JOURNAL OF combinatorial CHEMISTRY

Review

Solid-Phase Organic Synthesis: A Critical Understanding of the Resin

Andrew R. Vaino, and Kim D. Janda

J. Comb. Chem., **2000**, 2 (6), 579-596• DOI: 10.1021/cc000046o • Publication Date (Web): 25 October 2000

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Reviews

Solid-Phase Organic Synthesis: A Critical Understanding of the Resin[†]

Andrew R. Vaino and Kim D. Janda*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute,
10550 North Torrey Pines Road, La Jolla, California 92037

Received June 9, 2000

Introduction

Merrifield's 1963[1] contribution to science served to foment, decades later, an explosion in the field of solid-phase organic synthesis (SPOS).[2] Merrifield envisioned that anchoring peptides to insoluble supports should greatly improve peptide synthesis. With the desired molecule attached to an easily sequestered solid, purification becomes a trivial matter of simply cleaving the molecule from the support and removing the support from solution. While the idea sounds simple enough in hindsight, the actual implementation of the first solid-phase synthesis of a tetrapeptide required much effort.[3] With time, the idea of solid-phase organic synthesis has evolved from a flight-of-fancy to the present day where peptide synthesis has been reduced to an automated task. Recent advances in the field of small molecule combinatorial chemistry[4] have led to a dramatic growth in the use of solid-phase resins.

Literature reports of SPOS overwhelmingly depict resins used in reactions as little spheres with molecules appended to them. In an insightful 1998 monologue, Czarnik[5] challenged chemists with the notion that simply denoting the solid-phase resin as an opaque sphere is tantamount to omitting the solvent used in the reaction, a grave omission. Swollen cross-linked polymers are the solvents in which solid-phase organic reactions are performed. In the same sense that a thorough understanding of the properties of traditional solvents is essential for the design and implementation of traditional solution-phase chemistry,[6] a thorough understanding of the properties of the swollen polymeric matrix, the de facto solvent, is critical for the implementation of SPOS reactions. The main purpose of this review is to demonstrate the parallels between traditional solution-phase and swollen gel-phase solvents.

A surfeit of reviews devoted to resins employed in SPOS has appeared,[7] together with some reviews devoted to the physical properties of resins.[8] The present review will focus on physical aspects of the resins used for SPOS. In particular, an emphasis is placed on the kinetic motion of the polymer backbone and effects on this motion caused by changes in solvation. In solution, solvated molecules are able to move about and collide with one another. If the geometry and electrostatics of one of these collisions fulfills certain requirements imposed by the nature of the species involved, a new chemical bond is formed and a reaction is said to have occurred. The role of the solvent in this case is simply to permit these collisions to occur (by providing a kinetic milieu for the molecules involved) and to provide an electrostatic environment amenable to the reaction polarity. That swollen cross-linked polymers are the solvents in which SPOS reactions are performed will be demonstrated by depiction of techniques used to probe the interaction between cross-linked polymer, solution phase, and both bound and unbound molecules.

The polymeric support used by Merrifield, polystyrene cross-linked with divinyl benzene having pendant chloromethyl groups as points of attachment, has come to be known simply as Merrifield resin (MR), and is, to this day, the most commonly employed resin.[9] The structure of MR is not as simple as the structure of traditional liquid solvents, for example ethyl acetate. Thus, this review begins with a discussion of the inhomogeneities associated with MR and their origins. This will be followed by a brief discussion on the physical method used to prepare resins for SPOS, namely, suspension polymerization. Justifiably or not, the physical property of swelling is considered to be of the utmost importance in dealing with solid-phase resins. Clearly, the ability of a cross-linked polymer to take up solvents is of paramount importance. A discussion on swelling, including means to predict swelling ability based on thermodynamic solubility parameters, is included together with the effects of swelling on the polymer matrix itself. Polymer chemists have for decades realized that adding, or grafting, molecules off the main chains of macromolecules exerts a pronounced effect on the physical properties of the polymer. It is no surprise, then, that the same changes are observed by the addition of reagents to solid-phase resins, and a discussion addressing this is included.

A variety of spectroscopic techniques has been applied to the study of cross-linked polymers, each offering a different view of the matrix in question. The use of electron paramagnetic resonance, nuclear magnetic resonance, and fluorescence spectroscopies has found particular application to the study of the structures of solid-phase resins and their

---

[†] Dedicated to Professor Robert B. Bates on the occasion of his retirement.

10.1021/cc000046o CCC: $19.00

interaction with the solution phase. Infrared spectroscopy has been applied sparingly to this topic, but has come to prominence for monitoring the progress of solid-phase reactions. Cross-linked polymers are highly complex systems. Application of advanced spectroscopic techniques allows observation of certain aspects critical to the success of reactions on the solid phase, for example motion of the polymeric matrix. However, spectroscopy is still unable to answer all questions of relevance to the performance of the resin, for example, the average distance between nodal points or the actual degree of cross-linking.[10]

Solvents impact kinetics of reactions, site−site interactions, as well as reagent partitioning. Discussions of swollen resins on these areas are also included. While much effort has been devoted to so-called "on-bead" monitoring of solid-phase reactions, this area will only be mentioned in passing, and is a worthy topic for a review in itself.[11]

Cross-Linked Polystyrene

With very few exceptions polystyrene cross-linked with divinylbenzene is the polymeric support used in solid-phase organic synthesis.[9] Inhomogeneity in cross-linked polystyrene (CLPS) has long been known to hamper synthetic efforts.[12] Many reports have described in detail the kinetics of the copolymerization of styrene and divinylbenzene (DVB).[13] Owing to the lack of commercial availability of either of the pure isomers, *meta*- or *para*-divinylbenzene,[14] the synthesis of CLPS is performed with five different monomers, namely, *meta*- and *para*-divinylbenzenes, *meta*- and *para*-ethylstyrenes (present as impurities in commercial DVB), and styrene itself. If chloromethyl groups are to be included by copolymerization with vinylbenzyl chloride, often used as a mixture of the *meta*- and *para*- isomers, seven different monomers are now introduced into the reaction mixture. The main source of inhomogeneity of cross-linked polystyrene lies in the two different cross-linkers used, each having different reaction rates.[15] In general, the first vinyl group of the *meta* isomer reacts faster with styrene than does the first vinyl group of the *para* isomer. Data on the polymerization of pure *meta* and *para* isomers reveals that gelation of the *meta* isomer occurs before gelation of the *para* isomer. With the first vinyl unit incorporated, the pendant vinyl group reacts at a rate analogous to isopropylstyrene, which has been demonstrated to react more slowly than styrene.[16]

In copolymerization between both isomers of DVB and styrene, *para*-divinyl benzene reacts preferentially with itself such that *para*-divinyl benzene is more rapidly consumed from the reaction mixture. As such, most of the *para*-DVB is found in dense bunches. *meta*-Divinyl benzene does react more rapidly than styrene, but is better able to react with styrene and tends to be evenly incorporated into the polymer, resulting in evenly spaced cross-links. Thus, two distinct regimes are present in CLPS. Regions formed by reaction between *para*-divinyl benzene and styrene tend to be composed of dense regions, rich in cross-linker, strung together by long strands of styrene. Regions formed by reaction between *meta*-divinyl benzene and styrene tend to be more homogeneous, that is, the cross-linker and styrene are more evenly interspersed. Existence of different regions are further supported by pyrolysis data for CLPS prepared from pure *meta* and *para*-DVB which demonstrated that the rate of loss of *meta*-divinyl benzene from the polymer is greater than for *para*-divinylbenzene products.[17] This was correlated with clustering of the *para* units, letting them occupy less heat sensitive regions of the matrix.

Further sources of inhomogeneity of resins commonly used in SPOS arise from treatment of the cross-linked polymer after synthesis. Solvents used to remove unreacted monomers from the newly formed resin have a profound impact on pore size. Polymers dried from poor solvents show maximum porosity, while polymers dried from good solvents show collapsed pores upon drying.[18]

Suspension Polymerization

Suspension polymerization (often referred to as a "black art"[19]) is the preferred mode of formation of resins for SPOS. While bulk polymerization followed by grinding and sieving of the polymer is an easier means to create resins, the near perfect spheres obtained by suspension polymerization are not achieved. Suspension polymerization comprises a two-phase system wherein the monomers and porogen (solvent for monomer mixture) exist in one layer, for CLPS formation an organic solvent, suspended in an excess of nonsolvent; for CLPS formation the nonsolvent is water. For water soluble polymers, for example, polyacrylamides, the nature of the phases is reversed. The most important feature of suspension polymerization is the formation of stable droplets of monomer suspended in the nonsolvent phase. Achieving reproducible droplet formation is most effectively accomplished with mechanical stirring. The shape and speed of paddle, together with shape of the reaction vessel, determine the size and quality of beads obtained by influencing the steady-state formation of these droplets. The nonsolvent phase may be thought of as providing millions of tiny "spherical molds" in which the polymer beads are formed constrained by surface tension. Stabilizers, typically a mixture of simple inorganic salts together with large, polar organic species, such as acacia gum or poly(vinyl alcohol), are added to the mixture to help reduce the surface tension of the droplets. This stabilization of the droplets prevents aggregation of the "molds" which leads to misshapen beads.[20]

Phase separation of the nascent cross-linked polymer is greatly affected by the nature of the organic solvent (or porogen) used. Poor solvents tend to result in earlier phase separation which may lead to shorter polymer chains and less actual cross-linking. As well, the nature of the porogen affects porosity of the polymer, which affects its morphology and can lead to heterogeneous swelling.[21]

Slower rates of stirring afford beads having greater diameters.[22] However, rates too low may not be sufficient to establish steady-state droplet size. There are limits to the control over particle size. The dependence of particle size on stirring rate requires increased amount of droplet stabilizer; in its absence, smaller drops coalesce earlier, producing large and odd shaped beads. In principle it is possible to control bead sizes in the range 0.2−2000 μm; in reality,

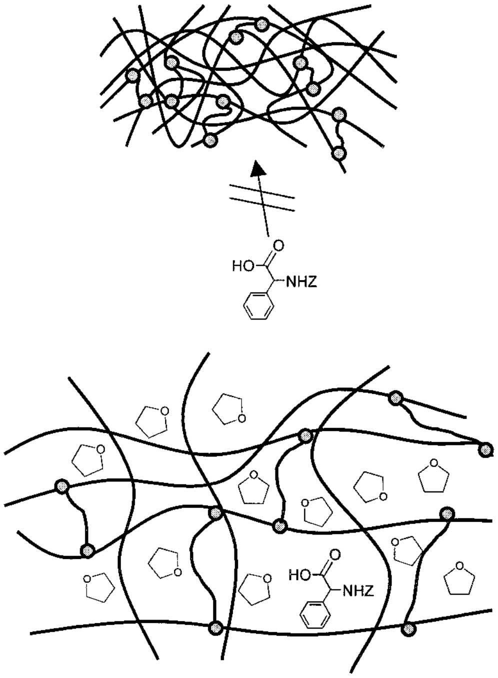

Figure 1. Cartoon depicting ability of compounds to enter nonswollen (top) vs swollen (bottom) cross-linked polymers.

production of beads having diameters smaller than 20 μm is challenging, although it has been reported.[23]

Swelling

Swelling of a cross-linked polymer is equivalent to solvation of a linear polymer.[24] Instead of dissolving and forming a solution the cross-links act as anchors to prevent excessive motion of the polymer chains required to form a solution. As a result, solvent is taken up by the cross-linked polymer resulting in an increase in volume as solvent molecules occupy positions between polymer chains (Figure 1). In most organic chemists' minds the dominant criteria for a solid-phase resin is the swelling of the reaction matrix. Swelling of a polymer resin is a prerequisite for any reaction to occur within a permeable polymeric support. A cross-linked polymer[25] that does not swell when imbued in solvent provides little opportunity for reagents to interact, precluding reaction. It must be noted, however, that swelling is not in itself a panacea. As the polymer swells, it now assumes the role traditionally associated with the solvent; that is, the swollen polymer *is* the solvent, with the important distinction of having a substantially greater viscosity.

