Ai miei genitori

UNIVERSITY OF SOUTHAMPTON

Synthesis and Evaluation of New Solid Supports for Solid Phase Organic Synthesis

by

Sonia Maria Alesso

Doctor of Philosophy

Department of Chemistry
Faculty of Science

September 2002

UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

DEPARTMENT OF CHEMISTRY

Doctor of Philosophy

SYNTHESIS AND EVALUATION OF NEW SOLID SUPPORTS FOR SOLID PHASE CHEMISTRY

by Sonia Maria Alesso

ABSTRACT

The aims of this project were the preparation, characterisation and application of a series of non-grafted polystyrene resins containing a styrenic poly(ethylene glycol) derivative. The resins were designed to balance swelling and solvation with improved handling issues. The monomer, 1-[2-(2-methoxyethoxy)ethoxy]-4-vinyl-benzene, containing an inert phenyl ether linkage designed to provide wide chemical compatibility and stability, was prepared on a large scale following a four-step procedure. A range of chloromethylstyrene functionalised resins with DVB cross-linking from 2% to 7% and PEG monomer loadings from 5% to 40% were prepared by suspension co-polymerisation. A multiple-parallel approach to the preparation of resins in suspension polymerisation was applied using a novel system that allowed fast optimisation and preparation of new solid supports. The resulting resins were tested in terms of swelling properties in a range of different solvents and chemical stability to strong acid/base conditions. The synthetic performance of the new resins were compared to those of TentaGel™, ArgoGel™ and aminomethyl PS by performing the synthesis of small model peptides, biaryl derivatives *via* Suzuki coupling and arylethers *via* Mitsunobu reaction.

Relative to traditional PEG-grafted resins, these PEG-based resins have a much higher loading capacity while maintaining broad solvent compatibility. The co-polymers were stable to strongly acidic/basic conditions and a range of physical manipulations. The new resins proved to be highly suitable for peptide synthesis, Suzuki and Mitsunobu couplings having improved performance over TentaGel™ and Merrifield type resins.

Table of Contents

Preface

The research described in this thesis was carried out under the supervision of Prof. Mark Bradley at University of Southampton between October 1998 and October 2001. No part of this thesis has been previously submitted at this or any University.

Acknowledgements

Firstly, I would like to thank my supervisor, Prof. Mark Bradley for his guidance, enthusiasm and optimism throughout my Ph.D. Thanks also to David Pears, Don Wellings, Paul Worthington and Richard Luke, my industrial supervisors, for their ideas and support during the collaboration with Avecia, AstraZeneca,and Syngenta.

A special 'thankyou' goes to Dr. Zhanru Yu for his support, optimism and patience during the past three years.

Thanks are due to the analytical section: John and Julie for mass spectrometry and Joan and Neil for extensive NMR work and run-time.

Thanks to all my lab mates from the last few years – for sharing everyday life in the lab and outside, coffee breaks, and...the Staff Club!

I would like to thank the Bradley group members past and present for making the time in during my PhD. enjoyable. Special thanks also to Mizio Matteucci, Mark Dixon, Katie MacNamara, Karin Johnson and James Merrington, for helping me with proof reading and Julia Queen-Parson for her help. It was really appreciated.

I would like to thank my 'mate' Mariangela Demarcus for her friendship, enthusiasm and support and for sharing all the good and bad times over the last three years of PhD. and even after!!

I would especially like to thank Mum, Dad and my brother Giuseppe for all the love and support over the years.

ABBREVIATIONS

AIBN	2,2'-Azobis(2-methylpropionitrile)
AM-TG	Aminomethyl TentaGel
AM-AG	Aminomethyl ArgoGel
^{13}C	Carbon-13
^{1}H	Proton
aq.	Aqueous
CMS	Chloromethylstyrene
d	Doublet
DCM	Dichloromethane
DIC	*N,N'*-Diisopropylcarbodiimide
DIOX.	Dioxane
DMF	*N, N'*-Dimethylformamide
DVB	Divinylbenzene
ES-MS	Electrospray Mass Spectrometry
Et_2O	Diethylether
EtOAc	Ethyl Acetate
EtOH	Ethanol
eq	Equivalents
Fmoc	9-Fluorenylmethyloxycarbonyl
HOBt	1-Hydroxybenzotriazole
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
IR	Infra-Red
J	Coupling Constant
L_i	Initial Loading
L_f	Final Loading
m	Multiplet
MeCN	Acetonitrile
MeOH	Methanol

min.	Minutes
M.p.	Melting point
MS	Mass Spectrometry
MW	Molecular weight
NMR	Nuclear Magnetic Resonance
PEG	Polyethylene glycol
Pet	Petroleum ether
ppm	Parts per million
PS	Polystyrene
PVA	Polyvinyl alcohol
q	Quartet
R_f	Retention factor (movement of compound/solvent front)
Rink	Rink linker (4-[(*R,S*)-α-[1-(9*H*-fluoren-9-yl)-methoxyformido]-2,4-dimethoxybenzyl]-phenoxyacetic acid
RP-HPLC	Reverse-Phase High Performance Liquid Chromatography
RT	Room temperature
rpm	Revolution per minute
s	Singlet
SPOC	Solid Phase Organic Chemistry
SPOS	Solid Phase Organic Synthesis
SPPS	Solid Phase Peptide Synthesis
t	Triplet
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
TLC	Thin Layer Chromatography
UV	Ultra Violet

Standard 3 letter abbreviations for amino acids

CHAPTER ONE

1.1 Combinatorial Chemistry and Solid Phase Chemistry

Combinatorial chemistry has been recently defined by Czarnik,[1] as a subfield of chemistry that aims to combine a number of chemical reagents, in all combinations defined by a given reaction scheme, to yield a large number of well-defined products in a form that is easy to screen for properties of interest. It can also be considered as a tool rather than an end in itself, which allows the rapid synthesis of large numbers of compounds with diverse features (structural and physicochemical properties). The dramatic growth of combinatorial chemistry has been mainly catalyzed by the continuously growing need for novel pharmaceutical agents. The generation of libraries has played an important part in the drug discovery process and combinatorial chemistry lends itself well to this process[2,3] facilitating the synthesis of a large number of compounds, which can be tested for biological activity. Organic combinatorial chemistry is generally divided into two tactical domains: solid and solution phase synthesis. In the solid phase approach, the solid support provides a simple means for the separation of the desired product from excess reactants by simple filtration and rinsing of the solid-supported product with appropriate solvents. The final product is then cleaved from the solid support. In the solution phase approach the reactions and technique to be employed for the generation of libraries are already familiar to the chemists; no additional steps for attachment to or detachment from supports are required and thus only the particular reaction of interest needs to be developed.

Solid phase chemistry has its earliest origins in solid phase peptide synthesis. The use of polystyrene based resins in the synthesis of peptides, was introduced by Merrifield[4] in 1963 who successfully carried out the synthesis of a tetrapeptide. Traditional solution phase synthesis of peptides was a laborious process with purification needed between synthetic steps and huge problems of solubility and protecting group manipulation. Merrifield showed this very laborious task could be simplified by the use of a solid

support. The solid phase strategy employed polystyrene resin beads as the initial "*C*-terminal protecting group" of the first amino acid, synthesis (elongation) of the growing peptide chain using the amino acid monomers in the N- direction was then possible. The total solid phase synthesis of the enzyme Ribonuclease A by Gutte and Merrifield[5] in 1971, was one of the most significant achievements in the application of this technology to biological chemistry.

The synthesis of small molecule libraries,[6] in particular the synthesis of oligonucleotides,[7,8] peptoids,[9,10] oligocarbamates[11] and the synthesis of small molecules such as the benzodiazepines,[12] diketopiperazines,[13] tetrahydrofurans[14] and 1,3-diols[15] are all examples of how Solid Phase Organic Chemistry (SPOC) has shed its "peptide synthesis" stereotype.

1.2 Comparison between Solid Phase and Solution Phase Chemistry

The advantages of solid phase organic synthesis relative to solution phase synthesis are a manifestation of many of the characteristics associated with the technique: ease of purification, high yields, multi-step synthesis amenable to automation.[16] The substrates, once tethered to the resins can be treated with high concentrations of reactants therefore allowing reactions to be driven to completion. After synthesis, excess reagents can be washed away from the resin, removing the need for lengthy and costly purification procedures. Convenience is achieved by means of simplified purification steps and by more efficient chemical reactions. As a consequence, the use of polymer resins as an aid to synthesis, is becoming an increasingly common feature in both academic and industrial laboratories.[17] A disadvantage of solid phase chemistry compared to solution phase chemistry is that resin-bound by-products remain on the resins during synthesis and cannot be separated from the desired products thus it is very important to achieve the highest possible yields in a particular reaction. Monitoring of reactions on the solid phase also becomes more difficult than in solution while it is also important that SPOC reactions

are carried out in solvents that are compatible with the solid support, the attached substrate and reagents.

The reactions rates carried out on the solid phase can differ from those carried out in solution. Due to the chemical characteristics of the polymeric matrix, reactions carried out on solid phase occur within different microenvironments to those carried out in solution. Therefore the matrix might be expected to affect the rate of chemical reactions. Differences in reaction rates would be expected to manifest themselves in homogeneous/ heterogeneous reaction condition comparisons. In 1971 Grubbs and Kroll[18] reported an example of the differences between homogeneous and heterogeneous catalysis by performing olefin hydrogenation with heterogeneous and homogeneous Rhodium (I) catalysts (Figure 1.1).

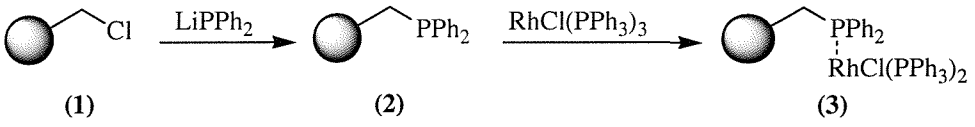


Figure 1.1 *Heterogeneous rhodium catalyst.*

They found that the rates of hydrogenation were lower in the heterogeneous system and also depended on alkene size. Lower rates of hydrogenation were observed for larger molecules, while cyclic alkenes were found to be more subject to diffusional hindrance within the polystyrene beads (compared to acyclic ones). The solid phase may also affect the selectivity of chemical reactions. For example Trost and Keinan[19] compared the selectivity in allylic aminations of $Pd(PPh_3)_4$ of a resin bound catalyst compared to the solution phase analogue. The solution phase catalyst afforded a mixture of *cis-* and *trans-* diethylamino-5-methoxycarbonylcyclohex-1-enes (Figure 1.2) whilst the benzyl-diphenylphosphine palladium catalyst supported on a PS-DVB (2%) resin afforded completely stereospecific amination of both *cis-(n)* and *trans-* 3-acetoxy-5-methoxycarbonylcyclohex-1-ene (n) with diethylamine.

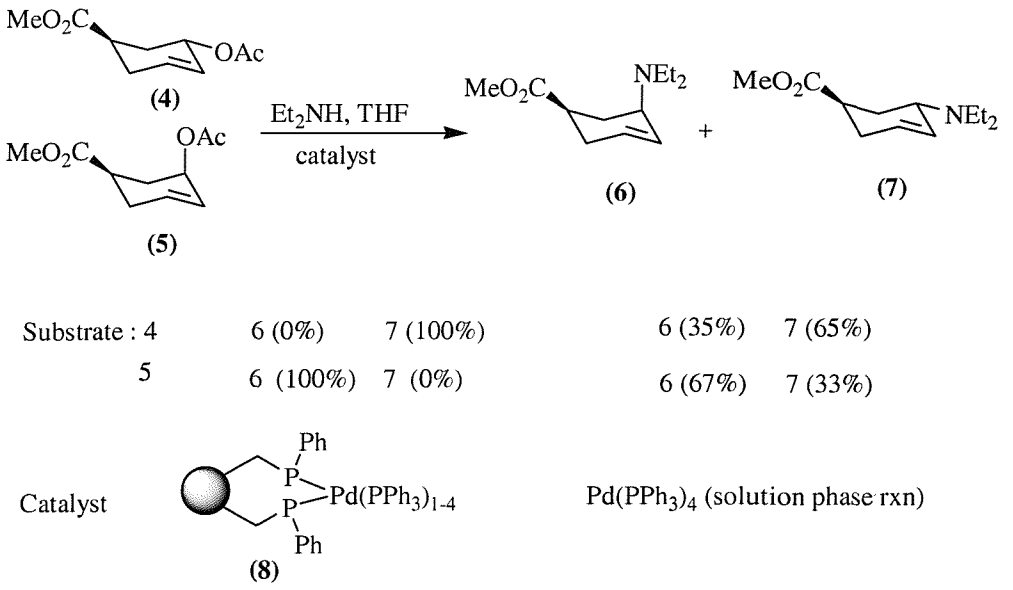


Figure 1.2 *Effect of catalyst resin immobilisation on the selectivity of allylic aminations.*

However, the drawbacks of solid phase synthesis discussed above are considered by many not to be relevant when compared to the many advantages that solid phase applications allow, hence the popularity of solid phase chemistries today.

1.3 Modes of polymerisation

Solid phase chemistry (SPS) has its origins in chemical processes that were discovered over 150 years ago. In 1839 Simon[20] performed the first laboratory polymerisation of styrene and in 1838 the nitration of cellulose[21] was the first reported example of a chemically modified polymer. Adsorption of the enzyme amylase onto an insoluble starch was carried out in one of the first examples of a polymer-supported catalyst reported in the literature in 1910 by Sarkstein.[22]

Polymeric solid supports for solid phase chemistry can be synthesised using a number of different polymerisation techniques. Four main modes of polymerisation have been identified; bulk,[23, 24] dispersion,[25, 26] emulsion,[27, 28] and suspension polymerisation.[29, 30, 31] Large-scale industrial bulk polymerisation to produce plastics is a well-known example of bulk polymerisation process, an example being the production of PVC. In bulk polymerisation, very high molecular weight polymers are generated by co-polymerising the monomers often in the absence of solvent. In practice, a range of additives, including anti-oxidants, dyes and plasticisers, are included in the initial mixture.[32] Bulk polymerisation has also recently found application in molecular imprinted polymers (MIPs),[33] although for the preparation of these polymers very high levels of cross-linker are used.

Dispersion polymerisation is a good method to generate beads of less than 5μm in diameter. In dispersion polymerisation, the polymers have a very low intrinsic solubility in the medium. Ethanol can be used as solvent when monomers similar in nature to styrene are employed in the polymerisation. As the polymerisation starts, the growing polymer particles reach the limit of their solubility in the ethanol solution and coalesce to form unstable nuclei. When further adsorption occurs the nuclei become stable and form particles of uniform size distribution (mono-dispersed particles).

Emulsion polymerisation can be used to produce spherical particles in the size order 0.01μm -1μm in diameter and is commonly applied to the synthesis of latexes. During emulsion polymerisation an organic phase is suspended in an aqueous phase in the presence of a water-soluble initiator and a surfactant (soap). Inorganic persulfates can to be used as initiators as they are readily soluble in the aqueous phase. In the laboratory, sodium lauryl (dodecyl) sulfate (SDS) is generally used as a surfactant. The soap forms micelles around the organic phase monomers and polymerisation occurs when the radical initiator diffuses through the aqueous phase and makes contact with the micelle.

Suspension polymerisation is similar in practice to emulsion polymerisation except that the radical initiator used is soluble in the organic phase and is present in the droplets in suspension. Surfactants are not used in this mode of polymerisation, but a stabilizer is required. Polymerisation occurs when the droplets reach a critical temperature and radical

initiation occurs. Chain-elongation events compete with chain termination events and a growing polymer chain can be terminated by impurities in the reaction mixture or by radical quenchers such as oxygen. Thus as a result of a suspension polymerisation reaction, a range of bead sizes may be produced (generally spherical particles of between 5μm –4mm in diameter are produced). Suspension polymerisation technique and the physico-chemical parameters involved in this process will be discussed in more details in Chapter 3.

1.4 Resins for Solid Phase Organic Chemistry

In the early days of solid phase chemistry, 2% cross-linked polystyrene supports were the most commonly used, nowadays 1% cross-linked polystyrene resin is the most widely used in solid phase chemistry. Besides the classical 1% cross-linked chloromethylated polystyrene (Merrifield resin), a large variety of other polymeric supports have been developed and are in use. From a chemical and physical view point, solid supports can be classified into three main groups:[34]

(i) 'Gel type' polymer supports;
(ii) macroporous supports and composite supports;
(iii) surface modified polymeric supports.

The first group includes the well-known 1% cross-linked polystyrene resins in which functional groups are introduced with either functional styrene monomers or in a post-functionalisation step. The cross-linked acrylamides introduced by Sheppard [35] as well as the ultrahigh loaded (up to 5 mmol/g) Core Q-resins,[36] which are also polyacrylamide-based, can be included in the first group of resins. The typical loading capacity of these resins is in the range of 0.1-1.5 mmol/g. Low cross-linked resins swell in the reaction solvent providing a 'gel' which represents the polymeric network accessible to the

reactants. This type of resins is more suitable for batch processes than continuous flow processes as the network swells to variable amounts in solvents.

Resins resulting from grafting of hydrophilic moieties such as poly-ethyleneglycol (PEG) onto polystyrene are another class of useful resins for SPOS. Tentagel™,[37] consisting of a polystyrene core with PEG chains grafted onto them and ArgoGel™ [38] are very well-known examples of this class of resins. One of the main features of both PEG-grafted resins is that they swell in polar protic solvents and aqueous media. Resins such as PEG-PS[i] or JandaJel™,[39] resulting from non-grafting approach, are prepared by suspension co-polymerisation of specific monomers (e.g. vinyl functionalised monomers and cross-linkers or polytetrahydrofuran cross-linkers) and may also be associated with the first class of resins. CLEAR[40] (cross-linked ethoxylate acrylate based resin) is one example of PEG based resins which like TentaGel™ and ArgoGel™ swells in polar solvents like water. CLEAR was designed for peptide synthesis and may be suitable for SPOS applications, as indicated by its successful application in Suzuki cross-coupling reaction.[41]

Macroporous resins are a class of resins having a permanent well-developed porous structure even in the dry state. The backbone can be highly cross-linked polystyrene or glass (CPG-glass). This type of resins shows a permanent porosity and no swelling is necessary for the chemistry to be performed on them. Macroporous polystyrene resins are prepared by carrying out polymerisation in the presence of a non-reactive organic solvent (diluent or porogen) that phase separate during the polymerisation and defines the pore structure. Macroporous resins can have much higher surface areas (ranging from ~50 to ~1000 m^2/g) in the dry state than gel-type resins. In macroporous resins reagents are transported through the pore structure rather than through spaces within a polymer gel. ArgoPore™ [42] is an example of macroporous polystyrene successfully used in reactions requiring polar protic media, for example, the $TiCl_3$-mediated nitro reduction in aqueous media, analogous to those applied in solution. Some characteristics of macroporous resins such as accessibility of their pore structure to essentially all solvents, rapid solvent removal *in vacuo*, make them particularly suitable for automated synthesis. Composite supports consist of a rigid skeleton such as kieselguhr (Pepsyn resin) or Polyhipe[43, 44] in

which low, cross-linked polyacrylamide is polymerised within the matrix. These solid supports are stable to pressure but very fragile and their use is restricted to continuous flow processes. Synthesis on composite supports takes place in the polyacrylamide part of the resin and typical capacities are in the range 0.1-0.5 mmol/g.

Another development in solid phase technology is the functionalisation of the surface of materials by radiation grafting methods (gamma radiation is often used as the radiation source[45]) and it results in surface functionalisation of the desired polymer. Radiation grafting of a range of monomers has been carried out with polyethylene and polypropylene crowns of which the aminomethyl polystyrene grafts are the most suitable for SPOS. The loading of the largest crowns is on the order of 40μmoles, indicative of a less spatially efficient format relative to polymer beads. The advantages of this system over traditional solid supports is that the solid phase is much more easily handled but the main disadvantage is that the chemistry is not as routinely developed as on other solid phase support. In response to resin handling issues, Houghten[46] enclosed pre-determined amount of PS-DVB beads in mesh polypropylene, forming. 'tea-bags', which represents an example of support technologies based on macroscopic objects that can be handled. Polyolefin pins are another example of this type of solid support format. The pins are of modular design and are mounted with SynPhase™ [38] crowns, which vary in size and loading.

1.5 Properties of a good solid support for solid phase chemistry

In all polymeric supported reactions the solid support represents the reaction space where the chemical reaction takes place. Using polymer beads for synthesis the reaction space is divided into small, individual reaction compartments. Therefore, resin parameters like cross-linking, swelling properties, bead size distribution have to be taken into account, as each individual bead represents a microreactor. In order to perform a diverse range of synthetic steps, a solid support must be chemically stable and not interfere with the

[i] PEG-PS resins will be discussed in more details in Chapter 2.

intended chemistries. The resin must be mechanically stable for its intended purpose. Mechanical breakdown into smaller irregular shaped particles may cause clogging of frits and other normal separation media. Mechanically unstable resin should not therefore be applied to automated synthesisers. There is also a relationship between the mechanical stability and rigidity of a swollen resin and the cross-linking parameter.[47, 48, 49] A lightly cross-linked material will physically, be more solution-like once swollen, than a more heavily cross-linked one. A heavily cross-linked resin will tend to be more rigid. PEG grafted supports have been reported to be unstable to TFA,[50] whilst linear PEGs at <0.5 wt % have been found in filtrates after exposing ArgoGel-OH and ArgoGel-NH_2 to a 95:5 mixture of TFA: water.[51] Newer PS-PEG resins are continually being developed, which aim to suppress the limitations of the classical PS-PEG systems.[52] The chemical loading of the resin is also an essential descriptive parameter and is generally chosen in accord with the chemical applications and conditions required. It is also important to be able to access a wide range of functional groups from the starting resin, without the need for elaborate or costly procedures. From a commercial point of view, an ideal resin is one that can be synthesised and disposed of inexpensively with respect to both financial and environmental costs. Synthetically useful resins are generally required to have excellent swelling properties. Swelling is a property associated with the cross-linking of the polymer. It occurs when a solvent enters a receptive polymeric matrix and partially solvates it. Swelling increases the volume of the resin by up to 10 times if a suitable solvent is chosen. Swelling is important because it allows chemistry to occur between the solution and the solid phase. Physically, in a swollen resin there is more room for access and diffusion of the substrates into the resin to the functional/ active sites, which results in the enhancement of reaction rates. 1% cross-linked PS-DVB resin was reported by Meldal,[53] in 1992 as the resin offering the best compromise between mechanical stability and compatibility in different organic solvents.

1.6 Functional polymers and scavenger resins

Functional polymers have been deployed for standard SPS and in two areas of combinatorial chemistry: as reagents and catalysts and as purification devices.[54] Many supported reagents have been developed[55] for conducting a variety of reactions in solution. A vast number of such reagents are available to date. For examples immobilised triarylphosphanes,[56] hydrogenation catalysts, oxiding and reducing agents, chiral auxiliaries ,[57] resins for use in acylation,[58] alkylation,[59] amination,[60] aromatic substitution,[61] Baylis Hillman,[62] condensation,[63, 64] cross coupling,[65] cyclisation,[66] electrocyclic [2+3],[67] organometallic,[68] radical,[69] ring opening,[70] transesterification[71] and reduction[72] reactions.

Inherent in any approach to produce chemical libraries is the need to rapidly purify, isolate, and manipulate chemical library members during their synthesis. One of the major impediments to parallel solution-phase synthesis of large numbers of individual organic molecules is the time and effort required for purification of the reaction products at each synthetic step, particularly in the case where a large number of diverse products are involved (100-1000 compounds). Methods that are well established for the purification of single compounds such as crystallisation, extraction, and flash chromatography will not be applicable. For these reasons, during the past several years chemists have been investigating hybrid solid/solution-phase synthesis techniques involving polymer-supported reagents which combine the purification advantages of SPOS with the flexibility of solution-phase chemistry. Reactions with polymer-bound reagents are reactions in which the dissolved substrate is allowed to react with chemical reagents, which are bound to solid supports. Polymer supported reagents simplify the performance of reactions and in special way the work up of reaction mixtures. Synthesis using polymer-supported reagents is different from traditional SPOS in that the purpose of the solid phase is to retain undesired or unreacted materials, the desired product being deposited directly into solution. Polymer supported materials can be divided into three main classes (Figure.1.3). While the first two participate in the formation of product, scavengers are typically added post-reaction solely for removal of impurities (by quenching excess

reagents added to drive a reaction to completion or a known impurity from a mixture) and offering an efficient means to obtain organic molecules of suitable purity. Generally the choice of scavengers depends on the nature of both the impurity and the desired product: one can choose which reagent to use in excess and adjust the quenching resin accordingly and they can entrap impurities by forming covalent or ionic bonds.

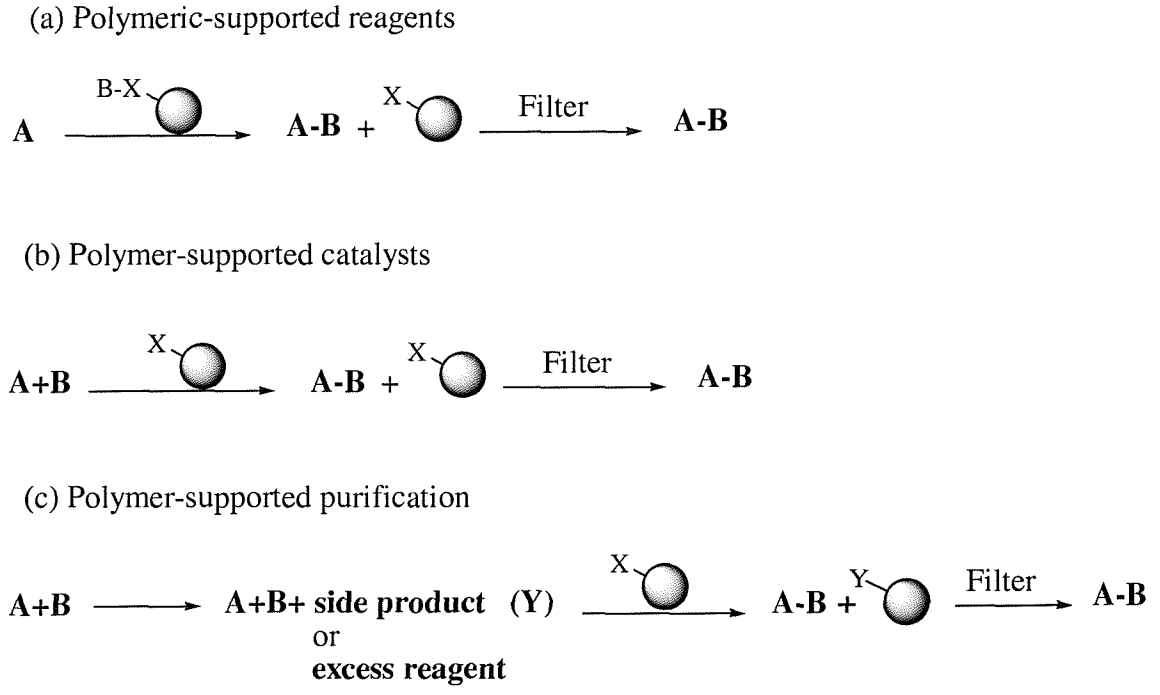


Figure 1.3 *Solid Supported reagents.*

This method has been called polymer-supported quench (PSQ) purification. PSQ has an inherent advantage over traditional purification methods since it focuses on the chemical and not on the physical properties of the contaminants. Moreover it allows the use of less solvent, requires less solid support and eliminates the need to collect multiple fractions. A multiplicity of quenching reagents may be added concurrently to remove a multiplicity of impurities and excess reagents since reactive groups on separate polymer-supports are known not to react (mutual site isolation).[73] The small quantity of PSQ resin and solvent

employed combined with labour saving make PSQ purification a very convenient method compared to flash chromatography. Additionally PSQ reagents are easily made[74] in large quantity from some of the least expensive resin starting materials and each PSQ reagent has many possible applications in the selective removal function.

Both covalent and ionic scavenger resins have found a wide application in the last few years, for example covalent scavengers have been employed for pharmaceutical lead discovery by Kaldor[75] in the construction of a library of 4000 ureas and screened it for antirhinovinal activity (the human rhinoviruses are the primary cause of the common cold). Furthermore, a series of polymer-supported nucleophiles and electrophiles for the selective removal of reaction impurities has been developed by Kaldor and Siegel:[76] covalent scavengers that are selective for the removal of electrophiles in the presence of non-electrophiles, nucleophiles in the presence of non-nucleophiles, and primary amines in the presence of secondary amines (Figure 1.4).

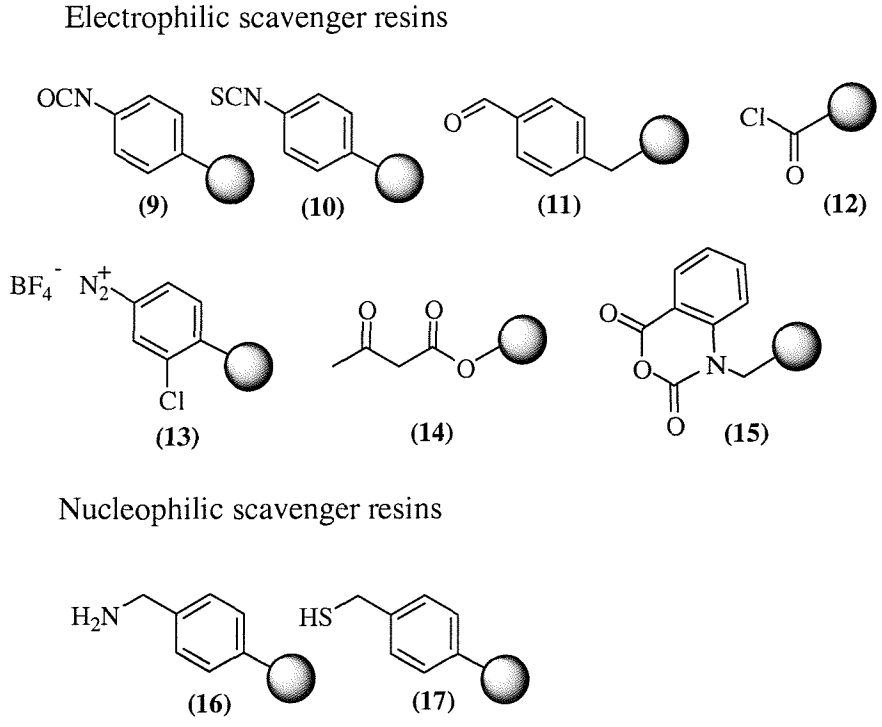


Figure 1.4 *Two main classes of scavenger resin.*

Booth and Hodges[77] found that polystyrene beads bearing reactive groups which mimic the limiting reagent of a reaction can be used to remove the remainder of excess reagent/s from crude product solutions and are thereby convenient and effective tools for performing rapid purification (Figure 1.5).

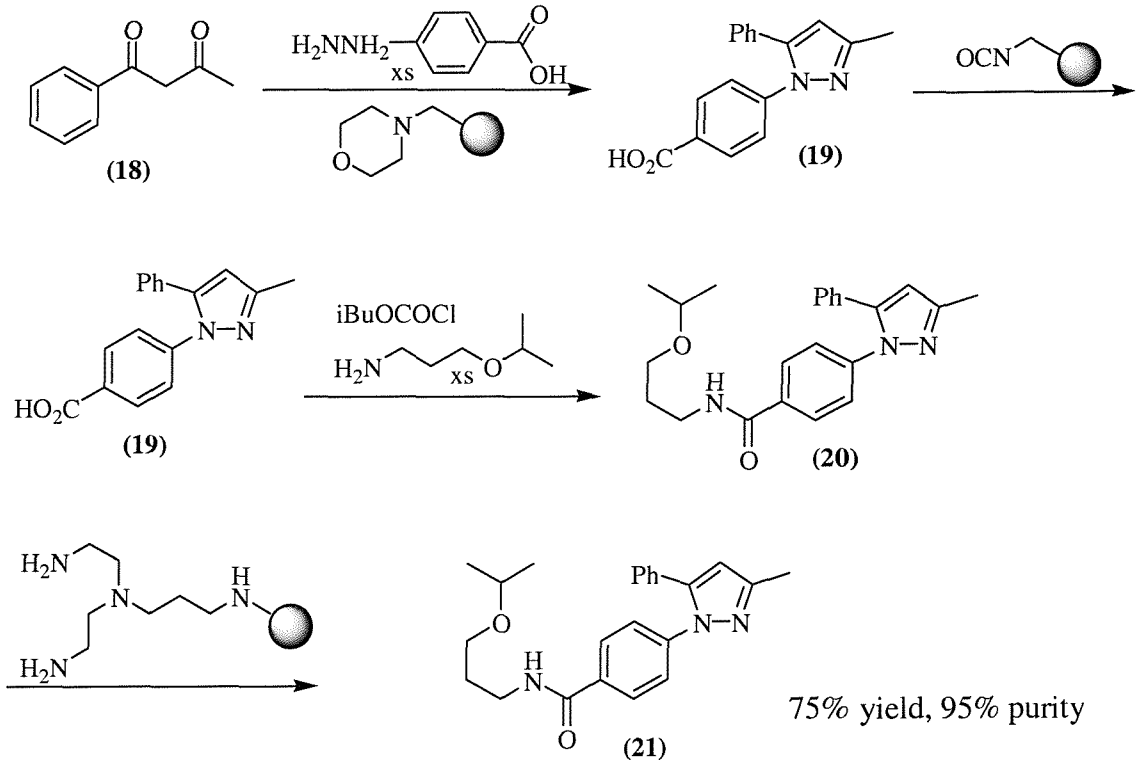


Figure 1.5 *Solution phase synthesis of pyrazoles using solid phase scavengers.*

Recently Yu[78] and co-workers developed a new ketoester methacrylate resin (Figure 1.6) for selective scavenging of primary amines in the presence of secondary amines. They demonstrated the utility of this scavenger resin with a range of reductive amination chemistries using both mono- and diamines. The resin's specificity was based on the removal of the primary amine *via* their enamines, with the enamino ester derived from the

primary amine being more stable than the one derived from the secondary amine due to H-bonding.

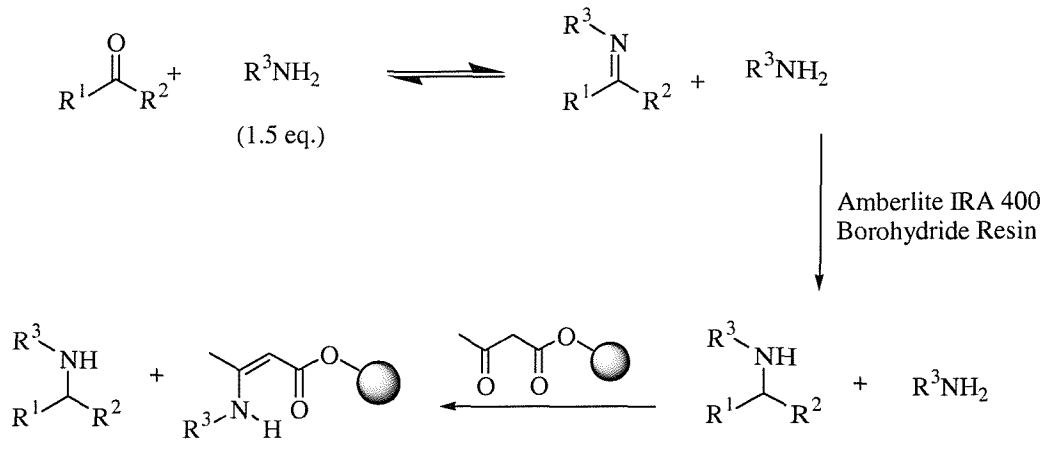


Figure 1.6 *Solution phase synthesis of secondary amines using ketoester methacrylate resin for the removal of excess primary amine.*

The power of ionic scavenger purification technique lies in its potential generality: nearly any ionisable molecule can be rapidly separated from non-ionisable impurities. Ion exchange resins were introduced during the 20 years between 1945 and 1965 after Adams and Holmes[79] in 1935 found that polar groups could be introduced into polymers. An ion exchange resin allows the replacement of a specific ion, usually contained in the solvent/ eluent, with an ion that is initially polymer bound. The two main types of these resins are shown in Figure 1.7.

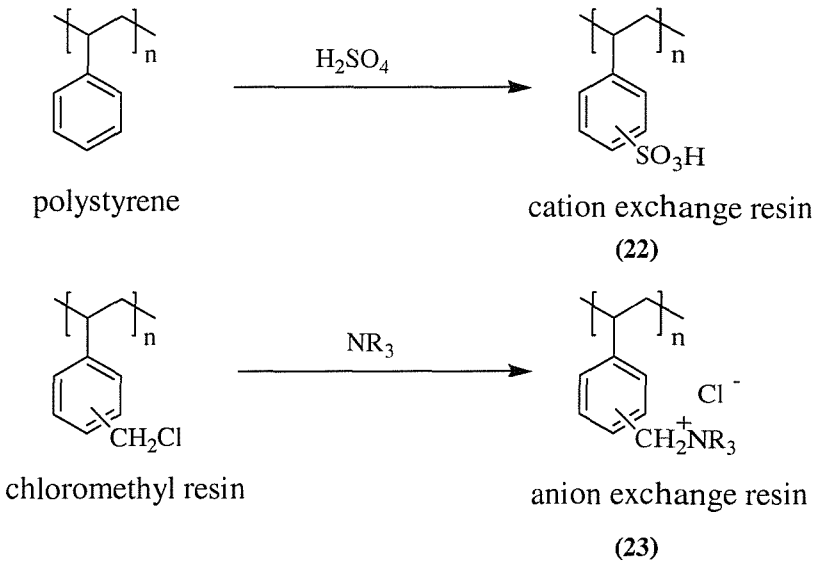


Figure 1.7 *Synthesis of simple ion exchange resins.*

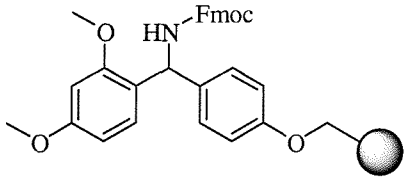
The use of ionic scavengers has also been demonstrated by L.M.Gayo and M.J.Suto.[80] They used basic ion-exchange resins for the synthesis and purification of various amide-derivatives. The excess acid chlorides were hydrolysed to the corresponding acids and selectively removed from solution by ionic interaction. Bolton and Jackson[81] in 1983 introduced thermally regenerable ion-exchange resins consisting of polyamine micro-particles encased in an acidic matrix.

The general utility of complementary molecular reactivity and recognition (CMR/R) purification strategy has employed artificially tagged reagents or reactants where there may not be the routine opportunity to separate products from excess reagents or by-products.[8] Typically, reactions requiring catalysts and/or reagents to effect transformation can be performed using tagging of catalysts or reagents with desired functionality which allow for a quite general (and highly controllable) strategy for their purification and separation. The choice of molecular recognition tags presents some limitations: the tag functionality must be inert to the other reactant and reagent species, must not interfere with the performance of the encoded reactant/reagent. The parallel Moffatt oxidation of hydroxyethylamines using the amine-encoded diimide is an example of employment of

artificially-imparted molecular recognition.[8] The examples reported above demonstrate how combination of solution synthesis and PSQ purification provides a convenient alternative to solid-phase synthesis in the practise of combinatorial and classic organic chemistry.

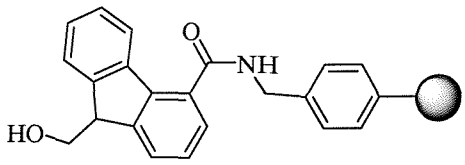
1.7 Linkers

Some of the early methods employing polystyrene resins required chemically very harsh conditions (NaOH or HF[82]) to cleave the products from the resin. Unfortunately many molecules, especially those being developed as potential drug leads, and those containing sensitive functional groups are not stable under these severe conditions. For this reason the use of linkers to facilitate synthesis has become very common in solid phase synthesis. Linkers are bi-functional molecules that are vital components in any library synthesis, as they allow controlled facile cleavage of product from the resin. Linkers can be chosen to release product under acidic, basic or neutral conditions depending on the compound being synthesised and the chemical strategy being employed. The linker needs to be orthogonal to the chemistry used to modify the attached compound that is it must not be affected for example by the conditions applied for the removal of any protecting group present on the compound. The best linkers allow attachment to the solid support in essentially quantitative yield. Furthermore, cleavage should proceed rapidly, cleanly and in good yield. Finally, it is an advantage if the cleavage step can introduce new diversity or is a key step in the formation of the products.[83] The number of linkers that have been reported to date is vast, estimated at approximately 600.[84] Some of the most commonly used linkers are shown in figure 1.8; Rink[85] and Siebers[86] amide linkers belonging to the class of acid cleavable linkers, 9-(hydroxymethyl)fluorene-4-carboxamidomethyl as a base cleavable linker, ortho-nitrobenzyl [87,88] is a photolabile linker and finally the Kenner[89] linker which is a safety catch linker .



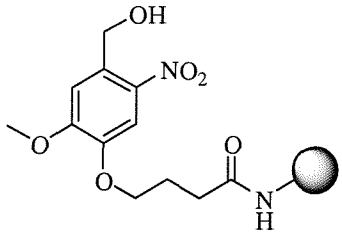
Rink amide linker

(24)



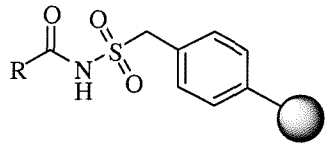
9-(hydroxymethyl)fluorene-4-carboxamidomethyl linker

(25)



Affymax Research Institute Photolabile Linker

(26)



Kenners safety catch linker

(27)

Figure 1.8 *The main classes of linkers in SPOC.*

1.8 Protecting group strategies-general methods

The solid support can be regarded as a protecting group in itself because of its insoluble and un-reactive nature. The development of new protecting group strategies has resulted in the creation of well-defined sets of orthogonal protecting groups. The principle of orthogonality is described in the schematic synthesis of a dipeptide and subsequent cleavage from the resin (Figure 1.10).

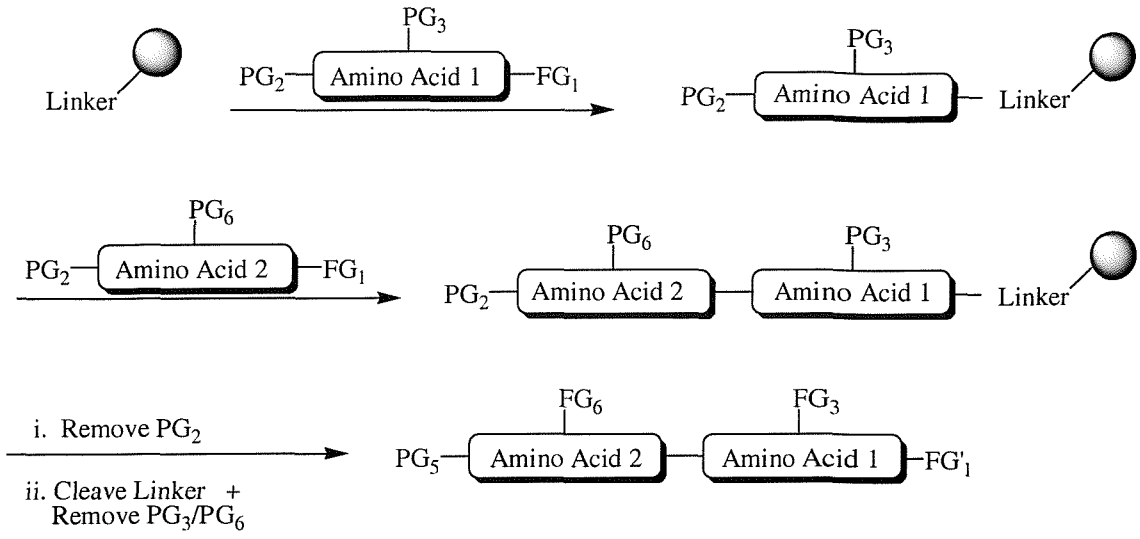


Figure 1.10 *Principle of orthogonality applied to the solid phase synthesis of a dimer. Note PG = protecting group, FG = functional group.*

In practice, the final steps in the synthesis, the removal of protecting groups PG_3 and PG_6 tend to be combined with the cleavage of the product from the linker. The functional group FG_1 needs to be activated towards attack before the amino acid residues can be loaded onto the peptide. On the solid phase, the residues bearing protecting group PG_2 are removed before the next amino acid can be loaded. PG_6 and PG_3 may be regarded as the side chain protecting groups of the small amino acid residues. After synthesis, these *PGs* can be removed as necessary. Orthogonality must be achieved because the protecting group PG_2 and PG_3 and the new amino acid must be loaded onto the solid phase in the presence of the original one. Once on the resin PG_2 can be removed quite independently of PG_6.

