

Chapter – III

Results and Discussion

CHAPTER III

RESULTS AND DISCUSSION

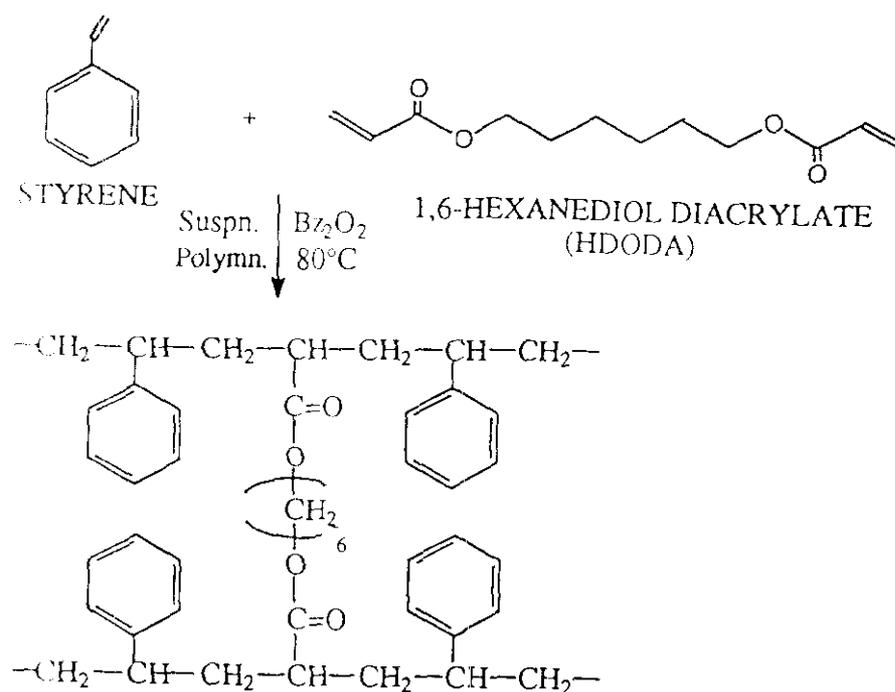
The first and foremost requirement for solid phase peptide synthesis is the development of the polymer support. Chemical and topographical nature determines the physicochemical properties of the polymer.¹⁷⁵ Topography of the polymer matrix is determined by the chemical nature of the monomer, mole percentage of the crosslinker, type of diluent and monomer diluent ratio.^{176,177} Temperature, rate of stirring, molecular weight of suspending agent and even shape of the vessel have considerable effect on the size and morphological characteristics of the polymer^{178,179}. The solid support should be inert to the chemical reagents used for peptide synthesis. Polymeric beads of size ranging 200-400 μm are suitable for peptide synthesis.

In the present study we used an effective support with optimum hydrophobic-hydrophilic balance and mechanical stability by copolymerizing the hydrophobic styrene and hydrophilic flexible hexanediol diacrylate (HDODA). Some model peptides and highly hydrophobic fragments of rubber elongation factor protein were synthesized using this support in nearly quantitative yield. The synthesised peptides were purified and characterised by various analytical methods.

3.1 PREPARATION OF POLYMER SUPPORTS AND FUNCTIONALIZATION

3.1.a Preparation of 2%, 1,6-hexanediol diacrylate (HDODA) - crosslinked polystyrene.

Styrene (after destabilization) and 1,6-hexanediol diacrylate were taken in 98:2 mole ratio and subjected to suspension polymerization.¹⁸⁰ Benzoyl peroxide was used as initiator and polyvinyl alcohol (Mol.wt. 72,000) as the suspending agent. The polymerization was carried out at 80°C using toluene as diluent (Scheme III.1)



Scheme III.1. Suspension polymerization of 1,6-hexanediol diacrylate and styrene

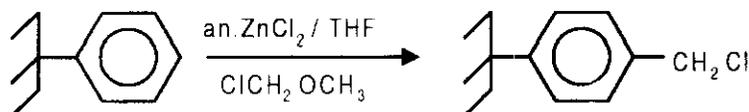
Polymer beads were separated out, washed and soxhletted with different solvents to remove soluble linear polymers and other additives. IR spectroscopy was used to characterize the resin.

IR (KBr): 1720, 1490 cm^{-1} (ester C=O); 2910, 2850 cm^{-1} (CH_2 str. of HDODA and polystyrene); 3020, 700 cm^{-1} (C-H of benzene).

3.1.b Functionalization of the Polymer

The first step in solid phase peptide synthesis is attachment of C-terminal amino acid of the desired peptide to the polymer support. Functionalization of polymer is necessary for this. The functional group acts as a linker of the peptide chain to polymer support and at the same time acts as carbonyl protecting group for the C-terminal amino acid.¹⁸¹ For the synthesis of peptides described in this thesis, chloromethyl group was used as anchoring group.

The introduction of chloromethyl group on 2% HDODA-PS was achieved by Blanc's chloromethylation using chloromethyl methyl ether (CMME).¹⁸² The chloromethyl methyl ether was prepared from methanol and formaldehyde in the presence of dry HCl.¹⁸³ Bis(chloromethyl) ether evolved along with CMME was highly carcinogenic. Hinderman *et al.*,¹⁸⁴ now developed a new method for the synthesis of CMME, free from this carcinogen. Here chloromethyl groups were introduced on the support using chloromethyl methyl ether and anhydrous ZnCl_2 (Lewis acid catalyst) in THF¹⁸⁵ (Scheme III.2).



Scheme III.2 Chloromethylation of HDODA-crosslinked polystyrene resin

HDODA-PS resins having chlorine capacities 1.7 and 1.9 mmol/g were prepared by this method. These resins were used for the synthesis of various peptides. Characterization of the functionalised support was done by IR spectroscopy. IR(KBr) : 668, 1420 cm^{-1} (C-Cl).

3.1.c Functional Group Assay

Several chemical and physical methods can be used for functional group determination.^{186,187} Reaction specific to functional groups and titrimetry are chemical methods and IR, NMR, X-ray scattering, fluorescent labelling, elemental analysis are physical methods.¹⁸⁸ The degree of chloromethylation was determined by converting the covalently bound chlorine to soluble pyridinium chloride by treating with pyridine. The chloride thus liberated was estimated titrimetrically by modified Volhard's method.¹⁸⁹

3.2 SYNTHESIS OF PEPTIDES

The following model peptides were synthesized for the purpose of testing the performance of the solid phase system.

- (a) Ala-Pro-Ala
- (b) Ala-Ala-Pro-Ala
- (c) Ala-Ala-Ala-Ala-Ala
- (d) Ala-Ala-Pro
- (e) Ala-Ala-Ala-Pro-Ala
- (f) Ala-Pro-Gly-Pro-Arg

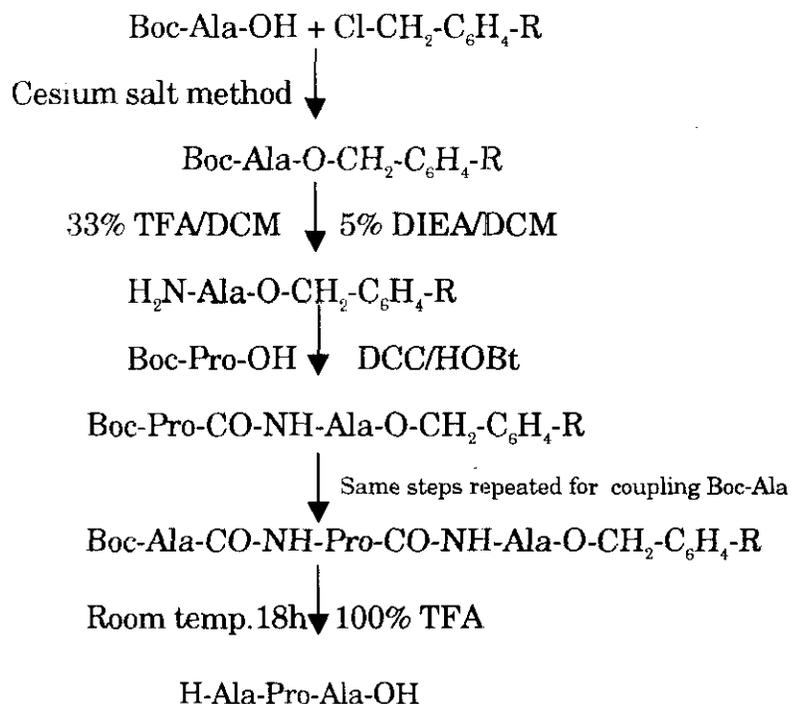
The partial sequences of REF protein; synthesised using 2% HDODA-crosslinked polystyrene are :

- (a) Gln-Gln-Gly-Gln-Gly (7-11)
- (b) Val-Gln-Asp-Ala-Ala-Thr-Tyr-Ala (20-27)
- (c) Pro-Leu-Gln-Pro-Gly-Val-Asp-Ile-Ile-Gln-Gly-Pro (44-55)
- (d) Val-Lys-Asn-Val-Ala-Val-Pro (56-62)
- (e) Tyr-Ile-Pro-Asn-Gly-Ala-Leu-Lys-Phe-Val-Asp-Ser-Thr-Val-Val-Ala (69-84)
- (f) Gln-Thr-Lys-Ile-Leu-Ala-Lys-Val-Phe-Tyr-Gly (125-135)

3.2.a Synthesis of Model Peptides

i. Ala-Pro-Ala

Boc-Ala is attached to chloromethylated resin by cesium salt method and the substitution level of Boc amino acid was found to be 1.9 mmol/g by picric acid method. After the removal of Boc deprotection and neutralisation, successive Boc-amino acids were coupled by HOBt active ester method till the target sequence was obtained. Finally the peptide was cleaved from the support using neat TFA. TFA was removed under reduced pressure and peptide was precipitated by adding ice cold ether. The crude peptide was obtained in 97% yield. The purity of the peptide was checked by TLC using 1-butanol : acetic acid: water (4:1:5) as the solvent system. A single spot was obtained. The synthetic procedure is represented in Scheme III.3.

