Materials and Methods

The special chemicals required for the work are procured from the following firms.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-amino acids</td>
<td>Merck, Germany</td>
</tr>
<tr>
<td>Side chain protected L-amino acids</td>
<td>Sigma Chemical Company, USA</td>
</tr>
<tr>
<td>Boc Carbazate, HOBt, DCC, NMP and Boc ON</td>
<td>Sigma Chemical Company, USA</td>
</tr>
<tr>
<td>2% Crosslinked Merrifield resin (200 - 400 mesh size, 2.0 mmol of Cl/g), HDODA, TFA, DIEA, TEA and styrene</td>
<td>Aldrich Chemical Company, WI, USA.</td>
</tr>
</tbody>
</table>

All (L) amino acids used were Boc protected. The various side chain protecting groups used are given below.

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>Trp</td>
</tr>
<tr>
<td>Acm</td>
<td>Cys</td>
</tr>
<tr>
<td>2Cl(Z)</td>
<td>Lys</td>
</tr>
<tr>
<td>Mts</td>
<td>Arg</td>
</tr>
<tr>
<td>Z</td>
<td>Glu, Asp, Ser, Thr, Tyr</td>
</tr>
</tbody>
</table>

Purification of Solvents and Reagents

Dichloromethane (DCM): DCM was purified by keeping over anhydrous sodium carbonate and then distilled (B.P. 41° C). Kept in dark coloured bottle.

N-Methyl-2-pyrrolidone (NMP): Distilled under reduced pressure and kept over molecular sieve (2 Å) in dark coloured bottle.
Benzene: Dried using anhydrous calcium chloride and distilled.

Tetrahydrofuran (THF): THF was freed from peroxide by shaking with basic alumina, dried using sodium wire, till all the water was removed, distilled and kept in dark coloured bottle.

Trifluoroacetic acid (TFA): Distilled over 10% concentrated (v/v) sulphuric acid, to eliminate water and extraneous substances (Repeated distillation was avoided considering the chance of formation of anhydride). Kept in amber coloured bottle.

Diethyl ether: Dried over fused calcium chloride over night.

Ethyl acetate: Commercial ethyl acetate was distilled and used for extraction purposes.

Diisopropylethylamine (DIEA)/ Triethylamine (TEA): Distilled over ninhydrin and kept in dark coloured bottle.

1-Hydroxybenzotriazole (HOBt): HOBt was crystallized from hot water or 70% ethanol.

Preparation of Boc azide

$t$-Butyl carbazate (30 g) was dissolved in glacial acetic acid (27 ml) and water (37.5 ml). This was cooled in an ice bath with constant stirring, sodium nitrite (17.4 g) was added slowly over a period of 15 min. After 90 min., the oily layer formed was separated. The aqueous layer was extracted with ether (3 x 20 ml). The ether extracts were mixed with oil fraction, washed with water
and saturated solution of sodium bicarbonate and dried over sodium sulphate. On evaporating ether under reduced pressure, Boc azide was obtained as golden yellow liquid. This was used without any further purification and stored at 4°C. Yield:30 ml.

**Preparation of Boc Amino acids**

*Schnabel's procedure*302

The amino acid (10 mmol) was suspended in 1:1 dioxane-water mixture (10 ml) and Boc azide (10 ml) was added to it. The solution was stirred at room temperature and the pH was brought to 8-10 by the addition of 4N NaOH (Particulars of individual amino acid are given below.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>10</td>
</tr>
<tr>
<td>Leu</td>
<td>10</td>
</tr>
<tr>
<td>Pro</td>
<td>8-9</td>
</tr>
<tr>
<td>Ala</td>
<td>10</td>
</tr>
<tr>
<td>Gin</td>
<td>8-9</td>
</tr>
<tr>
<td>Phe</td>
<td>9-10</td>
</tr>
<tr>
<td>Ile</td>
<td>10</td>
</tr>
<tr>
<td>Val</td>
<td>10</td>
</tr>
</tbody>
</table>

After 24 hours, water (25 ml) was added to the mixture and ether washings were done. The aqueous layer was acidified to pH 2 with 2N HCl, and Boc amino acid was extracted with ethyl acetate (3 x 20 ml). For Boc Leu, diethyl ether was used instead of ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed. Boc amino acids,
obtained as oil, precipitated on trituration with petroleum ether (B.P. range 40 - 60°C). The amino acids were dried and stored at room temperature. Boc Val and Boc Phe failed to crystallize, and they stored as their dichloromethane solution at 4°C.

**t-Butyloxy carbonyloximino-2-phenylacetonitrile (Boc ON) Method**

This method is used for the amino protection of amino acids having amide side chain groups i.e. Gln and Asn

Amino acid (10 mmol) was added to a mixture of Boc ON (11 mmol), TEA (11 mmol) and 1:1 dioxane-water mixture (12 ml). The mixture was stirred at room temperature for 12 hours. The reaction mixture was diluted with water (20 ml) and washed with ethyl acetate (25 ml x 2). The aqueous layer was acidified to pH 2.0 with 2N HCl and extracted with ethyl acetate (20 ml x 2). The organic layer was dried over sodium sulphate and evaporated to obtain Boc Gln. Yield 80%.

**Purity of Boc Amino Acids**

Purity of all protected amino acids was checked by comparing the physical constants (tlc and melting points) with authentic samples. Tlc was done on silica plates (solvent system CHCl₃-MeOH-AcOH, 85:10:5 v/v). Amino acids were visualized by ninhydrin spray after their exposure to HCl vapour for 10 minutes.

**Preparation of 2% HDODA-crosslinked Polystyrene**

Styrene (10 ml) was destabilized by washing with 1% NaOH solution (3 x 15 ml), washed with water (3 x 10 ml) and dried over anhydrous calcium
chloride. One % solution of PVA (Average mol. wt. 75,000) in water (110 ml) was prepared and kept stirred in a polymerization vessel at 8 °C with a constant flow of N\textsubscript{2} through the solution. A mixture of 1,6-hexanediol diacrylate (0.45 ml, 2 mol%), styrene (11.33 ml, 98 mol%), toluene (2.31 ml, 20 vol% of monomer ratio) as diluent and dibenzoyl peroxide (600 mg) was prepared and added to the PVA solution. The stirring continued for 8 hours. Polymer beads were collected by filtration through a sintered disc G3, and washed thoroughly with hot water (3 x 20 ml, 3 min.) to remove PVA, acetone (20 ml x 3, 3 min.) and methanol (20 ml x 3, 3 min.). The polymer was then Soxhletted using acetone, DCM, and methanol to remove linear polymers. Yield: 9 g. The polymer beads were sieved. Beads of 200 - 400 mesh sizes were used for peptide synthesis.