1.9 Amide bond formation on solid phase

In order to facilitate amide bond formation between an amine and a carboxylic acid avoiding the formation of the salt of the protonated amine and the deprotonated acid, the acid is typically activated to allow nucleophilic attack by the amine. Various activation strategies have been reported. The acid can be converted into the corresponding acid chloride with thionyl chloride,[90] the symmetrical anhydride,[91,92] the unsymmetrical anhydride with isobutyl chloroformate[93] or can be activated *via* conversion into active ester using DCC,[94,95] pentafluorphenol,[96,97] *p*-nitrophenol.[98,99] Probably for solid phase synthesis applications the most successful combination involves the addition of the diisopropylcarbodiimide (DIC)[100] (Figure 1.11).

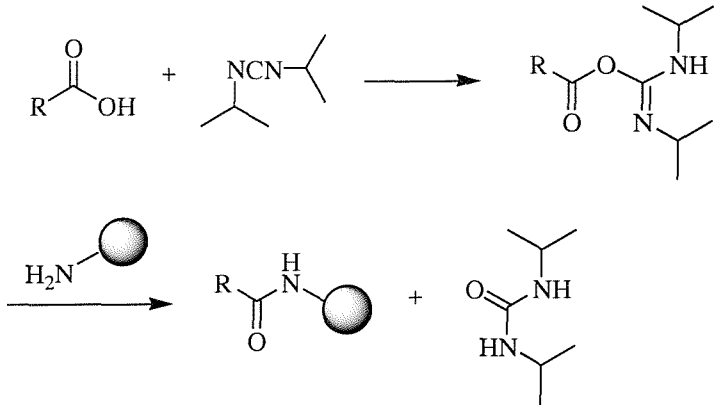
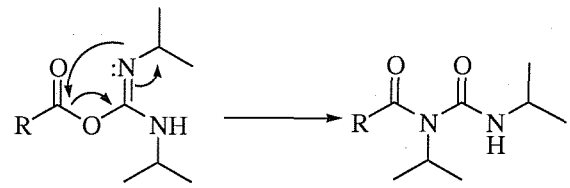


Figure 1.11 *Use of DIC to facilitate amide bond formation.*

The carboxylate reacts with the carbodiimide to form an activated *O*-acyl isourea. During this process the amine as the free base can consequently react with the activated acid to form the corresponding amide and urea by-product. But often racemisation and acetyl tranfer forming the unreactive *N*-acylurea are observed.



O-Acylisourea *N*-Acylisourea

These side reactions can be prevented by adding a selected nucleophile that reacts faster than the acyl transfer and generates an intermediate still active enough to couple with the amine. Such nucleophiles can be DMAP or HOBt [101,102,103] (Figure 1.12).

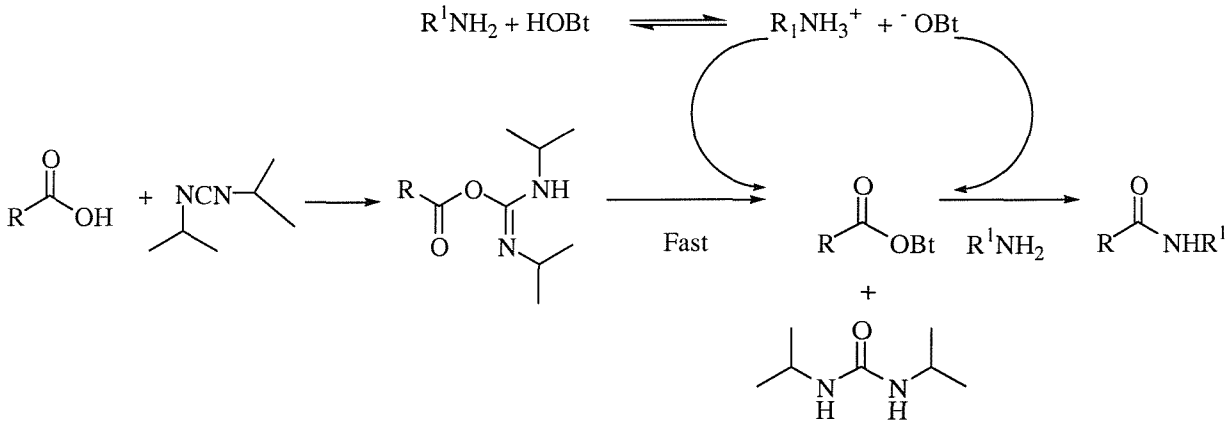


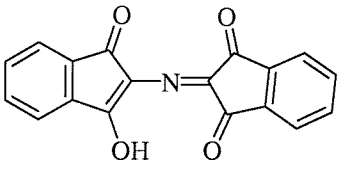
Figure 1.12 *Use of HOBt as a nucleophile for amide bond formation.*

1.10 Monitoring solid phase reactions

As mentioned in section 1.2 reaction monitoring can represent a major issue in solid phase chemistry. There is a tremendous need for analytical methodologies in various aspects of solid phase chemistry in order to meet the needs of today. In the early days of solid phase chemistry, the only way to identify the nature of a product was to cleave it from the resin and apply traditional solution phase characterisation techniques (NMR, MS, HPLC, UV,

IR etc.). It has recently been possible to apply some of these tests directly to the resin bound product.

Colourimetric tests have greatly facilitated monitoring of solid phase reactions. The ninhydrin reaction developed by Moore and Stein[104] in 1948 was adapted for use with solid phase systems by Kaiser,[105] but this test was semi-quantitative. Sarin[106] formulated conditions for the rapid and quantitative determination of free amino groups on the resin. The amount of free primary amine on the resin can be determined (by the Beer Lambert Law) from the concentration of the complex Ruhemann's purple formed (Figure 1.12).

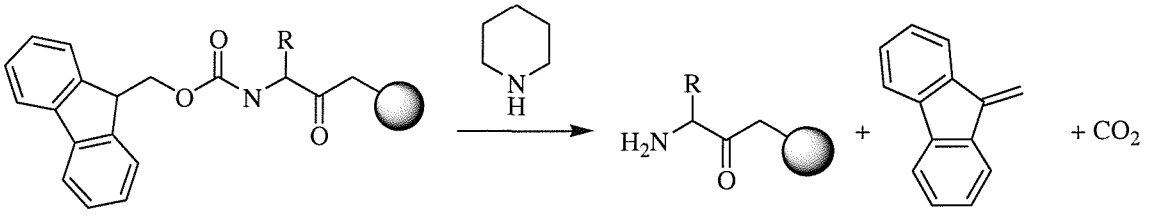


(28)

Figure1.12 *The Ruhemann's Purple Complex.*

The ninhydrin test can be used only for primary amines, for secondary amines Bromophenol blue[107,108,109] can be used on the resin. Bromophenol blue binds to amines on the resin and following acylation it is displaced. Chloranil[110, 111] can also react with free amines to produce a chromogenic compound.

The concentration of Fmoc group[112] on resin may be established by measuring the UV absorbance of a fulvene-piperidyl chromophore **(29)** (Figure 1.13) released on treatment with base.



(29)

Figure1.13 *The Fmoc test.*

The sequence of peptide chains on the solid support can be determined by Edman degration.[113, 114,115] Elemental (combustion) analysis is also a commonly used tool in solid phase analysis but due its destructive nature and the fact that the resin itself is included in the combustion analysis data, the presence of atoms or functional groups specific only to the resin bound molecule is essential for viable analysis. It is therefore not always applicable to use elemental analysis for routine characterization during solid phase peptide synthesis. When possible the above on resins tests are carried out but still, by far the most common method of analysis involves cleavage from the resin and traditional solution phase characterisation.

Infra-red spectroscopy can also be applied to the solid phase. In practice though it may be difficult to obtain more than just qualitative data from the IR analysis as the signals from the polymeric matrix can dominate the spectra. Single bead infra-red analysis has been used to investigate the course and the kinetics or organic reactions. Mass spectrometry (MS) is becoming a prolific analytical tool in combinatorial chemistry.[116] Resins can be cleaved *in situ* and MS analysis performed using MALDI-TOF MS.[117,118] This technique removes the need for separate cleavage and work-up steps, usually employed in the popular ES-MS[119] thereby reducing analysis times. Fast Atom Bombardment (FAB-MS)[120] has also been applied to the sequencing of peptides.

NMR analysis of homogenous solution phase compounds is routine, but with modifications, this technique has also been applied to the solid phase. The resin must be swollen, and for this reason the techniques is called Gel Phase NMR spectroscopy. In its swollen state the compound has increased molecular movement, which results in narrower bandwidths and increased signal resolution compared to the dry state. ^{1}H Gel Phase NMR is possible by the use of a magic angle spinning (MAS) nano probe. The sample spins in the magnetic field at the optimal angle (the magic angle) for increased spectral line resolution.[121] This technique has been determined to be most effective with resins that contain long PEG chains i.e., resins that can be most easily swollen. Gel Phase NMR spectroscopy can be applied to ^{13}C analysis without using custom probes.[122] Even though the bandwidths for a ^{13}C NMR spectrum are broader than from a ^{1}H NMR the overall much wider distribution of chemical shifts means that structural information can be

obtained by this method. A disadvantage though is that the technique is very insensitive (as the relative abundance of ^{13}C is 1%) and long NMR experiments times are necessary for good spectral resolution.

CHAPTER 2

Synthesis of vinyl-functionalised oligo-ethylene glycol derivatives as co-monomers in the preparation of resin beads

2.1 Introduction

Since Merrifield's pioneering work[4] on peptide synthesis, considerable efforts have been directed towards the development of new supports for solid phase synthesis with differing compositions and properties,[123] ranging from novel beads to new methods of bead encapsulation and handling.

Polystyrene (PS)-based resins are currently the most widely used supports in solid phase synthesis.[38,124] However such resins suffer from an incompatibility with polar solvents, which induces restricted swelling, and as a consequence permits only limited accessibility of reagents into the beads.[125] One method of manipulating the hydrophobic nature of PS is to graft a poly(ethylene glycol) (PEG) moiety onto the polymer backbone, and hence generate a highly solvatable environment. A second role of PEG in this case is also as a "spacer" separating the starting point of solid-phase synthesis from the PS core (Figure 2.1).

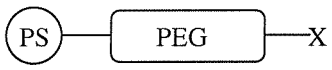
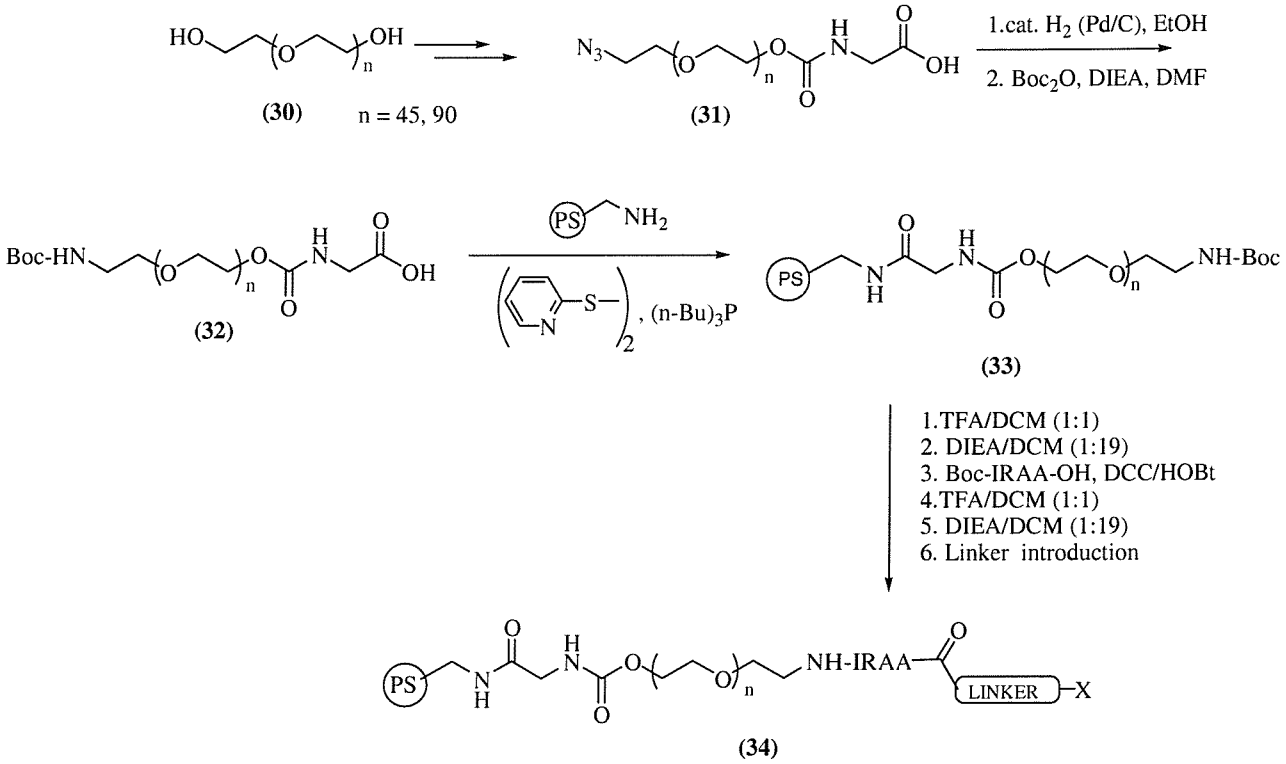


Figure 2.1 *General structure for PEG-PS supports ("Spacer model"). PS = cross-linked polystyrene; PEG = poly(ethylene glycol); X= starting point for general organic synthesis.*

Grafting of PEG onto PS was carried out first in the 1970's and early 1980's by Inman,[126] Regen,[127] Patchornik,[128] Sherrington,[129] Mutter[130] and Rapp.[131] Independent works were reported in the mid-1980's while the commercial advantages of PEG-containing supports

(including Meldal's PEGA[132]) were realized in the 1990's. Barany's general approach for the preparation of PEG-PS supports relied on the covalent attachment *via* amide linkages of heterobifunctional PEG derivatives of defined molecular weight onto suitable amino-functionalised microporous PS[133] (Scheme 2.1).

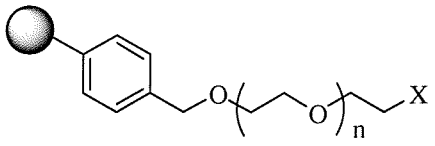


Scheme 2.1 *Preparation of heterobifunctional PEG derivatives and subsequent use to generate PEG-PS support. IRAA= internal reference amino acid; X = starting point for synthesis.*

Barany's method for the synthesis of PEG-PS resin **(34)** started from commercially available homobifunctional PEG **(30)** which was firstly converted into the corresponding azide **(31)** and successively transformed into the N^{ω}-Boc-protected PEG-ω-aminoacid **(32)** by hydrogenolysis and Boc-protection. The following steps were coupling of **(32)** onto

aminomethyl-PS and deprotection/coupling cycles to introduce an "internal reference" amino acid, usually norleucine, and a linker such as Fmoc-PAL-OH (5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid). This grafting methodology allowed the final weight ratio of PEG/PS, as well as the final loading, to be controlled by both the starting loading of amino group on PS and the molecular weight of PEG. Typical loadings obtained were 0.25-0.30 mmol/g. Such a family of resins swelled in a broad range of polar and non-polar solvents. Their physical and mechanical properties were excellent for both batchwise and continuous-flow systems. The general value of PEG-PS resins was demonstrated by the syntheses of numerous difficult peptide sequences by Fmoc chemistry. Versions of PEG-PS resins were successively prepared with a "high load" (0.35-0.6 mmol/g)[134] and/or with the acid-stability required for use along with Boc chemistry. [135]

Bayer and Rapp[34] provided an alternative way to prepare another version of PEG-PS commercially known as TentaGel™ (Figure 2.2).



n = 70
X = OH, NH_2 or linker

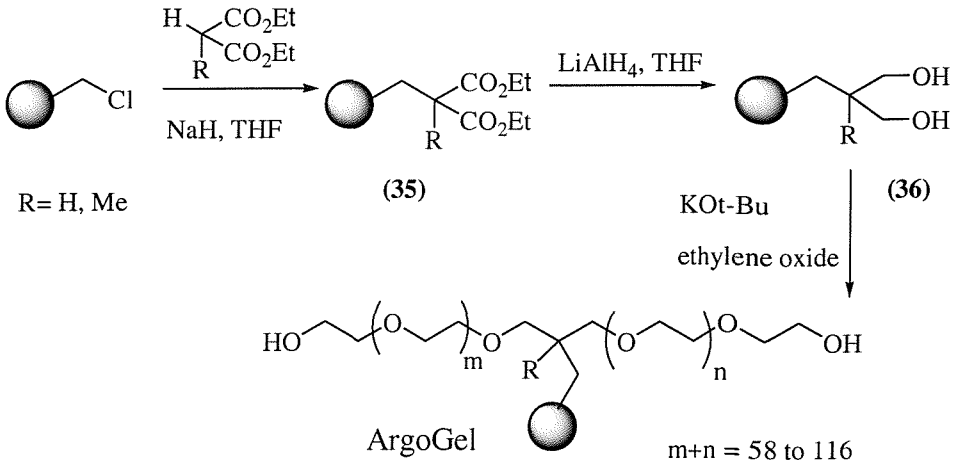
Figure 2.2. *TentaGel™ resin.*

TentaGel™ is a well-known example of a PS-PEG grafted resin. Rapp and co-workers reported that the simplest immobilisation procedure was, according to the classical ether synthesis,[136] thus coupling PEG *via* one of its terminal hydroxy groups to chloromethylated PS. However, when long chain PEGs (>800 Da) are used the yields are unsatisfactory. They found that by means of anionic graft polymerisation of ethylene oxide onto a PS-bound initiator, PEG chains can be set up step by step directly on the matrix; PEG chains of molecular masses up to 20KDa have been attached onto

functionalised cross-linked PS.[137] Graft copolymers with chains of about 3000 Da, proved to be optimal with respect to compatibility with polar solvents, mobility, resin capacity and kinetic rates. The properties of TentaGel™ are strongly dominated by the properties of PEG since the polymer contains about 60-70% of PEG by weight. The rate constants for the reaction of the active ester Boc-Gly-ONp to both low molecular compounds and the graft-copolymer were of the same order of magnitude[138] and gave evidence that the reactive sites were located at the end of the spacer-arms, and behaved kinetically as if they were in solution. The high flexibility and good solvation capacity of the PEG-spacers (as indicated by the values of ^{13}C NMR relaxation times, T_1) allows high quality MAS ^{1}H and ^{13}C NMR spectra to be obtained for these beads.[121, 122, 131c, 139]

In spite of all the advantages mentioned above, TentaGel resin suffers from some significant drawbacks that need to be considered. Resin substitution is significantly reduced due to the mass of the PEG attached to the beads reducing the initial loading. Swelling is also often too large to be useful for solid-phase organic synthesis. There are also potential problems with the long PEG chain complexing Lewis acids as well as problems of PEG leakage following cleavage and the not inconsiderable cost of the grafted PS-PEG supports.

In 1999, Gooding and co-workers[140] reported the synthesis and characterisation of a new improved series of poly(styrene-oxyethylene) graft copolymers commercially known as ArgoGel™ (Scheme 2.2). The main aims of their approach were the preparation of a novel resin with improved acid stability of the PS-grafted linkage and increased functional group loading with respect to TentaGel™. Graft co-polymerisation of ethylene oxide onto bifurcated 1,3-diol modified polystyrene **(36)** (Scheme 2.2) afforded PEG-PS resin beads in which benzylic ether linkages were replaced with a more stable aliphatic linkage while loading was increased through bifurcation. Typically a loading of 0.4-0.5mmol/g was obtained for the resin with the methyl substitution on the bifurcation. ArgoGel™ also showed good stability under acidic conditions.



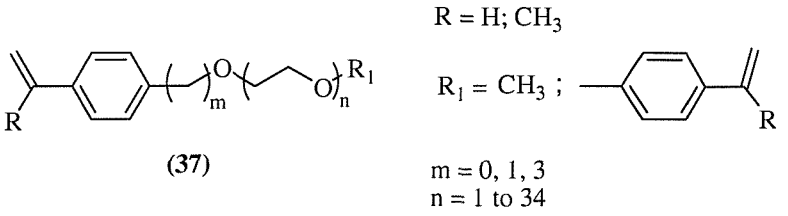
Scheme 2.2 *Synthesis of ArgoGel™ resin via anionic polymerisation of ethylene oxide. Also made by grafting on pre-formed PEG.*

2.2 Non-grafting approach toward the synthesis of PEG-PS resins

An alternative approach to post polymerisation grafting is the inclusion of vinyl-functionalised PEG or tetrahydrofuran[141] monomers into the copolymer formulation. The advantage of co-polymerisation of PEG derivatives in suspension polymerisation is that it should afford highly predictable and reproducible loadings, as long as issues such as relative reaction coefficients and water partitioning are taken into account. The nature of the monomers and cross-linkers, the PEG length and the percentage of the incorporated monomer will strongly influence the physical properties of resulting polymer networks.[142] The inclusion of vinyl-functionalised oligo-ethylene glycol monomers in the formulation should overcome, to some extent, the hydrophobicity of polystyrene resins.[143] These resins swell, for example, dramatically in non-polar solvents since the resins are still predominantly hydrophobic in nature, although the crosslinker is highly polar. A major problem faced with many supports is their excessive swelling which complicates resin handling and synthesis due to the large volumes of solvent needed for washing and the large volumes of reagents needed to promote efficient synthesis.

2.3 *Use of vinyl-functionalised oligo-ethylene glycols as cross-linkers and monomers in bead synthesis*

The design of chemically inert and mechanically hardy PS-based supports, possessing enhanced swelling and reagent accessibility in highly polar media has stimulated an interest in the properties of oligo-ethylene glycol cross-linkers and monomers[144] when incorporated into PS resin beads. Bifunctional, aromatic vinyl ether derivatives of the general type **(37)** are attractive compounds in this sense since they can be cationically or radically polymerised to produce polymer beads.



(37)

Figure 2.3 *Bifunctional, aromatic vinylbenzyl ether derivatives.*

The relative reactivity of the monomer derivatives of the type shown in Figure 2.3 towards cationic polymerisation was studied by Crivello and Ramdas,[145] using differential scanning photocalorimetry, and found to be similar to vinyl ethers while at the same time having the good mechanical properties associated with the aryl groups.

A large number of bifunctional vinylbenzyl ether derivatives have been synthesised over the past few years and their application in the synthesis of new improved polymeric supports for SPS has been thoroughly investigated. In 1990 the cross-linking agent **(38)** (Figure 2.4), which contains an oligo-ethylene glycol chain, was synthesised by Frechet[146] and used in the preparation of a regeneratable chiral auxiliary for use in the enantioselective catalytic alkylation of aldehydes. The new cross-linked polystyrene resin contained chiral primary amino alcohol moieties, which react with aldehydes to form a Schiff bases, which catalyses the addition of dialkylzinc to aldehydes, leading to optically

active secondary alcohols. Treatment of tetraethylene glycol (n = 3) with 4-vinylbenzyl chloride under Williamson conditions as shown in scheme 2.3 gave the desired compound **(38)**. The same synthetic strategy was followed by Renil and Meldal[16] in 1996 for the synthesis of the longer PEG chain (34 units) analogue of **(38)**. They obtained a mixture of di- and mono-alkylated compounds, which were bulk homo-polymerised to give a PEG-PS support. In the reported example the resin worked well as a support for the synthesis of a pentapeptide.

In 1994 the synthesis of a new cross-linker was reported by Pillai.[23] Tetraethyleneglycol diacrylate (TTEGDA) **(39)** was co-polymerised with styrene to provide a highly solvating copolymer which was used as a solid support for the synthesis of the hydrophilic C-terminal 18-residue peptide of pardaxin from *Pardachirus Pavoninus*.

An octamethylene chain was introduced in a similar di-styryl cross-linker **((40),** Figure 2.4) by Kamahori,[147] using a cross-coupling reaction of dibromohexane with 4-vinylbenzylmagnesium chloride in the presence of Li_2CuCl_4 in very good yields. The flexible structure of cross-linkers **(39)** and **(40)** provided, in both cases, good mechanical stability of the resulting co-polymers at cross-linking ratios of 2% and 10% respectively.

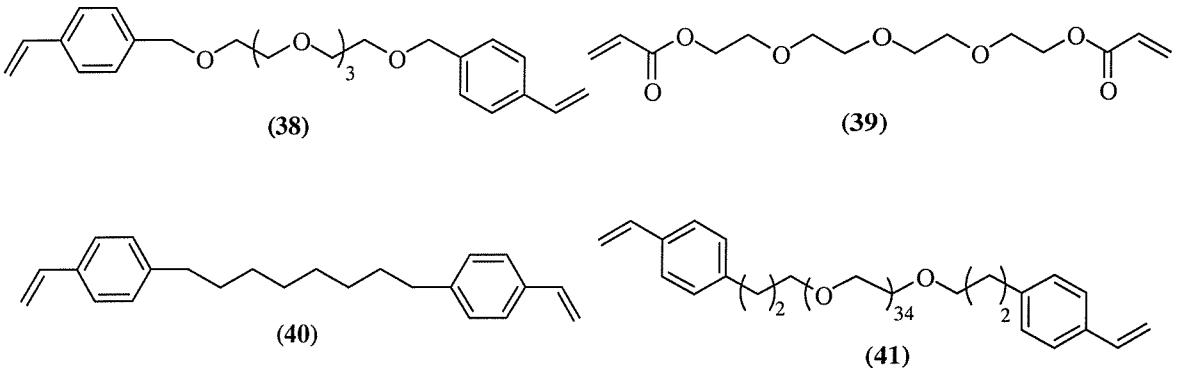
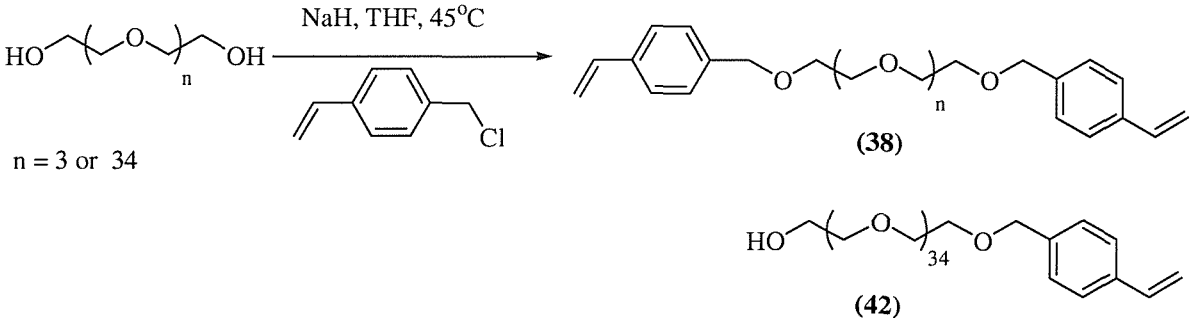


Figure 2.4 *Some example of monomers used for the preparation of cross-linked polymers.*

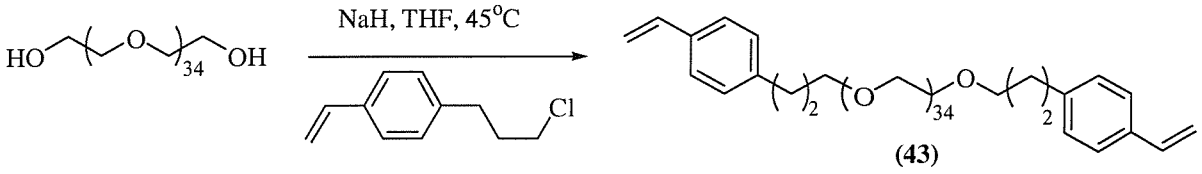
Cross-linker **(38)** and analogues containing a longer PEG chain have found application in polymer resin formulations providing PEG-PS resins in which PEG is attached to the polystyrene backbone by a benzylic ether bond. However, the benzyl ether linkage is

susceptible to cleavage under strongly acidic conditions (PEG leakage is the main issue). Kamahori[148] for example found the benzylic linkage was unstable during chlorosulfonylation reactions used for the preparation of polymer supported chiral *N*-sulfonylamino acids for asymmetric Diels-Alder reactions. Moreover the use of Lewis acids or hydrogenolytic conditions were found to destroy the resins by cleaving the benzylic ether bonds.[149] Considering the extensive use of benzyl ether protection of hydroxyl groups,[150] a non-benzylic attachment to the PEG molecules was thus desired.



Scheme 2.3 *Synthesis of cross-linker (38).*

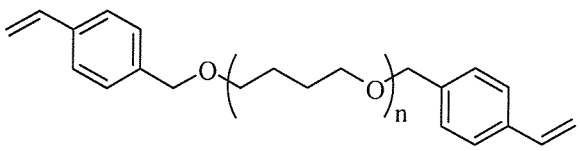
Cross-linker **(41)** was reported by Meldal[149] in 1998 with three methylene groups interposed between the PEG moiety and the styrene. Synthesis (Scheme 2.4) involved the reaction of sodiated PEG 1500 derivative with an alkyl halide prepared from 4-vinylbenzyl chloride[151] in a two step procedure. The chemical stability of the resin containing monomer **(43)** under acidic conditions (trifluoromethanesulfonate and acetic anhydride) was found to be greater than benzylic PEG-PS resins.



Scheme 2.4 *Synthesis of cross-linker (43).*

2.4 Polytetrahydrofuran (PTHF) resins

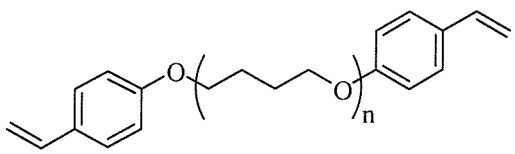
One of the functions of the resin is to solvate attached substrates. To this aim Janda[152] thought it might be beneficial to use cross-linker derivatives in the polymer formulation which closely resembles solvents used in traditional solution phase synthesis. Unlike the PEG-based cross-linkers of Itsuno,[146] Pillai[143] and Meldal[16] previously discussed, these resins incorporate a flexible polar polytetrahydrofuran (PTHF) cross-linker derivative (Figure 2.5). The resin beads were prepared using the same suspension polymerisation technique used by Kurth[144] (Scheme 2.5) and exhibited remarkable swelling in the most commonly used solvents for SPOS. The length of the cross-linker, surprisingly, did not affect the swelling properties of the resultant resins as a consequence **(45a)**, which is the simplest and most economical to prepare was chosen for use in the preparation of commercial JandaJel™ (2 mole percent of **(45a)** were co-polymerised).



(44a): n = 1

(44b): n = 3.5

(44c): n = 9.5



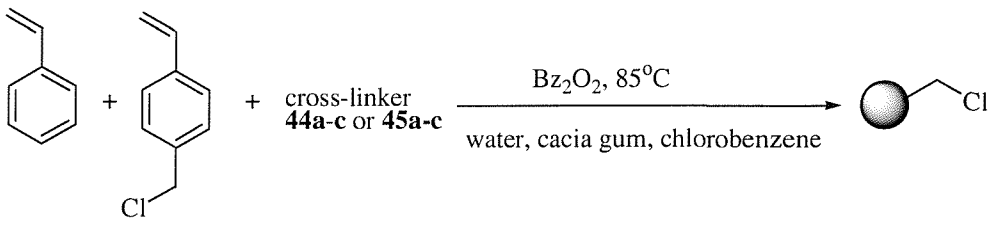
(45a): n = 1

(45b): n = 3.5

(45c): n = 9.5

Figure 2.5 *Polytetrahydrofuran cross-linkers.*

The synthetic utility of JandaJel™ was demonstrated with the preparation of a library of phthalide compounds using directed ortho-lithiation reactions;[153] polytetrahydrofuran resins afforded higher yields than did conventional DVB-PS resin. When a chiral (salen)Mn complex was attached to JandaJel™ the solid supported catalyst was used in asymmetric epoxydations, with styrene and *cis*-β-methylstyrene affording the desired epoxides with enantiomeric excesses that were nearly equivalent to those achieved using the homogeneous catalyst.[154] Another application of JandaJel™ was the preparation of a modified version of Sherrington's[155] monolithic polystyrene forms. These discs were prepared by incorporating cross-linker (**45a**) in monoliths of different shapes in which the shape itself served as an encoding element for the deconvolution of split and mix combinatorial compound libraries.[156]



Scheme 2.5 *Preparation of JandaJel™ resin.*

2.5 PEG-based resins

PEG-based resins are another class of solid supports, which have found application in solid phase enzyme chemistry. A number of resins of this type have been reported over the past few years. In 1996 Barany and Kempe reported on a novel family of highly cross-linked polymeric supports named CLEAR[40] (cross-linked ethoxylated acrylate resin). This new resin had a high molar ratio of the tri-cross-linker trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**46**) (Figure 2.6) which was co-polymerised (either in suspension or in bulk mode) with various combinations of amino functionalised

monomers such as allylamine (**47**) and 2-aminoethylmethacrylate·HCl (**48**). Non-functionalised monomers and cross-linkers such as poly(ethylene glycol-400) dimethacrylate (**49**), poly(ethylene glycol-400) ethyl ether methacrylate (**50**) and trimethylolpropane trimethacrylate (**51**) were also used to give five different CLEARs. All of these beaded supports or co-polymers had hydrophilic PEG-like character, even though individual oligo(ethylene oxide) chains were relatively short compared to conventional PEG-PS. The loading of the resins (0.1-0.3 mmol/g) was affected by the amount of the amino-functionalised monomer used. CLEAR resins showed excellent mechanical stability and swelling properties in a broad range of solvents. Their usefulness in the synthesis of challenging peptides was demonstrated by successfully carrying out comparative syntheses in parallel with other solid supports.[40]

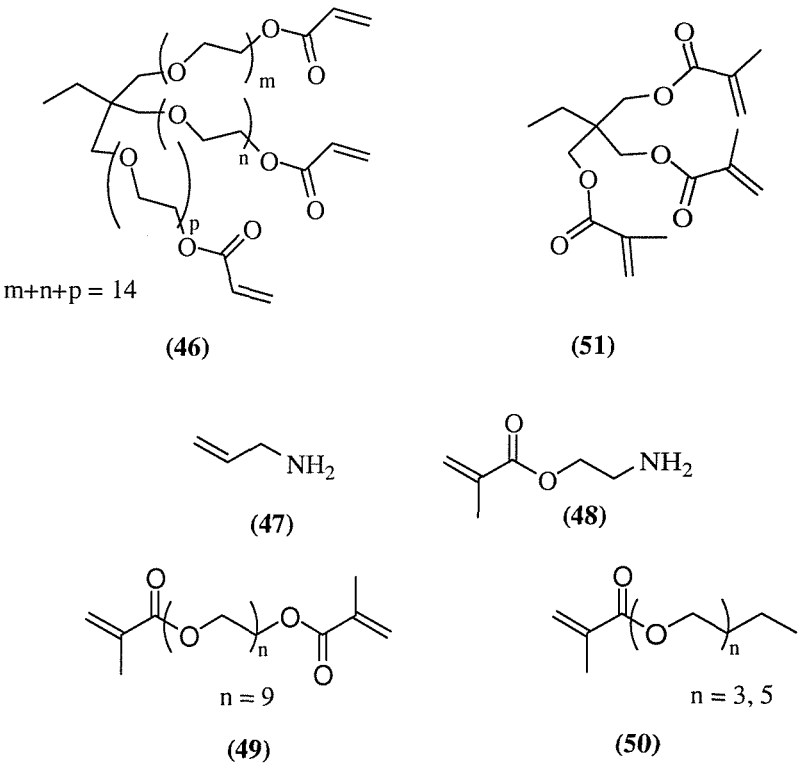
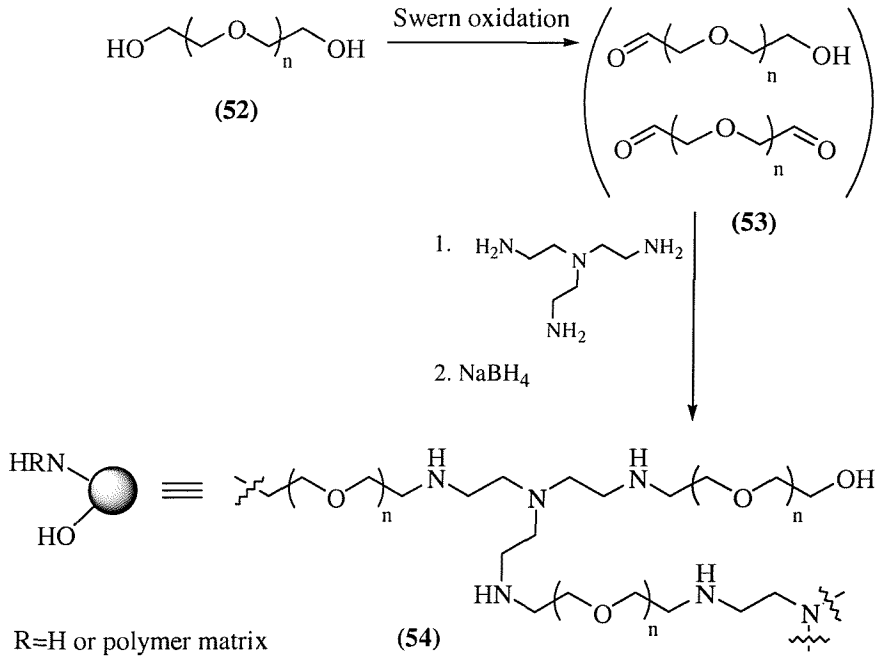


Figure 2.6 *Cross-linker and monomeric building blocks of CLEAR supports.*

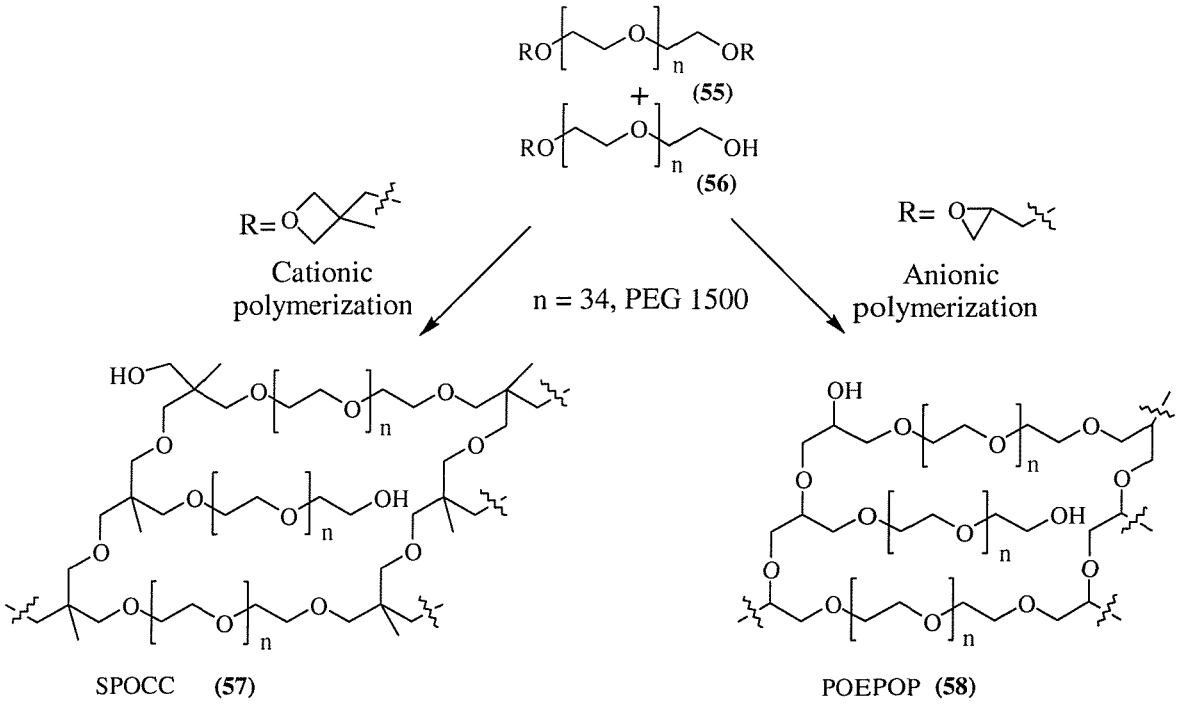
A bifunctional polar PEG-based resin named HYDRA was recently reported by Meldal and co-workers.[157] Such resin was synthesised by reductive amination of mixtures of mono- and dialdehyde PEG 1500 (**53**) and the branched cross-linker tris(2-aminoethyl)amine (Scheme 2.6). For the first time two functional groups (OH and NH_2) were present throughout the polymer matrix and were used to synthesise an octa- and a decapeptide independently on the different functionalities. The resin loading was varied (0.33-0.80 mmol/g OH and 0.24-0.88 mmol/g NH_2) by varying the ratio of monomers in the polymerisation mixture. The solid support was stable towards strongly acidic and basic conditions for weeks and was fully permeable to a 27 kDa protease.



Scheme 2.6 *Synthesis of HYDRA resin via reductive amination of PEG aldehyde mixture. Note: ñ=32.*

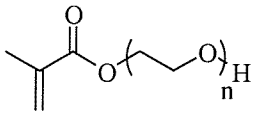
In 2001, Meldal and his co-workers[158] developed a novel (PEG)-based resin suitable for solid-phase organic synthesis, solid-phase enzymatic chemistry and on-bead screening.

The polymerisation reactions were carried out with either 3-methyloxetanylmethyl PEG 1500 macromonomers (SPOCC, (**57**)) or methyloxirane PEG 1500 macromonomers (POEPOP, (**58**)) with 75% incorporation of oxetanyl or oxiranyl groups, respectively. These resins were prepared by bulk polymerisation and afforded irregular, fragile particles. Ring-opening polymerisation of PEG-based monomers initiated either by a Lewis acid (cationic ring opening) or potassium t-butoxide (anionic ring opening) (Scheme 2.7) was performed to obtain polymer beads in high yields. A polymer of acrylate esters containing pentamethyldisiloxane and PEG prepared by radical polymerisation was used as a surfactant, which was efficient in stabilising the suspension of the PEG-based macromonomers, initiator and solvent in the silicon oil used as the suspension agent.



Scheme 2.7 *Synthesis of SPOCC and POEPOP.*

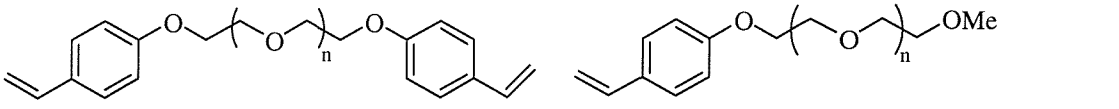
Another new lightly cross-linked resin in this category was reported by Fréchet in 2001.[159] The hydrophilic support was prepared from commercially available oligoethylene mono-methacrylates (Figure 2.7) and ethylene dimetacrylate (EDMA) using a radical suspension polymerisation process in which the partition of monomers in the organic phase was ensured by supplementing the aqueous phase with a co-solvent (cyclohexanol) and by choosing specifically designed polymerisation mixtures. The resin was shown to be resistant to both acid and base hydrolysis even though the ester group of PEG-MA might not be compatible with some reagents. The potential of these beads was demonstrated in the solid phase synthesis of a small library of hydantoins.



