1.1. General

The identification and optimization of new drug candidates are greatly facilitated by combinatorial and parallel synthesis and these techniques have become commonplace in modern organic chemistry [Frecentese et al., 2010; Jung, G. 1996]. These methods often employ polymeric supports to immobilize either the substrate or the reagent in order to simplify compound manipulation and purification. Insoluble polymer resins are most commonly used for such applications, [Yü et al., 2002; Thompson., 2000; Xiang et al., 2009] but soluble polymers have also proven useful [Chen et al., 2008; Holmes et al., 2008]. The insoluble polymer resin of choice has been the one originally introduced by Merrifield, divinylbenzene cross-linked polystyrene (PS-DVB), and this resin is commercially available derivatized with a wide variety of functional groups for substrate attachment [Merrifield., 1963]. Merrifield’s invention of solid phase organic synthesis revolutionized the field of peptide chemistry and opened a new era in organic synthesis especially in the field of polypeptide and oligonucleotide synthesis [Cooke et al., 2006; Kojima et al., 2009; Sethi et al., 2009; Roice et al., 2003; Clark et al., 2006].

Merrifield’s basic concept was to covalently attach the first amino acid to an insoluble support and to elongate the peptide chain from this support bound residue. Following the incorporation of desired number of monomers through a series of coupling and deprotection steps, the peptide was finally removed from the support and purified and characterized in free solution. The separation processes are quick and simple, and can be machine aided. There is an enormous time and labor advantage over the corresponding operations in solution chemistry which commonly involve techniques of
solvent extraction, filtration, evaporation and crystallization. These often results in substantial loss of material so that solid phase synthesis may be much more effective in this sense also. Retention of resin bound peptide in the same reaction vessel at all times also minimizes physical loss, as soluble reagents can be so easily removed by filtration, large excess can be used encouraging high efficiency in the various chemical steps. The enabling insights of solid phase methodologies are that reactions may be driven to completion by use of excess reagents, and that the only purification required after each step is simple washing away of excess reagents. The majority reactions of solid phase peptide synthesis (SPPS) are repetitive and proceed in high yield, hence the process can be automated, and even a non-specialist can produce the peptides in good overall yields and purities. Though the idea sounds simple, its actual implementation in the early stages of development required much effort. Lack of suitable N-terminal and side chain protected amino acids and the coupling reagents made polypeptide synthesis really a tough job for peptide chemist at that time. Further improvements to the chemistry, polymer matrices, linkers and protecting groups led to the development of automated peptide synthesizers by which dozens of peptides could be constructed simultaneously in just a few days [Teague., 2003; Geysen et al., 2003]. Recent developments in the field of human genome mapping, and with the rapid advances in cell biology, material science and new screening methods, it can be confidently predicted that the use of peptides and their derivatives are going to play a pivotal role in disease diagnosis and drug design [Gadek et al., 2003; Houseman et al., 2002; Holmes., 2002; Bolhassani., 2011; Tan et al., 2010; Leuschner et al., 2004].
The polymeric support introduced by Merrifield, divinylbenzene cross-linked polystyrene (PS-DVB), later came to be known as Merrifield resin and is still the most commonly used polymer support for solid-phase synthesis because of the advantages such as good mechanical and chemical stabilities and the facility of derivations with a wide variety of functional groups for substrate attachment. Although the polymer supported polypeptide synthesis strategy has many advantageous, it has several shortcomings due to the heterogeneous reaction conditions such as low reaction rate, difficulty to monitor the real time of reactions compared to solution phase chemistry, the extra steps required to attach and release the substrate from the resin and the presence of part of the resin attachment often found along with the final product. The non-linear kinetic behavior and non-uniform distribution of functional sites within the resin can also affect the purity and yield of the final product. The use of PS-DVB resin in many cases is accompanied by difficulty during synthesis. This is primarily due to the high hydrophobic character of the matrix, the short and rigid cross-linker (DVB) connecting the PS backbone and the presence of local higher cross-link density regions because of higher reactivity of DVB relative to styrene in the free radical copolymerization. The addition of polar solvents to the highly hydrophobic macromolecular PS-DVB supports imparts a negative influence, thereby reducing the possibility of effective interaction between reactants and the active sites within the polymer matrix. This phenomenon leads to the formation of truncation and deletion of peptide sequences [Barany et al., 1987; Bayer et al., 1970]. The non-accessibility of the resin bound functional site to different chemical reaction conditions, solvation problems and the incompatibility between polymer network and the growing
peptide chain of the PS-DVB resin led several laboratories to peruse alternative strategies
to develop new polymer matrices which are highly compatible with the growing peptide
chain.