Kinetics of reactions on polymer supports (vide supra) has demonstrated that even though one polymer may swell to a greater extent than another, rates of reaction do not necessarily reflect swelling. Merrifield realized early on that most (>99%) of sites of attachment of solid-phase resins are *inside* the swollen beads.[26] Indeed, this is self-evident simply by comparison of the surface area-to-volume ratio for a sphere. An interesting report contrary to this notion has appeared concerning the distribution of functional groups through different matrixes commonly employed for SPOS.[27] A fluorescent rhodamine dye was affixed to the aminomethyl functionalized ends of either Agrogel, controlled-pore glass (CPG), CLPS, or TentaGel (TG).[28] Optical analysis, which detects the fluorophore's emission directly from the point source together with emission from a diffraction pattern originating from inside the bead, was used to determine the degree of substitution throughout the bead. Essentially this technique allows visualization of slices of the bead. Treatment of the data from the diffraction pattern to a mathematical algorithm permits assignment of the source of emission. Interestingly, and contrary to conventional wisdom, it was determined that even distribution of the rhodamine dye throughout the bead was only observed for the CPG and macroporous resin. For the two supposedly swellable polymers, little interpenetration of the dye was noted. It is unclear, then, how reported loadings of CLPS type resins, measured for example by cleavage of terminal Fmoc groups, can be obtained as high as they are if only a small portion of the bead, that near the surface, is loaded. The above finding is in stark contrast both to conventional wisdom and to observation of β particle emission from the interior of resin beads doped with ^{3}H. Audioradiograph experiments with Boc-[^{3}H]Val-(LAGV-OMPA)$_6$ attached to CLPS unambiguously demonstrated incorporation of amino acid *into* CLPS after careful slicing of the bead.[47]

The driving force for swelling arises from contributions from entropy and enthalpy changes associated with mixing of solvent and solute together with changes in configurational entropy due to dilution of flexible chains.[29] Differences due to cross-linking include a decrease in configurational entropy of polymer chains held in the network resulting from the chains being forced to assume more elongated, less readily attainable, configurations as the network expands. Thus, increasing the degree of cross-linking affects the degree of configurational entropy dissipated, rendering swelling more difficult. The thermodynamics of mixing (swelling in the case of a cross-linked polymer) of a polymer with a solvent is governed primarily by the enthalpy of mixing which varies as the difference in molar attractiveness between solute and solvent.[30] For a cross-linked polymer, the degree of swelling is described by the Flory−Rehner theory.[31] Here, the swelling ability of a gel in a specific solvent is estimated by comparison of the differences in Hildebrand solubility parameters (δ)[32] between polymer and solvent. The Hildebrand solubility parameter represents the heat of vaporization per milliliter of material. As polymers will degrade before vaporization occurs, δ is not conveniently measured. Values of δ can be obtained by matching solubilities of polymers with solvents of known δ. Gee studied the swelling of rubber by immersing cubes of rubber in various solvents and measuring changes in their volume over several weeks.[33] Based on observed degree of swelling, he was able to estimate δ for the cross-linked polymer based on knowledge of δ for the best-swelling solvents. For a polymer, δ is estimated from eq 1[34]

$$\delta = \rho \sum G/M \tag{1}$$

where ρ is the density of the polymer, M is the mer molecular weight of the polymer, and $\sum G$ is the sum of group molar attractivities; G is calculated based on experimentally determined values for vapor pressure and heats of vaporization. A complementary approach to the calculation of δ that

Table 1. Hildebrand Solubility Parameters for a Range of Solvents

solvent	δ (cal/mL)$^{1/2}$	solvent	δ (cal/mL)$^{1/2}$
acetone	9.9	ethyl acetate	9.1
acetonitrile	11.9	ethyl ether	7.4
benzene	9.2	ethyl alcohol	12.7
chloroform	9.3	n-hexane	7.3
DMF	12.1	THF	9.1
DMSO	12.0	toluene	8.9
1,4-dioxane	10.0	water	23.4

takes nonbonded interactions between elements of the polymer chain into account has also been reported.[35] This approach is particularly useful for polymers containing pendant carboxylic acids or alcohols.

While the calculation of δ as described above is not completely rigorous, it serves as a useful guide to swelling ability of cross-linked polymers. Calculation of δ for linear PS by the method of Small[34] gives a value of 9.05 (cal/mL)$^{1/2}$, remarkably close to one of the reported values. There are several examples where calculation of δ for a cross-linked polymer was found to be within 10% of experimentally determined values, for example in cross-linked polyesters,[36] in epoxy networks,[37] and in vinylester networks.[38]

Recent publications[39] concerning δ for Merrifield resin have quoted a value of 9.1 (cal/mL)$^{1/2}$ using an experimental determination of δ for linear polystyrene obtained by a turbidimetric titration.[40] In fact, this report gives two different values (9.07 and 8.72) for δ, depending on which solvents were employed in the titration. An alternate publication concerning δ for linear polystyrene provides a value of 8.56 (cal/mL)$^{1/2}$.[41] A range of 8.6−9.7 (cal/mL)$^{1/2}$ has been suggested for cross-linked polystyrene.[42]

Correlation of swelling ability of CLPS with solvents encompassing a range of δ values has been reported.[39a] In general, species are considered miscible when $|\delta_1 - \delta_2| < 1$.[43] Comparison of resin swelling to solvent δ values demonstrated that maximum solvation occurs with solvents having δ values between 9 and 10, consistent with δ for CLPS as being 8.6−9.7. As well, it was pointed out that δ values for mixed solvent systems could be estimated using a weighted average of δ values from the respective solvents. Values of δ[44] for solvents commonly employed in SPOS are presented in Table 1. Judging from the values of δ given in Table 1 it is clear that CLPS will not be swollen by solvents that are too polar, for example water, or solvents that are not sufficiently polar, for example hexane. A similar approach to the prediction of polymer swelling based on acceptor and donor numbers[45] has been reported.[46] However, the advantage of this approach over the use of the more traditional Hildebrand solubility parameters is unclear.

Many methods have been reported for determination of degree of swelling, for example, measurements of changes in volume for a single bead,[47] a syringe method,[48] and methods measuring changes in weight after centrifugation.[49] An extensive survey of the swelling properties of some of the most commonly used resins in a range of solvents has been compiled[50] and serves as a useful guide for selection of resin/solvent pairs.

A delicate balance exists between the degree of cross-linking and swelling. Reducing cross-link density causes an increase in swelling, but at a cost of mechanical stability; beads broken by agitation can be difficult to filter. Conversely, increasing cross-link density results in greater mechanical strength, but at a cost of swelling ability. If reagents cannot interact with each other in a swollen medium, no reaction will occur. The chemical nature of the cross-link can have a profound effect on mechanical strength. For example, while polystyrene cross-linked with DVB is readily pulverized by magnetic stirring, polystyrene cross-linked with bis(vinylbenzyl)oligoethylene glycol units ($n = 3$) results in beads that readily withstand magnetic stirring.[51]

Glass Transition Temperature

The glass transition temperature (T_g), the temperature at which substantial motion of the polymer chains begins, is one of the defining properties of a polymer. For amorphous linear polymers, heating above T_g allows the polymer to flow under stress. Cross-links anchoring the chains of a polymer network do not permit such motion, so a cross-linked polymer will not flow. However, above T_g substantial motion of the cross-linked polymer chain is now possible. For CLPS, a highly amorphous polymer, T_g is lowered from 110 °C in the dry state to ambient temperature upon interaction with a good solvent.[52] A good solvent for a cross-linked polymer may be considered analogous to a plasticizer. That is, the polymer chain is able to move about more freely at a lower temperature. The glass transition temperature, in simile with swelling ability, is known to be proportional to the degree of cross-linking.[53]

A study of changes in the glass transition temperature of CLPS on swelling with toluene, chloroform, DMF, and THF has been reported.[54] Rates of molecular diffusion decrease markedly upon cooling through T_g, providing a guide to the useable temperature range of the resin. Polystyrenes having 2, 6, and 10% cross-linking, respectively, were examined by differential scanning calorimetery (DSC) in sealed vessels. Interaction of the polymer with all solvents examined resulted in T_g depression. Further, a comparison of methods of the calculation of T_g based on knowledge of the T_g of the solvent and T_g of the bulk dry polymer to experimental values was performed. Four different equations for the calculation of T_g from above data were examined.[55] Only eq 2[55a] gave satisfactory agreement with experimental values:

$$\ln T_g = m_1 \ln T_{g2} + m_2 \ln T_{g2} \tag{2}$$

where T_{g1} and T_{g2} refer to glass transtion temperatures of the pure solvent and polymer and m_1 and m_2 refer to the mass fractions of the pure solvent and polymer. Experimentally determined values for T_g in toluene are presented in Table 2. As cross-linking increases and swellability decreases, T_g increases implying that a greater temperature is required to attain the same degree of backbone mobility.

Effects of Loading of the Polymer

Modification of polymeric resins, for example by adding pendant molecules or grafts, greatly affects the thermodynamic properties that give rise to swelling. To study the effect of increased substitution on the swelling ability of resins,

Table 2. Glass Transition Temperatures for MR Mixed with Toluene[a]

% DVB	T_g (K)
0	371
1	378
2	382
4	384
6	389
8	387
10	404
12	405

[a] Degree of cross-linking is noted.

resin bound peptides composed of between 1 and 12 units of Leu-Ala-Gly-Val-oxymethylphenylacetic (OMP) acid were prepared.[47] The size of the dry beads and peptide-grafted beads were measured individually using a microscope. It was noted that the diameter of a dry bead roughly doubled as the content of the peptide approached 80%. Swelling was also measured by direct observation of changes in size of individual beads using a microscope. In CH_2Cl_2 the volume of the swollen, unloaded resin was 1.84 times greater than the dry resin. Addition of peptides had a dramatic effect on solvation. As the peptide content increased, the swelling ratio in the nonpolar CH_2Cl_2 decreased. With 2.5 g of peptide per gram of resin, the swelling ratio was reduced to 1.33 for CH_2Cl_2. The opposite trend was observed for the more polar solvent DMF, where the swelling ratio increased as the amount of bound peptide residues, with greater polarity, increased. The attached peptide, now an integral part of the polymer backbone, clearly makes a substantial contribution to the physicochemical properties of the swollen gel phase. A further example of the effect of backbone grafting of cross-linked polymers on solubilizing ability is seen in a report on the swelling behavior of solid-phase resins in a range of solvents.[50] Swelling of resins with different grafts off a cross-linked polystyrene backbone was examined in a range of solvents. The grafts ranged from large PEG oligomers, TentaGel, to simple linkers attached to the starting CLPS, for example Merrifield resin (chloromethyl groups grafted), Wang resin (hydroxymethylphenyl methyl ether groups grafted), MBHA (4-methylbenhydrylamine groups grafted), Trityl resin (diphenyl chloride groups grafted), and Rink resin (*N*-Fmoc-2,4-methoxybenzhydrylamine methyl ether). The addition of these grafts (small molecules) caused definitive shifts in the swelling properties of the resins, a facet that should be borne in mind when problems arise during synthesis on resins.

The solubilizing ability of the polymer backbone has a tremendous effect on the solvation of peptides that normally would be insoluble in the same liquid phase. For example, there are cases where a resin-peptide ensemble is solvated while together, but the cleaved peptide is insoluble in the same liquid solvent.[56] This solubilizing effect further demonstrates that the swollen lightly cross-linked resin, not the bulk liquid solvent, is the de facto solvent.

Naïvely, it may seem plausible that carrying out reactions further from the polymer backbone should be beneficial due to increased mobility. An elegant experiment describing the effect of tethering reagents off of the polymer backbone has been reported.[57] The synthesis of a well-characterized peptide (Leu-Ala-Gly-Val) was performed at the end of tethers of various length from the polymer backbone. The spacers were either [Leu-Ala-Gly-Val-OMP]$_n$ or [Leu-Ala-Gly-Val]$_n$. Even with 10 OMP and 48 amino acids (a segment as polar as a PEG chain) separating the site of reaction from the polymer backbone, no appreciable difference in synthetic efficiency was observed.

Spectroscopic Techniques

Electron Paramagnetic Resonance. Electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) observe similar phenomena, the response of a dipole perturbed from its equilibrium in a magnetic field by application of radio frequency radiation. As resonance spectroscopy observes an indirect response to an excitation, it differs from forms of spectroscopy that measure responses directly, for example absorption or emission of photons. This sets resonance spectroscopies apart from the others and imparts complementary, dynamic information.

What can be gleaned from an EPR experiment is the relaxation of an unpaired electron. Nitroxides are stable sources of radical and have found application in EPR. Relaxation of the unpaired electron is seen as a triplet, with the $I = 1$ ^{14}N nucleus giving rise to the observed splitting pattern. The degree to which the probe is rotating is reflected in the difference in the line widths of the signal.[58] Narrower line widths indicate the probe is able to rotate more freely. This speed of rotation varies directly with a correlation time (τ_C), which may be thought of as being the time required for the probe to travel an arc of 40°.[59] Nitroxide spin probes have been attached to linear polystyrene,[60] which readily dissolves in a variety of solvents. It was noted that the correlation time varied inversely with temperature. As viscosity of solutions, particularly polymer solutions, is known to vary with temperature, that the rotational speed of the probe should be decreased at lower temperatures is not unexpected.

Application of EPR to the study of cross-linked polymer resins was first reported by Chesnut and Hower.[61] Their approach was to study the interaction of a radical probe, 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl, with a range of ion-exchange resins. The resins themselves were beads of cross-linked polyacrylamide known to swell in polar solvents. The probe in solution was equilibrated with the resin, and the resin was then thoroughly washed such that only probes ionically attached to the resin would be observed in the EPR cavity. Concentrations of the radical probe were kept low, $<10^{-3}$ M, to avoid complications arising from excessive spin−spin coupling or Heisenberg exchange which affords artificial broadening.[62] Resins having 2, 4, 8, 10, and 12% cross-linking were examined. It was noted that as the degree of cross-linking increased, the rotational ability of the probe decreased, consistent with a decreasing ability to rotate in a denser medium. However, a noticeable exception to this was that the 12% cross-linked material exhibited rotation on par with that observed for the 4% cross-linked species. Experiments were duplicated to ensure reproducibility. A proposed explanation of this anomalous behavior was that the inhomogeneity of the resin itself resulted in an unequal distribution of the probe such that there was a preference for the probe to occupy areas that were easier to access, i.e., areas of less density.

A further application of EPR to the elucidation of polymeric network properties involved the covalent attachment of a nitroxide probe to cross-linked polyurethane.[63] As in the previously described report, concentrations of the radical were kept low. It was felt that the rotation of the bound probe would be determined by the free volume and local morphology of the immediate environment of the probe. Again, τ_C was found to vary inversely with temperature, and in the swollen state the probe was found to have much greater rotational freedom than in the unswollen state, consistent with enhanced motion of the polymer chains caused by T_g depression.

The nitroxide used in the above study of ion-exchange resins, 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl, was, in a subsequent study, covalently attached to 2% cross-linked CLPS by chloride displacement.[64] Correlation between observed line widths and swelling capability in a range of solvents demonstrated that as the degree of swelling increased, τ_C also decreased. On this basis it was suggested that solvents that swell PS the best allow for the greatest mobility of substrates in CLPS.

A similar study examined the distribution of nitroxide probes, in solution, free to partition themselves either in the liquid or the gel phase. If, instead of covalently attaching the nitroxide probe via the chloromethyl groups of the resin, the probe, 2,2,6,6-tetramethyl-4-benzyloxy-piperidine-1-oxyl, was simply equilibrated with the resin, distribution of radical inside and outside the bead could be observed.[65] The radical probes inside the polymer, but not bound to it, had a greater rotational mobility than did the bound probe by a factor of 10; however, rotation was still slower than the probe free in solution. The mobility of the imbued probe was reduced by increased cross-linking as was the bound probe. Thus, cross-linking influences not just bound molecules, but results in an overall increase in viscosity of the swollen gel phase decreasing rates of mass-transfer. Also evident from this study was that the noncovalently attached species prefers to reside in the free solvent phase rather than in the swollen polymer matrix. Differentiation of the species inside and outside the gel phase was readily distinguishable by the line shapes of the differently relaxing species in the EPR spectra.

