Enhanced Enantioselectivity of Molecularly Imprinted Polymers Formulated with Novel Cross-Linking Monomers

Martha Sibrian-Vazquez and David A. Spivak*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

Received October 4, 2002; Revised Manuscript Received April 15, 2003

ABSTRACT: To enhance the performance of molecularly imprinted polymers (MIPs), three new cross-linking monomers were designed, synthesized, and evaluated as matrix elements for molecularly imprinted polymers. Hybrid cross-linking monomers incorporating methacrylamide/methacrylate (NOBE), vinyl ketone/methacrylamide (NAG), and vinyl ketone/methacrylate (MVK) polymerizable groups, were synthesized and used to prepare MIPs and compared to a traditional MIP formulated with EGDMA. The resulting macroporous MIPs were evaluated by HPLC for their ability to separate the enantiomers of a chiral template. The MIP formulated with the cross-linking monomer NAG showed the highest enantioselectivity and complete baseline resolution for a racemic mixture of the template. Enhancement in selectivity in this MIP may be attributed to the presence of the amide group which can promote hydrogen bonding via favorable 1,3 donor−acceptor interactions with the template, the morphology of the polymeric material obtained due to differences in reactivity between the polymerizable groups and improved resolution in cavity formation as a consequence of shorter cross-linker size.

Introduction

Research toward improving molecularly imprinted polymer (MIP) materials has focused primarily on the choice of template or functional monomer of the prepolymer complex. One aspect of MIPs that has been overlooked is that approximately 80−90% of the MIP's matrix is composed of the cross-linking monomer, with the remaining 10−20% comprised of functional monomers. The large percentage of cross-linking monomer materials in imprinted polymers presents the possibility of a commensurate improvement in polymer properties. Thus, we have redirected our focus on the design and synthesis of cross-linking monomers for molecular imprinting. Improving the binding site behavior in MIPs by improving the scaffold materials finds analogy with enzymatic systems, where active-site interactions are influenced by the rest of the protein structure.[1] The design, synthesis, and evaluation of new classes of cross-linked polymers that can optimize the performance of molecularly imprinted polymers are presented in this report.

The first cross-linking monomer to be employed for molecular imprinting in organic polymers was divinylbenzene (DVB, **1**),[2] which is still a useful cross-linker today. An early comparison of ethylene glycol dimethacrylate (EGDMA, **2**) and butanediol dimethacrylate (BDMA, **4**) to DVB quickly determined the best cross-linking matrix to be EGDMA.[3] EGDMA has been the cross-linker of choice ever since. A relatively new cross-linker that has shown some improvement over EGDMA is the trifunctional monomer 2,2-bis(hydroxymethyl) butanol trimethacrylate (TRIM, **5**), which is commercially available.[4] This was also found to be true of the similar cross-linker pentaerythritol triacrylate (PETRA) but not of the tetrafunctional cross-linker pentaerythritol tetraacrylate (PETEA).[5] A number of bis(acrylamide) and bis(methacrylamide) cross-linkers have been synthesized and shown to be successful as imprinting matrices.[6,7]

* Corresponding author. Telephone: (225) 578 2868. Fax: (225) 578 3458. E-mail: David_Spivak@chem.lsu.edu.

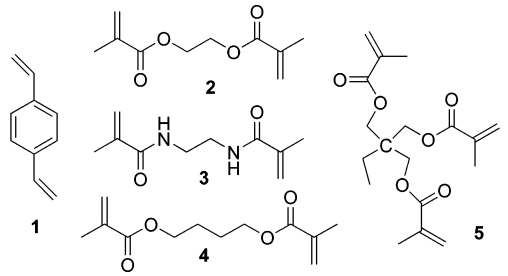


Figure 1. Cross-linking monomers commonly used for molecular imprinting.