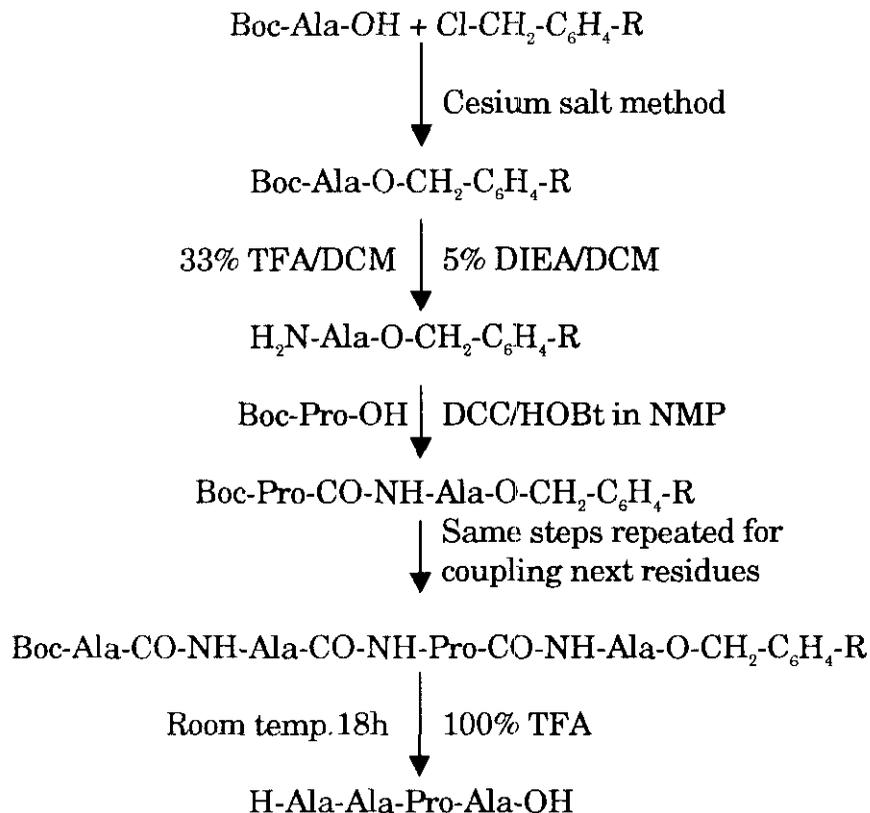


Scheme III.3. Solid phase synthesis of Ala-Pro-Ala on a 2% HDODA-PS chloromethyl resin (R: HDODA-PS)

ii. Ala-Ala-Pro-Ala

The C-terminal amino acid, Boc-Ala was esterified to the 2% chloromethylated resin by cesium salt method and the substitution level of Boc-amino acid was found to be 1.9 mmol/g by picric acid method. Boc protecting group was removed by 33% TFA/DCM followed by neutralisation with 5% DIEA/DCM. It was then thoroughly washed with DCM. The successive Boc-amino acids were coupled by HOBt active ester method using NMP as the solvent. Finally the peptide was cleaved by neat TFA. TFA was removed under reduced pressure and peptide was precipitated by adding ice cold ether. The crude peptide was obtained in 98% yield. Purity of the peptide was confirmed by

TLC using 1-butanol : acetic acid : water (4:1:5) as the solvent system. The synthetic steps involved are shown in Scheme III.4.

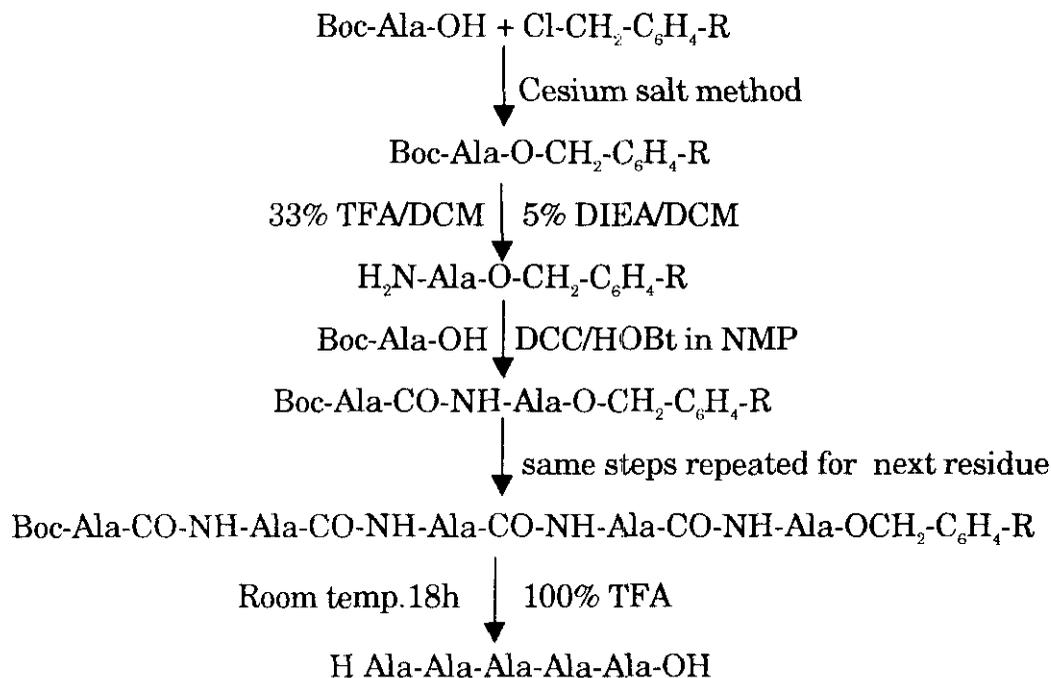


Scheme III.4. Solid phase synthesis of Ala-Ala-Pro-Ala on a 2% HDODA-PS chloromethyl resin (R: HDODA-PS)

iii. Ala-Ala-Ala-Ala-Ala

Boc-Ala was attached to the chloromethyl resin by Gisin's cesium salt method. The amino capacity was found to be 1.7 mmol/g. The next Boc-amino acid, Boc-Ala was coupled to aminoacyl resin, obtained after deprotection and neutralisation, using DCC/HOBt in NMP. After the removal of DCU, a second coupling was done to ensure completion of reaction. The next Boc-amino acids, were similarly attached. Finally the peptide was cleaved from the support using

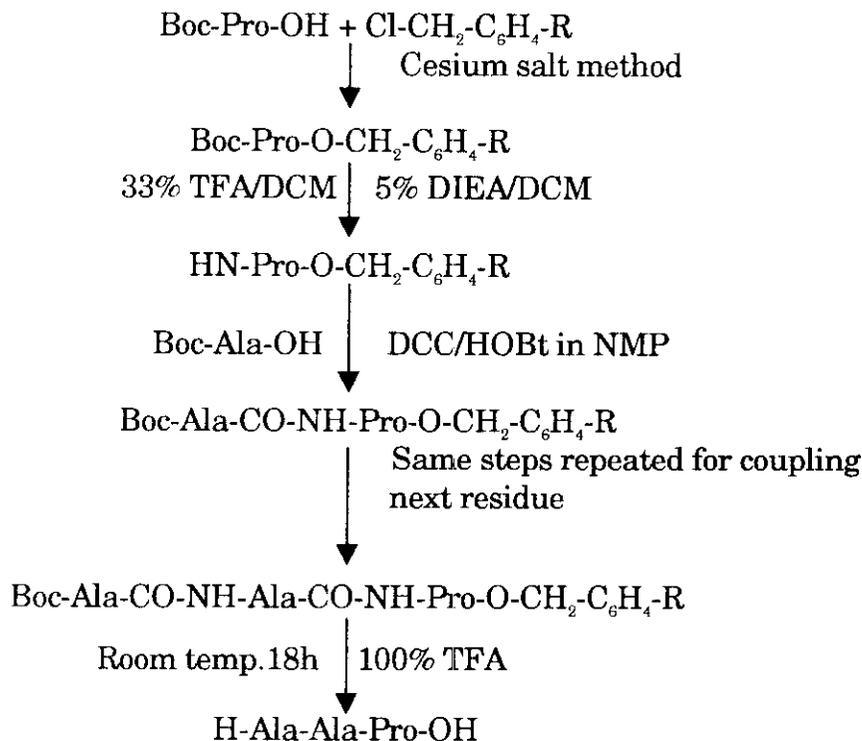
neat TFA. The purity was checked by TLC using 1-butanol : acetic acid : water : pyridine (4:1:1:1) as the solvent system. The crude peptide was obtained in 98% yield. The synthetic procedure is represented in Scheme III.5.



Scheme III.5. Steps involved in solid phase synthesis of Ala-Ala-Ala-Ala-Ala on a 2% HDODA-PS chloromethyl resin (R: HDODA-PS)

iv. Ala-Ala-Pro

The model synthesis of this tripeptide was carried out on a chloromethylated 2% HDODA-PS resin with a chlorine capacity of 1.9 mmol/g. DCC/HOBt coupling was used for the attachment of next amino acids. NMP was used as coupling solvent. The coupling and deprotection steps were monitored by Kaiser reagent. The peptide after synthesis was cleaved from the resin using neat TFA. The synthetic protocol is outlined in Scheme III.6.



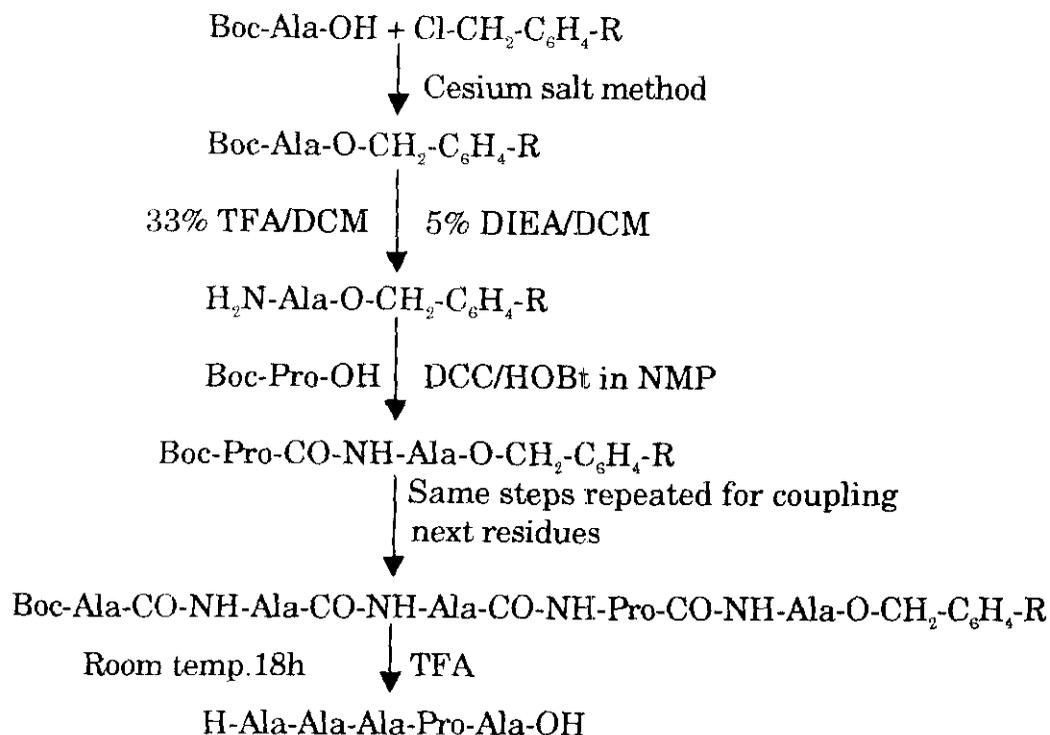
Scheme III.6. Solid phase synthesis of Ala-Ala-Pro on chloromethyl resin (R: HDODA-PS)

The purity was checked by TLC using 1-butanol : acetic acid : water (4:1:5) as the solvent system.

v. Ala-Ala-Ala-Pro-Ala

Synthesis of this pentapeptide was carried out on a 2% HDODA-crosslinked polystyrene resin. Boc-Ala was anchored to the resin by cesium salt method. The remaining amino acids were coupled to the Boc-Ala resin by DCC/HOBt method in NMP. After attachment of all amino acids, the final peptide was cleaved from the resin by TFA. The protocol for synthesis is

given below Scheme III.7.



