Pergamon

TETRAHEDRON

Tetrahedron 57 (2001) 3151–3158

Photocleavable 2-nitrobenzylamino anchored polystyrene-butanediol dimethacrylate supports for the synthesis of protected peptides, peptide amides and peptide *N*-alkyl amides

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Received 10 October 2000; revised 12 January 2001; accepted 1 February 2001

Abstract—2-Nitrobenzylamino-type anchoring groups were incorporated on 2% butanediol dimethacrylate cross-linked polystyrene support. The resultant photolabile polymeric supports were used for the synthesis of fully protected peptides and their final cleavage as C-terminal peptide acids, peptide amides and peptide *N*-alkyl amides by photolysis under mild neutral conditions. The photosensitive chromophore has a dual function of serving as an anchoring linkage between the support and the growing peptide chain and as a latent reagent for the conversion of a C-terminal carboxyl group into the modified form during photolytic cleavage. The C-terminal modified peptides were obtained by irradiating the peptidyl resin in a TFE/DCM mixture at 350 nm. The efficacy of the new resin is illustrated by synthesizing the protected derivatives of the peptides in very high yield and purity. The identity of the peptides was checked by amino acid analysis, TLC and MALDI TOF MS. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Synthetic peptides and their C-terminal amides or *N*-alkyl amides are powerful tools in modern biological research. Since the C-terminal peptide acid function ionizes at physiological pH, peptide segments of proteins prepared as their amides or *N*-alkyl amides for most of the immunological studies[1]. Stabilization of a particular conformation at physiological pH also requires the introduction of C-terminal amides or *N*-alkyl amides into a peptide.[2] Final purity of the peptide cleaved from the support depends upon the cleavage conditions. Since a complex molecule like a peptide contain several types of side chains, chiral centers, acid and base sensitive amino acids, their cleavage from the support using acid or base can result in several side reactions.[3] One can achieve a milder method for the solid-phase synthesis of peptides or their C-terminal derivatives by introducing a photosensitive attachment (anchoring) of the C-terminal residue to the polymeric support. This technique can help the final cleavage of the peptide or its C-terminal derivative from the polymeric support under neutral conditions, for example by irradiating the peptidyl resin in TFE/DCM solvent mixture at room temperature with 350 nm light without affecting the side chain protection or the N^a-protecting groups.

1,4-Butanediol dimethacrylate cross-linked polystyrene (PS–BDODMA) was recently introduced as a new polymer support for the solid-phase synthesis of peptides.[4,5] The PS–BDODMA supports are stable under all peptide synthetic conditions. The optimum hydrophilic–hydrophobic balance of the resin results in high swelling in different nonpolar and polar solvents, and therefore the compass of chemistry that could be conducted on the support makes it an efficient support for different organic reactions. The ease of preparation, functionalization and work-up procedure are the advantages of this resin over prevailing polymer supports. The physicochemical attunement of the macromolecular support and the growing peptide chain can help to synthesize peptides of very high purity and homogeneity. The improved coupling rate during peptide bond formation, high subtlety in monitoring the coupling reactions, and high yield and purity of the product peptides are the additional advantages of using the PS–BDODMA support. 1% PS–BDODMA resin, however, has low mechanical stability when used in synthesis; the resin become powdered usually when the number of residues exceeds eight. Resins having more than 3% cross-linking density are comparatively rigid and show low swelling character in solvents used for the synthesis. A 2% PS–BDODMA resin showed comparable mechanical strength and high solvent absorption to that of commercially available Merrifield resin. The

Keywords: polymer support; photosensitive anchoring group; SPPS; peptide amides and *N*-alkyl amides.
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PII: S0040-4020(01)00173-9

higher swelling properties of the support can help the easy interaction of the reactants with reactive centers in the polymer matrix. The macromolecular environment of the polymer seems to be highly compatible with the growing peptide chain as evident from the purity of the synthesized peptide.

This paper reports the incorporation of photolabile 2-nitrobenzyl anchoring groups into PS–BDODMA polymer support and its use in the synthesis of various C-terminal modified peptide derivatives. All the peptides and their derivatives obtained from the resin are in fully protected form and therefore can be used in segment condensation for the synthesis of long chain peptides.