Introduction of a spacer between the polymer backbone of CLPS and species of interest was examined as a means to enhance the mobility of the bound species. In this instance, a bis-decanamide linker was installed to the terminal chloromethyl group of CLPS as a tether. To examine the mobility of the tethered molecule, a spin label was attached to the amino terminus, which was compared to a spin-label bound to the benzylic carbon. It was noted that the nitroxide at the end of the tether exhibited narrower line widths in the EPR spectrum than did the benzylic nitroxide, consistent with enhanced mobility. Again, a small amount of the spin label was employed in the experiment.[66] Consistent with this result, rotation of a nitroxide probe at the ends of TG in solution have been observed to be as fast as free in solution.[67] On one hand this may imply free-solution-like behavior of TG; a more plausible explanation is that the small amount of probe used was bound only to the most accessible (exterior) tentacles. Clearly this should not be extrapolated to infer binding sites inside the resin bead will exhibit the same free rotation. The inability to uniformly add nitroxide throughout the resin must be considered as an important caveat.

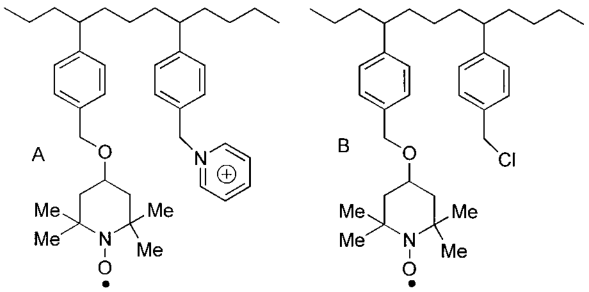

Figure 2. (a) Nitroxide bound pyridinium MR, 96% pyridine ring substitution, 0.04% nitroxide ring substitution. (b) MR, 0.04% nitroxide substitution. Reproduced with permission from: Regen, S. L. *J. Am. Chem. Soc.* **1975**, *97*, 3108. Copyright 1975 American Chemical Society.

A study which utilized EPR to monitor rotational ability of a nitroxide probe compensated for the tiny amount of probe that could be added[68] by modification of the manner of attachment of the nitroxide probe has been described. In this case 2,2,6,6-tetramethyl-4-oxopiperidin-1-oxyl oxime was the stable source of radicals. By using a mixture of a small amount of the nitroxide oxime together with cyclopentanone oxime, more uniform loading of the radical probe in the resin could be obtained. This is a valuable, although not completely rigorous, approach due to different dipole moments of the radical and nonradical oximes. Relaxation time varied inversely with the degree of cross-linking in dichloromethane. This study also examined a range of solvents, looking at differences between probes attached to cross-linker and to the monomer. The monomer bound probe was observed to rotate faster in all the solvents examined, save for DMF.

The use of EPR to evaluate the morphology of resins was examined in further detail by Regen.[69] An interesting extension involved the introduction of cationic groups onto the backbone of CLPS by treatment of the chloromethyl groups with pyridine. Addition of the pyridinium group acted to markedly increase the polarity of the resin. The proportion of nitroxide radicals (Figure 2) was kept low to avoid complications. The pyridinium derivatized resin was found to exhibit swelling “opposite” to underivatized CLPS. That is, for straight CLPS swollen in ethanol, very slow tumbling of the radical probe was noted, whereas in the pyridinium form of CLPS fast rotation of the probe was observed in ethanol. The converse was true for nonpolar, aprotic solvents: carbon tetrachloride afforded a faster rotation rate on underivatized CLPS, but this slowed with the pyridine derivatized CLPS. This is another example of how grafting onto the polymer backbone, in this case much smaller than a large peptide chain, can impact swelling properties.

The above study went further to examine the motion of a radical bound to the surface of silica, a nonswellable support. Here it was noted that for aprotic solvents such as CCl_4, the EPR spectra resembled that of the dry powder spectrum. With

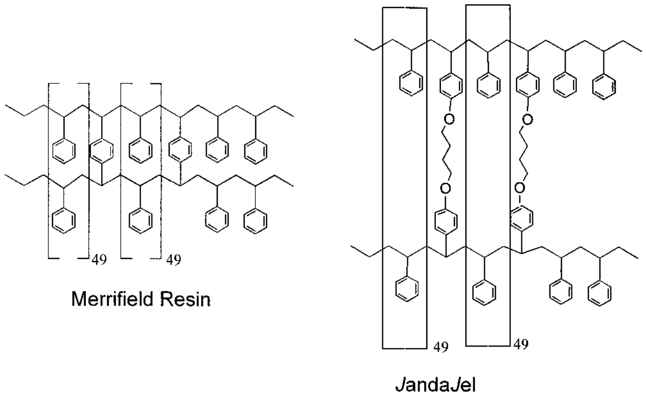

Figure 3. Structures of cross-linked polystyrene and *J*anda*J*el.

protic solvents a well-resolved spectrum resulted, consistent with better solvation of the radical despite no possibility of swelling the inorganic SiO_2. A proposed explanation is that the nitroxide participates in hydrogen bonding with adjacent hydroxyl groups on the silica which is obviated by the introduction of a hydrogen bond accepting solvent. Even so, rotation of the silica bound probes in the hydrogen bonding solvents was lower than for the permeable PS resins.

Introduction of an amino acid mimetic incorporating a spin label, 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-carboxylic acid,[70] has been achieved. This label was used to advantage in the study of the motion of peptide chains growing off CLPS. The amino acid mimic was incorporated into the CLPS, keeping the level of radicals low. A resin heavily loaded with peptide was noted to exhibit an order of decreasing τ_C which was the opposite of that for unfunctionalized resin. That is, rotation was faster in solvents where it was otherwise slower, similar to the change in swelling properties previously noted on adding long peptides to CLPS.[57] This further points to the dominating effect the pendant chains off the polymer backbone exert on the swelling properties of the material itself.[71]

An attempt has been made to relate τ_C with rates of reaction.[72] It was noted that the rate of a reaction, peptide coupling on CLPS, varied inversely with τ_C. Further, an examination was made to relate this to the viscosity of the swollen polymer gel. A relationship between correlation time and intrinsic viscosity of the swollen polymer, $\tau_C \cong \eta T$, has been suggested.[73]

*J*anda*J*el[74] is polystyrene cross-linked with 1,4-bis(vinylphenoxy)-butane in place of divinyl benzene. An interesting aspect of *J*anda*J*el (JJ) is its swelling ability, which is double that of the corresponding CLPS. Structures of JJ and CLPS are depicted in Figure 3. The enhanced swelling of JJ as compared to CLPS is somewhat surprising given the slight difference in structure between CLPS and JJ. For example, the 2% resins differ by just two cross-links in 98 styrene units. Calculation of δ for JJ reveals it to be 9.4 (cal/mL)$^{1/2}$, remarkably close to accepted values for CLPS—further pointing to what should be a slight difference. To examine the singular swelling ability of JJ, EPR studies were conducted in conjunction with fluorescence spectroscopic studies.[75] While the fluorescent studies (with dansyl probe added during polymerization, ensuring homogeneity) confirmed that the macroscopic observation of enhanced swelling of JJ was, in fact, due to closer interaction between polymer and solvent, not merely enhanced filling of porous structures, correlation times as measured by EPR were faster for the corresponding CLPSs. That is, the probe was better able to rotate in CLPS. Previous studies on the application of EPR to cross-linked polymers[63−70,73] have all concluded that τ_C is a function of swelling ability or viscosity, and that correlation time decreases as the viscosity decreases. As such, it was initially assumed that τ_C should be faster for a resin with a greater swelling capacity—the opposite of what was observed. This dichotomy was rationalized in that the enhanced heterogeneity of the CLPS, caused by the two species of cross-linkers used, allows for creation of areas of high and low cross-linking density. As only a tiny proportion of nitroxide is used, it will preferentially react at more easily accessible sites. Hence, attachment of the probe occurs in regions of lower cross-linking with their incumbent lower viscosity. In the initial investigation using fluorescence spectroscopy, the probe was uniformly incorporated into the polymer during polymerization.[76] In order to test the idea that the mode of incorporation of the probe was responsible for the observed disparity between τ_C and swelling ability, small amounts of a dansyl probe was added to both CLPS and JJ after polymerization by chloride displacement. Examination of the emission maxima of the post-polymerization modified resins revealed a greater shift in emission maxima for the dye added after polymerization to CLPS as compared with the same observations on JJ. That is, the means of attachment of the probe had a greater effect on the observed response for CLPS. This likely arises from greater homogeneity of the JJ, which has a higher overall, but more consistent, viscosity.

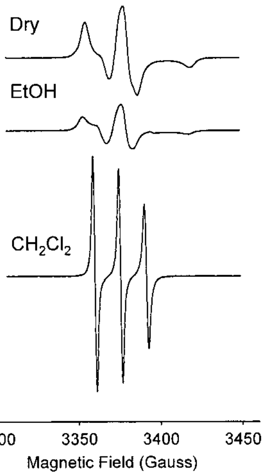

Figure 4. EPR spectra of 2% crosslinked *J*anda*J*el dry, with EtOH, with CH_2Cl_2.

An important aspect of the above study was that conclusions drawn from EPR data should be considered valid only for a small portion of the resin, in other words, only for regions where the nitroxide probe is attached. This also points to the importance of the mode of attachment of the probe. Some examples of EPR spectra of polymer bound TEMPO are presented in Figure 4. Of note is the lack of any interaction between EtOH and the resin, evidenced in the similarity of its spectrum to the dry polymer spectrum. By contrast, interaction with CH_2Cl_2, known to be a good solvent for CLPS, results in a well-resolved spectrum as the nitroxide is now able to rotate more freely.

Nuclear Magnetic Resonance. The first report of the application of NMR spectroscopy to a cross-linked polymer was a 1962 report on the ^{1}H NMR spectrum of an ion-exchange resin in which separate resonances were noted for water inside and outside the resin.[77] Duch and Grant[78] were able to demonstrate narrow ^{13}C NMR spectral lines for polybutadienes and polyisoprenes above T_g due to substantial movement of the polymer chains. A review detailing early work on the application of NMR to cross-linked polymers has appeared.[79]

Narrow signals in NMR (and EPR) spectra are only achieved if magnetic interactions between neighboring nuclei are averaged by random motion that is faster than the internuclear dipolar relaxation pathways.[80] This is readily attained in solution, but when affixed to large, static molecules, rapid motion of the dipoles becomes difficult. An advantage of using NMR spectroscopy, as compared to EPR, is that concentrations of the species to be observed may be much higher, permitting more uniform permeation of the matrix by the probe.

A report on line widths in NMR spectra of probes, in this case salts containing ^{23}Na and ^{19}F, inside of polymeric matrices was reported by Creekmore and Reilley.[81] Both cation-exchange resin (Dowex 50W) and anion-exchange resin (Dowex 1) were examined. Increases in the line width of the ^{23}Na or the ^{19}F spectra were observed as the degree of cross-linking increased; this was attributed to a decrease in τ_C caused by a reduced ability of the probe to move in a denser environment. Further examination of these polystyrene sulfonic acid polymers cross-linked with divinyl benzene was performed using amino acids ionically attached to the resins. Both 4% and 8% cross-linked resins were studied.[82] An effect of binding the amino acid to the resin was a substantial decrease in spin−lattice relaxation time (T_1) for the resin bound species, permitting a shorter relaxation delay thus allowing transients to be collected more rapidly. All the resin bound amino acids showed an increase in line width of 2−3 times on going to the 8% cross-linked as compared to the 4% cross-linked resin. These early studies make clear that motion of small molecules in the swollen gel-phase, while muted as compared to motion in solution phase, is still very much evident.

Ford and Balakrishnan[83] analyzed cross-linked PS gels using ^{13}C NMR spectroscopy. It was noted that spectra of the least cross-linked polymers displayed the narrowest lines. This is consistent with the idea that cross-linking puts extra restrictions on the motion of the polymer backbone. Degree of swelling (known to vary inversely as cross-link density) was also found to be important. A poorly solvated resin does not allow motion of the polymer backbone; thus, line broadening due to anisotropic relaxation processes are observed. ^{13}C spectroscopy can be of more benefit than ^{1}H NMR spectroscopy as the ^{13}C dipole exhibits a broader spectral range. By comparing values of T_1 for a range of PSs having 0−10% cross-linking, it was noted that a minimum occurred at cross-linking of 4%. However, the difference between the largest and smallest values were within experimental error, suggesting this was not the best way to make comparisons. Some examples of ^{13}C NMR spectra of CLPS are presented in Figure 5.

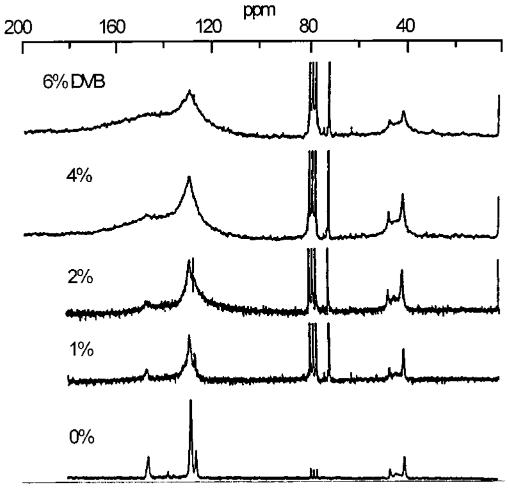

Figure 5. Gated decoupled ^{13}C NMR spectra of MR. The degree of cross-linking is indicated. Reproduced with permission from Ford, W. T.; Balakrishnan, T. *Macromolecules* **1981**, *14*, 284. Copyright 1981 American Chemical Society.