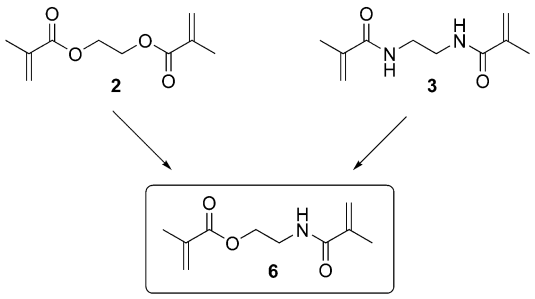


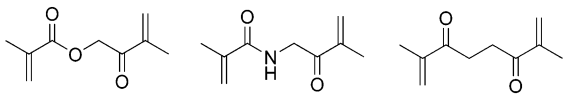
Figure 2. Hybridization of monomer **2** and **3** to form a new cross-linking monomer.

Since imprinted polymer formulations have predominantly used EGDMA (**2**), the majority of functional monomers have been developed with polymerizable groups compatible with the reactivity of the methacrylate moiety of EGDMA. Therefore, it was of interest to develop new cross-linking monomers that maintained compatibility with previously developed functional monomers. In designing a new cross-linking monomer, EGDMA was taken as the lead compound. Various compatible dimethacrylamide compounds have been previously investigated with encouraging results for MIP development. However, the new dimethacrylamides reported, including the EGDMA analogue ethylenediamine dimethacrylamide shown in Figures 1 and 2 (**3**), were found to be primarily soluble in polar organic solvents such as DMF and methanol. In general, the

10.1021/ma025710z CCC: $25.00
Published on Web 06/20/2003

use of polar solvents interferes with the formation of noncovalent prepolymer complexes in the molecular imprinting process.[8] Therefore, in an effort to obtain a cross-linker with the positive traits of both lead compounds **2** and **3**, the first design investigated was a product of "molecular breeding" the dimethacrylate species with the dimethacrylamide species to give compound **6** (NOBE) shown in Figure 2. Once synthesized (discussed in the next section), the hybrid monomer **6** was indeed found to be soluble in nonpolar organic solvents such as chloroform and methylene chloride.

A second design principle to be applied in this investigation stemmed from an idea put forth by a report by Wulff and co-workers, who noticed the smaller EGDMA gave MIPs with better enantioselectivity than MIPs made with the larger BDMA (**4**).[3] It is often overlooked that the templates and the monomers imprinting these templates are roughly on the same order of magnitude in size. Thus, spatial resolution of template by the cavities formed in the matrix is limited to the cross-linker dimensions. Therefore, it can be reasoned that smaller cross-linking monomers would improve the ability of the matrix to more snugly wrap around a template during polymerization. If EGDMA is taken as the lead compound, shortening the distance between the two polymerizable methacrylate groups can be accomplished by either eliminating the oxygen atom or the carbon atom of the glycol unit. Since elimination of one of the glycol carbon atoms in EGDMA would involve unlikely formation of a central acetal spacer, the design of choice was narrowed to either elimination of one of the oxygen atoms or elimination of one oxygen and one carbon atom of the glycol backbone of the monomer. The latter, compound **7** (MVK), was chosen due to its shorter length. An alternative to compound **7**, yet still employing the same distance parameters, is compound **8** (NAG), where the methacrylate moiety of compound **7** is replaced by a methacrylamide group. The amide group contributes changes in interactive functionality as well as conformational rotor flexibility, which influence the binding results discussed later. The contribution of the newly introduced vinyl ketone polymerizable moiety can be investigated using cross-linker **9** (EDVK), which maintains the same distance parameters. All of these cross-linking compounds were targeted for investigation toward improvement of MIP matrices.

