Organic Synthesis on Soluble Polymer Supports: Liquid-Phase Methodologies

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Received August 28, 1996 (Revised Manuscript Received January 13, 1997)

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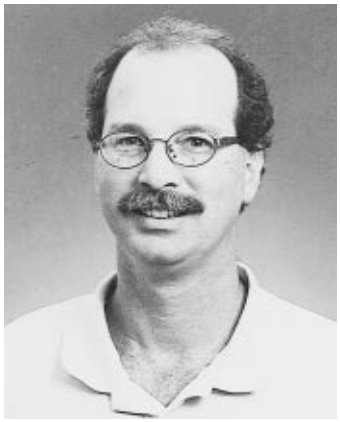

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I. Introduction

Since the introduction of the Merrifield method for peptide synthesis,[1] insoluble polymer supports have been incorporated into numerous synthetic methodologies to facilitate product purification.[2−4] Although highly successful, solid-phase synthesis still exhibits

S0009-2665(96)00064-7 CCC: $28.00

several shortcomings due to the nature of heterogeneous reaction conditions. Nonlinear kinetic behavior, unequal distribution and/or access to the chemical reaction, solvation problems, and pure synthetic problems associated with solid-phase synthesis have led several labs to pursue alternative methodologies to restore homogeneous reaction conditions. By replacing insoluble cross-linked resins with *soluble* polymer supports, the familiar reaction conditions of classical organic chemistry are reinstated, and yet product purification is still facilitated through application of macromolecular properties. This methodology, termed liquid-phase synthesis, in essence avoids the difficulties of solid-phase synthesis while preserving its positive aspects.

The term "liquid-phase" synthesis was first used to contrast the differences between solid-phase peptide synthesis and a method of synthesis on soluble polyethylene glycol.[5,6] Although "soluble polymer-supported" synthesis might be a less ambiguous label than "liquid-phase" synthesis, the latter term is more prevalent in the literature. In keeping with previous reviews,[7−10] the phrases "classical" or "solution" synthesis will be used to describe homogeneous reaction schemes that do not employ polymer supports while "liquid-phase" synthesis will be reserved for methodologies incorporating a soluble macromolecular carrier to facilitate product isolation. It should be noted that although the starting material and subsequent product is kept soluble by the attached polymer, some reactions in liquid-phase synthesis may in fact be heterogeneous due to the presence of insoluble catalysts or reagents (e.g. catalytic hydrogenation).

Our lab has undertaken a research program to develop liquid-phase methods for the synthesis of small molecule libraries in a combinatorial format.[11−14] To this end, a historical account of multistep synthesis on soluble supports provides substantial insights for method development and avoids potential problems by heeding the lessons learned and reported by past successes and failures.

The body of literature dealing with organic and polymer chemistry relevant to liquid-phase synthesis is enormous, and several criteria had to be imposed during the compilation of this review. To focus on methodologies toward multistep synthesis using soluble polymer supports, only polymers that completely dissolve in the reaction solvent are included, and consequently, cross-linked polymers that swell in organic solutions are not discussed. Cited literature must describe the chemical manipulation of a moiety attached to a soluble polymer with the intention of cleaving that moiety for subsequent isolation as the targeted product molecule; in other words, the polymer serves as a macromolecular protecting group. Thus, polymer-supported reagents or catalysts are not included. Furthermore, the requirement for product isolation after cleavage from the support limited the inclusion of a significant amount of polymer−small molecule conjugate chemistry; however, it is acknowledged that such research could be developed into methods for liquid-phase synthesis.

II. Overview of Polymers in Liquid-Phase Synthesis

A. Properties of Soluble Polymer Supports

Polymers employed as soluble supports for liquid-phase synthesis must: (1) be commercially available or rapidly and conveniently prepared, (2) demonstrate good mechanical and chemical stabilities, (3) provide appropriate functional groups for easy attachment of organic moieties, and (4) exhibit high solubilizing power in order to dissolve molecular entities with low solubilities and permit the development of a general synthetic methodology independent of the physicochemical properties of target compounds.

Additionally, it should be realized that polymer supports bought or prepared in the laboratory exhibit not one discrete molecular weight but instead consist of macromolecules with variable sizes. As polymer properties vary with chain length, the molecular weight range of the support should be narrow (i.e. polydispersity approaching unity). Soluble supports in general should have molecular weights high enough to be solid or crystalline at room temperature and yet not excessively high such that solubilities in a variety of solvents is limited.

The polymeric carrier must withstand the reaction conditions used in solution-phase synthesis, and consequently most soluble supports used in liquid-phase synthesis possess hydrocarbon or alkyl ether backbone structures. By variation of terminal and pendant functional groups of these two backbone structures, polymer properties are determined and may provide sites for attachment of organic moieties. If the conditions of polymerization and choice of monomer allow for suitable polymer functionalization, then anchoring of the initial synthetic structure may be made directly to the support for liquid-phase synthesis; however, often a linking group must be employed to ensure anchor stability throughout synthesis, improve accessibility to reagents, and facilitate cleavage for product recovery.

Chosen polymers for liquid-phase synthesis must also provide a reasonable compromise between loading capacity and solubilizing power. The loading capacity of a polymer support is a measure of the number of anchoring sites per gram of polymer and is expressed in units of millimoles per gram (mmol/g). High loading capacities are advantageous to reduce the total expenditure for polymer supports and to allow manageable amounts of material in medium- or large-scale applications. Solubilizing power can be defined as the ability of the macromolecular carrier to maintain a homogeneous solution of the polymer-bound organic moiety; this property is especially important in cases where the unbound moiety is insoluble in the reaction medium. High solubilizing power is desirable to ensure homogeneous reactions and high yields throughout the synthetic scheme. Generally, solubilizing power decreases as loading capacity increases because as the polymer is further loaded, the solubility properties of the polymer−organic moieties conjugate are increasingly determined by the properties of the attached compounds. Thus, it is important to achieve

a compound-to-support ratio that limits solubility changes and yet provides economic and manageable synthesis.

Furthermore, in choosing a polymer with high loading capacity, one must consider the influence of neighboring anchoring sites. The multiple attachment of compounds to a polymer support may result in nonequivalent reactivities of bound moieties distributed unequally along the polymer backbone. In some situations, excess reagents or longer reaction times may allow near-quantitative reaction of anchored compounds on heavily laden polymers; however, other reactions may require linkage exclusively to polymer termini to provide adequate accessibility to polymer-bound reagents or enzymes.

B. Methods of Separating Polymers from Reaction Mixtures

Several methods have capitalized on macromolecular properties in order to achieve product separation in liquid-phase synthesis. Most frequently, the homogeneous polymer solution is simply diluted with a solvent that induces precipitation of the macromolecular support. Analogous to solid-phase synthesis, the resulting heterogeneous mixture is filtered to isolate the polymer−product conjugate while excess reagents and impurities are rinsed away. Some polymers may be amenable to recrystallization to minimize inclusion complexes that may form during precipitation, and proper choice of solvents and temperature must be made for satisfactory recovery and purification.[11,15]

Although precipitation/crystallization is the fastest and most common mode of product separation, other methods have been used to isolate macromolecular supports from low molecular weight impurities. Dialysis using a semipermeable membrane has achieved polymer purification.[16] This procedure becomes less time-consuming in ultrafiltration (also called diafiltration or membrane filtration) when pressure gradients speed the separation of polymer from the reaction supernant using a membrane; additionally, centrifugation methods allow convenient isolation of biomolecules and could be applied to more general polymer separations. Gel permeation chromatography and adsorption chromatography have also been suggested as means to remove excess reagents and byproducts away from polymeric products.[7]

It is important to realize that these macromolecule-based methods isolate polymer-bound products from soluble impurities, but do not generally purify the product from other polymer-bound byproducts. Such byproducts arise from incomplete reactions or side reactions; and in classical solution chemistry, similar byproducts are removed during product purification at each step of a multistep synthesis. Although avoiding the multiple, laborious purification steps of classical chemistry, support-based methodologies in large part do not provide for the purification of intermediates. Instead, reactions must be optimized and driven to completion to avoid a complicated final mixture of products. Some liquid-phase methods, however, have been developed to achieve high purity of products without quantitative reaction yields (discussed in sections III.C.8 and VI.C).[17−22]

C. Analytical Methods in Liquid-Phase Synthesis

Not hindered by the heterogeneity intrinsic to solid-phase systems, liquid-phase synthesis permits product characterization on soluble polymer supports by routine analytical methods. UV−visible, IR, and NMR spectroscopies and even TLC may be used to monitor reactions without requiring preliminary cleavage from the polymer support.[8,11,15] Reaction monitoring, purity assessment, and conformational studies of peptides were performed using ^{1}H NMR or ^{13}C NMR spectroscopy.[23,24] Moreover, aliquots taken for characterization may be returned to the reaction flask upon recovery from these nondestructive analytical methods. Chemical methods such as titration and derivatization can be routinely performed and allow subsequent characterization in the presence of the bound soluble support. Peptide coupling reactions have been monitored without cleavage from polyethylene glycol by using ninhydrin or fluorescamine reactions or potentiometric titration;[25] however, potentiometric titration gave unreliable results for peptides longer than hexapeptides.[26]

Circular dichroism (CD) measurements were made at each step in the peptide synthesis of substance P and of a hydrophobic peptide corresponding to myoglobin 66−73.[27] CD reported formation of secondary structures and the influence of peptide protecting groups on structure. The polyethylene glycol support did not interfere with measurements due to its UV cutoff at 190 nm, and the solubilizing power of the polymer support allowed solution measurements in many solvents, achieving homogeneity in solvents that the free peptide displayed limited solubility.

D. Listing of Polymers

Soluble polymers that have been used in liquid-phase synthesis are listed in Table 1 and Figure 1. The brevity of this list indicates the need for additional research in this field of support-based chemistry. For some entries, only a few examples were reported in the literature, and for others, encouraging demonstrations of soluble polymer-supported synthesis have yet to be followed up and further extended. On the basis of past achievements in liquid-phase chemistry, new research in soluble support-based methodologies has the potential to make significant advancements.

Other soluble polymers not listed in Table 1 have found utility as macromolecular catalysts and reagents.[28−35] Linkage of metal catalysts to soluble polymer supports allows for simplified methods of product purification and catalyst recovery and reuse while retaining homogeneous reaction conditions. In particular, phase separation systems based on polyethylene $-(CH_2CH_2)_n-$ exhibit homogeneity in toluene at temperatures above 90 °C in cyclization,[30] cyclopropanation,[32,33] and hydrogenation reactions;[34] wherein, upon cooling to room temperature, the macromolecule precipitates and is easily recovered by filtration. This demonstration of polyethylene as soluble carriers of catalysts and reagents indicates

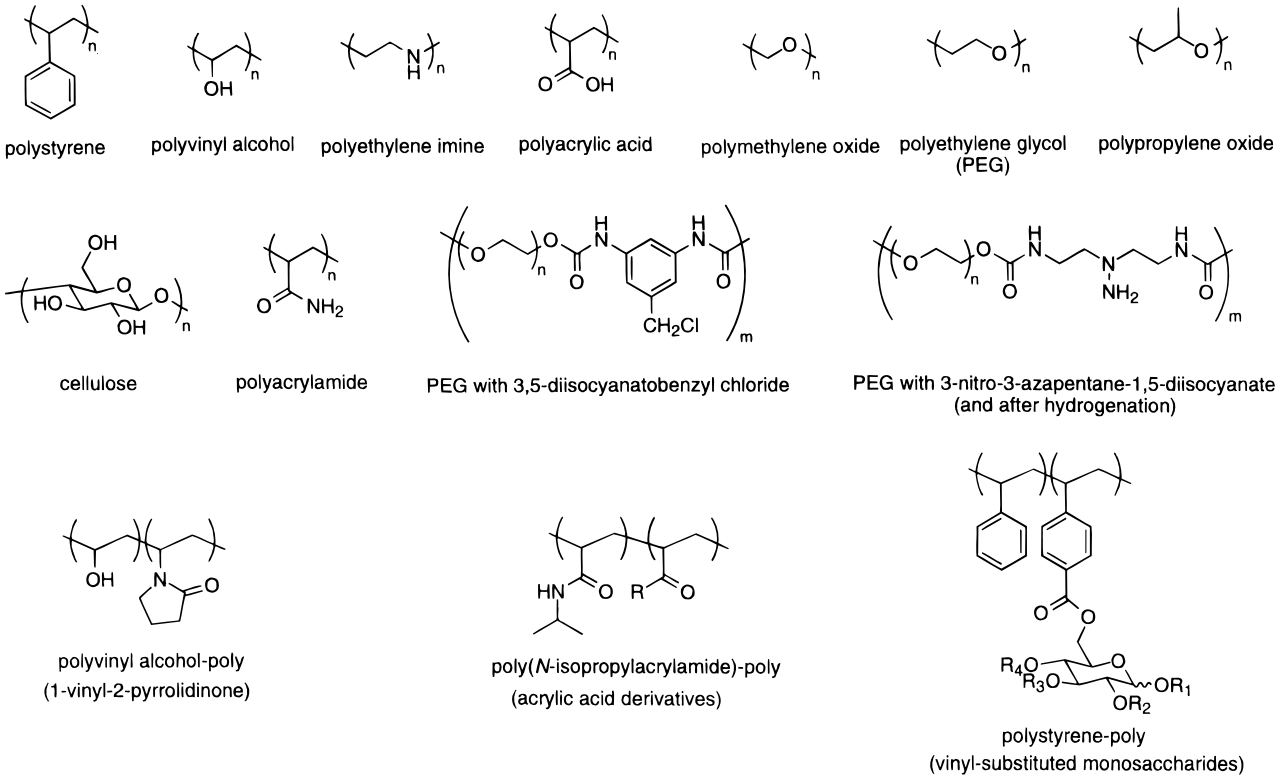


Figure 1. Soluble polymers utilized in liquid-phase synthesis.