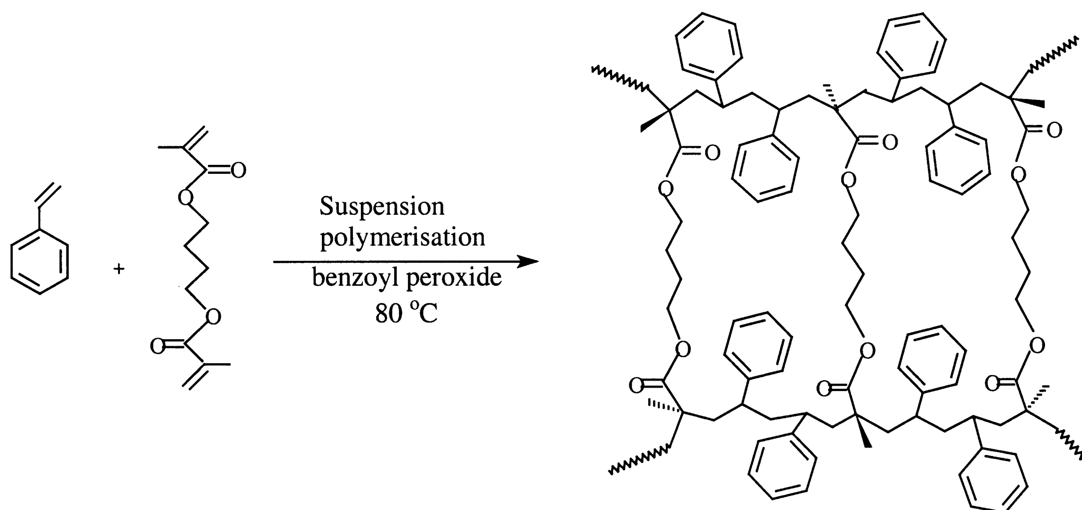
2. Results and discussion

Butanediol dimethacrylate cross-linked polystyrene was prepared by the aqueous suspension polymerization of butanediol dimethacrylate and styrene at 80°C using dibenzoyl peroxide as radical initiator (Scheme 1). Polymer synthesis was carried out using toluene as the diluent and 1% polyvinyl alcohol (M_r~75,000 Da) as the suspension stabilizer. The polymerization reaction was allowed to take place in an inert N_2 atmosphere. The polymer obtained is over 90% yield and as spherical uniform beads with size 100–200 μm.[4,5] Reproducible results were obtained by adjusting the various parameters like aqueous-organic phase ratio, amount of stabilizer, geometry and shape of the polymerization flask and stirring rate. The polymer was characterized by IR and ^{13}C CP-MAS-NMR spectroscopy.

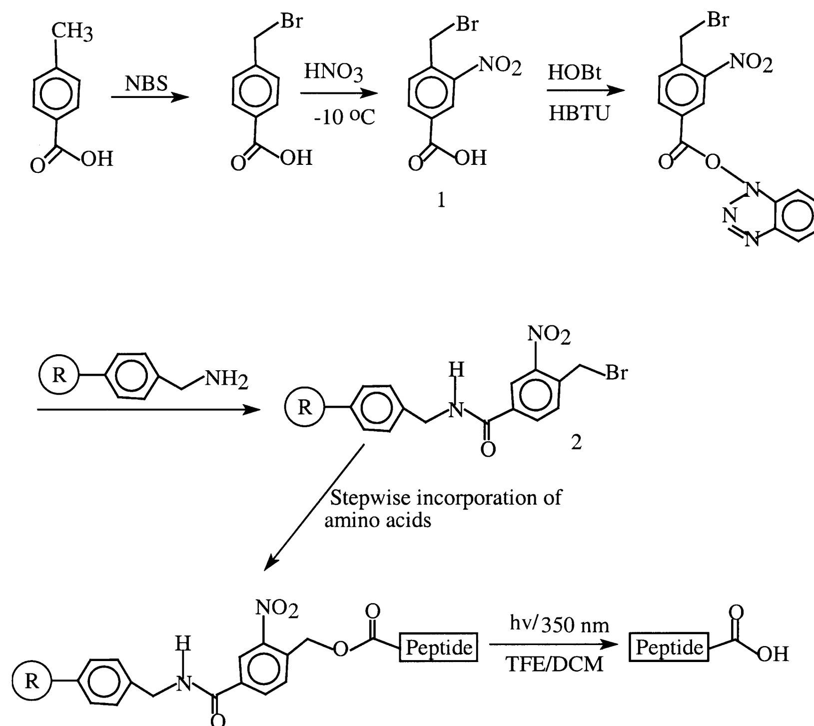
The range of chemistry performed on a macroporous resin is directly linked to its solvent absorption since the rate of diffusion of solution phase reagent to polymer bound functional site totally depends upon the swelling characteristics of the resin in various solvents. The solvent absorption of 2% PS–BDODMA resins was determined by centrifuge method.[4] The results showed that the 2% PS–BDODMA resin exhibits higher swelling in both polar and nonpolar solvents compared to 2% PS–DVB resin. The solvent absorption by unit weight of dry 2% PS–BDODMA and 2% PS–DVB (in parentheses) in different solvents are, acetone, 2.6 (2.5); MeOH, 1.1 (1.8); DCM, 8.5 (4.3); THF, 7.7 (5.2); toluene, 6.2 (4.7); NMP, 8.6 (5.2); chloroform, 8.5 (4.3); DMF, 7.2 (2.8); dioxane, 6.1 (3.5) and pyridine, 6.7 (4.2). The swelling behavior of the 2% PS–BDODMA resin in solvents DCM, NMP and DMF (commonly used solvents in SPPS) is almost double that of PS–DVB of the same cross-linking density. As a consequence mechanical resistance to the reagents by the cross-linked polymer network decreases and allows the reagents to reach the reaction sites easily, enhancing the reaction rate.

