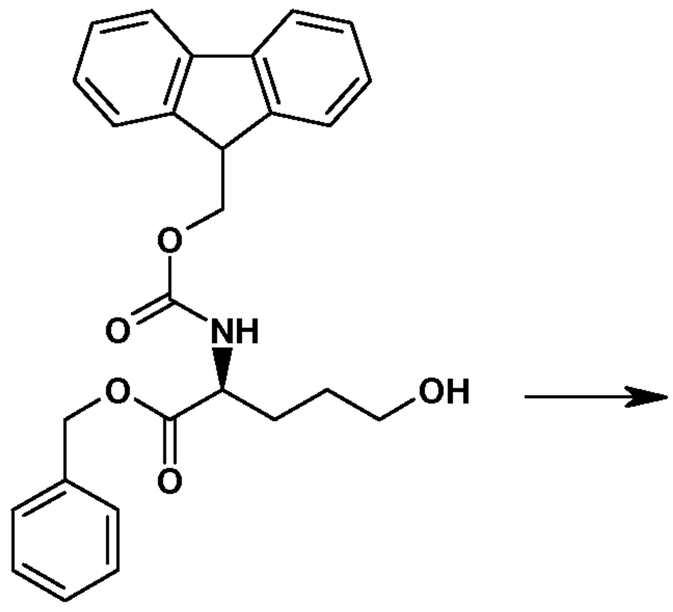


The TBDPS-Synthon (compound **11**) was prepared starting from commercially available Fmoc-Glu-OBzl in 3 steps (scheme 4).

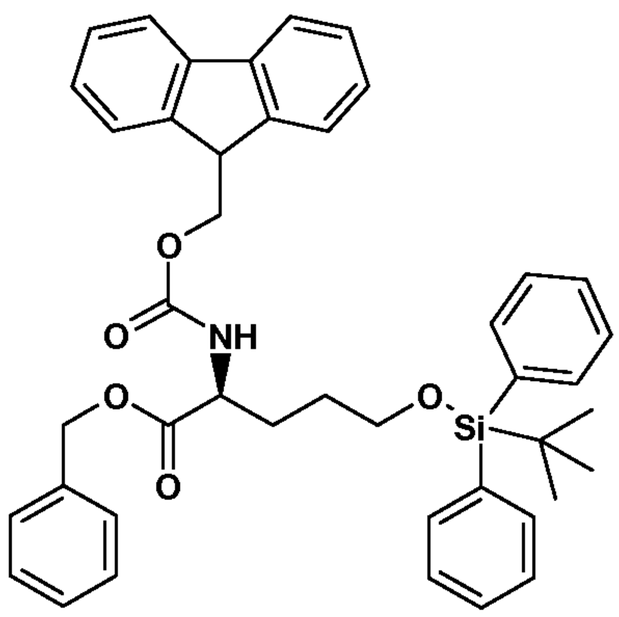
Scheme 4:



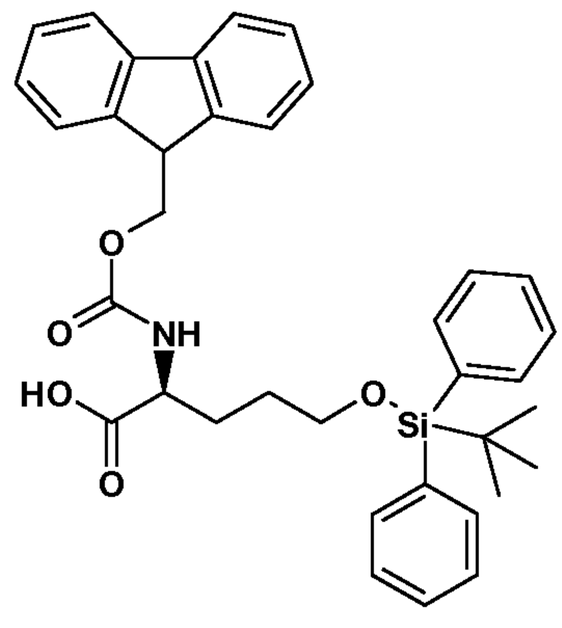
Fmoc-Glu-OBzl (Commercial)



Compound 22



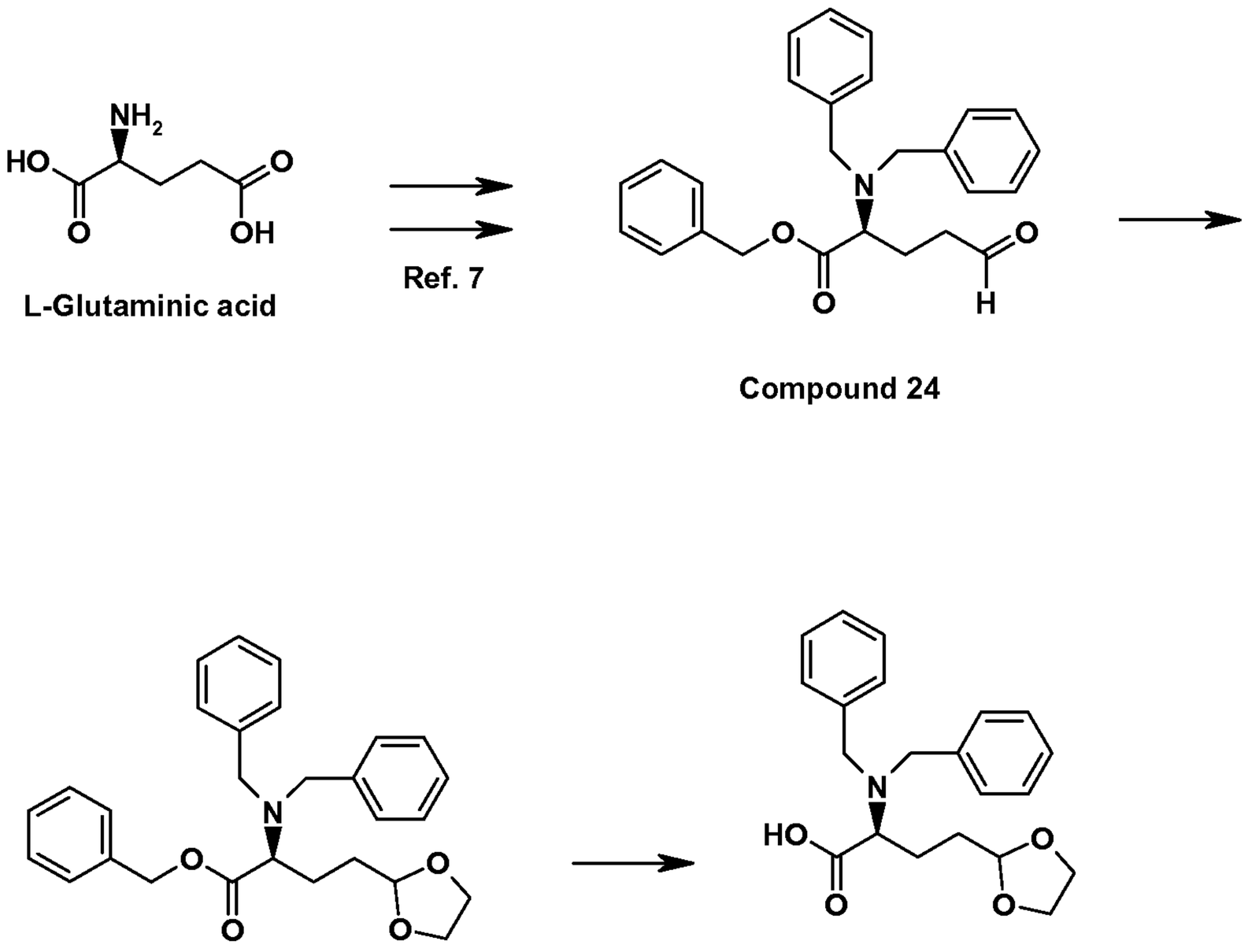
Compound 23



TBDPS-Synthon (Compound 11)

The Acetale-Synthon (compound **17**) was synthesized starting from compound **24** (Scheme 5), which can be prepared from L-Glutaminic acid according to a literature procedure described in: M. Rodriguez, M. Taddei, Synthesis 2005, 3, 493-495.

Scheme 5



L-Glutaminic acid

Compound 24

Compound 25

Acetale-synthon (Compound 17)

Experimental

Synthesis of BOC-Thr-Leu-OBzl (Compound 1)

N-Boc-Thr-OH (15.0g, 68.4 mmol) was dissolved in acetonitrile (90 mL). HATU (26.0g, 68.4 mmol) was added in several portions and the reaction mixture was stirred for 10 min at room temperature. To this mixture, a solution of H-Leu-OBz.HCl (17.6g, 68.4 mmol) and N,N-Diisopropyl-ethylamine (17.7g, 136.8 mmol) in acetonitrile (90 mL) was added within 15 min. The resulting reaction mixture was stirred at room temperature for 90 minutes, after which time the cloudy solution was clear filtered. The solvent was partly evaporated to obtain 78.5g of a highly viscous crude product. The crude product was purified by flash-chromatography on silica gel with ethyl acetate/hexanes (1:1 v/v) as mobile phase to obtain 78.53g product (compound **1**). Yield: 96.1%

^{1}H-NMR and IR confirmed the proposed structure.

HR-MS: Calculated for $C_{22}H_{34}N_2O_6$ $(M+H)^+$: 423.24896; $(M+Na)^+$: 445.23091. Found $(M+H)^+$: 423.24884; $(M+Na)^+$: 445.23083.

Synthesis of Thr-Leu-OBzl (Compound 2)

Compound **1** from previous step (27.5g, 65.084 mmol) was dissolved in dichloromethane (215mL) and the solution was treated with trifluoroacetic acid (149.9g) which was added within 5 minutes. The reaction mixture was stirred for additional 25 minutes at room temperature to complete the reaction. For work-up, the solution was diluted with dichloromethane (300 mL) and was treated slowly with half saturated aq. Na_2CO_3-solution (660 mL). The biphasic mixture was intensively stirred for 15 minutes and the phases were separated. The aqueous phase was extracted with dichloromethane (300 mL) and the organic phases were combined. The combined organic phase was washed with water (300 mL) and the solvent was evaporated under reduced pressure at 40-45 °C to obtain 21.72g crude product, Thr-Leu-OBzl (100% crude yield) as foam. The crude product (compound **2**) was sufficiently pure to use for the next step without additional purification.

^{1}H-NMR and IR confirmed the proposed structure.

HR-MS: Calculated for $C_{17}H_{26}N_2O_4$ $(M+H)^+$: 323.19653; $(M+Na)^+$: 345.17848. Found $(M+H)^+$: 323.19656; $(M+Na)^+$: 345.17834.

Synthesis of Isobutyryl-Gln(Trt)-OH (Compound 3)

Trityl-linker-resin (78g, prepared from Aminomethyl-polystyrene resin crosslinked with 1% divinyl benzene and 4-(diphenylhydroxymethyl)benzoic acid) was swollen with toluene (300 mL) and acetylchloride (34mL) was added. After stirring for 3h at room temperature, the mixture was sucked off and the procedure was repeated. The linker-resin was then washed sequentially five times with toluene and with dichloromethane. A mixture of Fmoc-Gln(Trt)-OH (97.6g) and N-Methylmorpholine (15 mL) in dichloromethane (200 mL) was added to the linker-resin and the mixture was stirred over night. Then the solvent was removed by filtration and the resin was washed with several portions of dichloromethane and dimethylformamide. For the cleavage of the Fmoc-protecting group, a solution of piperidine (300 mL of a 20% solution in dimethylformamide) was added and the mixture was stirred for 10 min at room temperature. The resin was isolated by filtration and the treatment with

piperidine was repeated. The resin was washed with dimethylformamide and isopropanol and finally with dimethylformamide to prepare for isobutyrylation. PyBop (99.0g) was dissolved in dimethylformamide (300 mL) and isobutyric acid (17.6 mL) was added, followed by the addition of EDIPA (67 mL). The mixture was stirred for 5 min and was added to the resin. The mixture was stirred over night at room temperature. The resin was isolated by filtration, washed subsequentially with dimethylformamide and dichloromethane and was dried in vacuo for 3h. The dry resin was suspended in a mixture of acetic acid (475 mL) and water (25 mL) and the mixture was stirred for 4h at room temperature. The resin was isolated by filtration and the product containing filtrate was stored. The resin was added again to a mixture of acetic acid (475 mL) and water (25 mL) and the mixture was stirred over night at room temperature. After filtration, the combined filtrates were partially evaporated to a final volume of ca. 200 mL and water (600 mL) is slowly added. The product precipitated. The mixture was stirred for additional 20 min at room temperature and the product was isolated by filtration. The filtercake was washed with water (100 mL) in several portions and dried overnight at 10 mbar and room temperature to obtain 26.82g of compound **3**. Note the compound can also be prepared using alternative ways with pure solution phase synthesis.

Amino-acid analysis revealed the presence of less than 0.2% D-Enantiomer.

HR-MS: Calculated for $C_{28}H_{30}N_2O_4$ $(M+H)^+$: 459.2284; Found $(M+H)^+$: 459.2285

Synthesis of Isobutyryl-Gln(Trt)-Thr-Leu-OBzl (Compound 4)

Thr-Leu-OBzl (compound **2**) from previous step (13.36g, 41.44 mmol) was dissolved in dichloromethane (380 mL) and the solution was cooled to 0 °C. In a second 4-necked round bottomed flask, N-Isobutyryl-Gln(Trt)-OH (**Compound 3);** 19.0g, 41.43 mmol) was dissolved in dichloromethane (380 mL) and the solution was cooled to 0 °C. EDIPA (5.06g, 41.43 mmol) and HATU (15.91g, 41.425 mmol) were added at 0 °C under intense stirring and the formed cloudy suspension was added to the cooled solution of Thr-Leu-OBzl maintaining the temperature at 0 °C. The reaction mixture was stirred for additional 90 min at 0 °C to complete the conversion. For work-up, the reaction mixture was extracted sequentially with half-saturated aq. $NaHCO_3$-solution (650 mL), 1N HCl (650 mL), half-saturated aq. $NaHCO_3$-solution (650 mL) and half-saturated NaCl-solution (650 mL). The aqueous phases were extracted with dichloromethane (250 mL) and the dichloromethane-phases

were combined. The dichloromethane solution was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain 29.2g crude product. The crude product was purified by flash chromatography on silica gel and crystallized from dichloromethane/hexanes to obtain 23.44g of Isobutyryl-Gln(Trt)-Thr-Leu-OBzl (compound **4**). Yield: 74.1%.
^{1}H-NMR confirmed the proposed structure.
LC-MS: $(M+H)^+$:763; $(M-H)^-$:761.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-OBzl (Compound 5)

Isobutyryl-Gln(Trt)-Thr-Leu-OBzl (Compound **4**) (10.0g, 13.107 mmol), Fmoc-Ile-OH (6.95g, 19.66 mmol) and DMAP (0.24g, 1.97 mmol) were dissolved in dichloromethane (300 mL) and the solution was cooled to 0 °C. EDC.HCl (3.85g t.q, 3.769g 100%, 19.66 mmol) was added to the solution at 0 °C and the temperature was allowed to rise to room temperature. The reaction mixture was stirred for 4.5h at room temperature, after which time the reaction mixture was poured onto water (300 mL). The mixture was intensively stirred and the phases were separated. The organic phase was extracted sequentially with 1N HCl (300 mL), aq. saturated $NaHCO_3$ (300 mL) and brine (300 mL). The aqueous phases were extracted with dichloromethane (100 mL) and the organic layers were combined. The organic phase was dried on anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain 21.65g crude product. Purification by flash chromatography on silica gel gave 11.22g of Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-OBzl (compound **5**). Yield: 77.7%. Additional 2.76g product (19.2% yield; 89.3 area% HPLC-purity) was isolated from side fractions.
^{1}H-NMR confirmed the proposed structure.
HR-MS: Calculated for $C_{66}H_{75}N_5O_{10}$ $(M+H)^+$: 1098.55867; $(M+NH_4)^+$: 1115.58522; $(M+Na)^+$: 1120.54062. Found $(M+H)^+$: 1098.55865; $(M+NH_4)^+$: 1115.58498; $(M+Na)^+$: 1120.53988.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-H)-Leu-OBzl (Compound 6)

Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-OBzl (compound **5**; 10.0g, 9.11 mmol) was dissolved in DMF (100 mL) and the solution was treated with TBAFx3H_2O (5.93g t.q., 5.75g 100%, 18.22 mmol). The reaction mixture was stirred for 1h at room temperature and water (300 mL) was added. The product was extracted with iso-

propyl acetate (800 mL) and the isopropyl acetate phase was washed sequentially with aq. saturated $NaHCO_3$ (2x400 mL) and water (400 mL). The aqueous phases were extracted with isopropyl acetate (400 mL) and the organic phases were combined. The organic phase was dried on anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain 9.31g crude product. Purification of the crude product by flash chromatography on silica gel provided 7.45g of Isobutyryl-Gln(Trt)-Thr(Ile-H)-Leu-OBzl (compound **6**). Yield: 93.4%.

^{1}H-NMR confirmed the proposed structure.

HR-MS: Calculated for $C_{51}H_{65}N_5O_8$ $(M+H)^+$: 876.49059; $(M+Na)^+$: 898.47254. Found $(M+H)^+$: 876.49036; $(M+Na)^+$: 898.47211.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-OBzl (Compound 7)

N-Fmoc-N-Methyl-Tyrosin-*t*-butylether (4.03g, 8.51 mmol) was dissolved in dichloromethane (100 mL) and the solution was cooled to 0°C. EDIPA (2.2g, 17.02 mmol) was added, followed by the addition of HATU (3.268g t.q., 3.236g 100%, 8.51 mmol). To this solution, a pre-cooled solution of isobutyryl-Gln(Trt)-Thr(Ile-H)-Leu-OBzl (compound **6**; 7.45g, 8.51 mmol) in dichloromethane (200mL) was added and the reaction mixture was stirred at 0°C for 2h15min, after which time an IPC indicated complete conversion. The reaction mixture was then diluted with dichloromethane (200 mL) and the dichloromethane solution was extracted sequentially with half saturated aq. $NaHCO_3$ (400 mL), 1N HCl (400 mL), half saturated aq. $NaHCO_3$ and half saturated aq. NaCl solutions. The water phases were extracted with dichloromethane (200 mL) and the organic phases were combined. Drying on anhydrous magnesium sulfate and evaporation of the solvent gave 12.29g crude Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-OBzl (compound **7**) as a colorless foam, which was used for the next step without purification assuming quantitative yield.

