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(54) Title: PROCESS FOR THE SYNTHESIS OF PEPTIDES

(57) Abstract: A process for the solid-phase synthesis of a peptide is provided which comprises attaching an α -nitrogen protected α -carboxy protected amino acid to a solid support via its side chain, removing the α -nitrogen protecting group, and assembling a peptide chain on said α -nitrogen. Also novel amino acid analogues and solid-phase supports are provided.



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PROCESS FOR THE SYNTHESIS OF PEPTIDES

This invention relates to a process for the solid-phase synthesis of peptides and to supports for peptide synthesis.

Peptides may be synthesised by solid-phase synthesis that commences from the carboxy-terminal end of the peptide using an α -amino protected amino acid. The two most widely used protocols employ *tert*-butyloxycarbonyl (Boc) or 9-fluorenylmethoxycarbonyl (Fmoc) as amino protecting groups.

The synthesis of C-terminal peptide acids by the Fmoc/Boc approach has conventionally required the use of an ester type linker, preferably 4-hydroxymethylphenoxy acetyl (HMPA) between the solid support and the peptide moiety. Introduction of the first amino acid to the solid support involves a *p*-dimethylaminopyridine (DMAP) catalysed esterification protocol. This procedure has been shown to promote both incidental Fmoc removal and the racemisation of susceptible amino acids at the C-terminal (Atherton, E. *et al.* (1981) J. Chem. Soc. Chem. Commun. p336). In particular, the cysteine residue is very susceptible to the vigorous esterification conditions leading to chiral inversion. Furthermore, the conditions required for Fmoc removal and thus peptide elongation have been shown to promote racemisation of the C α -cysteine residue following the first amino acid introduction. As a result the synthesis of C α -Cys terminal peptides via the Fmoc/Boc approach is problematic using established methodologies.

The present invention provides a process for the solid-phase synthesis of a peptide which comprises attaching an α -nitrogen protected α -carboxy modified amino acid to a solid support via its side chain, removing the α -nitrogen protecting group, and assembling a peptide chain on said α -nitrogen.

Preferably the assembled peptide is then cleaved from the solid support.

The α -nitrogen protecting group is preferably a base labile protecting group. More preferably the α -nitrogen protecting group is Fmoc.

Preferably the α -carboxy is modified by reaction with a protecting group.

Thus a preferred embodiment provides a process for the solid-phase synthesis of a peptide which comprises attaching an α -nitrogen protected α -carboxy protected amino acid to a solid support via its side chain, removing the α -nitrogen protecting group, and assembling a peptide chain on said α -nitrogen.

Preferably the α -carboxy is protected by the formation of a carboxy ester group, more preferably by the formation of an alkyl ester especially a *tert*-butyl ester (tBu).

It will be recognised that the α -carboxy protecting group is selected to be orthogonal to the α -nitrogen protecting group. The α -nitrogen protecting group can therefore be removed without deprotection of the α -carboxy.

Preferably the α -nitrogen protected α -carboxy modified amino acid comprises a heteroatom, such as N, O or S, especially S, in its side chain.

More preferably the α -nitrogen protected α -carboxy modified amino acid is derived from Cys, Arg, Ser, Tyr, Thr, Lys, Orn, Asp, Glu, Trp, His, Pen (penicillamine), Dpr (2,3-diaminopropionic Acid) and Dab (2,4-diaminobutyric acid).

It is particularly preferred that the α -nitrogen protected α -carboxy modified amino acid is derived from cysteine.

Thus, an especially preferred α -nitrogen protected α -carboxy modified amino acid is Fmoc-Cys-O-tBu.

Fmoc-Cys-O-tBu may be prepared by reacting the commercially available disulphide (H-Cys-OtBu)₂·2HCl with Fmoc to yield (Fmoc-Cys-O-tBu)₂ which may be hydrolysed to yield Fmoc-Cys-O-tBu.

Therefore a preferred embodiment of the first aspect of the invention the process comprises attaching Fmoc-Cys-O-tBu to a solid support by its thiol side chain.