Of particular note in the above report is a discussion that, in contrast to EPR measurements, line widths and spin−lattice relaxation cannot be rationalized by a single component, for example τ_C for motion of the backbone carbon atoms.[84] As such, results obtained from NMR on the matrix itself must only be taken qualitatively. The difficulty with interpretations based on T_1 is that they are based on peak intensities at the maxima of broad spectral bands arising from the nature of the observed signal which is a weighted average of all the different ^{13}C spins contributing to a particular resonance. Those carbon spins having the narrowest line width contribute most to the intensity at this maximum, and those with the greatest line width contribute the least. Thus, reports on T_1 for ^{13}C are skewed disproportionately toward faster relaxing carbons.

Ludwick and co-workers[85] examined line widths of Boc protected polyglycine-d_2 by observation of quadropole echo ^{2}H NMR. The advantage of the deuterium labeled glycine is that it allows observation of NMR spectra unfettered by excessive background from ^{1}H or ^{13}C dipoles. In the dry state, the powder spectrum line broadening was clearly observed consistent with reduced movement of the dipole in the polymer matrix. Even heating the dry samples, in an attempt to increase backbone motion, up to 112 °C (near T_g for CLPS) did not result in a resolved spectrum. Upon introduction of a good solvent for CLPS, DMF, a sharp singlet was noted, consistent with newfound mobility imparted by the now swollen gel phase. As the length of the polyglycine-d_2 chain began to slowly increase, at $n = 7$ some broadness was again evident, the opposite of what was implied by EPR measurements.[61] This broadening may be caused by the free volume of the CLPS being occupied by amino acids, rendering free rotation more difficult. Figure 6 depicts the ^{2}H NMR spectra for tethers of seven and nine Gly-d_2 units over a range of temperatures. Of note is that broadening for the Gly$_7$ tether is eventually compensated for by increased

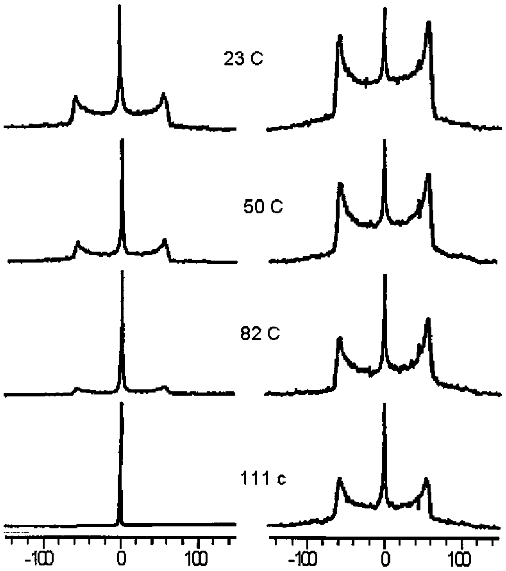

Figure 6. Quadropole echo ^{2}H NMR spectra of DMF swollen MR. The left column is (Gly-d_2)$_7$; the right column is (Gly-d_2)$_9$. The temperatures are indicated. Reproduced with permission from Dumais, J. J. *J. Am. Chem. Soc.* **1986**, *108,* 6493. Copyright 1986 American Chemical Society.

temperature, i.e., T_g is reached, whereas for Gly$_9$ line broadening is always observed. DMF was the only solvent examined.

A comparison of ^{13}C NMR spectra of three resins has been published.[86] The supports examined were poly(ethyleneglycol) (PEG), CLPS, and TG. An amino acid, namely, Boc-Gly-Pro-Pro-OH, was attached to the resins as a probe. Spin−lattice relaxation times (T_1) were measured by the standard inversion−recovery technique (180-z-90-acqu). Also, the line widths of the tripeptide ester were measured both attached to the support and free in solution. In solution, spin−lattice relaxation times were measured; a dramatic decrease in relaxation time was noted on going from the free methyl ester to the polymer bound species in C_6D_6. The fastest T_1 (times for the boc, the α proton of Gly, and the α proton for Pro were used) was observed for CLPS, followed by TG and PEG. Differences for the T_1 values varied markedly depending on which proton was observed; however, in all cases the same trend was observed. Looking at line widths of PRO-C$_\alpha$, CLPS always displayed the widest lines, followed by TG. The PEG bound probe displayed the same properties as free in solution, consistent with the ability of PEG to more closely approximate free solution behavior.[87] That the CLPS should have had less mobility was thought peculiar given it had a greater free volume than the TG, which had its free volume otherwise occupied by PEG grafts. This suggests enhanced motion of the amino acid at the end of the PEG tether. A comparison of rates of peptide coupling using an *ortho*-nitrophenyl active ester demonstrated that the rate of the PEG reactions, either grafted to PS or free in solution, was negligibly affected by the length of the PEG chain; PEG 2000/4000 for liquid-phase organic synthesis (LPOS), and PEG 2000/3300 for TG were used. Some representative spectra are included in Figure 7.

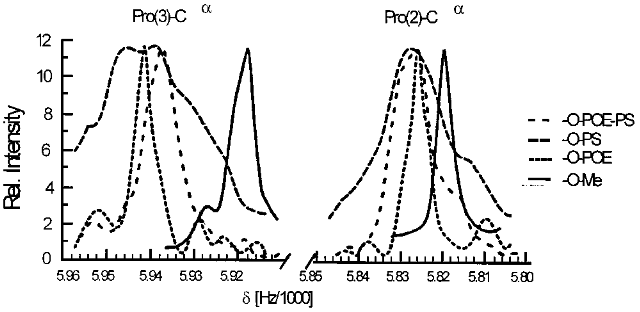

Figure 7. Line widths of α carbons of Pro (2) and Pro (3) of tripeptide esters bound to supports as indicated. Reproduced with permission from Bayer, E.; Albert, K.; Willisch, H.; Rapp, W.; Hemmasi, B. *Macromolecules* **1990**, *23,* 1937. Copyright 1990 American Chemical Society.

An extensive study of swollen resins by comparison of ^{1}H line widths has been measured[88] using a special probe, dubbed a Nanoprobe.[100] Wang resin, Novasyn TGA, Novasyn TGT, CLPS, PAM, MBHA, and Rink MBHA were examined in CD_2Cl_2, DMF-d_7, DMSO-d_6, $(CD_3)_2CO$, CD_3OD, and D_2O. It was suggested that the line width of a small organic molecule would serve as an excellent measure of spectral quality, a probe. As there is not a limitation on amount of dipole that may be introduced for NMR experiments (as is the case for EPR), sufficient probe may be introduced to ensure full permeation of the resin. The probe of choice was the singlet observed from the methyl group of a *tert*-butyl ester. A comparison was made between spectral quality, qualitated on a scale from best to worst spectrum for a given solvent. In certain cases no resolvable spectrum was noted. The results are summarized in Table 3.

Of note in Table 3 is the observation that little correlation exists between observed resolution of the spectra, i.e., mobility of the probe, and swelling ability. Resins with grafted PEG chains off the PS backbone, TGA and TGT, appeared to possess the greatest mobilities judging from the narrowness of the line widths. Interestingly, the Wang resin with its substantially shorter tether off the PS backbone, a single hydroxybenzyl alcohol unit, exhibited good to excellent mobility in the nonprotic solvents examined. ^{1}H NMR spectra are included in Figure 8. The difference between a poorly resolved spectrum, for example CLPS in CD_2Cl_2 and a well-resolved spectrum, TG in CD_2Cl_2, is clear.

An extensive report on the properties of TG has appeared.[89] Penetration of a fluorescein-labeled enzyme into the beads was examined using a confocal scanning laser microscope and by NMR spectroscopy. As well, the ready observation of the ^{31}P nucleus by NMR was exploited. α-Triphenylphosphonium *para*-toluic acid bromide salt (referred to as Compound P) was studied as a ^{31}P containing probe. That this was the only ^{31}P nucleus in the system provides for easy-to-interpret spectra. T_1 times were obtained through an inversion−recovery sequence.

Compound P was coupled to amino TG via an anhydride. ^{31}P NMR spectra of this ensemble in a variety of solvents were obtained. It was noted that line widths of the phosphonium salt free in solution were very narrow, but broadened on binding to the resin. Interestingly, the line widths of Compound P immersed in a solution containing TG, but not attached to the resin, were identical to the broadening of the polymer bound Compound P. This was

Table 3. Comparison of Spectral Quality,[a] Line Width,[b] and Swelling Ability[c] between Various Resins and Solvents

resin	CD_2Cl_2	DMF-d_7	DMSO-d_6	$(CD_3)_2CO$	C_6D_6	CD_3OD	D_2O
Wang	A	B	E	B	B	F	F
	10.0	8.7	nm	10.2	13.9	nm	nm
	×3	×4	×1	×2	×4	×1	×1
NovaSyn KA 100	C	C	C	E	E	B	E
	14.9	14.3	16.0	nm	nm	20.6	nm
	×2	×1	×1	×1	×1	×1	×1
NovaSyn PA 500	B	A	A	C	E	B	E
	13.0	11.6	11.5	28.8	nm	23.4	nm
	×3	×1	×1	×1	×1	×2	×1
NovaSyn TGA	A	A	A	B	C	B	E
	8.4	10.0	8.8	12.5	25.1	13.2	nm
	×3	×1.25	×2	×1	×1.5	×1.75	×1
NovaSyn TGT	A	A	A	B	C	B	E
	4.1	5.9	5.8	11.0	19.4	3.8	nm
	×4	×4	×3	×3	×3	×2	×2
MR	D	D	F	D	D	F	F
	12.6	17.3	nm	16.9	19.4	nm	nm
	×4	×3	×2	×2.5	×4.5	×1	×1
PAM	D	D	D	D	D	E	F
	12.1	10.7	16.8	13.5	15.1	nm	nm
	×3	×3	×1	×1.5	×1	×1	×1
MBHA	D	D	F	D	E	F	F
	25.7	27.1	16.8	28.0	26.2	nm	nm
	×4	×4	×1	×2	×6	×1	×1
Rink-MBHA	B	A	E	D	E	F	F
	12.5	8.0	24	16	nm	nm	nm
	×4	×3	×1.5	×2	×3	×1	×1

[a] Qualitative spectral quality (A−F) with A being the best. [b] Linewidth of half-peak-height of *tert*-butyl singlet, expressed in Hz; nm refers to not measured. [c] Volume increase induced by qualitative observation of solvent swelling, expressed as a factor of volume increase.

ascribed to magnetic-field inhomogeneity caused by the matrix itself rather than the probe being in a solvent of substantial viscosity. This conclusion implies that observation of line widths may be deceiving, but did not take supramolecular interactions causing anisotropic motion of the dipole into account. Relaxation times were also examined. No significant difference was noted between line widths of the noncovalently attached Compound P and the covalently bound Compound P.

A further report examining a variety of resins using NMR spectroscopy has recently appeared.[90] The resins examined were TentaGel, ArgoGel, CLPS, macroporous aminomethyl polystyrene, together with three novel resins synthesized by the authors: POEPOP, SPOCC, and POEPS-3. Structures of the resins are depicted in Figure 9. Not surprisingly, the degree of swelling was found to be a function of the polymers physical characteristics. As the length of the PEG chains increased, the swollen volumes increased. The resins with the greater proportion of PEG were found to exhibit narrower lines in protic solvents, better than even TG. This is consistent with the introduction of hydrophilic PEG chains without decreasing the free volume of the resin by graft polymerization off of the CLPS backbone, as is the case with TG. A higher proportion of the hydrophilic PEG afforded better resolved spectra in polar solvents, while still permitting good solvation in organic solvents such as dichloromethane and chloroform.

NMR spectroscopy has been applied to determination of the structure of cross-linked resins. Using ^{13}C NMR, Manatt and co-workers[91] were able to discern that chloromethylation of CLPS by chloromethyl methyl ether occurred primarily (>99%) in the 4 position. This is consistent with reports that introduction of a chloromethyl group to alkyl benzenes by Friedel−Crafts alkylation with chloromethyl methyl ether affords solely the *para*-isomer.[92] Ford and Yacoub[93] published an interesting report on a comparison of line widths between chloromethylated cross-linked polystyrene prepared by treatment of CLPS with chloromethyl methyl ether and CLPS prepared by copolymerization of styrene, vinylbenzyl chloride (VBC), and divinyl benzene. The ^{13}C NMR spectra of CLPS prepared by chloromethylation of CLPS depicted greater line broadening than did the resin prepared by copolymerization of VBC, DVB, and styrene having the same nominal degree of cross-linking. The greater line broadening is consistent with hindered rotation due to extra cross-links, and is also consistent with reports that treating linear polystyrene with chloromethyl methyl ether affords an insoluble cross-linked product in high yield.[94] Despite the difference in observed line widths, no substantial difference in swelling properties was observed, within experimental limitations, showing the NMR approach to be more sensitive than simple comparisons of changes in volume. That there was no decrease in swelling ability is in contradiction with results to be described.

