



Synthesis of Peptide-N-alkylamides on a New PS-TTEGDA Polymer Support using Photolabile Anchoring Group

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Abstract: Peptide-N-alkylamides were synthesised on a new highly solvating copolymer of 4% tetraethyleneglycol diacrylate-cross-linked polystyrene (PS-TTEGDA) support. The polymer was synthesised by suspension polymerisation using a free radical initiator. The synthesis of C-terminal peptide-N-alkylamide involve prior incorporation of a photolabile linker, 3-nitro-4-bromomethylbenzoic acid to the aminomethylated support. The N-alkylamino group act as an anchoring group for the peptide as well as a latent function for the C-terminal modification of the attached peptide. Irradiation of the peptide-resin with 350 nm light in TFE/DCM resulted in the release of peptide-N-alkylamides. Synthetic utility of the new support was demonstrated by the synthesis of Boc-amino acid-N-alkylamides and C-terminal peptide-N-alkyl amides in 75-80% yields and with high purity.

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INTRODUCTION

Ever since the introduction of solid phase peptide synthesis (SPPS) by Merrifield several improvements have been made to generalise this technique.^{2,3} The success of all these efforts depend up on the choice of polymeric support, its mechanical stability, swelling characteristics, compatibility between the support and the growing peptide chain and the nature of the anchoring group that connect the peptide to the support.⁴ In this context several solid supports like polyethyleneglycol-polystyrene (PEG-PS), polyamides, polyethylene glycol-polyacrylamide (PEGA) and cross-linked ethoxylate acrylate (CLEAR) were introduced and tested for their efficiency.⁵⁻⁸ In an effort to develop an efficient solid support, hydrophobic styrene was cross-linked with a hydrophilic flexible tetraethyleneglycol diacrylate (TTEGDA). The PS-TTEGDA polymer was synthesised by radical copolymerisation.^{9,10} A 4% TTEGDA crosslinked polystyrene resin showed optimum hydrophobic-hydrophilic balance, mechanical stability, excellent swelling properties and performance in the stepwise synthesis of medium to large peptides.

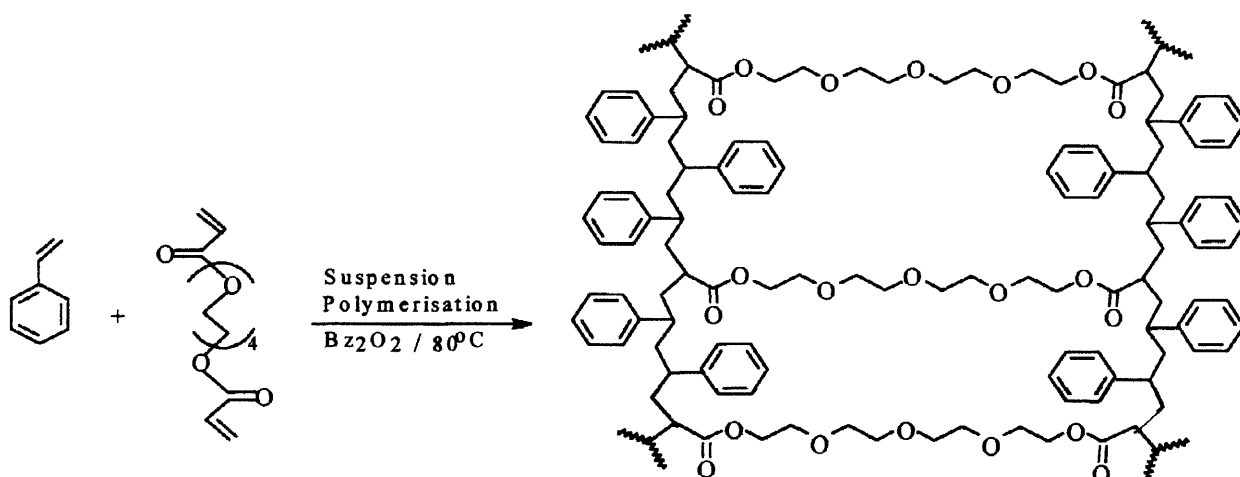
Introduction of N-ethyl or methylamides into peptides is a commonly used tool for bio-activity studies. The resulting peptide-N-alkylamides sometimes shows enhanced biological activity and/or stabilisation of a particular conformation of the peptide.^{11,12} Since peptide acids ionise at physiological pH, the partial sequences of a protein required for immunological studies are probably best when prepared as their C-terminal amides or N-alkylamides. Another problem with the SPPS is the final cleavage of the peptide from the support. Since peptides are complex molecules containing several side chain protecting groups, chiral centres, acid and base sensitive amino acids, their cleavage from the resin using acid or base can result in several side reactions.¹³ Incorporation of a photolytically cleavable anchoring linker between the resin and the growing peptide chain can help the final cleavage of the peptide under neutral conditions by irradiation with 350 nm light.¹⁴⁻¹⁶ A 3-nitrobenzamido anchoring linkage present between the polymer support and the peptide can help the release of the peptide amide by photolysis in TFE/DCM at room temperature without affecting the side chain protections or the N-protecting group.¹⁷

In this paper we report the use of a photolytically cleavable 3-nitro-4-bromomethylbenzamidomethyl PS-TTEGDA support for the solid phase synthesis of amino protected amino acid N-alkylamides and fully protected peptide-N-alkylamides. PS-TTEGDA resin swells and solvates much more than the Merrifield resin in all solvents that are used for the SPPS. This nature of the support can help the easy interaction of the

reactants with the reactive centres present within the resin matrix. This new support results in the release of peptide-N-alkylamide under mild neutral and non-destructive photolytic conditions, thus avoiding the direct interaction with TFA. All the peptides obtained are in the fully protected form, which therefore can also be used for the segment condensation. This method also offers the possibility of obtaining N-alkylamides of peptides containing Asp and Glu without any damage to the side chain benzyl ester protection.