(59) n=3, 7, 12

Figure 2.7 *PEG-MA monomer.*

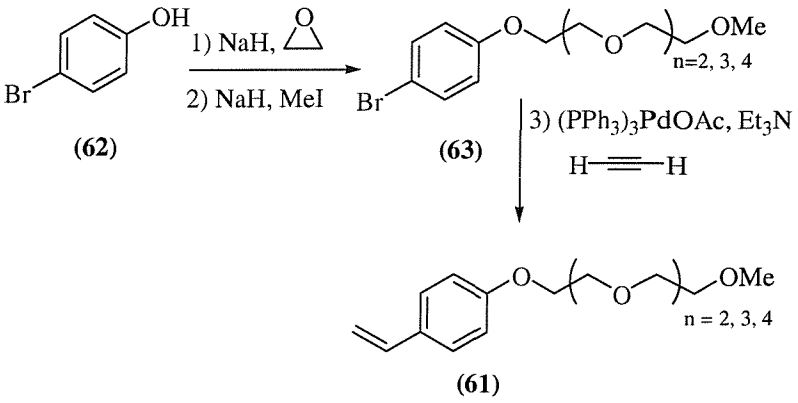
Given the literature examples presented in the previous sections, the bi- and mono-styrene ether derivatives **(60)** and **(61)** (Figure 2.8) represented attractive synthetic targets. They contain a stable phenyl ether linkage designed to provide broad chemical compatibility. The short PEG chain (n = 1, 3, 7) offers an attractive alternative to the long PEG chain found in TentaGel™ and thus a much higher resin loading.



(60) **(61)** n = 1, 2, 3, 4, 7, 11

Figure 2.8 *Bi- and mono-styrene ether monomers containing a stable phenyl ether linkage.*

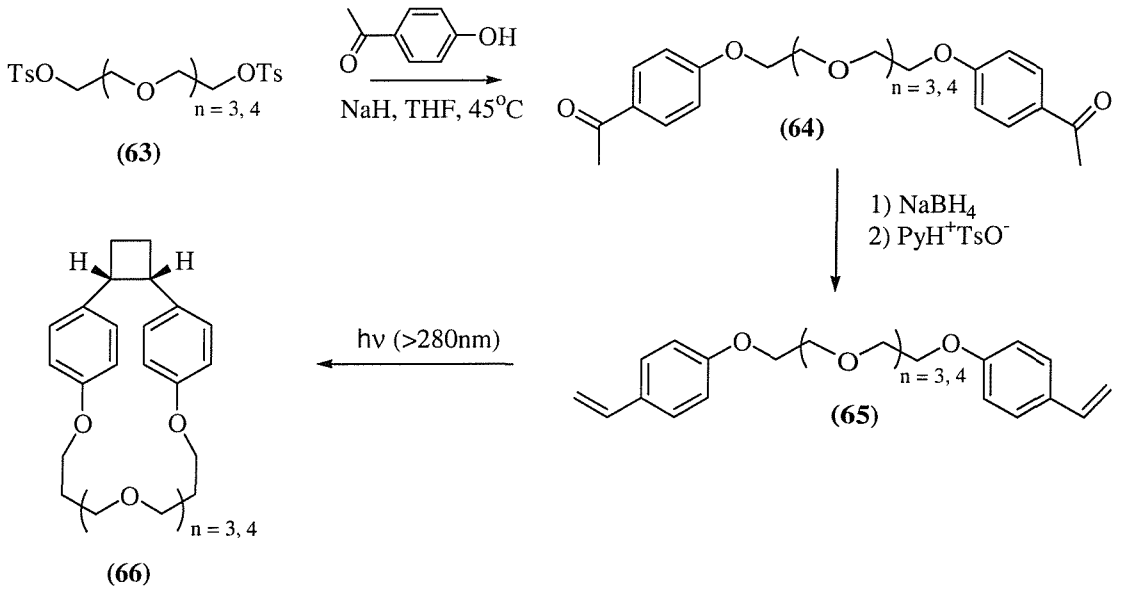
The synthesis of monomer **(61)** was described by Hallensleben and Lucarelli[160] in 1996. The strategy (Scheme 2.8) followed by these authors used *p*-bromophenol as a starting material for the preparation of compounds **(63).** A mixture of bromides with different side chain lengths was obtained. The vinyl group was established in a Heck reaction, although the overall yields for this reaction were very poor (5% to 15%).



Scheme 2.8 *Synthesis of monomer (61) via Heck reaction.*

Interestingly in 1990 Inokuma[161] published the synthesis and photoreaction of monomer **(65)** for the preparation of new crown ethers (Scheme 2.9).

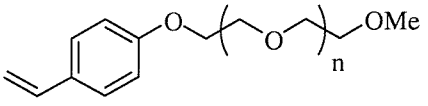
The synthetic strategy followed in the work described in this thesis was based on this synthesis since it appeared to be the most amenable to large scale synthesis involving the use of inexpensive and readily available starting materials and reagents.



Scheme 2.9 *Synthesis of monomer (65) for the preparation of new crown ethers.*

2.6 Results and Discussion

The first objective was the synthesis of the methoxy styrene monomer **(61)** (Figure 2.9) needed for suspension polymerisation. An important factor for the choice of synthetic strategy was the amenability to large scale preparation as well as the use of readily available and inexpensive starting materials.



(61)

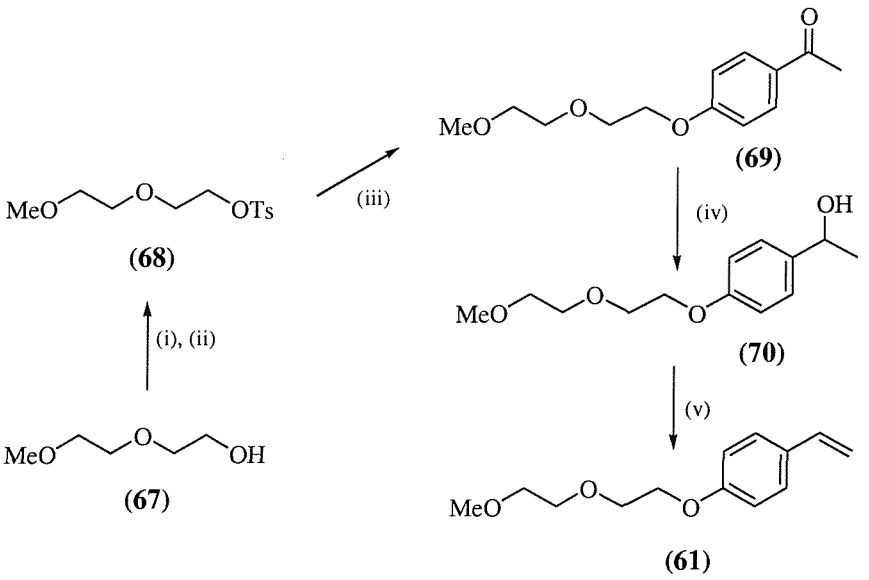
n = 1, 3, 7, 11

Figure 2.9 *Methoxy styrene monomer (61).*

The use of scavenger resins for purification as an alternative to chromatography was investigated since it would represent, for the first time, a tool for purification on a large scale. The third objective was the synthesis of a series of styrene analogues with different PEG chain lengths.

Monomers n = 3, 7 and 11 were chosen in order to provide a range of styrene monomers to compare the different properties of the relative polymer resins. For this purpose, the different reactivity of the starting monomethoxy polyethylene glycols was investigated.

2.7 *Synthesis of 1-[2-(2-methoxyethoxy) ethoxy]-4 vinyl benzene (61)*



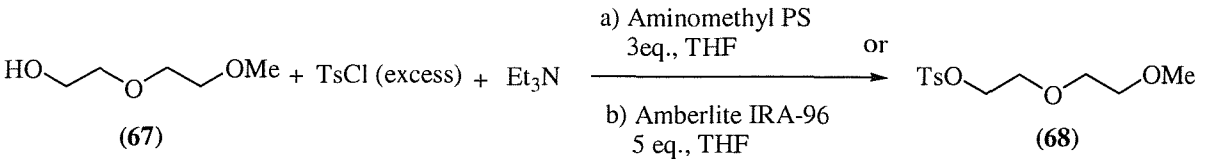
(i) TsCl (1.5 equiv.), NEt_3 (1.5 equiv.), DMAP (cat.), DCM; (ii) Amberlite IRA 96 (Scavenging); (iii) p-hydroxy-acetophenone (1.2 equiv.), K_2CO_3 (1.2 equiv.), CH_3CN, reflux; (iv) $NaBH_4$ (1 equiv.), THF/EtOH 1/1; (v) Pyridinium Tosylate (cat.), toluene, reflux.

Scheme 2.10 *General synthetic strategy followed for the synthesis of monomer* ***(61)***.

Styrene derivative **(61)** was prepared by tosylation of the alcohol **(67)**, Williamson ether formation to give **(69)**, reduction and dehydration to give **(61)**. *p*-Toluenesulfonylation of

commercially available diethylene glycol monomethyl ether (n = 1) was the first step toward the synthesis of the styrene derivative **(61)**. The presence of a *p*-toluene sulfonate as leaving group promoted ether bond formation. Among a number of methods for the tosylation of alcohols,[162] *p*-toluenesulfonyl chloride/pyridine reagent have been traditionally employed. However this method suffers from tedious procedures to remove pyridine and undesirable loss of the tosylates to their chlorides often occurs. A facile and practical method for the tosylation of alcohols employing TsCl/Et_3N with TMEDA (*N,N,N',N'*-tetramethylethylenediamine) as a catalyst was reported by Yoshida.[163] Compared to the traditional pyridine-solvent method, this method had the advantage of a higher rate of reaction, the suppression of undesirable side reactions and the operational simplicity and economy. According to the literature examples, when the reaction was performed using Et_3N as a base in THF or DCM no catalyst was needed. The reaction was driven to completion using an excess of tosyl chloride (typically 2 eq). The crude was initially purified by chromatography on silica.

Due to the large scale of the reaction, attention was addressed on investigating a purification method as an alternative to classic chromatography. Scavenger resins (see Chapter 1 for a more detailed description of scavenger resins) were used for this purpose (Scheme 2.11). The first purification attempt was made using aminomethyl resin as a covalent scavenger expecting the formation of a resin bound sulfonamide. The results were encouraging since only the desired tosylate, with no trace of excess *p*-toluene sulfonyl chloride, were observed. The main drawback of this method being that aminomethyl-PS resin was expensive and thus not suitable for large scale preparation purposes. This problem was addressed by using a macroreticular polyamine resin Amberlite IRA 96. This PS polyamine scavenger resin was synthesised by Yamshkov[164] and co-workers in 1979. Amberlite IRA 96 (capacity 5.6 eq/g, mesh: 16-50) was chosen for our purpose, since it is inexpensive and can therefore be used in large excess (typically 5 equivalents), covalently binding any remaining tosyl chloride and tosic acid by ion-pairing.



(67) (68)

Scheme 2.11 *Scavenger resins for purification of compound* ***(68)***.

A comparison of the three purification methods (chromatography on silica, aminomethyl polystyrene and Amberlite IRA96) showed very good efficiency and the ease of use of the Amberlite resin. Aminomethyl polystyrene, gave the compound in a purity of 83% while the macroreticular scavenger gave a 93% pure tosylate. Moreover reproducible results were obtained when the reaction was scaled up.

Table 2.1 *Scale-up reactions for the synthesis of* ***(68)***

Starting material (g)	**Product (g)**	**Purification method**	**Yield (%)**
5	10	column chromatography	82
10	20	Amberlite	89
30	65	Amberlite	95

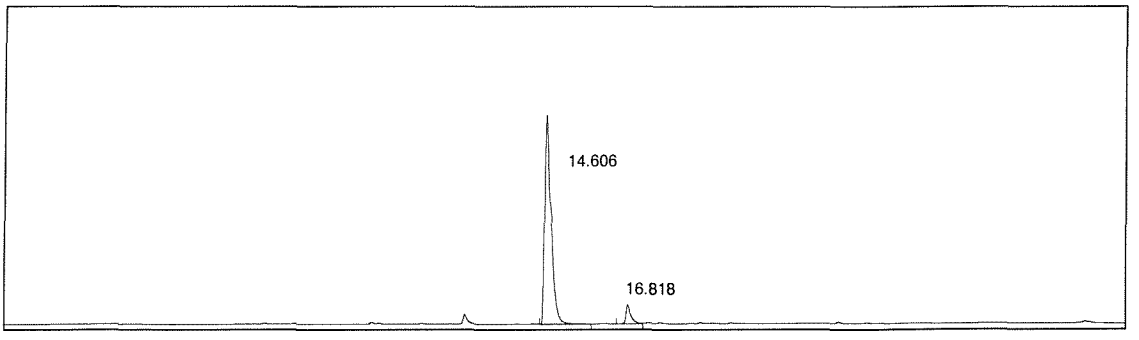
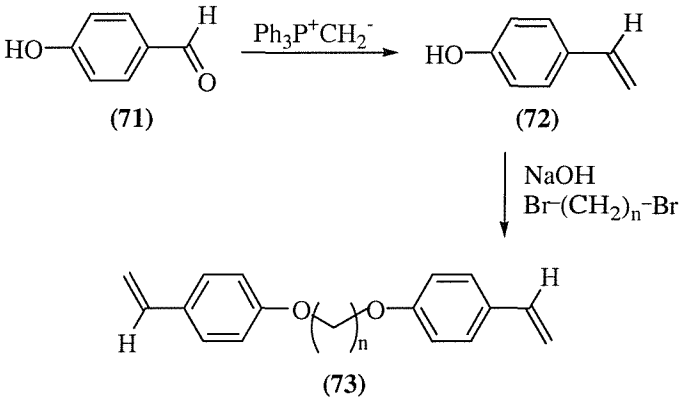


Figure 2.10 *RP-HPLC (λ=254nm) trace of tosylate* ***(68)*** *following treatment with IRA 96.*

A review of the literature to find alternative methods to the one reported by Inokuma,[161] showed that Ericcson[165] and Woods[166] had employed the synthetic approach shown in Scheme 2.12 for the synthesis of cross-linker **(73)**.



Scheme 2.12 *Synthesis of cross-linker (73) via a Wittig reaction.*

The first step of the synthetic strategy in Scheme 2.12 suggested coupling 4-hydroxy benzaldehyde to the tosylate **(68)** (shown in Scheme 2.10) and a Wittig reaction to give the desired vinyl moiety. Although the synthetic route was straightforward, the overall yields for **(73)** were low (between 25% and 66%) and additionally, the Wittig reaction was considered impractical for other than small scale chemistry. As a consequence, tosylate **(68)** was coupled to *p*-hydroxyacetophenone. The synthesis was firstly attempted using potassium carbonate as a base and acetonitrile as a solvent at 50°C. The desired compound was formed but starting material and *p*-hydroxyacetophenone were also recovered. Replacing potassium carbonate with sodium hydride and acetonitrile with THF did not improve the results. Use of potassium iodide and raising the temperature to 80°C gave the best results; the reaction went to completion and the product was isolated in 85% yield. For the reasons explained earlier in this section, attention was paid to the most convenient and easiest purification methods. Purification by means of a scavenger resin (anion exchange-quaternary alkylamine) to remove the excess of *p*-hydroxyacetophenone was

attempted. Purification of **(69)** was performed using NSA100 (ionic form: Cl). The resin was converted into its hydroxy form by washing with an aqueous solution of sodium hydroxide as reported by Parlow.[167] Unfortunately the purification was not successful. Therefore, the compound was purified by washing the crude with an aqueous solution of sodium hydroxide (pH=11). The reaction was scaled-up from 1 to 50 grams with yields from 81% to 89% (Table 2.2).

Table 2.2 *Scale-up reactions for the synthesis of* ***(69)***

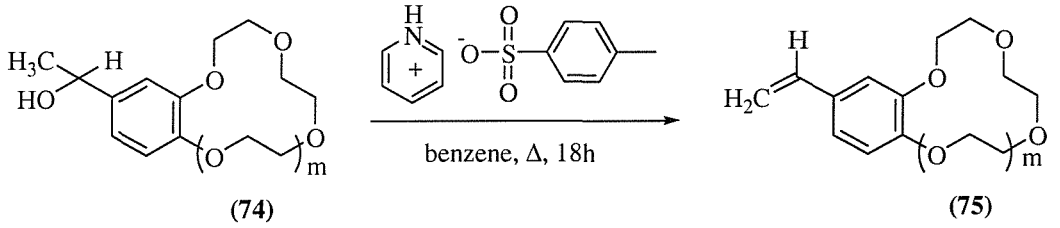
Compound (68) (g)	**Product** (g)	**Yield** (%)
1	0.8	87
2	1.5	85
5	4	89
10	7	81
65	49	86

The reduction of 1-{4-[2-(2-methoxyethoxy) ethoxy] phenyl}-1-ethanone **(69)** was performed using sodium borohydride in ethanol. This reaction gave very clean alcohol that could be used for the last step without further purification. As in previous reaction, this was scaled-up from 0.2 to 47 grams with yields from 40% to 96% (Table 2.3).

Table 2.3 *Scale-up reactions for the synthesis of* ***(70)***

Compound (69) (g)	**Product** (g)	**Yield** (%)
0.5	0.2	40
1	0.9	95
3	2.9	88
49	47	96

The high reactivity of the final vinyl derivative **(61)** towards polymerisation suggested that particular attention had to be paid to the choice of the catalyst employed for the dehydration reaction. Pyridinium tosylate has been found to be an excellent catalyst. In 1979 Sterzycki[168] reported the use of this salt to catalyse the formation of dioxolane-type acetals from ketones. Another example was reported by Nitz and Paquette[169] in which a hindered pyridinium salt (2,4,6,-collidinium *p*-toluenesulfonate) was used to promote the chemoselective acetalization of α,β-unsaturated ketones in the presence of saturated carbonyl groups. The hypothesis of possible polymerisation of the vinyl derivative under strongly acidic conditions was confirmed by Talma and co-workers.[170] They found that the dehydration reaction of 4'-(hydroxyethyl)-benzo-27-crown-9 (Scheme 2.13) with *p*-toluene sulfonic acid in benzene gave polymerisation while the use of less acidic catalysts like pyridinium tosylate as a dehydrating agent promoted the formation of the corresponding vinyl derivative without polymerisation in 90% yield.



(74) (75)

Scheme 2.13 *Dehydration reaction of 4'-(hydroxyethyl)-benzo-27-crown-9 using pyridinium tosylate.*

The last example led to the dehydration step being performed with pyridinium tosylate. The reaction was first performed in benzene giving the desired compound in good yield (83%). However, to avoid the use of benzene, the same reaction was performed in toluene with good results (according to ^{1}H and ^{13}C NMR) with yields of 90-95%. This reaction was scaled-up to 37 grams with yields from 42% to 95% (Table 2.4).

Table 2.4 *Scale-up reactions for the synthesis of* ***(61)***

Compound (70) (g)	Product (g)	Yield (%)
0.1	0.08	42
0.14	0.1	83
0.16	0.14	95
1	0.8	91
4.6	4	91
45	37	90

2.8 500g-scale synthesis of 1-[2-(2-methoxyethoxy) ethoxy]-4 vinyl benzene (61)

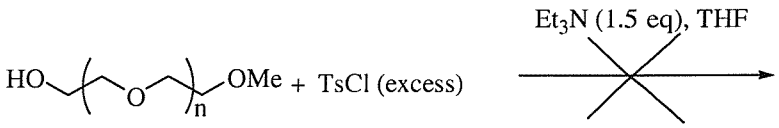
The large-scale synthesis of this compound presented some technical and chemical problems. The tosylation reaction was performed under the same conditions as those used for the small-scale preparation (40g) but DMAP was used as a catalyst to improve reaction rate and yield. The previously reported work-up and purification method were modified (see experimental section for details). Treatment of the crude with the polyamine scavenger resin Amberlite IRA-96 (macroreticular 5.6meq/g) did not remove, as expected, the excess tosyl chloride. The presence of some triethylammonium chloride salt in the crude mixture was believed to inhibit the reactivity of the NH_2 sites on the resin by protonation. Thus aqueous washing were used to remove the triethylammonium salt but even after this treatment the scavenger did not quench the excess tosyl chloride. Attempts to hydrolyse the excess tosyl chloride by acidic aqueous acid washings (HCl 2N) followed by basic washings (saturated aqueous solution of Na_2CO_3) failed. Finally treatment of the crude with a large excess of anhydrous potassium carbonate was successful. ^1H and ^{13}C NMR confirmed the results showing only traces of remaining tosyl chloride.

The second step of the synthesis was performed under the same conditions as those described in section 2.2 to give the resulting O-alkylated derivative **(69)**. Attempts to separate the product from the excess of *p*-hydroxyacetophenone by reduced pressure distillation were unsuccessful because of the similar boiling points of product and *p*-hydroxyacetophenone, thus basic aqueous wash (5% NaOH in water) was used, giving the

desired product. After basic aqueous work-up, ^{1}H and ^{13}C NMR showed the presence of two unidentified by-products. The reduction step was performed on the crude compound **(69)** to provide alcohol **(70)**, used in the following step without further purification. The dehydration step was initially performed on 30g of crude product **(70)** to give the final compound. After reduced-pressure distillation of the crude **(61)** the final vinyl derivative was obtained with an HPLC purity of 94% and a yield of 50%. Unfortunately when the reaction was repeated on a 300g scale the results were not reproduced! Several unidentified by-products were formed (^{1}H and ^{13}C NMR). The crude product obtained from this reaction was distilled in the presence of a radical stabiliser (phenothiazine) to prevent polymerisation of the monomer at high temperature. Only 25g of compound **(61)** (HPLC purity: 73%) was obtained, the remainder of the crude was recovered as a black sticky oil; presumably a variety of polymerisation reactions had occurred!

2.9 Synthesis of PEG stytyl monomers with n= 3, 7 and 11 (61a, 61b and 61c)

The tosylation of polyethylene glycol monomethyethers was attempted using the same conditions as those applied to the preparation of monomer **(61)** with n = 1.



Scheme 2.14 *Tosylation reaction of polyethylene glycol monomethyethers (n = 3, 7, 11).*

Unfortunately the reactions were very slow under these conditions (reaction time: 5 days) and tosylate derivatives were only present in traces. The starting materials were very hygroscopic and they had a low reactivity in the reaction according to the increasing length of the PEG chain. In order to find the most suitable conditions, a range of different

conditions were investigated. Table 2.5 summarises conditions applied for the monomers having 3, 7 or 11 ethylene glycol units. Reactions were monitored by HPLC. When using DMAP as a catalyst and triethylamine as a base in dry DCM for three days the best results were achieved in terms of reaction rates. Yields for the monomers with n = 3 and n = 7 were 80% and 70% respectively. Conditions for alkylation, reduction, dehydration and purification were identical to those used for the synthesis of monomer with n=1. The synthesis of the methoxy styrene monomer with n = 3 was performed on a 5g scale. The results relative to each step leading to the final compound are summarised in Table 2.6.

Table 2.5 *Conditions applied on the tosylation of PEG monomers having 3, 7 and 11 units.*

Base	Conditions
Et_3N (7eq)	DMF, 3 days
Et_3N (7eq)	Et_3N, 3 days
Pyridine	Pyridine, 3days
Et_3N (7eq), DMAP	**DCM, 3 days**
Pyridine (7eq)	DCM, 3 days
Pyridine (7eq)	DMF, 3days

Purity of the final compound was 95% (according to ^{1}H and ^{13}C NMR). For the synthesis of methoxy styrene monomer with n=7 differences in reactivity and cleanliness of the reactions were evident in each step. Monitoring the reaction by TLC and purification of the compounds were difficult for the first two steps. Starting material and product were found to have very similar retention factors. Purification by extraction was found to be insufficient and column chromatography on silica or precipitation (from DCM/petroleum ether) was required. Yields are reported in Table 2.6. Tosylation for monomer with n=11 did not give good results, difficulties in monitoring and driving the reactions to completion were encountered. Some of these problems arose from the starting material

characteristics; PEG550 monomethyl ether is a mixture of oligomers, it is very hygroscopic and water and organic soluble.

Table 2.6 *Yields for the four-step synthesis leading to monomers* ***(61b)*** *and* ***(61c)***.

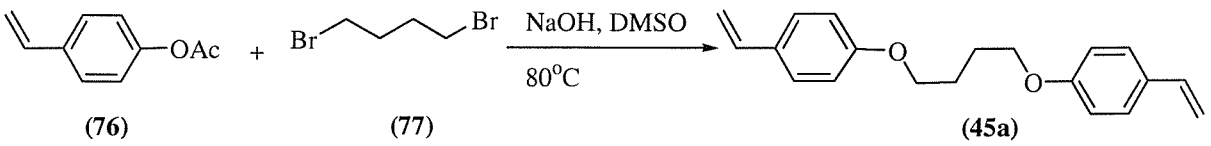
Reaction	Yield (%)	
	n = 3	**n = 7**
Tosylation	80	70
Alkylation	90	70
Reduction	84	98
Dehydration	98	85

2.10 Alternative methods towards the synthesis of monomer (61)

The synthesis of monomer **(61)** following the four steps sequence shown in scheme 2.10 was optimised and scaled up (as described in the previous sections), giving overall yields of ~60%. In order to find alternative shorter synthetic procedures leading to the target compound, two other synthetic methods were investigated.

2.11 Synthesis of (69) from 4-acetoxystyrene and tosylate (68)

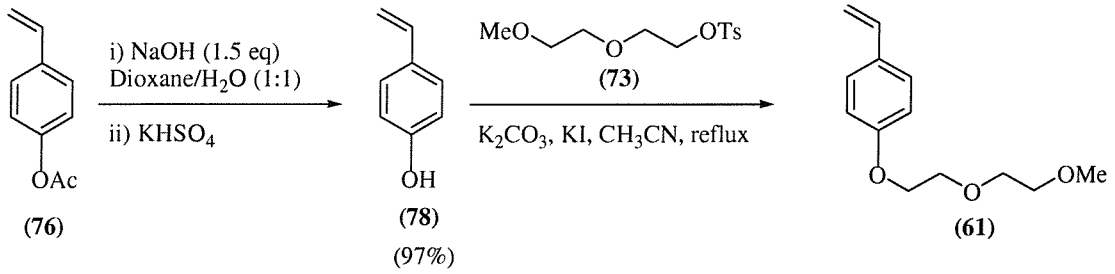
The synthesis of monomer **(45a)** described in scheme 2.15 was reported by Crivello and Ramdas.[145] In this paper a series of analogues were prepared by condensation of 4-acetoxystyrene (readily available from commercial sources) with α-ω-dihaloalkanes in the presence of a base or alternatively of a phase-transfer catalyst (tetra-n-butylammonium bromide).



(76) (77) (45a)

Scheme 2.15 *Synthesis of monomer (45a).*

Following this, a similar procedure was used for the synthesis of monomer **(61)**. The tosylate **(68)** was reacted with 2 equivalents of 4-hydroxystyrene (obtained by hydrolysis of 4-acetoxystyrene[39]) in the presence of potassium carbonate and a catalytic amount of potassium iodide in acetonitrile (Scheme 2.18). The desired compound was formed as shown by HPLC (Figure 2.11). Unfortunately excess 4-hydroxy-styrene could not be completely removed by aqueous extraction (NaOH, pH=11).



(76) (78) (97%) (61)

Scheme 2.16 *Preparation of monomer (61) from 4-acetoxystyrene and tosylate (68).*

The same reaction was performed using one equivalent of each reagent. Compound **(61)** was formed in good yield but purification by chromatography on silica was necessary but this was difficult due to the poor solubility of the crude material in the suitable eluent for separation.

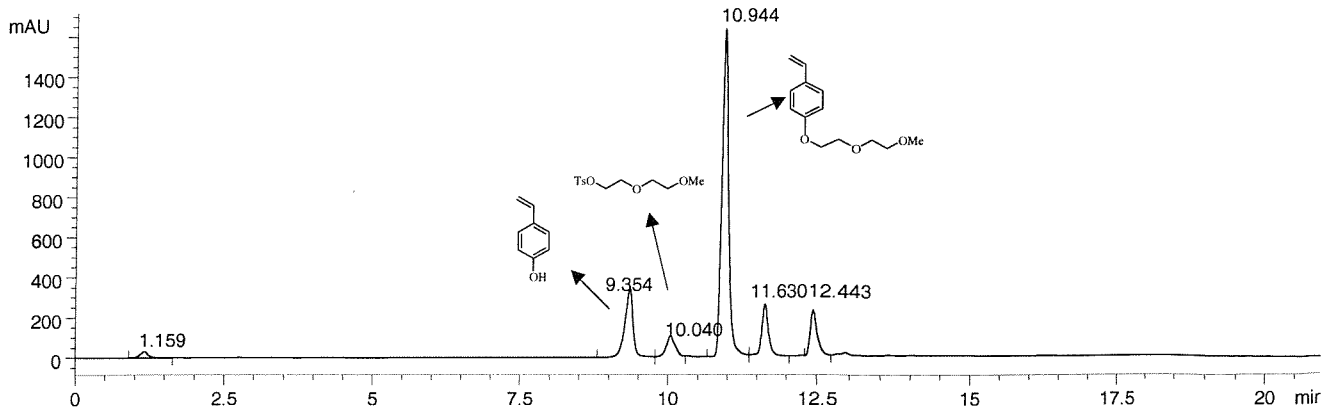
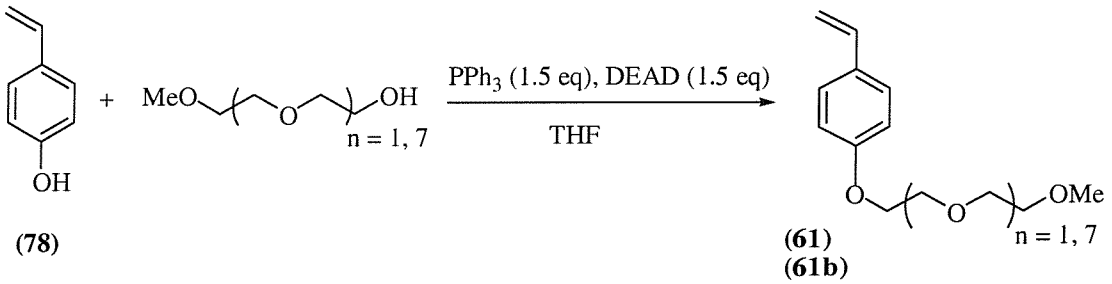


Figure 2.11 *HPLC (ELSD) trace of monomer* ***(61)*** *crude after aqueous work-up.*

2.12 Synthesis of (61) via Mitsunobu Coupling

A third attempt to synthesise monomer **(61)** following a straightforward method used readily available diethyleneglycol monomethylether **(67)** and 4-hydroxystyrene **(78)**. The two compounds were coupled under Mitsunobu[171] conditions using diethylazodicarboxylate (DEAD) and triphenylphosphine (Scheme 2.17). The reaction did not go to completion even after 3 days as shown by TLC and HPLC. Purification by chromatography was difficult in this case due to the presence of triphenylphosphine oxide (9.8 min. in HPLC trace in Figure 2.10).



Scheme 2.17 *Synthesis of monomer* ***(61)*** *via Mitsunobu coupling.*

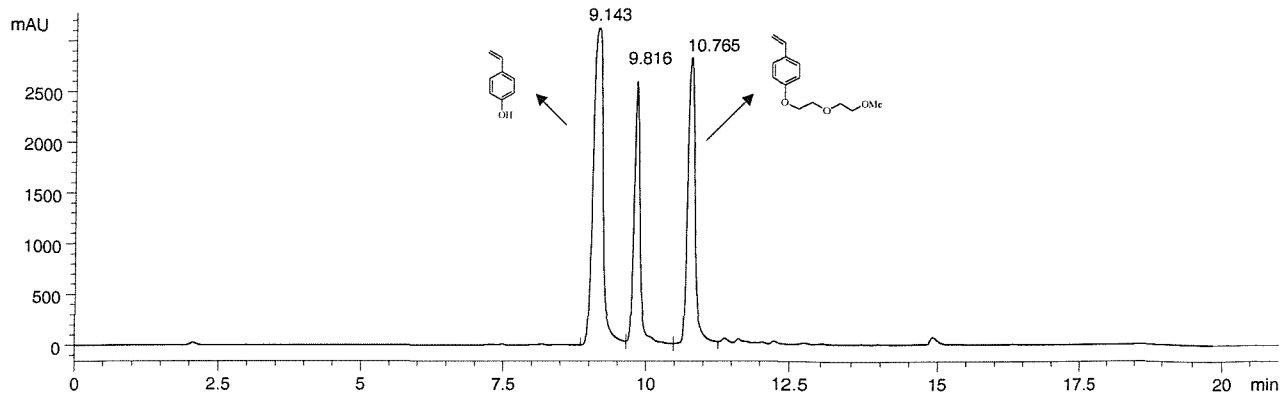


Figure 2.12 *HPLC (ELSD) trace of monomer* ***(61)*** *prepared under Mitsunobu conditions.*

The synthesis of the analogue PEG monomer with n = 7 **(61b)** following the same approach appeared to be very attractive since it did not require the preliminary tosylation of the alcohol which, as described previously, was very slow due to the low reactivity of the starting material. Unfortunately no product was formed when PEG350 was coupled with 4-hydroxystyrene under Mitsunobu conditions. The hygroscopicity of the polyethylene glycol was believed to be the major problem. However, even when the starting materials were dried using a Dean-Stark trap the reaction was not successful. Triphenylphosphine oxide and 4-hydroxystyrene were recovered after chromatographic separation

2.13 Conclusions

Attempts to synthesise monomer **(61)** using 4-hydroxystyrene and tosylate **(68)** under Williamson conditions were not completely successful and not amenable to large-scale synthesis due to the toxicity of 4-acetoxystyrene with the instability of its hydrolysed form being a major issue. The second alternative synthesis exploiting the straightforward

Mitsunobu coupling between ethylene glycols and 4-hydroxystyrene did not give the desired results, and would have been prohibitively expensive.

In conclusion, the four-step synthesis via the Inokuma sequence was the most successful method of preparing the desired PEG monomers with different PEG lengths. This method had all the requirements for a large-scale synthesis, the overall yields were uniformly good and materials were inexpensive. Compared to diethyleneglycol, the reactivity of polyethyleneglycols (PEG 350 and PEG 550) towards the synthesis of the target molecules was found to be very low and the reactions inefficient, in particular when tosylation or Mitsunobu reactions were attempted. The use of scavenger resins was shown to be a valid tool for purification.

CHAPTER 3

Multiple parallel polymerisation

3.1 Suspension polymerisation

The discovery and exploitation of ion exchange resins during the 1950s, the investigation on the use of suspension polymerised styrene-DVB sulfonic acid resins as heterogeneous catalysts,[172] the introduction in 1963 of Merrifield's solid phase peptide synthesis methodology[4] and later the explosion of solid phase combinatorial chemistry in drug discovery has made suspension polymerisation methodology one of the most efficient techniques employed for free radical polymerisation in industry.[173] This technique allows the ready preparation of polymeric beaded material having suitable size, shape and uniformity for many specific applications. Mainly for technical reasons, this method is less suitable than solution polymerisation for small-scale synthesis. Nevertheless, it is ideal for the preparation of beaded polymeric materials employed for SPOS or polymer supported reagents.[174] Like some other modes of polymerisation (see Chapter 1 for examples), suspension polymerisation can be carried out in water. The main feature of this mode of polymerisation is the maintenance of a suspension of the monomers in an immiscible solvent (suspension medium) during the polymerisation process. The suspension medium helps to achieve complete polymerisation since it acts as an efficient heat transfer agent for the droplets in suspension. A mixture of monomers such as styrene and DVB containing a radical initiator is dispersed as spherical liquid droplets in an excess of an immiscible water phase. The suspension is maintained by continuous stirring and the reaction is typically heated to initiate polymerisation.[17] Under these conditions the suspended droplets collide with each other and coalesce into larger ones. Re-division of the coalesced (larger) droplets becomes gradually more difficult as a result of polymerisation (beginning of the sticky period). However, the individuality of the polymerised droplets is maintained by using a small amount of a suitable suspension agent

(stabiliser or coagulation inhibitor) and progress of the polymerisation reaction leads to gradual hardening of the droplets. At a certain stage (end of the sticky period), the hardened droplets will no longer coalesce. When reaction is complete the resin particles can be collected by filtration and traces of non-reacted monomer, initiator and other organic fragments removed by extraction. The particles are finally vacuum dried.[175] Water-insoluble and water-soluble monomer mixtures are the two empirically developed suspension polymerisation systems. Polymerisation in both systems is similar, however the process is easier in the case of water-soluble monomers because of the relative higher rates of polymerisation achieved in the system. In fact, increased rates shorten the duration of the sticky period minimising accidental coagulation. On the other hand suspension polymerisation of water-insoluble monomers is easier in the presence of higher concentrations of cross-linking monomers that lead to faster hardening of the droplets (shorter sticky period).

3.2 Radical induced suspension polymerisation: the chemistry

Free radicals are formed by homolytic bond cleavage with each fragment keeping a single electron.[176] The homolysis of the bond can occur by thermal or photochemical cleavage. The radical initiator used for the preparation of the PS and PS-PEG resins in this project was an azo compound: 2,2'-azobis(2-methylpropionitrile) (AIBN). When thermal fragmentation of the C-N bond present in this initiator occurs, methylpropionitrile radicals and nitrogen are generated (figure 3.1). Addition of the radical **(84)** to the double bond of the vinyl group generates radical **(85)**. This reaction is called initiation. Chain elongation (chain/ polymer propagation) occurs when the new free radical reacts further with styrene. When each end of the divinylbenzene monomer react, cross-linking occurs. Chemical functionality can be introduced into the polymer by incorporation of chloromethylstyrene. A chain termination event ends the polymerisation process when two radicals quench each other by reaction or by disproportionation. When the rate of termination exceeds the rate of initiation the polymerisation reaction slows down and stops.

The resins described in this project were synthesised using a radical initiated suspension polymerisation process. Merrifield (chloromethyl) and chloromethylated PS-PEG resins were synthesised by incorporating the functional monomer **(61)** (described in chapter 2) into the suspension co-polymerisation mixtures.

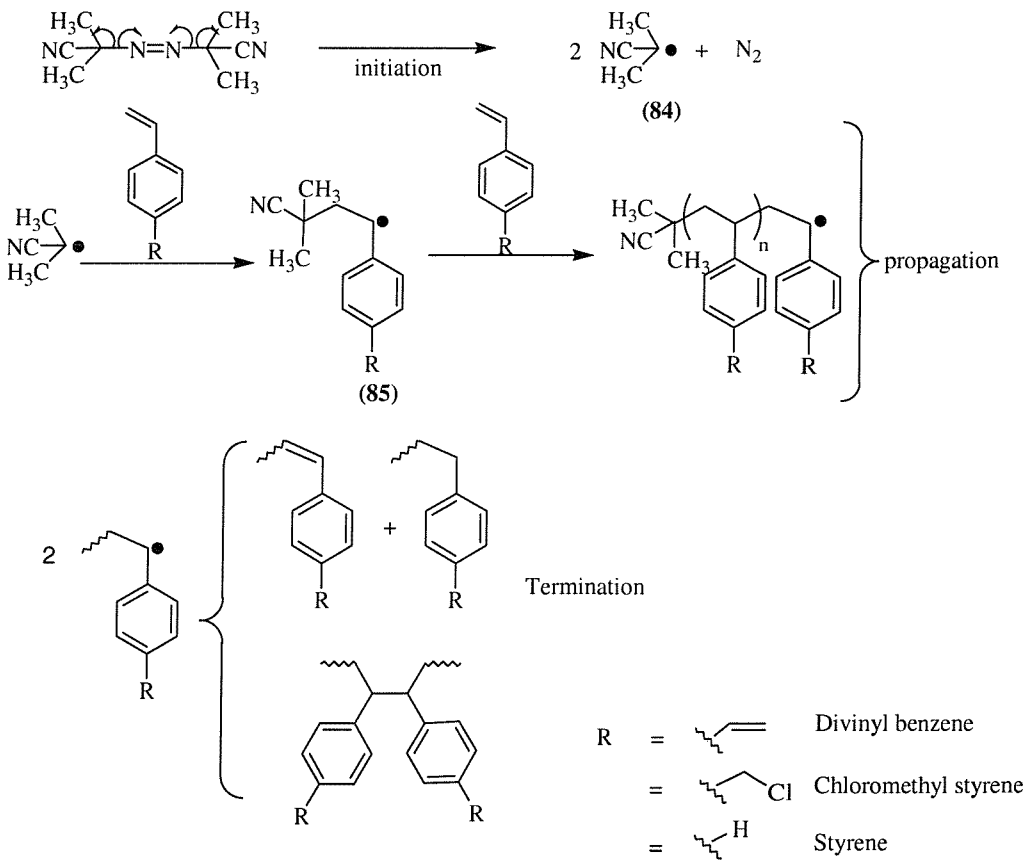


Figure 3.1 *Synthesis of polystyrene resin via radical induced suspension co-polymerisation.*

3.3 Suspension polymerisation reaction parameters

The chemical and physical parameters in suspension polymerisation reactions are of importance since they are very closely connected to each other. These parameters can in fact be adjusted in order to design and optimise the synthesis of special types of polymer materials. In first place the physical properties of the monomers (e.g. hydrophilicity/hydrophobicity and thermal stability) strongly affect the choice of parameters such as suspension medium, monomer diluent and temperature. Reactor and stirrer geometry, stirring speed, suspension agent, composition of organic phase, are all crucial parameters that need to be considered.

3.3.1 Suspension agent

When a polymerisation system (e.g. styrene/divinylbenzene/chloromethylstyrene) is insufficiently suspended, mass coagulation during the sticky period is very likely to occur. Minimising the surface tension of the droplets and the collision forces in the suspension is one solution to this problem. The latter depends on the apparatus design (magnitude and distribution of the stirring force) while the former is achieved by the addition of a water-soluble polymer to the aqueous phase that helps to stabilise the droplets produced in suspension. A large number of water-soluble stabilisers for suspension polymerisation reactions have been reported. The choice of stabiliser is usually an empirical one. Gelatin, polyvinyl alcohol (PVA), dodecyl benzene sodium sulfonate and tri-calcium phosphate, gum Arabic, polyvinyl pyrrolidone and cellulose ethers are all commercially available effective suspension agents. PVA is commonly used in industry since it is biodegradable and produced by hydrolysis of cheap polyvinyl acetate. It is available in varying molecular weight ranges with specific degrees of hydrolysis. Relatively small quantities of suspending agent might not be sufficient to stabilise the droplets during the equilibrium phase or more importantly during the sticky phase. Adding a large excess of suspending agent permits stabilisation of micro-droplets, but it is very difficult to remove from resin

beads due to low solubility. The amount of suspending agent required for a system should generally be a compromise between desired beads size and ease of removal after suspension polymerisation.