Differences arising from the method of introduction of chloromethyl groups were further examined by Mohanraj and Ford.[95] As alluded (vide infra), the customarily reported, nominal, degree of cross-linking does not necessarily represent the actual cross-linking. Introduction of chloromethyl groups by copolymerization with VBC does not introduce any extra cross-links. The authors earlier examined introduction of a mixture of *meta*- and *para*-vinylbenzyl chlorides. A soluble (linear) copolymer of *meta*- and *para*-vinylbenzyl chloride displayed discernible peaks, at 137 and 135 ppm in

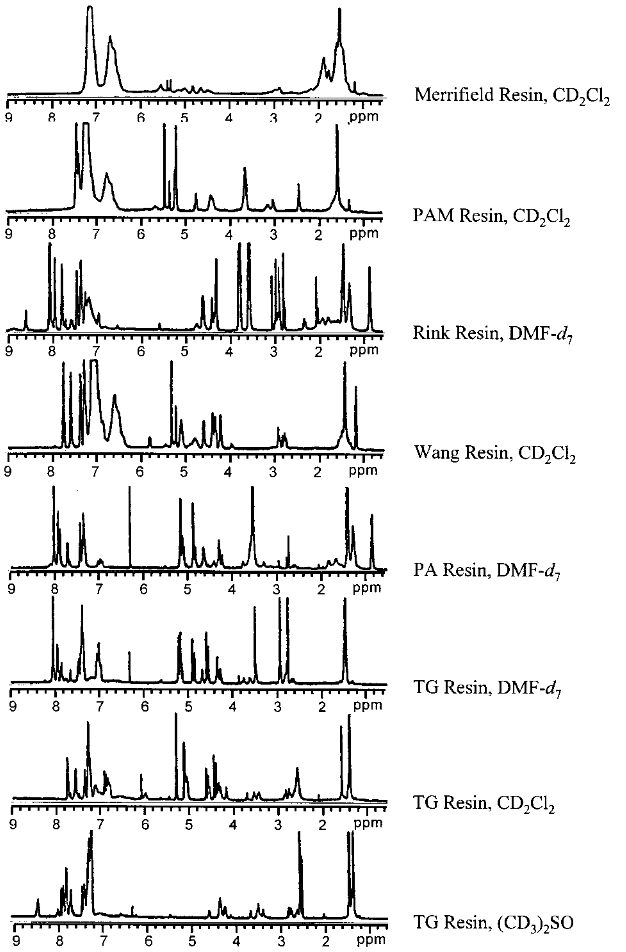

Figure 8. 500 MHz ^{1}H NMR spectra of various resins swollen in solvent as indicated. Reproduced with permission from Keifer, P. A. *J. Org. Chem.* **1996**, *61*, 1558. Copyright 1996 American Chemical Society.

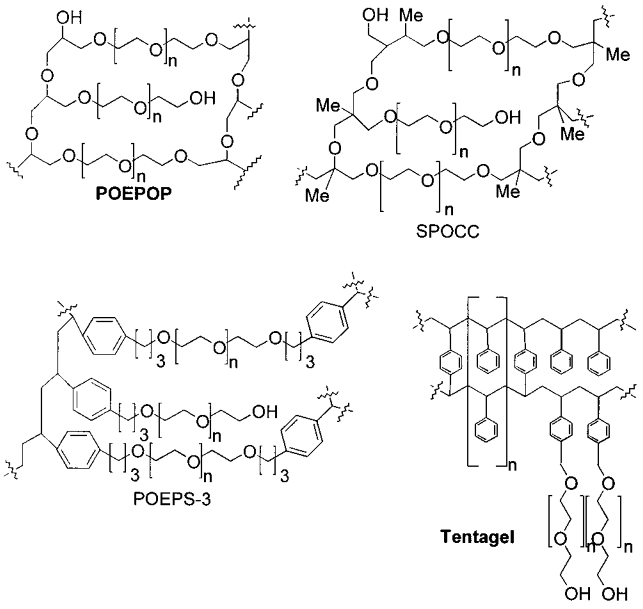

Figure 9. Chemical structures of a variety of resins.

the ^{13}C NMR spectra, respectively, for the *meta*- and *para*-isomers of vinylbenzyl chloride. Introduction of cross-linking in the form of as little at 0.5% divinyl benzene broadened the spectrum to the point that observation of the distinct resonances of the *meta*- and *para*-isomers was not possible. Upon further examination of the effect of treating lightly cross-linked PS with chloromethyl methyl ether it was noted that increased reaction time resulted in decreased chlorine content and increased the degree of cross-linking as determined by line width analysis. This increase in cross-linking of CLPS by treatment with chloromethyl methyl ether was first observed in the 1950s.[96]

Recently, the enhanced swelling of polymers prepared by copolymerization of styrene, divinyl benzene, and solely the *para*-isomer of vinylbenzyl chloride has been reported to result in swelling up to double that of conventionally prepared CLPS having the same nominal degree of cross-linking.[97] This enhanced swelling is simply a consequence of less actual cross-linking.

On-Bead Analysis. Traditionally, following the course of SPOS reactions required cleavage of a portion of the resin and examination of the cleaved aliquot by traditional methods. This can be time-consuming and wasteful of material. The use of NMR for the study of species bound to solid-phase resins is clearly of great benefit for reaction monitoring in a medium where TLC is not possible. Some examples of on-bead analysis using NMR are very briefly mentioned here. ^{31}P NMR has been applied to reaction monitoring; the advantage of the ^{31}P nucleus is the lack of interfering background signals together with a strong signal.[98] This technique is, of course, limited to compounds bearing phosphorus. Using MAS and ^{1}H HMQC even complex structures on a single bead could be elucidated if the sample was enriched with ^{13}C.[99] There are other reports on the use of MAS[100] and MAS ^{13}C,[101] as well as CP-MAS[102] for on-bead analysis. A comparison of methods for on-bead reaction monitoring using NMR has appeared, with tradeoffs for MAS vs non-MAS noted. MAS affords better resolution but requires more specialized equipment.[103] The use of ^{19}F NMR in conjunction with a fluorine containing resin, the amount of fluorine on the resin known from combustion microanalysis, has been reported to be an effective means to monitor reactions.[104] Looking at so-called fast ^{13}C NMR of labeled compounds on the solid phase, it was noted that good spectra could be obtained using TG without the need for preswelling.[105] Swelling CLPS was found to be critical for obtaining resolved spectra even by MAS HMQC.[106] Using Wang resin it was noted that DMSO markedly improved NMR spectral quality. No other solvents or resins were examined. A three-step Heck sequence on solid support was monitored using MAS HMQC.[107] As well, the use of a nanoprobe has been reported for single bead analysis using MAS.[108]

The first report of on-bead analysis using normal ^{13}C NMR did not take advantage of the lower T_1 imparted by the swollen gel phase.[109] By exploiting this reduced T_1, which allows faster pulsing of the sample, acceptable ^{13}C NMR spectra could be obtained without resorting to MAS in seven and a half minutes in a normal 300 MHz spectrometer.[110] The best solvent was found to be 20% (v:v) of a deuterated solvent (either C_6D_6, 1,4-dioxane-d_8, or DMSO-d_6) in CCl_4. Carbon tetrachloride was ideal as its ^{13}C has a long relaxation time. The choice of deuterated cosolvent depends on what is bound to the resin.

Diffusion through Beads. Transport of molecules in solution is critical to the undertaking of any reaction. Simply put, a molecule unable to reach its desired site of action, regardless of the medium, is unable to react. For molecules free in solution the barrier to this interaction is negligible due to rapid Brownian motion. Upon attachment of the same species to a solid support, with incumbent increase in viscosity, Brownian motion is diminished and mass transfer becomes critical.

The use of pulsed-field-gradient spin–echo (PGSE) NMR[111] has been applied to the study of solvent diffusion through CLPS.[112] The use of the PGSE method allows measurement of the rate of solvent diffusion within the swollen polymer. Diffusion of toluene in solutions of LPS was measured. As weight percent LPS increased, the rate of toluene diffusion decreased, from 31.2×10^{-10} m^2/s in free solution to 1.25×10^{-10} m^2/s in a solution of 0.691 wt % LPS. The same measurements on swollen cross-linked PS demonstrated that increasing degree of cross-linking decreased diffusion rates, for example from 4.57×10^{-10} m^2/s in nominally 5.7% CLPS to 1.45×10^{-10} m^2/s in 40% CLPS. Thus, in 0.15 s toluene travels 12 μm in 5.7% CLPS. It should pointed out that the decrease in rate of diffusion is proportional to the decrease in swelling ability as cross-linking increases.

Further study of diffusion through the swollen gel phase[113] reported the use of what was dubbed magnetization transfer NMR (MT-NMR).[114] The basis for this method is that ^{13}C NMR spectra of swollen CLPS often shows two peaks for each carbon of the solvent, one corresponding to solvent inside the swollen matrix and the other corresponding to solvent outside the polymer. Peak assignment is made based on spin–lattice relaxation times, known to be much faster inside the dipole-laden swollen polymer, as well as based on increased line broadening inside the polymer. Plots of peak intensity for the two solvent peaks vs delay time in selective inversion–recovery experiments depicts qualitative transfer of magnetization from the selectively inverted site to the other site, allowing calculation of rates of diffusion. Again, a decrease in diffusion rate is seen with increased cross-linking density. A caveat with this work is that beads in the NMR tube were tightly packed, which is not typically the case in SPOS. Also, vigorous spinning of the sample is not possible in the NMR spectrometer, which will create problems with mass transfer across the turbulent Nernst layer (vide infra).

A similar approach to monitoring the different environments for chloroform inside and outside CLPS has been reported.[115] The relative intensity of the chloroform resonance inside the polymer was compared with the resonance of chloroform outside the polymer. It was noted that most rapid exchange of solvent from inside the bead to outside the bead occurred with beads of the smallest diameter. This is consistent with flux varying as surface area–smaller spheres have a higher surface area-to-volume ratio. By extrapolation it was predicted that free exchange, that is the polymer would not impede motion of the solvent, should occur with beads having a diameter of 3.5 μm, below the limit of what is easily filtered.

One of the benefits of solid-phase synthesis is the ability to isolate reagents from each other, a technique of particular advantage for monofunctionalization of bifunctional molecules, for example diamines or dihydroxylated compounds.[116] To further examine this, the kinetics of an intraresin reaction were examined.[117] Merrifield resin was modified such that some of the chlorides from the chloromethyl groups were replaced by ammonium acetate (Figure 10). Observed rates of displacement of the chloride by acetate (the appearance of ionic chloride in free solution was monitored) showed little dependence on the length of alkyl chain or solvent used, implying that the resin/solvent pair, the gel-phase solvent, exerted the dominating effect. In contrast to the free solution reaction, the intraresin reaction exhibited a rapid initial rate, followed by a plateauing not seen in solution. Increasing the degree of cross-linking decreased reaction rates, due to lower solvation and restricted movement of the polymer chains.

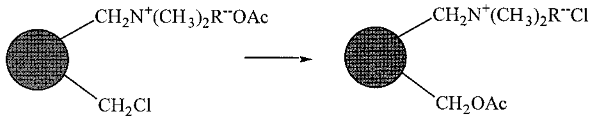

Figure 10. An intraresin displacement reaction.

Phase transfer catalysis involving the intermediacy of a charged quaternary salt is a reaction readily amenable to solid-phase chemistry. The use of polymer supported tri-*n*-butylphosphonium salts as a phase transfer catalyst has been applied to the chloride displacement of *n*-decyl methanesulfonate.[118] The rate of displacement of the sulfonate by aqueous chloride was monitored. The rate of reaction was found to increase as surface area-to-volume ratio increased, i.e., as bead size decreases.

Tomoi and Ford[119] have studied the effects of immobilization of a catalyst in a triphasic (aqueous, organic, polymer) reaction. Insoluble phase transfer catalysts were employed for the reaction of 1-bromooctane with aqueous sodium cyanide in toluene. In the absence of the quaternary ammonium or phosphonium salts as phase transfer catalysts the reaction did not progress at all. It was noted that the rate of reaction varied directly as the speed of stirring up to 600 rpm. Above 600 rpm the rate was as fast as that obtained with a vibromixer or an ultrasonic probe. The size of the beads had an effect on rate of reaction. Rates were found to vary inversely with bead diameter, that is, as the surface area-to-volume ratio decreased so did mass transfer of reagents into the bead.

A subsequent report examined more closely the use of polymer-supported benzyl tri-*n*-butyl phosphonium and polymer-supported benzyl tri-*n*-butylammonium as phase transfer catalysts.[120] The fundamental kinetic steps are depicted in Figure 11. The maximum rate of reaction was found to occur with beads having a diameter of 20 μm, near the lower limit of particle size easily prepared by suspension polymerization and near the limit of size that is readily filterable. The effect of solvent quality was found to be important. As the degree of swelling decreased, so too did the rate of reaction. Rates were found to vary inversely with degree of cross-linking, as was noted in three solvents. The sequence of events predicated for reaction to occur were discussed at some length. Transfer of the bromooctane to

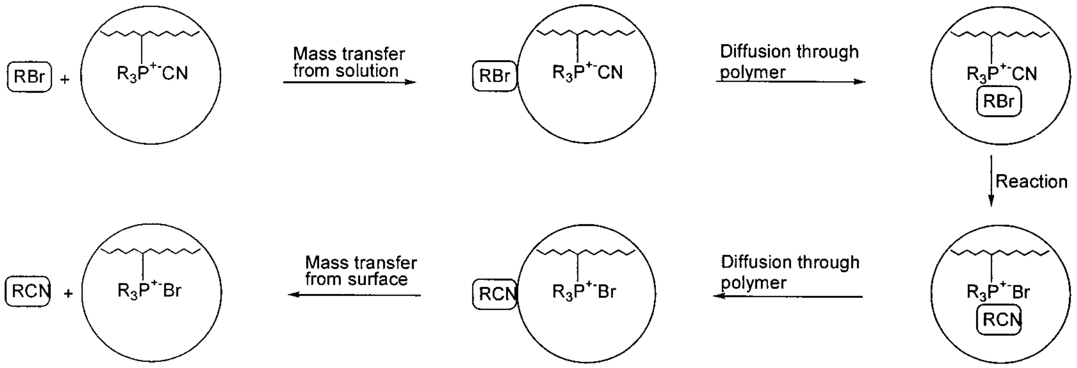

Figure 11. Steps required for reaction of polymer bound catalytic reaction.

the interior of the polymer required contact with the surface. The solution phase closest to the surface of the resin exhibits a turbulent layer of solvent with slow movement similar to a Nernst layer at an electrode, referred to as a "quiet layer." The magnitude of this quiet layer will depend on the rate of agitation, the faster the stirring the narrower the turbulent film at the solid−liquid interface. Diffusion of the reactant through the polymer matrix is the next requisite step and may limit the rate of reaction when mass transfer of the reactant through the Nernst layer is quick. Cross-linking affects diffusion by controlling swelling and, hence, internal viscosity of the polymer. In general, the greater the volume of solvent taken up by the polymer, the less the viscosity. Once the reactant reaches the active site, the rate of reaction with the bound species is critical. This will be different from the rate in free solution, in the same way rates of reaction are altered by changes in solvent polarity. After completion of the reaction the reverse sequence of steps leading up to the reaction are required, specifically, for diffusion of the product through the polymer and crossing the Nernst layer to get back into free solution.