^{1}H-NMR of a purified analytical sample confirmed the proposed structure.

LC-MS: $(M+H)^+$: 1331.7; $(M+NH_4)^+$: 1348.7.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-OBzl (Compound 8)

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Obzl (12.29g crude compound **7** from previous step; 8.51 mmol) was dissolved in DMF (113 mL) and the solution was treated with TBAFx3H_2O (5.53g, 17.01 mmol). The reaction mixture was stirred for 2h at RT to complete the conversion. The reaction mixture was then diluted with water (400 mL) and the product was extracted with isopropylacetate (800 mL). The isopropylacetate-phase was washed sequentially with half saturated aq. $NaHCO_3$ (2x400 mL) and water (400 mL). The aqueous phases were extracted with isopropylacetate (400 mL) and the organic phases were combined. The organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain 11.19g crude product. Purification of the crude product by flash-chromatography on silica gel provided 9.74g of isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-OBzl (compound **8**) as colourless foam. The product comprised low amounts of solvent. Quantitative yield is assumed also for this step.

^{1}H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{65}H_{84}N_6O_{10}$ $(M+H)^+$: 1109.63217; $(M+Na)^+$: 1131.61412.
Found $(M+H)^+$: 1109.63230; $(M+Na)^+$: 1131.61318.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Obzl (Compound 9)

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-H)-Leu-OBzl from previous step (9.73g, 96.8 a% HPLC purity corresponding to 9.42g pure compound **8**; 8.49 mmol) was dissolved in dichloromethane (118 mL). Fmoc-Ile-OH (6.01g, 17.0 mmol) was added followed by the addition of EDIPA (4.394g, 34 mmol). The solution was treated with HATU (6.53g, 17 mmol) and was stirred for 18.5h, after which time a second portion of HATU (1.63g, 4.2 mmol) was added. The reaction mixture was stirred for additional 2h and was diluted with dichloromethane (120 mL). The dilute solution was sequentially extracted with saturated aq. $NaHCO_3$ (240 mL), 1M HCl (240 mL), saturated aq. $NaHCO_3$ (240 mL) and brine (240 mL). The aqueous phases were extracted with dichloromethane (100 mL) and the dichloromethane phases were combined. The combined organic phase was dried on anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain 19.6g of a crude product as a foam. The crude product was purified by chromatography on silica gel

using ethyl acetate/hexanes (6:4) as eluent to obtain Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-OBzl (compound **9**) as a foam. Yield: 11.01g (89.6%).

^{1}H-NMR confirmed the proposed structure.

HR-MS: Calculated for $C_{86}H_{105}N_7O_{13}$ $(M+H)^+$: 1444.78431; $(M+NH_4)^+$: 1461.81086.

Found $(M+H)^+$: 1444.78503; $(M+NH_4)^+$: 1461.81055.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-OBzl (Compound 10)

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-OBzl (compound **9**) from the previous step (11.01g, 7.626 mmol) was dissolved in DMF (110 mL) and TBAFx3H_2O (4.81g, 15.25 mmol) was added. The mixture was stirred for 1.5h and demineralized water (300 mL) was added, followed by the addition of isopropylacetate (600 mL). The mixture was stirred for 10 min and the phases were separated. The organic phase was washed sequentially with half saturated aq. $NaHCO_3$-solution (2x300 mL) and demineralized water (300 mL). The aqueous phases were extracted with isopropylacetate (300 mL) and the organic layers were combined. The combined organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain 15.15g of a solid, which was purified by filtration over silica gel using dichloromethane/methanol (100/0 to 95/5) as eluent. Yield: 8.55g (91.8%) of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-OBzl (compound **10**).

^{1}H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{71}H_{95}N_7O_{11}$ $(M+H)^+$: 1222.71623; $(M+Na)^+$: 1244.69818.

Found $(M+H)^+$: 1222.71624; $(M+Na)^+$: 1244.69743.

Synthesis of (2*S*)-5-{[*tert*-butyl(diphenyl)silyl]oxy}-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}pentanoic acid (= Compound 11 = TBDPS-Synthon)

Compound **23** (for synthesis see further below) (66g, 96.6 mmol) was suspended in ethanol/isopropylalcohol/water (89:5:6; 3000 mL) and the suspension was heated to IT 45 °C to obtain a solution. The solution was cooled down to IT 30 °C. After inertization with Argon, palladium-catalyst (10% on barium sulfate; 6.6g) was added to the solution under an argon stream. The product was then hydrogenated under a hydrogen pressure slightly above the atmospheric pressure at 30-35 °C. The hydro-

genation was completed after 1.5h according to HPLC. The reaction mixture was filtered over a cellulose based filter aid (Cellflock 40; cellulose based filtering aid) and the filter aid was washed with ethanol/isopropanol/water (89:5:6; 600 mL). Evaporation of the solvent under reduced pressure at 45-50 °C gave 59.58g foam as crude product. The crude product was purified by chromatography on silica gel in 2 portions (2x1 kg silica gel 60) using dichloromethane/methanol 95:5 to 80:20 as mobile phase. Yield: 52g (90.6%) TBDPS-Synthon (compound **11**).

^{1}H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{36}H_{39}NO_5Si$ $[M+H]^+$: 594.26703; $[M+NH4]^+$: 611.29358; $[M+Na]^+$: 616.24897; Found: $[M+H]^+$: 594.26743; $[M+NH4]^+$: 611.29385; $[M+Na]^+$: 616.24900.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon(TBDPS)-fmoc)-Leu-OBzl (Compound 12) = benzyl *N*^{2}-(2-methylpropanoyl)-*N*^{5}-(triphenylmethyl)-L-glutaminyl-*O*-{*N*-[(2*S*)-5-[(*tert*-butyldiphenylsilyl)oxy]-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}pentanoyl]-L-isoleucyl-*O*-(*tert*-butyl)-*N*-methyl-L-tyrosyl-L-isoleucyl}-L-threonyl-L-leucinate

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-H)-Leu-OBzl (compound **10**) from the previous step (8.55g, 6.99 mmol) was dissolved in dichloromethane (170 mL) and TBDPS-Synthon (compound **11**; 4.98g, 8.39 mmol) was added at room temperature. EDIPA (2.17g, 16.78 mmol) and HATU (3.19g, 8.39 mmol) were added and the reaction mixture was stirred for 1.5h at room temperature. For work-up, the reaction mixture was diluted with dichloromethane (200 mL) and the dilute solution was extracted sequentially with aq. $NaHCO_3$ (400 mL), 1M HCl (400 mL), aq. $NaHCO_3$ (400 mL) and NaCl-solution (400 mL). The aqueous phases were extracted with dichloromethane (200 mL) and the dichloromethane phases were combined. The dichloromethane solution was dried over magnesium sulfate and the solvent was evaporated at reduced pressure to obtain 14.76g of a foam as crude product. Purification of the crude product by flash chromatography on silica gel using ethyl acetate/hexanes as eluent gave the desired Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon(TBDPS)-fmoc)-Leu-OBzl (compound **12**). Yield: 12.46g (99%).

^{1}H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{107}H_{132}N_8O_{15}Si$ $(M+H)^+$: 1797.96542; $(M+NH_4)^+$: 1814.99197; $(M+Na)^+$: 1819.94737. Found $(M+H)^+$: 1797.96565; $(M+NH_4)^+$: 1814.99245; $(M+Na)^+$: 1819.94629.

Synthesis of *N*^{2}-(2-methylpropanoyl)-*N*^{5}-(triphenylmethyl)-L-glutaminyl-*O*-{*N*-[(2*S*)-2-amino-5-hydroxypentanoyl]-L-isoleucyl-*O*-(*tert*-butyl)-*N*-methyl-L-tyrosyl-L-isoleucyl}-L-threonyl-L-leucinate
(Compound 13)

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Synthon(TBDPS)-fmoc)-Leu-OBzl (compound **12**) from the step described above (12.46g, 6.93 mmol) was dissolved in DMF (125 mL) and TBAFx3H_2O (6.56g, 20.79 mmol) was added. The reaction mixture was stirred for 3h at room temperature and was diluted with demineralized water (350 mL). The aqueous mixture was extracted with isopropyl acetate (700 mL) and the organic phase was washed sequentially with aq. $NaHCO_3$ (2x350 mL) and demineralized water (350 mL). The aqueos phases were extracted with isopropyl acetate (350 mL) and the organic phases were combined. The organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The crude product (12.65g) was purified by column chromatography on silica gel using dichloromethane/methanol (95:5 to 90:10) as eluent to obtain compound **13**. Yield: 8.34g (90%).

^{1}H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{76}H_{104}N_8O_{13}$ $(M+H)^+$: 1337.77956; $(M+Na)^+$: 1359.76151. Found $(M+H)^+$: 1337.77942; $(M+Na)^+$: 1359.76147.

Synthesis of *N*^{2}-(2-methylpropanoyl)-*N*^{5}-(triphenylmethyl)-L-glutaminyl-*O*-{*N*-[(2*S*)-2-amino-5-hydroxypentanoyl]-L-isoleucyl-*O*-(*tert*-butyl)-*N*-methyl-L-tyrosyl-L-isoleucyl}-L-threonyl-L-leucine
(Compound 14)

Compound **13** (8.34g, 6.23 mmol) from the previous step was dissolved in ethanol/water (95:5; 85mL) and 10% palladium on barium sulfate (1.67g) was added. The suspension was stirred for 75 min under a hydrogen atmosphere at room temperature. For work-up, the reaction flask was purged with nitrogen and the suspension was filtered over a cellflock-layer. The filter residue was washed in several portions with ethanol/water (95:5; 400 mL). The filtrate was concentrated

under reduced pressure and the crude product (8.6g) was purified by flash-chromatography on silica gel using dichloromethane/methanol (9:1 to 8:2) as eluent to obtain compound **14**. Yield: 7.6g (97.7%).

^{1}H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{69}H_{98}N_8O_{13}$ $(M+H)^+$: 1247.73261; $(M+Na)^+$: 1269.71456. Found $(M+H)^+$: 1247.73281; $(M+Na)^+$: 1269.71433.

Synthesis of (2*S*)-*N*-[(3*S*,6*S*,9S,12*S*,15*S*,18*S*,19*R*)-3,9-di[(2*S*)-butan-2-yl]-6-{[4-(*tert*-butoxy)phenyl]methyl}-12-(3-hydroxypropyl)-7,19-dimethyl-15-(2-methylpropyl)-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl]-2-(2-methylpropanamido)-*N'*-(triphenylmethyl)pentanediamide

(Compound 15)

Compond **14** from the previous step (7.0g, 5.61 mmol) was dissolved in acetonitrile (350 mL) and EDIPA (0.87g) was added. A solution was formed. This solution was added within 15 min to a solution of HATU (2.56g, 6.73 mmol) and EDIPA (0.87g) in acetonitrile (350 mL). The reaction mixture was stirred for additional 15 min at room temperature to complete the reaction. The reaction mixture was then treated with brine (1.4L) and isopropyl acetate (1.4L). The phases were separated and the organic phase was washed again with brine (1.4L). The layers were separated, the organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The crude product (8.95g) was purified by flash-chromatography on silica gel using ethyl acetate/methanol (95:5) as eluent to obtain compound **15**. Yield: 6.58g (95.4%).

^{1}H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{69}H_{96}N_8O_{12}$ $(M+H)^+$: 1229.72205; $(M+NH_4)^+$: 1246.74860; $(M+Na)^+$: 1251.70399. Found $(M+H)^+$: 1229.72205; $(M+NH_4)^+$: 1246.74756; $(M+Na)^+$: 1251.70325.

Synthesis of (2*S*)-*N*-[(3*S*,6*S*,9S,12*S*,15*S*,18*S*,19*R*)-3,9-di[(2*S*)-butan-2-yl]-6-{[4-(*tert*-butoxy)phenyl]methyl}-7,19-dimethyl-15-(2-methylpropyl)-2,5,8,11,14,17-hexaoxo-12-(3-oxopropyl)-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl]-2-(2-methylpropanamido)-*N'*-(triphenylmethyl)pentanediamide

(Compound 16)

Compound **15** (5.5g, 4.47 mmol) from the previous step was dissolved in tetra-hydrofuran (600 mL) and DMSO (200 mL) was added. This solution was treated with IBX (11.13g of a 45 %g/g sample, corresponding to 5.01g 100% IBX, 17.89 mmol). The reaction mixture was stirred for 4h at room temperature and aq. $NaHCO_3$ (1250 mL) was added, followed by the addition of dichloromethane (650 mL). The layers were separated and the aequos layer was extracted again with dichloromethane (650 mL). The dichloromethane phases were combined and were washed with demineralized water (2x650 mL). The dichloromethane phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The crude product (7.2g) was purified by flash-chromatography on silica gel using ethyl acetate/isopropanol (95:5) as eluent to obtain compound **16**. Yield: 5.136g (93.5%).

The purified compound **16** is a mixture of the desired aldehyde and corresponding 5-ring and 6-ring hemi-aminales.

HR-MS: Calculated for $C_{69}H_{94}N_8O_{12}$ $(M+H)^+$: 1227.70640; $(M+NH_4)^+$: 1244.73295; $(M+Na)^+$: 1249.68834. Found $(M+H)^+$: 1227.70679; $(M+NH_4)^+$: 1244.73315; $(M+Na)^+$: 1249.68762.

Synthesis of (2*S*)-4-(1,3-dioxolan-2-yl)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}butanoic acid (= Acetale-Synthon = Compound 17)

Compound **25** (26.8g; 60.15 mmol) was dissolved in dioxane (250 mL). LiOH (10.1g; 241.05 mmol) and water (150 mL) were added and the mixture was stirred for 72h at room temperature. The formed suspension was treated with water (200 mL) and acetic acid (32g) to obtain 2 clear phases. The biphasic mixture was diluted with ethyl acetate (500 mL) and the phases were separated. The aqueous phase was separated and was extracted with ethyl acetate (300 mL). The organic phases were combined and washed with water (300 mL). Evaporation of the solvent under reduced pressure gave 29g crude product as a viscous liquid. The crude product was purified by flash chromatography on silica gel with dichlorome-thane/isopropanol (9:1) as eluent to obtain 17.3g product comprising ca. 10 mol% isopropanol according to ^{1}H-NMR. Residual isopropanol was removed from the product by dissolving in isopropyl acetate (200 mL) and extraction of the isopropyl acetate solution with water (3x50 mL). Finally, the solvent was removed under

reduced pressure and the product was dried in vacuo at 70 °C to obtain Compound **17** (16g; 74.8% yield).

^{1}H- and ^{13}C-NMR Spectra of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{21}H_{25}NO_4$ $[M+H]^+$: 356.18564; $[M+Na]^+$: 378.16758. Found: $[M+H]^+$: 356.18586; $[M+Na]^+$: 378.16748.

Synthesis of benzyl N^2-(2-methylpropanoyl)-N^5-(triphenylmethyl)-L-glutaminyl-O-{N-[(2S)-2-(dibenzylamino)-4-(1,3-dioxolan-2-yl)butanoyl]-L-isoleucyl-O-(*tert*-butyl)-N-methyl-L-tyrosyl-L-isoleucyl}-L-threonyl-L-leucinate (Compound 18)

Compound **10** (1.83g, 1.497 mmol) was dissolved in dichloromethane (35 mL). To the solution was added Acetale-Synthon (compound **11**) (0.638g, 1.795 mmol), EDIPA (0.464g, 3.590 mmol) and HATU (0.683g, 1.796 mmol) and the reaction mixture was stirred for 75min at room temperature. The reaction mixture was diluted with dichloromethane (40 mL) and was extracted sequentially with aq. NaHCO3 (80 mL), 1M HCl (80 mL), aq. NaHCO3 (80 mL) and half-saturated sole (80 mL). The aqueous phases were extracted with dichloromethane (40 mL) and the dichloromethane phases were combined. The dichloromethane phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. Purification of the crude product (3.23g) by flash-chromatography on silica gel using ethyl acetate/hexanes (6:4 to 7:3) gave compound **18**. Yield: 2.13g (91.2%).

^{1}H-NMR spectrum of the product confirmed the proposed structure.

HR-MS: Calculated for $C_{92}H_{118}N_8O_{14}$ $[M+H]^+$: 1559.88403; $[M+Na]^+$: 1581.86597 Found: $[M+H]^+$: 1559.88318; $[M+Na]^+$: 1581.86572.

Synthesis of *N*^{2}-(2-methylpropanoyl)-*N*^{5}-(triphenylmethyl)-L-glutaminyl-*O*-{*N*-[(2*S*)-2-amino-4-(1,3-dioxolan-2-yl)butanoyl]-L-isoleucyl-*O*-(*tert*-butyl)-*N*-methyl-L-tyrosinyl-L-isoleucyl}-L-threonyl-L-leucine (Compound 19)

Compound **18** (2.10g, 1.282 mmol) was dissolved in isopropanol/water (95:5; 60 mL) and the flask with the solution was inertized with a stream of argon. The solution was treated with 10% Palladium on charcoal/water (1:1) (2.0g). The reactor

was inertialized again with argon and purged with hydrogen. The solution was stirred under an atmospheric hydrogen pressure at 40 °C for 7h 40min. The suspension was then filtered and the filter residue was washed with isopropanol. The solvent was evaporated at 45 °C under reduced pressure to obtain crude compound **19**. Crude yield: 1.55g (93.8%).

Crude compound **19** was converted into compound **20** without further purification. ^{1}H-NMR spectrum of a purified analytical sample confirmed the proposed structure. HR-MS: Calculated for $C_{71}H_{100}N_8O_{14}$ $[M+H]^+$: 1289.74318; $[M+Na]^+$: 1311.72512. Found: $[M+H]^+$: 1289.74243; $[M+Na]^+$: 1311.72485.

Synthesis of (2*S*)-*N*-[(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,19*R*)-3,9-di[(2*S*)-butan-2-yl]-6-{[4-(*tert*-butoxy)phenyl]methyl}-12-[2-(1,3-dioxolan-2-yl)ethyl]-7,19-dimethyl-15-(2-methylpropyl)-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazacyclononadecan-18-yl]-2-(2-methylpropanamido)-*N*'-(triphenylmethyl)pentanediamide (Compound 20)

4-DMAP (0.284g, 2.325 mmol) was dissolved in dichloromethane (100 mL) and HATU (0.590g, 1.552 mmol) was added. The cloudy suspension was cooled down to 0 °C. To this suspension, a solution of crude compound **19** (1.0g, 0.775 mmol) in dichloromethane (100 mL) was added over 30 min, maintaining the temperature at 0-4 °C. The reaction mixture was stirred for additional 30 min at 0-4 °C. The reaction was the quenched by addition on sole/water (1:1, 200 mL) and the phases were separated. The organic phase was washed again with sole/water (1:1, 200 mL) and the phases were separated. The organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain crude compound **20** (1.31g). Purification of the crude product by column chromatography on silica gel with ethyl acetate as eluent gave compound **20** in 97.7 a% purity according to HPLC. Yield: 0.634g (64.3% over 2 steps).

