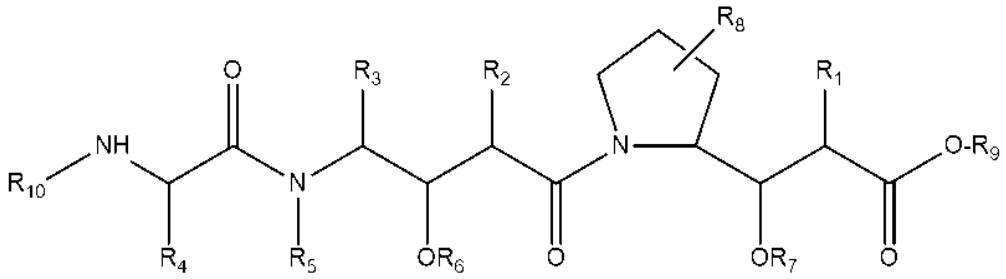
~~The invention claimed is:~~Claims

1. A method for making a dolastatin, auristatin or related compounds comprising the steps of:

providing a compound of Formula I, or salt thereof,



I

wherein

R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl,

R_6 and R_7 are each individually H or C_1-C_4 alkyl,

R_9 is H or an acid protecting group, and

R_{10} is H or an amino protecting group;

if R_9 is an acid protecting group, deprotecting the C-terminal carboxylic acid group, reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond;

if R_{10} is an amino protecting group, deprotecting the N-terminal amine, reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond.

2. The method of claim 1 wherein the steps of

if R_9 is an acid protecting group, deprotecting the C-terminal carboxylic acid group, ~~then~~

reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond;

are performed before the steps of

if R_{10} is an amino protecting group, deprotecting the N-terminal amine, ~~then~~

reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond.

3. The method of claim 1 wherein the steps of

if R_{10} is an amino protecting group, deprotecting the N-terminal amine, ~~then~~

reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond

are performed before the steps of

if R_9 is an acid protecting group, deprotecting the C-terminal carboxylic acid group, ~~then~~

reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond.

4. The method according to any of claims 1 to 3 wherein the amine (A) is selected from the group consisting of alkylamines, alkanolamines, arylalkanolamines, amino acids, amino acid derivatives, peptides and peptide derivatives,

wherein the amine (A) may have one or more substituents, and

wherein the amine (A) may have a protecting group.

5. The method according to any of claims 1 to 4 wherein the amine (A) is selected from the group consisting of phenylalanine, phenylalanine derivatives, substituted phenylalanine, substituted phenylalanine derivatives, tryptophan, tryptophan derivatives, substituted tryptophan, substituted tryptophan derivatives, phenylpropanolamine, protected phenylpropanolamine, substituted phenylpropanolamine, protected substituted phenylpropanolamine, dolaphenine and protected dolaphenine, substituted dolaphenine, protected substituted dolaphenine, dolaphenine derivatives, protected dolaphenine derivatives, substituted dolaphenine derivatives, protected dolaphenine derivatives.

6. The method according to any of claims 1 to 5 wherein the carboxylic acid (CA) is selected from the group consisting of amino acids, amino acid derivatives, peptides and peptide derivatives,

wherein the carboxylic acid (CA) may have one or more substituents, and

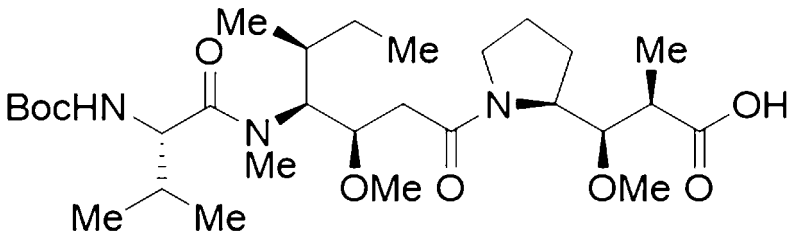
wherein the carboxylic acid (CA) may have a protecting group.

7. The method according to any of claims 1 to 6 wherein the carboxylic acid (CA) is selected from the group consisting of valine, protected valine, substituted valine, protected substituted valine, valine derivatives, protected valine derivatives, substituted valine derivatives, protected substituted valine derivatives, alanine,

protected alanine, substituted alanine, protected substituted alanine, alanine derivatives, protected alanine derivatives, substituted alanine derivatives and protected substituted alanine derivatives.