RESULTS AND DISCUSSION

The support was synthesised by suspension polymerisation of styrene with four mole percent of tetraethyleneglycol diacrylate. The monomer was mixed with the diluent toluene and then suspended in water containing 1% polyvinyl alcohol (MW~70,000) which was used as suspension stabiliser. Benzoylperoxide was used as radical initiator. The polymer was obtained in uniform spherical beads of 200-400 mesh size (Scheme 1). The physico-chemical properties of a polymer support determined by the chemical nature of the monomers and the mole percentage of the crosslinker. Four mole percent PS-TTEGDA resin can provide the desired mechanical stability to the support. Reproducible results may be obtained by adjusting the monomer-diluent ratio, the amount of stabiliser, geometry of the vessel and the stirring rate. The IR spectrum of powdered resin (KBr pellet) shows intense peaks at 1720 cm^{-1} corresponding to ester carbonyl and 1150 cm^{-1} corresponding to the ether linkage of the cross-linker besides the usual peaks of polystyrene. Solid state ^{13}C -CP-MAS NMR spectrum of the resin shows an intense peak at 127.89 ppm corresponding to the aromatic polystyrene ring and a small peak at 145.65 ppm corresponding to the C-3 carbon of the polystyrene ring. The backbone methylene carbon of the polymer appears as a single peak at 40.34 ppm. The methylene carbons of ether linkage of the cross-linking agent appears as a small peak at 70.65 ppm



SCHEME 1. Synthesis of PS-TTEGDA Polymer Support

A 4% PS-TTEGDA resin shows higher effective swelling in both polar and nonpolar solvents when compared to a 2% divinylbenzene-crosslinked polystyrene (PS-DVB) resin. The swelling volumes of 4% PS-TTEGDA resin and PS-DVB resin (in brackets) were 6.8 ml/g (4.3 ml/g) in chloroform, 8.6 ml/g (5.2 ml/g) in THF, 7.2 ml/g (4.7 ml/g) in toluene, 6.5 ml/g (3.5 ml/g) in dioxane, 8.4 ml/g (4.3 ml/g) in DCM, 6.5 ml/g (2.8 ml/g) in DMF, 7.8 ml/g (3.6 ml/g) in NMP and 2.8 ml/g (1.8 ml/g) in MeOH. Though the swelling characteristics of lower cross-linked PS-TTEGDA supports are high compared to 4% cross-linked support, their mechanical and morphological characters will change when encountered with different solvents and

reagents that are used in SPPS. Lightly cross-linked support become powdered when the resin was used for the synthesis of peptides medium to large peptides. Higher cross-linked supports are found to be rigid and their swelling characteristics in various solvents are poor compared to 4% PS-TTEGDA resin. In this context a 4% cross-linked PS-TTEGDA resin which shows high mechanical stability, excellent swelling properties and optimum hydrophobic-hydrophilic balance was selected as the best support for the SPPS. In solvents like DCM, DMF and NMP the polymer swells like a gel form in which the cross-linking network becomes extremely distant, mechanical resistance to the reagents due to the polymer net work decreases and thus allow the reagents to reach the reaction sites easily and enhance the reaction rate. Functionalisation of this support will not change swelling characteristics of the support. The support was extremely stable under all conditions of peptide synthesis. The ester groups that present in the resin was stable enough to withstand the nucleophilic cleavage by piperidine and bases stronger than piperidine. This was investigated by analysing the IR spectrum of a 4% PS-TTEGDA support before and after suspending 48 h in neat TFA and in different basic solutions like 1M NaOH, NH_4OH and 2M NH_2OH in aqueous MeOH (fig. 1). All these investigations revealed that the cross-links are stable enough to withstand the various reaction conditions employed in the SPPS.