^{1}H- and ^{13}C-NMR Spectra of the product confirmed the proposed structure. HR-MS: Calculated for $C_{71}H_{98}N_8O_{13}$ $(M+H)^+$: 1271.73261; $(M+NH_4)^+$: 1288.75916; $(M+Na)^+$: 1293.71456. Found $(M+H)^+$: 1271.73267; $(M+NH_4)^+$: 1288.75867; $(M+Na)^+$: 1293.71472.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-alloc)-Leu-OBzl (Compound 21)

To a solution of Alloc-Ile-OH (6.2g, 28.8 mmol) in dichloromethane (300 mL) were added compound **4** (10g, 13.11 mmol) and DMAP (0.16g, 1.3 mmol). The mixture was stirred at room temperature until a solution was formed. EDC.HCl (5.53g, 28.8 mmol) was added in portions to the stirred solution and the reaction mixture was stirred for 24h at room temperature. For work-up, the reaction mixture in dichloromethane was extracted sequentially with demineralized water (300 mL), 1M HCl (300 mL), aq. $NaHCO_3$ (300 mL) and brine (300 mL). The dichloromethane phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain crude compound **21**. Purification of the crude product (17.24g) by flash chromatography on silica gel using dichloromethane/methanol as eluent gave compound **21**. Yield: 11.23g (89.2%).
^{1}H-NMR of the product confirmed the proposed structure. The spectrum also indicated ca. 10% racemization on Alloc-Ile-OH during esterification.

Synthesis of Isobutyryl-Gln(Trt)-Thr(Ile-H)-Leu-OBzl (Compound 6)
Compound **21** (2.0g, 2.083 mmol) was dissolved in dichloromethane and the solution was cooled down to 0 °C. $PdCl_2(PPh_3)_2$ (0.073g, 0.104 mmol) was added to the solution, followed by the addition of acetic acid (0.188 g, 2.083 mmol). Finally, Bu_3SnH (1.213g, 4.166 mmol) was added and the solution was stirred for additional 75 min at 0 °C. The dichloromethane solution was washed sequentially with demineralized water (2x200 mL), aq. $NaHCO_3$ (2x100 mL) and demineralized water (100 mL). The organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain 2.37g of crude compound **6**. An analytical sample was purified by suspending in ethyl acetate/hexanes (1:9) and isolation of the precipitate by filtration.
^{1}H-NMR of the purified product confirmed the proposed structure.
HR-MS: Calculated for $C_{51}H_{65}N_5O_8$ $(M+H)^+$: 876.49059; $(M+Na)^+$: 898.47254. Found $(M+H)^+$: 876.49048; $(M+Na)^+$: 898.47199.

Crude compound **6** from this experiment was successfully converted into compound **7** in 85% overall yield over both steps using the procedure described above for the conversion of compound **6** into compound **7**.

One-pot synthesis of benzyl (2*S*)-5-{[*tert*-butyl(diphenyl)silyl]oxy}-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}pentanoate

(Compound 23)

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

The suspension was poured onto t-butyl-methylether (600 mL) and the reaction flask was washed with water (600 mL), which was added to the product mixture (2-phases). The phases were separated, the water phase was extracted with t-butyl-methylether (600 mL) and the organic phases were combined. The organic phase was washed with water (2x600 mL), dried over anhydrous magnesium sulfate (200g) and the solvent was removed under reduced pressure until a final volume of 1L was achieved. The solution was diluted with dimethyl-formamide (600g) and the solvent was evaporated under reduced pressure, until a final volume of 400 mL is achieved. The solution of compound **22** thus obtained was transferred into a round bottomed flask. Imidazole (14.4g, 211.524 mmol) was added to the resulting DMF solution of compound **22** (benzyl (2*S*)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-5-hydroxypentanoate) and the mixture was stirred for 5 minutes at rt. Finally, TBDPS-Cl (39.6g, 144.07 mmol) is added dropwise during 20 minutes at 20-25 °C and the reaction mixture is stirred for an additional hour at this temperature.

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

The crude product (106g) was dissolved in Ethanol/Isopropanol/Water (89:5:6; 1200 mL) at 40-50 °C and seed crystals (0.5g compound **23**) were added (the seed crystals were obtained by storage of a purified sample of compound **23** (viscous oil) in a refrigerator which resulted in cyrstals which were then used as seed crystals to crystallize compound **23** from hexanes/isopropylacetate 9:1, resulting in crystals used here as seeds for crystallization) . The mixture was allowed to cool down to room temperature and stirred for 17 hrs at rt. The suspension was cooled to -20 °C and stirred for 2 hrs at -20 °C. The product was isolated by filtration, the filter cake was washed with the solvent mixture Ethanol/Isopropanol/Water (89:5:6; 3x200 mL) and dried at 40 °C under reduced pressure to obtain 66.5g of compound **23** (74.6% yield over 2 steps). HPLC indicated >99 a% purity for the product.
HR-MS: calculated for $C_{43}H_{45}NO_5Si$: $[M+H]^+$: 684.31398; $[M+NH_4]^+$: 701.34053; $[M+Na]^+$: 706.29592. Found: $[M+H]^+$: 684.31430; $[M+NH_4]^+$: 701.34073; $[M+Na]^+$: 706.29577.

Additional product can be isolated from the mother liquor (34g after evaporation of the solvent), which contains ca. 30 a% compound **23** according to HPLC.

Synthesis of benzyl (2*S*)-2-(dibenzylamino)-4-(1,3-dioxolan-2-yl)butanoate (Compound 25)

To a solution of compound **24** (29g; 72.23 mmol) in dichloromethane (700 mL), ethylene glycol (133g, 2.14 moles), p-toluene-sulfonic acid monohydrate (15g; 78.86 mmol) and molecular sieves (3Angström, 40g) were sequentially added. The reaction mixture was stirred for 18 h at room temperature. The molecular sieve was removed by filtration, the filter cake was washed with ethyl acetate and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (1L), extracted with water (3x300mL) and the organic phase was evaporated under reduced pressure to obtain 33.3g crude product. The crude product was purified by chromatography on silica gel with ethyl acetate/hexanes (4:6) to obtain 28.0g of pure Compound **25** (87% yield).
1H-NMR of the product confirmed the proposed structure.
HR-MS: Calculated for $C_{28}H_{31}NO_4$ $[M+H]^+$ =446.23259. Found: 446.23248.

Synthesis of Compound A from compound 16

Compound A= (2*S*)-*N*-[(2*S*,5*S*,8*S*,11*R*,12*S*,15*S*,18*S*,21*R*)-2,8-di[(2*S*)-butan-2-yl]-21-hydroxy-5-[(4-hydroxyphenyl)methyl]-4,11-dimethyl-15-(2-methylpropyl)-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl]-2-(2-methylpropanamido)pentanediamide (alternatively named: (S)-N^1-((2S,5S,8S,11R,12S,15S,18S,21R)-2,8-di-(S)-*sec*-butyl-21-hydroxy-5-(4-hydroxybenzyl)-15-isobutyl-4,11-dimethyl-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl)-2-isobutyramidopentanediamide)**:**

Compound **16** (2.0g, 1.63 mmol) was dissolved in dichloromethane (400 mL) and the solution was cooled to 0 °C. TFA (115.9g) was added to the solution at 0-4 °C and the reaction mixture was stirred for 4h at this temperature. Dichloromethane (400 mL) was added to the reaction mixture, followed by the addition of demineralized water (20 mL). The reaction mixture was heated to room temperature and was stirred for additional 5h at this temperature. The reaction mixture was the poured onto a solution of NaOAc (165.1g) in demineralized water (800 mL). Etyl acetate (400 mL) was added to the mixture and the layers were separated. The organic phase was washed with demineralized water (2x200 mL) and the aqueous phases were extracted with ethyl acetate (200 mL). The organic phases were combined and the resulting solution was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure and the crude product (2.183g) was purified by RP-Chromatography on a RP Silica Kromasil 100-10-C8 column (Eka Chemicals AB, Bohus, Sweden), with a gradient of acetonitrile/water. The main product containing fractions were collected, the organic solvent (acetonitrile) was evaporated under reduced pressure and ethyl acetate was added to the formed suspension. The layers were separated and the solvent of the organic layer was evaporated under reduced pressure to obtain purified Compound A.
Yield: 0.895g (59.1%).
^{13}C- and ^{1}H-NMR of the product confirmed the proposed structure and were comparable to the spectra of compound A prepared from compound 20, see below..

HR-MS: Calculated for $C_{46}H_{72}N_8O_{12}$ $[M+H]^+$: 929.53425; $[M+NH_4]^+$: 946.56080; $[M+Na]^+$: 951.51619. Found: $[M+H]^+$: 929.53460; $M+NH_4]^+$: 946.56090; $[M+Na]^+$: 951.51643.

Synthesis of Compound A from compound 20

Compound A= (2*S*)-*N*-[(2*S*,5*S*,8*S*,11*R*,12*S*,15*S*,18*S*,21*R*)-2,8-di[(2*S*)-butan-2-yl]-21-hydroxy-5-[(4-hydroxyphenyl)methyl]-4,11-dimethyl-15-(2-methylpropyl)-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl]-2-(2-methylpropanamido)pentanediamide

Compound **20** (0.400g, 0.315 mmol) was dissolved in dichloromethane (80 mL) and the solution was cooled down to 0-5°C. Trifluoroacetic acid (23.06g) was added under intensive stirring and the reaction mixture was stirred for additional 3.5h at this temperature. The solution was then diluted with dichloromethane (80 mL) and treated with demineralized water (4.0g). The reaction mixture was allowed to warm up to room temperature and was stirred for additional 19.5h at room temperature. For work-up, the reaction mixture was poured onto a solution of sodium acetate (32.6g) in demineralized water (160 mL) and ethyl acetate (80 mL) was added. The phases were separated and the lower organic phase was washed with demineralized water (2x40 mL). The aqueous phases were extracted with ethyl acetate (80 mL) and the organic phases were combined. The combined organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to obtain crude compound **A**. The crude product was purified by column chromatography on silica gel with ethyl acetate/isopropanol (9:1) as mobile phase. Collection of product fractions and evaporation of the solvent under reduced pressure gave compound **A**. Yield: 0.22g (75.3%).

^{1}H- and ^{13}C-NMR Spectra of the product confirmed the proposed structure and were comparable to the spectrum of compound **A** obtained from compound **16**.

^{13}C (150MHz, d_6-DMSO): δ (ppm): 10.36, CH_3; 11.21, CH_3; 13.88, CH_3; 16.02, CH_3; 17.71, CH_3; 19.53, CH_3; 19.53, CH_3; 20.92, CH_3; 21.77, CH_2; 23.32, CH_3; 23.77, CH_2; 24.24, CH; 24.52, CH_2; 27.36, CH_2; 29.81, CH_2; 30.11, CH_3; 31.51, CH_2; 33.21, CH; 33.27, CH_2; 33.80, CH; 37.41, CH; 39.23, CH_2; 48.89, CH; 50.76, CH; 52.15, CH; 54.21, CH; 54.74, CH; 55.36, CH; 60.70, CH; 71.93, CH; 74.01, CH; 115.36, CH; 115.36, CH; 127.37, Cq; 130.38, CH; 130.38, CH; 156.32, Cq; 169.18, Cq; 169.35, Cq; 169.41, Cq; 169.83, Cq; 170.70, Cq; 172.42, Cq; 172.58, Cq; 173.96, Cq; 176.45, Cq.

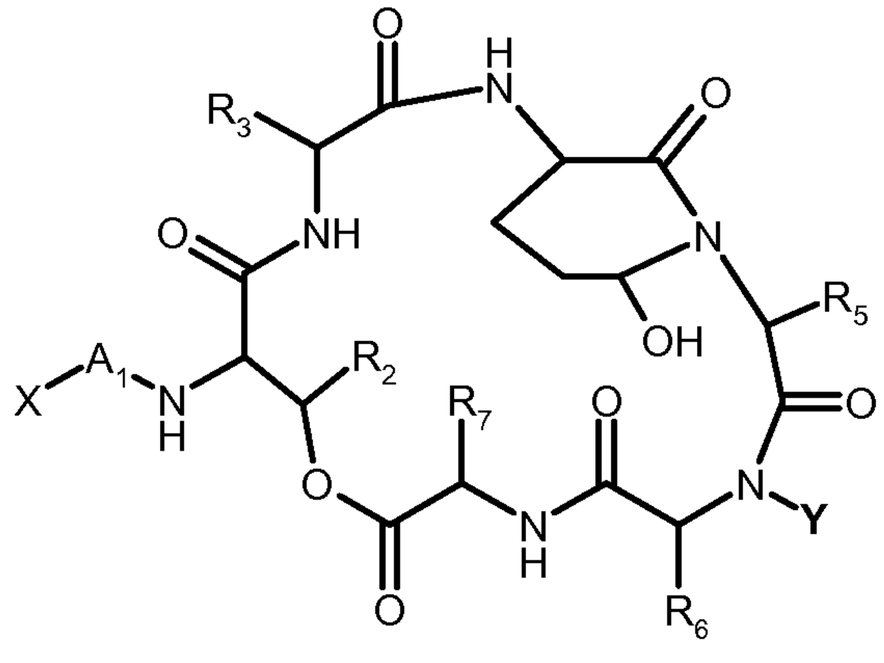
^{1}H (600 MHz, d_6-DMSO): δ (ppm): -0.11, (3H, d, J=6.2Hz); 0.63, (3H,m); 0.63, (1H,m); 0.77, (3H,d, J=6.2Hz); 0.80, (3H,t, J=7.5Hz); 0.84, (3H,d, J=7.0Hz); 0.88, (3H,d,

J=6.6Hz); 1.02, (3H,d, J=6.7Hz); 1.02, (1H,m); 1.03, (3H,d, J=6.7Hz); 1.09, (1H,m); 1.20, (3H,d, J=6.2Hz); 1.24, (1H,m); 1.39, (1H,m); 1.51, (1H,m); 1.72, (1H,m); 1.73, (2H,m); 1.75, (1H,m); 1.76, (1H,m); 1.78, (1H,m); 1.83, (1H,m); 1.92, (1H,m); 2.13, (2H,m); 2.47, (1H,q); 2.58, (1H,m); 2.67, (1H,dd, J=14.2, 11.4Hz); 2.70, (3H,s); 3.16, (1H,d, J=14.2Hz); 4.30, (1H,m); 4.34,(1H,m); 4.42, (1H,d, J=10.6Hz); 4.45, (1H,m); 4.61, (1H,d, J=9.2Hz); 4.70, (1H,dd, J=9.5, 5.5Hz); 4.93, (1H,s); 5.05, (1H,dd, J=11.4, 2.6Hz); 5.48, (1H,m); 6.08, (1H,d, J=2.6Hz); 6.64, (1H,d, J=8.4Hz); 6.64, (1H,d, J=8.4Hz); 6.72, (1H,s); 6.99, (1H,d, J=8.4Hz); 6.99, (1H,d, J=8.4Hz); 7.26, (1H,s); 7.36, (1H,d, J=9.2Hz); 7.65, (1H,d, J=9.5Hz); 7.72, (1H,d, J=9.2Hz); 8.02, (1H,d, J=7.7Hz); 8.42, (1H,d, J=8.8Hz); 9.18, (1H,s).

HR-MS: Calculated for $C_{46}H_{72}N_8O_{12}$ $[M+H]^+$: 929.53425; $[M+NH_4]^+$: 946.56080; $[M+Na]^+$: 951.51619. Found: $[M+H]^+$: 929.53379; $[M+NH_4]^+$: 946.56036; $[M+Na]^+$: 951.51537.

