CHAPTER 4

RESULTS AND DISCUSSION
It was generally believed that in the solid phase peptide synthesis, the support would act only as an inert carrier. However, many investigations during the last two decades dealing with the quantitative aspects of polymer-supported resins have shown that the insoluble support has a significant role in solid phase synthesis. The commercially available Merrifield resin has been widely used in polymer supported reactions due to its commercial availability and mechanical stability. However, the synthesis of long peptides using this resin is still a challenging problem. An efficient polymeric support should have an optimum hydrophilic-hydrophobic balance compatible with the peptide being synthesized. The success of solid phase synthesis depends on the swelling characteristics of the polymer and the solvation of the peptidyl resin in different solvents.\textsuperscript{237} It was shown that the use of a flexible polymer support enhances the reactivity due to enhanced solvation characteristics.

A new resin 1,6-hexanediol diacrylate crosslinked polystyrene has been developed in our laboratory for the synthesis of peptides. This support is highly flexible due to the six carbon atoms of HDODA crosslinks thus ensuring enhanced solvation.\textsuperscript{238} Compared to PS-DVB support, PS-HDODA resin is slightly hydrophilic.
Table 4.1. Swelling capacity (ml/g of resin) of PS-DVB and PS-HDODA resins in solvents used for peptide synthesis.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>PS-DVB</th>
<th>PS-HDODA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>5.2</td>
<td>10</td>
</tr>
<tr>
<td>DMF</td>
<td>3.5</td>
<td>7.2</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.95</td>
<td>2.0</td>
</tr>
<tr>
<td>NMP</td>
<td>4.0</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Investigations on swelling (table -4.1) and reactivity studies of this support showed that a 2% crosslinked system is suitable for peptide synthesis.239

The efficiency of Merrifield resin and PS-HDODA support were compared by synthesizing somatostatin analogue peptides. These analogues are precursor for radiopharmaceuticals which are used in the detection and treatment of carcinoid tumors. Hence we have selected these peptides for synthesis.

4.1 Preparation of the Solid Support

4.1.1 Preparation of 1,6-hexanediol diacrylate crosslinked polystyrene support

2% HDODA-Polystyrene support was prepared by the co-polymerisation of styrene and, 1,6-hexanediol diacrylate in molar ratio (98:2) in presence of a diluent toluene using benzoyl peroxide as initiator. Polyvinyl alcohol was used as the suspension stabilizer. (Scheme-4.1) The yield of the polymer was
around 90% after proper washing and soxhleting with different solvents to remove soluble linear polymers and other additives.

\[
\text{Suspension polymerization}
\]

Benzoyl peroxide
Polyvinyl alcohol
Toluene, 80°C, 6h

**Scheme 4.1** Preparation of PS-1, 6-HDODA support

2% crosslinked beads, having 200-400 mesh size were separated and characterized by IR spectroscopy (Fig.4.1). The incorporation of the crosslinking agent was confirmed. IR spectrum gave peaks corresponding to the ester carbonyl (1719 cm\(^{-1}\) & 1490 cm\(^{-1}\)) aromatic C-H stretching (3024 cm\(^{-1}\)) and C=C skeletal vibrations (1492 cm\(^{-1}\), 1450 cm\(^{-1}\) & 1600 cm\(^{-1}\)).
4.1.2 Chloromethylation of the resin

\[
\begin{align*}
\text{anhydrous ZnCl}_2 \quad \text{THF} \\
\text{DCM, } 40^\circ\text{C} \\
\text{4h.}
\end{align*}
\]

Chloromethyl groups were introduced by Friedel-Craft's reaction. Anhydrous ZnCl\textsubscript{2} in THF was used as the catalyst.