8. The method according to any of claims 1 to 7 wherein the carboxylic acid (CA) comprises one or more of a spacer, a linker and an attachment group.

9. The method according to any of claims 1, 2 or 4 to 8 wherein the compound of Formula I is



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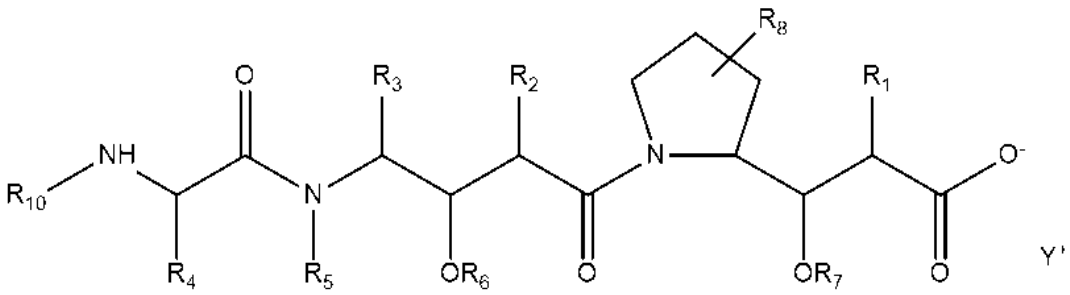
10. An isolated salt of Formula II:



II

wherein

R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H and C_1-C_6 alkyl,

R_6 and R_7 are each individually H or C_1-C_4 alkyl,

R_{10} is H or an amino protecting group; and

Y^+ is counterion.

11. The isolated salt ~~according to~~ of claim 10, wherein

R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, and iso-butyl;

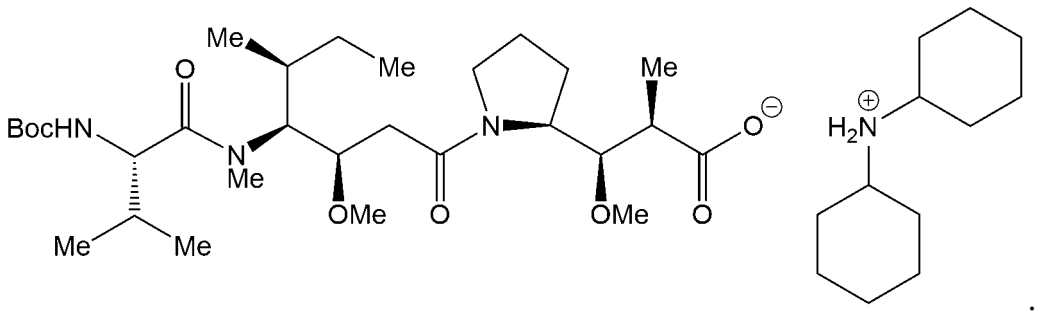
R_6 and R_7 are each individually H or methyl,

R_{10} is H or tert-butoxycarbonyl (Boc), and

Y^+ is an ammonium ion of the formula $N^+HR_{13}R_{14}R_{15}$ wherein R_{13} is selected from the group consisting of optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; R_{14} and R_{15} are independently selected from the group consisting of H, optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; wherein each optional substituent, if present, is selected from the group consisting of alkyl and aryl.

12. The isolated salt according to either of claims 10 or 11 wherein Y^+ is selected from the group consisting diethylammonium ion, dibutylammonium ion, dicyclohexylammonium ion, methylcyclohexylammonium ion and methylbenzylammonium ion.

13. The isolated salt according to any of claims 10 to 12, wherein the salt is



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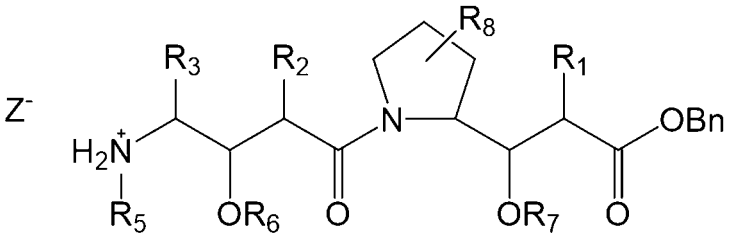
14. A compound of Formula III:



III

wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H and C_1-C_6 alkyl,

R_6 and R_7 are each individually selected from the group consisting of H or C_1-C_4 alkyl, and

Z^- is a counterion.

