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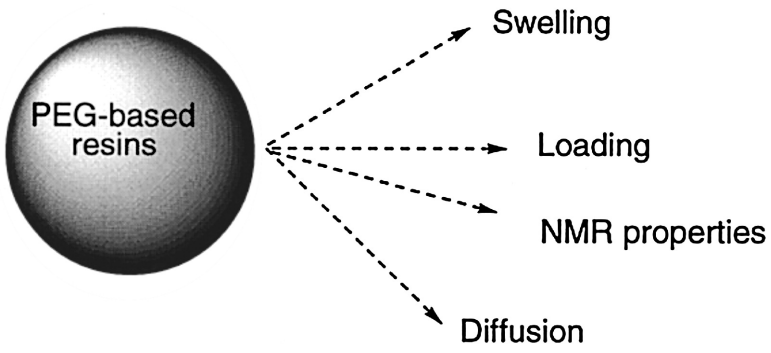

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Articles

Physical Properties of Poly(ethylene glycol) (PEG)-Based Resins for Combinatorial Solid Phase Organic Chemistry: A Comparison of PEG-Cross-Linked and PEG-Grafted Resins

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Three series of poly(ethylene glycol) (PEG)-based polymers were synthesized and characterized with respect to their physical properties. Polyoxyethylene-polyoxypropylene (POEPOP), polyoxyethylene-polyoxetane (SPOCC), and polyoxyethylene-polystyrene (POEPS-3) were synthesized respectively by anion polymerization, cation polymerization, and radical polymerization. Both bulk and suspension modes were used to synthesize the polymers from derivatized PEG monomers (PEG 400, PEG 900, and PEG 1500). The three supports were compared with two commercially available PEG-grafted supports (TentaGel S OH, ArgoGel-OH) and two polystyrene supports (aminomethylated polystyrene [PS-NH_2] and macroporous aminomethylated polystyrene [PLAMS]) with respect to their swelling properties, loading, NMR spectral quality, as well as solvent and reagent accessibility. Loadings of 0.3−0.7 mmol/g were obtained for the PEG-based resins. Swelling of the PEG-based resins was determined to be higher than that of the PEG-grafted resins and polystyrene supports. The PEG-based resins gave better resolved high-resolution NMR spectra than the PEG-grafted resins when examined by magic angle spinning nanoprobe (MAS) NMR spectroscopy. Moreover, fluorescence quenching of polymer bound 2-amino-benzoate by protonation with *p*-toluenesulfonic acid showed moderate to fast diffusion through the polymer depending on the solvent and the polymer matrix.

Introduction

Combinatorial techniques have become increasingly successful in producing libraries of molecularly diverse compounds for analysis in various screening protocols.[1] Polymer-supported approaches have found particular favor because they simplify product isolation and allow the use of excess reagents to help force reactions to completion. One strategy that has been widely used for the assembly of compound libraries is the "split synthesis" method.[2] In this approach, the support bound starting material is divided into a number of portions that are each treated separately with a different reagent. The individual samples are pooled, mixed, and split into a number of portions that are treated in a second chemical step to produce series of portions, each containing a mixture of compounds. In principle, the use of beaded polymers in this strategy results in a solid-supported library in which each bead carries only one compound.[3] This strategy offers the attractive prospect of assaying individual beads for biological activity while the compound is still covalently attached to the resin. Approaches for synthesizing one-bead-one-compound libraries depend on the selected polymer carrier. Beaded polymers must fulfill certain criteria depending on the synthesis method and screening strategy. Substitution homogeneity and the resistance of the resin to the formation of clusters are important for the analysis of one-bead-one-compound libraries. The ability of the resin to swell in aqueous media is especially important when on-bead assays are used for screening.

Polystyrene-based supports are presently used in a variety of solid phase organic chemistries; however, due to the hydrophobic nature of this polymer, polar reagents may fail to enter the matrix.[4] Polystyrene can be modified by grafting poly(ethylene glycol) to the hydrophobic core to produce a polymer that swells in both nonpolar and polar solvents.[5−7] Among these PEG-grafted polystyrene supports TentaGel has been used extensively in solid phase synthesis because of the mechanical stability of the beads and swelling properties in organic and aqueous media.[6] ArgoGel displays similar characteristics to TentaGel yet swells more extensively because of a higher PEG content.[8] The flexible PEG grafts have been synthesized to provide a solution-like environment for bound molecules.[9,10]

The PEG-grafted resins have, however, shown limitations

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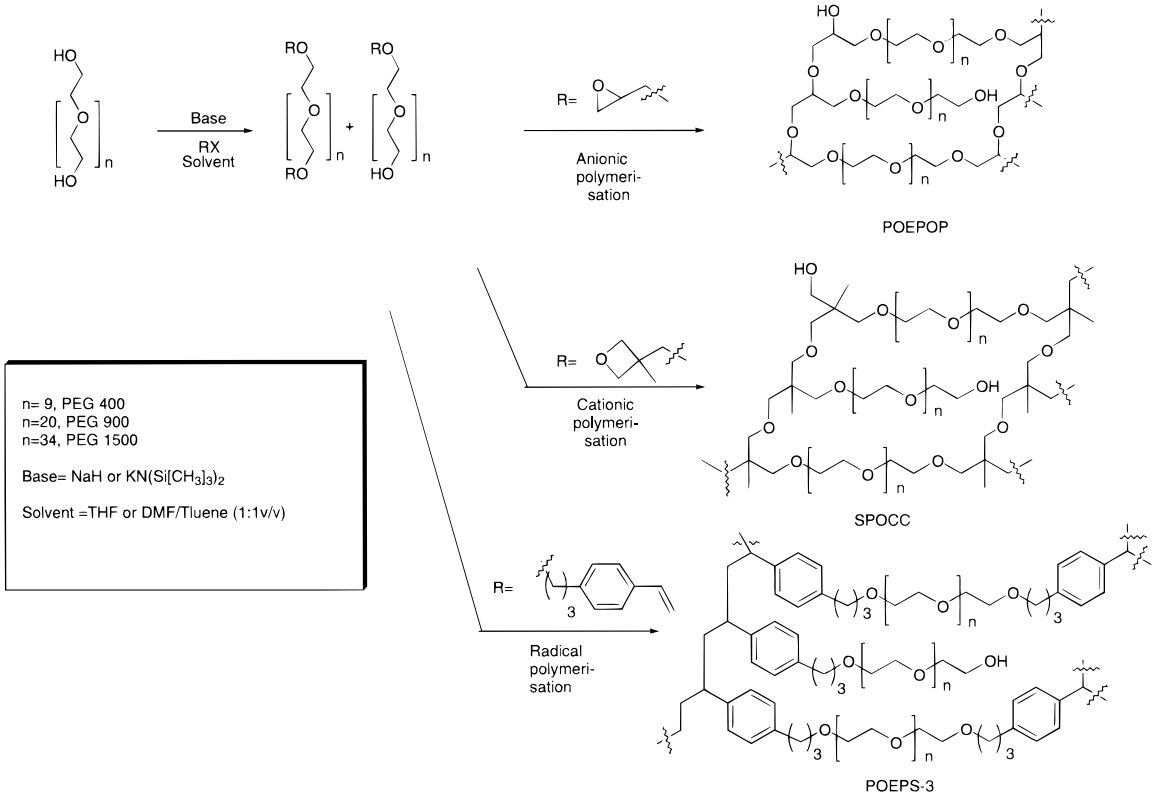
Scheme 1. Preparation of PEG Macromonomers and Polymerization To Afford POEPOP, SPOCC, and POEPS-3



with regard to their use in aqueous solution and enzymatic chemistry.[11] In contrast, a support based on using poly(ethylene glycol) as the major component of the resin can be more compatible with aqueous solution as first demonstrated with the poly(ethylene glycol)-poly(acrylamide) (PEGA) resin.[12] Because many organic reactions are not compatible with the amide functionalities present in the PEGA resin, several new PEG resins (POEPOP,[13] SPOCC,[14] and POEPS-3[15]) were developed that contain ether bonds.

