Chapter 1

Introduction and Objectives
The last two decades witnessed an exponential growth in the design and development of synthetic peptides and the unlimited variation of the covalent structure of the polypeptide chain with the objective of understanding the molecular basis of protein function or for creating function for novel biotechnological applications. Advances in the methods used for the chemical synthesis of peptides, combined with molecular cloning have enabled a rapid expansion in the field due to its increased 'easiness', possibility of structural modification and yield. Chemistry also promises the ability to systematically tune the property of a protein molecule in a completely general fashion.

Chemical synthesis of peptides can be achieved either by solution phase or solid phase method. Emil Fischer who developed the classical solution phase method in the beginning of the 20th century wrote, "My entire earning is directed towards the first synthetic enzyme. If its preparation falls in to my lap with the synthesis of a natural protein material, I will consider my mission is fulfilled". In the decades since then, the challenge of applying the methods of chemistry to study of the protein action has stimulated numerous advances in the synthetic methods. Historically, these advances included the use of novel reversible protecting group by Bergmann et al., novel activation methods for the formation of amide bonds and the development of new polymer resins as supports for the polypeptide synthesis.

In classical method, synthesis of peptides containing more than a few amino acids is a laborious process. Other serious problems include the poor solubility of some of the intermediate protected peptides in organic solvents and the racemisation of the activated C-terminal amino acids. As an effort to combat these limitations R.B. Merrifield introduced the new concept of polymer supported polypeptide synthesis which revolutionised the field of synthetic organic chemistry and protein research. The technique of this method involves the covalent anchoring of the N'-protected C-terminal amino acids to a solid support usually a cross-linked polymer, followed by the
deprotection and sequential incorporation of activated N\textsuperscript{\textprime} \textprime\textprime-protected amino acids until the target sequence is obtained. One of the main advantages of this method is the lenience of the purification procedure and the ease of automation. This technique later known as solid phase peptide synthesis (SPPS) laid also the foundation of phase transfer catalyst, enzyme immobilisation and combinatorial chemistry which is faster, more efficient and cheaper methods than conventional chemistry, later made a significant impact on the drug discovery process.

The solid support, 2% divinylbenzene cross-linked polystyrene polymer introduced by Merrifield which later evolved into a 1% cross-linked beaded resin are still used in batch synthesis of a number of peptides and proteins. Some problems inherent to SPPS have not been eliminated. These problems generally depend on polymer structure, poor solvation property of the resin and the resin bound peptide in various solvents and the physico-chemical incompatibility of the polymer network with the growing peptide chain.\textsuperscript{21-22} These drawbacks could lead to incomplete coupling and deprotection reactions in various stages of the synthesis that results in very low yield of the target peptide along with a large amount of various truncated and deletion sequences.\textsuperscript{23} The rate of incorporation of a particular amino acid residue decreases with increase in chain length. This phenomenon has been attributed to steric hindrance at various functional sites at the heterogeneous networks. The incompatibility can also influence the mass transport of the reagents, solvation of the polymer as well as peptide and the rates of coupling and deprotection reactions. All these drawbacks demand the development of improved supports and strategies in SPPS. The supports introduced and tested over the years includes polyamides, polyethylene glycol-polystyrene (PEG-PS and Tentagel) graft resin, PEGA, CLEAR, CLPSER and SPOCC resins.\textsuperscript{9-19} Though the amide type supports are highly efficient in peptide synthesis, the mechanical stability is much less compared to styrene-based supports.
Extensive research on solid supports has shown that the success of solid phase polypeptide synthesis especially for longer sequence crucially depends on the property of the solid supports. An ideal polymeric support for solid phase peptide synthesis should be chemically inert to a broad range of reaction conditions; it should have very high swelling characteristics, it must be mechanically stable and applicable in different solvents of varying polarity. A sort of hydrophobic/hydrophilic balance of the macromolecular matrix is thus found to be essential criterion for an efficient solid support. A number of new supports have been developed in recent years by introducing various hydrophilic cross-linkers in place of hydrophobic DVB cross-linker in the polystyrene network.\(^{24-27}\) These new cross-linkers have been designed to increase the swelling characteristics of the polystyrene resin without compromising its mechanical stability and it gives enough flexibility of the polymer backbone in order to allow higher diffusion of the reactive intermediates to resin bound reaction centres throughout the resin matrix. These supports are later substituted with different functionalities inorder to use them for various organic syntheses.\(^{27-31}\)

This thesis discusses the development of a new polymer support and introduces a varied approach in polymer supported polypeptide synthesis. The polymer was developed by the aqueous suspension polymerisation of styrene with tri(propyleneglycol) glycerolate diacrylate (TRPGGDA). Here the secondary hydroxyl functionality of the cross-linker is used for the incorporation of the C-terminal amino acid and the subsequent growth of the peptide. In the swollen state the functional group present in the flexible cross-linker can stay away from the hydrophobic polystyrene pocket which results in enhanced efficiency in C-terminal amino acid incorporation and subsequent coupling and deprotection reactions. Another major problem associated with the similarly cross-linked polystyrene support is the difficulty associated with initial functionalisation of the support with various functional groups. One of the most widely used functionalisation procedures is chloromethylation using chloromethylmethyl ether (CMME) which is a potent carcinogen. CMME can
also introduce additional cross-linking between phenyl rings of the polystyrene backbone: that resulting in high rigidity of the resin reducing the swelling properties of the resin. Another problem is the incomplete conversion of chloromethyl group to other functional groups. All these additional step increase the cost of production of functionalised resin considerably. Taking all these factors into consideration the new PS-TRPGGDA polymeric support is highly cost-effective. The new PS-TRPGGDA posses an optimum hydrophobic/hydrophilic balance. It is mechanically and chemically stable to strong acid and base. The requirement of the initial functionalisation step using various reagents can be avoided. Excellent control over the degree of functionalisation can be achieved by polymerising the required content of TRPGGDA cross-linker with polystyrene.

After a brief discussion on the recent developments in SPPS, the thesis deals with the following studies:

- Synthesis and characterisation of tri(propyleneglycol) glycerolate diacrylate cross-linked polystyrene (PS-TRPGGDA) supports with various contents of cross-linking; the solvation and swelling studies of the polymer in a wide range of solvents and investigation of the chemical stability of the polymer under various reaction conditions

- The functional group capacity of the resin, functional group interconversion and the incorporation of various functional groups in the support.

- A systematic study on the nature of cross-linker, effect of cross-linking density on the swelling and reaction rates and optimization of various peptide synthetic conditions. A kinetic comparison of the supports under peptide bond formation is also carried out with the commercially available Merrifield resin.
The efficiency of new support in polypeptide synthesis by the synthesis of various standard peptide sequences and the comparison of the results with Merrifield, Pam and Sheppard resins.

Application of the new support in polypeptide synthesis by the synthesis of various biologically active peptides and C-terminal modified peptides using both Boc and Fmoc-chemistry.
References