**Functionalisation of support - Chloromethylation**

**Preparation of chloromethyl methylether\textsuperscript{242}**

To a mixture of formaldeyde (66 ml) and methanol (33 ml), kept at 0 °C, passed a constant flow of dry HCl. The formation of ether was indicated by the appearance of oily layer, after one hour. Administration of HCl was continued for half an hour more till the ether was separated clearly from the aqueous phase. The oily layer was separated and dried over calcium chloride. Yield: 45 ml. This was used without further purification.

**Preparation of 1M Zinc chloride in THF\textsuperscript{244}**

Anh. ZnCl\textsubscript{2} (1.5 g) was placed in an Erlenmeyer flask. Conc. HCl (3 drops) and water (5 drops) were added. The solution was heated till the solid was dissolved completely. Heating was continued till a solid mass of ZnCl\textsubscript{2} was
left which was melted on further heating. When it became a mobile liquid the flask was kept in a desiccator, and allowed to cool. The solid was dissolved in THF (10 ml) and kept sealed.

**Chloromethylation of the polymer support**

The dry resin beads (2 g.) was kept in DCM (20 ml) in an R.B. flask. After half an hour CMME (12 ml) and a solution of ZnCl₂ (1M, 0.4 ml) in THF was added to the swollen resin. The mixture was kept at 50 °C for 5 hours. The resin was filtered, washed with THF (20 ml x 3, 3 min), THF/4N HCl (20 ml x 3, 3 min.) THF-water (1:1 v/v)(20 ml x 3, 3 min.), water (20 ml x 3, 3 min.) and finally with methanol. The resin was dried under vacuum.

**Estimation of chlorine capacity: pyridine fusion method.**

To the chloromethylated resin (200 mg) added 5 ml of pyridine and kept at 110 °C for 5 hours. The mixture was quantitatively transferred with acetic acid-water (1:1 v/v 30 ml) and diluted with water (25 ml). Conc. HNO₃ (7 ml) and AgNO₃ (0.1 N, 10 ml) were added to this solution and titrated against standard ammonium thiocyanate solution (0.1 N) using ferric alum as indicator. A blank was also performed.

**General Methods of Solid Phase Peptide Synthesis**

*Attachment of first amino acid*

**Cesium salt method**

Boc amino acid (4.6 mmol) was dissolved in ethanol (10 ml). By the slow addition of saturated solution of cesium carbonate the pH of the solution was brought to 7. The ethanol was evaporated under vacuum, and water was
removed by azeotropic distillation with dry benzene, till white powder of cesium salt of amino acid was obtained.

The cesium salt of Boc amino acid was dissolved in 10 ml of NMP and the dried chloromethylated resin was added. The suspension was kept at 50 °C in an oil bath, with occasional shaking, for 48 hours. The resin was filtered, washed with NMP, NMP-water mixture (1:1 v/v), methanol and DCM. The amino acid resin was dried under vacuum and the weight was noted.

*Estimation of first amino acid attachment*

*Picric acid method*\(^\text{305,306}\)

Two mg of deprotected resin was taken in a 5 ml sintered funnel or Pasteur pipette with cotton at the narrow end. It was treated with 0.1 M DCM solution of picric acid for 5 min. The unbound picric acid was washed off with DCM washings. The picric acid bound to the resin was separated and collected with 5% TEA in DCM (2 x 2 ml, 2 min.) followed by DCM wash (3 x 2 ml, 2 min.). 0.2 ml of the eluate was dissolved to 2 ml with 95% ethanol, and the absorbance at 358 nm was measured. TEA picrate complex in ethanol has \( \varepsilon_{358} = 14,500 \). From the OD of the picrate solution, the amount of amino acid substituted on the resin could be calculated.

*Deprotection procedure*

Amino protected amino acid or peptide bound to polymer was treated with 30% TFA (in DCM) for 30 min. The TFA solution was filtered and the resin was washed with DCM. This was then treated with 5% DIEA in DCM
(5 min.) and 5% DIEA in NMP-DCM mixture (1:1 v/v) to get the free amino acid resin or peptide bound resin.

**Coupling methods**

Symmetrical anhydride method and HOBT active ester method were used for the formation of peptide bond.

**Anhydride method**

A mixture of 2 mmol of amino acid and 1 mmol of dicyclohexyl carbodiimide (DCC) was added to one mmol of amino resin in DCM. The mixture was shaken for 1/2 hour, and washed with MeOH-DCM mixture (33:66 v/v, 5 x 3 ml, 2 min.) and DCM (3 x 5 ml, 2 min.).

**HOBT-active ester method**

Active esters of amino acid were prepared by adding 2.5 mequiv. of HOBT and 2.5 mequiv. of DCC to a solution of 2.5 mequiv. of amino acid in NMP. This was stirred for 5 min., and the HOBT ester of amino acid was added to the amino resin/peptidyl resin (1.0 mequiv.). The mixture was shaken for 45 minutes. DMSO (15% of total volume of coupling medium) was added to the mixture and shaken for 15 minutes. At the end of 15 minutes DIEA (3.8 mequiv.) was added. Filtered off the solution after 5 minutes, washed the resin with DCM-MeOH (67:33 v/v), (5 ml x 3, 2 min.), DCM (5 ml x 3, 2 min.) and NMP (5 ml x 3, 2 min.).
Cleavage of the Peptide from the Support:

TFA/Thioanisole method

The peptide resin (100 mg) was suspended in 10 ml TFA containing 1 ml each of thioanisole, m-cresol, and ethanedithiol. The mixture was gently stirred for 12 - 15 hours. The TFA solution was filtered through a sintered funnel, and the resin was washed with TFA (2 ml x 3). The filtrate and the washings were collected. TFA was removed under reduced pressure. The peptide solution thus obtained was cooled, and ice cold ether was added to it. The precipitated peptide was washed with ether 8 -10 times, to remove the low molecular weight organic impurities. The peptide was dried and the yield was noted. The resin was subjected to a second cycle of cleaving to ensure complete removal of peptide from the support.

