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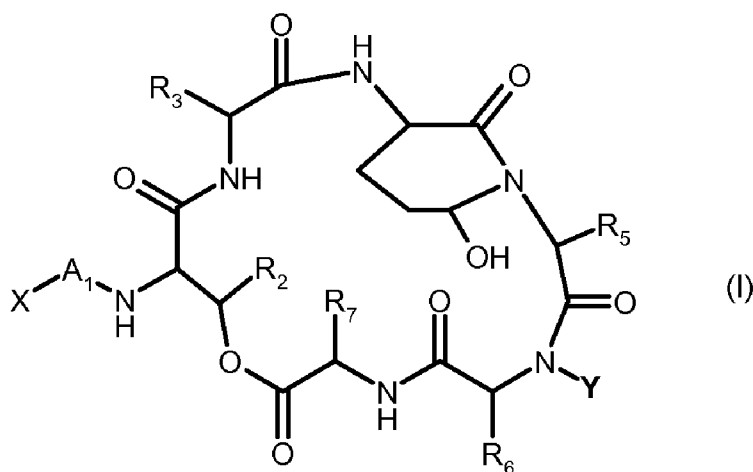
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(54) Title: ALDEHYDE ACETAL BASED PROCESSES FOR THE MANUFACTURE OF MACROCYCLIC DEPSIPEPTIDES AND NEW INTERMEDIATES



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

**Aldehyde Acetal Based 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 depsi-
5 peptides, to new intermediates and their manufacture, as well as related invention embodi-
ments.

Background of the Invention

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

Nostopeptin BN920, formerly isolated from the cyanobacterium Nostoc, was isolated
also from Microcystis. Nostopeptin BN920 inhibited chymotrypsin with an IC₅₀ value of
20 31 nM (see J. Nat. Prod. 68(9), 1324-7 (2005)).

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

Hitherto, the synthesis of such compounds was based on solution chemistry approaches
30 or in copending PCT application No. PCT/IB2012/051977 by a combination of solid
phase and solution peptide chemistry.

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A critical step is the formation of the ahp-substructure. This, according to published prior art, is mainly formed by oxidation of the open chain precursor amino acid 2-amino-5-hydroxy-pentanoic acid in the closed macrolactone ring by oxidative treatment via a labile aldehyde intermediate (see e.g. Yokohama et al., Tetrahedron 61 (2005), pp. 1459-80, compound 23; Yokohama et al., Pept. Sci. 38 (2002). Pp. 33-36; and Yokohama et al., Tetrahedron Lett. 42 (2001), 5903-8).

The aldehyde is too instable to be isolated. Therefore its direct use and synthesis are not recommended.

Aldehyde derivatives, such as acetals, are also known to be instable, in particular in the case where acetal and (especially free) carboxylic acid functions are present simultaneously or under (even only slightly) acidic conditions.

There is a need to find higher yielding processes and processes that are easier in handling for the manufacture of macrolactone ring systems comprising ahp moieties.

It has now been found that it is possible to replace the precursor with the 2-amino—5-hydroxypentanoic acid building block and use its 5-oxo-analogue in acetal form instead.

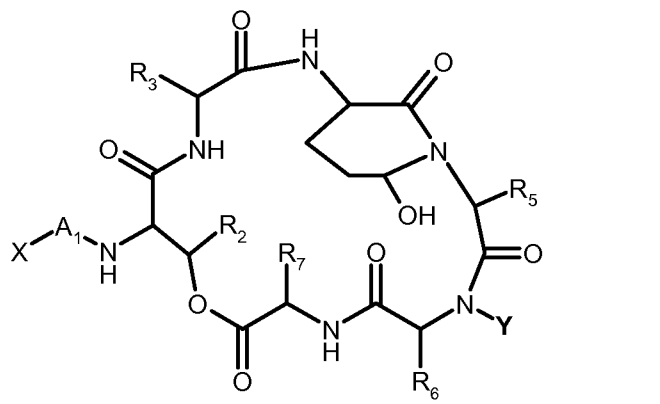
The present invention thus relates to processes or methods that allow obtaining such cyclic depsipeptides with increased yield and/or in good purity and with a lower number of steps.

In view of the many risks, such as racemization, tautomerization and the like, in the synthesis of a complex molecule with many possible isomers, it has been possible to find a manufacturing process, preferably comprising a mixture of solid phase peptide synthesis and reactions in solution, that allows to produce cyclic depsipeptides of formula I in good yield and/or with the required stereoisomeric purity, especially both, and that avoids the steps of oxidation of a hydroxyl group in the precursor molecule. 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. No synthesis has so far come to our attention making use of solid phase

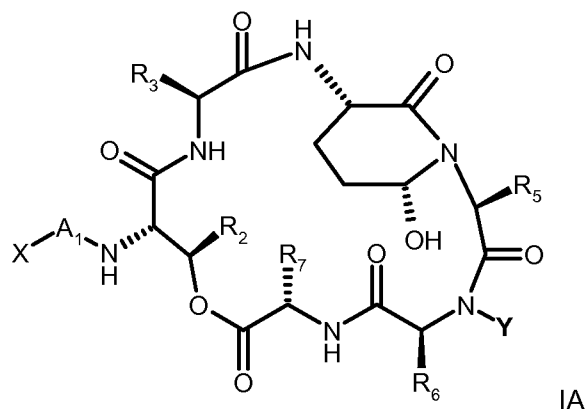
- 3 -

peptide synthesis in this field. In addition, the elimination of the oxidation step allows using N-Me-Tyrosine or analogues instead of the protected variants such as t-butyl-ethers or analogues thereof which are expensive and difficult to prepare.

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



especially of the formula IA



- 10 wherein
- A_1 is a bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group, especially asparagine or glutamine, and is bound at its right hand side in formula I via a carbonyl (preferably the carbonyl of an α -carboxyl group thereof) to the rest of the molecule; or is C_{1-8} -alkanoyl or phosphorylated hydroxy- C_{1-8} -alkanoyl;
- 15 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;

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R₅ is the side chain of an amino acid, preferably of phenylalanine, leucine, isoleucine or valine;

R₆ is the side chain of a hydroxy amino acid, especially of tyrosine;

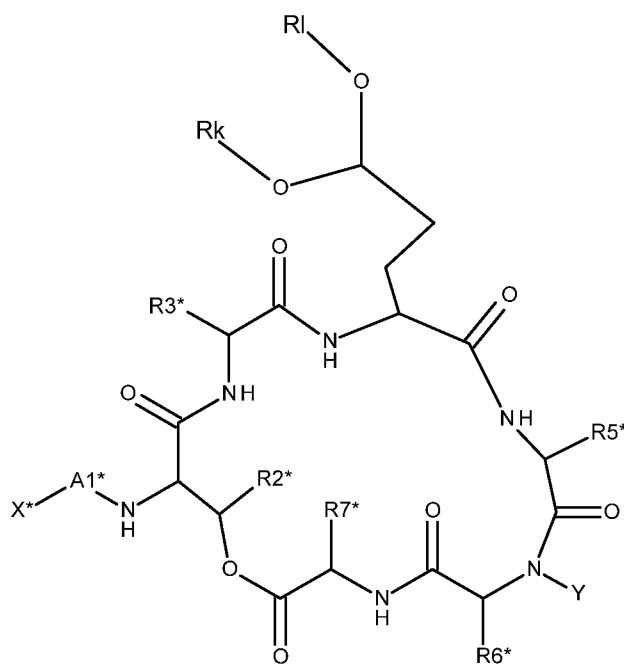
R₇ is the side chain of an amino acid, preferably of the amino acid leucine, isoleucine or valine; and

Y is hydrogen or C₁₋₈-alkyl;

or a salt thereof,

said method comprising

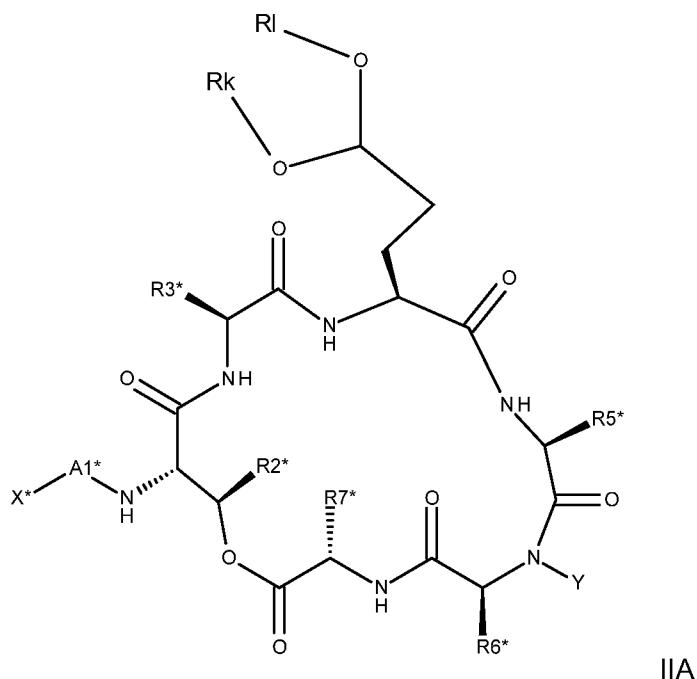
10 deprotecting a compound of the formula II



II

especially of the formula IIA

- 5 -



wherein the aldehyde protecting group(s) Rk and RI 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 RI then preferably forming an unsubstituted or substituted alkylene bridge, especially unsubstituted or substituted ethylen, such as $-\text{CH}_2-\text{CH}_2-$ or $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 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 ($-\text{O}-\text{P}(=\text{O})(\text{OH})_2$) are present in protected form at least if they could participate in undesired side reactions, to result in a compound of the formula 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, or especially IA, into the corresponding compound of the formula I, or especially IA.

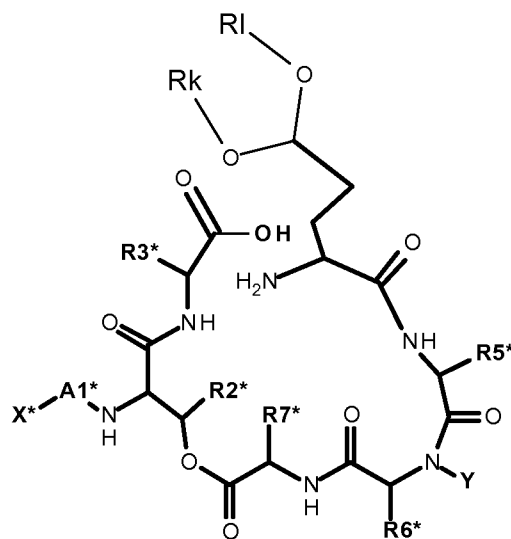
- 6 -

(ii/a) A further embodiment of the invention refers to the method or process described above, in addition comprising manufacturing the compound of the formula II or especially IIA by a combination of Solid Phase Peptide Synthesis (especially for synthesis of the precursor of formula III or III* or especially IIIA or IIIA* given below, and Solution Phase synthesis (especially from the compounds just mentioned to the final product) from the corresponding starting amino acids and side chain precursors.

(i/b) 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, 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, preferably using Solution Phase chemistry.

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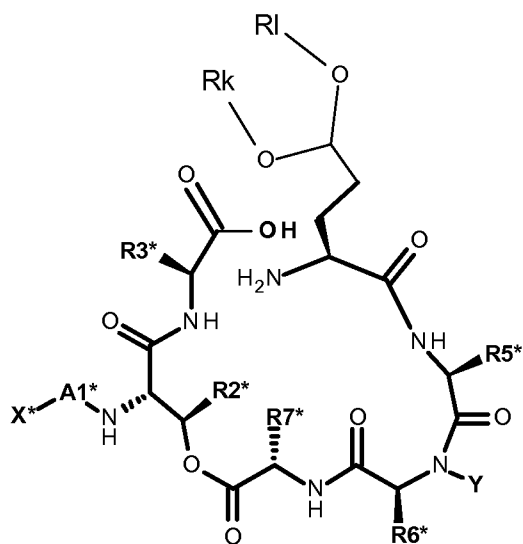
(ii/b) A further embodiment of the invention relates to the method or process according to the preceding paragraph (i/b), where the linear precursor peptide is of the formula III,



III

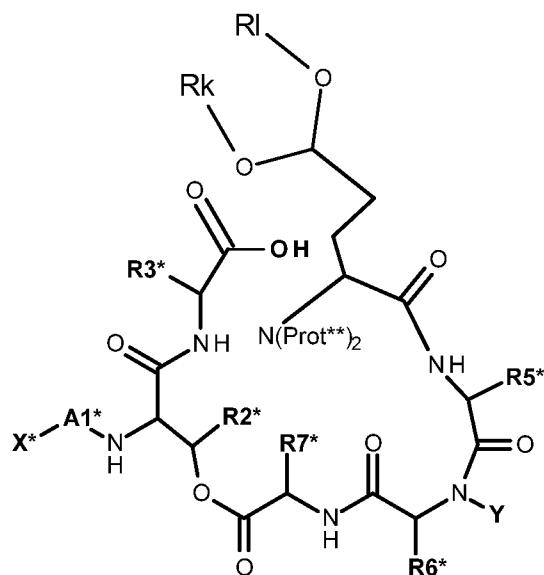
20 especially IIIA,

- 7 -



IIIA

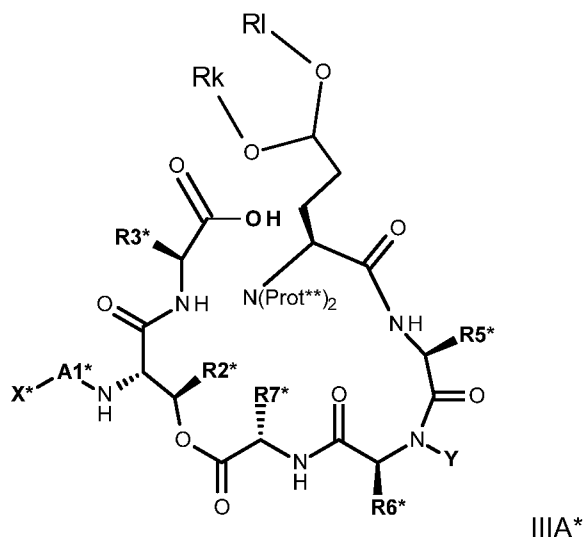
wherein Rk, R1, X*, A1*, R2*, R3*, R5*, R6* and R7* are as defined for a compound of the formula II above, which can be obtained directly from solid phase peptide synthesis (e.g. as described under step (iii/b) or by deprotection from the corresponding compound of the formula III*,



III*

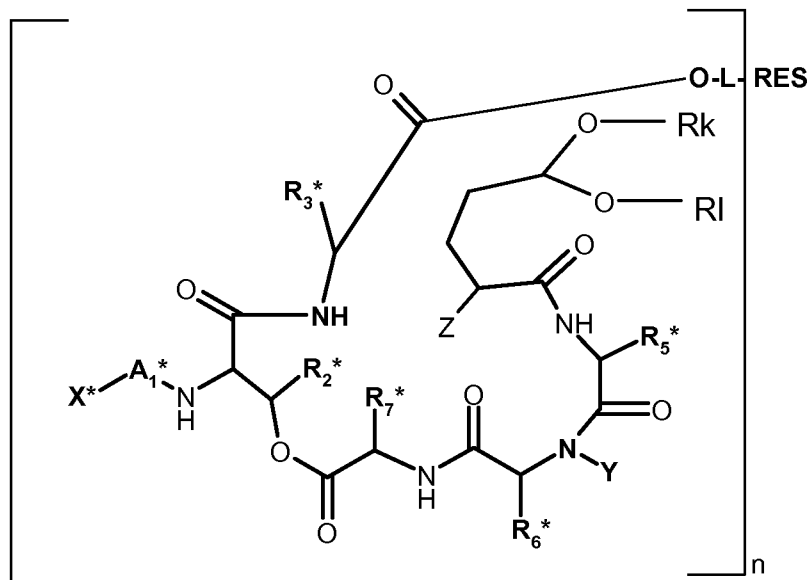
especially IIIA*,

- 8 -



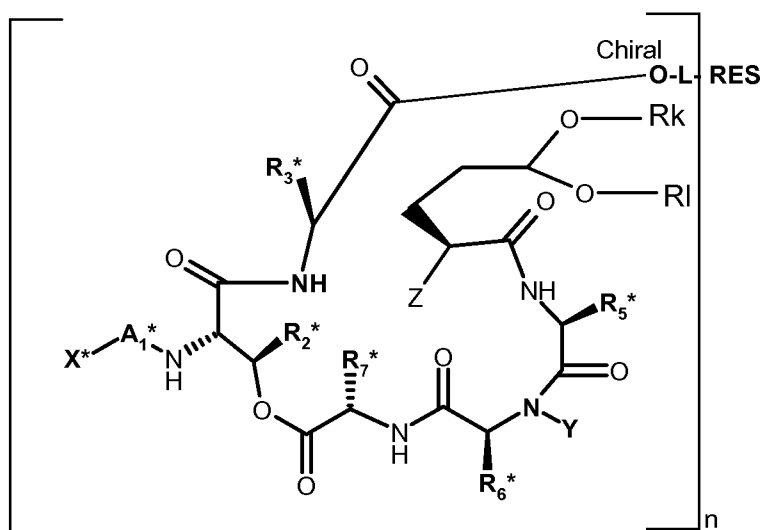
wherein Rk, Rl, X*, A₁*, R₂*, R₃*, R₅*, R₆* and R₇* are as defined for a compound of the formula II above and wherein each of the Prot** moieties is a protecting group that can preferably must) be removed under conditions different from those of the cleavage under
 5 (iii/b), especially each is an arylalkyl amino protecting group, as defined for a compound of the formula IV, by deprotecting the protected amino group.

(iii/b) Another embodiment refers to the method or process according to the preceding paragraph (ii/b), further comprising, for the synthesis of the compound of the formula III
 10 or especially IIIA, or of a compound of the formula III*, especially IIIA*, cleaving a compound of the formula IV,



IV

especially IVA,



IVA

5 wherein R_k , R_l , X^* , A_1^* , R_2^* , R_3^* , R_5^* , R_6^* and R_7^* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, n is a natural number not including 0 and

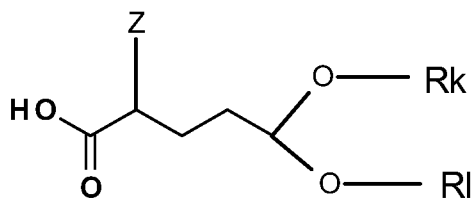
Z is a protected amino group either of the formula $NHProt^*$ wherein $Prot^*$ is a protecting group that is removed before or during the cleaving reaction or further subsequently to it

10 $[?]$ to yield a compound of the formula III, especially IIIA; or Z is a protected amino group of the formula $N(Prot^{**})_2$ wherein each $Prot^{**}$ is an amino protecting group that can (in

- 10 -

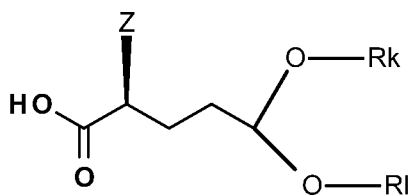
particular can only) be removed under conditions different from those of the cleaving reaction, especially each is an arylalkyl amino protecting group, to yield the compound of the formula III*, especially IIIA*.

- 5 (iv/b) A further embodiment of the invention relates to the method or process according to the preceding paragraph (iii/b), further comprising, for the synthesis of the compound of the formula IV, especially IVA, coupling an amino acid of the formula V,



V,

especially VA,



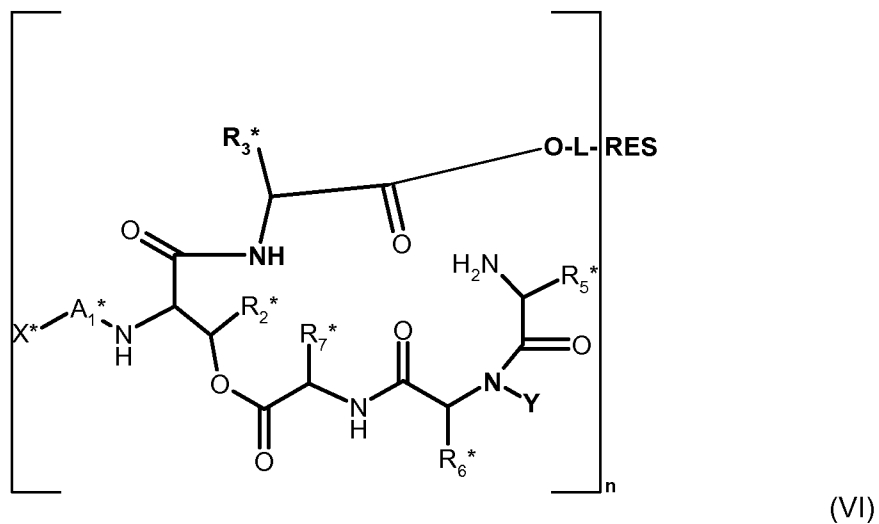
VA

10

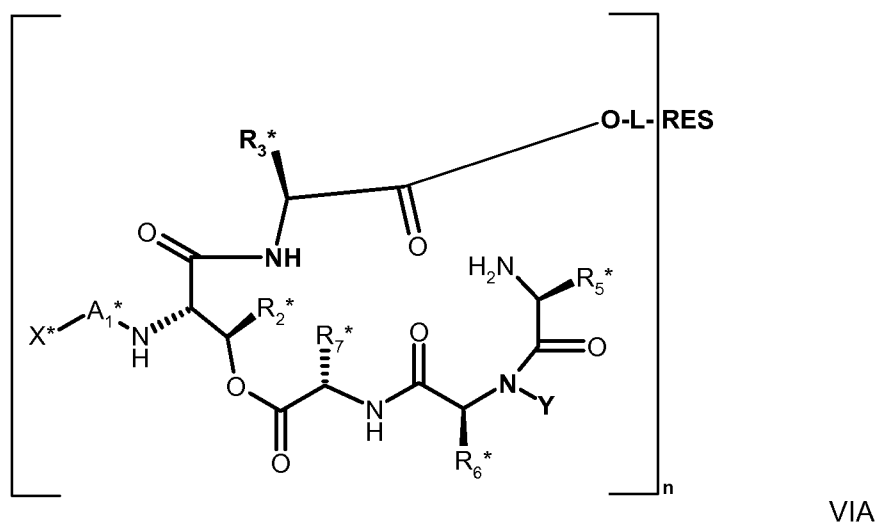
wherein Rk and RI are as defined for a compound of the formula II above and Z is a protected amino group either of the formula NHProt* wherein Prot* is a protecting group that can be (and is) removed before or during the cleaving reaction under (iii/b) or further subsequently; or Z is a protected amino group of the formula N(Prot**)₂ wherein each

15 Prot** is an amino protecting group that can be removed under conditions different to those of the cleaving reaction under (iii/b), especially each is an arylalkyl amino protecting group; or an activated derivative of said amino acid of the formula V or VA, with a compound of the formula VI,

- 11 -



especially VIA,

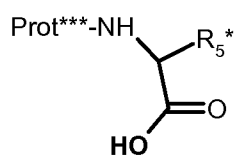


5

wherein X^* , A_1^* , R_2^* , R_3^* , R_5^* , R_6^* and R_7^* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0.

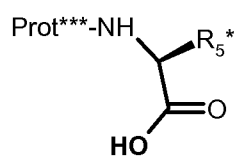
- 10 (v/b) Yet a further embodiment of the invention relates to the method or process according to the preceding paragraph (iv/b), further comprising, for the synthesis of the compound of the formula VI, especially VIA, coupling an amino acid of the formula VII

- 12 -



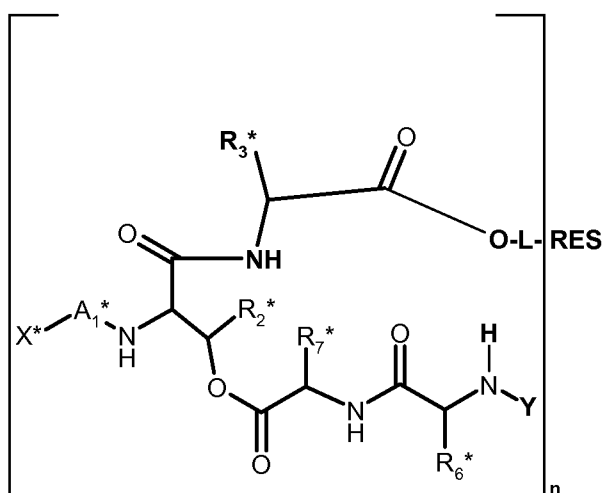
VII,

especially VIIA,



VIIA

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

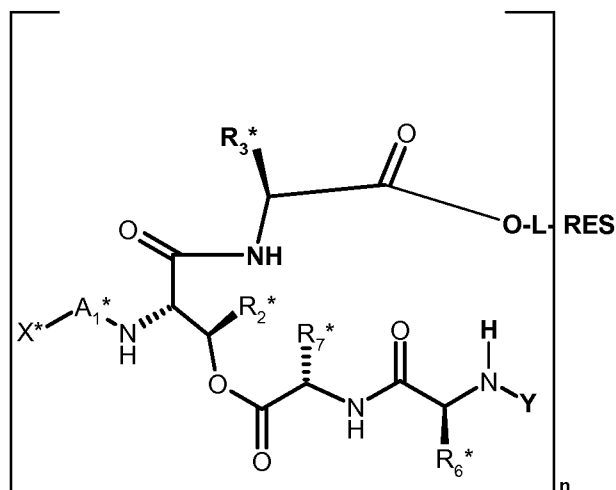


VIII

10

especially VIIIA

- 13 -

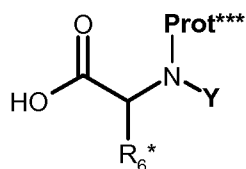


VIII A

wherein X^* , A_1^* , R_2^* , R_3^* , R_6^* and R_7^* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0, and removing the protecting group Prot***.

