CHAPTER I

INTRODUCTION AND OBJECTIVES

Synthetic and structural studies of peptides are important for the development of new methods for the preparation of peptides and also for understanding the reactions in which proteins participate in living systems. Peptide synthesis has also been proven indispensable for the structural elucidation of many recently isolated natural products which possess a peptide structure such as hormones, neuropeptides and antibiotics.

In the classical method of peptide synthesis two approaches are possible. (a) Stepwise chain building starting at the C-terminus; (b) Condensation of peptide segments. In these approaches the coupling is carried out in solution and the reaction can be monitored easily by analysis and characterisation of intermediate peptides. In the stepwise elongation, activated N-blocked amino acids are added stepwise to the growing peptide chain with the correct choice of protecting groups and coupling procedure. The chemical methods are so selected that recemization
is reduced to a minimum. In the segment condensation approach, peptide segments are first synthesised in the stepwise manner or by condensation of shorter segments which are then linked using various techniques. The conventional method of peptide synthesis has been extensively reviewed\textsuperscript{1,2}.

By the conventional method of synthesis the preparation of peptides containing more than just a few amino acids is an arduous task. Introduction of solid phase peptide synthesis by R.B. Merrifield in 1963 resulted in a revolutionary development in the field of peptide and protein research\textsuperscript{3}. In this method an N\textsuperscript{\textalpha}-protected amino acid is attached to an insoluble polymer matrix through the C-terminal; deprotection and stepwise incorporation of activated N\textsuperscript{\textalpha}-protected amino acid residues are then continued till the desired sequence is completed. The final peptide can be selectively cleaved from the support employing suitable chemical methods (Scheme I.1). The polymeric support used in the original strategy was chloromethylated 2\% divinylbenzene (DVB)-crosslinked polystyrene (PS). The operational simplicity of
Scheme I.1 General Scheme for Merrifield's Solid Phase Peptide Synthesis

- Protected amino acids;
- DVB-crosslinked polystyrene support
this polymer-supported procedure for peptide synthesis over conventional method has induced tremendous activity in peptide chemistry and biology. The impact and magnitude of this contribution was recognized by the award of the 1984 Nobel Prize in chemistry to R.B. Merrifield.

A number of peptides and proteins were prepared using the DVB-crosslinked polystyrene support. However there were a few problems associated with the original strategy of solid phase peptide synthesis. In the case of long peptides the rate of incorporation of a particular amino acid residue has been found to decrease with increasing chain length. The physicochemical incompatibility of the polystyrene matrix with the attached peptide and the development of unfavourable conformational characteristics of peptides and protein sequences are the factors responsible for the decrease in yield and purity in solid phase peptide synthesis. This led to the development of new supports and strategies in solid phase peptide synthesis. Polyacrylamide supports were introduced as an alternative to the most commonly used divinylbenzene (DVB)-crosslinked polystyrene (PS) support with a
view to increasing the hydrophilic nature of the support. However they undergo only poor swelling in non-polar solvents which are also extensively used in peptide synthesis. DVB-crosslinked polystyrene, on the other hand, is highly rigid and hydrophilic and hence less reactive in polar solvents.

An effective polymeric support for repetitive synthesis of biopolymers, particularly peptides, should facilitate the different types of organic reactions occurring in both polar and non-polar medium. This is possible only in the case of a macromolecular matrix with optimum hydrophobic-hydrophilic balance. In addition, the crosslinked polymer matrix should be mechanically stable to withstand the multitude of synthetic operations. The DVB-crosslinked polystyrene has the necessary mechanical stability whereas the polyacrylamide system lacks this. In order to provide the above essential characteristics of an effective polymeric support in one matrix the feasibility of developing a crosslinked polymer support consisting of hydrophobic polystyrene chain and flexible hydrophilic crosslinking agents was considered. The preparation of such a type of
polymer support which contains polystyrene backbone and tetraethyleneglycol diacrylate (TTEGDA) crosslinks, their functionalization with different anchoring groups, swelling and stability studies and illustration of the application of these resins in peptide synthesis form the theme of this thesis.

Objectives of the Present Work

The main objectives of the present work are:

1. Preparation of a new class of polymer support by copolymerizing the non-polar hydrophobic styrene with polar hydrophilic tetraethyleneglycol diacrylate (TTEGDA). The idea behind this was to increase the flexibility of macromolecular matrix and thereby increasing the pore size/volume and accessibility of the soluble reactants with the reactive site. Styrene-based supports
have the added advantage of good mechanical stability and ease of functionalization. The presence of hydrophilic and hydrophobic structural units in the crosslinking agent renders the support more compatible with the growing peptide chain.