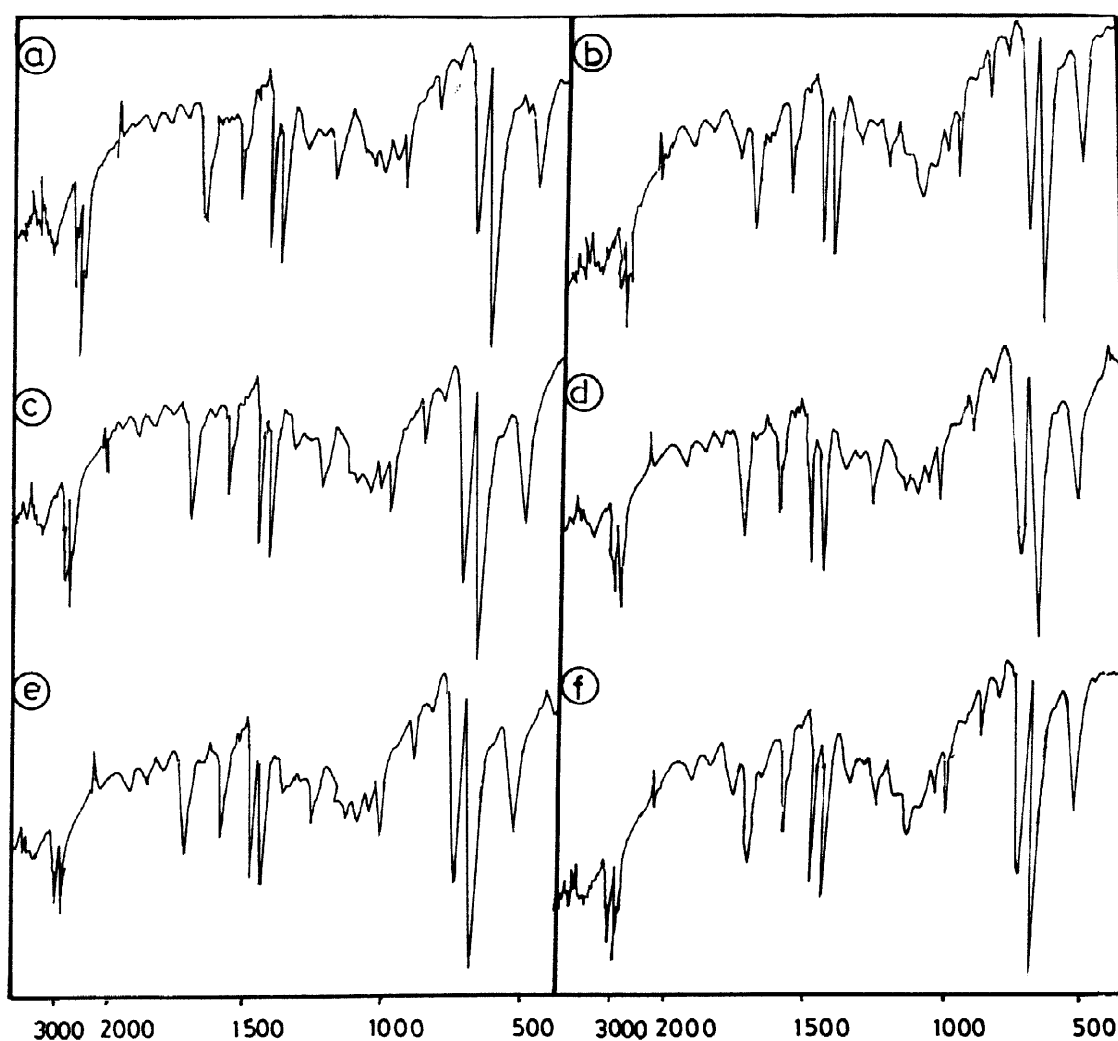
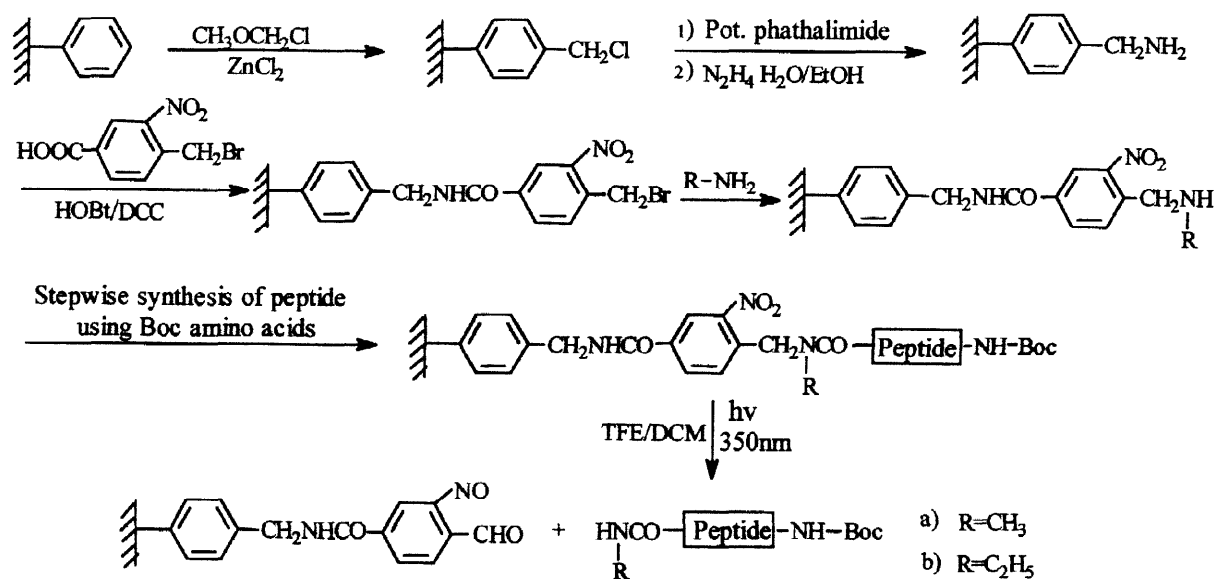


FIGURE 1. IR spectra of PS-TTEGDA (a) original; (b) after treatment with neat TFA for 48 h; (c) after 14 h irradiation at 350nm light; (d) after treatment with 2M aqueous NaOH for 48 h; (e) after treatment with 2M NH_2OH in aqueous MeOH for 48 h; (f) after treatment with piperidine for 48 h.

PS-TTEGDA was functionalised with a chloromethyl group by Friedel-Crafts type electrophilic substitution of the aromatic ring. Chloromethylation of the styrene ring was carried out using chloromethyl methylether (CMME) in the presence of anhydrous ZnCl_2 solution in THF (Scheme 2). Since CMME is a good swelling agent for the polymer, chloromethylation proceeds easily. The reaction can be controlled to produce chloromethyl resin of desired chlorine capacity by varying the amount of CMME, catalyst, temperature and duration of reaction.¹⁸ The degree of chloromethylation in the resin was determined by Volhardt's method¹⁹.

The resin was then converted to aminomethyl resin by treating it with potassium phthalimide followed by hydrazinolysis. To the aminomethyl resin a photosensitive 3-nitro-4-bromomethylbenzoic acid linker was attached. The anchoring group 3-nitro-4-bromomethylbenzoic acid was prepared from *p*-toluic acid by a two step reaction. In the first step *p*-toluic acid was converted to 4-bromomethylbenzoic acid by the treatment with *N*-bromosuccinimide. In the second step 4-bromomethylbenzoic acid was nitrated with fuming nitric acid at -10°C to obtain 3-nitro-4-bromomethylbenzoic acid. Since this polymer support contains only the required number of nitro groups that are essential for the photochemical reaction, the physico-chemical properties of the supports were not affected by the attachment of the functional group. The resin shows characteristic IR (KBr) bands at 1346 cm^{-1} , 1542 cm^{-1} (NO_2), 1650 cm^{-1} (NHCO) and 3450 cm^{-1} (broad) of the NH group.

3-Nitro-4-bromomethylbenzamidomethyl PS-TTEGDA resin was suspended in DMF, dry methyl or ethylamine gas was passed through the suspension at 0°C and the reaction mixture was shaken at room temperature for 24 h (Scheme 2). The *N*-alkyl resin obtained was purified and dried in vacuum. The resin shows IR(KBr) absorption at 1346 cm^{-1} , 1542 cm^{-1} (NO_2), 3450 cm^{-1} (broad) (NH) and 1646 cm^{-1} (CONH). The amino capacity of the resin was estimated by the picric acid method.²⁰ The possibility of forming tertiary amines or quaternary ammonium salt during this reaction was negligible since a large excess amine was used.



SCHEME 2. Functionalisation and solid phase synthesis of peptide *N*-alkylamides using photolabile 3-nitro-4-bromomethylbenzamidomethyl PS-TTEGDA resin.