The solid support may be any support known in the art which is suitable for use in solid-phase peptide synthesis, optionally with a linker group, able to react with a side chain of an amino acid, more preferably a nucleophilic amino acid side chain, to form a bond which is stable during the acylation and deprotection cycles involved in assembling a peptide.

Preferably the solid support comprises a polystyrene or polydimethylacrylamide polymer. More preferably the support comprises a copolymer of styrene with about 0.5 to 2% divinyl benzene as a cross-linking agent or a polydimethylacrylamide polymer comprising N,N-dimethylacrylamide, N,N-bisacryloylethylenediamine and acryloylsarcosine methyl ester monomers. Details of these preferred supports and other suitable supports may be found in Chan and White "Fmoc Solid-Phase Peptide Synthesis" Oxford University Press, 2000 which are included herein by reference.

The preferred linker group on the solid support comprises a trityl moiety, more preferably a 4-methoxytrityl moiety.

Based on the above preferences a particularly preferred solid support is 4-methoxytrityl polystyrene.

A preferred process for the solid-phase synthesis of a peptide comprises the steps of:

- (a) attaching an α -nitrogen protected α -carboxy protected amino acid comprising a heteroatom in its side chain to a solid support via said side chain;
- (b) deprotecting the α -nitrogen of the attached first amino acid by removing the α -nitrogen protecting group under conditions such that the attached first amino acid remains connected to the solid support and coupling an additional α -nitrogen protected amino acid to the unprotected α -nitrogen of the attached amino acid to yield an attached peptide with a protected N-terminus;
- (c) deprotecting the N-terminus of the attached peptide by removing the α -nitrogen protecting group under conditions such that the attached peptide remains connected to the solid support and coupling an additional α -nitrogen protected

amino acid to the unprotected N-terminus of the attached peptide and repeating until the desired peptide is assembled on the solid support;

(d) removing the α -nitrogen protecting group from the N-terminus of said peptide and optionally reacting with a non-amino acid N-terminal residue;

5 (e) cleaving the link between the side chain of the first α -carboxy protected amino acid of the peptide and the solid support so that the peptide is released from the solid support; and

(f) removing the α -carboxy protecting group and any side chain protecting groups.

Step (a) may be carried out under the same conditions and in the same solvents
10 as are commonly used in linking amino acids via their C α -carboxyl to solid supports in peptide solid-phase synthesis. These methods are well known in the art and are described in many standard texts on the subject such as Atherton and Sheppard, "Solid-Phase Peptide Synthesis A Practical Approach", IRL Press at Oxford University Press, 1989 and Chan and White "Fmoc Solid-Phase Peptide Synthesis" Oxford University
15 Press, 2000, which are incorporated herein by reference.

When the resin is 4-methoxytrityl polystyrene and the α -nitrogen protected α -carboxy protected amino acid is Fmoc-Cys-O-tBu, step (a) typically comprises dissolving Fmoc-Cys-O-tBu in a suitable solvent such as N,N-dimethylformamide then adding, with mixing, N,N-diisopropylethylamine. This solution is then added to the resin
20 and allowed to react before collecting the resin and washing with a suitable solvent such as N,N-dimethylformamide.

The coupling and deprotection cycles of steps (b), (c) and (d) may be carried out using any standard conditions for peptide solid-phase synthesis well known to one skilled in the art. For further detail reference is made, for example, to Atherton and Sheppard,
25 "Solid-Phase Peptide Synthesis A Practical Approach", IRL Press at Oxford University Press, 1989 and Chan and White "Fmoc Solid-Phase Peptide Synthesis" Oxford University Press, 2000 which are incorporated herein by reference. When the protecting group used is Fmoc, it is particularly favoured that Fmoc removal is effected by treating with a solution of piperidine in N,N-dimethylformamide, more preferably 20%v/v piperidine
30 in N,N-dimethylformamide.

Amino acid activation is preferably carried out in N,N-dimethylformamide in the presence of 1-hydroxybenzotriazole and diisopropylcarbodiimide.