Table 1. Soluble Polymers Utilized in Liquid-Phase Synthesis

	application[a]			
polymer	A	B	C	D
Homopolymers				
polystyrene (non-cross-linked)	√	√	√	
polyvinyl alcohol	√	√	√	√
polyethylene imine	√			
polyacrylic acid	√			
polymethylene oxide	√			
polyethylene glycol (PEG)	√	√	√	√
polypropylene oxide	√			
cellulose		√		
polyacrylamide			√	
Copolymers				
PEG with 3,5-diisocyanatobenzyl chloride	√			
PEG with 3-nitro-3-azapentane 1,5-diisocyanate	√			
polyvinyl alcohol-poly(1-vinyl-2-pyrrolidinone)	√	√		
polystyrene-poly(vinyl-substituted monosaccharides)			√	
poly(*N*-isopropylacrylamide)-poly(acrylic acid derivatives)				√

[a] Applications include: (A) peptide synthesis, (B) oligonucleotide synthesis, (C) oligosaccharide synthesis, (D) synthesis of small molecules and other compounds.

that the use of polyethylene as a macromolecular protecting group in combinatorial liquid-phase synthesis is very likely to be successful.

Of the polymer supports listed in Table 1, polyethylene glycol has been most often used for liquid-phase synthesis; consequently, a brief discussion of this polymer is justified.

E. Polyethylene Glycol (PEG)

Polyethylene glycol (PEG), polyethylene oxide (PEO), polyoxyethylene (POE), and polyoxirane all represent the same linear polymer formed from the polymerization of ethylene oxide. By convention, PEG usually indicates the polyether of molecular weight less than 20 000; PEO signifies polymers of higher molecular weights, and POE and polyoxirane have been applied to polymers of a wide range of molecular weights.[15] The term PEG will be used throughout this review as only polyethylene glycols of 2000 to 20 000 molecular weight have been utilized as supports. These limits have been set by the physical properties of the polymer: PEGs of molecular weight 2000 to 20 000 are crystalline with loading capacities of 1 to 0.1 mmol/g; lower molecular weight PEGs exist as liquids at room temperature, and higher molecular weight PEGs have low loading capacities. Macromolecular size will be reported in this review using the notation PEG 6000 to represent polyethylene glycol of molecular weight 6000. It should again be emphasized that polymers exist as a distribution of molecular weights; however, the polydispersity of commercial PEGs is quite narrow.[15]

Depending on polymerization conditions, PEG termini may consist of hydroxyl groups or may be selectively functionalized. Commercially available PEG is produced through anionic polymerization of ethylene oxide to yield a polyether structure possessing either hydroxyl groups at both ends, or a methoxy group at one end and a hydroxyl group at the other. In this review, PEG will be used to represent polyethylene glycol with hydroxyl functionalities at both ends. Similarly, MeO-PEG (polyethylene glycol monomethyl ether) will designate the polyether terminated by a methoxy group at one end and a free hydroxyl at the other. The polymer MeO-PEG is considered monofunctional as typically the methoxy group of MeO-PEG remains unchanged throughout chemical manipulations; and for identical chain

lengths, the loading capacity of PEG is twice that of MeO-PEG as two hydroxyl groups serve as anchoring sites on PEG. Finally, a common PEG derivative contains terminal amino groups instead of hydroxyl functionalities, and this polymer will be represented as diaminoPEG.

As will be seen shortly, many successful applications of the liquid-phase method have resulted from the use of polyethylene glycol as the polymeric support. Employed as a protecting group, this linear homopolymer exhibits solubility in a wide range of organic solvents and water. PEG is insoluble in hexane, diethyl ether, and *tert*-butyl methyl ether, and these solvents have been used to induce PEG precipitation. Careful precipitation conditions or cooling of polymer solutions in ethanol or methanol yields crystalline PEG due to the helical structure of the polymer that produces a strong propensity to crystallize.[15] Thus, as long as the polymer backbone remains unaltered during liquid-phase synthesis, then purification by crystallization can be utilized at each reaction step. Furthermore, the solubilizing power of PEG not only allows homogeneous reactions under numerous reaction conditions, but these solubility properties permit individual reactions steps to be monitored without requiring cleavage of product from the polymer support. The characterization of PEG-bound organic moieties is often straightforward as the polymer does not interfere with spectroscopic or chemical methods of analysis; additionally, MeO-PEG contains a single methoxy group ($\delta = 3.38$ ppm; ethyl protons of PEG backbone $\delta = 3.64$ ppm)[36] that provides an internal standard for easy monitoring of reactions by ^{1}H NMR spectroscopy.

It should be noted that although "solution-like" properties are obtained by grafting PEG onto cross-linked polystyrene (e.g. TentaGel),[37] the heterogeneous reaction conditions that persist still lead to certain limitations that are overcome through use of soluble PEG supports. In fact, the differences in molecular structure between TentaGel and PEG were found to be the dominant factor influencing percent conversion and enantiomeric excess in asymmetric dihydroxylation reactions.[38]

III. Peptide Synthesis on Soluble Polymer Supports

In both methodologies of solid-phase and liquid-phase peptide synthesis, the polymer support is linked to the C-terminus of the growing peptide and thus serves as a carboxylic acid protecting group. This macromolecular protecting group also provides a "handle" to simplify purification after each coupling step by capitalizing on the intrinsic differences between the polymer−peptide conjugate and low molecular weight impurities. Additionally, in liquid-phase synthesis the macromolecular appendage keeps the growing peptide in solution to provide homogeneous reaction conditions even with longer or hydrophobic peptides.

The design of polymer-supported peptide synthesis must plan for attachment of the first amino acid to the support, coupling cycles for peptide elongation, and cleavage of peptide from the support. Of foremost consideration is the linkage of peptide to the polymer support as it must be stable to the coupling chemistry, but yet allow facile and selective cleavage at the end of synthesis. The cleavage step may also be designed to simultaneously remove side chain protecting groups from the peptide or developed to leave blocking groups intact. The coupling cycle must be analyzed for reaction completion and allow for the removal of reagents and byproducts. Furthermore, complete attachment of the first amino acid to the polymer or blocking of unreacted sites must be accomplished to prevent the synthesis of truncated sequences. Fortunately, the soluble support accommodates traditional analytical techniques for monitoring of coupling reactions as well as facilitates reaction workup by polymer-based procedures. Suitable linker chemistry has been developed to enable stable attachment of peptides to polymer supports as well as provide controlled release at the completion of peptide synthesis.

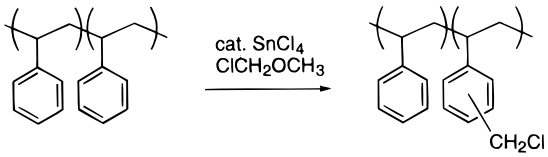


Figure 2. Chloromethylation of (linear) polystyrene.

Consideration should also be made regarding loading capacity and solubilizing power of the chosen polymer support. The polymer should provide sufficient solubilizing power to keep the peptide soluble throughout the synthesis cycle in all solvents and conditions required, and yet possess a loading capacity that satisfactorily provides economy, manageability, and noncrowded anchoring sites.

A. Peptide Synthesis on Polystyrene

To address the problems encountered with heterogeneous reactions using insoluble polystyrene resins, several labs turned to linear, soluble polystyrene to achieve familiar homogeneous reaction conditions while attempting to preserve many aspects of solid-phase chemistry.[9,39−50] Non-cross-linked polystyrene of 200 000 molecular weight served as a soluble support in the synthesis of glycylglycyl-L-leucylglycine (Gly-Gly-Leu-Gly) with an overall yield of 65%.[39] Linkage sites were made by chloromethylation of the polymer (Figure 2),[43] achieving a loading capacity of 0.91 mmol of glycine/g. Homogeneous reactions were conducted in dioxane or dimethylformamide (DMF), and the polymer was precipitated by addition of water. However, the precipitation of polystyrene resulted in some coprecipitation of reagents.[6] Furthermore, phase separation of the polymer during coupling reactions was observed after several synthetic steps due to the cross-linking of chloromethyl groups that remained after incomplete esterification of the first amino acid to the polymer support.

After optimizing reagent and solvent conditions, the synthesis of the hexapeptide Val-Tyr-Val-His-Pro-Phe proceeded in 61% yield on linear polystyrene.[40−42] Increasing losses of polymer-peptide was noted as the peptide elongated due to incomplete precipitation from dioxane by addition of water.

To increase the loading capacity, linear polystyrene was extensively chloromethylated to provide a poly-

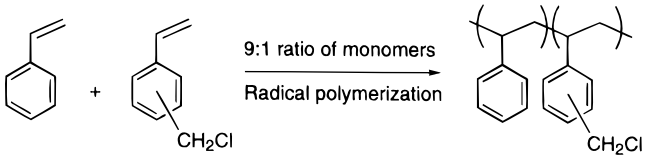


Figure 3. Chloromethylated polystyrene obtained by copolymerization.

mer support, demonstrating 1.49 mmol of glycine/g loading capacity; however, the resulting glycine-terminated polymer was insoluble in DMF and chloroform and subsequent peptide synthesis was achieved under heterogeneous conditions.[44]

To begin to address several of the limitations encountered above, kinetic studies served to optimize conditions for production of a soluble chloromethylated polystyrene with a very low degree of cross-linking.[43] With special precautions to minimize residual chloromethyl groups, a pentapeptide was successfully synthesized in 42% yield. Greater purification at each peptide coupling step was achieved through two precipitations performed by pouring a 10% polymer solution in DMF into a NaCl solution. However, the method was impeded by material losses due to incomplete precipitation as peptide elongation was also observed to cause the attached hydrophobic support to become increasingly more soluble in water.

Fewer material losses were realized by using gel filtration to separate low molecular weight impurities during synthesis of peptides linked to a chloromethylated polystyrene of molecular weight 20 400.[45] To maintain solubility in CH_2Cl_2 throughout the synthesis, the loading capacity could not exceed 0.5 mmol/g. Kinetic investigations of coupling reactions revealed nonlinearity attributable to the nonequivalence of anchoring sites.

A chloromethylated polystyrene for liquid-phase peptide synthesis was prepared by copolymerizing a 9:1 ratio of styrene and chloromethylstyrene (7:3 *m*- and *p*-isomers, Figure 3).[46−49] In contrast to functionalizing polystyrene with chloromethyl groups, the procedure of copolymerization resulted in virtually no cross-linking. Using this support, the effect of various reagents on coupling rates and yields were measured. Yields varied from 86−99% for coupling of a tetrapeptide to polymer-bound leucine with relative rates approximately the same for coupling of single amino acids. Reactions were performed in DMF with polymer precipitation using methanol in >93% recovery yields.

