Perspective

Matrix Assisted Synthetic Transformations: A Mosaic of Diverse Contributions. I. The Pattern Emerges

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Matrix Assisted Synthetic Transformations: A Mosaic of Diverse Contributions. I. The Pattern Emerges

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"Not much is impossible."
—Steve Williams, when at Industrial Light & Magic, San Rafael, CA

Introduction

One of the joys of my life in research, and on a personal level, has been my fortune in being able to perceive connections between people and observations and to use these to make what has seemed to me common-sense decisions. This process can be seen very clearly, both in my involvement with this Perspective, which follows closely in the footsteps of Michal Lebl's contribution on classical papers in combinatorial chemistry,[1] and in the complex interrelations that exist between all the contributors, which will become apparent as the reader progresses.

In truth, this is the closest thing to a review that I've ever written, having abhorred the labor in the past. My task, however, is immensely simplified, since I can rely on the originators of many of the supports and studies to tell the story themselves. I gave very loose, but identical, instructions to all the authors: to give their own personal perspective on their contributions, focusing on how the nature of the support(s) influences their suitability. They are thanked very warmly for their help. Where areas have escaped this attention and to place the pieces of my mosaic in their correct setting, I have attempted to set them together with some personal comments and mini-reviews to fill in blanks. It was my original intention to make this just a single article, but practical considerations (as well as those connection ideas) have lead to its present two-part format. Table 1 provides a summary listing of supports that are considered in part I; Table 2 provides a preview of the materials to be presented in part II, which, additionally, with the generous help of Bing Yan, will try to bring the whole topic to a satisfactory conclusion.

I have also elected to use the term "matrix assisted synthetic transformations", henceforth referred to by the acronym MAST,[2] for the purposes of this Perspective so that many diverse directions can be included.[3] There are manifest advantages to using a matrix, be it soluble or insoluble, beaded or of an alternative morphology or geometry, whether supporting the substrate or the reagent. Three advantages are of most importance: reactions can be driven to completion by use of excess, the components of the reaction are readily separated, and automation is simplified.

It is clear to me that the time is now right for this audience to consider some less conventional alternatives to 1 or 2% cross-linked polystyrene gel form beads for MAST. As we all know, transferring a reaction from solution phase to one mediated on a solid support is a nontrivial process in many cases. This is especially true of reactions that require exact stoichiometry of reactants; in such a case a macroreticular or porous support might be advantageous, since it can readily be freed of solvent and induced to "imbibe" the appropriate

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Table 1. Selected "Gel" Supports Used for MAST Discussed in This Perspective, Part 1[a]

Type	Common Features	Representatives	Comments	Originators (Contribution in **bold**)
Gel (microporous)	High capacity Differential solvent swelling Reagent access by diffusion Uniform sites Most unsuitable for column reactors	1 or 2% crosslinked polystyrene,	Useful for peptide, and MAST in genera	**Merrifield, Glettig***
		Pepsyn, low crosslinked polyacrylamides	Peptide, and bead based libraries	Atherton, **Sheppard,** Arshady
		"Sparrow" resin	V. similar to Pepsyn, useful for direct antibody production	**Sparrow**
		Polyacryloylmorpholines	Peptide synthesis	Epton
		PEGA, SPOCC resins,	Based on crosslinked PEG; excellent aqueous compatibility	**Meldal**
		Sephadex LH 20	Based on crosslinked dextran	Erickson and **Merrifield**
		CLEAR	Highly polar resin	Kempe and **Barany***
Copolymers	May be gel or macroporous	Amphiphilic poly(styrene-acrylamide)s	General solvent and substrate compatibility, potential not fully realized.	**Arshady** (see also Merrifield)
Encapsulated Gel	Lower Capacity Column compatible Unstable to agitation	Pepsyn K (acrylamide resin encapsulated in inorganic "cage")	Excellent for flow though Fmoc mediated peptide synthesis	**Sheppard**
		PolyHIPE (similar resin encapsulated in PS "shell")	Advocated for high-load peptide synthesis	**Sherrington** &Small

[a] References to be found in associated text. Asterisk (*) indicates contribution to be described in part 2.

amount of reactant, as with the "spot" method of Frank (also known as "inclusion volume solid-phase synthesis"). These supports, additionally, may be suitable for "nontraditional" procedures, e.g., reactions mediated by vapor-phase reagents (as with aminolytic cleavage). The role of the support in influencing the outcome or efficiency of a transformation is beginning to be more and more appreciated; the effects are still poorly understood but include steric hindrance, rate of reagent diffusion, partition of a reactant from solvent to support phase, and the environment of the attached substrate. Some reactions, e.g., those using highly reactive electrophilic reagents, cannot be applied on polystyrene matrixes. Controlled pore glass has obvious potential (being stable to many aggressive reagents) and is being used more and more for just such applications, but it suffers from considerable fragility. Examples of many alternative supports can be found in the literature, which stretches back many years, and I regard these as a "gold mine" to those seeking "new" ideas.

Many other reviews exist,[4] and much of the relevant early literature is included in a collation of papers, including the original papers by Bruce Merrifield as well as the parallel work by Letsinger, with insightful comments provided by the editors, Blossey and Neckers.[5] The breadth of applications addressed up to that time, 1976, may be surprising to many newcomers who consider solid-phase organic chemistry to be of recent origin. I draw particular attention to the brilliant work of Clifford Leznoff, who, with a variety of co-workers over many of the intervening years, has transferred many standard organic chemistry reactions to their solid-phase equivalents using polystyrene gel beads. The study of 1,3 cycloaddition and Diels−Alder reactions is a true classic.[6]

It may come as quite a shock to some readers to learn that, during the first decade of the solid-phase method (the 1960s), the majority of organic chemists were extremely skeptical of the idea. It took the brilliant achievement of Gutte and Merrifield, the synthesis of ribonuclease A, ribonuclease S, and analogues, to convince many of the virtue of the procedure. These accomplishments were achieved with the first automated synthesizer, an equally seminal contribution of John Stewart, Merrifield, and Nils Jernberg (who provided some clever valve engineering). This is the direct ancestor of today's massive parallel robotic solid-phase synthesizers capable of producing thousands of compounds for screening purposes. Knowing the magnitude of John Stewart's many contributions, it was with considerable trepidation that I approached him to evaluate the original Biosearch's first peptide synthesizer (1983); I was delighted when he agreed and ecstatic when he gave his favorable opinion. The prototypes of this machine, the SAMs (standing for synthesis automation module or machine), performed Fmoc-flow-through chemistry, DNA synthesis,[7] as well as various organic transformations (e.g., oxidations and reductions) performed by resin immobilized reagents.

Low-Cross-Linked "Gel" Type Resins

As we shall see, Bruce Merrifield clearly appreciated both the critical role that the support and chemistry have on

Table 2. Selected Modified Supports Used for MAST, To Be Discussed in Part 2 of This Perspective

Type	Common Features	Representatives	Comments	Originators (Contribution in **bold**)
Composite Particles	Reactions may be subject to hindrance by physical limitations of either component, and of the tightly packed nature if linear polymer coat	PS coated Kel-F	Early support for peptide synthesis produced by radiation grafting	**Tregear**
		PS coated PE	PE-PS, analogous material on PE particles	**Hudson**
			Big Beads for MAST	ParaMatrix, Tucson
		Composite containing magnetic particles	Product of Solid-Phase Sciences	Sucholeiki
Pellicular beads	Very Low loading	PS	Useful for DNA synthesis	PE-Biosystems
Graft Copolymers	Lower Capacity More uniform swelling Pressure stable Column compatible	PEG-PS	Prepared by addition of PEG block	**Barany**
		Tentagel	Prepared by polymerization of ethylene oxide onto resin, monosized beads useful for library applications	Rapp Polymere Bayer and **Rapp**
		Argogel	PEG attached via branched diol support	**Gooding, Labadie, Porco** (Argonaut)
		Champion, NovaGel	Various formulations prepared from PEG blocks	Biosearch Technologies Calbiochem-NovaBiochem, **Hudson**
		Dendrogel	Branched PEG chains	
		Dendritic polymers	Prepared by assembly of dendrimers on standard PS gels	Bradley
Rigid MacroPorous Supports	Capacity and efficiency depend on specific surface area, mean pore volume, pore distribution, and mean pore size	High-crosslinked PS	Small pore versions excellent for MAST (e.g. ArgoPore),	**Gooding, Labadie, Porco** (Argonaut)
			1000Å Pore versions good for DNA synthesis	**Andrus**
		Polymethacrylates	Bead based peptide libraries	**Buettner**
			DNA synthesis	**Reddy, Zhong**
		CPG, silicas	Support of choice for DNA synthesis,	**Adams**, Koster
			Useful for MAST when PS incompatible.	**Morgan, Petter**
		Poros, "Perseptive Biosystems" Primer, Pharmacia	Novel HPLC materials which have been used for DNA and peptide synthesis	**Kates**
		Aspect	Porous PE produced by vigorous oxidation	Cook & **Hudson**
Modified Surfaces	Low capacity except if coated with highly substituted functional polymer	PE-PS films	Can use film handling techniques for processing	Merrifield, Tam
		Derivatized linear PS	Modified Microtiter plates	**Clark**
		PS coated PE/PTFE MicroTubes	Used with Rf transponder for tracking	**Zhao**, Irori
		Cellulose	"Spot" method of peptide synthesis, epitope mapping	Frank
		Cotton	In centrifugal reactors	**Lebl**
		Glass	Array Applications (Peptide and DNA)	Affymax, **Holmes, Hoeprich**
		Plasma aminated PE	"	Beckman
		Grafted PE "pins" and "crowns	Epitope mapping, SPOS	Geysen, **Maeji, Rodda**
		Dextran coated PE discs	Pilot library technique	**Hudson**
		Membranes	DNA and peptide synthesis	**Daniels**, Millipore

synthesis efficiency and the problem of transferring solution chemistry to the solid phase. This realization, too, has been paramount in the thinking of other contributors to this section.

The complete *Life During a Golden Age of Peptide Chemistry* is a must read, but for the benefit of this audience and with his generous permission and guidance, I have recompiled some selected passages from this autobiographic work,[8] with additional material from a further review of his[9] as well as some remarks from him especially for this audience. For my part, I have simply eliminated a few phrases (indicated by ellipsis points "..."), added some links (*in italics*), and organized the selections. This segment focuses solely on Bruce's background resin and chemistry development work, which is, I believe, little known and is highly relevant to this subject! I have included NO mention of his more widely recognized synthetic accomplishments. His text constitutes a remarkable testimony to his vision and persistence and a shining example of how a research project should be pursued.

Bruce Merrifield.[10] The Concept and Development of Solid-Phase Synthesis

I have never been able to reconstruct the moment when the idea came to me, but probably it was at night, just as other ideas often come into one's head. It was obviously a result of having recognized a direct need and having thought about the general problems for some weeks. ...Although I cannot recall the exact time I had the idea, I do know when I recorded the basic concept in my notebook: May 26th 1959. ...A few days later, I had organized my thoughts enough to propose the plan to Dr. Woolley.[11] At first he did not respond, but the next day he said, "That may be a good idea, why don't you go ahead." Conceptually, the new feature of this idea was the use of a polymer to assist in the synthesis of another compound. There was, of course, a vast literature on polymer chemistry, and reactions had been done on many preformed polymers to change their properties. However, polymers had not been used as supports while chemistry was being done to prepare another class of compounds that could later be removed from the solid phase and isolated in the free state. ...It took me a long time to find a suitable polymeric support and to work out a set of chemical reactions that would accomplish my goal, but I enjoy this kind of experimental bench work and derived a great deal of satisfaction from the undertaking. It was soon apparent that each of the variables, the support, the solvents, the anchoring bond, the N^{α} protection and deprotection, the activation and coupling steps, and the cleavage step, had to be studied in detail and optimized. However, because they were not independent, optimization of all steps had to come together at the same time. A good coupling reaction in the wrong solvent or on the wrong support would not do, or poor chemistry applied to a potentially useful support would be ineffective. It is mainly for this reason that ... the first successful synthesis of a peptide was not achieved until 3 years later, in 1962, and was not published in a full paper until 1963. None of my early developmental experiments were ever published; I have decided ... to describe the various attempts that I made, to show what my thinking was and how it evolved.

The first requirement was to find a suitable solid support for the chemical reactions. I selected cellulose initially because it had been used successfully for the chromatographic separation of proteins. This meant that there was room inside the polymer matrix to accommodate a large molecule. The following experiments were done *with* Whatman cellulose powder: *coupling of Z-Phe via mixed anhydride and active ester methods, coupling of glycine via its acid chloride, and addition in the presence of toluenesulfonic acid and phosphoric acid*. ... No reaction occurred under any condition examined. The sodium salt of cellulose was prepared ... and treated with Z-Phe-ONp in dioxan. Cleavage of the ester occurred, but no phenylalanine remained on the cellulose. The reaction was also attempted by flowing the activated amino acid through packed columns of cellulose and sodium cellulose; *the latter eluted the appropriate amount of nitrophenol*, but only 6 mg of Z-Phe per gram of cellulose were retained. *After much further work, a regenerated cellulose was loaded, via addition of Z-leucine by a mixed anhydride method, to 0.2 mmol/g.* At this point I decided to go to N^{α}-trityl ... derivatives because of their lability toward acid. The ... reactions *shown in Figure 1* were eventually carried out.

By this time it was clear that cellulose was not going to be a satisfactory support, even though a dipeptide had been produced. The yields were low, and the reaction conditions were too drastic for this carbohydrate. I had used the carbobenzoxy group because it was the standard group for peptide synthesis at that time and because it was available. The trityl group offered certain advantages of sensitivity, but it is so bulky it has limited application. More acid-labile groups were just beginning to be developed, and I eventually did turn to them, even though they were still not commercially available. Ten years later I examined LH Sephadex, an even more labile carbohydrate, and was able to synthesize a tetrapeptide on it with no difficulty. The difference, of course, was that the chemistry had changed: the reagents and conditions were milder and better understood.