5

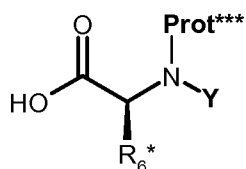
(vi/b) In yet a further embodiment, the invention relates to the method or process according to the preceding paragraph (v/b), further comprising, for the synthesis of the compound of the formula VIII, especially VIII A, coupling an amino acid of the formula IX,



IX

10

especially IXA

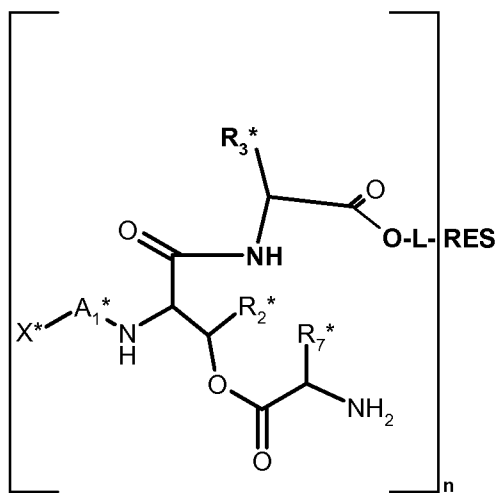


IXA

15

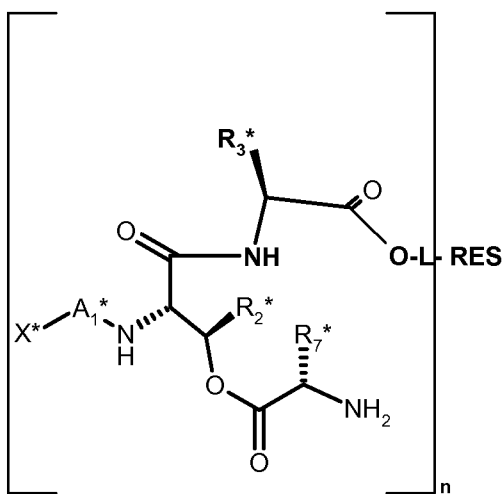
in which R_6^* and Y are as defined for a compound of the formula II above and Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the coupling product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula X,

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X,

especially XA,



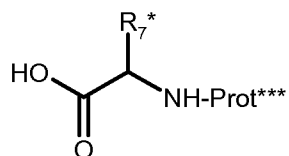
XA

5

wherein X^* , A_1^* , R_2^* , R_3^* and R_7^* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0, and removing the protecting group Prot^{***} .

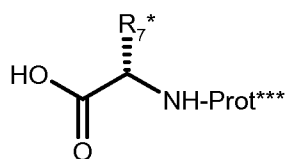
- 10 (vii/b) Another embodiment of the invention relates to the method or process according to the preceding paragraph (vi/b), further comprising, for the synthesis of a compound of the formula X, especially XA, reacting an amino acid of the formula XI,

- 15 -



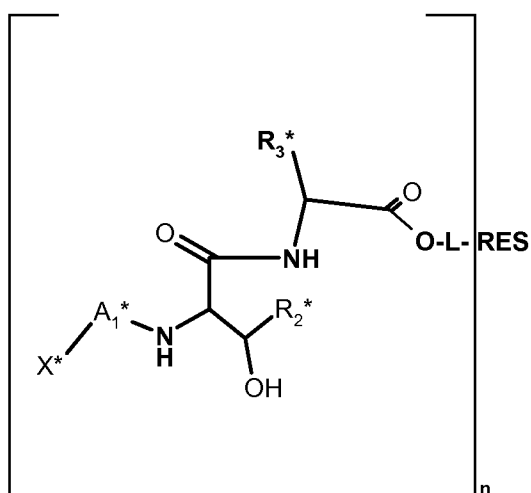
XI

especially XIA,



XIA

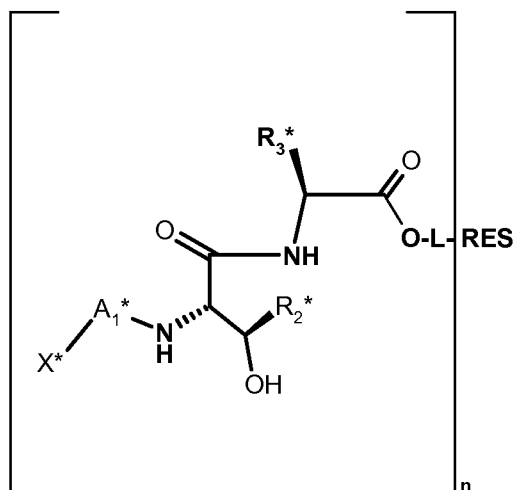
- 5 wherein Prot*** is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R₇* is as defined for a compound of the formula II above, or a reactive derivative of said amino acid,
- 10 with the hydroxyl group of a compound of the formula XII,



XII

especially XIIA,

- 16 -



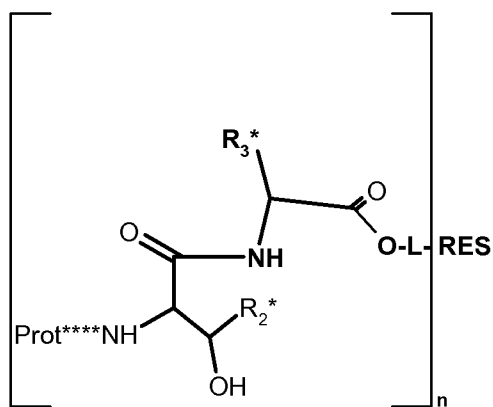
XIIA

wherein X^* , A_1^* , R_2^* and R_3^* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0; and removing the protecting group Prot^{***} .

5

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

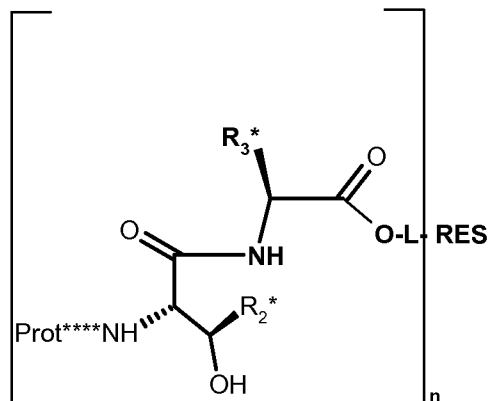
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XIII

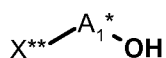
especially XIII A

- 17 -



XIII A

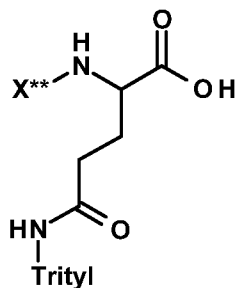
in which Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present in a compound of the formula II as defined above and with the product remaining on the resin, R_2^* and R_3^* are as defined for a compound of the formula II above, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0, after removal of the protecting group Prot**** via the thus obtainable free amino group, with an amino acid of the formula XIV,



XIV

10

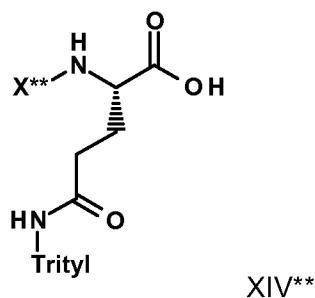
in particular of the formula XIV*,



XIV*

more particularly of the formula XIV**,

- 18 -



wherein X** is an amino protecting group or is X*, and wherein X* and A₁* are as defined for a compound of the formula II above, or a reactive derivative of said acid;

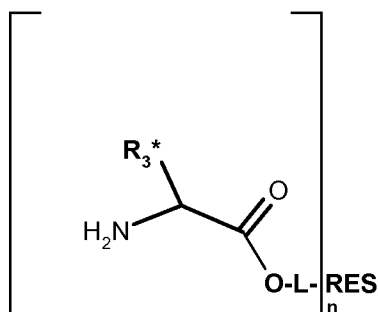
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and, if X** is an amino protecting group, removing said amino protecting group X** to yield the derivative of formula II wherein, instead of X*, H is present and coupling the resulting amino group with an acyl group X* using the corresponding acid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

10

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

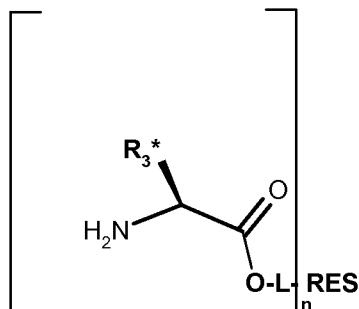
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XV

especially XVA,

- 19 -

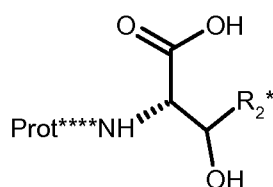


XVA

wherein R_3^* is as defined for a compound of the formula II above, L is a cleavable linker,

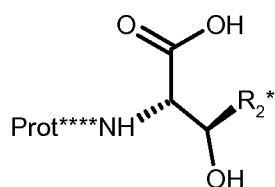
5 RES is a solid resin, and n is a natural number not including 0,

with an amino acid of the formula XVI,



XVI,

especially XVIA,

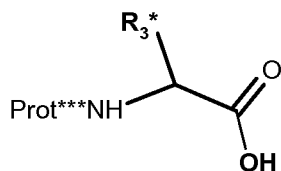


XVIA

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

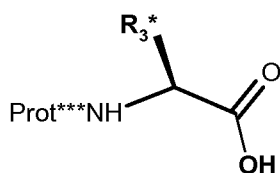
15 (x/b) A further embodiment of the invention relates to the method or process according to the preceding paragraph (ix/b), further comprising, for obtaining the resin bound amino acid of the formula XV, especially XVA, coupling an amino acid of the formula XVII,

- 20 -



XVII

especially XVIIA,



XVIIA

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

10

(i/c) Another embodiment of the invention relates to the method or process according to any one of the preceding paragraphs (i/a) to (x/b) where the symbols A_1 , 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,

15

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

R_2 is methyl;

20

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

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

R_6 is 4-hydroxybenzyl;

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

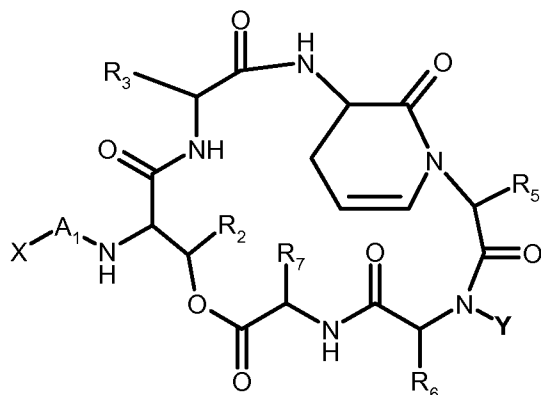
X is acetyl or isobutyryl, or is absent if A_1 is 2S-(2-hydroxy-3-phosphonooxy)-propionyl

25

and

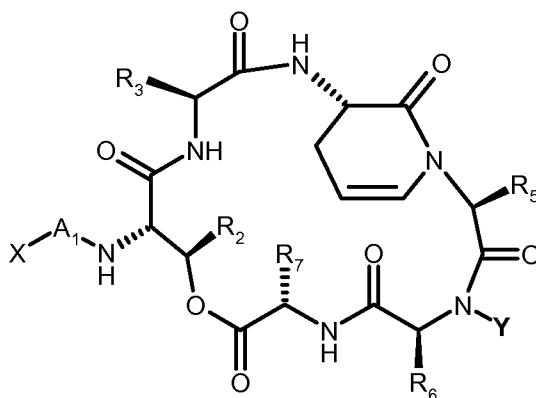
Y is methyl.

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



XVIII,

especially XVIIIA,



XVIII A

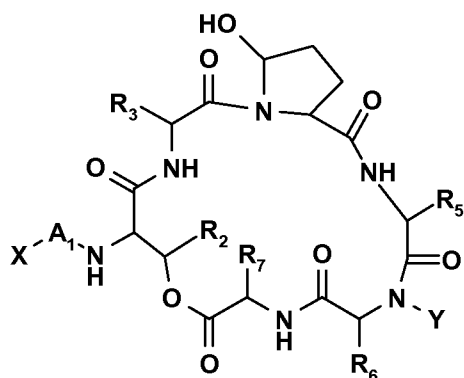
10

in which Y, X, A₁, R₂, R₃, R₅, R₆ and R₇ are as defined for a compound of the formula I above;

15

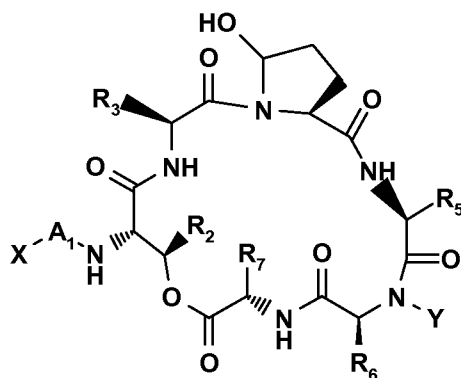
or especially a method or process for shifting the equilibrium of a mixture of a compound of the formula I and its corresponding dehydrate, and/or its corresponding hemiaminal analogue with a five-ring instead of the ahp structure in formula I which may also be formed as byproduct and has the formula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

- 22 -



XIX

especially the formula XIXA,



VIXA,

5

in which Y, X, A₁, R₂, R₃, R₅, R₆ and R₇ are as defined for a compound of the formula I above, respectively;

10 in favor of the compound of the formula I,

said method or process comprising using an aqueous acid as reactive solvent to drive the reaction. This method is especially used in addition to the other processes or methods described above and below to increase the yield or to re-convert a compound of the formula V, especially VA, and/or the analogue with a five-membered ring instead of the ahp structure in formula I, into the corresponding compound of the formula I.

15

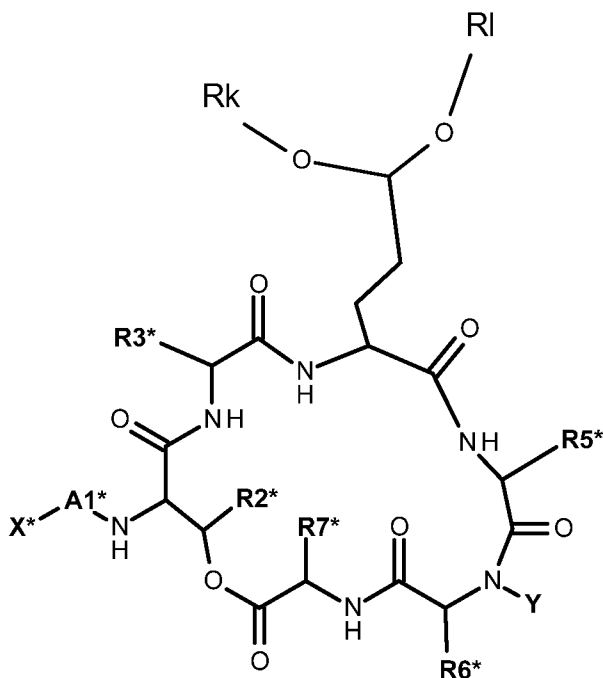
The method described for the conversion of the dehydrate and /or the five ring analogue (always regarding the desired ahp ring) into the desired compound of the formula I or es-

pecially IA, e.g. of Compound A -dehydrate from Example 3 B into Compound A, enables a straight-forward synthesis of this class of compounds. Up to now, an acidic treatment as final step had to be circumvented in order to avoid the dehydration of the product.

5

(i/e) A further embodiment of the invention relates to the method according to the preceding paragraph (i/d), wherein the acid is a carboxylic acid, especially a halo substituted C₁₋₈alkanoic acid, more especially trifluoroacetic acid or trichloroacetic acid.

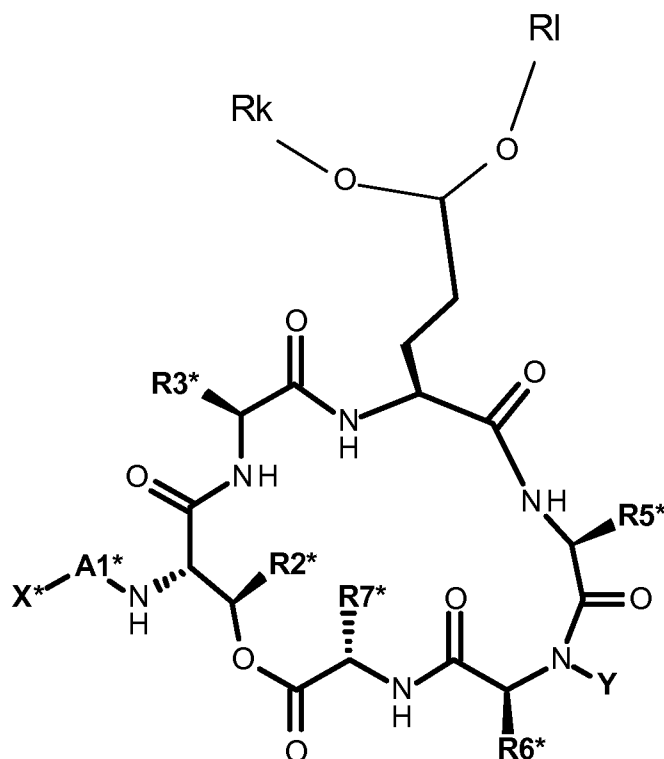
10 (i/f) The invention, in yet a further embodiment, relates to a compound of the formula II,



II

especially of the formula IIA,

- 24 -



XXA

wherein Rk and RI are as defined for a compound of the formula II above, Y is as defined for a compound of the formula I in the first instance above or in particular as defined above under (i/c) and X*, A1*, R2*, R3*, R5*, R6*, and R7* correspond to X, A1, R2, R3, R5, R6, and R7 in formula I as defined above or below or in paragraph (ia) or especially under (i/c) given above, respectively, however with the proviso that reactive functional groups on these moieties are preferably present in protected form.

(i/g) In a further embodiment, the invention relates to a novel compound selected from the group consisting of compounds of the formula II, III, III*, IV and V, and especially of the formula IIA, IIIA, IIIA*, IV and VA yet more especially to the group consisting of the following (especially enantiomerically enriched or pure) compounds: Compound 2*, Compound 3*, compound 5*, preferably Compound 2A*, Compound 3A*, Compound 4A*, , Compound 5A*, especially in the form given in the examples: Compound 2, Compound 3, Compound 5, Compound 6, Compound 7, Compound 8, Compound A, Compound 9 and Compound 10 and enantiomerically enriched or especially pure Compound 4, as well as compounds 12, 14, 15, 16 and 17. Also preferred are the compounds of the formulae 12*, 12A*, 14*, 14A*, 15*, 15A*, 16*, 16A*, 17* or 17A*

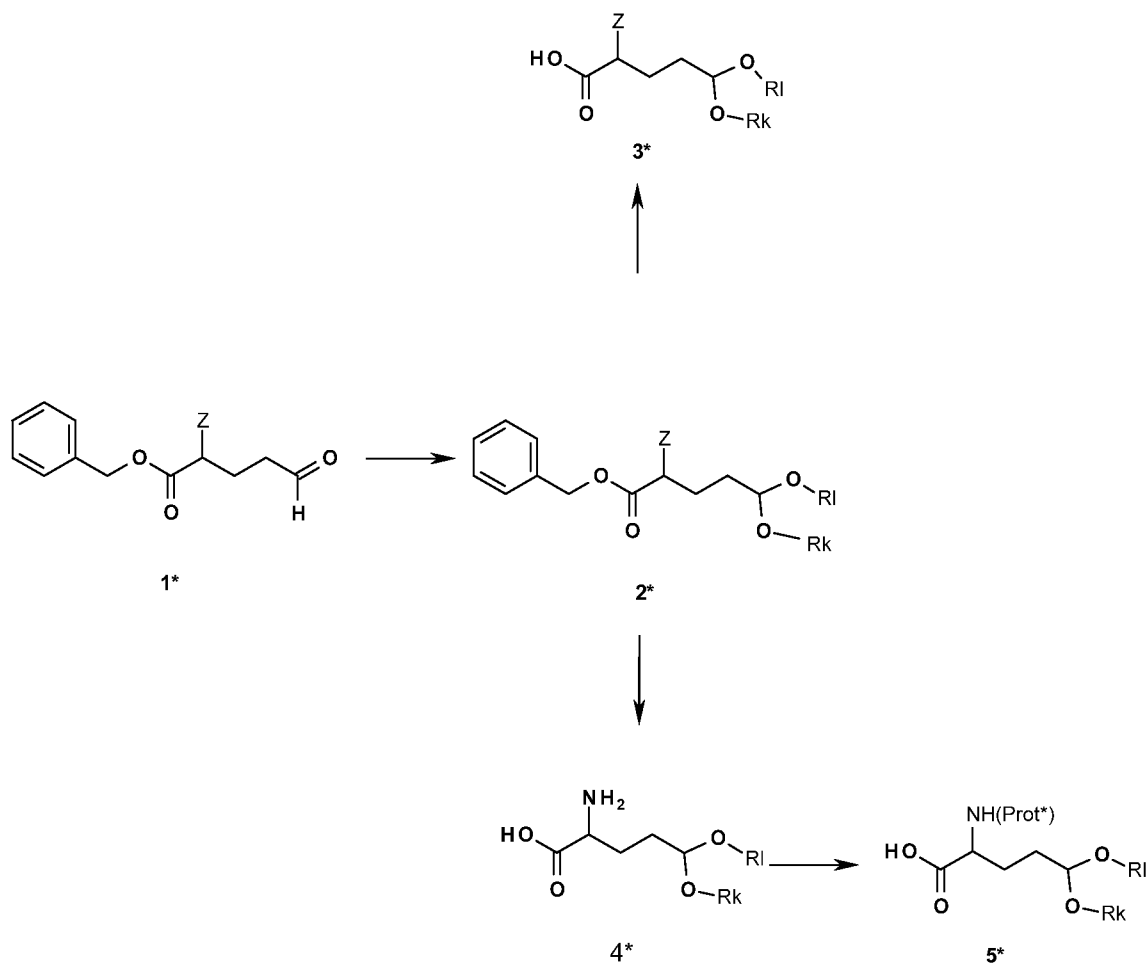
- 25 -

given below, in which *Rl* and *Rk* independently of each other are 1-alkyl, such as 1-(C₆-C₁₂aryl)-C₁-C₇alkyl, more especially benzyl, as well as the corresponding compounds wherein *Rl* and *Rk* together form an unsubstituted or substituted alkylene, especially –CH₂-CH₂- or –CH₂-CH₂-CH₂-.