Checking the Homogeneity of the Peptide

Thin layer chromatography (tlc)

Shorter peptides upto the size of five residues were characterized by tlc. The purity was assessed from the number of spots (visualized by ninhydrin spray) on the tlc plate. Solvent system used was pyridine-acetic acid-water = 50:15:30 v/v.

Fast protein liquid chromatography (FPLC)

The crude peptide was dissolved in methanol and loaded into the column of the FPLC (analytical silica, reversed phase C18 column - Pep RPC 5/5 from Pharmacia Fine Chemicals, AB, Sweden). Detection was done at 214 nm (UV detector). The chromatogram was taken using binary gradient
solvent system with a flow rate of 0.5 ml/min. (CH₃CN and water containing 0.1 TFA).

High performance liquid chromatography (HPLC)

The crude peptide was dissolved in methanol and loaded in the column of HPLC (Lichrosorb RP - 18) and the solvent system was water and acetonitrile containing 0.1 TFA. Detection at 220 nm, flow rate 1 ml/min.

Amino acid analysis

Amino acid analysis was used for the characterization of peptides. A known amount of peptide (0.1 - 1mg) was taken in a hydrolysis tube, 300 μl of TFA-6N HCl (1:2) was added and the tube sealed under vacuum and kept for overnight at 110 °C (for hydrophobic peptides the time may extends up to 48 hours). The tube was broke open and dried over NaOH and P₂O₅ in a desiccator under vacuum. The residue was dissolved in amino acid analysis loading buffer and aliquot was loaded in the analyzer, such that every amino acid was expected in 3-10 mmol range. Amino acid standards were run after every 5 runs and compared for reproducibility. Amino acid analyses were performed on LKB 4151 ALPHA PLUS amino acid analyzer using ninhydrin detection.

IR studies peptidyl resin

Peptidyl resin (2 mg), was pulverized giving little shear stress. KBr (300 mg) was also added to it and triturated. Little pressure was given for mixing. When homogeneous solid solution was obtained it was pressed in hydraulic press to get the disc. The disc was dried in a desiccator, containing P₂O₅. The
region under investigation was 1700 - 1600 cm\(^{-1}\) (amide I). The spectra were recorded on a Shimadzu -470 A double beam spectrophotometer.

**NMR studies of the peptide**

The peptide was dissolved in TFA as it was insoluble in all other solvents. All the NMR experiments were performed in a 400 MHz JEOL - GSX Supercon NMR spectrophotometer. The experiments were carried out at 25 °C. NOESY, DQF COSY & TOCSY experiments were performed to get information about the 3D structure of the 31 residue peptide. The sample had maximum NOE transfer at 25 °C with mixing time of 350 ms. TOCSY was performed for a spin lock period of 60 ms. and 100 ms. All NMR experiments were carried out in a phase sensitive mode with States et al. procedure.\(^{269}\)

**Solid Phase Syntheses of Peptides**

**Synthesis of (Ala)\(_3\)** on chloromethylated HDODA -PS.

**Attachment of Boc Ala on chloromethylated support**

Boc Ala (1 mmol) was dissolved in 1 ml ethanol. The pH of the solution was brought to 7 by the slow addition of saturated solution of cesium carbonate. The solution was stirred for one hour maintaining the pH at 7. Ethanol was removed by rotary evaporation and water by azeotropic distillation with benzene. White powder of cesium salt of Boc Ala thus obtained was kept in a vacuum desiccator containing P\(_2\)O\(_5\). Chloromethylated HDODA-PS (0.2 gm, 0.462 mmol of Cl) was added to the dried cesium salt of Boc Ala dissolved in NMP (3 ml). The mixture was gently stirred at 50 °C for 36 hours. The resin was filtered through a sintered funnel (G2), washed with NMP (5 ml x 3, 3 min.), NMP-Water (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.) NMP-water
mixture (1:1 v/v) (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.), DCM (5 ml x 3, 3 min.) and methanol (5 ml x 3, 3 min.). The resin was kept in a vacuum desiccator containing P2O5 over night. 250 mg of dried resin was obtained.

Estimation of the substitution level of Boc Ala on the support.

Two milligram of Boc deprotected resin was weighed accurately and transferred to a Gisin's tube. The resin was swollen with DCM. 0.1 M Picric acid (1 ml x 3, 5 min.) was added and the excess picric acid was washed with DCM. One millilitre of 10% TEA (in DCM) was added to the resin in portion, and the liberated picric acid was collected. The resin was washed with 95% ethanol. The solution was made upto 10 ml and optical density (OD) of the solution was measured at 358 nm. From the OD the substitution of amino acid was calculated.

NH₂ capacity of the resin: 2 mmol/g.

Coupling of second and third Alanine

200 mg of Boc Ala was taken in a peptide synthesizer. It was subjected to the following cycle of operation.

1. Washed the resin with DCM (5 ml x 3, 2 min.).
2. Added 30% of TFA (in DCM) (5 ml), shaken for 30 min.
3. Filtered off the TFA solution.
4. Washed the resin DCM (5 ml x 6, 2 min.).
5. Added 5% DIEA in DCM (5 ml), shaken for 5 min.
6. Filtered off the DIEA solution, washed the resin with DCM (5 ml x 6, 2 min.).
7. Added 2 ml DCM, Boc Ala (0.8 mmol), and DCC (0.4 mmol).

8. Filtered off the solution, washed with DCC-MeOH mixture (66:37 v/v, 5 ml x 6, 3 min.).


10. Removed 2 - 3 beads and tested for free -NH₂ group (Ninhydrin test) negative.

11. Steps 1 to 10 for the coupling of third alanine.

Weight of (Ala)₃ resin: 305 mg.