By adjusting the amount of catalyst, the temperature of the reaction medium and the time, resins having varying capacities were prepared. IR spectrum of the functionalised resin is given in (Fig.4.2). The peak at 680 cm\textsuperscript{-1} corresponds to the C-Cl vibration.
Fig. 4.2 IR Spectrum of Chloromethylated PS-HDODA resin
4.1.3 Determination of the chlorine capacity of the resin

Volhard's method was used for the determination of chlorine capacity. The resin was heated with 5ml pyridine at 110 °C for 5h and the capacity estimated. Resins having capacities in the range 0.8-1.2 mmoles/g were used for the synthesis of peptides.

Chloromethylated PS-DVB resin

2% PS-DVB resin having a chlorine capacity of 0.8mmol/g, purchased from Sigma chemical company, USA was used for the synthesis of peptides inorder to compare the efficiency of both resins.

4.2 Preparation of Protected Amino Acids for Peptide Synthesis

In peptide synthesis, preparation of side chain and amino protected amino acids in good yield and purity is very important. Synthesis of all the amino acids were done by the standard procedure. Amino acids were obtained in good yield in pure form.

4.2.1 Protection of side chain functions

4.2.1.1 Blocking the sulphydryl group of cysteine as S-Acetamidomethyl-Cys

Preparation of N-hydroxymethyl acetamide

\[ \text{H}_3\text{C} \equiv \text{C} \equiv \text{NH}_2 \xrightarrow{\text{K}_2\text{CO}_3, \text{HCHO}} \text{H}_3\text{C} \equiv \text{C} \equiv \text{NH} \equiv \text{CH}_2 \equiv \text{OH} \]
N-hydroxymethyl acetamide was prepared by the reaction of acetamide, $\text{K}_2\text{CO}_3$ and HCHO. The product was obtained as a colourless oil. Purity was checked by tlc and was characterized by IR spectroscopy. The spectrum is given in Fig.4.3.
Fig. 4.3. IR Spectrum of $\text{H}_3\text{C}-\text{CO-NH-CH}_2\text{-OH}$
The broad peak at 3364 cm\(^{-1}\) is due to the merging of peaks corresponding to the N-H stretching and O-H stretching. Amide Carbonyl gives a peak at 1656 cm\(^{-1}\). Peak at 1295 cm\(^{-1}\) corresponds to the C-O stretching of the C-OH bond. Peak at 2968 cm\(^{-1}\) corresponds to the C-H stretching of –CH\(_3\) group.

**Preparation of S-Acm-Cys**

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CH} \quad \text{COOH} \\
\text{CH}_2 & \quad \text{SH} \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 & \quad \text{C} \quad \text{NH} \quad \text{CH}_2 \quad \text{OH} \\
\text{TFA} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CH} \quad \text{COOH} \\
\text{CH}_2 & \quad \text{S} \quad \text{CH}_2 \quad \text{NH} \quad \text{CO} \quad \text{CH}_3
\end{align*}
\]

Cysteine hydrochloride, N-hydroxymethyl acetamide and TFA were treated at room temperature to get S-Acm-Cys. It was recrystallised from 2-propanol. Purity was checked by tlc using the solvent system, butanol: acetic acid: water (6:1:4) Melting point was noted (167 °C).
4.2.1.2 Protection of $\epsilon$-amino group of lysine

$N^\epsilon$-benzyloxycarbonyl lysine

Lysine monohydrochloride in water was heated with cupric carbonate to form the copper complex. Then the free amino group was protected with bezyloxycarbonyl chloride. The copper complex was decomposed with $H_2S$ to get the $N^\epsilon$-benzyloxycarbonyl lysine. Checked tlc using butanol: acetic acid: water (6:1:4) solvent system.

4.2.1.3 Blocking hydroxyl group of threonine

$O$-benzyl threonine
Threonine and copper sulfate pentahydrate were treated to get the copper complex of threonine. To the copper complex was added benzyl bromide to get the O-benzyl threonine copper complex. This copper complex was decomposed by \( \text{H}_2\text{S} \) to get the O-benzyl threonine.

TLC was checked in the solvent system, butanol: acetic acid: water (6:1:4) Melting point was 119°C.

