(a polar solvent) changes the C–N bond character slightly along the backbone chain. This causes additional freedom of backbone rotation in C_6F_5OH–CCl_4 compared with that in benzene.

Finally, it is interesting to compare the helix–coil transition behaviour observed in polypeptides and poly(*N*-methyl alanine) with that observed in PBIC. In the random-coil state of the polypeptide chain, the amide group remains *trans*, although the degree of freedom of the rotation around the backbone N–C^{α} and C^{α}–C bonds increases[16]. For poly(*N*-methyl alanine), the presence of both *trans* and *cis* C–N bonds in the polymer chain converts the polymer into a disordered state in a coil solvent such as trifluoroacetic acid[17]. However, an increase in the degree of freedom around the C–N bonds will cause formation of random coil PBIC.

The ^1H and ^{13}C spin–lattice relaxation times of PBIC would give a more quantitative information on the motions of the main chain and the side chain.

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Synthesis and gelation properties of crosslinked polymers of 4-(*N*-acryloyl-L-phenylalanyl)-morpholine, 4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine and 4-(*N*-acryloyl-L-tyrosyl)-morpholine

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Polymer networks which are suitable for application as supports for solid (gel) phase peptide synthesis must necessarily undergo gelation (constrained dissolution) in a range of suitable reaction solvents. To date, most polymer-supported peptide syntheses have been effected using supports based on crosslinked polystyrene. These networks have poor gelation properties in solvents of high polarity. Because the latter can be most useful in peptide synthesis, efforts have been made to devise alternative supports able to undergo gelation in such solvents.

Atherton *et al.*[1] and Arshady *et al.*[2] have described the synthesis of alternative supports based on crosslinked poly(*N,N*-dimethylacrylamide) and Smith *et al.*[3] the synthesis of supports based on crosslinked and poly(*N*-acryloylpyrrolidine). Most recently we have described alternative supports based on crosslinked poly(acryloylmorpholine)[4]. All of these matrices compare favourably with those based on polystyrene.

During the course of our work, we have prepared other polymer networks which are able to undergo gelation even more efficiently in the range of reaction solvents most useful in peptide synthesis. Our approach has been to synthesize these networks from suitable single monomers which combine selected polar and non-polar features. Three such networks, derived from amino-acid morpholine amides, are the subject of this report.

EXPERIMENTAL

Synthesis of N-*acryloyl*-L-*phenylalanine*

L-Phenylalanine (165.2 g, 1 mol) was dissolved in 1.5 M aqueous NaOH (1 dm^3) and the solution maintained, stirring mechanically, at 0°C. Acryloyl chloride (181 g, 162.4 cm^3, 2 mol) was added over 1 h while maintaining the pH at 11–11.5 by addition of aqueous 4 M NaOH. The reaction mixture was stirred for a further 1 h and the pH adjusted to 1–1.5 by addition of aqueous 11 M HCl. The resulting precipitate was collected by filtration, washed with distilled water, dried and recrystallized from chloroform/petroleum

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ether (40°–60°C) to give *N*-acryloyl-L-phenylalanine (160 g, 73%), m.p. 126°–127°C; $[\alpha]_D^{20}$ = +54.46° (methanol); ν_{max} (KBr disc) 1710 (acid C=O str), 1650 (amide C=O str) and 1600 cm^{-1} (C=C str); δ ($CDCl_3$ 60 MH_3) 3.15 (2H, d, CH_2CH), 4.84 (H, m, $NHCHCH_2$), 5.61 (H, m, $CH_2=CHCO$), 6.04 (2H, m, $CH_2=CHCO$), 6.4 (H, d, $NHCH_2=CH$) and 7.09 ppm (5H, s, C_6H_5). (Found: C, 65.92; H, 5.94; N, 6.42%. $C_{12}H_{13}NO_3$ requires: C, 65.74; H, 5.98 and N, 6.39%).

