Tetrahedron Vol. 44, No. 21, pp. 6661 to 6666, 1988
Printed in Great Britain.

0040-4020/88 $3.00 + .00

POLYMER-SUPPORTED SYNTHESIS OF PROTECTED PEPTIDE[1] SEGMENTS ON A PHOTOSENSITIVE o-NITRO(α-METHYL)BROMOBENZYL RESIN

A. AJAYAGHOSH[a+] and V.N. RAJASEKHARAN PILLAI[*b]

a. Department of Chemistry, University of Calicut, Kerala 673 635, India.
b. Department of Polymer Chemistry, Mahatma Gandhi University, Kottayam 686 001, Kerala, India.

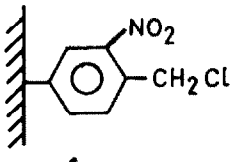
(*Received in UK* 9 *August* 1988)

Abstract - *The preparation of a new polystyrene support containing the photodetachable o-nitro(α-methyl)benzyl anchoring group and its application in the solid phase synthesis of fully protected C-terminal peptides are described. The preparation of the photosensitive resin involves a 4-step polymer-analogous reaction starting from 1%-divinyl benzene crosslinked polystyrene. Amino acid units were incorporated into this resin following standard solid phase peptide synthetic methodology and the peptides were cleaved from the support by photolysis under neutral conditions at 350 nm in 40-50% overall yield. The attachment of the peptide through a secondary ester linkage in o-position to the nitro group is the factor permitting the photolytic cleavage of the peptides from the support.*

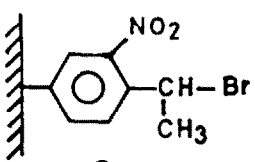
INTRODUCTION

The use of crosslinked polystyrene supports incorporating a photolytically cleavable anchoring linkage between the polymer support and the growing peptide chain was first reported by Rich et al in 1973[2-4]. The unique advantage of this method is that it avoids the drastic conditions of the acid- and the base-catalysed cleavages of the finished peptide from the support involved in the final cleavage step in the solid phase method of peptide synthesis[5,6]. The photolytic cleavage method under neutral conditions at room temperature offers the possibility of obtaining fully protected peptide fragments that can be subsequently used for segment condensation in solution or in the solid phase[7-10]. A number of polymeric supports with different types of photodetachable anchoring linkages for the synthesis of peptides on insoluble and soluble polymeric supports have been described[11-16]. They facilitate the preparation of protected C-terminal peptides, peptide amides and substituted peptide amides under neutral conditions[4,17,18].

During the photolysis of the peptides from the o-nitrobenzyl polymers, the photo-byproduct, polymeric o-nitrosobenzaldehyde formed act as an internal light filter[19,20]. This will ultimately affect the photolytic cleavage of the peptides from the support. In order to overcome this limitation we have modified the support (1) by introducing a methyl group at the α-position of the side chain to form the support (6).



1



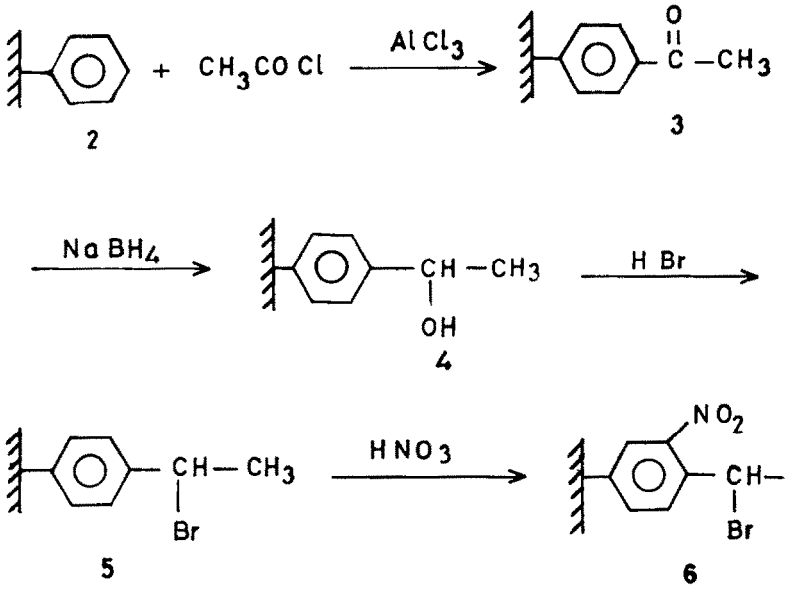
6

+ Present address: Regional Research Laboratory, Trivandrum 695 019, Kerala, India.