Instead of stepwise elongation of peptides, the synthesis of larger peptides was explored by condensing peptide fragments on chloromethylated polystyrene obtained by copolymerization.[45,46,50−52] Using pentapeptide or decapeptide fragments as building blocks, peptides were repeatedly coupled to polymer supports of various loading capacities. Polymer supports with higher loading capacities (3% or 30% chloromethylstyrene) exhibited reduced yields at each coupling step due to decreased solubility of the peptide-bound support. However, three cycles of coupling proceeded in yields greater than 77% using a polymer support containing 1% chloromethylstyrene. Coupling efficiencies of oligopeptides of 3−10 residues were found to be similar to monomeric

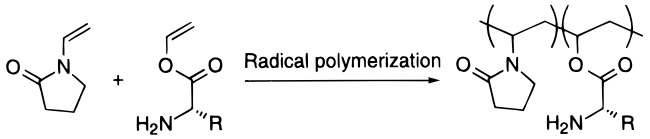


Figure 4. Support prepared by copolymerization of 1-vinyl-2-pyrrolidinone with alkenyl esters of amino acids.

building blocks when coupled to soluble chloromethylated polystyrene supports. This is in contrast to results for coupling to cross-linked polystrene resins in which coupling yields decreased as oligomer length increased; such effects were attributed to the increased inability of longer peptides to penetrate the cross-linked polymer matrix.[52]

B. Peptide Synthesis on Hydrophilic Polymers

The limited solubilizing power of linear polystyrene has motivated the search for more hydrophilic supports for liquid-phase peptide synthesis. Peptide synthesis on polyethyleneimine of molecular weight 30 000−40 000 has been accomplished.[53,54] Attachment of arginine was made through an amide bond to the secondary amine of the support with a loading of 18 mol % (2.6 mmol/g). Ultrafiltration was used to isolate the polymer-peptide after each coupling step, and after the synthesis of Ala-Trp-Ile-Arg, the peptide was enzymatically cleaved from the support using trypsin.

Attempts to use polyacrylic acid and polyvinyl alcohol for peptide synthesis have been made, but these homopolymers exhibited poor solubilities in reaction solvents.[8] However, copolymers of polyvinyl alcohol have been more successfully employed in liquid-phase peptide synthesis.[55,56] Using vinyl acetate and 1-vinyl-2-pyrrolidinone as comonomers, a copolymer of molecular weight 33 000 was made and hydrolyzed that exhibited suitable solubilities to accomplish the synthesis of tetrapeptides in overall yields of about 60%.[55] Ultrafiltration allowed the separation of polymer from excess reagents, and hydrolysis of vinyl acetate residues provided hydroxyl groups for peptide anchoring sites. However, the copolymer exhibited limited solubilizing power and was useful only for the synthesis of smaller oligopeptides.

Polyvinyl alcohol-poly(1-vinyl-2-pyrrolidinone) copolymers were made by polymerizing a series of alkenyl esters of amino acids with 1-vinyl-2-pyrrolidinone to give water-soluble polymers for peptide synthesis (Figure 4).[56] With the use of amino acid monomers obtained by vinyl exchange with vinyl acetate, copolymers were obtained containing the first amino acid preattached. Although coupling of a second residue to the support was demonstrated, no further work employing these polymer supports has been reported.

In addition to the hydrophilic polymers reported above, PEG has also served as a soluble support for liquid-phase peptide synthesis and has become the most popular choice due to its high solubilizing power and ease of precipitation. PEG has been chosen over other polyethers such as polymethylene oxide and polypropylene oxide because of more favorable solubility and crystallization properties.[8]

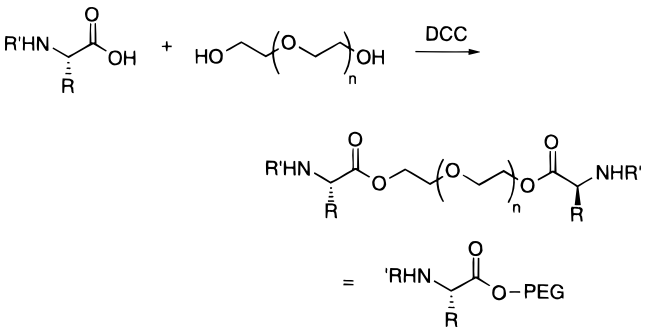


Figure 5. Direct esterification of PEG with amino acids.

C. Peptide Synthesis on Polyethylene Glycol

1. Linkage by Direct Esterification

The liquid-phase method of peptide synthesis on PEG succeeded in the synthesis of numerous peptides, including sparingly soluble oligomers.[51,57−61] To validate the method, a pentapeptide was synthesized on PEG 10 000.[6] Linkage was made by esterification of the first amino acid to PEG mediated by 1,3-dicyclohexylcarbodiimide (DCC) (Figure 5), and peptide coupling yields were greater than 99% as determined by ninhydrin and dansyl methods. No racemization was observed by gas chromatography. Initially, PEG−peptides were purified by ultrafiltration,[5,6,62] but it was soon realized that purification by precipitation/crystallization was faster and more efficient; polymer recovery was achieved by simple filtration after addition of greater than 5-fold excess diethyl ether to PEG solutions in methylene chloride.[63,64]

Optimization of soluble polymer-supported peptide synthesis succeeded in the stepwise synthesis of a 14-mer on PEG 6000.[51] After accounting for an average loss of 0.5% per precipitation step and a 64.6% yield for cleavage from support, 32.7% yield of analytically pure peptide was reported.

The synthesis of several peptides including a 20-mer has been performed using MeO-PEG 5000 or PEG 20 000.[65] Quantitative coupling was confirmed by fluorescamine or amino acid analyses, and products were obtained in high purity. The conformation of the growing peptide had a significant effect on solubility and coupling kinetics. PEG-bound oligomers reporting β-structure by circular dichroism (CD) displayed decreased solubility and reactivity unlike PEG−peptides with unordered or α-helical structures. To destabilize ordered structures, proline or glycine insertion in β-forming peptides led to increased solubility of these PEG-bound peptides. During synthesis of $[$L-Glu(OBzl)$]_{20}$ (OBzl = benzyl ester) on either MeO-PEG 5000 or PEG 20 000, the polymer−peptide conjugate displayed a decrease in solubility and reactivity at the hexapeptide to octapeptide stage, but an increase in solubility and reaction rate upon further elongation that correlated with CD measurements indicating aggregated β-like structures at intermediate peptide lengths prior to the induction of an α-helical conformation exhibited for longer peptides of Glu(OBzl). Evidently, PEG does not influence the conformation of bound peptides. Therefore, although increasing the molecular weight of PEG may help keep longer single-chain peptides in solution, the solubilizing power of PEG may not be strong enough to prevent aggregation of peptide chains.

A series of homoglycine peptides of 1−9 residues on PEG 10 000 were synthesized for conformational studies.[66] The synthesis of higher homologs was hindered by incomplete coupling. Additional homo-oligopeptides for conformational studies were also successfully constructed on PEG 10 000.[67] Oligoalanine (1−8 residues) or oligovaline (1−6 residues) linked to glycine−PEG were restricted in length as analytically pure peptides could not be obtained beyond certain lengths without significant changes in reaction conditions.

Partial sequences of the peptide antibiotic alamethicin were prepared along with analogs containing α-aminoisobutyric acid (Aib = 2-methylalanine) residues with sequence lengths up to 14.[68,69] Good yields and high purity of peptides were obtained on PEG 6000 and PEG 10 000. In order to drive reactions to completion, coupling of the sterically hindered α-aminoisobutyric acid residue required large excesses of reagents. Reaction monitoring and conformational studies were performed using ^{13}C NMR spectroscopy, and the presence of PEG did not induce conformational changes.[23]

The undecapeptide substance P was synthesized by the liquid-phase method and displayed full biological activity.[70] This endeavor demonstrated that purification by ether-induced precipitation instead of ultrafiltration enabled faster preparation times while maintaining high yields and purity. High coupling yields (>99.5%) were achieved with only 4−6-fold excess of *tert*-butyloxycarbonyl (Boc)-protected amino acids, and the ester linkage between the finished peptide and PEG was cleaved using NH_3/CH_2Cl_2. This lengthy peptide was prepared on PEG 15 000 to maintain homogeneous reaction conditions.

The stepwise synthesis of a peptide corresponding to the insulin segment B 13−20 was performed on PEG 3000.[71] The use of this relatively low molecular weight PEG required changing the solvent from CH_2Cl_2 to DMF during peptide elongation to maintain homogeneity. Coupling reactions were monitored by ^{13}C NMR spectroscopy of support-bound peptides. The onset of α-helical structure was observed by circular dichroism measurements beginning with the polymer-bound hexapeptide. Alkaline saponification released peptide from support in 94% crude yield.

Peptide fragments were synthesized on a soluble support in order to prepare the peptide secretin by fragment condensation.[72−74] Peptides up to 13 residues in length were prepared on PEG 10 000 and cleaved by liquid NH_3 in DMF/methanol or by hydrazine in methanol. In another example of fragment condensation, peptides obtained by classical methods were condensed on PEG 10 000 to provide the biologically active insulin A-chain.[57]

Analytically pure oligopeptides of glutamate and deuterated analogs were prepared on PEG 5000 up to the heptapeptide.[24] Coupling reactions required 10−50% DMF in CH_2Cl_2 to solubilize longer chain length peptides, and purity at each step after ether precipitation was assessed by ^{1}H NMR. Peptide cleavage was detected in low levels during synthesis,

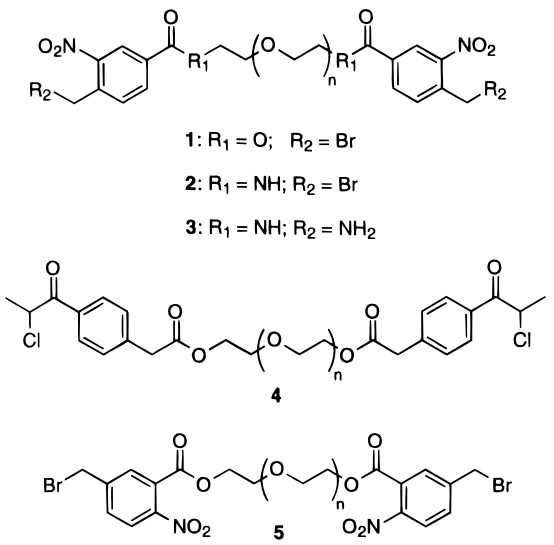


Figure 6. Photolabile PEG-based supports used in liquid-phase peptide synthesis.

and studies indicated that the presence of 1-hydroxybenzotriazole (HOBt) may displace the peptide from PEG during DCC-mediated coupling. Thus, a direct ester linkage between PEG and peptides is not completely stable under these reaction conditions.

Under certain coupling conditions, esterification of PEG with amino acids has provided a straightforward means of initiating liquid-phase peptide synthesis. In fact, acid-catalyzed esterification of PEG 6000 with neutral amino acids without protecting the amino group provided nearly quantitative PEG esters after refluxing in benzene over molecular sieves, although lengthy reaction times (2−3 days) were required.[75] However, different linkages have been sought to provide increased stability during peptide synthesis and greater versatility for selective cleavage of peptides from PEG.

2. Linkages Cleaved by Photolysis and/or Hydrogenolysis

Although observing higher coupling yields, a major disadvantage of the early use of liquid-phase peptide synthesis relative to solid-phase methods was low cleavage yields to obtain final peptides. Attempts to drive the cleavage reaction to completion using strenuous conditions or prolonged reaction times may cause racemization and other side reactions.[76] Incomplete cleavage and/or side reactions spurred research to find linkages other than direct esterification of PEG to peptides; however, a recent publication described transesterification conditions that accomplished quantitative cleavage of peptides directly linked to the soluble support.[77]

A derivatized PEG support was prepared that permitted photolytic release of peptides after synthesis.[78−80] Cleavage yields of heptapeptides synthesized on 3-nitro-4-(bromomethyl)benzoyl-PEG (**1**) proceeded in 87−96% (Figure 6).[79] A comparative study between solid- and liquid-phase synthesis using a model tetrapeptide found that photolytic release of the peptide from PEG proceeded in 98% yield compared to 69% yield of released peptide from a cross-linked polystyrene support containing the same linker. Catalytic hydrogenation may also be used to accomplish peptide release and removal of side chain protecting groups in a single step in 75−81% yield.[81] After esterification of PEG 6000 with 3-nitro-4-(bromomethyl)benzoic acid, the solubility of this PEG derivative in CH_2Cl_2 was somewhat less relative to unmodified PEG; however, solubility improved after the first amino acid was attached. The ester linkage was stable to all phases of peptide synthesis, and photolytic release was achieved by irradiation at 350 nm in anhydrous, oxygen-free methanol or DMF. A higher yielding method (from 60% to 85.8% yields) prepared **1** from the acyl chloride and catalyzed esterification of the first amino acid using the potassium, not cesium, salt in the presence of 18-crown-6.[82] In order to react with all bromomethyl groups of the linker and to avoid employing large excesses of reagent and prolonged reaction times that may lead to side reactions or racemization, the reaction sequence was modified such that the first residue was added to the linking unit prior to attaching linker to the polymer support.[83] Increased stability was realized by changing the ester linkage to an amide bond by using diaminoPEG 6000; carrier **2** enabled the synthesis of a tetrapeptide with photolytic cleavage in 95% yield.[84] After diaminoPEG was linked to 3-nitro-4-(bromomethyl)benzoyl chloride to yield a photolabile support, it was derivatized to provide an amino group for attachment of the first amino acid through an amide bond (support **3**).[85]

A nonapeptide corresponding to bovine insulin B_{22-30} was synthesized and photolyzed from PEG 15 000 terminated in [3-nitro-4-(bromomethyl)benzoyl]glycyl moieties.[86] The glycyl spacer was introduced to achieve higher reactivity and greater coupling yields of linker to soluble support due to increased nucleophilicity of the amino group over the hydroxyl groups of PEG (98% versus 30% yield, respectively). Furthermore, the glycyl spacer was used as an internal standard during amino acid analyses.