5

(i/h) In yet a further embodiment, the invention relates to a method or a process for the synthesis of a compound of the formula V, especially of the formula VA mentioned above, according to either

- (a) (especially) in the case of the synthesis of a compound of the formula V wherein *Rk* and *Rl* together form an unsubstituted or substituted lower alkylene bridge, especially –CH₂-CH₂- or –CH₂-CH₂-CH₂-, the following reaction scheme, alternatively via the route (i) 1* -> 2* -> 3*, (ii) 1* -> 2* -> 4* -> 5*, or (iii) 1* -> 2* -> 3* -> 5*:



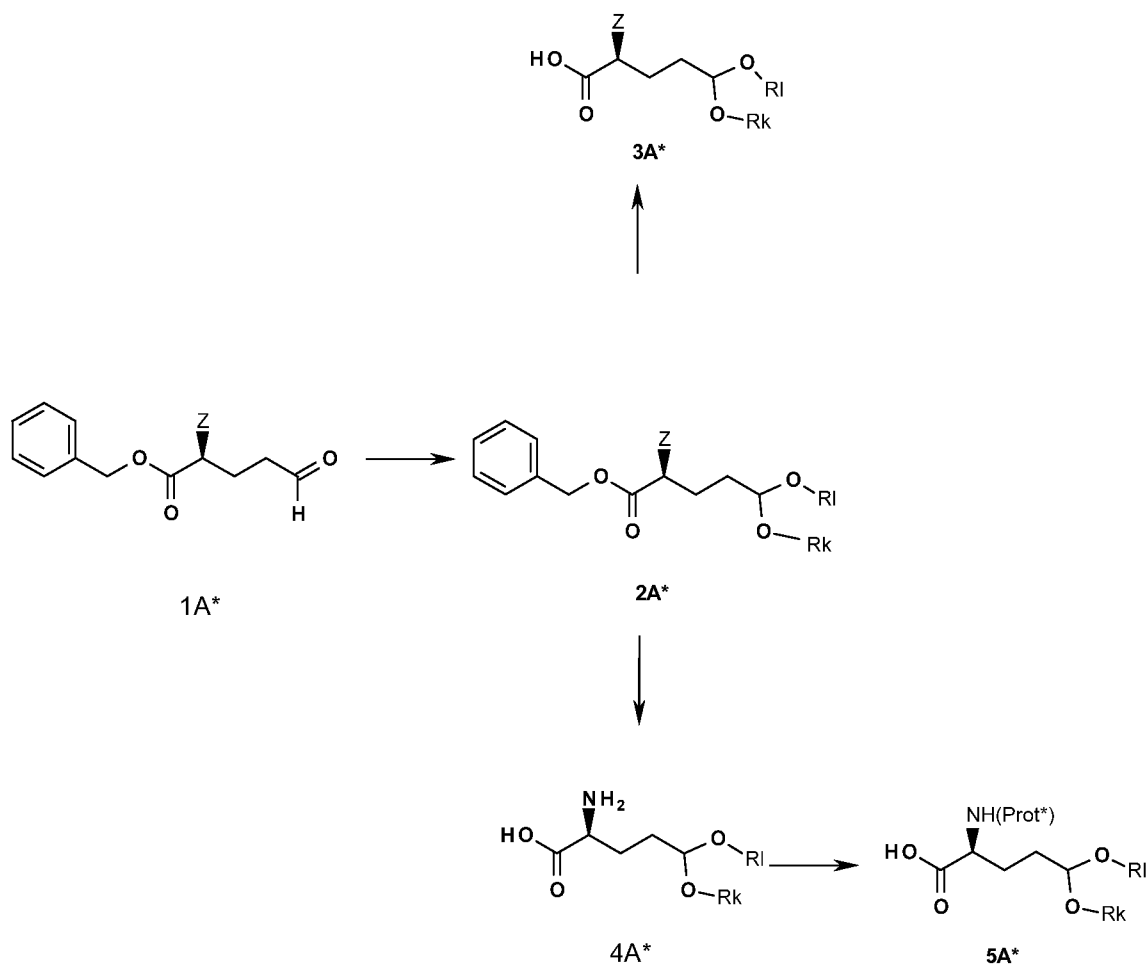
15

- 26 -

(wherein R_k, R_l have the meanings just indicated and Z has the meanings mentioned above for a compound of the formula V, especially in the compounds 1*, 2* and 3* being N(Prot**)₂ as defined for a compound of the formula V, especially 1-(C₆-C₁₂-aryl)- C₁-C₆-alkyl, especially benzyl, and in the compound 5* being NHProt* as defined for a compound of the formula V, Prot* especially being an acyl protecting group, e.g. fluoren-9-yl-methoxycarbonyl, and the compounds of formula 3* and 5* each correspond to a compound of the formula V;

or especially, to obtain a compound of the formula VA, the following scheme,

alternatively via the route (i) 1A* -> 2A* -> 3A*, (ii) 1A* -> 2A* -> 4A* -> 5A*, or (iii) 1A* -> 2A* -> 3A* -> 5A*:



(wherein R_k, R_l have the meanings just indicated and Z and Prot* have the meanings mentioned above for a compound of the formula V, especially Z in the compounds 1A*, 2A* and 3A* being 1-(C₆-C₁₂-aryl)- C₁-C₆-alkylamino, especially benzylamino, and Prot*

- 27 -

in the compound 5A* being an acyl protecting group, e.g. fluoren-9-yl-methoxycarbonyl, where the compounds of formula 3A* and 5A* each correspond to a compound of the formula VA;

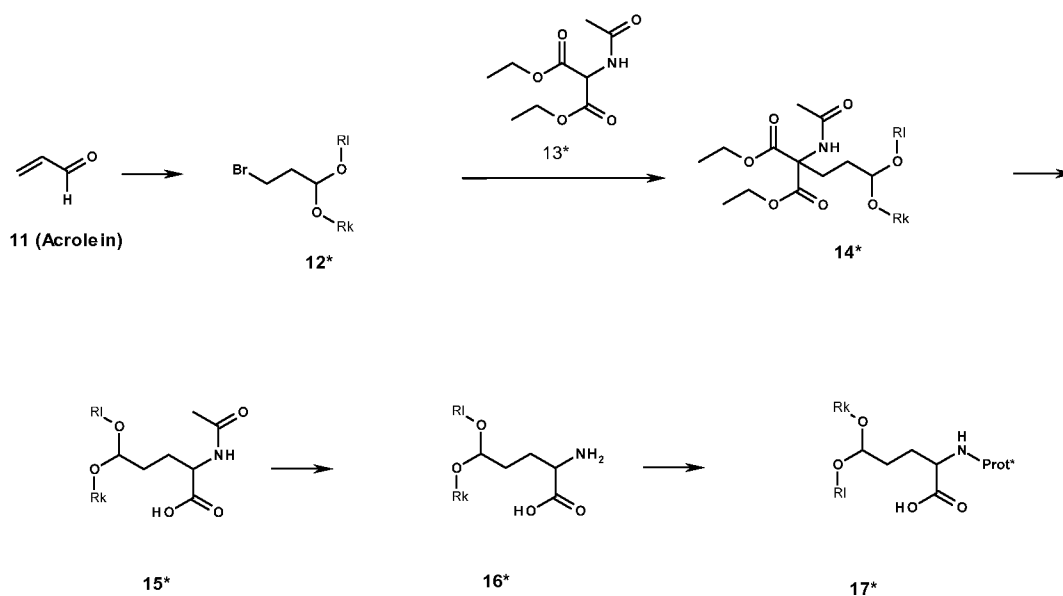
- 5 where the reaction of 1* or 1A* to 2* or 2A* is an acetal formation reaction with an unsubstituted or substituted lower alkylendiol, especially ethylene glycol, e.g. in an appropriate solvent, such as dichloromethane, in the presence of an acid, such as toluene sulfonic acid, in the presence of e.g. molecular sieve; the reaction of 2* or 2A* to 3A or 3A* by hydrolysis in the presence of a base, such as an alkali metal hydroxide, 10 e.g. LiOH, in an ether, e.g. dioxane, and water; or the alternative reaction from 2*, especially 2A* to 4*, especially 4A*, under deprotection of the carboxy and the amino group is made by e.g. catalytic hydrogenation, e.g. hydrogenation with a noble metal catalyst, e.g. Pd or Pt, e.g. on a carrier such as aluminium oxide or carbon, in an appropriate solvent, e.g. an alcohol, such as methanol, ethanol or isopropanol, followed 15 by reintroduction of an amino protecting group Prot**, especially an acyl protecting group, such as fluoren-9-yl-methoxycarbonyl, e.g. under acylation conditions or in the presence of a coupling agent as mentioned below for amino acid or acid couplings to amino groups, especially using the (e.g. Fmoc-) HOSU-ester, a tertiary base, e.g. triethylamine, and an appropriate solvent, e.g. water and/or acetonitrile; where 20 alternatively the compound of formula 4*, especially 4A*, may be obtained from a compound of the formula 3*, especially 3A*, by catalytic hydrogenation as just mentioned;

- and where the compound 1* or 1A* may be obtained by or in analogy to the method 25 mentioned in Rodriguez and Taddei, Synthesis 2005, 3, pp. 493-495);

or

- (b) (especially in the case of the synthesis of a compound of the formula V, especially VA mentioned above, wherein each of R_k and R_l is an unsubstituted or 30 substituted alkyl moiety, especially 1-aryl, such as 1-(C₆-C₁₂aryl)-C₁-C₇alkyl, more especially benzyl) according to the following reaction scheme:

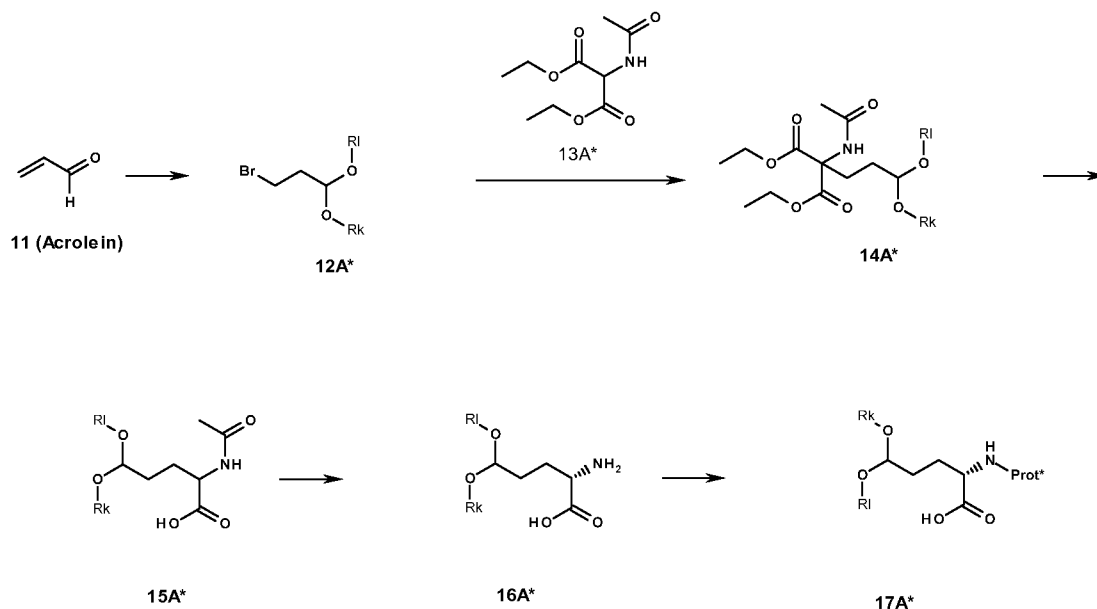
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(wherein Rk, RI have the meanings just indicated and Prot* has the meanings mentioned above for a compound of the formula V, especially in the compounds 17* being an acyl
 5 protecting group, e.g. fluoren-9-yl-methoxycarbonyl, and the compounds of formula 17* correspond to a compound of the formula V;

or especially, to obtain a compound of the formula VA, the following scheme:

- 29 -



I

(wherein R_k , R_l have the meanings just indicated and Prot^* has the meanings mentioned above for a compound of the formula V, especially in the compounds 17A* being an acyl protecting group, e.g. fluoren-9-yl-methoxycarbonyl, and the compounds of formula 17A* correspond to a compound of the formula VA;

where preferably the reaction of 11 with 12 or 12A* takes place in an appropriate solvent, e.g. methylene chloride, with a brominating agent, e.g. trimethylbromosilane, followed by the addition of compounds of the formula $\text{R}_k\text{-OH}$ and $\text{R}_l\text{-OH}$ (which are preferably identical) and a mixture or tertiary base, e.g. pyridine and N,N-dimethylaminopyridine, and an acid anhydride, e.g. acetic anhydride; reaction of the compound 12* or 12* with a compound of the formula 13* or 13A* in an appropriate solvent, e.g. an acid amide, such as dimethylformamide, a strong base, e.g. potassium tert-butoxide; hydrolysis of the resulting compound of the formula 14*, especially 14A*, with an alkali metal hydroxide, e.g. potassium hydroxide, in an appropriate solvent, e.g. an alcohol, such as ethanol, and subsequent warming up for decarboxylation to give a compound of the formula 15*, especially 15A*, removal of the acetyl group in an appropriate buffer, e.g. in the range of pH 7 to 10, e.g. in aqueous citrate buffer titrated with an alkali metal hydroxide, such as sodium hydroxide, with an acylase, if required in the presence of cofac-

tors such as cobalt chloride, to give a compound of the formula 16*, especially 16A*; and introduction of a protecting group Prot**, e.g. acyl, e.g. 9-fluorenylmethoxycarbonyl, with an appropriate reagent, e.g. Fmoc-OSU, in an appropriate solvent, e.g. water and/or acetonitrile.

5

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:

10

In all reactions, protecting gas may be used, such as nitrogen or Argon, where appropriate or necessary, and the temperatures are as known to the person skilled in the art, e.g. in the range from -25 °C to the reflux temperature of the respective reaction mixture, e.g. from -20 to plus 90 °C.

15

If Rk and RI are each independently of each other unsubstituted or substituted alkyl, this refers especially to C₁-C₇-alkyl or especially 1-alkyl, such as 1-(C₆-C₁₂aryl)-C₁-C₇alkyl, more especially benzyl.

20

If Rk and RI 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 RI then preferably form an unsubstituted or substituted alkylene bridge, especially unsubstituted or substituted ethylen, such as -CH₂-CH₂-), where the substituent(s) may preferably be selected from C₁-C₇-alkyl, especially two such substituents, such as methyl, ethyl, n-propyl or isopropyl.

25

A bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group is preferably an alpha-carbamoyl or carboxyl-C₁₋₈-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.

30

C₁₋₈-alkanoyl or phosphorylated hydroxy-C₁₋₈-alkanoyl (C₁₋₈-alkanoyl carrying both a hydroxyl and a phosphono (-O-P(=O)(OH)₂) group) A₁ is e.g. 2,3-dihydroxy-propanoyl (preferably in S-form) or 2-hydroxy-3-phosphono-propanoyl (preferably in S-form).

R₂ and R₂^{*} are C₁₋₈-alkyl, especially methyl wherever mentioned.

5 R₃ is the side chain of an amino acid, especially of a natural amino acid. Preferably, it is C₁₋₈alkyl which may be branched or linear. Most especially, C₁₋₈alkyl is n-(2-methyl)propyl (isobutyl), n-(1-methylpropyl (sec-butyl) or methyl, that is, the amino acid carrying the moiety is leucine, isoleucine or valine.

10 R₃^{*} 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 C₁₋₈alkyl which may be branched or linear, especially as defined in the preceding paragraph.

A "side chain of an amino acid" may be selected from any moiety, e.g. a mono- or polycyclic, linear, saturated, unsaturated (e.g. with conjugated double bonds) or partially
 15 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 stan-
 20 dard 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 cyclization including the alpha-amino group).

25 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

R₅ is the side chain of an amino acid, preferably a standard amino acid. Preferably, it is C₁₋₈alkyl which may be branched or linear and which is unsubstituted or substituted by phenyl. Most especially it is benzyl, n-(2-methyl)propyl, isobutyl or methyl, that is, the amino acid carrying the moiety is phenylalanine, leucine, isoleucine or valine.

R₆ is the side chain of a hydroxy amino acid, especially of tyrosine.

R₇ is the side chain of an amino acid, especially of a natural amino acid. Preferably, it is C₁₋₈alkyl which may be branched or linear. Most especially it is n-(2-methyl)propyl (isobutyl), n-(1-methyl)propyl (sec-butyl) or methyl, that is, the amino acid carrying the moiety is leucine, isoleucine or valine.

C₁₋₈-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 or trifluoroacetic acid, while acidic groups may form salts with positive ions, e.g. ammonium, alkylammonium, triethylamine, N-methylmorpholine, dimethylaminopyridine, alkali or alkaline-earth metal salt cations, e.g. Ca, Mg, Na, K or Li cations, or the like, or zwitterionic salts or inner salts of the compounds may be present.

“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.

Acetal protecting groups are highly sensitive to acidic conditions, especially in the presence of water. Cleavage of the acetal protecting group during the solid phase peptide synthesis or during cleavage from solid support would generate the free aldehyde function, which could react with the free amino group and undergo other side reactions. Therefore, it is important to keep the acetal protecting group until the cyclization of the oligopeptide is performed to obtain the macrocyclic compound II or IIA.

The protecting groups Prot^{**}, Prot^{***}, Prot^{****} and any further protecting groups present on the moieties A^{*}, R₂^{*}, R₃^{*}, R₅^{*}, R₆^{*}, R₇^{*}, X^{*}, where ever mentioned throughout the present description and claims, are selected so that they allow for orthogonal protection.

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

Preferably, the protecting groups are selected as follows:

- 34 -

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

Prot** is a protecting group that can be removed (especially from a compound of the formula III* or especially IIIA*) under conditions that are different to those of the cleaving reaction under (iii/b) especially arylalkyl, especially 1-(C₆-C₁₂aryl)-C₁-C₄alkyl, more especially benzyl, which can be removed e.g. by catalytic hydrogenation, e.g. with hydrogen in the presence of a noble metal catalyst, such as Pd or Pt which may be on a carrier, such as aluminium oxide or especially carbon.

Prot *** (an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin) is selected from those mentioned for Prot*, e.g. fluorenylmethoxycarbonyl (Fmoc), each of which can be removed e.g. as mentioned above or below.

Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present, especially as defined for Prot***.

The preferred orthogonal synthesis method in this case makes use of the Fmoc-protecting group strategy known in general for peptide synthesis using solid phase and liquid phase peptide synthesis.

The aldehyde protecting group(s) R_k and R_l (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
5 alkanolic acid, such as trifluoroacetic acid or trichloroacetic acid.

Other protecting groups present as well as the binding linker to a resin RES where present are preferably not removable under conditions under which Prot*, Prot**, Prot***
10 and Prot**** can be removed, e.g. in A*, carbamoyl can be N-protected e.g. with trityl (triphenylmethyl) (cleavage e.g. with trifluoro acetic acid (TFA); (e.g. in R₆*) a tyrosine hydroxy can be Boc (tert-butoxycarbonyl) protected, or protected by tert-butyldimethylsilyl, methoxymethyl or arylacetate (cleavage e.g. with TFA) and more preferably under conditions under which the bond to the linker to the Resin RES is preferably not cleaved
15 or (where simultaneous deprotection and cleavage from the resin to the bond is desired) also cleaved (e.g. cleavage with acid, such as TFA).

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

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

It is recommended to avoid too basic conditions (though the bases described for Fmoc cleavage, such as piperidine, are usually allowable) to avoid cleavage of the depsipeptide (ester) bond.
30

An appropriate solvent or solvent mixture useful during the deprotection steps may, e.g., be selected from customary solvents, e.g. an N,N dialkylformamide, such as dimethylformamide, a halogenated hydrocarbon, e.g. dichloromethane, alkanols, such as etha-

nol, propanol or isopropanol, nitriles, e.g. acetonitrile, alkanolic acid amides, such as dimethylformamide or diethylformamide, or further an aromatic hydrocarbon, e.g. toluene, or mixtures of two or more, also water may be present. The temperatures may be ambient temperature or lower or higher, e.g. in the range from -20 °C to 50 °C.

5

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

- 10 - Gel-type supports without or with spacer: These are highly solvated polymers with an equal distribution of functional groups. This type of support is the most common, and includes:
Polystyrene: Styrene cross-linked with e.g. 1-2% divinylbenzene; Polyacrylamide or polymethacrylamide: as hydrophilic alternative to polystyrene; Polyethylene glycol (PEG): PEG-Polystyrene (PEG-PS) is more stable than polystyrene and spaces the site
15 of synthesis from the polymer backbone; PEG-based supports: Composed of a PEG-polypropylene glycol network or PEG with polyamide or polystyrene (these already include a spacer, PEG);
- Surface-type supports: Materials developed for surface functionalization, including controlled pore glass, cellulose fibers, and highly cross-linked polystyrene.
- 20 - Composites: Gel-type polymers supported by rigid matrices.

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

25

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

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

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

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

The introduction of linker groups and their coupling with amino acids can be conducted essentially as described or in analogy to the Examples. For example, in the case of trityl ester formation, the resin (e.g. divinylbenzene cross-linked aminomethylpolystyrene resin) may be suspended in an appropriate solvent, such as a dialkyl acid amide, e.g. dimethylformamide, and/or an alcohol, such as ethanol, propanol or isopropanol, and reacted with a hydroxyaryl-acid linker, e.g. 4-(diphenylhydroxymethyl)-benzoic acid, in the presence of a coupling agent, e.g. mentioned below for the coupling of acids, e.g. 1-hydroxybenzotriazole and dicyclohexylcarbodiimide; or, for the manufacture on chloro-(2'chloro)trityl-polystyrene resin, the resin is suspended in an appropriate solvent, e.g. dichloromethane, addition of a chlorinating agent, e.g. acetyl chloride, and then reaction with the carboxyl group of an amino acid (this term always including unprotected or protected amino acids), e.g. in the presence of a base, e.g. a tertiary amino base, such as N-methyl-morpholine.

The cleavage of completed (protected or unprotected) peptides, e.g. to achieve the linear precursor peptide of formula III, especially IIIA, or III*, especially III*A, can then be conducted under mild acidic conditions, e.g. in the presence of an organic alkanoic acid, such as acetic acid, in an appropriate solvent, e.g. in dichloromethane or trifluoroethanol.

The cleavage conditions from the solid support must be selected such that the other protecting groups present in the molecule such as the trityl-, t-butyl- and in particular the acetal-protecting groups are not cleaved. Acetal protecting groups are highly sensitive to acidic conditions, especially in the presence of water. Cleavage of the acetal protecting group during the solid phase peptide synthesis or during cleavage from solid support would generate the free aldehyde function, which could react with the free amino group and undergo other side reactions.

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.

- 5 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
10 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
15 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 metha-
20 naminium (HATU), benzotriazol-1-yl-oxytripyrrolidinophosphonium 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-tetrame-
25 thyluronium -tetrafluoroborate (TNTU), O-[(cyano(ethoxycarbonyl)methyliden)amino]-1,1,3,3-tetramethyluronium-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-
30 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-oxoethylideneaminoxy-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), chlorotripyrrolidinophosphonium hexafluorophosphate (PyCloP), or the like;

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

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

azide forming agents, e.g. diphenyl phosphoryl azide,
10

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)formamidine tetrafluoroborate or hexafluorophosphate,

15 chloro-N,N,N',N'-tetramethylformamidine hexafluorophosphate, fluoro-N,N,N',N'-tetramethylformamidine hexafluorophosphate, fluoro-N,N,N',N'-bis(tetramethylene)formamidine hexafluorophosphate,

or the like, or mixtures of two or more such agents.
20

Also for the ester coupling of compounds of the formula XI or XIA with those of the formula XII or XIA, respectively, the corresponding reactive carboxyl compounds can be used or formed in situ. Here, especially MSNT is preferred as coupling agent as this allows for the maintenance of high stereospecificity.
25

For the macrolactonization of a compound of the formula III, especially IIIA, also coupling reagents and conditions as described for the coupling of amino acids can be used.

The reactions may, in each case, where appropriate, be conducted in the presence of a
30 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

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N,N dialkylformamide, such as dimethylformamide, a halogenated hydrocarbon, e.g. dichloromethane, N-alkylpyrrolidones, such as N-methylpyrrolidone, nitriles, e.g. acetonitrile, ethers, such as dioxane or tetrahydrofuran, or further an aromatic hydrocarbon, e.g. toluene, or mixtures of two or more, where, provided an excess of coupling agent is present, also water may be present. The temperatures may be ambient temperature or lower or higher, e.g. in the range from -20 °C to 50 °C.

The amino acids of the formula VII, VIIA, IX, IXA, XI, XIA, XV, XVA, XVI, XVIIA, XVII, XVII (obtainable e.g. by Solution Phase peptide synthesis) 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 XIV, 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.

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

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

Abbreviations

aq.	aqueous
Boc/BOC	tert-Butoxycarbonyl
25 brine	sodium chloride solution in water (saturated at RT)
Bzl	benzyl
COMU	1-cyano-2-ethoxy-2-oxoethylideneaminoxy-dimethylamino-morpholino-carbenium hexafluorophosphate
DCM	dichloromethane
30 DIPEA	N,N-diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-dimethylformamide
Fmoc/FMOC	9-fluorenylmethoxycarbonyl
Fmoc-OSu	N-(9-Fluorenylmethoxycarbonyloxy)succinimide

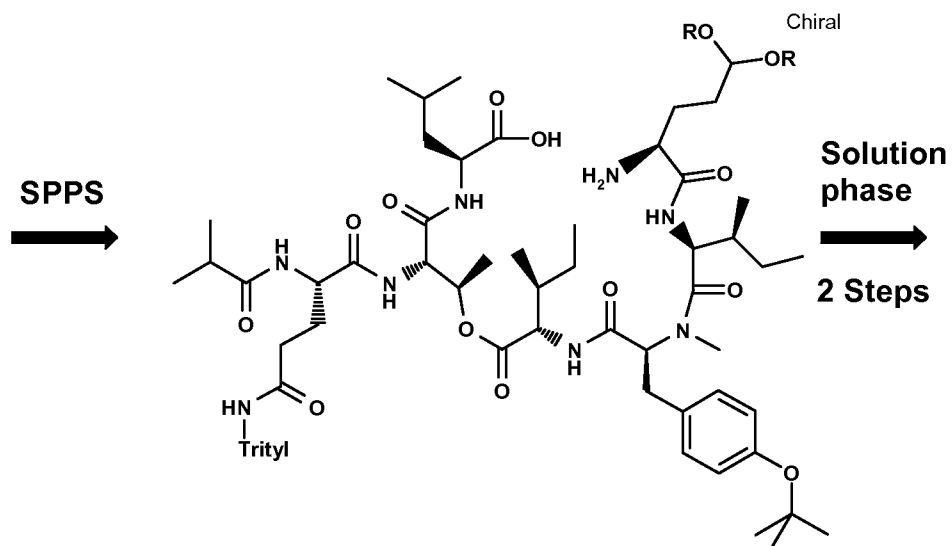
- 41 -

	Et	ethyl
	h	hour(s)
	HATU	2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyluronium hexafluorophosphate Methanaminium
5	HOSU	N-Hydroxysuccinimide
	HPLC	High Performance Liquid Chromatography
	HR-MS	High Resolution Mass Spectroscopy
	IPC	In-Process Control
	IR	Infrared Spectroscopy
10	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.
15	Me	methyl
	MED	Dichloromethane
	MS	Mass Spectroscopy
	MSNT	1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole
	NMR	Nuclear Magnetic Resonance Spectroscopy
20	PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
	RP	Reversed Phase
	RT/rt	room temperature
	SPPS	Solid Phase Peptide Synthesis
25	TBME	tert-butyl methyl ether
	TFA	trifluoroacetic acid

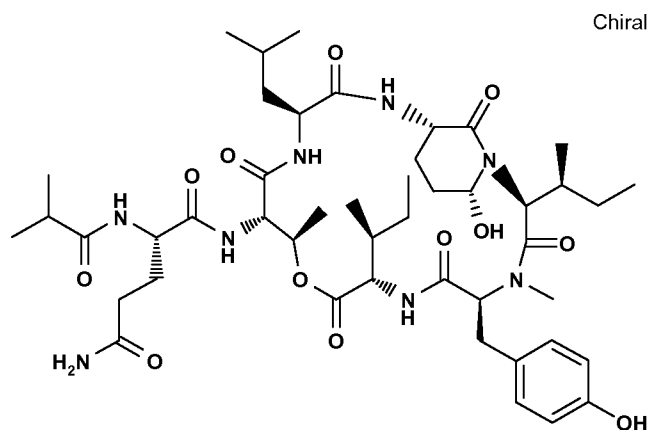
For amino acid abbreviations see the table above.