As part of a research program directed toward the design and preparation of PEG-based resins for organic chemistry and enzymatic reactions on solid phase, a systematic study of three series of PEG-based polymers (POEPOP, SPOCC, and POEPS-3) is presented. Single resin bead analysis played an important role in the development of this technology, and several techniques were used to analyze compounds attached to single resin beads, including NMR spectroscopy,[16,17] where spectral quality and resolution were very dependent on the resin.

For comparison we also examined the commercially available supports, TentaGel, ArgoGel, PS-NH_2, and macroporous aminomethyl polystyrene. Swelling properties, loading, and diffusion through the polymer matrix were studied with the resins, in addition to their ability to furnish high-quality ^{1}H magic angle spinning (MAS) nanoprobe NMR spectra.

Results and Discussion

Synthesis of Solid Supports. End-modified PEG macromonomers were synthesized and used as starting materials for polymer preparation. Alkylation of commercially available PEGs (400, 900, and 1500) with epichlorohydrin, 3-methyl-oxetan-3-yl-4-methyl toluenesulfonate, or chloropropyl-styrene resulted in mixtures of non-, mono-, and bis-alkylated PEG monomer that were used in the polymerization reactions (Scheme 1). By varying the equivalents of alkylating agent, PEG monomers with various amount of functionalization were obtained. The degree of functionalization (Df) was quantified by ^{1}H NMR (Tables 1−3) in solution. POEPOP,[13] SPOCC,[14] and POEPS-3[15] were synthesized respectively by anion polymerization, cation polymerization, and radical polymerization. Polymerization of highly functionalized PEG monomers resulted in polymers with a high degree of cross-linking. The bulk polymerized material was subsequently ground, washed, dried, and sieved to an appropriate particle size (300−500 μm). These were used in the swelling and loading assays as well as in the NMR studies.

POEPS-3 was polymerized in beaded form by inverse suspension polymerization.

Although suspension and dispersion polymerization methods for producing beads from acrylic monomers are well established,[18] in most cases, water or a highly polar organic solvent is required as a continuous phase for the relatively hydrophobic monomers. Because these solvents are incompatible with cationic or anionic ring-opening polymerization, a different approach was required that avoided the use of dispersants which interfere with the polymerization. This led to the development of a suspension polymerization technique based on emulsions of the PEG monomers formed in silicon oil.[14] Beads of SPOCC 400 (300−500 μm) were obtained by adding a solution of monomers (Table 2, entry **2**) and Et_2OBF_3 in acetonitrile (MeCN) to silicon oil at ambient temperature with stirring at 200 rpm. The PEG 900- and 1500-based macromonomers polymerized at a slower rate

Table 1. Reagents for the Synthesis of POEPOP 400, POEPOP 900, and POEPOP 1500

resin		POEPOP 400				POEPOP 900						POEPOP 1500			
entry		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
PEG	g:	4	4	4	4	9	9	9	9	9	9	7.5	7.5	7.5	7.5
	mmol:	10	10	10	10	10	10	10	10	10	10	5	5	5	5
CMO[a]	mL:	0.78	1.06	1.56	1.96	0.78	1.06	13.7	1.56	1.88	1.96	0.39	0.53	0.78	0.98
	mmol:	10	13.5	20	25	10	13.5	17.5	20	24	25	5	6.75	10	12.5
NaH	g:	0.4	0.54	0.8	1.0	0.4	0.54	0.7	0.8	0.96	1.0	0.2	0.27	0.4	0.5
	mmol:	10	13.5	20	25	10	13.5	17.5	20	24	25	5	6.75	10	12.5
inc.[b]	%:	34	49.5	77	97.5	36	51	60.5	73	86.5	98	33.9	52	74	98
	equiv:	0.68	0.99	1.54	1.95	0.72	1.0	1.21	1.46	1.73	1.96	0.68	1.04	1.48	1.96
*t*BuOK	(g) beads:	0.095	0.095	0.095	0.095	0.045	0.045	0.045	0.045	0.045	0.045	0.028	0.028	0.028	0.028
	(g) bulk:	0.445	0.445	0.445	0.445	0.535	0.535	0.535	0.535	0.535	0.535	0.270	0.270	0.270	0.270
Yr[c]	g:	0.51	0.53	0.47	0.56	0.11	0.11	0.15	0.18	0.31	0.33	0.10	0.10	0.17	0.29
	beads %:	73	0.76	0.67	80	16	16	21	26	44	47	0.14	0.14	0.24	0.41
Yr[c]	g:	2.61	2.84	2.71	2.87	7.23	7.30	7.35	8.03	7.6	8.20	5.61	5.83	6.31	6.24
	bulk %:	79	86	82	87	89	83	85	90	83.5	87	81	85	87	88

[a] 2-(Chloromethyl)oxirane. [b] Amount of incorporated CMO measured by ^{1}H NMR. [c] Yield resin.

Table 2. Reagents for the Synthesis of SPOCC 900 and SPOCC 1500

resin		SPOCC 400	SPOCC 900				SPOCC 1500			
entry		**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
PEG	g:	4	9	9	9	9	15	15	15	15
	mmol:	10	10	10	10	10	10	10	10	10
MOTS[a]	mL:	6.15	3.8	4.62	5.13	6.18	3.84	4.6	5.1	6.15
	mmol:	24	14.8	18	20	24.1	15	18	19.9	24
KHMDS[b]	g:	4.80	2.95	3.59	3.99	4.79	2.99	3.59	3.97	4.79
	mmol:	24.1	14.8	18	20	24	15	18	19.9	24
inc.[c]	%:	98	63	76	87	98	59	74	85	99
	equiv:	1.96	1.26	1.52	1.74	1.96	1.18	1.48	1.7	1.98
Yr[d]	g:	0.61		0.12	0.21	0.31		0.13	0.25	0.30
	beads %:	87		17	30	44		19	36	43
Yr[d]	g:	2.23	6.72	6.73	6.64	7.04	12.6	12.3	11.6	11.6
bulk %:		89	84	84	83	88	90	88	83	83

[a] 3-Methyl-oxetan-3-yl-methyl 4-toluenesulfonate. [b] Potassium hexamethyl-disilazane. [c] Amount of incorporated MOTS measured by ^{1}H NMR. [d] Yield resin.

Table 3. Reagents for Synthesis of POEPS-3

resin		400	900	1500		
entry		**24**	**25**	**26**	**27**	**28**
PEG	g:	4	9	7.5	7.5	7.5
	mmol:	10	10	5	5	5
CPVB[a]	mL:	4.96	4.96	1.51	2.48	3.8
	mmol:	28	28	8.5	14	21.5
NaH	g:	1.12	1.12	0.34	0.56	0.86
	mmol:	28	28	8.5	14	21.5
inc.[b]	%:	48	54	36	53	74
	equiv:	0.96	1.08	0.72	1.06	1.48
Yr[c]	beads g:	0.86	1.78	0.77	0.80	0.81
	%:	86	89	77	80	81
Yr[c]	bulk g:	1.64	3.95	4.2	4.05	3.8
	%:	82	79	84	81	76

[a] 1-Chloropropan-4-vinylbenzene. [b] Amount of incorporated CPVB measured by ^{1}H NMR. [c] Yield resin.

compared to the PEG 400 monomers and gave significant amounts of aggregation. Only macromonomers having a high Df (Table 2, entries **17**, **18**, **21**, and **22**) gave small amounts of beads with a reasonable uniformity in the size range between 150 and 500 μm in addition to aggregates. Beads of POEPOP 400 were obtained by addition of *t*BuOK to the PEG 400 macromonomers (Table 1, entries **1**−**4**) at 45 °C with stirring and exclusion of moisture followed by dropwise addition of the macromonomer mixture to silicon oil at 130 °C, with stirring. The size of the beads could be tuned by varying the stirring rate during emulsion formation. Stirring at 500 rpm appeared to be very efficient and gave good uniformity and reproducibility in the size range 300−500 μm (Figure 1a). Emulsion formation at 130 °C with PEG 900 and PEG 1500 macromonomers (Table 1, entries **5**−**8** and **11**−**13**) resulted in large amounts of aggregation. Attempts to increase the polymerization temperature did not avoid aggregation of the beads. The highly functionalized macromonomers (Table 1, entries **9**, **10**, **14**) gave mainly aggregation and only small amounts of beads with reasonable homogeneity when the polymerization was conducted in a larger volume of oil. New and improved procedures for beading of SPOCC and POEPOP are currently under development in our laboratory.

