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Synthesis of fully Protected Peptides on a Tetraethyleneglycol Diacrylate (TTEGDA)-Crosslinked Polystyrene support with a Photolytically Detachable 2-Nitrobenzyl Anchoring group

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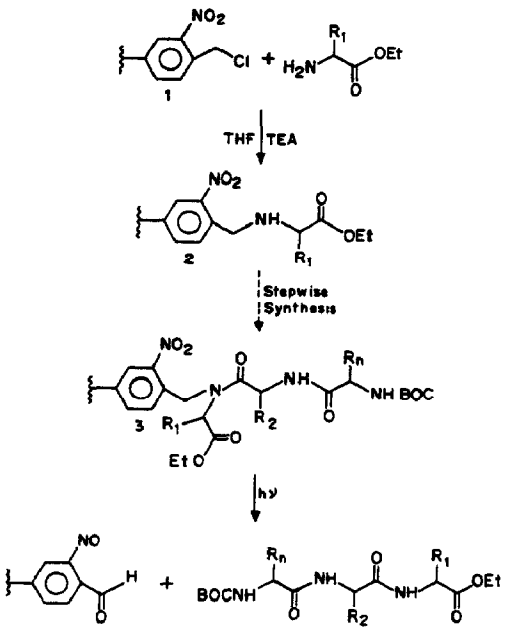
Abstract: 1-Chloromethyl-2-nitro tetraethyleneglycol diacrylate (TTEGDA)-crosslinked polystyrene resin was prepared by nitration of chloromethyl (4%) TTEGDA-crosslinked polystyrene resin and used as a photosensitive solid support for the preparation of fully protected peptides. C-protected amino acid esters were attached to resin (1) using large excess of amino acid ester and equivalent amount of triethylamine (TEA). After estimation of first amino acid attachment, the stepwise synthesis of the peptides was carried out using the symmetric anhydride procedure. The fully protected peptides were cleaved from the support by photolysis. The crude product was purified on a silica gel column and characterised by amino acid analysis and tlc.

The synthesis of large peptides or small proteins represents one of the most challenging goals in peptide synthesis. A convergent or fragment condensation approach is one of the most promising strategies for synthesis of large peptides. Photochemical cleavage from the polymer support permits the preparation of N-amino and side chain protected peptides which are useful in segment condensation. The 2-nitrobenzyl ester linkage finds wide-spread application for the C-terminal protection and anchoring in the polymer-supported method of peptide synthesis.[1] The introduction of the anchoring group between the solid support and the growing peptide chain is a convenient strategy for the mild non-destructive cleavage of peptides in solid phase peptide synthesis. The anchoring linkage should be stable under the conditions of the various reactions which are repeated and at the same time it should be cleaved finally by mild procedure which does not affect the finished peptide. The principle of photolytic deprotection of functional groups has been used to provide mildly and selectively cleavable anchoring linkages between the first aminoacid and the polymer support. 2-Nitrobenzyl type of anchoring group has been used for the final cleavage of peptides in free carboxyl form[2], peptide amide[3] or as N-alkyl peptide[4] amide form, but not in ester form.

Carboxyl ester protected peptides can be conveniently used in segment condensation approach by azide coupling or enzyme catalysed coupling reaction[5]. C-terminal ester peptides also find application in structure-activity studies of bioactive peptides. Trans-esterification is the only method in use at present for the cleavage of peptide as C-terminal esters from the solid support. This method has its own disadvantage in the case of sterically hindered amino acids like Val or Ile and side chain functional amino acids like Asp and Glu. To overcome these difficulties, we have designed a new method for the preparation of fully protected peptides for their possible use in segment condensation method. In this method, the first amino acid is attached to the solid



Scheme 1: Synthesis of fully protected peptides

support via N-terminal and C-protected ester form. After the synthesis, the final product is cleaved photolytically to obtain fully protected peptide. Preliminary studies have shown that small peptides can be photolytically cleaved from the solid support in high yield and purity.

4% TTEGDA-crosslinked polystyrene resin was prepared in beaded form by suspension polymerization technique.[6] The resin was extracted using Soxhlet extractor for 72 hr in acetone and then refluxed with trifluoroacetatic acid for 18 hr to remove any linear soluble polymeric impurity. The resin beads of uniform bead size (100-200 mesh) were screened and chloromethylated according to standard procedure.[7] Chloromethyl resin was nitrated at -10°C using fuming nitric acid.[8] All these resins were characterised by IR spectroscopy and elemental analysis.[9] The ester linkage in the crosslinking agent was found to be stable under all these conditions[10] as revealed by IR bands at 1720 cm^{-1} (ester), and 1150 cm^{-1} (ether).

Five to six fold excess of amino acid esters were allowed to react with resin (**1**) in THF in the presence of a base like triethylamine (TEA) or diisopropylethylamine (DIPEA) at 50-60°C for 30 hr.[11] The resulting amino acid incorporation of the resin (**2**) was determined by picric acid[12] method or amino acid analysis.[8] The possibility of formation of tertiary and quarternary amino acid salt during the reaction of resin (**1**) with amino acid ester was negligible since a large excess of the amino acid was used.