Fluorescence Spectroscopy. Resonance spectroscopies are dynamic techniques in that they examine events that unfold over a relatively long time, relaxation of a dipole perturbed from an equilibrium state. Fluorescent and infrared spectroscopies provide static information resulting from direct observation of the effect of photons on the observed transitions. Thus, static fluorescence spectroscopy provides an ideal complement to the dynamic resonance techniques.

Fluorescence spectroscopy has been applied to the study of interaction between polymer backbone and solvent. The fluorescence of the dansyl group is known to be highly dependent on its solvation.[121] Thus, dansylated styrene monomers were prepared and copolymerized into cross-linked polystyrene.[122] By comparing the emission maxima of the dansyl probe free in solution to the emission maxima of the dansyl probe on the backbone of the swollen cross-linked polymer, the interaction between solvent and probe can be compared to that free in solution. Subsequent analysis of the fluorescence emission of the swollen cross-linked polymer then provides a glimpse of the interaction, at a molecular level, between solvent and probe. While still qualitative, data obtained in this way provides more meaningful information than merely recording swelling ability based on observation of macroscopic properties such as a change in volume or an increase in weight; both properties are susceptible to spurious results due to variability in the porosity of the material. Emission maxima anticipated by Taft solvent parameters was compared to observed emission maxima of the polymer bound fluorescent probe in a variety of solvents. As the degree of cross-linking increased, the degree of interaction between the solvent and the fluorescent probe decreased, as evidenced in Figure 12 by deviation from the expected behavior in free solution, noted as the diagonal line. When ethanol, a known nonsolvent for CLPS, was employed, the value of emission maxima obtained was the same as that for the dry polymer, indicating no interaction between the solvent and the probe, as is to be expected.

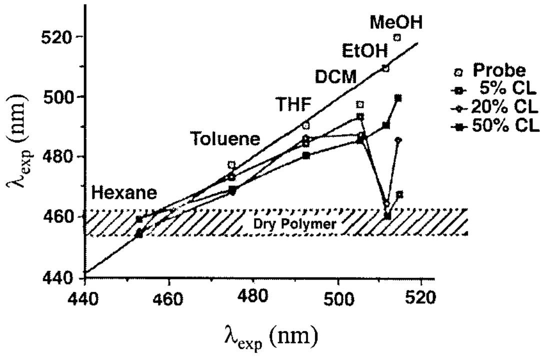

Figure 12. Emission maxima of free dansyl monomer vs emission maxima of dansyl monomer polymerized in MR. Degree of crosslinking is noted. Reproduced with permission from Shea, K. J.; Sasaki, D. Y.; Stoddard, G. J. *Macromolecules* **1989**, *22*, 1722. Copyright 1989 American Chemical Society.

An examination of the diffusion of ionic species into CLPS has appeared.[123] Strong electrophiles, namely $Et_3O^+BF_4^-$ and $Ph_3C^+BF_4^-$ were left to diffuse into the dansylated polymer in a mixture of dichloromethane/hexane. Quenching of the dansyl fluorescence by these Lewis acids in free solution is nearly instantaneous. As cross-linking increased, the rate of quenching of the polymer bound dansyl probe decreased due to impeded transport of the electrophile to the site of reaction.

Fluorescent probes have been used to examine the morphology of cross-linked polystyrene.[124] Dansyl groups were appended to styrene as previously described. In addition, *meta*-divinylbenzene modified by addition of a dansyl group was copolymerized into the polymer. The probes were independently incorporated into polystyrene, and their emission maxima examined. In a range of solvents, the emission maxima of the styrene bound dansyl probe more closely mimicked free solution behavior than did the dansyl probe attached to DVB. From this it was realized that the dansyl probe situated on the cross-linker was less accessible to solvent even in the swollen state, suggesting that substantial nodulation exists in the CLPS matrix. The same study, but with a dansyl probe attached to *para*-divinyl benzene, would have provided more meaningful data on commonly used CLPS.

A similar approach has been reported by Kurth and coworkers.[125] In this approach, trityl ethers, as mono- or divinyl monomers, were incorporated in the polymer attached to monomers or cross-linkers. Pyrene was attached to the trityl ethers by introduction of 4-(1-pyrenyl)butanol. Pyrene was used as it exhibits an excimer when in close proximity to another pyrene unit. The excimer to monomer emission intensity ratio (I_e/I_m) was measured. Shifts in the relative intensities provides information as to the ability of the cross-linked polymer chains to move about in the swollen state. Excimer formation requires the encounter of an excited pyrene with a ground-state pyrene at a distance of 3−3.7 Å.[126] It was noted that the ratio I_e/I_m was markedly lower for polymers having pyrenes solely as the cross-linkers, further implying greater difficulty in forming the requisite associations. The solvents used were CH_2Cl_2 or THF; the degree of cross-linking was 6%. For the trityl cross-link, solely *para*-vinyl groups off the phenyl ring were used. In good solvents, I_e/I_m was noted to increase; in poor solvents, it was reduced. Oddly, the cross-linked pyrene exhibited greater I_e/I_m in DMF than did the styrene bound pyrene. A similar phenomenon has been reported using radical probes bound to both styrene and cross-linker exhibiting greater mobility solely in DMF.[68] No good explanation was offered except to mention that it cast aspersions on the observation that simple steric crowding causes the differences.

A similar experiment was performed using dansyl-doped PS resins cross-linked with PEG.[127] It was noted that while PS-DVB was less swollen by a range of common solvents than PS-PEG, the overall trend regarding cross-linking was the same. Interestingly, swelling did not appear to be a function of PEG tether length at low (∼2%) cross-link percent. At higher nominal degree of cross-linking (20%) solvent uptake increased with increasing PEG length. The effect of mixtures of THF and water were examined. Both DVB and PEG as cross-linkers displayed nearly identical swelling below 50% THF. In all cases, as the proportion of THF dropped below 50% poor swelling was observed for all resins having 2% CL, a consequence of the small overall contribution of the polar PEG moiety to the polymeric matrix as a whole. Also, the fluorescence quenching of a dansyl probe as previously installed was monitored. In this instance the PEG cross-linked resin demonstrated faster quenching than did the DVB cross-linked resin. The enhanced solvent polarity imparted by the PEG aids in the quenching with the polar electrophile.

A study on the physical properties of TG examined the interaction of fluorescein-isocyanate labeled porcine pancreatic trypsin with amino TG. As a control, per-acetylated amino TG was also treated with the fluorescent isocyanate to account for nonspecific interaction between the large enzyme and the resin. Interaction between the fluorescent enzyme isocyanate and the amino TG showed a marked increase in fluorescence throughout the bead in water.[89] The acetamide capped TG subjected to the same conditions displayed some residual fluorescence due to noncovalent interactions with the fluorophore, but this was muted. This is of use in that the FICT-PPT is an enzyme with a molecular weight greater than 23.5 kDa and it was seen even in the center of a 90 μm bead, providing evidence that TG is amenable to on-bead biological analysis in water.

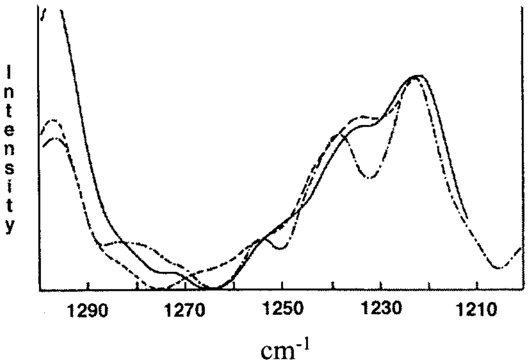

Figure 13. FTIR spectra of polymer bound Fmoc-(Ala)$_6$-Lys(Boc)-OR. Solid line in dry state, dashed line solvated with DMSO. Reproduced with permission from Larsen, B. D.; Christensen, D. H.; Holm, A.; Zillmer, R.; Nielsen, O. F. *J. Am. Chem. Soc.* **1993**, *115*, 6247. Copyright 1993 American Chemical Society.

An analytical method using fluorescence spectroscopy for the on-bead analysis of terminal aldehydes has been described. By introduction of a fluorescent dansyl hydrazine the corresponding hydrazone is formed.[128] As the hydrazone is formed, fluorescence is lost, which is readily observed. A similar approach to reaction monitoring using UV−vis spectroscopy has been reported.[129] In this report, the reagent nitro phenylisocyanate-*O*-trityl (NPIT) was affixed to amines with concomitant release of a trityl cation, which was readily seen as an absorbance at 498 nm.

Infrared Spectroscopy. The effect of swelling on peptide chains attached to a cross-linked polyacrylamide resin has been noted using IR. The IR spectrum of dry polymer bound Fmoc-(Ala)$_6$-Lys(Boc)-OR was nearly identical to the spectrum in DMSO, a poor solvent for polyacrylamide. The same peptide-resin mixture in DMF was markedly better resolved, an consequence of easier chain mobility arising from swelling.[130] This effect is depicted in Figure 13.

On Bead Analysis by IR. Infrared spectroscopy has been extensively applied to the monitoring of reactions on solid phases for decades.[131] There exists a surfeit of examples in the literature of the use of infrared spectroscopy for reaction monitoring, either as simple admixtures in standard KBr disks,[132] as single beads in a FTIR microspectrometer,[133] photoacoustic FTIR,[134] diffuse reflectance FTIR,[135] FT Raman spectroscopy,[136] or flow-through cell IR.[137] An excellent paper comparing and contrasting currently existing methods has appeared.[138] As with direct on-bead analysis by NMR, IR approaches the utility of TLC for SPOS with the advantage of requiring less time.

The use of FTIR monitoring was applied to a study of site-separation on solid supports.[139] Wang resin was treated with diacid chlorides of varying length. If site isolation is in effect, then, after introduction of the first acid chloride to form an ester the second, equally highly reactive acid chloride, should not be able to react further to form a new cross-link. The shift in carbonyl vibrations for the respective ester and acyl chloride groups are readily discernible. Time course analysis demonstrated that all reactions were complete within 10 min. When a 2-fold excess of diacid chloride was employed, extra cross-links were introduced. Using a 10-fold excess of reagent, no extra cross-linking was observed. This lack of additional cross-linking was attributed to the large excess of diacid chloride consuming all available sites for reaction prior to cross-linking. The length of the diacid chloride, from C-2 to C-6, was found to exert no influence.

Another interesting approach to on-bead analysis has been the development of an on-line FTIR spectrometer capable of monitoring resin beads directly from the reaction mixture and analyzing them.[140] The flow-through cell described allows for rapid sampling without the need to form a KBr disk or remove a bead from the reaction mixture.

The flow-through cell method was further used to examine partitioning of solvents into swollen beads.[141] Reagent partitioning refers to inclusion or exclusion of reagent by the swollen phase and is equivalent to partitioning between immiscible liquid phases in a separatory funnel. The direction and degree of partitioning are functions of the relative solubility of the reagent in the swollen bead and in the bulk solvent. For typical beads the solvent exerted an effect on the partitioning of the solute into the gel phase. For a series of imides and amides, increased uptake into beads occurs with increasing substitution of nitrogen in DMF or THF, while the opposite trend was observed in CH_2Cl_2. For alcohols, a decrease in reagent uptake was noted as the length of alkyl chain increased in all solvents. Simple filling of pores was accounted for by noting differences in vibrational frequencies for species bound to resin, and those merely in solution, or in the liquid-phase inside a pore. That entry of small organic moleclues into the beads is not controlled by size exclusion is an important result and points further to the solvent behavior of the swollen resin.

Kinetics of Solid-Phase Reactions

A study of the kinetics of coupling reactions of peptides on a solid phase has been reported.[142] A vessel able to extract aliquots of a solution and measure the UV spectrum of the solution above a resin bed was constructed. A disadvantage of this apparatus is that only disappearance of starting material is observable, and appreciable amounts of unreacted reagent will be sequestered inside the swollen resin, skewing results. Reactions were noted to be over in as little as 14 s for coupling via either an active anhydride or DCC. Adding tethers off the polymer backbone had no effect on reactivity. It should be noted that the scale on which the reaction was performed was not mentioned.

A further report on the kinetics of amide bond formation, in this case attachment of a Knorr linker to a terminal amine, has been reported.[143] Disappearance of the amine was monitored by removal of aliquots and Fmoc estimation of the free amine content remaining. The reaction, DIEA/PyBOP coupling, on the same size bead was nearly 3 times as fast when CH_2Cl_2 was the solvent than when DMF was the solvent. Consistent with other studies, the rate of reaction varied inversely with bead size. Reactions were also performed on resins with PEG grafts. The PEG resins afforded substantially faster reactions. Swollen volume was not taken into account when comparing resins. A similar report examining the kinetics of reactions on polystyrene grafted onto polypropylene has appeared.[144]

One of the main advantages of single bead monitoring by FTIR is the ability to readily perform kinetic experiments. This was exploited to great benefit in the monitoring[145] of tetra-*n*-propylammonium perruthenate (TPAP)[146] oxidation of alcohols, an early report of the application of TPAP to the oxidation of a polymer bound hydroxyl group. Oxidation of an alkyl hydroxyl group was found to be 98% complete in 40 min, similar to the time required in solution.[147] This is beneficial in that it points out that polymer-supported reactions can indeed be quite quick, often not requiring the extended reaction times commonly reported in the literature.