After abandoning cellulose, I looked at a few other polymers, including poly(vinyl alcohol), PVA. *On substitution*, the Leu-PVA contained 20 mol % Leu (43 wt %), a result indicating that one in five of the OH groups had become esterified. The product was soluble in water at pH 4.5 and was 40% dialyzable through a membrane that would retain 5000 Da material. Although the substitution could have been controlled, I decided not to pursue the use of this polymer.[12]

On January 8, 1960, I turned to a commercial ion-exchange resin and chose Rohm and Haas Amberlite IRC-50, 200−300 mesh, called XE-64. This copolymer of 94% methyl methacrylate and 6% divinylbenzene has been saponified to liberate all the carboxyl groups. The equivalent weight was calculated to be 94.3. By suspending the resin in 1 N NaOH and back-titrating with HCl I found a value of 97.5 which indicated that these reagents could reach all of the reactive groups within the polymer. At this point I first realized how few of the functional groups are actually on

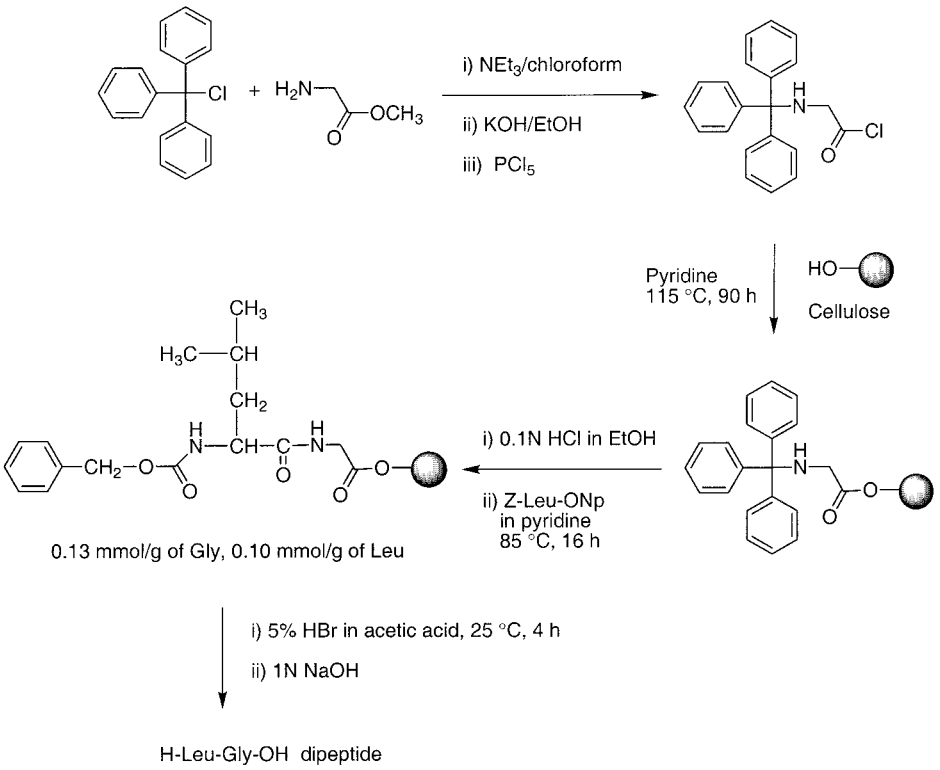


Figure 1. Synthesis of a dipeptide on cellulose.

the surface of the resin particle. This factor proved to be very important in the later development of the method. Assuming smooth beads with an average diameter of 50 μm, I estimated that only about 0.003% of the carboxyl groups would be on the surface. This number is linear with bead diameter, so grinding the resin into finer mesh particles would not increase the value to a usable level. For example 5 μm beads would still have only 0.03% of the carboxyl groups on the surface. It now seemed apparent that we should be looking for solid supports that would be porous enough to allow ready access of amino acid derivatives and reagents into their interiors, where most of the synthesis reactions would occur. Full derivatization of the carboxyl's of XE-64 would give a substitution of approximately 10 mmol/g, whereas surface substitution would give only 0.0003 mmol/g. From this calculation it looked as if I should be aiming for a resin loading of about 0.3 mmol/g, which means derivatization of roughly 1/30 of the carboxyl groups. The carboxyl groups of the XE-64 resin were then activated by conversion to an acid chloride, which could then react with the aminophenyl ester of carbobenzoxyglycine. Removal of the Z protecting group, and neutralization with tertiary amine gave the Gly-resin, which was ready for reaction with the next protected and activated amino acid, Z-Leu-ONp. This series of reactions was used to test further the free flow synthesis columns. The main problems were disruption of the column by CO_2 bubbles generated during acid decomposition of the Z group and plugging of the sintered glass filters by fine resin particles. Both of these problems were finally overcome by proper choice of solvents and flow rates. Nonetheless, the stability of the phenyl ester was poor, and the coupling reaction with nitrophenyl esters was slow and incomplete. The inadequacy of the chemistry had made the testing of the flow-through system premature. At this point, I decided to switch to a benzyl ester anchoring bond.

On April 7th Z-Phe-4-aminobenzyl ester was prepared, coupled to the acid chloride of the XE-64 resin, deprotected and extended to the dipeptide as shown (Figure 2).

This was the first successful synthesis of a dipeptide by a solid-phase procedure. I was excited and much encouraged by this result, after having faced a year of continual disappointment and frustration. Unfortunately, efforts to extend the chain to the tetrapeptide stage were not very successful, so my elation was short lived. The deficiencies of this approach were apparent, and I decided once more to change the strategy and search for better methods. As it is for everyone, the work does not always move quickly enough or in the right direction, and then discouragement begins to take over. The high points are relatively rare, but they sustain you for a long time. However, when the gaps between peaks get too long, you begin to wonder what to do. *At this stage Merrifield was sustained by friendship.*

On July 7th Merrifield embarked on a new plan to examine sulfonic acid derivatives of styrene and divinylbenzene, starting either with the commercial ion-exchange resins such as Dowex 50 or with the underivatized copolymer. I expected several advantages. First, the functional groups would be added after polymerization, rather than before, as with the polymethacrylate ester in XE-64 resins. ... *A tripeptide resin* was prepared, *but gave a* level of peptide ... clearly too low to be useful. By then it was obvious that high cross-linking was not the way to go and also that a carbobenzoxy group and benzyl esters were not compatible if the stepwise scheme was to be done in acid. *Merrifield then devized a complicated linkage to a 4% resin which yielded a dipeptide amide of good purity on sodium in liquid ammonia cleavage.*

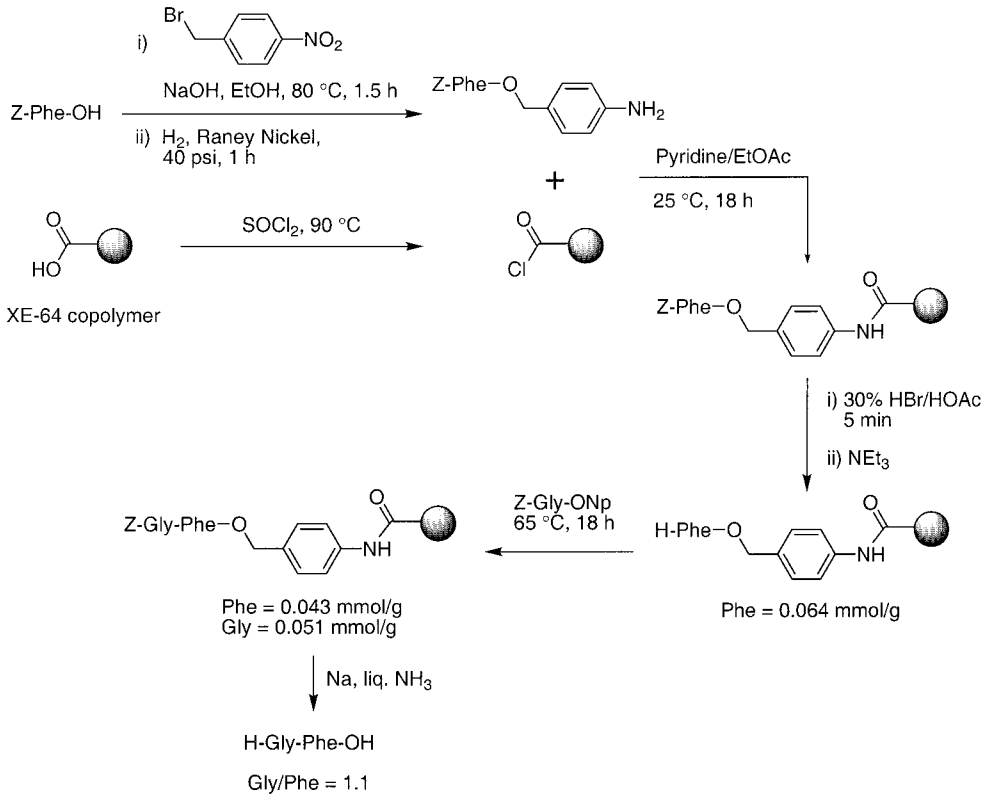


Figure 2. Dipeptide synthesis using a copolymer of methyl methacrylate and divinylbenzene. Similar, less satisfactory, transformations were also investigated using *p*-nitrophenol, rather than *p*-nitrobenzyl bromide, giving a phenyl ester attachment.

At this point Merrifeld investigated the preparation of chloromethylcopoly(styrene−divinylbenzene) and attachment of the first amino acid as a benzyl ester by direct esterification of a protected amino acid salt. ... I began with reaction of the resin with paraformaldehyde under acid or Friedel−Crafts catalysis *giving a substitution of 0.3 mmol/g*. I considered the results unsatisfactory at the time, but in retrospect they might have been useful. The cross-linking was probably too high, however. The conditions may have led to transient chloromethylation, which, subsequently, produced further cross-linking and loss of chlorine. Finally, I found a method using chloromethyl methyl ether that really worked (*a 4% resin, with $SnCl_4$ at 60 °C for 1 h, gave 4.65 mmol/g*). This first successful run actually yielded a higher substitution than I thought desirable because about one-half the rings were derivatized and overcrowding was expected. However, this reaction could easily be adjusted by diluting the chloromethyl methyl ester with chloroform, lowering the temperature, or decreasing the concentration of catalyst. Several years later we found that the reaction was best controlled by using $ZnCl_2$ ...This route to chloromethyl resin soon became the standard procedure for solid-phase synthesis and is still frequently used. *The resin could be converted* to the primary benzylamine by reaction with ammonia..., but addition of Z-Gly-ONp in benzene at 25 °C for 1 h gave only 0.023 mmol/g of glycine. ... The solution to the attachment problem finally came (*2*/*27*/*61*) when I carried out a direct esterification of an N-protected amino acid triethylamine salt (in ethyl acetate at 80° for 24 h, 95% incorporation). The Na salt of the Z-amino acid did not react. However, many years later Balz Gisin found that the cesium salt was reactive, and this became the method of choice.[13] The Z-Gly-OCH_2-resin was found to be stable to glacial acetic acid (100 °C, 18 h) ... triethylamine in benzene (25 °C, 18 h), but all the glycine was removed by 1 N NaOH within 1 h. Deprotection of the carbobenzoxy group in 30% HBr in acetic acid also removed 80% of the glycine benzyl ester. However, treatment with 10% HBr in acetic acid removed all the Z and only 0.36 mmol/g of ester. This analysis does not look too good now, but at the time it was so much better than anything I had previously obtained that I was very encouraged. I then found that the HBr:Gly-resins could be converted to the free amine (neutralized) by 20% triethylamine in EtOH. However, the reaction was slow and required three 30 min treatments. ...I still had not fully realized the importance of using solvents that solvate and swell the resin. Thus ... reaction with NEt_3 in CH_2Cl_2 is complete in only a few minutes.

Merrifield next discusses improvements to the para-nitrophenyl ester coupling method resulting in a reasonable tripeptide synthesis. The picture had become much clearer, and I was convinced that the solid-phase idea would succeed. It was obvious that something had to be done about the coupling reaction, but I persisted with the nitrophenyl ester method ... as well as the classical carbobenzoxy group that I did not try to change at this time. Instead, I decided to reduce the cross-linking of the resin and to make the anchoring benzyl ester bond more resistant to acid. Up to this time the work had been done with either 16 or 4% divinyl benzene (DVB) cross-linking. Since the extent of reaction was so much greater with the low cross-linking where the gel matrix was not so tight, I decided to drop to 2% DVB resins. The material was obtained through Dow Chemical Company through the courtesy of John Vanderhoff. ... *At this junction Merrifield makes a series of studies, mono-*

and di-nitrating, as well as brominating the resin benzyl ester; with the mononitro resin he performed the first successful LAGV synthesis, the product being liberated with NaOH hydrolysis.

There was, of course, much to be done. At that time, when resin penetration and capacity were still in question and it was clear that limiting the synthesis to the surface of the S-DVB beads was not useful, publications appeared by Kunin of Rohm and Haas and, a little later, by Millar at the Permutite Company in England on the preparation of copolymers with vastly larger surface areas, called macroreticular or macroporous resins. These resins were prepared from styrene with a very high proportion of divinylbenzene (25−75%) in a solvent that was good for the monomers but not for the polymers. They contained fixed pores and channels that allowed solvents and reagents to penetrate the beads and to come into contact with about 500 times as much surface as would be found on the outside of the previously high-cross-linked gel-like bead. Both Kunin and Millar generously gave me samples, and I applied my best procedure for the synthesis of the test peptide. The materials could be derivatized satisfactorily, and the first amino acid attached quite well. Even the first peptide bond formed in good yield, but then the yields began to fall. Eventually, after considerable effort, I was forced to drop this approach. A porous copolymer produced by Dow also did not work for me. Several years later, by using new less cross-linked resins and improved peptide chemistry, at least two groups succeeded with macroporous polymers, although the new supports never showed real advantages over the original amorphous beads.

An alternative idea for the preparation of polymer supports with increased surface and minimum of steric interference with the approach of reactants to the functional sites was to grow "whiskers" onto the outside of highly cross-linked beads. First, I initiated the polymerization of styrene with butyllithium. That "living polymer" was coupled to chloromethyl copoly(styrene−16%-divinyl benzene) beads and then quenched. These pendant polystyrene chains were attached at only one end and were not cross-linked. If they would solvate as expected, they should provide the desired support. The second attempt to grow whiskers involved using a phase-transfer reaction to produce a polystyrene anion with metallic lithium, and then to initiate chains of polystyrene on the surface of the bead. In the third procedure I soaked 2% cross-linked styrenedivinylbenzene copolymer beads in styrene and initiated polymerization with benzoyl peroxide and heat. The first two procedures resulted in very small but significant increases in weight due to addition of polystyrene chains to the coarse polymer beads. The third procedure lead to a 3-fold increase in weight. The added material was not removed by boiling in benzene or CCl_4 and was considered to be covalently bonded to the core. The beads had their normal appearance under the microscope but were larger in diameter. However, the swelling of the beads did not increase in the way that I had expected. None of these preparations showed any improvements as supports for peptide synthesis, and this approach was set aside. Later such polymers were prepared by radiation-induced grafting of polystyrene onto small Teflon particles.[14] The materials were used for the preparation of peptides, although the synthetic efficiency was found to be lower than that for the amorphous S-DVB beads. *Merrifield then returned to the 2% resin, but used DCCI for coupling with much improvement. At this junction Merrifield also studied other attachment chemistries, including sulfonyl hydrazides removed by oxidation as well as using hydrogenation to cleave the support benzyl ester.* Unfortunately I decided the last idea was no good, gave up on it, and, of course, never published these results. About 14 years later Mazur and his group had the same idea, but they made it work! *Merrifield then returned to looking at alternatives to the Z protecting group and tried Schiff bases and formyl protection, but the real breakthrough came with his investigations of Boc protection, as is well known to this audience.*

With these key components, resin, benzyl ester linkage, tBoc N protection, and strong acid deprotection, Merrifield then succeeded in the syntheses of several peptides, including bradykinin. I really missed the boat on combinatorial peptide synthesis. In 1965, shortly after my SP (solid-phase) synthesis of bradykinin, I seriously considered a synthesis in which multiple residues were coupled at each cycle in order to find new agonists or antagonists, and I thought of an iterative assay method in which simpler and simpler mixtures were produced until the most active component was identified. But, alas, I talked myself out of it for fear that there would be counteracting activities that would confuse the interpretation. We did a couple of experiments in the 1970s, but without much success. Twenty-five years later when the time was ripe this has been done, of course, and the field has exploded.[15]

In the very early days of solid-phase synthesis it was believed that the solid support effectively isolated the peptide sites, leading to reactions that resembled those at infinite dilution and thereby avoiding intermolecular reactions between separate resin-bound sites, which in turn would promote intramolecular reactions.[16] It was soon found, however, that there is very significant polymer chain motion even on the cross-linked resins. This was demonstrated by electron spin resonance (ESR) and NMR measurements. The motional rates for the aromatic side chains and the aliphatic backbone atoms of 1% cross-linked polystyrene in CH_2Cl_2 are high (10^8 s^{-1}) and equivalent to those of soluble polystyrene, which indicates that the polymers are highly solvated. For pendant peptides the rates were as high as 10^{10} s^{-1}. Chemical experiments also showed significant polymer chain motion of 200−300 Å.

It was demonstrated early on by autoradiography that the peptide chains were uniformly distributed throughout the matrix of lightly cross-linked copoly(styrene−divinylbenzene) beads (Figure 3). The coupling reactions proceeded by rapid second-order rates (99% reaction within 10 to 100 s), and the mass transfer was at least an order of magnitude faster than the initial coupling rate.