[4-(2-Chloropropionyl)phenyl]acetic acid coupled to PEG 6000 also provided the photolabile support **4** that enable synthesis of a pentapeptide in 92% yield with quantitative release from the support.[87]

In addition to peptide release via hydrogenolysis, products synthesized on 2-nitro-5-(bromomethyl)-benzoyl-PEG 6000 **5** may be cleaved by reduction with sodium dithionite.[76] A tetrapeptide was synthesized by the liquid-phase method and constituted 87.6% of the crude yield of cleaved peptide; less than 0.1% of a dipeptide was racemized during the reductive cleavage step.

3. Acid- or Base-Labile Linkages

Urethane linkages enable cleavage of peptides from the support using acidic or basic conditions and form readily by reaction of isocyanates with the hydroxyl groups of PEG.[85] Polymer-peptide linkages derived from 2-chloroethyl isocyanate proved unsatisfactory as β-elimination of HCl from the PEG derivative occurred during attachment of the first amino acid; however, 6-chlorohexyl isocyanate produced a stable PEG support that enabled synthesis of a PEG-bound tetrapeptide in 75% yield. Peptide release was achieved through mild basic hydrolysis. But as alkaline conditions allow the possibility of racemiza-

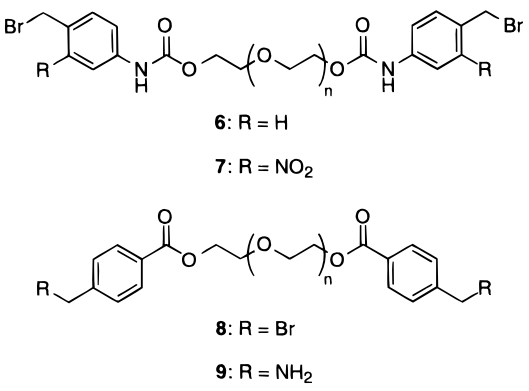


Figure 7. Acid- or base-labile linkages to PEG.

tion, other linkers were pursued. Breakdown of the urethane linkage between 4-(bromomethyl)phenyl isocyanate and PEG 6000 occurred during attempts to remove the Boc group of the amino acid residue bound to **6** using 1.2 N HCl/HOAc; however, transformation of the support to include a nitro group ortho to the bromomethyl moiety increased acid stability of **7** (Figure 7). After peptide synthesis, acid hydrolysis (HBr/HOAc or HF) or catalytic hydrogenolysis cleaved the peptide from the nitro-containing support. 4-(Bromomethyl)benzoyl chloride generated a versatile PEG derivative **8** that also released test peptides using acid or H_2/Pd. Conversion of **8** to the amino-terminated linker (overall conversion of hydroxyl groups in 80% yield) permitted direct coupling of the first amino acid to **9** in quantitative yields.

Several supports using MeO-PEG 5000 contained hydrazine functionalities that enabled peptide synthesis in the presence of different side chain protecting groups.[88] Thus, an acid-labile linker enabled the synthesis of several peptides containing aspartic acid or glutamic acid protected with benzyl-type esters; peptides with *tert*-butyl-based side chain protecting groups were anchored by linkages cleavable by catalytic hydrogenation. The use of MeO-PEG 5000 derivatized with hydroxybenzyl end groups also allowed cleavage of synthesized peptides using trifluoroacetic acid and a similar derivative permitted photolytic cleavage.[89,90]

4. Thiol-Labile Linkages

Synthesis of glutathione was accomplished by using PEG 10 000 as a thiol protecting group.[91] Linkage was made through selective reaction of PEG 4-(2-nitrovinyl)benzoate with cysteine.

PEG 6000 was derivatized with 4-phenoxy-3,5-dinitrobenzoyl chloride to generate a crystalline solid with a measured loading capacity of 0.1−0.2 mmol/g.[92] In aqueous solutions of pH 7, reversible attachment of peptides was realized through the thiol group of cysteine (Figure 8) with peptide release achieved by addition of 2-mercaptoethanol. Thus, semisynthesis of peptides may be completed by selective attachment of peptides without masking of other functional groups.

5. Peptide Synthesis in Water

A novel approach to peptide synthesis by extension from the carboxyl end under completely aqueous

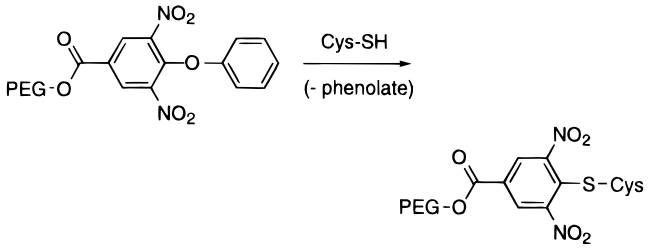


Figure 8. PEG used as a thiol protecting group for semisynthesis of peptides.

conditions has been demonstrated.[93] PEG 6000 functionalized with terminal carboxyl groups served as the support for coupling of methyl ester-protected amino acids mediated by water-soluble 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC). Reaction workup was accomplished by repeated chloroform extraction of the polymer-peptide from water. After evaporation of chloroform, the polymer-bound peptide was dissolved in water and treated with immobilized carboxypeptidase Y to free the carboxyl group for another round of elongation. The final pentapeptide was released using CNBr for selective cleavage at the carboxyl side of a methionine residue and obtained in 64% yield.

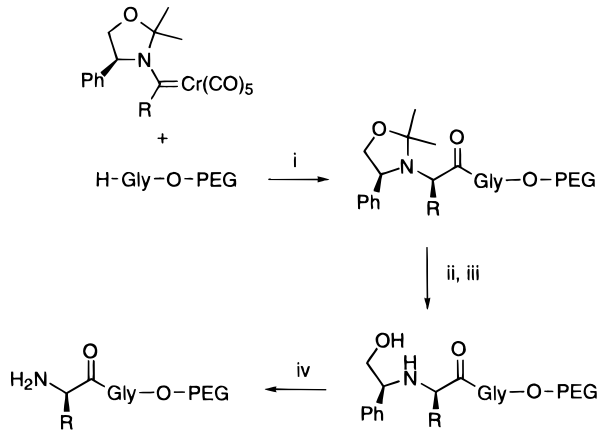
6. Copolymers of PEG

To obtain a PEG-based support with increased loading capacity, block copolymers were made by linking PEG with 3,5-diisocyanatobenzyl chloride or 3-nitro-3-azapentane-1,5-diisocyanate (Figure 1).[94] The diisocyanide-containing moieties provided the additional anchoring groups for peptide synthesis through either the chloromethyl group or hydrazino group obtained by hydrogenation of the nitro group after copolymerization. Polyether blocks were made from PEG 400, 1000, 6000, or 10 000; and final copolymers ranged between 50 000 and 1 000 000 molecular weight. Only copolymers containing PEG blocks of 6000 or 10 000 molecular weight were crystalline while others were highly viscous oils. The copolymer containing PEG 1000 was used as a liquid-phase support for synthesis of the tetrapeptide Ile-Ala-Val-Gly in 52% overall yield with purification achieved at each step by ultrafiltration.

7. Peptides Containing Non-Natural Amino Acids

The elongation of peptides bound to PEG using chromium aminocarbene complexes led to the preparation of peptides containing non-natural amino acid residues.[77] Reactions were initially attempted on a solid-phase support; however, the heterogeneous conditions resulted in problematic removal of the chiral auxiliary on the new introduced residue. Instead, after PEG 4000 or 8000 was esterified to glycine, several di- and tripeptides were synthesized using a coupling cycle consisting of photolysis of the carbene complex to add a residue and additional chemical manipulations to unmask an amino group for continued peptide elongation (Scheme 1). Transesterification allowed quantitative cleavage, but overall yields were reduced due to incomplete diastereoselectivity and less than quantitative coupling yields. Nevertheless, after two photochemical coupling cycles, a tetrapeptide was obtained in 58% yield after

Scheme 1[a]



[a] Reagents: (i) *hν*/CO, THF; (ii) 1 N HCl/MeOH; (iii) *N*-methylmorpholine; (iv) H_2, $Pd(OH)_2/C$.

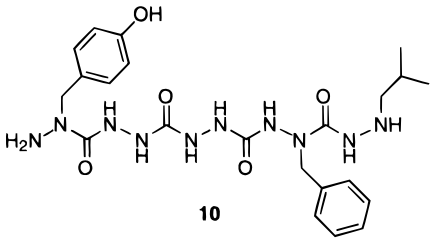


Figure 9. Peptidomimetic azatide Tyr[a]-Gly[a]-Gly[a]-Phe[a]-Leu[a] prepared by liquid-phase synthesis (superscript a refers to aza−amino linkage).

purification to a single diastereoisomer, whereas under solid-phase conditions, only 18% overall yield was realized.

Liquid-phase synthesis has also been applied to the construction of peptidomimetics consisting of oligomers of "α-aza amino acids" or "azatides".[12] These peptide-like molecules are built of amino acids wherein the α-carbon of each residue has been replaced by a nitrogen atom. Lacking the asymmetrical structure of normal peptides, azatides may serve to explore peptide structure as well as to provide a source of bioactive molecules with improved pharmacokinetic properties. After developing general synthetic procedures to obtain a new alphabet of α-aza amino acid monomers, bispentafluorophenyl carbonate-mediated coupling allowed the synthesis of the azatide pentamer **10** (Figure 9) in 56.7% overall yield; cleavage was performed by catalytic hydrogenolysis of a benzyl ester linkage to MeO-PEG 5000. Synthesis on MeO-PEG was monitored by ^{1}H NMR and ninhydrin analysis.

8. Peptide Synthesis Incorporating the Purification of Intermediates

In most cases, polymer-supported peptide synthesis drives reactions to completion as incomplete conversion and/or side reactions result in the accumulation of failure sequences that cannot be separated from desired product until after peptides are cleaved from the support. To obtain pure peptides without the need for quantitative coupling yields, an alternating solid−liquid phase scheme was developed (Figure 10).[17,18,21] In this method, peptide elongation takes place on PEG carriers; however, the amino acid building blocks are supplied by an insoluble polymeric reagent. During the coupling step, peptides are increased in length by one residue and simultaneously attached to the solid support. Upon filtration, any PEG−peptide that failed to couple is rinsed away. Afterward, the PEG-bound peptide elongated by one residue is cleaved from the solid support. Also cleaved from solid support is uncoupled amino acid which is separated from PEG−peptide by gel permeation chromatography or precipitation methods; thus each coupling cycle results in both chain elongation and purification not only from excess reagents but also from failed sequences. Upon final cleavage from the PEG support, the peptide is obtained in pure form without additional purification steps. PEG 4000 was chosen for the liquid-phase support as a compromise between the faster reaction rates of shorter PEGs with the solid-phase reagent and the solubilizing power and ease of purification/crystallization of higher molecular weight PEGs. Coupling rates and yields decreased when using PEG carriers of 10 000 and 20 000 molecular weight.[19] This alternating liquid−solid phase peptide synthesis method was used to prepare calcitonin M (28−32) pentapeptide.[20] The concepts of the alternating liquid−solid phase method have merit for applications employing reactions that do not proceed to completion.

In another approach that combined the use of soluble and insoluble polymers, PEG−peptides were coupled with activated amino acid esters on a solid support without covalent linkage between polymeric components. Up to 15 residues was built onto PEG 10 000 before the solubility properties of PEG were significantly diminished.[95] PEG−peptides were coupled quantitatively by three treatments of 5−10 molar excess of the polymer reagent, and simple purification from excess reagent was performed by filtration of the solid support. Unlike the previous method, some impurities were detected with the peptides after cleavage from PEG.

9. Automated Liquid-Phase Peptide Synthesis

An automated peptide synthesizer employing liquid-phase synthesis has been described.[26] Depending on the identity of amino acid to be coupled, the synthesizer required 1−5 h for completion of each coupling cycle and included a recrystallization step that was performed after precipitation of the PEG−peptide. Solvents and reagent solutions were fed through tubes under nitrogen pressure into a reaction vessel resembling a rotary evaporator that provided mixing and temperature control during reactions and subsequent work up. Precipitation/recrystallization was performed by concentrating the reaction mixture, adding diethyl ether, mixing and cooling, and filtering through a porous glass frit by 90° rotation of the apparatus. Purity and coupling yields of peptides compared similarly to previous manual operations of liquid-phase synthesis; however, precipitation/recrystallization steps were faster and higher yielding. Linear reactions rates for PEG-supported peptide synthesis (see below) permitted kinetic analysis, rather than chemical or spectroscopic analysis, to determine coupling completion.