A study on the effects of resin on rates of reactions as monitored by single bead FTIR provides useful insight into the dynamics of polymer-substrate interactions.[148] In particular, the rates of various reactions both on CLPS and on TG were examined. TentaGel is CLPS onto which has been grafted long PEG oligomers (50−60 ethylene oxide units). Once formed, the PEG content of the resin approaches 70 wt %. Thus, the influence of the PEG on the physical properties of the resin is profound. This is particularly evident in that reactions can be performed in water, a solvent to which CLPS is completely impervious. TPAP oxidation was found to be four times faster on TG than on CLPS (1% CL was used for each). DIC/DMAP esterification was found to be the same on both resins. Synthesis of hydrazones was 2.2 times faster on CLPS. Opening of a lactone was 18 times faster on CLPS. Thus, it is self-evident that rates of reaction are not necessarily faster on the supposedly free-flowing PEG tethers. It should be noted that as the grafting of the PEG chains onto the CLPS occurs after polymerization, the actual effect is to reduce the free volume of the cross-linked polymer. Also, the idea of free flowing chains does not take entanglements between the linear oligomers into account; in the confined space they find themselves additional “virtual cross-links”, are no doubt in effect. These kinetic studies did not consider a fundamental variable in any rate study, namely, concentration. This study did not investigate different swollen volumes, the effective volume which the reactant is in, effecting the actual molarity of the bound substance. As reaction rates are concentration dependent, this is clearly a key point when comparing rates of reaction on different resins.

The above paper discusses debunking the idea that TG is more “solution-like”. So-called “solution-like” behavior was thought to arise from the supposed floating nature of the PEG tethers. As the comparison of the reactions (vide supra) demonstrates, while naïvely this tethering should result in uniformly more rapid kinetics, this is not observed. Reaction with TPAP, a highly polar salt, may well be expected to proceed at a higher rate owing to the hydrophilicity imparted to the matrix by the PEG grafts. However, in no other case were faster rates observed. These experimental results provide the first report of the realization that the matrix of the swollen polymer *is* the solvent, not merely "solution-like".

Miscellaneous Methods

The use of small angle neutron scattering, (SANS), has been applied to the study of highly cross-linked polystyrene. The polymers studied were not eligible for use in SPOS, having been prepared with large proportions of cross-linker; however, the results provide some insight into the physicochemical changes incumbent on solvent swelling. It was noted that for a nonsolvent, ethanol, the proportion of porogen employed during polymerization increased the pore size, the pores of which were closed to ethanol, a nonsolvent for CLPS.[149] By contrast, with the use of a good solvent for CLPS, for example toluene, it was noted that the pores opened up, relaxing their structure and permitting toluene to enter.[150] The pore structure of the dry polymer is clearly different from that of the swollen polymer. SANS permits the study of changes in resin porosity. The experimental protocol adopted to note differences was to use a solvent with a high contrast of neutron scattering between solid and liquid, i.e., deuterated solvents.

Lithium salts have been implicated in increasing yields of peptide coupling reactions in solution by modification of challenging conformations.[151] In a study of the coupling efficiency of peptides on some resins, CLPS and TG, it was noted that for some peptides the rate of swelling is increased by the presence of 0.4 M LiCl. The kinetics of the reaction were also affected, although not in a predictable fashion. In the more polar TG resin coupling efficiency was markedly improved by addition of salt.[152]

A method has been devised to directly examine the mechanical strength of individual swollen beads—a critical concern.[153] Swelling of the beads was found to decrease the penetration modulus by 100 times for cellulose. The modulus depended on the radius (r) of the beads. When $r > 0.4$ mm the penetration modulus was independent of radius, when $r < 0.4$ mm the moduli increased with decreasing r. In the swollen state, moduli for Amberlite were comparable to cellulose; however, no comparison to the dry beads was made.

Conclusion

A myriad of physical studies have been performed on resins used in SPOS. No single technique will adequately address a system as complex as a cross-linked polymer network. However, by judicious choice of spectroscopic techniques fundamental questions of importance to the practice of SPOS can indeed be answered. Knowledge of the internal motions of the polymer backbone, of the direct interaction between polymer and solvent, and of degrees by which movement changes depending on the physicochemical properties of both the resin and the solvent provides the scientist with enough information to successfully plan experiments. Taken with kinetic data and being cognizant of the effects of swelling and reagent partitioning, there is no reason SPOS should not be as readily performed as traditional organic synthesis. The admixture of cross-linked polymer and solvent, the swollen polymer, *is* the solvent as surely as a mixture of THF and benzene *is* a solvent, not merely a liquid having solvent-like properties.

Acknowledgment. This work was supported by funding from the Skaggs Institute for Chemical Biology and the National Institutes of Health (GM56154). A.R.V. thanks NSERC (Canada) for a postdoctoral fellowship. The authors thank Dr. Martin R. Tremblay for pithy discussions.

References and Notes

(1) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

(2) (a) Brown, R. D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3293. (b) Baxter, A. D. *Curr. Opin. Chem. Biol.* **1997**, *1*, 79. (c) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. *Tetrahedron* **1996**, *52*, 4527.

(3) Merrifield, B. In *Life During a Golden Age of Peptide Chemistry*; Seeman, J. I., Ed.; Profiles, Pathways and Dreams: Autobiographies of Eminent Chemists; American Chemical Society: Washington, DC, 1993.

(4) (a) Ellman, J. A. *Acc. Chem. Res.* **1996**, *29*, 132. (b) Ellman, J. A.; Thompson, L. A. *Chem. Rev.* **1996**, *96*, 555. (c) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.

(5) Czarnik, A. W. *Biotechnol. Bioeng. (Comb. Chem.)* **1998**, *61*, 77.

(6) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1.

(7) (a) Felder, E. R. *Comb. Chem. Technol.* **1999**, 35. (b) Renneberg, B.; Labadie, J. W. *Chim. Oggi* **1999**, *17*, 7. (c) Sherrington, D. C. *J. Chem. Soc., Chem. Commun.* **1998**, 2275. (d) Labadie, J. W. *Curr. Opin. Chem. Biol.* **1998**, *2*, 346. (e) Hodge, P. *Chem. Soc. Rev.* **1997**, *26*, 417. (f) Svec, F.; Fréchet, J. M. J. *Science* **1996**, *276*, 782. (g) Crowley, J. I.; Rapoport, H. *Acc. Chem. Res.* **1976**, *9*, 135.

(8) (a) Yan, B. *Comb. Chem. High Throughput Screening* **1998**, *1*, 215. (b) Meldal, M. In *Methods in Enzymology: Solid-phase peptide Synthesis;* Fields, G., Ed.; Academic press: London, 1997; p 83. (c) Fréchet, J. M. J.; Darling, G. D.; Itsuno, S.; Lu, P.-Z.; de Meftahi, M. V.; Rolls, W. A., Jr. *Pure Appl. Chem.* **1988**, *60*, 353. (d) Guyot, A.; Bartholin, M. *Prog. Polym. Sci.* **1982**, *8*, 277.

(9) Even TentaGel, arguably the second most common resin, is based on cross-linked polystyrene. For a review covering alternatives to cross-linked polystyrene, see: Bergbreiter, D. E. *Med. Res. Rev.* **1999**, *19*, 439.

(10) (a) Dusek, K.; MacKnight, W. J. In *Cross-linked Polymers Chemistry, Properties, and Applications;* Dickie, R. A., Labana, S. S., Bauer, R. S., Eds.; ACS Symposium Series 367; American Chemical Society: Washington, 1998. (b) Hild, G. *Prog. Polym. Sci.* **1998**, *23*, 1019.

(11) An excellent case study describing application of many techniques for on-bead analysis has recently appeared: Grice, P.; Leach, A. G.; Ley, S. V.; Massi, A.; Mynett, D. M. *J. Comb. Chem.* **2000**, *2*, 491.

(12) Andreatta, R. H.; Rink, H. *Helv. Chim. Acta* **1973**, *56*, 1205.

(13) See, for a few examples: (a) Mikos, A. G.; Takoudis, C. G.; Peppas, N. A. *Polymer* **1987**, *28*, 998. (b) Hild, G.; Okasha, R. *Makramol. Chem.* **1985**, *186*, 93. (c) Kwant, P. W. *J. Polym. Sci.: Part A: Polym. Chem.* **1979**, *17*, 1331.

(14) Both *meta*- and *para*-divinyl benzene have been isolated[15] and synthesized[16] separately.

(15) Wiley, R. H. *Pure Appl. Chem.* **1975**, *43*, 57.

(16) Wiley, R. H.; Jin, J. *J. Macromol. Sci.: Chem.* **1969**, *A3*, 835.

(17) Wiley, R. H.; DeVenuto, G.; Martin, F. E. *J. Macromol. Chem.* **1966**, *1*, 137.

(18) Haeupke, H.; Pientka, V. *J. Chromatogr.* **1974**, *102*, 117.

(19) Attributed to D. C. Sherrington in: Hudson, D. *J. Comb. Chem.* **1999**, *1*, 333.

(20) (a) Arshady, R. *J. Chromatogr.* **1991**, *586*, 181. (b) Arshady, R.; Ledwith, A. *React. Polym.* **1983**, *1*, 159.

(21) Galina, H.; Kolarz, B. N.; Wieczorek, P. P.; Wojcznska, M. *Br. Polym. J.* **1985**, *17*, 215.

(22) (a) Arshady, R. *J. Chromatogr.* **1991**, *586*, 199. (b) Ahmed, S. M. *J. Dispersion Sci. Technol.* **1984**, *5*, 421.

(23) (a) Lewandowski, K.; Svec, F.; Fréchet, J. M. J. *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20*, 227. (b) Li, W.-H.; Li, K.; Stover, H. D. H. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 2295.

(24) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.

(25) This definition clearly does not include highly cross-linked polymers such as Agropore wherein reactions occur solely at the surface of the porous material.

(26) Attributed to R. B. Merrifield in ref 19.

(27) McAlpine, S. R.; Schreiber, S. L. *Chem. Eur. J.* **1999**, *5*, 3528.

(28) Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 113.

(29) Flory, P. J. *Macromolecules* **1979**, *12*, 119.

(30) Barton, A. F. M. *Chem. Rev.* **1975**, *75*, 731.

(31) Flory, P. J.; Rehner, J. *J. Chem. Phys.* **1943**, *11*, 521.

(32) Hildebrand, J. H.; Scott, R. L. *The Solubility of Non-Electrolytes*, 3rd ed.; Reinhold: New York, 1950.

(33) Gee, G. *Trans. Faraday Soc.* **1942**, *38*, 418.

(34) Small, P. A. *J. Appl. Chem.* **1953**, *3*, 71.

(35) Hoy, K. L. *J. Paint Technol.* **1970**, *42*, 76.

(36) Deslandes, N.; Bellenger, V.; Jaffiol, F.; Verdu, J. *J. Appl. Polym. Sci.* **1998**, *69*, 2663.

(37) Morel, E.; Bellenger, V.; Bocquet, M.; Verdu, J. *J. Mater. Sci.* **1989**, *24*, 63.

(38) Bellenger, V.; Verdu, J.; Ganem, M.; Montaigne, B. *Polym. Polym. Compos.* **1994**, *2*, 9.

(39) (a) Fields, G. B.; Fields, C. G. *J. Am. Chem. Soc.* **1991**, *113*, 4202. (b) Cilli, E. M.; Oliveira, E.; Marchetto, R.; Nakaie, C. R. *J. Org. Chem.* **1996**, *61*, 8992. (c) Wilson, M. E.; Paech, K.; Zhou, W.-J.; Kurth, M. J. *J. Org. Chem.* **1998**, *63*, 5094.

(40) Suh, K. W.; Clarke, D. H. *J. Polym. Sci. Part A-1* **1967**, *5*, 1671.

(41) Bristow, G. M.; Watson, W. F. *Trans. Faraday Soc.* **1958**, *54*, 1742.

(42) Errede, L. A. *Macromolecules* **1986**, *19*, 1522.

(43) Sperling, L. H. *Introduction To Physical Polymer Science*; Wiley-Interscience: Toronto; 1986.

(44) Burrell, H. In *Polymer Handbook*, 2nd ed.; Brandup, J., Immergut, E. H., Eds.; Wiley-Interscience: New York, 1975.

(45) Gutmann, V. *Electrochim. Acta* **1976**, *21*, 661.

(46) Cilli, E. M.; Oliveira, E.; Marchetto, R.; Nakaie, C. R. *J. Org. Chem.* **1996**, *61*, 8992.

(47) Sarin, V. K.; Kent, S. B. H.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 5463.

(48) Auzanneau, F.-I.; Meldal, M.; Bock, K. *J. Peptide Sci.* **1995**, *1*, 31.

(49) Stamberg, J.; Sevcík, S. *Collect. Czech. Chem. Commun.* **1966**, *31*, 1009.

(50) Santini, R.; Griffith, M. C.; Qi, M. *Tetrahedron Lett.* **1998**, *39*, 8951.

(51) Itsuno, S.; Sakurai, Y.; Maruyama, T.; Nakahama, S.; Fréchet, J. M. J. *J. Org. Chem.* **1990**, *55*, 304.

(52) Ferry, J. D. *Viscoelastic Properties of Polymers*; John Wiley & Sons: New York, 1961.

(53) Ueberreiter, K.; Kanig, G. *J. Chem. Phys.* **1950**, *18*, 399.

(54) Gutierrez, M. H.; Ford, W. T. *J. Polym. Sci.: Part A: Polym. Chem.* **1986**, *24*, 655.

(55) (a) Pochan, J. M.; Beatty, C. L.; Pochan, D. F. *Polymer* **1979**, *20*, 879. (b) Fox, T. G. *Bull. Am. Phys. Soc.* **1956**, *1*, 123. (c) Couchman, P. R.; Karasz, F. E. *Macromolecules* **1978**, *11*, 117. (d) Brinke, G. T.; Karasz, F. E.; Ellis, T. S. *Macromolecules* **1983**, *16*, 244.

(56) Tjoeng, F. S.; Tam, J. P.; Merrifield, R. B. *Int. J. Pept. Protein Res.* **1979**, *14*, 262.

(57) Sarin, V. K.; Kent, S. B. H.; Mitchell, A. R.; Merrifield, R. B. *J. Am. Chem. Soc.* **1984**, *106*, 7845.