**Cleavage of the peptide from the support.**

To 100 mg of peptidyl resin, 10 ml of TFA and 1 ml of thioanisole were added and stirred the mixture at room temperature. After 12 hours the peptide solution was filtered, the resin was washed with TFA (2 ml x 2). The TFA washings and the filtrate were combined. TFA was removed under reduced pressure. The thick fluid thus obtained was cooled, and cold ether was added to this. The precipitated peptide was washed with cold ether (5 ml x 6), and dried. Yield :35 mg (~95%)
tlc on silica plate: Rf = 0.85 (Pyridine-Water-Acetic acid::50:15:30 v/v), Single spot.

**Synthesis of Ala-Ala-Gly (PepH-1)**

*Attachment of Boc Gly on the chloromethylated HDODA-PS.*

To Boc Gly (0.19 g, 1.2 mmol) in ethanol (5 ml), a saturated solution of cesium carbonate was added till the pH of the solution reached 7.0. The
solution was stirred for 2 hours, ethanol was rotaevaporated under reduced pressure. Water was removed by azeotropic distillation with dry benzene. The white powder of cesium salt of Boc-Glycine was dried under vacuum in presence of $\text{P}_2\text{O}_5$. The salt was dissolved in NMP (5 ml), and to this added 300 mg of chloromethylated resin (capacity 2.05 mmol/g). The mixture was kept at 50 °C for 36 hours. The resin was filtered, washed with NMP (5 ml x 3, 3 min.), NMP-water (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.) NMP-water mixture (1:1 v/v) (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.), DCM (5 ml x 3, 3 min.) and methanol (5 ml x 3, 3 min.). The resin was kept in a vacuum desiccator containing $\text{P}_2\text{O}_5$.

Weight of the resin : 370 mg. The substitution level : 1.96 mmol of Glycine/g of the resin (by picric acid method).

**Synthesis of Ala-Ala-Gly**

300 mg of Boc glycine bound resin (0.59 mmol of $\text{NH}_2$) was used for the synthesis of the tripeptide. The protocol involves the following cycle of operations.

1. Washed the resin with DCM (10 ml x 3, 2 min.).
2. Added 30 % of TFA (in DCM) (10 ml), shaken for 30 min.
3. Filtered off the TFA solution.
4. Washed the resin DCM (10 ml x 6, 2 min.).
5. Added 5% DIEA in DCM:NMP (1:1, v/v) (10 ml), shaken for 5 min.
6. Filtered off the DIEA solution, added 5% DIEA (in NMP, 10 ml).
7. Filtered off the solution, washed the resin with NMP (10 ml x 3, 2 min.).
8. Added active esters of Boc-alanine with HOBt (prepared by stirring 1.475 mmol of Boc-Ala, 1.475 mmol of HOBt and 1.475 mmol of DCC in 3 ml of NMP. DCU formed during the reaction was filtered off before the addition of active ester to the resin). Shaken for 45 min.

9. Added DMSO (0.75 ml) solvent, shaken for 15 min.

10. Added 3.8 mequiv. of DIEA, shaken for 5 min.

11. Filtered off the solution, washed with MeOH-DCM (33:67)-(10 ml x 6, 5 min.) and with NMP (10 ml x 3, 3 min.).

12. Repeated steps 8 to 11 (second coupling).


14. Repeated steps 1 to 13 for coupling next Boc Ala.

Weight of the tripeptide resin (PepH1): 450 mg.

*Cleavage of Ala-Ala-Gly from the Support*

Peptidyl resin (100 mg) was treated with TFA (5 ml) and thioanisole (0.5 ml). The solution was gently stirred for 12 hours at room temperature. The TFA solution was filtered, washed the resin with TFA (2 ml x 3). The washings and TFA solutions were combined. TFA was removed under reduced pressure. Cold ether was added to the oily liquid. The precipitated peptide was washed with cold ether (5 ml x 6). The peptide was dried under vacuum. Yield 38 mg (82%). tlc (Silica plate) : Rf = 0.77, single spot.

(solvent system : Pyridine- Water- Acetic acid, 50: 15: 30 v/v).
**Synthesis of Val-Ala-Ala-Gly (PepH 2)**

300 mg of PepH1 resin (capacity 0.42 mmol of NH₂) was used for the incorporation of Val. Boc group of the tripeptide (Ala-Ala-Gly) resin was removed using 30% TFA (10 ml x 30 min.). The neutralization was employed using 5% DIEA solution (DCM:NMP mixture, 1:1 v/v, 10 ml). Active esters of Boc Val (1.05 mmol each of Boc Val, DCC, HOBT were stirred together in 3 mol of NMP, the solution was added to the resin after filtering off the DCU) was added to the resin and shaken for 45 min. DMSO (0.75 ml) was added and shaken for 15 min., DIEA (3.8 mequiv) was added at the end of one hour. The resin was washed with MeOH-DCM (33:67 v/v, 10 ml x 3, 5 min.) and NMP (10 ml x 3, 2 min.). The second coupling of Val was again conducted. Weight of peptidyl resin:330 mg.

**Cleavage of tetrapeptide from the support**

To the peptidyl resin (100 mg), TFA (5 ml) and thioanisole (0.5 ml) was added and the mixture was kept for 12 hours at room temperature. The TFA solution was filtered, and the resin was washed with TFA (2 ml x 3). The washings and the TFA solutions were combined and distilled at reduced pressure to remove the TFA. Cold ether (10 ml) was added to the peptide solution. The precipitated peptide was washed with cold ether (10 ml x 5). Yield of peptide: 38 mg.

**tlc (silica plate)** Rf = 0.833 (pyridine: water: acetic acid, 50:15:30) single spot.
Synthesis Val-Ala-Val-Ala-Ala-Gly (PepH 3)

PepH 2 resin (100 mg, capacity 0.12 mmol of NH₂) was taken for the attachment of Ala and Val sequentially. The protocol for a cycle of operation is given below.