For the synthesis of medium to large peptides the linkage between the peptide and the resin should be stable under the various conditions encountered during the synthesis. Boc deprotection with 30% TFA in

DCM resulted in no harm to the peptide resin linkage. Twenty-four hour treatment of Gly-resin with 30% TFA in DCM resulted in no apparent change in its amino capacity. This result shows that the peptide resin linkage was stable enough to withstand the repeated acid treatment encountered during the removal of the Boc protecting group. Irradiation of peptide-resin with 350 nm light in a trifluoroethanol/DCM mixture about (10-12 h) resulted in the release of the attached carboxyl function of the C-terminal amino acid as peptide-N-alkyl amide. Fourteen hour photolysis of the resin in TFE/DCM mixture results no apparent change in its IR spectrum. Photolysis of the resin bound peptide does not facilitate the release of Boc protection from the N-terminal amino acid or the protecting group of the side chain functional groups. After the photolysis the resin bound 2-nitrosobenzaldehyde, the photolysis by-product, can interact with the free amino group of the peptide-N-alkylamide if protection of N-terminal amino acid is removed. This can reduce the overall yield drastically. This side reaction could be avoided by adding an aldehyde scavenger like semicarbazide hydrochloride or sulphuric acid to the reaction mixture.

The photolytic cleavage mechanism of *o*-nitrobenzyl and related systems has been well established.²¹⁻²³ The primary photochemical process responsible for the deprotection of the carboxylic group was an intramolecular hydrogen abstraction by the excited nitro group followed by an electron redistribution to the aci-nitro form which rearranges to the nitroso derivatives. The unstable nitroso derivative undergoes cleavage yielding resin bound 2-nitrosobenzaldehyde and the N-alkyl amide. Here further transformation of the resin bound 2-nitrosobenzaldehyde to an azocompound which can act as an internal light filter is very low because of the low probability of polymer-polymer interactions.

The synthetic capability of the new photolabile polymer supports was demonstrated by synthesising model amino acid N-alkylamides and peptide N-alkylamides. The peptides were assembled on 3-nitro-4(alkyl) aminomethylbenzamidomethyl PS-TTEGDA support using preformed HOBt active esters of Boc-amino acids. All acylation reactions were completed with a single coupling in 50 min as shown by the Kaiser test.²⁴ Side reactions like the formation of diketopiperazine (DKP), though highly sequence dependent was not observed during the synthesis. However, coupling of the third amino acid was followed immediately after the deprotection of the second amino acid. After synthesis the target peptide N-alkylamide was cleaved from the support by photolysis. The crude amino protected amino acid N-alkylamide or peptide-N-alkylamides were obtained in 75-80% yield as judged by amino acid analysis of the peptide-resin after the photolysis. The amino acid N-alkylamides or peptide-N-alkylamides obtained by photolysis were purified by elution through sephadex G-10 columns using an acetic acid-water mixture. FPLC profile of the crude peptide showed only one major peak corresponding to the target peptide (Fig 2). Comparison of the FPLC retention times of the protected peptide segments synthesised shows that the peptide N-ethylamide was somewhat more polar than peptide N-methylamide. The amino acids or peptide-N-alkylamides were characterised by IR, amino acid analysis and ¹H NMR techniques. The amino acid or peptide-N-alkylamides that were synthesised and their spectral characteristics were shown in Table 1.

EXPERIMENTAL

Materials. Styrene, TTEGDA, polyvinyl alcohol (MW ~75000) were purchased from Aldrich Chemical Company, USA., dicyclohexyl carbodiimide (DCC) and side chain protected Boc amino acids were purchased from Novabiochem Ltd., UK. Chloromethylmethyl ether (CMME) was prepared using the literature procedure²⁵. All solvents used were purchased from E. Merck (India), BDH (India), and SISCO Chemicals (Bombay). The solvents were of reagent grade, some of them were further purified as follows. DCM was distilled over P₂O₅ and then Na₂CO₃, THF was refluxed over metallic sodium for 1 h and distilled, ethanol