This paper describes the different steps involved in the synthesis of the modified photoremovable support (6) and its application in the solid phase synthesis of protected peptides which can be subsequently used for segment condensation.

RESULTS AND DISCUSSION

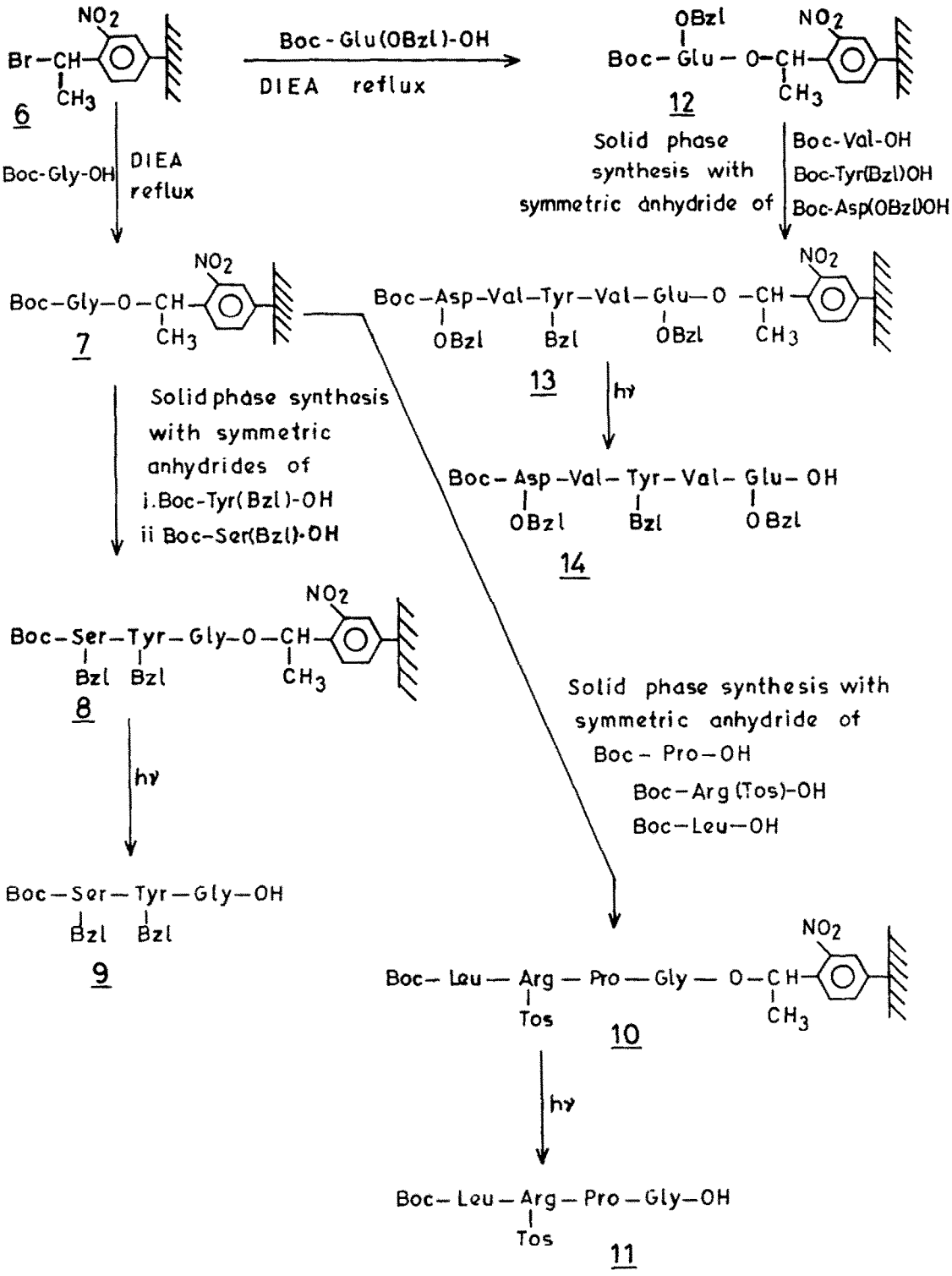
The photocleavable support 6 was prepared from commercially available 1%-divinylbenzene cross-linked polystyrene (200-400 mesh, Fluka) by a four-step polymer-analogous reaction (Scheme I). The styrene-divinylbenzene copolymer on acetylation with acetyl chloride in the presence of anhydrous $AlCl_3$ gave the acetyl resin 3. This resin showed IR band at 1690 cm^{-1} indicating the presence of a carbonyl group. The keto group of the resin 3 was reduced to the hydroxyl group with $NaBH_4$ in diglyme to give the hydroxy resin 4. The reduction was followed by the disappearance of the peak at 1690 cm^{-1} and appearance of the peak at 3600 cm^{-1} characteristic of the OH group. The resin 4 on treatment with dry gaseous HBr yielded the α-methylbromobenzyl resin 5. The capacity of the resin 5 was determined by estimating the amount of bromine using the Volhard titration method. The α -methylbromobenzyl resin was nitrated with fuming nitric acid at -5°-$0^{\circ}C$ to yield the photosensitive support 6. The nitrated resin 6 showed IR peaks at 1350 and 1530 cm^{-1} characteristic of the nitro group. Elemental analysis gave 5.6 mmol of nitro group/g of the resin.