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagent</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCM</td>
<td>5 ml x 3, 2 min.</td>
</tr>
<tr>
<td>2</td>
<td>30% TFA in DCM</td>
<td>10 ml, 30 min.</td>
</tr>
<tr>
<td>3</td>
<td>DCM</td>
<td>10 ml x 3, 2 min.</td>
</tr>
<tr>
<td>4</td>
<td>DIEA (5% in DCM:NMP, 1:1 v/v)</td>
<td>5 ml, 5 min.</td>
</tr>
<tr>
<td>5</td>
<td>DIEA (5% in NMP)</td>
<td>5 ml, 5 min.</td>
</tr>
<tr>
<td>6</td>
<td>NMP wash</td>
<td>10 ml x 3, 2 min.</td>
</tr>
<tr>
<td>7</td>
<td>HOBut ester of Boc Ala (0.3 mmol each of Boc Ala, DCC, HOBut in NMP)</td>
<td>5 ml, 45 min.</td>
</tr>
<tr>
<td>8</td>
<td>DMSO</td>
<td>0.75 ml, 15 min.</td>
</tr>
<tr>
<td>9</td>
<td>DIEA</td>
<td>3.8 mequiv., 5 min.</td>
</tr>
<tr>
<td>10</td>
<td>MeOH-DCM (33:67 v/v)</td>
<td>10 ml x 4, 2 min.</td>
</tr>
<tr>
<td>11</td>
<td>NMP</td>
<td>5 ml x 3, 2 min.</td>
</tr>
<tr>
<td>12</td>
<td>Repeat step 7-11</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Kaiser Test</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Repeat step 1-13 for Boc Val</td>
<td></td>
</tr>
</tbody>
</table>

Weight of peptidyl resin: 118 mg.

Cleavage of peptide was done by TFA/thioanisole method. Peptide (100 mg) was treated with TFA (5 ml) and thioanisole (0.5 ml). The mixture was kept for 15 hours. TFA solution was collected and evaporated under reduced pressure. Upon addition of cold ether (10 ml) peptide got precipitated. It was washed with cold ether (10 ml x 6) to remove organic impurities. Yield of peptide: 42 mg.
The peptide was dissolved in methanol and loaded in hplc (C18, rpc, flow rate 0.5 ml/min., detection at 210 nm, solvent system: water-acetonitrile, containing 0.1% TFA)

Synthesis of Glu-Val-Glu-Leu-Gly (PepH 4)

Glycine bound resin (200 mg, 1.96 mmol of NH2/g) was used for the synthesis of this sequence and for PepH 5. The general protocol for a cycle of operation involves the following steps.

1. Washed the resin with DCM (5 ml x 3, 2 min.).
2. Added TFA (30 % in DCM, 5 ml), shaken for 30 min.
3. Filtered off the TFA solution.
4. Washed the resin DCM (5 ml x 5, 2 min.)
5. Added 5% DIEA in DCM-NMP (1:1 v/v) (5 ml), shaken for 5 min.
6. Filtered off the DIEA solution, added 5% DIEA (in NMP, 5 ml).
7. Filtered off the solution, washed the resin with NMP (5 ml x 3, 2 min.).
8. Added active ester of Boc-Leu with HOBt (prepared by stirring 0.980 mmol each of Boc-Leu, HOBt and DCC in NMP. DCU formed during the reaction was filtered off before the addition of active ester to the amino acid resin). Shaken for 45 min.
9. Added DMSO (15% of total volume of the NMP used as coupling solvent), shaken for 15 min.
10. Added 3.8 mequiv. of DIEA, shaken for 5 min.
11. Filtered off the solution, washed with MeOH-DCM (33:67 v/v, 5 ml x 6, 5 min.) and with NMP (5 ml x 3, 3 min.).
12. Repeated steps 8 to 11.
14. Repeated steps 1 to 13 for Boc Glu, Boc Val and Boc Gln.

Weight of peptidyl resin: 405 mg.

**Cleavage of the peptide**

PepH4 resin (100 mg) was treated with TFA (5 ml) and thioanisole (0.5 ml) and stirred at room temperature for overnight. The TFA solution was collected and the resin was washed with TFA (3 ml x 2) and combined the washings with TFA solution. TFA was removed under reduced pressure. Cold ether was added to the solution. The precipitated peptide was washed with cold ether (10 ml x 10). Yield of peptide: 45 mg. tlc (silica plate) Rf. 0.68 (single spot); solvent system, pyridine-water-acetic acid:: 50:15:30 v/v.

**Synthesis of Gln-Val-Gly-Gln-Val-Glu-Leu-Gly (Pep H5)**

200 mg of PepH4 resin (capacity 0.98 mmol of NH2/g) was used for the subsequent attachment of Gly, Val and Gln. The deprotection was carried out with TFA (30% TFA in DCM, 10 ml) and neutralization with 5% DIEA (in DCM-NMP mixture 1:1 v/v) and 5% DIEA (in NMP). Boc amino acid (2.4 mmol) in active ester form with HOBT was used for coupling reaction. The protocol was exactly similar to that used earlier synthesis.

**Cleavage of the peptide**

Peptidyl resin (100 mg) was treated with TFA (5 ml), thioanisole (0.5 ml), m-cresol (0.5 ml) and ethanedithiol (0.5 ml). The mixture was stirred at room temperature for 20 hours. The resin was filtered and the TFA was removed under reduced pressure. Cold ether was added to precipitate the oily liquid.
thus obtained. The peptide was washed with cold ether (10 ml x 10) to remove organic impurities. Yield of peptide: 50 mg.

The peptide was dissolved in methanol and loaded in an hplc column. (analytical C18 rpc, Flow rate 0.5 ml/min. Solvent system, Water:acetonitrile, containing 0.1% TFA.).

**Solid -Phase Synthesis of Peptides on Merrifield Support**

**Synthesis of Ala-Ala-Gly (pepM 1)**

**Attachment of Boc Gly on Merrifield System**

Boc Gly (0.19 g, 1.2 mmol) was dissolved in ethanol (5 ml) and the pH of the solution was brought to 7 by the slow addition of saturated solution of cesium carbonate. The solution was stirred for 2 more hours, keeping the pH at 7. Ethanol was removed by rotaevaporation and water by azeotropic distillation with dry benzene under reduced pressure. The cesium salt of Boc Gly was dried under vacuum in presence of phosphorous pentoxide. The salt was dissolved in NMP (5 ml) and Merrifield resin (2% DVB crosslinked, chlorine capacity 2.02 mmol/g, 200 - 400 mesh size.) was added to it. The mixture was gently stirred at 50 °C for 48 hours. Filtered the resin, washed with NMP (5 ml x 3, 3 min.), NMP-water (1:1 v/v, 5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.), DCM (5 ml x 3, 3 min.) and methanol (5 ml x 3, 3 min.). Dried over phosphorous pentoxide. Weight of the resin: 350 mg.