In steps (b), (c) and (d) side chain protecting groups may be used to protect susceptible side chains which could otherwise be modified in the coupling and
35 deprotection cycles. Examples of amino acids with susceptible side chains in steps (b) and (c) are Cys, Asp, Glu, Ser, Arg, Har, Tyr, Thr, Lys, Orn, Pen, Trp, Asn and Gln. Alternatively, a post solid-phase synthesis chemical modification of the peptide may be carried out to yield a desired side chain.

The precise conditions required to cleave the peptide from the solid support vary
40 with the nature of the side chain of the α -nitrogen protected α -carboxy modified amino

acid and the linker group on the support and are similar to those known in the art. Typically with the preferred combination of Cys attached via its side chain with 4-methoxytrityl polystyrene the peptide may be released by treating the peptide resin with 10% (v/v) ethanedithiol in trifluoroacetic acid.

5 Suitable conditions for the removal of various carboxy protecting groups in step (f) are described in Chan and White "Fmoc Solid-Phase Peptide Synthesis" Oxford University Press, 2000 on pages 20-21 which is incorporated herein by reference and in Atherton and Sheppard, "Solid-Phase Peptide Synthesis A Practical Approach", IRL Press at Oxford University Press, 1989 which is also incorporated herein by reference.

10 Step (f), removal of the α -carboxy protecting group, may be carried out before or after step (e), cleavage of the peptide from the resin. However, preferably step (e) and (f) are carried out as a single process. It is also preferred that when step (d) does not involve reaction with a non-amino acid N-terminal residue that: the α -nitrogen protecting group is removed from the N-terminus, the link between the solid support and peptide (step (e)) and removal of the α -carboxy protecting group and any side chain protecting groups is carried out by a single process (step(f)).

15 Isolation and purification of the peptide may be achieved using standard procedures and techniques that would be well known to one skilled in the art. These methods include precipitation of the peptide in a solvent that will not affect the integrity of the peptide, such as diisopropylether, followed by preparative HPLC and salt exchange. The peptide isolated may be subjected to further processing, either before or after purification

20 The processes of the present invention may use batch or continuous flow synthesis techniques or any automated synthesizer following the instructions provided by the manufacturer.

25 A second aspect of the invention provides a support for solid-phase synthesis which comprises an α -nitrogen protected α -carboxy modified amino acid attached to a solid phase support through the side chain of the amino acid.

30 The solid phase support is as described in the first aspect of the invention and preferably is based on a polystyrene or polydimethylacrylamide polymer with a trityl linker.

 The α -nitrogen protecting group on the attached α -nitrogen protected α -carboxy protected amino acid is preferably a base labile protecting group. More preferably the α -nitrogen protecting group is Fmoc.

 Preferably the α -carboxy is modified by a protecting group.

35 Preferably the α -carboxy is protected by the formation of a carboxy ester group, more preferably by the formation of an alkyl ester especially a *tert*-butyl ester (t-Bu).

 A preferred support for solid-phase synthesis in the second aspect of the invention comprises a polystyrene or polydimethylacrylamide polymer with a trityl linker to which a Fmoc- α -nitrogen-C α -carboxy *tert*-butyl ester amino acid is attached by its side chain

wherein the amino acid is selected from the group consisting of Cys, Arg, Ser, Tyr, Thr, Lys, Orn, Asp, Glu, Trp, His, Pen, Dpr and Dab, especially Cys.

An especially preferred support for solid-phase synthesis in the second aspect of the invention comprises a polystyrene or polydimethylacrylamide polymer with a trityl linker to which Fmoc-Cys-O-tBu is attached by its thiol side chain.

According to a third aspect of the invention there is provided an α -nitrogen protected α -carboxy protected cysteine wherein the α -nitrogen protecting group is a base labile protecting group, preferably Fmoc and the α -carboxy is protected by the formation of a carboxy ester group, more preferably by the formation of an alkyl ester especially a *tert*-butyl ester (t-Bu).