Scheme I Preparation of o-Nitro (α-methyl) bromobenzyl resin

The use of the resin 6 in solid phase peptide synthesis was illustrated by the synthesis of several protected peptide fragments, as outlined in Scheme-II. The C-terminal amino acid unit was incorporated by refluxing the resin with the Boc-amino acid in the presence of diisopropylethylamine in ethylacetate. The amino acid incorporation in the resin was determined by the estimation of the amino acid from the acid hydrolysate. The peptides were assembled by the stepwise incorporation of Boc-amino acids using the symmetric anhydride procedure. The symmetric anhydrides of Boc-amino acids were prepared by reaction with dicyclohexylcarbodiimide in 2:1 molar ratio in CH_2Cl_2. The progress of the coupling reaction was monitored by the semi-qauantitative ninhydrin method[21]. 4N HCl-dioxane was used for the Boc group deprotection and 10% DIEA/CH_2Cl_2 solution was used for the neutralization. Since the photosensitive nitro resins are reported to have increased tendency to form diketopiperazine[22] at the dipeptide stage, the second and third amino acid units were incorporated by the in situ coupling procedure.

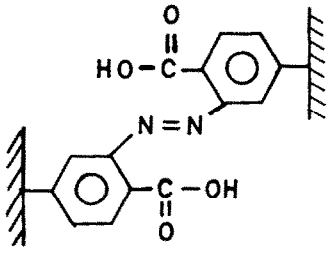
The finished peptides were removed from the support by photolysis at 320-350 nm. The solvent used for photolysis has a major role in the rate of photolytic cleavage. The photolyses were carried out in different solvents like anhydrous EtOH, CH_2Cl_2, DMF and binary mixtures of these solvents. The rate of cleavage increases in solvents which permits maximum swelling of the peptide resin matrix.



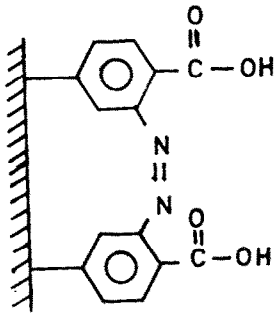
Scheme II : Synthesis of protected peptides on the o-nitro(α-methyl) bromobenzyl resin.

In a previous report a 20% TFE/CH_2Cl_2 was used for the photolytic cleavage of the toxin II of Androctonus australis Hector from a photosensitive nitrobenzamidobenzyl-resin (Nbb-resin)[20]. We have used a 1:1 mixture of anhydrous EtOH and CH_2Cl_2 in which the peptide resin possesses good swelling and facilitate the photolytic cleavage.