The beaded resins were sieved (300−500 μm), and the fraction smaller than 300 μm was suspended in methanol and repetitively sedimented and decanted to remove beads < 90 μm. Beads with an approximate size of 100 μm were selected and used in the diffusion studies.

Swelling Properties. Swelling of the resin in both organic solvents and enzyme buffers is essential for use of resins in combinatorial synthesis as well as in solid phase assays. The swelling of the resins was measured in tetrahydrofuran (THF), MeCN, dimethylformamide (DMF), water, and dichloromethane (DCM) by the syringe method (Figures 2 and 3).[12] Our results show that the swelling characteristics of the three different resins depended on the degree of cross-

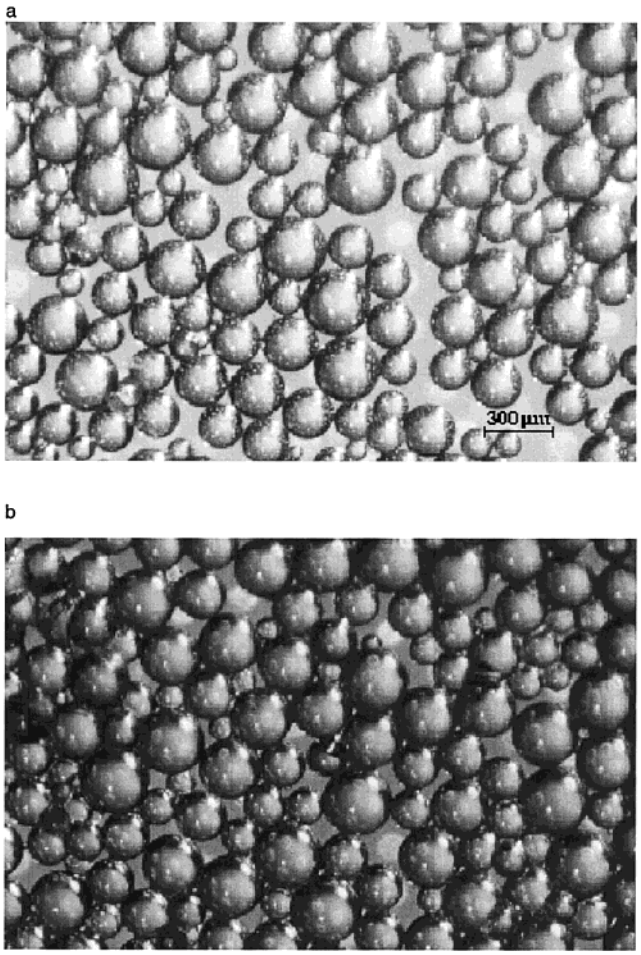


Figure 1. Beaded POEPOP 400 (a) and SPOCC 400 (b).

linking, the length of the PEG chain, and the chemical nature of the backbone in the resins. Swelling was strongly influenced by the length of the PEG chain in the starting monomer as well as the amount of cross-linking. For example, the PEG-1500 based resins expanded to larger swelling volumes than the PEG 900 and PEG 400 resins. Swelling decreased significantly with increased cross-linking. The PEG-based resins swelled best in CH_2Cl_2, somewhat less in DMF and water, and much less in THF and MeCN. The SPOCC resins swelled slightly more than POEPOP resins made up of the same PEG chain length, and the POEPS-3 resins exhibited a significantly lower swelling capacity than both SPOCC and POEPOP.

In comparison, all three PEG-based resins showed more swelling than the commercial ArgoGel-OH, TentaGel S OH, PS-NH_2, and PLAMS resins. For example, the PEG 1500- and PEG 900-based resins having low to medium cross-linking expanded to larger swelling volumes than ArgoGel-OH, TentaGel S OH, PS-NH_2, and PLAMS in all solvents used. Resins with a high degree of cross-linking showed similar swelling properties as PS-NH_2 and PLAMS. The PEG-based resins displayed almost equal swelling properties in DMF and water, in contrast to ArgoGel-OH, TentaGel S OH, PS-NH_2, and PLAMS which exhibited a higher degree of swelling in DMF than in water.

A key advantage of the PEG-based resins is their ability to swell sufficiently in aqueous solvent to allow on-bead binding assays with large biomolecules.[14,19] Although TentaGel and ArgoGel swell in water, they were found to restrict the access of enzymes toward resin bound substrates.[11,19]

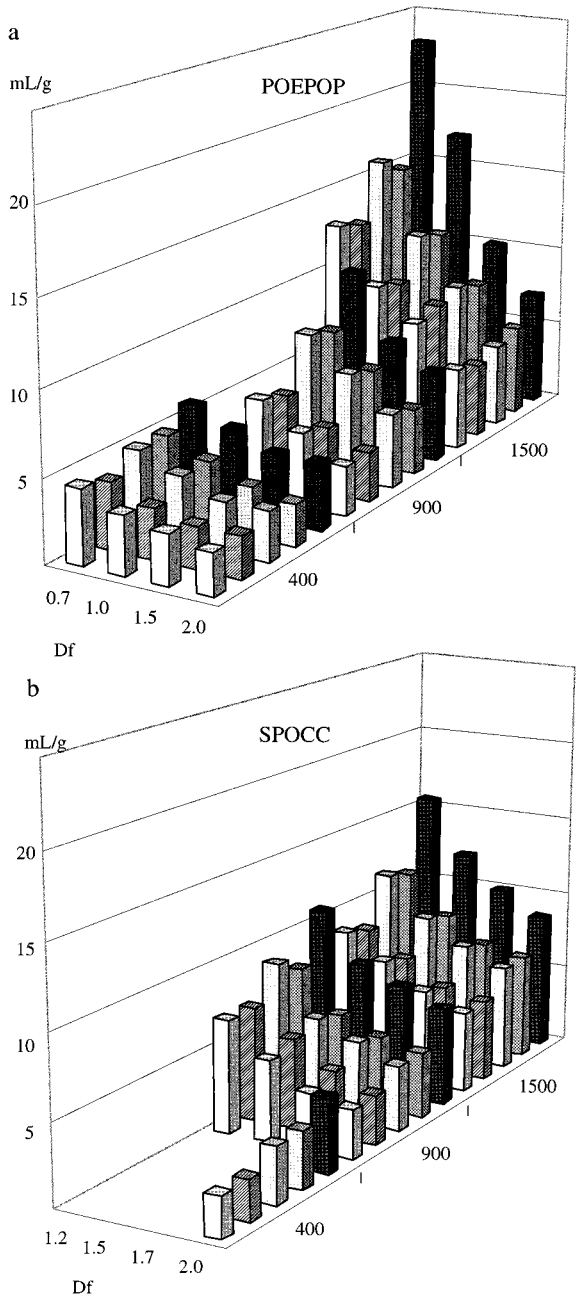


Figure 2. Swelling of POEPOP and SPOCC in different solvents (THF = white, MeCN = slants, DMF = dots, water = cross-hatched, CH_2Cl_2 = black) as a function of tether length and cross-linking. Df = degree of functionalization of the PEG monomer measured by ^1H NMR.