Commercially available supports and linkers are originally developed primarily for
biopolymer synthesis and may not be ideal for broader range of conditions required for
small molecule synthesis. The continued adaptation of chemistry to the solid phase
requires new supports and linkers which will tolerate the full spectrum of reaction
conditions and reagents developed for chemistry in solution over the last century.
Extensive research on solid supports has shown that the success of polypeptide synthesis
especially for longer sequences depend on the various properties of the solid supports. An
ideal polymeric support for SPPS should be chemically inert, mechanically stable, should
have high swelling ability and must be physico-chemically compatible with the growing
peptide chain being synthesized and to the medium in which reaction is carried out. The
non-accessibility of the resin bound functional site to different chemical reaction
conditions, solvation problems and the incompatibility between the polymer network and
the growing peptide chain led several laboratories to pursue alternative strategies to
develop new polymer matrices which are highly compatible with the growing peptide
chain. Lightly cross-linked gel-type polystyrene (GPS) has been most widely used due to its
common availability and inexpensive cost. GPS beads which are functionalized with
chloromethyl, aminomethyl and a variety of linkers are commercially available from a
variety of sources. A prominent characteristic of GPS beads is their ability to absorb large
relative volumes of certain organic solvents (swelling). This swelling causes a phase change
of the bead from a solid to a solvent-swollen gel, and therefore, the reactive sites are accessed by diffusion of reactants through a solvent-swollen gel network.

1.2. Peptide synthesis

Proteins formed together with carbohydrates, lipids and nucleic acids are the 'molecules of life'. Currently, biotechnology-based initiatives like the Human Genome Project [Falcón de Vargas., 2002; Gisler et al., 2011] as well as the improved understanding of fundamental biological processes provide a huge number of new protein sequences [Austin., 2003; Frenkel et al., 2009]. This leads to a rapid increase in the number of novel or important targets for drugs and agricultural applications. Proteinaceous biomolecules such as antibodies, antigens, growth factors and bioactive peptides are well-known for their therapeutic potential in various diseases [Yadav et al., 2011]. These therapeutic proteins and peptides are potential target for the development of therapeutic nanoprotein and peptides because of their nanoscale dimension, highly specific therapeutic importance and numerous other specific enzymatic activities [Astier et al., 2005; Armon-Omer et al., 2004; Kennedy et al., 2008; Leader et al., 2008]. Therefore, there is a high demand for these new targets in at least micro to multi-milligram quantities. Obviously, access to these proteins should be provided within the shortest possible time frame. Mainly three different approaches are used to fulfill these requirements which are,

1.2.1. From natural sources

Isolation of native peptides from natural sources [Li et al., 2008; Wilson et al., 2011], however, is often problematic. In many cases, the concentration of peptide
mediators ranges from $10^{-15}$ to $10^{-12}$ mol per mg fresh weight of tissue. Therefore, only high sensitive assay methods such as immunohistochemical techniques which render cellular localization are possible. Although not all relevant bioactive peptides occur in such low concentrations, isolation methods generally suffer from disadvantages, such as limited availability of human tissue sources. Complicated logistics during collection or storage of the corresponding organs, e.g., porcine or bovine pancreas for insulin production, additionally impose difficulties on the utilization of natural sources. Possible contamination of tissue used for the isolation of therapeutic peptides and proteins with pathogenic viruses is an enormous health hazard.

1.2.2. By recombinant expression

Nowadays, many therapeutic peptides and proteins are produced by recombinant expression of proteins in genetically engineered microorganisms or animals [Li., 2011]. Immunological incompatibilities of peptide drugs obtained from animal sources have also been observed. Consequently, the development of processes for the synthesis of peptide drugs must be pursued with high priority. Although most of the proteins have been obtained by recombinant methods, these approaches often suffer from the time used to generate milligram quantities. Formation of inclusion bodies, misfolding, and low expression levels further decrease the efficiency of this method [Rudolph et al., 1996]. In addition, expressed heterogeneity and biological contamination (e.g. DNA impurities or endotoxins), may affect their use or activity.
1.2.3. By chemical synthesis

A good solution to circumvent the above mentioned drawbacks is provided by their chemical synthesis [Albericio., 2004; Johnson et al., 2007; Parra et al., 2009]. Chemical peptide synthesis is the classical method which has been mainly developed during the past five decades, although the foundations were laid in the early 20\textsuperscript{th} century by Theodor Curtius and Emil Fischer. Chemical synthesis is probably the most practical way of providing sufficient amount of material, and in addition, allows the systematic variation of structure necessary for the development of peptides for various biochemical studies. Besides avoiding biological contaminations, such a purely synthetic approach offers the possibility to incorporate unnatural amino acids or other chemical modifications that may improve protein efficacy. Synthetic peptides find applications in all areas of biomedical research including immunology, neurobiology, pharmacology, enzymology and molecular biology [Jones., 2000; Rajanbabu et al., 2010]. Nowadays, the synthetic approach allows only the routine synthesis of small proteins with up to 50 amino acids. There are few examples of longer chains, such as ribonuclease A (124 residues) [Jones., 2000] and human immune deficiency virus (HIV)-1 TaT (86 residues) [Gutte et al., 1971] or the green fluorescent protein, a 238-residue peptide chain [Nishiuchi et al., 1998].