3.3.2 Reactor geometry

The symmetrical configuration of the apparatus used for polymerisation reactions has been found to be one of the most important factors for product homogeneity. The suspension polymerisation reactor in Figure 3.2 was described by Arshady and Ledwith[177] in 1983. In an extensive series of empirically modelled experiments involving vessels of varying geometry they found that homogeneous mixing of the entire content of the vessel was of critical importance for successful suspension polymerisation. It was therefore concluded that standard laboratory vessels and stirrers were not suitable for this purpose as the distribution of stirring forces was not uniform. Symmetry considerations indicated, however, that maximally homogeneous mixing could be achieved in a stirred cylindrical vessel. Sample withdrawal using this type of vessel provides a practical means of estimating both rates of polymerisation and polymer compositions at various stages of conversion. An additional important feature of this apparatus is associated with scaling up operations. The variable scale capacity of the system has important implication in the development of new polymer supports since it usually involves a large number of suspension polymerisations for evaluation and optimisation of the polymer. In general scaling up of suspension polymerisation is complicated due to the interrelated problems of mixing efficiency, stability of the suspension, particle size distribution, and reactor dimension and geometry.[178] Large-scale preparations often produce higher yields nevertheless the symmetrical configuration of this apparatus allowed the authors to obtain satisfactory yields and products of good quality even for small-scale (ca.5g) preparations. A 1987 European Patent application[179] described the use of a complicated arrangement of a cylindrical reactor with conical upper and lower portions with inlets at the top and bottom of the system, whilst 'goldfish bowls' and simple Morton flasks have also been

used to hold various suspension mixtures.[180] The use of a two-piece round bottomed cylindrical flask was reported by Erbay[181] (figure 3.3) in 1993 and represents a more sophisticated version of Arshady[177] and Ledwith's reaction vessel. Vessel geometry is therefore a significant parameter in the optimisation of a suspension polymerisation system and it is closely connected with other important parameters in the reaction.

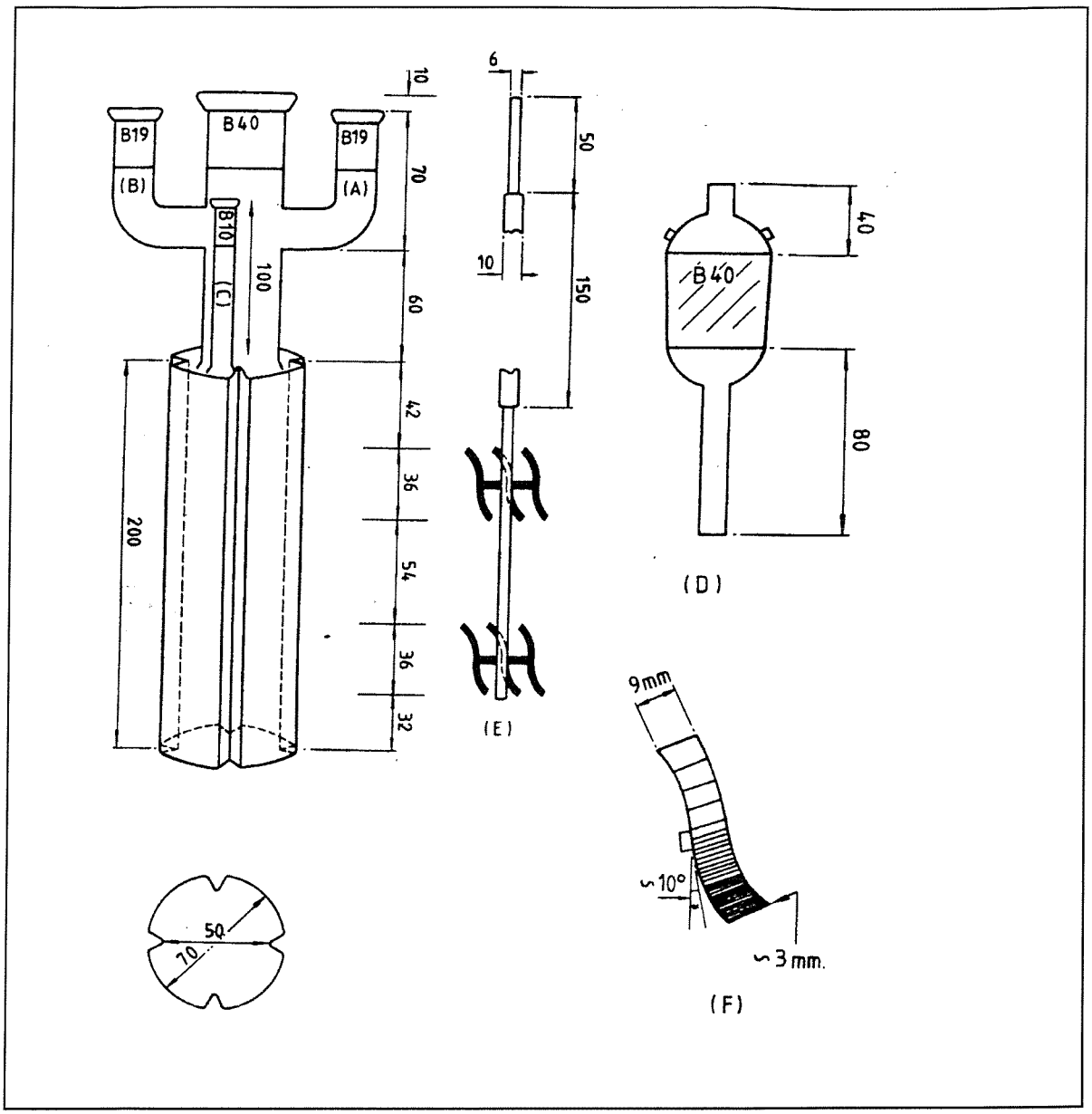


Figure 3.2 *Suspension polymerisation apparatus employed by Arshady (Reactive Polymers, 1, 1983, 159). A and B are access points for a reflux condenser and nitrogen inlet, C is a sampling arm, D is a stirrer guide, E is the stirrer with its position indicated for full scale operation. F is an expanded drawing of a single stirrer blade indicating the curvature at both ends and the position and angle of attachment to the stirrer rod. All dimensions are in mm.*

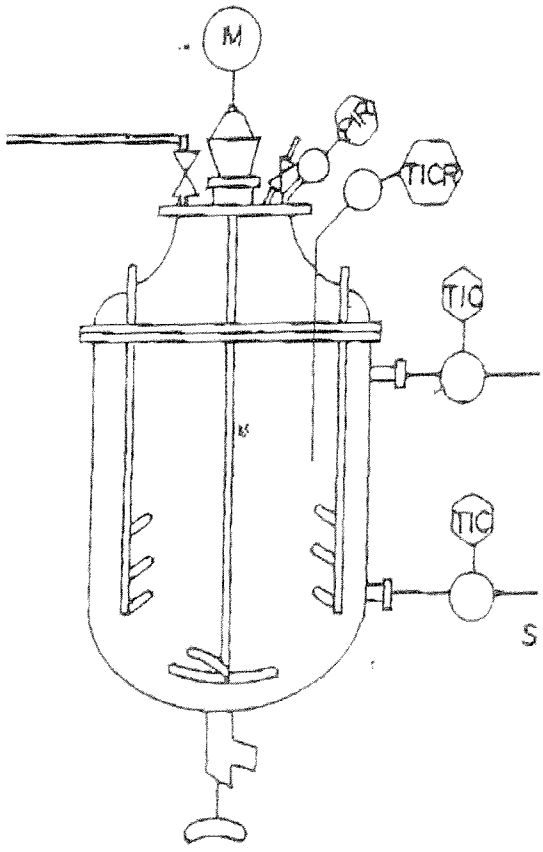


Figure 3.3 *2 piece round bottomed cylindrical flask employed during the suspension polymerisation reaction by Erbay.*[181]

3.3.3 Stirring speed and stirrer geometry

Among various factors influencing the particle size of droplets/beads in suspension, adjustment of the stirring speed is a convenient mean of controlling size distribution. The mean bead diameter can be reduced or increased by respectively increasing or reducing the speed of stirring. However there are limits within which particle size can be controlled by adjusting the stirring speed. These limits depend on the size and the configuration of the polymerisation reactor and the amount of suspension agent (a qualitative trend is

described in Figure 3.4). The slower the stirring of the suspension, and the larger the droplets, the more difficult it is to keep the droplets well suspended, whereas too vigorous stirring may exceed the tolerance of the whole apparatus. Moreover smaller droplets produced by fast stirring require correspondingly increased concentration of stabiliser. In the laboratory, careful adjustment of speed with a careful control of the suspending agent is the most practical way of achieving a narrow bead size distribution.[182]

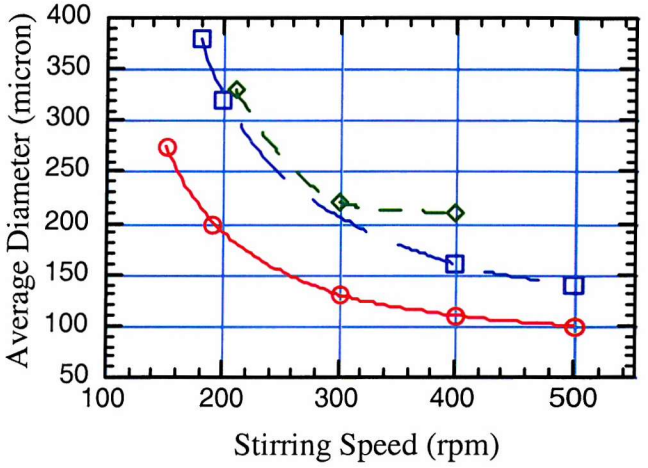
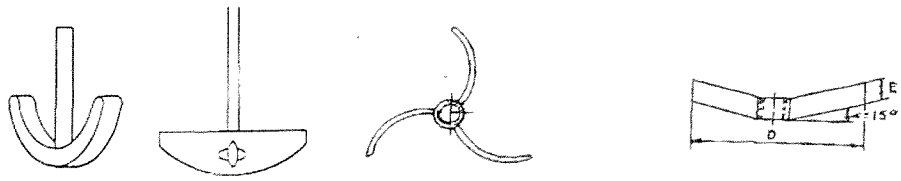


Figure 3.4 *The effect of stirring speed on size of beads produced by suspension polymerisation. Stabiliser: (green) 0.2%; (blue) 0.3%; (red) 0.4% (adapted from Ahmed, S. M. Dispersion Sci. Technol. 5, 1984, 421).*

The position of the stirrer in the suspension and its relationship to the vessel is an issue which has not been extensively reported in the literature nevertheless it can have an important effect upon the suspension. A typical effect of a stirring process is the generation of turbulent forces and local currents around the stirrer. These forces can be empirically evaluated by performing trial polymerisations. Also the size of the stirrer in proportion to the rest of the vessel can play an important role in producing a positive effect. Thus a compromise between speed/ height and shear-force must be achieved.

In suspension polymerisation droplets must be sufficiently suspended and well stirred in the aqueous medium without exerting too many deforming or coagulation forces on them. To this purpose, the shape and geometry of the stirrer used to create and maintain the suspension can be important. A wide range of stirrers can be used to maintain suspensions. Traditional designs supplied by the commercial laboratory suppliers (stirrers a) and b) in figure 3.5) are very widely used in optimised polymerisation experiments. Alternatively, Erbay reported the use of a three blade curved propeller c) in Figure 3.5).



a) b) c)

Figure 3.5 *Traditional designs available stirrers from the commercial laboratory suppliers (figures a) and b) respectively); Erbay's three blade curved propeller (figure c).*

According to the specific features required for the polymeric support, any of these stirrers may be chosen to achieve good beaded products. The materials used in the manufacture of stirrers can be glass, Teflon coated steel or steel. Practically, glass stirrers are prone to shattering on vibration and collision. In metallic stirrers the metal surface can act as a radical quencher during the polymerisation and this can be a major drawback.[183] Teflon coated stirrers do not suffer from this drawback although they are more expensive.

3.3.4 Organic phase composition

Functional groups can be introduced into the polymer either *via* post-polymerisation functionalisation of pre-formed polymers or by co-polymerisation of styrene with a

number of cross-linking or functional monomers (some examples are shown in Figure 3.6). Post-polymerisation functionalisation is very often accompanied by a number of problems. The loading of the resins is very difficult to control accurately, characterisation of the structural changes which occur is difficult, not all the reagents easily penetrate into the polymer network and furthermore, possible side-reactions occurring following this approach may lead to undesirable polymers characteristics.[174a]

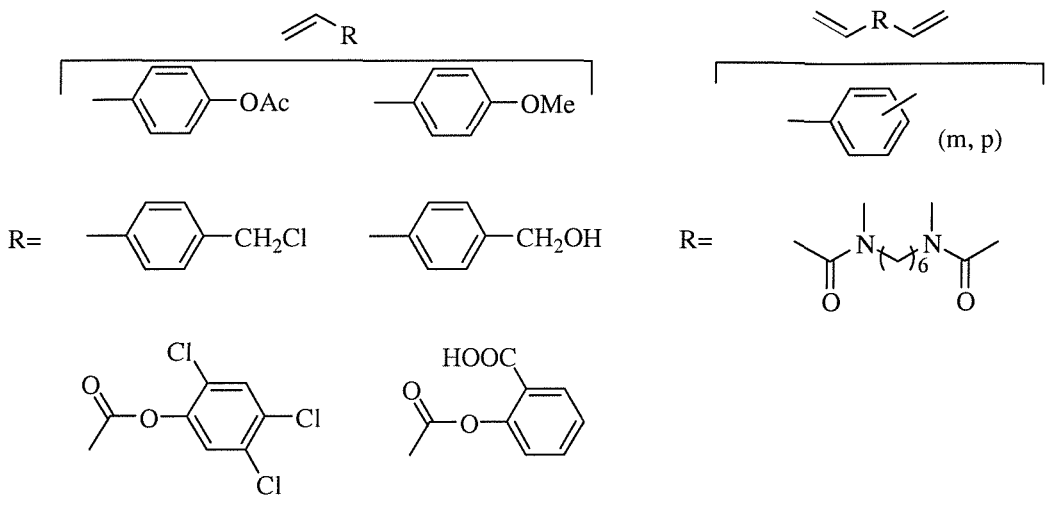
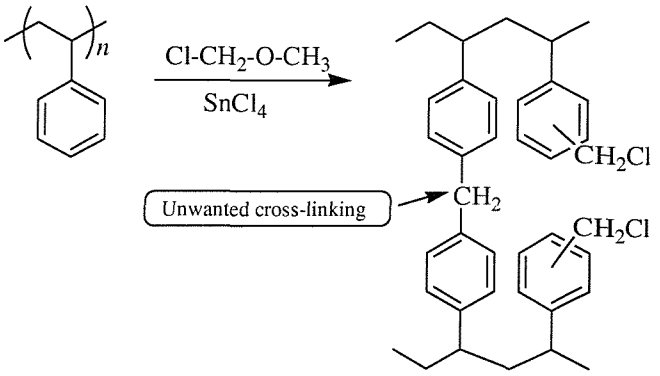


Figure 3.6 *A few examples of mono- and di- vinyl functionalised monomers.*

Nevertheless, chloromethylation of polystyrene[184] *via* Friedel-Craft alkylation using chloromethyl methylether is a very useful and versatile route to functionalised polymers allowing a wide range of loadings to be achieved. The main drawback of this method is that it suffers from intra-resin side-reactions which introduce additional cross-linking with a consequent reduction in the accessibility of reaction sites (Scheme 3.1). This occurs by initial chloromethylation and/or inter-polymeric electrophilic aromatic substitution to give higher cross-linked products. This side-reaction is known as methylene bridging and becomes more significant with high degrees of chloromethylation.



Scheme 3.1 *Friedel-Craft alkylation of polystyrene. Preparation of chloromethyl resin* [185] *and cross-linking.*

A second reported side reaction is the presence of hydroxymethyl groups in some examples of chloromethylated polystyrene,[186] due to hydrolysis of the chloromethyl residues.

Suspension co-polymerisation provides a very efficient tool for the synthesis of functionalised polymer supports since properties of the polymer particles such as functional group loading can be readily controlled by varying the composition of the organic phase. On the other hand, the technical requirements of the suspension polymerisation and preparation of the respective functional monomers can be complicated in some cases, which might appear to make post-polymerisation functionalisation more convenient.[187]

3.3.5 Organic monomer diluent

For beaded co-polymer supports obtained by suspension polymerisation the three-dimensional structure of the polymer network takes shape according to the conditions used during the formation of the resin beads. The nature and percentage of monomer diluent is also found to have a very strong influence on the polymer network. Arhady and Ledwith[177] reported that for a given degree of cross-linking, the swelling factors are

dependent on the nature of the monomer diluent used during matrix formation. In general, the higher the percentage of a good monomer diluent, the larger the swollen volume of the resin will be. As a result the swelling of a polymer can be maintained at a relatively constant level by simultaneously increasing both the degree of polymer cross-linking and percentage of polymer diluent.

The size distribution of the polymer particles is largely influenced by factors such as initial monomer viscosity and rate of polymerisation. The presence of a good solvent in the monomer mixture is believed to increase the stability of the suspension system by reducing the overall viscosity during the sticky period. Suspension polymerisation of water-insoluble monomers is considerably easier in the presence of proportionally higher concentrations of cross-linking monomers and/or good solvents.[177] The chemical performance of the polymer can then be improved by modest changes of both cross-linking percentage of monomers and the amount of a good solvent in conjunction.

3.3.6 Other parameters

The ratio of the organic phase/aqueous phase is another important parameter in the nature of the equilibrium of the organic phase droplets. A low ratio gives very small beads as a result of the organic phase distribution throughout the aqueous phase. When the ratio is very high, the equilibrium becomes more rapid as there is less space between the droplets. Thus, when the suspension enters the sticky phase it is not sufficiently stable, and as a consequence larger non-bead type particles and aggregated materials form.

The overall yield of the reaction is generally reduced if high concentrations of initiator are used. In this case, in fact the synthesis of a greater number of lower molecular weight polymers takes place and the polymeric chains are not sufficiently cross-linked to give insoluble beads. The suspension polymerisation reaction is also dependent on traditional parameters, which influence chemical reactions. Stability of suspensions is generally influenced by temperature chosen in relation to specific sets of experimental conditions.

Gradually increasing the temperature of a reaction causes an increase in reaction rate. The yield of polymerisation also increases[188] when the reaction time is increased.

3.4 Results and Discussion

3.4.1 Synthesis of resins via multiple-parallel suspension polymerisation

Traditionally, polymer supports have been made by suspension polymerisation. Optimisation of suspension polymerisation conditions is often very time-consuming, an issue which becomes important when research on new polymerisation systems is carried out. Moreover, as discussed in the previous sections, suspension polymerisation process parameters can significantly influence particle size and distribution. Therefore, in most polymerisation systems carried out on a bench-scale (as in our case), particular attention has to be paid to these parameters and their reproducibility. The novel instrument, designed in our laboratories (Figure 3.7), is an example of a multiple-parallel synthesis system which we have successfully used in suspension polymerisation reactions and represents a valid alternative to single polymerisation reactions. This methodology facilitates the systematic study of the correlation between polymer structure, properties and performance. The new system offers the possibility of changing several parameters of a polymerisation recipe, e.g. the composition of organic and aqueous phases but with constant vessel, and agitation rate. This equipment facilitates the optimisation of both specific polymer compositions for particular applications as well as the suspension polymerisation conditions (initiator, temperature, colloid stabilisers, agitator design). The apparatus shown in Figure 3.7 comprises a stand to support 6 glass reaction vessels counterpoised on a vertical column; the system is free to move around the column and up and down by 120mm and designed to be mounted in a standard water bath. At the top of the system is a low voltage brushless DC motor, which drives the six stirrers through a gearbox and a multishaft belt coupled to six stirrers in the reactions vessels. Motor speed is fully adjustable without the risk of any significant reduction in speed as the load is

increased. Further details about the parallel polymerisation system are given in the appendix of this thesis.

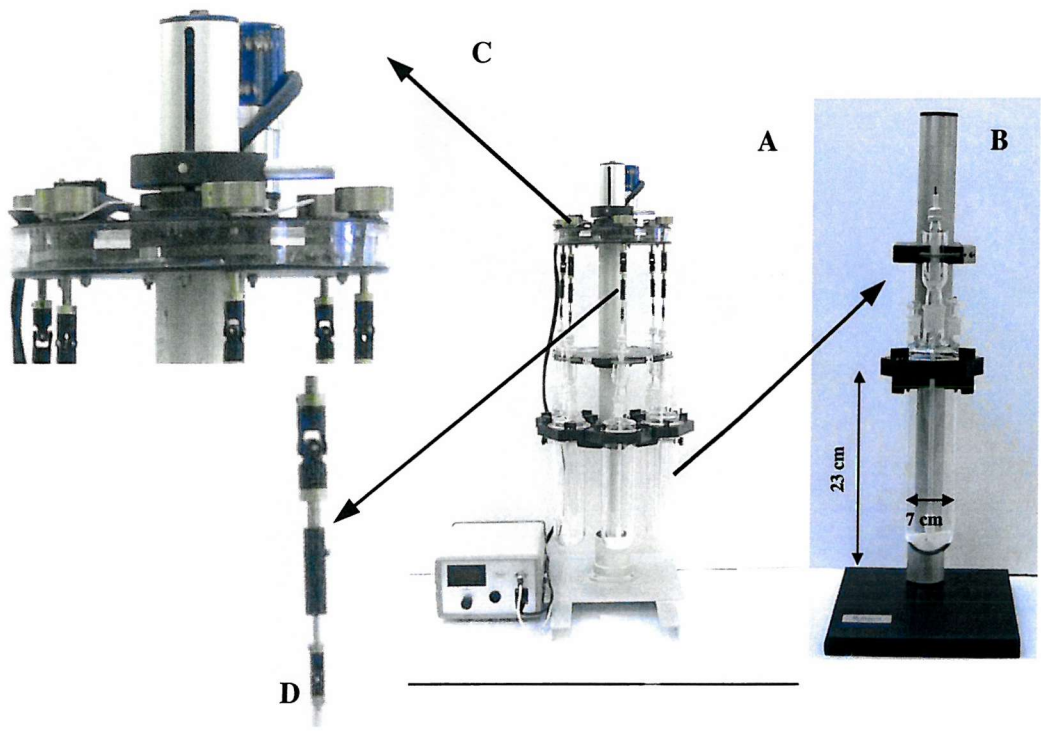


Figure 3.7 *(a) Multiple-Parallel Polymerisation System showing, (b) Multiple-Parallel Polymerisation 500mL reaction vessel, with half-moon stirrer, (c) Magnified view of stirring motor assembly and drive-belt system, (d) Magnified view of movable joint connector to join driving spindle to stirrer bar.*

3.5 Preliminary studies on the effects of cross-linking and monomer diluent on resin bead size distribution

As mentioned in section 3.3.5, suspension polymerisation of water-insoluble monomers is easier in the presence of proportionally higher concentrations of cross-linking monomers and/or good solvents. We tested the apparatus described in section 3.4.1 performing the multiple-parallel synthesis of a set of chloromethyl polystyrene resins with different DVB cross-linking percentages (from 1mol% to 15mol%), ratio (v/v) organic monomers / diluent and aqueous phase compositions. The particle size distribution was then studied in relation to the variation of the first two parameters in the polymerisation process. A series of fourteen polymerisation reactions on a 20g scale were carried out and worked-up simultaneously. Table 3.1 shows the results of the first three batches of resin.

Table 3.1 *Size distribution of 1% to 15% DVB chloromethyl PS resins. Full composition of the suspension polymerisation organic phase is reported in Table 7.6, Chapter 7.*

Entry	ratio*	Size distribution/ μm (%)							Yield** (%)
		DVB (%)	>500	500-355	355-250	250-125	125-75	75-45	
1A	2/1	1	56	21	10	11	1	1	98
2A	2/1	2	6	23	26	40	4	1	85
3A	2/1	6	7	30	6	56	1	/	87
4A	2/1	8	49	27	2	22	/	/	99
5A	2/1	15	62	21	4	12	/	/	97
1B	1/1	1	79	11	5	5	/	/	67
2B	1/1	2	68	7	3	12	5	5	56
3B	4/1	2	86	9	3	2	/	/	68
4B	1/1	15	29	20	15	31	3	2	78
1C	5/1	1	12	25	38	23	1	1	84
2C	4/1	3	34	31	23	10	1	1	93
3C	2/1	3	43	22	20	14	1	/	87
4C	3/1	2	22	21	26	27	2	2	85
5C	5/1	2	37	19	27	14	2	1	93

*Ratio (v/v) between organic monomers and diluent (toluene); ** Yield of beads after sieving..

These studies were carried out varying one polymerisation parameter while keeping the second one fixed. The diagram in Figure 3.8 shows the bead size distribution percentages (measured by sieving the resin beads, see section 7.4.1 for experimental details) of a set of three 1% DVB cross-linked resins prepared using three different percentages of monomer diluent. Entries 1A and 1B, having ratio (v/v) organic monomers/diluent 2/1 and 1/1 respectively, afforded a very high percentage of beads in the size range of > 500μm and 500-355μm and a poor percentage in the other size ranges. Size distribution data relative to resin 1C, having a ratio (v/v) of 5/1 shows that low DVB cross-linked resins require lower percentages of diluent to give a regular size distribution. The diagram in Figure 3.9 shows again the size distribution of resin beads when the DVB cross-linking percentage was increased to 2% and the organic monomers/ diluent ratios varied from 5/1 to 1/1. Entries 2B and 3B (ratio (v/v) 1/1 and 4/1 respectively) afforded a very irregular size distribution; entries 4C and 5C (ratio (v/v) 3/1 and 5/1) afforded a reasonable but flat size distribution. No systematic trend in the size distribution of the 2% DVB resins was observed, nevertheless these results showed that ratio 2/1 relative to the resin 2A was the most suitable to afford the highest percentage of beads (40%) in the desired size range (250-75μm).

Figure 3.10 shows the size distribution trend when the ratio was fixed at a value of 2/1 and the DVB cross-linking percentage was varied from 1% to 15%. Also in this case the trend in size distribution was not systematic. Entries 1A, 4A and 5A (cross-linking 1%, 8% and 15% respectively) did not afford a regular size distribution presumably because the ratio 2/1 is too high for a 1% DVB cross-linked resin and too low for 8% and 15%. Interestingly, entry 3A (6% DVB cross-linked resin) showed a distribution rich in beads in the size range of 250-75μm. Finally resins 2C and 3C (3% DVB, see Table 3.1) both afforded a quite similar distribution (rich in beads of large size) in spite of the different ratios used (4/1 and 2/1 respectively).

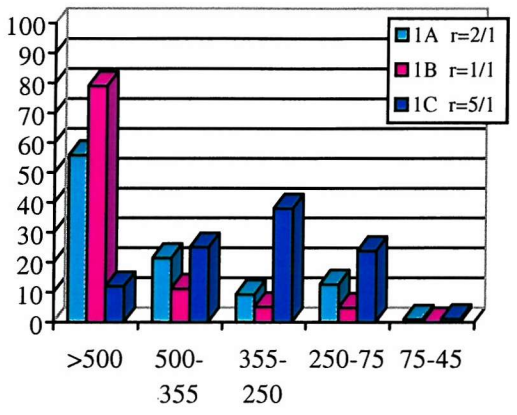


Figure 3.8 *Size distribution (%) of 1% DVB cross-linked chloromethyl PS resins prepared using different ratios (v/v) of organic monomers/ diluent.*

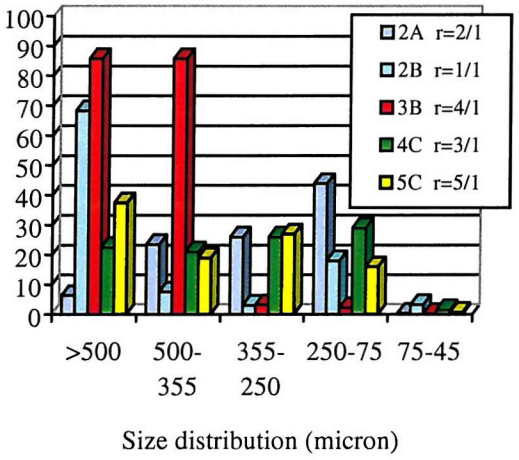


Figure 3.9 *Size distribution (%) of 2% DVB cross-linked chloromethyl PS resins prepared using different ratios (v/v) of organic monomers /diluent.*

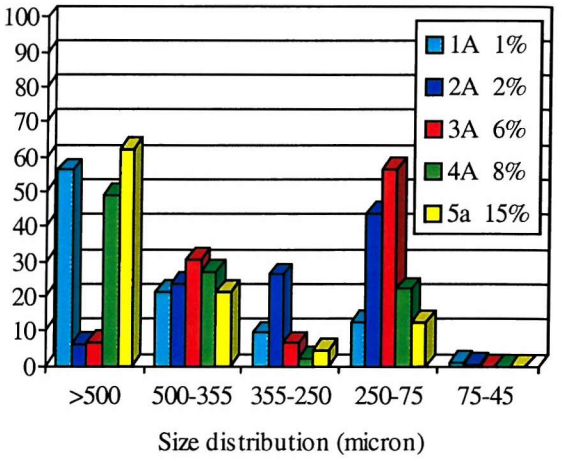


Figure 3.10 *Size distribution (%) of 1% to 15% DVB cross-linked chloromethyl PS resins prepared using a 2/1 ratio (v/v) of organic monomers/diluent.*

3.6 The reproducibility of the multiple-parallel polymerisation system

The reproducibility of conditions and corresponding results for each of the six reaction vessels was tested by synthesising a set of polystyrene resins with 2% DVB cross-linking in parallel. Suspension polymerisation reactions on a 20g to 40g scale of organic monomers (500mL vessels) were conducted and worked-up simultaneously. Results of the first batch of six 2% DVB cross-linked PS resins prepared in parallel are reported in Figure 3.11, showing the reproducibility of the system in terms of bead size distribution and yields (see figure 3.14 and Chapter 7 for details of yields).

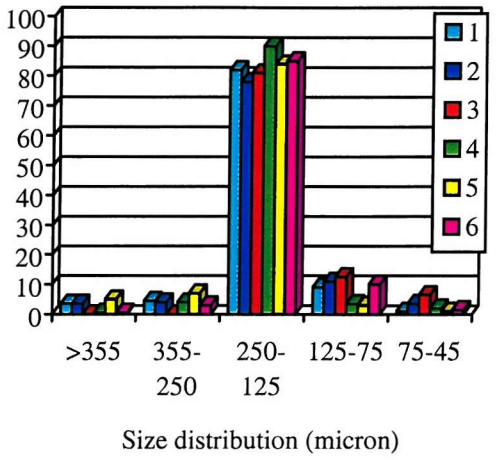


Figure 3.11 *Reproducibility of size distribution percentage of 2% DVB-PS resins prepared with comonomer/diluent ratio =2/1.*

Once the reproducibility of the new system had been tested, two additional batches of resins were prepared: one without and one with chloromethylstyrene. In the preparation of these resins the level of DVB, ratios (v/v) of organic monomers/diluent and the loading of the chloromethyl group were varied across the whole set. For the preparation of these resins (see Table 3.2) the choice of the ratio to use was generally based on results obtained from the preliminary studies discussed in section 3.5. A comparison between Table 3.1 and Table 3.2 nevertheless shows two exceptions: entries 7 and 14 of Table 3.2. For the 1% DVB chloromethyl resin (entry 7) a 3/1 ratio of organic monomers/diluent was used and the resulting size distribution was as good as the one obtained using a 5/1 ratio (Table 3.1). A narrow size distribution was also obtained for the 2% DVB chloromethyl resin (entry 14) in spite of the higher ratio of organic monomers/diluent used (3/1 instead of 2/1). The size distributions of the whole set of resins are given in figures 3.12 and 3.13. Narrow bead size distributions were produced with variable DVB levels (Figure 3.12). A clear trend of enhanced yields (Tables 7.9 and 7.10, Chapter 7) with increased percentage cross-linking and a good size distribution were obtained with this polymerisation system across the whole array.

When chloromethylstyrene was included in the polymerisation system to prepare resins with loading of 1 or 2 mmol/g an unexpected difference in the surface appearance was observed. This effect was observed even carrying out a series of polymerisation reactions under different combinations of conditions such as different percentages of PVA in the suspension, variation of agitation speed and stirring agitator geometry.

It has been reported that the presence of chloromethylstyrene dramatically increases the acidity of the suspension during the polymerisation process, possibly because of the hydrolysis of chloromethylstyrene in PVA. The increase in acidity was found to affect the surface appearance of the beads.[189] Following these considerations, the pH of the suspension polymerisations was controlled, performing the reaction in the presence of dibasic sodium phosphate as a buffer. The nature of the beaded product was monitored using an optical microscope and the beads were found to be visually perfect displaying a very smooth surface as shown in Figure 3.15.

Table 3.2 *Composition of 1% to 15% DVB PS and DVB chloromethyl PS resins.*

Entry	DVB (%)	ratio* (v/v)	Entry	DVB (%)	loading (mmol/g)	ratio* (v/v)
7	1	3/1	**13**	1	1	3/1
8	3	2/1	**14**	2	1	3/1
9	4	2/1	**15**	2	2	2/1
10	8	1/1	**16**	3	2	2/1
11	10	1/1	**17**	4	2	2/1
12	15	1/1	**18**	8	1	1/1

* Volume ratio between organic monomers /diluent.

Excellent size distribution and yields were obtained while varying DVB ratios and incorporating chloromethylstyrene (see Figure 3.13 and Figure 3.14) once the system was buffered.

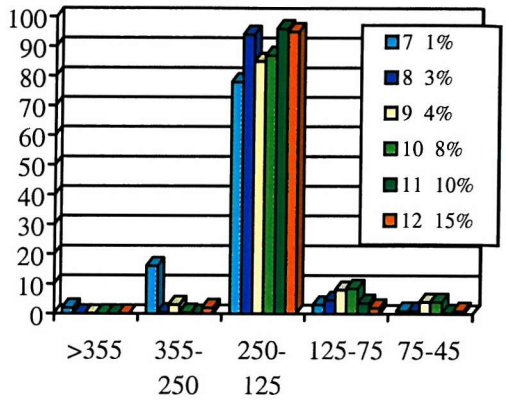


Figure 3.12 *Size distribution (%) of 1 % to 15% DVB PS resins. DVB cross-linking percentages of the resins are given in the diagram legend. Detailed compositions of entry 7 to 12 are given in Table 3.2 and Table 7.8 in Chapter 7.*

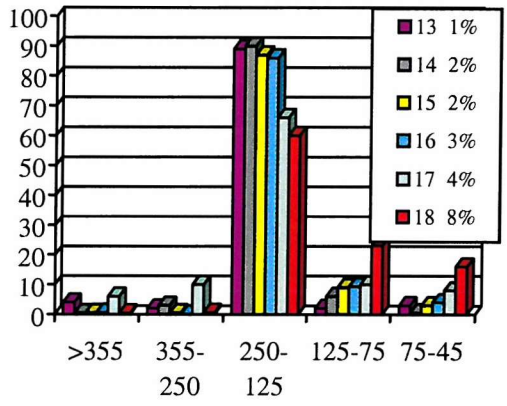


Figure 3.13 *Size distribution (%) of 1 % to 8% DVB PS chloromethylated resins. DVB cross-linking percentages of the resins are given in the diagram legend. Detailed compositions of entry 13 to 18 are given in Table 3.2 and Table 7.11 in Chapter 7.*

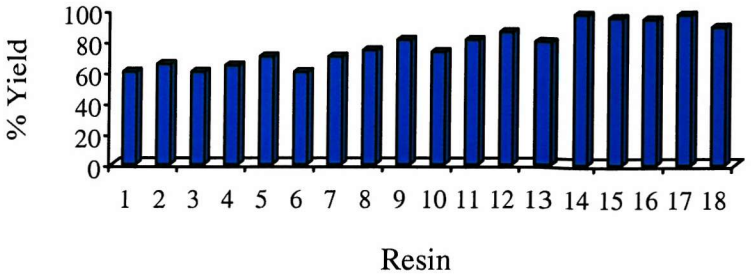


Figure 3.14 *Yields of resin (entry 1 to 18 of Table 3.2) having DVB cross-linking percentages from 1% to 15%.*

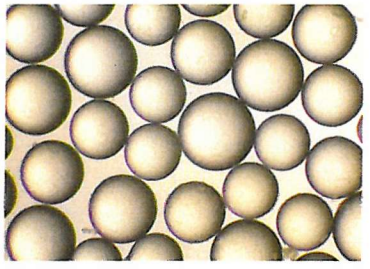


Figure 3.15 *Example of 1% DVB PS resin beads (size: 250-125μm) in DMF*

3.7 Conclusions

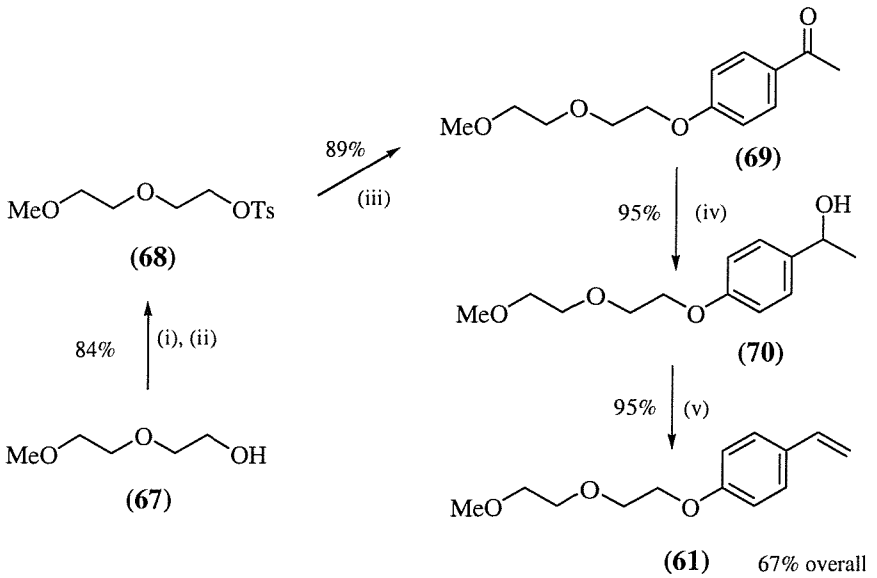
In conclusion the results clearly illustrate that a multiple-parallel approach is a valid method for suspension polymerisation and represents a powerful tool to both rapidly design new bead supports and/or optimise polymerisation conditions. Considering the time frame and problematic consistency associated with suspension polymerisation this opens up the path to parallel bead production thus allowing unique new polymer resins to be synthesised and evaluated.

CHAPTER 4

Preparation of PS-PEG resins via suspension polymerisation

4.1 Introduction

The preparation and characterisation of a series of resins containing the styryl poly(ethylene glycol) (PEG) derivative **(61)** will be discussed in this chapter. These resins were designed to balance swelling and solvation with improved handling issues. The PEG moiety was introduced *via* suspension polymerisation rather than by grafting. 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene **(61)** was prepared as shown in Scheme 4.1, following a procedure for the preparation of α,ω-bis(*p*-vinylphenyl)oligo(oxyethylenes) described by Inokuma.[161] Scavenging using Amberlite IRA-96 resin was carried out to aid purification. Monomer **(61)** was obtained following the sequence of tosylation of diethylene glycol monomethyl ether, Williamson ether formation with *p*-hydroxyacetophenone, reduction and dehydration in an overall yield of 67% (as detailed in Chapter 2).



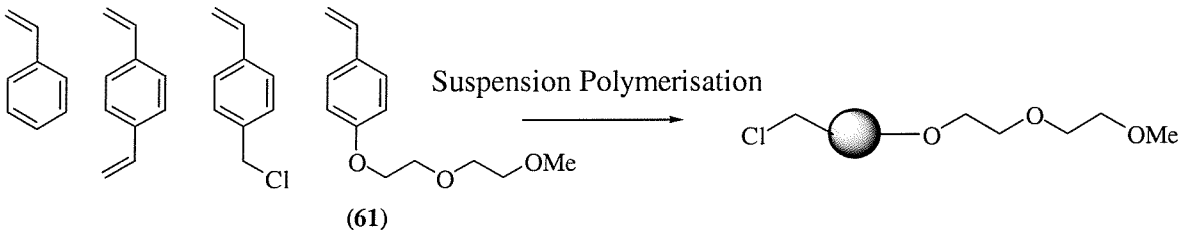
(i) TsCl (1.5 equiv.), NEt_3 (1.5 equiv.), DMAP (cat.), DCM; (ii) Amberlite IRA 96 (Scavenging); (iii) p-hydroxy-acetophenone (1.2 equiv.), K_2CO_3 (1.2 equiv.), CH_3CN, reflux; (iv) $NaBH_4$ (1 equiv.), THF/EtOH 1/1; (v) Pyridinium Tosylate (cat.), toluene, reflux.

Scheme 4.1 *Synthesis of styryl poly(ethylene glycol) (PEG) derivative* ***(61)****.*

The monomer, 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene **(61)**, contained an inert phenyl ether linkage designed to provide wide chemical compatibility and stability, yet imparting all the favourable properties of the PEG group into the new resin, and still maintaining a high loading capacity. The physico-chemical properties of the new resins were compared to those of TentaGel™, ArgoGel™ and aminomethyl PS.

4.2 Results and Discussion

The PS-PEG resins were prepared by incorporating monomer **(61)** into a suspension co-polymerisation of styrene, divinylbenzene (DVB) and chloromethyl styrene, following the procedure described in Chapter 3 (Scheme 4.2).



(61)

Scheme 4.2 *Synthesis of PEG-PS resin containing monomer (61).*

In order to look for the optimal conditions to obtain cross-linked resins having a broad solvent compatibility a series of resins with different compositions (variable percentages of **(61)** and DVB) were synthesised in the first place using a traditional "single-resin preparation" method. A multiple parallel approach for the preparation of the PS-PEG resins was then successively developed and applied.

4.3 PEG-PS resins containing 12% of 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene (61)

The first set of resins was prepared using 12% of **(61)** while varying the percentage of DVB. Initially a highly cross-linked system (8% DVB) was chosen in order to investigate the reactivity of **(61)** towards polymerisation and the ability to obtain resin beads. Two different sets of polymerisation conditions were used in order to investigate the most suitable polymerisation parameters. The first polymerisation was performed at 65°C, stirring at 380 rpm with an aqueous phase of 5% Na_2SO_4 and 0.75% of a suspension agent PVA (polyvinyl alcohol). The second polymerisation was performed at higher temperature (85°C) with a stirring rate of 360 rpm and an aqueous phase containing higher percentages of Na_2SO_4 (10%) and PVA (1.5%). In both cases the monomers were dissolved in 40mL of toluene (ratio in volume 1/1). Figure 4.1 shows the general size, shape and uniformity of the resin beads **(R1)** produced under the first set of conditions. The beads surface appeared smooth and the shape was regular. The resin was obtained in 36% yield and 60% of the beads had size between 250 and 125μm.

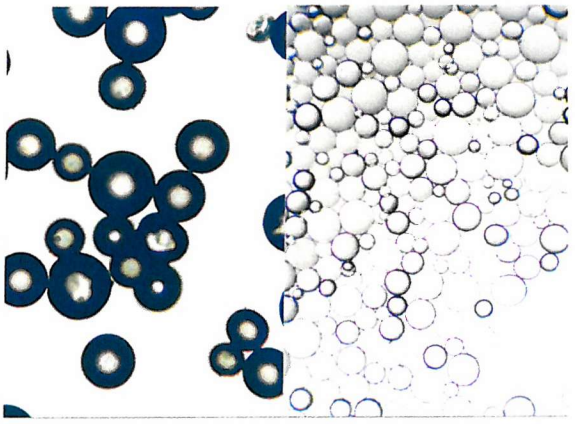


Figure 4.1 *Resin **R1**. 125-45 μm dry (left) and 125-45 μm swollen beads (right).*

The quality of resin beads **(R2)** resulting from the polymerisation under the second set of conditions was also good (shape and uniformity) with a yield of 60%. The percentage of monomer **(61)** incorporated into the resin beads, indicated from the percentage of oxygen

present in the resins, was calculated using elemental analysis. Based on the fact that there were no nitrogen atoms in any of the comonomers, the percentage of oxygen was the remaining having subtracted the percentages found for carbon, hydrogen and chlorine in the sample. Elemental analysis showed that the percentage of monomer **(61)** incorporated into the beads was higher for the resin obtained under the second set of conditions, a possible reason being the higher polymerisation temperature. The stability of suspensions and reaction rate can be in fact favourably influenced by an increase of the reaction temperature. The most suitable polymerisation parameters were those used for the second polymerisation (temperature 85°C, stirring rate of 360 rpm, aqueous phase containing 10% of Na_2SO_4 and 1.5% of PVA, comonomers/toluene ratio in volume = 1/1), and therefore they were used as a general method in the preparation of resins **(R1)**-**(R7)**.