The swelling of POEPOP 900 resins obtained from suspension polymerization (300−500 μm) and bulk polymerization (300−500 μm) was also compared (data not shown) because variation in particle size and particle size distribution may confer different packing characteristics. Only small variations between the granulated resin and the beaded resin were observed, typically in the range between 3 and 6%. Because ArgoGel-OH (120−230 μm), PS-NH_2 (75−150 μm), and TentaGel S OH (130 μm) have a smaller bead size than the PEG-based resins (300−500 mm) and PLAMS (400−500 μm), direct comparison of the swelling properties is difficult to make with the different resins. Clearly,

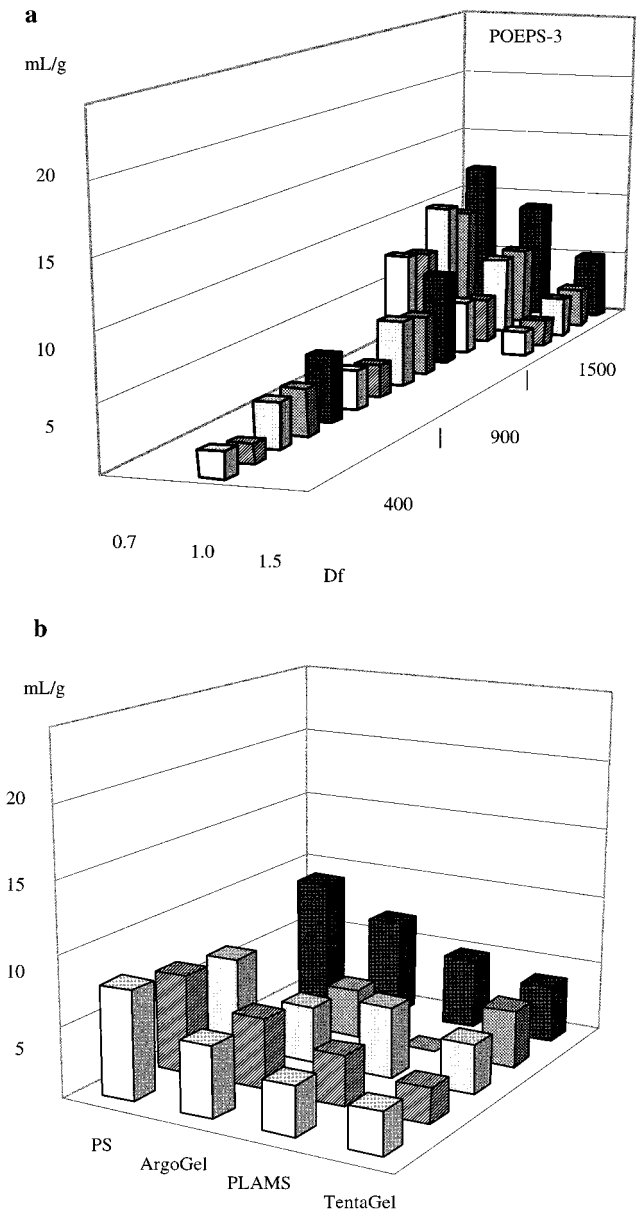


Figure 3. Swelling of POEPS-3 1500 as a function of cross-linking and commercial resins in different solvents (THF = white, MeCN = slants, DMF = dots, water = cross-hatched, CH_2Cl_2 = black). Df = degree of functionalization of the PEG monomer measured by ^{1}H NMR.

however, all three series of PEG-based resins possessed excellent swelling characteristics.

Loading. Control of the amount of available sites for substrate attachment (loading) is important in combinatorial chemistry where concentration of resin bound ligands may influence sensitivity of assays and on-bead analysis. The loading was determined as described in the Experimental Section and presented in Table 4. Loading decreased moderately as the amount of bis-alkylated PEG material was increased in the polymerization, indicating that the major part of the hydroxyl functionality originates from initiation and termination sites in the anion and cation catalyzed reactions. Loading varied with resin composition. For example, POEPS-3 resin possessed a significantly lower loading than the corresponding SPOCC and POEPOP resins. The POEPS-3 resins loading were comparable to TentaGel S OH, typically in the range of 0.2−0.3 mmol/g. ArgoGel-OH had a loading of 0.43 mmol/g. The SPOCC and the POEPOP resins exhibited loadings between 0.4 and 0.7 mmol/g. The polystyrene-based resins (e.g., PLAMS and PS-NH_2) typically had the highest loadings in the range of 0.5−2 mmol/g.

Table 4. Loading as a Function of Cross-Linking for POEPOP, SPOCC, and POEPS-3

eq.aa[a]	0.7	1	1.2	1.5	1.75	2	
POEPOP 1500	0.56	0.55		0.52		0.51	
POEPOP 900	0.48	0.46	0.45	0.45	0.4	0.32	
POEPOP 400	0.59	0.57		0.49		0.49	
POEPS-3 1500		0.25	0.22	0.19			
POEPS-3 900		0.27					
POEPS-3 400		0.25					
SPOCC 1500			0.54	0.48	0.43	0.45	
SPOCC 900			0.6	0.56	0.65	0.52	
SPOCC 400						0.6	
TentaGel-OH							0.23
ArgoGel-OH							0.43
PS-NH_2							1.8
PLAMS							1.9

[a] Df = degree of functionalization of the PEG monomer measured by ^{1}H NMR.

NMR. Proton NMR may be sufficiently sensitive for structure determination of chemical compounds on a single bead; however, the typically broad line widths exhibited by molecules bound to the solid phases limits data interpretation. This problem can be overcome by using a combination of MAS and high-resolution probe technology (nanoprobe NMR spectroscopy).[20] The quality of the NMR spectra taken of resin bound compounds is dependent mainly upon the achievable line width[21] and sensitivity is not a limiting factor, such that NMR spectra of even single beads can be obtained.[16,17] The achievable NMR line widths depend primarily upon the choice of resin used. Resins that provide the greatest mobility of the bound compounds produce more narrow ^{1}H NMR line widths.[9] The choice of solvent then becomes the second most important issue. Narrow NMR resonances can be generated only if both the resin bound compound and the resin itself are well solvated.

All of the resins were derivatized with 2-(*N*-*tert*-butoxycarbonylamino)benzoic acid (Boc-Abz) and characterized by nanoprobe MAS ^{1}H NMR spectroscopy in order to characterize the PEG-based resins and evaluate the influence of the tether length, the degree of cross-linking, the polymer matrix, and the solvent on the overall quality of the NMR spectra.

Although POEPS-3 and SPOCC constitute exclusively primary ethers and alcohols, this is not the case for POEPOP. It was initially assumed that the oxirane ring in the POEPOP macromonomers would be attacked in basic solution by an S_N2 mechanism at the less highly substituted carbon to produce a secondary alcohol. Two signals were observed in the MAS ^{1}H NMR of a POEPOP 1500 sample treated with trifluoroacetic anhydride in pyridine which demonstrated that equal amounts of primary (4.25 ppm) and secondary hydroxyl groups (5.15 ppm) were present in the resin.

Varying amounts of vinylic signals from resin bound unreacted styrene groups were observed in the MAS ^{1}H

Table 5. Spectal Quality,[a] Line Width (L),[b] and Multiplet Resolution (R)[c] for POEPOP and SPOCC Resins

		toluene-d_8	CD_2Cl_2	CD_3Cl	acetone-d_6	DMF-d_7	DMSO-d_6	CD_3OD	D_2O
POEPOP 1500 (**13**)		E	A	A	B	B	B/C	B	C
	L	11.7	1.2	1.2/1.3[d]	4.5	3.6/11.2[d]	1.62	3.1/11.1[d]	2.8
	R	s	0.08	0.08	0.30	0.31	0.44	0.50	wd
POEPOP 900 (**8**)		F	A	A	B	B	B/C	C	D
	L	24.1	3.0[d]	1.6/2.6[d]	6.25[d]	5.3	2.1	5.4	10.0
	R	s-nd	0.09	0.11	0.53	0.42	0.60	s-d	br s
POEPOP 400 (**3**)		F	A/B	A/B	C	D	D	E	F
	L	19.1	1.34[d]	2.1/2.9[d]	7.3[d]	6.4	3.4	5.3	12.2
	R	br s-nd	0.24	0.34	s-nd	s	s	s	br s
SPOCC 1500 (**21**)		E	A	A	A	B	B	B	D
	L	8.9	5.1/1.1[d]	1.8	1.1	3.0	2.5	2.7	7.8
	R	s	0.1	0.15	0.1	0.24	0.31	0.38	s
SPOCC 400 (**15**)		F	A/B	A/B	C	D	D	E	F
	L	21.7	2.2/3.7[d]	3.5	9.3	8.7	8.4	9.1	32.7
	R	br s	s-d	br s	s	s-nd	s	s	nd

[a] Spectral quality on a scale from A−F where A = high-resolution spectra, B = acceptable spectra, C = ok spectra, D = poor spectra, E = unresolved spectra, and F = no visible spectrum. The scale only compares for a solvent series within one specific resin and cannot be taken as a figure for comparing the different resins. [b] The line width (in hertz) of the Boc singlet at 1.5−1.6 ppm. [c] This is measured for the Abz aromatic proton doublet at 8.4 ppm of the degree of separation between the doublet peaks. In case a value could not be computed, the character is indicated: s−singlet, d−doublet, nd−nondetectable, wd−weak doublet, br s−broad singlet. [d] The Boc singlet was too close or overlapping with the water or other resonances and could not be unambiguously identified.

