CHAPTER-1

SOLID PHASE
PEPTIDE SYNTHESIS
Solid phase methods have taken a major place in peptide synthesis within the last thirty years. A multitude of biologically active peptides have been discovered and characterized. Many of them have been found in both neuronal and nonneuronal tissues. Representative examples include Somatostatin, substance P, cholecystokinin, endorphin, enkephalin, angiotensin II and endothelin. After binding to their membrane bound receptors, these neurotransmitters, neuromodulators and hormones influence cell-cell communication and control a series of vital functions such as metabolism, immune defence, digestion, respiration, sensitivity to pain, reproduction, behaviours and electrolyte levels.

Peptide chemistry is of great importance in pharmaceutical research, with major impacts on immunology-preparation of synthetic vaccines and the study of biologically active molecules like enzymes and hormones. Since they are of enormous medical interest, there have always been a search for better methods of peptide synthesis.

Chemical synthesis of peptides is achieved either by the solution phase or by the solid phase method. The classical method for the synthesis of peptide in solution begins with Fischer at the beginning of the 20th century, when he first synthesized the first peptide and coined the word peptide.
Chemical synthesis of peptide involves the blocking of carboxyl group of one amino acid and the amino group of the second amino acid. Then by activating the free carboxyl group the peptide bond can be formed. Selective removal of the protecting groups results in the dipeptide. Even though this method gives high purity peptides, it suffers from drawbacks like longer synthesis time, insolubility of the intermediate peptide and reduction in yield due to large number of operative steps.

In 1963, Merrifield introduced the method of solid phase peptide synthesis (SPPS).

In this method, the first amino acid residue of the peptide to be synthesized is bound to polystyrene beads through an ester linkage formed using the carboxyl group of the C-terminal amino acid. As a consequence, the peptide subsequently synthesised is attached to the beads via the carboxyl terminus. The polystyrene beads are crosslinked and are therefore totally insoluble in all organic solvents. Thus at each stage of the synthesis, the supported peptide can be separated cleanly and easily from the other species present. At the end of the synthesis the peptide produced is detached from the polymer support by cleaving the ester linkage. Merrifield first synthesized a tetra peptide using this approach, but he had soon developed a machine for automated peptide synthesis and synthesized ribonuclease A, an enzyme with 124 amino acid residues. This novel approaches so revolutionised peptide synthesis and influenced
other methods in organic chemistry and analytical chemistry. The 1984 Nobel Prize for chemistry was awarded to R.B. Merrifield considering the significance and impact of this work. Simultaneously, Letsinger and Kornet described an alternative solid phase peptide synthesis procedure in which the first amino acid was bound to the polymer support, through the amino group. This research work was subsequently developed into a basis for solid phase oligonucleotide synthesis and this general synthetic approach has proved invaluable for synthesizing oligonucleotide for many applications in genetic engineering.

1.1 Basic principle of Merrifield’s solid phase peptide synthesis

The basic principle of Merrifield’s SPPS is to assemble a peptide chain in a stepwise manner while it was attached at one end to a solid support. With the growing chain covalently an insoluble matrix at all stages of the synthesis, the peptide would also be completely insoluble and furthermore would be in a suitable physical form to permit rapid filtration and washing after completion of each of the synthetic reaction. The intermediate peptides in the synthesis would thus be purified by a very simple, rapid procedure. In the synthesis of a long peptide, the saving in time, effort and material could be very large. The fact that all of the steps just described are heterogeneous reactions between a soluble
reagent in the liquid phase and the growing peptide chain in the soluble solid phase led to the introduction of the name "solid phase peptide synthesis".

The general scheme for solid phase peptide synthesis using the Boc/Bzl strategy is outlined in scheme 1.