- 30 If not mentioned otherwise, reactions are carried out at room temperature.
The synthesis of the compound A mentioned below is made in solution according to the
following simplified scheme, more details are given below:

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Compound 6 (R and R together form ethenyl (-CH₂CH₂-)
or Compound 9 (each R is benzyl)



Compound A

The two steps in Solution phase are macrocyclization and deprotection/equilibration.
Details of this and preceding reactions are given in the following example:

5

The names of the compounds according to the 2004 IUPAC recommendations given in the reaction schemes and examples below are as follows:

Compound 1: benzyl (2S)-2-(dibenzylamino)-5-oxopentanoate;

Compound 2: benzyl (2S)-2-(dibenzylamino)-4-(1,3-dioxolan-2-yl)butanoate;

Compound 3: (2S)-2-(dibenzylamino)-4-(1,3-dioxolan-2-yl)butanoic acid;

Compound 4: (2S)-2-amino-4-(1,3-dioxolan-2-yl)butanoic acid;

5 Compound 5: (2S)-4-(1,3-dioxolan-2-yl)-2-[[*(9H*-fluoren-9-ylmethoxy)carbonyl]amino]-butanoic acid;

Compound 6: *N*²-(2-methylpropanoyl)-*N*⁵-(triphenylmethyl)-L-glutaminy-O-{*N*-[(2S)-2-(dibenzylamino)-4-(1,3-dioxolan-2-yl)butanoyl]-L-isoleucyl-O-(*tert*-butyl)-*N*-methyl-L-tyrosinyl-L-isoleucyl}-L-threonyl-L-leucine;

10 Compound 7: *N*²-(2-methylpropanoyl)-*N*⁵-(triphenylmethyl)-L-glutaminy-O-{*N*-[(2S)-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 8: 2S)-*N*-[(3S,6S,9S,12S,15S,18S,19*R*)-3,9-di[(2S)-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;

15 Compound 9: *N*²-(2-methylpropanoyl)-*N*⁵-(triphenylmethyl)-L-glutaminy-O-{*N*-[(2S)-2-amino-5,5-bis(benzyloxy)pentanoyl]-L-isoleucyl-O-(*tert*-butyl)-*N*-methyl-L-tyrosinyl-L-isoleucyl}-L-threonyl-L-leucine;

20 Compound 10: (2S)-*N*-[(3S,6S,9S,12S,15S,18S,19*R*)-12-[3,3-bis(benzyloxy)propyl]-3,9-di[(2S)-butan-2-yl]-6-[[4-(*tert*-butoxy)phenyl]methyl]-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 12: 1,1'-[(3-bromopropane-1,1-diyl)bis(oxymethanediyl)]dibenzene;

25 Compound 13: 1,3-diethyl 2-acetamidopropanedioate;

Compound 14: 1,3-diethyl 2-acetamido-2-[3,3-bis(benzyloxy)propyl]propanedioate;

Compound 15: 2-acetamido-5,5-bis(benzyloxy)pentanoic acid;

Compound 16: (2S)-2-amino-5,5-bis(benzyloxy)pentanoic acid;

Compound 17: (2S)-5,5-bis(benzyloxy)-2-[[*(9H*-fluoren-9-ylmethoxy)carbonyl]amino]-pentanoic acid;

30

Compound A: (2S)-*N*-[(2S,5S,8S,11*R*,12S,15S,18S,21*R*)-2,8-di[(2S)-butan-2-yl]-21-hydroxy-5-[(4-hydroxyphenyl)methyl]-4,11-dimethyl-15-(2-methylpropyl)-3,6,9,13,16,22-

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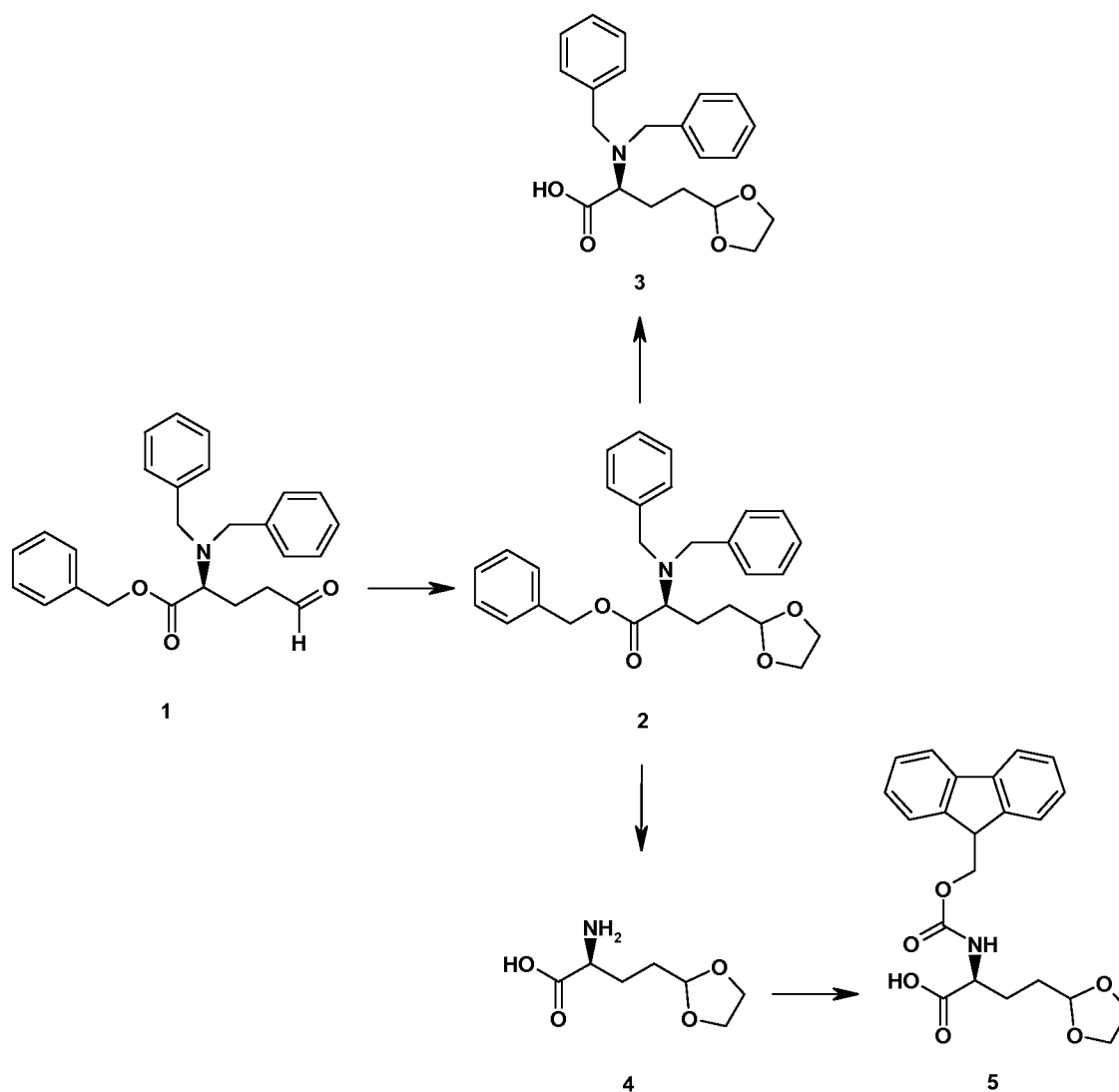
hexaoxo-10-oxa-1,4,7,14,17-pentaazabicyclo[16.3.1]docosan-12-yl]-2-(2-methylpropanamido)pentanediamide.

Example 1: Synthesis of Compound A

5

1 A) Synthesis of ethyleneglycol-acetale synthons compound **3** and compound **5**:

Reaction scheme 1



10

The unnatural amino acid with the ethyleneglycol-acetale protecting group was synthesized starting from the known aldehyde-intermediate **1** (scheme 1), which was

prepared according to the procedures described in [Rodriguez and Taddei, Synthesis 2005, 3, pp. 493-495]. Aldehyde **1** was converted into the ethyleneglycol-acetale **2** by treating with ethyleneglycol in the presence of p-TsOH as catalyst and molecular sieves. The benzyl-ester in compound **2** was hydrolysed with LiOH to obtain the free acid compound **3**, which was used for the preparation of the corresponding oligopeptide, compound **6**. Alternatively, the benzyl-protecting groups in compound **2** were cleaved by hydrogenation using palladium on charcoal (10%) and the free amino-acid intermediate **4** was treated with commercially available Fmoc-HOSU ester to obtain the N-Fmoc-protected ethyleneglycol-acetale of the unnatural amino acid (compound **5**). It is noteworthy to mention that no stability issues were faced with regard to stability of compounds **3** and **5**. This stability cannot be taken granted in such a molecule comprising an acetale and a free carboxylic acid function.

a) Synthesis of compound 2:

To a solution of Compound 1 (29g; 72.23 mmol) in DCM (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 **2** (87% yield).

¹H-NMR of the product confirmed the proposed structure.

HR-MS: Calculated for C₂₈H₃₁NO₄ [M+H]⁺ =446.23259. Found: 446.23248.

b) Synthesis of compound 3:

Compound **2** (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 DCM/isopropanol (9:1) as eluent to obtain 17.3g product comprising ca. 10 mol% isopropanol according to ¹H-NMR.

- 5 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 **3** (16g; 74.8% yield). ¹H- and ¹³C-NMR Spectra of the product confirmed the proposed structure.

10

HR-MS: Calculated for C₂₁H₂₅NO₄ [M+H]⁺: 356.18564; [M+Na]⁺: 378.16758. Found: [M+H]⁺: 356.18586; [M+Na]⁺: 378.16748.

c) Synthesis of compound 4 from compound 3:

- 15 Compound **3** (2.7g; 7.596 mmol) was dissolved in isopropanol (60 mL) and the catalyst (10% Pd on charcoal; 300mg) was added. The reaction mixture was hydrogenated for 23h at room temperature under atmospheric pressure, after which time the hydrogenation was completed. The reaction mixture was diluted with water (60 mL) and stirred for 1h at room temperature to dissolve the precipitated product. The
20 reaction mixture was then filtered to remove the catalyst and the filter cake was washed with water/isopropanol (1:1) (45 mL). Evaporation of the solvent under reduced pressure and subsequent drying of the product *in vacuo* at 40 °C overnight gave compound **4** (1.24g; 93.18% yield). ¹H- and ¹³C-NMR-Spectra confirmed the proposed structure for compound **4**.

25

HR-MS: Calculated for C₇H₁₃NO₄ [M+H]⁺: 176.09174; [M+Na]⁺: 198.07368. Found: [M+H]⁺: 176.09173; [M+Na]⁺: 198.07362.

d) Synthesis of compound 4 from compound 2:

- 30 Compound **4** was prepared by hydrogenation of compound **2** using 10% palladium on charcoal as catalyst under atmospheric hydrogen pressure, in ethanol/water (1:1 v/v) as solvent at room temperature. For work-up, the catalyst was removed by filtration and the solvent was evaporated under reduced pressure. Subsequent drying of the product *in vacuo* at 45 °C gave compound **4** in quantitative yield.

The product was identical to compound **4** obtained from compound **3** above.

e) Synthesis of compound 5:

Compound **4** (1.2g; 6.85 mmol) was dissolved in water (7mL) and triethylamine
5 (0.692g) was added. To this stirred mixture, a solution of Fmoc-HOSU-Ester (= (9H-Fluoren-9-yl)methyl 2,5-dioxopyrrolidin-1-yl carbonate) (2.31g; 6.85 mmol) in acetonitrile (6g) was added and the reaction mixture was stirred for ca. 1h at room temperature. The pH value of the resulting reaction mixture was adjusted to 8.5-9.0 by addition of triethylamine in several portions. In total, addition of ca. 0.7g triethylamine
10 was necessary to maintain a pH of 8.5-9.0. For work-up, the reaction mixture was subjected to flash chromatography on silica gel by direct transfer of the reaction mixture on a silica gel column. Elution with ethyl acetate/acetic acid (98:2), combination of product fractions and evaporation of the solvent gave wet compound **5**. The wet product was suspended in hexanes, stirred for 1h at room temperature and
15 the precipitate was isolated by filtration. The precipitate was dried *in vacuo* at 50 °C over night to obtain a product comprising ca. 20 mol% of acetic acid. This product was dissolved in ethyl acetate (50 mL) at 60 °C and the solution was cooled down to room temperature. Seed crystals (compound **5**) were added at room temperature and the suspension was stirred until a thin suspension was formed. The volume of
20 the suspension was reduced to ca. 15mL by partial evaporation of the solvent at 40 °C under reduced pressure, and hexanes (89 mL) was added to the suspension over 30 minutes at room temperature. The suspension was stirred for 1 additional h at room temperature and the product was isolated by filtration. The product was dried *in vacuo* at 50 °C overnight to obtain Compound **5** (2.31g; 84.85% yield).

25 HR-MS: Calculated for $C_{22}H_{23}NO_6$ $[M+H]^+$: 398.15982; $[M+NH_4]^+$: 415.18636; $[M+Na]^+$: 420.14176. Found: $[M+H]^+$: 398.15991; $[M+NH_4]^+$: 415.18655; $[M+Na]^+$: 420.14183.

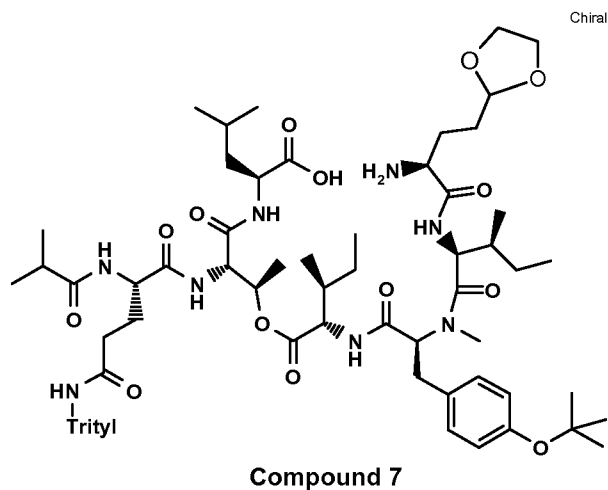
1H -NMR (600 MHz, d_6 -DMSO): δ ppm 1.64 (2H, m); 1.68 (1H, m); 1.81 (1H, m);
30 3.76 (2H, m); 3.87 (2H, m); 3.98 (1H, m); 4.22 (1H, m); 4.27 (2H, m); 4.79 (1H, m); 7.33 (2H, t, $J=7.3$ Hz); 7.42 (2H, t, $J=7.3$ Hz); 7.66 (1H, d, $J=8.1$ Hz); 7.73 (2H, d, broad); 7.89 (2H, d, $J=7.3$ Hz); 12.59 (1H, s, broad).

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^{13}C -NMR (150 MHz, d_6 -DMSO): δ ppm 25.46 (CH_2), 29.93 (CH_2), 46.67 (CH), 53.61 (CH), 64.27 ($2\times\text{CH}_2$), 65.65 (CH_2), 103.12 (CH), 120.12 ($2\times\text{CH}$), 125.30 ($2\times\text{CH}$), 127.08 ($2\times\text{CH}$), 127.67 ($2\times\text{CH}$), 140.71 ($2\times\text{C}$), 143.79 ($2\times\text{C}$), 156.14 (C), 173.68 (C).

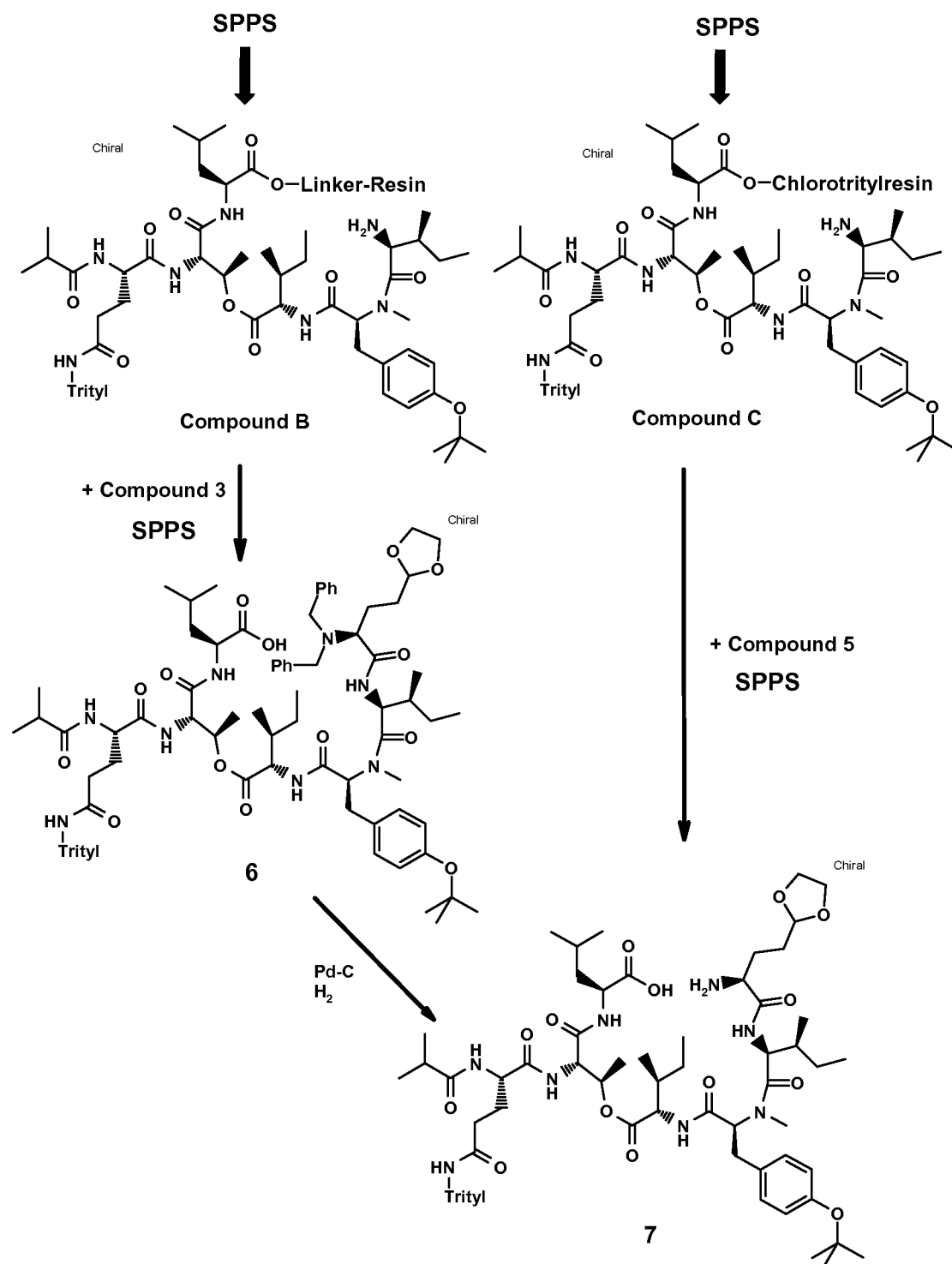
5 IR: 3345, 3321, 3063, 3021, 2974, 2963, 2949, 2767, 1950, 1914, 1878, 1741, 1691, 1682, 1610, 1541, 1525, 1477, 1464, 1451, 1403, 1367, 1323, 1285, 1270, 1249, 1225, 1188, 1138, 1104, 1087, 1055, 1033, 1008, 983, 963, 939, 925, 873, 836, 798, 782, 759, 740, 648, 622.

10 1 B) Synthesis of compound 7 by SPPS



Compound **7** is synthesized by solid phase peptide synthesis (SPPS), the last steps being represented in short in the following simplified reaction scheme 2:

Reaction scheme 2



In alternative reactions, both the N-dibenzyl-protected compound **3**, as well the N-Fmoc-protected compound **5** were used for the SPPS synthesis of the corresponding oligopeptides **6** and **7** respectively, comprising the unnatural amino

- 50 -

acid with the aldehyde function, protected as ethyleneglycol-acetale(scheme 2). The benzyl-protecting groups of compound **6** were removed by hydrogenation with palladium on charcoal (10%) in solution to obtain compound **7** (scheme 2). Alternatively, compound **7** was also obtained directly from SPPS on a chlorotriptyl-resin, when compound **5** was used as synthon for the unnatural amino acid in the last coupling step. Surprisingly, no problems were faced with regard to stability of the acetal protecting group during SPPS and subsequent acid catalyzed cleavage of the oligopeptides **6** and **7** from solid support. Details are given below:

10 **Equipment:**

Peptide reactor with a filter cloth at the bottom. A nitrogen manifold allows to empty the reactor via filter cloth and bottom valve .

15 The linker-resin bound Compound **B** was manufactured as follows:

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

200g of Aminomethyl-polystyrene resin (crosslinked with 1% divinyl benzene, loading of aminomethyl groups 1mmol/g) (supplier: Senn Chemicals AG, Dielsdorf/Switzerland) were stirred alternatedly with several portions of dimethylformamide (1600mL) and isopropanol (1600mL) After two final washes with dimethylformamide, the resin was treated with a previously prepared solution of 4-(diphenylhydroxymethyl)-benzoic acid (91.3g 300mmol), 1-hydroxy-benzotriazole monohydrate (45.9g, 300mmol) and diisopropylcarbodiimide (75.7g, 600mmol) in dimethylformamide (1600mL). The reaction mixture was stirred for 1.5h and a Ninhydrin test was performed. The test still showed free amino groups and thus further diisopropylcarbodiimide (7.6g, 60mmol) was added and the reaction stirred overnight. A subsequent ninhydrin test in the morning was negative and the reaction mixture was filtered off. The resin was washed alternatingly with dimethylformamide and isopropanol. The resin was dried in vacuo and yielded 257g of dry linker-resin. The material was used for the next synthesis step without further analysis.

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

Preparation of Fmoc-Leu-Linker-Resin

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Linker-Resin from the preceding step (190g, 147.8mmol) was swollen by stirring in toluene (1400mL). The solvent was filtered off and replaced by a solution of toluene (1400mL) and acetyl chloride (53mL, 1478mmol). This mixture was stirred for 2h, filtered off, replaced by an identical mixture which was stirred for another 2h before filtering off.

5 The chlorinated resin was washed twice with toluene and three times with dichloromethane.

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

15 1 B) (C) Coupling of Fmoc-Thr-OH

Preparation of Fmoc-Thr-Leu-Linker-Resin

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

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

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

In a round bottomed flask a solution of Fmoc-Thr-OH (112.1g, 328mmol), hydroxylbenzotriazole monohydrate (51.25g, 334mmol) and diisopropylcarbodiimide (51mL, 655mmol) in tetrahydrofuran (600mL) was prepared

30 The solution was added to the resin and the pH checked immediately (pH= 6.5). The reaction mixture was stirred for 1.5h until a ninhydrin test showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly. A small sample of the resin was dried and sent for amino acid analysis (0.13% D-Leu, <0.1% D-Thr, <0.1% L-allo-Thr, <0.1% D-allo-Thr), the bulk of the material was subjected to the next step without further drying.

1 B) (D) Coupling of Fmoc-Gln(Trt)-OH**Preparation of Fmoc-Gln(Trt)-Thr-Leu-Linker-Resin**

The Fmoc-Thr-Leu-Linker-Resin from the previous step was swollen by stirring in two
5 subsequent portions of dimethylformamide (1100mL) for 30min each.

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

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

In a round bottomed flask a solution of Fmoc-Gln(Trt)-OH (138.6g, 226mmol), HATU
(86.2g, 226mmol) and ethyldiisopropylamine (58.4g, 452mmol) in dimethylformamide
15 (400mL) was prepared.

The solution was added to the resin and the pH checked immediately (pH= 10). The
reaction mixture was stirred for 3h until a ninhydrin test showed complete reaction. The
solution was filtered off and the resin was washed with dimethylformamide and
isopropanol alternately.

20 The resin was dried in vacuo and yielded 170g of dry Fmoc-Gln(Trt)-Thr-Leu-Linker-
Resin. The loading with Fmoc-groups was determined at 0.60 mmol/g indicating a yield
of 102mmol (94% of theory over the last two steps). Amino acid analysis at an external
contractor led to the following values: (0.13% D-Leu, <0.1% D-Thr, <0.1% L-allo-Thr,
<0.1% D-allo-Thr, <0.8% D-Gln).