Claims

1. A method or process for the preparation of a cyclic depsipeptide compound of the formula I solely by solution phase synthesis,



(I)

wherein

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

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

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

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

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

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

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

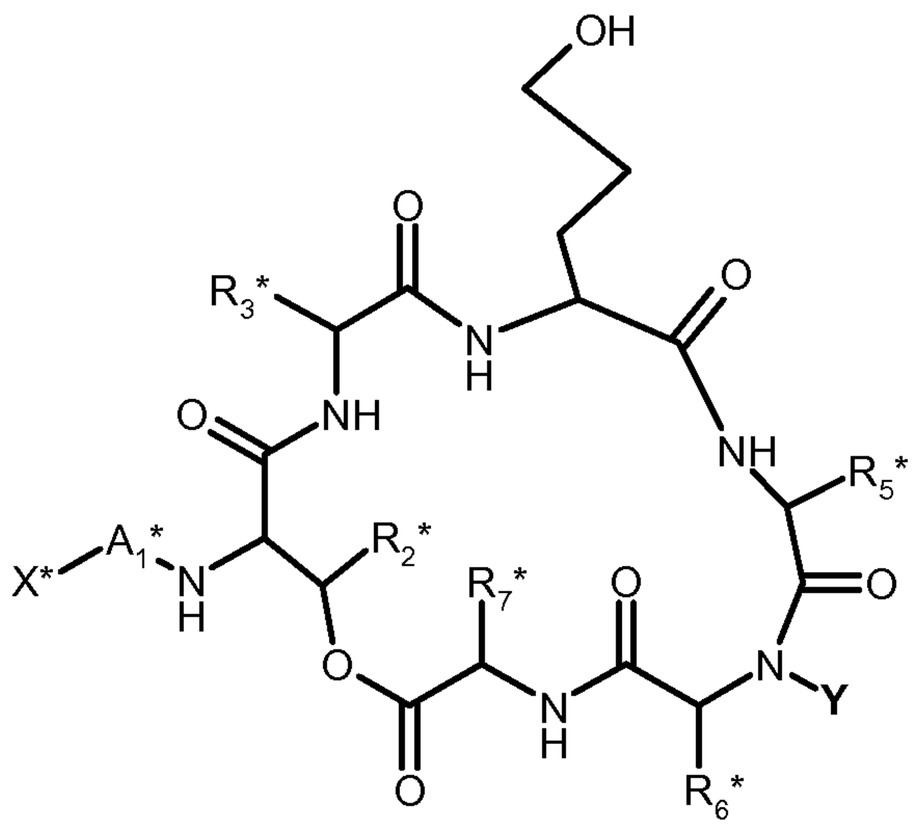
Y is hydrogen or C_{1-8}-alkyl;

or a salt thereof,

said method comprising

in a first variant (A)

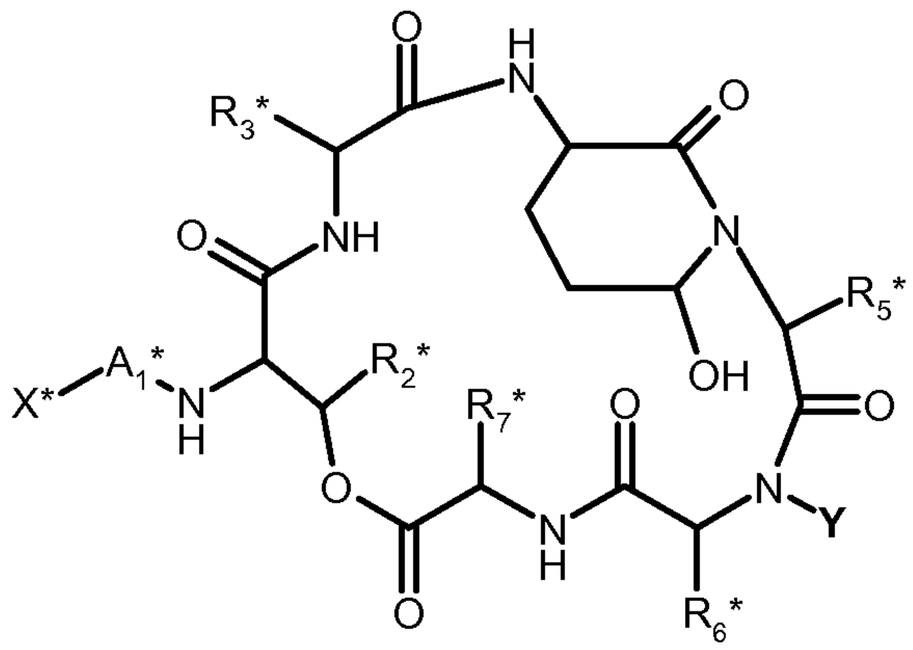
reacting the free hydroxyl group of a compound of the formula (II),



(II)

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

under oxidizing conditions to form a compound of the formula III,



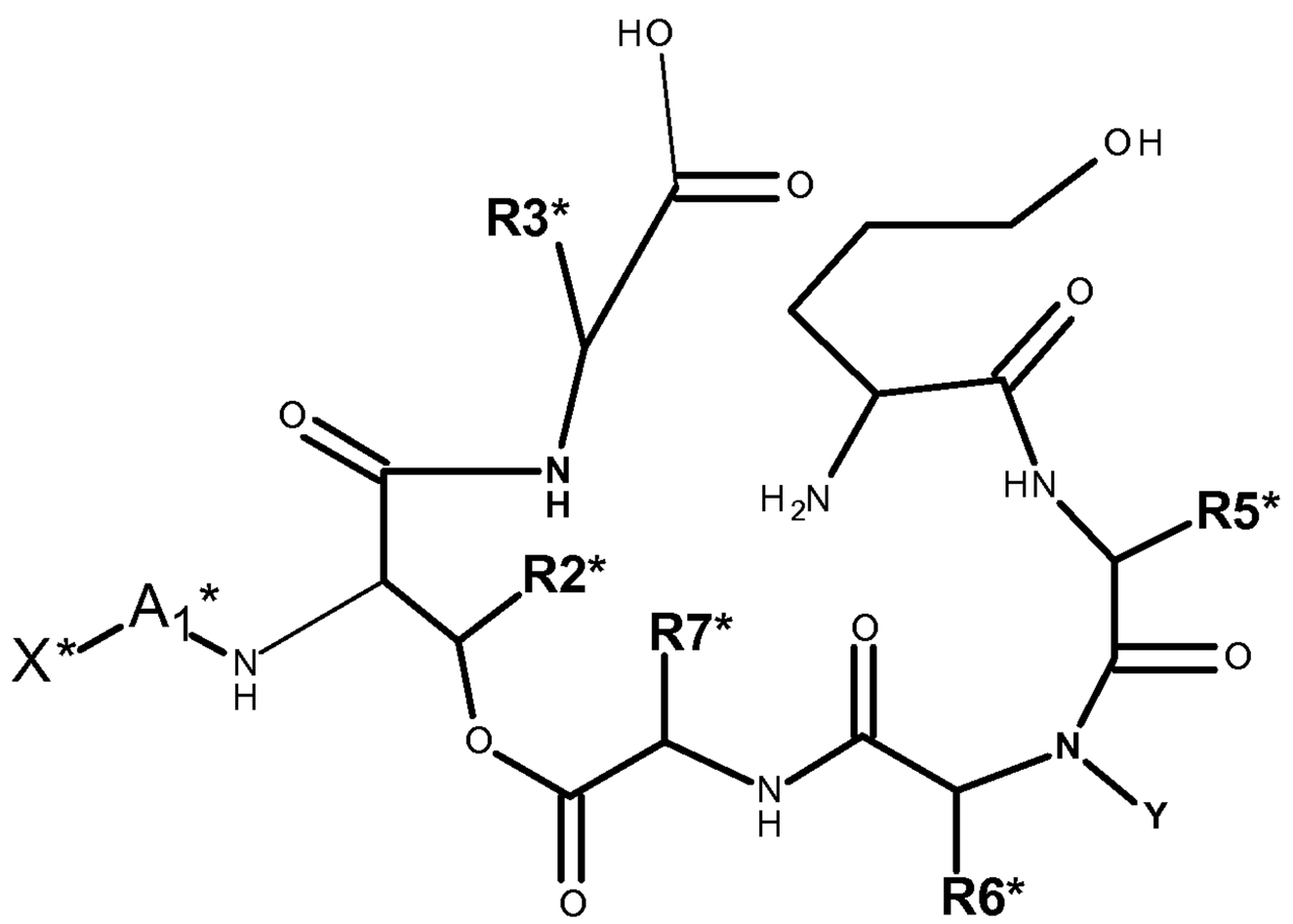
(III)

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

and, if desired, converting a free compound of the formula I into a salt, a salt of a compound of the formula I into a different salt of a compound of the formula I or into the free compound of the formula I and/or and/or converting a dehydrate analogue

and/or five ring analogue of a compound of the formula I formed as by-product into the corresponding compound of the formula I;

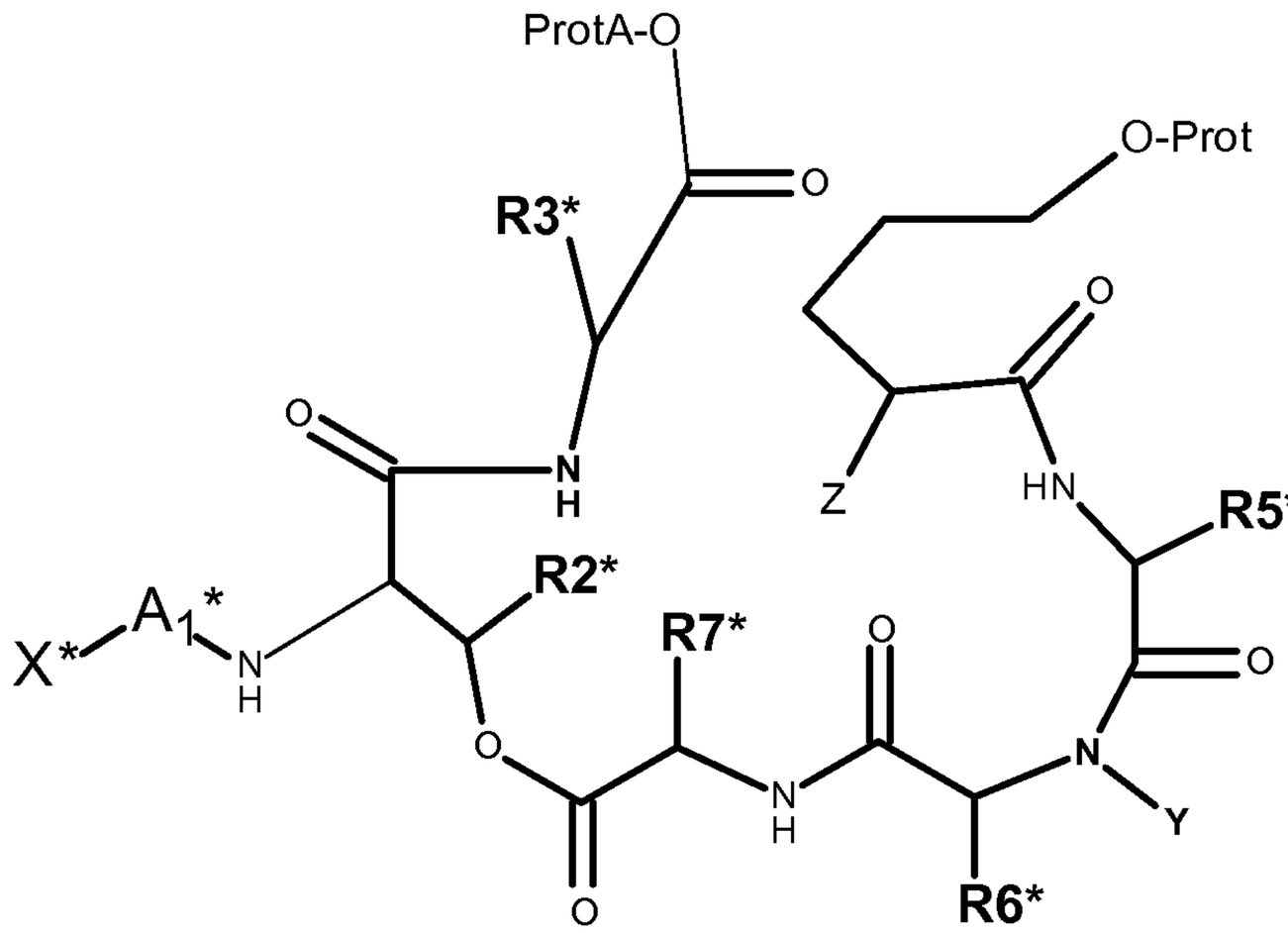
where the compound of the formula III is prepared by macrolactamization of a compound of the formula IV,



(IV),

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

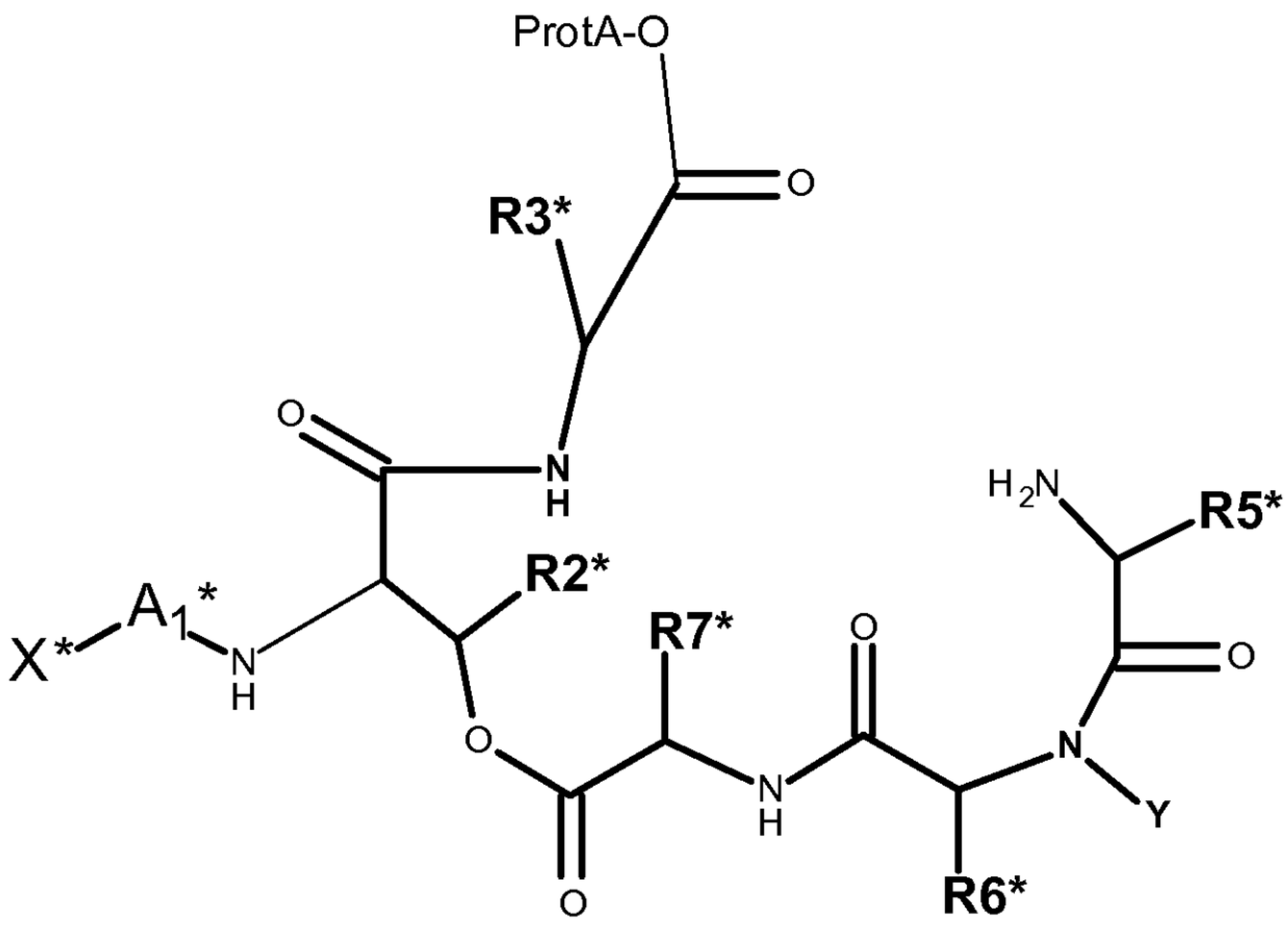
where, in a further embodiment, the method or process described above, is further comprising manufacturing the compound of the formula IV in solution phase by simultaneously or sequentially removing the protecting group ProtA, the protecting group(s) of protected amino group Z and the protecting group Prot from a compound of the formula V,



(V)

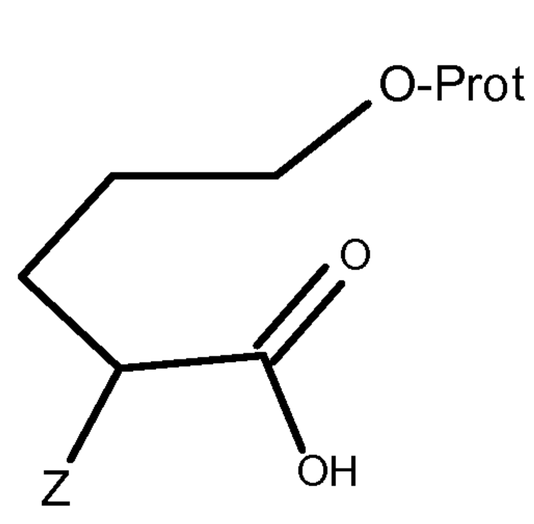
wherein ProtA is carboxy protecting group, Prot is a hydroxyl protecting group, Z is a protected amino group either of the formula NHProt** wherein Prot** is an amino protecting group; or Z is a protected amino group of the formula $N(Prot^*)_2$ wherein each Prot* is an amino protecting group; and Y, R_2*, R_3*, R_7*, R_6* and R_5*, X* and A_1* are as defined for a compound of the formula II;

the method or process mentioned above preferably further comprising manufacturing the compound of the formula V in solution phase by coupling a compound of the formula VI,



(VI),

wherein ProtA is as defined above for a compound of the formula V and Y, R_2*, R_3*, R_7*, R_6* and R_5*, X* and A_1* are as defined above for a compound of the formula II, with an amino acid of the formula VII,