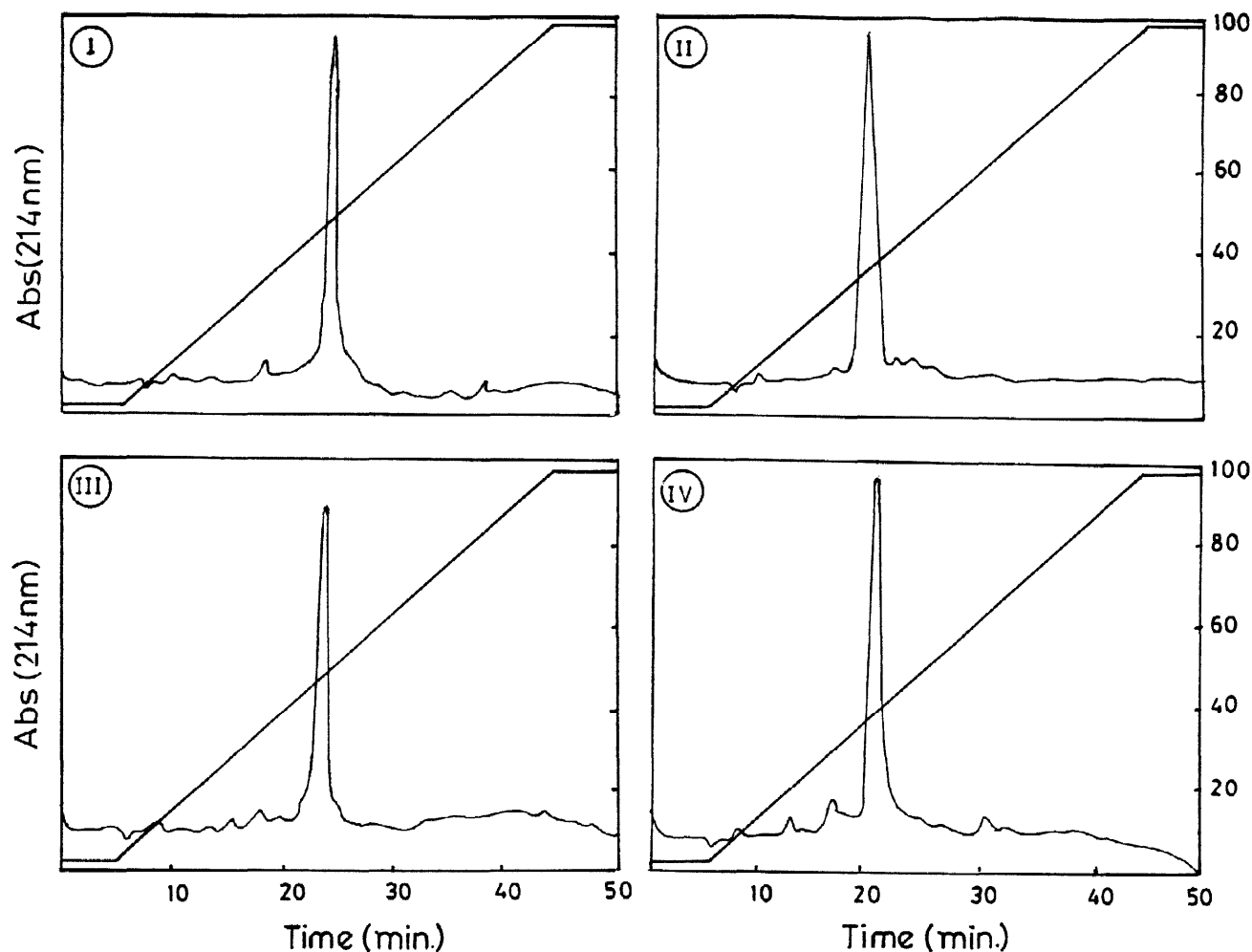


FIGURE 2. FPLC time-course analysis of peptide-N-alkylamides using the buffer A: 0.5 ml TFA in 100 ml water; B: 0.5 ml TFA in 100 ml acetonitrile; Flow rate: 0.5 ml/min; Gradient used: 0% B in 5 min, 100% B in 45 min, 100% B in 50 min. (I) Boc-Leu-Ala-Val-NHET; (II) Boc-Val-Leu-Ala-Val-NHMe (III) Boc-Val-Leu-Ala-Val-NHET (IV) Boc-Gly-Ile-Cys (AcM)-Pro-NHET

Table 1. Yield, photolytic duration and spectral characterisation of amino acid or/peptide-N-alkylamide.

Amino acid/peptide-N-alkylamide	Yield	Solvent mixture TFE:DCM	Photolytic duration	Spectral Characteristics
Boc-Val-NHMe	83	(1:3)	12 h	IR (KBr): 1654 cm^{-1} (amide), 1726 cm^{-1} (urethane); $^1\text{H NMR}$ (350MHz, d_6 DMSO): 1.40 (s, Boc), 2.00-2.10 (m, β -H, γ -H Val), 2.91 (d, NH-methyl CH_3), 4.40 (m, α -H Val), 8.10 (m, N-methylamide-H).
Boc-Ala-NHMe	80	(1:3)	12 h	IR (KBr): 1650 cm^{-1} (amide), 1720 cm^{-1} (urethane); $^1\text{H NMR}$ (350 MHz, d_6 DMSO): 1.39 (s, Boc), 1.86 (m, β -H Ala), 2.56 (d, NH-methyl CH_3), 4.20 (m, α -H Ala), 8.40 (m, N-methylamide-H).
Boc-Phe-NHMe	76	(1:3)	12 h	IR (KBr): 1653 cm^{-1} (amide), 1590 cm^{-1} (Aromatic), 1722 cm^{-1} (urethane); $^1\text{H NMR}$ (350 MHz, d_6 DMSO): 1.42 (s, Boc), 2.56 (d, NH-methyl CH_3), 4.90 (m, α -H Phe), 8.00 (m, Aromatic Phe), 8.60 (m, N-methylamide-H).
Boc-Leu-NHMe	75	(1:3)	12 h	IR (KBr): 1647 cm^{-1} (amide), 1715 cm^{-1} (urethane); $^1\text{H NMR}$ (350MHz, d_6 DMSO): 1.10 (d, γ -H Leu), 1.38 (s, Boc), 2.60 (d, NH-methyl CH_3), 3.36 (m, β -H, Leu), 4.60 (α -H, Leu), 8.60 (m, N-methylamide-H).