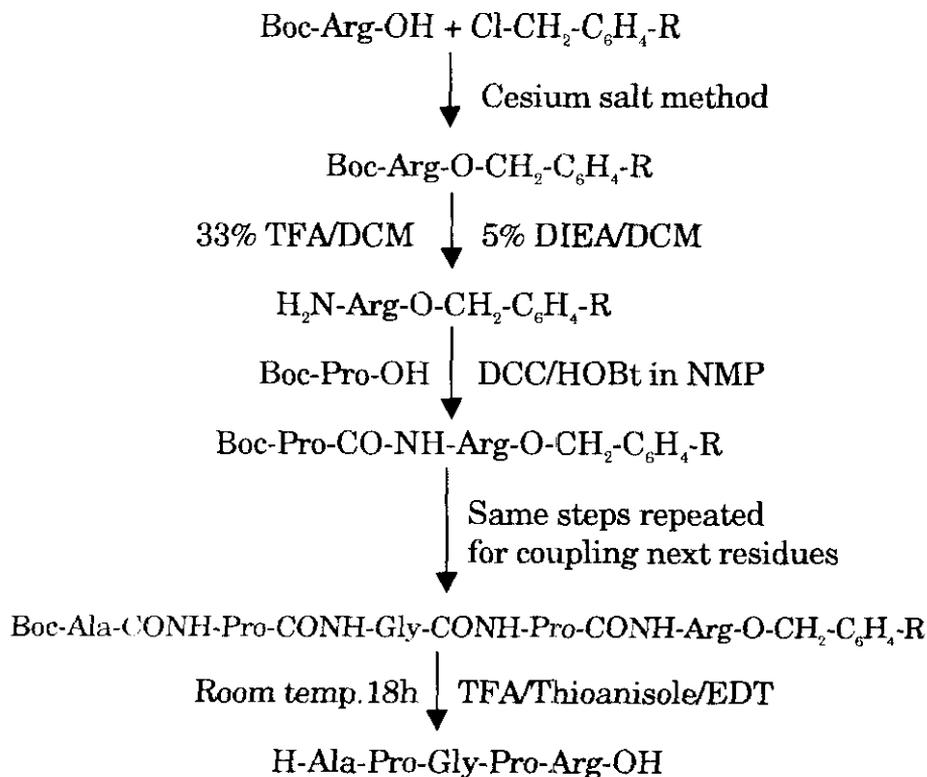
Scheme III.7. Solid phase synthesis of Ala-Ala-Ala-Pro-Ala on a 2% HDODA-PS chloromethyl resin (R: HDODA - PS)

vi. Ala-Pro-Gly-Pro-Arg

The C-terminal amino acid Boc-Arg was esterified to 2% chloromethylated resin by cesium salt method. The substitution level was found to be 1.6 mmol/g by picric acid method. Boc protection was removed by 33% TFA/DCM followed by neutralisation with 5% DIEA/DCM. Next couplings were carried out by DCC / HOBt method.

NMP was the solvent used. A second coupling was done to ensure maximum

reaction. The progress of the reaction was monitored by Kaiser test. After synthesis, the peptide was cleaved from the support using neat TFA in presence of thioanisole and 1,2-ethanedithiol.^{190,191} The protocol for synthesis is given below in Scheme III.8.



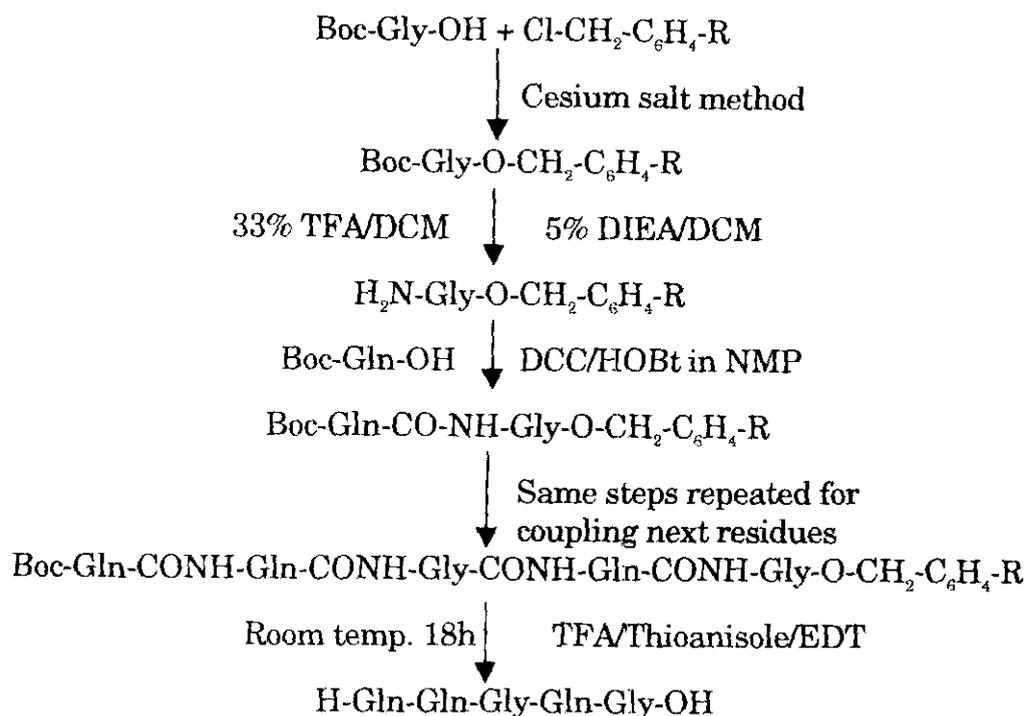
Scheme III.8. Solid phase synthesis of Ala-Pro-Gly-Pro-Arg on a 2% HDODA-PS chloromethyl resin (R: HDODA-PS)

The purity was checked by TLC using chloroform : methanol (9:1) as the solvent system. The crude peptide was obtained in 90% yield.

3.2.b Synthesis of Short Fragments of Rubber Elongation Factor (REF) Protein

i. Gln-Gln-Gly-Gln-Gly (7-11)

This sequence was synthesized using chloromethylated 2% HDODA-PS resin having a chlorine capacity of 1.9 mmol/g. Boc-Gly was esterified to the resin by Gisin's cesium salt method and the amino capacity was found to be 1.8 mmol/g by picric acid method. DCC/HOBt coupling in NMP was used for the attachment of all remaining amino acids. The progress of the coupling reactions was monitored by Kaiser test. The first coupling time was 45 min and for each amino acid, an increment of 5 min was given. The DCU precipitated was washed off with 33% MeOH/DCM followed by washing with DCM. Double coupling was done at every stage for maximum completion of reaction. After the attachment of all the amino acids, the peptide was cleaved off using neat TFA in presence of scavengers, thioanisole, and 1,2 ethanedithiol. The cleaved peptide was precipitated by ice cold ether. The protocol adopted for synthesis is given below in Scheme III.9.



Scheme III.9. Solid phase synthesis of Gln-Gln-Gly-Gln-Gly on 2% chloromethyl PS-HDODA (R: HDODA-PS)

Analytical HPLC of the crude peptide showed only one major peak (Figure III.1a). This was further purified on reverse phase column, (Biorad, Hi-Pore RP-18, 25 x 215 cm) using a solvent system of 0.1% TFA in water and acetonitrile. Homogeneity was checked on analytical column (Figure III.1b). Amino acid analysis was used for the characterisation of the peptide. The fraction collected from HPLC was evaporated, hydrolysed with 6N HCl at 100°C for 24h and analysed. The amino acid analysis gave values which agreed with the expected values (Table III.1).

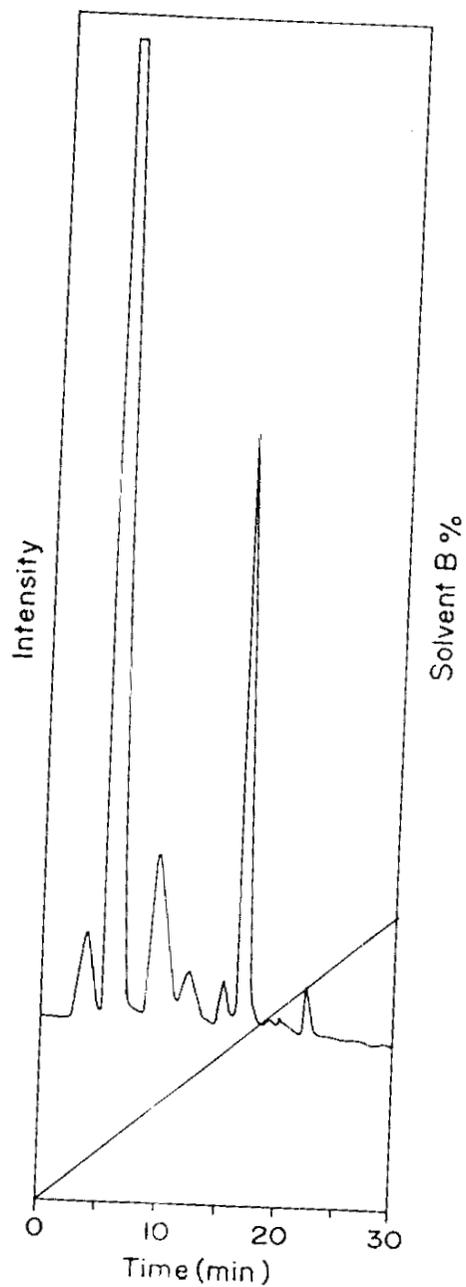


Figure III.1a HPLC trace of crude Gln-Gln-Gly-Gln-Gly
Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 230nm