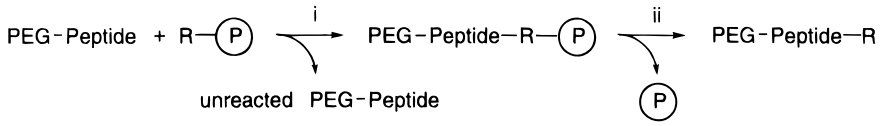


Figure 10. Elongation cycle in liquid-phase synthesis using solid-phase reagents in order to produce pure peptides in the absence of quantitative coupling. Reactions: (i) coupling of PEG-bound peptide to polymeric reagent results in a solid-phase product that is filtered to remove unreacted PEG-bound peptides; (ii) cleavage and filtration steps to obtain pure PEG-bound peptides lengthened by one residue.

Table 2. Measured Rates of *p*-Nitrophenol (Np) Release from Boc-Ala-ONp by H_2N-Gly-OCH_2R in $CHCl_3$[45]

R	k_2 (M^{-1} s^{-1})
phenyl	0.081
polystyrene, soluble (molecular weight 20 400)	0.047
polystyrene, insoluble (cross-linked)	0.030

Table 3. Measured Rates of *p*-Nitrophenol (Np) Release from Boc-Ala-ONp by H_2N-Gly-OR in CH_3CN[96]

R	k_2 (M^{-1} s^{-1})
ethyl	0.013
2-methoxyethyl	0.008
PEG (2000)	0.018
PEG (4000)	0.019
PEG (6000)	0.016
PEG (10 000)	0.014
PEG (20 000)	0.014

Table 4. Measured Rates of *p*-Nitrophenol (Np) Release from Boc-Ala-ONp by H_2N-Gly-OR or H_2N-$(Gly)_3$-OR in CH_3CN[96]

peptide	R	k_2 (M^{-1} s^{-1})
Gly	ethyl	0.013
$(Gly)_3$	ethyl	0.019
Gly	PEG (20 000)	0.014
$(Gly)_3$	PEG (20 000)	0.031

D. Comparative Kinetic Studies of Support-Based Peptide Synthesis

The substantial differences in reaction rates initially observed between heterogeneous solid-phase reactions and classical solution chemistry stimulated the research of liquid-phase methodologies. Table 2 illustrates the differences found among classical, liquid-phase, and solid-phase methodologies for polystyrene-based systems and were attributed to variable degrees of steric hindrance of the reactive sites for compounds free in solution or linked to a soluble linear polymer or insoluble cross-linked polymer.[45] Restricted permeability was also the proposed cause for low yields in fragment condensations on cross-linked polymers.[52]

Unlike polystyrene, PEG-supported reactions demonstrate similar kinetic behavior to classical solution-phase synthesis.[10,64,96−97] To delineate the effect of polymer molecular weight on the reactivity of PEG−peptide esters, kinetic studies found no significant differences between high and low molecular weight amino acid esters (Table 3). In fact, reaction rates were slightly faster for PEG esters, and rate constants showed little dependence on polymer chain length (Tables 3 and 4).

The finding that rate constants were higher for PEG esters compared to a truncated analog (2-methoxyethyl ester) was explained by polymer-induced, local environmental changes in solvation or the effective dielectric constant.[8] Hydrogen bonding of an oxygen atom of PEG to the terminal amino group of peptide PEG esters was also suggested as a mechanism for increasing the nucleophilicity of the terminal amino group and increasing rates of reaction.[8] Rate constants did not differ significantly among esters of PEG 2000 to 20 000 molecular weight, and second-order kinetics were observed throughout reaction up to 80% completion without deviation from linearity. Although a slight rate increase was observed for the ethyl esters upon increasing from one to three glycine residues, the rate increase for the corresponding PEG 20 000 esters was significantly greater (Table 4).[96]

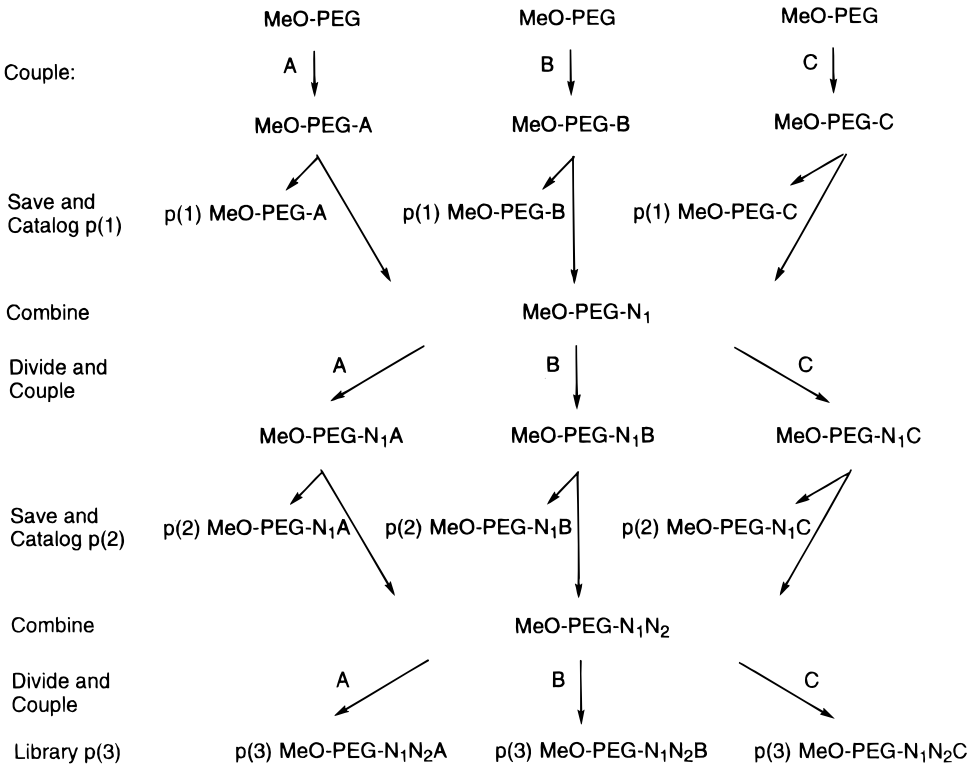
In other research using PEG as a soluble support, the kinetics of peptide coupling was measured and found to be linear; rate constants were slightly higher than classical solution-phase peptide synthesis.[64] In order to optimize coupling reactions, competition experiments were performed to obtain relative reaction rates for peptide bond formation for PEG-bound amino acids.[97] The measurement of relative reaction rates for 20 amino acids found a strong dependence on the steric requirements of each amino acid with relative reaction rates differing by up to factors of 50 among individual amino acids. Although the molecular weight of PEG had no influence on the kinetics of the coupling reaction, reaction rates were influenced by solvent and conformation of the growing peptide chain. Therefore, although PEG largely determined solubility properties of the PEG−peptide conjugate, the macromolecular protecting group had little effect on peptide reactivity; a prerequisite for the successful application of liquid-phase synthetic schemes.

E. Liquid-Phase Combinatorial Synthesis (LPCS) of Peptides

Combinatorial chemistry is an intense area of research driven in large part by the need of the pharmaceutical industry to obtain new drug candidates for high-throughput screening assays. The synthesis of pools of numerous molecules, or libraries, is currently the limiting step in the drug discovery process,[13] and efforts are underway to develop larger and more diverse libraries to enhance discovery of new leads for drug development.

For the synthesis of libraries, insoluble polymer supports have been used to facilitate product purification through simple filtration and rinsing. Although successful, solid-phase synthesis has several shortcomings that arise by the nature of the heterogeneous reaction conditions. To overcome these limitations, liquid-phase combinatorial synthesis (LPCS) has been developed as an alternative methodology for the construction of small molecule librar-

Scheme 2



ies wherein the use of a *soluble* polymer support provides both the advantages of solid-phase synthesis and the benefits of classical solution-phase organic chemistry.[11] Liquid-phase synthesis is amenable to combinatorial techniques; in fact, this solution-phase method permits all manipulations, including portion mixing or split synthesis, to be performed under homogeneous conditions. Successful results indicate that LPCS should have general utility for the construction of small molecule libraries of high chemical diversity.[11,12,14] The validity of this method was demonstrated by the synthesis and screening of a peptide-based library that led to the identification of several members found to bind an anti-β-endorphin monoclonal antibody.[11]

1. Library Synthesis

To demonstrate the utility of LPCS, a combinatorial library of 1024 pentapeptides composed of four amino acids (Tyr, Gly, Phe, Leu) with five partial libraries were synthesized.[11] By using a technique known as recursive deconvolution, partially synthesized libraries are obtained by saving and cataloging aliquots of reaction mixtures during the synthesis of the combinatorial library. The amount saved and catalogued is dependent on the library degree; for a final library consisting of pentapeptides, five sublibraries are required. These partial libraries are later utilized in the screening process to identify novel library members with targeted properties.

A variation of the split synthesis approach was employed to allow recursive deconvolution of the combinatorial library to identify peptides with high binding properties.[11,98,99] To illustrate this method, Scheme 2 outlines the hypothetical synthesis of a tripeptide library containing an alphabet of three different amino acids: A, B, and C. Coupling each member (A, B, C) of the alphabet to MeO-PEG 5000 in segregated reaction vials establishes three channels of synthesis. After diethyl ether-induced precipitation, filtration, and dissolving in methylene chloride, a portion of the polymer solution from each reaction vial is removed, set aside, and labeled as partial library p(1) consisting of the three members MeO-PEG-N_1 (where N_1 can represent A, B, or C). The remaining solutions of each reaction vial were then combined and divided among the reaction vials. After another round of coupling, portions of the purified PEG-bound peptides were again sampled to generate a sublibrary labeled p(2). This process of coupling, saving/cataloging, and randomizing was repeated until the desired peptide library p(n) was obtained.

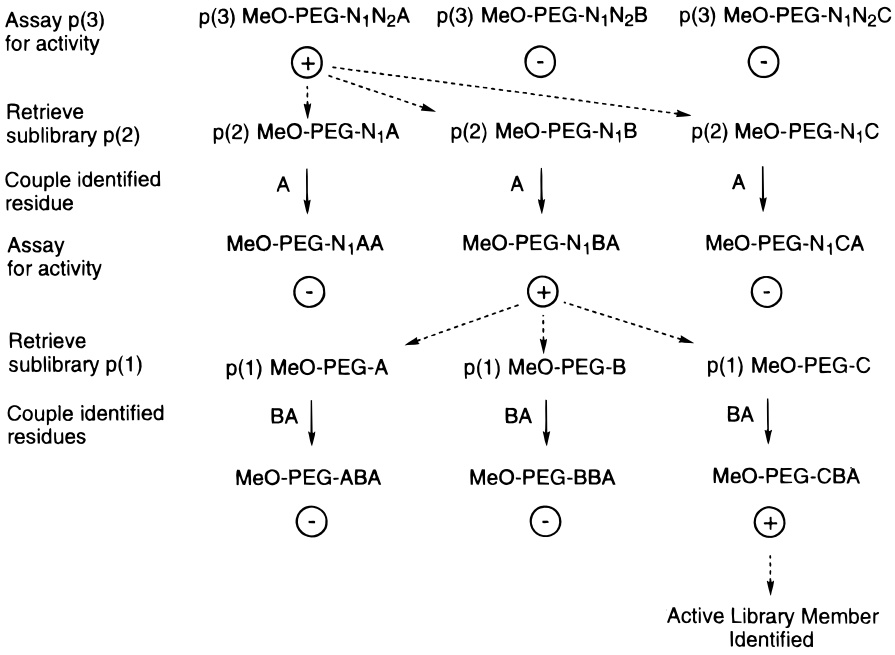
2. Library Screening

By using an affinity assay, the pentapeptide library was screened for high-affinity ligands to a monoclonal antibody that binds the β-endorphin sequence Tyr-Gly-Gly-Phe-Leu with great affinity ($K_d = 7.1$ nM).[100] Library screening was accomplished through a recursive deconvolution method that is illustrated with a hypothetical tripeptide library in Scheme 3. In this method, the final peptide pools (sublibrary p(n)) obtained after peptide synthesis were each screened for binding by a competitive ELISA assay. After identifying the peptide pool containing the best binding ligands, the terminal residue common to that pool was coupled to each of the catalogued pools of the p($n-1$) sublibrary. This process of screening and coupling was then repeated until the full identities of the active peptides were found.