*Synthesis of 4-(*N*-acryloyl-L-phenylalanyl)-morpholine*

N-acryloyl-L-phenylalanine (150 g, 0.685 mol) was suspended in dry dichloromethane (3 dm^3) and the mixture maintained, stirring mechanically, at 0°C. A solution of morpholine (59.5 g, 59.5 cm^3, 0.685 mol) in dry dichloromethane (100 cm^3) was slowly added, followed by a solution of dicyclohexylcarbodiimide (141.1 g, 0.685 mol) in dry dichloromethane (100 cm^3). The mixture was stirred mechanically for 24 h at ambient temperature after which glacial acetic acid (0.1 cm^3) was added. After a further 1 h, dicyclohexylurea was removed by filtration and the filtrate washed with aqueous M Na_2CO_3 (2 × 500 cm^3), aqueous M HCl (2 × 500 cm^3) and distilled water (2 × 500 cm^3), dried over $MgSO_4$ and the solvent removed under reduced pressure. The resulting solid was repeatedly extracted with hot acetone which upon cooling deposited white prismatic crystals of 4-(*N*-acryloyl-L-phenylalanyl)-morpholine (79.7 g, 40.4%), m.p. 147–150°C; $[\alpha]_D^{20}$ = +3.94° (methanol); ν_{max} (KBr disc) 1670 (amide C=O str), 1610 (C=C str) and 1110 cm^{-1} (C–O str); δ ($CDCl_3$, 60 MHz) 3.08 (2H, d, CH_2CH), 3.53 [8H, s, $CON(CH_2CH_2)_2O$], 5.23 (H, m, $NHCHCH_2$), 5.61 (H, m, $CH_2=CHCO$), 6.2 (2H, m, $CH_2=CHCO$) and 7.26 ppm (5H, s, C_6H_5). (Found: C, 66.16; H, 6.85; N, 9.53%. $C_{16}H_{20}O_3N_2$ requires C, 66.5; H, 6.99; N, 9.71%).

Synthesis of N,O-*diacryloyl-*L-*tyrosine*

L-Tyrosine (181.2 g, 1 mol) was dissolved in 2 M aqueous NaOH (1 dm^3) containing hydroquinone (10 mg) and the solution cooled with vigorous mechanical stirring to 0°C. Crushed ice (0.5–1.01 g) was added directly to the solution and the reaction vessel maintained, cooled by means of an external ice–salt bath, while adding acryloyl chloride (271.5 g, 243.8 cm^3, 3 mol) over a period of 10–15 min. The pH was maintained at 11–11.5 by judicious addition of aqueous 4 M NaOH. After a further 5 min the pH of the reaction mixture was adjusted rapidly to 1–1.5 by addition of aqueous 11 M HCl. The aqueous layer was decanted off and the remaining oil extracted repeatedly with aliquots (200 cm^3) of boiling water which, on cooling, deposited crystals of *N,O*-diacryloyl-L-tyrosine (74 g, 25%) m.p. 113–114°C; $[\alpha]_D^{20}$ = –17.94° (methanol); ν_{max} (KBr disc) 1730 (ester C=O str) 1720 (acid C=O str) 1655 (amide C=O str) and 1600 cm^{-1} (C=C str); δ ($CDCl_3$; 60 MHz) 3.13 (2H, d, CH_2CH), 4.82 (H, m, $NHCHCH_2$), 5.4–6.5 (6H, cm, $CH_2=CHCONH$ and $CH_2=CHCO_2$), and 7.02 ppm (4H, dd, $CH_2C_6H_4O$). (Found: C, 61.95; H, 5.17; N, 4.83%. $C_{15}H_{15}O_5N$ requires C, 62.27; H, 5.22; N, 4.84%).