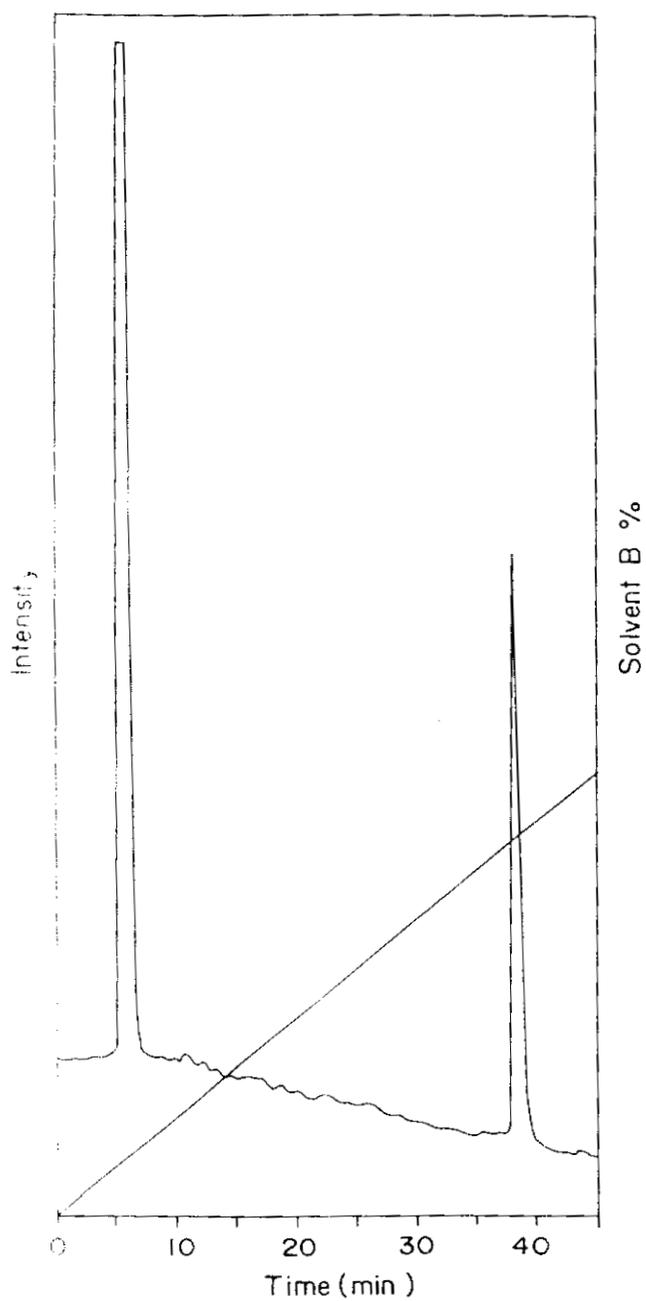


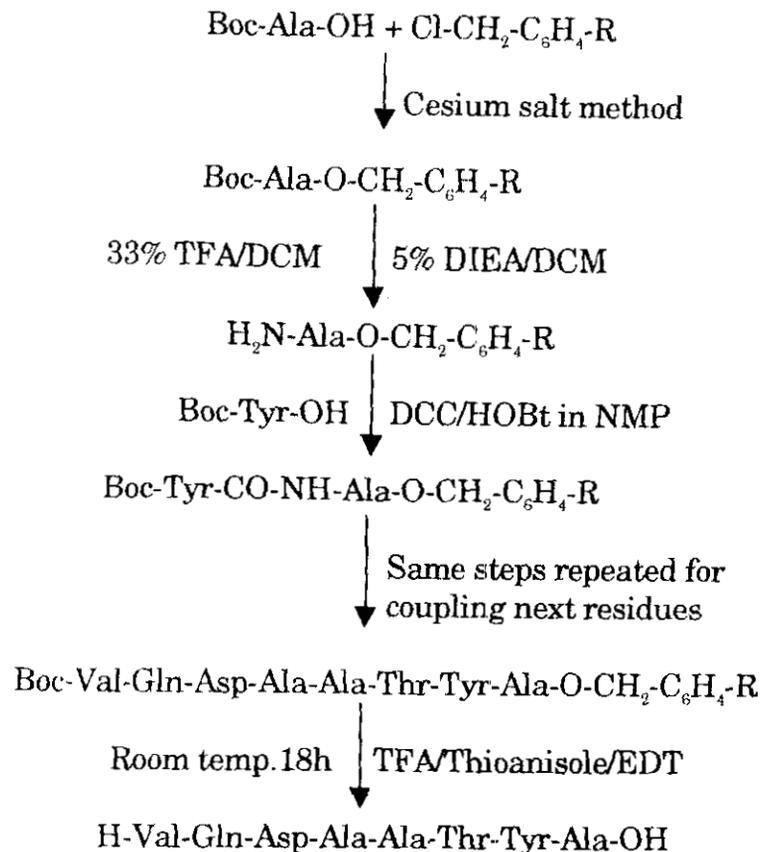
Figure III.1b HPLC trace of pure Gln-Gln-Gly-Gln-Gly
Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 230nm

Table III.1. Details of the amino acid analysis of Gln-Gln-Gly-Gln-Gly (7-11)

Amino acid	Actual value	Calculated value
Glu	3	2.7
Gly	2	1.6

ii. Val-Gln-Asp-Ala-Ala-Thr-Tyr-Ala (20-27)

Chloromethylated HDODA-PS (chlorine capacity 1.9 mmol/g) was used for the synthesis of this peptide. The first amino acid Boc-Ala was attached to the chloromethylated resin by Gisin's cesium salt method. The substitution was found to be 1.8 mmol of NH₂/g of the resin, by picric acid method. Subsequent attachment of amino acids were performed by active ester method. HOBt was used as acyl carrier reagent. The progress of the coupling reaction was monitored by Kaiser test. For all residues a second coupling was given to ensure the completion of the reaction. The coupling of Thr to Tyr was incomplete even after second coupling. Hence a third coupling was also performed. The peptide was cleaved from the support by neat TFA in presence of thioanisole and ethanedithiol. Thioanisole in addition to promoting the acidolysis, plays the role of an acceptor of benzylic group of polymer during cleavage. The peptide was precipitated after removing TFA, using ice cold ether. The yield of the peptide was found to be 92% calculated on the basis of first amino acid substitution level. The protocol for synthesis is given below in Scheme III.10.



Scheme III.10. Solid phase synthesis of Val-Gln-Asp-Ala-Ala-Thr-Tyr-Ala on a 2% chloromethylated resin (R: HDODA-PS)

The peptide was dissolved in 50% CH_3COOH and loaded in analytical HPLC column using binary solvent system (0.1% TFA in water (A) and 50% CH_3CN in 0.1% TFA (B)). The chromatogram shows two main peaks of equal intensity (Figure III.2a). This was further purified on reverse phase column. The two main fractions collected from HPLC was evaporated in a speed vac. and hydrolysed with 6N HCl at 110°C for 24 h and analysed. Homogeneity of this peptide was checked on analytical column (Figure III.2b). Amino acid analysis of one of them gave values which agreed with the expected values (Table III.2).

Table III.2. Details of the amino acid analysis of Val-Gln-Asp-Ala-Ala-Thr-Tyr-Ala (20-27)

Amino acid	Actual value	Calculated value
Val	1	0.8
Glu	1	0.9
Asp	1	1.1
Ala	3	2.6
Thr	1	1.2
Tyr	1	0.9

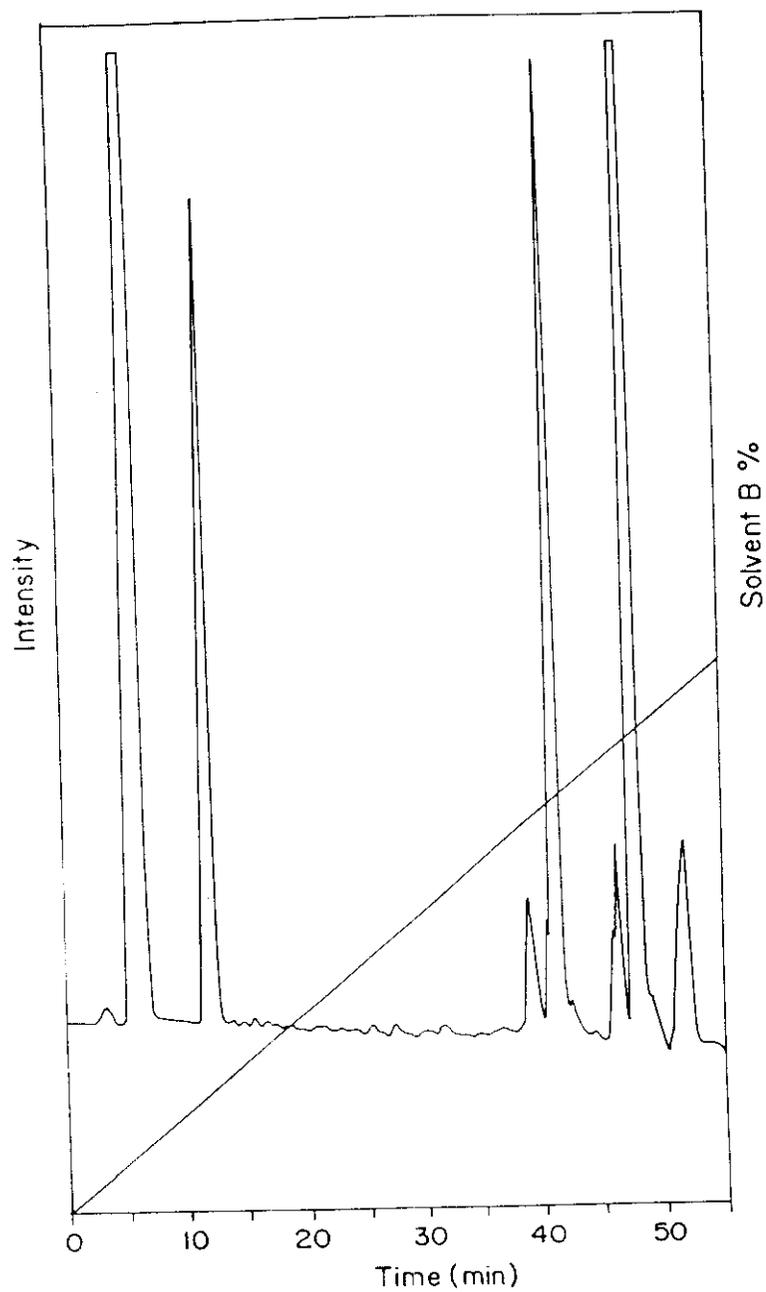


Figure III.2a HPLC trace of crude Val-Gln-Asp-Ala-Ala-Thr-Tyr-Ala
Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 280nm