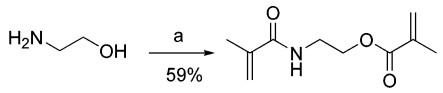


7 **8** **9**

Results and Discussion

Synthesis of Cross-Linkers. Synthesis of 2-(Methacryloylamino)ethyl 2-Methyl Acrylate (NOBE). Synthesis of 2-(methacryloylamino)ethyl 2-methyl acrylate (**6**) has been reported by Chan[9] who prepared this monomer by acylation of ethanolamine in a two-step route with an overall yield of 46%. Our approach for the synthesis of **6** took as reference the one-pot procedure reported by Anderson and Mosbach for the synthesis of *N*,*O*-bisacryloyl-L-phenylalaninol,[10] using equimolar amounts of ethanolamine to methacryloyl chloride and triethylamine (Scheme 1). The yield for this reaction

Scheme 1[a]



10 **6**

[a] Reaction conditions: (a) $H_2C{=}C(CH_3)COCl/Et_3N/CH_2Cl_2$, 40 °C/23 h.

was 20% after 40 h at room temperature; optimization of this procedure gave a 59% yield by using 2.5 equiv of methacryloyl chloride and triethylamine to ethanolamine at 40 °C/23 h.

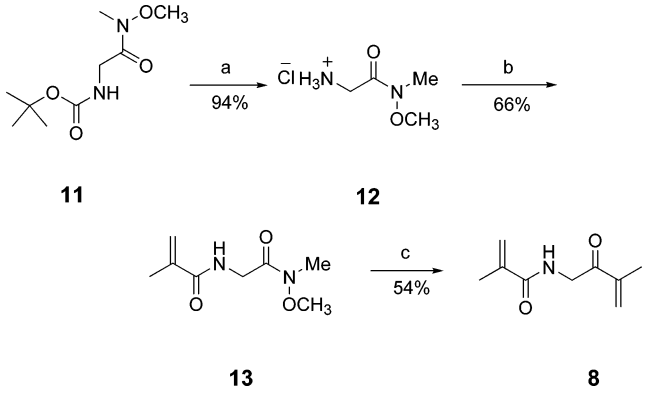
Synthesis of 2-Methyl-*N*-(3-methyl-2-oxobut-3-enyl)acrylamide (NAG). Several attempts for the synthesis of 2-methyl-*N*-(3-methyl-2-oxobut-3-enyl)acrylamide (**8**) were made using the acyl chloride derived from *N*-Boc-glycine. The nucleophilic addition of isopropenylmagnesium bromide and isopropenyl manganese iodide to the acyl chloride derived from *N*-Boc-glycine was unsuccessful. An alternative approach for the synthesis of the monomer **8** entailed the nucleophilic addition of isopropenylmagnesium bromide to *N*-Boc-glycine *N*-methoxy-*N*-methyl amide. The reaction between *N*-methoxy-*N*-methyl amides and nucleophiles such as Grignard reagents or alkyllithiums followed by acidic hydrolysis has been used successfully in the synthesis of ketones. In his studies, Weinreb[11] showed that a variety of *N*-methoxy-*N*-methyl amides undergo nucleophilic addition to provide a variety of ketones in good yields. These reactions are highly selective, and the formation of alcohols by overaddition of the nucleophile is rarely observed, which is one of the main drawbacks in the reaction of acyl halides and esters with Grignard reagents. Another advantage of this reaction is that no racemization products have been found in the synthesis of ketones derived from *N*-protected amino acids.[12]

Reaction of the *N*-Boc-glycine *N*-methoxy-*N*-methyl amide with isopropenylmagnesium bromide to give *tert*-butyl 3-methyl-2-oxobut-3-enylcarbamate initially gave a low yield (32%), accompanied by a large number of side products. The procedure was modified, by lowering the reaction temperature from 0 to −15 °C, adding the nucleophile quickly, and reducing the reaction time, to avoid isomerization of the isopropenylmagnesium bromide and other side reactions which culminated in an 83% yield.

It was anticipated that deprotection of the amino group of *tert*-butyl 3-methyl-2-oxobut-3-enylcarbamate by acid hydrolysis using HCl/Et_2O[13] would give the corresponding hydrochloride, which would be easily isolated from the reaction mixture; however, the analysis by ^1H NMR and IR showed that the compound obtained from the hydrolysis of *N*-Boc-methacryloyl glycine did not correspond to the 3-methyl-2-oxobut-3-en-1-aminium chloride. Instead, the product of an intramolecular Michael addition was obtained via the protonated enone after loss of the *tert*-butoxycarbonyl unit.[14] Considering these results, a new strategy using *N*-methoxy-*N*-methyl amides as a protecting group for the carboxylic acid was used in the synthesis of this monomer.