4.2.1.4 Blocking hydroxyl group of tyrosine

O-benzyl tyrosine

Tyrosine and \( \text{CuSO}_4 \cdot 5\text{H}_2\text{O} \) were treated and to the complex was added benzyl bromide. The copper complex was then treated with \( \text{HCl} \) to get the crude O-benzyl-tyrosine.

TLC was checked using the solvent system butanol: acetic acid: water (6:1:4)

Purified by column chromatography and characterized by melting point (268 °C).
4.2.1.5 Protection of indole nitrogen of tryptophan

Dry HCl gas was bubbled into a solution of tryptophan in formic acid. At about hourly intervals the reaction mixture was taken (0.5ml) and diluted with distilled water to 10ml. 1ml of this was further diluted to 100ml and UV spectrum recorded. The $\lambda_{\text{max}}$ at 278 nm, characteristic of tryptophan, decreased with time and after a certain time a new peak emerged at 298 nm corresponding to the absorption of Trp(N-CHO). Intensity of this peak increased with time. The reaction was over in 3 h when there was no further increase in the absorption at 298 nm. The UV spectra are given in Fig.4.4.

**Fig.4.4.** UV spectrum of formylation of Trp 1) after 30 min 2) after 3 h
Solvent was removed and added dry ether to get crystals of Trp (N-CHO). Dried and checked TLC in butanol: acetic acid: water (6:1:4). Purified by column chromatography and checked the melting point [218 °C].

4.2.2 Preparation of Boc-amino acids

Amino group of the side chain protected amino acids were protected by the Boc group using Boc-anhydride. TLCs were checked using the solvent system, chloroform: MeOH: acetic acid (99:1:0.1) and wherever necessary column chromatography was used to purify the compound.

IR spectra of the compounds were recorded.

The values are

1. **Boc-Cys (S-Acm) (Fig.4.5)**
   
   3501 cm$^{-1}$ (N-H stretching)

   2984 cm$^{-1}$, 2940 cm$^{-1}$ & 2907 cm$^{-1}$ (Aliphatic C-H stretching)

   2392 cm$^{-1}$ (C-S Stretching)

   1741 cm$^{-1}$ (carbonyl stretching)

   1215 cm$^{-1}$ (Boc skeletal vibration)

2. **Boc-\textsuperscript{N\textprime}-benzyloxyCarbonyl Lysine (Fig.4.6)**

   3019.26 cm$^{-1}$ (Aromatic C-H stretching)

   2933.98 cm$^{-1}$ (Aliphatic C-H stretching)

   3335.1 cm$^{-1}$ (N-H stretching)
1721 cm\(^{-1}\) (Ester carbonyl)

1215 cm\(^{-1}\) (skeletal vibrations of Boc group)

3. **Boc-O-benzyl threonine (Fig. 4.7)**

3435 cm\(^{-1}\) (N-H stretching)

3073 cm\(^{-1}\) (Aromatic C-H stretching)

2934 & 2979 cm\(^{-1}\) (Aliphatic C-H stretching)

1713 cm\(^{-1}\) (Carbonyl C=O of COO)

1720 cm\(^{-1}\) (ester carbonyl)

1215 cm\(^{-1}\) (skeletal vibrations of Boc group)

4. **Boc-O-benzyl tyrosine (Fig. 4.8)**

3018 cm\(^{-1}\) (Aromatic C-H stretching)

2981 & 2932 cm\(^{-1}\) (Aliphatic C-H stretching)

1712 cm\(^{-1}\) (Ester carbonyl)

1230 cm\(^{-1}\) (C-O stretching of C\(_6\)H\(_5\)-O-bond)

1215 cm\(^{-1}\) (skeletal vibrations of Boc group)

1025 cm\(^{-1}\) (C-O stretching of CH\(_2\)-O-bond)