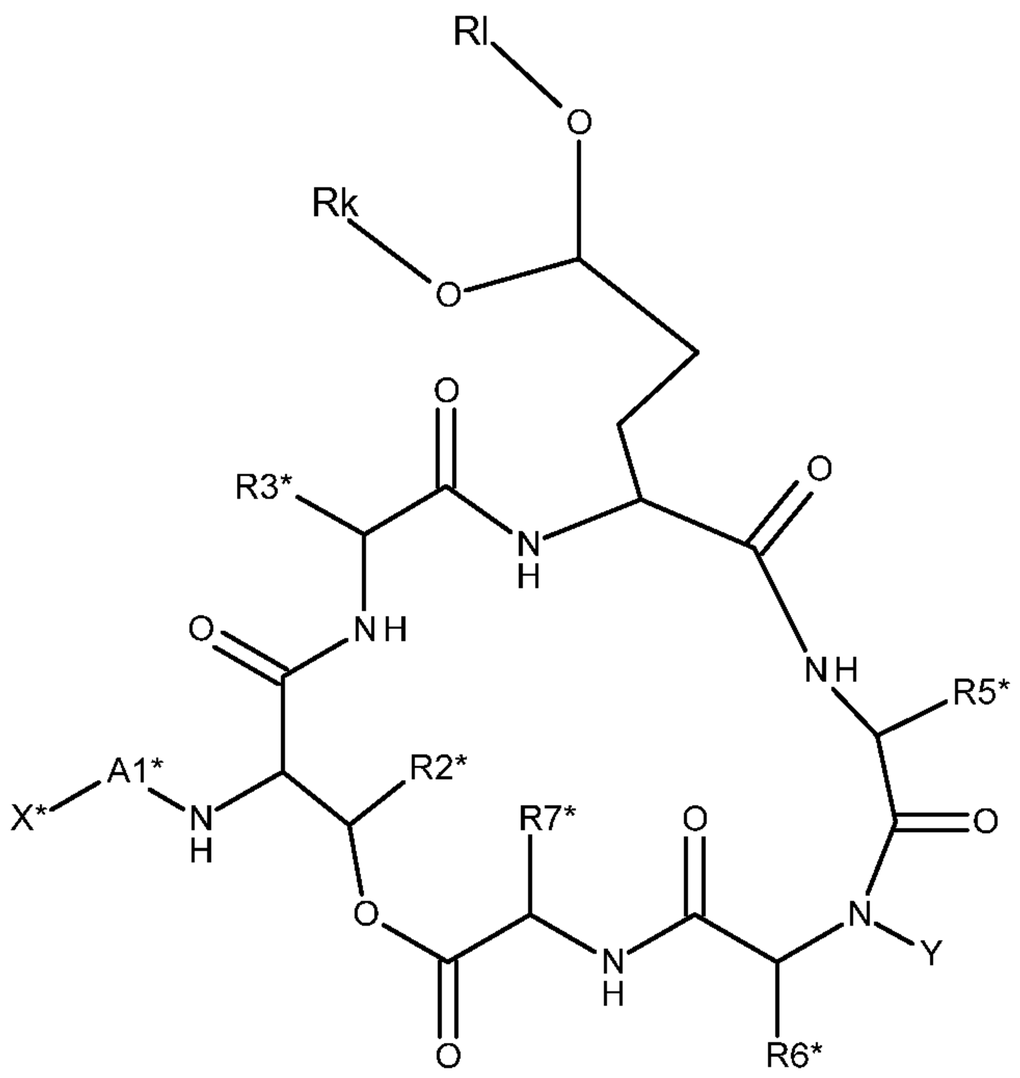


(VII)

wherein ProtA and Z (which is preferably Prot**) are as defined above for a compound of the formula V, or an activated derivative thereof (end of variant A);

and/or in parallel or alternatively, in a variant (B),

deprotecting a compound of the formula II*



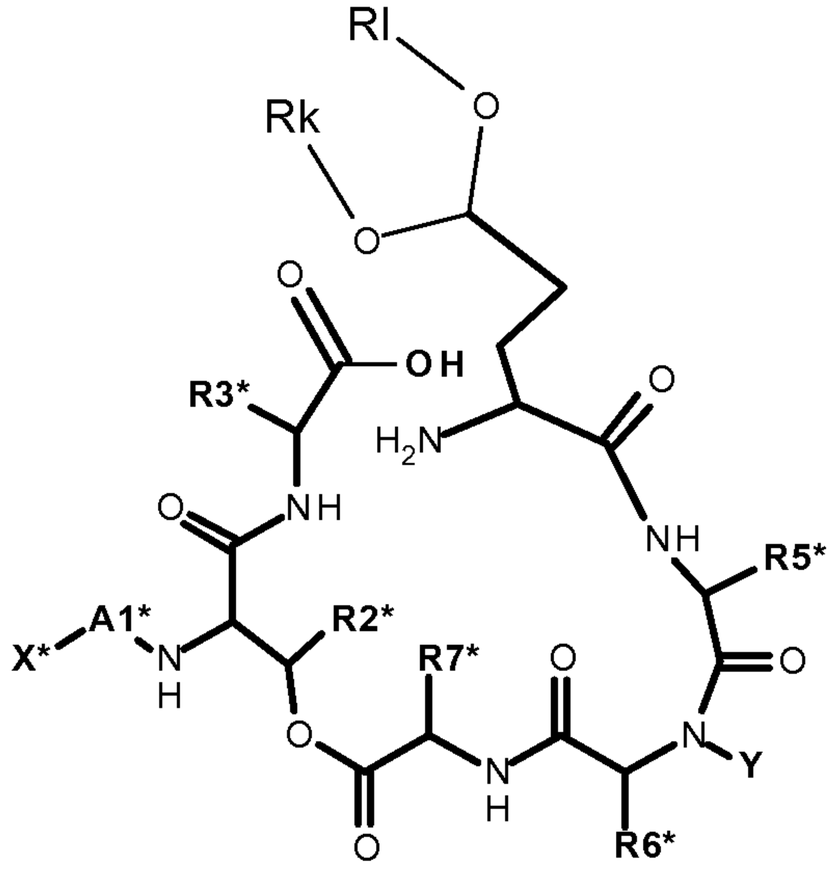
II*

wherein the aldehyde protecting group(s) Rk and Rl are independently of each other unsubstituted or substituted alkyl or together with the two binding O atoms and the carbon atom to which the two O atoms are bound form a ring that is unsubstituted or substituted, Y is as defined for a compound of the formula I and X*, A_1*, R_2*, R_3*, R_5*, R_6*, and R_7* correspond to X, A_1, R_2, R_3, R_5, R_6, and R_7 in formula I, respectively, but with the proviso that reactive functional groups on these moieties are present in protected form at least if they could participate in undesired side reactions, to result in a compound of the formula I, especially IA;

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

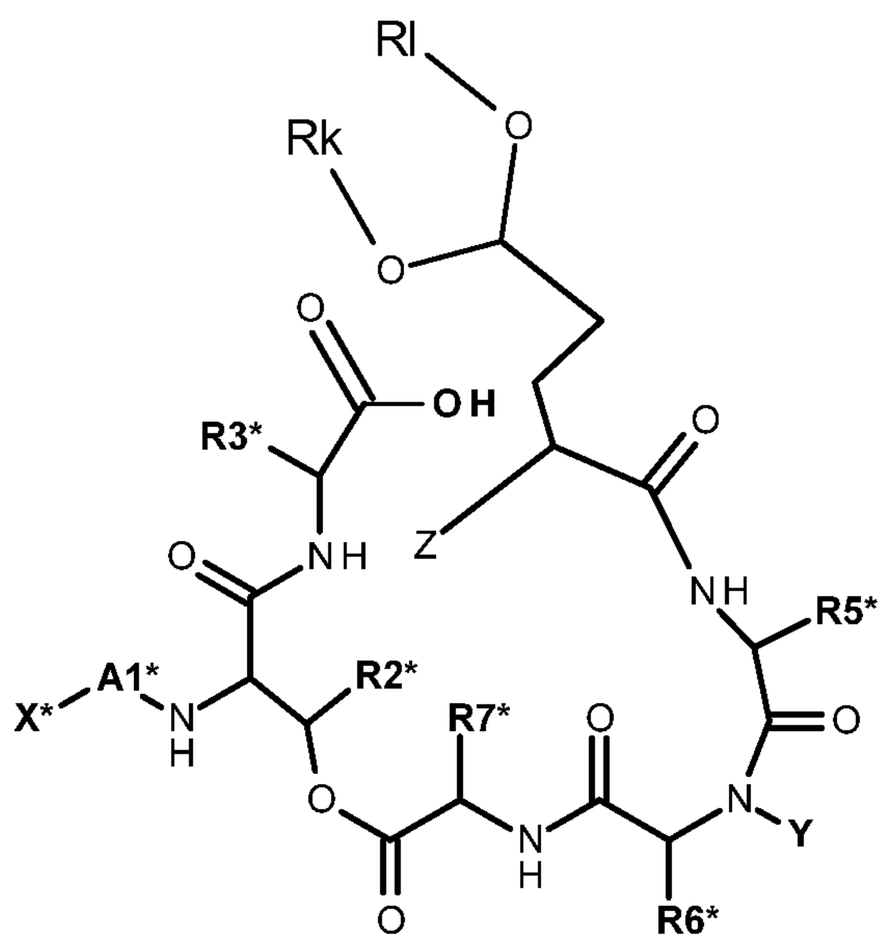
2. A method or process as described in claim 1 under variant (B), further comprising, for the synthesis of a compound of the formula II* cyclization under lactamization of a linear precursor peptide of the compound of the formula II*, carrying

an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that allow for the formation of an amide bond from said amino and said carboxy group, using Solution Phase chemistry,
where the linear precursor peptide is of the formula IV*,



IV*

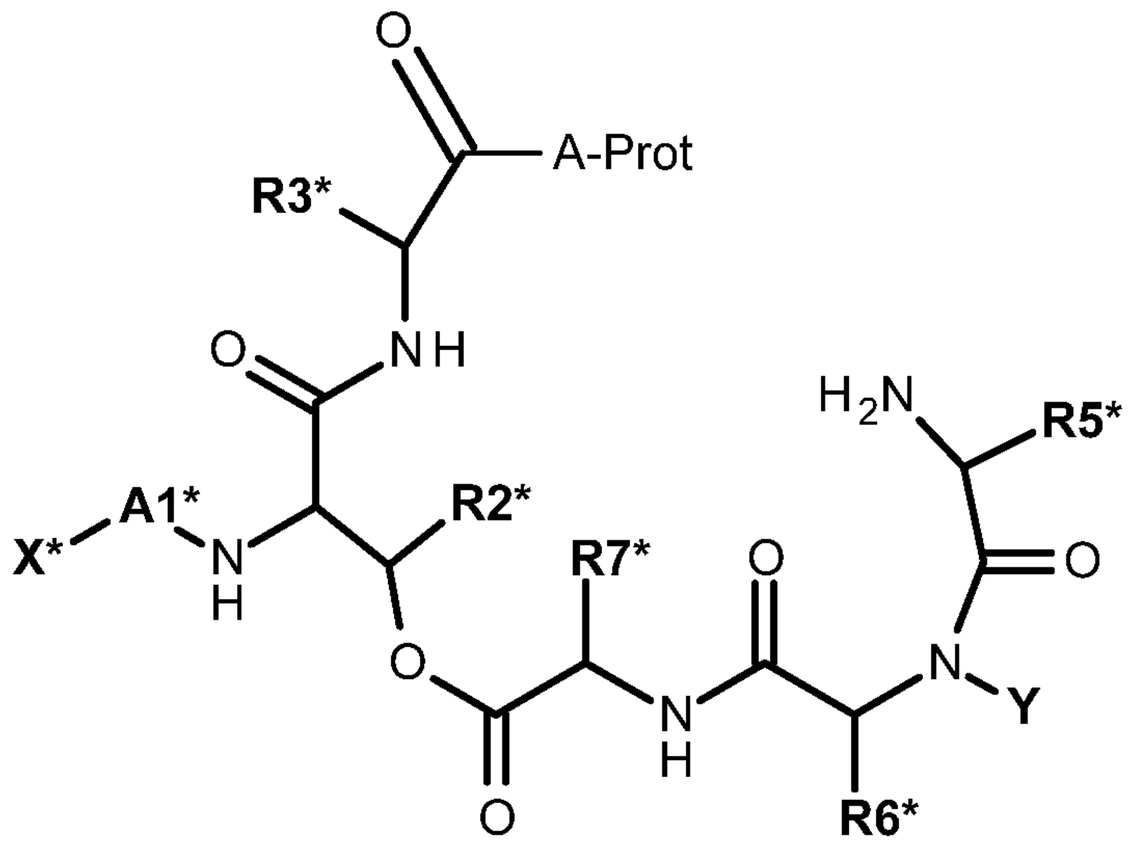
wherein Rk, Rl, X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II* above, which can be obtained by deprotection from the corresponding compound of the formula V*,



V*

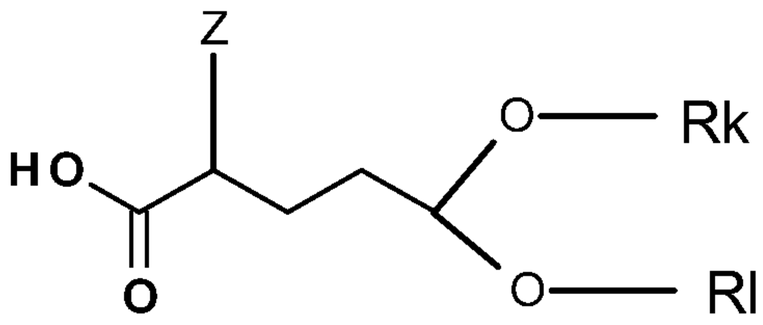
wherein Rk, Rl, X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II* above and wherein Z is as defined for a compound of the formula V in claim 1, by deprotecting the protected amino group Z;

the method or process preferably further comprising, for the synthesis of the compound of the formula V*, reacting a compound of the formula VI



VI

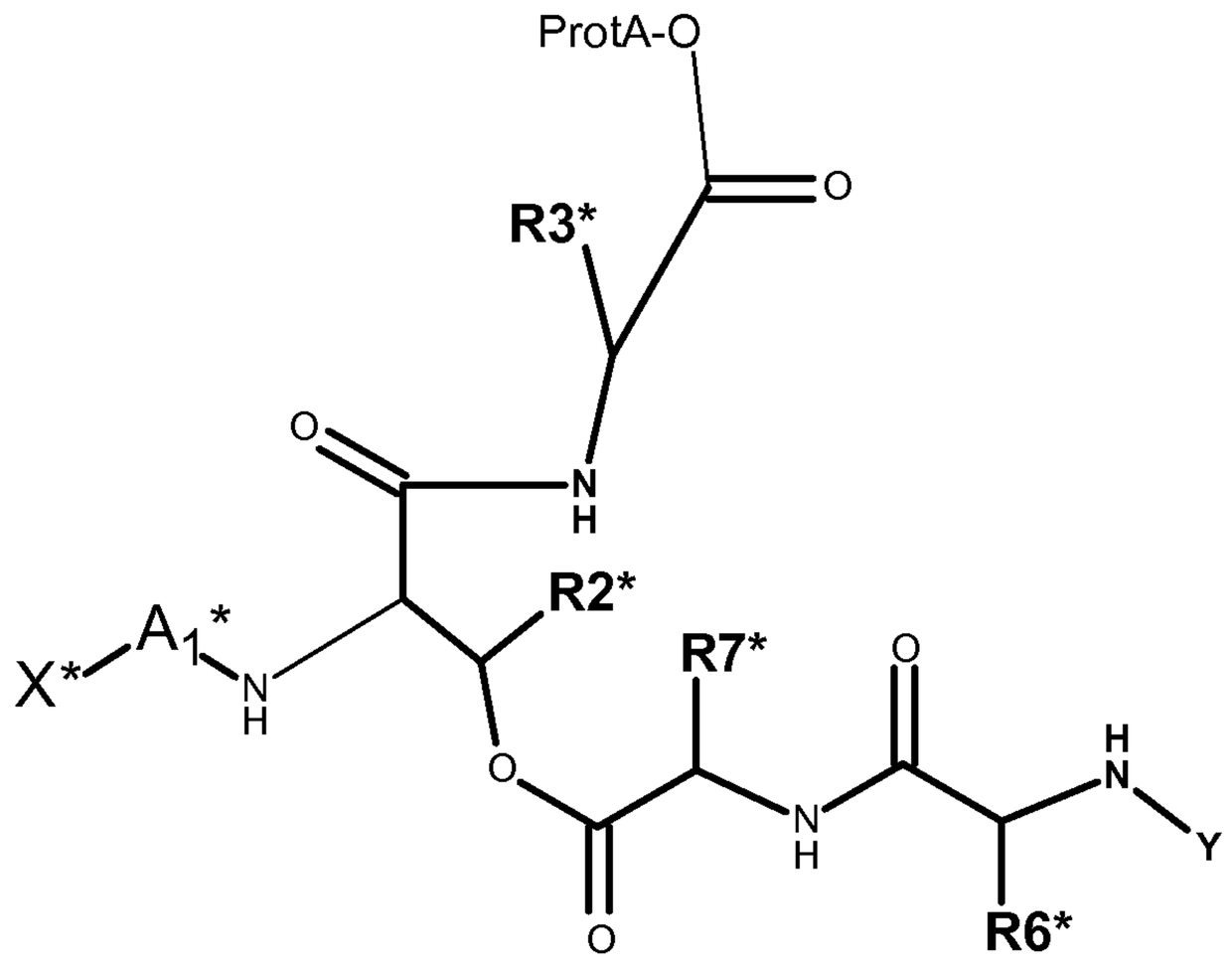
wherein Rk, Rl, X*, A_1*, R_2*, R_3*, R_5*, R_6* and R_7* are as defined for a compound of the formula II* and Prot-A is a carboxy protecting group by coupling an amino acid of the formula VII*,



VII*,

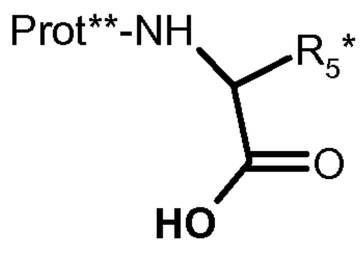
wherein Rk and Rl are as defined for a compound of the formula II* and Z is as defined in claim 1 for a compound of the formula V*; or an activated derivative of said amino acid, to said compound of the formula VI* or VIA* **(end of variant (B)).**

3. The method or process according to claim 1 or claim 2, including either variant (A) or variant (B), not excluding that both are used, further comprising manufacturing the compound of the formula VI used in both variants in solution phase by coupling a compound of the formula VIII



(VIII),

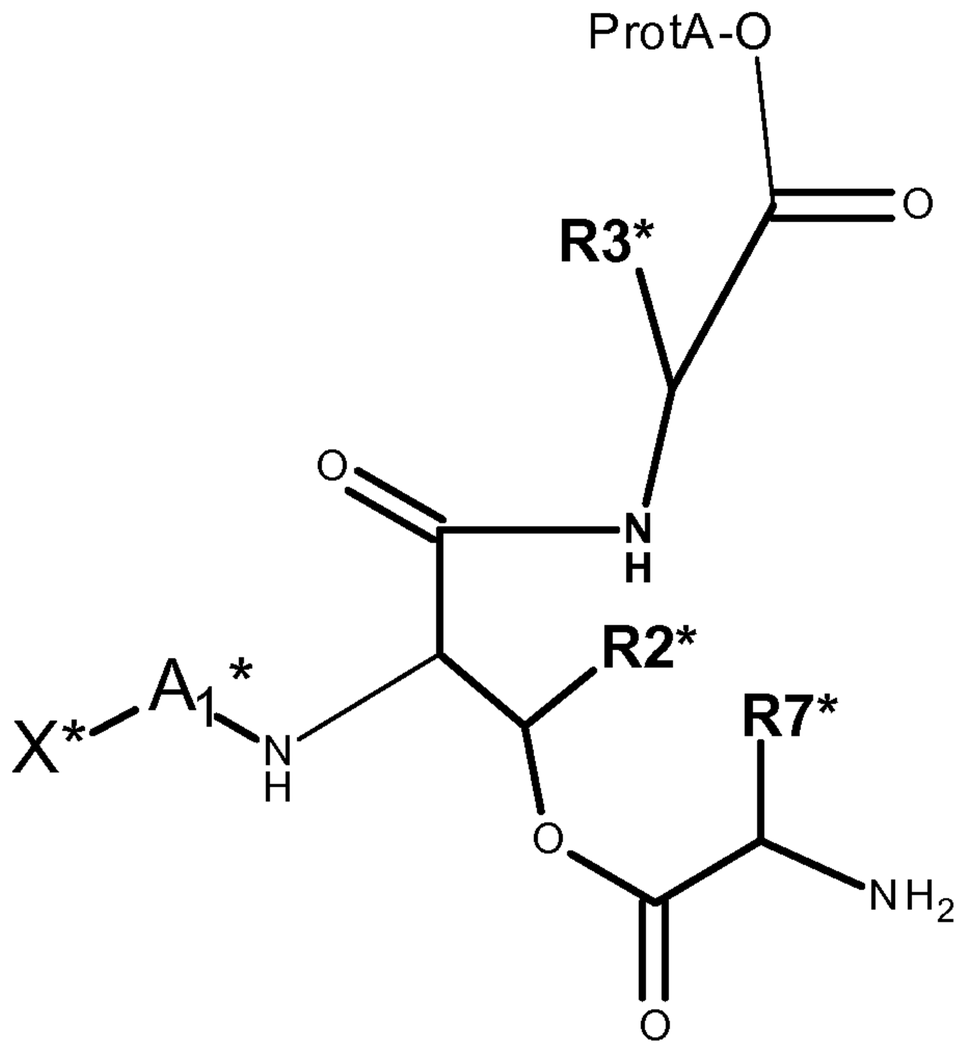
wherein ProtA is as defined above for a compound of the formula V in claim 1 and Y, R_2*, R_3*, R_7*, R_6* and X* and A_1* are as defined above for a compound of the formula II in claim 1, with an amino acid of the formula IX,



(IX)

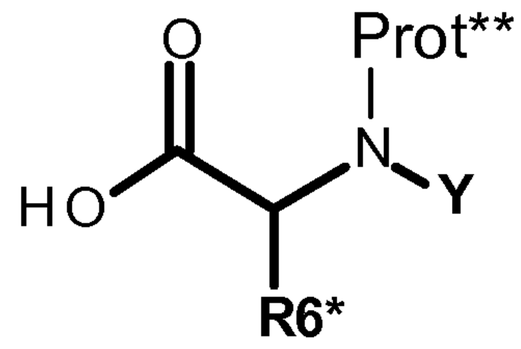
wherein Prot** is as defined for a compound of the formula V in the definition of Z in claim 1 and R5* is as defined for a compound of the formula V in claim 1, or a reactive derivative of said amino acid.