Boc-Ile-NHMe	80	(1:3)	12 h	IR (KBr): 1652 cm ⁻¹ (amide), 1720 cm ⁻¹ (urethane); ¹ H NMR (350MHz, d ₆ DMSO): 0.80 (m, δ-H, Ile), 1.00 (m, γ-H Ile), (1.42 (s, Boc), 2.78 (d, NH-methyl), 4.43 (m, α-H Ile), 7.89 (d, amide-H, Ile), 8.20 (m, N-methylamide-H)
Boc-Thr (Bzl)-NHMe	78	(1:2)	10 h	IR (KBr): 1660 cm ⁻¹ (amide), 1590 cm ⁻¹ (Aromatic), 1724 cm ⁻¹ (urethane); ¹ H NMR (350MHz, d ₆ DMSO): 1.0 (d, γ-H Thr), 1.42 (s, Boc), 2.56 (d, NH-methyl CH ₃), 3.86-3.96 (m, β-H Thr), 4.80 (m, γ-H Thr), 8.00 (m, Aromatic Thr(Bzl), 8.60 (m, N-methylamide-H).
Boc-Gly-NHMe	80	(1:3)	12 h	IR (KBr): 1640 cm ⁻¹ (amide), 1712 cm ⁻¹ (urethane); ¹ H NMR (350MHz, d ₆ DMSO): 1.30 (s, Boc), 2.60 (d, NH-methyl CH ₃), 3.36 (m, β-H, Gly), 4.32 (m, α-H Gly), 7.00 (m, N-methylamide-H), 7.23 (t, amide-H, Gly).
Boc-Ser (Bzl)-NHMe	79	(1:3)	12 h	IR (KBr): 1646 cm ⁻¹ (amide), 1726 cm ⁻¹ (urethane); ¹ H NMR (350MHz, d ₆ DMSO): 1.34 (s, Boc), 2.76 (d, NH-methyl-H), 3.7 (m, β-H, Ser), 4.32-4.11 (m, α-H Ser, Ser(Bzl)), 7.00 (m, N-methyl amide-H), 8.25 (m, amide-H)
Boc-Cys (Acm)-NHMe	80	(1:3)	12 h	IR (KBr): 1639 cm ⁻¹ (amide), 1722 cm ⁻¹ (urethane); ¹ H NMR (350MHz, d ₆ DMSO): 1.36 (s, Boc), 1.83 (s, Me Acm), 2.77 (d, NH-methyl CH ₃), 3.45 (m, β-H, Cys), 4.40 (m, α-H Cys), 7.14 (m, N-methylamide-H), 7.65 (d, amide-H Cys), 8.50 (t, amide-H, Acm).
Boc-Lys (ClZ)-NHMe	81	(1:3)	10 h	IR (KBr): 1646 cm ⁻¹ (amide), 1718 cm ⁻¹ (urethane); ¹ H NMR (350MHz, d ₆ DMSO): 1.36 (s, Boc), 1.67-2.00 (m, α-H Lys, δ-H Lys), 2.80 (d, NH-methyl CH ₃), 3.46 (ε-H, Lys), 4.40 (m, α-H Lys), 5.16 (s, benzylic Lys ClZ), 8.60 (m, N-methylamide-H)
Boc-Pro-NHMe	79	(1:2)	10 h	IR (KBr): 1650 cm ⁻¹ (amide), 1721 cm ⁻¹ (urethane); ¹ H NMR (350MHz, d ₆ DMSO): 1.35 (s, Boc), 1.86 (m, γ-H Pro), 2.68 (d, NH-methyl CH ₃), 2.95 (m, β-H, Pro), 3.45 (m, δ-H Pro), 4.44 (α-H Pro), 8.28 (m, N-methylamide-H)
Boc-Leu-Ala-Val-NHMe	79	(1:3)	10 h	IR (KBr): 1660 cm ⁻¹ (amide), 1726 cm ⁻¹ (urethane); Amino acid analysis: Ala, 1.00 (1); Leu, 1.09 (1); Val, 1.12 (1); ¹ H NMR (350 MHz, d ₆ DMSO): 1.10 (d, γ-H Leu), 1.38 (s, Boc), 1.80-2.50 (m, γ-H Val, β-H Ala, Leu, Val), 2.90 (d, NH-methyl CH ₃), 4.20-4.80 (m, α-H Ala, Leu, Val), 7.00-8.34 (m, amide-H Leu, Ala, Val, N-methylamide-H).
Boc-Leu-Ala-Val-NHEt	78	(1:3)	10 h	IR (KBr): 1648 cm ⁻¹ (amide), 1721 cm ⁻¹ (urethane); FABMS <i>m/z</i> 449.53 [(M+Na) ⁺ 76%] C ₂₁ H ₄₀ N ₄ O ₅ requires 428.51; ¹ H NMR (350 MHz, d ₆ DMSO): 1.2 (d, γ-H Leu), 1.43 (s, Boc), 2.00-3.00 (m, γ-H Val, β-H Ala, Leu, Val, N-ethyl), 3.20 (d, N-H-ethyl), 3.70-4.72 (m, α-H Ala, Leu, Val, N-ethyl), 6.80-8.60 (m, amide-H Leu, Ala, Val, N-methylamide-H).
Boc-Val-Leu-Ala-Val-NHMe	80	(1:3)	10 h	IR (KBr): 1650 cm ⁻¹ (amide), 1720 cm ⁻¹ (urethane); FABMS <i>m/z</i> 534.91 [(M+Na) ⁺ , 100%]; 514.84 (M+1) ⁺ , 12%; C ₂₅ H ₄₇ N ₅ O ₆ requires 513.67 Amino acid analysis: Ala, 1.00 (1); Leu, 1.04 (1); Val, 1.96 (2); ¹ H NMR (350MHz, d ₆ DMSO): 1.10 (d, γ-H Leu), 1.30 (s, Boc), 2.00-3.46 (m, β-H, Leu, Val, Ala, NH-methyl H), 3.20-4.50 (m, α-H Leu, Val, Ala), 7.30-8.60 (m, amide-H Ala, Val, Leu, N-methylamide-H)
Boc-Val-Leu-Ala-Val-NHEt	82	(1:3)	10 h	IR (KBr): 1640 cm ⁻¹ (amide), 1717 cm ⁻¹ (urethane); FABMS <i>m/z</i> 548.67 [(M+Na) ⁺ 100%], 528.24 (M+1) ⁺ , 12%; C ₂₆ H ₄₉ N ₅ O ₆ requires 527.67 ¹ H NMR (350 MHz, d ₆ DMSO): 1.11 (d, γ-H Leu), 1.33 (s, Boc), 2.00-3.20 (m, γ-H Val, β-H Ala, Leu, Val, N-ethyl), 3.20 (d, N-H-ethyl), 3.80-4.82 (m, α-H Ala, Leu, Val, N-ethyl), 7.00-8.80 (m, amide-H Leu, Ala, Val, N-methylamide-H).
Boc-Gly-Ile-Cys (Acm)-Pro-NHEt	79	(1:3)	10 h	IR (KBr): 1650 cm ⁻¹ (amide), 1716 cm ⁻¹ (urethane); FABMS <i>m/z</i> 609.22 [(M+Na) ⁺ , 100%], 587.18 (M+1) ⁺ , 38%; C ₂₆ H ₄₇ N ₆ O ₇ S requires 586.67; Amino acid analysis: Gly, 1.00 (1), Ile, 0.98 (1), Pro, 0.65 (1), Cys, 1.23 (1); ¹ H NMR (350 MHz, d ₆ DMSO): 0.80 (m, δ-H, Ile), 1.00 (m, γ-H Ile), 1.42 (s, Boc), 1.86 (s, Me, Acm), 1.81-1.89 (m, γ-H Pro), 2.66 (m, β-H Pro), 2.80 (d, methyl NHCH ₃), 3.55-3.58 (m, β-H Cys, Gly, δ-H Pro), 4.61-4.10 (m, α-H Cys, Gly, Ile, Pro, CH ₂ Acm), 6.68 (m, N-methylamide-H), 7.01 (t, amide-H Gly), 7.55 (d, amide-H, Ile Cys), 8.40 (d, amide-H, Cys, Ile), 8.50 (t, amide-H Acm)