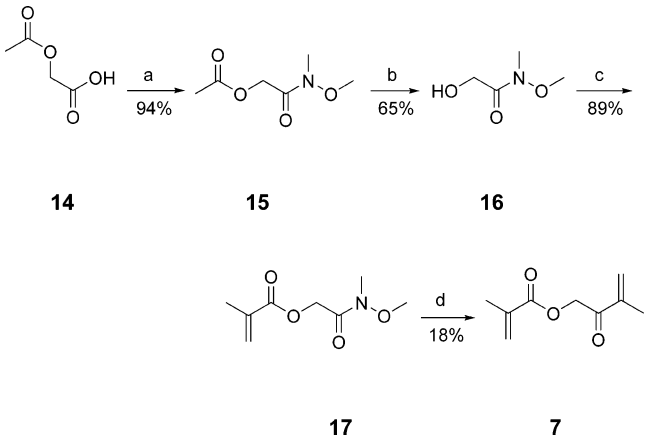
N^1-methoxy-N^1-methylglycinamide chloride (**12**) was synthesized by acidic hydrolysis of *N*-*tert*-Boc-Glycine *N*-methoxy-*N*-methyl amide (**11**) using HCl/Et_2O in 94% yield. 2-Methyl-*N*-(3-methyl-2-oxobut-3-enyl)acrylamide

Scheme 2[a]



[a] Reaction conditions: (a) HCl/Et_2O, N_2, 0 °C/6 h; rt/18 h; (b) $H_2C{=}C(CH_3)COCl$/DMAP/Et_3N/CH_2Cl_2, rt/48 h; (c) C_3H_5MgBr, THF/N_2, −15 °C/10 min, rt/25 min.

Scheme 3[a]

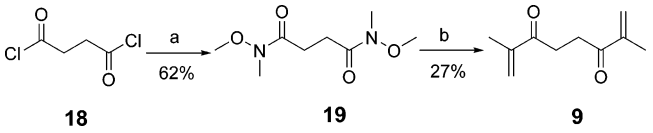


[a] Reaction conditions: (a) NMM/*i*-$BuCO_2Cl$/HCl.HN(OCH_3)(CH_3)/CH_2Cl_2/N_2, −15 °C/1 h, rt/12 h; (b) LiOH/THF/MeOH, 0 °C/20 min; (c) $H_2C{=}C(CH_3)CO_2H$/DCC/DMAP/CH_2Cl_2, rt/7 d; (d) C_3H_5MgBr, THF/N_2, −78 °C/30 min, 0 °C/30 min.

(**13**) was synthesized employing the methodology used in the synthesis of **6**. Since the starting material in this case corresponds to a hydrochloride, a preliminary neutralization of the HCl with Et_3N was necessary. The best reaction conditions were those in which the Et_3N was in a high excess; however, the yield was relatively low (44%). Under the same conditions, adding a 10% mol of DMAP as catalyst, the yield for this reaction was improved from 44 to 66%. 2-methyl-*N*-(3-methyl-2-oxobut-3-enyl)acrylamide (**8**) was synthesized by nucleophilic addition of isopropenylmagnesium bromide to **13** to give a 54% yield. The low yield is attributed to the formation of side products due to polymerization, isomerization of the isopropenylmagnesium bromide, and nucleophilic attack on the amide functionality. Scheme 2 shows the overall synthesis for monomer **8**.