During the photolysis of resin (1) any chance for the inter- or intramolecular formation of the secondary photo product (1a) and/or (1b) may hinder further photolysis, by masking of the light especially

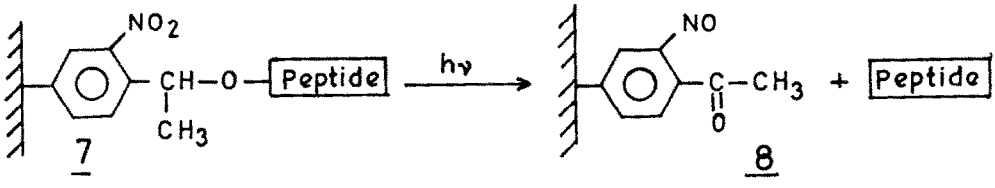


1a



1b

when a large quantity of the resin was used. The colour of the resin that was yellowish initially, turned red on irradiation and the intensity of the colour increases with photolysis. This can be attributed to the formation of the polymeric azobenzenedicarboxylic acids 1a or 1b, the secondary photo-product of the resulting o-nitrosobenzaldehyde. This side reaction was overcome in the case of the modified support (6) which form a polymeric nitrosoacetophenone (8) as the photo-byproduct that has less tendency for photodimerization. (eqn. 1).



Eqn-1

Using these approach fully protected peptide fragments, Boc-Ser(Bzl)-Tyr(Bzl)-Gly-OH, Boc-Leu-Arg(Tos)-Pro-Gly-OH, Boc-Asp(OBzl)-Val-Tyr(Bzl)-Val-Glu(OBzl)-OH were prepared in 40-50% overall yield. However the synthesis beyond a penta peptide was not found promising due to the low photolytic cleavage yield. This appears to be due to the poor swelling of the peptide resin which is attributable to the overloading with nitro group[3]. Each phenyl ring of the resin may contain a nitro group as indicated by the nitrogen analysis, which increases the polarity of the resin. However the resin finds application for the solid phase synthesis of fully protected small peptide sequences which can be used for segment condensation.

EXPERIMENTAL

Copoly(styrene-divinylbenzene) beads (1% DVB-crosslinked, 200-400 mesh) were purchased from Fluka. All the solvents used were of reagent grade and purified according to the literature procedure. Melting points were recorded on a hot-stage melting point apparatus and are uncorrected. IR spectra were recorded on a Pye Unicam SP 3-300 spectrophotometer. Micro analyses were obtained from the Central Drug Research Institute, Lucknow. Photolyses were carried out in an immersion-type photochemical reactor equipped with a Philips HPK 125 W medium-pressure mercury lamp.

General Procedure for Peptide Synthesis

The Boc group was deprotected with 4N HCl-dioxane (10 mL/g of resin for 30 min.). A 10% solution of DIEA/CH_2Cl_2 (10 mL/g of resin for 8 min.) was used for the neutralization of the hydrochloride. A symmetric anhydride of the Boc amino acid containing 3 fold molar excess was used for each coupling.

Third amino acid was incorporated by adding the corresponding Boc amino acid anhydride to the hydrochloride resin followed by addition of 2 equivalents of DIEA. The extent of coupling was monitored by semiquantitative ninhydrin test. During filtration, the resin was thoroughly washed with CH_2Cl_2 and methanol.

Photolytic Cleavage of the Peptide from the Support

A suspension of the peptide resin (1 g) in a mixture of anhydrous ethanol and CH_2Cl_2 (1:1) (100 mL) in a water-cooled immersion-type photochemical reactor was flushed with dry N_2 for 3 h to remove any dissolved oxygen. The mixture was then irradiated with a Philips HPK 125 W medium-pressure mercury lamp for 24 h, with gentle magnetic stirring. A saturated solution of $CuSO_4$ was circulated through the outer jacket of the photoreactor to filter out wavelengths below 320 nm. After photolysis the resin was filtered and washed with CH_2Cl_2 and EtOH. The solvent was evaporated from the combined filtrate and washings in vacuo. The crude peptide was collected and purified by chromatography on a sephadex LH-20 column (2.5 x 80 cm) using methanol. Thin layer chromatography was done in the following two solvent systems. A: (1-butanol-acetic acid-water-ethyl acetate, 1:1:1:1), B: (1-butanol-acetic acid-water, 4:1:5).

Preparation of Acetyl Polystyrene Resin (3)

Polystyrene-1% DVB copolymer resin (10 g) was suspended in nitrobenzene (50 mL) at 0°C. Acetyl chloride (2.1 mL, 30 mmol) was added followed by slow addition of anhydrous $AlCl_3$ (6 g, 45 mmol). The reaction mixture was stirred at room temperature for 2 h. The resin was filtered and thoroughly washed with nitrobenzene, dioxane, dioxane-4N HCl (3:1), dioxane-water (1:1), water, ethanol, and methanol. The resin was dried overnight under vacuum. The capacity of the resin was 1.6 mmol C=O/g as determined by the nitrogen analysis of the corresponding oxime resin. IR (KBr) 1690 cm^{-1} (C=O).