Table 6. Spectal Quality,[a] Line Width (L),[b] and Multiplet Resolution (R)[c] for POEPS-3 1500, PEGA 1900, TentaGel, ArgoGel, and PLAMS

		toluene-d_8	CD_2Cl_2	CD_3Cl	acetone-d_6	DMF-d_7	DMSO-d_6	CD_3OD	D_2O
POEPS-3 1500 (**26**)		E	A	A	B	B	D	D	F
	L	18.0	7.1/2.7[d]	2.2/4.4[d]	5.5	5.2	5.9	5.0	
	R	br s	0.21	0.18	0.46	0.61	s	s-d	nd
PEGA 1900		F	A	A	C	B	C	C	E
	L	22.0	1.6	4.4/1.8[d]	5.91	3.4	2.8	2.6[d]	3.3
	R	nd	0.20	0.23[d]	0.67	0.37	0.34	0.78	s
TentaGel		E/F	A	A	C	B	C	D	E
	L	13.9	3.8/3.1[d]	2.9	5.1	4.8	5.4	4.4	
	R	s	0.21	0.17	0.93	0.69	0.51	br s	nd
ArgoGel			A	A	C	B	C	D	
	L		3.46[d]	2.8	5.3	4.9	7.4	6.4	
	R		0.30	0.24	0.67	0.71	0.87	br s	nd
PLASM		F	E	E	E	E	F	F	F
	L	19.9		17.4	14.3	13.0	nd	nd	nd
	R	nd	nd	br s	br s	s	nd	nd	nd

[a] Spectral quality on a scale from A−F where A = high-resolution spectra, B = acceptable spectra, C = ok spectra, D = poor spectra, E = unresolved spectra, and F = no visible spectrum. The scale only compares for a solvent series within one specific resin and cannot be taken as a figure for comparing the different resins. [b] The line width (in hertz) of the Boc singlet at 1.5−1.6 ppm. [c] This is measured for the Abz aromatic proton doublet at 8.4 ppm of the degree of separation between the doublet peaks. In case a value could not be computed, the character is indicated: s−singlet, d−doublet, nd−non detectable, wd−weak doublet, br−broad singlet. [d] The Boc singlet was too close or overlapping with the water or other resonance and could not be unambiguously identified.

NMR spectra for the different POEPS-3 resins, indicating the polymerization to be incomplete.

One of the fundamental aspects of sample performance was the obtainable resolution. As a common measure of resolution, the width at half-height of the *t*Bu singlet near 1.5 ppm measured in hertz (Tables 5 and 6) was used. Another factor that was used to evaluate spectral quality wasthe baseline separation of the Abz aromatic H-3 proton doublet at 8.4 ppm (Tables 5 and 6). Finally, the resolution of the residual resin signals after presaturation was evaluated. These three parameters were combined to develop a spectral quality ranking criteria for each member in the resin series (Tables 5 and 6).

(a) Solvents. Solvent significantly influenced the quality of the NMR spectra (Figure 4). Best quality spectra were typically obtained in $CDCl_3$ and CD_2Cl_2, although good quality spectra could be obtained in hexadeuteriodimethyl sulfoxide (DMSO-d_6) with the PEG-based resins. Lower quality spectra with reduced resolution and broader resonances were obtained for the PEG-grafted resins. Considerable broadening of the resonances was seen in D_2O and toluene, making these two solvents unsuitable, and acetone-d_6, DMF-d_7, and CD_3OD all afforded spectra of intermediate quality.

One explanation for this influence of solvent is that narrow NMR resonances can be generated only if both the compound and the polymer matrixes are well solvated. Spectral quality may thus depend on the solvent as well as compound bound to the resin and the resin itself.

Although it is tempting to presume that greater swelling leads to narrowing in the NMR line widths because of increased motion within the swollen resin,[22] in the present study the quality of the NMR data from different resins did not correlate with their swelling. For instance, the swelling

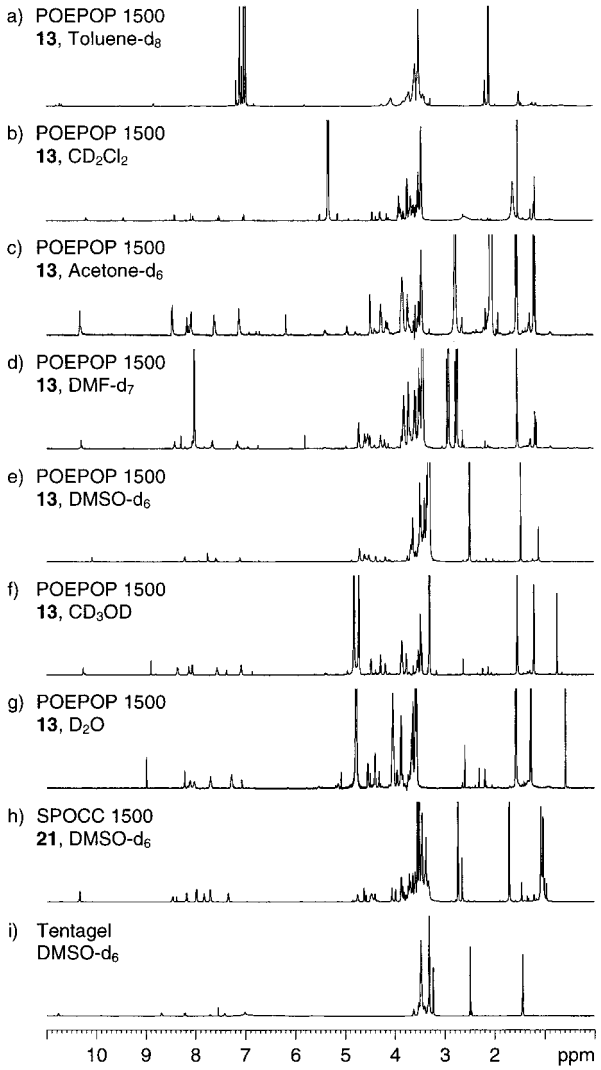


Figure 4. MAS ^{1}H NMR (500 MHz) spectra of POEPOP 1500 in different solvents and SPOCC 1500 and TentaGel in DMSO.

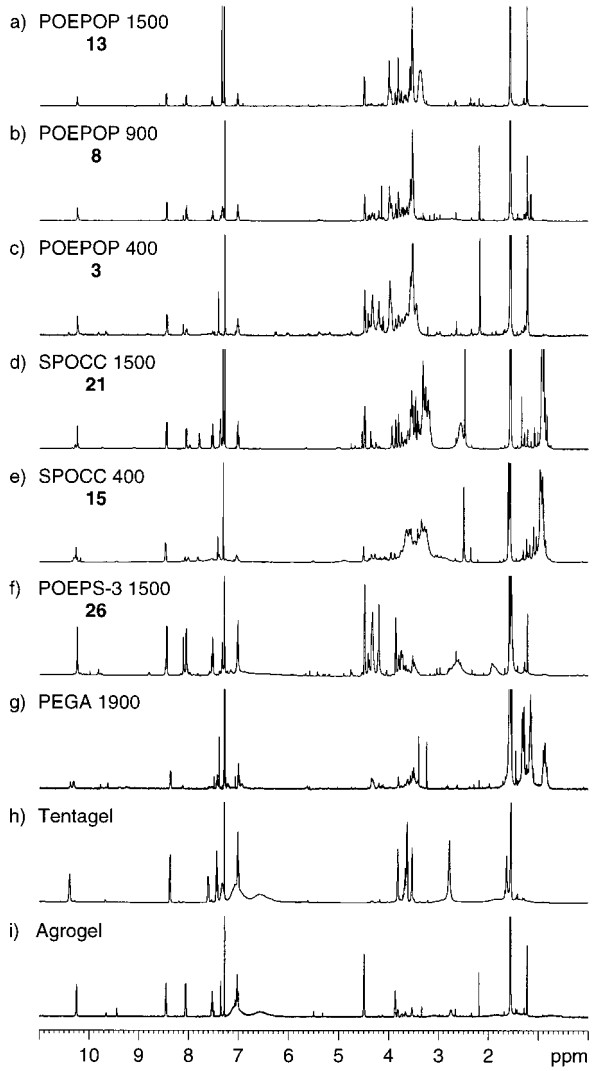


Figure 5. MAS ^{1}H NMR (500 MHz) spectra of different resins in $CDCl_3$.