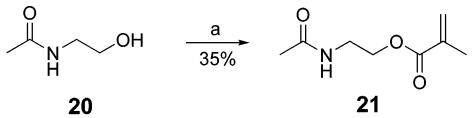
Synthesis of 2-Methylacrylic Acid 3-Methyl-2-oxo-but-3-enyl Ester (MVK). As shown in Scheme 3, the first intermediate **15** was synthesized from (acetyloxy)acetic acid (**14**) using a protocol involving the initial formation of a mixed anhydride with isobutyl chloroformate followed by nucleophilic attack of the amine to produce the amide.[15] Basic hydrolysis[16] of the ester group using an equimolar amount of LiOH produced the 2-hydroxy-*N*-methoxy-*N*-methylacetamide (**16**). Better yields (63−65%) were obtained when LiOH solid was added directly to a solution of the amide in THF/MeOH, rather than adding 1 N LiOH/MeOH to a solution of the amide in THF; (yields 30−40%). Esterification of the hydroxyl group to give compound **17** can be done using either protocols involving the use of DCC/DMAP[17] or the acyl chloride/Et_3N,[10] with comparative yields in the range of 67−89%. Nucleophilic addition of isopropenylmagnesium bromide to the Weinreb amide **17** in order to produce the vinyl ketone (**7**) gave a low yield (18%), under the best conditions.

Scheme 4[a]



[a] Reaction conditions: (a) HCl.HN(OCH_3)(CH_3)/Et_3N/CH_2Cl_2/N_2, rt/16 h; (b) C_3H_5MgBr, THF/N_2, −78 °C/30 min, 0 °C/30 min.

Scheme 5[a]

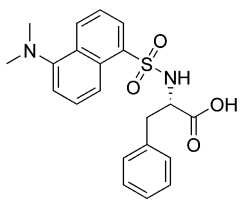


[a] Reaction conditions: (a) $H_2C{=}C(CH_3)CO_2H$/DCC/DMAP/$CHCl_3$, rt/4 d.

Synthesis of 2,7-Dimethyl-octa-1,7-diene-3,6-dione (EDVK). Synthesis of 2,7-dimethyl-octa-1,7-diene-3,6-dione (**9**) was based in the work published by Sibi[18] and co-workers, who successfully prepared asymmetric 1,2-diketones using *N*-methoxy-*N*-methyl diamides derived from oxalyl chloride. As shown in Scheme 4, the *N*,*N*′-dimethoxy-*N*,*N*′-dimethyl-succinamide (**19**) derived from succinoyl dichloride (**18**) was prepared according to the procedure reported by Uchiyama.[19] Nucleophilic addition of 2 equiv of isopropenylmagnesium bromide to **19** gave the 1,4-diketone (**9**) with yields in the range of 23−27%.

Synthesis of 2-(Acetylamino)ethyl 2-Methacrylate (21). As shown in Scheme 5, compound **21** was synthesized by acylation of **20** with MAA using DCC as the coupling reagent in a 35% yield.

Formation of Imprinted Polymers. To evaluate the performance of the new cross-linking monomers, dansyl-L-phenylalanine (**22**) was chosen as the template. Dansyl-L-phenylalanine was chosen because it has a single chiral center allowing for evaluation of chiral recognition as a diagnostic of polymer performance. Three functional groups, a sulfonamide, a tertiary amine, and a carboxylic acid provide three points for electrostatic and hydrogen-bonding interactions for the complex prior to polymerization.



22

Molecularly imprinted polymers were synthesized using the L- enantiomer of dansyl phenylalanine complexed with the functional monomer methacrylic acid (MAA) in acetonitrile. Recovery of the template after Soxhlet extraction with methanol was in the range of

Table 1. Chromatographic Results for Dansyl-L-Phenylalanine and Dansyl-D-phenylalanine on Imprinted Polymers Using the New Cross-linking Monomers[a]

entry	cross-linking monomer	template extracted, %	t_{RL}(av), min	t_{RD}(av), min	k'_L	k'_D	α
1	EGDMA, **2**	90	3.87	3.58	0.24	0.14	1.7
2	NOBE, **6**	98	9.20	5.66	2.09	0.90	2.3
3	NAG, **8**	80	15.6	6.95	4.2	1.32	3.2
4	MVK, **7**	56	4.10	4.01	0.29	0.26	1.1
5	EDVK, **9**	85	4.18	4.08	0.29	0.27	1.1
6	EGDMA + **21**[b]	77	9.17	8.58	1.22	1.08	1.1
7	EGDMA + **21**[c]	79	7.65	6.51	0.88	0.60	1.5