Reduction of the Acetyl Polystyrene Resin (3): Preparation of the Alcoholic Resin (4)

To the keto resin 3 (9 g) in diglyme (100 mL) a solution of $NaBH_4$ (2 g, 52.85 mmol) in diglyme (30 mL) was added. The reaction mixture was stirred at 50-55°C for 16 h. The suspension was cooled to 0°C and conc. HCl (20 mL) was added. The resin was filtered, washed with hot water, hot ethanol, methanol and dried in vacuo. yield 9 g. IR (KBr) 3600 cm^{-1} (OH). The capacity of the resin was determined by the acetylation of the OH group with aceticanhydride-pyridine followed by the back titration of the excess acetic acid. Capacity: 1.58 mmol OH/g.

α-Methylbenzylbromide Resin (5)

Dry HBr gas was passed through a suspension of the alcohol resin 4 (8 g, 1.58 mmol OH/g) in CH_2Cl_2 (100 mL) kept at 0°C for 4 h. The reaction mixture was stirred at room temperature for one more hour. After filtration and thorough washing with CH_2Cl_2 the resin was dried under vacuum. Yield: 9 g. An aliquot of the polymer was digested with pyridine for 6 h and the bromine content was determined by Volhard titration. Br: 1.3 mmol/g.

o-Nitro-(α-methyl)benzylbromide Resin (6)

Fuming nitric acid (50 mL) was placed in a 100 mL round-bottom flask and cooled to -5°C. The α-methylbenzylbromide resin (5) (8 g) was added slowly and the mixture was stirred at -5°-0°C for 1 h. The suspension was poured into crushed ice and the product resin was collected by filtration. After thorough washing with cold water and methanol the resin was vacuum-dried. Elemental analysis indicated 5.6 mmol of NO_2/g and 1 mmol Br/g of resin. IR (KBr) 1350 and 1530 cm^{-1} (NO_2).

Boc-Gly-OCH(CH_3)C_6H_4(NO_2)-Resin (7)

The nitrobromobenzyl resin (6) (3 g, 3 mmol) was added to a solution of Boc-Gly-OH (2.1 g, 12 mmol) in EtOAc (20 mL) containing DIEA (1.56 g, 12 mmol). The suspension was gently heated under reflux for 48 h with mild stirring. The resin was filtered, washed with EtOAc, MeOH, CH_2Cl_2 and MeOH and dried in vacuo. Yield 3 g. The product resin contained 0.89 mmol of glycine/g of resin.

Boc-Glu(OBzl)-OCH (CH_3)C_6H_4(NO_2)-Resin (12)

The nitrobromobenzyl resin (6) (2 g, 2 mmol) was esterified with Boc-Glu(OBzl)-OH (2.7 g, 8 mmol) following the above procedure. The resulting resin contained 0.72 mmol of Glu/g of resin. The resin was further esterified by refluxing with excess of acetic acid to block any unreacted bromobenzyl group.

Boc-Ser(Bzl)-Tyr(Bzl)-Gly-OH

The peptide resin (8) was synthesized from Boc-Gly-resin (7) using symmetric anhydrides of Boc-Ser(Bzl)-OH and Boc-Tyr(Bzl)-OH according to the general procedure (Scheme-II). The tripeptide Boc-Ser(Bzl)-Tyr(Bzl)-Gly-OH was obtained from the support by photolysis for 24 h. The crude peptide was purified by chromatography over a sephadex LH-20 column in methanol at a flow rate of 30 mL/h. The purified peptide was obtained in 48% yield, based on the starting Boc-Gly-resin. mp 135-137°C. Tlc R_f (A) 0.71, R_f (B) 0.56.
Amino acid analysis: Gly, 1.1; Tyr, 0.9; Ser, 0.98; Anal. Calcd. for $C_{33}H_{39}N_3O_8$: C, 65.44; H, 6.49; N, 6.94; Found C, 65.18; H, 6.39; N, 7.1.

