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Synthesis of substance P partial sequential peptides on a photolytically removable 4-aminomethyl-3-nitrobenzoylaminomethyl polystyrene support

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Abstract. Sequential peptides corresponding to substance P (6–11) were synthesised on a photocleavable 4-aminomethyl-3-nitrobenzoylaminomethyl polystyrene resin. This photoremovable polymeric support, useful for the synthesis of C–terminal peptide amides, was prepared from 4-bromomethyl-3-nitrobenzoylaminomethyl polystyrene by treatment with potassium phthalimide followed by hydrazinolysis. Amino acid residues were incorporated onto this support following the standard solid-phase methodology of peptide synthesis. The protected peptide amides were cleaved from the resin by photolysis with light of wavelength 350 nm. The resin was used for the synthesis of peptides including the partial sequence corresponding to substance P (9–11) which was further subjected to stepwise synthesis to prepare substance P (6–11) in an overall yield of 88%. This approach combines the advantages of the photochemical deprotection of the 4-aminomethyl-3-nitro carboxyl protecting group, the polymer-supported peptide synthesis and the polymer-analogous functionalisation procedure to obtain the C–terminal peptide amide under mild conditions.

Keywords. Peptide synthesis; substance P; solid-phase synthesis; photoremovable polymeric supports.

1. Introduction

Peptides play an imporant role as structural and functional elements in biochemistry and pharmacology. Peptides and their structural analogues are generally difficult to obtain from natural sources. Several biologically active peptides are in the C–terminal peptide amide form. The synthesis of C–terminal peptide amides is important for structure-activity (Bodanszky and Sheehan 1966; Takashima *et al* 1968; Schally *et al* 1971; River *et al* 1972) correlations and conformational studies (Maser *et al* 1984). In the conventional method of peptide synthesis, the C–terminal peptide amides are generally prepared by converting the N–protected C–terminal amino acid to the amide by treating with ammonia (Chambers and Carpenter 1955; Izeboud and Beyerman 1978) and the stepwise incorporation of amino acid residues toward the amino end. Peptide amides are also obtained by the ammonolysis of peptide benzyl or methyl esters.

In the solid-phase polymer-supported method of Merrifield (1963, 1986) these C–terminal peptide amides are usually synthesised by ammonolysis or transesterific-

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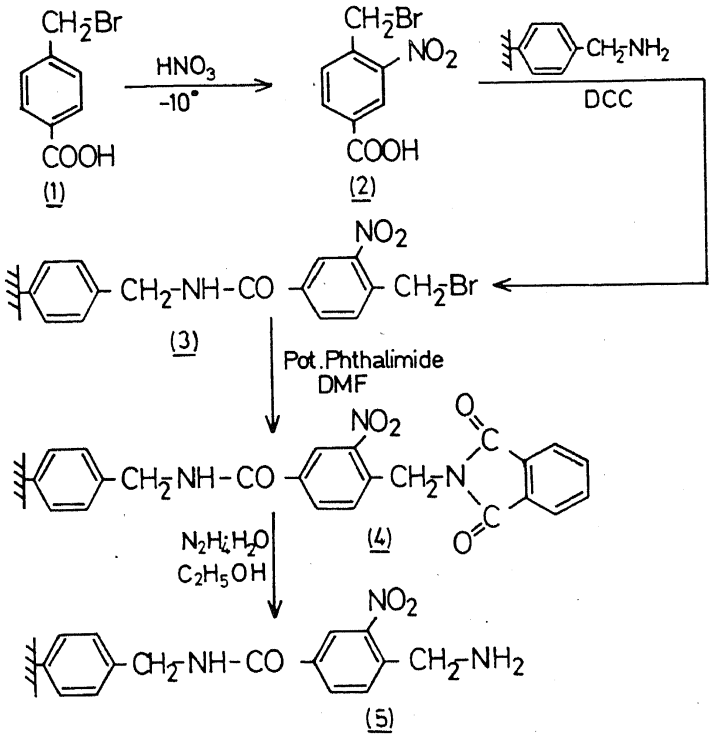
ation of the peptidyl polymer (Beyerman *et al* 1968; Manning 1968). The introduction of anchoring groups between the solid support and the growing peptide chain is a convenient strategy solving or minimising various limitations of solid-phase method. The anchoring linkage should be stable under the conditions of the various reactions which are repeated. However, the anchoring linkage should be cleavable finally by mild and selective reaction which does not affect the finished peptide. The compromise between a very stable attachment which can withstand all the synthetic procedures in peptide synthesis and a mild cleavage of the attachment after the synthesis is a problem often encountered in the solid-phase method (Barany and Merrifield 1979). The use of different types of anchoring groups with varying stability between the polymer support and the first amino acid facilitates the attachment of the first residue and the final cleavage of the peptide (Pillai and Mutter 1982). Polymer supports containing an amino group which facilitate the coupling of the C–terminal amino acid of the desired peptide through an amide linkage and the final release of the peptide in C–terminal amide form by acidolysis is one synthetic approach (Orlowski *et al* 1976; Penke and Rivier 1987). However, these conditions necessitate the use of side chain protecting groups which are resistant to ammonolysis or acidolysis and therefore restrict the selection of acid-labile protecting groups. Furthermore, in the case of peptides with hindered C–terminal residues such as valine, the attachment and final cleavage are difficult. In this context, the principles of photolytic deprotection of functional groups have been exploited to provide mildly and selectively cleavable anchoring linkages between the first amino acid and the polymer support (Bellof and Mutter 1984; Ajayaghosh and Pillai 1987, 1988; Pillai 1987). Photochemical cleavage from the polymer support permits the preparation of α-amino and side chain-protected peptides which are useful in segment condensation (Tjoeng *et al* 1978; Giralt *et al* 1982, 1986; Albericio *et al* 1987). The amino group incorporated onto the supports facilitates the coupling of the first amino acid through its carboxyl group and serves as a *latent reagent function* for the conversion of the carboxyl group into the corresponding amide.