The amino capacity was found to be 1.85 mmol of NH_2/g by picric acid method.
Synthesis of Ala-Ala-Gly (Pep M1)

The procedure adopted was exactly similar to that used for the synthesis of Pep H1. The synthesis started with 300 mg of Boc Gly resin. Only double coupling was given without considering the Kaiser test.

Weight of peptidyl resin: 1.425 mg.
Yield of peptide: 20 mg (from 100 mg of Pep M1 resin).

\[ \text{tlc (silica plate), single spot } R_f = 0.76 \text{ (pyridine-water-acetic acid::50:15:30 v/v).} \]

Synthesis of Val-Ala-Ala-Gly (Pep M2)

300 mg of Pep M1 (0.43 mmol NH$_2$) was used for attaching Boc Val to the tripeptide resin. The protocol used was same as that used for synthesizing Pep H2. Weight of peptidyl resin: 305 mg.

Yield of peptide: 20 mg (from 100 mg of Pep M2 resin).

\[ \text{tlc (silica plate)} R_f = 0.767 \text{ (major spot). Small spots were present at higher } R_f. \]

Solvent system used: pyridine-water-acetic acid, 50:20:30 v/v.

Synthesis of Val-Ala-Val-Ala-Ala-Gly (Pep M3)

100 mg of Pep M2 resin (capacity: 0.153 mmol of NH$_2$) was used for the synthesis. The procedure adopted was similar to that used for the synthesis of PepH 3. Weight of peptidyl resin: 110 mg. Yield of peptide: 28 mg. Little of the peptide was dissolved in methanol and loaded in hplc column (analytical C18 rpc, flow rate 0.5 ml/min. Solvent system used: water-acetonitrile containing 0.1% TFA.).
Synthesis of Gln-Val-Glu-Leu-Gly (Pep M4)

Boc Gly-bound Merrifield resin (200 mg, 1.85 mmol of NH₂/g) was used for the synthesis. For active ester formation 0.925 mmol each of DCC, HOBt and Boc amino acids were used. NMP along with DMSO and DIEA was used as the coupling medium. The method followed was same as that used for the synthesis of PepH 4. Weight of peptidyl resin: 380 mg.

Weight of peptide (crude): 36 mg (from 100 mg of Pep M4 resin).

tlc (Silica plate) Rf = 0.68 (major spot) solvent system, pyridine-water-acetic acid = 50:15:30.

Synthesis of Gln-Val-Gly-Gln-Val-Glu-Leu-Gly (PepM 5)

Pep M4 (200 mg, cap 0.98 mmol of NH₂/g) was used for the subsequent attachment of Gly, Val, and Gln. The procedure adopted was same as that used for the synthesis of PepH 5. Weight of peptidyl resin: 212 mg.

Yield of peptide: 32 mg from 100 mg of PepM 5 resin. Part of the peptide was dissolved in methanol and loaded in an in hplc (analytical C18rpc, Flow rate 0.5 ml/min. Solvent system, Water:acetonitrile, containing 0.1% TFA.).

Synthesis of Acyl Carrier Protein fragment (ACP -65 - 74) on HDODA-PS Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly

Attachment of Boc Gly on the Chloromethylated HDODA-PS

To 0.19 gm of Boc Gly (1.2 mmol) in ethanol (5 ml) a saturated solution of cesium carbonate was added till the pH of the solution reached 7.0. The solution was stirred for 2 hours. The ethanol was evaporated under reduced pressure. Water was removed by azeotropic distillation with dry benzene. The
white powder of cesium salt of Boc-Glycine was dried under vacuum in the presence of P2O5. The salt was dissolved in NMP (2 ml), and to this 0.3 gm of chloromethylated resin (Capacity 2.01 mmol/g) was added. The mixture was kept at 50 ºC for 48 hours. The resin was filtered, washed with NMP (5 ml x 3, 3 min.), NMP-water (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.) NMP-water mixture (1:1 v/v) (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.), DCM (5 ml x 3, 3 min.) and methanol (5 ml x 3, 3 min.). The resin was kept in a vacuum desiccator containing P2O5. Weight of the resin: 370 mg.

The substitution level: 1.9 mmol of Glycine/g of the resin (by picric acid method). For the entire synthesis 100 mg of Boc Glycine resin (0.19 mmol of NH2) was used.

The protocol used for the synthesis is depicted below.

1. Washed the resin (100 mg) with DCM (5 ml x 3, 2 min.).
2. Added TFA solution (30 % in DCM) (5 ml, 30 min.).
3. Filtered off the TFA solution, washed with DCM (5 ml x 5, 2 min.).
4. Added 5% DIEA solution (in DCM-NMP mixture, 1:1 v/v), 5 ml, shaken for 5 min.
5. Filtered off DIEA solution, added 5% DIEA in NMP, 5 ml and shaken for 5 min.
6. Filtered off the solution, washed with NMP (5 ml x 3, 2 min.).
7. Added active esters of Boc Asn (0.475 mmol) with HOBut, shaken for 45 min.
8. Added DMSO (15 % of total volume of NMP used), shaken for 15 min.
9. Added DIEA (3.8 mequiv.), shaken for 5 min.
10. Filtered off the solution, washed with MeOH-DCM (33:67 v/v), 5 ml x 6, 5 min.
11. Repeated steps 7 -10.
12. Performed Kaiser test, if positive repeated step 11.
13. Repeated steps 1-12 for Boc Ile, Boc Tyr, Boc Asp(OBzl), Boc Ala, Boc Gln and Boc Val.

Weight of peptidyl resin : 270 mg.
Amino acid analysis of peptidyl resin.
Val 0.61(1), Ala 2.07(2), Glu 0.825(1), Ile 1.967(2), Asp 2.043 (2), Tyr 0.982 (1), Gly 0.938 (1).

Cleavage of the peptide

80 mg of ACP-resin was treated with neat TFA (10 ml), thioanisole ( 1 ml), m-cresol ( 1 ml). The mixture was stirred at room temperature for 48 hours. TFA solution was collected and the TFA was removed under reduced pressure. On adding cold ether peptide got precipitated. It was washed several times with cold ether and dried. Yield: 45 mg (82 %). Portion of the peptide was dissolved in MeOH and loaded in hplc column C18 rpc analytical column, solvent system: CH₃CN:Water, containing 0.1% TFA. Flow rate 0.5 ml/min.

