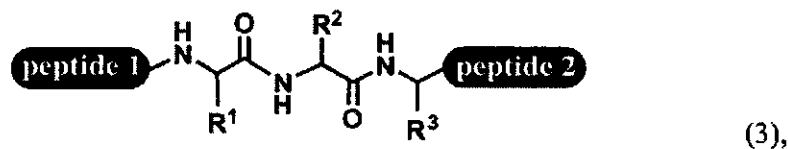
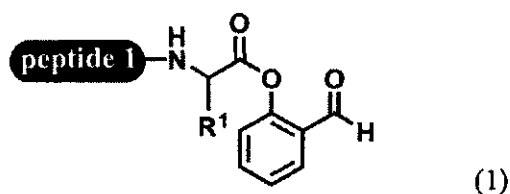


Claims

1. A method of preparing a polypeptide or protein of formula (3)



comprising reacting a C-terminal salicylaldehyde ester peptide of formula (1)



with an aminoacyl-N-hydroxyl peptide of formula (2)



wherein the reaction is carried out in a pyridine/organic acid mixture to form an intermediate compound having a 1,2,5-oxadiazinane ring structure; and

adding a reducing agent to cleave the 1,2,5-oxadiazinane ring structure of the intermediate compound to form the polypeptide or protein of formula (3),

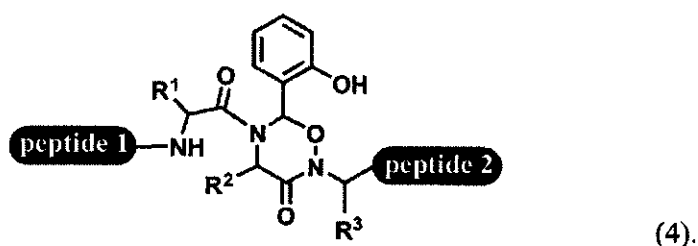
wherein each of R^1 , R^2 and R^3 is independently selected from the group consisting of H, a substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{15} alkenyl, substituted or unsubstituted C_2 - C_{15} alkynyl, substituted or unsubstituted C_3 -

C₁₅ cycloalkyl, substituted or unsubstituted C₃-C₁₅ cycloalkenyl, substituted or unsubstituted C₃-C₁₅ heterocycloalkyl, substituted or unsubstituted C₃-C₁₅ heterocycloalkenyl, substituted or unsubstituted C₆-C₁₅ aryl, or substituted or unsubstituted C₆-C₁₅ heteroaryl, and a side chain of an amino acid; or

R¹ is C₁-C₁₀ alkyl that forms together with the nitrogen of the adjacent backbone imino group, a heteroalicyclic ring.

2. The method of claim 1, wherein at least one of R¹ and R³ is independently selected from the group consisting of H, CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, (CH₂)₂-S-CH₃, CH₂-SH, CH₂-OH, CHOH-CH₃, CH₂-(3-indolyl), CH₂-phenyl, (CH₂)₄-NH₂, (CH₂)₃-NH-C(=NH)NH₂, CH₂-1*H*-imidazol-4-yl, CH₂-(*p*-hydroxy-phenyl), CH₂-C(O)NH₂, (CH₂)₂-C(O)NH₂, CH₂-COOH, and (CH₂)₂-COOH, or R¹ is (CH₂)₃ that forms together with the nitrogen of the adjacent backbone imino group a 5-membered heteroalicyclic ring.
3. The method of claim 1 or 2, wherein R² is selected from the group consisting of H, CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, (CH₂)₂-S-CH₃, CH₂-SH, CH₂-OH, CHOH-CH₃, CH₂-(3-indolyl), CH₂-phenyl, (CH₂)₄-NH₂, (CH₂)₃-NH-C(=NH)NH₂, CH₂-1*H*-imidazol-4-yl, CH₂-(*p*-hydroxy-phenyl), CH₂-C(O)NH₂, (CH₂)₂-C(O)NH₂, CH₂-COOH, and (CH₂)₂-COOH.
4. The method of any one of claims 1 to 3, wherein the reducing agent is samarium (II) iodide (SmI₂) solution.