The present approach combines the advantage of the photochemical deprotection of the 4-aminomethyl-3-nitro carboxyl protecting group, the solid-phase polymer-supported peptide synthesis and the polymer analogous functionalization procedures to obtain the C–terminal peptide amides under mild conditions (Rich and Gurwara 1975a). The synthesis of protected tripeptides Boc–Pro–leu–Gly–NH_2 and Boc–Gly–Leu–Met–NH_2 are carried out on 4-aminomethyl-3-nitrobenzoylaminomethyl polystyrene prepared from the already reported 4-bromomethyl-3-nitrobenzoylaminomethyl polystyrene resin (Rich and Gurwara 1975b).

2. Results and discussion

2.1 *Synthesis of 4-aminomethyl-3-nitrobenzoylaminomethyl polystyrene* (5)

The amino group present in this new resin facilitates the attachment of the first amino acid through an amide bond and the final cleavage of the peptide from the resin by photolysis. The synthesis of this polymer support is shown in scheme 1. 4-Bromomethylbenzoic acid (1) was nitrated with fuming nitric acid below $-10°C$ to get 4-bromomethyl-3-nitrobenzoic acid (2). Treatment of aminomethyl polystyrene with the nitro acid (2) in presence of dicyclohexylcarbodiimide in dimethylformamide



Scheme 1. Preparation of 4-aminomethyl-3-nitrobenzoylaminomethyl polystyrene.