D.H. comments: I first had the chance to get acquainted with the technique of solid-phase synthesis in the Liverpool University laboratories of Prof. George Kenner, in the early 1970s. As part of a major project to assemble a lysozyme analogue, George asked me to look into using a phenolic

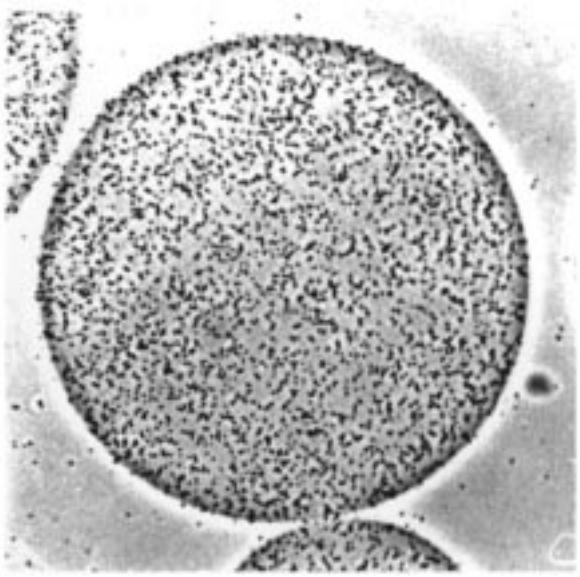


Figure 3. Autoradiograph of a thin cross-section of a bead containing a synthetic tritiated peptide. Reproduced with permission from ref 8. Copyright 1997 American Chemical Society.

resin for the assembly of partially protected peptides for the convergent solid-phase synthesis of that protein. Part of the concept was to use the remarkable peroxide catalysis of phenyl ester cleavage discovered by John Seeley. I had many false starts, trying to modify "plain" polystyrene and to convert it to an appropriately derivatized form. Eventually, I had successfully rediscovered how to nitrate polystyrene in a controlled manner as well as how to cleanly reduce it to an amino form. In fact, by attachment of a phenolic derivative, I did "prediscover" the "handle" methodology. However, as Reza Arshady describes later, I was saved from having to develop this further, as Reza prepared a custom resin formed by copolymerization of the necessary functional monomers and the cross-linking reagent. This study provided me an introduction to the fact that resin modification methods produce side reactions which compromise the performance of the product, and copolymerization of functionalized monomers with the other constituents remains the best way to obtain such products, e.g., chloromethyl-polystyrene. I quickly demonstrated peroxide catalysis did occur with peptide phenyl ester resins, and had made one peptide fragment, when I moved to Hammersmith Hospital to start making peptides "for real!". Although I, for the most part, used Merrifield resin,[17] I was able to use the phenolic resin in some nice enkephalin syntheses (which included the very first example where isosteric replacements were made for every bond in a single peptide),[18] and with the availability of some radiolabeled amino acids, I was able to put the finishing touches to the work started with George. I wrote this up as my tribute to a great man, after his untimely and tragic death.[19] Looking back, I only now realize how important this work was: it was the first occasion when I used side-by-side comparisons to gain insight into methodological issues, and the in situ neutralization programs developed then still have much merit today.

Returning to the topic of polystyrene (PS) beads, then, the autoradiograph shown in Figure 3 indicates not only that peptide chains were uniformly distributed throughout the interior of the beads but that resin beads obtained from suspension polymerization are highly spherical. They normally are obtained in quite a wide particle size distribution, and can be separated, by the process of air classification, into two size ranges, 100−200 mesh (75 to 150 μm), and 200−400 mesh (38 to 75 μm). Monosized beads can be obtained by a variety of processes. Larger beads are possible, either from careful classification of the crude polymerization product (e.g. 70−90 mesh resin) or by seeding of the polymerization. Beads of 600 μm diameter have been produced for library applications but appear to have unfavorable properties, due to heterogeneity of the sites. Recent studies with a variety of bead sizes, showing slower reaction kinetics, confirm that diffusion of reagents into larger beads is far slower than for standard beads. The practicality of such big beads is limited by their stability, both to mechanical forces and to shear induced by shrinking and swelling. Nevertheless, they can be highly useful in single-compound single-bead libraries.

Numerous workers have appreciated the importance of swelling, both of the initial resin and during the course of synthesis. The phenomenon is not only relevant to peptide chain assembly, where interpeptide aggregation can lead to significant reductions in accessibility and reaction rates, but for all forms of MAST; favorable swelling in dipolar aprotic solvents, for example, can facilitate nucleophilic displacement reactions. This topic is discussed further by Bing Yan in part II of this Perspective.

Clearly the solvation of low-cross-linked polystyrene beads is relatively inefficient. Merrifield comments, "the extent of swelling of low-cross-linked polystyrene beads is determined by the counteracting effects of polymer solvation and the restraining force due to extending the cross-linking bonds, as we have discussed in detail.[20] We calculated that the maximum swelling with all bonds fully extended could reach approximately 200 mL/g, whereas for a good quality 1% cross-linked resin, a value of ~9 mL/g in DCM is actually obtained. When heavily loaded with non-cross-linked peptide, the equilibrium shifts to greater solvation without added restraining force, and larger volumes are observed. However, the resin network is still not fully extended (even at a ratio of 4:1 [wt:wt] peptide:resin)."

A further important concern is minimization of resin modification during functionalization. Many procedures, e.g., the production of high levels of chloromethylation, result in an increase in cross-linking, and consequent diminished swelling and reactivity. In multistep modifications incompletion of any reaction, too, can generate problems. This has been especially apparent during the aminolysis step via the Leukhardt reaction in the production of MBHA resins. The presence of unreacted keto groups can lead to blocking of reactions, resulting in temporary blocking of subsequently introduced amino functionality. Variations of the original procedures circumvent these problems and give improved product performance and swelling.[21]

Little use is now made of these resins for DNA synthesis because of incompatibilities with the acetonitrile used in the current cyanoethylphosphoramidite coupling chemistry. Nevertheless, gel resins are well suited for the phosphate triester

methodology, as implemented by Itakura,[22] since longer reaction times are required, and pyridine is the solvent of choice for coupling. The 1 and 2% cross-linked resins have become the work horse of MAST for the mass production of organic and template libraries to facilitate screening programs. As noted in the introductory remarks, the translation of organic reactions from solution phase to support-bound phase can require much investigation to optimize the reaction yields. In general reactions which require, or tolerate, excess of one reagent, can be transferred quite straightforwardly; however, reactions which require stoichiometric amounts of reagents seldom work well on a solid support. Many organic reactions generate side reactions and colored impurities (a fact well known to all lab chemists); if these arise from a matrix-bound component, a complex work-up procedure will be required. Many workers have believed that PEG−PS graft copolymers provide a more generally useful support for MAST because of the improved solvent compatibility as well as from spacer arm and environment effects. However, recent studies bring this generality into question. The topic, close to this reviewer's heart, will be discussed by Bing Yan and myself in part II. It is also apposite to add that some reactions, such as Freidel−Crafts acylation reactions, are fundamentally incompatible with PS supports, of any type, since the aromatic rings of the polymers will be acylated and alkylated under the reaction conditions. These considerations led to a current program at Biosearch to investigate Aspect for this application. The search for a truly inert support stable to aggressive reaction conditions is further complicated by the requirement that both handle and linker used for substrate attachment, too, be absolutely inert. Morten Meldal has made a remarkable step in that direction with the new resins that he reports on.

The development of the first custom-prepared nonpolystyrene resin, Pepsyn, is described in the next article by Bob Sheppard, who also describes a kieselguhr encapsulated version, Pepsyn K. Many variations on these themes have been developed, and a representative selection are presented in the following section. I was happy to give some minor advice to Roger Epton in some very early work in developing an acryloylmorpholine-based resin, which had excellent solvation characteristics and worked well at very high load.[23] Jim Sparrow next discusses a further variant that proved to be an excellent immunogenic carrier. It is believed that these materials are more flexible than polystyrene, and excellent swelling is obtained (ca. 20 mL/g) even with higher degrees of cross-linking. The aqueous compatibility of these resins confer suitability for "one-bead/one-compound" type libraries, for solid-phase enzyme assays, as well as for the direct productions of antibodies. These beaded variants still have many useful applications in peptide synthesis, but they have been little used for solid-phase organic synthesis (SPOS), since, although they lack aromatic reactivity, they are rich in amide bonds which confer a whole new level of reaction incompatibilities. Arshady discusses the development of a range of hybrid materials, including both gel and macroporous copolymers, usually formed between acrylamide and styrene monomers. These possess some interesting characteristics midway between those of the parent resins.

Dave Sherrington, in a multifaceted mini-Perspective, cunningly pulls together most of the topics of this article from his personal experience as well as including the important contributions of others too reticent to blow their own trumpet! Exciting developments are obviously still in store for us from his fertile laboratories, as is equally true for the work of Morten Meldal, who was undaunted by the need to learn polymer chemistry techniques and has produced some highly promising PEG-based materials of unique capabilities. A related resin, CLEAR (the acronym standing for PEG cross-linked ethoxylate acrylate resin), developed by Kempe and Barany, will hopefully be included in George Barany's contribution in part II.

Bob Sheppard.[24] Tailor-Made Supports for Solid-Phase Synthesis

So how does a classically trained organic chemist set up a peptide chemistry group in a laboratory of molecular biology in 1971? It was soon clear that the slow and labor-intensive solution methods of synthesis with which we were familiar would not suffice in the new environment. A reliable accelerated synthesis technique of one sort or another had to be established. The literature already contained details of several such techniques, of which the 1963 solid-phase method of R. B. Merrifield[25] was of course the most prominent. Yet there were recognized problems in contemporary solid-phase chemistry,[26] which seemed to limit the potential of the method to shorter peptide sequences. Molecular biologists were, by definition, more interested in proteins and nucleic acids than peptide hormones and the like which had so far provided the most fertile ground for the solid-phase method. The only acceptable course was to look afresh at the underlying chemistry of solid-phase synthesis: to consider, in turn, the special requirements of the solid phase, protecting groups, and reaction conditions; and this is what we did.

This article is concerned only with the solid phase. As every organic chemist would have done, Merrifield had begun by examining a number of commercially available polymers for their suitability as supports.[27] We followed his lead by examining a few more. An invited review lecture[28] at the 11th European Peptide Symposium had provided opportunity to review the literature and to think, inter alia, about the role of solvation in the gel phase. Solvation of reactants and intermediates is usually held to be important in organic chemistry. But in contemporary solid-phase peptide synthesis there was a clear paradox. The hydrocarbon polystyrene gel support required a relatively apolar medium such as dichloromethane to swell (solvate) it maximally. Experience from solution chemistry, however, indicated that such media were rather poor solvents for many protected peptide sequences. Furthermore, they were not always kinetically the best solvents for carrying out peptide bond-forming reactions. We concluded that perhaps the way forward was to focus on more polar gel supports than had been previously used. Supports which would be well solvated by polar media known to be both good solvents for protected peptide sequences and suitable reaction media for the chemistry of peptide synthesis. For some years, dimethyl-

formamide (DMF) had headed the list of peptide chemists' favored solvents, and it was an obvious first choice. It was a short step from there to think in terms of polyamide supports for peptide synthesis, visualizing a peptide−polymer−solvent system with common structural features and compatible solvation properties.[29]

Polyacrylamide gel was a very well-known reagent in molecular biology laboratories, and the beaded resin was commercially available. My colleague, Eric Atherton, spent many months studying its use in solid-phase synthesis. Substantial chemical modification was required to reduce the very strong internal amide−amide hydrogen bonding which caused the starting resin to be swollen essentially only by water. Replacement of a large proportion of the primary amide hydrogen atoms by hydrazinolysis, diazotization to the azide, and reaction with dimethylamine provided poly(*N*,*N*-dimethylacrylamide) (PDMA) which was freely solvated (swollen) by DMF. It was used successfully in solid-phase synthesis, but not reliably. There were frequent failures.

We persevered with this support for perhaps longer than we ought. In the back of our minds we knew that we might well be doing the wrong thing. The commercially available polyacrylamide was designed for chromatography, not peptide synthesis. We knew that it was cross-linked with methylene bisacrylamide which could conceivably liberate formaldehyde during chemical manipulation, and formaldehyde reacts with peptides and proteins! We had no knowledge of how "pure" the polymer was, i.e., what other chemical structures might have been incorporated inadvertently during the polymerization process or afterward. Experience elsewhere many years later showed how important this consideration could also be in the polystyrene series. Clearly polyacrylamide or better polydimethylacrylamide itself specifically designed and prepared for peptide synthesis was required. This realization marked the end of our use of commercially available polymers originally designed for other purposes. As soon as we had sufficiently learned the art of polymer chemistry, polydimethylacrylamide with all the chemical attributes required for solid-phase synthesis was prepared by direct polymerization from pure monomers.[30] It swelled some 10−15-fold in DMF, contained completely stable cross-links derived from ethylene, not methylene, bisacrylamide, and sites specifically built in for conversion under very mild reaction conditions to growth points for the peptide chain. The skill of an experienced polymer chemist was required before it could be obtained in the final, nearly uniformly beaded form.[31] It was and remains an extremely successful and reliable support for solid-phase peptide synthesis.

The moral of this tale is simple enough. There is much to be gained by use of tailor-made solid-phase supports. There may be much to lose through prolonged use of polymers designed for other purposes unless their constitution is fully known and is completely appropriate. Impurities generated at polymerization or through subsequent chemical manipulation usually stay with the polymer. Small molecular proportions of reactive impurities may be disastrous in a field such as solid-phase peptide (or oligonucleotide) synthesis where success or failure depends on the nearness to which quantitative conversion can be consistently achieved. Critically in a field noted for its reliance on chemical catalogs and general conservatism, the new support soon became commercially available, initially under the name Pepsyn, and achieved widespread popularity. More than 20 years after its invention it is still being used successfully, especially notably in the United Kingdom by Zeneca Specialities (CRB) for large scale (multi-kilogram) peptide synthesis.

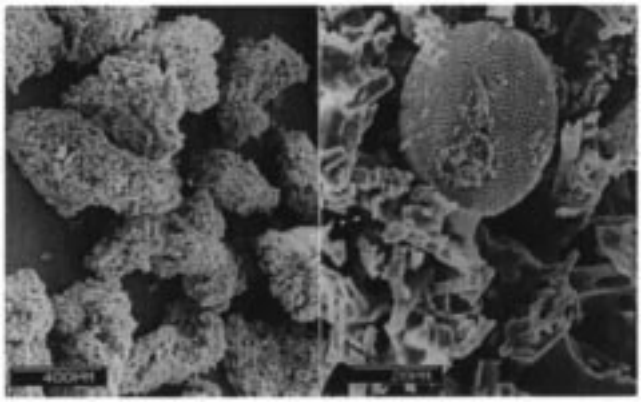


Figure 4. Fabricated macroporous kieselguhr support for the preparation of Pepsyn K. Photomicrographs reproduced with permission from Dr. Wolgang Rapp.