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Functionalisation

Cl CH₂-O-CH₃
anh.ZnCl₂

Coupling of first amino acid

Deprotection

30% TFA/DCM

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Scheme 1: General scheme for solid phase peptide synthesis using the Boc/Bzl strategy.
Scheme 1: General Scheme for Solid Phase Peptide Synthesis

The carboxyl terminal amino acid is blocked at the amino end by a protecting group and is covalently attached to the resin
support as a benzyl ester by way of chloromethyl group. Side chain functional groups must also be blocked usually with benzyl based derivatives. The synthesis depends on the differential sensitivity of these two classes of protecting groups to acid, which is greater than 1000:1. The Boc group is completely removed by 30% TFA in DCM with minimal loss of the anchoring bond or of the other protecting groups. The resulting α-amine salt is neutralised with a tertiary amine and the free amine of the resin-bound amino acid is then ready to couple with a second Boc-amino acid, which must be activated for the reaction to occur. For activation DCC method, HOBt active ester method or anhydride method can be used. All these reactions are carried out under non-aqueous conditions in organic solvents that swell the resin and accelerate the rates.

To extend the peptide chain the deprotection, neutralisation and coupling steps are repeated for each of the succeeding amino acids until the desired sequence has been assembled. Finally, the completed peptide is deprotected and cleaved from the solid support. This is accomplished by treatment with a strong anhydrous acid such as HF, TFA etc. The free peptide is then purified by suitable procedures.

1.2 The Support for SPPS

The selection of solid support is crucial in SPPS. An ideal support should have the required mechanical stability, should be
stable under the reaction condition, could be functionalised easily and should have very good swelling characteristics.

For effective swelling of the resin and solvation of the peptide, the polymer should have optimum hydrophilic-hydrophobic balance. Structure-reactivity, structure-property correlation in crosslinked polymer systems helped to design new supports with optimum reactivity, mechanical stability and other essential requirements of a polymeric support.

Physicochemical incompatibility of the growing peptide chain and the rigid hydrophobic macromolecular environment created by the divinyl benzene cross linked polymeric support has been one of the major problems associated with Merrifield's peptide synthesis. Sheppard introduced a polar polydimethyl acrylamide resin which is structurally similar to peptide backbone. Although this helps easy solvation of the peptidyl resin in polar solvents, their swelling property in non polar solvents is very poor. Chemical stability was less compared to that of polystyrene support.

A new resin 1,6-hexanediol diacrylate (HDODA) crosslinked polystyrene support with greater functional group reactivity due to the greater chain mobility of the flexible oligoethyleneglycol diacrylate crosslinks, can be used for the stepwise synthesis of biologically active peptides.
1.3 Advantages & Disadvantages of SPPS

1.3.1 Advantages

1. During the synthesis, the peptide remains attached to the insoluble polymer support. Therefore, it is easy to separate the soluble byproducts by filtration. Intermediates can thus be purified after each step avoiding the time consuming purification methods.

2. Excess reagents can be used to force the reaction to completion. This excess reagent can be removed by filtration.

3. Mechanical loss of the material is avoided because the series of reaction steps are carried out in the same reaction vessel.

4. The method is very fast and simple.

5. The ability to purify, after each reaction by simple filtration and washing and the fact that all reactions can be conducted within a single reaction vessel, made this method ideal for automation.

1.3.2 Disadvantages

1. Incompatibility of the growing peptide chain and the polymer support.

2. Reaction can’t be monitored by the standard method.

3. Incomplete reaction may lead to deletion sequences.

4. During the deprotection step, at least 1% of the resin-peptide bond is also cleaved, resulting in an overall decrease in yield.
1.4 Organisation of the Thesis

The thesis consists of six chapters. The first chapter is a note on solid phase peptide synthesis with an introduction to the thesis. Second chapter is a review on peptidomimetics. Third chapter is the experimental section and fourth comprises of the results and discussion part. Fifth Chapter is the summary of the work. This is followed by the Sixth Chapter, a list of references.

1.5 Present work

In the present work, 1,6-hexanediol diacrylate crosslinked polystyrene support is being used for the synthesis of three somatostatin analogues, sandostatin\textsuperscript{181-185}, TOC\textsuperscript{168,182} and RC\textsuperscript{160\textsuperscript{186-191}}.

a. Sandostatin

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

b. TOC

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

c. RC 160

\[
\text{D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp}
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In order to test the efficiency of the new support the syntheses of these peptides were also conducted using standard 2% PS-DVB resin and the results were compared.