**Melting points of Boc- amino acids**

1. Boc-Phe-88°C

2. Boc-Cys (Acm) 112°C

3. Boc-Thr (O-Bzl)-115°C

4. Boc-Trp (N-CHO)-126°C

5. Boc-Val-79 °C

6. Boc-Lys (z) - 41 °C
**Fig. 4.5.** IR Spectrum of Boc-Cys(S-Acm)
Fig. 4.6. IR spectrum of Boc-Lys(Z)
Fig. 4.7. IR Spectrum of Boc-Thr (O-Bzl)
Fig. 4.8. IR Spectrum of Boc-Tyr(O-Bzl)
4.3 Synthesis of Somatostatin Analogues on 2% PS-HDODA Support

4.3.1 Synthesis of D-Phe\textsuperscript{1}-Octreotide

\[ \text{D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr} \]
\[ \text{S} \quad \text{S} \]

The first amino acid Boc-Thr (O-Bzl) was attached to the chloromethylated resin having capacity 1mmol/g, by cesium salt method. The amino capacity was determined by the picric acid method and was found to be 0.9mmol/g. In the next step, the Boc-group was removed by 30% TFA/DCM and neutralised with 5% TEA/DCM. Checked with ninhydrin. The second amino acid Boc-Cys (S-Acm) was coupled to the amino acyl resin in NMP by the HOBt active ester method. 3 molar excess of amino acid, HOBt and DCC were used. DCU was removed by 33% MeOH/DCM. The resin was checked with ninhydrin.

Subsequent amino acid units were assembled by the same procedure. Finally, the peptide was cleaved by TFA in presence of thioanisole, 1,2-ethane dithiol and m-cresol.

The synthetic procedure is given in scheme 4.2.
Scheme 4.2. Synthetic procedure for the synthesis of D-Phe-Cys-Phe-Trp-Lys-Thr-Cys-Thr

TFA was concentrated in vacuo and added ice-cold dry ether to precipitate the crude peptide. TLC was checked in CHCl₃:
MeOH (90:10). Yield is 95%. HPLC was recorded using the solvent system 20% CH₃CN and 80% water containing 0.1% TFA.

Then it was subjected to hydrogenation and again checked the HPLC. It gave two peaks showing that hydrogenation was incomplete. Subjected to hydrogenation for further 24h. Single peak obtained in HPLC (Fig.4.9).

NMR was recorded in DMSO at 200 MHz. (Fig.4.10) NH peaks were found to be merging.

![HPLC trace of D-Phe-Cys-Phe-Trp-Lys-Thr-Cys-Thr](image)

**Fig 4.9.** HPLC trace of D-Phe-Cys-Phe-Trp-Lys-Thr-Cys-Thr
Solvent A. Water containing 0.1% TFA
Solvent B. CH₃CN containing 0.1% TFA
Gradient used: 5 to 45% CH₃CN/H₂O in 45 min
flow rate 0.5ml/min
Fig. 4.10. NMR Spectrum of D-Phe-Cys-Phe-Trp-Lys-Thr-Cys-Thr in DMSO at 200 MHz
Cyclisation via disulfide formation

The pure linear peptide was subjected to cyclisation using a solution of iodine in methanol under nitrogen atmosphere.

\[ \text{D-Phe -- Cys -- Phe -- D-Trp -- Lys -- Thr -- Cys -- Thr} \]
\[ \text{S-Acm} \quad \text{S-Acm} \]
\[ \text{I}_2/\text{MeOH} \]
\[ \text{N}_2 \]
\[ 4\text{h} \]

\[ \text{D-Phe -- Cys -- Phe -- D-Trp -- Lys -- Thr -- Cys -- Thr} \]
\[ \text{S} \quad \text{S} \]

Very dilute solution of the peptide was used to ensure intramolecular cyclisation. Deprotection of the Acm group and coupling occurs in the same step. After 4 h, the reaction was quenched by adding ascorbic acid solution.
HPLC of the crude reaction mixture is given in Fig. 4.11

**Fig 4.11.** HPLC trace of crude reaction mixture of the peptide D-Phe-Cys-Phe-Trp-Lys-Thr-Cys-Thr after cyclisation
Solvent A. Water containing 0.1% TFA
Solvent B. CH₃CN containing 0.1% TFA
Gradient used: 5 to 45% CH₃CN/H₂O in 45 min
flow rate 0.5ml/min