*Synthesis of 4-(*N,O-*diacryloyl-*L-*tyrosyl)-morpholine*

A solution of *N,O*-diacryloyl-L-tyrosine (14.45 g, 0.05 mol) in dichloromethane (50 cm^3) was stirred at 0°C and a solution of dicyclohexylcarbodiimide (10.3 g, 0.05 mol) in dichloromethane (25 cm^3) added. After 30 min a solution of morpholine (4.35 g, 4.35 cm^3, 0.05 mol) in dichloromethane (25 cm^3) was added dropwise and the mixture was stirred at room temperature for 24 h. Dicyclohexylurea was removed by filtration, hydroquinone (10 mg) added to the filtrate and the solvent removed under pressure. The residue was repeatedly extracted with aliquots (10 cm^3) of boiling acetone, which were filtered and diluted with petroleum ether (40°–60°C) when a precipitate was deposited. The precipitate was crystallized from water to give 4-(*N, O*-diacryloyl-L-tyrosyl)-morpholine as a hydrate. (15.4 g, 85.9%) m.p. 118°–119°C; $[\alpha]_D^{20}$ = –185.35° (methanol); ν_{max} (KBr disc) 1735 (ester C=O str), 1660 (amide C=O str), 1610 (C=C str) and 1110 cm^{-1} (C–O str); δ ($CDCl_3$; 60 MHz) 3.08 (2H, d, CH_2CH), 3.50 (8H, s, $CON(CH_2CH_2)O$), 5.19 (H, m, $NHCHCH_2$), 5.5–6.6 (6H, cm, $CH_2=CHCONH$ and $CH_2=CHCO_2$) and 7.12 ppm (4H, dd, $CH_2C_6H_4O$). (Found: C, 62.65; H, 6.34; N, 7.66%; $C_{19}H_{22}N_2O_5 \cdot \frac{1}{2}H_2O$ requires C, 62.11; H, 6.16; N, 7.63%).

*Synthesis of 4-(*N*-acryloyl-*L*-tyrosyl)-morpholine*

The monomer 4-(*N, O*-diacryloyl-L-tyrosyl)-morpholine (10 g, 0.028 mol) was dissolved in 4 M aqueous NaOH (30 cm^3) which had been deoxygenated by nitrogen bubbling. After stirring for 1 h at room temperature under nitrogen the reaction mixture was chilled to 0°C and acidified to pH 1–1.5 by dropwise addition of 18 M H_2SO_4. The oil which separated was taken up in acetone, dried over sodium sulphate, filtered, and the solvent removed under reduced pressure. The oil was then applied to a silica gel column and eluted with chloroform (1 bed volume) then with acetone/chloroform (50:50 v/v). The acetone/chlorofrom eluant was concentrated by rotary evaporation and chilled to give crystals of 4-(*N*-acryloyl-L-tyrosyl)-morpholine (3.50 g, 41.7%) m.p. 168°–170°C; $[\alpha]_D^{20}$ = –3.66° (methanol); ν_{max} (KBr disc) 1610 (amide C=O str), 1610 (C=C str) and 1110 cm^{-1} (C–O str); δ (CD_3OD; 60 MHz) 2.95 (2H, d, CH_2CH), 3.5 (8H, s, $CON(CH_2CH_2)_2O$), 5.05, (H, t, $NHCHCH_2$) 5.65 (H, m, $CH_2=CHCO$), 6.29 (2H, m, $CH_2=CHCO$) and 6.92 ppm (4H, dd, $CH_2C_6H_4O$). (Found: C, 62.68; H, 6.73; N, 8.75%. $C_{16}H_{20}N_2O_4$ requires C, 63.14; H, 6.63; N, 9.20%).

*Synthesis of 4-(*O*-acetyl-*N*-acryloyl-*L*-tyrosyl)-morpholine*

A solution of 4-(*N*-acryloyl-L-tyrosyl)-morpholine (2.5 g, 0.0082 mol) in 2 M NaOH (8.2 cm^3, 0.0164 mol) was chilled to 0°C and shaken vigorously with acetic anhydride (1.26 cm^3, 0.0124 mol) for 10 min. The white precipitate which formed was collected quickly by filtration, washed with water and dried over P_2O_5 *in vacuo*. Recrystallization from acetone gave hexagonal plates of 4(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine (2.4 g, 84%) m.p. 79°–81°C; $[\alpha]_D^{20}$ = –35.54° (methanol); ν_{max} (KBr disc) 1750 (ester C=O str), 1660 (amide C= str) and 1110 cm^{-1} (C–O str); δ(CD_3OD, 60 MHz) 2.26 (3H, s, CH_3CO), 3.05 (2H, d, CH_2CH), 3.5 (8H, s, $CON(CH_2CH_2)_2O$), 5.2 (H, m, $NHCHCH_2$), 5.62 (H, m, $CH_2=CHCO$), 6.36 (2H, m, $CH_2=CHCO$), and 7.2 ppm (4H, m, $CH_2C_6H_4O$). (Found: C, 62.17; H, 6.85; N, 6.96%. $C_{18}H_{22}N_2O_5 \cdot C_3H_6O$ requires: C, 62.37; H, 6.93; N, 6.93%).