The chemical stability of the resin in various solvents and reagents is another factor that determines the efficiency of the polymer support in peptide synthesis. 2% PS–BDODMA resin is found to be highly stable even after vigorous functionalization conditions. IR spectral studies revealed that the multiple ester bonds present in the PS–BDODMA resin are highly stable to nucleophilic cleavage by strong acid like TFA and bases like piperidine, 2 M aqueous NaOH, 2 M NH_2OH in aqueous MeOH and liquor ammonia even after 48 h incubation in the respective reagents. Stability of the resin was tested again by irradiating the TFE suspended resin with 350 nm light. The IR spectrum of the resin after 48 h irradiation showed no additional peaks compared to the original IR spectrum of the resin. All these observations revealed that the cross-links are stable enough to withstand the various reaction conditions employed in solid-phase peptide synthesis.

The PS–BDODMA resin was functionalized to the aminomethyl group by using *N*-chloromethylphthalimide in the presence of anhydrous $ZnCl_2$ in THF as catalyst. The resulting phthalimidomethylated resin was converted to aminomethyl resin by hydrazinolysis with hydrazine hydrate. The reaction can be easily controlled and aminomethyl resin with the desired amino capacity could be synthesized by varying any of the parameters like the amount of *N*-chloromethylphthalimide, catalyst, temperature and duration of reaction. The degree of substitution was estimated by the picric acid titration method.[6] The IR spectrum of the resin showed characteristic absorptions at 1720 and 1480 cm^{-1}



Scheme 1. Synthesis of PS–BDODMA support.

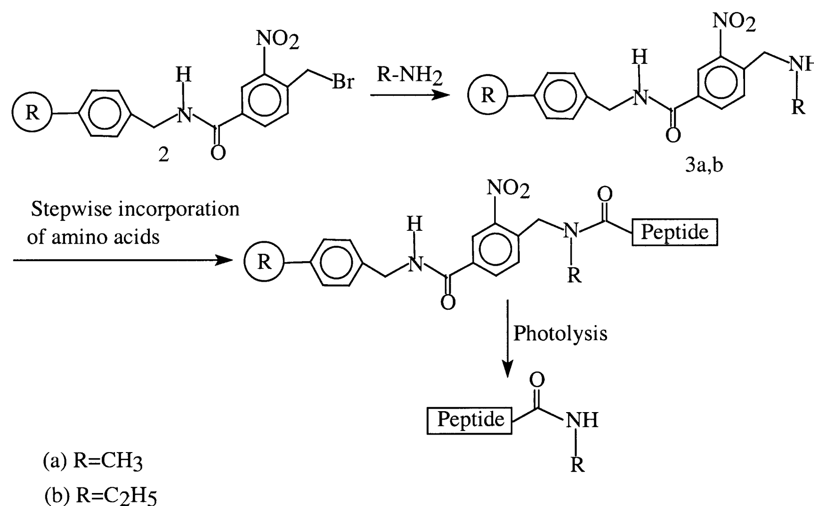


Scheme 2. Preparation and use of 4-bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin.

corresponding to the ester carbonyl group and 1520 cm^{-1} corresponding to the amino group. The ^{13}C CP-MAS NMR spectrum showed a peak at 58.93 ppm for methylene carbon of aminomethyl group and a small peak at 136.42 ppm for C-6 of the polystyrene ring.

The photolabile-anchoring group 4-bromomethyl-3-nitrobenzoicacid (**1**) was prepared by a two-step reaction from *p*-toluic acid. *p*-Toluic acid was converted to 4-bromomethyl benzoic acid by treatment with *N*-bromosuccinimide (NBS). Nitration with fuming nitric acid at -10°C of 4-bromomethylbenzoic acid yielded 4-bromomethyl-3-nitrobenzoic acid. The pre-swollen aminomethyl resin in NMP was treated with the HOBt active ester of 4-bromo- methyl-3-nitrobenzoic acid to yield the photolabile 4-bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (**2**; Scheme 2). Estimation of the bromine capacity by Volhardt's method indicates the quantitative reaction (bromine capacity= 0.64 mmol/g).[7] The resin showed IR (KBr) bands at 1340 and 1540 cm^{-1} (NO_2) and 1650 cm^{-1} (NHCO).

4-Aminomethyl-3-nitrobenzamidomethyl PS–BDODMA resin was prepared from 4-bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (**2**) by refluxing with potassium phthalimide in NMP followed by hydrazinolysis. Amino capacity estimation by the picric acid method indicates a



Scheme 3. Preparation and use of 4-*N*-alkylaminomethyl-3-nitrobenzamidomethyl PS–BDODMA resin.