5. The method of claim 4, wherein the reducing agent is SmI_2 solution in tetrahydrofuran (THF) or SmI_2 solution in trifluoroethanol (TFE).
6. The method of any one of claims 1 to 5, wherein the organic acid is acetic acid, propionic acid, isobutyric acid or butyric acid.
7. The method of any one of claims 1 to 6, wherein the intermediate compound has the following formula (4):



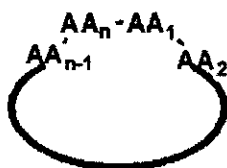
8. The method of any one of claims 2 to 7, wherein at least one of R^1 , R^2 and R^3 is independently selected from the group consisting of CH_3 , CH_2 -phenyl, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, $\text{CH}(\text{CH}_3)_2$ and H.
9. The method of claim 8, wherein:
 - R^1 is CH_3 and R^2 is CH_3 ;
 - R^1 is CH_3 and R^2 is H;
 - R^1 is CH_3 and R^2 is CH_2 -phenyl;
 - R^1 is CH_3 and R^2 is $\text{CH}_2\text{CH}(\text{CH}_3)_2$;
 - R^1 is CH_2 -phenyl and R^2 is CH_3 ;
 - R^1 is $\text{CH}_2\text{CH}(\text{CH}_3)_2$ and R^2 is CH_3 ;
 - R^1 is $\text{CH}(\text{CH}_3)_2$ and R^2 is CH_3 ;

R^1 is $\text{CH}(\text{CH}_3)_2$ and R^2 is H;

R^1 is $\text{CH}_2\text{CH}(\text{CH}_3)_2$ and R^2 is $\text{CH}_2\text{CH}(\text{CH}_3)_2$; or

R^1 is CH_2 -phenyl and R^2 is $\text{CH}_2\text{CH}(\text{CH}_3)_2$.

10. A method of preparing a cyclic peptide of formula (5)

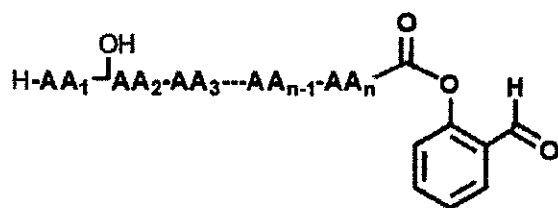


(5),

where AA_n represents an amino acid at a n^{th} position of the peptide,

comprising:

reacting the C-terminal salicylaldehyde ester peptide of formula (1A)



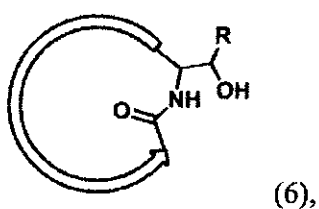
(1A)

in a pyridine/organic acid mixture, where "---" represents additional amino acids in the chain of the peptide; and

adding a reducing agent to form the cyclic peptide of formula (5).

11. The method of claim 10, wherein the reducing agent is SmI_2 solution.

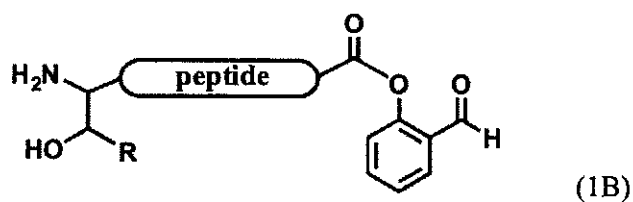
12. The method of claim 11, wherein the reducing agent is SmI_2 solution in THF or SmI_2 solution in TFE.
13. The method of any one of claims 10 to 12, wherein the organic acid is acetic acid, propionic acid, isobutyric acid or butyric acid.
14. A method of preparing a serinyl- or threonyl-containing cyclic peptide of formula (6)



where the peptide contains serine (Ser) or threonine (Thr),

comprising:

reacting the C-terminal Ser- or Thr-containing salicylaldehyde ester peptide of formula (1B)