4. The method or process according to claim 3, further comprising manufacturing the compound of the formula VIII in solution phase by coupling a compound of the formula X,



(X)

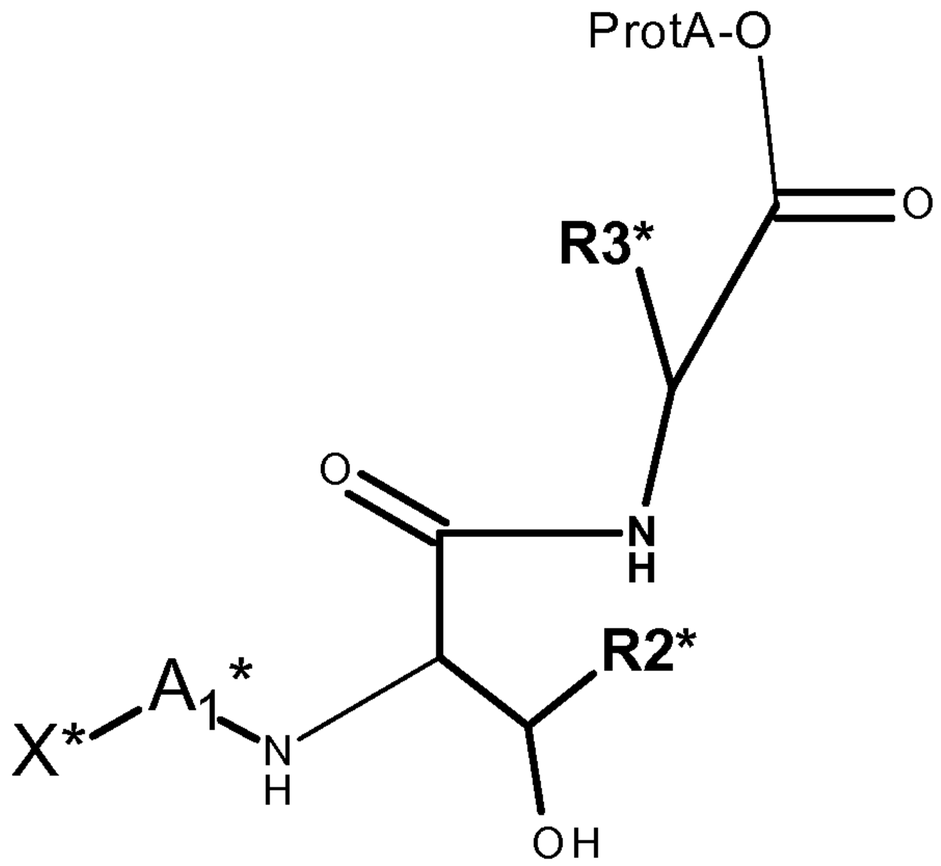
wherein ProtA is as defined in claim 1 for a compound of the formula V and R_2*, R_3*, R_7*, X* and A_1* are as defined in claim 1 for a compound of the formula II, with an amino acid of the formula XI,



(XI)

wherein Prot** and R_6* are as defined in claim 1 for a compound of the formula V and Y is as defined in claim 1 for a compound of the formula I, or a reactive derivative of said amino acid.

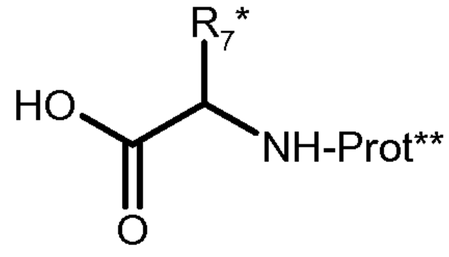
5. The above method or process according to claim 4, further comprising manufacturing the compound of the formula X in solution phase by esterifying a compound of the formula XII,



(XII)

wherein ProtA is as defined in claim 1 for a compound of the formula V and R_2*, R_3* X* and A_1* are as defined in claim 1 0for a compound of the formula II,

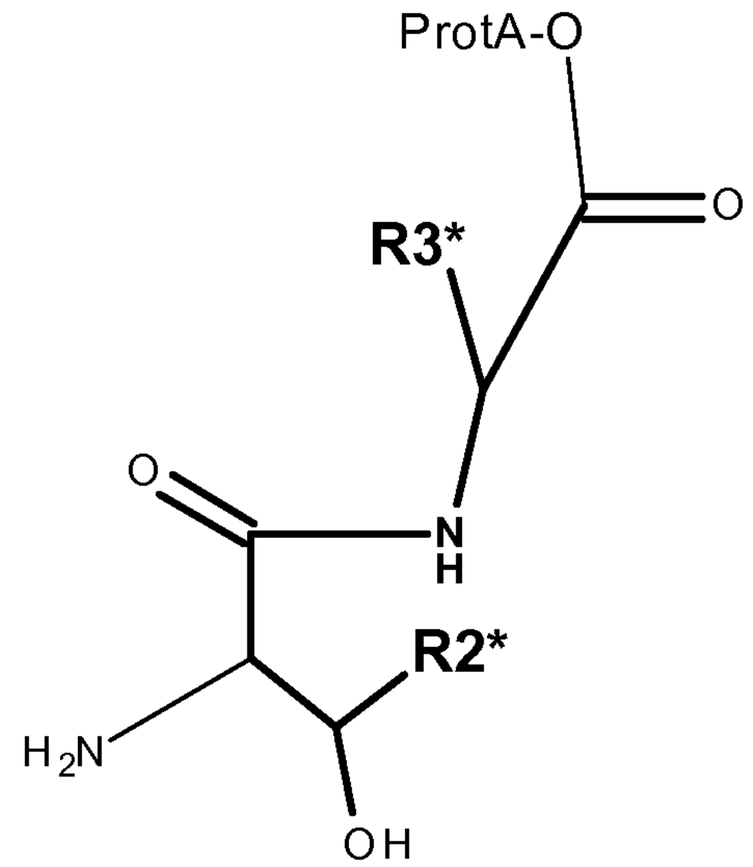
with an amino acid of the formula XIII



(XIII)

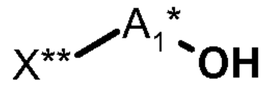
wherein Prot** and R_7* are as defined in claim 1 for a compound of the formula V, or a reactive derivative of said amino acid.

6. The method or process according to claim 5, further comprising manufacturing the compound of the formula XII in solution phase by coupling a compound of the formula XIV,



(XIV)

wherein ProtA is as defined above for a compound of the formula V and R_2* and R_3* are as defined in claim 1 for a compound of the formula II, with an acid of the formula XV,

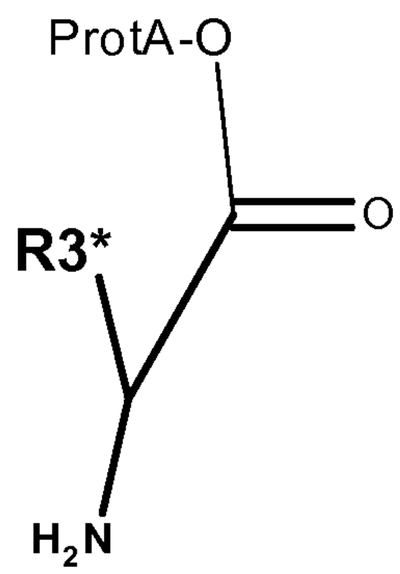


XV

or a reactive derivative thereof,

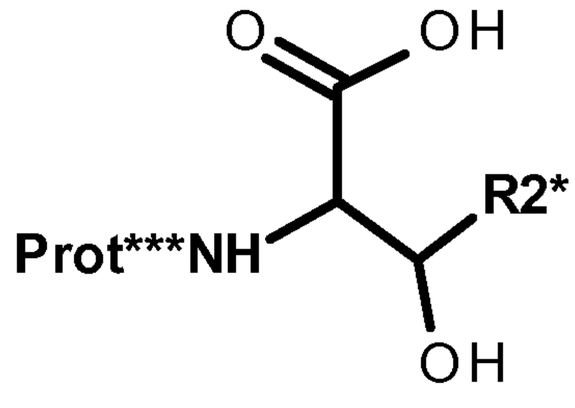
wherein X** is an amino protecting group or is X*, and wherein X* and A_1* are as defined above for a compound of the formula II; and, if X** is an amino protecting group, removing said amino protecting group X** to yield H instead of X* and coupling the resulting amino group with an acyl group X* using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

7. The method or process according to claim 6, further comprising manufacturing the compound of the formula XV by coupling an amino acid of the formula XVI



(XVI),

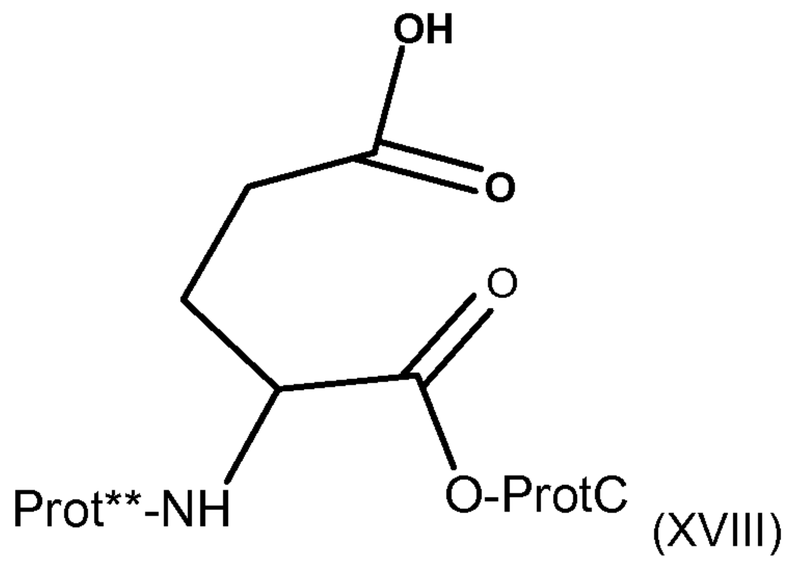
wherein ProtA is as defined above for a compound of the formula V and R_3* is as defined above for a compound of the formula II, with an amino acid of the formula XVII,



(XVII)

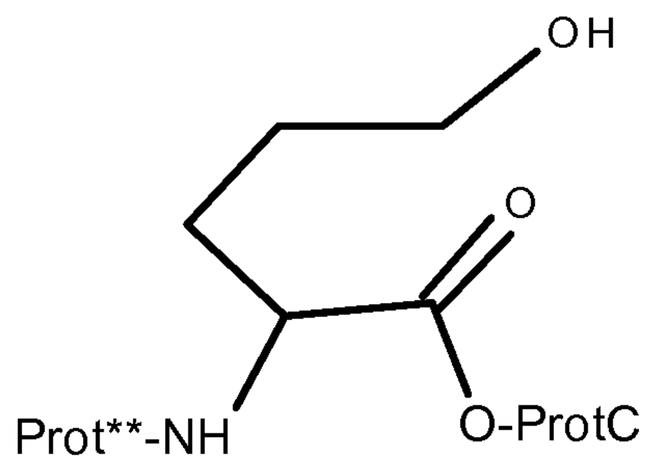
wherein Prot*** is an amino protecting group that can be cleaved off without removal of the protecting group ProtA and R_2*is as defined above for a compound of the formula II, or a reactive derivative of said amino acid,
and removing the amino protecting group Prot***.

8. The method according to claim 7, further comprising manufacturing the compound of the formula VII by reducing the free carboxyl group in a compound of the formula XVIII



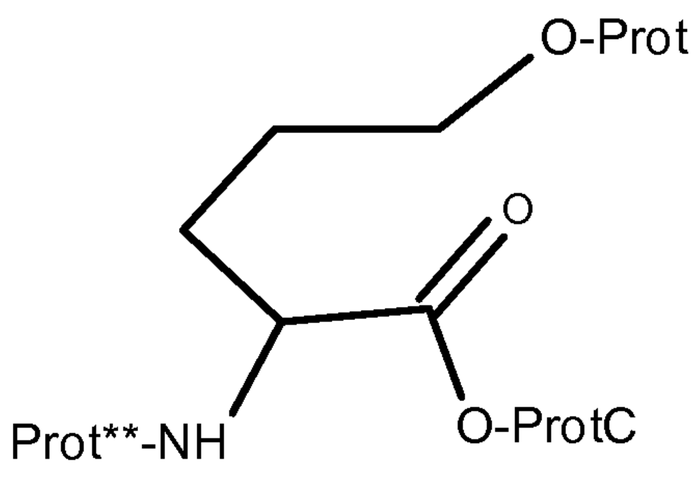
(XVIII)

wherein ProtC is a carboxyl protecting group and Prot** is as defined in claim 1 for a compound of the formula V, to the corresponding alcohol of the formula XIX,



(XIX)

wherein Prot** and ProtC are as just defined, protecting the free hydroxyl group with a reagent introducing a hydroxyl protecting group Prot to give a compound of the formula XX,

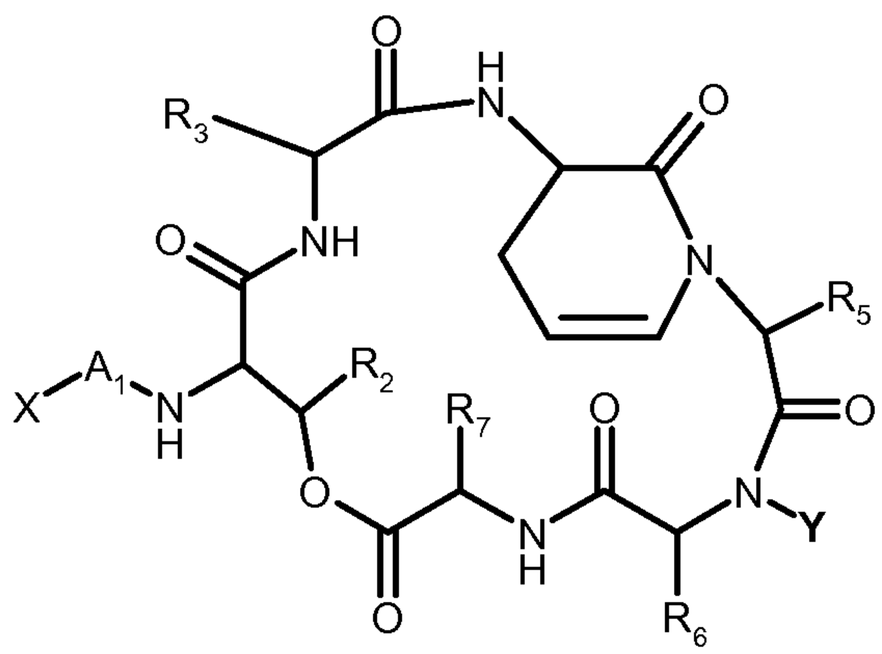


(XX)

wherein Prot**, ProtC and Prot are as just defined,

and removing the protecting group ProtC to give the compound of the formula VII.

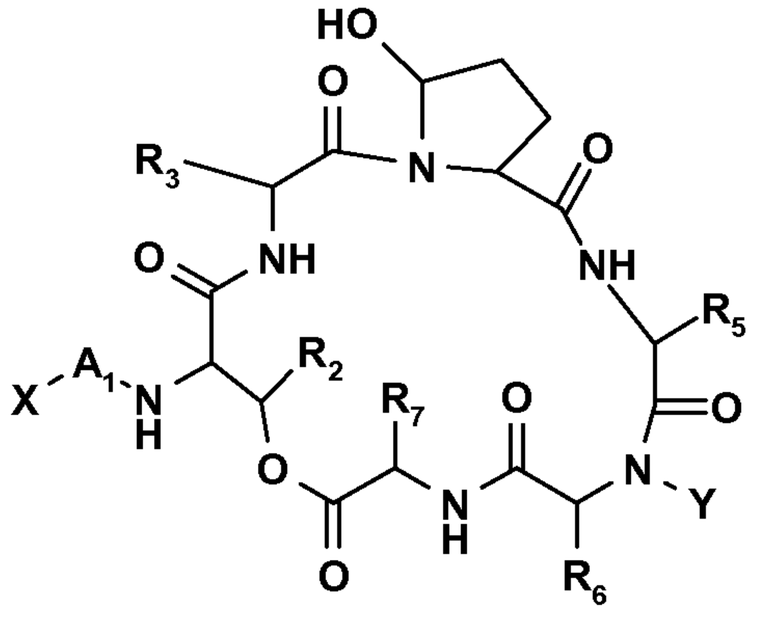
9. The process or method for the manufacture of a compound of the formula I, comprising converting a dehydrate of a compound of the formula I (especially given in claim 1 or with the substituents as defined in claim 10) into the corresponding compound of the formula I, where the dehydrate has the formula XXI,



(XXI),

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

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



(XXII*)

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

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

10. The method according to any one of the preceding claims, wherein where present

the symbols A_1, R_2, R_3, R_5, R_6, R_7, X and Y or the corresponding unprotected or protected moieties R_2*, R_3*, R_5*, R_6*, R_7*, X* and Y* are selected so that, in the resulting compound of the formula I, or a salt thereof,

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

R_2 is methyl;

R_3 is isopropyl, isobutyl or benzyl;

R_5 is sec-butyl or benzyl,;

R_6 is 4-hydroxybenzyl;

R_7 is isopropyl or sec-butyl;

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

and

Y is methyl.