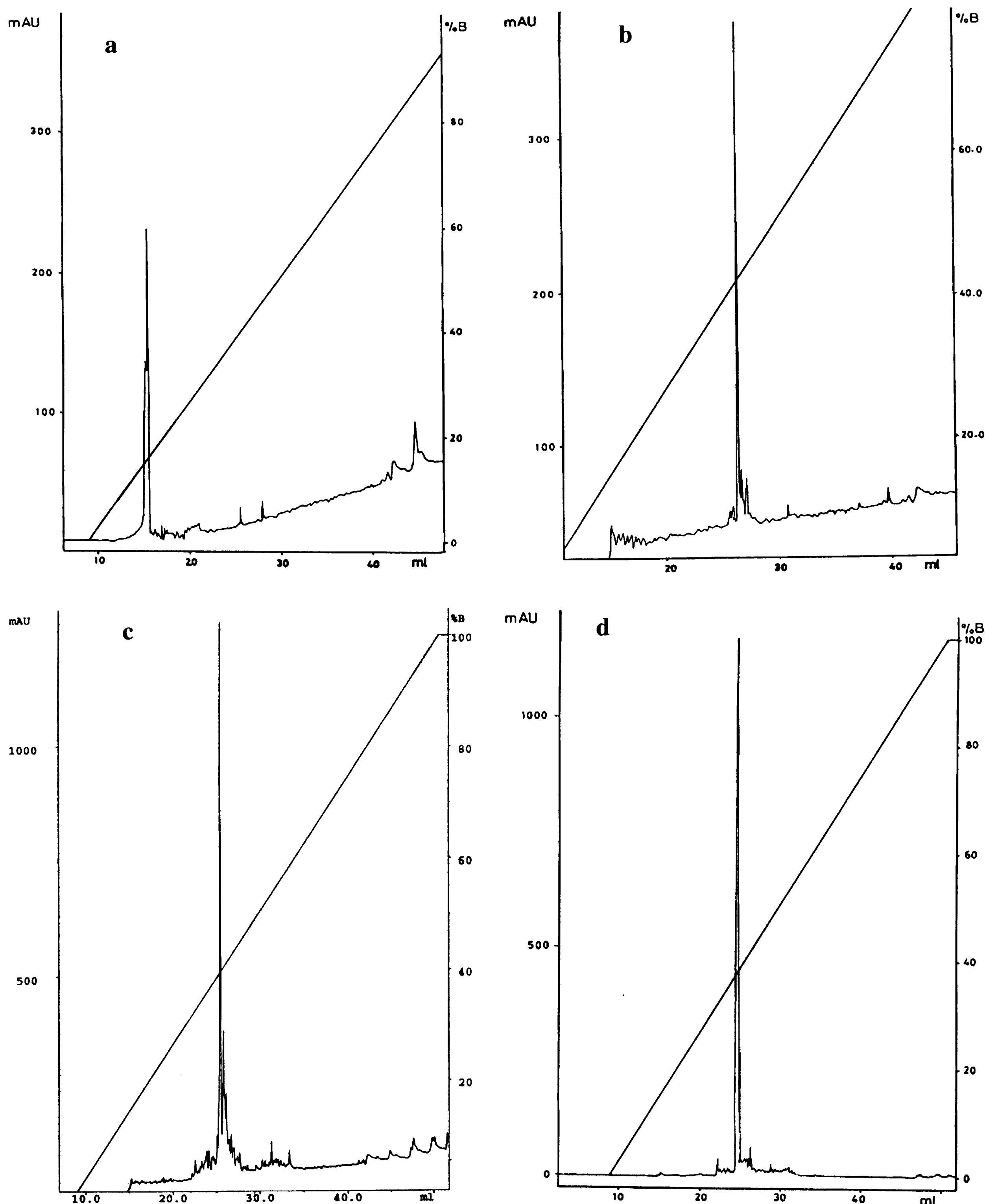


Figure 1. HPLC profile of the peptides using the buffer: (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 100 mL acetonitrile/water (4:1); flow rate: 0.5 mL/min; gradient used: 0% B in 5 min and 100% B in 50 min. (a) Boc-NH-Gly-Leu-Ala-Leu-Ala-Gly; (b) Fmoc-NH-Leu-Asp(OBu^t)-Leu-Gly-Ala-Gly; (c) Boc-NH-Ala-Gly-Leu-Ile-Gly-NH_2; (d) Fmoc-NH-Ala-Gly-Leu-Ile-Gly-NH_2.

quantitative reaction. The resin showed characteristic IR (KBr) bands at 1342 and 1548 cm^{-1} (NO_2), 1680 cm^{-1} (NHCO) and 3450 cm^{-1} (broad) (NH).

4-Bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (**2**) was suspended in DMF, dry methylamine or ethylamine gas was passed through the suspension at 0°C and the reaction mixture was shaken at room temperature for 24 h (Scheme 3). The *N*-alkylated resin (**3a**,**b**) was purified by washing and dried under vacuum. The resin showed IR (KBr) absorption at 1342, 1540 cm^{-1} (NO_2), 3430 cm^{-1} (broad) (NH) and 1640 cm^{-1} (NHCO). The amino capacity