The use of this new resin (**2**) in solid phase synthesis was demonstrated by the synthesis of a few dipeptides and tripeptides (**Scheme 1**). The first amino acid was anchored to the solid support through the N-terminal and the peptide chain was elongated from the secondary amino group by Boc-amino acid symmetric anhydride procedure. The removal of the Boc-group was effected by treatment with 4N HCl-dioxane. The progress of the coupling reaction was monitored by the semi-quantitative ninhydrin test. After the solid phase synthesis, the

protected peptides were removed from the support by photolysis. The photolysis was carried out in trifluoroethanol (30%) CH_2Cl_2 mixture under nitrogen atmosphere[13] for 18-25 hr. The peptides after purification on silica gel column was characterised by comparison with authentic samples (tlc) and amino acid analysis. The yields of the peptides are given in **Table 1**.

The mechanism of photolytic cleavage of 2-nitrobenzyl and related system is well-documented.[14] This involves a light-induced internal oxidation-reduction reaction of aromatic nitro compounds containing a carbon-hydrogen bond *ortho* to the nitro group. The mechanism of the photolytic cleavage of peptides from the modified peptide resin (**3**) is analogous to that of the low molecular weight 2-nitrobenzyl system. The foregoing observations illustrate the applicability of the modified TTEGDA-polystyrene resin as a photo-removable polymeric support for the solid phase synthesis of fully protected peptides. The method has the unique advantage of avoiding the unwanted side reaction in 2-nitrobenzyl ester linkage, the diketopiperazine formation and of *trans*-esterification procedure, thus increasing the overall yield of the peptide. In comparison with the *trans*-esterification method, photolytic cleavage can be conveniently employed for peptides containing sterically hindered C-terminal amino acids like Val.

Table 1: *Details of Peptides Synthesised Using Resin* (**2**)

Peptide	Time of photolysis(h)	R_f^*	Yield %	Aminoacid analysis
Boc-Met-Leu-Ala-OMe	23	0.56	70	Leu 1.01(1); Ala 0.95(1); Met 0.95(1)
Boc-Ala-Gly-Val-OEt	20	0.65	77	Ala 1.05(1); Val 0.98(1); Gly 1.01(1)
Boc-Met-Leu-Phe-OMe	19	0.68	62	Leu 1.2(1); Met 0.95(1); Phe 1.05(1)
Boc-Gly-Val-OEt	18	0.85	78	Gly 1.01(1); Val 0.99(1)
Boc-Phe-Gly-OEt	19	0.75	76	Gly 1.05(1); Phe 0.98(1)

*MeOH:$CHCl_3$ (1:9)

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9. TTEGDA-Polystyrene Resin: IR (KBr) : 1720, 1490 cm^{-1} (ester); 1150 cm^{-1} (ether); 690 and 755 cm^{-1} (aromatic);
Chloromethyl resin: IR (KBr) : 1720 and 1480 cm^{-1} (ester); 1258 cm^{-1} (CH_2Cl); 1150 cm^{-1} (ether);
Nitro resin (1) : IR (KBr) : 1535 and 1350 cm^{-1} (NO_2); 1720 and 1490 cm^{-1} (ester); 1150 cm^{-1} (ether); 1258 cm^{-1} (CH_2Cl).
Elemental analysis indicated 5.6 mmol of NO_2/g and 2.4 mmol Cl/g of resin.
The nitration need not take place exclusively on the same aromatic ring as chloromethylated one. Due to the presence of the CH_2Cl group nitration could be preferentially on the chloromethyl ring. However the peptide chain, if any incorporated on the non-nitrated chloromethylated ring, will not undergo cleavage under the photolytic condition which are selective for the *o*-nitrobenzyl system.
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11. THF (5ml) was added to Ala.OMe HCl (0.85g, 6 mmol) and neutralized using TEA. A mixture of 1-chloromethyl-2-nitro-TTEGDA-crosslinked polystyrene resin(1) (0.5g, 2.4 mmol Cl/g) in THF (10ml) and TEA (0.1ml, 1.2 mmol) was added to this and refluxed for 30hr. The resin was filtered and washed with THF/water (1:1) (5ml x 6). DMF (5ml x 6), MeOH (5ml x 6) and dried under vacuum at 50°C. The product resin (0.58g) gave intense blue colour with ninhydrin reagent. Picric acid estimation indicated incorporation of 1.6 mmol of Ala OMe/g of resin. There was no residual chlorine after the reaction.
IR (KBr) : 1530 and 1350 cm^{-1} (NO_2) 1710 cm^{-1} (ester), 3400 cm^{-1} (NH).
(Similar procedure was adopted for other amino acids also).
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13. The peptide resin (1.5g) was suspended in a mixture of 30% trifluoroethanol (TFE) in CH_2Cl_2 (150ml) and placed in an immersion-type photochemical reactor. The suspension was deaerated for 1hr with dry nitrogen and irradiated with Philips HPK 125W medium pressure mercury lamp at 340-350 nm for 18-24hr. A saturated solution of $CuSO_4$ was circulated through the outer jacket of the photochemical reactor to filter out wave lengths below 320 nm. After the photolysis, the spent resin was removed by filtration, washed with TFE and CH_2Cl_2. The combined filtrate and washings were rotary-evaporated. The residue was collected and purified by chromatography on a silica gel column using 5% MeOH in $CHCl_3$ as eluent.
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