Boc-Leu-Arg(Tos)-Pro-Gly-OH

The synthesis of the peptide resin (10) was carried out according to the general procedure, using the Boc-Gly-resin (7) (1 g, 0.89 mmol) (Scheme-II). The tetrapeptide Boc-Leu-Arg(Tos)-Pro-Gy-OH was cleaved from the peptide resin by photolysis for 24 h. The crude peptide was purified by chromatography on sephadex LH-20 column using methanol. Yield. 45%. mp 122-124°C. Tlc R_f (A) 0.93; R_f (B) 0.45;
Amino acid analysis: Leu, 1.02; Arg, 1.0; Pro, 0.98; Gy 1.04; Anal. Calcd. for $C_{31}H_{49}N_7SO_9$: C, 53.51; H, 7.10; N, 14.09; S, 4.61; Found C, 53.56; H, 7.20, N, 14.3; S, 4.65.

Boc-Asp(OBzl)-Val-Tyr(Bzl)-Val-Glu(OBzl)-OH

The protected peptide-resin (13) was assembled stepwise, starting from Boc-Glu(OBzl)-resin (12) (1.5 g, 0.72 mmol/g) according to the general procedure. The fully protected pentapeptide was obtained from the resin (12) on photolysis for 24 h following the general method of photolysis. After purification on sephadex LH-20 column the peptide was obtained in 40% yield. mp 222-226°C. Tlc R_f (B) 0.77.
Amino acid analysis: Asp, 1.00; Val, 2.03; Tyr, 0.91, Glu, 1.00.

ACKNOWLEDGEMENT

The authors thank the Council of Scientific and Industrial Research, New Delhi, for the award of a Senior Research Fellowship to A.A.

REFERENCES AND NOTES

1. Nomenclature follows the tentative rules of the IUPAC-IUB Commission on Biochemical Nomenclature, Biochem. J. 219, 345 (1984); Eur. J. Biochem. 138, 9 (1984). Abbreviations used: Boc, tert-butyl-oxycarbonyl; DCC, dicychlohexylcarbodiimide; DIEA, N,N'-diisopropylethylamine; DVB, divinylbenzene; TFE, 2,2,2-trifluoroethanol.
2. D.H. Rich and S.K. Gurwara, J. Chem. Soc. Chem. Commn. 610 (1973).
3. D.H. Rich and S.K. Gurwara, J. Am. Chem. Soc. 97, 1575 (1975).
4. D.H. Rich and S.K. Gurwara, Tetrahedron Lett. 301 (1975).
5. V.N.R. Pillai, Synthesis, 1 (1980).
6. V.N.R. Pillai, 'Organic Photochemistry', (Ed. A. Padwa), Marcel Dekker, Inc, New York, 1987, Vol.9, pp 225-312.
7. S.S. Wang and R.B. Merrifield, Int. J. Pept. Protein Res. 4, 309 (1972).
8. H. Yajima and Y. Kiso, Chem. Pharm. Bull. 1087 (1974).
9. J.P. Tam, F.S. Tjoeng and R.B. Merrifield, J. Am. Chem. Soc. 102, 6117 (1980).
10. Solid phase fragment synthesis has also been referred to as convergent solid phase peptide synthesis by Pedroso et al., Tetrahedron 38, 1183 (1982).
11. S.S. Wang, J. Org. Chem. 41, 3258 (1986).
12. F.S. Tjoeng and G.A. Heavner, J. Org. Chem. 48, 355 (1983).
13. A. Ajayaghosh and V.N.R. Pillai, J. Org. Chem. 52, 5714 (1987).
14. F. Albericio, C. Granier, C. Labbe-Julie, M. Seager, F. Couraud and J. Van Rietschoten, Tetrahedron 40, 4313 (1984).
15. F.S. Tjoeng, E.K. Tong and R.S. Hodges, J. Org. Chem. 43, 4190 (1978).
16. V.N.R. Pillai, M. Mutter, E. Bayer and I. Gatfield, J. Org. Chem. 45, 5364 (1980).
17. V.N.R. Pillai, M. Mutter and E. Bayer, Tetrahedron Lett. 3409 (1979).
18. A. Ajayaghosh and V.N.R. Pillai, British Polym. J. (submitted).
19. A. Patchornik, B. Amit and R.B. Woodward, J. Am. Chem. Soc. 92, 6333 (1970).
20. E. Giralt, R. Eritja, E. Pedroso, C. Granier and J. Van Rietschoten, Tetrahedron 42 ,691 (1986).
21. V.K. Sarin, S.B.H. Kent, J.P. Tam and R.B. Merrifield, Anal. Biochem. 117, 147 (1981).
22. E. Giralt, R. Eritja and E. Pedroso, Tetrahedron Lett. 22, 3779 (1981).