11. The method or process according to any one of the preceding claims, wherein where present

the symbols A_1, R_2, R_3, R_5, R_6, R_7, X and Y or the corresponding unprotected or protected moieties R_2*, R_3*, R_5*, R_6*, R_7*, X* and Y* are selected so that, in the resulting compound of the formula I, or a salt thereof,

A_1 is the moiety of L-glutamine bound via the carbonyl of its α-carboxyl group to the amino group at the right of A_1 in formula I and via its α-amino group to X;

R_2 is methyl;

R_3 is isobutyl;

R_5 is sec-butyl;

R_6 is 4-hydroxybenzyl;

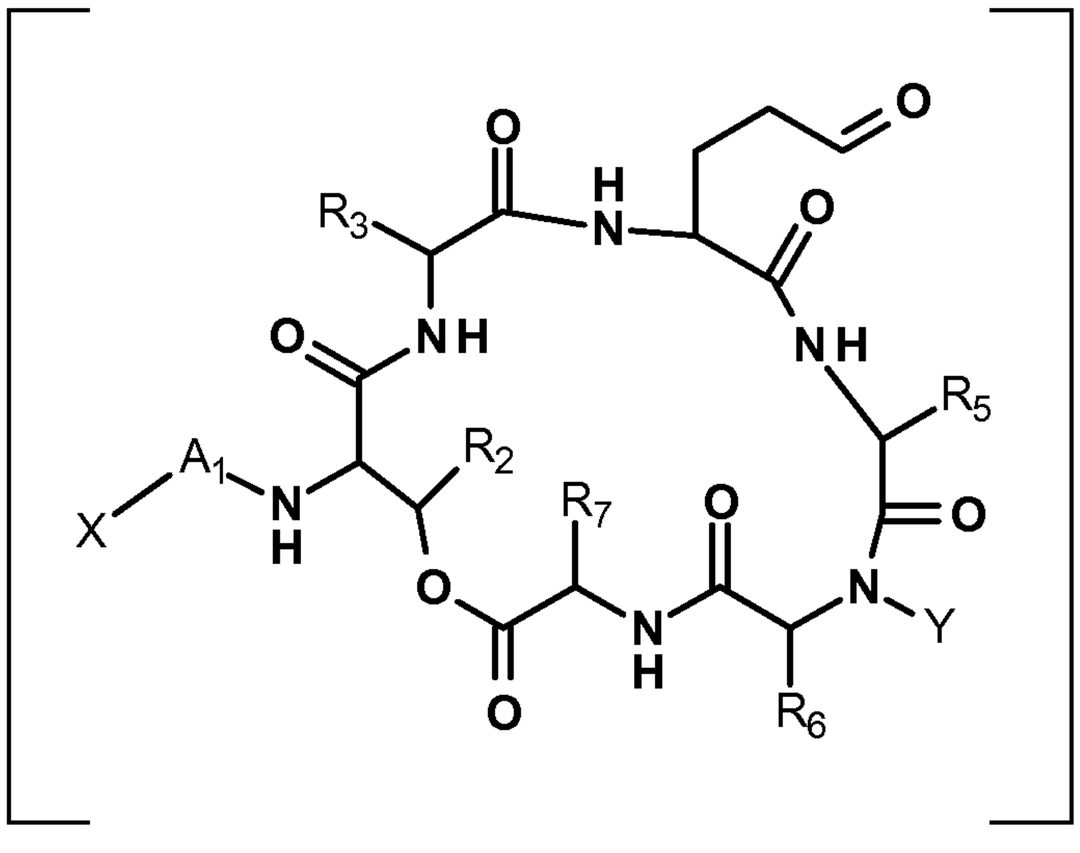
R_7 is sec-butyl;

X is isobutyryl;

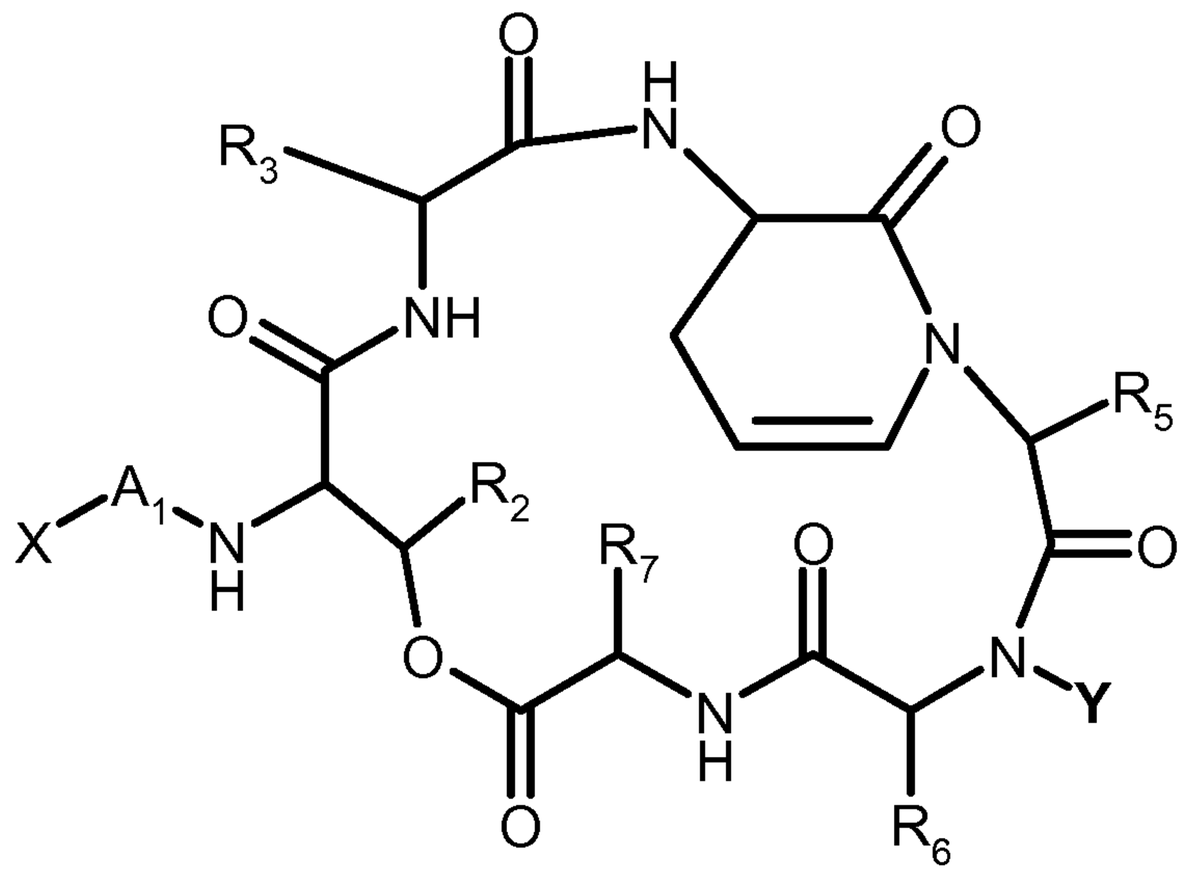
and

Y is methyl.

12. The method or process according to any one of the preceding claims, where the reaction after variant A or variant B leads via a compound or compound mixture including one or more compounds represented by the formula XXIII-1, XXIII-2 and XXIII-3,

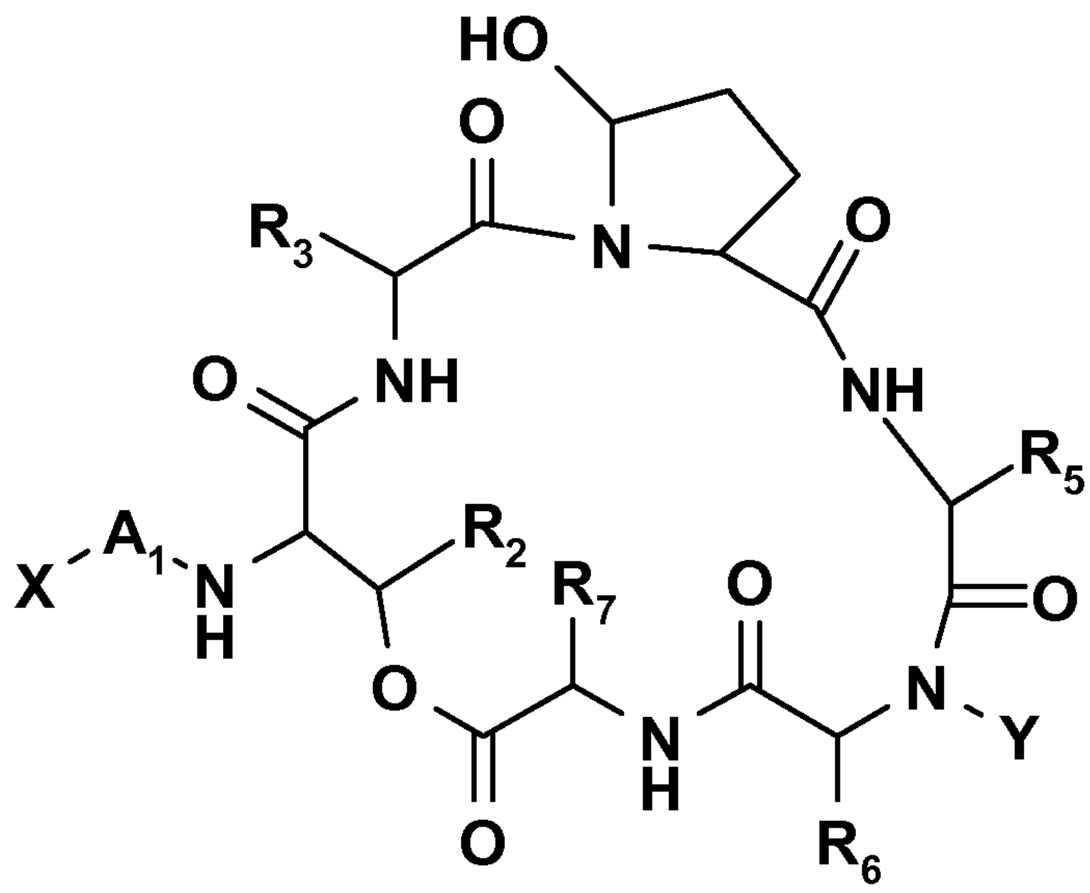


XXIII-1,



XXIII-2,

and/or XXIII-3

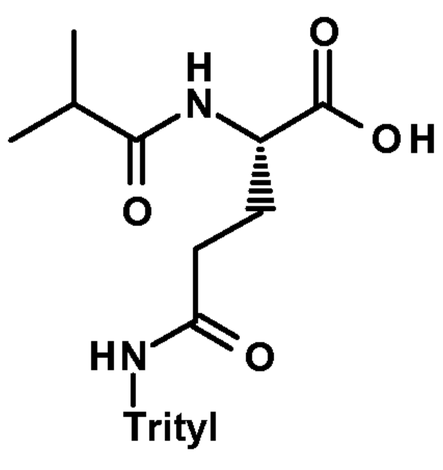


XXIII-3,

wherein the symbols A_1, R_2, R_3, R_5, R_6, R_7, X and Y have the meanings defined in claim 1 for the compound of formula I.

13. The method or process according to any one of the preceding claims, where each intermediate, independently of the others, is used or present in free or salt form.

14. A compound selected from the group consisting of compounds with the following formulae, or a salt thereof:



Trityl

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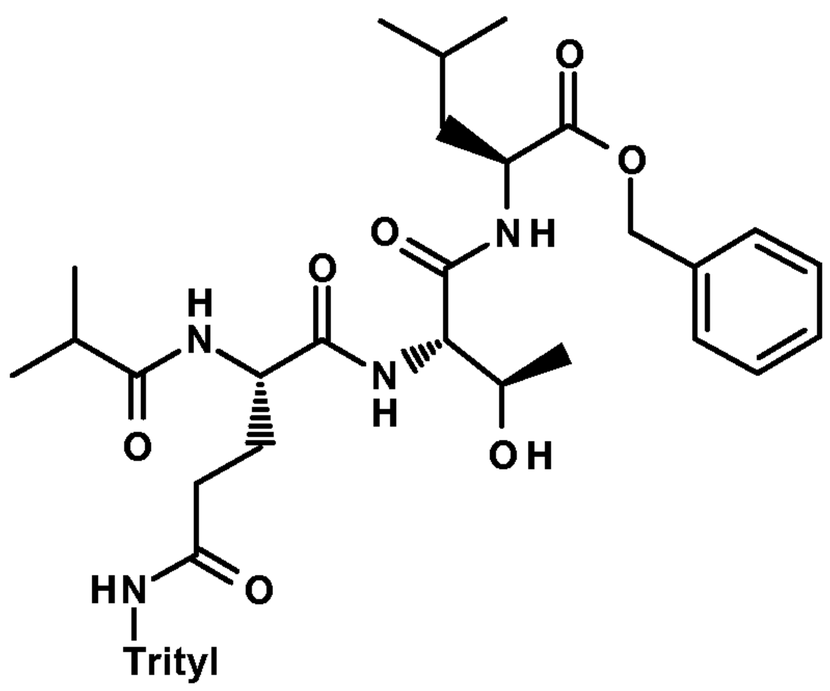