Thus, in a preferred embodiment of the third aspect of the invention the α -nitrogen protected α -carboxy protected cysteine is of formula Fmoc-Cys-O-tBu.

According to a fourth aspect of the invention there is provided α -nitrogen protected α -carboxy protected cysteine disulphide linked dimer wherein the α -nitrogen protecting group is a base labile protecting group, preferably Fmoc and the α -carboxy is protected by the formation of a carboxy ester group, more preferably by the formation of an alkyl ester especially a *tert*-butyl ester (t-Bu).

Thus, in a preferred embodiment of the fourth aspect of the invention the α -nitrogen protected α -carboxy protected cysteine disulfide linked dimer is of formula (Fmoc-Cys-O-tBu)₂.

The invention is now illustrated, but not limited, by the following Examples.

Example 1

Synthesis of c[Mpr-Har-Gly-Asp-Trp-Pro-Cys]-OH

Reactants

Abbreviations

	DCM	Dichloromethane
	DIC	Diisopropylcarbodiimide
	DIPEA	N,N-Diisopropylethylamine
	DMF	N,N-Dimethylformamide
	EDT	1,2-Ethanedithiol
	Fmoc	9-Fluorenylmethoxycarbonyl
	Fmoc-O-Su	N-(9-Fluorenylmethoxycarbonyloxy) succinimide
	Har	Homoarginine
	HOBt	1-Hydroxybenzotriazole
	IPP	Diisopropylether
	Mpr	3-Mercaptopropionic acid

[Mpr-OH] ₂	3-Mercaptopropionic acid disulphide
TIPS	Triisopropylsilane
TFA	Trifluoroacetic acid

5 Stage 1Synthesis of (Fmoc-Cys-O-tBu)₂

(H-Cys-OtBu)₂.2HCl (5.00g, 0.012 moles) (from Bachem) was dissolved in an aqueous solution of 0.2M NaOH (360ml, 0.072 moles). This solution was stirred and Fmoc-O-Su (8.90g, 0.026 moles) (from Novabiochem) in acetone (360ml) was added. The resultant cloudy suspension was stirred at room temperature for 4hours. The reaction mixture was then transferred to a separating funnel and washed with diethyl ether (300ml). A solid precipitate immediately formed. This solid was filtered and washed with diethyl ether. The aqueous fraction was then separated and washed a further 2 times with diethyl ether (2 x 200ml). The solid was then triturated with diethyl ether to afford the intermediate (Fmoc-Cys-O-tBu)₂ as a dark yellow gum. To confirm the identity of the crude product a small sample (10mg) was treated with TFA/TIPS in order to remove the O-tBu carboxyl protection. After evaporation of the TFA the residue was reconstituted in CH₃CN/H₂O (1:1) and analysed by RP-HPLC against a standard of (Fmoc-Cys-OH)₂ (from Bachem). The elution time of the product was identical to that of (Fmoc-Cys-OH)₂ confirming the identity of the crude product as (Fmoc-Cys-O-tBu)₂.

Stage 2Synthesis of Fmoc-Cys-O-tBu

(Fmoc-Cys-O-tBu)₂ (5.00g, 6.28 mmoles) from stage 1 was suspended in a mixture of ethyl acetate / 5% w/v aqueous NaHCO₃ (1:1, 100ml) within a suba-sealed 250ml round bottomed flask. The flask was then purged with nitrogen to displace any air within the vessel. This solution was vigorously stirred and tributylphosphine (3.5ml, 14 mmoles) was added by positive displacement. The reaction mixture was then agitated over a 2-hour period. At the end of this time the reaction was quenched by the careful addition of 1M aqueous KHSO₄. The reaction mixture was then transferred to a separating funnel and the crude product was extracted into ethyl acetate (3 x 100ml). The organic fractions were then combined and evaporated to virtual dryness to afford a viscous brown oil. The crude oil was purified by semi-preparative RP-HPLC, using the following conditions:

Buffer A	0.1% v/v TFA in H ₂ O
Buffer B	0.1% v/v TFA in MeCN
Column	Kromasil C8 250 x 21.2mm
Gradient	45 to 65% Buffer B in Buffer A over 60 mins,

		increasing to 65 to 75% Buffer B in Buffer A over 20 mins
	Load	1g/450ml in 40% Buffer B in Buffer A
	Flow rate	7.5ml/min
5	Wavelength	230nm
	Temperature	Ambient
	Fraction collection	2 mins

Fractions of purity >94% by peak area were pooled and lyophilised. The resultant oil was then reconstituted in ethyl acetate. Precipitation from n-hexane was unsuccessful and the combined organics were evaporated to dryness to afford a dark yellow oil. This oil was then dried 'in-vacuo'. RP-HPLC determined the purity of the product as 97.42%. The product was confirmed as Fmoc-Cys-O-tBu by electrospray MS (theoretical 399.5, found 399) and proton NMR.

Stage 3

Introduction of Fmoc-Cys-O-tBu to 4-methoxytrityl polystyrene resin

Fmoc-Cys-O-tBu (0.503g, 1.31 mmoles) from stage 2 was dissolved, with stirring, in DMF (3 ml). DIPEA (0.457ml, 2.63 mmoles) was added to the resultant solution and mixed for one minute. The Fmoc-Cys-O-tBu solution so formed was then added to 4-methoxytrityl polystyrene resin (0.500g, scale of assembly 0.875 mmoles) (supplied from CBL-Patros, Greece) as a single aliquot. The reaction mixture was mixed for two hours and then charged to a reactor vessel that allowed removal of the solvent by filtration. The resin with Fmoc-Cys-O-tBu attached was then washed seven times with DMF (7 x 10ml). The washed resin was suspended in 10% v/v DIPEA in methanol (10ml) for 5 minutes before filtration and subsequent washing 7 times with DMF (7 x 10ml). The Fmoc protecting group was then removed by treating the resin twice with 20% v/v piperidine in DMF (2 x 10ml). In the first treatment the resin and piperidine/DMF mixture were agitated gently for 3 minutes before removing the piperidine/DMF by filtration. In the second treatment the resin and piperidine/DMF mixture was agitated gently for 7 minutes before removing the piperidine/DMF by filtration. The resin was then washed 7 times with DMF (7 x 10ml) removing the solvent by filtration.

Stage 4

Assembly of the peptide Mpr-Har-Gly-Asp-Trp-Pro-Cys-OH

Fmoc-Pro-OH (0.443g, 1.31 mmoles) and HOBt (0.402g, 2.63 mmoles) were dissolved in DMF (5 ml) and cooled to less than 10°C in an ice bath. DIC (0.272ml, 1.75 mmoles) was added to the reaction mixture as a single aliquot. The mixture was then agitated for 6 minutes before being charged to the damp resin from stage 3. The coupling reaction was allowed to proceed for 6 hours at ambient temperature. Coupling efficiency

was tested by the Kaiser test. The peptide -resin was then washed with DMF (10ml) and the protecting Fmoc-group removed in an identical manner to that as described previously. The process described above was then repeated coupling Fmoc-Trp-OH (0.560g, 1.31 mmoles) to the resin bound proline except that the chloranil test for secondary amines was used in place of the Kaiser test. Fmoc-Asp(O-tBu)-OH (0.540g, 1.31 mmoles) and Fmoc-Gly-OH (0.390g, 1.31 mmoles) were coupled using the above protocol as described for Fmoc-Pro-OH. Fmoc-Har-OH (0.598g, 1.31 mmoles) was coupled using the same protocol as Fmoc-Pro-OH except that additional HOBt (0.603g, 3.94 mmoles) was used to protonate the unprotected guanidino group. After the Har coupling and following the Fmoc-group removal and DMF washes (the volume of each wash being increased to approximately 12ml) a solution of 10% w/v HOBt in DMF (10ml) was added to the resin which was stirred for 5 minutes and then filtered. [Mpr-OH]₂ (0.276g, 1.31 mmoles) was coupled using the same protocol as for Fmoc-Pro-OH except that the volume of DMF used in the washes was increased from 10 to 12ml. Upon completion of the assembly the peptidyl resin was collapsed using DCM (5 x 20ml). The resin was then air dried for 1 hour (resin yield 1.096g)