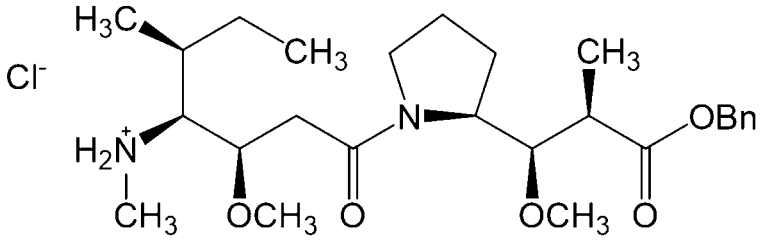
15. The compound of ~~Formula III according to~~ claim 14, wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

R_6 and R_7 are each individually selected from the group consisting of H and methyl, and

Z^- is selected from the group consisting of halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, trifluoroacetate, oxalate, and citrate.

16. The compound of ~~Formula III~~ according to either of claims 14 or 15, wherein the compound is:



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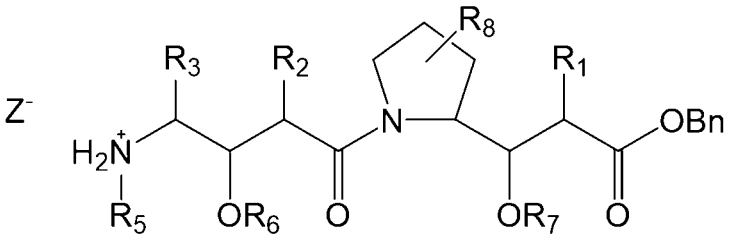
17. A method of coupling an amino acid to a compound of Formula III



III

wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H and C_1-C_6 alkyl,

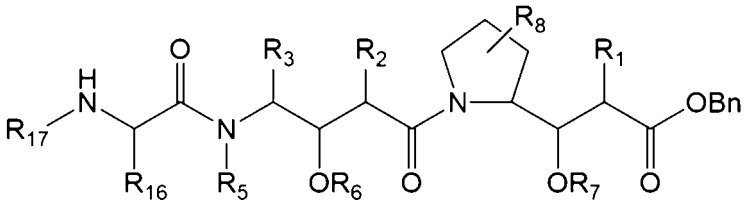
R_6 and R_7 are each individually selected from the group consisting of H or C_1-C_4 alkyl, and

Z^- is a counterion

comprising the steps of

contacting the compound of Formula III with an aqueous base to remove the counterion, and

contacting the compound of Formula III with an N-protected amino acid N-carboxyanhydride to yield a compound of Formula IV:



IV

wherein

R_1, R_2, R_3, R_5, R_6, R_7 and R_8 are defined as above,

R_{16} is an amino acid side chain, and

R_{17} is a protecting group.

18. The method ~~according to~~ of claim 17 wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

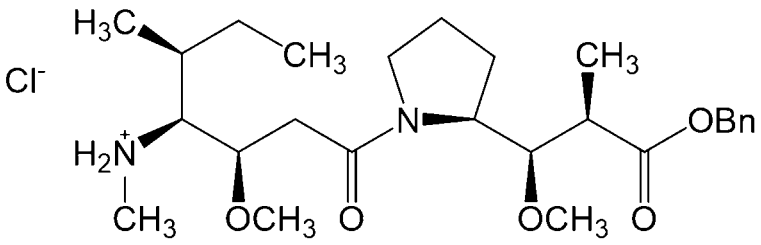
R_6 and R_7 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

Z^- is selected from the group consisting of halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, trifluoroacetate, oxalate, and citrate;

the aqueous base is selected from the group consisting of Na_2CO_3, $NaHCO_3$, NaOH, Na_2HPO_4, and Na_3PO_4,

the N-protected amino acid N-carboxyanhydride is a Boc- protected amino acid N-carboxyanhydride.