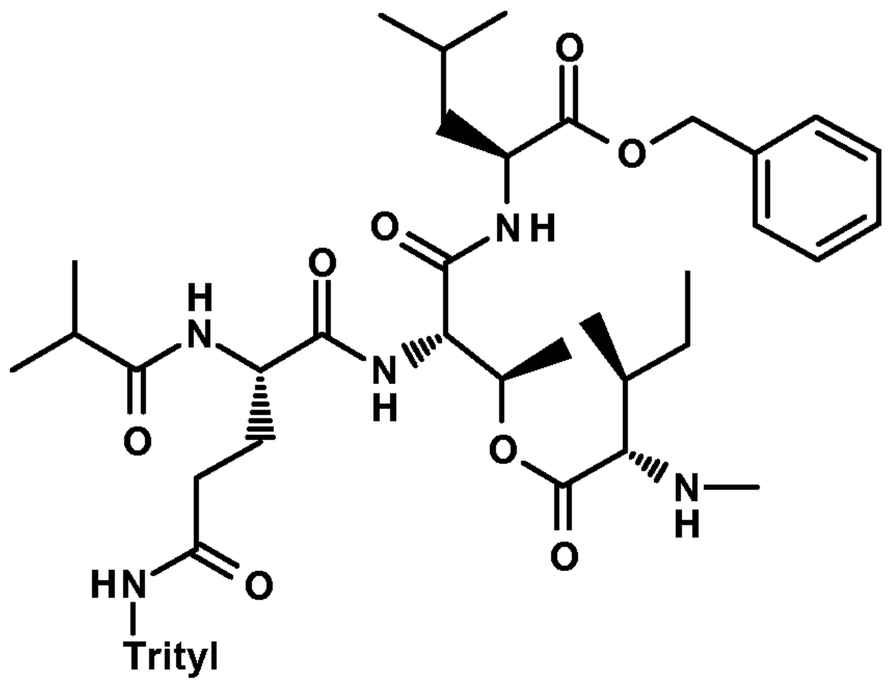

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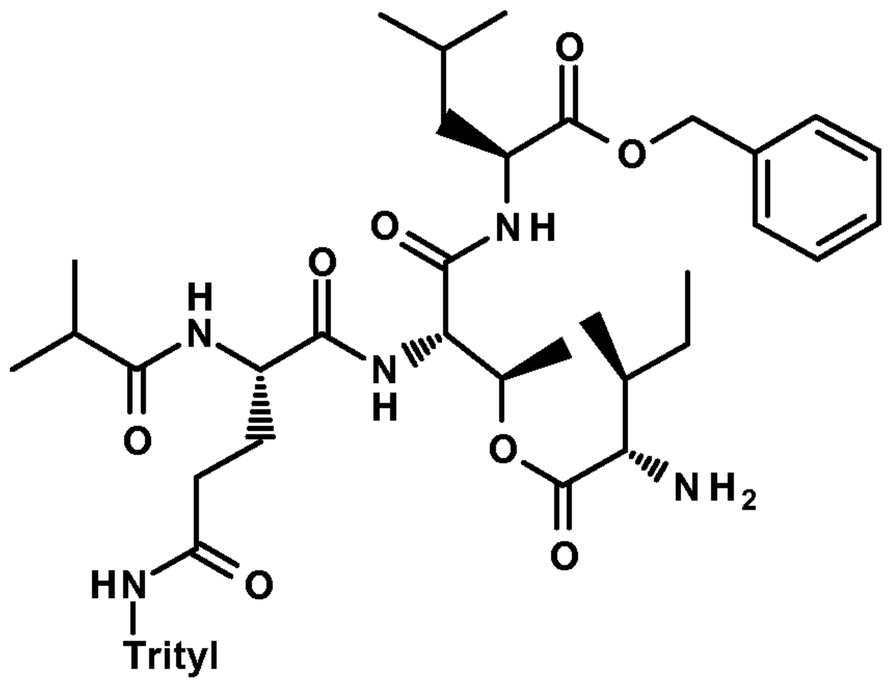
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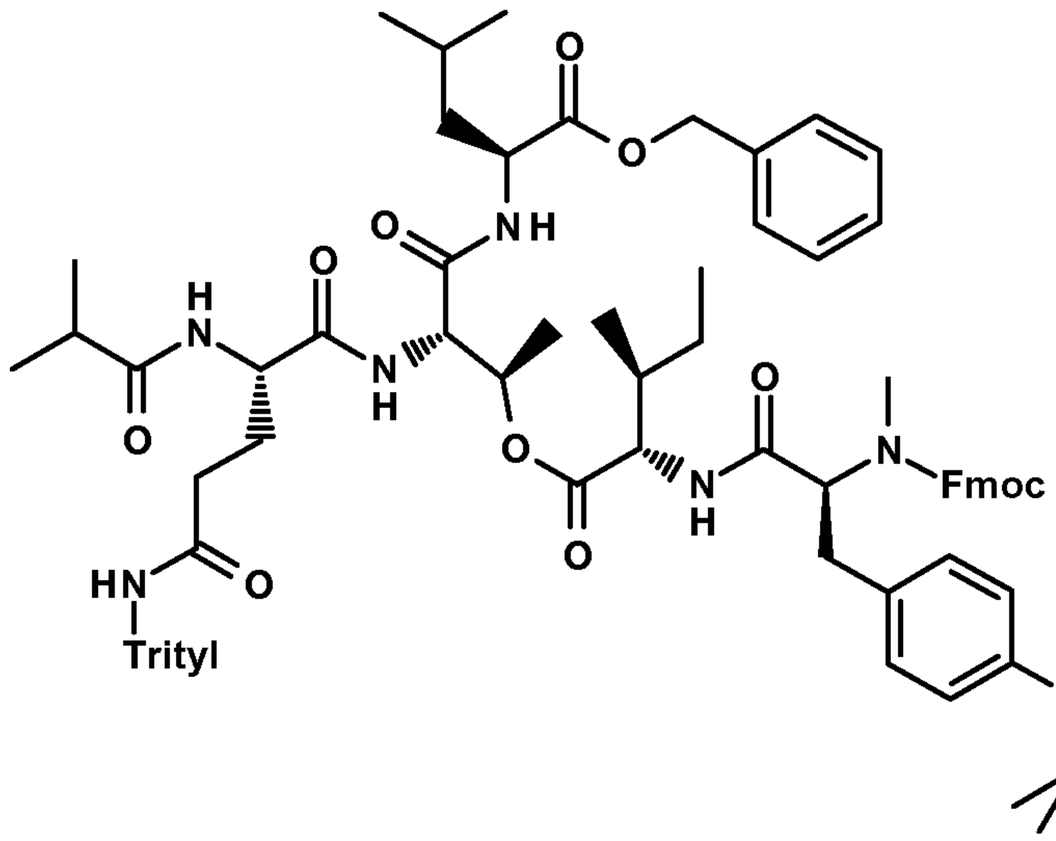
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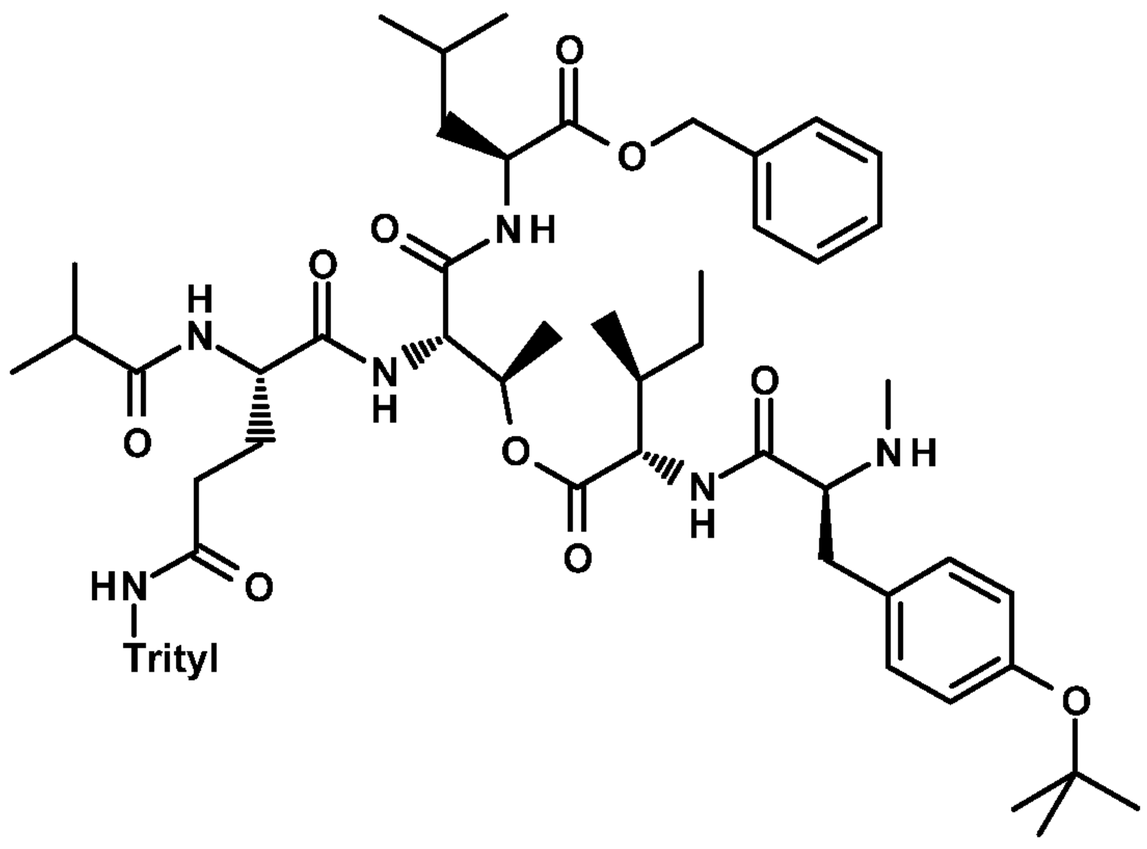
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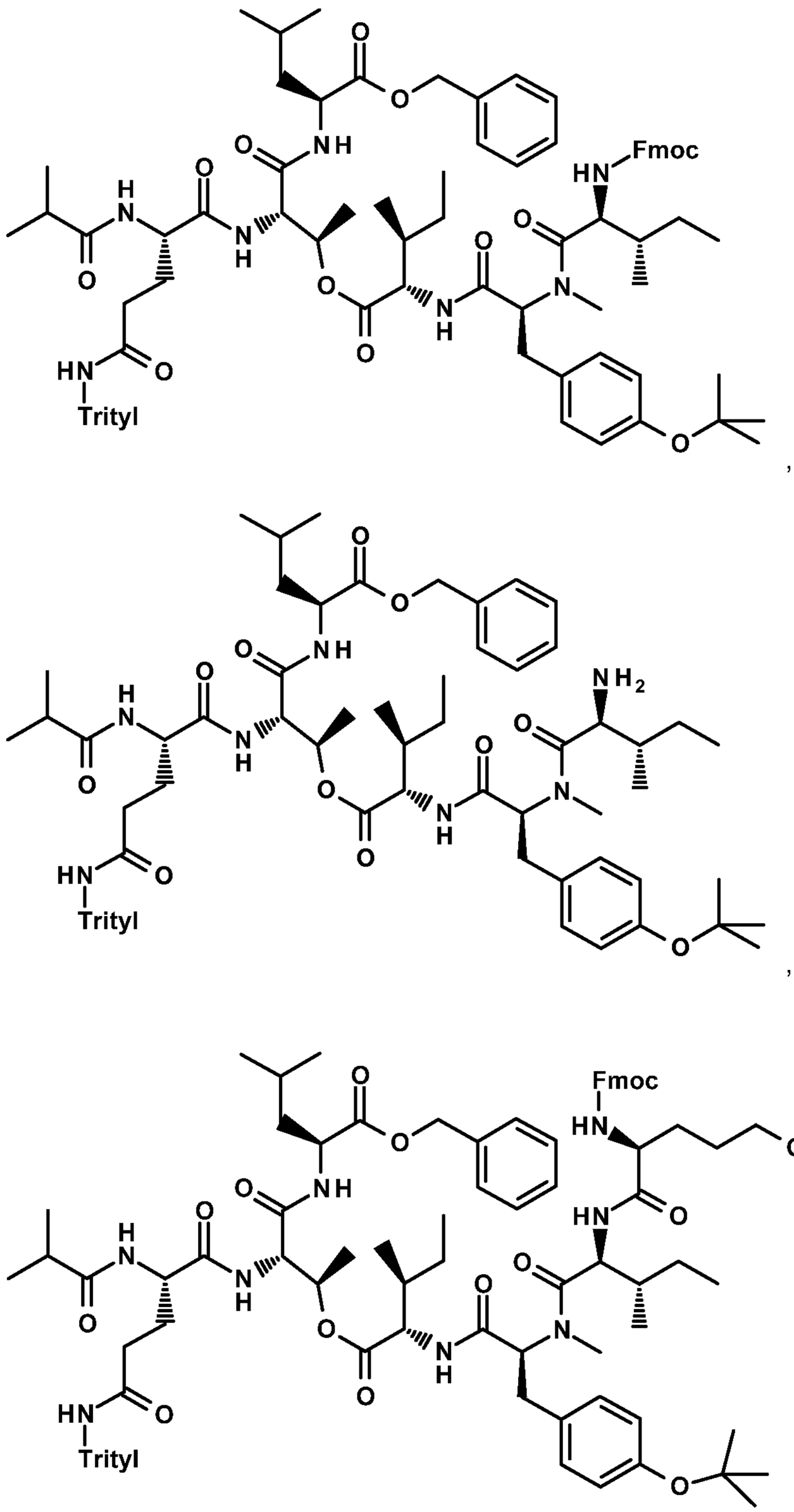
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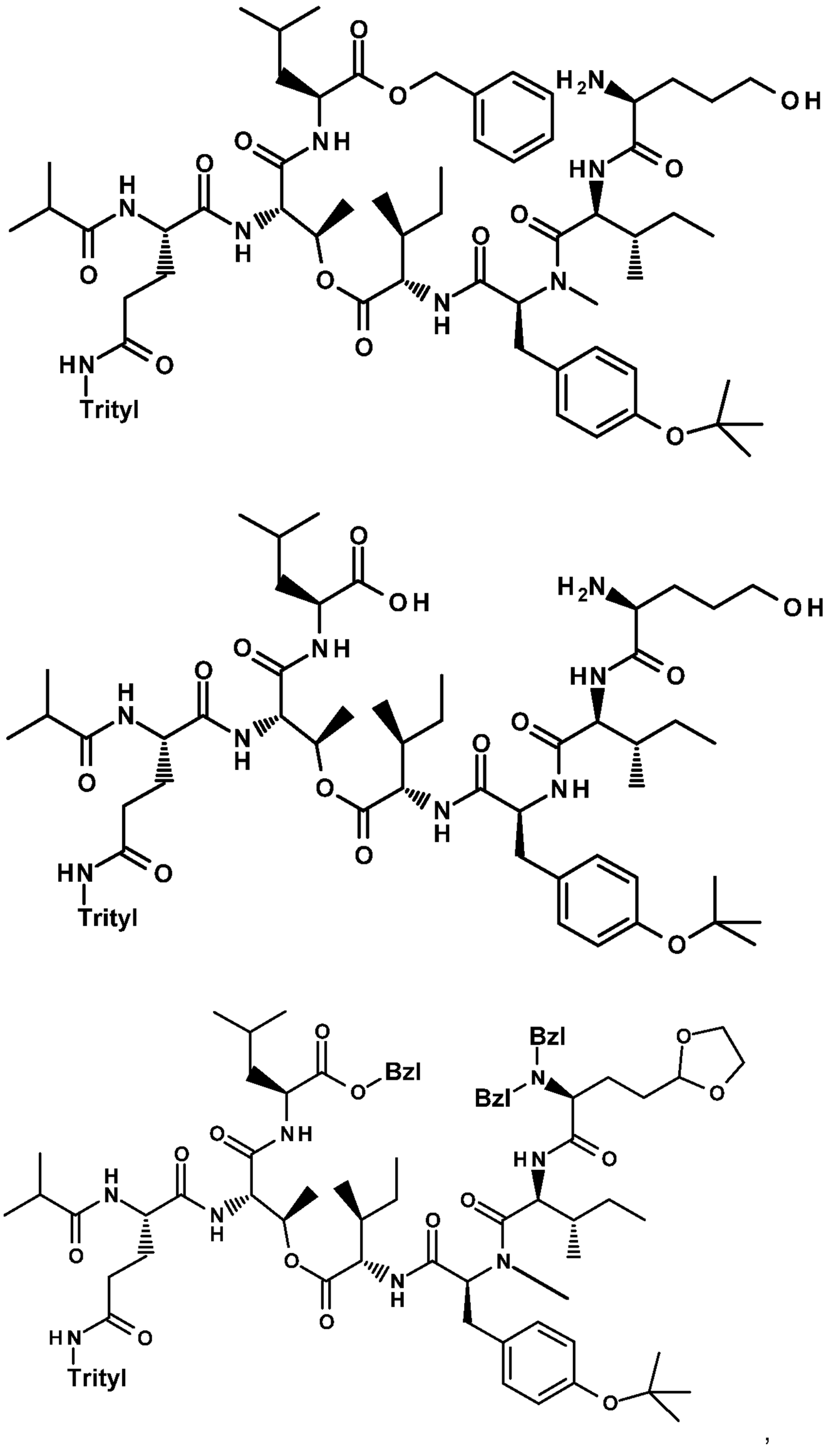
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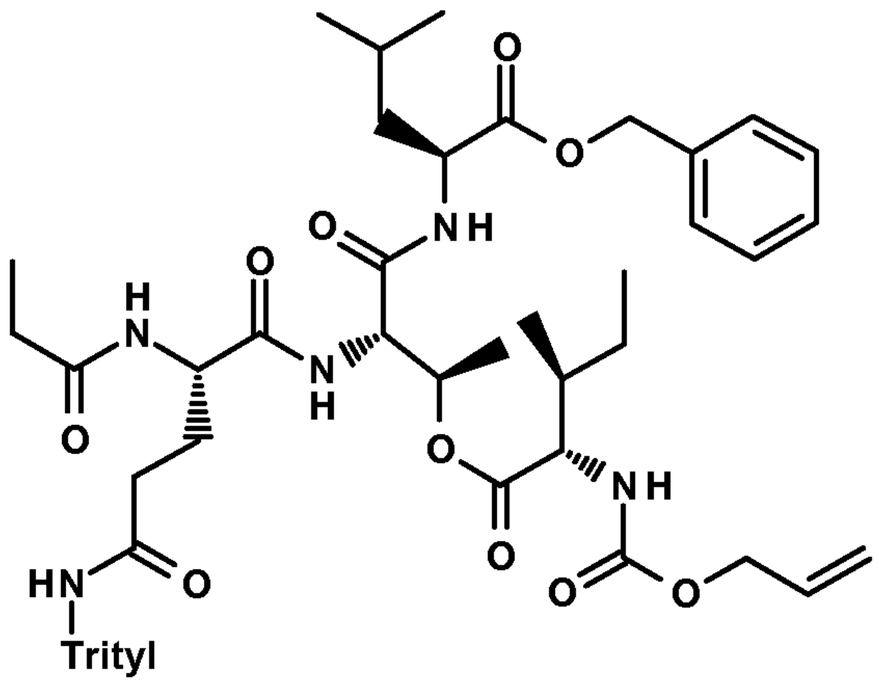
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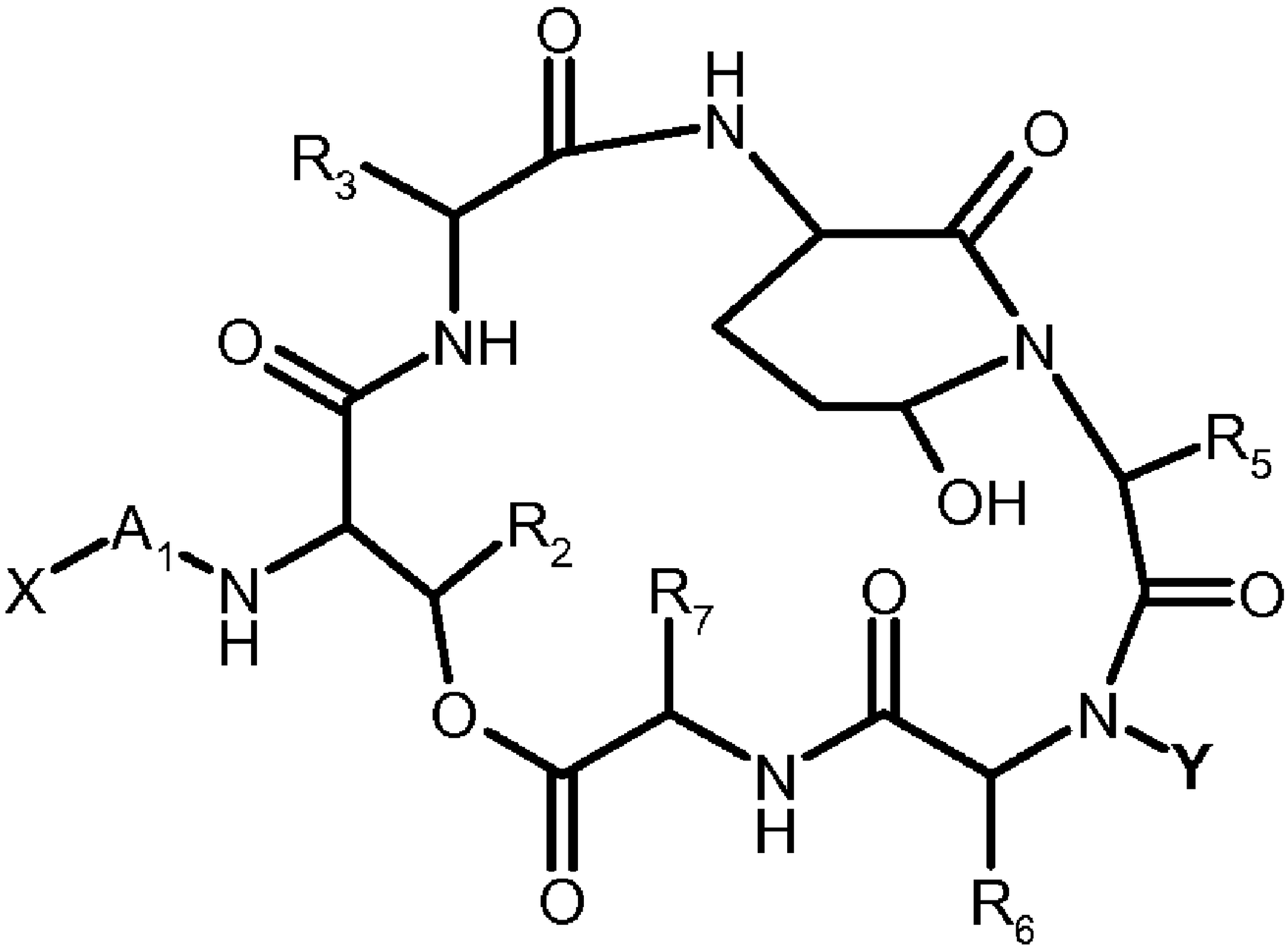

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