Chapter 7

Summary
An important objective of the modern biochemical research is to understand the molecular basis of the numerous and intricate biological activities of peptides and proteins and therefore able to predict and control these activities. The importance dramatically increased today because of the explosive success of the genome-sequencing project, which revealed hundreds of thousands of new proteins, but only as predicted sequence data. The chemical synthesis and elucidation of the biological function of a predicted protein molecule is thus a challenge of great significance. The introduction of unnatural chemical groups into the covalent structure of a peptide or protein molecule can provide valuable insight into questions about peptide/protein functions and enzyme activity. The most efficient way of introducing these unnatural amino acids into peptides and small proteins through the complementary approach of total chemical synthesis is by the stepwise solid phase peptide synthesis. The yield and the purity of these products are depending upon various factors of which the most important one is the choice of solid support, its mechanical and chemical stability, swellability and compatibility with a range of solvents with different polarity. For a successful synthesis, the reaction should go to completion in each step at a higher rate. The physicochemical incompatibility of the support with the growing peptide chain is considered as another major factor that affects the performance of the resin in polypeptide synthesis. The design and synthesis of an efficient polymer support depends on the judicious selection of monomers, their structure and polarity and the net hydrophobic/hydrophilic balance of the polymer. The work presented in the thesis describes the development of a new TRPGGDA cross-linked polystyrene support with a view to approaching the above mentioned problems.

A brief overview of the key characteristics of various solid supports and their modification with suitable linkers, protection strategies, carboxyl activation reagents and the difficulties encountered in SPPS are described in the first and second chapters. This review aims to give a comprehensive outline of the recent developments and refinements in the field of polymer
supported polypeptide synthesis and various factors involved in optimising the purity and the yield of the synthetic peptide.

The third chapter describes the synthesis of the new polymer support, PS-TRPGGDA. This has been designed to increase the flexibility of the polymer backbone allowing better diffusion of reagents through the matrix. The polymer is synthesised by the aqueous free radical suspension polymerisation of styrene and TRPGGDA. The resultant polymers have propyleneglycol-like character, which provides a spacer effect from the rigid polystyrene core, and the glycerol moiety present in the cross-linker serves as the growth site for the polypeptide synthesis. The hydrophilicity of the cross-linker and its content in the resin determines the hydrophobic/hydrophilic balance of the new polymer. It also results in the very high swelling of the polymer in a wide range of solvents. The support was stable to the various chemistries employed during the synthesis. The support was characterised by CP-MAS $^{13}$C NMR and FT-IR techniques. The shape, size and morphological features of the crosslinked polymer beads were analysed by scanning electron microscopy. SEM analysis of the polymer showed that they are uniform spherical beads. This chapter also describes the functional interconversion of the hydroxy resin to various other derivatives like chloro and amino resins. The support was also functionalised with various commercially available linkers like HMPA, HMPB and Rink amide so that the peptide or its derivative can be cleaved from the support in short time.

The fourth chapter describes the optimisation of various chemical reaction conditions encountered during polypeptide synthesis when the new resin is used as the support for the synthesis. A systematic study of the nature and the percentage of the cross-linker present in the resin on its swelling behaviour in various solvents were carried out. This chapter also critically evaluates the various parameters like optimum reaction conditions for the C-terminal amino acid incorporation, Nα-deprotection, coupling reaction and final cleavage of the target peptide from the support. A kinetic comparison of
the amide bond formation was carried out on the new support with Merrifield resin. The quantification of these closely related parameters was found necessary for the judicious selection of the optimised reaction conditions.

The fifth chapter discusses a comparative synthetic study of some difficult peptide sequences, which are considered as the test peptides to evaluate the capability of a new support with commercially available Merrifield, Sheppard and Pam resins. Various techniques like HPLC, amino acid analysis and MALDI-TOF-MS were employed to analyse the purity of the synthetic peptides. The high yield and purity of the peptides synthesised on the new support reveal the ability of the resin to assist solvation and break the aggregation of peptide by direct amphipathic interaction between the polymer and the peptide chain. This study revealed the superiority of the new support over various commercially available supports.

The sixth chapter discusses the utility of the new resin in the synthesis of a number of different biologically active peptides in good yield and purity using either by Boc- or by Fmoc chemistry. PS-TRPGGDA supports with various cross-linking densities were utilised for the synthesis. These sequences include the NR2B and NR2A peptide substrates and their mutations of Ca$^{2+}$/calmodulin binding protein and various peptides from the non-structural regions of hepatitis C viral polyprotein and nuclear export signals. The purity of these synthetic peptides were analysed by RP-HPLC using C18 column and further confirmed by amino acid analysis and MALDI-TOF-MS. Circular dichroism measurements were also carried out on selected peptides.

The work presented in this thesis introduces a new class of polymer support for solid phase peptide synthesis. This new highly flexible and chemically inert PS-TRPGGDA support, combining with the high mechanical stability of the hydrophobic polystyrene matrix, makes it a better polymer support than most of the commercially available supports. The oxypropylene chains together with ester functionality’s and hydroxyl groups of the cross-linker confer a hydrophilic character to the polymer. By selecting the
percentage of cross-linker in the polymer an optimum hydrophobic/hydrophilic character can be imparted to the resin. The high swelling characteristics of the resin in various solvents employed in the polypeptide synthesis can result in an effective interaction between the resin bound functionality and the reactive species in the solvent. Since the secondary hydroxyl group in the resin is selected as the peptide growth point, the drastic initial functionalisation steps involved in polystyrene type polymers with various reagents like chloromethylmethyl ether can be avoided in the new resin. The degree of functional groups available for C-terminal amino acid incorporation can be easily controlled by selecting the degree of cross-linking in the resin. This makes the resin more economical when compared to most of the other commercially available resins. The performance of PS-TRPGGDA resin in polypeptide synthesis, especially in the case of sterically hindered peptide sequences and those which tends to form a secondary structure during the synthesis, makes it a better choice when compared to various styrene-based supports.