Merrifield's solid-phase technique utilized beaded polystyrene gel in a shaken or stirred reaction vessel, and Pepsyn was used similarly. With the publication of Merrifield's autobiography in 1993, we now know that he originally envisaged a free flowing system. Others later followed this path, but no truly practical system emerged and none of these early attempts have survived. The potential advantages of continuous flow synthesis in which reagents are pumped continuously through a stationary resin bed are easily seen, but again there are conflicting requirements. The rather soft, loosely cross-linked gel resins which seemed to be chemically most suitable were physically too fragile. Compression of the resin bed easily occurs with collapse of the internal open gel matrix, hindering reagent penetration, restriction of the liquid flow, and the generation of unacceptably high pressures. A new support was required which retained the excellent chemical properties of polydimethylacrylamide gel but was physically stronger to resist compression under flow conditions. Such a support was first prepared by polymerizing the Pepsyn gel monomer mixture within the pores of rigid macroporous particles (a process technically much easier than the carefully controlled suspension polymerization required to afford the beaded gel). Macroporous kieselguhr, fabricated by sintering the powdered material in the presence of an organic binder, was found suitable. The photomicrograph in Figure 4 shows that some of the neatly perforated fossilized diatom skeletons were retained intact in the composite resin. Presumably in their undamaged state they themselves would have made quite ideal rigid containers for the polydimethylacrylamide gel. This physically supported resin, which we originally called Pepsyn K, provided the first practical continuous flow synthesis support.[32] Later, an alternative, extraordinarily attractive, rigid porous polystyrene matrix (Figure 5) was devised by David Sherrington and his colleagues. This also seemed a near ideal physical support

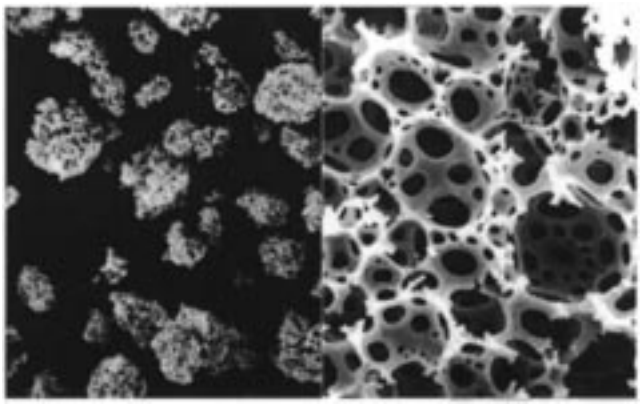


Figure 5. Rigid macroporous polystyrene support for the preparation of PolyHIPE resins. Photomicrographs reproduced with permission from National Starch.

for soft gels, though the first composite resin (PolyHIPE)[33] proved disappointing after inital success. Almost certainly the difficulties observed were due to covalent attachment of the polydimethylacrylamide gel through chemical modification of the polystyrene support. This superficially attractive idea required a series of reactions on the polystyrene leaving unknown and variable resin-bound side products. The situation was just what we had experienced in our early attempts to modify commercial polyacrylamide. A second composite prepared by simple polymerization of the polydimethylacrylamide monomer mixture within the polystyrene matrix (as with Pepsyn K) proved more satisfactory and provided another useful continuous flow support (Polyhipe SU).

Continuous flow synthesis proved very popular and commercial synthesizers (CRB Pepsynthesiser I and II, LKB 4175 and 4170 (Biolynx), Milligen 9020 and 9050, Novabiochem Gem and Crystal, and the Perkin-Elmer Pioneer) soon became available.[34] The development of continuous flow synthesis together with introduction of the Fmoc-*tert*-butyl technique enabled substantial advances in the practice of solid-phase synthesis. Real time reaction monitoring and even automated feedback control became possible.

A third tailor-made support for solid-phase synthesis has so far proved markedly less successful.[35] With the steady improvements in technique, many peptides were now being prepared with such high efficiency that they were cleaved from the resin sufficiently pure for some biological applications. Probably most solid-phase peptide synthesis in the past decade or so has been for immunization and antibody production. This is an area where the biologists have not always been concerned to obtain the most highly pure immunogens. It seemed that there was scope for a synthesis support on which the synthetic peptide would be permanently retained (i.e., not detached for purification) and which would also function as a high molecular weight carrier for the immunogen. Our experiments with solid carriers were not encouraging, and we therefore devised a solid support for peptide synthesis which could be solubilized in a final step without detachment of the peptide. All that was required was a cross-link labile to acid; cleavage would convert the insoluble cross-linked resin to a soluble, linear polymer. A variety of Pepsyn K was therefore prepared in which the cross-linking reagent ethylene bisacrylamide in the monomer mixture was replaced by one containing an acid-labile dimethylketal function.[36]

Although we obtained quite encouraging immunological results ourselves with this solubilizable polymer, to our knowledge it was probably only tried in two laboratories outside our own. In large part this must have been due to lack of commercial availability. Both laboratories which did use it obtained their material from us. Chemical manufacturers were either not impressed or found the newly acquired entrepreneurial policies of our parent organization too difficult to accommodate. The new resin was patented, and immediately attempts were made to sell licenses to potential manufacturers. Not surprisingly, the latter were more cautious and waited to see further and more widespread applications, which of course did not come because of lack of availability of the new support!

Jim Sparrow.[37] Improved Dimethylacrylamide-Based Resins

In the early 1970s when we were attempting to synthesize fragments of the serum apolipoproteins to determine their lipid binding and enzyme activation properties,[38] we frequently encountered difficulty in coupling during the synthesis of these hydrophobic amphiphilic sequences. I reasoned that part of the problem was the intrinsic structural nature of these peptides and, in addition, that the pore volume of the polystyrene support might be at fault particularly during the synthesis of the larger peptides that had a high helical potential and a natural tendency to aggregate. In 1976, I published a paper on an improved polystyrene support for peptide synthesis which included the first description of the phenylacetamidomethyl (PAM) linkage.[39] This more stable linker was attached at the amino terminus of two molecules of 11-aminoundecanoic acid coupled to aminomethylpolystyrene, thereby displacing the synthetic position of the peptide from the polystyrene backbone. Electron spin resonance measurements of the amino terminus of a series of apolipoprotein fragments prepared on this support versus those of the same peptides prepared on a normal polystyrene support indicated that, indeed, the peptide was more mobile on the new support and that DMF/DCM mixtures dramatically increased peptide chain mobility for all peptide lengths.[40] I also found that the yield and purity were greatly improved for a wide variety of peptides.

Shortly thereafter, Sheppard and his group[30−32] reported on the use of polyamide supports for peptide synthesis, followed several years later by Walter and Smith's polyamide support.[41] I reasoned that the more polar nature of these polyamide materials might decrease the interactions of the apolipopeptide with itself and with the nonpolar polystyrene support that I believed were interfering with high-yield synthesis. I undertook a research effort to incorporate the best features of the Sheppard and Walter/Smith resins and develop a support that had high loading, good stability, and larger pore volume than other supports. We used *N*,*N*-dimethylacrylamide as the backbone monomer, as did Sheppard. However, in our support, we incorporated Msc allylamine as a protected monomer to prevent alkylation

during the polymerization which we believed was occurring with the Walter/Smith support. We also prevented the possibility of further cross-linking which could occur in Sheppard's resin when it is functionalized with ethylenediamine. We used *N*,*N*′-bisacrylyl-1,3-diaminopropane as a more flexible cross-linker which could be readily synthesized in high purity and yield by an improved procedure.[42] In addition, for the detergent to form the emulsion, we chose sorbitan monolaurate instead of sorbitan oleoate to prevent the incorporation of the unsaturated detergent during the polymerization.[43−47]

Our support proved to be mechanically stable and highly swollen in most polar solvents, including alcohol and water, and had a loading of about 0.7 mequiv of amino group per gram of dry resin. It proved to be very useful for high-yield peptide synthesis by Boc chemistry. However, our goal of a support with a larger pore volume was not immediately realized. We found that, like polystyrene and Sheppard's support, the exclusion volume of our material was about 20 kDa when measured by exclusion chromatography with peptides and proteins of known molecular weight.[48] During this investigation, we also determined that the exclusion limit decreased rapidly as a short hydrophilic peptide was synthesized on the support. Considering the molecular weight of most activated protected amino acids used in both Boc and Fmoc synthesis, we reasoned that they can be quickly excluded from the innermost regions of the presently used supports, thus leading to deletion and/or terminated peptides.

Using our new support, we were able to show that it could be used as a vehicle to produce antibodies to peptides and proteins.[49,50] The peptide was synthesized directly on the support without a linker. By injecting the deprotected peptidyl resin, high titers of antibodies could frequently be obtained, particularly if a known T-cell epitope from another protein was synthesized on the amino terminus of the protein fragment of interest. In addition, since the peptidyl resin was swollen in water, the resin could also be used to determine antibody titers as well as epitopes of the synthetic peptide or the native protein. To ensure the integrity of the injected material, we developed an improved procedure to sequence peptidyl resins.[51] By preparing larger amounts of the peptidyl resin, an affinity column could be prepared and used to purify specific antibodies.[52]

We made several attempts to obtain a polymer with larger pores by incorporating proteins during the polymerization and then trypsinizing the resulting support to remove the protein. Although most of the protein was removed by this treatment as determined by amino acid analysis of the polymer, these resins did not meet our requirements for a stable support and were abandoned.[53]

About this time we found that alkylation could be prevented by carrying out the polymerization at pH 6 using *N*-acrylyl-1,6-diaminohexane hydrochloride or, more conveniently, using the commercially available *N*-methacrylyl-1,3-diaminopropane hydrochloride as an unprotected functional monomer.[54,55] Since the water solubility of *N*-methacrylyl-1,3-diaminopropane hydrochloride is higher than that of the diaminohexane, supports with loading as high as 1.4 mequiv of amino group per gram could be obtained. All of these polymers proved to be equally useful for peptide synthesis. We also discovered that by increasing the volume of the aqueous phase used to dissolve the monomers for the emulsion polymerization we could control the pore volume of the resulting support. Using this technique, we prepared supports with exclusion limits of 50 kDa, 125 kDa, and 250 kDa. We have used the 50 kDa support extensively for peptide synthesis, and it is commercially available from Advanced ChemTech as SPAR-50. Recently, we have shown that this support gives high yields and purity of peptides synthesized by Fmoc chemistry.[56] In some cases, the peptide of interest could not be prepared by Fmoc synthesis using a polystyrene support. The supports with exclusion limits of 125 and 250 kDa have not been tested thoroughly for peptide synthesis.

We found that the SPAR-50 resin could be used for combinatorial chemistry by the one-peptide/one-bead approach using Fmoc chemistry.[57] If Boc chemistry and HF cleavage were used, we found that the resin became fluorescent which interfered with our fluorescence detection system for isolating labeled antibody or protein bound to the swollen beads. Using our improved sequence methodology, we could sequence the peptide on the bead as others have done; we obtained from 50 to 100 pmol of released amino acid per resin bead.

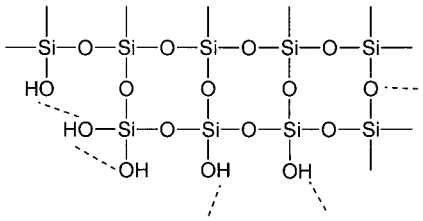
In closing, I would like to thank my wife, Doris, and my many research associates and collaborators whose hard work over the years brought this research to fruition.

Reza Arshady.[58] Amphiphilic Copoly(styrene−acrylamide) Supports[59]

This article presents a brief overview of the author's work on the development of amphiphilic polymer supports, which are based on approximately alternating copolymers of styrene and acrylamides and hence encompass the solvent and substrate compatibility of both polystyrene and polyacrylamide. This general solvent and substrate compatibility is discussed in terms of polymer chemical structure, texture of the cross-linked matrix, and polymer−solvent−substrate interactions, illustrating their balanced physicochemical features ideally suitable for solid-phase synthesis.

Polymer Synthesis and Structure. Beaded polymer supports,[60,61] like other functional polymers in general,[62] can be derived from inorganic oxides (e.g., silica and glass), polysaccharides (e.g., cellulose), and synthetic organic polymers (e.g., polystyrene and polyacrylamides) (Figure 6). The generation of functional groups (anchoring points) on polymer supports is also conventionally achieved in two different ways: direct bead copolymerization of functional monomers with structural monomers, or functionalization of preformed nonfunctional polymer beads. The introduction of solid-phase peptide synthesis by Bruce Merrifield in the early 1960s was based on the polystyrene structure, and chloromethylation, as shown in Figure 6. However, alternative polymer structures and synthetic routes have also been explored since the inception of the solid-phase method.[63]

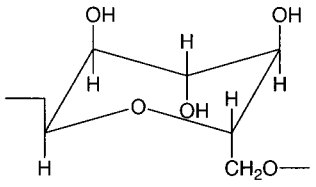
But the adoption of a given polymer support, like any other product, depends strongly on the technology and economics of its production. It is, therefore, not surprising that many



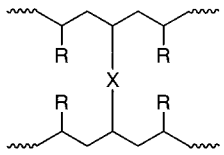
1

$(SiO_2)_m(B_2O_3)_n(Na_2O)_p \cdot x\ H_2O$

2



3



4, R = C_6H_5, X = C_6H_4 (meta, para)
5, R = $CONH_2$, X = $CONHCH_2NHCO$

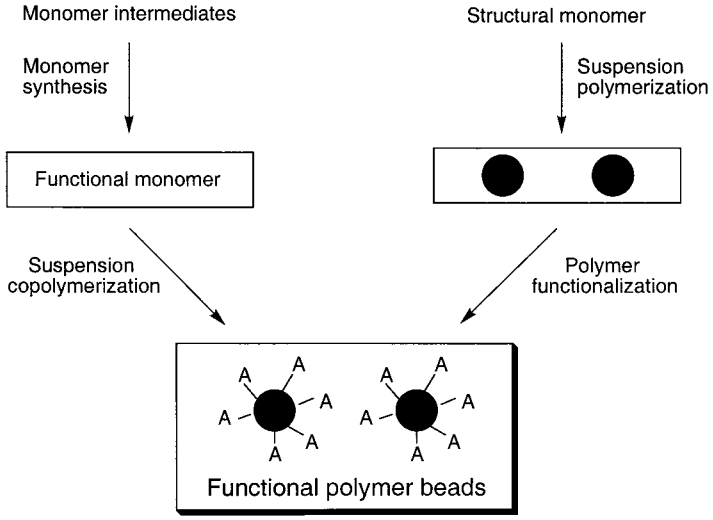


Figure 6. Basic chemical structures and conventional routes to beaded polymer supports. Bead formation is achieved by suspension polymerization in both routes, but the anchoring points (functional groups) are introduced onto the beads by either (co)polymerization of a functional monomer or functionalization of preformed particles. Reproduced in modified form from ref 59 with permission of Citus Books (copyright 1999).

of the commercially available polymer supports owe their popularity largely to their established technology of production, rather than necessarily on their current synthetic merit or functional performance. However, a number of interesting polymer supports based on modified polystyrenes or other polymers have been developed during the past 20 years, as discussed in this Perspective. Here I wish to present a brief description of a new type of amphiphilic copolymer support based on both styrene and acrylamide residues, combining the solvent and substrate compatibility of both polystyrene and polydimethylacrylamide supports. These new amphiphilic polymers are produced by an efficient suspension polymerization system with full control of particle size, cross-linking, porosity, and degree of swelling, and they are the closest that can be presently envisaged to an ideal polymer support for solid-phase synthesis. Fuller details of these and related polymer supports (and microspherical polymeric materials in general), including their preparation and numerous applications in the chemical and life sciences, biotechnology, and medicine, can be found in the cited references from Citus Books.

As mentioned above, polymer supports can be largely characterized by their "chemical structure" and "production route". The idea of amphiphilic polymers also evolved gradually from our work on these two fronts, i.e., production of polystyrene supports by the "copolymerization route" during the 1970s and the introduction of an "alternative polymer structure", polydimethylacrylamide, during the 1980s. Accordingly, an outline of this work is also provided here as a background to the idea and synthesis of copoly(styrene−acrylamide) supports.

Phenolic Resins by Copolymerization. The copolymerization approach can, in principle, be employed to introduce various functional groups into the polymer by (co)polym-

erization of respective functional monomers,[64] but it does not mean the copolymerization approach is itself problem free. In particular, preparation of well-defined beaded polymer supports requires a high degree of polymer science expertise and resources that are seldom readily accessible to practitioners of solid-phase synthesis, hence the general appeal of collaboration between practitioners of solid-phase synthesis and polymer scientists in developing improved and new polymer supports.

One such collaborative program was my Ph.D. work in Liverpool University (1972) with the cooperation of Derek Hudson and the late George Kenner (peptide), and Tony Ledwith (polymer) and with the aim of developing styrene-based polymer supports carrying phenolic hydroxy groups by direct copolymerization of 4-hydroxystyrene (4HS, or rather its acetyl-protected form) with styrene.[65] This was an example of a highly successful collaborative program, and the resulting phenolic resins, as we called them, proved highly useful for peptide synthesis in both Liverpool and London,[18,19] and as I later learned from Derek Hudson the resins could be recycled and reused repeatedly for a number of times. An interesting byproduct of this work was also a specifically designed laboratory scale suspension polymerization reactor which has since been found generally useful for production of beaded polymer supports in relatively narrow particle size ranges.