25

1 B) (E) Coupling of Isobutyric acid**Preparation of Isobutyryl-Gln(Trt)-Thr-Leu-Linker-Resin**

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

Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in
dimethylformamide (1300mL) for 5min and 15min respectively. The resin was washed by
several alternating washes with dimethylformamide and isopropanol. Phenolphthalein and

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water were added to a sample of the final wash solution. The absence of pink colour proved successful removal of piperidine.

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

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

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

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

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

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

Isobutyryl-Gln(Trt)-Thr-Leu-Linker-Resin (wet from preceding step, 101mmol) was swollen by stirring in three subsequent portions of dichloromethane (1200mL) for 20min each.

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

30 1 B) (G) Coupling of Fmoc-N-methyl-Tyr(tBu)-OH

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

Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Linker-Resin from the preceding step (172g, 72mmol) was swollen by stirring in two subsequent portions of dimethylformamide (1300mL) for 30min each.

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Fmoc protecting group was cleaved by two subsequent washings of 20% piperidine in dimethylformamide (1400mL) for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylformamide and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink colour
5 proofed successful removal of piperidine.

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

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

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

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

Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Linker-Resin (linker-resin bound Compound B).

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

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

30 In a round bottom flask a solution of Fmoc-Ile-OH (103.9g, 294mmol) COMU (125.9g, 294mmol) and Ethyldiisopropylamine (76g, 588mmol) in dichloromethane (440mL) and dimethylformamide (440mL) was prepared.

The solution was added to the resin and the reaction mixture stirred for 20h. After that time a ninhydrin test was done, showing complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly. The resin was dried in vacuo and yielded 185.5g of dry Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Linker-Resin. The loading with Fmoc groups was determined to be 0.4 mmol/g. Thus a quantitative yield of 74mmol resulted.

1 C) Solution Phase Synthesis of Compound 7

1 C) (A) Synthesis of Compound 6

a) **Coupling of Compound 3** Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Linker-Resin from the previous step (10.4g, 4.37 mmol) was swollen by stirring in two subsequent portions of dimethylformamide (50mL) for 30min each. The Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in dimethylformamide (50mL) for 5min and 15min respectively. The resin was washed with several portions of dimethylformamide and isopropanol in an alternating sequence. Finally, the resin was washed with dimethylformamide (2x50mL) to prepare for the next coupling step. In a 100mL glass bottle, a solution of compound **3** (3.1g, 8.74mmol) PyBOP (4.55g, 8.74mmol) and ethyl-diisopropylamine (2.2g, 17.0mmol) in dimethylformamide (50mL) was prepared. The basic solution (pH11) was agitated for 5min before adding it to the resin. The reaction mixture was stirred for 3h. Then the solution was filtered off and the resin was washed with dimethylformamide and isopropanol. Finally the resin was washed three times with dichloromethane (50mL) to prepare for the cleavage of the peptide.

b) Cleavage from resin

To the wet peptide-resin obtained after coupling of compound **3** above, a mixture of acetic acid (40mL) and dichloromethane (10mL) was added and the suspension stirred for 5h. The suspension was filtered and the filtrate collected in a round bottomed flask (filtrate 1). The resin was washed twice with dichloromethane (20mL) and the washes were combined with filtrate 1. The resin was treated with a fresh portion of acetic acid (40mL) and dichloromethane (10mL) and the mixture was stirred overnight. The suspension was filtered and the filtrate collected in a round bottomed flask (filtrate 2). The resin was washed twice with dichloromethane

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(20mL) and the washes combined with filtrate 2. All filtrates were combined and concentrated in a rotary evaporator. The residual acetic acid was removed by azeotropic distillation with toluene (100mL). Complete evaporation of the solvent yielded 4.5g of a glassy red-brown residue as crude product. The crude material was purified by RP-chromatography, using a YMC ODS-AQ column. The fractions were assessed using HPLC, rich fractions were concentrated in a rotary evaporator and the concentrate freeze dried. Yield: 2.86g Compound **6** (44% for the last two steps).

HR-MS: Calculated for $C_{85}H_{112}N_8O_{14}$ $[M+H]^+$: 1469.83708. Found: 1469.83691
 1H -NMR-Spectrum confirmed the proposed structure.

1 C) (B) Synthesis of compound 7 (variant with deprotection of Compound 6)

Compound **6** (0.5g, 0.34 mmol) was dissolved in isopropanol/water (95:5 v/v, 15mL) and the solution was flushed with argon. Palladium on charcoal (10% Pd; 0.25g) was added under a stream of argon and the suspension was heated to IT 35-40 °C. Hydrogen gas was introduced into the gas phase and the mixture was stirred under atmospheric pressure of hydrogen, until an IPC (HPLC) indicated the completion of the reaction (<5% starting material).

For work-up, the catalyst was removed by filtration and the filter residue was washed with isopropanol. The filtrate was evaporated to obtain 500mg crude product. The crude product was purified by flash chromatography on silica gel using ethyl acetate/methanol (93:7) to obtain 348mg compound **7**. Yield: 79.3%.

HR-MS: Calculated for $C_{71}H_{100}N_8O_{14}$ $[M+H]^+$:1289.74318; $[M+Na]^+$:1311.72512. Found: $[M+H]^+$:1289.74308; $[M+Na]^+$:1311.72487.

1 D) SPPS Synthesis of Compound 7 (variant with coupling of compound 5)

The Chlortritylresin bound Compound **C** was manufactured and used as follows:

1 D) (A): Coupling of Fmoc-Leu-OH to Chlortrityl-resin - Preparation of Fmoc-Leu-Chlortritylresin

Chlor-(2'-chlor)trityl-polystyrene resin (47.15g, 50 mmol) was swollen by stirring in dichloromethane (350mL) over night. The solvent was filtered off and replaced by a solution of dichloromethane (330mL) and acetyl chloride (19.75g, 250mmol). This mixture was stirred for 3h45min, filtered off and replaced by an identical mixture which was stirred for another 90min before filtering off. This treatment was repeated once again for 1h 45min. The chlorinated resin was washed three times with dichloromethane.

In a round bottom flask, a solution of Fmoc-Leu-OH (35.4g, 100mmol) and of N-methylmorpholine (15.2g, 150mmol) in dichloromethane (120mL) was prepared. This solution was added to the resin and stirred overnight. In the morning, the solution was filtered off and the resin was washed with dichloromethane and isopropanol alternatingly. The resin was dried in vacuum and yielded 69.1g of dry Fmoc-Leu-Chlortritylresin. The loading with Fmoc-groups was determined at 0.74mmol/g what led to a yield of 51mmol (quantitative yield)..

1 D) (B): Coupling of Fmoc-Thr-OH - Preparation of Fmoc-Thr-Leu-Chlortritylresin

Fmoc-Leu-Chlortritylresin from the preceding step (67.1g, 49.7mmol) was swollen by stirring in two successive portions of dimethylformamide (400mL and 350mL) for 30min and 75min respectively.

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

The resin was washed with tetrahydrofurane (330mL) three times to prepare for the following coupling step.

In a round bottomed flask a solution of Fmoc-Thr-OH (33.95g, 99.4mmol), hydroxylbenzotriazole monohydrate (23.31g, 152mmol) and diisopropylcarbodiimide (37.6g, 298mmol) in tetrahydrofurane (285mL) was prepared

The solution was added to the resin and the reaction mixture stirred overnight. HPLC showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly. Finally the resin was washed 5 times

with TBME, dried in vacuum and yielded 67.5g of dry Fmoc-Thr-Leu-Chlortritylresin. The loading with Fmoc-groups was determined at 0.58mmol/g leading to a yield of 39mmol (79% of theory).

5 **1 D) (C): Coupling of Fmoc-Gln(Trt)-OH - Preparation of Fmoc-Gln(Trt)-Thr-Leu-Chlortritylresin**

Fmoc-Thr-Leu-Chlortritylresin (49.4g, 28.7mmol) from the previous step was swollen by stirring in two subsequent portions of dimethylformamide (350mL each) for 30min and 70min respectively.

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

15 The resin was washed with dimethylformamide (250mL) three times to prepare for the following coupling step.

In a round bottomed flask a solution of Fmoc-Gln(Trt)-OH (34.4g, 57.3mmol), HATU (21.5g, 57.3mmol) and ethyldiisopropylamine (14.8g, 114.6mmol) in dimethylformamide (190mL) was prepared.

20 The solution was added to the resin and the pH checked immediately (pH= >8). The reaction mixture was stirred for 2h30min before HPLC in process control showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly.

The resin was not dried but directly introduced into the next step.

25

1 D) (D): Isobutyrylation - Preparation of Isobutyryl-Gln(Trt)-Thr-Leu-Chlortritylresin

Fmoc-Gln(Trt)-Thr-Leu-Chlortritylresin (wet from preceding step, 28.7mmol) was washed with dimethylformamide (300mL) for 30min.

30 Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide (400mL) for 5min and 15min respectively. The resin was washed by several alternating washes with dimethylformamide and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink colour proved successful removal of piperidine.

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The resin was washed with dimethylformamide (250mL) three times to prepare for the following coupling step.

In a round bottom flask a solution of Isobutyric acid (5.01g, 56.9mmol), PyBOP (29.33g, 56.4mmol) and Ethyldiisopropylamine (15.55, 120mmol) in dimethylformamide (190mL) was prepared.

The solution was added to the resin and the pH checked immediately (pH= 11). The reaction mixture was stirred for 1h before HPLC in process control showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly.

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

1 D) (E): Coupling of Fmoc-Ile-OH (esterification) - Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Chlortritylresin

Isobutyryl-Gln(Trt)-Thr-Leu-Chlortritylresin (wet from preceding step, 28.7mmol) was washed with two portions of dichloromethane at room temperature and two additional portions of cold (0°C) dichloromethane under nitrogen.

In a round bottom flask a solution of Fmoc-Ile-OH (37.65g, 106.5mmol) N-methyl imidazole (10.00g, 121.8mmol) and MSNT (31.65g, 106.8mmol) in MED (210mL) was prepared at 0°C under nitrogen. The coupling solution was added to the cold resin and the reaction mixture was stirred for 30min before an additional amount of N-methyl imidazole (5.33g, 64.9mmol) was added. The reaction was stirred for further 30min before HPLC in process control showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide isopropanol alternatingly. Finally the resin was washed 5 times with TBME, dried in vacuum and yielded 55.7g of dry Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Chlortritylresin. Fmoc loading was determined to be 0.43mmol/g leading to a yield of 24.2mmol (84% over the last three steps).

1 D) (F): Coupling of Fmoc-N-Me-Tyr(tBu)-OH - Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Chlortritylresin

Dry Isobutyryl-Gln(Trt)-Thr(Ile-Fmoc)-Leu-Chlortritylresin from the preceding step (55.5g, 24.1mmol) was swollen by stirring in two subsequent portions of dimethylformamide (400mL each) for 30min each.

Fmoc protecting group was cleaved by two subsequent washings of 20% piperidine in dimethylformamide (450mL) for 5min and 15min respectively. The resin was washed by

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several alternating washes with dimethylformamide and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink colour proofed successful removal of piperidine.

5 The resin was washed with dimethylformamide (280mL) three times to prepare for the following coupling step.

A solution of Fmoc-N-methyl-Tyr(tBu)-OH (22.12g, 47.7mmol) and HATU (17.81g, 46.8mmol) and ethyldiisopropylamine (12.09g 93.5mmol) in dimethylformamide (280mL) was prepared and added to the resin. The pH of the reaction mixture was checked immediately after addition of the coupling solution (pH >8) The solution was stirred for 2h
10 before HPLC in process control showed complete reaction. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly. The batch was directly subjected to the next step without drying and further analysis.

1 D) (G): Coupling of Fmoc-Ile-OH - Preparation of Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Chlortritylresin
15

The wet Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Fmoc)-Leu-Chlortritylresin from preceding step, (24.1mmol) was washed with dimethylformamide (400mL) for 30min. Fmoc protecting group was cleaved by two subsequent washings with 20% piperidine in dimethylformamide (450mL) for 5min and 15min respectively. The resin was washed by
20 several alternating washes with dimethylformamide and isopropanol. Phenolphthalein and water were added to a sample of the final wash solution. The absence of pink colour proved successful removal of piperidine.

The resin was washed with dimethylformamide (280mL) three times to prepare for the following coupling step.

25 In a round bottom flask a solution of Fmoc-Ile-OH (33.01, 93.4mmol) COMU (39.99g, 93.4mmol) and Ethyldiisopropylamine (24.16g, 187mmol) in dichloromethane (110mL) and dimethylformamide (110mL) was prepared.

The solution was added to the resin and the pH checked (pH>8). The reaction mixture was stirred for 2h. After that time HPLC in process control showed complete reaction.

30 The solution was filtered off and the resin was washed with dimethylformamide and isopropanol alternatingly. Finally the resin was washed 5 times with TBME.

The resin was dried in vacuum and yielded 61.1g of dry Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Chlortritylresin. The loading with Fmoc groups was

determined to be 0.30mmol/g. Thus a yield of 18.3mmol resulted, corresponding to 76% over the last two steps.

1 D) (H) Coupling of compound 5: preparation of compound 7

5

a) coupling

Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Chlortritylresin (6.5g, 1.89mmol) was swollen by stirring in two subsequent portions of dimethylformamide (45mL and 40mL) for 30min each.

- 10 The Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in dimethylformamide (50mL and 55mL) for 5min and 15min respectively. The resin was washed with several portions of dimethylformamide and isopropanol in an alternating sequence. A color test with phenolphthalein/water confirmed absence of base. Finally, the resin was washed with dimethylformamide (3x30mL) to prepare for the
- 15 following coupling step.

- In a round bottom flask, a solution of compound **5** (1.50g, 3.77mmol) PyBOP (1.96g, 3.77mmol) and ethyl-diisopropylamine (0.98g, 7.54mmol) in dimethylformamide (20mL) was prepared. The basic solution (pH8) was agitated for 5min before adding it to the resin. The reaction mixture was stirred for 2h and the progress of the reaction was monitored by cleaving a sample from the resin. HPLC of the sample showed complete conversion. The solution was filtered off and the resin was washed with dimethylformamide and isopropanol in an alternating sequence. From the dimethylformamide wet resin, the Fmoc protecting group was cleaved by two subsequent treatments with 20% piperidine in dimethylformamide (60mL) for 5min and 15min respectively. The resin was washed
- 20 with several portions of dimethylformamide and isopropanol in an alternating sequence. The effectiveness of washing was checked with phenolphthalein / water. Finally the resin was washed three times with dichloromethane (40mL) to prepare for the cleavage of the peptide.

30 b) Cleavage from resin

To the wet Isobutyryl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Compound **5**-H)-Leu-Linker-Resin a mixture of dichloromethane (40mL), acetic acid (4.99mL) and trifluoroethanol (4.96mL) were added and the suspension was stirred for 3h. The suspension was filtered and the filtrate collected in a round bottomed flask. The cleavage step was repeated twice with

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identical amounts of cleavage cocktail for 2h each time. All filtrates were combined. The resin was washed three times with toluene (40mL) and the washes combined with the filtrates.

The solution containing the product was evaporated to dryness in a rotary evaporator.

- 5 The residual solid was dissolved in acetonitrile/water and freeze dried. 2.95g of lyophilized powder was isolated as crude product.

The crude material was purified by RP-chromatography, using a Kromasil RP 4 10um column (Eka Chemicals AB, Bohus, Sweden). The fractions were assessed using HPLC, product-rich fractions were combined and concentrated in a rotary evaporator and the
10 concentrate was freeze dried to obtain 1.0g compound **7** (41% yield for the last two steps). Purity: 92 %a.

¹H-NMR spectrum of the product confirmed the proposed structure as a mixture of rotamers.

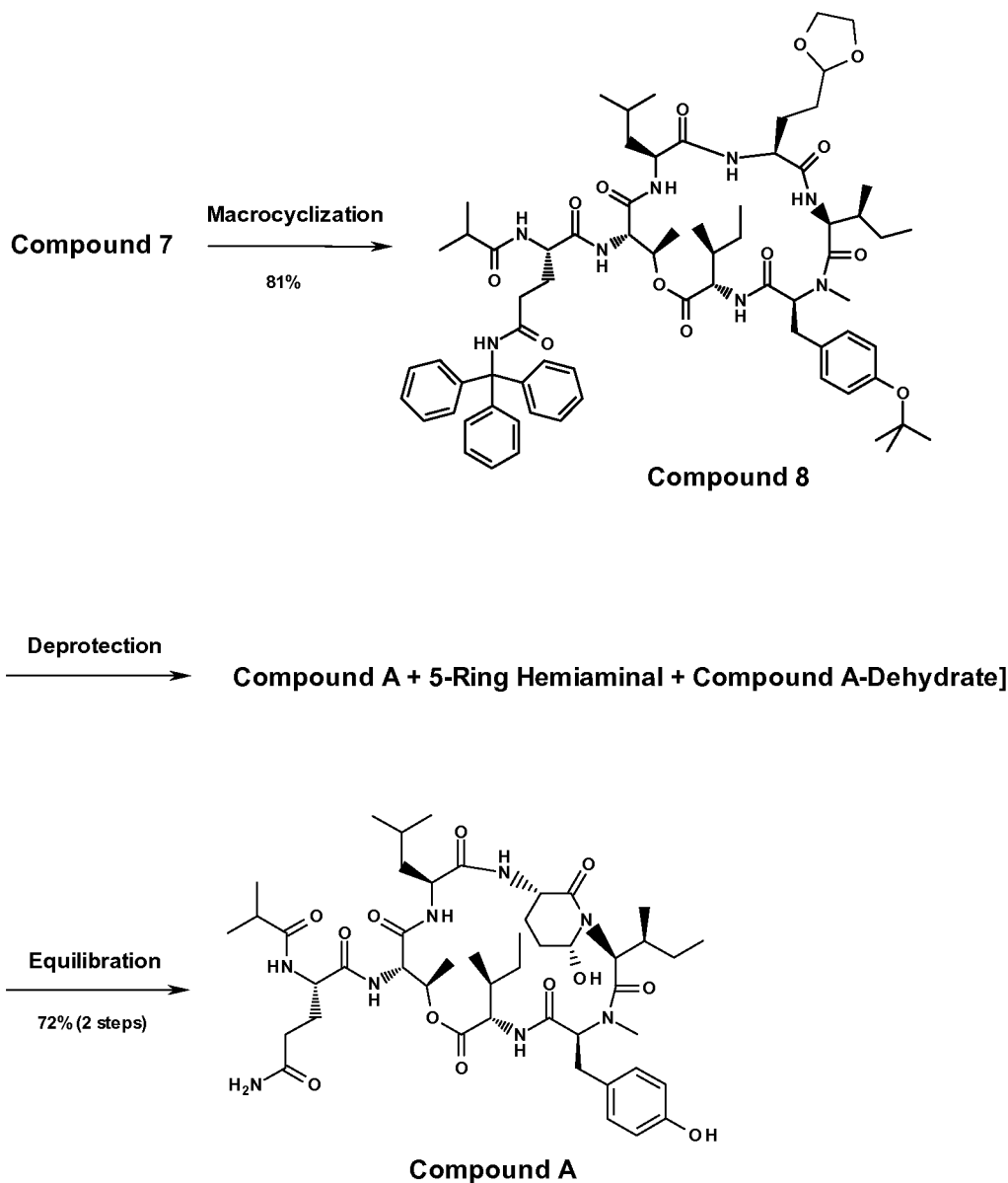
HR-MS: Calculated for C₇₁H₁₀₀N₈O₁₄ [M+H]⁺:1289.74318; [M+Na]⁺:1311.72512. Found:

- 15 [M+H]⁺:1289.74292; [M+Na]⁺:1311.72473.

1 E) Synthesis of Compound A

The final steps in the synthesis are shown in the subsequent scheme:

Reaction scheme 3:



In detail the synthesis is described in the following:

5

1 E) (A) **Macrocyclization: preparation of compound 8**

4-DMAP (100 mg, 0.82 mmol) and HATU (205.4 mg, 0.54 mmol) were dissolved in acetonitrile (15 mL). The solution was cooled to 0 °C. To this solution, a solution of compound **7** (348 mg, 0.27 mmol) in acetonitrile (20 mL) was added dropwise within 10 minutes. The reaction mixture was stirred for additional 10 minutes at 0 °C to complete

10

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the cyclization. For work-up, the reaction mixture was poured onto water (50 mL) and isopropyl acetate (150 mL) was added, followed by the addition of brine (10 mL). The phases were separated, and the organic phase was extracted again with a dilute aqueous NaCl-solution (60 mL). The organic phase was separated and the solvent was evaporated in rotavap under reduced pressure to obtain 523mg crude product. The crude product was purified by flash chromatography on silica gel with ethyl acetate as mobile phase to obtain 278.9 mg compound **8** (81.3% yield).

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: $[M+H]^+$: 1271.73210; $[M+NH_4]^+$: 1288.75820; $[M+Na]^+$: 1293.71478.

1H -NMR (600 MHz, d_6 -DMSO): δ ppm 0.47 (3H, m); 0.73 (3H, m); 0.75-0.87 (12H, m); 0.97-1.05 (8H, m); 1.10 (3H, m); 1.13 (1H, m); 1.25 (9H, m); 1.33 (1H, m); 1.46 (1H, m); 1.56 (6H, m); 1.65 (2H, m); 1.79 (1H, m); 1.96 (1H, m); 2.30 (2H, m); 2.43 (1H, m); 2.68 (4H, m); 3.29 (1H, m); 3.73-3.88 (4H, m); 4.17 (1H, m); 4.19 (1H, m); 4.24 (1H, m); 4.30 (1H, m); 4.42 (1H, m); 4.57 (1H, m); 4.73 (1H, m); 5.17 (1H, m); 5.26 (1H, m); 6.86 (2H, m); 7.12-7.27 (18H, m); 7.90 (1H, m); 7.94 (1H, m); 8.18 (1H, m); 8.51 (2H, m); 8.82 (1H, m).

IR: 3637, 3400, 3306, 3059, 3031, 2968, 2934, 2877, 1738, 1658, 1509, 1469, 1449, 1414, 1388, 1367, 1339, 1239, 1162, 1100, 1066, 1037, 968, 946, 851, 767, 753, 741, 702, 637, 625.

1 E) (B) Deprotection and Equilibration

Compound **8** (200mg, 0.157 mmol) was dissolved in dichloromethane (40 mL) and the solution was cooled to 0 °C. Trifluoroacetic acid (11.5g) was added dropwise to the solution at 0°C and the reaction mixture was stirred for additional 3h at 0 °C. The reaction mixture was then diluted with dichloromethane (40 mL) and water (2mL) was added. The reaction temperature was elevated to 20-25 °C and the mixture was stirred for 16h at this temperature. The reaction mixture was then poured onto a solution of sodium acetate (16.3g) in water (80 mL) and ethyl acetate (40 mL) was added. The biphasic mixture was stirred intensively and the phases were separated. The organic phase was washed with water (2x20 mL) and the water phases were extracted with ethyl acetate (40 mL). The organic phases were combined and dried over magnesium sulfate. Evaporation of the solvent at 40-45 °C under reduced pressure gave 270mg of crude product. The cru-

- 65 -

de product was purified by flash chromatography on silica gel using ethyl acetate/isopropanol (9:1) as eluent to obtain 105mg of compound **A**. Yield: 71.8%.

The structure was confirmed by NMR data.