A second batch of PS-PEG resins was synthesised, this time having cross-linking percentages of 3%, 2% and 1% but keeping the percentage of monomer at 12%. For the 3% DVB PS-PEG resin **(R3)** stirring problems occurred during the polymerisation causing a very low yield and a heterogeneous size distribution. Figure 4.2 shows that the beads surface was not smooth for the 3% DVB cross-linked resin. However, the best quality beads with sizes between 125 and 250 μm were selected by sieving and used to assess bead properties.

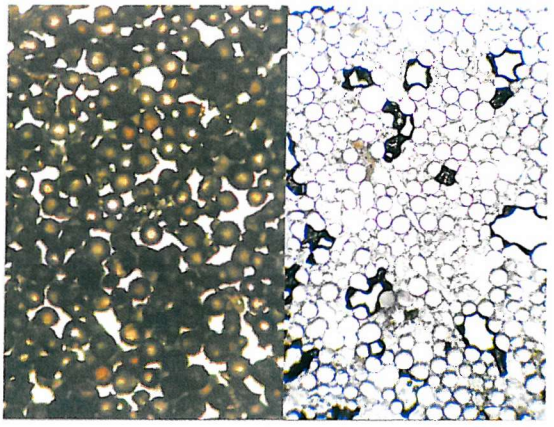


Figure 4.2. *Resin **R3**. 75-45μm dry (left) and swollen (right) beads.*

A 2% cross-linked set of beads was prepared (**R4**). This resin differed from resin (**R3**) in that the stirring rate (400 rpm) was increased to address the aggregation problem that occurred in the preparation of the 3% cross-linked resin. In spite of the enhanced stirring rate, the quality of the resulting beads was not good and traces of non-beaded polymer seemed to be present. Size distribution was not regular.

Attempts to prepare 1% DVB PS-PEG resins were not successful. Polymerisation under the same set of conditions as for the more highly cross-linked analogues failed to give resin beads. A very sticky polymer with a few beads inside was obtained. Removal of PVA and the sticky polymer was not possible in spite of washings with hot water, methanol or THF. Extractions in a Soxhlet with MeOH, EtOH/water and THF gave the same result.

4.4 Chloromethyl functionalised PS-PEG resins containing 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene (61)

As a result of the previous investigations, good quality beads were obtained for the more highly cross-linked system (8% DVB) containing 12% of (**61**). As soon as the cross-linking percentages of the resins were lowered to 3%, 2% or 1%, the polymeric system afforded lower quality beads or non-beaded polymerised material only. For this reason a batch of resins containing a lower percentage of the PEG monomer (6%) was prepared, while varying the level of DVB cross-linking and co-polymerising chloromethylstyrene along with the three monomers. To this end 8% and 3% cross-linked chloromethyl functionalised resins were prepared.

In order to have a reference resin to assess the effects of chloromethyl styrene inclusion into the polymerisation system containing a lower percentage of the monomethoxy-diethoxy styrene derivative (61), a non-functionalised resin was prepared. For resin (R5) the percentage of (61) was fixed at 6% and DVB at 3%. The yield was 73% but unfortunately size distribution was not good with most beads between 500 and 355 μm. Figure 4.3 shows resin (R5): size was fairly regular and the surface quite smooth.

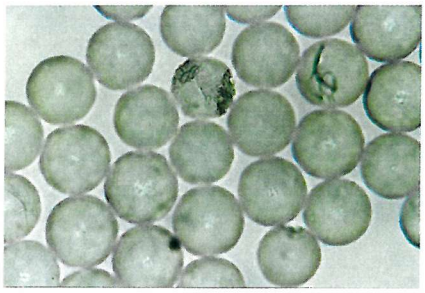


Figure 4.3. *Resin **R5**. 250-125 μm dry beads.*

3% DVB cross-linked chloromethyl functionalized resin **(R6)** was prepared by co-polymerisation of **(61)** and chloromethylstyrene with styrene and DVB to give a loading of 1 mmol/g. The polymerisation was carried out at 65°C while stirring at 470 rpm. The yield of beads was 90% but only 20% of the beads were between 250 and 125μm. Figure 4.4 shows the beads obtained: the size was fairly regular but the surface was not smooth. A possible explanation for the small particles surrounding the beads' surface is that the polymeric system was not sufficiently stabilised.

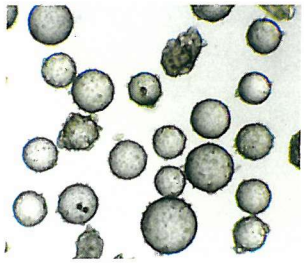


Figure 4.4. *Resin **R6**. 250-150μm swollen beads.*

8% DVB cross-linked chloromethyl functionalised resin **(R7)** was prepared using the same aqueous phase composition as above and a stirring rate of 490 rpm. In this case the yield (83%) and size distribution were good (64% of beads between 250 and 125μm). The surface of the 8% DVB cross-linked beads was very smooth and shape was regular as shown in Figure 4.5.

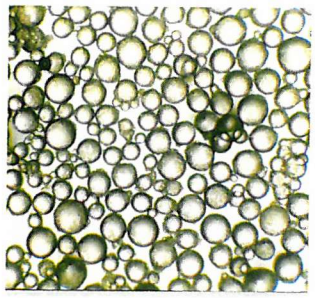


Figure 4.5. *Resin **R7**. 250-150μm swollen beads.*

These studies confirmed the tendency, under the investigated polymerisation conditions, of the polymeric system to afford good quality beads only for more highly cross-linked resins in spite of the decreased percentage of PEG monomer **(61)** used and the different compositions of aqueous phase employed for the suspension polymerisation. These results led us to consider the investigation of other possible polymerisation parameters to optimise in order to find out the suitable conditions for the preparation of more lightly cross-linked resins.

Table 4.1 *Composition of pre-mixed organic phase in suspension polymerization series of DVB PEG-PS resins containing monomer **(61)**.*

Resin	DVB (%)	Monomer[a] (61) (mmol/g)	CMS (mmol/g)	Loading of Monomer (61)[b] (mmol/g)
R1	8	0.99	/	0.57
R2	8	0.99	/	0.98
R3	3	0.99	/	N/A[c]
R4	2	0.99	/	N/A[c]
R5	3	0.50	/	N/A[c]
R6	3	0.45	1	N/A[c]
R7	8	0.45	1	N/A[c]

[a] Feed percentage of monomer **(61)**; [b]Loading calculated by elemental analysis; [c] The loadings of monomer **(61)** and/or of chlorine were not experimentally determined for these resins since it was not retained of major interest for the purposes of those particular studies.

4.5 Chloromethyl functionalised PS-PEG resins via multiple-parallel polymerisation

As discussed in Chapter 3 optimisation of suspension polymerisation reactions are often very time-consuming. In order to study the relation between physico-chemical properties and molar percentage of incorporated monomer in the new resins, the first objective was to prepare a batch of six resins (1% and 2% DVB cross-linked) containing 7 %, 14%, 24% and 43% of monomer **(61)**. A parallel approach using the multiple-parallel polymerisation system was chosen to allow the resins to be synthesised in parallel in a very short time (two days). The resins were prepared using the multiple-parallel polymerisation reactor designed in our laboratories.[190] Various mole fractions (from 7% to 43%) of monomer **(61)** were incorporated, by suspension copolymerisation [181,191] into the resin beads along with styrene, 4-chloromethylstyrene (1mmol/g) and DVB as described in Scheme 4.2.

4.6 2% DVB chloromethyl functionalised resins containing from 7% to 43% of 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene (61)

The influence of the ratio (v/v) between monomers and diluent on the quality and size distribution of polystyrene resin beads has been discussed in Chapter 3. This parameter was also considered relevant for the optimisations of the PEG-PS resins under study here. In order to look for the most convenient ratio (v/v) between organic phase (monomers) and diluent (toluene) to use in the suspension polymerisation of this polymeric system, the polymerisation reactions were performed using two different ratios (Table 4.2) to give two resins shown in Figure 4.6.

Table 4.2 *Ratio (v/v) of organic monomers/toluene used for the preparation of resins **R8** and **R9**.*

Resin	Org.Phase /Toluene (v/v)	Yield (%)
R8	1:1	64
R9	2:1	66

Resin **(R9)** afforded better quality beads and slightly higher yield than resin **(R8)**. Beads on the right hand side of figure 4.6 showed a good shape and uniformity in contrast to the beads on the left hand side which were quite rough and included some polymerised monomer not incorporated into the resin. This result indicated that the volume of solvent used affected the formation of the resin beads and that the ratio 2:1 was the best to prepare the 2% DVB cross-linked resins of this type. The percentage of monomer incorporated into the resin beads (0.68 mmol/g) and the loading (1 mmol Cl/g) of resin **(R9)** were both calculated by elemental analysis. 70% of beads were between 250-125μm.

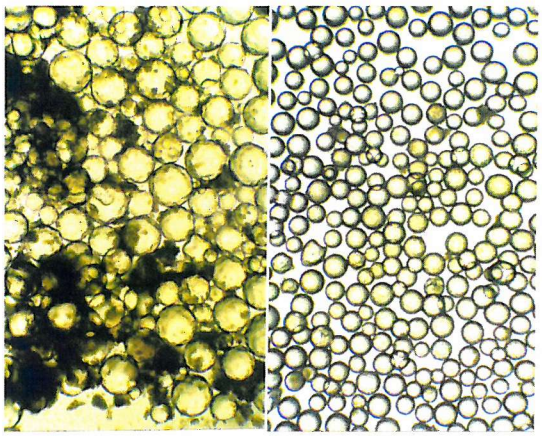


Figure 4.6. *Resins **R8** (left) and **R9** (right). 250-125μm and 125-45μm swollen beads respectively.*

Using 14% of monomer **(61)** and the same volume of solvent **(R10)** was produced, with beads showing a smooth surface and regular shape (Figure 4.7). The yield for resin **(R10)** was 60%, and 90% of the beads were between 125-45μm. From elemental analysis the percentage of monomer incorporation was 1.1 mmol/g and the loading was 1 mmol Cl/g, in agreement with that expected.

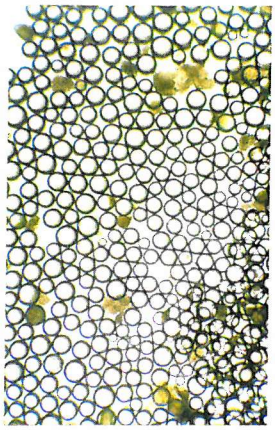


Figure 4.7. *Resins **R10**. 125-75μm swollen beads.*

When the percentage of monomer was raised to 24% **(R11)** and 43% **(R12)** the quality of beads was still good, but only a small quantity of resin was recovered because aggregation between the resin beads occurred (Figure 4.8). By elemental analysis, the percentage of monomer incorporation for resin **(R11)** was 1.87 mmol/g and 0.89 mmol Cl/g, and 2.7 mmol/g of monomer and 0.96 mmol Cl/g for resin **(R12)**.

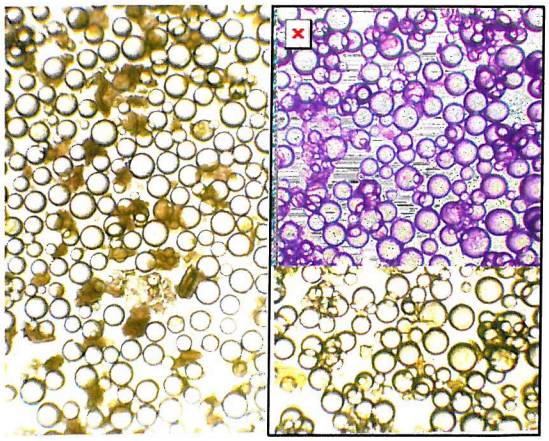


Figure 4.8. *Resins **R11** (left) and **R12** (right). 125-75μm swollen beads.*

Attempts to prepare a 1% cross-linked resin were unsuccessful. The synthesis of 1% DVB PEG-PS resin was performed changing polymerisation parameters such as: the

composition of the aqueous phase, quantity of diluent (toluene) etc., unfortunately none afforded resins of good quality.

4.7 Physical properties

Swelling parameters are a very important physical property for resin beads, as reactive site accessibility is directly related to resin swelling. Swelling studies were carried out to assess the influence of the styrene derivative **(61)** on the properties of the resins. Swelling studies were performed in solvents typically used for organic synthesis and compared with three commonly used supports of the same bead size (125-250 μm): 2% cross-linked aminomethyl polystyrene (made in-house, 1mmol/g), TentaGel™ (0.25 mmol/g) and ArgoGel™ (0.35 mmol/g) resins.

The dry volumes for each of the resins were found to be between 1.8 and 2.0 mL/g. 1g of each resin was swollen in a range of solvents and the swelling factors were measured.

The swelling factors of the PEG-PS resins prepared in this study are compared in Table 4.3 and Figure 4.9, going from 2% to 8% DVB and from 0% to 38% **(61)**. It is noteworthy observing that solvents such as dichloromethane, tetrahydrofuran, dioxane, and dimethylformamide commonly used in SPOC were amongst the "good" solvents, which swelled the resins most. The general trend in figure 4.9 is that for a fixed DVB percentage the swelling factors increased with the percentage of inclusion of monomer **(61)**. Swelling values of 7% DVB cross-linked resin containing 12% of **(61)** were found to be lower than the corresponding 2% cross-linked analogues. This is in accord with the trend that with increasing cross-linking, the percentage increase in volume, on transition from the dry to the swollen state, becomes smaller. The series of 2% DVB PEG-PS resins showed good solvation characteristics across the whole range of solvents studied. Resin swelling was found to be broadly dependent on the percentage of PEG monomer with a 7% level of incorporation observed to impart comparable swelling to TentaGel™ resin. However, none of the resins prepared in-house could match the level of swelling in water and alcohols displayed by the commercial resins.

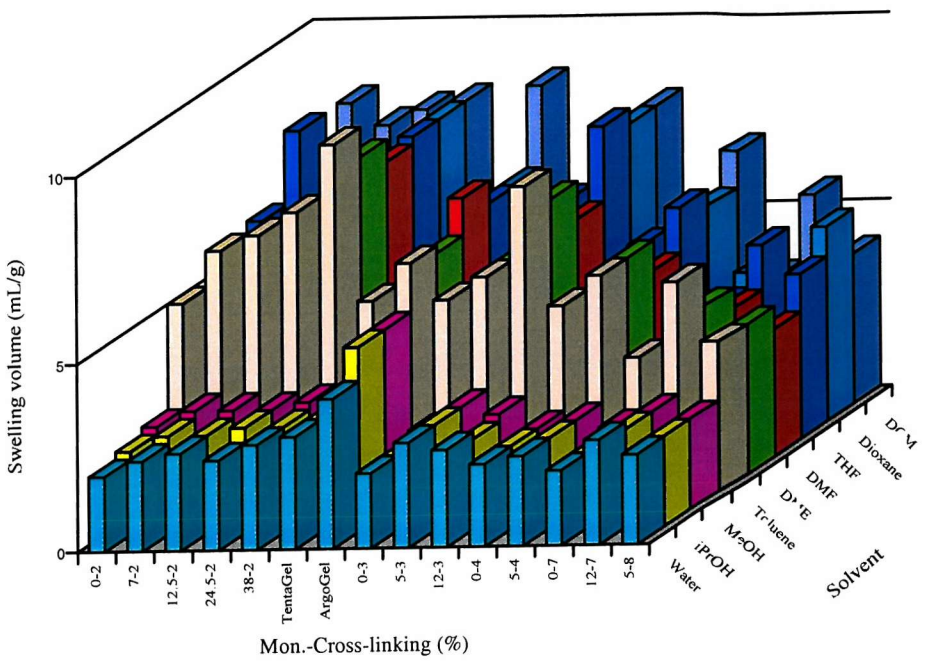


Figure 4.9. *Effect of PEG monomer* ***(61)*** *on the swelling properties of a series of 2% to 8% DVB-PS resins.*

Table 4.3 *Resin swelling experiments in a range of solvents. (Data quoted in the diagram are in units of mL per gram of resin ± 0.20mL/g). Dry resins volumes were determined to be 1.8-2.0 mL/g; c= chloromethyl functionalised resin.*

Mon. (%)	DVB (%)	DCM	Diox.	THF	DMF	DME	Toluene	MeOH	iPrOH	H_2O
0	2c	6.6	6.0	7.0	5.2	6.2	6.4	2.4	2.4	2.2
7	2c	8.2	7.0	8.4	5.8	5.8	6.6	2.8	2.6	2.4
14	2c	7.6	6.8	7.2	5.8	5.8	7.0	2.8	2.6	2.6
24	2c	8.0	6.8	6.8	6.6	6.6	7.6	2.8	2.8	2.4
43	2c	8.2	8.2	8.2	8.0	8.6	9.4	3.0	2.8	2.8
TG	1	5.5	3.9	3.9	4.0	4.0	5.2	3.0	3.0	3.0
AG	1	8.6	6.4	6.4	7.0	6.0	6.2	4.9	4.9	4.0
0	3	5.0	5.0	5.2	4.2	4.8	5.2	2.2	2.0	2.0
5	3c	6.8	6.0	6.4	5.0	5.8	5.8	2.8	2.8	2.8
12	3	8.0	8.0	8.4	6.4	7.4	8.2	2.6	2.4	2.6
0	4	4.8	5.0	5.2	4.0	4.4	5.0	2.2	2.2	2.2
5	4c	6.8	5.8	6.2	5.0	5.8	5.8	2.4	2.4	2.4
0	7	3.4	4.0	3.6	2.8	3.2	3.6	2.2	2.0	2.0
12	7	5.6	5.0	5.2	4.0	4.5	5.6	2.6	2.6	2.8
5	8c	4.0	5.2	4.4	3.4	4.0	4.0	2.4	2.4	2.4

TG= TentaGel resin; AG= ArgoGel resin; Diox.= Dioxane.

4.8 Stability test

The chemical and physical stability of the 2% DVB PS-PEG resins containing from 7% to 43% of monomer **(61)** was observed to be very good. Thus when the resins were treated for 2-12 hours at room temperature with a variety of acids (TFA, 6N HCl), bases (piperidine, 2N NaOH), common chemical reagents (Ac_2O, MeI) and mechanical shaking, magnetic stirring and vacuum drying over a 48h period, the beads were found to be

chemically and physically stable, with no PEG fragments being observed in ^{1}H and ^{13}C NMR.

4.9 Conclusions

In conclusion a series of novel chloromethylated PS-PEG resins were prepared using a co-polymerisation strategy involving a styryl PEG comonomer. Relative to traditional PEG-grafted resins, the PEG-PS resins had a higher loading capacity while maintaining broad solvent compatibility. The co-polymers were stable to strongly acidic/basic conditions and a range of physical manipulations. The resin containing 7% of the styrene diethoxylated monomer **(61)** was found to be the best compromise in terms of quality of beads and swelling properties.

CHAPTER 5

Site distribution studies on resin beads by Confocal Raman Spectroscopy

5.1 Introduction

The conversion from solution-phase chemistry to solid-phase chemistry is often not straightforward, one of the reasons being the lack of information on reactions occurring on a solid support. Many complexities associated with the solid support itself such as, solvation in media with different polarity, steric constraints within the bead or the partitioning of reagents between the different environments are some of the reasons why solid phase conditions often need to be modified from the existing solution literature. As a consequence physico-chemical characterisation of the resins, such as their swelling in different solvents, the influence of solvation on polymer-supported reactions and an understanding of resin based kinetics are important parameters in predicting the most suitable characteristics of any new solid support. Numerous techniques have been used over the past few years to study the chemical and physical properties of the most commonly used resins in solid phase synthesis. For example, the pore structure of 1-12 mol% PS-DVB resins have been investigated by Sherrington and co-workers.[192] Resins prepared with <10 mol% DVB collapsed to clear glassy particles on drying from dichloromethane or toluene. However, some opacity was retained when these resins were dried from acetone, methanol and $scCO_2$. Resins containing ≤6 mol% DVB and dried from the former two solvents had negligible surface areas and superficially appeared as gel-type resins. For the remaining resins cross-linked with less than 12 mol% DVB, the surface area depended on the solvent from which the resin was dried. Treatment with suitable solvents allowed the porous morphology to collapse, which could be easily re-established.

Studies on the influence of cross-linking level of polystyrene-divinylbenzene (PS-DVB) and on the efficiency of solid-phase chemistry were carried out by Rana.[193] Results on peptide synthesis and Suzuki coupling indicated that the choice of the optimal resin for

solid-phase synthesis was greatly related to the chemistry being carried out. Furthermore highly cross-linked materials were found to limit the rate of diffusion into the polymeric matrix, with the solvent still playing an important role.

The diffusion rates of reagents in macro beads (>570 μm) has been studied by Meldal.[194] The extent of diffusion of various acylating reagents into amino-functionalised macro beads at different times was determined by treating beads with a staining reagent (2,3,5,6-tetrachloro-1,4-benzoquinone, Chloranil) which colored the non-permeated regions of the beads. A comparison between the volume of the unstained and stained regions of the bead allowed the calculation of the diffusion rate constants. The results showed, unsurprisingly, that increased temperature, good swelling of the resin and small reagents all promoted diffusion more than sonication or mechanical agitation.

The distribution and relative accessibility of reactive functional groups within a given bead type is also of interest, to understand better the properties of solid-phase supports. In 1980 Merrifield[195] and co-workers found evidence for the homogeneous distribution of functional groups throughout 1% cross-linked resin beads, using autoradiography as a tool for the investigation. Autoradiographs of cross sections of peptide-resin beads containing tritium-labeled valine showed a uniform distribution of peptide chains throughout the polymeric support, indicating that synthesis occurred throughout the swollen peptide-resin. The uniformity of site distribution on polystyrene resin beads was confirmed a few years later by Fréchet.[196] These studies were carried out on solid supported α-olefines polymerization catalysts which were prepared by treating lightly cross-linked chloromethylated polystyrene with a secondary amine followed by an ammonium salt of a weakly coordinating anion and finally a neutral dialkyl metallocene. Mapping of the boron concentration in a cross section of a catalyst bead, by time of flight secondary ion mass spectrometry (TOF-SIMS) showed that the borate, and hence the metallocene cations were homogeneously distributed throughout the particle.

Recently, an optical analysis method, previously used for analysing biological structures, to visualise functional group distribution within a number of solid-support beads was reported by McAlpine and Schreiber.[197] This optical analysis technique allowed the visual cross sectioning (slicing) in 5-10 μm increments of fluorophore labeled beads, in which

rhodamine was covalently attached to the beads. The distribution of the dye moieties, and hence of the pendant functional groups, could be determined by collection of the fluorescence emissions of the fluorophore on each bead cross section. Studies on gel-type polystyrene and TentaGel™ resin beads gave evidence of a higher effective concentration of functional groups at the 'surface' of both resins. The thickness of this functionalised outer layer was found to depend on the degree of cross-linking and on the precise conditions under which the supports were prepared and functionalised. These results were clearly in disagreement with those previously observed by Merrifield [195] and Fréchet.[196] The same technique was used again by McAlpine and co-workers[198] for the investigation of "Rasta resins", a resin having long polymer chains bearing the desired functional groups attached to the functional sites of a traditional resin. A uniform functional group distribution was found throughout the entire bead of "Rasta resins".

Fluorescence microscopy (confocal and non-confocal) was used by Rademann and co-workers[199] to detect both the spatial distribution of products and the formation of product gradients in representative reactions. Single bead analysis on physical slices of labeled polystyrene beads revealed a homogeneous distribution of the fluorophores, covalently attached to the aminomethyl functional group of the resin. The progress of an acylation and a nucleophilic substitution reaction was examined, as a function of the bead size and the equivalents of reactant. These results indicated that solid phase reactions were not diffusion-controlled and that the bead size had no significant influence on the reaction progress. However, fluorescence experiments on fluorophore loaded beads[200] is not a straightforward method. Absorption of the incident radiation and re-absorption of the fluorescence and therefore an apparent nonlinear response of the fluorescence intensity with the bead loading were generally the consequence of "optical thickness"[125] (high concentration of fluorophore on the bead accompanied by the high absorption of dyes).

An alternative to fluorescence based methods was reported by Kress and co-workers in 2000.[125] Confocal Raman spectroscopy[201], a non-fluorescent method, was used in order to study the distribution of the functional sites within a number of commercial resins such as 1% cross-linked polystyrene and TentaGel™. 4-Cyanobenzoic acid was loaded onto aminomethylated PS and TentaGel resin. A variation in site loading was obtained by

quenching the reaction at different times. The spatial distribution of the nitrile functional group within the bead was determined by analysis of the strong Raman CN stretching vibrational transition at 2230 cm^{-1}. The studies were carried out on both dry and swollen beads. Polystyrene and TentaGel™ resins both showed a completely uniform distribution of functional sites across the bead when fully loaded. No evidence for the formation of 'clusters', either on the bead surface, or the interior, was found. However, time course experiments indicated that not all sites were kinetically equivalent, as differences in the rate-limiting step were found for polystyrene and TentaGel™ resins.

5.2 Results and Discussion

In order to investigate the site distribution of the PEG-PS resins prepared in Chapter 4, confocal Raman spectroscopy studies similar to the ones performed in our research group by Kress[125] were carried out. A series of four aminomethylated PEG-PS resins containing 7%, 14%, 24% and 43% of 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene **(61)** (Table 5.1) having loadings in the range of 0.63-0.77 mmol/g were fully loaded with 4-cyanobenzoic acid, using 1,3 diisopropylcarbodiimide as the coupling reagent and dimethylformamide as a solvent (the extent of loading was determined by ninhydrin assay). When the reaction was complete the resins were thoroughly washed with dichloromethane, methanol and dried from diethylether. In order to compare the site distribution of resins having the same level of cross-linking as the PEG-PS resins under study, 2% cross-linked polystyrene was included amongst the resins investigated.

Table 5.1 *Loading of aminomethyl PS resins used for the preparation of samples for Raman Analysis. Aminomethyl resins were prepared from chloromethyl-PS resins.*

Resin	Monomer (61) %	Loading (mmol NH_2/g)
AM-R9*	7	0.77
AM-R10*	14	0.63
AM-R11*	24	0.70
AM-R12*	43	0.67
2% DVB-AM-PS*	0	0.70

*In-house prepared resin. Compositional details for this resin are shown on Table 7.11 (entry 14), Chapter 7.

Figures 5.1 to 5.5 show maps of a slice through the equatorial region of the beads. The different intensities of the 2230 cm^{-1} band are assigned to different colours (from blue to red). Slices through each map in the *x* and *y* directions (as indicated by the lines over the area maps) are also shown in the diagrams on the top right and bottom left of each figure.

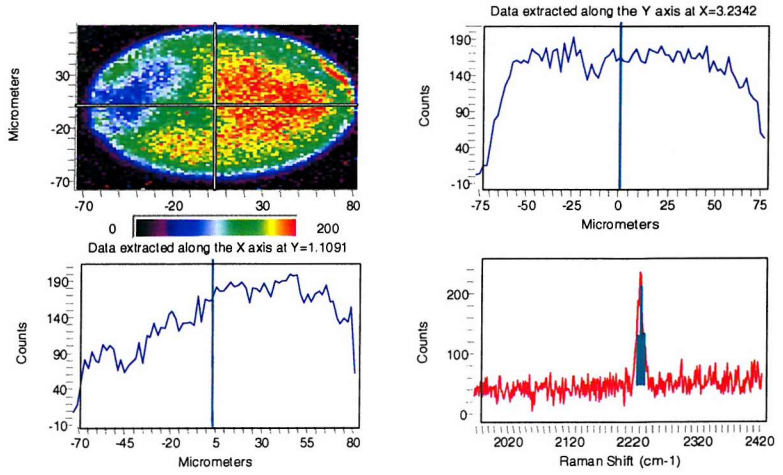


Figure 5.1 *Raman intensity map of the 2230 cm^{-1} CN band of a, fully loaded 2% cross-linked, DVB-PS resin.*

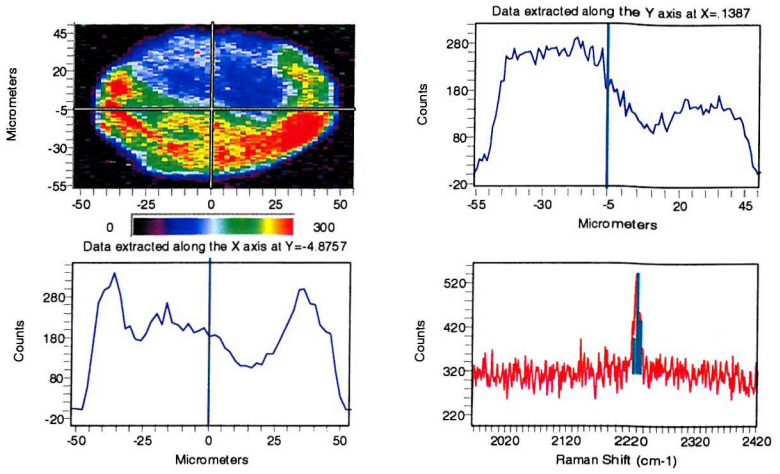


Figure 5.2 *Raman intensity map of CN band of a, fully loaded 2% cross-linked, PEG-PS resin containing 7% of monomer* ***(61)****.*

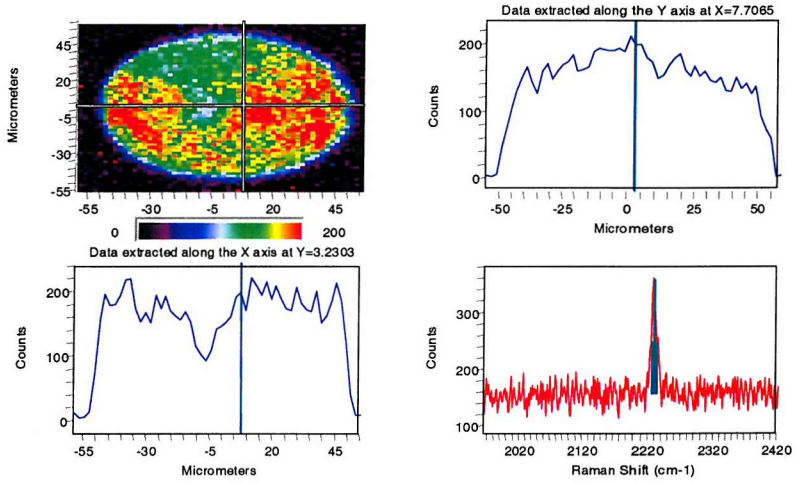


Figure 5.3 *CN group intensity map of a, fully loaded 2% cross-linked, PEG-PS resin containing 14% of monomer* ***(61)*** *through the equatorial plane of the bead.*

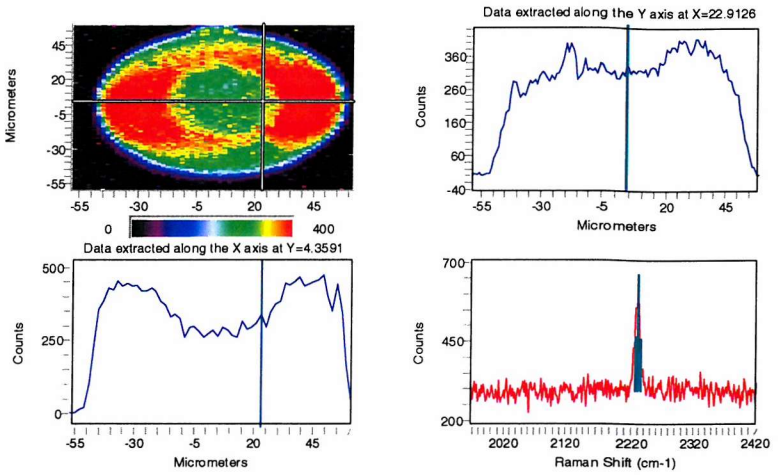


Figure 5.4 *CN group intensity map of a, fully loaded 2% cross-linked, PEG-PS resin containing 24% of monomer* ***(61)****.*

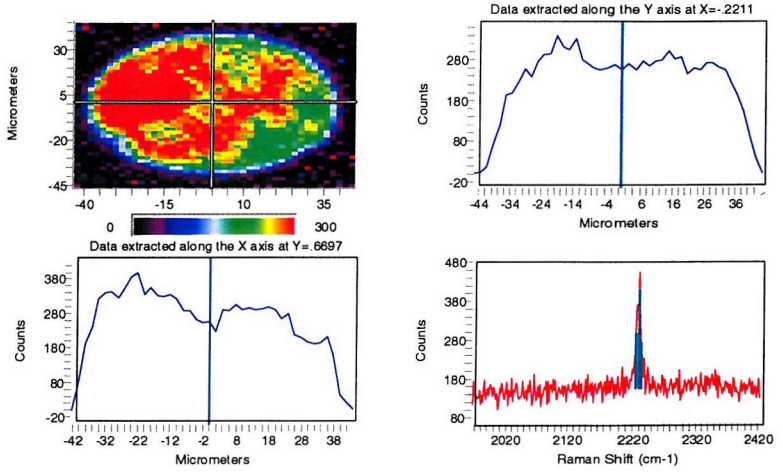


Figure 5.5 *Raman intensity map of the CN band of a, fully loaded 2% cross-linked, PEG-PS resin containing 43% of monomer* ***(61)****.*

The Raman intensity map of the 2% cross-linked resin beads showed a uniform site distribution, although it appeared lopsided on the *x*-axis. Raman intensity maps on the

whole set of PEG-PS resins showed evidence of an unequal site distribution across the beads. Segregation of reactive sites and hollow cavities were observed throughout the beads. The presence of "clusters" across the beads was also observed.

The poor level of solvation of the polyethylene glycol pendant chains in diethylether (used as last wash after the coupling reaction) was believed to be one of the possible reasons for the presence of "clusters". When similar experiments were carried out on aminomethylated resin beads loaded with 4-cyanobenzoic acid dried from tetrahydrofuran, a "good solvent" for the polyethylene glycol chains, a fairly regular site distribution was observed. Figure 5.6 shows that the intensity stays almost constant within the bead while an increase in relative intensity is observed as soon the edge of the bead is reached. Clearly, as demonstrated by Sherrington[192], the last solvent wash can have a major impact on the physical morphology of the resin beads.

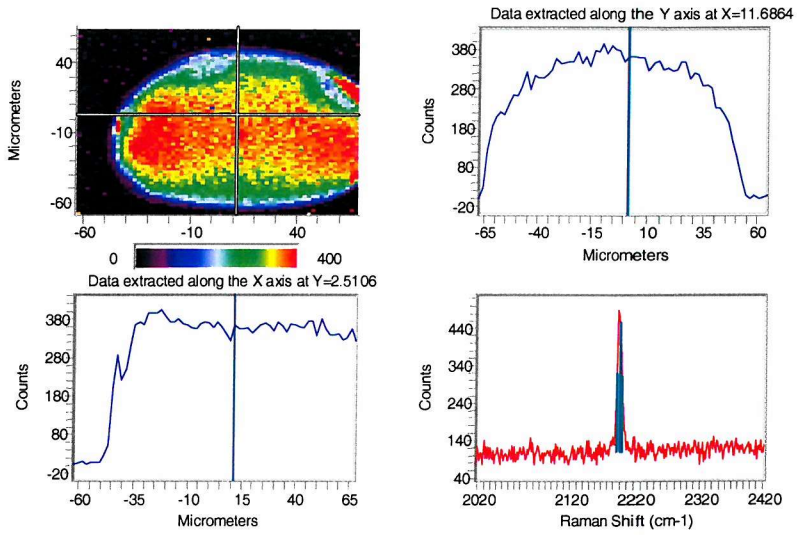


Figure 5.6 *Raman intensity map of the 2230 cm^{-1} CN band of a, fully loaded 2% cross-linked, PEG-PS resin bead containing 24% of monomer* ***(61)****. The resin bead was dried from tetrahydrofuran.*

CHAPTER 6

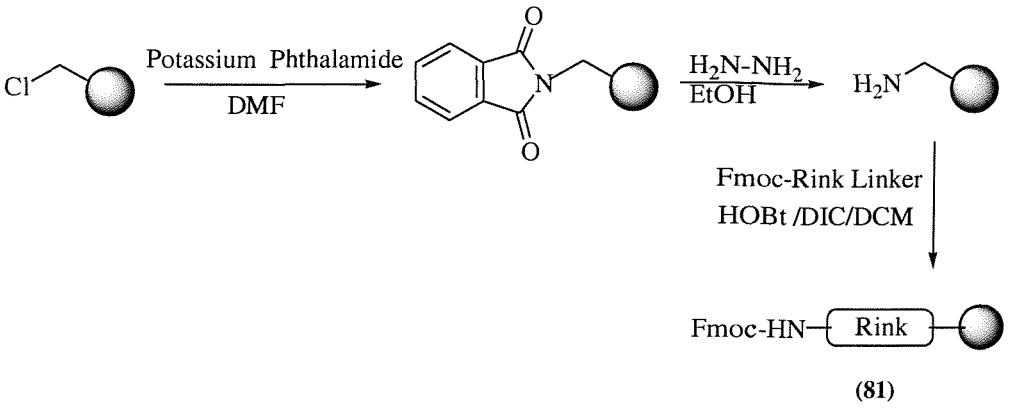
Applications of PS-PEG resins in solid phase peptide synthesis and solid phase organic synthesis

6.1 Introduction

The applications of the new PEG-PS resins under study in widely used reactions in organic chemistry will be discussed in this chapter. The behaviour of the novel resins will be compared to that of TentaGel™ and Merrifield supports by performing reactions that could be influenced by the presence of PEG chains of the co-polymerised monomer **(61)**. Parallel syntheses of small model peptides, biaryl derivatives *via* Suzuki coupling and arylether derivatives *via* Mitsunobu reactions were chosen. In order to investigate the relationship between chemical performance and percentage of monomer **(61)** co-polymerised in the new resins, a set of four PEG-PS resins was chosen. The resins were 2% DVB cross-linked and contained 7% **(R9)**, 14% **(R10)**, 24% **(R11)** and 43% **(R12)** of monomer **(61)** respectively.

6.2 *Synthesis of PEG-PS aminomethyl resins*

Chloromethyl PEG-PS resins were initially converted into the corresponding aminomethylated derivatives using potassium phthalamide followed by hydrazine (Scheme 6.1).



(81)

Scheme 6.1 *Preparation of aminomethyl PEG-PS resins and loading of Fmoc-protected Rink Linker.*

The initial loading of the resins was 1.0 mmol Cl/g. After aminomethyl derivatisation loadings[106] (calculated by coupling Fmoc-Gly-OH onto the resins and performing a Fmoc quantitative test[98]) were in the range 0.63-0.77 mmol/g (Table 6.1).

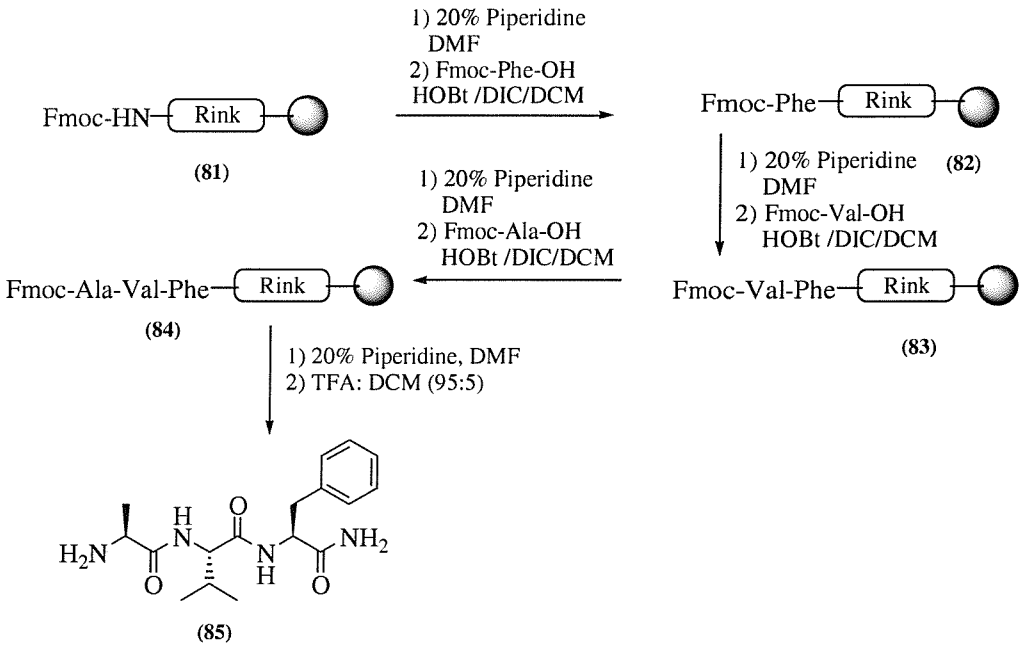
Table 6.1: *Loadings (mmol/g) of aminomethyl PEG-PS resins.*

Resin	loading (mmol NH_2/g)
R9	0.77
R10	0.63
R11	0.70
R12	0.67

The resins were then loaded with Fmoc-protected Rink amide linker[85] using DIC/HOBt in DCM.

6.3 *Synthesis of Ala-Val-Phe-NH_2 (89): preliminary studies*

In order to test the suitability of the resin for peptide coupling, the synthesis of the tripeptide Ala-Val-Phe-NH_2 **(85)** was first performed on resin **R9** (loading 0.77 mmol/g, beads size: 250-125μm). This resin was selected as it had high quality beads and good solvent compatibility across the whole set of PEG-PS resins. The synthetic route followed is shown in Scheme 6.2. After Fmoc deprotection using 20% piperidine in DMF, Fmoc-Rink amide aminomethyl PEG-PS resin **(81)** was coupled with three different Fmoc-protected aminoacids: phenylalanine, valine and alanine using HOBt and DIC as coupling agents and DCM as a solvent. The peptide was then cleaved from the resin by treatment with TFA:DCM (95:5) for 3 hours. The resin was then filtered, the solution concentrated and added to cold diethylether. The crude compound was essentially pure (95% HPLC purity, Figure 6.1) and the overall yield was 85%.



Scheme 6.2 *Solid phase synthesis of tripeptide (85).*

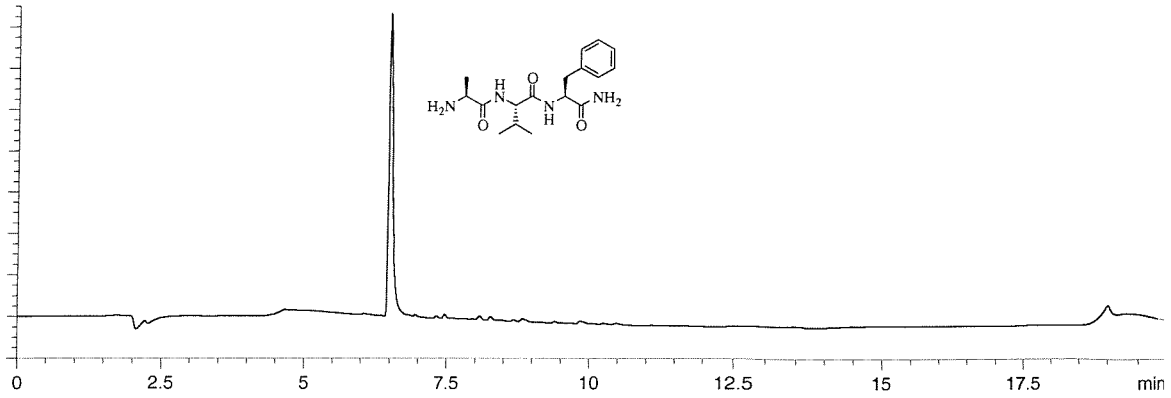


Figure 6.1 *HPLC profile of crude tripeptide Ala-Val-Phe-NH_2 (85) synthesised on resin R9.*

6.4 Parallel synthesis of Ala-Val-Phe-NH_2 (85)

After the promising results achieved using resin **R9** as a solid support, the small model tripeptide was synthesised in parallel using the whole set of PEG-PS resins, TentaGel™ and aminomethyl-PS (AM-PS) following the same route described above (Scheme 6.2). Synthesis of peptide **(85)** was also performed using a 2% DVB polystyrene resin in order to compare the behaviour of the PEG-PS resins to a resin having the same cross-linking level. Results summarised in Table 6.2 show that the new resins generally behave as well as normal TentaGel™ or aminomethyl-PS and in some cases even better. Resin **(R9)** containing 7% of monomer **(61)** afforded a better yield than 2% AM-PS and better yield and purity than TentaGel™. This resin was the best of the four analogue resins affording a good compromise between yield and purity.