Solution phase technique has been used for the synthesis of small peptides composed by only a few amino acid residues and the classical approach of solution phase peptide synthesis has yielded impressive successes in the preparation of several biologically active peptides [Vigneaud et al., 1953; Merrifield., 2006; Hofmann., 1961]. But
this method developed by Fischer was laborious and time-consuming because the intermediate peptides have to be removed, purified and characterized before the next coupling step [Dahmen et al., 2001;]. Insolubility of the intermediate peptide in solvents used for the synthesis and mechanical losses are other problems associated with this method. Methods such as chromatography and crystallization required during the synthesis results in considerable reduction of the overall yield of the peptides. Therefore, a new approach was needed if large amounts of peptides were required or if larger and more complex peptides were to be made.

1.3. Solid phase peptide synthesis

Merrifield introduced the concept of solid phase synthesis to achieve more efficiency in the synthesis of peptides and described the preparation of the tetra peptide ‘Leucyl-Alanyl-Glycyl-Valine’ by successive addition of benzoyloxycarbonyl amino acids to polystyrene resin. Simultaneously with Merrifield, Letsinger and Kornet reported synthesis of a dipeptide, L-leucyl-glycine on a "Popcorn polymer support" using a different chemical strategy [Letsinger et al., 1963].

Stepwise SPPS introduced by Merrifield has fundamental physical and chemical advantages over the solution phase approach. The reactions can be driven to completion using excess soluble low molecular weight reagents and final products are obtained in good yield. The excess reagents can be easily separated from the polymer-bound peptide by simple filtration. As a result, the laborious and cumbersome purification of intermediate peptide is avoided and this results in a tremendous saving of time. Since it is possible to carry out all the reactions in a single reaction vessel, manipulations and
attendant losses involved in the repeated transfer of materials can be avoided. After synthesis, the spent resin can be recycled as such or with some chemical modifications. So the process is economical. In short, the advantageous of solid phase organic synthesis (SPOS) relative to solution phase synthesis are generally manifestation of six characteristics associated with the techniques which can be summarized as following points.

1.3.1. Advantageous of SPPS

(a). The peptide is synthesized while its C-terminus is covalently attached to an insoluble polymeric support. This permits the easy separation of the growing peptide from any by-products or excess unused amino acid components (b). Reactions are driven to completion by using an excess of reactants and reagents (c). No mechanical loss occurs because the growing peptide is retained on the polymer in a single reaction vessel throughout the synthesis (d). Final peptide is detached from the polymer support by a single cleavage step at the end of synthesis. The side chain protecting groups can also be cleaved in the same reaction in order to simplify the work-up and the isolation of the final peptide. The cleavage step does not degrade the assembled peptide (e). Physical operations involved in the synthesis are simple, rapid, and amenable to automation (f). Spent resin can be recycled. The wide spread applications of solid phase synthesis has expanded from peptides to other biomolecules, including DNA [Zatsepin et al., 2005], peptide nucleic acids (PNA) [Richter et al., 1995; Sato et al., 2003] and oligosaccharides [Lam et al., 2002; Agoston et al., 2009; Guillaumie et al., 2003] and the basic principles
have been exploited to create the emerging field of combinatorial chemistry [Renault et al., 2001; Opatz et al., 2004; Díaz-García et al., 2006].

1.3.2. Shortcomings of PS-DVB resin

The polymeric support developed by Merrifield has been the most commonly employed resin for SPOS because of the advantages such as good mechanical and chemical stabilities and the facility of derivations with a wide variety of functional groups for substrate attachment [Vaino et al., 2000]. But the use of PS-DVB resin in many cases is accompanied by difficulty during synthesis. Aromatic substitutions of the styrene ring and Friedel crafts alkylation or acylation in presence of appropriate Lewis acid catalysts have served as the principle approaches for the initial introduction of functional groups in to the polystyrene resins [Pepper et al., 1953]. A major problem in many of these methods is the potential for introduction of new cross-links in the polymeric matrix, such as methylene or sulfone bridges during chloromethylation or sulfonation respectively that leads to a chemically ill-defined support. Inhomogeneity in cross-linked polystyrene has long been known to hamper synthetic efforts [Arshady et al., 1992]. This difficulty is primarily due to the high hydrophobic character of the matrix, the short and rigid cross-linker (DVB) connecting the PS backbone and the presence of local higher cross-link density regions [Bedford et al., 1992; Lu et al., 1990]. PS-DVB is completely hydrophobic in nature, whereas the growing peptide chain is much more hydrophilic and this difference induces a chain folding effect in which the peptide satisfies its own hydrogen bond requirements rather than being solvated. This can severely limit the synthetic access to the exposed end of growing chains.
1.3.3. Characteristics of ideal solid supports