The first peak corresponds to ascorbic acid, the second small peak to the linear peptide and the third peak to the desired cyclic peptide. It was purified by preparative tlc using the solvent system CHCl₃:MeOH (90:10) HPLC of the pure cyclic peptide and linear peptide is given in fig (4.12).
Fig 4.12. HPLC trace of the peptide
D-Phe-Cys-Phe-Trp-Lys-Thr-Cys-Thr
a) Linear b) Cyclic
Solvent A. Water containing 0.1% TFA
Solvent B. CH₃CN containing 0.1% TFA
Gradient used: 5 to 45% CH₃CN/H₂O in 45 min
flow rate 0.5ml/min

4.3.2 Synthesis of Tyr³-octreotide (TOC)

D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr

To the chloromethylated resin having capacity 1mmol/g, Boc-Thr(-O-Bzl) was coupled. The amino capacity was found to be 1mmol/g. The next amino acid Boc-Val was coupled by the HOBT active ester method using DCC. 3fold excess of amino acid HOBT and DCC were used for coupling. After coupling all the subsequent amino acids the peptide has been cleaved by TFA in presence of scavengers thioanisole, 1, 2-ethane dithiol and m-cresol. Yield is 92%.
The synthetic protocol is shown in scheme 4.3.

Scheme 4.3. Synthetic protocol for the synthesis of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr

Checked tlc and HPLC and subjected to hydrogenation. NMR was also recorded. HPLC is given in Fig.4.13 and NMR in Fig.4.14. NMR was recorded in DMSO at 200 MHz. The NH peaks were found to be merging.
**Fig 4.13.** HPLC trace of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr
Solvent A. Water containing 0.1% TFA
Solvent B. CH3CN containing 0.1% TFA
Gradient used: 5 to 45% CH3CN/H2O in 45 min
flow rate 0.5ml/min

**Cyclisation of the linear peptide**

The linear peptide was dissolved in methanol and added I₂ in methanol under nitrogen. Very dilute solution of the peptide in MeOH was used to avoid intramolecular reactions.
HPLC of the crude cyclised peptide was checked (Fig. 4.15) and purified by preparative tlc using the solvent system chloroform: methanol (90:10).

Fig 4.15. HPLC trace of the crude reaction mixture of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr after cyclisation Solvent A. Water containing 0.1% TFA Solvent B. CH$_3$CN containing 0.1% TFA Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min flow rate 0.5ml/min
HPLC traces of linear and cyclic peptides are given in fig (4.16).

**Fig 4.16.** HPLC traces of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr
a) Linear b) Cyclic
Solvent A. Water containing 0.1% TFA
Solvent B. CH₃CN containing 0.1% TFA
Gradient used: 5 to 45% CH₃CN/H₂O in 45 min
flow rate 0.5ml/min

4.3.3 Synthesis of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp

**RC160**

Boc-Trp (N-CHO) has been attached to the resin having chlorine capacity 1mmol/g, by the cesium salt method. Aminocapacity was determined by picric acid method and was found to be 0.9mmol/g. Subsequent amino acids were coupled by the HOBT active ester method. Double coupling was done to
ensure completion of coupling. The peptide was cleaved by TFA and washed with ether. Yield is 85%.

The synthetic steps are given in Scheme 4.4.