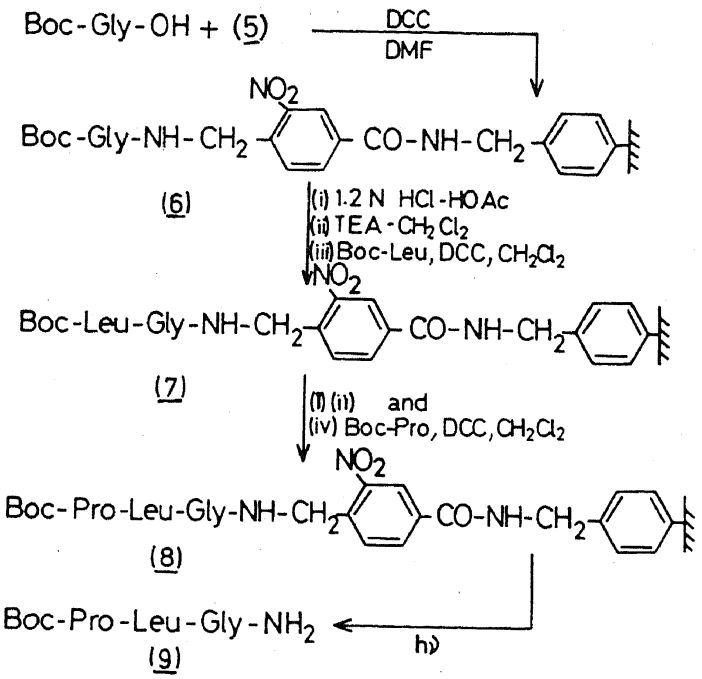
followed by acetylation of the residual amino groups by acetic anhydride and triethylamine gave 4-bromomethyl-3-nitro-benzoylaminomethyl polystyrene (3) (Rich and Gurwara 1975b; Barany and Albericio 1985). The product contained 0·49 m mol of bromine/g of resin and no detectable free amino groups. This resin was then converted to the phthalimide resin (4) which on treatment with potassium phthalimide in DMF (Mitchel and Merrifield 1976) afforded 4-aminomethyl-3-nitrobenzoyl-aminomethyl polystyrene (5) which contained 0·4 m mol of NH_2/g as shown by the picric acid method (Gisin 1972). The resin swells in chloroform and other solvents used in solid-phase synthesis to the same extent as the original Merrifield resin.

2.2 *Synthesis of peptide on 4-aminomethyl-3-nitrobenzoylaminomethyl polystyrene* (5)

N–protected amino acids can be attached to the amino resin (5) by dicyclohexyl-carbodiimide-mediated coupling. The α-Boc group can be deprotected using 50% TFA/CH_2Cl_2, 4 N HCl/dioxane or 1·2 N HCl/AcOH. The stepwise coupling was done following the general method of Merrifield as modified by Hancock and coworkers to include a second coupling step for each amino acid in a second solvent system (Hancock *et al* 1973). The coupling step was monitored using the ninhydrin method (Kaiser *et al* 1970). The protected tripeptides were removed from the resin by photolysis in a water-cooled immersion-type photochemical reactor using a Philips HPK 125W mercury–quartz lamp. The peptide was finally purified by chromatography.

2.3 *Synthesis of Boc–Pro–Leu–Gly–NH_2* (9)

The synthetic sequence is depicted in scheme 2. The C–terminal glycine residue was attached to the resin (5) using dicyclohexylcarbodiimide in DMF. The amino acid



Scheme 2. Solid-phase synthesis of Boc–Pro–Leu–Gly–NH_2 on a photocleavable support.

analysis gave 0·38 m mol Boc–Gly on the resin. The Boc group was deprotected with 1·2 N HCl-AcOH and neutralized with 5% solution of triethylamine in CH_2Cl_2. The next two amino acids were incorporated by the symmetrical anhydride method, first in CH_2Cl_2 and then in DMF media, to get the resin (8). Finally the tripeptide, Boc–Pro–Leu–Gly–NH_2 (9) was obtained by photolysis of the peptidyl resin (8).

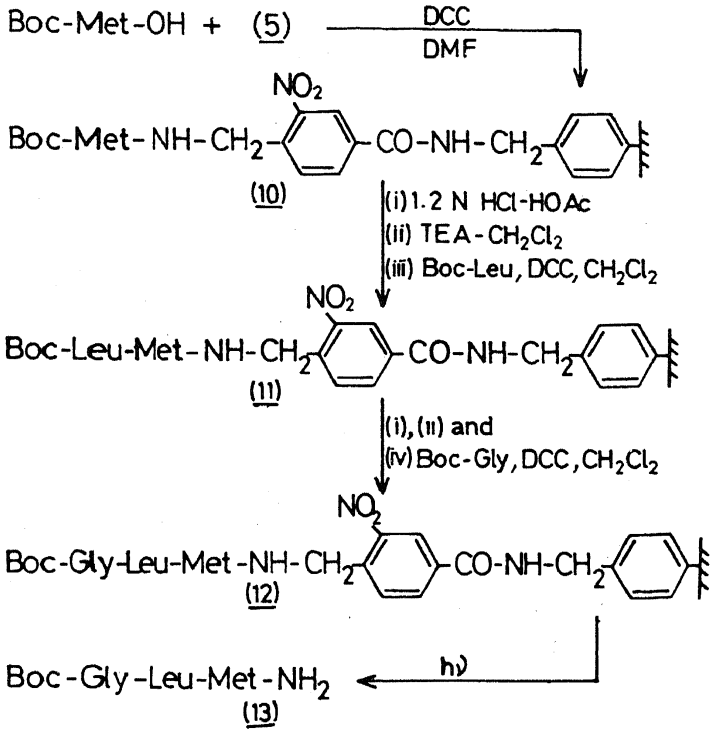
2.4 *Synthesis of Boc–Gly–Leu–Met–NH_2 (13)*

