CHAPTER 8

SUMMARY AND OUTLOOK
The work presented in this thesis describes the development of new 1,4-butanediol dimethacrylate cross-linked polystyrene and its application as a support for solid phase synthesis of polypeptides. Preparation, functionalisation of the support with various functional groups and incorporation of different anchoring groups that enable the cleavage of the peptide or peptide derivatives under selected conditions are discussed. Solvation, swelling and stability of the support under different synthetic conditions were compared with PS-DVB support. The coupling and deprotection reactions were optimized. The synthetic efficiency of the new support was demonstrated by comparative synthesis of few peptides with commercially available Merrifield and Sheppard resins under the same synthetic conditions. The utility of the support was further demonstrated by stepwise synthesis of several biologically active peptides with high purity and yield.

A review on recent developments in solid phase peptide synthesis including various resins used as solid support for peptide synthesis, the effect of different macromolecular network of the polymer in various peptide synthetic reactions, application of different acid, base and photo-labile anchoring groups, carboxyl activation using different activating groups and a brief summary of the organization of the thesis are described in the first and second chapters. The review gives an idea about the important developments in solid phase peptide synthesis and the physicochemical properties of the polymer supports.

The third chapter describes the synthesis and characterization of the new 1,4-butanediol dimethacrylate cross-linked polystyrene support, prepared by the aqueous suspension polymerization of styrene with 1,4-butanediol dimethacrylate. The hydrophilicity of the cross-linker provides flexibility and polarity to the support compared to PS-DVB resin. The hydrophobic styrene gives the desired mechanical stability to the resin. The support was functionalised with chloromethyl, aminomethyl and hydroxymethyl groups using various reagents under controlled conditions. The time and temperature dependent chloromethylation reaction using CMME was conducted and optimized. A comparative swelling study with PS-DVB resin in various solvents showed that BDODMA cross-linker had a marked influence on the swelling behaviour. Chloromethylation of the support is not influenced in the swelling character of the resin.
IR spectral studies showed that the support is highly stable in various reagents even after 48 h suspension. This chapter also describes the optimization of C-terminal amino acid incorporation, deprotection of temporary Nα-amino protection, coupling reaction and the peptide cleavage from the support using TFA.

The efficiency of the new support in polypeptide synthesis was compared with Merrifield and Sheppard resin by synthesizing a few model peptides under identical conditions; this is described in the fourth chapter. The purity of the peptides was analyzed by HPLC and the results showed the superiority of PS-BDODMA resin over PS-DVB resin; it is equally efficient as the Sheppard resin in peptide synthesis. The following peptides were synthesized for comparative studies:

1. Leu-Ala-Gly-Val (Merrifield's model peptide)
2. Leu-Gly-Ala-Leu-Gly-Ala
3. Ala-Ala-Ala-Ala
5. Leu-Ile-Asn-Thr-Asn-Ala-Ser-Trp-His-Ala-Asn-Arg-Thr-Ala-Leu-Ser-Asn-Asp-Ser-Lys-Leu-Asn-Thr-Gly-Ala-NH₂ (25-residue peptide selected from NS 1 region of hepatitis C viral polyprotein).
6. Leu-Ile-Asn-Thr-Asn-Ala-Ser-Trp-His-Ala-Asn-Arg-Thr-Ala-NH₂ (14-residue peptide selected from NS 1 region of hepatitis C viral polyprotein).
7. Leu-Asn-Cys(Acm)-Asn-Asp-Ser-Leu-Asn-Thr-Ala-NH₂ (10-residue peptide selected from NS 1 region of hepatitis C viral polyprotein).

The support was employed for polypeptide synthesis using Fmoc and Boc-chemistry. Different acid and base labile anchoring groups such as HMPA, HMPB, HMBA and Rink amide groups were incorporated to the resin prior to peptide synthesis. After the synthesis the target peptide was cleaved under controlled conditions so that side reactions can be avoided. Peptide purity was tested by HPLC and characterized by amino acid analysis and MALDI TOF MS. In the fourth and fifth chapter we discuss the various conditions used for the synthesis of the following peptides and the secondary structure of these synthetic peptides in phosphate buffer (pH=7) were analyzed by circular dichroism.