Stage 5

Peptide cleavage, α -carboxy protecting group removal and side chain protecting group removal

A premixed solution of 90% TFA /10% EDT (7.5ml) was added to a sample of the peptide resin (0.500g) from stage 4. Thirty minutes after the initial charge the crude linear peptide solution was filtered from the resin. The resin was washed 3 times with TFA (3 x 5ml). The combined filtrates were pooled and concentrated to a thin oil by rotary evaporation (bath temperature <35°C). The resultant oil was gradually added drop wise to a stirred media of IPP (20ml). The mixture was agitated for a further 10 minutes and the resultant precipitated peptide filtered through a 0.65 μ m DVPP membrane (Millipore). The resultant solid was then washed 3 times with IPP (3 x 10ml) taking care not to draw excessive amounts of air through the material. The crude linear peptide was then dried overnight 'in-vacuo' at 20°C to constant weight (0.232g). Confirmation to the identity of the crude linear peptide acid was achieved by electrospray MS (theoretical 939.1, found 938.7)

Stage 6

Peptide cyclisation

A sample of peptide (10.1mg) from stage 5 was suspended in CH₃CN (1ml), and water (1ml) was added with agitation to provide a solution. This solution was then added to water (8.1ml) with mixing. A solution of ammonia in water (3.5%w/v, 0.1 ml) was added to the peptide solution to adjust the pH to 9.0 $\pm$ 0.25. The amount of ammonia required was approximately 1.0ml/g crude peptide. If the pH exceeded pH 9.25 acetic acid was

added to bring the pH value into the required range. The resultant solution was stirred in air and monitored by RP-HPLC until the reaction was deemed complete (18 hours). The elution profile clearly indicated the absence of the linear starting material and the formation of a new single entity (purity 92%) assumed cyclic peptide acid. The sample was then co-injected with a standard of the peptide acid affording a single peak confirming the identity of the crude peptidic product. Additional confirmation to the identity of the crude peptidic material was achieved by electrospray MS (theoretical 833.0, found 832.8).

The RP-HPLC analysis method used to monitor the cyclisation was as follows:

Buffer A	6mM HCl/water
Buffer B	MeCN
Column	Waters Symmetry Shield RP ₈ 3.5µm 100Å 150 x 4.6mm
Gradient	10 to 35% Buffer B in Buffer A over 30 minutes
Flow rate	1cm ³ /min
Wavelength	204nm
Injection volume	10µl
Temperature	30°C
Peak width	1.0
Peak sensitivity	0.4

Stage 9

Chiral Analysis of Starting Materials and Products from Novel Route – Assessment of Racemisation

To assess the chiral inversion of the susceptible C α Cysteine residue each of the reagents or intermediates at various stages in the assembly were submitted to chromatography by C.A.T. GmbH and Co. for optical purity analysis. The method gives an unambiguous quantitation of racemate utilising hydrolysis by 6N D₂O/DCl. The standard deviation of the result is validated as <+/- 0.1%, the limit of detection as 0.1%.

Product of	Sample	% Cys d-isomer
Starting material stage 1	(H-Cys-OtBu) ₂ .2HCl	0.19%
Stage 2	Fmoc-Cys-O-tBu	0.32%
Stage 5	Linear peptide	0.50%
Stage 6	Cyclic peptide	0.72%

The results clearly indicate that the cysteine residue had undergone minimal chiral inversion due to the solid phase methodology. An abundance of 0.32% d-Cys increasing to 0.72% in the final crude product indicating an overall increase of 0.4% (+/- 0.1%). This level of racemisation is well within acceptable boundaries.