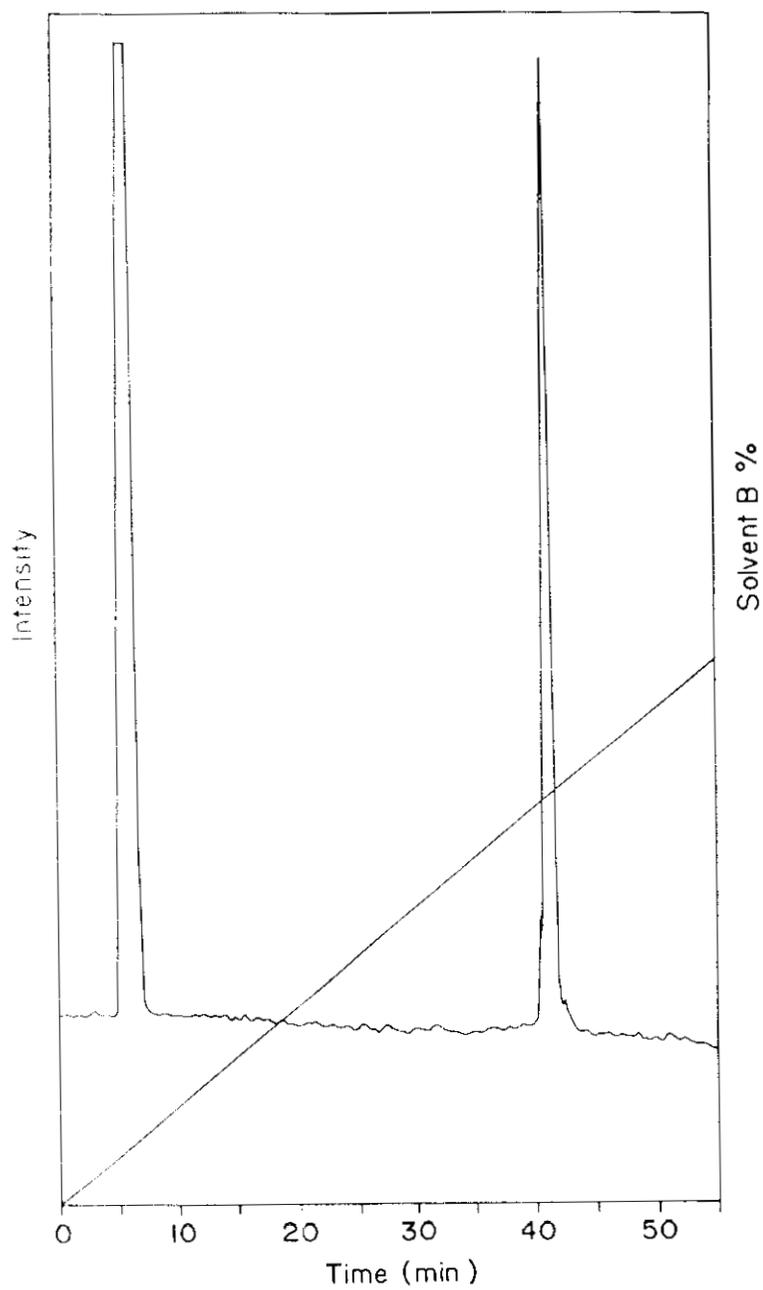
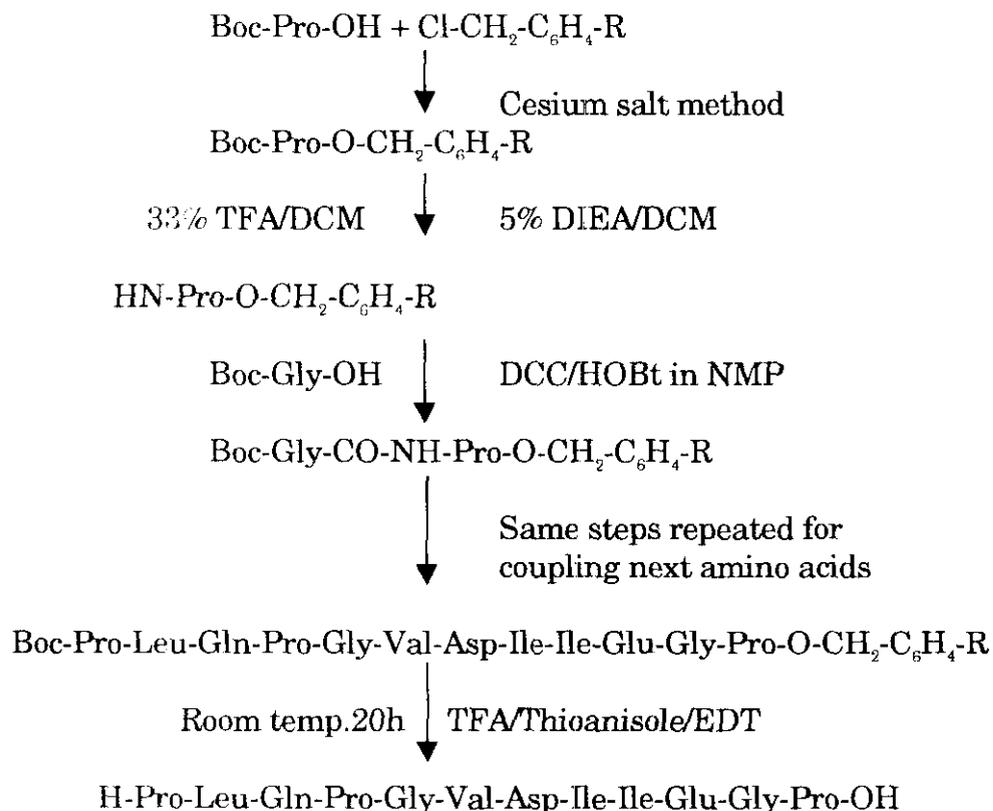


Figure III.2b HPLC trace of pure Val-Gln-Asp-Ala-Ala-Thr-Tyr-Ala
Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 280nm

iii. **Pro-Leu-Gln-Pro-Gly-Val-Asp-Ile-Ile-Glu-Gly-Pro (44-55)**

Boc-Pro was anchored to 2% HDODA-crosslinked chloromethyl resin by cesium salt method. DCC/HOBt was used for the coupling all amino acids. NMP was the solvent used. A double coupling was performed for all the attachments. The coupling of Ile to Ile was incomplete even after a second coupling. Hence a third coupling was also carried out. This is due to the bulk nature of side chain. The completion of coupling and deprotections were monitored by Kaiser test. When the desired sequence was attained, the peptide was cleaved from the support using neat TFA, thioanisole and EDT. The protocol for synthesis is given in Scheme III.11.



Scheme III.11. Solid phase synthesis of Pro-Leu-Gln-Pro-Gly-Val-Asp-Ile-Ile-Glu-Gly-Pro (44-55) on 2% chloromethylated resin (R: HDODA-PS)

The peptide was dissolved in 50% CH₃COOH, from this 10 µl was made up to 1 ml by 0.1% TFA in water and 20 µl loaded in the analytical column. The chromatogram showed one main peak (Figure III.3a). This was further purified on reverse phase column using 0.1% TFA in water and CH₃CN. Homogeneity was checked on analytical column (Figure III.3b) using 50% CH₃CN in 0.1% TFA (B) and 0.1% TFA in water (A) as the solvent system. Amino acid analysis was used for characterisation of peptide. Fraction collected from HPLC was evaporated and hydrolysed with 6N HCl at 110°C for 24h and analysed. The amino acid analysis gave values, almost agreed with the expected values (Table III.3).

The synthesized peptide was analysed by ESI MS (Figure III 3c). The observed molecular weight was in good agreement with the calculated value.

Table III.3 Details of the amino acid analysis of Pro-Leu-Gln-Pro-Gly-Val-Asp-Ile-Ile-Glu-Gly-Pro (44-55).

Amino acid	Actual value	Calculated value
Pro	3	2.6
Leu	1	0.9
Glu	2	1.7
Gly	2	1.5
Val	1	0.82
Asp	1	1.1
Ile	2	1.6