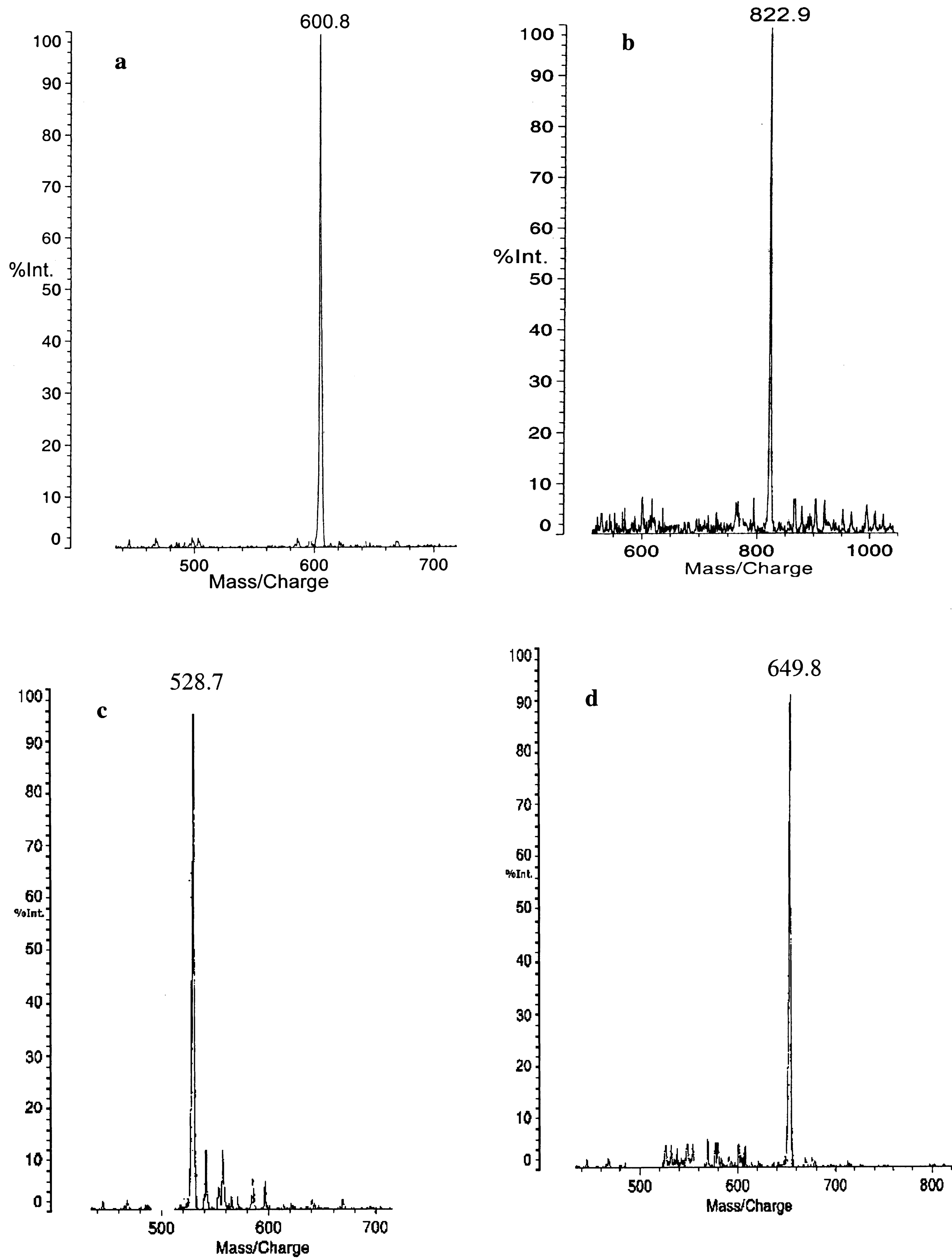


Figure 2. MALDI TOF MS of (a) Boc-NH-Gly-Leu-Ala-Leu-Ala-Gly; (b) Fmoc-NH-Leu-Asp(OBu^t)-Leu-Gly-Ala-Gly; (c) Boc-NH-Ala-Gly-Leu-Ile-Gly-NH_2; (d) Fmoc-NH-Ala-Gly-Leu-Ile-Gly-NH_2.

of the resin was estimated by the picric acid titration method. Side reactions like the formation of tertiary amine and quaternary ammonium salt of resin can be eliminated by the use of a large excess of amine.

The resin-anchoring linkage was found to be stable in both Fmoc and Boc synthetic strategies. Treatment of the Gly resin with 30% TFA in DCM and 20% piperidine in DMF for 24 h did not result in any change in its amino content indicating that the resin peptide linkage was stable enough to withstand the repeated acid and base treatment in the various solid-phase peptide synthetic conditions. Irradiation of the peptide-resin with 350 nm light in TFE/DCM mixture for 12 h released the protected derivatives of the peptides in excellent yields.