Table 6.2 *Results of peptide synthesis on a set of PEG-PS containing monomer* ***(61)****, TentaGel and aminomethyl-PS.*

Resin [a]	Monomer (61) (%)	Loading [b]	Overall Yield (%)	Purity [c] (%)
R9	7	0.77	80	94
R10	14	0.63	50	94
R11	24	0.70	65	85
R12	43	0.67	58	89
TentaGel	0	0.25	43	83
AM-PS (1%)	0	1.13	72	95
AM-PS (2%)	0	1.0	56	95

a) Beads size 125-75μm
b) Loading of aminomethyl resin (mmol/g)
c) RP HPLC (λ =220) purity of crude peptides

6.5 Synthesis of N-(4'-phenylbenzoyl)glycinamide) (98) via Suzuki[202] coupling: preliminary studies

Peptide chemistry was considered as a preliminary test for the new PEG-PS resins. The successful synthesis of tripeptide Ala-Val-Phe-NH_2 **(85)** proved that these new resin beads could be routinely utilised for amide-bond formation reactions, Fmoc-deprotection and acidic cleavage. The influence of the pendant PEG chains on reactions involving metals as catalysts (such as palladium), and the stability of PEG-PS resins to relatively high temperatures were not investigated. It was thought that the Suzuki reaction would have been an interesting test.

The Suzuki reaction is a very commonly used reaction in solid phase chemistry. This palladium catalyzed carbon-carbon bond forming reaction[203,204,205] between an iodoaryl or iodoalkenyl derivative and a boronic acid has become very important in synthetic organic chemistry[206] in the last few years. The main advantage compared to the solution phase

version is that a larger excess of palladium catalyst can be used without the usual problems associated with purification. The Suzuki coupling mechanism is closely related to that of the Stille[207,208] coupling reaction. Nevertheless, the exact nature of the structures in the transition state catalytic cycle of the reaction[209] on the solid phase is not very clear but in solution, the reaction can be represented as shown in Figure 6.2; presumably the two mechanisms are related. Pd(II) is initially generated by oxidative addition of the halide component with the palladium (0) species. The leaving groups relative rate of reactivity decreases in the order of I > OTf > Br > Cl. The experimental conditions usually involve a temperature of 80-100°C and the presence of a base for successful completion of the Suzuki reaction. Sodium or potassium carbonate is commonly used, but these bases are barely soluble in popular solvents of choice such as DMF. One equivalent of base is required in the displacement, which forms palladium hydroxide **(86)** and the other is required to form the boronate **(87)**.

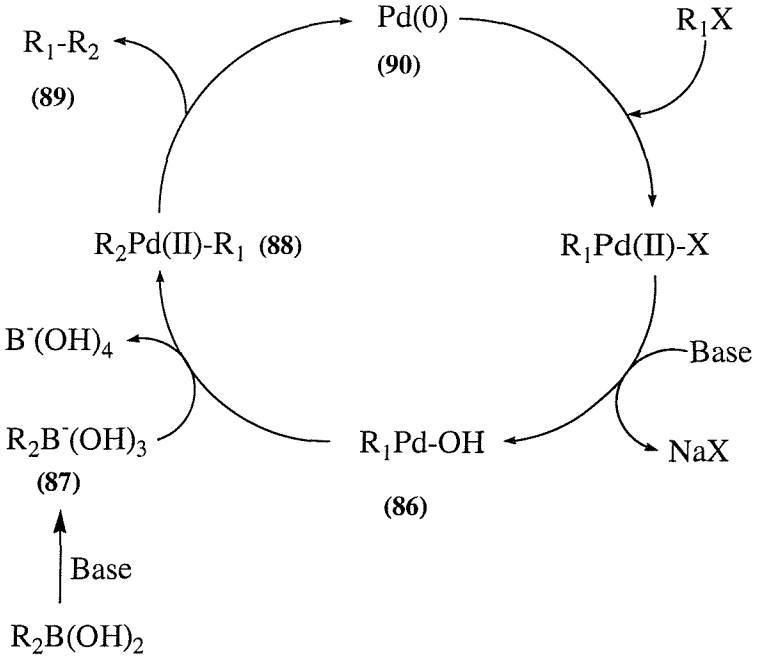
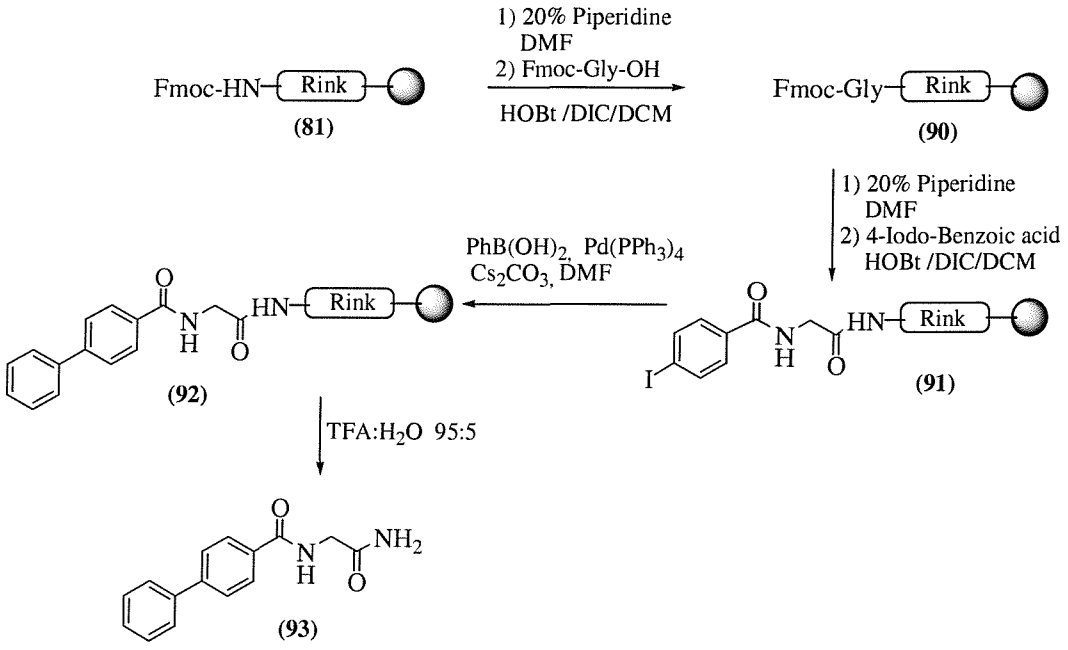


Figure 6.2 *Mechanism of the Suzuki reaction.*

Tranfer of the sustituent R_2 from the boron to the palladium center generates the palladium (II) species **(88)** that contains both substituents that are to be coupled (transmetallation). Reductive elimination then occurs to yield product **(89)** and to regenerate the catalytically active Pd(0) complex **(90)**.

In analogy with the tripeptide synthesis, the biaryl derivative **(93)** was firstly synthesized using resin **R9** (loading 0.77 mmol/g beads size: 250-125μm). The synthetic route followed is outlined in Scheme 6.3. The synthesis started from the Rink-PEG-PS resin **(81)** previously described, and after Fmoc deprotection continued with the couplings of Fmoc-glycine and 4-iodobenzoic acid in that order. The glycine appendage was introduced to help the ionisation of the final compound for MS analysis. A first attempt to perform the Suzuki coupling was carried out using 0.1 equivalents of $Pd(PPh_3)_4$ and 2 equivalents of potassium carbonate (relative to the amount of 4-iodobenzoic acid) in DMF at 90°C for 24 hours. Unfortunately only starting material and a very small amount of biaryl derivative **(93)** were collected after cleavage. When the reaction was repeated using 2 equivalents of cesium carbonate in DMF at 100°C for 24 hours and the reagents added under nitrogen atmosphere better results were achieved. Although at the end of the reaction the resin was completely black, the desired compound was isolated with a 99% HPLC purity and 97% overall yield.



Scheme 6.3: *Solid-phase synthesis of biaryl derivative (93).*

6.6 Parallel synthesis of biaryl derivative (93)

Once the most suitable conditions for the Suzuki coupling had been found, the parallel synthesis of compound **(93)** was performed on the four PEG-PS resins, TentaGel and aminomethyl-PS. The resins were reacted in the presence of phenylboronic acid, palladium tetrakis-triphenylphoshine and cesium carbonate in DMF at 100°C to generate the resin bound product **(92)** in Scheme 6.3. The course of the reaction was monitored by RP-HPLC (Figure 6.3) and MS analysis of the crude cleavage product from each of the resins. Results of the parallel synthesis are summarised in Table 6.3. The rate of formation of the biaryl product **(93)** was in general the same (24 hours) for each of the resins. A longer reaction time was required in the case of resins **R10** and **R12**. In both cases reactions were complete after 48 hours. With each resin the Suzuki reaction occurred successfully. It was therefore concluded that the presence of PEG chains contained into the resins was compatible with the use of palladium as a catalyst and that the new PEG-PS resins were stable at relatively high temperatures.

Table 6.3 *Results of parallel synthesis of biaryl derivative* ***(93)*** *via Suzuki coupling.*

Resin[a]	Monomer (61) (%)	Loading [b]	Overall Yield (%)	Purity [c] (%)
R9	7	0.77	97	99
R10	14	0.63	80	95
R11	24	0.70	74	63
R12	43	0.67	60	84
AM-PS	0	1.65	71	97
TentaGel	0	0.28	94	84

a) Beads size 125-75μm
b) Loading of to aminomethyl resin (mmol/g)
c) RP-HPLC (λ=254) purity of crude products

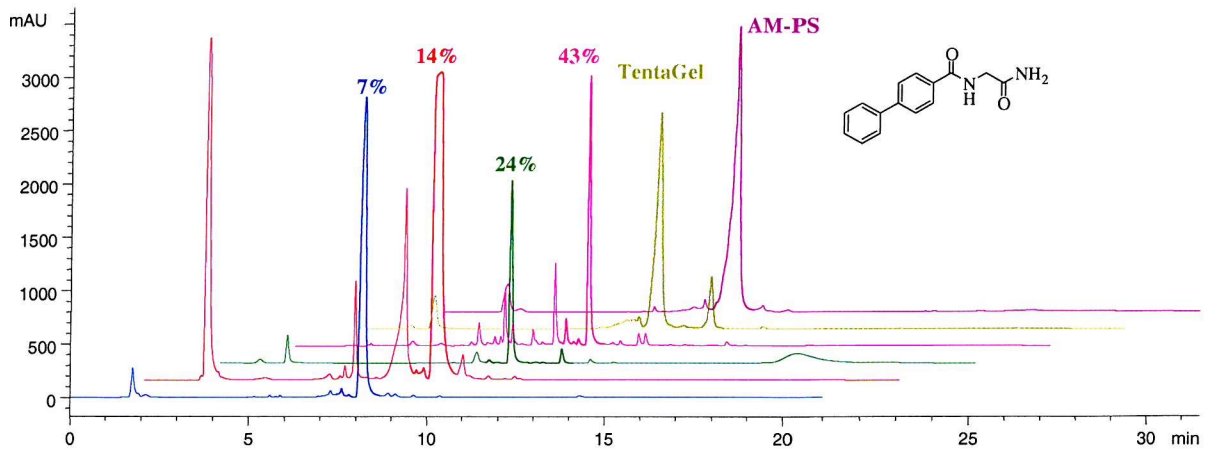


Figure 6.3 *Parallel Suzuki coupling on novel resins: HPLC profiles of crude biaryl derivative* ***(93)****.*

6.7 *Synthesis of 2-(acetylamino)-3-[4-(benzyloxy)phenyl]propanamide (96) via Mitsunobu[210] condensation*

The major application of the Mitsunobu reaction[211] is the conversion of a chiral secondary alcohol into an ester with concomitant inversion of configuration at the secondary carbon center. In a second step the ester can be hydrolysed to yield the inverted alcohol. Using appropriate nucleophiles, alcohols can be converted to other classes of compounds such as azides, amines or ethers. The mechanistic pathway[212] (Figure 6.4) can be divided into three steps: 1. formation of the activating agent from triphenylphosphine and diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD); 2. activation of the substrate alcohol; 3. bimolecular nucleophilic substitution (S_N2) at the activated center.

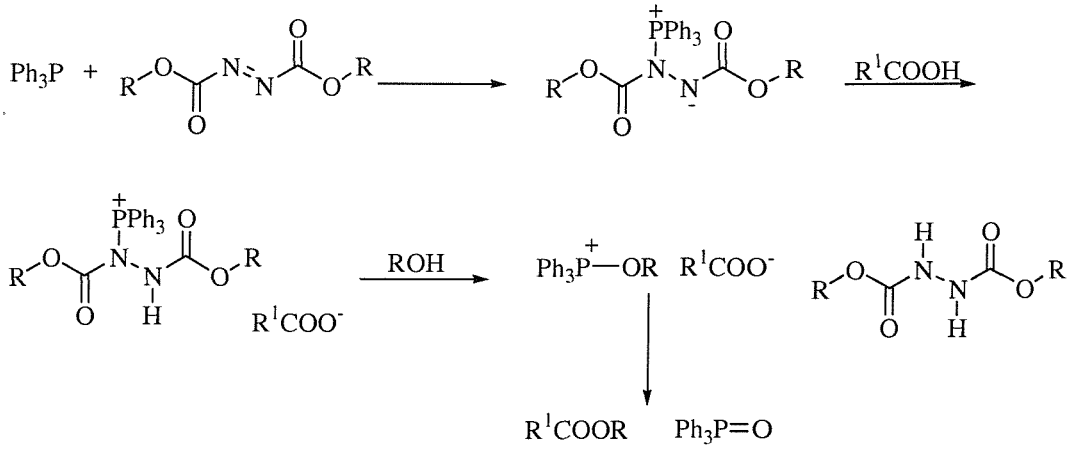
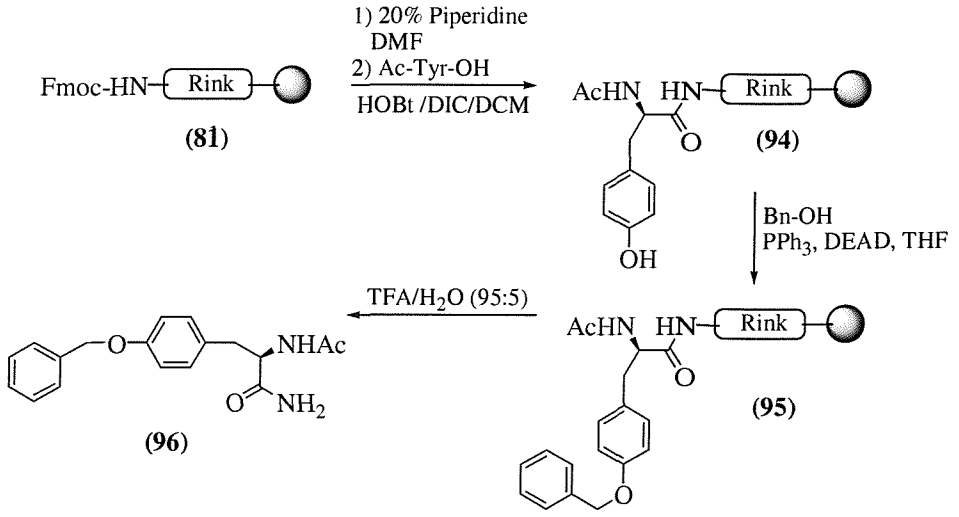


Figure 6.4 *Mitsunobu reaction: general mechanism.*

Preliminary studies on Mitsunobu condensations were conducted following the synthetic pattern described in Scheme 6.4 and using resin **R9** as a solid support.



Scheme 6.4 *Solid-phase synthesis of aryether (96) via Mitsunobu condensation.*

After Fmoc deprotection of Rink-PEG-PS resin **(81)**, *N*-acetyl tyrosine was loaded onto PEG-PS resin **R9** under standard peptide coupling conditions. The resin was washed three times with freshly distilled THF before the reaction with benzyl alcohol (10 equivalents), DEAD (5 equivalents) and triphenylphosphine (5 equivalents). The reaction was routinely performed at room temperature but the portionwise addition of azodicarboxylate to the mixture of benzyl alcohol, triphenylphosphine and resin-bound alcohol was found to be of crucial importance for the success of the coupling. After a first cycle a small amount of compound was cleaved from the resin. HPLC analysis showed that the reaction was 80% complete. After a second cycle the reaction was complete. After cleavage from the resin, the compound was isolated in good purity (80% by HPLC) and yield (88%).

6.8 Parallel synthesis of arylether (96)

Once the best conditions for the coupling had been found, it was decided to investigate the suitability of the other PEG-PS resins to the Mitsunobu reaction conditions. The parallel synthesis of compound **(96)** was also in this case performed in parallel with normal

TentaGel resin and aminomethyl-PS. All the reactions were straightforward and went to completion after the first cycle. Compounds synthesised on the different resins were characterised by HPLC (Figure 6.5), MS, ^{1}H NMR and ^{13}C NMR. Results listed in Table 6.4 show that, in terms of purity and yield, resins **R9, R10** and **R11** worked better than both TentaGel and AM-PS. During the parallel synthesis of the compound **(96)** the robustness of PEG-PS resins was also proven since agitation was generated by using magnetic bars. Although this method was rather harsh, no physical degradation of the beads was observed.

Table 6.4 *Results of Mitsunobu coupling on a set of three PEG-PS, TentaGel and aminomethyl-PS.*

Resin[a]	Monomer (61) (%)	Loading [b]	Overall Yield (%)	Purity [c] (%)
R9	7	0.77	88	80
R10	14	0.63	77	67
R11	24	0.70	77	70
AM-PS	0	1.65	43	51
TentaGel	0	0.28	65	68

a) Beads size 125-75μm
b) Loading of aminomethyl resins (mmol/g)
c) HPLC purity of crude products

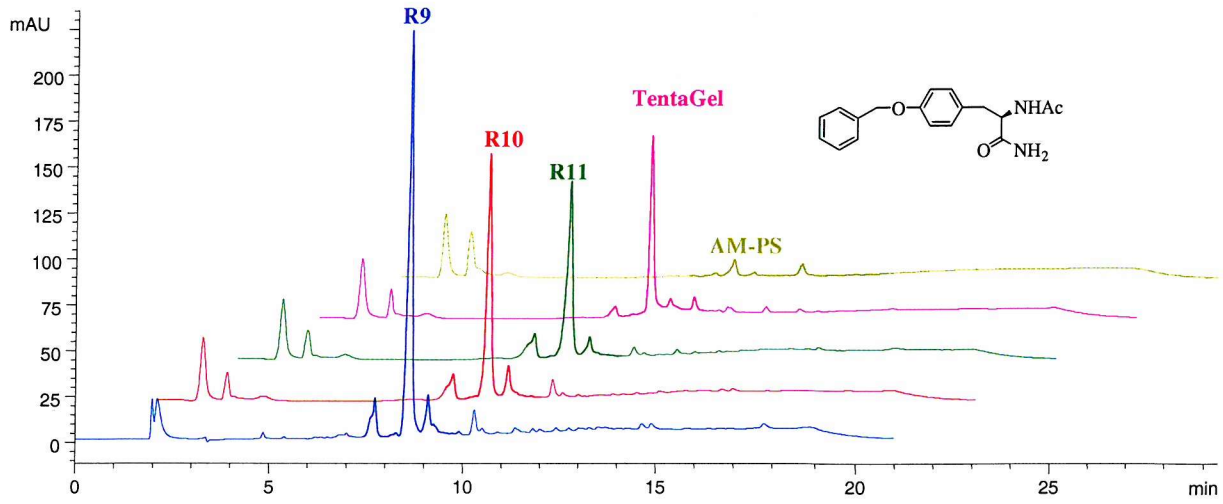


Figure 6.5 *Parallel Mitsunobu condensation on novel resins: HPLC profiles of crude arylether derivative* ***(96)***.

6.9 Conclusions

In conclusion the resins were suitable for peptide synthesis, Suzuki and Mitsunobu chemistry and found to behave as well as TentaGel and Merrifield resin. It is noteworthy to highlight that resin R9 containing 7% of monomer **(61)** afforded the best results amongst the set of four PEG-PS resins under all the chemical conditions investigated.

CHAPTER 7

7.1 General information

NMR spectra unless stated otherwise were recorded using a Bruker AC300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, or a Bruker DPX400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C (δ scale in parts per million).

ESI mass spectra were recorded using a VG Platform Quadrupole Electrospray Ionisation mass spectrometer, measuring mono-isotopic masses.

Infra-red spectra were recorded on a BIORAD Golden Gate FTS 135. All samples were run as either neat solids or oils. In the case of resin linked compounds, the resins beads were washed with DCM, Et_2O and then dried *in vacuo*.

Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected.

UV-VIS spectra were recorded using an 8452A Diode Array Spectrophotometer.

Analytical HPLC was accomplished using a Hewlett Packard HP1100 Chemstation, using a Phenomenex C_{18} prodigy 5 μm (150 mm x 3 mm) column. A gradient from water, 0.1% TFA to MeCN, 0.042% TFA was run over 20 minutes followed by 5 minutes with MeCN, 0.042% TFA.

Preparative HPLC was carried out using a Hewlett Packard HP1100 Chemstation with an automated fraction collector using a Phenomenex C_{18} prodigy 5μm (250 mm x 10 mm) column. The gradients used were based on water, 0.1% TFA and MeCN, 0.042% TFA.

Aluminium backed silica plates (0.25 mm layer of silica gel 60 with the florescent indicator Alugram SIL G/UV_{254}) were used for thin layer chromatography (TLC). UV (254 nm) was used to visualize compounds unless stated otherwise.

Resin beads were sieved using a Retsch AS 200 control Sieve Shaker.

7.1.1 Materials Section

All chemicals were of reagent grade and were used without further purification unless otherwise stated. Divinylbenzene used for the preparation of PS and PS-PEG resins was tech., 80%, mixture of isomers supplied by Aldrich. Amberlite IRA 96 macroreticular polyamine resin (capacity 5.6 eq/g, mesh: 15-60) used as a scavenger was supplied by Supelco. Amberlite IRA-900 (ionic form: Cl^-) strongly basic macroreticular resin was supplied by Aldrich. All amino acids used were the natural L configuration unless otherwise stated.

7.2 General experimental procedures for solid phase peptide synthesis and solid phase organic synthesis

7.2.1 Method A: Solid phase peptide coupling conditions[213]

The resin was swollen in a minimum amount of DCM/DMF (10:1) for 30 minutes. *N*-Fmoc-amino acid (2eq) and HOBt (2eq) were dissolved in DCM/DMF (10:1) for 10 minutes. DIC (2eq) was added and the mixture stirred for a further 10 minutes before addition to the resin. The resin was agitated for 3 hours. The resin was washed with DMF (x3), DCM (x3), MeOH (x3) and Et_2O (x2) and the resin was dried under vacuum for 30 minutes.

7.2.2 Method B: N-terminal Fmoc removal[214]

Fmoc removal was performed using 20% piperidine in DMF with two sequential treatment of 20 minutes. The resin was filtered, washed with DMF (x3), DCM (x3), MeOH (x3) and Et_2O (x2) and dried under vacuum for 30 minutes.

7.2.3 Method C: Quantitative Fmoc test [98]

A known quantity of resin (*ca.* 5mg) was treated with 20% piperidine in DMF (1ml) for 15 minutes. The solution was filtered through glass wool and the volume of filtrate made

up to 25 ml with 20% piperidine in DMF. The resin substitution was deduced from the following equation:

$$mmol/g = [(A_{302}xV)/(\varepsilon_{302}x\ W)]\ x10^3$$

where A_{302} is the absorbance at 302nm of the piperidyl-fulvene adduct, V is the total volume (mL), W = the weight of the resin sample (mg) and ε_{302} is the extinction coefficient of the adduct at 302nm ($7800M^{-1}cm^{-1}$).

7.2.4 Method D: Cleavage of peptide from resin[213]

TFA/DCM (95:5) (1mL/100mg of resin) was added to the resin and the mixture agitated for 3 hours. The resin was removed by filtration, the filtrate concentrated to *ca.* 1mL and added to cold Et_2O (25mL). The resulting precipitate was collected by centrifugation and washed with Et_2O (25mLx2).

7.2.5 Method E: Cleavage from the resin

TFA/H_2O (95:5) (1mL/100mg of resin) was added to the resin and the mixture agitated for 1 hour. The resin was removed by filtration and the TFA evaporated *in vacuo*.

7.2.6 Method F: Quantitative ninhydrin test[215]

A known mass of resin (<5 mg) was treated in a small test tube with 6 drops of reagent A (described below) and 2 drops of reagent B (described below) and heated at 110°C for 10 minutes. The tube was cooled and 60% aqueous ethanol (2mL) was added. The contents of the tube were filtered through a pipette charged with a plug of glass wool and the blue filtrate collected in a 25mL volumetric flask. The resin was washed using a solution of tetraethyl ammonium chloride (NEt_4Cl) (0.5 M in DCM, 2x0.5 mL) and the sample made up to 25mL using 60% aqueous ethanol. The absorbance at 570 nm was then measured against a reagent blank. The amount of primary amine present on the resin was then calculated using the equation:

$$Loading\ (mmol/\ g) = (A\ x\ V)/\ (\varepsilon_{570} = W)\ x\ 1000$$

Where ε_{570} is the molar extinction coefficient (15000/ $M^{-1}cm^{-1}$), V is the diluted volume (25 mL), W is mass of resin (mg) and A_{570} is the absorbance measured at 570 nm.

Reagent A

Solution 1 – Reagent grade phenol (40 g) was dissolved in absolute ethanol (10 mL) with warming and then stirred over Amberlite mixed-bed resin MB-3 (4 g) for 45 minutes. The mixture was then filtered.

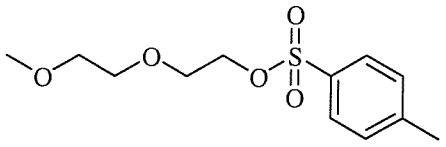
Solution 2 – Potassium cyanide (65 mg) was dissolved in water (100 mL). A 2 mL aliquot of this solution was diluted with pyridine (freshly distilled from ninhydrin) to 100mL and stirred over Amberlite mixed-bed resin MB-3 (4 g) for 45 minutes. The solution was filtered and mixed with solution 1 to form reagent A.

Reagent B:

Ninhydrin (2.5 g) was dissolved in absolute ethanol (50 mL).

7.3 EXPERIMENTAL TO CHAPTER 2

7.3.1 *Synthesis of 2-(2-methoxyethoxy)ethyl 4-methyl-1-benzensulfonate*[216] *(68)*



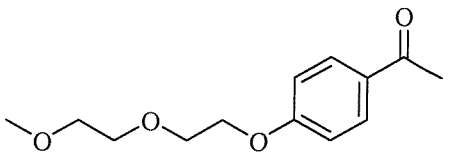
To an anhydrous THF solution (50mL) of diethylene glycol monomethyl ether (5g, 42mmol, 1eq) and dry triethylamine (8.48g, 84mmol, 2eq) *p*-toluene sulfonyl chloride (12g, 63mmol, 1.5eq) was slowly added over a 20 minutes period at 0°C. The mixture was allowed to warm to room temperature, stirred for 24 hours and monitored by TLC. The white solid ($Et_3N{\cdot}HCl$) present in the mixture was removed by filtration and the solvent removed *in vacuo* to give a yellow oil. The product was purified by column chromatography on silica gel using Pet:EtOAc (7:3) as eluent to give the pure title product as a yellow oil. Yield 9.4g, 82%. R_f = 0.35 (Pet:EtOAc 7:3); IR: ν=2878, 1598, 1451, 1353, 1108 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ=7.80 (d, J=8 Hz, 2H, ArH), 7.34 (d, J=8 Hz, 2H, ArH), 4.18 (m, 2H, CH_2OSO_2), 3.70 (m, 2H, CH_2O), 3.58 (m, 2H, CH_2O), 3.49 (m, 2H, CH_2O), 3.36 (s, 3H, OCH_3), 2.45 (s, 3H, $ArCH_3$); ^{13}C NMR (75MHz, $CDCl_3$): δ=144.9 (0), 133.1 (0), 130.0 (1), 128.0 (1), 71.9 (2), 70.8 (2), 69.4 (2), 68.8 (2), 59.2 (3); 21.8 (3); MS (AP^+): m/z (%): 275.1 (100) $[M+H]^+$, 297.0 (20) $[M+Na]^+$; RP HPLC (λ = 254): 14.6 min.

Table 7.1 *Scale-up reactions for the synthesis of* ***(68)***

Starting material (g)	Product (g)	Purification method	Yield (%)
5	10	column chromatography	82
10	20	Amberlite*	89
30	65	Amberlite*	95

* See section 7.4 for details about this purification method.

7.3.2 *Synthesis of 1-{4-[2-(2-methoxyethoxy)ethoxy]phenyl-ethane-1-one (69)*

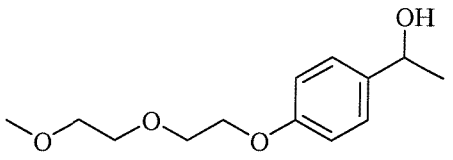


(68) (1.01g, 3.64mmol, 1eq) and a catalytic amount of potassium iodide (20mg) were dissolved in acetonitrile (15mL), and stirred for 5 minutes. A mixture of *p*-hydroxyacetophenone (0.74g, 5.50mmol, 1.5eq) and potassium carbonate (0.75g, 5.50mmol, 1.5eq) dissolved in acetonitrile (10mL), was added. The reaction mixture was refluxed for 24 hours. The white solid in the mixture was removed by filtration and the solvent was removed *in vacuo*. The crude product was dissolved in EtOAc (10mL) and washed with a 10^{-3}M solution of sodium hydroxide (20mL). The organic layer was dried over sodium sulphate and the solvent removed *in vacuo* to give 0.76g of the title compound as a yellow oil that needed no further purification. Yield 0.75g, 86%. R_f= 0.25 (Pet:EtOAc 7:3); IR: ν=2876, 1674, 1599, 1358 cm^{-1}; ^1H NMR: δ = (300 MHz, $CDCl_3$): 7.92 (d, 2H, J=9 Hz, ArH), 6.94 (d, 2H, J=9 Hz, ArH), 4.20 (m, 2H, CH_2OAr), 3.88 (m, 2H, CH_2O), 3.72 (m, 2H, CH_2O), 3.59 (m, 2H, CH_2O), 3.39 (s, 3H, OCH_3), 2.55 (s, 3H, $COCH_3$); ^{13}C NMR (75MHz, $CDCl_3$): δ=197.0 (0); 162.8 (0); 130.7 (0); 130.5 (1); 114.4 (1); 72.0 (2), 71.0 (2), 70.0 (2), 68.0 (2), 59.2 (3); 26.5 (3); MS (ES^+): m/z (%): 239.2 (100) $[M+H]^+$, 256.2 (25), $[M+NH_4]^+$, 261.2 (5), $[M+Na]^+$; HRMS calcd for $C_{13}H_{18}O_4$ $[M+H]^+$: 238.1205, found: 238.1204; RP HPLC (ELSD): 8.6 min.

Table 7.2 *Scale-up reactions for the synthesis of* ***(69)***

Compound (68) (g)	**Product (g)**	**Yield (%)**
1	0.8	87
2	1.5	85
5	4	89
10	7	81
65	49	86

7.3.3 *Synthesis of 1-{4-[2-(2-methoxyethoxy)ethoxy]phenyl}-1-ethane-1-ol (70)*

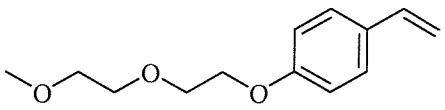


(69) (1.0g, 4.20mmol, 1eq) was dissolved in ethanol (10mL) and stirred at 0°C. Sodium borohydride (0.16g, 4.23mmol, 1eq) was slowly added in portions. The mixture was stirred for 3 hours and the excess $NaBH_4$ quenched with 2N HCl in ethanol. The solvent was removed by distillation, water was added and the compound was extracted with DCM (100mL). The organic layer was dried with sodium sulphate and the solvent removed *in vacuo* to give 0.95 g of the title compound as a yellow oil. Yield 0.95g, 95%. R_f = 0.2 (Pet:EtOAc 7:3); IR: ν= 3422, 2877 cm^{-1}; ^1H NMR: δ=(300 MHz, $CDCl_3$): 7.22 (d, 2H, J=9 Hz, ArH), 6.88 (d, 2H, J=9 Hz, ArH), 4.35 (q, 1H, J=7 Hz, C*H*CH_3), 4.14 (m, 2H, C*H*$_2$OAr), 3.85 (m, 2H, CH_2O), 3.72 (m, 2H, CH_2O), 3.58 (m, 2H, CH_2O), 3.39 (s, 3H, OCH_3), 1.40 (d, 3H, J=7 Hz, CHC*H*$_3$); ^{13}C NMR (75MHz, $CDCl_3$): δ=158.2 (0), 136.5 (0), 127.8 (1), 114.6 (1), 72.0 (1), 71.0 (2), 70.0 (2), 67.0 (2), 64.0 (2), 59.2 (3); 24.3 (3); MS (ES^+): m/z (%): 258.3 (30) $[M+NH_4]^+$, 263.2 (100) $[M+Na]^+$, 279.2 (10) $[M+K]^+$; HRMS calcd for $C_{13}H_{20}O_4$ $[M+H]^+$: 240.1362, found: 240.1365; RP HPLC (ELSD): 7.7 min.

Table 7.3 *Scale-up reactions for the synthesis of* ***(70)***

Compound (69) (g)	**Product** (g)	**Yield** (%)
0.5	0.2	40
1	0.9	95
3	2.9	88
49	47	96

7.3.4 Synthesis of 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene (61)[160]

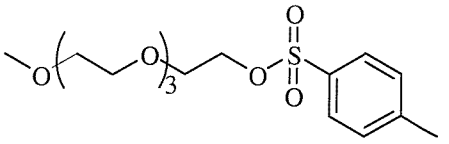


A solution of **(70)** (0.14g, 0.57mmol) and pyridinium tosylate (14mg, 0.056mmol) in toluene (30mL) was refluxed overnight at 120°C using a Dean-Stark trap. The cooled solution was washed with water (30mL) and with a saturated solution of sodium chloride (30mL). The toluene layer was dried with sodium sulfate and the solvent removed *in vacuo*. The product was purified by column chromatography on alumina eluting with Pet:EtOAc (7:3) to give the title product as a pale yellow oil. Yield 0.1g, 83%. R_f=0.48 (Pet:EtOAc 7:3) on alumina; IR: ν=2876, 1606, 1509 cm^{-1}; ^1H NMR: δ=(300 MHz, $CDCl_3$): 7.33 (d, 2H, J=8 Hz, ArH), 6.87 (d, 2H, J=8 Hz, ArH), 6.65 (dd, 1H, J_{trans}=18 Hz, J_{cis}=11 Hz, C*H*CH_2), 5.60 (d, 1H, J_{trans}=18 Hz, C$H_\alpha H_\beta$), 5.12 (d, 1H, J_{cis}=11 Hz, $CH_\alpha CH_\beta$), 4.14 (m, 2H, CH_2OAr), 3.86 (m, 2H, OCH_2), 3.72 (m, 2H, OCH_2), 3.58 (m, 2H, OCH_2), 3.39 (s, 3H, OCH_3); ^{13}C NMR (75MHz, $CDCl_3$): δ=158.6 (0), 136.4 (0), 130.7 (1), 127.5 (1), 114.7 (1), 111.7 (2), 72.0 (2), 71.0 (2), 70.0 (2), 67.5 (2), 59.2 (3); MS (ES^+): m/z (%): 240.2 (100) $[M+NH_4]^+$, 245.1 (18) $[M+Na]^+$, 261.1 (8) $[M+K]^+$, 286.2 (50) $[M+Na+CH_3CN]^+$); HRMS calcd for $C_{13}H_{18}O_3$ $[M+H]^+$: 222.1256, found: 222.1253; RP HPLC (λ = 254): 10.8 min.

Table 7.4: *Scale-up reactions for the synthesis of* ***(61)***

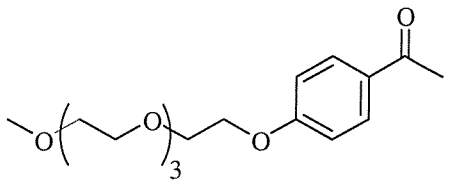
Compound (70) (g)	**Product (g)**	**Yield (%)**
0.1	0.08	42
0.14	0.1	83
0.16	0.14	95
1	0.8	91
4.6	4	91
45	37	90

7.3.5 *Synthesis of tetraethylene glycol 4-methyl-1-benzensulfonate (68a)* [217]



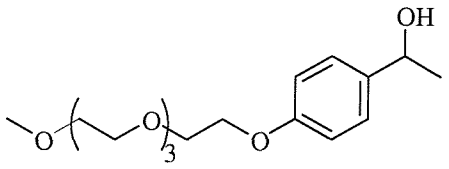
To a cooled solution of tetraethylene glycol monomethyl ether (5g, 24mmol, 1eq) in anhydrous DCM (50mL) and dry triethylamine (17g, 168mmol, 7eq), *p*-toluene sulfonyl chloride (23g, 116mmol, 5eq) and DMAP (100mg) were slowly added. The mixture was allowed to warm up to room temperature while stirring for 27 hours and monitored by TLC. The white solid ($Et_3N{\cdot}HCl$) was filtered and the solvent removed *in vacuo* to give a yellow oil. The product was purified by treating a solution of the reaction mixture in DCM (80mL) with 5 fold excess of polyamine resin (Amberlite IRA-96) overnight, followed by filtration of the scavenger resin and removal of the solvent *in vacuo*. The title product appeared as a yellow oil that needed no further purification. Yield 7g, 80%. R_f = 0.40 (Pet:EtOAc 4:6); IR: ν= 2872, 1597,1453, 1352, 1095 cm^{-1}; ^1H NMR: δ= (300 MHz, $CDCl_3$): 7.80 (d, 2H, J=8 Hz, ArH); 7.35 (d, 2H, J=8 Hz, ArH), 4.20 (m, 2H, CH_2OSO_2), 3.65 (m, 14H, CH_2O), 3.38 (s, 3H, OCH_3), 2.45 (s, 3H, $ArCH_3$); ^{13}C NMR (75MHz, $CDCl_3$): δ= 145.0 (0), 133.3 (0), 129.9 (1), 128.1 (1), 72.0 (2), 70.9 (2), 70.7 (2), 70.6 (2), 69.4 (2), 68.8 (2), 59.2 (3); 21.8 (3); MS (ES^+): m/z (%): 385.3 (100) $[M+Na]^+$, 401.3 (30) $[M+K]^+$; RP HPLC (ELSD): 9.8 min.

7.3.6 Synthesis of 4'-(methoxy-tetraethoxy)-acetophenone (69a)



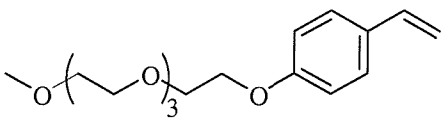
(68a) (1.03g, 2.84mmol, 1eq) and a catalytic amount of potassium iodide (20mg) were dissolved in acetonitrile (15mL), and stirred for 5 minutes at room temperature. A mixture of *p*-hydroxyacetophenone (0.97g, 7.11mmol, 2.5eq) and potassium carbonate (0.98g, 7.11mmol, 2.5eq) dissolved in acetonitrile (10mL), was added. The reaction mixture was refluxed (80°C) for 24 hours. The solvent was removed *in vacuo* and water (30mL) was added. The crude product was dissolved in EtOAc (15mL) and washed with a 10^{-3}M aqueous solution of sodium hydroxide (45mL). The organic layer was dried with sodium sulphate and the solvent removed *in vacuo* to give 0.96g of the title compound as a brown oil. Yield 0.8g, 90%. R_f = 0.30 (Pet:EtOAc 4:6); IR: ν= 2874, 1673, 1599, 1357 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.92 (d, 2H, J=9 Hz, ArH), 6.94 (d, 2H, J=9 Hz, ArH), 4.18 (m, 2H, CH_2OAr), 3.87 (m, 2H, CH_2O), 3.70-3.50 (br m, 12H, CH_2O), 3.37 (s, 3H, OCH_3), 2.55 (s, 3H, $COCH_3$); ^{13}C NMR (75MHz, $CDCl_3$): δ= 196.9 (0); 162.8 (0); 130.7 (0); 130.5 (1); 114.4 (1); 71.0 (2), 70.7 (2), 70.6 (2), 69.6 (2), 67.7 (2); 59.2 (3); 26.5 (3); MS (AP^+): m/z (%): 327.3 (100) $[M+H]^+$, 365.4 (5) $[M+K]^+$; HRMS calcd for $C_{17}H_{26}O_6$ $[M+Na]^+$: 349.1627, found: 349.16216; RP HPLC (ELSD): 8.5 min.

7.3.7 *Synthesis of 1-[4-[2-(2-methoxy-tetraethoxy) phenyl]-ethanol (70a)*



(69a) (0.85g, 2.58mmol, 1eq) was dissolved in ethanol (10mL) and stirred at 0°C. Sodium borohydride (0.20g, 5.30mmol, 2eq) was slowly added in portions. The mixture was stirred for 4 hours and the excess $NaBH_4$ quenched with 10% NH_4Cl in water. The solvent was removed *in vacuo*, water was added and the compound was extracted with DCM. The organic layer was dried with magnesium sulphate and evaporated to give 0.71 g of the title product as yellow oil. Yield 0.7g, 84%. R_f = 0.25 (Pet:EtOAc 4:6); IR: ν= 3420, 2877, 1511 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.28 (d, 2H, J=9 Hz, ArH), 6.88 (d, 2H, J=9 Hz, ArH), 4.83 (q, 1H, J=7 Hz, C*H*CH_3), 4.11 (m, 2H, C*H*$_2$OAr), 3.84 (m, 2H, CH_2O), 3.65-3.50 (m, 12H, CH_2O), 3.36 (s, 3H, OCH_3), 1.45 (d, 3H, J=7 Hz, CHC*H*$_3$); ^{13}C NMR (75MHz, $CDCl_3$): δ= 158.3 (0), 138.4 (0), 127.0 (1), 114.7 (1), 72.0 (1), 70.9 (2), 70.7 (2), 70.6 (2), 70.0 (2), 67.5 (2), 59.0 (3), 25.2 (3); MS (ES^+): m/z (%): 346.4 (20) $[M+NH_4]^+$, 367.4 (100)$[M+K]^+$; HRMS calcd for $C_{17}H_{28}O_6$ $[M+Na]^+$: 351.1779, found: 351.1778; RP HPLC (ELSD): 7.7 min.