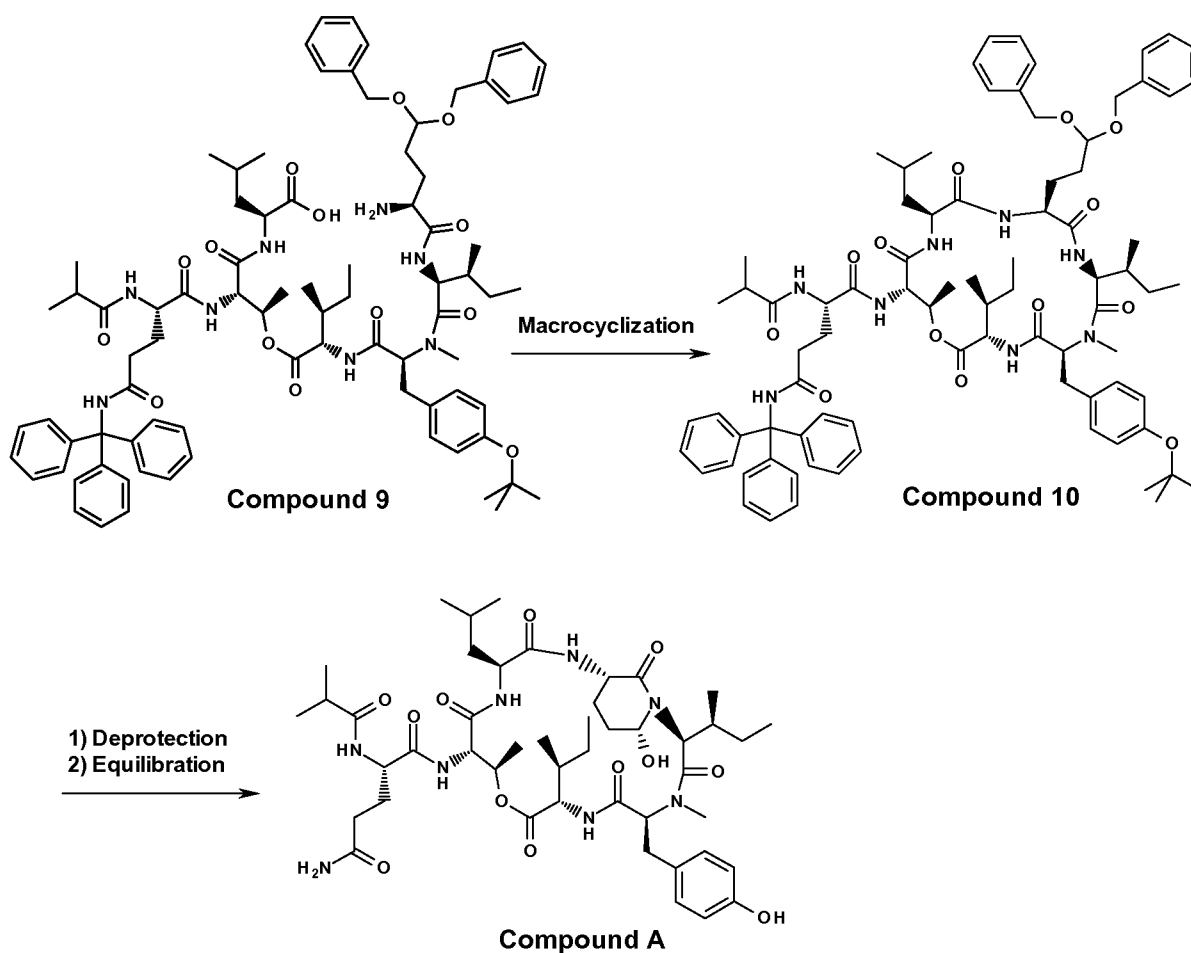
HR-MS: Calculated for $C_{46}H_{72}N_8O_{12}$ $[M+H]^+$: 929.53425; $[M+NH_4]^+$: 946.56080; $[M+Na]^+$:

5 951.51619. Found: $[M+H]^+$: 929.53412; $[M+NH_4]^+$: 946.56110; $[M+Na]^+$: 951.51611.

**Example 2: Synthesis of Compound A using an acyclic acetal protecting group:
Employing of the dibenzyl acetale**

10 The following reaction scheme shows the use of compound **9** as an alternative to Compound **7** in Example 1:

Reaction scheme 4:



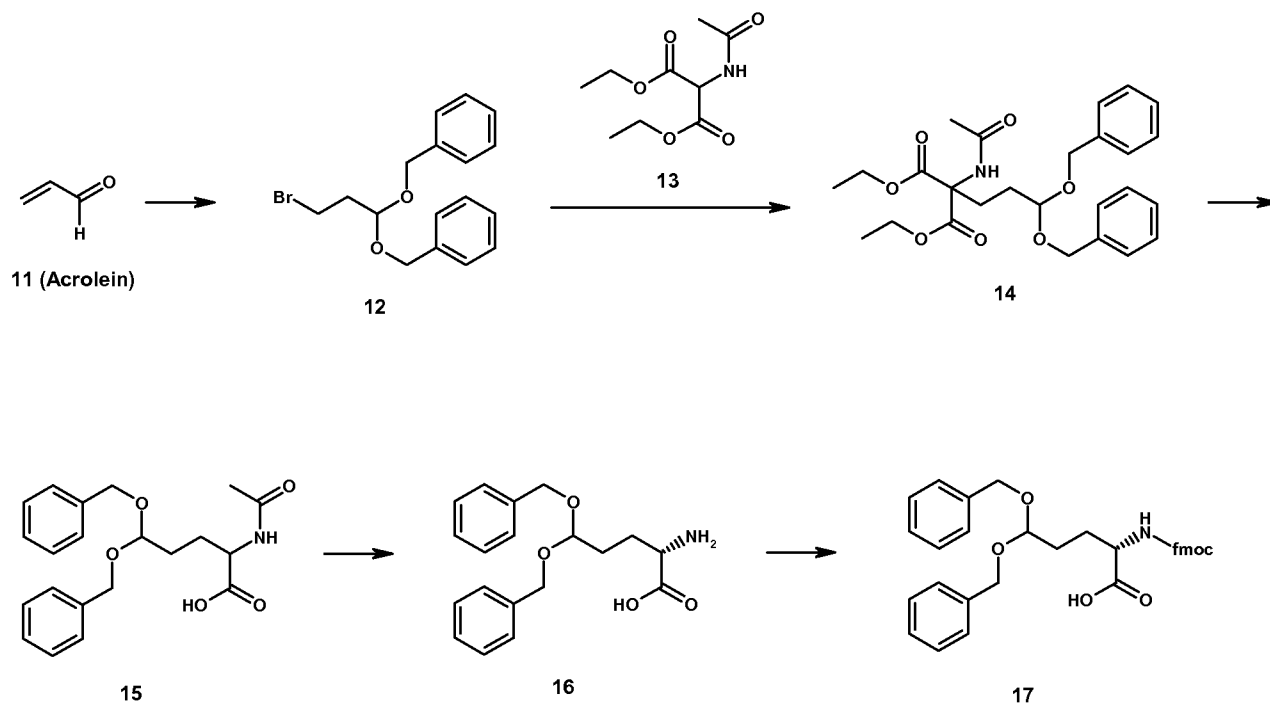
In detail, the corresponding precursors and the reactions in the scheme are realized as follows:

2 A) Synthesis of the dibenzyl-acetale synthon (compound 17)

5

Compound **17** was prepared according to the synthesis scheme shown below:

Reaction scheme 5:



10

2 B) Synthesis of compound 12:

In a 3L-reactor under nitrogen at 0°C, dried acrolein (105.3 g, 1.78 mol) in methylene chloride (1.15 kg) was introduced. Trimethylbromosilane (281.7 g, 1.78 mol) was added dropwise over 30 min with a dropping funnel, keeping the temperature below 5°C. After 1 h 30 min of stirring, benzyl alcohol (311.1 g, 2.85 mol) was added dropwise over 45 min, and the mixture was stirred at 0°C for 16 h. To the orange solution, pyridine (29 g, 0.36 mol), acetic anhydride (38 g, 0.36 mol) and DMAP (4.4 g, 0.04 mol) were added. The mixture was stirred at room temperature for 24 h, cooled to 10°C, and NaHCO₃ 10% solution (750 mL) was added until pH=7. The organic layer was washed with water (2 x

20

500 mL), and dried over anhydrous sodium sulfate. Evaporation of the solvent gave Compound **12** (428 g, 90%) as a crude yellow oil which dissociates upon standing a few hours in the fridge. After removal of the viscous orange lower phase, 6% of benzyl acetate was still present in the crude material.

5 ^1H NMR (400 MHz, CDCl_3) δ ppm 2.2 (q, 2H), 3.4 (t, 2H), 4.5 (d, 2H), 4.6 (d, 2H), 4.9 (t, 1H), 7.2 (m, 10H).

2 C) Synthesis of compound 14:

10 In a 3L-reactor, diethylacetamidomalonate (Compound **13**, 275 g, 1.241 mol) was introduced in anhydrous DMF (800 g) under N_2 . Potassium tert-butoxide (144g, 1.241 mol) dissolved in anhydrous DMF (424 g) was added over 10 min. by a dropping funnel. The temperature rose to 45°C. The mixture was heated up to 80°C, and crude Compound **12** (428 g, 1.034 mol) was added dropwise over 1h. Additional DMF (125 g) was used, and the mixture was stirred for 2 h at 80°C under inert atmosphere. The solvent was then

15 evaporated. Ethyl acetate (1.2 L) and water (2.5 L) were added. The organic layer was washed with aq. NaCl 10% (3 x 1.5 L), and the organic solvent was distilled off.

Crystallization of the residue, containing some residual ethyl acetate, from t-butyl-methyl ether/heptane gave Compound **14** in 87 % yield as a white solid (423 g, purity: 97%).

20 ^1H NMR (400 MHz, CDCl_3) δ ppm 1.2 (t, 6H), 1.6 (m, 2H), 2.0 (s, 3H), 2.5 (m, 2H), 4.2 (m, 1H), 4.55 (d, 2H), 4.65 (d, 2H), 4.7 (t, 1H), 6.8 (s, 1H), 7.3 (m, 10H).

2 D) Synthesis of compound 15:

In the 3L-reactor, Compound **14** (211 g, 434 mmol) and 950 mL ethanol 95 % were introduced. Potassium hydroxide (30 g, 477 mmol) in 150 mL ethanol 95 % was added over

25 25 min with a dropping funnel. The slurry became a clear solution. The mixture was stirred for 3h30min at RT. The complete conversion of the monoacid/monoester intermediate was established by HPLC. The mixture was heated to reflux for 2h30min for decarboxylation. Then, more potassium hydroxide (34 g, 564 mmol) in 150 mL ethanol 95 % was added, and the mixture was stirred for 2 h at 75°C. The ethanol was then distilled off

30 over 1h. The mixture was cooled to room temperature; water was added, followed by t-butyl-methyl-ether to extract insoluble by-products. Then, the aqueous layer was acidified with acetic acid to pH 5.0. and the product was extracted again with t-butyl-methyl-ether, the organic layer was washed with water, and concentrated in vacuo. Note that a mild concentration is required, more precisely a temperature below 50°C, with a good

vacuum, and quite fast; otherwise decomposition to the cyclic derivative occurs. The re-covered oil crystallizes very quickly. Recrystallization from t-butyl-methyl-ether/Heptane (1:2) gave 148.6 g of Compound **15** as a white solid (yield: 87%, purity: 98 %a).

¹H NMR (400 MHz, CDCl₃) δ ppm 1.8 (s, 3H), 1.9 (m, 2H), 2.0 (m, 2H), 4.5 (q, 1H), 4.6 (d, 2H), 4.7 (d, 2H), 4.8 (t, 1H), 6.4 (d, 1H), 7.3 (m, 10H).

2 E) Synthesis of compound 16:

In a 3L-reactor citrate buffer (1 L, pH=5) was introduced. The aminoacid, Compound **15** (146 g, 385 mmol) was added, and the pH was adjusted to 8.0-8.5 with sodium hydroxide 30 % (84 g) and additional citrate buffer (130 mL). Cobalt chloride 1 mM (150 mL) was added to get a concentration of cofactor of 0.1 mM. The enzyme (acylase "Amano" ACV12502 (Amano Enzyme Inc., Nagoya, Japan), 7.25 g, 5% w/w) was finally added at 30°C. The brown mixture was stirred until HPLC showed 50 % conversion (around 24h). The white slurry was acidified to pH 7.0, filtered off. Note that filtration of the crude solid was quite slow and required a large funnel to proceed properly. The solid was washed with water (3x) and acetone (3x), and eventually dried in vacuo. Recrystallisation in methanol/water gives 51.6 g of Compound **16** as a white solid (41% isolated yield, purity: 99%). mp 190 °C with decomposition.

¹H NMR (400 MHz, CDCl₃) δ ppm 1.8 (m, 4H), 2.5 (s, 2H), 3.1 (t, 1H), 4.5 (d, 2H), 4.6 (d, 2H), 4.7 (t, 1H), 7.3 (m, 10H).

2 F) Synthesis of compound 17:

In a 750mL-reactor, under inert atmosphere, was introduced Compound **16** (40.2 g, 120 mmol) in water (320 mL) and acetonitrile (80 mL). To this white slurry was added over 35min triethylamine (24.3 g, 240 mmol). After complete dissolution of the solid, Fmoc-OSu (40.5 g, 120 mmol) was added. The mixture was stirred at room temperature for 3h30. Extraction was carried out with ethylacetate. The organic layer was washed with water, and a large amount of solvent was distilled off. A small amount of water was added, and the mixture was acidified to pH 4.6. The organic layer was kept, washed with water (2x) and finally concentrated in vacuo. Recrystallization of the residue in t-butyl-methyl-ether/Heptane (1/3) gave Compound **17** as a white solid (58.2g, 88% yield, purity: 92 %a, ee: 100% established by chiral HPLC).

¹H NMR (400 MHz, DMSO) δ ppm 1.8 (m, 4H), 4.0 (m, 1H), 4.2 (q, 1H), 4.3 (d, 2H), 4.5 (d, 2H), 4.6 (d, 2H), 4.8 (t, 1H), 7.3 (m, 11H), 7.4 (d, 2H), 7.6 (d, 1H), 7.7 (d, 2H), 7.9 (d, 2H).

5 **2 G) Synthesis of compound 9:**

Compound **17** (8.388g, 15.21 mmol) was coupled on Isobutyl-Gln(Trt)-Thr(Ile-Tyr(tBu)Me-Ile-Fmoc)-Leu-Linker-Resin (10.3g, 4.33 mmol), the Fmoc protecting group was cleaved on the linker-resin-bound peptide and the product was cleaved from the linker-resin according to the procedures described above for the preparation of
10 compound **6**. The crude product was purified by RP-chromatography, using a YMC-ODS AQ column. The fractions were assessed using HPLC, product-rich fractions were combined and concentrated in a rotary evaporator and the concentrate was freeze dried to obtain 2.3g of compound **9** (36.8% yield for the last two steps). HPLC-Purity: 92.9 %a. ¹H-NMR confirmed the proposed structure as a mixture of rotamers.

15 HR-MS: Calculated for C₈₃H₁₁₁N₈O₁₄ [M+H]⁺: 1443.82143. Found: [M+H]⁺: 1443.82166.

2 H) Synthesis of compound 10 (macrocyclization):

4-DMAP (256 mg, 2.09 mmol) and HATU (527 mg, 1.39 mmol) are dissolved in acetonitrile (20 mL). The solution is cooled to 0 °C. To this solution, a solution of compound **9**
20 (1.00 g, 0.693 mmol) in acetonitrile (30 mL) is added dropwise within 10 minutes. The reaction mixture is stirred for additional 15 minutes at 0 °C to complete the cyclization. For work-up, the reaction mixture is poured onto water (100 mL) and isopropyl acetate (250 mL) is added, followed by the addition of brine (20 mL). The phases are separated and the organic phase is extracted again with a dilute aqueous NaCl-solution (60 mL).

25 The organic phase is dried over anhydrous magnesium sulfate and the solvent is evaporated at 40-45 °C under reduced pressure to obtain 1.08g crude product. Purification of the crude product by flash chromatography on silica gel using ethyl acetate as eluent and drying of the product in vacuo at 40-45 °C gave 0.62 g compound **10**.

HR-MS: Calculated for C₈₃H₁₀₈N₈O₁₃ [M+H]⁺: 1425.81086; [M+NH₄]⁺: 1442.83741;
30 [M+Na]⁺: 1447.79281; [M+K]⁺: 1463.76675. Found: [M+H]⁺: 1425.81140; [M+NH₄]⁺: 1442.83728; [M+Na]⁺: 1447.79248; [M+K]⁺: 1463.76685.

¹H-NMR (600 MHz, d₆-DMSO): δ ppm 0.47 (br. S., 1H), 0.47-0.54 (m, 1H), 0.63-0-70 (m, 2H), 0.73-0.81 (m, 9H), 0.85 (d, J= 6.6Hz, 3H), 0.97-1.05 (m, 7H), 1.08

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(d, J= 6.6Hz, 2H), 1.16-1.22 (m, 2H), 1.23-1.31 (m, 3H), 1.27 (m, 9H), 1.46-1.52 (m, 1H), 1.53-1.61 (m, 3H), 1.65-1.73 (m, 3H), 1.80-1.88 (m, 2H), 1.94-2.01 (m, 1H), 2.00 (s, 1H), 2.32 (m, 2H), 2.39-2.47 (m, 1H), 2.49-2.55 (m, 2H), 2.68-2.76 (m, 1H), 2.72 (s, 2H), 3.29 (dd, J= 14.3, 7.7Hz, 1H), 3.36 (m, 8H). 4.20-4.24 (m, 1H), 4.30-4.34 (m, 1H), 4.48-4.56 (m, 3H), 4.58-4.66 (m, 3H), 4.71 (m, 1H), 5.27-5.35 (m, 1H), 6.84-6.91 (m, 2H), 7.15-7.22 (m, 11H), 7.25-7.31 (m, 8H), 7.32-7.36 (m, 6H), 7.90-7.98 (m, 2H), 8.52 (s, 1H), 8.74-8.83 (m, 1H).

2 I) Synthesis of compound A from compound 10 (de-protection and equilibration):

Compound **10** (400 mg, 0.281 mmol) is dissolved in dichloromethane (80 mL) and the solution is cooled down to 0 °C. Trifluoroacetic acid (20.5g) is added under intense stirring at 0 °C. The reaction mixture is stirred for additional 3h at this temperature and diluted with dichloromethane (80 mL), followed by the addition of water (4.0 mL). The temperature was then allowed to rise to room temperature and the reaction was stirred intensively for 20h at room temperature.

The reaction mixture is then poured onto a solution of sodium acetate (29.3g) in water (160 mL) and ethyl acetate (80 mL) is added. The biphasic mixture is stirred intensively and the phases are separated. The organic phase is washed with water (2x40 mL) and the water phases are extracted with ethyl acetate (80 mL). The organic phases are combined and dried over magnesium sulfate. Evaporation of the solvent at 40-45 °C under reduced pressure gave 520mg of crude product. The crude product was purified by flash chromatography on silica gel using ethyl acetate/isopropanol (9:1) as eluent to obtain 171mg of Compound A. Yield: 65.5%.

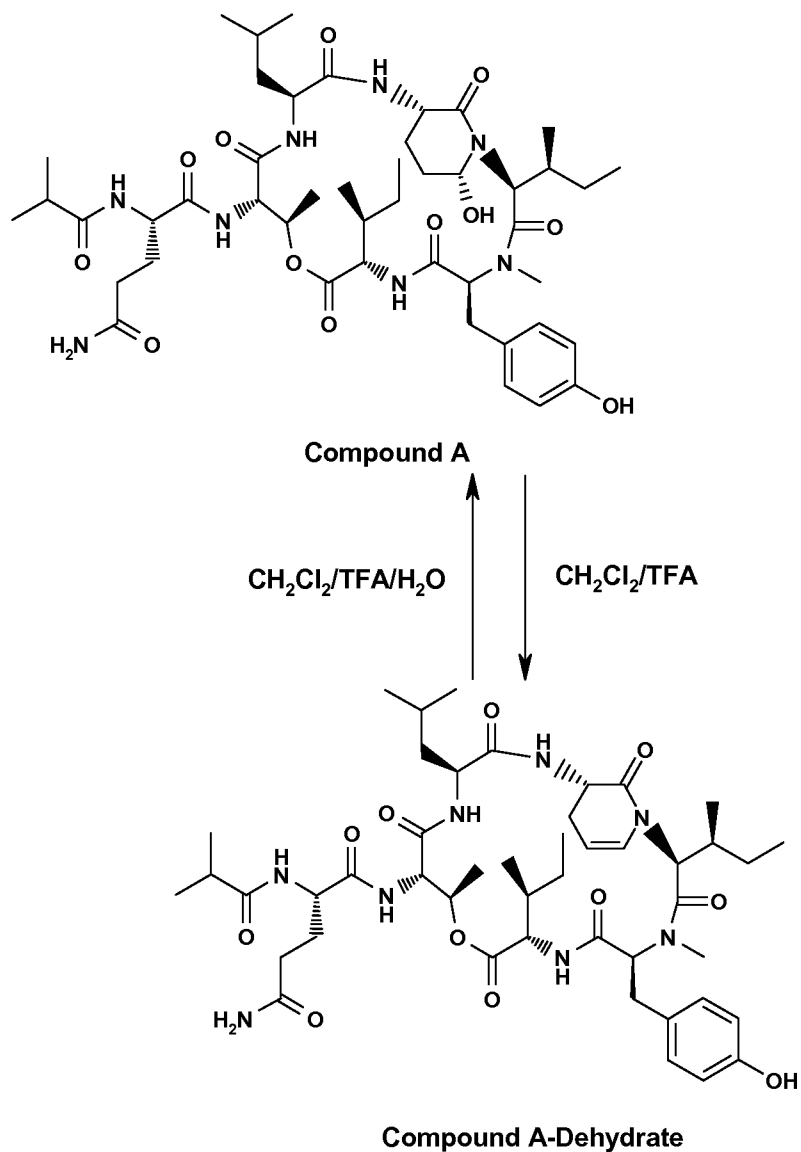
NMR and HR-MS data confirmed the proposed structure.

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: $[M+H]^+$: 929.53345; $[M+NH_4]^+$: 946.56045; $[M+Na]^+$: 951.51536.

Example 3: Shift of Equilibrium

A possible side reaction of the acetal cleavage and formation of ahp may result in the dehydrate form of Compound A. This can be converted easily (back) into Compound A using a simple procedure for hydration of the dehydrate form depicted in the following reaction scheme:

Reaction scheme 6:



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

For example, during cleavage of acid sensitive protecting groups in a compound comprising the ahp-subunit, e.g. Compound A in the Scheme before Example 1, the

- 10 formation of large amounts of the corresponding dehydrated byproduct (Compound A-Dehydrate) is observed. This byproduct is usually separated e.g. by chromatography and

disposed. This leads to loss of valuable product and to low yield for this step. For example, if the deprotection product(s) of Compound 8 are subjected to acidic conditions to cleave the trityl- and t-butyl protecting groups (scheme 4), significant amounts of Compound-A-Dehydrate are formed as byproduct. Depending on the acid concentration and reaction conditions, Compound-A-Dehydrate might be formed even as major product in this product mixture.

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

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

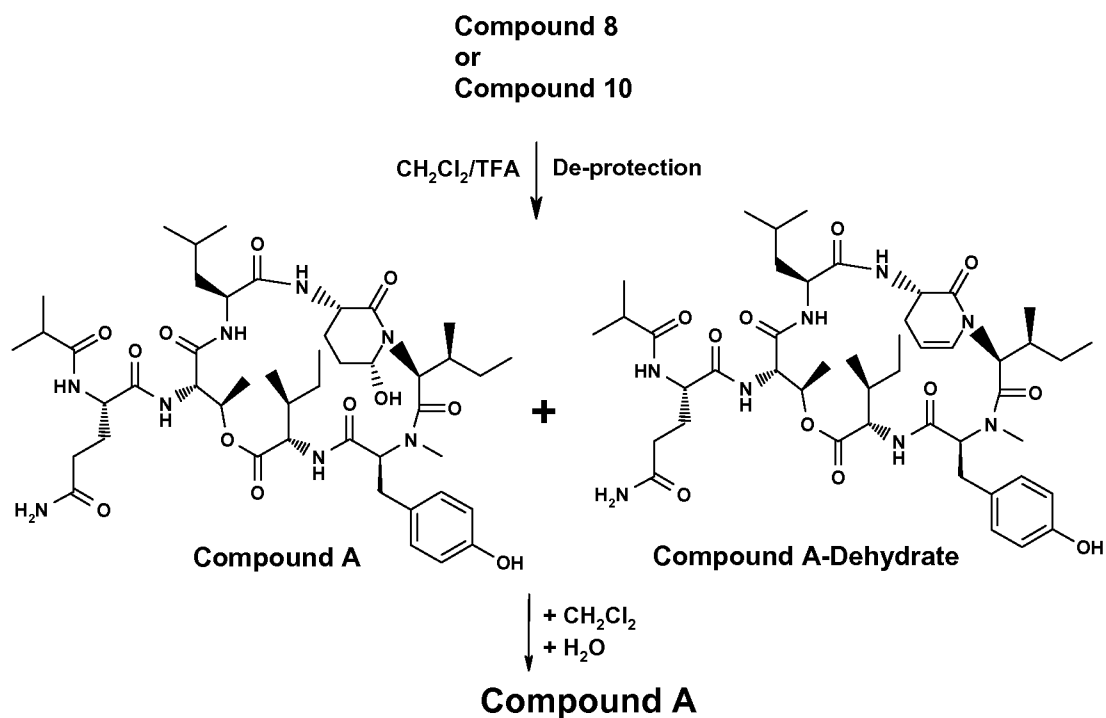
The formation of Compound A -Dehydrate from Compound A under acidic de-protection conditions was confirmed by conversion of pure Compound A into Compound A -Dehydrate using trifluoroacetic acid in DCM. Treatment of Compound A with 33% (v/v) TFA in DCM for 2h at room temperature gave a product mixture of Compound A -Dehydrate/Compound A in a ratio of 78:22 according to HPLC. The dehydration could be driven to >95% conversion, when water absorbing agents, such as molecular sieves were added to the reaction mixture. Thus, stirring of pure Compound A in a 1:2 mixture of TFA/DCM in the presence of molecular sieves gave the dehydrated-product in quantitative crude yield and ca. 96 area% HPLC purity (example 3 B). There were still ca. 4 area-% of Compound A present in the crude product.

Conversion of Compound A -Dehydrate from Example 3 B into Compound A was demonstrated by stirring Compound A-dehydrate in dichloromethane in the presence of

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trifluoroacetic acid and water (Example 3 C). The product thus obtained comprised 95.6 area% Compound A and only 4.4 area% Compound A -dehydrate according to HPLC.

5 Reaction Scheme 7:



For physical data of Compound A see Examples 1 E(B) and 2 I).

- 10 The equilibration also converts the 5-ring hemiaminal isomer into compound **A**, which is a 6-ring hemiaminal. The 5-ring hemiaminal isomer is formed in large amounts as kinetic product after de-protection of the aldehyde function (reaction scheme 3). This isomer is converted to the thermodynamically more stable compound A during equilibration.