The mechanism of photolytic cleavage of nitrobenzyl and related system is well documented.[8–11] The reaction involves a light induced internal oxidation–reduction reaction of the aromatic nitro compound containing a carbon–hydrogen bond *ortho* to the nitro group.[12,13] The reaction follows the reduction of nitro group to the nitroso group when oxygen is inserted to the carbon–hydrogen bond at the 2-position resulting in the oxidation of $-CH_2-$ group to $-CHO$ group.[14]

The synthetic utilities of 4-bromomethyl-3-nitrobenzamidomethyl PS–BDODMA, 4-aminomethyl-3-nitrobenzamidomethyl PS–BDODMA, *N*-methylaminomethyl-3-nitrobenzamidomethyl PS–BDODMA and *N*-ethylaminomethyl-3-nitrobenzamidomethyl PS–BDODMA resins are illustrated by the preparation of some representative peptide acids, amides and *N*-alkyl amides. The caesium salt method was used for the attachment of the C-terminal amino acid to the 4-bromomethyl 3-nitrobenzamidomethyl PS–BDODMA resin.[7] The peptides were assembled on the resin using the pre-formed HOBt active ester of Boc or Fmoc-amino acids. For 4-aminomethyl 3-nitrobenzamidomethyl PS–BDODMA, *N*-methyl aminomethyl 3-nitrobenzamidomethyl PS–BDODMA and *N*-ethylaminomethyl 3-nitro benzamidomethyl PS–BDODMA resins, the C-terminal amino acid was attached using its pre-formed HOBt active ester. After the incorporation of amino acids in the target sequence, the peptides were cleaved from the resin by photolysis in TFE/DCM solution at 350 nm. The peptides were purified by HPLC (Fig. 1) and characterized by amino acid analysis, TLC and MALDI TOF MS (Fig. 2; Table 1).

These results illustrate the applicability of the modified PS–BDODMA resin as a photo-removable polymeric support for solid-phase synthesis of fully protected peptide acids,

Table 1. Cleavage conditions, yield and characterization of protected peptides

Peptide acid/amide/*N*-alkyl amide	Photolytic duration (h)	Yield (mg)	R_f[a]	Amino acid analysis and MALDI TOF MS
Boc-NH-Gly-Leu-Ala-Leu-Ala-Gly	12	13.8 (72%)	0.71	Gly, 2.1 (2); Leu, 1.92 (2); Ala, 2.03 (2). *m/z* 600.8 [$(M+H)^+$, 100%], $C_{27}H_{48}N_6O_9$, requires M^+ 599.7
Boc-NH-Leu-Ala-Gly-Leu-Ala-Gly	12	14.4 (75%)	0.69	Gly, 2.0 (2); Leu, 1.98 (2); Ala, 1.95 (2)
Boc-NH-Gly-Ile-Cys (Acm)-Pro	12	11.7 (68%)	0.45	Gly, 1.0 (1); Ile, 0.87 (1); Pro, 0.87 (1); Cys, 0.78 (1)
Fmoc-NH-Ile-Leu-Ala-Gly	12	13 (68%)	0.78	Ile, 0.93 (1); Leu, 1.03 (1); Ala, 1.1 (1); Gly, 1.04 (1)
Fmoc-NH-Leu-Asp (OBu^t)-Leu-Gly-Ala-Gly	12	17 (65%)	0.40	Leu, 2.1 (2); Asp, 0.91 (1); Gly, 2.12 (2); Ala, 0.98 (1). *m/z* 822.9 [$(M+H)^+$, 100%], $C_{42}H_{58}N_6O_{12}$, requires M^+ 821.2
Ile-Ala-Val-Gly-NH_2	10	11 (69%)	0.85	Ile, 0.93 (1); Ala, 1.05 (1); Val, 0.92 (1); Gly, 1.1 (1)
Boc-NH-Pro-Val-NH_2	10	10.4 (74%)	0.81	Pro, 0.91 (1); Val, 1.0 (1)
Boc-NH-Gly-Phe-Pro-NH_2	12	15 (80%)	0.75	Gly, 0.96 (1); Phe, 1.0 (1); Pro, 0.98 (1)
Boc-NH-Leu-Ala-Gly-Val-NH_2	10	14.8 (71%)	0.67	Leu, 1.0 (1); Ala, 0.95 (1); Gly, 1.02 (1); Val, 0.97 (1)
Boc-NH-Ala-Gly-Leu-Ile-Gly-NH_2	12	17.5 (73%)	0.62	Ala, 1.12 (1); Gly, 2.02 (2); Leu, 0.91 (1); Ile, 1.04 (1). *m/z* 528.7 [$(M+H)^+$, 100%], $C_{24}H_{43}N_6O_7$, requires M^+ 527.626
Fmoc-NH-Ala-Gly-Leu-Ile-Gly-NH_2	12	22.5 (75%)	0.43	Ala, 1.06 (1); Gly, 1.92 (2); Leu, 1.01 (1); Ile, 1.1 (1). *m/z* 649.8 [$(M+H)^+$, 100%], $C_{34}H_{45}N_6O_7$, requires M^+ 648.759
Boc-NH-Leu-Ala-Val-NHMe	10	13.6 (72%)	0.49	Ala, 1.0 (1); Leu, 0.9 (1); Val, 1.12 (1)
Boc-NH-Val-Leu-Ala-Val-NHMe	12	15.9 (70%)	0.42	Val, 2.1 (2); Leu, 0.97 (1); Ala, 1.0 (1)
Boc-NH-Leu-Ala-Val-NHEt	10	12.4 (65%)	0.47	Leu, 1.1 (1); Ala, 1.02 (1); Val, 0.98 (1)
Boc-NH-Val-Leu-Ala-Val-NHEt	12	13.5 (64%)	0.43	Ala, 1.0 (1); Leu, 1.21 (1); Val, 1.98 (2)

[a] Solvent system used pyridine/acetic acid/water (15:35:50).

peptide amides and peptide *N*-alkyl amides. The method has the unique advantage of avoiding the formation of diketopiperazine and the unwanted side reactions in the *trans*-esterification procedure, thus increasing the overall yield of the peptide. Photolytic cleavage can be conveniently employed for the synthesis of peptides containing sterically hindered C-terminal amino acid like Val. The peptides obtained are in fully protected form, which can be applicable for segment condensation.