Boc–Met–OH was attached on the 4-aminomethyl-3-nitrobenzoylaminomethyl polystyrene (5) in presence of dicyclohexylcarbodiimide as in the previous case. The coupling of the next two amino acids was carried out by the symmetrical anhydride method. The tripeptide, Boc–Gly–Leu–Met–NH_2 (13) was obtained by the photolysis of the resin (12) in methanol. The steps involved are depicted in scheme 3.

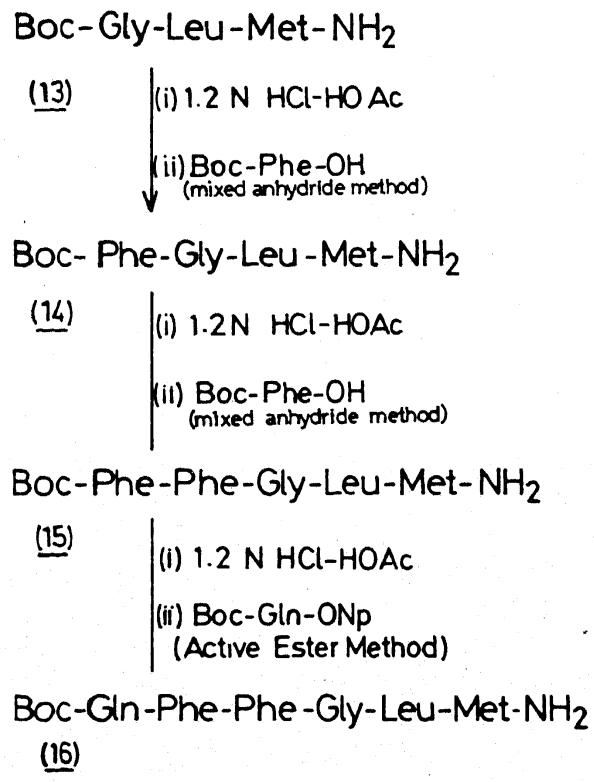
2.5 *Synthesis of substance P (6–11): Boc–Gln–Phe–Gly–Leu–Met–NH_2 (12)*

The synthesis of the substance P(6–11), Boc–Gln–Phe–Phe–Gly–Leu–Met–NH_2, starting from the tripeptide Boc–Gly–Leu–Met–NH_2 (13) is shown in scheme 4.

Boc–Gly–Leu–Met–NH_2 (13), obtained by the solid-phase method was deblocked with 1·2 N HCl–HOAc and the next Boc–amino acid, Boc–Phe–OH was coupled by the mixed anhydride method. The tetrapeptide Boc–Phe–Gly–Leu–Met–NH_2 (14) was characterised by amino acid analysis. The purity was checked on tlc. The Boc group was deprotected in the usual manner and the coupling with Boc–Phe–OH was repeated once again to get the pentapeptide Boc–Phe–Phe–Gly–Leu–Met–NH_2 (15). Finally Boc–Gln–OH was coupled to the pentapeptide, after deprotection, by the active ester method using Boc–Gln–ONp to get the hexapeptide, Boc–Gln–Phe–



Scheme 3. Synthesis of Boc–Gly–Leu–Met–NH_2.



Scheme 4. Solid-phase synthesis of substance P(6–11).

Phe–Gly–Leu–Met–NH_2 (16), the partial sequence of substance P(6–11). Characteristics of the peptide 14, 15 and 16 are given in table 1 and results of amino acid analysis are given in table 2.