The ‘solid support’ must possess certain physical and chemical parameters which are required for a support to be useful for organic synthesis. So the choice of polymer support plays an important role in the success of solid phase reactions [Labadie., 1998; Patrick et al., 2000] which depends on both its chemistry and the environment in which the support is used [Gerritz et al., 2000; Franzen., 2000]. So an ideal, efficient solid support should possess certain characteristic features which are, (a) Resin must be in particles of physical size and shape that permit ready manipulation and rapid filtration from liquids and hence it must be physically stable [Stewart., 1976]. (b) Resin should be inert in all reagents and solvents used during the synthesis. SPOC may require a resin that is in contact with a wide range of reagents (strong acidic or basic conditions; radical, carbonium, carbanion; reducing and oxidizing conditions; conditions of nitration or halogenations) and subjected to a variety of reaction conditions (variation in temperature and pressure) [Marshall et al., 2004; Boyle et al., 2002]. (c) As a polymer support, the swelling property is very important when used in organic synthesis and many chemists and biologists have noticed the importance of swelling properties of the polymer supports [Meldal., 1997; Kokufuta et al., 2011; Sarin et al., 1980]. The reactions are heterogeneous in nature, which means that if a reagent should react with any active site at the interior of the polymer matrix, it has to diffuse from the bulk phase to the resin matrix. High swelling properties of the polymer chain may induce the reagent to diffuse quickly inside the resin and the reactions proceeds smoothly in more homogenous phase. A cross-linked polymer that does not swell when imbued in solvent provides little opportunity for reagents to
interact, precluding reactions. For a cross-linked polymer, the degree of swelling is described by the Flory-Rehner theory [Flory et al., 1943]. The extent of swelling may be quantitated by a variety of techniques, including volumetric analysis, [Coin et al., 2007; Fields., 1991] gravimetric analysis after centrifugation or rapid filtration [Gregor et al., 1965; Pepper et al., 1952], direct microscopic examination [Shankman et al., 1949] and exclusion of high-molecular-weight substances [Itsuno et al., 1990]. (d) The resin must be readily modified to allow attachment of the first entity (i.e. an amino acid in SPPS or a variety of organic molecules in SPOC), by a covalent bond. The introduction of functional groups suitable for further chemical elaboration is usually accomplished by derivatization of preformed polymers, although, in a number of cases, a functionalized monomer unit may be copolymerized in the course of preparation of the support [Guyomard et al., 2004; Otero-Romani et al., 2009].

1.4. Classification of solid supports

Novel resins have been developed after Merrifield resin with different aims such as improving resin-peptide bond stability, solvent-resin product compatibility, support loading, coupling efficiency, cleavage of finished resin-peptide bond and synthesis of protected peptide fragments including peptide esters, amides or hydrazides. The recent interest in small molecule combinatorial libraries has led to rebirth of SPOC and subsequent need for both new types of resins and linkers with varied physical properties to accommodate a wide range of reagents and reactions. Based on physical properties, following classification of solid supports has been proposed.
1.4.1. Gel type supports:

Gel type supports are most often used for solid phase synthesis and feature equal distribution of functional groups throughout a highly solvated and inert polymer network ideal for the assembly of large molecules. The polymer network is flexible and the resin can expand or exclude solvent to accommodate the growing molecule within the Gel. There are four types of Gel resins.

1.4.1.1. Polystyrene (PS) resins

The hydrophobic polystyrene resins are produced from styrene backbone with varying mol percentages of different flexible cross-linkers. In order to circumvent the inherent problems associated with PS-DVB resins, new cross-linkers have been designed and used for polymerization [Sherrington, 1998; Patrick et al., 2001]. The strategy of using flexible cross-linkers to prepare polystyrene resins was first introduced by Itsuno [Itsuno et al., 1989]. Kurth and co-workers have studied the physical properties of resins cross-linked by the same molecule [Wilson et al., 1998]. The new cross-linkers have been designed to increase the flexibility of the polymer backbone to allow for better diffusion through the matrix and also to impart a variety of solvent-like properties to the resins [Sasikumar et al., 2003]. In addition to derivatives of PEG-diacylate cross-linked systems [Sherrington, 2001; Wang et al., 2006; Siyad et al., 2010], a number of other flexible cross-linkers have been designed to improve hydrophobic character of styrene based supports such as 1,4-butanediol dimethacrylate [Roice et al., 1999], tetraethyleneglycol diacrylate [Kumar et al., 2000], 1,6-hexanediol diacrylate [Arunan et al., 2000], and so on [Groth et al., 2000; Kita et al., 2001; Leena et al., 2001; Moss et al., 2002; Delgado et al.,
2002; Ajikumar et al., 2000; Arunan et al., 2003; Cavalli et al., 2003; Yokum et al., 2000; Kumar et al., 2004; Sasikumar et al., 2004; Roice et al., 2005; Krishnakumar et al., 2002] which were copolymerized with styrene to prepare polystyrene resins as supports for solid-phase synthesis. Efficiency of these resins was demonstrated by synthesizing large number of peptides in high purity and yield [Ajikumar et al., 2001; Kumar et al., 2001]. The optimum reactivity of these newly developed resins was due to the greater chain mobility of the cross-linker in solvents that enable effective interaction between the reactants and resin bound functional groups [Rademann et al., 2000].