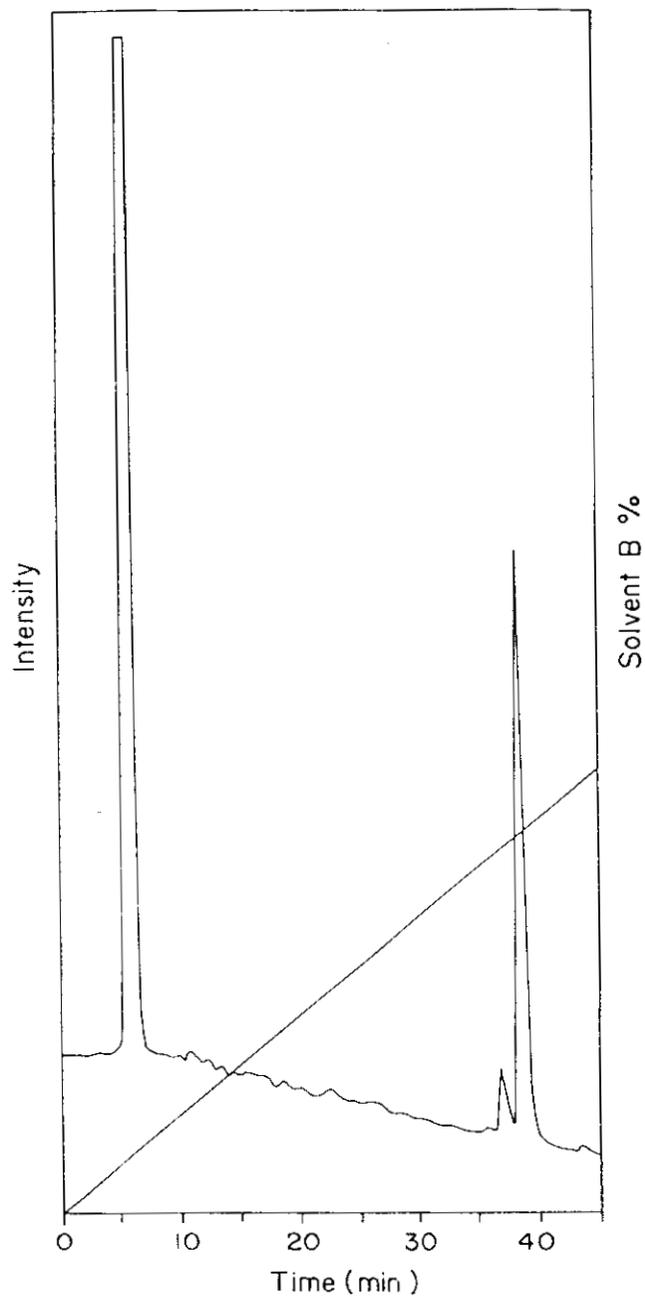


Figure III.3a

HPLC trace of crude Pro-Leu-Gln-Pro-Gly-Val-Asp-Ile-Ile-Glu-Gly-Pro

Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 230nm

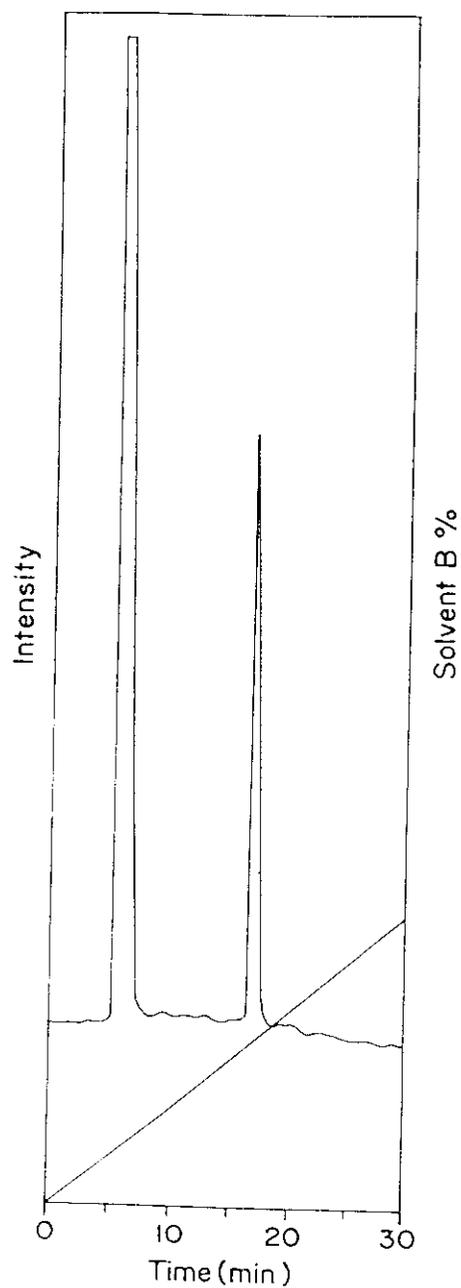


Figure III.3b

HPLC trace of pure Pro-Leu-Gln-Pro-Gly-Val-Asp-Ile-Ile-Glu-Gly-Pro

Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 230nm

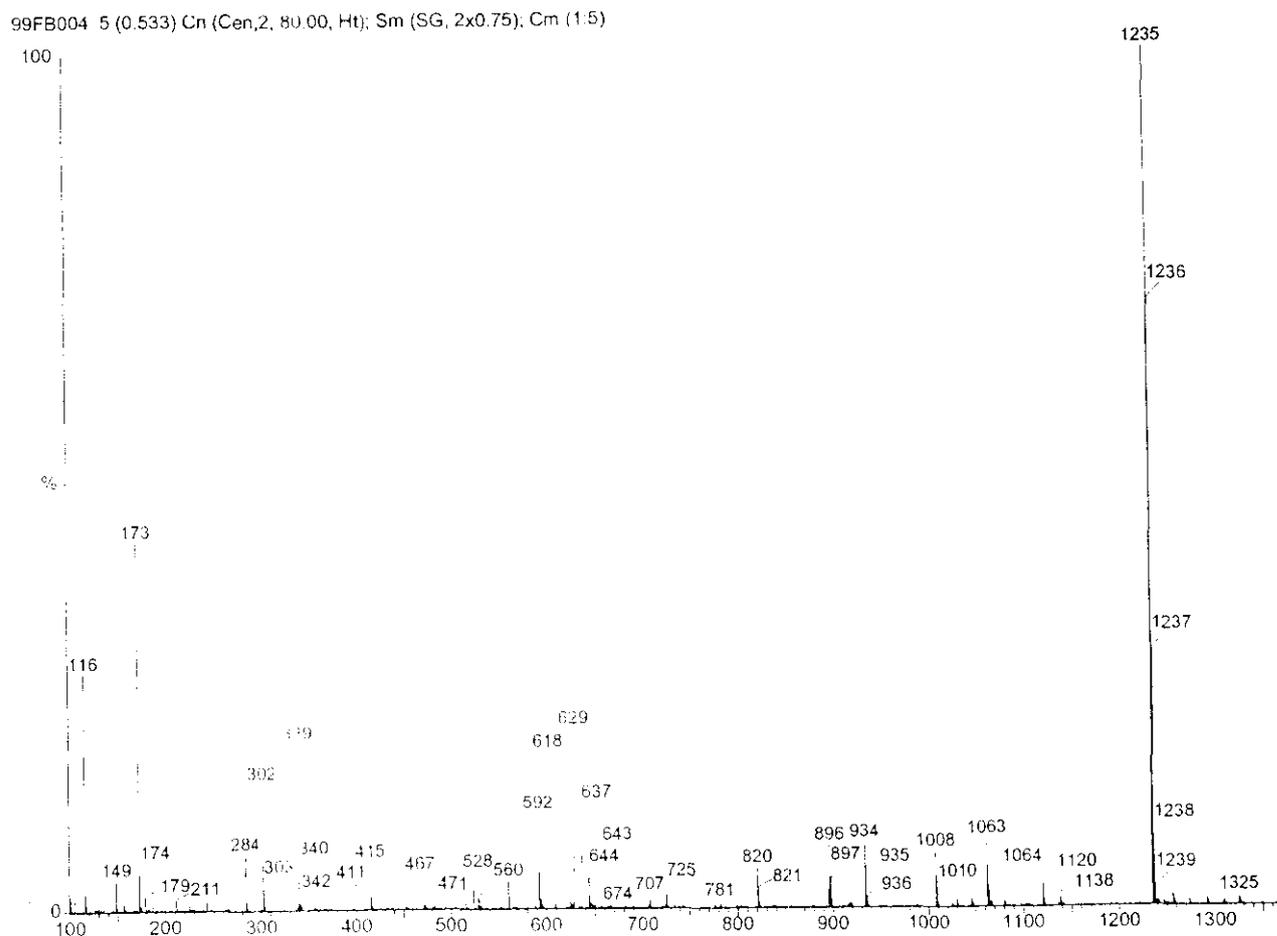
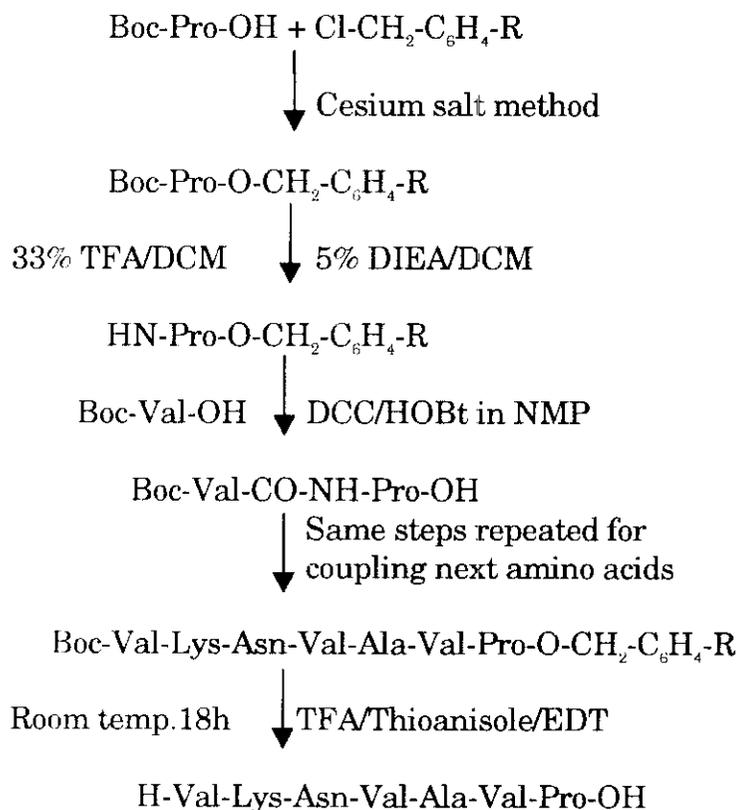


Figure III.3c

ESI MS of Pro-Leu-Gln-Pro-Gly-Val-Asp-Ile-Ile-Glu-Gly-Pro

iv. **Val-Lys-Asn-Val-Ala-Val-Pro (56-62)**

The synthesis of this heptapeptide was carried out on a chloromethylated 2% HDODA-PS resin with a chlorine capacity of 1.9 mmol/g. After attachment of first amino acid, the substitution level was found to be 1.7 mmol/g by picric acid method. All amino acids were attached by DCC/HOBt coupling in NMP. Double coupling was performed for each amino acid for maximum reaction. When the assembly of amino acids were over, the peptide was cleaved from the support using neat TFA in presence of thioanisole and 1,2-ethanedithiol. The synthetic steps involved are shown in Scheme III.12.



Scheme III.12. Solid phase synthesis of Val-Lys-Asn-Val-Ala-Val-Pro (56-62)

The purity of the peptide was checked on analytical HPLC using binary solvent system (0.1% TFA in water and 50% CH₃CN in 0.1% TFA). The chromatogram shows the extreme purity of peptide. The HPLC profile of crude and purified peptides are shown in Figure III.4a and Figure III.4b. The synthesized peptide was analysed by ESI MS (Figure III.4c). The observed molecular weight was in good agreement with the calculated value. Amino acid analysis was used for characterisation (Table III.4).

Table III.4 Details of the amino acid analysis of Val-Lys-Asn-Val-Ala-Val-Pro (56-62)

Amino acid	Actual value	Calculated value
Val	3	3.040
Lys	1	1.084
Asp	1	1.057
Ala	1	1.212
Pro	1	1.435

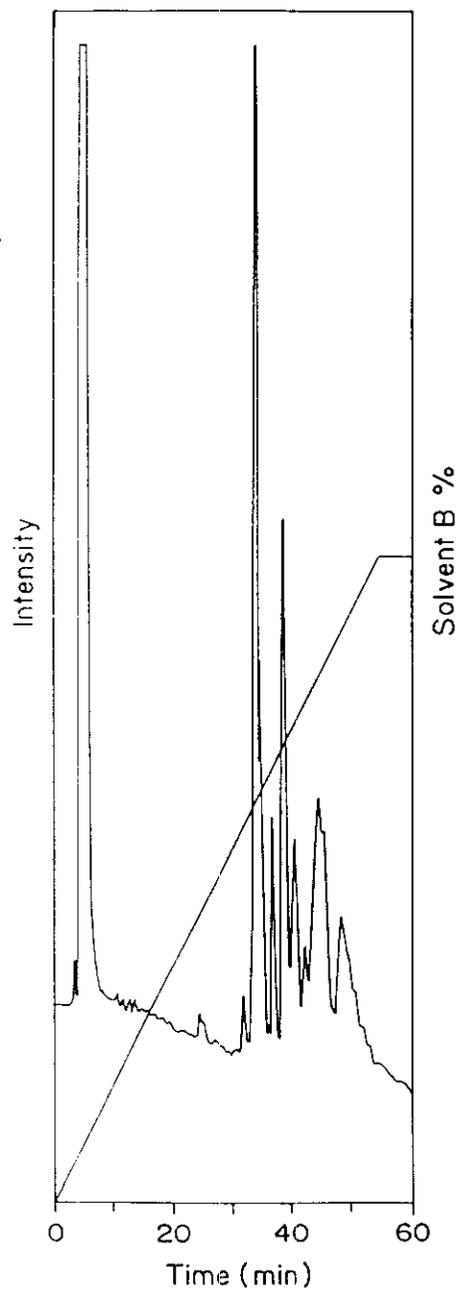


Figure III.4a

HPLC trace of crude Val-Lys-Asn-Val-Ala-Val-Pro
Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 230nm

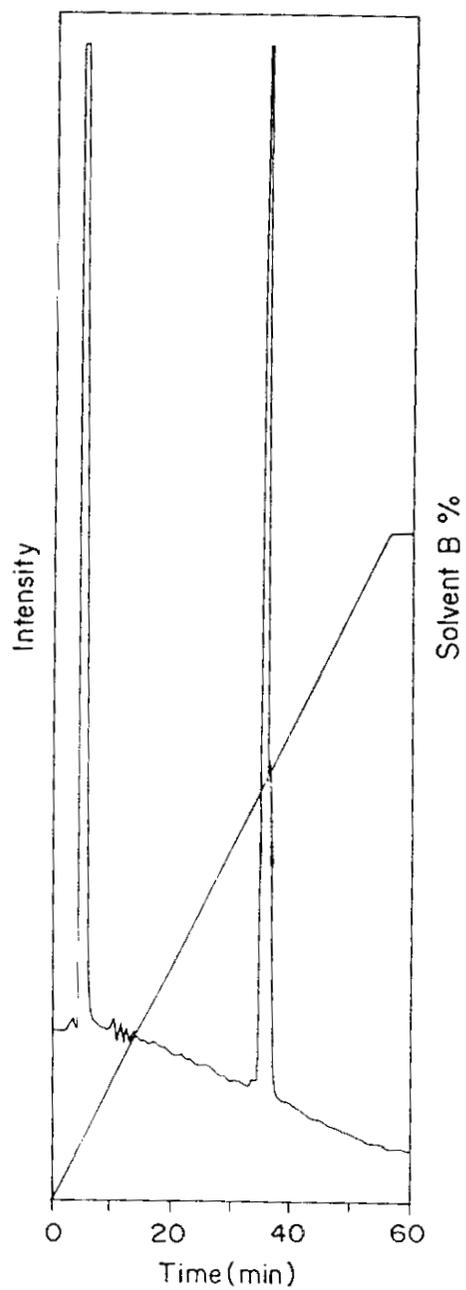


Figure III.4b

HPLC trace of pure Val-Lys-Asn-Val-Ala-Val-Pro
Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 230nm