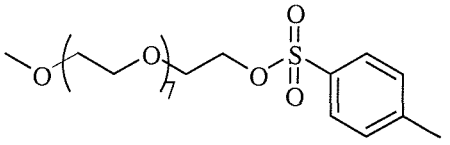
7.3.8 *Synthesis of 1-[2-(2-methoxyethoxy)ethoxy]-4 vinyl benzene (61a)*[160]



(70a) (0.67g, 2.02mmol) and pyridinium tosylate (50mg, 0.2mmol) in toluene (50mL) were refluxed for 24 hours at 120°C using a Dean-Stark trap. The cooled solution was washed with water (50mL) and with a saturated solution of sodium chloride (30mL). The organic layer was dried with sodium sulfate and toluene removed *in vacuo* to give 0.62g

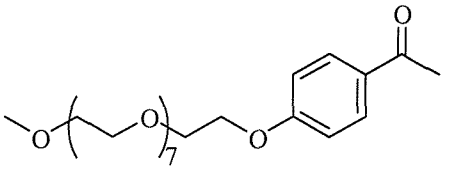
of the title product as a pale yellow oil. Yield 0.6g, 98%. R_f = 0.42 (Pet:EtOAc 4:6); IR: ν= 2869, 1605, 1510 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.33 (d, 2H, J=9 Hz, ArH), 6.87 (d, 2H, J=9 Hz, ArH), 6.66 (dd, 1H, J_{trans}=18 Hz, J_{cis}=11 Hz, C*H*CH_2), 5.60 (d, 1H, J_{trans}=18 Hz, C*H*$_\alpha$H$_\beta$), 5.12 (d, 1H, J_{cis}=11 Hz, CH$_\alpha$*H*$_\beta$), 4.13 (m, 2H, C*H*$_2$OAr), 3.85 (m, 2H, CH_2O), 3.70-3.60 (br m, 12H, CH_2O), 3.37 (s, 3H, OCH_3); ^{13}C NMR (75MHz, $CDCl_3$): δ=158.7 (0), 136.3 (0), 130.7 (1), 127.5 (1), 114.7 (1), 111.8 (2), 70.9 (2), 70.7 (2), 70.6 (2), 69.8 (2), 67.5 (2), 59.2 (3); MS (ES^+): m/z (%): 328.4 (5) $[M+NH_4]^+$, 349.3 (40) $[M+K]^+$; RP HPLC (ELSD): 10.1 min.

7.3.9 Synthesis of 4'-(methoxy-heptaethoxy)-methyl-1-benzensulfonate (68b)



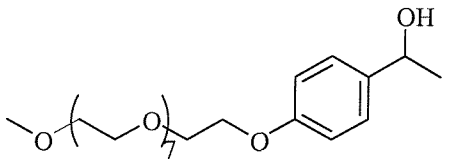
To a cooled solution of PEG350 monomethylether (1g, 2.86mmol, 1eq) and dry triethylamine (2.02g, 20.0mmol, 7eq) in anhydrous DCM (20mL), *p*-toluene sulfonyl chloride (2.84g, 14.3mmol, 5eq) and DMAP (20mg) were slowly added. The mixture was allowed to warm up to room temperature stirred for 7 days and monitored by TLC. The white solid ($Et_3N{\cdot}HCl$) present in the mixture was filtered and the solvent removed *in vacuo* to give a brown oil as crude material. The excess *p*-toluene sulfonyl chloride was removed by treating a solution of the crude in DCM (30mL) with 5 fold excess of polyamine resin (Amberlite IRA-96) overnight. The product was further purified by precipitation in DCM/Pet to give 1g of pure the product as a dark oil. Yield 70%. R_f = 0 (EtOAc:MeOH 8:2); IR: ν= 2873, 1597, 1453, 1352, 1095 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.80 (d, 2H, J=8 Hz, ArH), 7.35 (d, 2H, J=8 Hz, ArH), 4.20 (m, 2H, C*H*$_2$OSO$_2$), 3.65 (m, CH_2O), 3.38 (s, 3H, OCH_3), 2.45 (s, 3H, $ArCH_3$); ^{13}C NMR (75MHz, $CDCl_3$): 144.9 (0), 133.1 (0), 129.9 (1), 128.1 (1), 72.0 (2), 70.8 (2), 70.6 (2), 69.3 (2), 68.8 (2), 59.2 (3), 21.8 (3); MS (ES^+): m/z (%): 568.3 (70) $[M+Na+MeCN]^+$.

7.3.10 Synthesis of 4'-(methoxy-heptaethoxy)-acetophenone (69b)



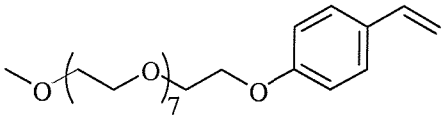
(68b) (0.5g, 1.0mmol, 1eq) and a catalytic amount of potassium iodide (10mg) were dissolved in acetonitrile (10mL), and stirred for 5 minutes. A mixture of *p*-hydroxyacetophenone (0.34g, 2.5mmol, 2.5eq) and potassium carbonate (0.34g, 2.5mmol, 2.5eq) dissolved in acetonitrile (10mL), was added. The reaction mixture was refluxed for 7 days. The solvent was removed *in vacuo* and water (30mL) was added. The crude product was dissolved in EtOAc (15mL) and washed with a 10^{-3} M aqueous solution of sodium hydroxide (45mL). The organic layer was dried with sodium sulphate and the solvent removed *in vacuo* to give 0.37g of the title compound as brown oil. Yield 70%. R_f = 0 (EtOAc:MeOH 8:2); IR: ν= 1739, 1602, 1357 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.93 (d, 2H, J=9 Hz, ArH), 6.96 (d, 2H, J=9 Hz, ArH), 4.19 (m, 2H, CH_2Ar), 3.88 (m, 2H, CH_2O), 3.80-3.50 (br m, CH_2O), 3.38 (s, 3H, OCH_3), 2.56 (s, 3H, $ArCH_3$); ^{13}C NMR (75MHz, $CDCl_3$): 173.9 (0), 162.9 (0), 130.7 (0), 130.5 (1); 114.4 (1); 72.0 (2), 71.0 (2), 70.7 (2), 69.6 (2), 67.7 (2), 59.2 (3), 26.5 (3); MS (ES^+): m/z (%): 532.0 (50) $[M+Na+MeCN]^+$; HRMS calcd for $C_{25}H_{42}O_{10}$ $[M+Na]^+$: 525.2670, found: 525.2678; RP HPLC (ELSD): 8.4 min.

7.3.11 Synthesis of 1-[4-methoxy-heptaethoxy) phenyl]-ethanol (70b)



(69b) (0.63g, 1.33mmol, 1eq) was dissolved in ethanol (5mL) and stirred at 0°C. Sodium borohydride (0.005g, 0.13mmol, 1eq) was slowly added in portions. The mixture was stirred for 5 hours and the excess $NaBH_4$ quenched with 10% NH_4Cl in water. The solvent was removed *in vacuo*, water was added and the compound was extracted with DCM. The organic layer was dried with magnesium sulphate and the solvent removed *in vacuo* to give 0.71 g of title product as yellow oil. Yield 98%. R_f = 0 (EtOAc:MeOH 8:2); IR: ν= 3420, 2873, 1511 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.29 (d, 2H, J=9 Hz, ArH), 6.90 (d, 2H, J=9 Hz, ArH), 4.85 (q, 1H, J=7 Hz, C*H*CH_3), 4.13 (m, 2H, C*H*$_3$Ar), 3.85 (m, 2H, CH_2O), 3.65-3.50 (m, CH_2O), 3.38 (s, 3H, CH_3O), 1.47 (d, 3H, J=7 Hz, CHC*H*$_3$); ^{13}C NMR (75MHz, $CDCl_3$): 158.2 (0), 138.0 (0), 126.7 (1), 114.7 (1), 72.0 (1), 70.9 (2), 70.6 (2), 70.0 (2), 69.9 (2), 67.6 (2); 59.2 (3), 25.2 (3); HRMS calcd for $C_{25}H_{44}O_{10}$ $[M+Na]^+$: 527.2827, found: 527.2836**;** RP HPLC (ELSD): 7.7 min.

7.3.12 Synthesis of 1-[2-(2-methoxy-heptaethoxy)ethoxy]-4 vinyl benzene (61b)



A solution of **(70b)** (0.61g, 0.13mmol) and pyridinium tosylate (3.0mg, 0.012mmol) in toluene (20mL) was refluxed for 64 hours at 120°C using a Dean-Stark trap. The cooled solution was washed with water (20mL) and with a saturated solution of sodium chloride (20mL). The organic layer was dried with sodium sulfate and toluene evaporated to give 0.50g of the title product as a pale yellow oil. Yield 85%. R_f=0 (EtOAc:MeOH 7:3); IR:

ν= 2869, 1606, 1509 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.34 (d, 2H, J=9 Hz, ArH), 6.88 (d, 2H, J=9 Hz, ArH), 6.66 (dd, 1H, J_{trans}=18 Hz, J_{cis}=11 Hz, C*H*CH_2), 5.61 (d, 1H, J_{trans}=18 Hz, CH_αCH_β), 5.12 (d, 1H, J_{cis}=11 Hz, CH_αCH_β), 4.14 (m, 2H, CH_2OAr), 3.86 (m, 2H, CH_2O), 3.70-3.60 (br m, CH_2O), 3.38(s, 3H, OCH_3); ^{13}C NMR (75MHz, $CDCl_3$): 159.0 (0), 136.3 (0), 130.7 (1), 127.5 (1), 114.7 (1), 111.8 (2), 70.9 (2), 70.7 (2), 69.9 (2), 67.6 (2), 59.2 (3); MS (ES^+): m/z (%): 516.2 (10) $[M+Na+MeCN]^+$; RP HPLC (ELSD): 10.1 min.

Scavenger resins as a tool for rapid purification

7.3.13 Purification of 2-(2-methoxyethoxy)ethyl 4-methyl-1-benzensulfonate (68) using PS-aminomethyl resin.

1% DVB PS-aminomethyl resin (bead size: 125-250μm, 990mg, 0.99mmol) was placed into a peptide vessel and swollen in THF (15mL). Crude 2-(2-methoxyethoxy)ethyl 4-methyl-1-benzensulfonate **(68)** (250mg) was dissolved in THF (5mL) and added to the resin together with triethylamine (2mL). The mixture was shaken for 48 hours and filtered. The resin was washed with THF (10mL) and the solvent evaporated *in vacuo* to give 0.21 g of the title compound **(68)** (yield: 84%). RP HPLC (λ = 254): 10.8 min. HPLC purity: 83%.

7.3.14 Purification of 2-(2-methoxyethoxy)ethyl 4-methyl-1-benzensulfonate (68) using Amberlite scavenger resin

Polyamine resin Amberlite IRA-96 (530mg, 3eq) was washed thoroughly with DCM (50mL), MeOH (30mL), Et_2O (30mL) and dried *in vacuo*. The resin was swollen with THF (5mL). 2-(2-methoxyethoxy)ethyl 4-methyl-1-benzensulfonate **(68)** (108 mg) was dissolved in THF (1mL) and added to the resin slurry. The mixture was then shaken overnight. The resin was washed with THF (5mL) and the solvent was evaporated *in vacuo* to give 85 mg of compound **(68)** (yield: 79%). HPLC (λ = 254) purity: 93%

Table 7.5 *Scavenging of excess p-toluene sulfonyl chloride used for the preparation of compound* **(68)**: *performance comparison between AM-PS, Amberlite polyamine resin and Silica gel chromatography.*

Diethylene glycol monomethyl ether (mmol)	Crude product (68) (mmol)	Purification method	Solvent	Yield (%)	HPLC Purity (%)
2.1	1.7	PS-AM*	THF	84	83
0.9	0.7	Amberlite *	THF	77	93
41.6	34	Column Chromatography	Pet/EtOAc	82	100
33.3	19.6	Amberlite*	THF	59	93
200	140	Amberlite*	THF	70	93

*A 5 fold excess of the resin was used with respect to excess of tosyl chloride (1.5 eq)

7.3.15 Large scale synthesis of 4'-(methoxy(ethoxy(ethoxy))-styrene (61)

To cooled a solution of diethylene glycol monomethylether (500g, 4.16mol,) and dry triethylamine (530g, 5.25mol, 1.3eq) in anhydrous DCM (500mL), a solution of *p*-toluene sulfonyl chloride (1190g, 6.24mol, 1.5eq) and DMAP as a catalyst (~1.0g) in DCM (500mL) were slowly added. The mixture was allowed to warm up to room temperature, stirred for 48 hours, and monitored by TLC until completion of the reaction. The white $Et_3N{\cdot}HCl$ salt present in the mixture was removed by filtration. The excess of *p*-toluene sulfonyl chloride was hydrolised by treating the reaction mixture with anhydrous K_2CO_3 (1355g, 9.80mol) and water (4mL) for 72 hours at room temperature. The reaction gave the expected product as shown by ^1H and ^{13}C NMR.

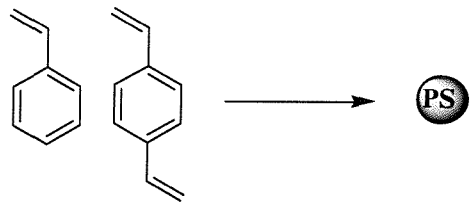
The solvent was removed from the mixture (product **(68)**, K_2CO_3 and DCM) by distillation and replaced with MeCN (500mL). *p*-hydroxy acetophenone (680g, 4.99 mol) was added in 2 portions and the mixture was stirred for 24 hours. Potassium carbonate was then removed by filtration and the solvent was removed *in vacuo*.

A solution of $NaBH_4$ (53.68g, 1446mmol) in EtOH (75mL) and THF (225mL) was added dropwise to a cooled solution of the starting crude product **(69)** (338g, 1.42mol) in THF:EtOH (1:1, 300mL). The reaction mixture was allowed to reach room temperature and stirred overnight. The reaction was then quenched using 5% aqueous NH_4Cl (100mL). Solvents were then removed by distillation. The residue was dissolved in DCM (1L) and washed with brine (x3) to give 283 g of crude compound **(70)**. The crude product was used in the following step without further purification.

The crude product **(70)** (283g) was dissolved in toluene (800mL). Pyridinium tosylate (8g) was added to the mixture and the reaction performed as described in section 7.3.4 to give 273g of crude product **(61)**. Purification was carried out under reduced pressure distillation to yield 43.5g of compound **(61)** (overall yield 10%).

7.4 EXPERIMENTAL TO CHAPTER 3

7.4.1 *Synthesis of DVB-PS resins via multiple parallel suspension polymerisation*[190]



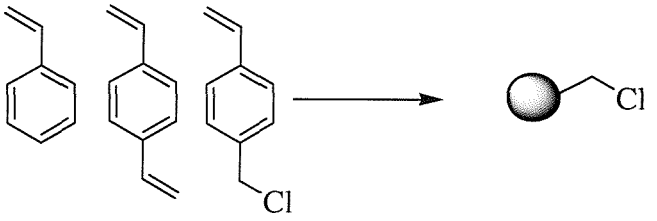
General procedure

Organic phases were typically prepared by mixing the appropriate amount of styrene, DVB and chloromethylstyrene. The proportion between the comonomers was calculated in mole percentage unless otherwise specified. The DVB mole percentage figures were adjusted to account for the real DVB isomer content of the commercial material (80% mol%). The pre-mixed organic phase was added to the aqueous phase (PVA (poly vinyl alcohol, 87-89% hydrolysed-Mr 85-146 kDA), Na_2SO_4, water) while stirring in a 500mL cylindrical reaction vessel equipped with overhead mechanical stirring bars connected to a central stirring motor (a detailed description of the multiparallel polymerisation system is reported in chapter 3) and purged with N_2. The suspensions were allowed to equilibrate for 30 minutes, and the temperature raised to 65°C for 16 h. The crude polymers were collected in 6 polypropylene filtration bags and washed with water, water/THF (1:1), THF, THF/MeOH (1:1), and MeOH. The resins were extracted with methanol *via* a 500mL Soxhlet in six cups for 24 hours. The beads were dried *in vacuo* and sieved to afford resins in 5 or 6 size ranges, (>500, 500-355, 355-250, 250-125, 125-75, 75-45μm).

Sieving: general procedure

The dried resin beads were poured into the first sieve (500μm) of the sieve shaker and left to separate over night. The fractions of beads of different size were collected from each sieve and weighed. The size distribution percentage was then calculated based on the amount of beads of each size range.

7.4.2 Multiple parallel synthesis of chloromethyl polystyrene resins: preliminary studies on the correlation between bead size distribution and ratio (v/v) organic monomers/solvent



A set of 14 resins was prepared following the general method described in section 7.7.1. The organic phase compositions are reported in Table 7.6. The aqueous phases were made up as follows: water (100mL), PVA (1.5%, 1.5g), Na_2SO_4 (5%, 5g). AIBN (1% w/w with respect to monomers, 0.2g) was used as the initiator.

Table 7.6 *Composition of pre-mixed organic phase in suspension polymerisation series of chloromethyl PS resins (chloromethylstyrene 3.75g, 24.6 mmol, loading =1 mmol/g).*

Vessel (batch)	DVB (%)	ratio*	DVB		Styrene		Toluene (mL)
			g	mmol	g	mmol	
1 (A)	1	2/1	0.2	1.5	12.5	120	9
2 (A)	2	2/1	0.5	3.8	15.5	150	11
3 (A)	6	2/1	1.3	10	14.5	140	11
4 (A)	8	2/1	1.8	14	14.5	140	11
5 (A)	15	2/1	3.2	25	12.5	120	11
1 (B)	1	1/1	0.2	1.5	12.5	120	21
2 (B)	2	1/1	0.5	3.8	15.5	150	21
3 (B)	2	4/1	0.5	3.8	15.5	150	5
4 (B)	15	1/1	3.2	25	12.5	120	21
1 (C)	1	5/1	0.2	1.5	12.5	120	4
2 (C)	3	4/1	0.6	5	15.5	150	5
3 (C)	3	2/1	0.6	5	15.5	150	10
4 (C)	2	3/1	0.5	3.8	15.5	150	7
5 (C)	2	5/1	0.5	3.8	15.5	150	4

*Volume ratio between organic monomers and solvent (toluene)

7.4.3 Multiple-parallel polymerisation: set of 1% to 15% DVB-PS resins

The resins were prepared following the general method described in 7.7.1. The water phases were made as follows: water (200mL), PVA (0.5%, 1g), Na_2SO_4 (2.5-5%, 5-10g).

Table 7.7 *Size distribution percentage and yields of chloromethyl-PS resins.*

Entry	Size distribution/ μm							Yield* (%)
	DVB (%)	>500	500-355	355-250	250-125	125-75	75-45	
1A	1	56	21	10	11	1	1	98
2A	2	6	23	26	40	4	1	85
3A	6	7	30	6	56	1	/	87
4A	8	49	27	2	22	/	/	99
5A	15	62	21	4	12	/	/	97
1B	1	79	11	5	5	/	/	67
2B	2	68	7	3	12	5	5	56
3B	2	86	9	3	2	/	/	68
4B	15	29	20	15	31	3	2	78
1C	1	12	25	38	23	1	1	84
2C	3	34	31	23	10	1	1	93
3C	3	43	22	20	14	1	/	87
4C	2	22	21	26	27	2	2	85
5C	2	37	19	27	14	2	1	93

* Yield of beads after sieving

Table 7.8 *Composition of pre-mixed organic phase in suspension polymerisation series of PS resins.*

Entry	Resin cross-linking (%)	Styrene		DVB		Toluene* (mL)
		g	mmol	g	mmol	
1 to 6	2	18.4	177	0.5	4	11
7	1	19.8	190	0.3	2	7
8	3	19.2	185	0.8	6	11
9	4	19	183	1.0	8	11
10	8	18	173	1.9	15	21
11	10	17.5	168	2.5	19	21
12	15	13	126	3.6	28	21

* Initiator AIBN (1.0% w/w) was added to the organic phase.

Table 7.9 *Size distribution percentages and yields of 2% DVB-PS resins. The volume ratio between organic monomers and solvent (toluene) used in the preparation of 2% DVB PS resins was 2/1.*

Entry	Size distribution/ μm					Yield* (%)
	>355	355-250	250-125	125-75	75-45	
1	4	4	82	9	1	60
2	4	4	78	11	3	65
3	/	/	81	12	7	60
4	/	4	90	4	2	64
5	5	7	84	3	1	70
6	1	3	85	10	1	60

*Yield of beads after sieving.

Table 7.10 *Size distribution percentages and yields of 1% to 15% DVB-PS resins.*

Entry	DVB (%)	ratio* (v/v)	Size distribution/ μm					Yield** (%)
			>355	355-250	250-125	125-75	75-45	
7	1	3/1	2	16	78	3	1	70
8	3	2/1	/	1	94	4	1	74
9	4	2/1	/	3	85	8	4	81
10	8	1/1	/	1	87	8	4	73
11	10	1/1	/	/	96	4	/	81
12	15	1/1	/	2	95	2	1	86

* Volume ratio between organic monomers and solvent (toluene)
** Yield of sieved beads

7.4.4 Synthesis of a set of 1% to 8% DVB chloromethyl PS resins (loading: 1 to 2 mmol/g) in buffered solution

The resins were prepared following the general method described in 7.7.1. The water phase consisted of water (200mL), PVA (0.5%, 1g), Na_2HPO_4, (2%, 4g, pH=8).

Table 7.11 *Composition of pre-mixed organic phase in suspension polymerisation series of DVB chloromethyl PS resins.*

Entry	Resin cross-linking (%)	Styrene		DVB		CMS		Toluene (mL)
		g	mmol	g	mmol	g	mmol	
13	1	16.0	154	0.3	2	3.8	25	7
14	2	15.8	152	0.5	4	3.8	25	10
15	2	13.5	129	0.5	4	6.0	40	10
16	3	13.0	125	0.6	5	6.0	40	10
17	4	13.2	127	0.9	7	6.0	40	10
18	8	14.4	140	2.2	14	3.8	25	22

Table 7.12 *Size distribution percentages of 1 % to 8% DVB chloromethyl-PS resins.*

Entry	loading (mmol/g)	DVB (%)	ratio* (v/v)	Size distribution/ μm				
				>355	355-250	250-125	125-75	75-45
13	1	1	3/1	4	2	89	2	3
14	1	2	3/1	/	3	90	6	1
15	2	2	2/1	/	1	87	9	3
16	2	3	2/1	1	/	86	9	4
17	2	4	2/1	6	10	66	10	8
18	1	8	1/1	/	1	60	23	16

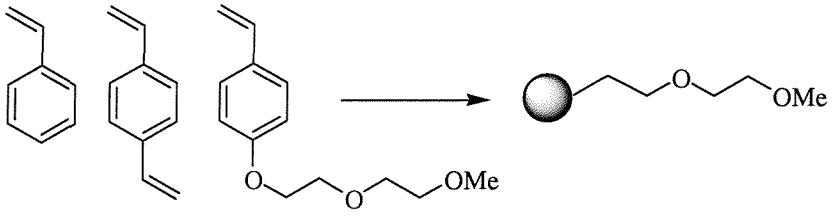
* Volume ratio between organic monomers and solvent (toluene)

Table 12a *Sieved beads yields of 1 % to 8% DVB CMS-PS resins.*

Entry	13	14	15	16	17	18
Yield (%)	80	98	96	95	98	90

7.5 EXPERIMENTAL TO CHAPTER 4

Synthesis of PEG-PS resins containing monomer (61)



(61)

7.5.1 General suspension polymerisation protocol (method 1)

Polyvinyl alcohol AIRVOL-540 (87-89% hydrolysed-Mr 85-146 kDA) (0.75%, 1.5g) and Na_2SO_4 (5%, 10g) were added to deionized water (200mL) in a reactor and stirred at room temperature until dissolution. Styrene, DVB and PEG monomer **(61)** were mixed with AIBN (1%, 0.4g, 2.4 mmol) and dissolved in toluene (10 to 40mL according to the appropriate organic comonomers/diluent ratio). The organic phase was added to the aqueous phase and stirred under nitrogen atmosphere at 400-450 rpm overnight at 65°C. The reaction mixture was allowed to cool to 30°C and filtered. The beads were washed with water (500mL), THF/water 1:1 (250mL), THF (250mL), MeOH/THF 1:1 (250mL), MeOH (100mL) and dried *in vacuo*. The beads were weighed and sieved.

7.5.2 General suspension polymerisation protocol (method 2)

For this method the same procedure as above was followed using different amount of polyvinyl alcohol AIRVOL-540 (1.5%, 3g) and Na_2SO_4 (10%, 20g).

Table 7.13 *Composition of pre-mixed organic phase in suspension polymerization series of DVB PEG-PS resins containing monomer* ***(61)****. The comonomers/toluene ratio in volume was 1/1 for all the resins in this table.*

Resin	DVB (%)	Monomer (61) (%)	Monomer (61)		Styrene		DVB		CMS	
			g	mmol	g	mmol	g	mmol	g	mmol
R1	8 a	12	8.8	40	27.5	264	3.6	27.7	/	/
R2	8	12	8.8	40	27.5	264	3.6	27.7	/	/
R3	3	12	8.8	40	30.0	287	1.3	9.6	/	/
R4	2	12	8.8	40	30.3	292	0.8	6.0	/	/
R5	3	6	4	18	30.4	292	1.6	12.3	/	/
R6	3	6	4	18	34.4	330	1.6	12.3	6	40
R7	8	6	4	18	32.0	307	4.0	31.0	6	40

a) Resin prepared following method (1)

Table 7.14 *Yields and size distribution percentages of 2 % to 8% DVB PEG-PS resins containing monomer* ***(61)****.*

Resin	DVB (%)	Yield * (%)	Size distribution/ μm				
			>500	500-355	355-250	250-125	125-45
R1	8	36	5.0	5.0	16.0	60.0	14.0
R2	8	60	9.0	1.0	4.0	60.0	26.0
R3	3	N/A**					
R4	2	25	94.0	2.0	2.0	/	/
R5	3	73	26.0	28.0	10.0	35.0	1.0
R6	3	90	40.0	20.0	12.0	20.0	8.0
R7	8	83	10.0	6.0	4.0	64.0	16.0

*Yields calculated after sieving

Size distribution percentage of resin **R3 could not be calculated due to aggregation among beads occurred during the polymerisation process.

7.5.3 Synthesis of chloromethyl functionalized DVB PEG-PS resins containing monomer (61) via multiple-parallel suspension polymerisation

The synthesis of this set of resins was performed using the multiple parallel polymerisation system described in chapter 3. The resins were prepared following the general method described in 7.7.1.

The aqueous phases were prepared as follows: water (200mL), PVA (0.5%, 1.0g), Na_2SO_4 (2.5-5%, 5-10 g). Stirring speed was 400 rpm.

Table 7.15 *Composition a series of 2% DVB chloromethyl functionalized resin containing PEG monomer (61). The comonomers /toluene ratio in volume was 1/1* ***R8*** *and 2/1 for* ***R9-R12.***

Resin	Monomer (64) (%)	Monomer (64)		Styrene		DVB		CMS	
		g	mmol	g	mmol	g	mmol	g	mmol
R8	7	2.7	12	14.0	133	0.5	3.5	3.8	25
R9	7	2.7	12	14.0	133	0.5	3.5	3.8	25
R10	14	5.0	22	11.0	104	0.5	3.5	3.8	25
R11	24	5.8	26	6.7	64	0.3	2.3	2.3	15
R12	43	8.9	40	3.6	35	0.3	2.3	2.3	15

Table 7.16: *Yields and size distribution percentages of 2% DVB PEG-PS resins containing monomer (61)*

Resin	Yield * (%)	Size distribution/μm				Elemental analysis (%)		
		>355	355-250	250-125	125-45	C	H	Cl
R8	64	1.0	4.0	75	20	N/A		
R9	66	2.0	2.0	70	26	85.20	7.60	3.91
R10	60	0.4	0.6	8	91	85.50	7.46	4.47
R11	78	N/A**				79.83	7.58	3.16
R12	71	N/A**				75.95	7.74	3.40

*Yields calculated after sieving; **Size distribution percentage of resin **R11** and **R12** could not be calculated due to aggregation among beads occurred during the polymerisation process.

7.5.4 Swelling experiments: general method

Swelling experiments were conducted by the addition of solvent to resin (1g) in 12 mL polypropylene tubes followed by application of a sharp tapping motion to the side of the tubes to remove trapped bubbles, solvent draining by vacuum and re-equilibration for 15 minutes with mechanical agitation. Readings were recorded when consistency between the following two sampling methods was achieved. The solvent was removed by gravity with agitation of the tubes and also by compression of a syringe barrel until the resin posed a resistance to the compression. The resin was then washed with DCM (3 x bed volumes), and the solvent drained by vacuum and Et_2O (2 x bed volumes) and then drained by vacuum, followed by the addition of the new solvent. Swelling studies were performed on resin beads having size between 75 and 125 micron. Results for the swelling parameters for each of the different cross-linked PS-DVB and PS-PEG resins in each solvent used are shown in Table 7.17.

7.5.5 Chemical stability test

200mg of each resin (incorporated monomer **(61)**: 7%, 12%, 24%, 43% respectively) was placed in a 5mL polyethylene tube. Reagents (>20mmol/g of resin) were added to each of the tubes and shaken over a period of 4 hours. The resins were the filtered and washed with DCM or water depending on the reagent used for the test. The reagents used for these experiments were: aqueous NaOH (2N) and HCl (6N) and neat MeI, Ac_2O, TFA/DCM (1:1), TFA/H_2O (95:5).

7.5.6 Mechanical stability test

100 mg of resin was suspended in DCM (10 mL), and shaken for 48 hours and an aliquot of the suspension removed from the vials for microscope analysis.

Table 7.17: *Effect of PEG monomer* ***(61)*** *on the swelling properties of a series of 2% to 8% DVB-PS resins. Resin swelling experiments in a range of solvents. (Data quoted in the table are in units of mL per gram of resin ± 0.20mL/g). Volumes of dry resins were determined to be 1.8-2.0 mL/g; c= chloromethyl functionalised resin.*

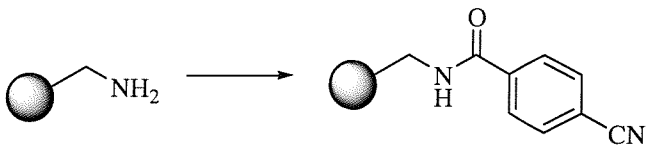
Mon. (%)	DVB (%)	DCM	Diox.	THF	DMF	DME	Toluene	MeOH	iPrOH	H_2O
0	2c	6.6	6.0	7.0	5.2	6.2	6.4	2.4	2.4	2.2
7.0	2c	8.2	7.0	8.4	5.8	5.8	6.6	2.8	2.6	2.4
14	2c	7.6	6.8	7.2	5.8	5.8	7.0	2.8	2.6	2.6
24	2c	8.0	6.8	6.8	6.6	6.6	7.6	2.8	2.8	2.4
43	2c	8.2	8.2	8.2	8.0	8.6	9.4	3.0	2.8	2.8
TG	1	5.5	3.9	3.9	4.0	4.0	5.2	3.0	3.0	3.0
AG	1	8.6	6.4	6.4	7.0	6.0	6.2	4.9	4.9	4.0
0	3	5.0	5.0	5.2	4.2	4.8	5.2	2.2	2.0	2.0
5	3c	6.8	6.0	6.4	5.0	5.8	5.8	2.8	2.8	2.8
12	3	8.0	8.0	8.4	6.4	7.4	8.2	2.6	2.4	2.6
0	4	4.8	5.0	5.2	4.0	4.4	5.0	2.2	2.2	2.2
5	4c	6.8	5.8	6.2	5.0	5.8	5.8	2.4	2.4	2.4
0	7.5	3.4	4.0	3.6	2.8	3.2	3.6	2.2	2.0	2.0
12	7b	5.6	5.0	5.2	4.0	4.5	5.6	2.6	2.6	2.8
5	8c	4.0	5.2	4.4	3.4	4.0	4.0	2.4	2.4	2.4

TG= TentaGel resin; AG= ArgoGel resin; Diox.= Dioxane.

7.6 EXPERIMENTAL TO CHAPTER 5

Site distribution studies on resin beads by Confocal Raman Spectroscopy

7.6.1 Preparation of resin samples for Raman Screening: 4-cyano-benzoic acid coupling



A small amount (20mg) of each resin in Table 7.18 (bead size: 75-125μm) was swollen in a minimum amount of DMF for 30 minutes. 4-cyano-benzoic acid (5eq) was dissolved in DMF (2mL), DIC (5eq) was added to the acid and the mixture stirred for 10 minutes before addition to the resins. The resins were agitated for 3 hours to effect coupling. The resins were washed with DMF (3x2mL), DCM (3x2mL), MeOH (3x2mL) and Et_2O (2x2mL) and dried under vacuum for 30 minutes. A qualitative ninhydrin test was negative.

Table 7.18 *Loading of aminomethyl PS resins used for the preparation of samples for Raman Analysis.*

Resin	Loading (mmol NH_2/g)
AM-R9*	0.77
AM-R10*	0.63
AM-R11*	0.70
AM-R12*	0.67
2% DVB-AM-PS*	0.70

*Prepared from chloromethyl-PS resin (see synthesis in section 7.7.1)

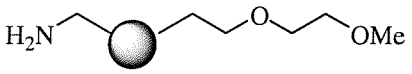
7.6.2 Microscopic manipulation[125]

A sample (2-3 mg) of resin fully loaded with 4-CN-benzoic acid was placed into the cavity of a microscope slide, swollen in DMF and covered with a 0.1 mm thick glass plate, which was fixed along the edges with three layers of parafilm, in order to prevent evaporation of the solvent during the measurement (~12 hours). An area of 148μm x 148μm in the *xy* plane through the equator of the bead was scanned in 2μm steps (giving a total of 5476 data points). Due to the confocal depth resolution this corresponds to approximately a 3μm slice through the bead. For the dry beads an area 70μm x 70μm was scanned in 1μm steps (4900 data points). In the mapping experiments a 50 x objective was usually used, the slit set to 10μm on the spectrograph entrance assembly and the CCD area set to a four pixel height, in contrast to a 20μm slit width and a 20 pixel height for a non-confocal experiment. A silicon flat was used to determine the thickness of the confocal optical section. The measured depth profile gave a FWHM resolution of 3μm. This results in a three-dimensional resolution of typically greater than about 1μm x1 μm x 3 μm as the bead samples have a lower refractive index than the Si flat. For a line map the spectra were collected with between 2300cm^{-1} and 900cm^{-1} to allow both the peak of interest (2230cm^{-1}) and the reference polymer peak (1001cm^{-1}) to be observed. The area maps were recorded with a static scan with a range of 453 cm^{-1} (with the 1800 groove grating) centred at the peak of interest. The area to be mapped is drawn in the software. The bead was focussed manually on the bead centre. The bi-directional scanning mode was used to avoid any jumps in the objective that may have caused the bead to move. For long scan (8h plus) "cosmic ray removal" was turned on to remove large sporadic noise peaks disturbing the data. The spectra of the loaded beads were analysed using the GRAMS curve fitting application and the maps were created using the mapping program supplied with WiRE (Windows-based Raman environment) program from Renishaw.

7.7 EXPERIMENTAL TO CHAPTER 6

Solid Phase Synthesis

7.7.1 Synthesis of aminomethyl PEG-PS resin (AM-R9, AM-R10, AM-R11, AM-R12)[218]



Phthalimido resin

The resins were stirred for 10 minutes in DMF. Potassium phthalimide (5eq, c=0.5M) was added portionwise at room temperature and the resulting mixture stirred overnight at 120°C. The resins were then filtered and washed with DMF (3x20mL), DMF/H_2O (1:1 v/v, 3x20mL), H_2O (20mL), Dioxane (20mL), MeOH (20mL) and Et_2O (see Table 7.19).

Table 7.19 *Reagents stoichiometry in the preparation of phthalimido-intermediate resins.*

Resin	loading (mmol Cl/g)	Quantity (g)	Potassium Phthalimide (mmol)	DMF (mL)
R9	1.0	5.0	27.5	56
R10	1.0	5.3	31.5	64
R11	1.0	3.2	14.0	34
R12	1.0	1.1	5.3	13

The phthalimido resins (Table 7.20) were suspended and stirred in ethanol. Hydrazine (15eq) was added and the mixture refluxed overnight. The resins were washed with hot DMF (3x10-20mL), hot water/DMF (1:1 v/v, 3x20mL), hot water, dioxane (3x20mL), MeOH (3x20mL) and Et_2O (20mL). Then dried *in vacuo*. The loading of the aminomethyl resins was calculated by Fmoc-Gly-OH coupling followed by quantitative Fmoc test according to method B and C respectively (see section 7.2).

Table 7.20 *Reagents stoichiometry in the preparation of resins* ***AM-R9, AM-R10, AM-R11 and AM-R12.***

Phthalimido Resin	Quantity (g)	Hydrazine (mmol)	EtOH (mL)	loading (mmol NH_2/g)
R9	5.50	90	50	0.77
R10	5.30	100	55	0.63
R11	3.18	42	23	0.70
R12	1.03	15	8	0.67

Parallel peptide synthesis on resins AM-R9, AM-R10, AM-R11, AM-R12, TentaGel, AM-PS (1% and 2%DVB)

7.7.2 Rink amide aminomethyl PS resin (81)

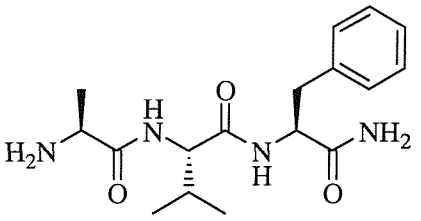
The Fmoc-Rink linker, *p*-[(*R,S*)-α-[1-(9*H*-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid (1.5eq) was coupled to the amino methyl resins (Table 7.21, beads size: 75-125□m) according to method A (described in section 7.2). The qualitative ninhydrin test was negative and the loading of the resins was calculated by quantitative Fmoc test according to method C. The Rink-PS resins were deprotected according to method B.

Table 7.21 *Reagents stoichiometry in the preparation of Rink amide aminomethyl PS resins.*

Resin	L_i (mmol/g)	Resin (g)	Fmoc Rink linker (mmol)	L_f (mmol/g)
AM-R9a)	0.68	0.6	0.6	0.65
AM-R9	0.77	0.2	0.3	0.56
AM-R10	0.63	0.2	0.2	0.48
AM-R11	0.70	0.2	0.2	0.54
AM-R12	0.67	0.2	0.2	0.50
TentaGel	0.25	0.4	0.2	0.20
AM-PS (1%)	1.13	0.2	0.3	0.75
AM-PS (2%)	1.0	0.2	0.3	0.70

a) beads size: 125-250μm; L_i= loading of aminomethyl resin; L_f= loading of Rink amide AM resins.

7.7.3 Solid Phase Synthesis of Ala-Val-Phe-NH_2 (85)



FmocNH-Rink amide PS-AM resins **(81)** were agitated in 20% piperidine in DMF (5mL) for 20 minutes and washed with DCM (3x10mL), DMF (3x10mL) and DCM (3x10mL). A qualitative ninhydrine test was positive. *N*-Fmoc-phenylalanine (2eq) and HOBt (2eq) were dissolved in 9:1 DCM: DMF (5mL) and stirred for 10 minutes. DIC (2eq) was added and the mixtures stirred for further 10 minutes before addition to the pre-swollen resins. The suspensions were agitated for 3 hours. After this period of time qualitative ninhydrin tests were negative. The resins were washed following the above mentioned cycle and the deprotection step repeated. Qualitative ninhydrin tests were positive.

N-Fmoc-Valine and *N*-Fmoc-Alanine respectively were coupled to Phe-Rink amide PS resins following the same method (described above) used for *N*-Fmoc phenylalanine coupling. The resins were washed with DCM (3x10mL), DMF (3x10mL), DCM

(3x10mL) and dried. The resins were agitated in 20% piperidine in DMF (5mL) for 20 minutes and washed using the usual washing cycle. TFA/DCM (95:5) (1mL/100mg of resin) was added to the resins and the mixtures agitated for 3 hours. The resins were removed by filtration, the filtrate concentrated to *ca.* 1mL and added to cold Et_2O (25mL). The resulting precipitates were collected by centrifugation and washed with Et_2O (2 x 25mL). Yields and purity relative to the entire set of resins are summarized in Table 7.22. R_f = 0.50 (DCM:MeOH 9:1); M.p. 215-216°C; IR: ν=3375-3299, 1671, 1632 cm^{-1}; ^1H NMR (400 MHz,$CD_3SO_2CD_3$): δ=8.34 (d, 1H, J=9 Hz, NH), 8.10 (d, 1H, J=8 Hz, NH), 7.43 (s, 2H, $CONH_2$), 7.23 (m, 5H, ArH), 7.10 (s, 2H, CH_3CHNH_2), 4.54 (ddd, 1H, J_1=5 Hz, J_2=9 Hz, J_3=9 Hz, $CHCH_2$Phe), 4.23 (m, 1H, $CHCH(CH_3)_2$), 3.94 (q, 1H, J=7 Hz $CHCH_3$), 3.04 (dd, 1H, J_1=5 Hz, J_2=14 Hz $CHCH_\alpha H_\beta$Phe), 2.85 (dd, 1H, J_1=9 Hz, J_2=14 Hz $CHCH_\alpha H_\beta$Phe), 1.99 (m, 1H, $CHCH(CH_3)_2$), 1.30 (d, 3H, J=7 Hz, CH_3CHNH_2), 0.87 (d, 6H, J=7 Hz, $CH(CH_3)_2$); ^{13}C NMR (100MHz, $CD_3SO_2CD_3$): 173.1 (0), 170.5 (0), 170.0 (0), 138.2 (0), 129.5 (1), 128.4 (1), 126.6 (1), 58.3 (1), 53.9 (1), 48.5 (1), 38.2 (2), 31.1 (1), 19.6 (3), 18.5 (3), 17.9 (3); MS (ES^+): m/z (%): 335.3 (100) $[M+H]^+$, 669.5 (5) $[2M+H]^+$; HRMS calcd for $C_{17}H_{26}N_4O_3$ $[M+Na]^+$: 357.1897, found: 357.1890; RP HPLC (λ = 220): 6.5 min.

Table 7.22 *Yields and purity of Ala-Val-Phe-NH_2 (85).*

Resin	Monomer (61) (%)	HPLC Purity (%)	Yield* (%)
R9 a)	7	94	85
R9	7	94	80
R10	12	94	50
R11	24	85	65
R12	38	89	58
TentaGel	0	83	43
AM-PS (1%)	0	95	72
AM-PS (2%)	0	95	56

a) beads size: 125-250μm
* Isolated yield of peptide **(85)**

Parallel synthesis of N-(4'- phenylbenzoyl)glycinamide via Suzuki Coupling on resins AM-R9, AM-R10, AM-R11, AM-R12, TentaGel, AM-PS (1%DVB)

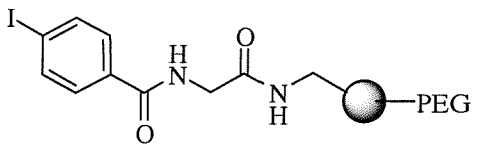
7.7.4 Gly-Rink AM-PS resin (90)

Rink amide-AM-PS resins **(81)** (Table 7.23, beads size: 125-75μm) were coupled to *N*-Fmoc-Glycine (5eq) according to method A. The loading of the resins was calculated by quantitative Fmoc test according to method C. Fmoc protecting group was removed according to method B.

Table 7.23 *Reagents stoichiometry in the preparation of Gly- Rink AM-PS resin (90)*

Resin	Resin (81) (mg)	Fmoc-Gly-OH, HOBt and DIC (mmol)	DCM/DMF (mL)
AM-R9	680	1.34	7
AM-R10	980	1.57	7
AM-R11	850	1.75	7
AM-R12	610	1.17	7
TentaGel	560	0.78	5
AM-PS (1%)	675	2.71	7

7.7.5 N-(4'-Iodobenzoyl)glycyl Rink PEG-PS resin (91)

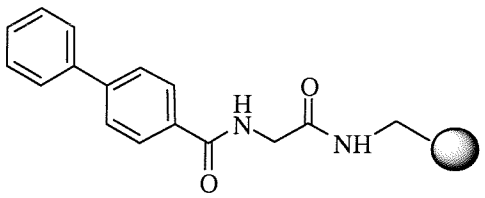


Gly-Rink AM-PS resins **(90)** (Table 7.24) were swollen in DCM/DMF (9:1) and after 5 minutes the solvent was drained. 4-Iodobenzoic acid (3eq) was coupled according to method A. Qualitative ninhydrin test was negative.