[a] HPLC conditions: particle size, 20−25 μm; column size, 75 × 2.1 mm; mobile phase, acetonitrile/acetic acid 99/1; analytes, 0.1 m M dansyl-L-phenylalanine, 0.1 mM dansyl-D-phenylalanine, and acetone (used to determine void volume); flow rate, 0.1 mL/min; wavelength detection, 330 nm; injected volume, 5 μL. [b] MIP formulated with 50% of **21**. [c] MIP formulated with 25% of **21**.

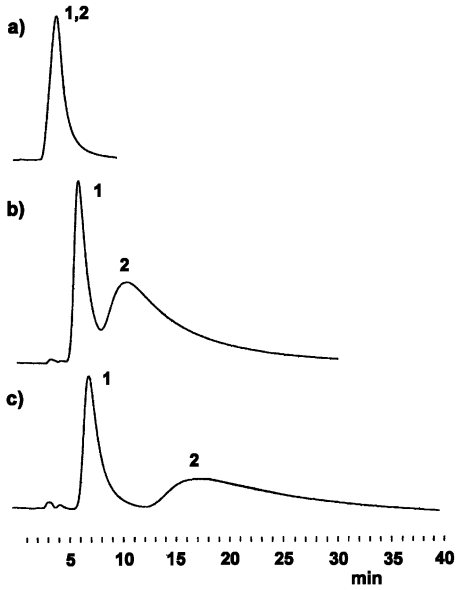


Figure 3. Elution profile of a racemic mixture of dansyl−phenylalanine [(1) D-enantiomer, (2) L-enantiomer] injected on columns (75 × 2.1 mm) packed with (a) MIP-EGDMA, (b) MIP−NOBE, and (c) MIP−NAG: particle size 20−25 μm; mobile phase acetonitrile/acetic acid 99/1; flow rate 0.1 mL/min; injected volume 5 μL; wavelength detection 330 nm.

56−98%, depending on the cross-linker used (Table 1). The selectivity of the polymers was determined by HPLC under isocratic conditions at room temperature. Capacity factors (k') were obtained in order to determine separation factors (α) of the L- and D-enantiomers of dansyl phenylalanine ($\alpha = k'_L/k'_D$). Table 1 shows the capacity and separation factors for the imprinted polymers using the new cross-linking monomers (entries 2−5) vs an imprinted polymer using the traditional polymer formulation with EGDMA as the cross-linking monomer (entry 1).

Table 1 shows that imprinted polymers formulated with cross-linking monomers in entries 1−3 showed a high selectivity for the original template. The imprinted polymer using NAG as the cross-linking monomer (entry 3) gave the highest separation factor, exhibiting a *2*-fold improvement vs the traditional formulation with EGDMA (entry 1). This improvement in molecular recognition of the template is emphasized by Figure 3, demonstrating the ability of the imprinted polymer in entry 3 to resolve a mixture of the two enantiomers of dansyl phenylalanine. Complete baseline resolution as shown in Figure 3 is obtained from the D-enantiomer in the first peak, and the L-enantiomer in the second, broader peak; a behavior often seen for MIPs. In comparison, the imprinted polymer using EGDMA (entry 1) as the cross-linking monomer did not give any chromatographic resolution of the dansyl−phenylalanine enantiomers and resulted in complete overlapping of the two peaks. This example shows that even though there is a significant α value from enantiomers injected separately, the very low retention times give rise to poor resolution of peaks in Figure 3 because the width of each peak at half-height is very large compared to retention time. Although there was some improvement in the binding affinity and specificity, as reflected in the capacity factors k' for imprinted polymers in entries 4 and 5 with respect to the traditional EGDMA (entry 1), these polymers also did not show chromatographic resolution of the enantiomers.