Table 1. Synthetic partial sequences of substance P.

Peptide	Yield (%)	m.p. (°C)	Thin-layer chromatography R_f^a	R_f^b	R_f^c	Elemental analysis (%) Found (calcd)* C	H	N
Boc–Phe–Gly–Leu–Met–NH_2 (14)	70	176–178	0·30	0·91	0·85	56·8 (57·2)	7·6 (7·6)	12·1 (12·4)
						($C_{27}H_{43}N_5O_6S$)		
Boc–Phe–Phe–Gly–Leu–Met–NH_2 (15)	75	226–230	0·61	0·99	0·92	60·0 (60·6)	7·1 (7·3)	11·6 (11·7)
						($C_{36}H_{52}N_6O_7S$)		
Boc–Gln–Phe–Phe–Gly–Leu–Met–NH_2 (16)	88	212–216	—	0·95	0·91	58·0 (58·5)	6·8 (7·1)	13·1 (13·3)
						($C_{41}H_{60}N_8O_9S$)		

[a]Chloroform: methanol (9:1); [b]butanol: acetic acid: water (4:1:1); [c]butanol: acetic acid: pyridine: Water (4:1:1:2); *the molecular formula is given in parentheses.

Table 2. Amino acid analysis of the synthetic partial sequences of substance P.

Residue	Tripeptide (13)	Residue	Tetrapeptide (14)	Residue	Pentapeptide (15)	Residue	Hexapeptide (16)
Gly	1·00 (1)	Phe	1·00 (1)	Phe	1·97 (2)	Glu (Gln)	1·10 (1)
Leu	1·05 (1)	Gly	0·96 (1)	Gly	0·98 (1)	Phe	2·04 (2)
Met	1·15 (1)	Leu	1·08 (1)	Leu	1·00 (1)	Gly	0·95 (1)
		Met	1·25 (1)	Met	1·10 (1)	Leu	1·00 (1)
						Met	1·05 (1)

3. Experimental

Melting points were determined on a hot-stage melting point apparatus and are uncorrected. Solvents were reagent grade and were distilled and purified by literature procedures. Microanalyses were performed at the Regional Sophisticated Instrumentation Centre. Central Drug Research Institute, Lucknow. IR spectra were recorded on a Pye-Unicam SP–300 spectrophotometer. Column chromatography was done on silica gel or on Sephadex LH–20. Amino acid analyses were obtained on a Biotronik amino acid analyser (chromatography system LC 6000 E) after hydrolysis of the samples with 6 N HCl in sealed evacuated tubes at 110°C for 24 hours. The irradiations were carried out with a Philips HPK 125 W mercury lamp in an immersion type water-cooled photochemical reactor. Thin-layer chromatography was carried out on precoated silica gel plates in three different solvent systems, R_f^a in chloroform: methanol (9:1), R_f^b in butanol:acetic acid:water (4:1:1) and R_f^c in butanol:acetic acid:pyridine:water (4:1:1:2). ^{1}H NMR measurements were carried out on a Perkin–Elmer spectrometer using tetramethylsilane as the internal standard.

3.1 *Synthesis of 4-bromomethyl-3-nitrobenzoic Acid (2)*

4-(Bromomethyl)benzoic acid (1, 5·4 g, 25 m mol) was added in portions over 30 min to fuming nitric acid 100 ml) at − 10°C. The suspension was stirred at − 10°C for an additional 2 h, by which time everything was in solution. The reaction mixture was poured onto crushed ice. The product was collected by filtration and washed with ice-cold water (4 × 50 ml) until washings were neutral. The dried product was crystallized from methylenechloride–petroleum ether. Yield: 5·2 g (80%). m.p.: 122–124°C (lit. m.p. 125–126°C). ^{1}H NMR ($CDCl_3$–DMSO–d_6) δ: ppm 8·6–7·9 (aromatic), 4·7 (Ar CH_2), IR (KBr): 2800–2300, 1680, 1600 (COOH), 1610 (aromatic), 1520, 1350 cm^{-1} (NO_2).