was distilled twice and HPLC grade DMF was used for the synthesis. IR spectra were recorded on a Shimadzu IR 470 spectrometer using KBr pellets. The ^{13}C -CP-MAS solid state NMR measurements were conducted on a Bruker 300 MSL CP MAS instrument operating at 75.47 MHz. The spectra were run with fine powder of polymer beads at room temperature and Kelf rotor was employed for MAS. The samples were rotated with a spectral width of 25,000 Hz, the CP time was 22 min and the number of scans was in the range of 200-300. Each sample was rotated with two different spin rates and by comparing the resultant spectra the spinning side bands were eliminated. FPLC was done on a Pharmacia instrument using C-18 reverse phase semi prep. FPLC column.

TTEGDA-crosslinked polystyrene support. Inhibitors are removed from styrene and TTEGDA by washing with 1% NaOH solution and distilled water and drying over calcium chloride. A four necked reaction vessel equipped with a thermostat, teflon-bladed stirrer, water condenser and nitrogen inlet was used as the reaction vessel. A net volume of 1% solution of polyvinyl alcohol (~75,000) was prepared by dissolving polyvinyl alcohol (2.6 g) in double distilled water (228 ml) at 80°C. A mixture of styrene (22.46 ml), tetraethylene-glycol diacrylate (1.09 ml) and benzoyl peroxide (500 mg) dissolved in toluene (12.45 ml) were added to the PVA solution by stirring the aqueous solution at 2000 rpm. The temperature was maintained at 80°C under a slow stream of nitrogen. After 6 h the copolymer were obtained as white beads of 200-400 mesh size. The polymer was washed thoroughly with hot water (to remove the stabiliser), benzene (3 x 50 ml), toluene (3 x 50 ml), MeOH (3 x 50 ml), DCM (3 x 50 ml) and with acetone (3 x 50 ml) and dried under vacuum.

Chloromethyl PS-TTEGDA resin. PS-TTEGDA resin (4 g) was swelled in CH_2Cl_2 in a 250 ml round bottom flask. After 1 h excess DCM was filtered off. CMME (24 ml) was added to the swelled resin and 1M ZnCl_2 in THF (0.6 ml) was added to the mixture. The suspension was kept at 50°C. After 3 h the resin was filtered using a sintered glass funnel and washed with THF (4 x 30 ml), THF/water (1:1; 3 x 30 ml), THF (3 x 30 ml), methanol (3 x 30 ml) and dried under vacuum. The chlorine capacity of the resin = 0.3 mmol/g as estimated by Volhardt's method.¹⁸ IR (KBr): 1720, 1480 cm^{-1} (ester), 1254 cm^{-1} ($\text{CH}_2\text{-Cl}$), 1150 cm^{-1} (ester).

Aminomethyl PS-TTEGDA resin. Chloromethyl PS-TTEGDA resin (4g, 0.3 mmol Cl/g) was swollen in DMF (50 ml). After 30 min potassium phthalimide (4.5g, 2.4 mmol) was added to the resin and the reaction mixture was stirred at 110-120°C. After 12 h the resin was filtered and washed with DMF (3 x 10 ml), DMF:H₂O (1:1; 3 x 10 ml), water:dioxan(1:1; 3 x 10 ml), EtOH (3 x 10 ml) and MeOH (3 x 10 ml) and dried in vacuum. Dried resin (4g) was suspended in EtOH (40ml) and refluxed with hydrazine hydrate (0.6ml, 18 mmol). After 3h the resin was filtered and washed with hot EtOH, DMF, EtOH and MeOH (all solvents 3 x 10 ml each) and dried in vacuum. Amino capacity of the resin = 0.2 mmol/g as determined by picric acid method.¹⁹

4-Bromomethyl benzoic acid. Benzoyl peroxide (200 mg) and N-bromosuccinimide (17.8 g, 100 mmol) were added to a suspension of *p*-toluic acid (13.6 g, 100 mmol) in dry benzene (100 ml) and the mixture was refluxed for 24 h. The solvent was removed under vacuum and the residue was suspended in boiling water (100ml) for 10 min. The precipitate was filtered and washed with boiling water (4 x 50ml). The crude product was recrystallised from a hot MeOH: CHCl_3 mixture. m.p = 226-229°C. IR (KBr) 2800-2400 cm^{-1} , 1686 cm^{-1} (COOH), 1560 cm^{-1} (aromatic); NMR (CDCl_3 , DMS- Cl_6), 4.61 (s, 2H), 7.8 (8.4, J = 8 Hz), 10.4 (s, 1H).

3-Nitro-4-bromomethyl benzoic acid.¹⁷ 4-Bromomethyl benzoic acid (5.4 g, 25 mmol) was added in portions over 30 min to fuming HNO_3 (100 ml) at -10°C. The suspension was stirred for 2 h at -10°C. The solution was then poured on to crushed ice. The precipitate was collected by filtration and washed with ice cold water until the washings were pH neutral. The precipitate was dried and recrystallised from petroleum ether. Melting point = 125-126°C. IR (KBr) 2800-2300 cm^{-1} , 1688 cm^{-1} (COOH), 1598 cm^{-1} (aromatic) and 1542 cm^{-1} , 1310 cm^{-1} (NO_2)

3-Nitro-4-bromomethylbenzamide PS-TTEGDA resin. 3-Nitro-4-bromomethylbenzoic acid (2.09 g, 0.8 mmol) and HOBt (2.16 g, 1.6 mmol) were dissolved in DCM (25 ml) and shaken with a DCC (1.65g, 0.8 mmol) solution in DCM (10ml). After 1 h, dicyclohexylurea was filtered off and the HOBt active ester formed was shaken with pre-swelled aminomethyl resin (1 g, 0.2 mmol NH/g) in DCM. After 1h the resin was filtered, washed with DCM (6 x 20 ml) and a second coupling was performed with HOBt active ester. The resin was collected by filtration, washed with DCM, DMF and MeOH (4 x 15 ml each). Bromine constant of the resin as determined by Volhardt's method = 0.2 mmol/g. IR (KBr) 1650 cm^{-1} , (NHCO), 1340 cm^{-1} , 1540 cm^{-1} (NO_2).