1.4.1.2. Polyacrylamide resins

In order to optimize the resin structure in SPPS, Sheppard introduced a polar polydimethyl acrylamide resin, which is structurally similar to peptide backbone [Atherton et al., 1975]. This helps easy solvation of the peptidyl resin and thus reduces the steric hindrance during deprotection and coupling reactions [Mitchell.,2008]. The polyacrylamide resins are well solvated in polar solvents such as DMF and have improved properties compared to polystyrene for synthesis under polar conditions. Sufficient solvation in aqueous buffers has allowed the development of solid-phase enzyme assays [Meldal et al., 1994]. The support shows effective swelling in polar solvents but in non-polar solvents it is very poor. The chemical stability of the resin was also less compared to that of polystyrene supports.

1.4.1.3. Poly (ethylene glycol) grafted resins

An alternative approach to impart polar solvent/aqueous compatibility of solid support is to graft larger linker units such as poly(ethylene glycol) directly onto the
polystyrene (PEG-PS) support [Zalipsky et al., 1994]. PEG-PS was developed as a mechanically more stable resin that spaces the site of synthesis from the polymer backbone and a highly promising class of solid support which is used successfully in peptide synthesis [Ede., 2002; Wang et al., 2010]. PEG-containing supports of these types are available commercially as TENTA-GEL and ARGO-GEL [Delgado et al., 2002] and have been used successfully for SPOC. Swelling, a sign of solvation of the resin, is good for polystyrene resins in non-polar solvents like DCM, whereas polyacrylamide resins swell much better in DMF. Mixed PEG-PS polymers show excellent swelling in common solvents such as THF, acetonitrile and alcohols [Park et al., 2000]. The weight ratio of PEG to polystyrene is often about 1:1 in these resins, and the presence of PEG results in a complete solvation behavior. PEG-PS resins are thus very useful in SPOC, where a wide variety of solvent conditions may be required for optimal results, and in solid phase synthesis of larger peptides. PEG-PS resins are also stable in flow systems. The uniformity of bead size makes these resins ideal for synthesis of libraries using the ‘one bead-on compound’ approach [Franz et al., 2003].

1.4.1.4. Poly(ethylene glycol) based resins

Optimal support properties can be achieved by using PEG as the major component of the resin [Rademann et al., 1999, García-Martín et al., 2006]. The PEG molecule has an amphipathic nature and is solvated well in both polar and non-polar solvents as well as mobility and reorganization of the PEG molecules create a highly dynamic process. The four important classes of cross-linked PEG based polymeric supports currently available are,
1.4.1.4.1. PEGA resins:

PEGA [Poly(ethylene glycol)-Poly(acrylamide)] resins are synthesized by partial acroylation of bis-2-aminopropyl PEG with acryloyl chloride followed by radical initiated inverse suspension polymerization yields resins that are well suited for continuous flow peptide synthesis [Meldal., 1992]. PEGA resins contain only polyether backbones and polyacrylamide backbones linked together by amide bonds that are stable and inert under all peptide synthesis conditions including cleavage with strong acids or aqueous base. The families of PEGA resins are flow stable and beaded, and they can be used as solid supports in split synthesis of libraries [Renil et al., 1995].

1.4.1.4.2. POEPS resin:

POEPS [Polyoxyethylene-polystyrene] resins are synthesized by partial alkylation of PEG with chloromethyl styrene that gives a mixture of non-, mono-, and bisalkylated PEG that can be polymerized in a beaded form by inverse suspension polymerization followed by swelling and granulation. The resin shows good solvation in water as well as in non-polar media such as toluene [Renil et al., 1996].

1.4.1.4.3. POEPOP resin:

POEPOP [Polyoxyethylene-polyoxypropylene] resins are synthesized by partial reaction of poly(ethylene glycol) with sodium hydride and epichlorohydrin yields a mixture of PEG mono and bismethyloxirane. This mixture can be bulk polymerized at high temperatures in the presence of tBuOK to yield an inert polymer with only ether bonds and hydroxyl groups as the functional groups [Renil et al., 1996].
1.4.1.4. CLEAR resin:

CLEAR [Cross-Linked Ethoxylate Acrylate Resin] is a highly cross-linked support that exhibits excellent solvation properties and performance in batch wise and continuous-flow syntheses [Kempe et al., 1996]. This support is prepared by radical co-polymerization of the branched cross-linker trimethoxypropane ethoxylate triacrylate with other monomers either by bulk polymerization or suspension polymerization results in adequate chemical and mechanical stability [Lee et al., 2009].

1.4.2. Miscellaneous supports

1.4.2.1. Controlled pore glass (CPG):

Controlled pore glass is a non-swelling, inorganic matrix composed of polar silanol groups mainly hydrophilic and non-deformable [Minganti et al., 1985]. The loadings on CPG are usually too low to be of practical use and problems associated with synthesis of long peptides on the rigid surface have not been evaluated. This support has been used in oligonucleotide [Adinolfi et al., 1998; Laurent et al., 2004] and peptide synthesis [Fathi et al., 1996]. CPG has been shown to prevent peptide chain aggregation during SPPS. The most serious problems of CPG are the presence of silica crude product and polymeric siloxanes released during cleavage and deprotection of oligonucleotides. Furthermore the silane coupling chemistry used to functionalize the inorganic surface of CPG bead is complex, leading to variation in substitution levels from preparation to preparation.