CLAIMS

1. A process for the solid-phase synthesis of a peptide which comprises attaching an
5 α -nitrogen protected α -carboxy modified amino acid to a solid support via its side chain, removing the α -nitrogen protecting group, and assembling a peptide chain on said α -nitrogen.
2. A process according to claim 1 wherein the assembled peptide is then cleaved
10 from the solid support.
3. A process according to any one of the preceding claims wherein the α -nitrogen protected α -carboxy modified amino acid comprises a hetero atom in its side chain.
- 15 4. A process according to any one of the preceding claims wherein the α -carboxy is modified by reaction with a protecting group.
5. A process according to any one of the preceding claims for the solid-phase synthesis of a peptide comprising the steps of:
20 (a) attaching an α -nitrogen protected α -carboxy protected amino acid comprising a heteroatom in its side chain to a solid support via said side chain;
(b) deprotecting the α -nitrogen of the attached first amino acid by removing the α -nitrogen protecting group under conditions such that the attached first amino acid remains connected to the solid support and coupling an additional α -nitrogen protected
25 amino acid to the unprotected α -nitrogen of the attached amino acid to yield an attached peptide with a protected N-terminus;
(c) deprotecting the N-terminus of the attached peptide by removing the α -nitrogen protecting group under conditions such that the attached peptide remains connected to the solid support and coupling an additional α -nitrogen protected amino acid to the
30 unprotected N-terminus of the attached peptide and repeating until the desired peptide is assembled on the solid support;
(d) removing the α -nitrogen protecting group from the N-terminus of said peptide and optionally reacting with a non-amino acid N-terminal residue;
(e) cleaving the link between the side chain of the first α -carboxy protected amino acid of
35 the peptide and the solid support so that the peptide is released from the solid support; and
(f) removing the α -carboxy protecting group and any side chain protecting groups.
6. A process according to claim 5 wherein step (e) and step (f) are carried out as a
40 single process.

7. A process according to any one of the preceding claims wherein the α -nitrogen protecting group is a base labile protecting group.
- 5 8. A process according to any one of the preceding claims wherein the α -nitrogen protecting group is Fmoc.
9. A process according to any one of the preceding claims wherein the α -carboxy is protected by the formation of a carboxy ester group.
- 10 10. A process according to claim 8 wherein the α -carboxy is modified by the formation of an alkyl ester group.
11. A process according to claim 9 wherein the α -carboxy is protected by the formation of a *tert*-butyl ester.
- 15 12. A process according to any one of the preceding claims wherein the α -nitrogen protected α -carboxy modified amino acid is derived from cysteine.
13. A process according to any one of the preceding claims wherein the α -nitrogen protected α -carboxy modified amino acid is Fmoc-Cys-O-tBu.
- 20 14. A process according to any one of the preceding claims wherein the solid support comprises a polystyrene or polydimethylacrylamide polymer.
- 25 15. A process according to any one of the preceding claims wherein the solid support comprises a linker group which comprises a trityl moiety.
16. A process according to claim 15 wherein the linker group comprises a 4-methoxytrityl moiety.
- 30 17. A support for solid-phase synthesis which comprises an α -nitrogen protected α -carboxy modified amino acid attached to the solid support through the side chain of the amino acid.
- 35 18. A support for solid-phase synthesis according to claim 17 which comprises a polystyrene or polydimethylacrylamide polymer with a trityl linker to which Fmoc-Cys-O-tBu is attached by its side chain.

19. An α -nitrogen protected α -carboxy protected cysteine wherein the α -nitrogen protecting group is a base labile protecting group and the α -carboxy is protected by the formation of a carboxy ester group.

5 20. An α -nitrogen protected α -carboxy protected cysteine according to claim 19 of formula Fmoc-Cys-O-tBu.

10 21. An α -nitrogen protected α -carboxy protected cysteine disulphide linked dimer wherein the α -nitrogen protecting group is a base labile protecting group and the α -carboxy is protected by the formation of a carboxy ester group.

22. An α -nitrogen protected α -carboxy protected cysteine disulfide linked dimer according to claim 21 of formula (Fmoc-Cys-O-tBu)₂.