Amino acid analysis of pure peptide
Val 0.62 (1), Ala 2.1(2) Glu 0.9(1) Ile 1.95(2), Asp 2.1(2), Tyr 0.99(1), Gly 1(1).
Synthesis of Acyl Carrier Protein Fragment 65 - 74 on Merrifield System

Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly

Attachment of Boc Glycine on Merrifield's resin

Boc-Glycine (1.2 mmol, 0.19 g) was dissolved in ethanol (5 ml). The pH of the solution was brought to 7 by the slow addition of saturated solution of cesium carbonate. The solution was stirred for 2 hours. The solution was rotaevaporated to remove ethanol and water. The white powder of cesium salt of Boc-Gly was dissolved in NMP (2 ml). Merrifield's resin (2% crosslinked 200 - 400 mesh, 0.3 g, 2.02 mmol of Cl/g of the resin) was added to the solution. The mixture was kept at 50 °C for 48 hours in an oil bath. The resin was filtered washed with NMP (5 ml x 3, 3 min.), NMP-water mixture (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.), NMP-water mixture (1:1 v/v) (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.), DCM (5 ml x 3, 3 min.) and methanol (5 ml x 3, 3 min.). The resin was kept in a vacuum desiccator containing P₂O₅.

Weight of the amino acid resin: 350 g.

Substitution level: 1.85 mmol of glycine/g of the resin (by picric acid method).

For the synthesis of peptide 100 mg of glycine resin was used.

The protocol used for the synthesis of ACP 65 - 74 is given below.

1. Washed the resin (100 mg) with DCM (5 ml x 3, 2 min.).
2. Added TFA solution (30% in DCM) (5 ml, 30 min.).
3. Filtered off the TFA solution, washed with DCM (5 ml x 5, 2 min.).
4. Added 5% DIEA solution (in DCM-NMP mixture, 1:1 v/v), 5 ml, shaken for 5 min.
5. Filtered off DIEA solution, added 5% DIEA in NMP, 5 ml and shaken for 5 min.
6. Filtered off the solution, washed with NMP (5 ml x 3, 2 min.).
7. Added active esters of Boc Asn (0.475 mmol) with HOBt, shaken for 45 min.
8. Added DMSO (15 % of total volume of NMP used), shaken for 15 min.
9. Added DIEA (3.8 mequiv.), shaken for 5 min.
10. Filtered off the solution, washed with MeOH-DCM (33:67 v/v), 5 ml x 6, 5 min.
11. Repeated steps 7 -10.
12. Performed Kaiser test, if positive repeated step 11.
13. Repeated steps 1-12 for Boc Ile, Boc Tyr, Boc Asp(OBzl), Boc Ala, Boc Gln and Boc Val.

Weight of peptidyl resin: 190 mg.

Amino acid analysis of peptidyl resin.

Val 0.506 (1), Ile 1.21 (2) Glu 0.479 (1) Ala 1.36 (2) Asp 1.465 (2), Tyr 0.858 (1) Gly 1 (1).

**Cleavage of the peptide**

The peptide was separated from the peptidyl resin (80 mg) by TFA/thioanisole (10 and 1 ml each) method. m-cresol (1 ml) was used as the acid scavenger. The mixture was stirred at room temperature for 48 hours. TFA solution was collected after removing the polymer support. TFA was removed under reduced pressure and the peptide was precipitated using cold ether. The peptide was washed with cold ether several times to remove organic
impurities. Yield of peptide: 25 mg (gum like). Portion of the peptide was dissolved in MeOH and loaded in a hplc column (analytical C18 rpc, flow rate 0.5 ml/min., Solvent system: water and acetonitrile containing 0.1% TFA).

Synthesis of a 31 Residue Peptide Corresponding to Vesicular Stomatitis Virus G (VSV G) Protein.

Trp Lys Ser Ser Ile Ala Ser Phe Phe Phe Ile Ile Gly Leu Ile Ile Gly Leu Phe Leu Val Leu Arg Val Gly Ile Lys Leu Cys(Acm) Ile Lys

Attachment of Boc Lys (2Cl Bzl) to chloromethylated HDODA-PS

414.8 mg of Boc Lys (2ClBzl) was dissolved in 1 ml ethanol. The pH of the solution was brought to 7 by the addition of saturated solution of cesium carbonate. The solution was kept at that pH for one hour. It was rotary evaporated to remove ethanol. Water was removed by azeotropic distillation with dry benzene. The cesium salt of Boc Lys was dried over P2O5 over night. 3 ml of NMP was added to the salt. Resin (420 mg, 2.32 mequiv of Cl/g ) was added to the solution, and the mixture was kept at 50 °C in an oil bath, with occasional shaking for 36 hours. The resin was filtered washed with NMP (5 ml x 3, 3 min.), NMP-water (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.) NMP-water mixture (1:1 v/v) (5 ml x 3 ,3 min.), NMP (5 ml x 3, 3 min.), DCM (5 ml x 3, 3 min.) and methanol (5 ml x 3, 3 min.). The resin was kept in a vacuum desiccator containing P2O5. Weight of amino acid resin: 620 mg.

Estimation of first amino acid substitution level

2 mg of resin after deprotection was treated with 0.1 M picric acid (in DCM). The excess unbound picric acid was removed by washing with DCM (3 ml x 5, 2 min.). The resin was treated with 10% TEA (in DCM) (1 ml) and
the free picric acid was collected. The resin was washed with 95% ethanol. The resin was washed again with 10% TEA (1 ml). All the washing were collected, and OD was noted. From the OD the substitution was calculated. Substitution level of Boc Lys: 1 mmol/g (~ 75%).

**Synthesis of 31-residue peptide**

300 mg of Lys resin (0.3 mmol) was used for the synthesis. 0.75 mmol of active esters of amino acid was used for the coupling of each amino acid. The total volume of the coupling medium (NMP) was minimized to 5 ml for the effective reaction. Second coupling was employed for all steps, and wherever ninhydrin test was positive third coupling was given. The amino acid and their position where a third coupling were required is given below.