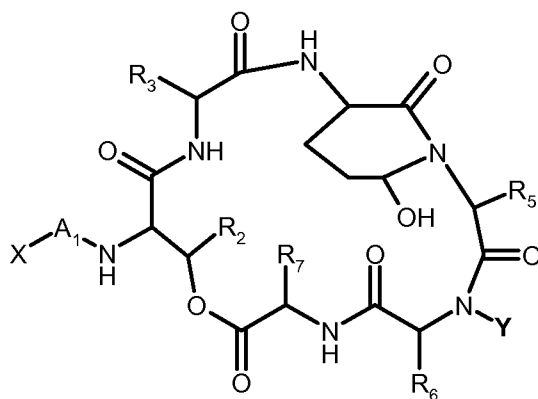
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Claims

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



5

wherein

A₁ is a bivalent moiety of an amino acid with a terminal carboxy or carbamoyl group and is bound at its right hand side in formula I via a carbonyl to the rest of the molecule; or is C₁₋₈-alkanoyl or phosphorylated hydroxy-C₁₋₈-alkanoyl;

10 X is bound via an N of A₁ and is acyl, or is absent if A₁ is C₁₋₈-alkanoyl or phosphorylated hydroxy-C₁₋₈-alkanoyl;

R₂ is C₁₋₈-alkyl;

R₃ is the side chain of an amino acid;

R₅ is the side chain of an amino acid;

15 R₆ is the side chain of a hydroxy amino acid;

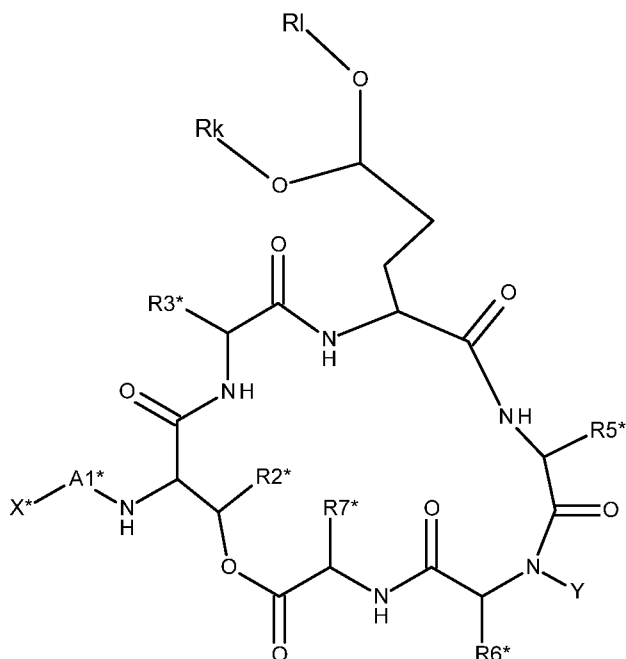
R₇ is the side chain of an amino acid; and

Y is hydrogen or C₁₋₈-alkyl;

or a salt thereof,

20 said method comprising
deprotecting a compound of the formula II

- 75 -



II

wherein the aldehyde protecting group(s) Rk and RI 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*, A₁*, R₂*, R₃*, R₅*, R₆*, and R₇* correspond to X, A₁, R₂, R₃, R₅, R₆, and R₇ 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;

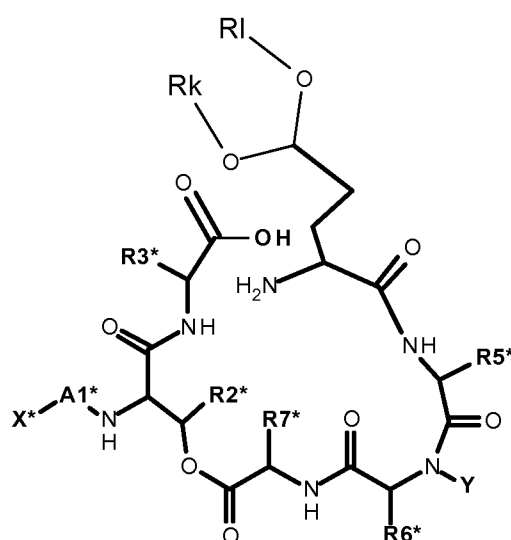
and, if desired, converting a free compound of the formula I into a salt, a salt of a compound of the formula I into a different salt of a compound of the formula I, or into the free compound of the formula I, and/or converting a dehydrate analogue and/or five ring analogue of a compound of the formula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

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

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3. The method or process according to claim 1 or claim 2, comprising, for the synthesis of the compound of the formula II given in claim 1, cyclization under lactamization of a linear, not yet cyclic, precursor peptide of the compound of the formula II, carrying an N-terminal amino group and a C-terminal carboxy group, under reaction conditions that
 5 allow for the formation of an amide bond from said amino and said carboxy group, preferably using Solution Phase chemistry.

4. The method or process according to claim 3, where the linear precursor peptide is of the formula III,

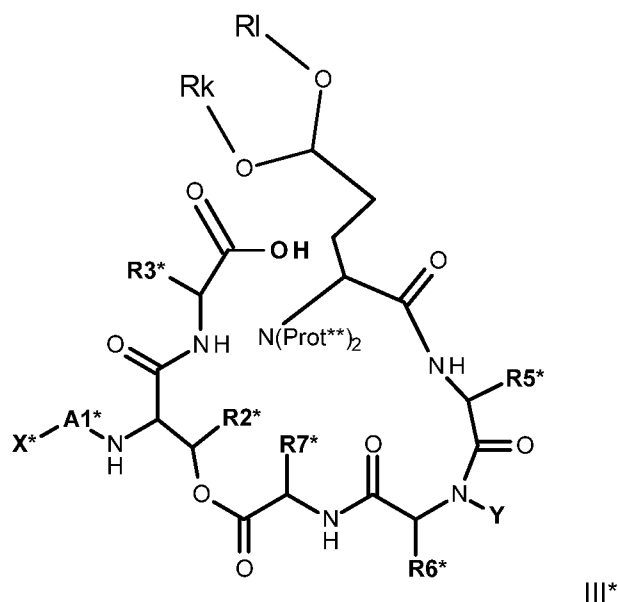


III

wherein Rk, RI, X*, A₁*, R₂*, R₃*, R₅*, R₆* and R₇* are as defined for a compound of the formula II in claim 1,

which can be obtained directly from solid phase peptide synthesis (e.g. as described
 15 under step (iii/b) or by deprotection from the corresponding compound of the formula III*,

- 77 -



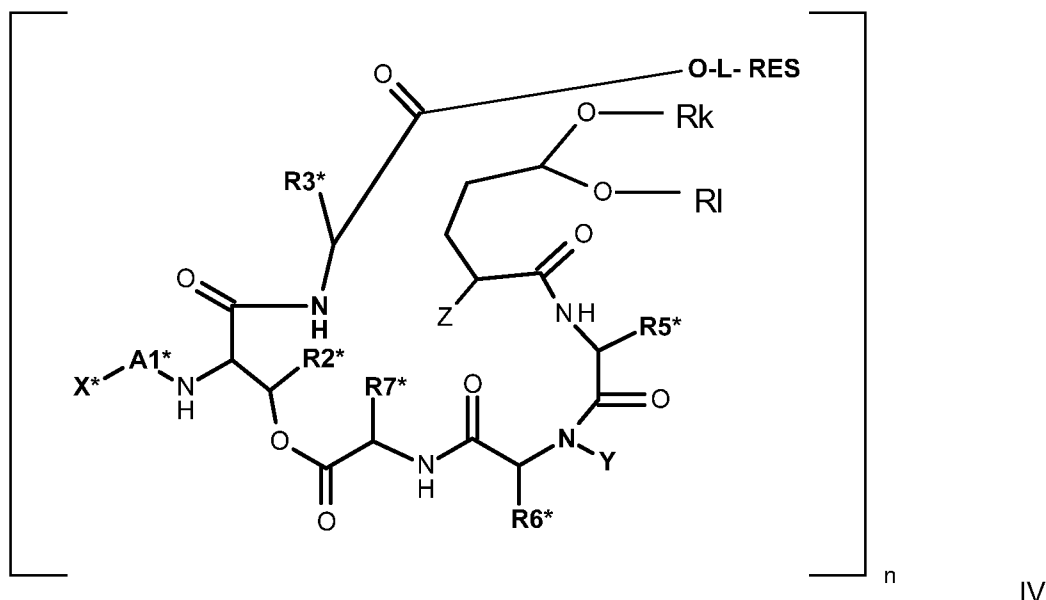
wherein Rk, RI, X*, A₁*, R₂*, R₃*, R₅*, R₆* and R₇* are as defined for a compound of the formula II above and wherein each of the Prot** moieties is a protecting group that can be removed under conditions different from those of the cleavage according to claim 5,

5 by deprotecting the protected amino.

5. The method or process according to claim 4, further comprising, for the synthesis of the compound of the formula III, or of a compound of the formula III*, cleaving a

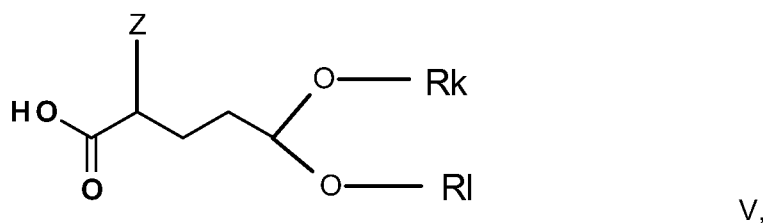
10 compound of the formula IV,

- 78 -



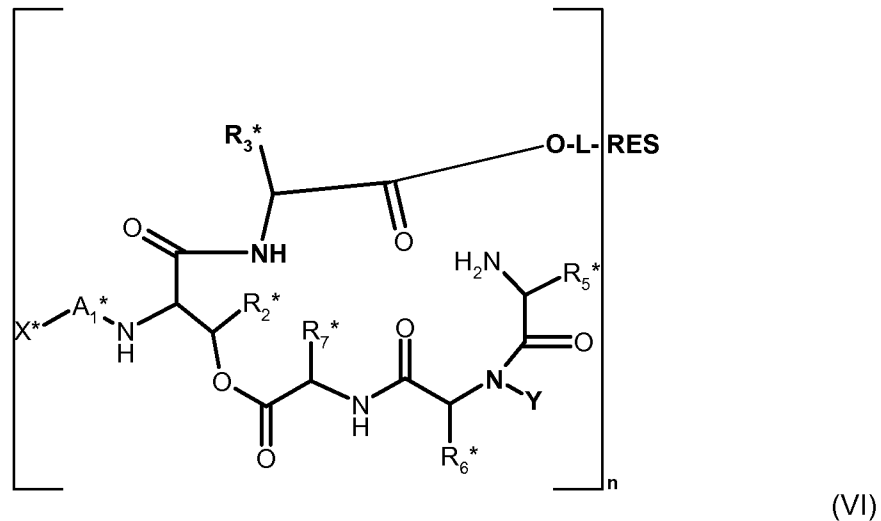
wherein Rk, RI, X*, A1*, R2*, R3*, R5*, R6* and R7* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, n is a natural number not including 0 and Z is a protected amino group either of the formula NHProt* wherein Prot* is a protecting group that can be (and is) removed before or during the cleaving reaction or further subsequently to it to yield a compound of the formula III; or Z is a protected amino group of the formula N(Prot**)2 wherein each one of Prot** is an amino protecting group that can be removed under conditions different from those of the cleaving reaction to yield the compound of the formula III*.

6. The method or process according to claim 5, further comprising, for the synthesis of the compound of the formula IV, coupling an amino acid of the formula V,



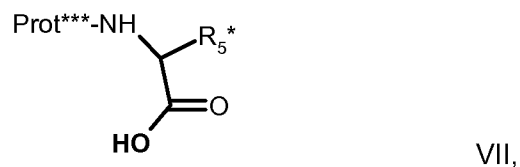
wherein Rk and RI are as defined for a compound of the formula II in claim 1 and above and Z is a protected amino group either of the formula NHProt* wherein Prot* is a protecting group that can be (and is) removed before or during the cleaving reaction under (iii/b) or further subsequently; or Z is a protected amino group of the formula N(Prot**)2 wherein each Prot** is an amino protecting group that can be removed under

conditions different to those of the cleaving reaction under in claim 5, or an activated derivative of said amino acid, with a compound of the formula VI,



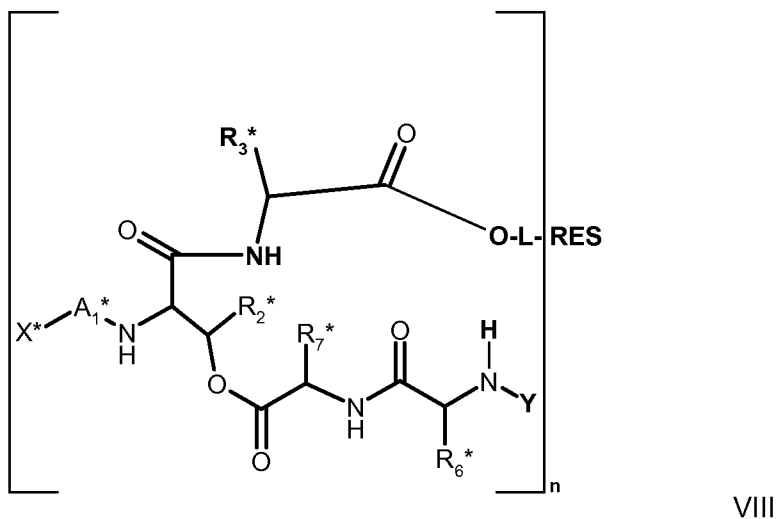
5 wherein X*, A₁*, R₂*, R₃*, R₅*, R₆* and R₇* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0.

7. The method or process according to claim 6, further comprising, for the synthesis of
10 the compound of the formula VI, coupling an amino acid of the formula VII



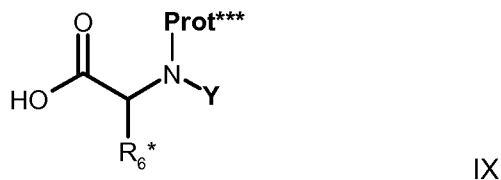
wherein R₅^{*} is as defined for a compound of the formula II in claim 1 and Prot^{***} is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula VIII,

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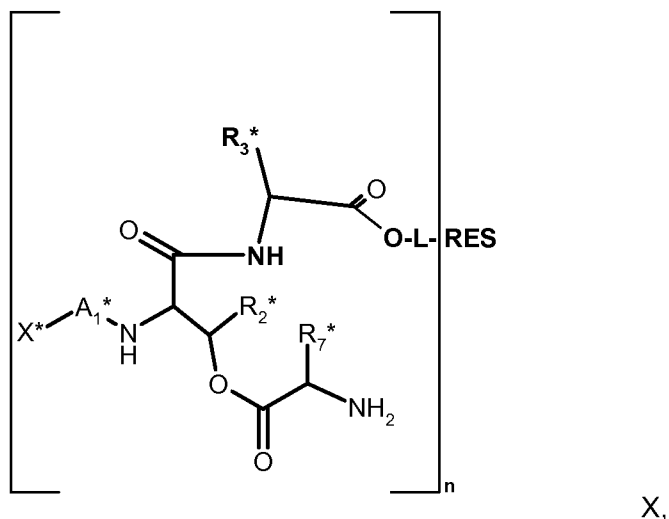
wherein X^* , A_1^* , R_2^* , R_3^* , R_6^* and R_7^* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0, and removing the protecting group Prot^{***} .

8. The method or process according to claim 7, further comprising, for the synthesis of the compound of the formula VIII, coupling an amino acid of the formula IX,



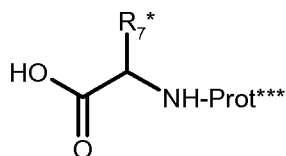
in which R_6^* and Y are as defined for a compound of the formula II in claim 1 and Prot^{***} is an amino protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, or a reactive derivative of said amino acid, with a compound of the formula X,

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wherein X^* , A_1^* , R_2^* , R_3^* and R_7^* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0,
 5 and removing the protecting group Prot***.

9. The method or process according to claim 8, further comprising, for the synthesis of a compound of the formula X, reacting an amino acid of the formula XI,



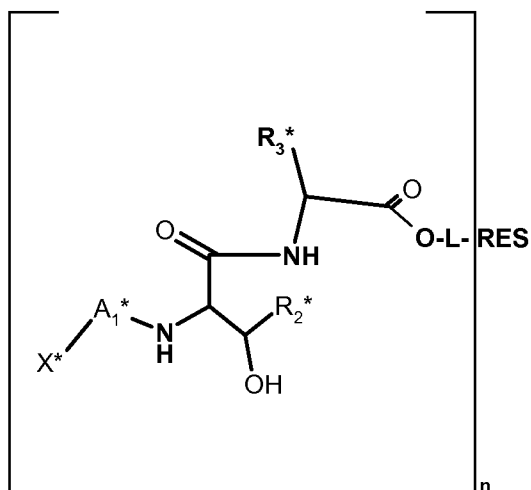
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XI

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

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

- 82 -

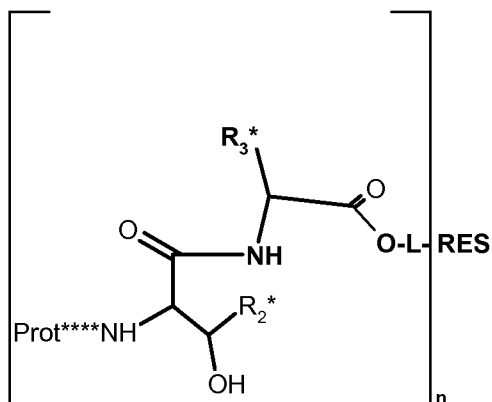


XII

wherein X^* , A_1^* , R_2^* and R_3^* are as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0; and removing the protecting group Prot***.

5

10. The method or process according to claim 9, further comprising, for the synthesis of a compound of the formula XII, coupling a resin bound dipeptide symbolized by the formula XIII,



XIII

10

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

- 83 -

is a natural number not including 0, after removal of the protecting group Prot**** via the thus obtainable free amino group, with an acid of the formula XIV,

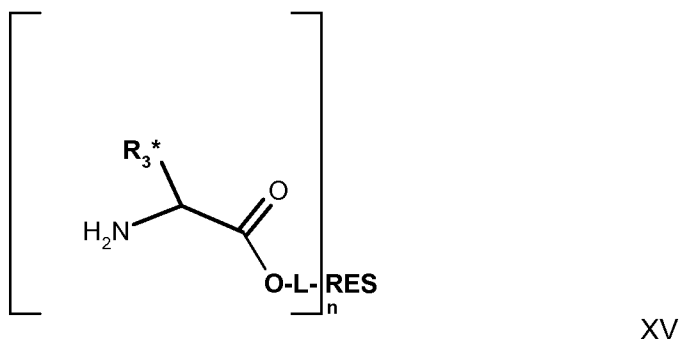


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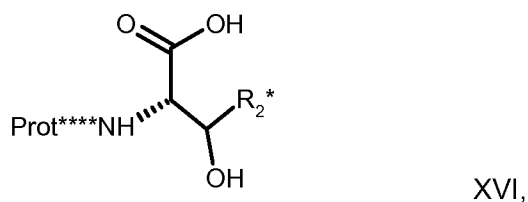
wherein X** is an amino protecting group or is X*, and wherein X* and A₁* are as defined for a compound of the formula II in claim 1, or a reactive derivative of said acid;

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

11. The method or process according to claim 10, further comprising, for the synthesis of a compound of the formula XIII, especially XIII A, coupling a resin bound amino acid symbolized by the formula XV,



wherein R₃* is as defined for a compound of the formula II in claim 1, L is a cleavable linker, RES is a solid resin, and n is a natural number not including 0, with an amino acid of the formula XVI,



wherein Prot**** is a protecting group that can be cleaved off selectively without affecting other protecting groups present and with the product remaining on the resin, and R₂* is as defined for a compound of the formula II in claim 1, or a reactive derivative of said amino acid.

5

12. The method or process according to any one of claims 1 to 11, where the symbols A₁, R₂, R₃, R₅, R₆, R₇, X and Y or the corresponding unprotected or protected moieties R₂*, R₃*, R₅*, R₆*, R₇*, X* and Y₃ are selected so that, in the resulting compound of the formula I, or a salt thereof,

10 A₁ is the bivalent radical of L-glutamine bound via the carbonyl of its α-carboxyl group to the amino group at the right of A₁ in formula I and via its α-amino group to X, or is 2S-(2-hydroxy-3-phosphonooxy)-propionyl;

R₂ is methyl;

R₃ is isopropyl, isobutyl or benzyl, especially isobutyl;

15 R₅ is sec-butyl or benzyl, especially sec-butyl;

R₆ is 4-hydroxybenzyl;

R₇ is isopropyl or sec-butyl, especially sec-butyl;

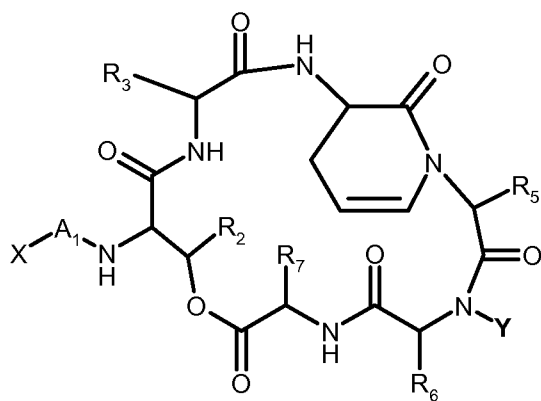
X is acetyl or isobutyryl, or is absent if A₁ is 2S-(2-hydroxy-3-phosphonooxy)-propionyl and

20 Y is methyl.

13. A method or process for converting a dehydrate of a compound of the formula I given in claim 1 or with the substituents as defined in claim 12, in each case obtained according to the method or process of claim 1, into the corresponding compound of the

25 formula I, where the dehydrate has the formula V,

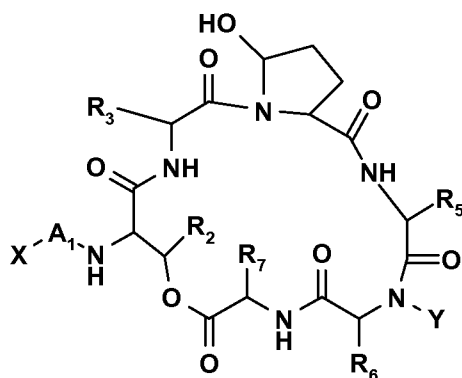
- 85 -



V,

in which Y, X, A₁, R₂, R₃, R₅, R₆ and R₇ are as defined for a compound of the formula I in any one of claims 1 and 11;

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



V*

10 in which Y, X, A₁, R₂, R₃, R₅, R₆ and R₇ are as defined for a compound of the formula I in any one of claims 1 and 17, respectively;

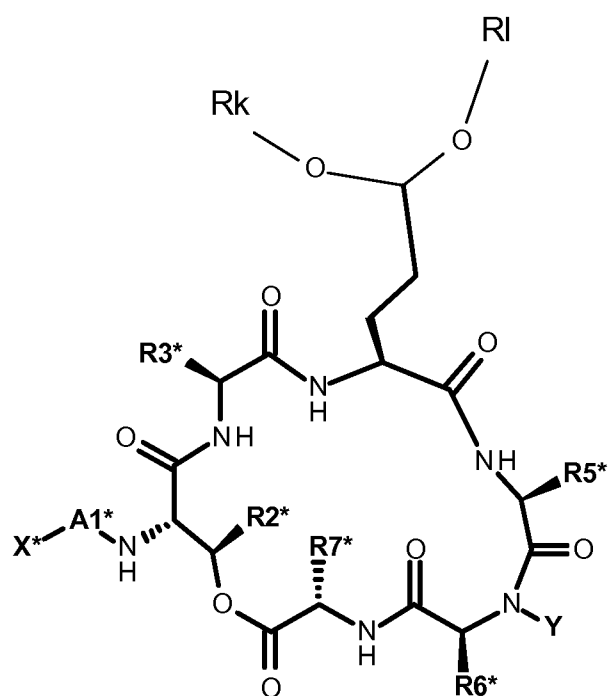
or a method or process for shifting the equilibrium of a mixture of a compound of the formula I obtained according to the method or process of claim 1 and its corresponding dehydrate and/or hemiaminal in favor of the compound of the formula I,