*Synthesis of crosslinked poly[4-(*N*-acryloyl-*L*-phenylalanyl)-morpholine]*

A solution of 4-(*N*-acryloyl-L-phenylalanyl)-morpholine (5.04 g, 0.0175 mol) and *N,N'*-bisacryloylethylenediamine (0.294 g, 0.00175 mol) was dissolved in dry dimethylacetamide (25 cm^3) and deoxygenated by bubbling with nitrogen

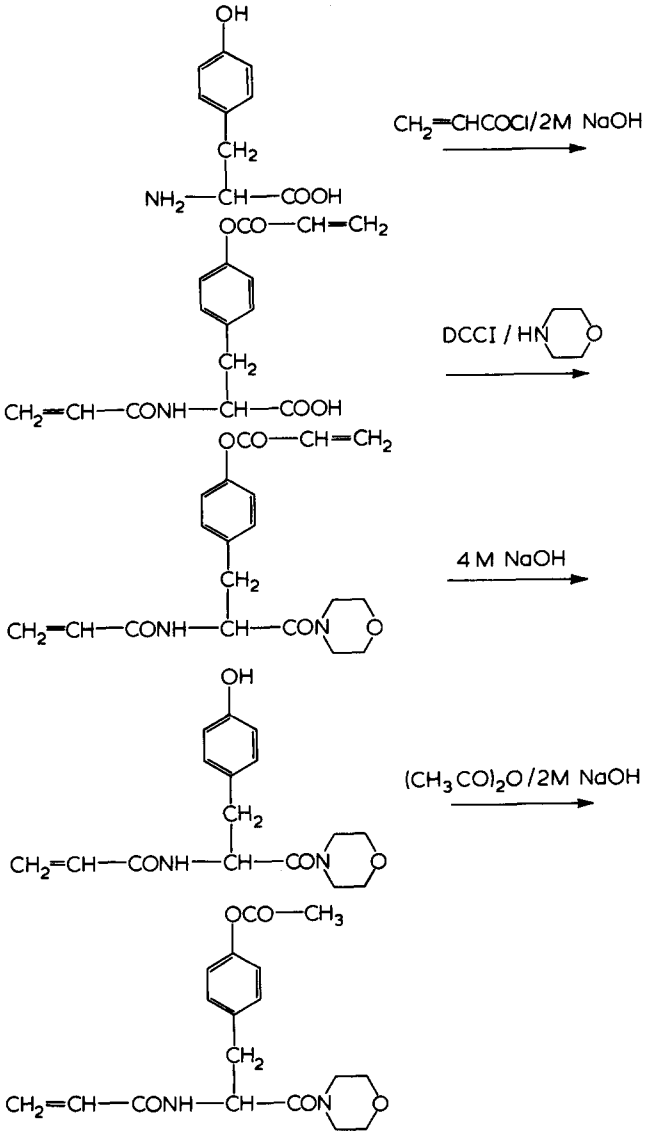

Figure 1 Synthetic route for the preparation of 4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine (d). Phenolic hydroxyl was protected as an *O*-acryloyl ester during DCCI coupling. Characterized intermediates include: a; *N,O*-diacryloyl-L-tyrosine; (b) 4-(*N,O*-diacryloyl-L-tyrosyl)-morpholine and (c) 4-(*N*-acryloyl-L-tyrosyl)-morpholine

for 1 h. The solution was transferred to the outer jacket of a water-cooled, 100 cm^3 capacity, concentric u.v. photoreactor fitted with a 100 watt lamp and nitrogen bubbler. The solution was irradiated for 20 h. The clear gel was then removed, washed with ethanol and ether, dried *in vacuo* and ground through a metal sieve (mesh size 150 μm^2) to give crosslinked poly[4-(*N*-acryloyl-L-phenylalanyl)-morpholine] (copolymer A) (yield 3.95 g, 74%) ν_{max} (KBr disc) 1670 (amide C=O str), 1620 (amide C= str) and 1110 cm^{-1} (C–O str).