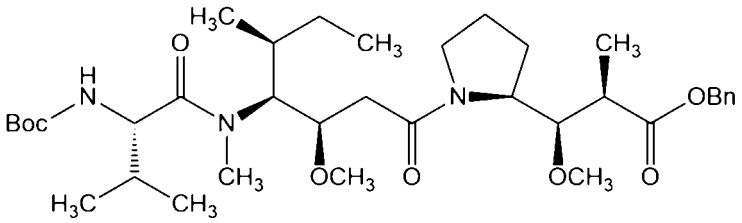
19. The method according to either of claims 17 or 18 wherein the compound of Formula III is



the aqueous base is Na_2CO_3,

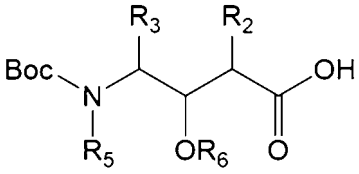
the N-protected amino acid N-carboxyanhydride is Boc-Val-NCA, and

the compound of Formula IV is:



~~20. A method for purifying a crude hydroxy acid of Formula V without using column chromatography, the method comprising the steps of~~

~~providing a crude hydroxy acid of Formula V~~



~~V~~

~~wherein~~

~~R_2, R_3, and R_5 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl, and~~

~~R_6 is selected from the group consisting of H and C_1-C_4 alkyl;~~

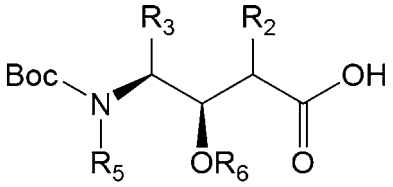
~~dissolving crude hydroxy acid in a soluble solvent,~~

~~adding an insoluble solvent,~~

~~initiating crystallization, and~~

allowing the crystallization to complete, yielding a purified hydroxy acid of Formula V.

21. The method according to claim 20, wherein the hydroxy acid of Formula V is



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R_2, R_3 and R_5 are each individually selected from the group consisting of H, methyl,ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

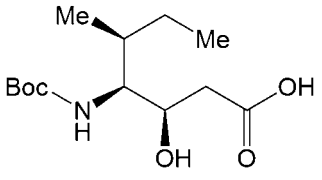
R_6 is selected from the group consisting of H and methyl,

the soluble solvent comprises tert-butyl methyl ether (MTBE),

the insoluble solvent comprises heptane, and

crystallization is initiated by seeding, by heat cycling or by a combination thereof.

22. The method according to either of claims 20 or 21 wherein the hydroxy acid of Formula V is



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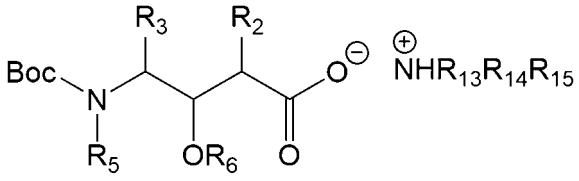
23. The method according to any of claims 20-22, further comprising the step of isolating the purified hydroxy acid of Formula V.

24. A compound of Formula VI:



VI

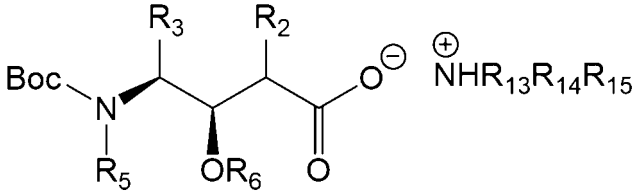
wherein

R_2, R_3, and R_5 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, $-OR_{11}$, $-NR_{11}R_{12}$, $-SR_{11}$ and halo; R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl, and

R_6 is selected from the group consisting of H and C_1-C_4 alkyl; and

~~R_{13} is selected from the group consisting of optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; R_{14} and R_{15} are independently selected from the group consisting of H, optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; wherein each optional substituent, if present, is selected from the group consisting of alkyl and aryl.~~

~~25. The compound according to claim 24, wherein the compound of Formula VI is~~



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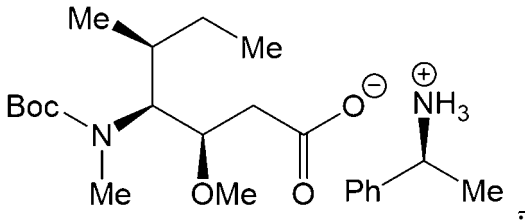
~~R_2, R_3 and R_5 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl, and~~