1.4.2.2. Silica Gel:

Silica Gel is rigid matrix that does not swell in common organic solvents [Maillard et al., 2009]. Silica Gel can be modified with amino propylsilane to yield a satisfactory
loading and synthetic performance for smaller peptides [Matteucci et al., 1981]. This support has been used in high yield (60-70%) enzyme conversions, such as those with glycotransferases to afford glycopeptides [Schuster et al., 1994].

1.4.2.3. Membranes:

(a) Cellulose membrane: Beaded cellulose was one of the first carriers tested by Merrifield for solid phase synthesis but was found to be unstable. However, it has subsequently been shown that peptides synthesized on cellulose support can be obtained in reasonable yield and purity [Bowman et al., 2004]. The large surface area of the rigid fibers provides a reasonable quantity of functional groups. The fibrous cellulose sheets or woven cotton pieces are easy to manipulate and are well suited for multiple combinatorial synthesis [Wong et al., 2004]. The rigid structure and low swelling properties of cellulose papers allow multiple coupling reactions to be carried out under low pressure continuous flow conditions. Cellulose type supports cannot be used with strong acids such as HF, owing to the cleavage of glycosidic bonds under such conditions. However they are surprisingly stable to TFA for short time periods. Both cotton and paper need a mild acid pre-treatment [Lipinsky, 1978] that affords limited and irreversible swelling of the cellulose matrix.

(b) Organic membrane: Polymeric membranes are monolithic, continuously porous materials. Membranes can be produced from numerous organic polymers including polyalkanes such as polyethylene or polypropylene and their fluorinated derivatives. Once formed, a membrane can be chemically functionalized by a number of methods including direct conversion of functional groups in the bulk polymer, coating of the surface with a
preformed polymer or graft co-polymerization of reactive monomers onto the membrane surface [Daniels et al., 1989].

1.4.2.4. Multipin supports:

A mobile polymeric surface suitable for organic synthesis can be radiation grafted to more rigid plastic (polypropylene or polyethylene) pins or small caps attachable to pins [Bray et al., 1990; Lutz et al., 2002]. The gel supported on an array of rigid pins is then used for multiple syntheses of up to 96 compounds simultaneously. The mobile polymeric surfaces used are polyacrylic and poly methacrylic acid, polystyrene, and poly-2-hydroxyethylmethacrylate (HEMA). Once the pins are grafted, further derivatization is performed.

1.4.2.5. ASPECTS:

Powdered polyolefin materials, such as ASPECTS (augmented surface polyethylene prepared by or used for chemical transformations), have been developed as solid supports [St Hilaire et al., 2000]. In order to functionalize native ASPECT particles and to obtain a higher surface area, an oxidative treatment is performed to introduce groups such as –COOH, -OH or –C=O. ASPECTS are mechanically, chemically and pressure stable and more hydrophilic than the native materials. This support is well suited for SPOC because of its inertness in conditions of numerous chemical reactions.

1.4.2.6. Chitin support:

Chitin is a β-1,4-linked polymer of N-acetyl-2-D-glucosamine found in the shells of crustaceans and the structural matter of insects and fungi. The average chain length of prepared chitin is 5200, corresponding to an average molecular weight of $1.0 \times 10^6$. 
Chitosan, a partially deacylated form of chitin, contains amino groups that can be used as sites for peptide synthesis [Rinaudo., 2006; Batista et al., 2006]. Peptide-chitin complexes resulting from peptide synthesis on chitin could prove useful for the introduction of antipeptide antibodies. Chitin is stable to the conditions of Fmoc chemistry including methods used to release peptides. The properties of chitin are similar to those of cellulose. Chitosan, unlike cellulose, does not have to be derivatized to create an amino group for attachment of the linker.

1.4.2.7. Sepharose and LH-20:

Sepharose and LH-20 [Blixt et al., 1997] have been used as supports for solid phase synthesis. Both are synthetic dextran polymer beads composed of fibers in a porous network. The fragile nature and acid lability of the glycosidic bonds of the dextran are serious limitations to the use of these materials as solid supports. However, they are perfectly compatible with aqueous buffers and can be used in combined chemo-enzymatic syntheses.