3.2 *Preparation of 4-bromomethyl-3-nitrobenzoylaminomethyl resin (3)*

To a solution of 4-bromomethyl-3-nitrobenzoic acid (2, 2·08 g, 8 m mol) in CH_2Cl_2 (10 ml) kept stirred in an ice-bath, DCC(0·82 g, 4 m mol) was added and the stirring was continued for 30 min. The precipitated dicyclohexylurea was filtered off. Aminomethyl polystyrene (1% crosslinked) (5 g, 3·25 m mol NH_2) and CH_2Cl_2 (50 ml) were added to the filtrate and the reaction mixture was shaken for 12 h. The product was filtered, washed successively with CH_2Cl_2, DMF, CH_3OH and CH_2Cl_2 (25 ml × 4 × 2 min) and dried *in vacuo*. The resin was suspended in CH_2Cl_2 (25 ml) and acetic anhydride (2·0 g, 20 m mol) and triethylamine (2·0 g, 20 m mol) were added. The suspension was stirred at room temperature for 1 h, washed with CH_2Cl_2 and CH_3OH (20 ml × 3 × 1 min) and dried *in vacuo* to give the resin 3 which was found to be ninhydrin-negative. Yield: 5·6 g; IR (KBr): 1560 and 1350 cm^{-1}(NO_2). The resin was found to contain 3·76% Br corresponding to a capacity of 0·47 m equiv of Br/g.

3.3 *Preparation of 4-phthalimidomethyl-3-nitrobenzoylaminomethyl resin (4)*

A suspension of 4-bromomethyl-3-nitrobenzoylaminomethyl resin (3, 5·0 g, 2·35 m equiv Br) and potassium phthalimide (5·5 g, 30 m mol) in DMF (60 ml) was heated at 100°C under a nitrogen atmosphere for 8 h. The resin was filtered and washed successively with hot DMF, DMF–H_2O (1:1, v/v), H_2O, H_2O-dioxane (1:1, v/v), dioxane and methanol (30 ml × 4 × 2 min) and dried *in vacuo*. Yield: 5·30 g; IR (KBr): 3200 (amide-A); 1660 (amide-I); 1780 and 1720 (C=O); 1530 and 1370 cm^{-1} (NO_2).

3.4 *Preparation of 4-aminomethyl-3-nitrobenzoylaminomethyl resin (5)*

4-Phthalimidomethyl-3-nitrobenzoylaminomethyl resin (4, 5·0 g) and hydrazine hydrate (6 ml) in absolute ethanol (60 ml) were heated under reflux for 6 h. After filtration, the residue was washed with hot ethanol, DMF, H_2O, CH_3OH and CH_2Cl_2 (30 ml × 4 × 2 min) and dried *in vacuo*. Yield: 4·8 g; IR (KBr): 3200 (amide-A); 1660 (amide-I); 1520 and 1350 cm^{-1} (NO_2). Picric acid method (Gisin 1972) of estimation of amino groups indicated a capacity of 0·38 m mol NH_2/g.

3.5 *Incorporation of Boc–Gly on 4-aminomethyl-3-nitrobenzoylaminomethyl resin: preparation of resin (6)*

To a solution of Boc–Gly–OH (0·87 g, 5 m mol) in CH_2Cl_2 (10 ml) kept at 0°C, an ice-cold solution of DCC (0·515 g, 2·5 m mol) in CH_2Cl_2 (70 ml) was added. The

mixture was stirred at 0°C for 1 h. This solution was filtered directly into a flask containing a suspension of 4-aminomethyl-3-nitrobenzoylaminomethyl resin (5, 2·0 g, 0·76 m mol NH_2 groups) in CH_2Cl_2 (20 ml) and the reaction mixture was stirred at room temperature overnight. The residue was collected by filtration and washed successively with CH_2Cl_2, MeOH and CH_2Cl_2 (4 × 20 ml × 4 times) and dried under vacuum. The coupling was repeated with Boc–Gly–OH (0·35 g, 2 m mol) in DMF (10 ml) and DCC (0·21 g, 1 m mol) in 10 ml of DMF. Yield: 2·094 g. The resin contained 0·37 m mol of Boc–Gly/g.