The following peptides were synthesized by Boc-chemistry:
1. Phe-Phe-Thr-Lys-Phe-Lys-Ser-Gln
2. Val-Gln-Gln-Gly-Pro-Trp-Gly-Gly-Ala-Ala-Val
4. (Val)_{10}
5. Val-Gln-Asn-Asn-Val-Val-Val-Val
6. Pro-Val-Val-Thr-Val-Val-Val-Val-Asn
7. Thr-Val-Val-Val-Val-Asn
8. Pro-Met-Leu-Phe-Val-Thr
9. Val-Met-Leu-Phe-Leu-Pro
10. Met-Leu-Phe-Tyr-Val-Gly
11. Boc-Met-Leu-Phe-Cys(Acm)-Lys(C1-Z)-Val-OMe
12. Boc-Pro-Met-Leu-Phe-Val-Thr-OMe
14. Boc-Met-Leu-Phe-Tyr(Bzl)-Val-Gly-OMe
15. Tyr-Gly-Gly-Phe-Leu (Leucine enkephalin)
16. Tyr-Pro-Phe-Pro-Gly-Pro-Ile (β-casomorphin, bovine)
17. Tyr-Pro-Phe-Val-Glu-Pro-Ile (β-casomorphin, human)
18. Val-Gly-Gly-Ser-Glu-Ile (77-82 fragment of C-reactive protein)
20. Tyr-Ala-Gly-Ala-Val-Val-Asp-Leu (inhibitor of Ribonucleotide reductase of HSV-type1)
21. Met-Leu-Gly-Tyr-Phe-Lys-Asp-Phe-Lys-Ala (Scyliorhinin 1 peptide)
22. Asn-Ser-Thr-Trp-Ser-Thr-Glu-Gly-Ser-Asn-Thr-Glu-Gly-Ser-Asp-Thr-Ile-Thr-Leu-Pro-Cys(Acm)-Arg-Ile-Lys-Gln-Ile-Ile-Asn-Met-Trp-Gln-Lys-Val-Gly-Lys-Ala-Met-Tyr-Ala-Pro-Pro-Ile (43-mer peptide from the CD-4 binding domain of Human Immuno Deficiency Virus envelope glycoprotein)
The following peptides were synthesized by Fmoc-chemistry:

3. Thr-Gly-Ile-Asp-Ile-Ala-Gly-Cys-Lys-Ile-Lys-Gly (33-44 fragment of Esculentin 1)
4. Thr-Gly-Ile-Asp-Ile-Ala-Ala-Cys-Lys-Ile-Lys-Gly (33-44 fragment of Esculentin 1 modified at Gly19 by Ala)

Synthesis of protected peptides is still a challenging problem in SPPS. The peptide cleavage from resin under strong acidic and basic conditions can result in various side reactions if the cleavage conditions are not properly selected. In chapter seven, we described the stepwise incorporation of the amino acids and the cleavage of peptide or peptide derivatives in the fully protected form from the resin under neutral conditions using principles of photochemistry. The photolabile anchoring groups like 4-bromomethyl 3-nitro benzamidomethyl, 4-aminomethyl 3-nitro benzamidomethyl, N-methyl aminomethyl 3-nitro benzamidomethyl and N-ethyl aminomethyl 3-nitro benzamido- methyl were introduced between the resin and the growing peptide. After incorporating all the amino acids, the peptide was cleaved by photolysis at 350 nm under neutral condition as protected peptide acid, amide or N-alkyl amide. These peptides were purified by various chromatographic techniques and characterized by MALDI TOF MS.

The following protected peptides and peptide derivatives were synthesized using the above technique:
The PS-BDODMA resin serves as a new class of polymeric support for solid phase peptide synthesis. The optimum hydrophobic-hydrophilic balance of the resin causes the high swelling in different non-polar and polar solvents. Consequently the range of chemistry that could be conducted on the support makes it an efficient support for different organic reactions. The ease of preparation, functionalisation and workup procedures are the advantages of the resin over conventional polymer supports. The physicochemical compatibility of the macromolecular support and the growing peptide chain help to synthesize peptides of very high purity and homogeneity. The enhanced coupling rate during peptide bond formation, high sensitivity in monitoring the coupling reactions, economical use of reactants and solvents and the high yield and purity of the peptides are the distinct features of the new PS-BDODMA support over the PS-DVB support. Peptide synthesis using PS-BDODMA resin showed that the support is flexible and suitably adopted for the conventional synthetic procedures.
The work presented in the thesis was published or is in the process of publication as detailed below.


   K. S. Kumar, M. Roice, P. G. Sasikumar and V. N. Rajasekharan Pillai, communicated.

   M. Roice, K. S. Kumar and V. N. Rajasekharan Pillai, communicated.

   M. Roice, K. S. Kumar and V. N. Rajasekharan Pillai, communicated.

6. Synthesis of Immunodominant Peptide Region of Hepatitis C Viral Pathogens Using PS-BDODMA Resin; A Single Peptide Derived from the Conserved Domain (E2/NS1) was Highly Effective in Detecting Anti-HCV-Antibody.
7. 1,4-Butanediol Dimethacrylate Cross-Linked Polystyrene Resin an Effective Support for Polypeptide Synthesis: A Comparison with PS-DVB Resin.

K. S. Kumar, M. Roice, P. G. Sasikumar and V. N. Rajasekharan Pillai, communicated.


K. S. Kumar, M. Roice, P. G. Sasikumar and V. N. Rajasekharan Pillai, communicated.


M. Roice, K. S. Kumar and V. N. Rajasekharan Pillai, communicated.