<table>
<thead>
<tr>
<th>Leu → Ile</th>
<th>Gly → Ile</th>
<th>Lys → Leu</th>
</tr>
</thead>
<tbody>
<tr>
<td>(14 → 15)</td>
<td>(25 → 26)</td>
<td>(27 → 28)</td>
</tr>
</tbody>
</table>

**Monitoring the progress of the synthesis**

When the 10th residue (Leu) was coupled, little of the peptidyl resin was withdrawn and subjected to amino acid analysis.

Gly 0.9 (1), Arg 1.01 (1), Leu 1.98 (2), Val 1 (1), Lys 1.99 (2) Ile 2.01 (2).

Cys got destroyed under acid hydrolytic condition.

When the 21st residue (Ile) was attached 3 mg of the peptidyl resin was removed from the synthesizer and the peptide was separated from the support and loaded in an FPLC column (analytical C18 rp column, solvent system: water and CH3CN containing 0.1% TFA). Flow rate: 2 ml/min. Two peaks, at 48:52 and 68:33 solvent ratio (Water and CH3CN). About one third of the
peptidyl resin (~350 mg) was used for the subsequent coupling of amino acids. When the last amino acid was coupled the peptidyl resin after proper washing was taken out from the synthesizer and dried under vacuum. Yield of 31 residue peptidyl resin: 430 mg.

**Cleavage of peptide**

130 mg of peptidyl resin was treated with TFA, thioanisole, m-cresol, and ethanedithiol (EDT) (10:1:1:1, v/v). Kept at 40 °C for 12 hours, in an oil bath. TFA was removed under reduced pressure. The peptide solution was cooled in an ice bath and to this added cold ether to precipitate the peptide. The peptide was washed with cold ether (8 x 5 ml) and petroleum ether (40-60 °C) and dried. Yield: 45 mg.

**Purification of the 31-residue peptide**

The free peptide was insoluble in most of the solvent tried and slightly soluble in methanol and acetonitrile. 40 mg of crude peptide was washed with methanol and the methanol fractions were collected. The methanol fraction was loaded in a FPLC and amino acid analysis of different peptides eluted was carried out. The residue was then washed with acetonitrile and the washings were collected and loaded in a FPLC column (C18 rpc, Solvent system: Water-Acetonitrile containing 0.1 % TFA).

The weight of insoluble peptide: 33 mg (85% of total).
Characterization of insoluble portion

0.1 mg of the insoluble peptide was hydrolyzed (HCl:TFA:Propionic acid mixture) in a hydrolysing tube at 100°C, for 48 hours, and diluted with buffer solution. An aliquot of the solution was loaded in an amino acid analyzer. Sequencing of the insoluble part was also conducted for checking the purity.

Synthesis of Modified Seminal Plasmin Fragment - a 13 residue peptide
Pro-Glu-Leu-Leu-Glu-Thr-Phe-Leu-Ser-Glu-Trp-Ile-Gly

Attachment of Boc Glycine on HDODA-PS support

250 mg of Boc Glycine was dissolved in ethanol (5 ml). The pH of the solution was brought to 7 by the slow addition of saturated solution of cesium carbonate. The solution was stirred for 1 hour, keeping the pH at 7.0. Ethanol was rotary evaporated and water was removed by azeotropic distillation with benzene. White powder of cesium salt of Boc Gly was separated. This was kept in vacuum desiccator over P2O5. The dried cesium salt was dissolved in 2 ml NMP. HDODA-PS (0.3 gm, 0.71 mmol of Cl) was added to the solution. The mixture was kept at 50°C with gentle stirring for 48 hours. The resin was filtered, washed with NMP (5 ml x 3, 3 min.), NMP-water (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.) NMP-water mixture (1:1 v/v) (5 ml x 3, 3 min.), NMP (5 ml x 3, 3 min.), DCM (5 ml x 3, 3 min.) and methanol (5 ml x 3, 3 min.). The resin was kept in a vacuum desiccator containing P2O5.

Glycine substitution: 2.02 mmol of -NH2/g of the resin.

148.0 mg of Gly-resin was used for the entire synthesis.
The synthesis involves the following cycle of operations.

<table>
<thead>
<tr>
<th>No</th>
<th>Operation</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Washed resin with DCM</td>
<td>10 ml x 6, 10 min.</td>
</tr>
<tr>
<td>2.</td>
<td>30% TFA in DCM</td>
<td>10 ml, 30 min.</td>
</tr>
<tr>
<td>3.</td>
<td>DCM wash</td>
<td>10 ml x 6, 10 min.</td>
</tr>
<tr>
<td>4.</td>
<td>5% DIEA (DCM-NMP, 1:1 v/v)</td>
<td>10 ml, 5 min.</td>
</tr>
<tr>
<td>5.</td>
<td>5% DIEA in NMP</td>
<td>10 ml, 5 min.</td>
</tr>
<tr>
<td>6.</td>
<td>Boc Amino acid in active ester form</td>
<td>40 min.</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>0.35 ml, 15 min.</td>
</tr>
<tr>
<td></td>
<td>DIEA</td>
<td>0.07 ml, 5 min.</td>
</tr>
<tr>
<td>7.</td>
<td>DCM-MeOH (67:33 v/v)</td>
<td>10 ml x 3, 5 min.</td>
</tr>
<tr>
<td>8.</td>
<td>Repeated steps 6-7</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>DCM wash</td>
<td>10 x 3, 5 min.</td>
</tr>
<tr>
<td>10.</td>
<td>Kaiser test</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Repeated steps 2-10 for next amino acid</td>
<td></td>
</tr>
</tbody>
</table>

Weight of peptidyl resin: 512 mg.

*Cleavage of peptidyl resin*

To 13-residue peptidyl resin (160 mg) a mixture of 0.5 ml of m-cresol, 0.5 ml thioanisole, 0.5 ml of ethanedithiol and 5 ml of TFA were added and kept at room temperature for 12 hours. The solution was filtered and TFA was removed by vacuum. Ether (10 ml) was added. The precipitated peptide was washed with diethylether and dried.

Weight of peptide: 75 mg.
CHAPTER 5

Summary and Outlook