3.6 *Deprotection of the Boc group from the peptidyl polymer: general procedure*

The Boc resin (1·0 g) was suspended in 1·2 N HCl–AcOH (30 ml) and stirred at room temperature for 1 h. The resin was filtered, washed successively with dioxane, water, dioxane, methanol and dried. The protonated resin was neutralised by stirring in a 5% solution of triethylamine in CH_2Cl_2 (20 ml) for 15 min. The product resin was filtered, washed with CH_2Cl_2, DMF, MeOH, CH_2Cl_2 and dried under vacuum.

3.7 *Coupling of Boc amino acids on the resins 6 and 10: general procedure*

The support resin was subjected to the following set of operations (for 1·0 g of the resin):

(i) Washing with CH_2Cl_2 (15 ml × 2 min, thrice);
(ii) Boc–deblocking with 1·2 N HCl–AcOH as given in the general procedure;
(iii) Coupling with preformed symmetrical anhydride of Boc–amino acids (2 m mol) in CH_2Cl_2 solution (1·5 h);
(iv) Filtration followed by washing successively with CH_2Cl_2, DMF, MeOH and EtOH (20 ml × 2 min, thrice);
(v) Repetition of steps (ii) and (iii);
(vi) Test for quantitative coupling;
(vii) Repetition of steps (ii), (iii), (iv) and (v).

3.8 *Boc–Pro–Leu–Gly–Resin (8)*

The Boc–Gly resin (6, 1·5 g) was deblocked as described in the general procedure and the coupling of Boc–Leu and Boc-Pro was as per the procedures described under 3·7, which afforded the peptidyl resin 8 (1·52 g).

3.9 *Cleavage of the peptide from the resin 8 by photolysis*

A suspension of the resin 8 in MeOH (150 ml) was irradiated for 24 h using light of wavelength 350 nm from a mercury lamp in an immersion-type water-cooled photochemical reactor under a nitrogen atmosphere. The suspension was kept stirred during irradiation. Upon completion of the photolysis, the resin was filtered and washed with MeOH (3 × 20 ml). The combined filtrate and washings were evaporated *in vacuo*. The crude product was purified by chromatography over silica gel using $CHCl_3$–MeOH (9:1, v/v); yield: 30 mg R_f^a: 0·41; R_f^b: 0·95; R_f^c: 0·90; m.p.; 125–127°C. Amino acid analysis: Pro, 1·1(1); Leu, 1·0(1); Gly, 0·98(1). Analysis found: C, 55·7; H, 8·0; N, 14·5%; calculated for $C_{18}H_{32}N_4O_5$: C, 56·2; H, 8·3; N, 14·8%.

3.10 *Incorporation of Boc–Met on 4-aminomethyl-3-nitrobenzoylaminomethyl resin: Preparation of resin 10*

To a solution of Boc–Met–OH (1·25 g, 5 m mol) in CH_2Cl_2–THF (2:1, v/v, 15 ml) kept at 0°C was added an ice-cold solution of DCC (0·515 g, 2·5 m mol) in CH_2Cl_2 (10 ml). The mixture was stirred at 0°C for 1 h. This solution was filtered directly into a flask containing a suspension of the 4-aminomethyl-3-nitrobenzoylaminomethyl resin (3·0 g, 1·14 m mol NH_2) in DMF (30 ml) and the reaction mixture was shaken at room temperature overnight. The residue was filtered, washed successively with DMF, MeOH and CH_2Cl_2 and dried under vacuum. The coupling was repeated with the anhydride of Boc–Met–OH (2·5 m mol); Yield: 3·16 g; capacity: 0·38 m mol of Boc–Met–OH/g.

3.11 *Boc–Gly–Leu–Met–Resin (12)*

Boc–Met resin (10, 3·0 g) was deblocked and the coupling of Boc–Leu and Boc–Gly was carried out as described in the general procedure. Yield: 3·035 g.