capacities of POEPOP 900 in DMF and in water were similar, yet acceptable NMR spectra were obtained only in DMF-d_6 and not in D_2O. Other studies have also pointed out that the observed line widths are not directly a function of swelling.[1b,9,21]

(b) Polymer Matrix. The resin structure was the dominant factor influencing the ^{1}H NMR line widths and the baseline separation (Tables 5 and 6 and Figure 5). For example, POEPOP 1500 and SPOCC 1500, which have long tethers and a flexible backbone, provided the best resolution and smallest line widths in $CDCl_3$ and CD_2Cl_2. The line widths and the baseline separation obtained for these two PEG-based resins were comparable; however, the residual PEG signals found in SPOCC 1500 were more complex and covered a wider ppm range compared to the POEPOP 1500 resin spectrum. This phenomenon was most obvious when evaluating the spectra acquired in $CDCl_3$ and CD_2Cl_2 and less pronounced for spectra acquired in DMSO-d_6. POEPS-3 1500 gave sharp resonances in the aromatic region and was well resolved in $CDCl_3$ and CD_2Cl_2. However, the PEG resonances were broader than those found for POEPOP 1500, although not to an extent that they can be removed using a T2 filter.[23] The PEGA 1900 resin spectra in $CDCl_3$ and CD_2Cl_2 generally gave well resolved peaks for the aromatic resonances of the Abz group and more broad resonances for the backbone part of the polymer. As for POEPS-3 1500 the residual PEG resonances were broader compared to the POEPOP 1500 PEG signals. TentaGel and ArgoGel in $CDCl_3$ and CD_2Cl_2 gave spectra with relatively sharp and well resolved resonances except for the signals from the styrene backbone that appeared very broad compared to that of POEPS-3 1500. These aromatic resonances could be removed using a T2 filter. The line widths of the Abz resonances were larger than those found for POEPOP 1500 and SPOCC 1500. The spectra of the conventional polystyrene resins exhibited only very broad unresolved signals, both for the polystyrene backbone and the Abz group attached.

Each of the PEG-based resins exhibited a characteristic set of multiple broad resonances arising from the tether. Presaturation of the main PEG signal was very effective and reduced most of the resonances arising from the resin.[21] While presaturation did reduce the main signal, the residual signals could still complicate spectral assignments. However, these sharp resonances can be distinguished from signals originating from the compound attached to the resin, and

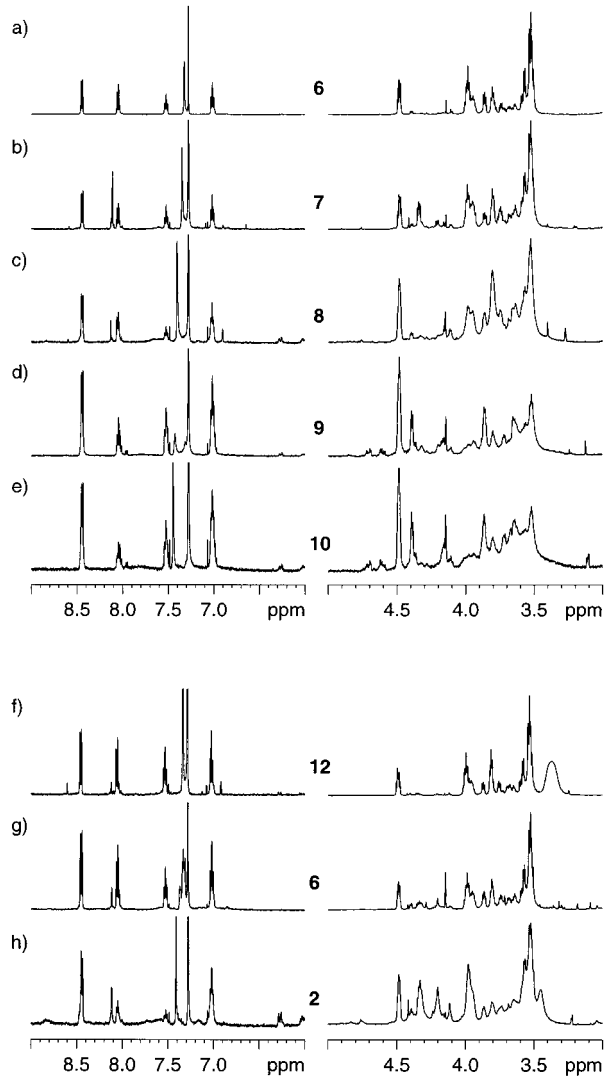


Figure 6. MAS nanoprobe ^{1}H NMR (500 MHz) spectra of POEPOP 900 with different amount of cross-linking (a−e, entries **6**−**10**) and a series of POEPOP resins with different lengths of the tether (f−h, entries **12**, **6**, **2**). All spectra were recorded in $CDCl_3$.

complete structure elucidation has been carried out on peptides and glycopeptides.[17,24]

(c) Tether Length. The length of the tether is an important factor influencing the quality of the spectra (Figure 6, entries f−h). This effect was independent of the solvent used. For example, POEPOP 1500 and POEPOP 900, which have long or medium sized tethers and therefore relative mobile moieties, provided sharp resonances for both the Abz group and for the PEG tether chain when irradiating the main PEG peak. The POEPOP 400 resins have a shorter tether and reduced regional mobility, and both the Abz aromatic resonances and the resonances from the PEG chains gave broad signals. A similar trend was observed for the SPOCC resins.

(d) Cross-Linking. Another series of experiments was conducted to investigate the influence of high cross-linking on the quality of the NMR spectra (Figure 6, entries a−e). The resolution of the aromatic signals (e.g., Abz) and the resin signals were reduced with increased cross-linking. This decrease in resolution was more pronounced for the PEG chain resonances than for the Abz resonances. A high degree of cross-linking may result in heterogeneity in PEG solvation and restrict local molecular motion within the resin and thereby broaden the NMR resonances.

Diffusion Studies. The diffusive properties of polymer supports can influence the kinetics of solid phase chemistry.[25] In microporous polystyrene beads, where pore size is related to swelling, reaction rates tend to increase with swelling and decrease with increased cross-linking.[26] Studying diffusion in PEG-cross-linked polystyrene, Wilson et al. observed relatively faster diffusion rates than with polystyrene cross-linked with divinyl benzene in poor swelling solvents and the reverse ratio of diffusion rates in high-swelling solvent.[27]

The rate of diffusion through the beaded resin was examined by measuring fluorescence quenching of polymer bound 2-amino-benzoate on protonation with *p*-toluenesulfonic acid using a confocal scanning laser microscope. Because the quenching reaction was instantaneous in solution, the rate of reduction of fluorescence intensity reflected the diffusion rate of *p*-toluenesulfonic acid into the resin.

The confocal microscope uses a pinhole aperture to screen reflections of polarized light passing through a translucent specimen in order to obtain an image that is limited to a single focal plane.[28] A precise image of the resin particles was obtained when the depth of this plane was set to around 60 μm. No bleaching effect was observed when the resins were exposed to the laser for 5 min. Initial fluorescence levels of the sample were determined before a *p*-toluenesulfonic acid containing solution was added to the resin. The measured diffusion rates are reported as the time it took to reduce the initial fluorescence to half of its final value ($T^1/_2$).

(a) Solvents. A pronounced solvent dependency on decreasing diffusion rates in the order of CH_2Cl_2 > water > DMF was observed with Abz functionalized POEPOP 1500 (entry **13**, Figure 7a). The rate of quenching did not follow a trend toward increased diffusion in better swelling solvents because the quenching rates for water were faster than those for DMF despite their similar degree of swelling.