~~R_6 is selected from the group consisting of H and methyl; and~~

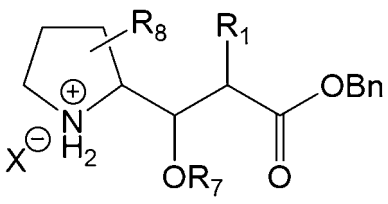
~~$NHR_{13}R_{14}R_{15}$ is selected from the group consisting of selected from the group consisting diethylammonium ion, dibutylammonium ion, dicyclohexylammonium ion, methylcyclohexylammonium ion and methylbenzylammonium ion.~~

~~26. The compound according to either of claims 24 or 25, wherein the compound of Formula VI is:~~



~~27. The compound according to any of claims 24-26 wherein the compound of Formula VI is a solid.~~

~~28. A compound of Formula VII~~



~~VII~~

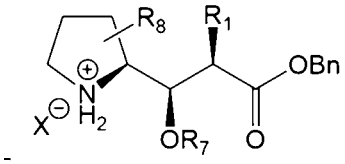
~~wherein~~

~~R_1 and R_8 are individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, $-OR_{11}$, $-NR_{11}R_{12}$, $-SR_{11}$ and halo; R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl;~~

~~R_7 is selected from the group consisting of H or C_1-C_4 alkyl; and~~

~~X^- is selected from the group consisting of halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, oxalate, and citrate.~~

~~29. The compound according to claim 28 wherein the compound of Formula VII is~~



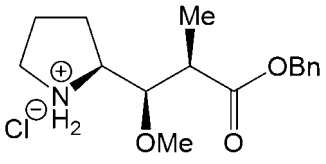
~~wherein~~

~~R_1 and R_8 are individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,~~

~~R_7 is selected from H and methyl; and~~

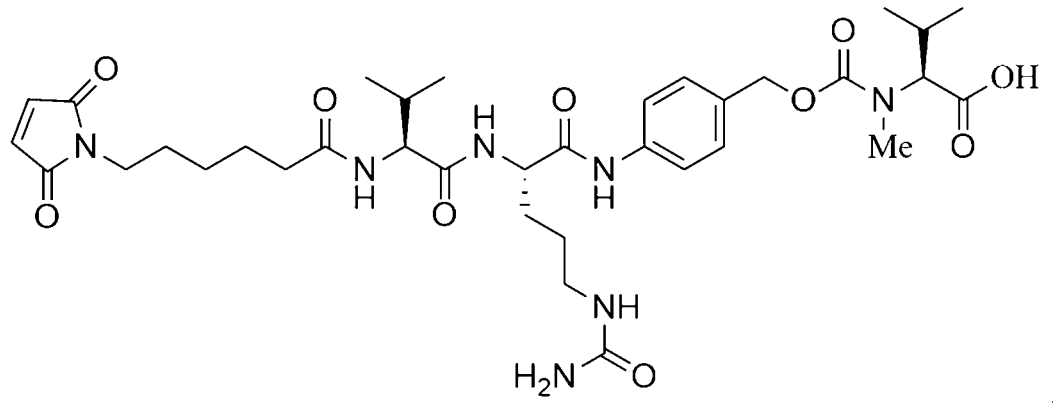
~~X^- is a halide.~~

~~30. The compound according to either of claims 28 or 29 wherein the compound of Formula VII is~~



~~31. The compound according to any of claims 28-30 wherein the compound of Formula VII is a solid.~~

~~32. A compound of Formula VIII:~~



~~VIII.~~

20. The method according to any of claims 1-9, with the proviso that when R_1, R_5, R_6 and R_7 are methyl, R_2, R_8 and R_9 are H, R_3 is *sec*-butyl, R_4 is isopropyl, R_{10} is a *tert*-butoxycarbonyl (Boc) protecting group, and amine (A) is a salt of phenylalanine, then carboxylic acid (CA) is not protected substituted valine.

21. The method according to any of claims 1-9, with the proviso that when R_1, R_5, R_6 and R_7 are methyl, R_2, R_8 and R_9 are H, R_3 is *sec*-butyl, R_4 is isopropyl, R_{10} is a *tert*-butoxycarbonyl (Boc) protecting group and carboxylic acid (CA) is protected substituted valine, then amine (A) is not a salt of phenylalanine.