Table 2. Chromatographic Results for Boc-L-tyrosine and Cbz-L-tryptophan on Imprinted Polymers Using the Hybrid Cross-Linking Monomers NOBE and NAG[a]

		Boc-L-tyrosine			Cbz-L-tryptophan		
entry	cross-linking monomer	k'_L	k'_D	α	k'_L	k'_D	α
1	EGDMA, **2**	0.41	0.23	1.78	0.78	0.33	2.36
2	NOBE, **6**	2.24	1.22	1.84	3.72	1.39	2.68
3	NAG, **8**	8.03	2.56	3.14	18.08	3.54	5.11

[a] HPLC conditions: particle size, 20−25 μm; column size, 100 × 2.1 mm; mobile phase, acetonitrile/acetic acid 99/1; analytes, 1 mM Boc-L-tyrosine, 1 mM Boc-D-tyrosine, 0.2 mM Cbz-L-tryptophan, 0.2 mM Cbz-D-tryptophan, and acetone (used to determine void volume); flow rate, 0.1 mL/min; wavelength detection, 270 nm for Boc-tyrosine and 260 nm for Cbz-tryptophan; injected volume, 5 μL.

The improvement of molecular recognition by the imprinted polymer using the cross-linking monomer NOBE (entry 2) vs the traditional EGDMA imprinted polymer must be attributed to the presence of the amide group. The generality of improved binding properties for various types of templates is verified by Table 2, which shows the results of imprinting Boc-L-tyrosine and Cbz-L-tryptophan.

The trends in Table 2 follow the same trends seen in Table 1; specifically, polymers made with NAG show the best selectivity, the highest binding affinity, and the only imprinted polymers to give baseline separation in chromatography. One possible reason for the improvement in enantioselectivity is participation of the amide group of NOBE in hydrogen-bonding interactions with the template. If this is the case, cross-linkers incorporating amide groups can be considered functional groups as well. Although amides generally form weak interactions with other functional groups, the capacity factors for NOBE show that considerable binding does in fact take place. This binding is due to the amide group, and it is not seen for the isosteric EGDMA. Another possible reason for the improvement in enantioselectivity by NOBE may be due to increased rigidity of the entire matrix, imparted by the amide bond in the cross-linker. Since amide bonds are more conformationally rigid than ester bonds, the introduction of the amide bond into the backbone of the cross-linking monomer may maintain binding site fidelity, in addition to possible conformational control within the matrix unavailable using the traditional EGDMA.

The NAG further shows improvement in selectivity over NOBE. Comparing these cross-linkers, both have a single amide bond capable of hydrogen-bonding in-

teractions with the template that can provide greater rigidity to the backbone of the polymer. Table 1 shows that the amide bond is responsible for the similar increase in binding affinities of NAG and NOBE vs EGDMA. Since this increase is of the same order of magnitude for NAG and NOBE, the increased selectivity of NAG is not due merely to the presence of the amide bond. There are two differences between these cross-linkers: (1) the spacer between the cross-linking points is reduced from six atoms to four, and (2) the methacrylate group of NOBE is substituted by a methacrolein group in the cross-linking monomer NAG. Simply comparing the monomer NAG to NOBE does not supply enough information to discern whether the size or change in polymerizable group is responsible for the enhancement in selectivity. Therefore, two new cross-linking monomers, MVK and EDVK, were investigated.