Two experimental elements of this work relevant to the later development of amphiphilic polymer supports were the removal of the polymer-bound acetyl groups by "hydrazinolysis" and acylation of polymer-bound hydroxy and amino residues by "activated esters".[66] Hydrazinolysis was employed because alkaline hydrolysis was almost totally ineffective owing to incompatibility of the strongly polar hydroxyl group [$HO^-(H_2O)_n$].

On the lighter side, too, I recall some intricacies of interdisciplinary research particularly relevant to Ph.D. and postdoctoral folks who must be careful when revealing their alliances, especially if they have to conduct themselves in a language other than their mother tongue. For example, during the final stage of my work in Liverpool I was running some acylation experiments in two different labs in two different departments, occasionally leaving a note on either of my benches reading *I am on the other side* (i.e., in the other lab). So one day came the question "...Tony [Ledwith] tells me you are on our side, and you're telling us you are on the other side, so who's side you are really on? ...." I had no immediate answer to this question, but later the note was changed to read *I am in the other lab*.

Polydimethylacrylamide Supports. The topic of polar acrylamide supports is discussed by Bob Sheppard and Jim Sparrow elsewhere in this Perspective. However, it is interesting that practically all acrylamide-based supports reported for solid-phase synthesis are produced by direct copolymerization of basic disubstituted acrylamides with functional acrylamides and diacrylamide cross-linkers. Although it is feasible to produce polyacrylamide supports with suitable functional groups by functionalization of preformed acrylamide-based resins, the scope of such work is rather limited. Thus, in the case of polydimethylacrylamide (PDMA) supports for solid-phase synthesis, the design of a special functional monomer, acrylyoylsarcosine methyl ester, and an efficient water in oil (w/o) suspension polymerization system enabled the reproducible production of well-defined beaded polymers.[31,67]

A number of other acrylamide-based functional monomers can be used to produce beaded acrylamide resins, but acryloylsarcosine methyl ester was chosen for its relative structural similarity with DMA. Another design element of the DMA supports is the mixture of water and dimethylformamide used as monomer diluent in suspension polymerization which plays a dual function: enhancing the solubility of the functional monomer in the monomer phase and controlling the matrix structure and swelling behavior of the beaded resin. This technology has also been adapted to produce related acrylamide−acrylate copolymer supports with a variety of other functional groups, including isocyano (isonitrile) functionality suitable for peptide synthesis by four component condensation (4CC, Ugi reaction) and complexation with transition metal catalysts.[68,69]

The development of PDMA supports in the late 1970s coincided with a period of active interest in the use of the fluorenylmethoxycarbonyl (Fmoc) protecting group in peptide synthesis, including its removal by basic polymeric reagents, i.e., polymer-bound piperazine and other secondary amines. Thus, beaded PDMA (and other acrylamide-based polymers) were examined in model experiments for deprotection of Fmoc-amino acids, simple dipeptides, and other substrates.[70] The results of these experiments were disappointing in the sense that PDMA was not suitable as a basic polymeric reagent for Fmoc deblocking. However, detailed analysis of the results indicated that the poor performance of PDMA relates to the incompatibility of this polar polymer with the strongly hydrophobic (aromatic) Fmoc residue. This observation was particularly interesting in light of the contrasting (but basically the same) incompatibility phenomenon observed in polystyrene-$HO^-(H_2O)_n$, system, and it provided a strong confirmation of the significance of polymer−substrate compatibility in solid-phase synthesis.

Synthesis of Amphiphilic Copoly(styrene−acrylamide)s. On reflection, the combination of the structural units of PS and PDMA described above into a suitable copolymer structure would appear logical enough. However, the realization of this idea initially met with two severe experimental obstacles. First, the reactivity ratios of STY and DMA (1.15 and 0.12, respectively)[71] are not favorable for the synthesis of well-defined copolymer compositions. Second, STY and DMA have opposite aqueous solubility, and hence the synthesis of well-defined beaded copolymers of STY and DMA by suspension polymerization is not practicable.

Put in other words, the two problems stated above simply mean that well-defined copoly(styrene−dimethylacrylamide) beads cannot be produced by copolymerization of the corresponding monomers. This was obviously disappointing, but not the end of the story. The idea needed more serious "polymer homework". Reactivity ratios[72] are derived mathematically from copolymerization kinetics and should not be confused with the relative reactivities of the two mono-

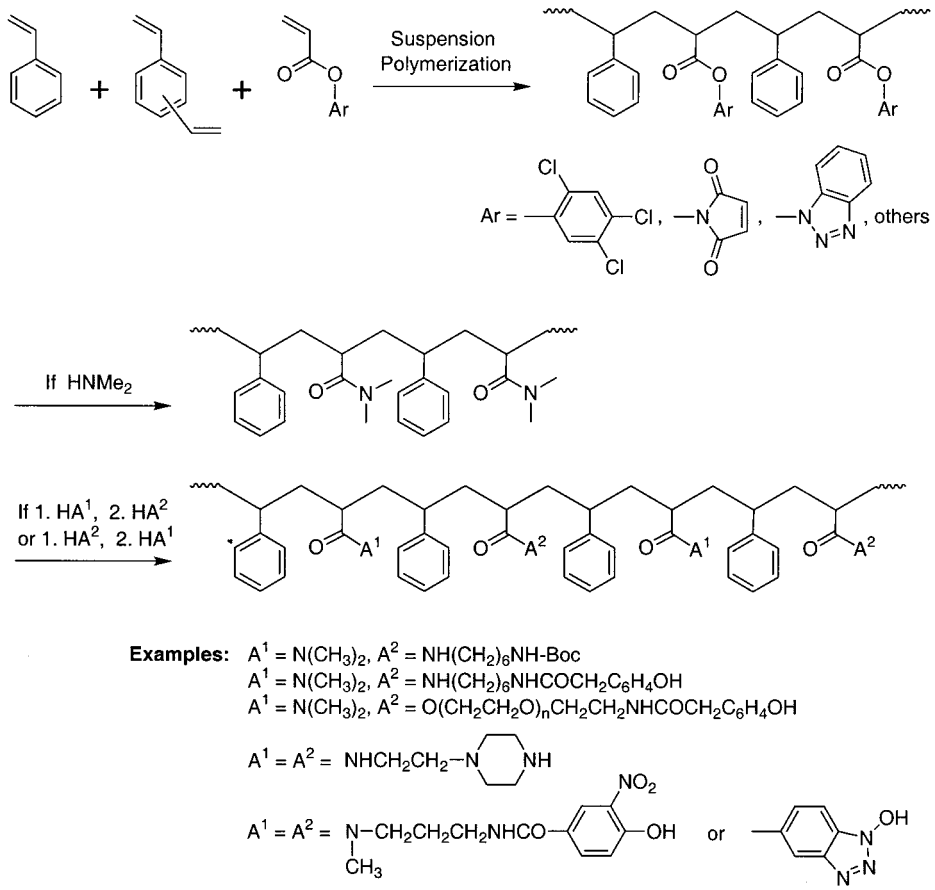


Figure 7. Synthesis of beaded copoly(styrene-dimethylacrylamide) polymer supports from the corresponding copolymers of styrene with activated acrylates. Reproduced in modified form from ref 59 with permission of Citus Books (copyright 1999).

mers. Briefly, the two monomers present in a polymerization mixture may copolymerize in a random, alternate, or blockwise fashion. It is also possible that one of the monomers may homopolymerize with little or no incorporation of the other. The copolymerizability patterns of a large number of vinyl monomer pairs are known (previous reference) or can be deduced from their Q−e values.[73] Some examples of functional−structural monomer pairs relevant to the synthesis of beaded polymer supports include styrene with 4-acetoxystyrene (as previously described), styrene with 2,4,5-trichlorophenyl acrylate,[74] and 1-vinylpyrrolidone with 2-hydroxyethyl methacrylate.[75] Of particular interest here was that equimolar copolymerization of monomer pairs with small reactivity ratios, e.g., that of styrene with 2,4,5-trichlorophenyl acrylate, produces approximately alternating copolymers.

The significance of alternating copoly(styrene−trichlorophenyl acrylate) in relation to the synthesis of copoly(styrene−dimethylacrylamide) became apparent by a mental linkage to the aminolysis of activated esters referred to above in the study of phenolic resins, and hence the "active ester" route to copoly(STY−DMA) was pursued (Figure 7).[76−78] On reflection, this link was also implicit in the routine use of activated esters in peptide synthesis. Whichever the initial clues to its potentials, this synthetic approach also resolved the problem of differential monomer solubility in suspension copolymerization and provided a highly efficient route to beaded amphiphilic polymer supports on the basis of the following criteria:

1. Copolymerization of styrene with activated acrylates under equimolar monomer conditions leads, after aminolysis, to approximately alternating copoly(STY−DMA)s with general solvent and substrate compatibility.

2. Suspension copolymerization of styrene with activated acrylates (especially 2,4,5-trichlorophenyl acrylate) proceeds very efficiently, can be scaled up easily, and the corresponding carboxyl-activated copolymers can be produced with various degrees of cross-linking with gel or porous morphology.

3. In addition to DVB, more flexible cross-linking units such as *N*,*N*′-dimethyl-1,6-hexanediacrylamide can also be used as cross-linking monomer for producing less tightly interlinked resin matrixes.

4. Conversion of the activated resin intermediates to the desired amphiphilic copoly(STY−DMA)s is accomplished by treatment with excess dimethylamine at room temperature.

5. Amphiphilic polymer supports with a wide variety of functional groups can also be produced by reaction with appropriate amine-ended functional residues.

As a note of general interest, protein synthesis in nature is also based on activated ester synthesis, and the active ester route shown in Figure 7 is suggested to provide a new dimension of creativity in macromolecular chemistry and polymer synthesis in general.[79]

Structure−Performance Relationships for Polymers. The course of chemical transformations on polymer supports

is influenced by the physicochemical nature of the polymer at the following four levels.

1. Microscopic structure and morphology (i.e., particle size and porosity).
2. Cross-linking and matrix structure.
3. Polymer−solvent compatibility.
4. Polymer−substrate compatibility.

These four criteria originate from both the polymer chemical structure and the physicochemical parameters under which the polymer matrix is formed and are all inter-related. Polymer−solvent and polymer−substrate compatibility should ideally be considered together in terms of polymer−solvent−substrate interactions. However, experimental observations can, with due care, be more conveniently interpreted in terms of two separate effects mainly related to "polymer−solvent" and "polymer−substrate" interactions.

Microscopic Structure. At the first level of structure−performance relationship, the particle size and porosity of the new amphiphilic polymer supports is controlled during the synthesis in basically the same way as those of PS and PDMA resins (loc. cit.). Porosity and surface area are also controlled during the manufacturing process. Figure 8 shows scanning electron micrographs of typical particles and a cross-section of a medium porosity copoly(styrene−2,4,5-trichlorophenyl acrylate) used for the synthesis of amphiphilic polymer supports according to Figure 7.

Cross-Linking and Matrix Structure. The frequency of cross-linking bridges between the polymer chains (cross-link density or degree of cross-linking) determines, in the first instance, mechanical strength and the extent to which the cross-linked polymer swells in a given solvent. However, the overall polymer robustness and swelling are also strongly dependent on noncovalent cross-linking (e.g., aromatic and H-bonding), the equivalent length of un-cross-linked polymer chains, and matrix morphology. In particular, the monomer diluent used during polymerization to control polymer porosity and surface area has a strong influence on polymer swelling. These parameters apply to the new amphiphilic resins in the same way as they do generally to other beaded resins, except that the question of noncovalent cross-links (which is based on chemical structure) assumes a special significance in copoly(STY−DMA), as described below.

Polymer−Solvent Compatibility. Typical swelling data for PS, polyacrylamide, PDMA, and copoly(STY−DMA) are listed in Table 3. The fact that different resins swell to different degrees in a given solvent can be interpreted in basically the same way as the swelling of a given polymer (e.g., PS or PDMA) in different solvents. Thus polymer swelling in general is best understood by looking at the chemical structures of the polymer and the solvent in relation to each other, and it is this relationship which determines polymer−solvent interactions and polymer swelling. The dependence of polymer swelling on chemical structure is best illustrated by the sharply contrasting swellability of polystyrene and polyacrylamide, and the general solvent compatibility of copoly(STY−DMA), as indicated.

Polystyrene forms a very tight network of aromatic interactions within its covalent network, and its swelling in a given solvent is determined by the extent to which that solvent can interrupt these intraresin aromatic interactions. In polyacrylamide resins, the amide residues ($CONH_2$ and $CONH-CH_2-NHOC$) similarly form a very tight H-bonded network of $-OCNH- - - H- - - OCNH- - - H- - - O-$ within the covalent matrix. As a result, acrylamide resins are permeated only by H-bond breaking solvents such as water,

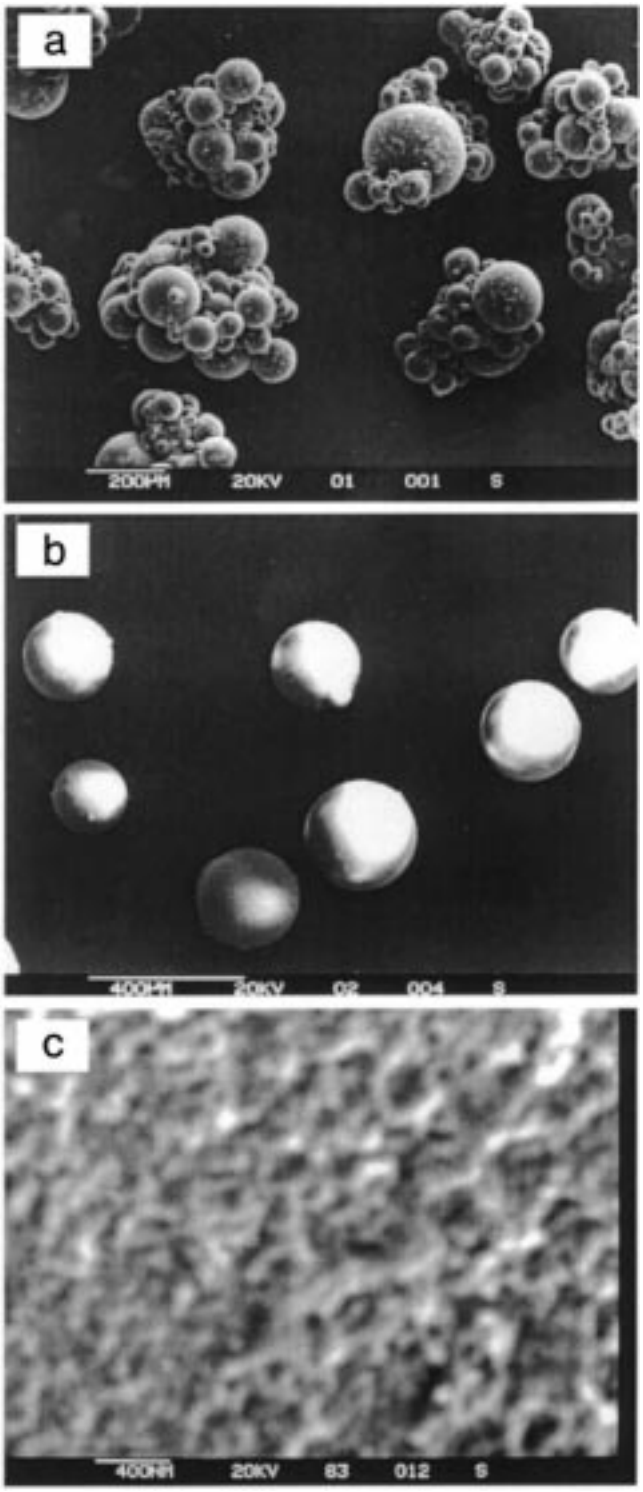
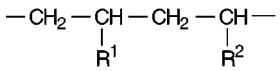


Figure 8. Scanning electron micrographs of (a) beads formed with an improperly stabilized suspension, (b) typical uniform beads of copoly(styrene−2,4,5-trichlorophenyl acrylate) from a correctly stabilized suspension, (c) cross-section of a copoly(styrene−2,4,5-trichlorophenyl acrylate) bead showing a moderately porous structure. Reproduced in modified form from ref 59 with permission of Citus Books (copyright 1999).