Synthesis of crosslinked poly[4-(O-acetyl-N-acryloyl-L-tyrosyl)-morpholine]

U.v. initiated copolymerization of 4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine (2.42 g, 0.008 mol) and *N,N'*-bisacryloylethylenediamine (0.134 g, 0.0008 mol) in dimethylacetamide (11.5 cm^3) was effected as described for copolymer A. The clear gel obtained was washed with ethanol and ether, dried and ground through a metal sieve (mesh size 150 μm^2) to give crosslinked poly[4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine] (copolymer B) (yield 2.01 g, 79%) ν_{max} (KBr disc) 1760 (ester C=O str), 1670 (amide C=O str), 1620 (C=O str) and 1110 cm^{-1} (C–O str).

Synthesis of crosslinked poly[4-(N-acryloyl-L-tyrosyl) morpholine]

A dry sample of copolymer B (1.00 g) was dispersed in an excess of dry morpholine and the dispersion of gel so obtained allowed to stand at ambient temperature for 2h. The gel particles were washed successively with water, ethanol and ether and dried *in vacuo* to give crosslinked poly[4-(*N*-acryloyl-L-tyrosyl)-morpholine] (copolymer C) (0.861 g, 100%) ν_{max} (KBr disc) 1670 (amide C=O str), 1620 (amide C=O str) and 1110 cm^{-1} (C–O str).

Synthesis of crosslinked poly(acryloylmorpholine)

U.v. initiated copolymerization of acryloylmorpholine (2.5 g, 0.0175 mol) and *N,N'*-bisacryloylethylenediamine (0.294 g, 0.00175 mol) in dry dimethylacetamide (25 cm^3) was effected as described for copolymer A. The gel obtained was washed with ethanol and ether and ground through a metal sieve (mesh size 150 μm^2) to give crosslinked poly(acryloylmorpholine) (yield 1.72 g, 61.5%).

Solvent imbibition measurements

Samples (~50 mg) (w_1 g) of copolymer were accurately weighed into Visking dialysis tubes (10 x 2.5 cm) (Medicell International) presealed at one end. Tubing and copolymer were again weighed (w_2 g) and immersed in the solvent under study. After 3 days, excess solvent was removed by centrifugation while suspended in a stoppered centrifuge tube. Tubing and polymer were again weighed (w_3, g). Solvent imbibition was then calculated from the expression:

$$S_I = \frac{(w_3 - w_2)\rho}{w_1} \text{ cm}^3 \text{ g}^{-1}$$

where ρ is the solvent density.

RESULTS AND DISCUSSION

The natural amino acids L-phenylalanine and L-tyrosine were selected as starting materials for monomer preparation. These molecules possess a hydrophobic aromatic ring and a carboxylate group which may be converted to its polar morpholine amide. The amino group provides a convenient residue which may be used to convert the whole molecule to a polymerizable substituted acrylamide.

The monomer 4-(*N*-acryloyl-L-phenylalanyl)-morpholine was prepared by aqueous acryloylation of L-phenylalanine to give *N*-acryloyl-L-phenylalanine followed by dicyclohexylcarbodiimide-mediated condensation of the latter with morpholine. A similar strategy was adopted to prepare the monomer 4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine. However, in this case, the synthesis was complicated by the necessity of protecting the phenolic hydroxyl group of L-tyrosine during the subsequent dicyclohexylcarbodiimide-mediated condensation with morpholine. Our reaction scheme is shown in *Figure 1.*

It was found convenient to convert the phenolic hydroxyl of L-tyrosine to its acrylate ester concurrently with the required acryloylation of the amino residue. The base lability of the phenolic ester necessitated careful pH control during this reaction. Nevertheless, we found this approach prefer-

Table 1 Solvent imbibition values (S_I) for the various copolymers in different solvents