3-Nitro-4-aminomethylbenzamidomethyl PS-TTEGDA resin. 3-Nitro-4-bromomethylbenzamide PS-TTEGDA resin (5 g, 1 mmol Br) was suspended in DMF. Potassium phthalimide (3.5 g, 2 mmol) was added and the reaction mixture was stirred at 110-120°C. After 12 h, the reaction mixture was filtered, washed with DMF (3 x 10 ml), dioxan (3 x 10 ml), EtOH (3 x 10 ml), MeOH (3 x 10 ml) and dried in vacuum. The dried resin was suspended in EtOH (100 ml) and refluxed with hydrazine hydrate (0.3 ml, 9 mmol) for 8 h. The resin was collected by filtration and washed with hot EtOH (3 x 10 ml) and MeOH (3 x 10 ml). The resin was then dried in vacuum. Amino capacity of the resin = 0.2 mmol/g, as determined by the picric acid method.¹⁹

3-Nitro-4-N-methylaminomethylbenzamidomethyl PS-TTEGDA resin. 3-Nitro-4-aminomethylbenzamidomethyl PS-TTEGDA resin (2 g, 0.4 mmol) was suspended in DCM (20 ml) in a 50 ml stoppered bottle and cooled to 0-5°C. Dry methylamine gas was bubbled through the suspension for 12 h. The reaction bottle was tightly stoppered and shaken for another 12 h at room temperature. The mixture was filtered and the resin washed with DCM (3 x 20 ml), THF (3 x 20 ml), H₂O (3 x 20 ml), MeOH (3 x 20 ml) and dried in vacuum. Amino content of the resin = 0.18 mmol/g, as determined by the picric acid method.¹⁹ IR(KBr) 1650 cm^{-1} (NHCO), 1340, 1530 cm^{-1} (NO_2), 3400 cm^{-1} (NH).

3-Nitro-4-ethylaminomethylbenzamidomethyl PS-TTEGDA. 3-Nitro-4-aminomethylbenzamidomethyl PS-TTEGDA resin was suspended in DCM (20 ml) in a 50 ml stoppered bottle and cooled to 0-5°C. Dry ethyl amine gas was bubbled through the suspension for 12 h. The reaction bottle was tightly stoppered and shaken for another 12 h at room temperature. The resin was collected by filtration, washed with DCM (3 x 20 ml), THF (3 x 20 ml), H₂O (3 x 20 ml), MeOH (3 x 20ml) and under vacuum. Amino content of the resin=0.18 mmol/g. IR (KBr) 1650 cm^{-1} (NHCO), 1340, 1530 cm^{-1} (NO_2), 3440 cm^{-1} (NH).

General procedure for solid phase peptide synthesis. Synthesis was carried out manually in a silanized glass reaction vessel of 15 ml volume. The resin bound N-alkyl derivative of the C-terminal amino acid was swollen in DCM. HOBt active esters of Boc-amino acids were prepared by reacting 2.5 equivalents acid (relative to resin capacity) of DCC, HOBt and Boc-amino acid for 1 h at room temperature. The precipitated DCU was filtered off and the active ester solution was added to the pre-swelled resin in DCM. The contents were shaken for 50 min, the resin was filtered and washed with DCM (6 x 10 ml). Each coupling was carried out for a second time with NMP as solvent to achieve 100% coupling. Each coupling step was monitored by semiquantitative ninhydrin test to ensure the completion of coupling.

After incorporating each Boc-amino acid, the resin was treated with 30% TFA in DCM for 30 min to remove the Boc protection. The resin was filtered and washed with DCM (6 x 10 ml). The deprotected resin was neutralised by adding 5% DIEA in DCM for 10 min and then washed with DCM (6 x 10 ml). The coupling, deprotection and washing were repeated until the desired sequence was achieved. Boc protection of the N-terminal amino acid was not removed.

General procedure for photolytic cleavage. The peptide resin (1 g) was suspended in a mixture of TFE and DCM (1:3), (100 ml) and placed in the inner jacket of a water-cooled pyrex immersion-type photochemical reactor. The reaction vessel was silylated before photolysis of peptide resin in order to prevent resin adhering

to the walls of the vessel. This is done by rinsing the reaction vessel 3 or 4 times with a 10% solution of Me_3SiCl in DCM followed by washing with absolute EtOH and drying. Nitrogen gas was bubbled through the suspension for about 1 h to remove the dissolved oxygen gas. The suspension irradiated with a Philips HPK 125 W medium pressure mercury-quartz lamp placed inside the inner jacket for about 10–12 h. A solution of CuSO_4 was circulated through the outer jacket of the photochemical reactor to filter out light wavelengths below 320 nm. After photolysis, the spent resin was filtered, washed with EtOH (3 x 20 ml), MeOH (3 x 20 ml) and DCM (3 x 20 ml). The combined filtrate and washing were evaporated on a rotary evaporator under pressure. The residue was collected and purified by column chromatography on a sephadex G-10 column using 5% acetic acid in water as the eluent.

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1. Abbreviations used for amino acids follow the IUPAC-IUB Commission of Biochemical Nomenclature in *Eur. J. Biochem.* **1984**, 138, 9-37; *J. Biol. Chem.* **1989**, 264, 663-673. The following additional abbreviations are used : DCM, dichloromethane; DMF, dimethylformamide; RP-FPLC, reverse phase-fast protein liquid chromatography; HOBt, 1-hydroxybenzotriazole; IR, infrared; MeCN, acetonitrile; MeOH, methanol; NMP, N-methyl-2-pyrrolidinone; EtOH, ethanol; NMR, nuclear magnetic resonance; CP-MAS, cross polarised-magic angle spin; TFA, trifluoroacetic acid; THF, tetrahydrofuran; KBr, potassium bromide, NBS, N-bromosuccinimide, Boc, t-butyloxycarbonyl; DIEA, diisopropylethylamine
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