Table 3. Swelling Behavior of Typical Examples of Polymer Supports Based on Polystyrene (PS), Polyacrylamide (PA), Polydimethylacrylamide (PDMA), and Copoly(STY−DMA)[a]



		bulk expanded volume[b] in different solvents[c−e]								
polymer type	R	TOL	EtAc	THF	DCM	DMF	DMSO	MeOH	AcOH	H_2O
PS	Ph	5.1	4.8	5.0	5.2	4.2	−	−	−	−
PA	NH_2	−	−	−	−	−	±	−	+	+
PDMA	$CONMe_2$	−	−	−	9.5	9.1	10	12	12	9
STY−DMA	Ph and $CONMe_2$	4.7	4.0	5.3	5.8	5.2	4.6	5.5	5.5	3.7
STY−DMA	Ph and $CONMe_2$	7.1	6.0	7.5	7.3	6.0	5.1	6.1	6.9	3.9
STY−DMA	Ph and $CONMe_2$	18	16	21	27	16	13	13	21	8.9

[a] Reproduced in modified form from ref 59 with permission of Citus Books (copyright 1999). [b] Given as mL/g dry resin. [c] Polymers with higher, or lower, swelling can be produced readily for all polymer types, but the pattern of solvent compatibility is the consequence of the chemical structure of the polymer, as can be clearly seen in this table. [d] TOL, toluene; THF, tetrahydrofuran; DCM, dichloromethane; DMF, dimethylformamide; DMSO, dimethylsulfoxide; ACOH, acetic acid. −, incompatible; +, swellable; ±, poor swelling. [e] Swelling data for the more commonly used PS and PDMA, and for differently cross-linked samples of copoly(STY−DMA).

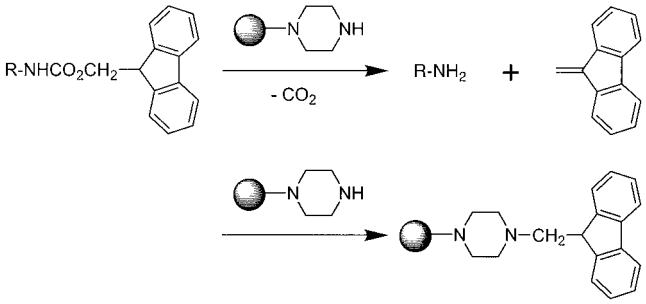


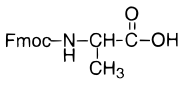
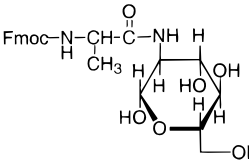
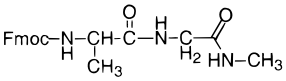
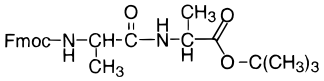
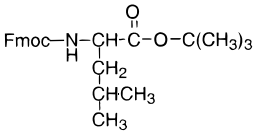
Figure 9. Removal of fluorenylmethoxycarbonyl (Fmoc) protecting group by a polymeric piperazine reagent. Reproduced in modified form from ref 59 with permission of Citus Books (copyright 1999).

acetic acid, and (to a limited extent) formamide and dimethyl sulfoxide (DMSO). When the two hydrogen atoms in polyacrylamide are displaced by two methyl groups, the resulting PDMA not only swells in solvents that do not swell polyacrylamide but it also has significantly higher swelling in water compared with polyacrylamide of similar cross-linking. In copoly(styrene−dimethylacrylamide), multicenter noncovalent interactions within the matrix are disrupted to some extent, and hence the extent of polymer swelling is more directly controlled by the degree of covalent cross-linking. And, because the matrix contains both STY and DMA residues, its solvent compatibility encompasses those of both PS and PDMA.

Polymer−Substrate Compatibility. Another fundamentally important, but less generally appreciated, level of structure−performance relationship in solid-phase chemistry is polymer−substrate compatibility. The incompatibility of polystyrene with $HO^-(H_2O)_n$, mentioned above in relation to phenolic resins, is probably the simplest of its kind. The second previously mentioned example, in relation to Fmoc deprotection by PDMA resins, is illustrated in Figure 9 and Table 4. This is a two-step reaction, involving the cleavage of Fmoc from a soluble peptide by the polymer-bound piperazine, followed by addition of the byproduct (dibenzofulvene, DBF) to the polymer, and hence its removal from the reaction mixture. These results clearly illustrate that efficiency of the first deprotection step is influenced strongly by the hydrophilic/hydrophobic character of the substrate. The carboxylic acid (or ionic carboxylate) substrate is the most polar, the most compatible with DMA (strong H-bond interactions), and hence the most efficiently deprotected. For all other substrates examined, deprotection becomes increasingly less efficient with increasing substrate hydrophobicity. It is particularly interesting that the second step in this reaction is not effective for any of the substrates examined, because DBF is strongly hydrophobic and incompatible with the polar polymer (irrespective of which substrate it was cleaved from). In the case of the carboxylic substrate, salt formation may also play a part in the higher reaction rate, but this only reinforces the argument for the significance of polymer−substrate interactions.

A practical solution to this and most other problems of polymer−substrate compatibility is offered by amphiphilic polymer supports and polymeric reagents. Because these polymers are composed of both H-bonding (polar, acryla-

Table 4. Effect of Chemical Structure on the Rate of Fmoc Removal by the Immobilized Hydrophilic Resin Reagent[a]

substrate	time for complete Fmoc cleavage	dibenzofulvene scavenging
	10 min[b]	none
	1−2 h	none
	4−5 h	none
	8−10 h	none
	15−24 h	none

[a] Reproduced in modified form from ref 59 with Permission of Citus Books (copyright 1999). [b] This is comparable to deprotection rates observed for piperazine in solution.

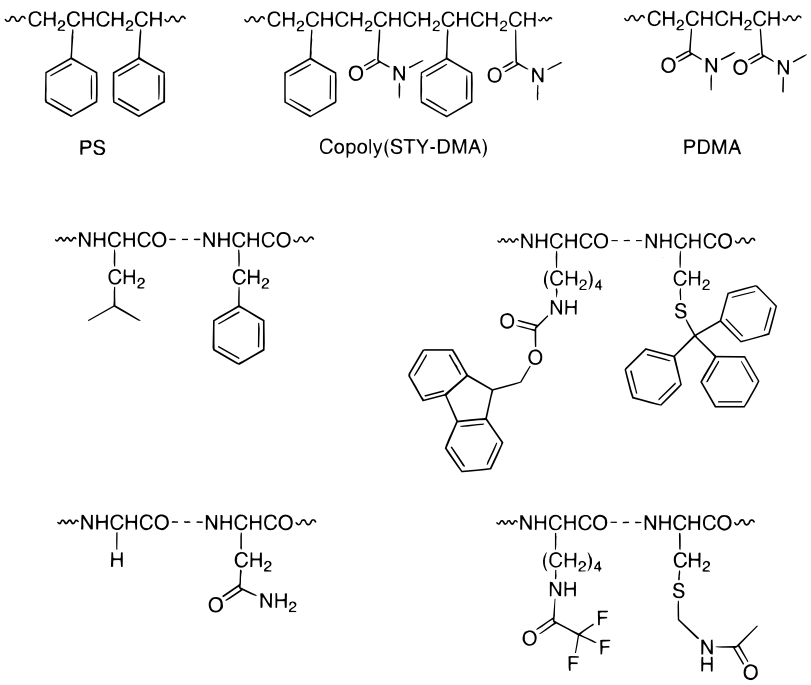
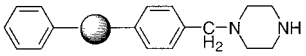
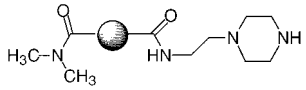
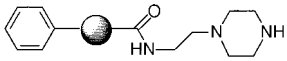


Figure 10. Structures of polymer supports based on styrene (nonpolar, hydrophobic), polydimethylacrylamide (polar, hydrophilic), and styrene−dimethylacrylamide (amphiphilic). Structures of nonpolar and polar amino acid residues are also shown to illustrate the basis of polymer−peptide incompatibility during peptide synthesis on polystyrene and polydimethylacrylamide. Note that diiferent side chain protected forms of amino acids can have widely different polarities [as with Cys (Acm or Trt), Lys (Fmoc or TFA)]. Amphiphilic polymer supports are expected to be compatible with both nonpolar and polar residues. Reproduced in modified form from ref 59 with permission of Citus Books (copyright 1999).

mide) and aromatic (styryl) side chains, they are capable of interaction with (i.e., compatible with) both hydrophilic and hydrophobic substrates. Thus, in analogy with their general solvent compatibility, the new amphiphilic polymers have also general substrate compatibility [previous two references]. Typical results for deprotection of relatively nonpolar Fmoc-substrates by polymeric piperazine reagents based on PS, PDMA, or copoly(STY−DMA) are shown in Table 5. These results clearly illustrate that the amphiphilic piperazine polymer provides the most efficient reagent for removal of the Fmoc group. The improved performance of the amphiphilic support, as compared with PS and PDMA, is evidently due to its amphiphilic copolymer structure and favorable compatibility with both the Fmoc-protected substrate and DBF.

In the more usual solid−phase methodology, where the peptide is always attached to the polymer support, the synthesis of relatively nonpolar sequences (e.g., leucine and phenylalanine in Figure 10) proceeds efficiently on the nonpolar polymer matrix, PS, but the assembly of sequences rich in polar residues (e.g., glycine and asparagine in Figure 10) is particularly difficult on this polymer.[80] This arises because strongly polar sequences are not compatible with the nonpolar polymer backbone (no effective H-bonding or aromatic interaction, no peptide−polymer interactions). Accordingly, strongly polar peptide sequences on polystyrene can only interact within themselves (leading to phase separation) and become inaccessible as a result of intrachain and interchain H-bonding.[81] As may be expected from the results presented in Table 4, an opposite problem of polymer−peptide incompatibility is observed in the case of strongly hydrophobic peptide sequences on PDMA for

Table 5. Removal of Fluorenylmethoxycarbonyl (Fmoc) Protecting Group from Relatively Hydrophobic Substrates and the Scavenging of the Byproduct Dibenzofulvene (DBF) by Different Polymeric Piperazine Reagents[a]

reagent	resin capacity (mmol/g)	100% Fmoc cleavage	DBF scavenging
10% Piperazine in DMF solution	-	10 min	+
	1.7–3.5	1–24 h	–
	3.0 h	1–24 h	–
	3.5	1–2 h	+

[a] Reproduced in modified form from ref 59 with Permission of Citus Books (copyright 1999).

exactly the same reason of peptide−polymer incompatibility. Strongly hydrophobic residues cannot interact with the polar acrylamide backbone, and as a result interact between themselves, leading to phase separation referred to as hydrophobic aggregation.[82]

The new amphiphilic polymers which carry both dimethylamide and styryl residues provide both H-bonding and hydrophobic sites for interaction with both polar and nonpolar sequences on the growing peptide chains, and hence they should overcome the problem of peptide truncation inside the polymer matrix. The new polymers are also expected to

provide a similarly favorable matrix environment for combinatorial synthesis of small molecules. Here, in addition to possible unfavorable compatibility of the soluble substrate as exemplified by the data in Table 4, high polymer loading may also lead to intraresin aggregation of the polymer-bound substrate on homopolymers, but not on the amphiphilic structure of the new polymers.

Concluding Remarks. During the early years of the solid-phase method, and up to early 1980s, there was a great deal of enthusiasm for multidisciplinary research between peptide chemists and polymer scientists in developing variously proposed polymer supports based on copolymers, core−shells, brushes, modified carbon blacks, gums, and so on. Not many long term programs of that nature actually materialized, but nevertheless a very wide range of different polymer types were reported, some of which are covered in this issue. But by then generally improved synthetic methodologies, and better understanding of the chemistry of solid-phase synthesis, meant that increasingly more efficient syntheses could be achieved by fine-tuning of synthetic protocols on most available polymer supports. This seemed to somewhat diminish the earlier enthusiasm for improved polymer supports.

More recently, however, the multidisciplinary quest for improved and new polymer supports appears to be picking up again for a variety of different tasks including synthesis on more and more highly loaded resins, large scale peptide synthesis, small molecule synthesis, and for wider solvent compatibility in the synthesis of combinatorial libraries. The new copolymer resins discussed in this article are expected to be universally suitable for all of these different tasks on the basis of their ease of production, amphiphilic structure, simple loading control, and general solvent and substrate compatibility. Their synthetic chemistry and polymer matrix characteristic have been extensively studied, and we at Citus welcome collaborative programs for exploring their utilization.

Morten Meldal.[83] Polar Inert PEG-Based Solid Supports

The solid support has a large influence on the outcome of solid-phase synthesis, in particular when the synthesis carried out becomes more difficult or chemically demanding.[84−86] Even though this was realized many years ago, surprisingly little has been done to improve and tailor the solid supports for special purposes.

Two observations were determining for our entry into the field of polymer chemistry. One was the fact that enzymes were not compatible with existing solid supports,[87,88] and the other was a report at the solid phase Symposium in Kent by George Barany.[89] He mentioned that the PEG in grafted PS−PEG resins, besides the function of spacing the reactants away from the PS-backbone, had an environmental effect on reactivity, diffusion, and swelling in the resin. This hypothesis was verified by functionalizing the resin at the junction between the PS and PEG graft and carrying out improved synthesis at these sites in the polymer.

In 1991 we rationalized that the high solvation potential of PEG in many solvents was due to the amphipatic nature of the PEG chain. Furthermore, we assumed that the PEG chain maintained a limited range of conformer populations due to the preference of gauche−gauche interactions of vicinal carbon−oxygen bonds. Together, these two properties were the basis of the design of the ideal synthesis resins containing almost exclusively PEG polymer. We imagined a polymer network, which instead of the short cross-linkers usually used in, e.g., polystyrene-based resins would contain cross-linkers of long chain PEG, derivatized at both ends with a small moiety allowing polymerization in a second dimension.[90] Variation of size and size distribution of the PEG incorporated and addition of copolymerizing additives would allow the fine-tuning of resin properties.[91] Functional groups could also be obtained with additives incorporated during polymerization[92] or they could be obtained through partial derivatization of the PEG to yield a mixture of mono and bis-derivatized PEG macromonomers.[9]

This was initially achieved by reaction of various sizes of linear bis- and branched tris-2-aminopropyl-PEG with acryloyl chloride and radical polymerization in inverse suspension by the method developed for polyamide resins by Arshady et al.[93,94] The uniformly beaded polymer immediately appeared to have some unusual properties. The beads swelled in all solvents ranging from toluene to aqueous buffers, and swelling could be completely controlled by selection of the proper average length of the PEG chains. Reactions in peptide synthesis, which were considered generally difficult, showed no sign of problems in this resin, and in a very stringent test[86] of synthesizing VNVNVQVQD without amide protection, completion of the synthesis was only achieved on this novel PEGA polymer. Furthermore, the mechanical properties of the resin were excellent despite the high degree of swelling, and the resin did not seem to change its properties even during synthesis of long peptides. It therefore performed well in continuous flow synthesizers. When fluorescence resonance energy transfer substrates[95] were synthesized on this resin and the resin-bound substrates were subjected to low concentrations of enzyme, rapid and complete hydrolysis was observed (loc. cit.). This indicated a rapid diffusion of even large biomolecules in the interior of the PEGA polymer network, as has recently been confirmed by confocal fluorescence microscopy. The observation prompted the development of PEGA resins particularly suited for assays of enzyme specificity[96−100] and inhibition[101,102] (see Figure 11). Furthermore, PEGA resins have been compared with other resins for the peptide and protein ligation techniques,[103] developed by the groups of James Tam[104] and Steven Kent.[105] In these investigations only the PEGA supports were found to be efficient and give proper purity and yield of the protein product.