Scheme 4.4. Synthetic Protocol for the synthesis of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp

TLC was checked in CHCl₃: MeOH (90:10)
Subjected to hydrogenation and HPLC was recorded (Fig. 4.17)

**Fig 4.17.** HPLC trace of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp
Solvent A. Water containing 0.1% TFA
Solvent B. CH$_3$CN containing 0.1% TFA
Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min
flow rate 0.5ml/min

NMR was recorded in DMSO at 200 MHz. (fig.4.18) and NH peaks were found to be merging.
Cyclisation

Cyclisation was done in very dilute solution of methanol using I₂/MeOH, under N₂.

\[
\begin{align*}
\text{D-Phe--Cys--Tyr--D-Trp--Lys--Val--Cys--Trp} \\
\text{S-Acm} & \quad \text{I₂/MeOH} & \quad \text{N₂} & \quad 3 \text{ h} \\
\text{D-Phe--Cys--Tyr--D-Trp--Lys--Val--Cys--Trp} \\
\text{S} & \quad \text{S-Acm}
\end{align*}
\]

HPLC was recorded. The first peak corresponding to ascorbic acid was separated by preparative tlc. Co-injection of linear and cyclic peptide gave two peaks whose Rf value difference is 0.8. The peak corresponding to the cyclic peptide was isolated.

HPLC of the linear and cyclic peptides are given in fig.4.19.

**Fig 4.19.** HPLC traces of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr
a) Linear b) Cyclic
Solvent A. Water containing 0.1% TFA
Solvent B. CH₃CN containing 0.1% TFA
Gradient used: 5 to 45% CH₃CN/H₂O in 45 min
flow rate 0.5ml/min
4.4 Synthesis of Somatostatin Analogs on 2% PS-DVB Resin

4.1.1 Synthesis of D-Phe\(^1\)-Octreotide

To the Chloromethylated PS-DVB resin having capacity of 0.8 mmol/g, Boc-Thr(O-Bzl) was attached by the cesium salt method. The amino capacity was determined by picric acid method and was estimated as 0.76 mmol/g. Rest of the amino acid were coupled by the HOBt/DCC active ester method in DCM/DMF.

After the coupling of eighth amino acid, the peptide was cleaved using TFA/thioanisole/1,2-ethane dithiol/m-cresol.

\[
\begin{array}{c}
\text{Boc-Thr-(O-Bzl)} \\
\text{CH}_2\text{Cl} \xrightarrow{\text{CS}_2\text{CO}_3} \text{DMF 50-60°C} \xrightarrow{\text{CH}_2} \text{Thr} \xrightarrow{\text{N}\text{H}_2} \text{Boc-Cys(Acm)} \xrightarrow{\text{HOBt/ DCC in NMP}} \\
\end{array}
\]

1) 30% TFA/DCM 30 min
2) 5% TEA/DCM 10 min

Repetition of deprotection neutralisation & Coupling to get the desired sequence
Scheme 4.5. Synthetic steps for the synthesis of D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr
The Synthetic steps are given in Scheme-4.5.

Fig 4.20. HPLC trace of D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr
Solvent A. Water containing 0.1% TFA
Solvent B. CH$_3$CN containing 0.1% TFA
Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min
flow rate 0.5ml/min

Yield is 67%
Subjected to hydrogenation.
Checked by HPLC (Fig.4.20)
Cyclisation

Pure linear peptide was subjected to cyclisation using I₂/MeOH under N₂.

\[
\text{D-Phe} - \text{Cys} - \text{Phe} - \text{D-Trp} - \text{Lys} - \text{Thr} - \text{Cys} - \text{Thr} \]

\[
\text{S-Acm} \quad \text{S-Acm}
\]

\[
\xrightarrow{\text{I₂/ MeOH}}
\]

\[
\xrightarrow{\text{N₂}}
\]

\[
\xrightarrow{2\text{h}}
\]

\[
\text{D-Phe} - \text{Cys} - \text{Phe} - \text{D-Trp} - \text{Lys} - \text{Thr} - \text{Cys} - \text{Thr}
\]

\[
\quad \text{S} \quad \text{S}
\]

Quenched the reaction by adding ascorbic acid. Checked HPLC (fig.18,a). Three peaks were obtained. The first peak corresponds to ascorbic acid, second to the cyclic peptide and third may be a cyclic dimer. The cyclic peptide was separated. HPLC of the pure linear compound and cyclic compound is given in (fig.4.21).
4.4.2 Synthesis of Tyr³-Octreotide (TOC)

Boc-Thr (O-Bzl) was attached to the chloromethylated resin having capacity 0.8mmol/g by the cesium salt method. Amino capacity was 0.76 mmol/g. Subsequent amino acids were coupled by the HOBr active ester method in DCC/DMF.