3. Experimental

3.1. Materials and methods

Styrene, BDODMA, polyvinyl alcohol (PVA, MW~75,000), caesium carbonate, trifluoroacetic acid (TFA), trifluoro ethanol (TFE), *p*-toluic acid, *N*-bromosuccinimide (NBS), potassium phthalimide, diisopropylethyl amine (DIEA) and chloromethyl phathalimide were purchased from Aldrich Chemical, USA. 4-Dimethylaminopyridine (DMAP), piperidine, 2-(1H-benzotriazol-1-yl) 1, 1, 3, 3-tetramethyluroniumhexafluoro phosphate (HBTU), 1-hydroxybenzotriazole (HOBt) and Boc and Fmoc-amino acids, were purchased from Novabiochem, UK. 4-Bromomethyl 3-nitrobenzoic acid was prepared by the literature procedure.[15] All solvents used were of HPLC grade. 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. HPLC was done on a Pharmacia Akta purifier instrument using C-18 reverse phase semi prep. column. The amino acid analysis was carried out on an LKB 4151 Alpha plus amino acid analyzer. The peptide was hydrolysed using 6N HCl in a pyrex glass tube fused under nitrogen for 15 h at 130°C. Mass spectra of peptides were obtained with a Kratos PC-Kompact MALDI TOF MS instrument.

3.1.1. Synthesis of PS–BDODMA resin. Inhibitors were removed from styrene and BDODMA by washing with 1% NaOH in water (2×30 mL) followed by distilled water (2×30 mL) and drying over anhydrous sodium sulphate. A four-necked reaction vessel equipped with a thermostat, Teflon stirrer, water condenser, and nitrogen inlet and a dropping funnel were used for the polymerization reaction. A net volume of 1% solution of polyvinyl alcohol (MW~75,000) was prepared by dissolving PVA (1.1 g) in double distilled water (110 mL) and added to the reaction vessel. The solution was deoxygenated by continuous bubbling of nitrogen. Styrene (11.4 mL), BDODMA (0.45 mL), and benzoylperoxide (0.5 g) were dissolved in toluene (10 mL) and the solution was added to the reaction vessel by stirring the solution at 2000 rpm. The reaction vessel was sealed using a rubber septum. The temperature of the reaction system was maintained at 80°C using a thermostated oil bath, and the reaction was allowed to continue for 6 h. The system was kept under a continuous flow of nitrogen till the solution became clear. The copolymer obtained as beads of 100–200 mesh size was washed thoroughly with hot water (to remove stabilizer), acetone (5×30 mL), benzene (5×30 mL), toluene (5×30 mL) and methanol (5×30 mL). The copolymer was further purified by Soxhlet extraction with DCM and methanol and dried under vacuum. IR (KBr): 1720, 1480, 755, and 700 cm^{-1}. ^{13}C CP-MAS NMR: 145.30, 130.48, 67.47, and 42.78 ppm.

3.1.2. Aminomethyl PS–BDODMA resin. The PS–BDODMA resin (5 g) was swollen in DMF (50 mL). After 1 h, excess DMF was removed and the swollen resin was placed in a three-necked 100 mL round bottom flask equipped with N_2 inlet, addition funnel, mechanical stirrer, and reflux condenser and heating mantle. Chloromethyl phthalimide (978 mg, 5 mmol) and anhydrous $ZnCl_2$ (0.1 M in dry THF, 1 mL) were dissolved in DMF (30 mL) and the resulting mixture was added to the resin. The suspension was refluxed for 3 h under the nitrogen atmosphere. The reaction mixture was cooled, filtered and the resin was washed with DCM (5×30 mL), dioxane (5×30 mL), ethanol (5×30 mL), and methanol (5×30 mL). The dried resin was suspended in ethanol (20 mL), and refluxed with hydrazine hydrate (100 μL, 2 mmol). After 8 h, the suspension was filtered and the resin was washed with ethanol (5×30 mL), methanol (5×30 mL) and dried in vacuum. Amino capacity of the resin was 0.69 mmol/g as estimated by the picric acid titration method.[14] IR (KBr): 1720, 1520, 1480, 755, and 700 cm^{-1}. ^{13}C CP-MAS NMR: 145.30 and 136 ppm.