During attempted solid-phase glycosylation of peptide templates and synthesis of resin-bound inhibitors, organic reactions were tested on PEGA resins with a poor result. The relatively high incidence of secondary amide bonds in the amide part of the polymer interfered with reactions involving carbon and carbenium ions.

Therefore, an effort was made to design a polymer using the same concept, which would still be polar but chemically more inert. This was achieved in three ways.

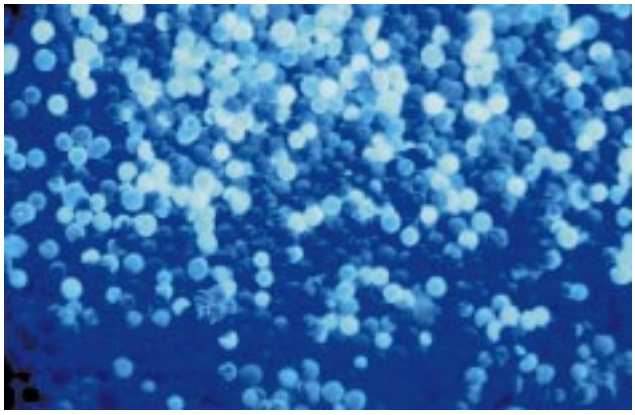


Figure 11. Beaded PEGA resin containing a library of fluorescent quenched substrates (Y(NO_2)/Abz) after hydrolysis with a dilute solution of trypsin.

A similar partial derivatization of HO−PEG−OH (1500) with vinyl phenyl methyl chloride or vinyl phenyl propyl chloride gave, after inverse suspension radical polymerization, two new polymers with properties very different from grafted PS−PEG resins.[106,107] As seen by MAS NMR, the PEG-based polymer contained relatively short PS chains (5−10% of the polymer) with only minor influence on the overall character of the resin.[108] The former resin was not stable to Lewis acids due to the benzylic linkage between the PEG and the PS while the latter resin was very stable, even to harsh reaction conditions, and performed well in synthesis. However, the synthesis of the starting vinyl phenyl propyl chloride was not trivial, and even the small amount of PS present in this polymer slightly decreased the favorable swelling observed for PEGA resins in polar solvents and buffers.

A second possibility to increase chemical stability and obtain an inert resin was to introduce only ether bonds in the polymer network, and this was first achieved by anion-catalyzed polymerization of PEG, partially derivatized with chloromethyloxirane.[109] Catalyzed by powdered potassium *tert*-butoxide at high temperature, the reaction was carried out as bulk polymerization requiring long curing. The resulting polyoxyethylene cross-linked polyoxypropylene (POEPOP) polymer was swelled and granulated through a sieve. Fairly uniform 500 μm particles suitable for library synthesis were obtained.[110,111] The resin was mechanically very robust with a relatively high loading of primary and secondary alcohols as functional groups. Organic reactions such as glycosylations, Horner−Wathsworth−Emmons, nitroaldol, and Sakurai reactions all gave a quantitative conversion on this new resin. It also presents favorable swelling in aqueous buffer and was as permeable to enzymes as the PEGA resin. Furthermore, the superior behavior of these resins in MAS solid-phase NMR spectroscopy yields spectra comparable to those obtained in solution, and complete structural elucidation of complex molecules attached to single beads was facilitated.[112] However, like benzylic linkages in PS−PEG, although less pronounced, the presence of secondary ether bonds at the central carbon atoms of the polyoxypropylene units yield a polymer which is not entirely stable to strong Lewis acids. Thus treatment with

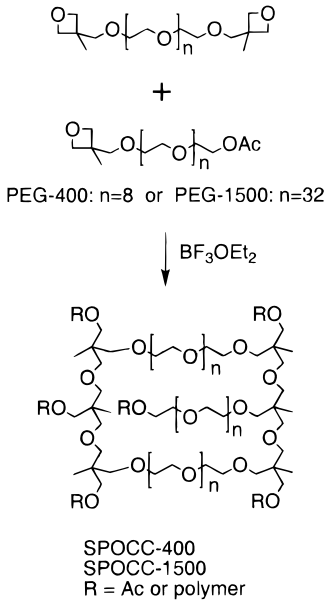


Figure 12. The polar polymer matrix of the SPOCC resin contains only primary ether and alcohol bonds in addition to the secondary and quaternary CC- and CH-bonds.

TMSOTf/acetic anhydride eventually dissolves the resin, and it is quite unstable to 35% HBr in acetic acid.[113]

Therefore, to improve the resin further, the SPOCC resin was developed (Figure 12).[114]

This resin is quite similar to the POEPOP resin. However, it contains no tertiary carbon atoms, and all ether bonds and functional alcohol groups are primary. The macromonomers are obtained under homogeneous conditions through simple quantitative alkylation of PEG chains with 3-methyl-oxetan-3-yl-methyl groups effected by a strong soluble base. After reprecipitation of the macromonomers, the bulk polymerization of the oxetanyl groups (PEG-1500) effected by Lewis acid under optimized conditions yielded a resin swelling of 6−9 mL/g in most solvents, ideal for synthesis. The resin was granulated and sieved to yield uniformly sized irregular particles suitable for library synthesis. However, for libraries, beaded resins are usually preferred and a novel method of beading polymers, which can be obtained only under cation- or anion-catalyzed reaction conditions, was therefore developed. Small droplets of macromonomer/catalyst solution were slowly added to a stirred silicon oil container. Zero gravitation conditions gave the beaded polymer shown in Figure 13. Excellent, high-resolution MAS NMR spectra of the SPOCC polymer showed some heterogeneity of oxetane-derived CH_2 groups. This may be ascribed to the rather short chains of polyoxetane obtained during the polymerization.

Diffusion studies using confocal fluorescence microscope techniques on PS-based and PEG-based resins, respectively, show that small molecules diffused into beads in seconds (i.e., not rate limiting) and in DMF diffusion was somewhat faster in macroporous polystyrene and Tentagel than in PEG-based resins. Macromolecules diffused with fast rates in the PEG-based resins and more slowly in Tentagel. In macroporous polystyrene the protein precipitated on the surface of the pores; however, slowly it could be observed in the interior, but most probably in its denaturated state.

In conclusion, a range of novel polymers has been

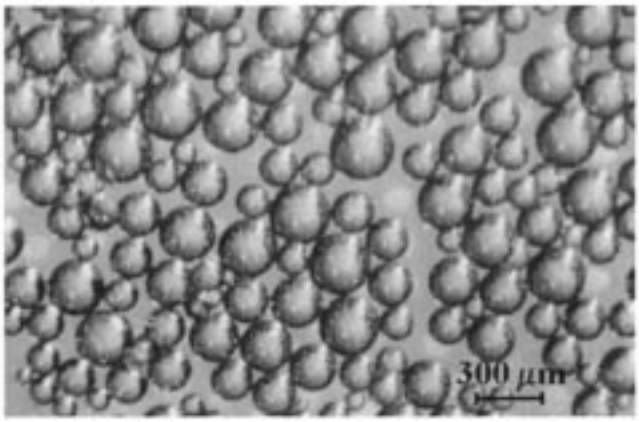


Figure 13. Beaded SPOCC resin obtained through Lewis acid-catalyzed suspension polymerization in silicon oil.

developed from easily available low-cost starting materials, and the simple process of partial derivatization afforded the polymer in a single step and ready for synthesis. The PEG-based resins have significantly enhanced mobility of polymer chains as determined by T1 measurements in NMR spectroscopy and high-resolution MAS NMR spectra of compounds on single beads may be obtained. The resins are all suitable for solid-phase enzyme assays as demonstrated with proteolytic enzymes, glycosyl transferases, and protein disulfide isomerase. PEGA supports are still the best of the PEG-based resins for peptide synthesis, mainly because of the low cost and ease of production and the fast quantitative peptide reactions observed on these resins. The most generally applicable of these polar resins is the SPOCC polymer, and in the Carlsberg Laboratory we are currently focusing efforts on the investigation of the applications of SPOCC resin for solid-phase organic combinatorial chemistry. In our opinion there is not much to improve with the SPOCC resin, although beading in silicon still is a problem on large scale.[115]

David. C. Sherrington.[116] Personal Perspectives on the Development of Solid-Phase Synthesis Supports

For my own polymer chemistry research group it is impossible to divorce our interest in, and contribution to, the development of solid-phase synthesis (SPS) supports from our wider activities involving polymer supports covering ion-exchange resins, metal ion chelating resins, polymeric sorbents, and supports for catalysts and reagents. Looking back on how this involvement developed has been fascinating for me, and not the least very rewarding, as the early ideas that I and my contemporaries had have gradually been vindicated and, indeed, have blossomed into a number of key methodologies. The development and exploitation of polymer supports continues with considerable vigor today and seems destined to form a major component of my own research activities for many years to come.

I joined the tenured academic staff at the Department of Pure and Applied Chemistry at the University of Strathclyde, Glasgow, in October 1971 having graduated with a Ph.D. in polymer physical organic chemistry from the group of Cecil Bawn and Tony Ledwith in Liverpool, England, in 1969. On my departure from Liverpool Tony had responded to my question "... what line of independent research should I pursue..." with the advice "...keep reading the literature and use your imagination...it will soon become apparent". I was recruited to Strathclyde by Alastair North who headed up a substantial polymer physics group. To keep me out of trouble Alastair asked me to take responsibility for the commissioning and operation of a new Waters AnaPrep GPC instrument. This massive machine, of which less than a handful (~3?) were ever purchased by U.K. institutions, was designed not only to carry out analytical GPC of polymers yielding $\bar{M}_w$, $\bar{M}_n$) n etc. data but, in principle, to achieve preparative fractionation of samples of up to ~25 g in mass. The core of the preparative technology was a large steel column ~3 in. diameter × 5 ft length packed with poly(styrene−divinylbenzene) Ps-DVB "gel resin". Despite great (intermittent) effort we failed to ever get the preparative facility working routinely, and during this period I became fascinated by the Ps-DVB column packing. The highly uniform resin particles prepared by suspension polymerization[117,118] had a beautiful symmetry (Figure 14) which I had not encountered before in all my earlier chemistry. GPC technology focused on macroporous Ps-DVB beads ~50−100 μm in diameter,[119−121] and following a lecture by John Knox, then the United Kingdom leading academic in liquid chromatography, I realized that we should be able to improve the performance of analytical GPC if we could produce and pack much smaller resin beads. This started my experimentation with suspension polymerization which has remained with my group since that time. What I did not realize was that the burgeoning liquid chromatography companies were already ahead of me in their thinking, and within a few short years analytical GPC columns began to shrink rapidly in size, while improving considerably the resolution offered, as the Ps-DVB resins used became smaller and of narrower particle size distribution. Frank Warner and Terry Croucher at Polymer Laboratories U.K. made a great contribution toward optimizing this analytical technique, and their company deservedly remains a world leader.

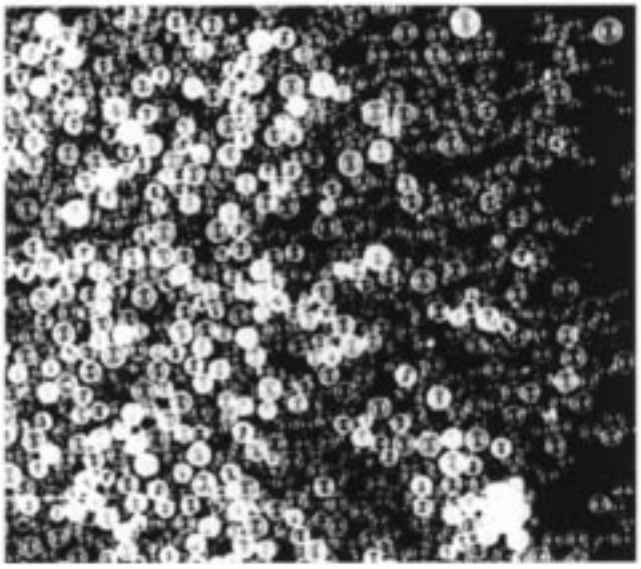


Figure 14. Photograph of polystyrene beads used for GPC and produced by suspension polymerization.

At this time my reading of the literature broadened to include the organic chemistry journals and I noticed papers

on supported phosphines from the laboratories of Joseph Castells[122] and Walter Heitz[123] and by McKinley and Rakshys.[124] These proved to be the earliest disclosures of the concept of polymer-supported reagents, and they impressed me enormously. Coincidentally, a young Australian organic chemistry colleague, George Meehan, pointed out the same papers to me, and over a coffee in the department one afternoon we agreed to throw our joint resources (one very young technician and two final year B.Sc. undergraduates!) behind a project aimed at synthesizing a Ps-DVB resin analogue of triphenylphosphine and using this as a stoichiometric reagent with CCl_4 to convert primary alcohols to primary alkyl chlorides. Our drive was largely curiosity: "could appropriate organic polymer resins be designed to have reactive functionality analogous to low molecular weight organic reagents (and later catalysts)?" The question is irrelevant today and the answer taken for granted by essentially all synthetic organic chemists. Our first paper appeared in *European Polymer Journal*[125] and together with seminal publications from Phil Hodge[126,127] represents the earliest contribution in this field from U.K. laboratories.

During this project I became aware of Professor Merrifield's work and the concept of polymer resin protecting groups and solid-phase peptide synthesis (see contribution by R. B. Merrifield, this Perspective). The collection of *benchmark papers* edited by Eric Blossey and Doug Neckers[5] was impressive, and this was probably the first book I bought as an independent academic. After I had left Liverpool, Reza Arshady and Tony Ledwith were working in a collaboration with George Kenner, helping to introduce SPPS into Kenner's peptide group and trying to improve on the then current resin technology. They introduced the first phenolic resins based on Ps-DVB,[128] and interestingly the relative lack of interest by the wider organic chemistry community meant that much of this earlier work appeared in polymer journals rather than in mainstream organic chemistry journals where it really had more relevance. Later, of course, Roger Epton considerably expanded the use of the phenolic function on the support with his acrylamido-based resins,[129] leading eventually to his ultrahigh-load method of gel-phase peptide synthesis.[130] Roger also demonstrated how powerful ^{13}C NMR spectroscopy could be for monitoring reactions on highly swollen gel type supports,[131] and in many respects he had led the way in the now more widespread use of this tool.[132] It was 1977 when I really became aware of the great debate that had been raging between the solution synthesizers and the users of SPPS methods. The latter seemed destined to win out as far as I could see. I visited Bob Sheppard and Eric Atherton at the Medical Research Council (MRC) Laboratories at Cambridge, and Bob explained his philosophy of utilizing a resin backbone which unlike Ps-DVB would have solvating requirements, and response to different solvents, similar to that which the growing peptide in SPPS would have. This all made great sense to me as a polymer chemist, and coupled with Sheppard's orthogonal protection strategy,[133] it seemed to me that the demise of PS-DVB in SPPS was imminent. However, the widespread move toward automation was now in progress. Though Merrifield had described his own instrument in 1966[132] and this seemed to be the sensible way forward, there was significant delay in the more widespread adoption of the SPPS instruments being made available. Sheppard's (and indeed Epton's) acrylamide-based supports expanded well in solvents such as DMF and so tended to be compressible when used in columns under continuous flow conditions in automatic synthesizers. Sheppard accepted this weakness very early on and devised his Kieselguhr-polydimethylacrylamide composite resin (loc. cit.) to provide the necessary balance of mechanical and chemical properties for use under pressure. My discussions with Bob focused on this issue and led to a joint project funded by the MRC. I was charged with producing a Ps-DVB resin or equivalent mechanically stable polymer particulate, grafted with chains of a second polymer which was more physicochemically compatible with oligopeptides. Our naïve view at that time was that rather short chains grafted to the external surface of Ps-DVB beads or other particles would serve the purpose. Sam Kingston joined my group in 1979 as my first postdoctoral fellow and established a procedure for the ^{60}Co γ-ray grafting of poly(acryloyl sarcosine methyl ester) onto inert polyethylene and polypropylene particulates.[135] We also succeeded in introducing a polyamide secondary network into macroporous poly(styrene-DVB) resins. Unfortunately our funding ran out before the materials could be properly assessed in continuous SPPS synthesis, but all the mechanical and solvation properties looked good. Likewise some rigid macroporous poly(*N*,*N*-dimethyl-*p*-vinylbenzamide) resins were produced[136] and held out good promise, but were never adequately screened.