(58) (a) Waggoner, A. S.; Griffith, O. H.; Christensen, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 1198. (b) Freed, J. H.; Fraenkel, G. K. *J. Phys. Chem.* **1963**, *39*, 326.

(59) Griffith, O. H.; Waggoner, A. S. *Acc. Chem. Res.* **1969**, *2*, 17.

(60) (a) Bullock, A. T.; Butterworth, J. H.; Cameron, G. G. *Eur. Polym. J.* **1971**, *7*, 445. (b) Bullock, A. T.; Cameron, G. G.; Smith, P. M. *J. Polym. Sci.: Polym. Phys. Ed.* **1973**, *11*, 1263.

(61) Chesnut, D. B.; Hower, J. F. *J. Phys. Chem.* **1971**, *75*, 907.

(62) Berliner, L. J. *Biological Magnetic Resonance: vol. 8*; Academic Press: New York, 1989.

(63) Ward, T. C.; Books, J. T. *Macromolecules* **1974**, *7*, 207.

(64) Regen, S. L. *J. Am. Chem. Soc.* **1974**, *96*, 5275.

(65) Regen, S. L. *Macromolecules* **1975**, *8*, 689.

(66) Sparrow, J. T. *J. Org. Chem.* **1976**, *41*, 1350.

(67) Vaino, A. R.; Goodin, D. B.; Janda, K. D. Unpublished results.

(68) Wilson, M. E.; Wilson, J. A.; Kurth, M. J. *Macromolecules* **1997**, *30*, 3340.

(69) Regen, S. L. *J. Am. Chem. Soc.* **1975**, *97*, 3108.

(70) Marchetto, R.; Schreier, S.; Nakaie, C. R. *J. Am. Chem. Soc.* **1993**, *115*, 11042.

(71) Cilli, E. M.; Marchetto, R.; Schreier, S.; Nakaie, C. R. *Tetrahedron Lett.* **1997**, *38*, 517.

(72) Cilli, E. M.; Marchetto, R.; Schreier, S.; Nakaie, C. R. *J. Org. Chem.* **1999**, *64*, 9118.

(73) Heitz, W. *Adv. Polym. Sci.* **1977**, *1*, 23.

(74) Toy, P. H.; Janda, K. D. *Tetrahedron Lett.* **1999**, *40*, 6329.

(75) Vaino, A. R.; Goodin, D. B.; Janda, K. D. *J. Comb. Chem.* **2000**, *2*, 330.

(76) Incorporation of the TEMPO during free-radical polymerization has been reported; however, it is found to result in the introduction of extra cross-links: Li, C.; He, J.; Li, L.; Cao, J.; Yang, Y. *Macromolecules* **1999**, *32*, 7012.

(77) Gordon, J. E. *J. Phys. Chem.* **1962**, *66*, 1150.

(78) Duch, M. W.; Grant, D. M. *Macromolecules* **1970**, *3*, 165.

(79) Ford, W. T.; Mohranraj, S.; Periyasamy, M. *Br. Polym. J.* **1984**, *16*, 179.

(80) Waugh, J. S. In *NMR and Biochemistry*; Opella, S. J., Lu, P., Eds.; Marcel Dekker: New York, 1980: 203.

(81) Creekmore, R. W.; Reilley, C. N. *Anal. Chem.* **1970**, *42*, 570.

(82) Sternlicht, H.; Kenyon, G. L.; Packer, E. L.; Sinclair, J. *J. Am. Chem. Soc.* **1971**, *93*, 199.

(83) Ford, W. T.; Balakrishnan, T. *Macromolecules* **1981**, *14*, 284.

(84) Ford, W. T.; Balakrishnan, T. *Adv. Chem. Ser.* **1983**, *203*, 475.

(85) Ludwick, A. G.; Jelinski, L. W.; Live, D.; Kintanar, A.; Dumais, J. J. *J. Am. Chem. Soc.* **1986**, *108*, 6493.

(86) Bayer, E.; Albert, K.; Willisch, H.; Rapp, W.; Hemmasi, B. *Macromolecules* **1990**, *23*, 1937.

(87) Wentworth, P., Jr.; Janda, K. D. *J. Chem. Soc., Chem. Commun.* **1999**, 1917.

(88) Keifer, P. A. *J. Org. Chem.* **1996**, *61*, 1558.

(89) Quarrell, R.; Claridge, T. W.; Weaver, G. W.; Lowe, G. *Mol. Diversity* **1995**, *1*, 223.

(90) Grøtli, M.; Gotfredsen, C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J. Ø.; Meldal, M. *J. Comb. Chem.* **2000**, *2*, 108.

(91) Manatt, S. L.; Horowitz, D.; Horowitz, R.; Pinnell, R. P. *Anal. Chem.* **1980**, *52*, 1529.

(92) Olah, G.; Tolgyesi, W. S. In *Friedel−Crafts and Related Reactions*; Olah, G. A., Ed.; Wiley-Interscience: New York, 1964; Vol. 2, pp 659−784.

(93) Ford, W. T.; Yacoub, S. A. *J. Org. Chem.* **1981**, *46*, 819.

(94) Pepper, K. W.; Paisley, H. M.; Young, M. A. *J. Chem. Soc.* **1953**, 4097.
(95) Mohanraj, S.; Ford, W. T. *Macromolecules* **1985**, *18*, 351.
(96) Jones, G. D. *Ind. Eng. Chem.* **1952**, *44*, 2686.
(97) *Advanced Chemtech Handbook of Combinatorial & Solid-Phase Organic Chemistry*; Bennett, W. D., Christensen, J. W., Hamaker, L. K., Peterson, M. L., Rhodes, M. R., Saneii, H. H., Eds.; Advanced Chemtech Inc.: Louisville, 1998; pp 115−116.
(98) Bardella, F.; Eritja, R.; Pedroso, E.; Giralt, E. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2793.
(99) Sarkar, S. K.; Garigipati, R. S.; Adams, J. L.; Keifer, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 2305.
(100) Fitch, W. L.; Detre, G.; Holmes, C. P. *J. Org. Chem.* **1994**, *59*, 7955.
(101) Anderson, R. C.; Jarema, M. A.; Shapiro, M. J.; Stokes, J. P.; Ziliox, M. *J. Org. Chem.* **1995**, *60*, 2650.
(102) Stöver, H. D. H.; Fréchet, J. M. J. *Macromolecules* **1991**, *24*, 883.
(103) Keifer, P. A.; Baltusis, L.; Rice, D. M.; Tymiak, A. A.; Shoolery, J. N. *J. Magn. Reson. Ser. A* **1996**, *119*, 65.
(104) Drew, M.; Orton, E.; Krolikowski, P.; Salvino, J. M.; Kumar, N. V. *J. Comb. Chem.* **2000**, *2*, 8.
(105) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588.
(106) Dhalluin, C.; Boutillon, C.; Tartar, A.; Lippens, G. *J. Am. Chem. Soc.* **1997**, *119*, 10494.
(107) Pop, I. E.; Dhalluin, C. F.; Déprez, B. P.; Melnyk, P. C.; Lippens, G. M.; Tartar, A. L. *Tetrahedron* **1996**, *52*, 12209.
(108) Gotfredsen, C. H.; Grøtli; Willert, M.; Meldal, M.; Duus, J. Ø. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1167.
(109) Epton, R.; Goddard, P.; Ivin, K. J. *Polymer* **1980**, *21*, 1367.
(110) Lorgé, F.; Wagner, A.; Mioskowski, C. *J. Comb. Chem.* **1999**, *1*, 25.
(111) (a) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288. (b) Stilbs, P. J. *Prog. NMR Spectrosc.* **1987**, *19*, 1.
(112) Pickup, S.; Blum, F. D.; Ford, W. T.; Periyasamy, M. *J. Am. Chem. Soc.* **1986**, *108*, 3987.
(113) Ford, W. T.; Ackerson, B. J.; Blum, F. D.; Periyasamy, M.; Pickup, S. *J. Am. Chem. Soc.* **1987**, *109*, 7276.
(114) Ford, W. T.; Periyasamy, M.; Spivey, H. O.; Chandler, J. P. *J. Magn. Reson.* **1985**, *63*, 298.
(115) Ogino, K.; Sato, H. *J. Appl. Polym. Sci.* **1995**, *58*, 1015.
(116) Stranix, Brent R.; Liu, Hua Qin; Darling, Graham D. *J. Org. Chem.* **1997**, *62*, 6183.
(117) (a) Regen, S. L.; Bolikal, D. *J. Am. Chem. Soc.* **1981**, *103*, 5248. (b) Kim, B.; Kirszensztejn, P.; Bolikal, D.; Regen, S. L. *J. Am. Chem. Soc.* **1983**, *105*, 1567.
(118) Regen, S. L.; Besse, J. J. *J. Am. Chem. Soc.* **1979**, *101*, 4059.
(119) Tomoi, M.; Ford, W. T. *J. Am. Chem. Soc.* **1980**, *102*, 7141.
(120) Tomoi, M.; Ford, W. T. *J. Am. Chem. Soc.* **1981**, *103*, 3821.
(121) (a) Li, Y.-H.; Chan, L.-M.; Tyer, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118. (b) Strauss, U. P.; Vesnaver, G. *J. Phys. Chem.* **1975**, *79*, 2426.
(122) Shea, K. J.; Sasaki, D. Y.; Stoddard, G. J. *Macromolecules* **1989**, *22*, 1722.
(123) Shea, K. J.; Stoddard, G. J.; Sasaki, D. Y. *Macromolecules* **1989**, *22*, 4303.
(124) Shea, K. J.; Stoddard, G. J. *Macromolecules* **1991**, *24*, 1207.
(125) Wilson, M. E.; Wilson, J. A.; Kurth, M. J. *Macromolecules* **1997**, *30*, 3340.
(126) Wang, U.; Marawetz, H. *Makromol. Chem.* **1975**, *1*, 283.
(127) Wilson, M. E.; Paech, K.; Zhou, W.-J.; Kurth, M. J. *J. Org. Chem.* **1998**, *63*, 5094.
(128) (a) Yan, B.; Li, W. *J. Org. Chem.* **1997**, *62*, 9354. (b) Yan. B.; Martin, P. C.; Lee, J. *J. Comb. Chem.* **1999**, *1*, 78.
(129) Chu, S. S.; Reich, S. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1053.
(130) Larsen, B. D.; Christensen, D. H.; Holm, A.; Zillmer, R.; Nielsen, O. F. *J. Am. Chem. Soc.* **1993**, *115*, 6247.
(131) Frechet, J. M. J.; Scuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492.
(132) (a) Crowley, J. I.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 3215. (b) Chen, C.; Ahlberg Randall, L. A.; Miller, R. B.; Jones, A. D.; Kurth, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 2261.
(133) (a) Yan, B.; Kumaravel, G.; Anjaria, H.; Wu, A.; Petter, R.; Jewell, C. F., Jr.; Wareing, J. R. *J. Org. Chem.* **1995**, *60*, 5736. (b) Yan, B.; Fell, J. B.; Kumaravel, G. *J. Org. Chem.* **1996**, *61*, 7467. (c) Marti, R. E.; Yan, B.; Jarosinski, M. A. *J. Org. Chem.* **1997**, *62*, 5615. (d) Sun, Q.; Yan, B. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 361. (e) Yan, B. *Acc. Chem. Res.* **1998**, *31*, 621.
(134) Gosselin, F.; Di Renzo, M.; Ellis, T. H.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 7980.
(135) Chan, T. Y.; Chen, R.; Sofia, M. J.; Smith, B. C.; Glennon, D. *Tetrahedron Lett.* **1997**, *38*, 2821.
(136) Ryttersgaard, J.; Larsen, B. D.; Holm, A.; Christensen, D. H.; Nielsen, D. F. *Spectrochim. Acta, Part A* **1997**, *38*, 7980.
(137) Pivonka, D. E.; Simpson, T. R. *Anal. Chem.* **1997**, *69*, 3851.
(138) Yan, B.; Gremlich, H.-U.; Moss, S.; Coppola, G. M.; Sun, Q.; Liu, L. *J. Comb. Chem.* **1999**, *1*, 46.
(139) Yan, B.; Sun, Q. *J. Org. Chem.* **1998**, *63*, 55.
(140) Pivonka, D. E.; Russell, K.; Gero, T. *Appl. Spectrosc.* **1996**, *50*, 1471.
(141) Pivonka, D. E.; Palmer, D. L. *J. Comb. Chem.* **1999**, *1*, 294.
(142) Merrifield, B. *Br. Polym. J.* **1984**, *16*, 173.
(143) Li, W.; Xiao, X.; Czarnik, A. W. *J. Comb. Chem.* **1999**, *1*, 127.
(144) Li, W.; Czarnik, A. W.; Lillig, J.; X. X.-Y. *J. Comb. Chem.* **2000**, *2*, 224.
(145) Yan. B.; Sun, Q.; Wareing, J. R.; Jewell, C. F. *J. Org. Chem.* **1996**, *61*, 8765.
(146) Griffith, W. P.; Ley, S. V.; Whitcomb, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625.
(147) Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639.
(148) Li, W.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092.
(149) Hall, P. J.; Machado, W. R.; Galan, D. G.; Barria, E. B.; Sherrington, D. C. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 2607.
(150) Hall, P. J.; Galan, D. G.; Machado, W. R.; Mondragon, F.; Barria, E. B.; Sherrington, D. C. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 463.
(151) (a) Seebach, D.; Thaler, A.; Beck, A. K. *Helv. Chim. Acta* **1989**, *72*, 857. (b) Kessler, H.; Gehrke, M.; Lautz, M.; Köck, D.; Seebach, D.; Thaler, A. *Biochem. Pharmacol.* **1990**, *40*, 169.
(152) Thaler, A.; Seebach, D.; Cardinaux, F. *Helv. Chim. Acta* **1991**, *74*, 628.
(153) Peska, J.; Hradil, J.; Ilavsky, M. *J. Chromatogr.* **1976**, *125*, 455.

CC000046O