3.1.3. 4-Bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (2). To the pre-swollen aminomethyl PS–BDODMA resin (1 g, 0.69 mmol) in DCM, a mixture of 4-bromomethyl 3-nitrobenzoic acid (**1**) (520 mg, 2 mmol), HOBt (270 mg, 2 mmol), HBTU (760 mg, 2 mmol) and DIEA (350 μL, 2 mmol) was added and kept at room temperature. After 1 h, the resin was filtered, washed with DCM (6×10 mL) and a second coupling was performed under exactly the same conditions. The resin was collected by filtration, washed with DCM (5×20 mL), DMF (5×20 mL) and MeOH (5×20 mL). Bromine content of the resin=0.64 mmol/g. IR (KBr): 1650 cm^{-1} (NHCO), 1340, 1540 cm^{-1} (NO_2).

3.1.4. 4-Aminomethyl-3-nitrobenzamidomethyl PS–BDODMA resin. 4-Bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (**2**) (500 mg, 0.32 mmol) was suspended in NMP (20 mL), potassium phthalimide (550 mg, 3.2 mmol) was added and the reaction mixture was kept at 110–120°C with occasional shaking for 12 h. The resin was filtered, washed with NMP (6×25 mL), dioxane (6×25 mL), EtOH (6×25 mL), MeOH (6×25 mL) and dried under vacuum. The dried resin was suspended in EtOH (100 mL) and refluxed with hydrazine hydrate (0.15 mL, 3.2 mmol) for 8 h. The resin was then filtered, washed with EtOH (6×25 mL) and MeOH (6×25 mL) and dried in vacuum. Amino capacity of the resin=0.63 mmol/g.

3.1.5. 4-Methylaminomethyl 3-nitrobenzamidomethyl PS–BDODMA resin (3a). 4-Bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (**2**) (100 mg, 0.064 mmol) was suspended in DCM (20 mL) in a stoppered bottle and kept at 0–5°C. Dry methylamine gas was bubbled through the reaction mixture for 12 h. The reaction flask was stoppered and shaken for 12 h at room temperature. The resin was filtered, washed with DCM (3×2 mL×3 min), THF (3×20 mL×3 min), water (3×20 mL×3 min), MeOH

(3×20 mL×3 min) and dried in vacuum. Amino capacity of the resin=0.60 mmol/g. IR (KBr): 1650 cm^{-1} (NHCO), 1340, 1530 cm^{-1} (NO_2), 3400 cm^{-1} (NH).

3.1.6. 4-Ethylaminomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (3b). 4-Bromomethyl-3-nitrobenzamidomethyl PS–BDODMA resin (**2**) (100 mg, 0.064 mmol) was treated with dry ethylamine following the same protocol as above. Amino capacity of the resin=0.60 mmol/g. IR (KBr): 1652 cm^{-1} (NHCO), 1340, 1530 cm^{-1} (NO_2), 3440 cm^{-1} (NH).

3.2. Synthesis of peptides

3.2.1. Synthesis of peptides using Boc-amino acids. Peptide synthesis was carried out manually in a silanized glass reaction vessel with a glass filter at one end and a calcium chloride guard tube at the other end. The C-terminal amino acid of the peptide acids were attached to the resin by caesium salt method and that of peptide amides and *N*-alkyl amides were incorporated by treating the resin with Boc-amino acids in the presence of HOBt and HBTU for 1 h. The first Boc-amino acid attached resin was taken in the reaction vessel and swelled in DCM. Boc-protection was removed by using 30% TFA in DCM and neutralization was achieved by 5% DIEA in DCM. The resin was washed thoroughly with DCM and DMF. Boc-amino acid (3 equiv. corresponding to the halogen capacity of the resin) along with HOBt (3 equiv.), HBTU (3 equiv.) and DIEA (3 equiv.) were added and kept for 1 h at room temperature. The coupling procedure was proceeded till the target peptide sequence was formed. The coupling was performed twice for 100% reaction. Each coupling step was monitored by Kaiser's test.[16]

3.2.2. Synthesis of peptides using Fmoc-amino acids. The C-terminal amino acid was incorporated to the resin by treating with Fmoc-amino acids in presence of HOBt and HBTU for 1 h. The Fmoc protection from the C-terminal amino acid attached resin was removed by 20% piperidine in DMF. The resin was washed thoroughly with DMF and the subsequent Fmoc-amino acids (3 equiv.) were coupled by using HOBt (3 equiv.) and HBTU (3 equiv.) in the presence of DIEA (3 equiv.) for 40 min. Each coupling step was monitored by the ninhydrin test.[16]

3.3. General procedure for photolysis

The peptidyl resin was suspended in a mixture of 30% TFE in DCM (100 mL) and inserted in an immersion type photochemical reactor. The suspension was degassed for 1 h with dry nitrogen and irradiated with Philips HPK 125 W medium pressure mercury lamp at 340–350 nm for 12 h. A solution of $CuSO_4$ was circulated through the outer jacket of the photochemical reactor to filter off light waves below 320 nm. After photolysis, the resin was filtered, washed with ethanol (3×25 mL) and DCM (3×25 mL). Combined filtrate and washings were evaporated on a rotary evaporator under reduced pressure. The residue was collected and purified by chromatography on a Sephadex G-10 column using acetic acid in water (5%) as eluent. The eluted fractions containing the peptide or its derivatives were pooled together and lyophilized to recover the peptide.

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