Fortunately at this time my group was joined by Ahmed Akelah on sabbatical leave from Tanta University. Ahmed proved to be a very hardworking and stimulating scientist. We got on very easily with each other and my wife and I grew to know his family well. Phil Hodge and I were embarked on the editing of our first book on supported chemistry[137] but Ahmed convinced me that there was also a good case for producing a different style of review, concentrating all the known literature on polymer-supported reagents, catalysts, and protecting groups together in a tabulated form. Out of this came our *Chemical Review*,[3,138] for which Ahmed did most of the spade work. I remain very grateful to him for his efforts.

With peptide chemists increasingly focusing on the solid-phase approach, somewhat of a lull appeared in resin development as one camp homed in on Ps-DVB/Merrifield methodology and the other on *N*,*N*-dimethylacrylamide/Sheppard methodology. For our part we focused our efforts on chelating ion-exchange resins and supported reagents and catalysts, increasingly convinced of their enormous potential and yet finding it hard to convince industrialists and indeed academic colleagues of their value. Funding for such work in the United Kingdom (and indeed elsewhere) was difficult to win—but behind the scenes the environmental lobby was starting to assemble!

By the early 1980s I was ready for some new stimulation. My worldwide colleagues in the supported reagent/catalyst field (Jean Frechet, Phil Hodge, George Gelbard, Alain Guyot, Ger Challa, Abraham Patchornik, Joseph Castells, Warren Ford, and many other good friends) had done a great

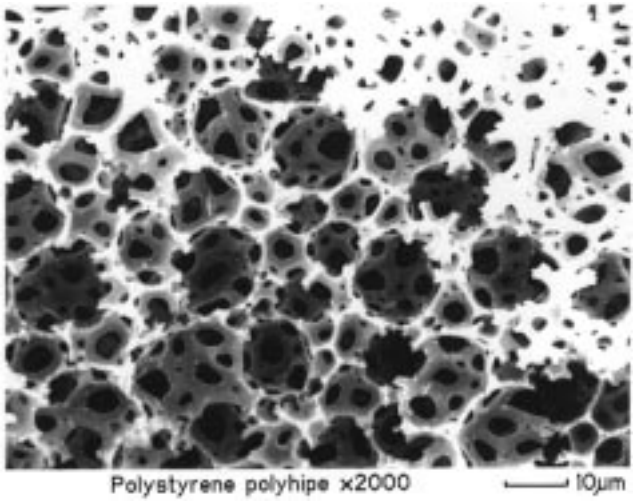


Figure 15. Photomicrograph of pore structure of polyHIPE base resin.

job in demonstrating feasibility and applicability, but the chemical world was still not ready for heterogenized systems. Consequently when Unilever Research invited me to join their research laboratories at Port Sunlight in the United Kingdom in 1984, I was delighted to do so. My knowledge and experience were broadened enormously in the three years I spent in the Port Sunlight lab, and I still retain interactive contact with some of the good friends I made during that period. I was given an open-ended remit to design novel polymers to deliver new benefits in the company's detergent and personal products. I also got to interact with most of the company's chemicals businesses. When I arrived at the Port Sunlight lab, the highly porous macrocellular Ps-DVB material known as PolyHIPE (Figure 15) had already been discovered and the material and its unique properties were well protected by patents.[139] A very large group was exploring many hundreds of ideas thrown up as potential products and application lines. Within a number of areas of interest to the company it was becoming clear that carefully designed oligopeptides, and indeed other stepwise tailored oligomers, produced rapidly by automated SPPS and SPS could be of great value. I therefore recruited Phil Small from Roger Epton's group, and Phil very quickly acquired, commissioned, and started to exploit a peptide synthesizer. Both he and I were still unhappy with the balance of physical and chemical properties offered by existing supports, and our management was keen to see greater productivity (i.e., resin loading) from the synthesizer. Together we realized that PolyHIPE particles offered a great opportunity for developing a high-load composite support, but initially we felt the management would not look kindly on our spending effort on improving the support per se. Fortunately, high-volume, low-value-added uses for PolyHIPE were proving elusive to pin down, and our imaginative boss responded positively to our idea of a low-volume, high-value-added outlet for the material. Phil soon demonstrated that Bob Sheppard's soft resin could indeed be located within the rigid protective macrocells of PolyHIPE and, furthermore, that a high-load of synthesis sites could be incorporated. We were quickly able to demonstrate high load capacity and efficiency in automated test oligopeptide syntheses and the PolyHIPE composite was protected by patent.[138139] It soon became clear that the existing manufacturing facilities and management structure of the company's many operating enterprises were not appropriate for commercializing PolyHIPE and products such as the SPPS composite which were likely to flow from it. Accordingly, therefore, a new entrepreneurial company, Microporous Materials, was established under the management of Jim Marshall to be based within the manufacturing facility of Unilever's newly acquired polymer company National Starch in Bridgewater, U.S. Don Gregory and Phil Small from the Unilever laboratory were seconded to the new company initially to produce and market the composite and also to explore other outlets for PolyHIPE monoliths, largely in the membrane and filtration areas. With the dominance of Ps-DVB/Merrifield methodology in the United States, and despite the practical drawbacks of this, establishing a sales foothold rapidly for the PolyHIPE composite proved very difficult, and after a couple of years the operation was moved back to the United Kingdom with the involvement of Phase Separations, Deeside, U.K. We continue to use it in our synthesizer at Strathclyde for producing materials-orientated oligopeptides, and until recently it was still available via Novabiochem.

I was given permission from the Unilever management to make the first public disclosure of the PolyHIPE composite in 1989 at the Oxford, U.K., conference on Solid Phase Synthesis organized by Roger Epton.[142] Frankly, I was rather nervous of the intimate relationships between some academics and some industrialists at that meeting, although in the field of SPPS, and indeed now in SPS more widely, the lapse time between academic discovery and commercial exploitation is so short that it is understandable that academics and industrials may easily become bedfellows. Although I did not recognize it at that time my disclosure of the PolyHIPE composite was accompanied by one which was to have much broader and long-lived relevance. At that meeting I listened to Wolfgang Rapp's description[143] of the PEG grafted Ps-DVB resin he had developed while studying under Ernst Bayer at the University of Tübingen.[144,145] This reminded me very much of our 1983 efforts to graft poly(*N*,*N*-dimethylacrylamide) chains onto polymer particulates but, whereas we ran out of steam, Bayer and Rapp were able to produce a brilliantly optimized resin with reportedly excellent physical and chemical characteristics for SPPS. I am not sure if they realized at that time what an impact their resin was to have in the wider area of SPS, but I suspect not. My impression then was that the whole area of combinatorial synthesis was still gathering momentum behind the development of rapid screening technologies within the pharmaceutical and related industries. The bottleneck in drug discovery programs was about to shift to the organic synthesis step, and together with the imminent demand for enormous structural diversity, rapid combinatorial synthesis methods were about to burst onto the scene.

The initial targets for large libraries from SPS were oligopeptides, for which existing supports had been optimized. However, as demand came to generalize combinatorial synthesis to include all facets of organic synthetic

methodology, some supports were clearly totally unsuitable. Ironically perhaps, Merrifield's lightly cross-linked, lightly chloromethylated, PS-DVB gel type resin displayed remarkable versatility, and new sources for these species started to spring up. This simple polystyrene-based resin, however, has one major drawback in that it will not swell in lower alcohols, water, or aliphatic hydrocarbons, and so a wealth of organic chemistry cannot be exploited in combinatorial chemistry on this support. Enter now the Bayer/Rapp resin again (and its mimics) with its remarkable ability to allow reactions to be performed on it in solvents as diverse as water and toluene!

On my return from Unilever to academia in October 1987 I was appointed full professor and asked to head the Organic Chemistry Section. This was a great honor for a mere polymer chemist, and I remain deeply grateful to my organic chemistry colleagues for the welcome and encouragement they gave me. It also made me realize that my chemistry heart was in organic synthesis and, in particular, the marrying of organic and polymer synthesis. Without being disrespectful to my gay friends, I have described this as me "coming out of my chemical closet"!

In parallel with the emergence of combinatorial SPS, in all its forms, the environmental (green) lobby I hinted at earlier has also come to the forefront and in the long run may arguably be a greater technology driver than library synthesis. As a result, funding resources for our own work on clean polymer-supported catalysts have increased substantially. Not only have we been able to carry out a great deal of fundamental work on the molecular structural and morphological aspects of key resins such as the Ps-DVB systems,[146−149] but we have also had the freedom and encouragement to develop entirely new supports and in particular spherical particulate species based on highly thermo-oxidatively stable species such as polysiloxanes,[150] polyimides,[151] and most notably polybenzimidazole.[152] The research group has developed a number of very effective polymer-supported metal complex alkene epoxidation catalysts for use in both the production of commodity epoxides,[153] and speciality chiral epoxides.[154,155] Currently we have active programs involving polymer immobilized Pt-, Pd-, Rh-, and Mn-based catalysts, as well as a renewed activity in novel resins for use in selective metal ion recovery.

Quite remarkably for my group the fields of solid-phase combinatorial chemistry and immobilized catalysts, reagents, and scavengers, have now converged. The difficulty of achieving good molecular structural characterization of molecules assembled on a support has been a constant frustration for classical synthetic organic chemists who need a high-quality high-resolution solution-phase ^{1}H NMR spectrum of a molecule before they are confident they have the correct species with adequate purity. Despite recent advances such as microscopic single-bead FTIR spectroscopy and gel-phase magic angle spinning ^{1}H NMR spectroscopy, this uncertainty remains. Turning the SPS methodology on its head solves this problem at a stroke. With such "inverse" solid-phase synthesis (ISPS), molecules are assembled in solution with all the reagents, catalysts, and scavengers heterogenized on polymers or other solid supports. At each step, therefore, in a synthesis, a sample of solution can be analyzed using conventional solution-phase techniques. Interestingly, in the very early days of SPPS Abraham Patchornik's group at the Weizmann Institute devised such an inverse methodology for oligopeptide sysntheses.[156] The level of what can be achieved in ISPS has been demonstrated very remarkably recently by Steve Ley's group at Cambridge, U.K.[157,158]

Figure 16. Photograph of 2 mm × 10 mm monolithic polystyrene disks for solid-phase synthesis applications. Shown on the left is a single disk fully swollen in toluene. To the right are a small stack of disks, composition 97% styrene, 3% DVB; note 25 wt % toluene was present in the polymerization to keep the monolith soft enough for cutting.

Currently we have a program in progress devising new formats for solvent versatile SPS supports. We have in press a report on rod and disk formats which can be prepared simply and cheaply in any organic chemistry laboratory without the need to tackle the black art of suspension polymerization.[159] Disks can be produced readily (∼2 mm thick and 10 mm diameter) with a functional group loading capable of yielding ∼1.0 mmol of compound per disk (Figure 16). Each macroscopic disk can be readily manipulated manually or robotically. We have also devised novel anion-exchange resin beads which can be degraded and dissolved at various pHs when required, potentially to release recovered species which have proved difficult to elute.[160] Other SPS support developments will be reported in due course. Marrying these support developments with our activity in polymer-supported catalysts potentially for use in ISPS and elsewhere is now a task upon which we are urgently engaged. We hope that some of these efforts will, in due course, prove of value in SPS and in ISPS, making a contribution both to the discovery of new "active" molecules and to the operation of chemical processes in a cleaner and less wasteful manner.

How will ISPS fair against SPS in a combinatorial chemistry context? The technique will certainly become more widely used as the range of resin-supported reagents, catalysts, and scavengers available commercially grows steadily. In many laboratories both methodologies seem destined to be used in parallel depending on the target synthesis. This may well have a profound effect on those automatic and robotic technologies that will survive, with those being adaptable for use in ISPS as well as SPS being considerably favored. This suggests that polymer resin-based technologies have a big edge, and those more exotic support formats that have been described, and some brought to the

market place, may well gradually disappear as a passing phase! The ISPS approach also offers considerable scope outside the limited area of, say, drug discovery and catalyst development programs. There has been much discussion of extending the use of SPS in the scale-up and indeed production of target molecules. Generally this has been viewed rather negatively because of the inherent inefficiencies of SPS, particularly the problems of slow and/or incomplete reactions. As in SPPS, these problems tend to give rise to impure products and the need for (extensive) downstream purification of cleaved products. With ISPS these effects will, in general, either be absent or of much lower significance, and so the extension of ISPS into scale-up and production stages looks more realistic. Bearing in mind that ISPS also allows the "greening" of many reagents and catalysts, the use of which is increasingly been frowned upon and in some instances legislated against, the case for the ISPS approach seems very strong indeed. In any event, the future for supported synthesis seems rosy indeed.

None of the developments I have described from my own laboratory would have been possible without the hard work and commitment of my young co-workers over the last 25 years; I am much indebted to all of them. Likewise to the more mature contemporaries with whom I have collaborated and indeed with whom have become good friends, I express my sincere thanks.

Derek Hudson. Interim Conclusions

We have seen how, after the seminal contribution of Merrifield, others have attempted to improve on his selection of low-cross-linked gel PS beads as the matrix for synthetic transformations. These contributions, I believe, were made without knowledge of the many alternatives that Merrifield himself explored, before settling on his selection. Would progress have been any different if these preliminaries had been common knowledge? In part II we will explore further ideas to improve or modify Merrifield's concept and expand to an even wider variety of applications. Of course, part II will conclude by trying to bring all this into perspective (as is only right for such an article).

Acknowledgment. I must take this opportunity, again, to thank my coauthors. It has been especially gratifying for me to see that all of them did, actually, enjoy the processes of recollection and analysis involved. I, too, have found the exercise rewarding. Our thanks are, therefore, due to Tony Czarnik for getting this idea started. I, additionally, would like to thank Michael Songster, at Biosearch Technologies, who simultaneously educated me in the technique of electronically transmitting and unstuffing words and images and helped reformat all of this into one, more or less consistent whole while juggling many other demands at the same time.

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(2) D.H. comments, "One of the contributors to this masterpiece clearly believes my most important contributions have been to supply apposite, nay, masterful, names and acronyms for resins, linkers, and apparatus. When the question discussed is solely that of performing organic chemistry transformations for the purposes of pharmaceutical development, on a solid-support, then the acronym SPOS is used."
(3) D.H. comments, "One such direction is the use of soluble polymers; however, none of the principle protagonists of this approach were swayed by my entreaties to participate. But the MAST variation in which reagents are immobilized on the matrix has been covered, in a minor masterpiece, by David Sherrington. Note, too, that his early review on the subject, ref 138, has been extremely influential and certainly deserves the title 'classic paper'."
(4) See references cited in ref 1; also the entire contents of issue 5 (Comprehensive Reports and Reviews, Issue Editor, Kates, S. A.) of *Pept. Sci.* **1998**, *47*.
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(14) D.H. comments, "Please refer to Geoff Tregear, in part II of this Perspective, scheduled to appear in issue 6 of *J. Comb. Chem.*"
(15) D.H. notes, "This passage is quoted directly from a personal letter to me, dated Jan 21, 1999."
(16) D.H. comments, "This effect has been much used in the synthesis of cyclic peptides and, in addition, has been used for a variety of organic transformations: Ford, W. T. in *Polymeric Reagents and Catalysts*; ACS Symposium Series 308; American Chemical Society: Washington, DC, 1986."
(17) D.H. notes, "Supplied by Bill Reich, of Lab Systems Inc., who was in many ways a pioneer of today's biotechnology supply industry. Bill had a clear vision of the commercial future of solid-phase processes and an endearing personal approach (he unfailingly addressed me in the vein 'Well, young man, ...'). He died quite recently, from pancreatic cancer. His inheritance, some very fine polystyrene, now resides in Biosearch Technologies inventories and is still some of the best material available commercially."
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