1.5. Strategies for peptide synthesis

The two types of strategies used for the solid phase synthesis of peptides are

1.5.1. Stepwise SPPS: Stepwise SPPS proceeds by repetitive coupling and deprotection steps to introduce individual amino acid building blocks. Once the desired peptide length has been obtained, the peptide is cleaved from the support, and side chain protecting groups are usually removed simultaneously. Stepwise SPPS is most efficient for short to medium length peptides, with the routine upper limit being approximately 40 residues. The acid labile t-butoxycarbonyl (Boc) groups or base labile fluorenlymethoxycarbonyl
(Fmoc) groups are used for N-α-protection. After removal of this protecting group, the next protected amino acid is added using either a coupling reagent or pre-protected amino acid derivative. The resulting peptide is attached to the resin, via a linker, through its C-terminus and may be cleaved to yield a peptide acid or amide, depending on the linking agent used. Side chain protecting groups are often chosen so as to be cleaved simultaneously with detachment of the peptide from the resin. Cleavage of the Boc protecting group is achieved by triflouro acetic acid (TFA) and the Fmoc protecting groups by piperidine. Final cleavage of the peptidyl resin and side chain deprotection requires strong acids such as hydrogen fluoride (HF) or triflouromethane sulfonic acid (TFMSA), in the case of Boc chemistry, and TFA in Fmoc chemistry. Dichloromethane (DCM) and N,N-dimethylformamide (DMF) are the primary solvents used for resin deprotection, coupling and washing. Peptide synthesis can be carried out in a batch-wise or continuous flow manner. In the former technique, the peptidyl resin is contained in a filter reaction vessel and reagents added and removed under manual or computer control. In the continuous flow method, the resin is contained in a column through which reagents and solvents are pumped continuously, again under manual or automatic control. A range of manual, semi-automatic or automatic synthesizers are commercially available for both batch-wise and continuous flow methods. Only the Fmoc strategy is fully compatible with the continuous flow method, depending on the instrument used, allowing for real time spectrophotometric monitoring of the progress of coupling and deprotection and general accounts of solid phase methods are extensively reviewed [Souza et al., 2004; Antopolsky et al., 2002; Antopolsky et al., 2000]
1.5.2. Convergent SPPS:

Convergent approaches are often preferred when embarking on the synthesis of longer sequences [Lloyd-Williams et al., 1993]. Convergent approaches exploit efficient step-wise SPPS to create short segments, which are then purified and joined together further to form the target peptide. Convergent approaches can be subdivided into protected segment couplings and chemical ligation. In the former, segments that are fully protected, except for the termini to be coupled, are condensed via traditional methods involving carboxyl activation. In the later highly specific reacting groups on unprotected peptide fragments are used [Arshady., 1982].

1.6. Dendrimers

1.6.1. General

Dendrimers are symmetric, highly ordered, highly branched polymers with a compact spherical structure (diameter ranging from 1.1 nm for the 1st generation (G1) to 9 nm for G8) and unique behavior [Vögtle et al., 2000; Caminade et al., 2005] with potential for a whole range of applications [Jang et al., 2009; Svenson., 2009; Kofod et al., 2005; Sadler., 2002]. The first successful attempt to create and design dendritic structures by organic synthesis was carried out by Vögtle and co-workers [Buhleier et al., 1978] in 1978. These relatively small molecules were initially named ‘cascade molecules’ and already then Vögtle and co-workers had seen the perspectives in using these polymers as, e.g. molecular containers for smaller molecules. However, after this first report, several years passed before the field was taken up by Tomalia’s group at Dow Chemicals. They had during the years developed a new class of amide containing cascade polymers, which
brought these hitherto quite small molecular motifs into well-defined macromolecular
dendritic structures.

Tomalia and co-workers [Tomalia et al., 1985] baptized this new class of
macromolecule ‘dendrimers’ built up from two Greek words ‘dendros’ meaning ‘tree’ or
‘branch’ and ‘meros’ meaning ‘part’ in Greek. Later refinement and development of
synthetic tools enabled the scientists also to synthesize macromolecular structures relying
on the original ‘Vögtle cascade motif’ [de Brabander-van den Berget al., 1993]. Parallel to
polymer chemists taking this new class of compounds into use, dendritic structures also
started to emerge in the ‘biosphere’, where J. P. Tam in 1988 developed intriguing
dendritic structures based on branched natural amino acid monomers thereby creating
macromolecular dendritic peptide structures commonly referred to as ‘Multiple Antigen
Peptide’ (MAP) [Tam., 1988]. Dendrimers are also sometimes denoted as ‘arboroles’,
arborescent polymers’ or more broadly ‘hyperbranched polymers’, although dendrimers
having a well-defined finite molecular structure should be considered a sub-group of
hyperbranched polymers.

1.6.2. Characteristics of dendrimers

Dendrimers are spheroid or globular nanostructures that are precisely engineered
to carry molecules encapsulated in their interior void spaces or attached to the surface.
Size, shape, and reactivity are determined by generation and chemical composition of the
core, interior branching and surface functionalities. The dendrimer diameter increases
linearly whereas the number of surface groups increases geometrically. Dendrimers are
very uniform with extremely low polydispersities, and are commonly created with
dimensions incrementally grown in approximately nanometer steps from 1 to over 10 nm. The control over size, shape, and surface functionality makes dendrimers one of the ‘smartest’ or customizable nanotechnologies commercially available. These features such as molecular structure, architecture, topology, controllable growth, and the presence of endo and exo-receptor properties among others have made these macromolecules intriguing and have contributed to the spectacular advances in the studies of a large number of dendrimers [Hourani et al., 2010; Janssen et al., 1999].