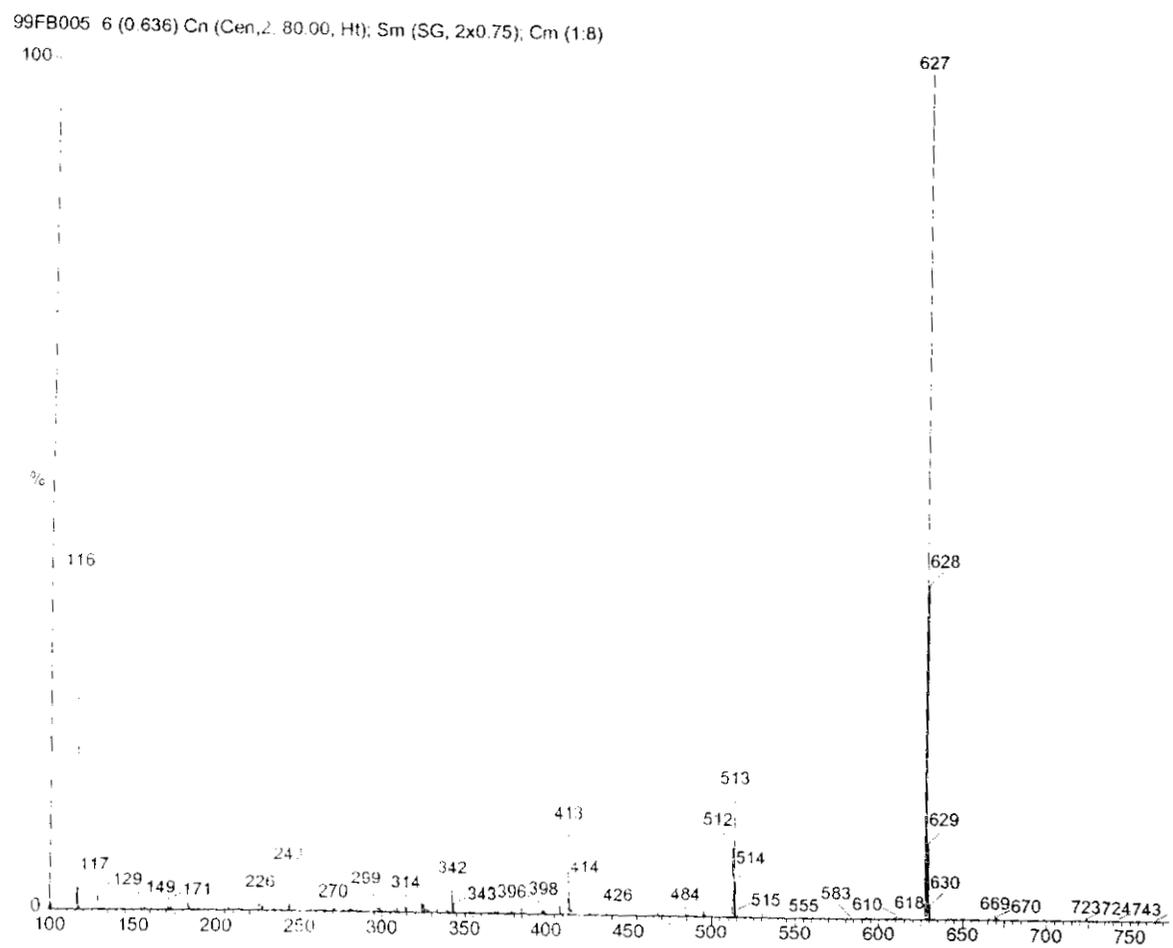
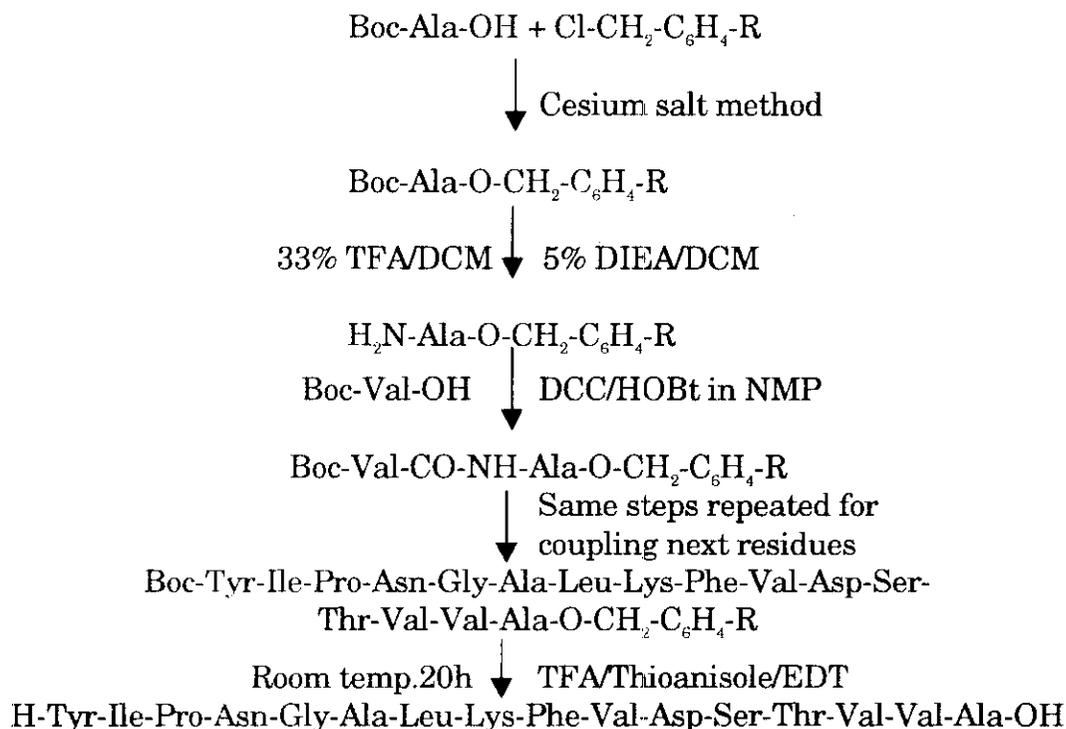


Figure III.4c ESI MS of Val-Lys-Asn-Val-Ala-Val-Pro

**v. Tyr-Ile-Pro-Asn-Gly-Ala-Leu-Lys-Phe-Val-Asp-Ser-Thr-Val-Val-Ala
(69-84)**

This sequence was synthesized using chloromethylated 2% HDODA-PS resin having a chlorine capacity of 1.9 mmol/g. Boc-Ala was esterified to the resin by Gisin's cesium salt method and the amino capacity was found to be 1.8 mmol/g by picric acid method. DCC/HOBt coupling in NMP was used for the attachment of all remaining amino acids. The progress of the coupling reaction was monitored by Kaiser test. The coupling time for the second amino acid was 60 min, and for each amino acid, an increment 5 min. was given. Rate of coupling was decreased much after 12th residue. The DCU precipitated was washed off with MeOH/DCM followed by washing with DCM. Coupling was done three times for each residue after 12th amino acid. Finally peptide was cleaved from the resin using neat TFA/thioanisole/EDT. The cleaved peptide was precipitated by ice cold ether. The protocol for synthesis is given below in Scheme III.13.



**Scheme III.13. Solid phase synthesis of 16 residue fragment (69-84)
of REF (R : HDODA - PS)**

The cleaved peptide was checked on analytical column using 0.1% TFA water and 50% CH₃CN in 0.1% TFA. Several deletion peptides are seen along with the target peptide. This was further purified on reverse phase column using TFA in water and CH₃CN. Because of solubility problem (sparingly soluble even in 100% CH₃CN, it is very difficult to purify whole peptide. Fraction collected from HPLC was subjected to amino acid analysis (Table III.5). Homogeneity of peptide was then checked on analytical column (Figure III.5).

Table III.5. Details of the amino acid analysis of Tyr-Ile-Pro-Asn-Gly-Ala-Leu-Lys-Phe-Val-Asp-Ser-Thr-Val-Val-Ala (69-84)

Amino acid	Actual value	Calculated value
Tyr	1	0.926
Ile	1	0.890
Pro	1	1.02
Asp	2	1.642
Gly	1	0.895
Ala	2	1.731
Leu	1	0.786
Lys	1	0.923
Phe	1	0.882
Val	3	2.653
Ser	1	0.836
Thr	1	0.942

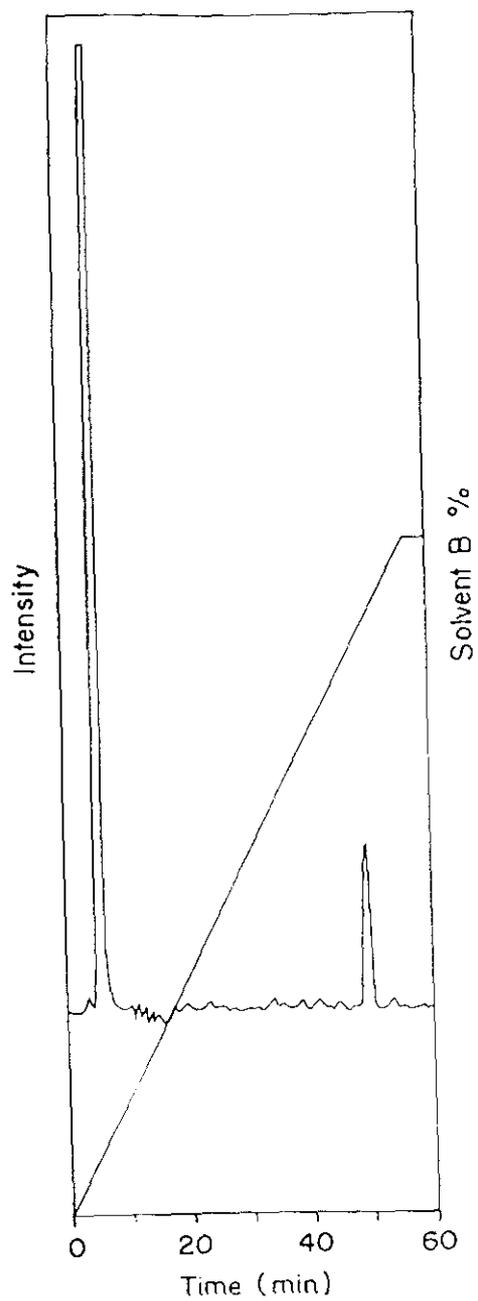


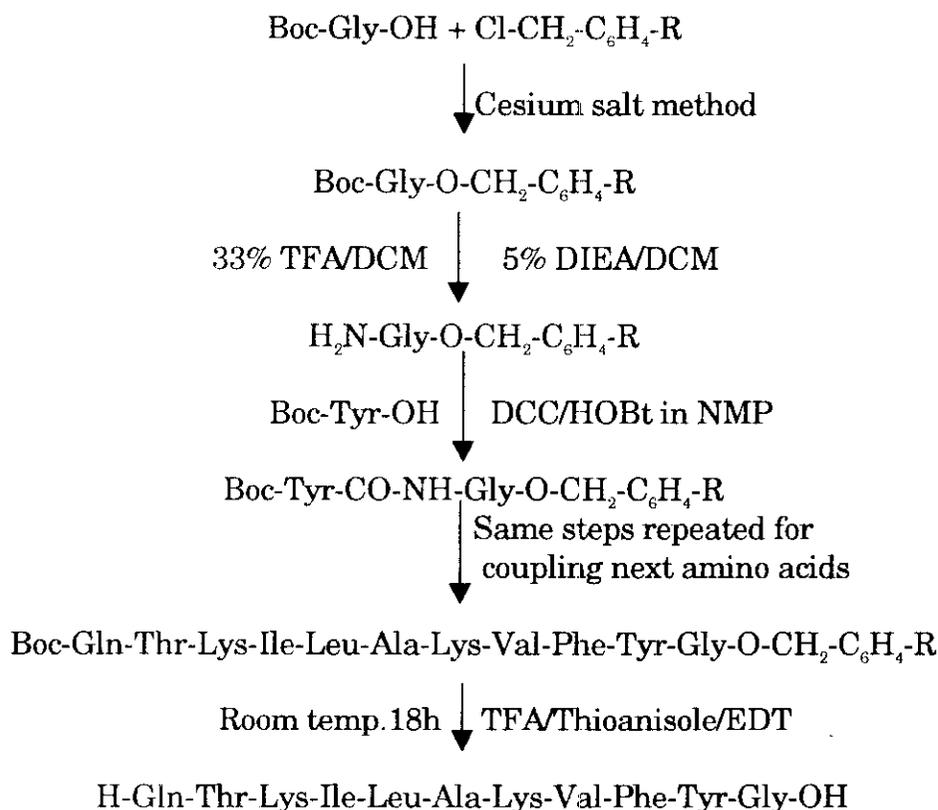
Figure III.5

**HPLC trace of pure Tyr-Ile-Pro-Asn-Gly-Ala-Leu-
Lys-Phe-Val-Asp-Ser-Thr-Val-Val-Ala**

**Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 280nm**

vi. Gln-Thr-Lys-Ile-Leu-Ala-Lys-Val-Phe-Tyr-Gly (125-135)

Boc-Gly was anchored to 2% HDODA-crosslinked chloromethyl polystyrene resin by cesium salt method and the amino capacity was found to be 1.8 mmol/g by picric acid method. Boc group was removed by 33% TFA in DCM followed by neutralisation. The remaining amino acids were attached to the amino acyl resin by HOBt active ester method. The coupling solvent used was NMP. A second coupling was carried out to ensure completion of reaction. The completion of coupling and deprotection were monitored by Kaiser test. When the desired sequence was attained, the peptide was cleaved from the support using TFA/thioanisole/EDT. The steps involved in the synthesis is given below in Scheme III.14.