3.12 *Photolysis of resin 12: Preparation of Boc–Gly–Leu–Met–NH_2 (13)*

A suspension of the resin (2·5 g) in anhydrous MeOH (150 ml) was irradiated in a photochemical reactor for 24 h using a 125 W mercury lamp. The dissolved air was previously removed from the suspension by passing nitrogen for 2 h. Upon completion of the photolysis, the reaction mixture was filtered and the resin was washed with MeOH (3 × 20 ml). The filtrate and washings were evaporated under vacuum and the crude product was purified chromatographically over silica gel using a $CHCl_3$–MeOH mixture (9:1). Yield: 160 mg, m.p. 138–140°C. R_f($CHCl_3$–MeOH, (9:1)): 0·35; R_f(BuOH–AcOH–H_2O, 4:1:1): 0·93; R_f(BuOH–AcOH–Py–H_2O, 4:1:1:2): 0·78. Amino acid analysis: Gly, 0·98(1); Leu, 1·00 (1); Met, 1·15 (1). Analysis found: C, 51·3; H, 8·0; N, 13·3%; calculated for $C_{18}H_{34}N_4O_5S$: C, 51·6; H, 8·1; N, 13·4%.

3.13 *Boc–Phe–Gly–Leu–Met–NH_2 (14)*

To a suspension of Boc–Phe–OH (0·11 g, 0·4 m mol) in dry THF (10 ml) and DMF (10 ml), kept in an ice-bath, N-methylmorpholine (0·045 ml, 0·4 m mol) followed by isobutylchloroformate (0·055 ml, 0·4 m mol) was added with stirring. After 15 minutes a solution of Gly–Leu–Met–NH_2·HCl (0·14 g, 0·4 m mol) (obtained by the Boc-deprotection of Boc–Gly–Leu–Met–NH_2 (13) with 1·2 N HCl–AcOH) was added to the reaction mixture and neutralised with Et_3N to pH 7. The reaction mixture was stirred at 0°C for 2 h and at room temperature for 18 h. The progress of the reaction was checked by tlc. The solvent was removed by rotary evaporation and the residue was taken in EtOAc, washed with $NaHCO_3$ (10%), citric acid (10%), and standard NaCl solutions and finally with water, and then dried over anhydrous Na_2SO_4.The solvent was removed and the tetrapeptide was obtained as a white powder. Yield:158 mg (analytical details: tables 1 and 2).

3.14 *Boc–Phe–Phe–Gly–Leu–Met–NH_2 (15)*

Boc–Phe–Gly–Leu–Met–NH_2 (14) (140 mg) was treated with 1·2 N HCl-AcOH (10 ml) at room temperature for 45 min. The completion of the deprotection was

checked by tlc. The excess reagent was removed under vacuum and the hydrochloride of the tetrapeptide was precipitated by the addtion of dry ether. The product was collected by filtration and dried. Boc–Phe–OH (0·067 g, 0·25 m mol) was dissolved in DMF (2 ml) and to this N-methylmorpholine (0·027 ml, 0·25 m mol) followed by isobutylchloroformate (0·032 ml, 0·25 m mol) was added while stirring in an ice-salt bath. After 15 min, a solution of Phe–Gly–Leu–Met–$NH_2 \cdot HCl$ (0·1004 g, 0·2 m mol) in DMF (4 ml) was added, after neutralising it to pH 7 by the addition of triethylamine, and the mixture stirred for 20 h. The solvent was removed and the residue suspended in EtOAc and filtered. The residue was washed with $NaHCO_3$ (10%), citric acid (10%), and saturated NaCl solutions and finally with water, and then dried over P_2O_5 in a vacuum desiccator. Yield: 98 mg (analytical details: tables 1 and 2).

3.15 *Boc–Gln–Phe–Phe–Gly–Leu–Met–NH_2 (substance P(6–11))* (16)

The pentapeptide 15 from the above step (75 mg) was deblocked by treating with 1·2 N HCl–AcOH (10 ml) for 30 min, evaporated under vacuum and the residue triturated with ether and dried. The deprotected pentapeptide (0·065 g, 1 m mol) was treated with Boc–Gln–ONp (0·050 g, 0·13 m mol), DMF (6 ml) and stirred for 72 h at room temperature. The solvent was removed by rotary evaporation and the residue was suspended in EtOAc and filtered. The residue was washed $NaHCO_3$, citric acid and NaCl solutions, and finally with water and dried. Yield of substance P (6–11): 70 mg. The analytical details, amino acid analysis and the R_f values of the peptide in different solvent systems are given in tables 1 and 2.

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