3. Results

The direct assay of PEG−peptide conjugates synthesized by LPCS combined with the recursive de-

Scheme 3



convolution screening strategy resulted in the discovery of ligands that inhibited the binding of leucine enkephalin to anti-β-endorphin monoclonal antibody 3E7. Screening the final sublibrary identified the native epitope and several other potent binders.[11] Noteworthy is the observation that PEG did not significantly affect binding as detected binding affinities were found to be similar between the p(5) sublibraries of MeO-PEG−peptide conjugates (51 μM) and free peptides (46 μM).[11]

IV. Oligonucleotide Synthesis on Soluble Polymer Supports

Analogous to polymer-supported peptide synthesis, covalent attachment of the growing chain of nucleotides to a macromolecular support also simplifies an otherwise arduous task of purification during oligonucleotide synthesis. The different reaction conditions required for oligonucleotide synthesis as compared to peptide synthesis poses new constraints on the polymer support. In oligonucleotide synthesis, pyridine and water are common solvents; thus although polystyrene was initially attempted as a soluble support, hydrophilic polymers were found to be more compatible for the preparation of oligonucleotides.[101]

A. Oligonucleotide Synthesis on Polystyrene

In an attempt to extend the techniques developed in the early phases of liquid-phase peptide synthesis, soluble linear polystyrene was also used as the support for oligonucleotide synthesis in pyridine. The hydrophobic macromolecule precipitated upon addition of water; however, the attached oligonucleotide conferred some degree of water solubility, and losses of 10−15% at each extension step were realized during precipitation.[102−104] Additionally, some crosslinking and consequent changes in solubility occurred after several chain elongation cycles.[105]

A trinucleotide was synthesized on linear polystyrene of molecular weight 170 000 derivatized to contain about 20% *p*-monomethoxytrityl chloride groups (Figure 11).[102] After acidic cleavage from the polymer, the trithymidine product was purified by chromatography and obtained in 11% yield. Phosphorylation and cleavage of thymidine bound to trityl-functionalized polystyrene was also performed.[106,107] Another group found conditions for homogeneous pyridine reactions using a similar trityl-containing polystyrene of molecular weight 270 000 (with 0.4 mmol/g measured loading capacity) that provided higher yields of oligonucleotides.[103,104] Although reaction rates were comparable to classical solution methods, product recovery was again problematic for longer oligonucleotides.[105] In an attempt to synthesize a pentadeoxyribonucleotide, the polystyrene-supported oligonucleotides had significant solubility in water that prevented significant precipitation of polymer from pyridine. As was seen in liquid-phase peptide synthesis, polystyrene was abandoned to be replaced by more hydrophilic polymer supports for oligonucleotide synthesis.

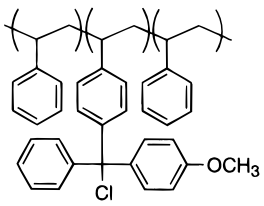


Figure 11. Trityl-functionalized polystyrene.

B. Oligonucleotide Synthesis on Polyvinyl Alcohol

For increased solubilizing power, better compatibility, and higher loading capacity, polyvinyl alcohol is a better soluble support than polystyrene for the synthesis of oligonucleotides. Polyvinyl alcohol possesses numerous anchoring sites due to the array of

hydroxyl groups that define the polymer; however, to avoid the synthesis of truncated sequences, all unoccupied sites must be blocked after attachment of the first nucleotide before continuing oligonucleotide synthesis. A potential problem of this support is overloading of the polymer such that pendant oligomer chains begin to dominate the solubility characteristics of the polymer−oligonucleotide conjugate or that neighboring chains interact and adversely affect the reactivity of the attached oligonucleotides.

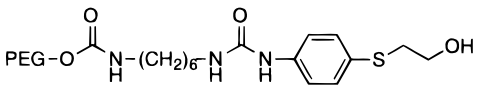
Oligothymidyl phosphates of 1−4 residues in length were synthesized on polyvinyl alcohol of molecular weight 70 000.[108,109] After esterification of ribouridylic acid to the soluble support, stepwise synthesis in pyridine/hexamethylphosphoramide (HMPT) and ultrafiltration purification produced the polymer product that was hydrolytically cleaved to yield free oligonucleotides and phosphorylated uridylic acid bound to the polyvinyl alcohol. Although a high loading of oligonucleotides was achieved, only short oligomers could be prepared before solubility properties began to be determined largely by the growing pendant chains. Thus, a loading capacity of up to 10 mmol of mononucleotide per gram of polymer was achieved by coupling in pyridine/HMPT mixtures with greater than 90% yields, but problematic separations occurred upon further elongation.[110] Polyvinyl alcohol also served as a soluble support for the synthesis of pentathymidine in 8% overall yield,[110] and another group found cleavage conditions that left nucleobase protecting groups intact.[111] Separation of the polymer product from low molecular weight compounds was suggested using dialysis, ultrafiltration, and Sephadex chromatography.[109]

C. Oligonucleotide Synthesis on Copolymers of Polyvinyl Alcohol

A hydrolyzed copolymer of polyvinyl acetate and poly(1-vinyl-2-pyrrolidinone) of molecular weight 42 000 (10−20% vinyl alcohol) provided a soluble support containing hydroxyl anchoring sites and possessing higher solubilizing power than polyvinyl acetate.[112,113] Nucleotides were attached at the 5′-hydroxyl group to the polymer by carbonate linkages with a loading capacity of 1 mmol/g. Oligothymidines of 1−4 residues in length were prepared; however, dialysis in water was insufficient for removal of impurities, a second step using ion chromatography was required to complete purification.

D. Oligonucleotide Synthesis on Cellulose

A derivative of cellulose acetate provided a soluble support for the synthesis of oligonucleotides using the phosphotriester approach.[114,115] This polymer was chosen because of pyridine solubility yet was precipitated by the addition of ethanol. Linkage between polymer and oligonucleotide contained a 2-sulfonylethyl group that allowed facile cleavage of product from the support using triethylamine in pyridine. With this method, a fragment condensation approach provided a ribonucleotide undecamer in 69% overall yield via three coupling steps using RNA fragments, and a deoxyribonucleotide octamer was synthesized in 78% overall yield also in three steps.



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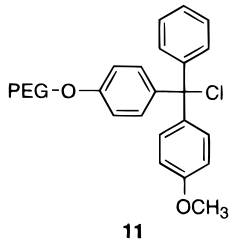

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Figure 12. Functionalized PEG supports for liquid-phase oligonucleotide synthesis.

E. Oligonucleotide Synthesis on Polyethylene Glycol

Early experiments in liquid-phase oligonucleotide synthesis used 2-hydroxyethyl phenyl thioether for attachment of PEG 6000 to a thymidine nucleotide (**10**, Figure 12).[116] This linkage allowed cleavage from support in a two step manner using *N*-chlorosuccinimide followed by NaOH; these mild cleavage reactions released products from PEG under conditions that kept protecting groups of the nucleobases intact.

PEG 20 000 was also employed as a macromolecular protecting group for oligonucleotide synthesis.[16] Linkage was made through a trityl-like functionality (**11**, Figure 12) or by phosphodiester attachment of PEG-OH to the 5′ end of uridine. Individual reaction yields were measured by UV−visible spectroscopy using calculated extinction coefficients; and since analyses were nondestructive, the material used for spectroscopy was returned to the reaction sequence.

PEG 6000 and 20 000 were used as hydrophilic phosphate protecting groups in the development of a liquid phase method of oligonucleotide synthesis.[111] DiaminoPEG 10 000 provided for triadenosine synthesis in 14% overall yield using ultrafiltration for product separation.[111]

The rapid and high-yielding synthesis of oligonucleotides using the High-Efficiency Liquid-Phase method (HELP) employed PEG as the soluble support.[117−119] Improvements in large-scale HELP using a phosphotriester procedure eliminated side reactions involving guanosine and employed less costly starting materials.[120] After MeO-PEG 5000 was esterified in 90−95% yield for a 0.18 mmol/g loading capacity, HELP yielded hundred milligram quantities of short oligonucleotides from gram quantities of MeO-PEG. After testing the efficiency of the method by synthesis of four DNA homodimers, an octanucleotide was synthesized in an overall yield of 79% with coupling yields at each step greater than 87%. PEG−nucleotides were analyzed by ^{1}H NMR for reaction completion, product verification, and purity assessment. Advantages of HELP over solid-phase methods include requiring a lower excess of reagents due to homogeneous reactions, obtaining large amounts of oligonucleotides in a single synthetic run, and easy reaction monitoring by nondestructive methods.

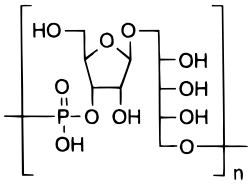

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Figure 13. Polysaccharide target for liquid-phase synthesis.

Phosphoramidite chemistry was later employed in a HELP methodology to allow the more efficient synthesis of longer oligomers by reducing reaction times and increasing coupling yields. Thus, an octanucleotide was synthesized using MeO-PEG 5000 with an overall yield of 93%.[121] The higher solubilizing power of MeO-PEG 12 000 was used to synthesize a 20mer in 85% overall yield with an average yield of 99% coupling at each step. Unfortunately, a small but constant weight loss was noted after each coupling step; weight reductions less than 1% of the total amount of support occurred during precipitation/filtration steps.

Performing the purification of intermediates using gel filtration instead of precipitation enabled quantitative recoveries of PEG oligomers in a modified HELP method to produce cyclic oligonucleotides.[122] To allow for cyclization, the oligonucleotide was not anchored by a phosphate linkage; instead, the exocyclic amino group of a cytidine derivative was linked to a succinic acid moiety attached MeO-PEG 5000. After chain assembly of linear homooligomers of 2′-deoxycytidylic acid containing up to 14 residues (92−96% coupling yields using dimeric building blocks), cyclization was performed in yields higher than those seen using classical solution chemistry under the same reaction conditions.

The introduction of a sulfurization step in a HELP method allowed the synthesis of phosphorothioate derivatives of oligonucleotides.[123] In a demonstration of the modified protocol, a 20mer phosphorothioate attached to PEG 12 000 was synthesized in 83% overall yield, with 46% isolated yield after deprotection and purification.

To address the need for kilogram quantities of synthetic oligonucleotides anticipated for therapeutic and diagnostic applications, a large-scale HELP method was developed.[124] Instead of phosphotriester and phosphoramidite chemistry, oligonucleotide synthesis employed H-phosphonates to allow for the recovery and recycling of the excess reagent in order to realize significant cost savings in large-scale applications. By performing only one oxidation step at the end of oligonucleotide synthesis, the new method eliminated a synthetic step during each coupling cycle and realized higher overall yields by reducing the number of precipitation steps in the overall synthetic procedure.

Phosphoramidite chemistry on MeO-PEG 5000 was also applied to the synthesis of a polyribosylribitol phosphate corresponding to a fragment of a capsular polysaccharide from *Haemophilus influenzae* (**12**, Figure 13).[125] The liquid-phase procedure was found to be less time consuming than classical methods and avoided the problems and inefficiencies of solid-phase

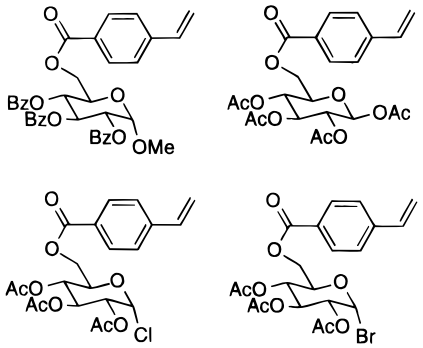


Figure 14. Monomers for copolymerization with styrene to obtain polystyrene-based supports for liquid-phase oligosaccharide synthesis.

procedures. Elongation cycles proceeded in greater than 90% with overall yields of 35% for a trimeric product, and compounds up to the hexamer was synthesized in 3−4-fold increase in yields over solid-phase methods.

V. Oligosaccharide Synthesis on Soluble Polymer Supports

The development of efficient methodologies for oligosaccharide synthesis is driven by the recognition of the essential role these molecules play in biological processes and by the pursuit of a novel class of therapeutics based on these entities.[126] It is apparent, however, that of the three classes of biopolymers, the synthesis of oligosaccharides by classical or support-based methods has proven to be the most difficult. Unlike peptides and oligonucleotides which have achiral linkages, oligosaccharides require stereochemical control during formation of the glycosidic bond. Furthermore, oligosaccharides display a greater number of similar functional groups that demand greater selectivity in bond-forming reactions; thus, elaborate protecting group schemes or enzyme-mediated synthesis is required. At present, polymer-supported synthesis of carbohydrates is not yet competitive with classical glycosylation methods; insufficient reactivity, incomplete stereoselectivity, instability of glycosyl donors, and lack of suitable anchoring groups have resulted in low yields and products contaminated with side products.[127] However, in trying to overcome these limitations, current research seeks to harness the advantages of polymer-supported synthesis. In particular, liquid-phase methodologies provide the dual advantages of facilitating efficient separation of products from reaction mixtures and permitting high reactivity due to homogeneous reaction conditions. Additionally, homogeneous conditions allow reaction kinetics and anomeric control to approach that observed in established non-support-based chemistry.