(b) Cross-Linking. The effect of cross-linking on the diffusion rate for the different resins is presented in Figure 7b. Because the density of cross-linking had a large influence on the swelling properties of SPOCC resins, it was anticipated that increased cross-linking would give decreased mass transport. However, three SPOCC resins with varying degrees of cross-linking (Table 2, entries **20**, **21**, and **23**) showed similar diffusion rates in DMF. Accordingly, even the highly cross-linked resin swelled enough to allow effective diffusion into the polymer matrix. Diffusion rates for PEG-based resins with PEG chains shorter than 400 has not yet been investigated.

(c) Tether Length. The influence of PEG chain length on the diffusion rates was examined on POEPOP 400 (Table 1, entry **3**), POEPOP 900 (Table 1, entry **8**), and POEPOP 1500 (Table 1, entry **13**), all possessing the same amount of cross-linking, by treating the resin with *p*-toluenesulfonic acid in DMF (Figure 7c). Diffusion rates were found to be similar for all three resins which swelled differently in DMF and indicate sufficient swelling to allow fast diffusion into the polymer matrix.

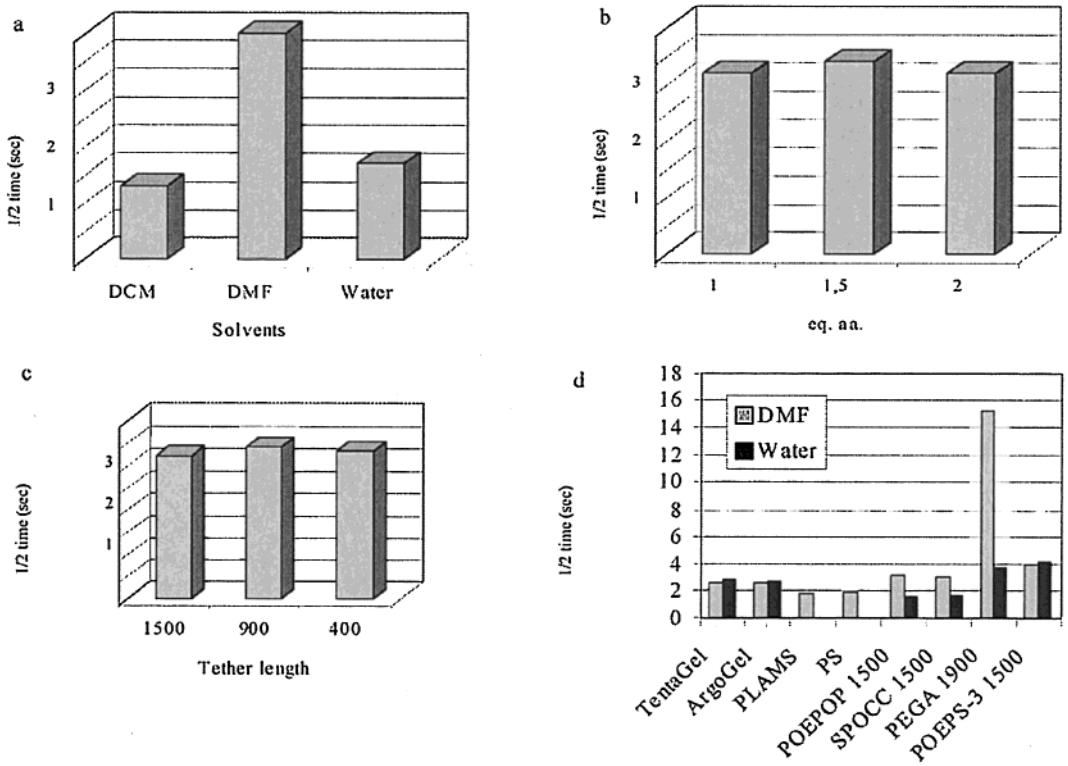


Figure 7. Fluorescence quenching of polymer bound 2-amino-benzoate by protonation with *p*-toluenesulfonic acid. (a) As a function of solvent: POEPOP 1500 (**13**) in DCE, DMF and water. (b) As a function of cross-linking: SPOCC 900 (**15**, **16**, **18**) in DMF. (c) As a function of tether length: POEPOP 400 (**2**), POEPOP 900 (**6**), POEPOP 1500 (**12**) in DMF. (d) As a function of polymer matrix: TentaGel, PLAMS, POEPOP 1500 (**12**), SPOCC 1500 (**19**), and POEPS-3 1500 (**26**) in DMF and water.

(d) Polymer Matrix. Examination of the rate of diffusion of *p*-toluenesulfonic acid into various Abz derivatized resins (Figure 7d) showed variations of quenching rate depending on the polymer matrix. In DMF, the rate of diffusion decreased in the order of PLAMS > PS > ArgoGel = TentaGel > POEPOP 1500 (Table 1, entry **13**) = SPOCC 1500 (Table 2, entry **21**) > POEPS-3 1500 (Table 3, entry **28**) ≫ PEGA 1900. In water the rate of quenching decreased in the order POEPOP 1500 (Table 1, entry **13**) = SPOCC 1500 (Table 2, entry **21**) > ArgoGel = TentaGel > PEGA 1900 > POEPS-3 1500 (Table 3, entry **28**). The polystyrene resins PS and PLAMS failed to exhibit normal quenching behavior in water, presumably because these resins do not swell in water due to their hydrophobic matrix.

Diffusion may be retarded by interaction of the *p*-toluenesulfonic acid with the polymer matrix in the case of the PEG-based polymers which may form strong ion−dipole interactions and hydrogen bond with the diffusant. Such a phenomenon could at least in part explain the differences in diffusion rates between the polystyrene-based resins and PEG-based resins in DMF. The rate of the quenching reaction may also be influenced by the location of the Abz group within the polymer matrix. Because TentaGel architecture consists of a core of cross-linked 1% DVB-polystyrene grafted with long PEG chains, the reactive sites are located at the end of long flexible spacers. In contrast, SPOCC and POEPOP, shorter less flexible PEG chains, may separate the reactant and the resin matrix. The rate of protonation of the Abz by sulfonic acid in these polymers may thus be slower relative to TentaGel.

Adams et al., who compared aminomethylated polystyrene and PEG-grafted aminomethylated polystyrene in several chemical transformations, concluded that PEG did not play an active role in promoting synthesis efficiency either by its influence on the microenvironment or by its spacer arm effect, but that good swelling characteristics are of great importance.[29] However, examination of the rate of diffusion of *p*-toluenesulfonic acid into various Abz derivatized resins showed no direct correlation between rate of diffusion and swelling. For example, SPOCC 1500 (Table 2, entry **21**) and POEPOP 1500 (Table 1, entry **13**) had larger swelling volumes than TentaGel and ArgoGel in DMF but showed slower diffusion of *p*-toluenesulfonic acid into the polymer matrix in DMF than that for the PEG-grafted resins. However, in all cases, diffusion was fast compared to reaction times generally observed in organic synthesis.

Conclusion

The physical properties of three families of PEG-based resins have been compared with the most common PEG-grafted resins (TentaGel and ArgoGel) and polystyrene resins. The three PEG-based supports POEPOP, SPOCC, and POEPS-3 were efficiently prepared by single step polymerization. Relative to the PEG-grafted resins, the PEG-based resins were shown to possess better loading capacity as well as a higher degree of swelling in a broad range of solvents, ranging from water to dichloromethane.

High-quality NMR spectra were obtained with the PEG-based resins which exhibited better resolution and narrow line widths compared to the spectra of PEG-grafted resins,

when examined by magic angle spinning nanoprobe NMR spectroscopy. Among the PEG-based resins, the best quality spectra were obtained with POEPOP 1500 and SPOCC 1500 in the widest variety of solvents.

Fluorescence quenching of polymer bound Abz by protonation with *p*-toluenesulfonic acid showed that diffusion was faster through TentaGel and ArgoGel than that of the PEG-based resins in DMF. In water, the PEG-based resinsshowed faster diffusion rates compared to TentaGel and ArgoGel.

The results presented demonstrate some of the excellent properties of PEG-based resins that will translate well for applications to solid phase synthesis and combinatorial libraries currently being investigated in our laboratory.