1.6.3. Dendrimer synthesis

Two conceptually different synthetic routes for the construction of high-generation dendrimers exist: divergent and the convergent approaches. In both approaches, dendrimer generations are created by the iterative repetition of a sequence of reactions. Dendrimers have been synthesized via a combination of the two methodologies. This strategy, referred to as the double-stage convergent approach is more flexible and adaptable than either of its component methods.

1.6.3.1. Divergent approach:

Divergent synthesis, based on the growth of the dendrimer from the central core to the periphery, is probably the most efficient and rapid procedure to construct dendrimers of high generation. The methods consist of two basic processes (1) coupling of the monomer and (2) deprotection or transformation of the monomer end-group to create a new reactive surface functionality and then coupling of a new monomer etc. This method is characterized by a rapid increase in the number of functional groups on the periphery. However, due to the exponential increase in the number of terminal functional
groups as well as the increasing steric congestion at the periphery during dendrimer growth, incomplete reactions can occur, especially at higher generations, which can produce structural defects [Vögtle et al., 1999]. Chromatographic separation of the desired dendrimer is not always possible because byproducts often exhibit similar physical properties to those of the target molecule. The other two major problems when dealing with divergent synthesis of dendrimers are (1) the number of reaction points increases rapidly throughout the synthesis of the dendrimer. This rapid increase in number of end-groups are to be functionalized, combined with the following rapid increase in molecular weight resulting in slower reaction kinetics, makes the synthesis of the dendritic network to create higher generation dendrimers increasingly difficult, even when using high yielding reactions. Therefore the divergent approach may lead to increasing deletions throughout the growth of the dendrimer, resulting in numerous defects in the higher generation dendrimer product. (2) When performing divergent synthesis, it is hard to separate the desired product from reactants or ‘deletion products’, because of the great molecular similarity between these by-products and the desired product. Despite these drawbacks being observed predominantly in the synthesis of high generation dendrimers, the divergent approach has been applied in the synthesis of a large variety of different dendrimer designs with great success [Seiler., 2002].

1.6.3.2. Convergent approach:

The convergent approach begins from the dendrimer periphery and progresses inward toward the core. A segment growing with each reaction step is coupled to only one branching unit. While this approach facilitates the removal of undesired byproducts, for
example, by gel permeation chromatography (GPC), steric hindrance in the reaction between segments and the growing core molecule limits the number of generations possible as compared to the case of the divergent method. In contrast to the divergent method, the convergent method construct a dendrimer so to speak from the surface and inwards towards the core, by mostly 'one to one' coupling of monomers thereby creating dendritic segments, dendrons, of increasing size as the synthesis progress. In this way the number of reactive sites during the proliferation process remains minimal leading to faster reaction rates and yields. Another advantage of this methodology is the large 'molecular difference' between the reactant molecule and the product, facilitating the separation of the reactants from the product during the purification process. The final part of the convergent synthesis ends up at the core, where two or more dendritic segments (dendrons) are joined together, creating the dendrimer, the convergent strategy thus generally has an inverse propagation compared to the divergent strategy and has been explored in various strategies [Beigi et al., 2011; Zhang et al., 2001].
Scheme 1.1. Schematic representation of Fmoc SPPS

1.6.4. Solid phase dendrimer synthesis

Majority of dendrimeric structures were prepared using orthodox solution phase chemistry and increasing attention has recently been diverted to dendrimer preparation on solid supports [Haag, 2001; Lebreton et al., 2001; Lebreton et al., 2003]. Solution phase synthesis of dendrimers is often challenging, requiring long reaction times and non-trivial purification. Solid phase methodology enabled reactions to completion by using a large excess of reagents. Solid-phase synthesis can also provide solutions to a number of
problems associated with dendrimer preparation and, particularly, the time-consuming purifications. Purification that would otherwise be very difficult simply becomes a matter of extensive washing of the resin. Solid-phase synthesis can also markedly improve the yield and homogeneity of the formed dendrimer. It is also realized that the dendrimeric materials can be functionalized conveniently upon the solid support, giving a route into a whole range of functionalized dendrimers that again benefit from the advantageous of solid phase synthesis. Over the past decade, a number of dendrimers have been prepared on solid support [Wells et al., 1998]. In addition to the polylysine dendrimers, used mainly as cores for MAP induced antibody elicitation (multiple antigenic peptides), a number of other polyamide dendrimeric systems have also been prepared on solid support [Basso., 2000].

1.7. Research hypothesis and plan of study

The present work focuses on development of novel classes of dendrimeric supports for solid phase organic synthesis based on the following aspects

**Hypothesis #1:** Synthesis of various poly(ethylene glycol) grafted polystyrene based poly(N,N-bisethylamine) dendrimers and its utilization in solid phase peptide synthesis.

**Hypothesis #2:** Synthesis of various poly(ethylene glycol) grafted polystyrene based poly(O-benzyl ether) dendrimers and its application in solid phase peptide synthesis.

**Hypothesis #3:** Comparative assessment of N,N-bisethylamine and O-benzyl ether dendrimers synthesized on poly(propylene glycol)dimethacrylate cross-linked polystyrene support.