Table 7.24 *Reagents stoichiometry in the preparation of N-(4'-Iodobenzoyl)glycyl Rink PEG-PS resin* ***(91)***

Resin	Resin (90) (mg)	4-Iodobenzoic acid, HOBt and DIC (mmol)	DCM/DMF (mL)
R9	640	1.48	7
R10	950	1.79	7
R11	930	1.95	7
R13	620	1.24	7
TentaGel	450	0.34	5
AM-PS (1%)	697	3.35	7

7.7.6 N-(4'- phenylbenzoyl)glycyl Rink PEG-PS resin (92)

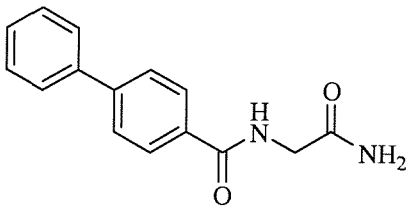


Resins **(91)** (Table 7.25) were suspended in DMF and Cs_2CO_3 (2eq), $Pd(PPh_3)_4$ (0.1eq) and phenyl boronic acid (1.5eq) were added under a N_2 atmosphere. The resulting suspensions were heated at 100°C for 24h. Resins were then filtered and washed with DMF (3x10mL), DCM (3x10mL), MeOH (2x10mL) and Et_2O (2x10mL).

Table 7.25 *Reagents stoichiometry in the Suzuki coupling for the preparation N-(4'-phenylbenzoyl)glycyl Rink PEG-PS resins* ***(92)****.*

Resin	Resin (91) (mg)	Phenyl boronic acid (mmol)	$Pd(PPh_3)_4$ (mmol)	Cs_2CO_3 (mmol)	DMF (mL)
R10	790	0.90	0.06	1.22	12
R11	1160	1.1	0.07	1.6	15
R12	990	1.0	0.07	1.4	14
R13	780	0.8	0.05	1.0	12
TentaGel	460	0.2	0.013	0.3	7
AM-PS (1%)	850	2.0	0.14	2.8	14

7.7.7 *N-(4'- phenylbenzoyl)glycinamide (93)*[219]



Resins **(92)** were then treated following method F. The crude materials were analysed by HPLC and ES-MS and purified by preparative HPLC. Yields and purity are presented in Table 7.26.

M.p.: 210-212°C; IR: ν= 3382, 3330, 1660, 1633 cm^{-1}; ^1H NMR: δ=(400 MHz, $CD_3SO_2CD_3$): 8.81 (t, 1H, J=6 Hz, CON*H*), 8.09 (d, 2H, J=8 Hz, ArH), 7.89 (d, 2H, J=8 Hz, ArH), 7.85 (d, 2H, J=7 Hz, ArH), 7.61 (t, 2H, J=7 Hz, ArH), 7.52 (t, 1H, J=7 Hz, ArH), 3.96 (d, 2H, J=6 Hz, COCH_2); ^{13}C NMR (100MHz, $CD_3SO_2CD_3$): δ=169.8 (0), 164.8 (0), 141.5 (0), 137.9 (0), 131.7 (0), 127.8 (1), 126.8 (1), 125.6 (1), 125.2 (1), 41.2 (2); MS (ES^+): m/z (%): 255.0 (30) $[M+H]^+$, 318.0 (100) $[M+Na+MeCN]^+$, 531.1 (80) $[2M+Na]^+$, 785.0 (20) $[3M+Na]^+$; HRMS calcd for $C_{15}H_{14}N_2O_2$ $[M+Na]^+$: 277.0950, found: 277.0947; RP HPLC (λ = 254): 8.1 min.

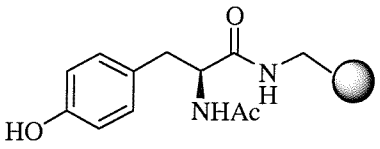
Table 7.26 *Yields and purity of compound* ***(93)***

Resin	Monomer (61) (%)	Compound (93) (mg)	HPLC Purity (%)	Overall Yield (%)*
R9	7	64	99	97
R10	12	70	95	80
R11	24	37	63	74
R12	38	40	84	84
TentaGel	0	27	84	94
AM-PS (1%)	0	50	97	71

* Calculated by HPLC

Parallel synthesis of 2-(acetylamino)-3-[4-(benzyloxy)phenyl]propanamide via Mitsunobu coupling on resins AM-R9, AM-R10, AM-R11, AM-R12, TentaGel, 1% DVB-AM-PS

7.7.8 N-Acetyltyrosyl Rink PEG-PS resin (94)

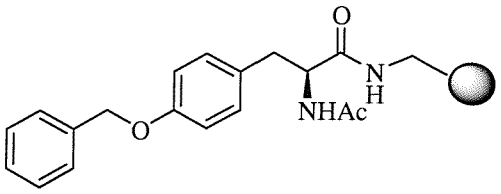


Rink amide-AM-PS resins **(81)** (Table 7.27, beads size: 75-125μm) were coupled to *N*-acetyltyrosine (5eq) according to Method A.

Table 7.27 *Reagents stoichiometry in the preparation of N-Acetyltyrosyl Rink PEG-PS resins* ***(94)***

Resin	Resin (81) (mg)	N-acetyltyrosine, HOBt and DIC (mmol)	DCM/DMF (mL)
AM-R9	298	1.2	3
AM-R10	316	0.1	4
AM-R11	310	1.1	4
TentaGel	448	0.6	4
AM-PS (1%)	350	2.9	4

7.7.9 2-(acetylamino)-3-[4-(benzyloxy)phenyl]propanamide-Rink AM-PS resin (95)

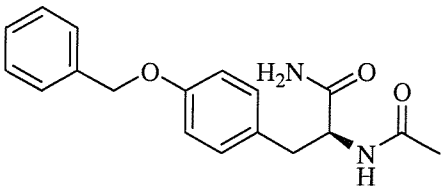


N-Acetyltyrosyl Rink PEG-PS resins (**94**) (Table 7.28) were washed with dry THF (3 x 7mL) and swollen in the same solvent. THF was drained after 5 minutes. A solution of PPh_3 (5eq) and benzyl alcohol (10eq) in dry THF was added. DEAD (5eq) was added portionwise at 5 minutes intervals. The resins were shaken overnight, washed and dried under vacuum. After performing small TFA cleavages on approximately 5mg of resin, the reactions were monitored by HPLC.

Table 7.28 *Stoichiometry of reagents in the Mitsunobu coupling for the preparation of resins (95).*

Resin	Resin (94) (mg)	Benzyl alcohol (mmol)	PPh_3 and DEAD (mmol)
R10	410	3.1	1.6
R11	446	2.8	1.4
R12	417	2.9	1.5
TentaGel	528	1.5	0.8
AM-PS (1%)	535	8.8	4.4

7.7.10 2-(acetylamino)-3-[4-(benzyloxy)phenyl]propanamide (96)[123c]



Resins **(95)** were then treated following method F. The crude materials were analyzed by HPLC and ES-MS and purified by preparative HPLC. Yields and purity are presented in Table 7.29.

IR: ν= 3373, 3317, 1660, 1639, 1510 cm^{-1}; ^1H NMR: δ=(400 MHz, $CD_3SO_2CD_3$): 8.06 (d, 1H, J=8 Hz, CON*H*), 7.48 (m, 5H, ArH), 7.25 (d, 2H, J=8 Hz, ArH), 7.09 (broad s, 2H, CONH_2), 7.01 (d, 2H, J=8 Hz, ArH), 5.16 (s, 2H, OCH_2Phe), 4.34 (m, 1H, CONHC*H*), 3.02 (dd, 1H, J_1=5 Hz, J_2=14 Hz, CH_αH$_\beta$Phe), 2.78 (dd, 1H, J_1=9 Hz, J_2=14 Hz, CH$_\alpha$$H_\beta$Phe), 1.85 (s, 3H, $COCH_3$); ^{13}C NMR (100MHz, $CD_3SO_2CD_3$): δ=173.8 (0), 169.4 (0), 157.3 (0), 137.7 (0), 130.7 (0), 130.5 (1), 128.8 (1), 114.8 (1), 69.6 (2), 54.5 (1), 37.3 (2), 23.0 (3); MS (ES^+): m/z (%): 313.2 (70) $[M+H]^+$, 335.0 (10) $[M+Na]^+$, 625.3 (10) $[2M+1]^+$, 647.3 (10) $[2M+Na]^+$; HRMS calcd for $C_{18}H_{20}N_2O_3$ $[M+H]^+$: 313.1553, found: 313.1547; RP HPLC (λ = 254): 8.6 min.

Table 7.29 *Yields and purity of 2-(acetylamino)-3-[4-(benzyloxy)phenyl]propanamide (96).*

Resin	Monomer (61) (%)	Compound (96) (mg)	HPLC Purity (%)	Overall Yield* (%)
R9	7	56	80	93
R10	12	40	77	63
R11	24	43	77	72
TentaGel	0	32	68	80
AM-PS (1%)	0	81	51	60

*Obtained by HPLC

References

[1] Czanik, A. W. *Anal. Chem.* **1998**, 378A.

[2] Floyd, C. D.; Lewis, C. N.; Whittaker, M. *Chemistry in Britain*, **1996**, 31.

[3] Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233.

[4] Merrifield, R. B. *J. Am. Soc.* **1963**, *85*, 2149.

[5] Gutte, B.; Merrifield, R. B. *J. Biol. Chem.* **1971**, *246*, 1922.

[6] Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555.

[7] Wyatt, J. R.; Vickers, T. A.; Roberson, J. L.; Buckheit Jr, R. W.; Klimkait, T.; DeBaets, E.; Davis, P. W.; Rayner, B.; Imbach, J. L.; Ecker, D. J. *Proc. Natl. Acad. Sci.* **1994**, *91*, 1356 and references therein.

[8] Flynn, D. J.; Crich, J. Z.; Devraj, R. V.; Hockerman, S. L.; Parlow, J. J.; South, M. S.; Woodard, S. *J. Am. Chem. Soc.* **1997**, *119*, 4874.

[9] Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci.* **1992**, *89*, 9367.

[10] Zuckermann, R. N,; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

[11] Cho, C. Y.; Moran, E. J.; Cherry, S. R.; Stephans, J. C.; Fodor, S. P. A.; Adams, C. L.; Sundaram, A.; Jacobs, J. W.; Schultz, P. G. *Science* **1993**, *261*, 1303.

[12] Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997.

[13] Ordon, D. W.; Steale, J. *Bio. Med. Chem. Lett.* **1995**, *5*, 47.

[14] Beebe, X.; Schore, N. E.; Kurth, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 10061.

[15] Kurth, M. J.; Ahlberg Randall, L. A.; Chen, C.; Melander, C.; Miller, R. B.; Mclister, K.; Reitz, G.; Kang, R.; Nakatssu, T.; Green, C. *J. Org. Chem.* **1994**, *59*, 5862.

[16] Renil, M.; Meldal, M. *Tetrahedron. Lett.* **1996**, 37, *34*, 6185.

[17] Sherrington, D. C. *Chem Commun*, **1998**, 2275.

[18] Grubbs, R. H.; Kroll, L. C. *J. Am. Chem. Soc.* **1971**, *93*, 3062.

[19] Trost, B. M.; Keinan, E. *J.Am. Chem. Soc.* **1978**, *100*, 7780.

[20] Simon, E. *Ann. Pharm.* **1839**, *31*, 265.

[21] Calmon, C. *React. Polym.* **1982**, *1*, 3.

[22] Sarkstein, E. *Biochem. Z.* **1910**, *24*, 21.

[23] Amos, J. L. *Polym. Eng. Sci.* **1974**, *14*, 1.

[24] R. H. M. Simon and D. C. Chappelear, in *Polymerisation Reactions and Processes* Henderson, J. N.; Bonton, T. C. Eds. ACS Symposium Series, 104, 71, American Chemical Society, Washington DC, **1979**.

[25] Carothers, W. H.; Arvin, J. A. *J. Am. Chem. Soc.* **1929**, *51*, 2559.

[26] Cameron, G. G.; Law, L. S. *Polymer.* **1981**, *22*, 272.

[27] Durst, H. P. *Tetrahedron Lett.* **1974**, 2421.

[28] Ugelstad, J.; Mørk, P. C. *Adv. Coll. Int. Sci.* **1980**, *13*, 101.

[29] Yuan, H. G.; Kalfas, G, Ray, W. H. J. *Macromol. Sci. Rev. Macromol. Chem. Phys.* **1991**, C31, 215.

[30] Vivaldo-Lima, E.; Wood, P. E.; Hamielec, A. E.; Penlidis, A. *Ind, Eng, Chem. Res.* **1997**, *36*, 939.

[31] Arshady, R. *Makromol. Chem.* **1984**, *185*, 2387.

[32] Cooper, A. J. *J. Mater. Chem.* **2000**, *10*, 207.

[33] Wulff, G. Angew. *Chem. Int. Ed. Engl.* **1995**, *34*, 1812.

[34] Rapp, W. Combinatorial Peptide and Nonpeptide Libraries. Jung, G (Eds). New York: VCH; **1996**, 425.

[35] Butwell, F. G. W.; Hawks, E. J.; Epton, R. *Macromol. Chem. Macromol. Symp.* **1986**, *19*, 69.

[36] Atherton, E.; Brown, E.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1981**, 1151.

[37] Bayer, E. Rapp, W.; Poly(ethylene Glycol) Chemistry : Biotechnical and Biomedical Applications, edited by J. Milton Harris, Plenum Press; New York, **1992**, 325.

[38] Labadie, J. W. *Curr. Op. Chem. Biol.* **1998**, *2*, 346.

[39] Toy, P. H.; Janda, K. D. *Tetrahedron Lett.* **1999**, *40*, 6329.

[40] Kempe, M.; Barany, G. *J. Am. Chem. Soc.* **1996**, *118*, 7083.

[41] Dariak, K.; Narayana, C.; Spatola, A. F. Proceeding of the 5th International Symposium on Solid Phase Synthesis and Combinatorial Libraries. Epton, R (Eds.) London: Mayflower.

[42] Argonaut Technical Bulletin: San Carlos; no DS-015.

[43] Small, P. W.; Sherrington, D. C. *J. Chem. Soc., Chem. Commun.* **1989**, 1589.

[44] Bayer, E.; Hemmasi, B.; Albert, K.; Rapp, W.; Dengler, M. Peptide: Structure and Function. Hruby, V. J.; Rich, D. H. Eds. Pierce Chemical Company, **1983**, 87.

[45] Geysen, H. M.; Meloen, R. H.; Barteling, S. *J. Proc. Nat. Acad. Sci.* USA. **1984**, *81*, 3998.

[46] Houghten, R. A. *Proc. Natl. Acad. Sci.* **1985**, *82*, 5131.

[47] Li, W.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092.

[48] Bui, C. T.; Rasoul, F. A.; Ercole, F.; Pham, Y.; Maeji, N. J. *Tetrahedron Lett.* **1998**, *39*, 9279.

[49] Li, W.; Xiao, X.; Czarnik, A. W. *J. Comb. Chem.* **1999**, *1*, 127.

[50] Larhed, M.; Lindeberg, G.; Halberg, A. *Tetrahedron Lett.* **1997**, *37*, 8213.

[51] Labadie, J. W.; Deegan, T. L.; Gooding, O. W.; Heisler, K.; Newcomb, W. S.; Porco Jr., J. A.; Tran, T. W.; van Eikeren, P. *Polym. Mater. Sci. Eng.* **1996**, *75*, 389.

[52] Cho, J. K.; Park, B-D.; Lee, Y. S. *Tetrahedron Lett.* **2000**, *41*, 7481.

[53] Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077.

[54] Shuttleworth, S. J.; Allin, S. M.; Sharma, P. K. *Synthesis,* **1997**, 1217.

[55] a) Bergbreiter, D. E. in *Functional Polymers* (Bergbreiter, D. E.; Martin, C. R. Eds.) Plenum, New York, **1989**, 143; b) Hodge, P. in *Synthesis and Separation Using Functional polymers* (Sherrington, D.C.; Hodge, P. Eds.), Wiley, Chichester, **1988**, 43; c) Akelah, A. *Reakt. Polym. Ion Exch. Sorbents* 1988, *8*, 273; d) Akelah, A. *Synthesis* **1981**, 413; e) Crosby, G. A. *Aldrichimica Acta* **1976**, 15.

[56] Akelah, A.; Abdel, G.; Fathy, M. *Phoshporous Sulfur Relat. Elem.* **1987**, *30*, 91.

[57] a) Hodge, P. in *Innovation and Perspectives in Solid Phase Synthesis* (Epton, R. Ed.), SPCC, Birmingam, **1990**, 273; b) Worster, P. M.; McArthur, C. R.; Leznoff, C. C. *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 221.

[58] Kim, D.Y.; Suh, K. H. *Synthetic Commun.* **1999**, *29*, 1271.

[59] Yim, A. –M. *Tetrahedron Lett.* **1999**, *40*, 4535.

[60] Bonnet, D. *Tetrahedron Lett.* **1999**, *40*, 7315.

[61] Arsequell, G. *Tetrahedron Lett.* **1999**, *40*, 7279.

[62] Richter, H. *J. Org. Chem.* **1999**, *64*, 1362.

[63] Hébert, N. *Tetrahedron Lett.* **1999**, *40*, 8547.

[64] Katritzky, A. R. *Org. Lett.* **1999**, *1*, 1189.

[65] Combs, A. P. *Tetrahedron Lett.* **1999**, *40*, 1623.

[66] Goff, D. A. *Tetrahedron Lett.* **1999**, *40*, 8741.

[67] Peng,G. *J. Org. Chem.* **1999**, *64*, 8342.

[68] Cavallaro, C. L. *Tetrahedron Lett.* **1999**, *40*, 2711.

[69] Caddick, S. *Tetrahedron Lett*, **1999**, *40*, 7285.

[70] Rossé, G. *J. Comb. Chem.* **1999**, *1*, 397.

[71] Karoyan, P. *Tetrahedron Lett.* **1999**, *40*, 71.

[72] Tremblay, M. R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2827.

[73] Cohen, B. J.; Patchornick, M. A. *J. Am. Chem. Soc.* **1981**, *103*, 7620.

[74] Yamskov, I. A.; Budanov, M. V.; Davankov, V. A. *Bioorg. Khim.* **1979**, *5*, 757.

[75] Kaldor, S.W.; Fritz, J. E.; *Bioorg. Med. Chem. Lett.* **1996**, *6,* 3041.

[76] Kaldor, S.W.; Siegel, S. W.; Fritz, J. E. *Tetrahedron Lett.* **1996**, *37*, 7193.

[77] Booth, R. J.; Hodges, J. C. *J. Am. Chem. Soc.* **1997**, 4882.

[78] Yu, Z.; Alesso, S.; Pears, D.; Worthington, P. A.; Luke, R. W. A.; Bradley, M. *J. Chem. Soc., Perkin Trans. 1.* **2001**, 1947.

[79] Adams, B. A.; Holmes, E. L. *J. Soc. Chem. Ind.* **1935**, *54*, 1.

[80] Siegel, M. G.; Hahn, P. J.; Dressman, B. A.; fritz, J.E.; Grunwell, J. R.; Kaldor, S. W. *Tetrahedron Lett.* **1997**, *38*, 3357.

[81] Bolton, B. A.; Jackson, M. B. *React. Polym.* **1983**, *1*, 119.

[82] Pedroso, E.; Grandas, A.; Saralegui, M. A.; Giralt, E.; Granier, C.; Van Rietschoten, J. *Tetrahedron.* **1982**, *38*, 1183.

[83] Terrett, N. K. Combinatorial Chemistry. Compton, R. G.; Davies, S. G.; Evans, J. Eds. Oxford University Press, **1998**, 120.

[84] Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, *100*, 2091.

[85] Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.

[86] Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 2107.

[87] Holmes, P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2318.

[88] Sternson, S. M.; Schreiber, S. L. *Tetrahedron Lett.* **1998**, *39*, 7451.

[89] Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. *J. Chem. Soc. Commun.* **1971**, 636.

[90] Carpino, L. A.; Cohen, B. J.; Stephens Jr., K. E.; Sadat-Aalace, S. Y.; Tien, J. H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732.

[91] Yamashiro, D.; Noble, R. L.; Li, C. H. *J. Org. Chem.* **1973**, *38*, 3561.

[92] Noble, R. L.; Yamashiro, D.; Li, C. H. *Int. J. Peptide Protein Res.* **1977**, *10*, 385.

[93] Chaturvedi, N.; Sigler, G.; Fuller, W.; Verlander, M.; Goodman, M. (1981) in Chemical Synthesis and Sequencing of Peptides and Proteins (Liu, T. Y, Schechter, A. N.; Heinrikson, R. L.; Condliffe, P. G. Eds).

[94] Fields, C. G.; Fields, G. B.; Noble, R. L.; Cross, T. A. *Int. J. Peptide Protein Res.* **1989**, *33*, 298.

[95] Eritja, R.; Ziehler-Martin, J. P.; Walker, P. A.; Lee, T. D.; Legesse, K.; Albericio, F.; Kaplan, B. E. *Tetrahedron*, **1987**, *43*, 2675.

[96] Kisfaludy, L.; Schön, I. *Synthesis*, **1983**, 325.

[97] Kisfaludy, L.; Otros Jr., L.; Schön, I.; Low. M. (1985) in *Peptides: Structure and Function* (Deber, C. M.; Hruby, V. J.; Kopple, K. D, Eds.), 221.

[98] Bodanszky, M, Bodanszky, A, Tolle, J. C, Bednarek, M. A, (1981) in *Chemical Synthesis and Sequencing of Peptides and Proteins* (Liu. T, Y, Schechter.; A. N, Heinrikson.; R. L.; Condliffe, P. G. Eds.) 165, Elsevier North Holland, Amsterdam.

[99] Atherton, E.; Logan, C. J.; Sheppard, R. C. *J. Chem. Soc. Perkin Trans. I* **1981**, 538.

[100] Hudson, D. *J. Org. Chem.* **1988**, 53, 618.

[101] Atherton, E.; Cameron, L. R.; Sheppard, R. C. *Tetrahedron,* **1998**, *44*, 843.

[102] Dryland, A.; Sheppard, R. C. *Tetrahedron,* **1985**, *44*, 859.

[103] Atherton, E.; Sheppard, R. C. *J. Chem. Soc. Chem. Commun.* **1985**, 165.

[104] Moore, S.; Stein, W. H. *J. Biol. Chem.* **1948**, *176*, 367.

[105] Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

[106] Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147.

[107] Krchnak, V.; Vagner, J.; Eichler, J.; Lebl, M. (1989) in Peptides 1988 (Jung, G. and Bayer, E. eds), 232, Walter de Gruyter and Company, Berlin.

[108] Krchnak, V.; Vagner, J.; Lebl, M. *Int. J. Peptide Protein Res.* **1988**, *32*, 415.

[109] Krchnak, V.; Vagner, J.; Safar, P.; Lebl, M. Coll. Czech. *Chem. Commun.* **1988**, *53*, 2542.

[110] Christensen, T. (1979) in *Peptides: Structure and Biological Function* (Gross, E.; Meienhofer, J. Eds.) 385, Pierce Chemical Company, Rockford, IL.

[111] Christensen, T. Villemoes, P. and Brunfeldt, K. (1977) in Peptides: Proceedings of the 5th American Peptide Symposium (Goodman, M. and Meinhofer, J. eds), 569, Halsted Press, New York.

[112] Eicher, J.; Bienert, M.; Stierandove, A.; Lebl, M. *Peptide Res.* **1991**, *4*, 296.

[113] Edman, P . *Acta Chem. Scand.* **1950**, *4*, 283.

[114] Edman, P. *Acta Chem. Scand.* **1956**, *10*, 761.

[115] Edman, P.; Begg, G. *European J. Biochem.* **1967**, *1*, 80.

[116] Siuzdak, G.; Lewis, J. K. Biotech. Bioeng. (Comb. Chem.) **1998**, *61*, 127.

[117] Karas, M.; Bahr, U.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion. Proc.* **1989**, *92*, 231.

[118] Egner, B. J.; Langley, G. J.; Bradley, M. *J. Org. Chem.* **1995**, *60*, 2652.

[119] Metzger, J. W.; Wiesmuller, K. –H.; Gnau, V.; Brunjes, J.; Jung, G. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 894.

[120] Aubagnac, J. –L.; Calmes, M.; Daunis, J.; El Amarani, B.; Jacquir, R.; Devienne, F. M.; Combarieu, R. *Tetrahedron Lett.* **1985**, *26*, 1497.

[121] Fitch, W. L.; Detre, G.; Holmes, C. P.; Shoolery, J. N.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 7955.

[122] Look, K. G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588.

[123] a) Sherrington, D. C. Preparation, Functionalisation and Characteristics of Polymer Supports. In: *Polymer-supported Reactions in Organic Synthesis.* (Sherrington, D. C.; Hodge, P. Eds. New York: Wiley & Sons, Inc; **1981**, 1-82; b) Frechet, J. M. J. *Tetrahedron.* **1981**, *37*, 663; c) Atrash, B.; Bradley, M.; Kobylecki, R.; Cowell, D.; Reader, *Angew. Chem. Int. Ed.* **2001**, *40*, 938.

[124] a) Yu, Z.; Bradley, M. *Curr. Opin. Chem. Biol.* **2002**, in press.

[125] Kress, J.; Rose, A.; Frey, J. G.; Brocklesby, W. S.; Ladlow, M.; Mellor, G. W.; Bradley, M. *Chem. Eur. J.* **2001**, *7*, 3880.

[126] Inman, K. J.; Du Bois, G. C.; Appella, E. In *Solid Phase Methods in Protein Sequence Analysis (Proceedings of the Second International Conference),* Previero, A.; Coletti-Previero, M. A., Eds.; North-Holland Publishing Co. Amsterdam, The Netherlands, **1977**, 81.

[127] Regen, S. L.; Dulak, L. *J. Am. Chem. Soc.* **1977**, *99*, 623.

[128] Warshawsky, A.; Kalir, R.; Deshe, A.; Berkovitz, H.; Patchornik, A. *J. Am. Chem. Soc.* **1979**, *101*, 4249.

[129] Heffernan, J. G.; MacKenzie, W. M.; Sherrington, D. C. *J. Chem. Soc. Perkin Trans. 1* **1981**, *2*, 514.

[130] a) Becker, H.; Lucas, H. W.; Maul, J.; Pillai, V. N. R.; Anzinger, H.; Mutter, M. *Makromol. Chem. Rapid Commun.* **1982**, *3*, 217; b) Hellerman, H.; Lucas, H. W.; Maul, J.; Pillai, V. N. R.; Anzinger, H.; Mutter, M. *Makromol. Chem.* **1983**, *184*, 2603.

[131] a) Bayer, E.; Hemmasi, B.; Albert, K.; Rapp, W.; Dengler, M. in *Peptides: Structure and Function, Proceeding of the Eight American Peptide Symposium*, Hruby, V. J.; Rich, D. H Eds. Pierce: Rockford, IL, **1983**, 87; b) Bayer, E.; Dengler, M.; Hemmasi, B. *Int. J. Peptide. Prot. Res.* **1985**, *25*, 178; c) Bayer, E. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 113; d) Bayer, E.; Rapp, W. in *Poly(ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications;* Harris, J. M., Ed. Plenum Press: New York, NY, **1992**, 325.

[132] a) Meldal, M.; *Tetrahedron Lett.* **1992**, *33*, 3077; b) Auzanneau, F. I.; Meldal, M.; Bock, K. *J. Peptide Science*, **1995**, *1*, 31.

[133] Zaplisky, S.; Chang, J. L.; Albericio, F.; Barany, G. *Reactive Polymers*, **1994**, *22*, 243.

[134] Kates, S. A.; McGuinness, B. F.; Blackburn, C.; Griffin, G. W.; Sole, N. A.; Barany, G.; Albericio, F. *Biopolymers*, **1998**, *47*, 365.

[135] Albericio, F.; Bacardit, J.; Barany, G.; Coull, J. M.; Egholm, M.; Giralt, E.; Griggin, G. W.; Kares, S. A.; Nicolas, E.; Sole, N. A. in *Peptides 1994. Proceedings of the Twenty-Third European peptide Symposium*, Maia, H. L. S. Ed.; ESCOM Science Publishers: Leiden, The Netherlands, **1995**, 271.

[136] Tsuchida, E.; Nishide, H.; Shimidazu, N.; Yamada, A.; Keneko, M. *Makromol. Chem. Rapid Commun.* **1981**, *2*, 621.

[137] a) Bayer, E.; Rapp, W. *German Pat. DSO 3 714 258*, **1988**; b) Bayer, E.; Hellstern, H.; Eckstein, Z. *Naturforsch*, **1987**, *42c*, 455.

[138] Bayer, E.; Albert, K.; Willisch, H.; Rapp, W.; Hemmasi, B. *Macromolecules,* **1990**, *23*, 1937.

[139] Bayer, E. *Angew. Chem. Engl. Ed.* **1991**, *30*, 2, 113.

[140] Gooding, O. W.; Baudart, S.; Deegan, T. L.; Heisler, K.; Labadie, J. W.; Newcomb, W. S.; Porco, J. A.; van Eikeren, P. *J. Comb. Chem.* **1999**, *1*, 113.

[141] Toy, P. H.; Janda, K. D. *Tetrahedron Lett.* **1999**, *40*, 6329.

[142] a) Erman, B.; Flory, P. J. *Macromolecules*, **1982**, *15*, 806; b) Grøtli, M.; Gotfredsen C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J. O.; Meldal, M. *J. Comb. Chem.* **2000**, *2*, 108.

[143] Renil, M.; Nagaraj, R.; Pillai, R. V. N. *Tetrahedron* **1994**, *22*, 6681.

[144] Wilson, M. E.; Paech, K.; Zhou, W. J.; Kurth, M. J. *J. Org. Chem.* **1998**, *63*, 5094.

[145] Crivello, J.; Ramdas, D. J. M. C. *Pure Appl. Chem.* **1992**, A29 (9), 753.

[146] Itsuno, S.; Sakurai, Y.; Ito, K.; Maruyama, T.; Nakahama, S.; Frechet, J. M. J. *J. Org. Chem.* **1990**, *55*, 304.

[147] Kamahori, K.; Ito, K.; Itsuno, S. *J. Org. Chem.* **1996**, *61*, 8321.

[148] Kamahori, K.; Tada, S.; Ito, K.; Itsuno, S. *Tetrahedron: Asymmetry.* **1995**, *6*, 2547.

[149] Burchardt, J.; Meldal, M. *Tetrahedron Lett.* **1998**, *39*, 8695.

[150] Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis.* 2nd ed. New York: John Wiley, **1991**, 47.

[151] Hirao, A.; Hayashi, M.; Nakahama, S. *Macromolecules,* **1996**, *29*, 3353.

[152] Toy, P. H.; Janda, K. D. *Tetrahedron Lett.* **1999**, *40*, 6329.

[153] Garibay, P.; Toy, P. H.; Hoeg-Jensen, T.; Janda, K. D. *Synlett*, **1999**, 1438.

[154] Reger, T. S.; Janda, K. D. *J. Am. Chem. Soc.* **2000**, *122*, 6929.

[155] Hird, N.; Hughes, I.; Hunter, D.; Morrison, M. G. J. T.; Sherrington, D. C.; Stevenson, L. *Tetrahedron*, **1999**, *19*, 451.

[156] Vaino, A. R.; Janda, K. D. *Proc. Natl. Acad. Sci.* U.S.A. **2000**, *97*, 7692.

[157] Groth, T.; Grotli, M.; Lubell, D. W.; Miranda, L. P.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1*, **2000**, 4258.

[158] Grotli, M.; Rademan, J.; Groth, T.; Lubell, W. D.; Miranda, L. P.; Meldal M. *J. Comb. Chem*, **2001**, *3*, 28.

[159] Kita, R.; Svec, F.; Frechet, J. M. J. *J. Comb. Chem.* **2001**, *3*, 564.

[160] Hallensleben, M. L.; Lucarelli, F. *Polymer Bulletin*, **1996**, *37*, 765.

[161] Inokuma, S.; Yamamoto, T.; Nishimura, J. *Tetrahedron Lett.* **1990,** *31*, 97.

[162] a) Sekera, V. C.; Marvel, C. S. *J. Am. Chem. Soc.* **1933**, *55*, 345; b) Roos, A. T.; Gilman, H.; Beaber, N. J. in Organic Synthesis, 2nd ed.; Gilman, H., Ed.; Wiley: New York, **1941**; Col. Vol. 1. 145-147; c) Sekera, V. C.; Marvel, C. S. Organic Synthesis; Wiley: New York, **1955**; col. Vol. 3, 366-367; d) March, J. Advanced Organic Chemistry; 4th ed.; Wiley: New York, 1992, 352-354; e) Kabalka, G. W.; Varma, R. S; Srivastava, P. C.; Knapp, F. F. Jr. *J.Org. Chem.* **1986**, *51*, 2386; f) Tanabe, Y.; Yamamoto, H.; Yoshida, Y.; Miyawaki, T.; Utsumi, N. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 297; g) Yoshida, Y.; Sakakura, Y.; Aso, N.; Tanabe, Y. *Tetrahedron.* **1999**, *55*, 2183.

[163] Yoshida, Y.; Shimonishi, K.; Sakakura, Y.; Okada, S.; Aso, N.; Tanabe, Y. *Synthesis,* **1999**, *9*, 1633.

[164] Yamskov, I. A.; Budanov, M. V.; Davankov, V. A. *Bioorg. Khim.* **1979**, *5*, 757.

[165] Ericcson, J.; Hult, A. *Polym. Bull.* **1987**, *18*, 289.

[166] Wood, J.; Rooney, J. M.; Harris, J. H. *U.S. Patent 4, 543*, 397, **1985**.

[167] Parlow, J. J. *Tetrahedron Lett.*, **1996,** *37*, 5255.

[168] Sterzycki, R. *Synthesis*, **1979**, 724.

[169] Nitz, T. J.; Paquette, L. A. *Tetrahedron. Lett.* **1984**, *25*, 3047.

[170] Talma, A. G.; van Vossen, H.; Dudholter, E. J. R.; van Eerden, J.; Reinhoudt, D. N. *Synthesis*, **1986**, 680.

[171] Manhas, M. S.; Hoffman, W. H.; Lal, B.; Bose, A. K. *J .Chem. Soc, Perkin Trans. 1* **1975**, 461.

[172] Widdecke, H. *Synthesis and Separations Using Functional Polymers*, Sherrington D. C. (Ed.), Wiley, Chichester, UK, **1980**, p. 157 and references cited therein.

[173] a) Bishop, R. B. *Practical Polymerisation for Styrene*, Cahners Publ. Co., New York, **1971**; b) Church, J. M. *Suspension polymerisation, Chem. Eng.*, **1966**, *73*, 79.

[174] a) Hodge, P. and Sherrington, D. C. (Eds)., *Polymer supported reactions in Organic Synthesis*, John Wiley and Sons, Chichester, **1980**; b) Mathur, N. K.; Narang, C. K.; Williams, R. E. *Polymers as Aids in Organic Chemistry*, Academic Press, New York, **1980.**

[175] Birr, C. *Aspects of Merrifield Peptide Synthesis*, Springer-Verlag, Heidelberg, **1978.**

[176] Harmony, *Methods Free-Radical Chem.* **1974**, *5*, 101.

[177] Arshady, R.; Ledwith, A. *React. Polym.* **1983**, *1*, 159.

[178] Kafarov, V. V.; Babanov, B. V. *J. Appl. Chem. USSR*, **1959**, *32*, 810.

[179] European Patent 87118837.1.

[180] Borhan, B.; Wilson, J. A.; Gasch, M. J.; Ko, Y.; Kurth, D. M.; Kurth, M. J. *J. Org. Chem.* **1995**, *60*, 7375.

[181] Erbay, E.; Bilgiç, T.; Karali, M.; Savasci, O. T. *Polym. Plast. Technol. Eng.* **1992**, *31*, 589.

[182] Ahmed, S. M. *Dispersion Sci. Technol.* **1984**, *5*, 421.

[183] Christian, P.; Giles, M. R.; Howdle, S. M.; Major, R. C.; Hay, J. N. *Polymer* **2000**, *41*, 1251.

[184] a) Pepper, K. W.; Paisley, H. M.; Young, M. A. *J. Chem. Soc.*, **1953**, 4097; b) Law, R. V.; Sherrington, D. C.; Snape, C. E.; Ando, I.; Korosu, H. *Ind. Eng. Chem.*, **1995**, *35*, 2740.

[185] a) Millar, R.; Smith, D. G.; Marr, W. E.; Kressman, T. R. E. *J. Chem. Soc.* **1963**, 218; b) Hudson, M. J. (Ed.), *Recent Developments in Ion Exchange*, Elsevier Applied Science, Barking, **1987**; c) Malinsky, J. A (Ed.), *Ion Exchange*, Dekker, M., New York, **1969**; d) Feinberg, R. S.; Merrifield, R. B. *Tetrahedron*, **1974**, *30*, 3209; e) Sparrow, J. T. *Tetrahedron Lett.* **1975**, *16*, 4637; f) Barron, R. E.; Fritz, J. S. *React. Polym.* **1983**, *1*, 215.

[186] Patterson, J. A. *Biochemical Aspects of Reactions on Solid Supports*, G.R. Stark (Ed.), Academic Press, New York, **1971.**

[187] Arhady, R.; Ugi, I. *Angew. Chem. Int. Ed. Eng.* **1982**, *94*, 367.

[188] Stranix, B. R.; Gao, J. P.; Barghi, R.; Salha, J. *J. Org. Chem.* **1997**, *62*, 8987.

[189] Rana, S. *PhD Thesis*, University of Southampton, **2000**, 44.

[190] Alesso, S.; Yu, Z.; Pears, D.; Worthington, P. A.; Luke, R. W. A.; Bradley, M. *J. Comb. Chem.* **2001**, *3*, 631.

[191] a) Arshady, R. *J. of Chromatography.* **1991**, 586, 199; b) Itsuno, S.; Sakurai, Y.; Ito, K.; Maruyama, T.; Nakahama, S.; Frechet, J. M. J. *J. Org. Chem.* **1990**, *55*, 304.

[192] Howdle, S. M.; Jerabek, K.; Leocorbo, V.; Marr, P. C.; Sherrington, D. C. *Polymer,* **2000**, *41*, 7273.

[193] Rana, S.; White, P.; Bradley, M. *J. Comb. Chem.* **2001**, *3*, 9.

[194] Groth, T.; Grotli, M.; Meldal, M. *J. Comb. Chem.* **2001**, *3*, 461.

[195] Sarin, V. K.; Kent, S. B. H.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 5463.

[196] Roscoe, S. B.; Frechét, J. M. J.; Walzer, J. F.; Dias, A. J. *Science.* **1998**, *280*, 270.

[197] McAlpine, S. R.; Schreiber, S. L. *Chem. Eur. J.* **1999**, *5*, 3528.

[198] McAlpine, S. R.; Lindsley, C. W.; Hodges, J.C.; Leonard D. M.; Filzen, G. F. *J. Comb. Chem.* **2001**, *3*, 1.

[199] Radermann, J.; Barth, M.; Brock, R.; Egelhaaf, H. J.; Jung, G. *Chem. Eur. J.* **2001**, *7*, 3884.

[200] Egner, B. J.; Rana, S.; Smith, H.; Bouloc, N.; Frey, G.J.; Brocklesby, W. S.; Bradley, M. *Chem. Commun.* **1997**, 735.

[201] a) Martens, B. R. *Confocal Microscopy-Milestone Series*, Vol. MS 131, Photo-Optical Instrumentation Engineers, **1996**; b) Rosacco, G. S. *Advances in Infrared and Raman Spectroscopy*, Vol 7, Clark R. J. H.; Hester, R. E, Eds, Wiley, Chichester, **1980**.

[202] Miyaura, N.; Yanagi, T.; Suzuki, A.; *Syn. Commun.* **1981**, *11*, 513.

[203] Suzuki, A.; Miyaura, N. *J. Chem. Soc. Chem. Commun.* **1979**, 866.

[204] Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.

[205] Martin, A. R.; Yang, Y. H. *Acta Chem. Scand.* **1993**, *47*, 221.

[206] Laue, T.; Plagens, A.; *Named Organic Reactions*; John Wiley and Sons: New York, **1999**, 4th Edn.; 262.

[207] Milstein, D.; Stille, J. K. *J. Am. Chem. Soc.* **1978**, *100*, 3636.

[208] Stille, J. K. *Angew. Chem. Int. Ed.* **1986**, *25*, 508.

[209] Nicolaou, K. C.; Sorensen, E. J.; *Classics in Total Synthesis – Targets, Strategies, Methods*; VCH Verlagsgesellschaft: Weinheim, **1996**.

[210] Mitsunobu, O. *Synthesis* **1981**, 1.

[211] a) Mitsunobu, O. *Bull. Chem, Soc. Jp.* **1967**, *40*, 4235; b) Hughes, D. L. *Org. React.* **1992**, *42*, 335.

[212] a) Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1988**, *110*, 6487; b) Crich, D.; Dyker, H.; Harris, R. J. *J. Org. Chem.* **1989**, *54*, 257.

[213] Atherton, E.; Sheppard, R. C. *Solid Phase Synthesis- a Practical Approach*; IRL Press. **1989**.

[214] Fields, G. B.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161.

[215] Kaiser, E.; Colescott, R.L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

[216] Iwata, S.; Matsuoka, H.; Tanaka, K. *J. Chem. Soc., Perkin Trans.* **1997**, *1*, 1357.

[217] Lauter, U.; Meyer, W. H.; Wegner, G. *Macromolecules*, **1997**, *30*, 2092.

[218] Carpino, L. A.; Cohen, B. J.; Stephens, Jr., K. E.; Sadataalee, S. Y.; Tien, J. H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732.

[219] Basso, A. **2000,** *PhD Thesis.*

APPENDIX

Multiple Parallel Polymerisation System

Description

The multiple parallel polymerisation system (Figure 1) comprises of 6 borosilicate glass round bottomed, stirred reactors (diameter 7cm, height 23cm) firmly mounted on a rigid central vertical column. A low voltage brushless DC motor (power consumption approx. 150W) drives the six stirrers through a gearbox and multishaft belt driven drive box (Figure 2). Motor speed is adjustable (from approx. 100 to 800 rpm). The whole system is small enough to stand in a conventional (300 mm x 300mm depth) thermostated water bath. The system can be rotated about the support column so that each reactor can be inspected. Further, the whole system is counterpoised so that it can be effortlessly lifted upwards (120mm) clear of the water bath.

Figure 1. *The reactor system. At the top right is the Brushless D.C. motor, beneath is the drive 'gearbox' system. Below is the set of drive shafts to the glass reactors in turn below. In the photograph only 3 reactors are mounted. Capacity is 6 reactors.*

Figure 2. *Close-up of the drive 'gearbox'. The motor is seen to the right top. Below, you will see the drive shafts and brass flywheels, to their right you will see grey levers - these are used to de-clutch individual drivers to the reactors. Thus, one reactor can be safely and easily removed or introduced without disturbing the others.*

To make filling, emptying and cleaning easy and safe, the closed reactor complete with its stirrer can be released from the stirrer drive system and removed from the system in seconds without the use of any tools. Once removed, the reactor can be mounted in a stand (Figure 3) which is part of the system. Once mounted on the stand, the reactor can then be opened and dismantled. The parallel polymerisation system has now become commercially available (Supplier: Ventacon Combichem Limited, UK).

Figure 3. *Reactor system held in the stand. The glass reactors, sealed and containing their stirrers (PTFE blade: 65mm diameter, stirrer: 500mm length from Aldrich) can be safely mounted and then filled or dismantled in the stand.*