Scheme III.14. Solid phase synthesis of Gln-Thr-Lys-Ile-Leu-Ala-Lys-Val-Phe-Tyr-Gly (R:HDODA-PS)

The cleaved peptide was checked on analytical column using 0.1% TFA in water (A) and 50% CH₃CN in A (Figure III.6a). This was further purified on reverse phase HPLC column using CH₃CN and 0.1% TFA in water. Homogeneity of peptide was checked on analytical column (Figure III, 6 b). The peptide was characterized by amino acid analysis (Table III.6). The synthesized peptide was analysed by ESI MS (Figure III.6c). The observed molecular weight was in good agreement with the calculated value.

Table III.6 Details of the amino acid analysis of Gln-Thr-Lys-Ile-Leu-Ala-Lys-Val-Phe-Tyr-Gly (125-135)

Amino acid	Actual value	Calculated value
Glu	1	1.37
Thr	1	1.23
Lys	2	2.32
Ile	1	1.05
Leu	1	1.32
Ala	1	1.26
Val	1	0.91
Phe	1	1.00
Tyr	1	1.27
Gly	1	1.29

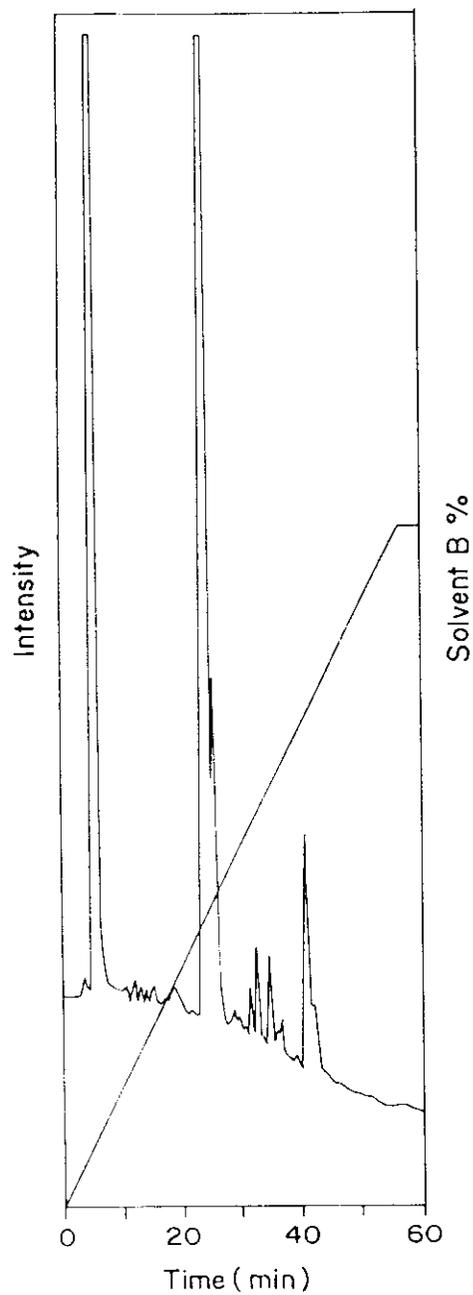


Figure III.6a

HPLC trace of crude Gln-Thr-Lys-Ile-Leu-Ala-Lys-Val-Phe-Tyr-Gly

**Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 280nm**

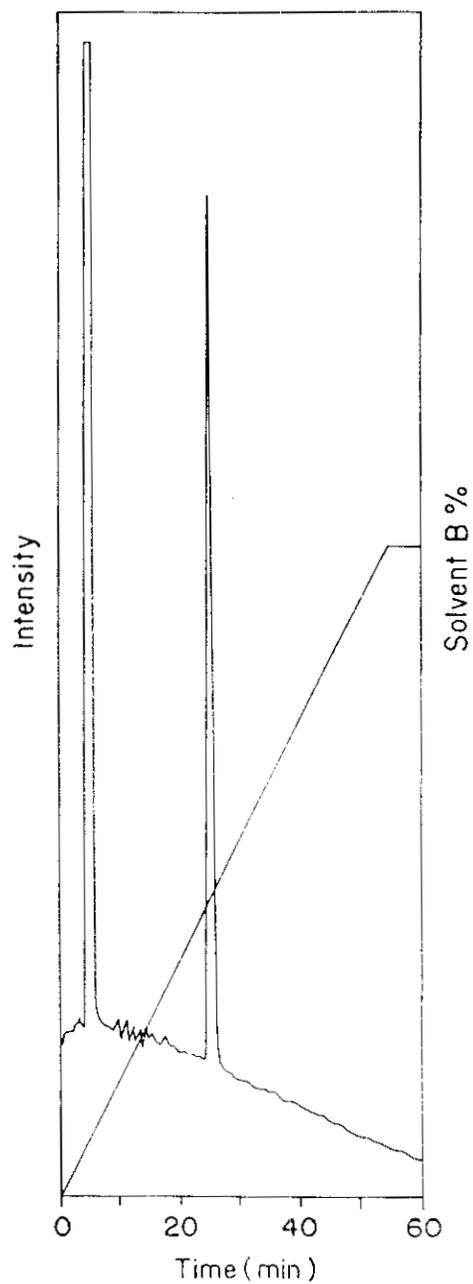
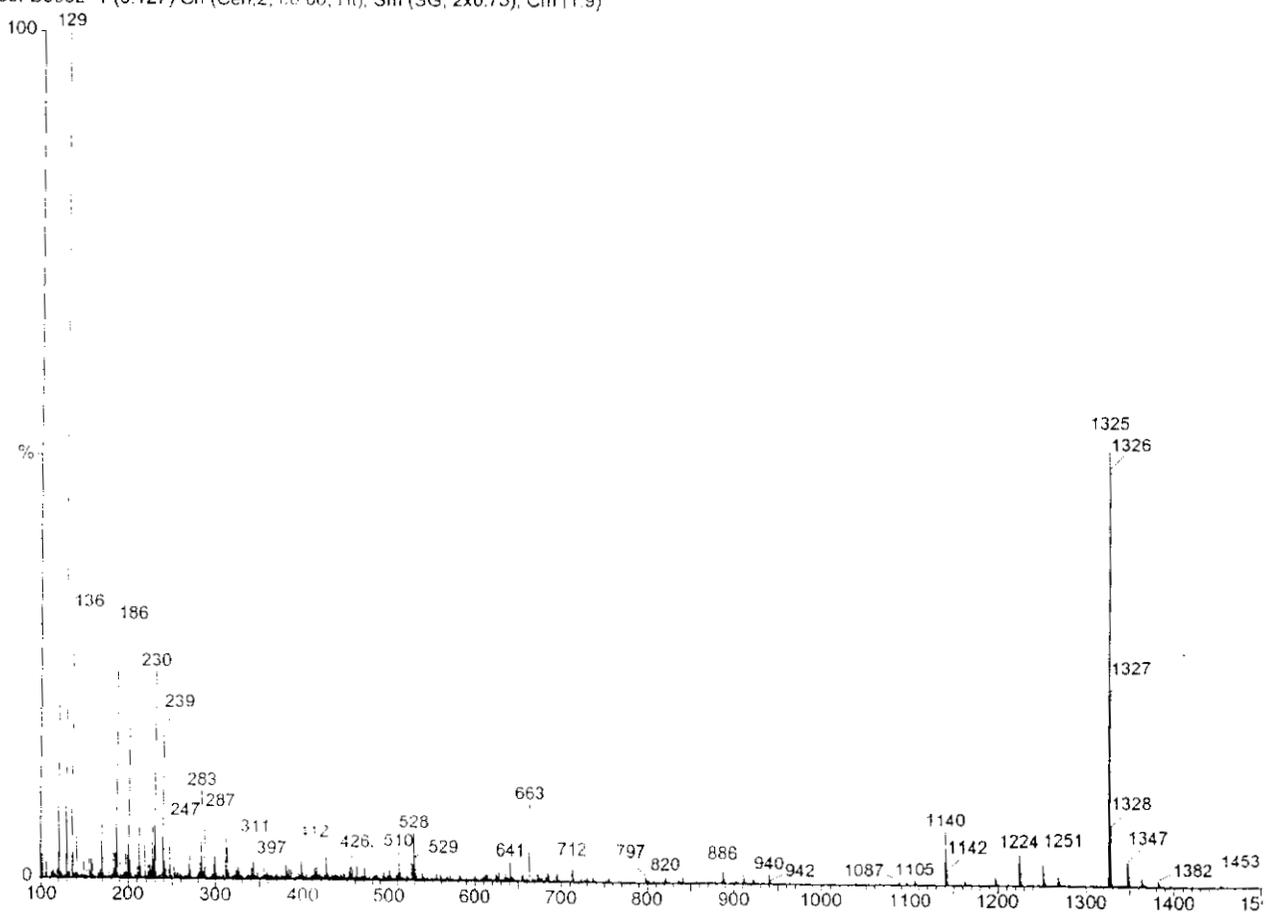


Figure III.6b

**HPLC trace of pure Gln-Thr-Lys-Ile-Leu-Ala-
Lys-Val-Phe-Tyr-Gly**

**Conditions: Solvent A (0.1% TFA in water),
Solvent B (50% acetonitrile in A) , 280nm**

99FB0062 1 (0.127) Cn (Cen,2, 80.00, Ht); Sm (SG, 2x0.75); Cm (1:9)

**Figure III.6c ESI MS of Gln-Thr-Lys-Ile-Leu-Ala-Lys-Val-Phe-Tyr-Gly**

From the foregoing investigation it is clear that HDODA-crosslinked polystyrene is an efficient support for solid phase peptide synthesis especially for hydrophobic peptides. Fragments of REF which suffer recovery problems due to its hydrophobic nature were synthesized in good yield and high purity by using 2 % HDODA crosslinked polystyrene support. It is clear that by the use of HDODA -PS and the right choice of the synthetic strategy, the synthesis of hydrophobic peptides can be effectively achieved.