15

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

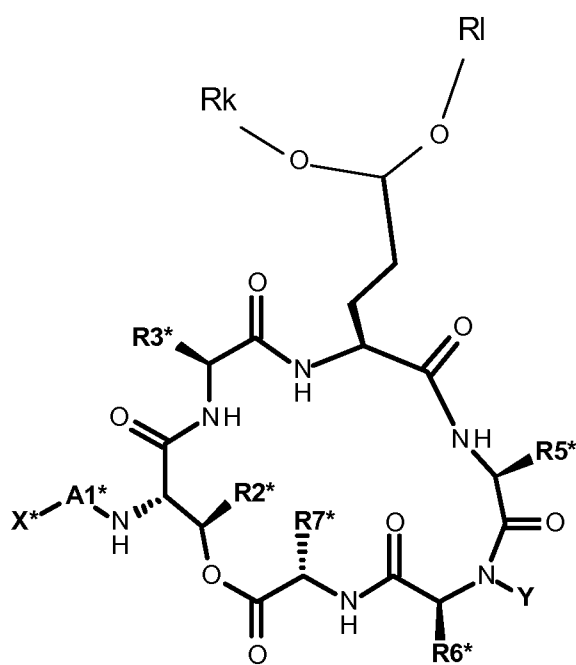
14. A compound of the formula XX,

- 86 -



XX

especially of the formula XXA,



XXA

- 87 -

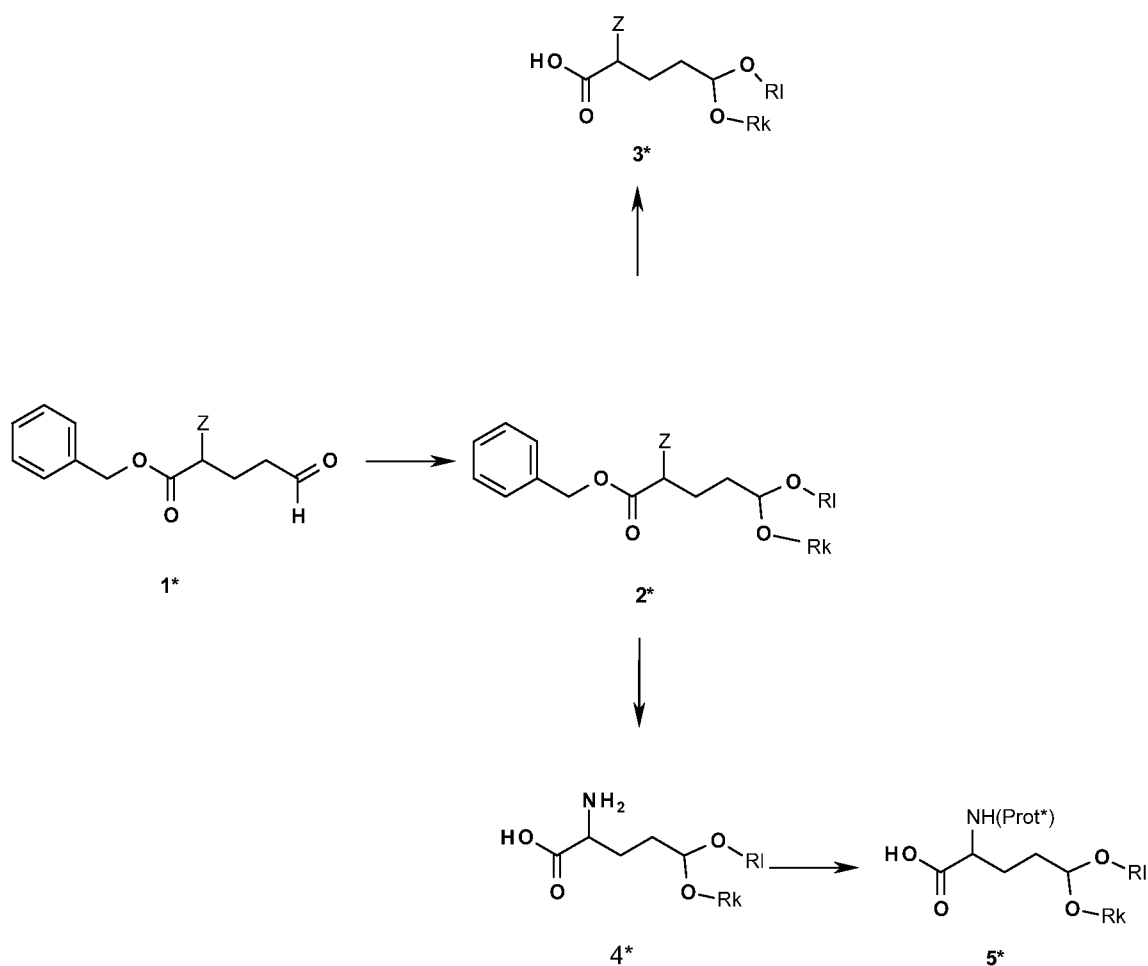
wherein R_k and R_l are as defined for a compound of the formula II in claim 1, Y is as defined for a compound of the formula I in claim 1 or in particular as in claim 14 and X*, A₁*, R₂*, R₃*, R₅*, R₆*, and R₇* correspond to X, A₁, R₂, R₃, R₅, R₆, and R₇ in formula I as defined in claim 1, respectively, however with the proviso that reactive functional groups on these moieties are present in protected form.

15. A method or a process for the synthesis of a compound of the formula V mentioned in claim 6, according to either

(a) in the case of the synthesis of a compound of the formula V wherein R_k and R_l

together form an unsubstituted or substituted lower alkylen bridge,

the following reaction scheme, alternatively via the route (i) 1* -> 2* -> 3*, (ii) 1* -> 2* -> 4* -> 5*, or (iii) 1* -> 2* -> 3* -> 5*:

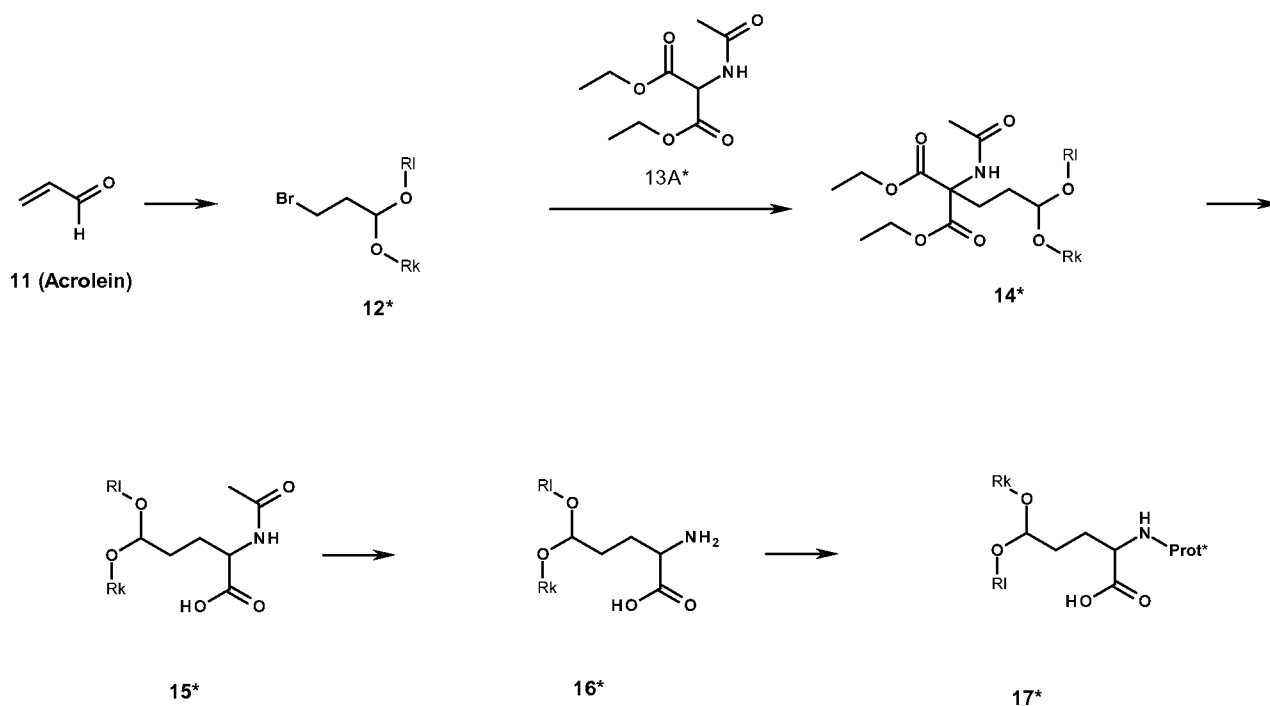


- 88 -

wherein Rk, RI have the meanings just indicated and Z has the meanings mentioned in claim 6 for a compound of the formula V;

or

- 5 (b) in the case of the synthesis of a compound of the formula V, wherein each of Rk and RI is an unsubstituted or substituted alkyl moiety, according to the following reaction scheme:



- 10 (wherein Rk, RI have the meanings just indicated and Prot* has the meanings mentioned in claim 6 for a compound of the formula V.

16. A compound selected from the following group of compounds:

a) a compound of the formula



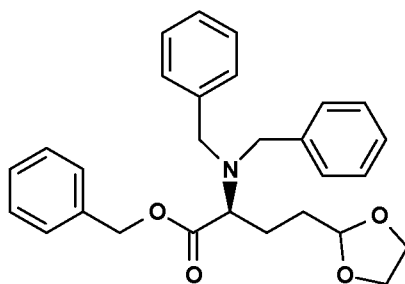
15

, especially

2A*

- 89 -

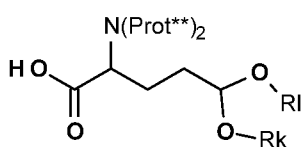
more especially



2

;

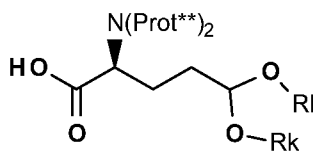
a compound of the formula



5

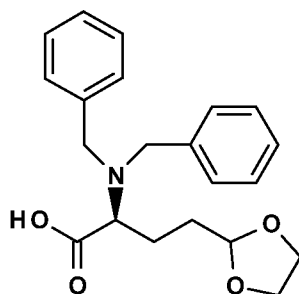
3*

, especially



3A*

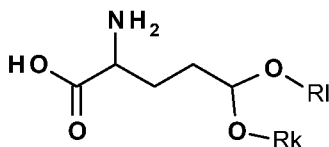
, more especially



3

;

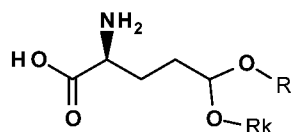
a compound of the formula



10

4*

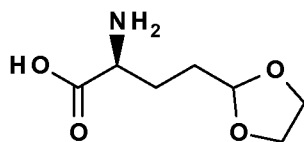
, especially



4A*

, more especially

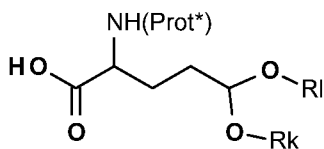
- 90 -



4

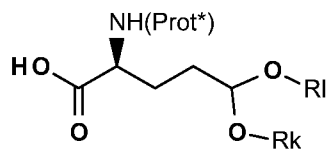
;

a compound of the formula



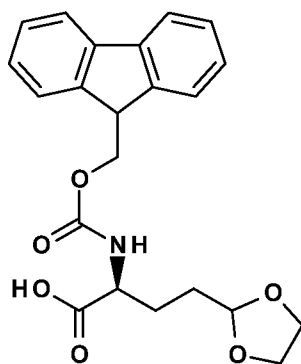
5*

, especially



5A*

, more especially



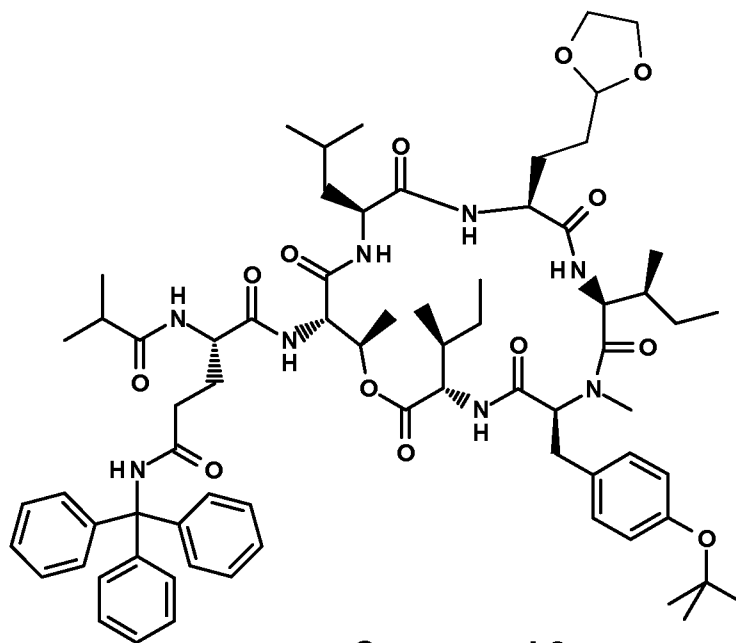
5

5

wherein where mentioned the moieties Prot*, Prot**, Rk and Rl are as defined for a compound of the formula V in claim 6;

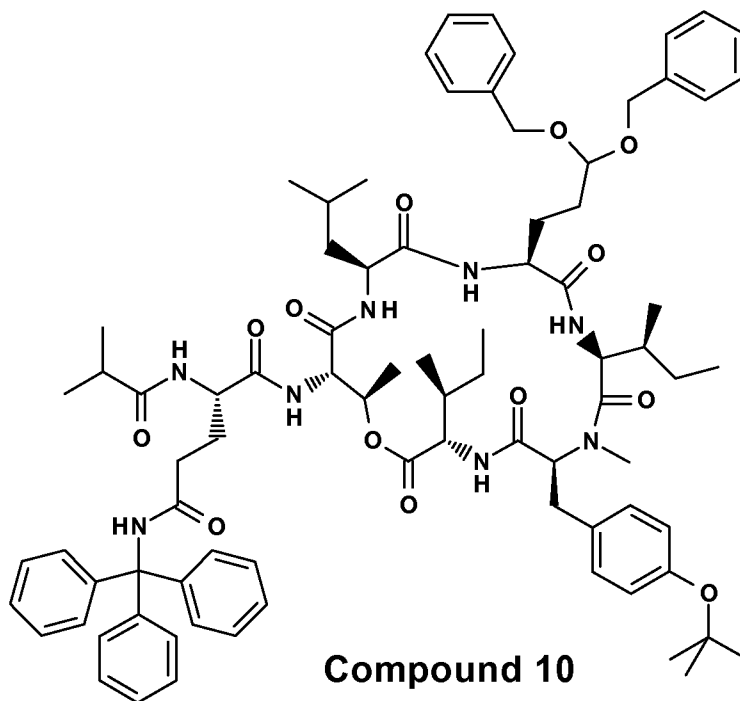
10 b) a compound of the formula II

wherein the substituents are as defined in claim 1, especially of the formula

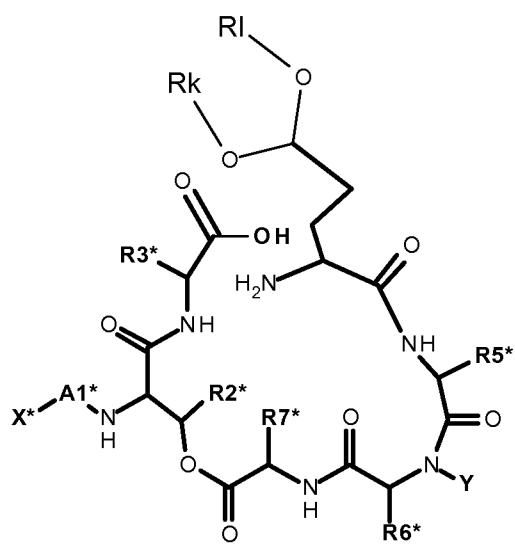


or of the formula

- 92 -

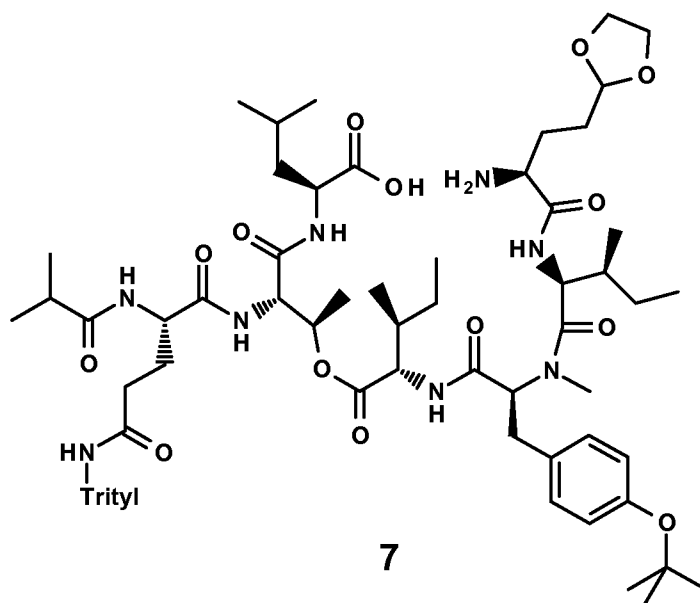


c) a compound of the formula III,

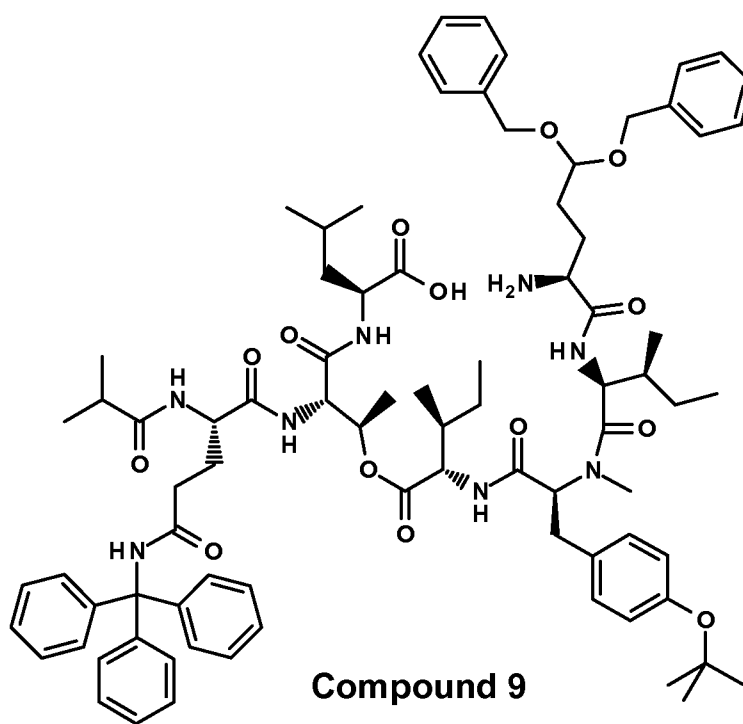


5 wherein the substituents are as defined in claim 3,
especially of the formula

- 93 -



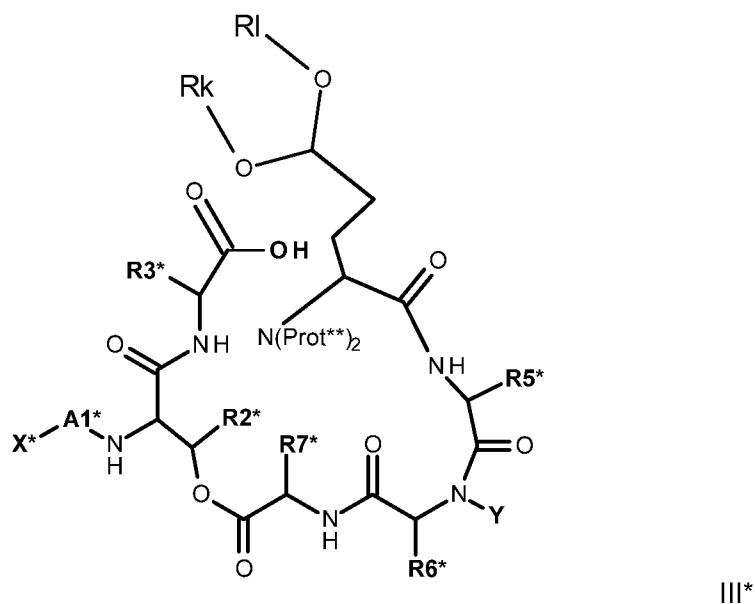
or of the formula



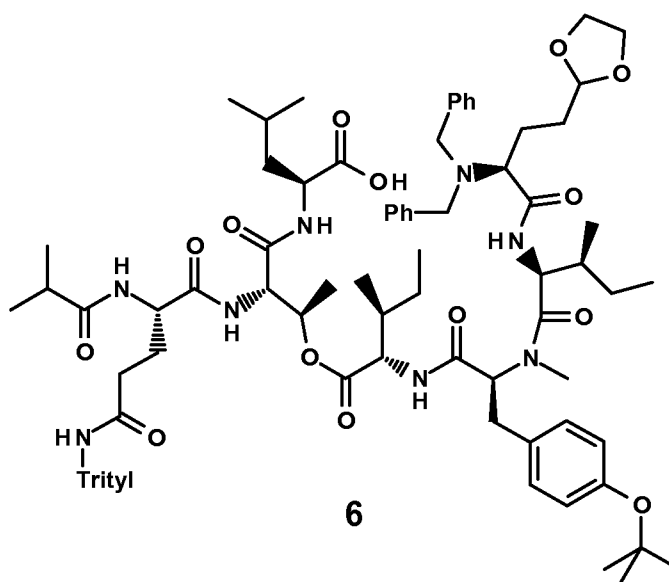
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and

d) of the formula III*,



wherein the substituents are as defined in claim 4, especially of the formula



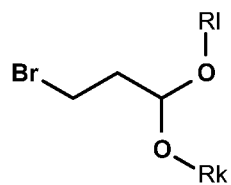
or in each case a salt thereof.

5

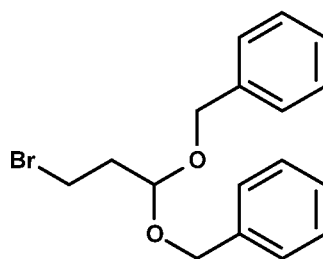
17. A compound selected of the group of the following compounds:

a compound of the formula

- 95 -

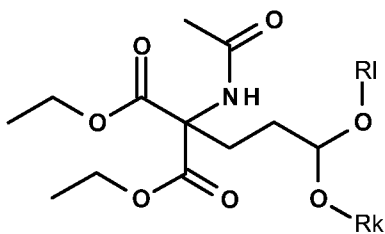
**12***

especially of the formula

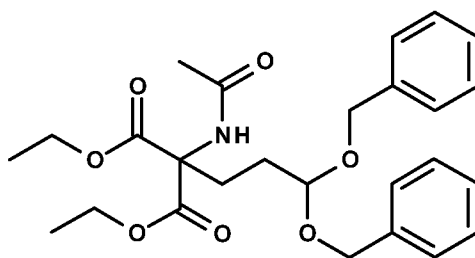
**12**

5

a compound of the formula

**14***

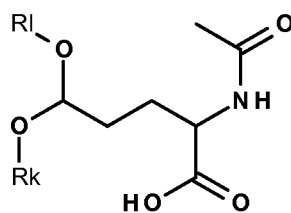
especially of the formula

**14**

10

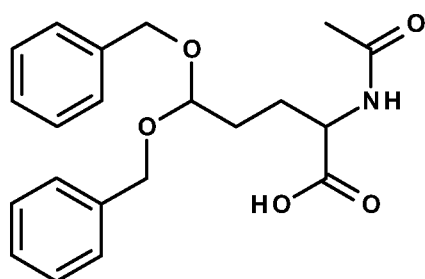
- 96 -

a compound of the formula



15*

especially of the formula

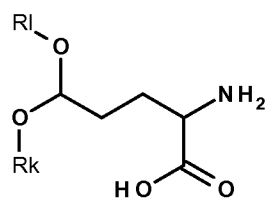


15

5

;

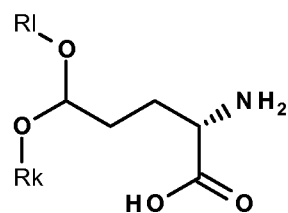
a compound of the formula



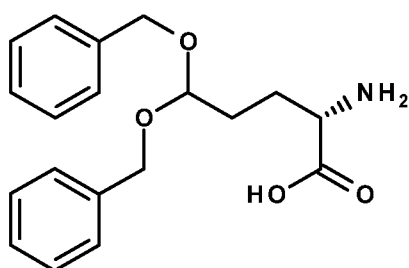
10

in particular of the formula

- 97 -

**16A***

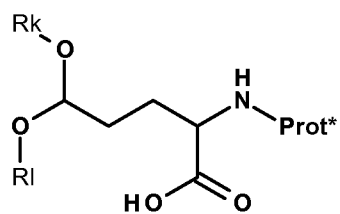
especially of the formula

**16**

5

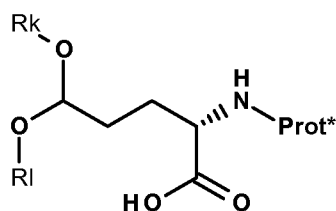
and

a compound of the formula

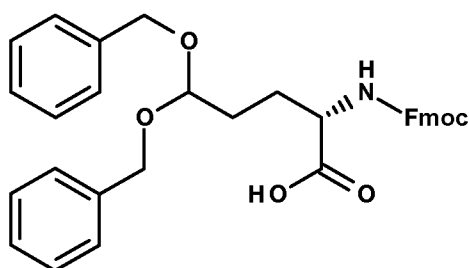
**17***

in particular of the formula

- 98 -

**17A***

especially of the formula

**17**

5 wherein where mentioned Rk and Rl are a substituted alkyl moiety, such as 1-arylalkyl; or together form an unsubstituted or substituted alkylene bridge, such as –CH₂–CH₂– or –CH₂–CH₂–CH₂–;

or in each case a salt thereof.