The peptide was cleaved from the resin by TFA/thioanisole/1,2-ethane dithiol/m-cresol.

The different steps in the synthesis are given in scheme 4.6.
Scheme 4.6. Synthetic protocol for the synthesis of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr

Washed with ether. Hydrogenated using Pd/Charcoal Catalyst and checked HPLC (Fig 4.22). Yield is 70%
**Fig 4.22.** HPLC trace of D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr  
\( a \) linear \( b \) Cyclic  
Solvent A. Water containing 0.1% TFA  
Solvent B. CH$_3$CN containing 0.1% TFA  
Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min  
flow rate 0.5ml/min

**Cyclisation**

The linear peptide was subjected to cyclisation using I$_2$/MeOH.

\[
\begin{align*}
\text{D-Phe} & \text{ Cys} \text{ Tyr} \text{ D-Trp} \text{ Lys} \text{ Val} \text{ Cys} \text{ Thr} \\
\text{S-Acm} & \text{ S-Acm}
\end{align*}
\]

\[
\begin{align*}
\text{I}_2/\text{MeOH} & \\
\text{N}_2 & \\
3 \text{ h} & \\
\text{D-Phe} & \text{ Cys} \text{ Tyr} \text{ D-Trp} \text{ Lys} \text{ Val} \text{ Cys} \text{ Thr} \\
\text{S} & \text{ S}
\end{align*}
\]
HPLC of the linear and cyclic peptides are given in (Fig. 4.23)

Fig 4.23. HPLC trace of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr
a) linear b) Cyclic
Solvent A. Water containing 0.1% TFA
Solvent B. CH$_3$CN containing 0.1% TFA
Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min
flow rate 0.5ml/min

4.4.3 Synthesis of RC 160

Boc-Trp (N-CHO) was attached to the chloromethylated resin having capacity 0.8 mmol/g by the cesium salt method. Amino capacity was determined. (0.7 mmol/g). Subsequent amino acids were coupled by the HOBT active ester method. Finally the peptide was cleaved.
**Fig 4.24.** HPLC trace of the peptide
D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp
Solvent A. Water containing 0.1% TFA
Solvent B. CH$_3$CN containing 0.1% TFA
Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min
flow rate 0.5ml/min

The synthetic protocol used is given in Scheme 4.7.

\[
\begin{align*}
&\text{Boc-Trp(N-CHO)} \\
\xrightarrow{30\% \text{ TFA/DCM}}
&\text{Boc-Cys(S-Acm)} \\
\xrightarrow{5\% \text{ TEA/DCM}}
&\text{neutralisation \& Coupling to get the sequence}
\end{align*}
\]
Scheme 4.7. Synthetic steps for the synthesis of
D-Phe-Cys-Tyr-D-Trp-Lys-Val-Qs-Trp

Yield was 68%

It was hydrogenated using pd/Charcoal Catalyst and HPLC was recorded. (Fig. 4.24)

Cyclisation

Cyclisation of the pure linear peptide using I₂/MeOH gave the desired product.
HPLC of the crude cyclised peptide is given in Fig. 4.25. The cyclic peptide was isolated.

**Fig 4.25.** HPLC trace of crude reaction mixture of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp after cyclisation
Solvent A. Water containing 0.1% TFA
Solvent B. CH$_3$CN containing 0.1% TFA
Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min
flow rate 0.5ml/min
Spectra of the linear and cyclic peptides are given in (Fig. 4.26).

**Fig 4.26.** HPLC traces of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp
a) Linear b) Cyclic
Solvent A. Water containing 0.1% TFA
Solvent B. CH$_3$CN containing 0.1% TFA
Gradient used: 5 to 45% CH$_3$CN/H$_2$O in 45 min
flow rate 0.5ml/min