A. Oligosaccharide Synthesis on Polystyrene

Initial attempts to develop a method of liquid-phase oligosaccharide synthesis created a soluble polystyrene support bearing the first sugar unit by copolymerizing one of four 6-*O*-vinylbenzoylglucopyranose derivatives with styrene (Figure 14).[128] Polymers of varying saccharide content were obtained and exhib-

ited solubilities similar to polystyrene. Methods for orthoester formation and bromine substitution at the anomeric position were achieved, and cleavage of the modified monosaccharide was completed by transesterification (sodium methoxide in dioxane). Reactions were terminated by precipitating the glycopolymer with methanol; however, as seen in other methodologies employing polystyrene, recovery yields are anticipated to decrease as the oligosaccharide elongates due to increased solubility of the sugar−polymer conjugate in polar solvents.

B. Oligosaccharide Synthesis on Polyvinyl Alcohol

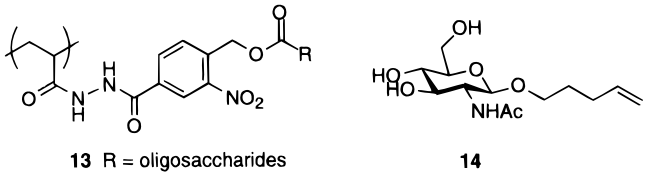
Water-soluble polyvinyl alcohol of molecular weight 10 000 was derivatized with amino pendant groups for the attachment of photolabile nitrobenzyl linkages carrying the first saccharide unit.[129] The polymer served as an acceptor for the enzyme galactosyltransferase, and 27% of the polymer was incorporated with galactose. The specificity of the enzyme provided for stereocontrol of the glycosidic bond and alleviated the need for protecting group chemistry. The resulting lactose was cleaved by UV irradiation and separated from the polymer support by ultrafiltration. On a similar polyvinyl alcohol support, the enzyme glycogen synthase provided very low yields (up to 4.23%) of glucose incorporation onto acceptor polymers.[130] Low yields suggest that the polymer support impeded enzyme interactions with the bound organic moiety.

C. Oligosaccharide Synthesis on Polyacrylamide and Copolymers

Used in parallel with polyvinyl alcohol supports, polyacrylamide of molecular weight 6000 containing monosaccharides via photolabile linkages also served as an acceptor in galactosyltransferase reactions.[130] Coupling yields were again low, but the polyacrylamide support allowed hydrolytic release of product using α-chymotrypsin in addition to the photolytic process. Use of an 1-*N*-L-phenylalanyl linkage also allowed selective cleavage by α-chymotrypsin of a trisaccharide bound to polyacrylamide after a galactosyltransferase reaction.[131]

Enzymes again provided selectivity and stereochemical control for the synthesis of glycosphingolipids.[132,133] A nitrobenzyl moiety with attached saccharide functionalities was linked to a polyacrylamide-poly(*N*-acryloxysuccinimide) copolymer to give a photolabile, sugar-bound support (**13**, Figure 15) soluble in water. This acceptor macromolecule served as a substrate for galactosyltransferase, and after incorporation of sphingosine, the glucosylspingosine−polyacrylamide conjugate was purified by ultrafiltration. In a separate series of reactions, an enzyme-catalyzed exchange of lactosyl to the original acceptor macromolecule proceeded in 36% yield, and after photolysis (350 nm) and purification, 54% yield of a lactosylsphingosine derivative was obtained.

The disaccharide *N*-acetyl-D-lactosamine was synthesized on water-soluble polyacrylamide containing the first sugar unit attached by a photolabile linkage.[134] An NMR method was developed to measure reaction progress at each step of polymer derivatization and product synthesis. However, yields of only 2% were realized using an immobilized enzyme system.



13 R = oligosaccharides **14**

Figure 15. Compounds employed in polyacrylamide-based liquid-phase oligosaccharide synthesis.

Polyacrylamide carriers containing photolabile linkages to oligosaccharides allowed facile separation of products from byproducts and excess reagents in product−distribution studies.[135] Immobilized cyclodextrin glucosyl transferase catalyzed the synthesis of a trisaccharide and higher oligosaccharides on a polymer-bound disaccharide. Other work employed glucose attached to the poly(acrylamide)-poly(*N*-acryloxysuccinimine) support to detect coupling and disproportionation products.[136] The immobilized enzyme also catalyzed cyclization of the saccharide reagent; however, these cyclodextrin byproducts were removed by ultrafiltration. Photolysis followed by ultrafiltration yielded free oligosaccharides that were identified and quantitated by HPLC.

Higher yielding enzymatic reactions on soluble support were realized by increasing the linker length between polymer and attached compounds. Copolymerization of acrylamide and a sugar residue containing a terminal olefin connected by a linear alkyl chain (**14**, Figure 15) provided a soluble support with *N*-acetyl-D-glucosamine (GlcNAc) pendant groups.[137] After enzymatic reaction with sialidase, unsialylated polymer side chains were removed by further enzymatic treatments to furnish a polyacrylamide containing 3′-sialyl-*N*-acetyllactosamine after purification by gel filtration. The alkyl linkage to the polymer was credited for providing increased flexibility and greater accessibility required for more favorable interactions with enzymes. In fact, quantitative yields were achieved in the enzymatic galactosylation of GlcNAc residues linked by a 3-carbon chain to water-soluble polyacrylamide.[137] Similarly, the polymer with attached sugar moieties formed from acrylamide copolymerized with *para*-substituted benzyl glycosides allowed galactosyl incorporation in 30% yield using bovine galactosyl transferase; however, near quantitative yields resulted after addition of a 5-carbon spacer unit.[139] Catalytic hydrogenolysis released the product *N*-acetyllactosamine in 95% yield. By using phenylalanyl linkages, product cleavage was also performed enzymatically with α-chymotrypsin, although in a lower yield of 72%.[140]

D. Oligosaccharide Synthesis on Polyethylene Glycol

Although a heptaglucoside was successfully synthesized using classical organic chemistry, the solution-phase method was laborious and time consuming and consequently spurred the development of a liquid-phase method.[141] Adaptation of the solution-phase method to a procedure employing MeO-PEG 5000 with a succinate linker was straightforward,

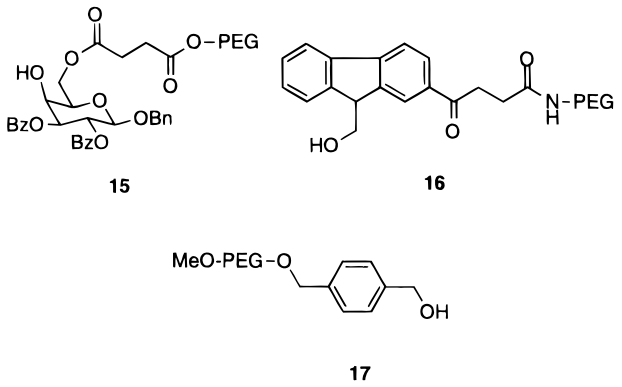


Figure 16. PEG-based supports for liquid-phase oligosaccharide synthesis.

and regioselective glycosylations were retained without interference by the polymer support. Saccharide elongations using mono- and disaccharide reagents allowed an overall yield of 11% of purified heptaglucoside having the same biological activity as previous prepared material.

Some attempts have been made to optimize glycosylation reactions in liquid-phase synthesis.[126] Glycosylation reactions on MeO-PEG 5000 were driven to completion by excess reagents as monitored by NMR.[36] After precipitation by diethyl ether or *tert*-butyl methyl ether and recrystallization from ethanol, PEG-bound oligosaccharides were cleaved by DBU-catalyzed methanolysis of the ester linkage or by hydrazinolysis when a phthalimido linkage was present. Anomeric control was provided by the use of reagents developed in classical methods, and the di- and trisaccharides were obtained free of unwanted isomers.

Liquid-phase methods allowed the synthesis of derivatives of a disaccharide motif recognized by bacterial adhesins: these glycoproteins bind GalpNAc(β1−4)Galp(β1−O) on host cells in order to facilitate colonization and bacterial infection.[142] Although the synthesis of GalpNAc(β1−4)Galp(β1−O) has been accomplished by classical methods, the scale up to gram quantities was unmanageable. Thus, a polymer-supported synthetic protocol was developed using MeO-PEG 5000 attached to the 6-OH of galactopyranoside via a succinate ester linkage (**15**, Figure 16). Glycosylation, purification, deprotection, and cleavage yielded the desired product in fewer chromatographic steps as compared to the original solution-phase synthesis.

DiaminoPEG 6000 modified with a linker containing a 9-(hydroxymethyl)fluorene moiety provided the base-labile protecting group **16** for oligosaccharide synthesis.[127] Efficient coupling using phenyl 1-thioglycopyranoside sulfoxides as glycosyl donors (80−86% coupling yields) generated a disaccharide that was cleaved from the support using triethylamine in CH_2Cl_2.

The direct linkage of MeO-PEG 5000 to the anomeric carbon was found be labile under certain glycosylation reaction conditions; stable attachment was made to other sugar hydroxyl groups using succinate ester linkages.[143] However, switching from the use of succinic acid to a linkage based on α,α′-dioxy-*p*-xylyl (DOX), allowed stable O-glycosidic linkage of **17** to the anomeric carbon as well as ether linkages to other sugar positions.[144] Cleavage by hydrogenolysis liberated an oligosaccharide with free hydroxyl, or under controlled conditions, a benzyl-like protecting group (*p*-tolylmethyl) remained at the point of previous polymer attachment. In development of a liquid-phase method, several reaction conditions were varied to achieve successful attachment of 2-acetamidoglycopyranosyl to MeO-PEG. Side reactions occurred including degradation of the linker support; however, through reaction optimization, conditions were found to yield pure polymer glycoside upon precipitation of the support. An iterative synthesis of D-mannopentose was achieved with stereocontrolled glycosylations. Cleavage by hydrogenolysis, acetylation, and purification provided the protected pentasaccharide without β-anomer formation.

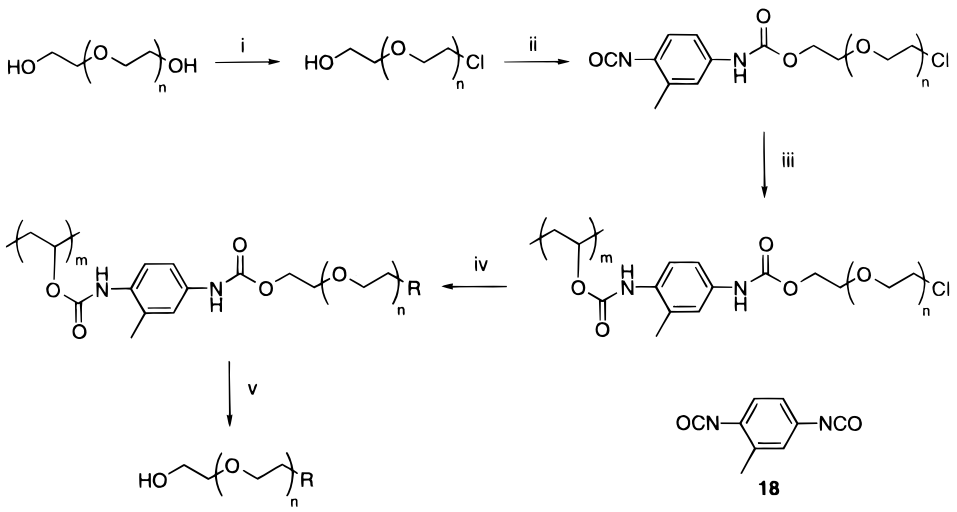
VI. Liquid-Phase Synthesis of Small Molecules and Other Compounds

Nearly all examples of liquid-phase synthesis presented thus far used a single reaction repetitively to synthesize oligomeric compounds; however, methods that employ multiple synthetic reactions can provide highly complex molecules. Classical organic chemistry has been the predominant method for small molecule synthesis, yet it is likely that the solubilizing power and facile purification aspects of liquid-phase chemistry could be applied toward more efficient synthesis of novel compounds. In fact, the liquid-phase synthesis of combinatorial libraries of small molecules has been realized.[11,13,14] Containing large numbers of molecules with various chemical functionalities, such libraries are sought by the pharmaceutical industry for high-throughput screening assays to discover and develop new drug compounds. The advantages of support-based methodologies for combinatorial library synthesis are clear: simplified purification methods allow the use of excess reagents so that optimum yields are easier to obtain. Producing homogeneous reaction conditions, liquid-phase methods have several advantages over solid-phase methodologies including the elimination of diffusion problems created by a polymer matrix, analytical control without intervening cleavage steps, and straightforward scaleup to large-scale synthesis. Thus, liquid-phase chemistry developed for peptide, oligonucleotide, and oligosaccharide synthesis may also be applied and extended into multistep synthetic methodologies.