2. Investigation of the solvation and swelling behaviour of the new resins in a range of solvents commonly used in peptide synthesis.

3. Investigation of the stability of the new resin under various conditions of peptide synthesis.

4. Functionalization of the new polymeric support with the following anchoring groups for peptide synthesis.

Chloromethyl group (1) was introduced into tetraethyleneglycol diacrylate (TTEGDA)-crosslinked polystyrene resin for the synthesis of biologically active peptides which can be cleaved from the support by acidolysis, trans-esterification or ammonolysis. Photolytically cleavable 4-chloromethyl-3-nitro
(II) anchoring linkage was introduced for the synthesis of protected peptides under mild conditions of cleavage. Aminomethyl group was introduced into the new resins for coupling of various anchoring groups. α-Bromopropionyl and α-aminopropionyl (V) groups were incorporated onto
the polystyrene resin for the synthesis of free peptides and C-terminal peptide amides by the photolytic cleavage approach. 4-Bromomethyl-3-nitro benzamido (VI) and 4-aminomethyl-3-nitro benzamido (VII) photolabile groups were introduced in TTEGDA-crosslinked polystyrene resin for its application in the synthesis of peptide acids and peptide amides under mild conditions. p-Alkoxybenzyl alcohol resin (VIII) was prepared for the synthesis of peptides under mild conditions of Fmoc strategy and cleavage of the finished peptide using trifluoroacetic acid.

5. Stepwise syntheses of model peptides, peptide amides and biologically important sequences following the solid phase methodology. Syntheses of the following peptides were investigated by making use of different strategies of protection, coupling, anchoring and cleavage.

a) Boc-Met-Leu-Ala-OMe
b) Boc-Ala-Val-Gly-OEt
c) Boc-Met-Leu-Phe-OMe
d) Boc-Val-Gly-OEt
e) Boc-Phe-Gly-OEt

f) Boc-Ala-Leu-Ala-Ala-Leu-Ala-Ala-Leu-Ala-Ala-Leu-Ala-Ala-OMe

g) Boc-Leu-Leu-Leu-Leu

h) Boc-Gly-Val-Ala-Leu

i) Boc-Val-Glu(0Bzl)-Ala-Leu-Tyr(Bzl)-Leu-NH₂

j) Boc-Met-Phe-Leu

k) Boc-Leu-Ala-Gly-Val-NH₂

l) Boc-Phe-Phe-Gly-Leu-Met-NH₂

m) Deltorphin:
   Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH₂

n) Gly-Arg-Gly-Asp-Ser-Pro

o) Gly-Arg-Gly-Glu-Ser-Pro

p) Ser-Thr-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-Cys(Acm)

q) Pardaxin 1-33:
   Gly-Phe-Phe-Ala-Leu-Ile-Pro-Lys-Ile-Ile-Ser-Ser-Pro-Leu-Phe-Lys-Thr-Leu-Leu-Ser-Ala-Val-Gly-Ser-Ala-Leu-Ser-Ser-Ser-Gly-Glu-Gln-Glu

r) Pardaxin 1-26:
   Gly-Phe-Phe-Ala-Leu-Ile-Pro-Lys-Ile-Ile-Ser-Ser-Pro-Leu-Phe-Lys-Thr-Leu-Leu-Ser-Ala-Val-Gly-Ser-Ala-Leu
Organization of the Thesis

The thesis comprises of seven major sections. After giving a brief introduction of peptide synthesis and stating the objectives of the present work the second section gives a critical review on the role of the macromolecular matrix in solid phase peptide synthesis. The third section describes the various experimental procedures employed in the development of tetraethyleneglycol diacrylate (TTEGDA)-crosslinked polystyrene support. The fourth section comprises of the description of the preparation, characterisation, functionalization and physicochemical studies on the newly developed polymer support. The solid phase synthesis, purification and characterization of model peptides and biologically important peptide sequences are included in the fifth section. Sixth section gives a summary of the work and further outlook. References are listed in the last section.
ROLE OF THE POLYMER SUPPORT IN THE SOLID PHASE SYNTHESIS OF PEPTIDES