Experimental Section

All solvents were purchased from Labscan Ltd. (Dublin, Ireland) and stored over molecular sieves. TentaGel was purchased from Rapp Polymere (Tübingen, Germany), and ArgoGel and PLAMS were purchased from Argonaut Technologies Inc. (San Carlos, CA) and Polymer Laboratories (Amherst, MA), respectively. Derivatization of the different solid supports were carried out using plastic syringes. Flat-bottom PE syringes were equipped with sintered Teflon filters (50 μm pores), Teflon tubing, and valves, allowing one to apply suction to the syringes from below. 4-Vinylphenylpropyl-PEG macromonomers were synthesized and polymerized according to a literature procedure (Table 3).[15] The amount of hydroxyl groups available for substrate attachment (loading) of the resins was determined by esterification with Fmoc-Gly, activated by MSNT in the presence of *N*-methyl imidazole in CH_2Cl_2, and subsequently measuring the UV absorbency of the adduct of dibenzofulvene and piperidine formed on treatment of a weighed polymer sample with 20% piperidine in DMF. Solution phase ^{1}H NMR spectroscopy was performed on a Bruker DPX 250 MHz instrument. Chemical shifts were calibrated relative to the signal of tetramethylsilane (0 ppm) or internal solvent signals (7.27 ppm for $CDCl_3$ and 2.49 ppm for DMSO-d_6).

General Synthesis of POEPOP Resins. The (methyloxirane)-PEG macromonomers were synthesized by a modified literature procedure.[13] Poly(ethylene glycol) (see Table 1) was dried by azeotropic evaporation of acetonitrile (2 × 25 mL) and dissolved in THF (10 mL) with stirring and exclusion of moisture. Sodium hydride [60 wt % dispersion in mineral oil (Table 1)] was added in small portions to the PEG solution with stirring and exclusion of moisture. The deprotonation reaction was stirred at room temperature (PEG 400 and 900) or at 40 °C for 2 h. Epichlorohydrin (Table 1) was added dropwise, and the reaction was stirred at 40 °C for 12 h. The solvent was evaporated in vacuo, and the residue was mixed with acetonitrile (25 mL). The precipitated sodium salt was separated by centrifugation at 8000 rpm for 20 min, and the supernatant was decanted and evaporated in vacuo. The products (PEG 400 and 900) were washed with heptane (3 × 25 mL) to remove mineral oil and dried under high vacuum. The PEG 1500 monomer was precipitated using diethyl ether (50 mL), filtered, and dried under high vacuum. The percentage of oxiranyl groups was quantified by ^{1}H NMR spectroscopy (Table 1).

(a) Bulk Polymerization. (Methyloxirane)-PEG macromonomers were heated to 50 °C with stirring and exclusion of moisture. *t*BuOK (Table 1) was added, and the mixture was stirred for 30 min. The temperature was increased to 130 °C, and the polymerization was left for 16 h. The polymer was cooled to room temperature and cut to pieces before it was swollen in dichloromethane (100 mL, 1 h) and then granulated through a 1 mm metal sieve. The granulated resin was washed with dichloromethane (3 × 50 mL), acetone (3 × 50 mL), and water (3 × 50 mL) before it was stirred in 4 M HCl for 2 h at room temperature. The resin was washed with water (6 × 50 mL), acetone (6 × 50 mL), DMF (6 × 50 mL), and dichloromethane (6 × 50 mL) and dried under high vacuum. Resin loading (Figure 4) was determined as described in the general procedures, and swelling volumes in different solvents were determined following a literature procedure[17] (Figure 2).

(b) Suspension Polymerization. (Methyloxirane)-PEG macromonomers (1.5 mmol) were warmed to 50 °C, and *t*BuOK (73 mg, 0.6 mmol) was added with stirring and exclusion of moisture. After 30 min, the resulting mixture was added dropwise to a beaker containing warm silicon oil (75 mL, 130 °C) with magnetic stirring (500 rpm). The reaction was allowed to proceed overnight. The slurry of beads was filtered off and treated as above. The beads were air-dried for 1 h and then placed in a vacuum line overnight. Yields are listed in Table 1.

General Synthesis of SPOCC Resins. The (3-methyl-oxetanylmethyl)-PEG macromonomers were synthesized by a literature procedure.[14] For amounts of reagents, see Table 2. The percentage of oxetanyl groups was quantified by ^{1}H NMR (Table 2).

(a) Bulk Polymerization. The [bis(3-methyl-oxetanyl-methyl)]-PEG macromonomers were polymerized following a modified literature procedure.[14] Oxetanylated PEG (Table 2) was dissolved in dry acetonitrile (5 mL) under argon and cooled on ice. Boron trifluoride diethyl etherate (0.4 equiv, Table 4) was added, and after 10 min the ice bath was removed. After 1 h at room temperature the temperature was increased to 70 °C, and the polymerization was continued for 16 h. Workup was carried out as for the POEPOP resins, but the incubation with 4 M HCl was changed to 1 M NaOH. The hydroxyl group capacity (Figure 4) and the swelling capacity was determined as above for the POEPOP resin (Figure 2).

(b) Suspension Polymerization. A solution of bis[(3-methyloxetan-3-yl)methyl]-PEG macromonomer (1.5 mmol) in dry acetonitrile (0.5 mL) under argon was cooled in an ice bath, treated with boron trifluoride diethyl etherate (0.4 equiv, Table 4), stirred for 10 min and allowed to warm to room temperature. The mixture was added dropwise to silicon oil (75 mL, 20 °C) with stirring (500 rpm). After being stirred overnight, the resulting slurry of beads was filtered and treated as described above. The beads were air-dried for 1 h and then placed on a vacuum line overnight. Yields are listed in Table 2.

Derivatization of Resins with Abz/Boc-Abz. Resin (100

mg) was swollen in CH_2Cl_2 and washed with CH_2Cl_2 (3 × 2 mL). A solution of Boc-Abz (3 equiv) and *N*-methylimidazol (2.9 equiv) in CH_2Cl_2 was treated with 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4 triazole (MSNT, 3 equiv) for 5 min and added to the resin. After 45 min at room temperature the resin was washed with CH_2Cl_2 (3 × 2 mL) and treated a second time with the above solution. The resin was washed with DMF (6 × 2 mL) and CH_2Cl_2 (6 × 2 mL) and dried under high vacuum. A sample (50 mg) of each resin was treated with 95% aqueous TFA for 2 h, filtered, washed with DMF (6 × 2 mL), and CH_2Cl_2 (6 × 2 mL), and dried under high vacuum.

^{1}H MAS Nanoprobe NMR. Resin particles were transferred into nanotubes, dried overnight in vacuo, and suspended in the desired solvent (40 μL), leaving a small air bubble in the tube.

All spectra were recorded on a Varian Unity Inova 500 MHz spectrometer equipped with a 4 mm ^{1}H-observe nano NMR probe, at 25 °C, using a spin rate of approximately 2 kHz. The spectra were acquired as one-pulse experiments with presaturation of the main PEG resonance. Acquisition data for the spectra were as follows: 2.0 s acquisition time, 2.0 s presaturation delay, sweep width of 8000 Hz, and sampled in 32000 points per 256 scans. All spectra were processed using a 0.5 Hz line broadening and zero-filled to 64 k using Bruker XWINNMR version 2.1.

Diffusion Studies. Confocal microscopy was performed on a Leica IRBE inverted fluorescence microscope with a Leica TCS SP confocal system. The attached laser setup was an 80 mW argon ion laser INNOVA Enterprise II model 653 (Coherent Inc Enterprises, Santa Clara, CA) with emission at 351−364 nm. The objective lens was a Pl Fluotar 10x/NA 0.30 dry UV transmitting lens. Full laser power was utilized, and the pinhole was 1.0 mm. Beads were mounted directly onto 0.17 mm coverslips and viewed with the stage position adjusted to scan the center of the beads. Experiments were initiated using the Leica NT Timelapse software. Emitted light was collected by photomultiplier tubes with SP filter settings at 410 to 520 nm. Images were manipulated using Leica TCS NT Physiology software. The full width at half-maximum (fwhm) was 2.62 ± 0.14 mm.

The resin particles were swelled in the desired solvent for 15 min, and excess solvent was removed by a pipet. The background level of fluorescence was monitored for 1 min before 20 μL of 0.13 M *p*-toluenesulfonic acid in the desired solvent was added onto the resin material and changes in fluorescence levels were monitored for 240 s.

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