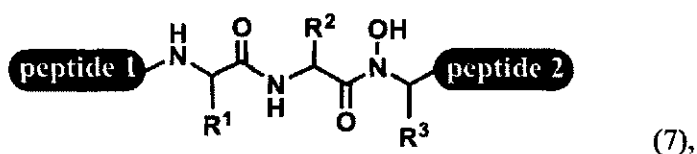
in a pyridine/organic acid mixture; and

adding an acidic deprotecting agent to form the serinyl- or threonyl-containing cyclic peptide of formula (6),

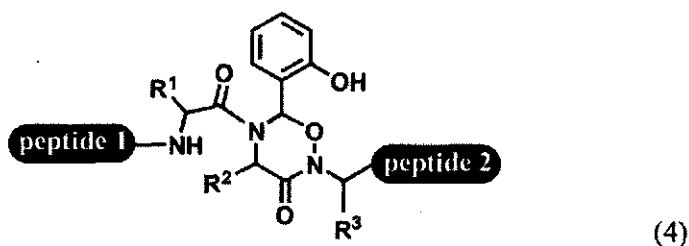
wherein R is a substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₅ alkenyl, substituted or unsubstituted C₂-C₁₅ alkynyl, substituted or unsubstituted C₃-C₁₅ cycloalkyl, substituted or unsubstituted C₃-C₁₅ cycloalkenyl, substituted or unsubstituted C₃-C₁₅ heterocycloalkyl, substituted or unsubstituted C₃-C₁₅ heterocycloalkenyl, substituted or unsubstituted C₆-C₁₅ aryl, or substituted or unsubstituted C₆-C₁₅ heteroaryl, or a side chain of an amino acid.

15. The method of claim 14, wherein the acidic deprotecting agent to deprotect the oxazolidine ring is trifluoroacetic acid (TFA).
16. The method of claim 14 or 15, wherein the organic acid is acetic acid, propionic acid, isobutyric acid, or butyric acid.
17. A method of preparing a C-terminal salicylaldehyde ester peptide in solid phase, comprising:
 - (i) mixing 2-hydroxycinnamic acid and 4-methylbenzhydrylamine hydrochloride salt (MBHA) resin in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and a tertiary amine to form a 2-hydroxycinnamamide linker on the resin;
 - (ii) coupling a Boc-amino acid to the 2-hydroxycinnamamide linker-resin, followed by standard Boc solid phase peptide synthesis to form a peptidyl 2-hydroxycinnamamide phenolic ester on the resin, where Boc represents tert-butoxycarbonyl protecting group;

- (iii) adding a cleavage agent to release the peptidyl 2-hydroxycinnamamide phenolic ester from the resin; and
- (iv) ozonizing the C=C bond of the peptidyl 2-hydroxycinnamamide phenolic ester to obtain the C-terminal salicylaldehyde ester peptide.
18. The method of claim 17, wherein the tertiary amine is diisopropylethylamine (DIEA).
19. The method of claim 17 or 18, wherein the cleavage agent is hydrofluoric acid or trifluoromethanesulfonic acid-trifluoroacetic acid mixture.
20. A method of preparing a polypeptide or protein of formula (7)



comprising reacting a compound of formula (4)



with trifluoroacetic acid (TFA),

wherein each of R¹, R² and R³ is independently selected from the group consisting of H, a substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₅ alkenyl, substituted or unsubstituted C₂-C₁₅ alkynyl, substituted or unsubstituted C₃-

C₁₅ cycloalkyl, substituted or unsubstituted C₃-C₁₅ cycloalkenyl, substituted or unsubstituted C₃-C₁₅ heterocycloalkyl, substituted or unsubstituted C₃-C₁₅ heterocycloalkenyl, substituted or unsubstituted C₆-C₁₅ aryl, or substituted or unsubstituted C₆-C₁₅ heteroaryl, and a side chain of an amino acid.