Solvent	Solvent imbibition value (S_I) ($cm^3\ g^{-1}$)			
	Crosslinked poly[4-(*N*-acryloyl-L-phenylalanyl)-morpholine] (Copolymer A)	Crosslinked poly[4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine] (Copolymer B)	Crosslinked poly[4-(*N*-acryloyl-L-tyrosyl)-morpholine] (Copolymer C)	Crosslinked poly(acryloyl-morpholine)
Benzene	3.36	4.69	2.72	0.13
Ether	0.08	1.35	2.05	1.29
Acetone	2.20	8.00	1.81	3.67
Dichloromethane	3.60	15.57	3.08	24.54
Dioxan	5.33	12.78	3.73	8.55
Ethyl alcohol	2.00	0.76	1.20	4.12
Methyl alcohol	4.02	1.63	2.68	5.92
Benzyl alcohol	15.14	8.49	8.62	2.87
Dimethylformamide	7.67	6.24	8.37	3.37
Dimethylacetamide	12.47	10.66	12.33	4.92
Acetic acid	13.21	15.60	5.42	16.24
Water	3.38	5.12	3.74	17.68

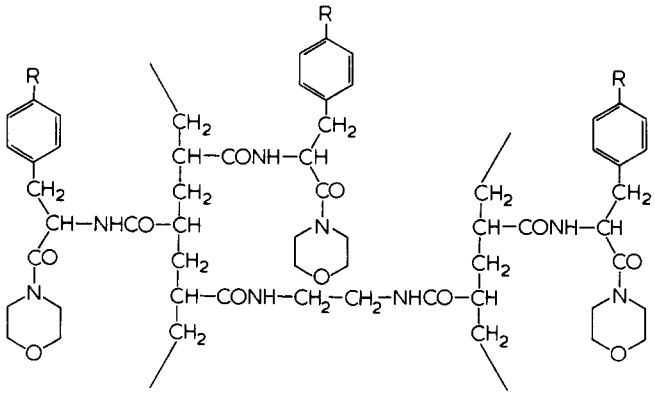

Figure 2 Schematic general representation of the three crosslinked copolymers based on amino-acid morpholine amides: R= —H, crosslinked poly[4-(*N*-acryloyl-L-phenylalanyl)-morpholine] (Copolymer A); R= $-OCOCH_3$, crosslinked poly[4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine (Copolymer B); R= —OH, crosslinked poly[4-(*N*-acryloyl-L-tyrosyl)-morpholine] (Copolymer C)

able to a two-stage strategy involving isolation of *N*-acryloyl-L-tyrosine because the latter proved to be difficult to purify and crystallize. Following dicyclohexylcarbodiimide-mediated condensation with morpholine, the phenolic acrylate group of 4-(*N,O*-diacryloyl-L-tyrosyl)-morpholine was easily hydrolysed selectively under alkaline conditions. The phenolic hydroxyl group so exposed was acetylated to give the desired monomer.

The two new monomers, 4-(*N*-acryloyl-L-phenylalanyl)-morpholine and 4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine, were each copolymerized with the crosslinker, *N,N'*-bis-acryloylethylenediamine, in the molar ratio 10/1, by u.v. irradiation in *N,N*-dimethylacetamide at a concentration of 0.75 mol dm^{-3}. Onset of gelation was apparent after 1 h. The networks so formed, copolymer A and copolymer B, respectively, were precipitated from the transparent gels by equilibration with ethanol followed by ether. The networks, shown in *Figure 2*, were subdivided and sieved in the dry state.

Crosslinked poly[4-(*O*-acetyl-*N*-acryloyl-L-tyrosyl)-morpholine], copolymer B, was readily de-*O*-acetylated quantitatively by treatment with morpholine to give poly[4-(*N*-acryloyl-L-tyrosyl)-morpholine] (copolymer C). This reaction was monitored by following the disappearance of the ester carbonyl peak at 1760 cm^{-1} in the infrared spectrum of the copolymer.

The swelling (gelation) properties of the three new polymer networks, together with a control consisting of crosslinked poly(acryloylmorpholine) prepared under similar reaction conditions, were investigated in a range of solvents. The results are shown in *Table 1*. It is evident that incorporation of both polar and non-polar features in the same monomer unit does produce interesting changes in gelation properties compared to the poly(acryloylmorpholine) networks which we have previously studied. Most remarkable are the enhanced relative swellings in dimethylformamide and dimethylacetamide, both of which are currently popular solvents for polymer-supported peptide synthesis[2]. Unfortunately, cost factors together with the difficulty of synthesizing the new networks in bead form preclude their further development as support matrices for this purpose.

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