A. Synthesis on Polyvinyl Alcohol

PEG itself served as the organic moiety for chemical modification in a liquid-phase method.[145] The functionalization of PEG 3400 with different terminal end groups was achieved using polyvinyl alcohol of 23 320 molecular weight that served as the macromolecular protecting group while polyethylene glycol acted as the bound entity targeted for chemical manipulation. The grafting of one hydroxyl of PEG to polyvinyl alcohol using tolylene 2,4-diisocyanate (**18**) allowed selective derivatization of the other terminal hydroxyl of PEG (Scheme 4). The copolymer

Scheme 4[a]



[a] Reagents: (i) 1.5−2 equiv of $SOCl_2$, Et_3N, toluene; (ii) **18**, CH_2Cl_2; (iii) cat. dibutyltin dilaurate, polyvinyl alcohol, DMSO; (iv) various reactions for selective monofunctionalization of PEG; (v) K_2CO_3, H_2O, reflux.

Table 5. Lower Critical Solution Temperatures (LCST) of Copolymers of *N*-Isopropylacrylamide and 5 mol % Acrylic Acid Derivatives[146]

acrylic acid derivative	LCST (°C)
p-acrylamidophenol	27
p-acrylamidobenzoic acid	29
acrylic acid	31
p-acrylamido(hydroxymethyl)benzene	38

was precipitated out of CH_2Cl_2 or from DMSO by methanol under conditions that left unbound PEG in solution. Hydrolytic cleavage of the isolated copolymer provided PEG with an hydroxyl group at one end and a different terminal group at the other end that included azide, amine, chloride, benzaldehyde, or products from benzoin condensations.

B. Synthesis on Copolymers of Poly(*N*-isopropylacrylamide)

The macromolecular characteristics of liquid-phase synthesis not only facilitate product purification; but in a novel application, the polymer support has enabled a temperature-controlled switch for catalytic hydrogenation.[146] In this method, a polymer-bound compound may react with a heterogeneous hydrogenation catalysis at room temperature; however, above a certain temperature, the polymer phase separates from solution and prevents further hydrogenation of the bound organic moiety. Carbobenzyloxy-protected glycine attached through the phenolic hydroxyl group of the copolymer made from 1:20 *N*-(*p*-hydroxyphenyl)acrylamide:*N*-isopropylacrylamide was inert to hydrogenolysis above 38 °C but was cleaved readily at 10 °C. Copolymers of poly(*N*-isopropylacrylamide) and acrylic acid or other derivatives were prepared (Figure 1) and found to differ in the temperature at which phase separation occurs; thus by altering polymer structure, the lower critical solution temperature (LCST) could be adjusted (Table 5). It was also noted that compounds bound to the commercially available triblock poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) also phase separated from water at elevated temperatures; however, the emulsion thus formed still exhibited some level of catalytic hydrogenation.

C. Synthesis on Polyethylene Glycol

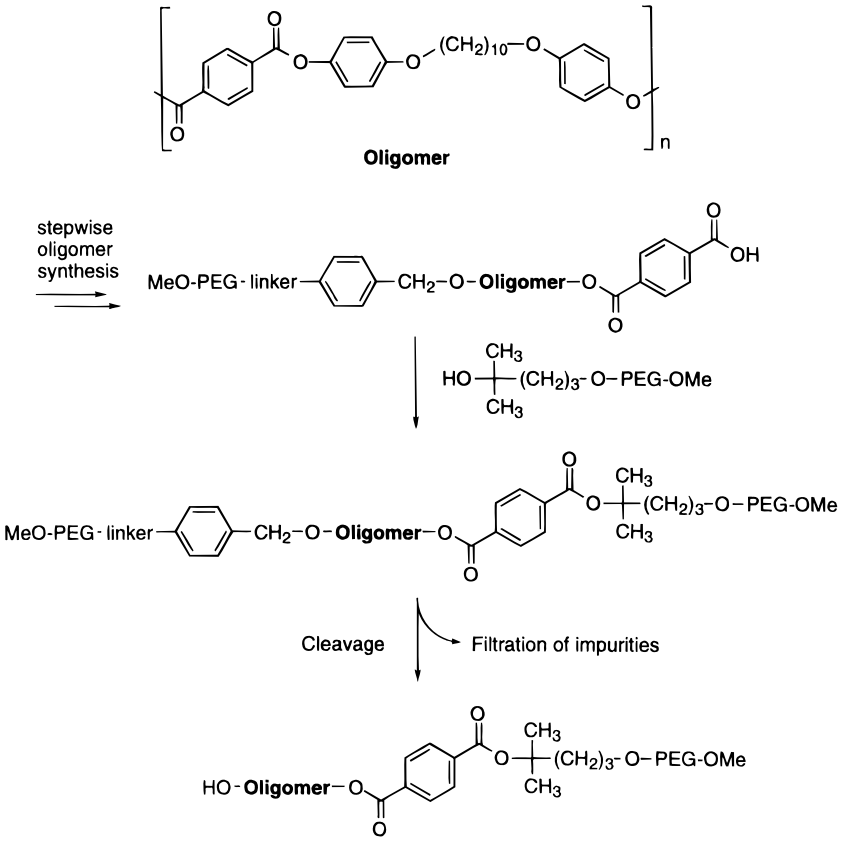
Heterogeneous catalytic hydrogenation of nitrophenyl groups linked to PEG has been performed.[147] Due to reaction heterogeneity and/or interaction with the macroscopic catalysts, both polymer size and choice of solvent influenced the reactivity of PEG-bound compounds toward hydrogenation.

Liquid crystalline oligomeric compounds derived from terephthalic acid and α-hydroxy-ω-hydroxy(oxy-1,4-phenyloxy-1,10-decamethyleneoxy-1,4-phenylene) (Scheme 5) were synthesized on MeO-PEG 2000.[22] Solution-based methods succeeded in the stepwise synthesis of oligomers only to length $n = 2$ due to low product solubility in all solvents; however, the solubilizing power of MeO-PEG enabled synthesis of higher oligomers. The synthesis and purification of the insoluble polyether esters were achieved by using two polymeric protecting groups, introduced and cleaved selectively. After synthesis was conducted on MeO-PEG, purification was begun by coupling a second MeO-PEG derivatized with a terminal tertiary alcohol group. Selective cleavage of the initial MeO-PEG carrier by catalytic hydrogenation releases MeO-PEG-product into solution, but truncated chains precipitate as they lack the second MeO-PEG carrier (coupling of the second MeO-PEG is prevented through a capping step performed after each coupling reaction). Thus, simple filtration yields a solution containing only the desired oligomer bound to MeO-PEG. The solubilizing power of MeO-PEG allowed product identity and purity to be verified by solution-based analytical methods before hydrolytic cleavage enabled isolation of the pure insoluble product.

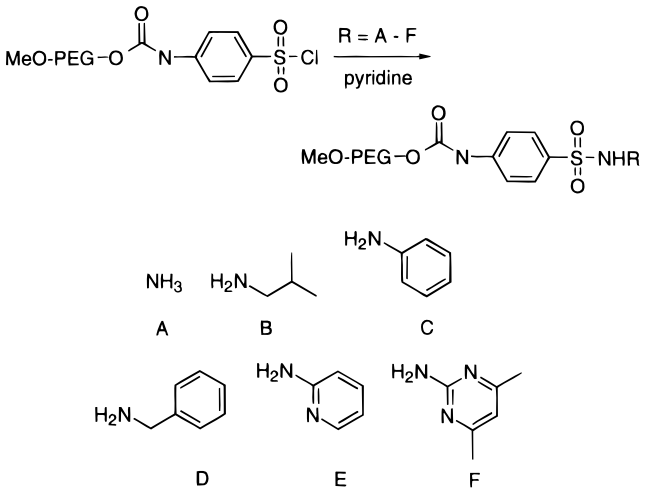
D. Liquid-Phase Combinatorial Synthesis (LPCS) of Sulfonamides

Liquid-phase combinatorial synthesis (LPCS) has been demonstrated as a successful, alternative methodology for the construction of peptide libraries.[11,13,14]

Scheme 5



Scheme 6



The LPCS method should permit the synthesis of any compound as long as the chemistry does not interact with or adversely affect MeO-PEG properties. To verify the utility of LPCS to generate nonpeptidyl compounds, a library of sulfonamides has been constructed by parallel synthesis.[11] The screening of combinatorial libraries of this class of compounds with known clinical bactericidal efficacy may lead to the discovery of new pharmaceutical agents.

A new route for the synthesis of sulfonamides was developed to allow attachment of the arylsulfonyl moiety to MeO-PEG and to provide for molecular diversity (Scheme 6). Attachment to the polymeric support was formed quantitatively by reaction of 4-(chlorosulfonyl)phenyl isocyanate with MeO-PEG in the presence of a catalytic amount of dibutyltin laurate. Splitting the MeO-PEG-arylsulfonyl chloride among six reaction vials containing different amines resulted in a library of MeO-PEG-protected sulfonamides. Hydrolytic cleavage of the urethane linkage under basic conditions yielded the final library of six members in analytically pure form in overall yields of 95−97%.[11] Reactions were monitored by ^{1}H NMR spectroscopy and allowed the reaction sequence to occur cleanly for the production of multimilligram quantities of each sulfonamide. The results indicate that LPCS should have general utility for the construction of small molecule libraries of high chemical diversity and in sufficient quantities to allow for multiple high-throughput screening assays.

VII. Conclusions

Liquid-phase methodologies provide alternative strategies for compound synthesis by incorporating the positive aspects of both classical and solid-phase chemistry. Enabling homogeneous reaction conditions and simplifying product separation, liquid-phase synthesis has demonstrated utility for peptide, oligonucleotide, oligosaccharide, and shows promise for use in small molecule synthesis. Research in combinatorial chemistry has led to the development of LPCS in order to simplify and speed the synthesis and screening of small molecule libraries for drug lead discovery. Certainly additional applications will be discovered for liquid-phase methodologies.

It is apparent after reviewing the literature that only a small number of known soluble polymers have been explored for use in liquid-phase synthesis. Given the many successful demonstrations of these

polymers in support-based methodologies, the potential for discovery of additional useful polymers is high. PEG has proven to be the most versatile and successful polymer support for liquid-phase synthesis; however, the need still exists for further research into soluble supports. Evidence of limitations in the solubilizing power of PEG has been observed during the synthesis of longer peptides,[66,67] and a desire for increased loading capacity has been stated.[94] One method has employed extraction to achieve purification of PEG-bound products;[93] but in general, the water solubility of PEG limits the use of aqueous extractions as a purification technique. Finally, PEG is insoluble in THF at low temperatures, and as the polymer is typically precipitated by addition of diethyl ether or *tert*-butyl methyl ether, reactions may not be conducted in these solvents.

In fact, the need and potential payoff for research into polymeric supports in liquid-phase synthesis was emphasized in a recent review.[7] It was stated that the majority of difficulties faced in repetitive-type syntheses are due to the macromolecular support. Compatibility problems, such as hydrophobic molecules built on hydrophilic supports, limit some applications.[43,46,47,50−52,101] For large-scale applications, the loading capacity must be high, but not excessive such that the macromolecule no longer determines solubility properties. As the polymer may influence reactivity, the optimization of some synthetic applications may require tailor-made soluble polymer supports. Interestingly, the discovery of novel polymers may lead to new applications; the property of a copolymer to phase separate above 38 °C was utilized to create a temperature-controlled switch for catalytic hydrogenation of polymer-bound organic moieties.[12] Thus, the investigation of new polymers should find utility in LPCS as well as in other important applications. To this end, the authors have initiated efforts directed at synthesizing functionalized block copolymers for the goal of discovering novel polymer supports for liquid-phase combinatorial synthesis. In addition to development into liquid-phase methodologies, novel block copolymers may have utility in other areas of material science as commercial block copolymers have been developed as elastomers, thermoplastics, surfactants, and emulsifiers.[148]

VIII. Acknowledgments

This work was supported in part by The Scripps Research Institute, the R. W. Johnson Pharmaceutical Research Institute, The Skaggs Institute for Chemical Biology, and the Alfred P. Sloan Foundation.

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CR960064L