Both MVK and EDVK monomers have the same length dimensions as NAG; however, the amide nitrogen in NAG is replaced with an oxygen for MVK and a carbon for EDVK. If size of the cross-linker was the key to enhanced selectivity, then polymers imprinted using these monomers should also exhibit improved separation factor values. Table 1 shows this is not the case, instead these monomers produced MIPs that were less selective than polymers imprinted using the cross-linkers NAG, NOBE, or EGDMA. Furthermore, if enhanced selectivity of NAG vs NOBE was due to the substitution of a methacrolein polymerizable group in place of the methacrylate polymerizable group, it would be reasonable to suggest the polymers imprinted using MVK and EDVK cross-linkers should show enhanced selectivity vs polymers imprinted using traditional EGDMA. However, the separation factors in Table 1 show that this is not the case, and incorporation of the methacrolein polymerizable group is not by itself responsible for increased selectivity seen by MIPs made with NAG cross-linker.

On the basis of these data, enhancement in selectivity for the imprinted polymer using NAG may be the result of *cooperative* interactions between functional groups in the monomer. This may result in a polymer with greater stability, capable of maintaining the fidelity of the original imprinted binding site. Another possibility is the close proximity of donor and acceptor functionality that can provide cooperative hydrogen-bonding interactions. NAG has a shorter distance between the nitrogen of the amide group and the carbonyl group of the methacrolein group, providing donor−acceptor interactions in a favorable 1,3 disposition that would promote chelating interactions. On the other hand, NOBE provides donor−acceptor in a less favorable 1,5 disposition, forcing the amide and carbonyl groups to act independently. Even if chelation is not at the root of binding improvement, the smaller spacer group between the donor and acceptor functionalities on NAG may provide more positive interactions per square angstrom of polymer in the vicinity of the template vs NOBE or other longer cross-linkers. The higher density of polymer functionality in the vicinity of the template may provide a general basis for improved molecular recognition for the new MIPs.

Analysis of Porosity and Surface Area. Most MIPs are part of a class of polymers known as macroporous polymers. The morphology of these materials is known and actually consists of aggregates of material with macro-, meso-, and micropores formed between aggregates.[20] Porosity is controlled by the interaction of the forming polymer matrix and the solvent (referred to as "porogen") as phase separation occurs. Data from BET analysis of the new materials by nitrogen adsorption−desorption porosimetry are shown in Table 3. The nitrogen adsorption−desorption isotherms indicate that all polymers have average pore diameters in the macroporous range, essentially in the same order of magnitude as the polymer made using the traditional MIP formulation with EGDMA. With the exception of MVK, the polymers also exhibited pore volumes essentially of the same order of magnitude. The polymer made with MVK had a low pore volume which is responsible also for low surface area. With the exception of polymers made with NOBE, MVK, and the MIP formulated with 50% of **21**, the surface areas for the other polymers were within the same order of magnitude. While low porosity is the origin of low surface area for the MVK polymer, the low surface area for the NOBE polymer is due to the larger pore size. As with earlier porosity studies on MIPs,[8] there is no direct correlation of surface area or porosity with MIP performance; however, the data presented here show that macroporous polymers made with the new cross-linkers are similar in this respect to the well-characterized EGDMA polymers.

Table 3. Surface Area and Pore Analysis on the Imprinted Polymers

entry	polymer	surface area (BET),[a] m^2/g	pore vol,[b] mL/g	pore diam,[c] Å
1	EGDMA, **2**	165	0.486	118
2	NOBE, **6**	51	0.510	402
3	NAG, **8**	154	0.488	127
4	MVK, **7**	92	0.191	83
5	EDVK, **9**	136	0.480	141
6	EGDMA + **21**[d]	91	0.728	319
7	EGDMA + **21**[e]	106	0.416	156

[a] Determined using the BET model on a seven-point linear plot. [b] BJH cumulative adsorption pore volume. [c] BJH adsorption average pore diameter. [d] MIP formulated with 50% of **21**. [e] MIP formulated with 25% of **21**.

Table 4. Unreacted Double Bonds Determined by Solid-State ^{13}C CP-MAS NMR

entry	cross-linking monomer	unreacted double bonds, %
1	EGDMA, **2**	17
2	NOBE, **6**	23
3	NAG, **8**	24
4	MVK, **7**	15
5	EDVK, **9**	19

^{13}C CP-MAS NMR Analysis. Quantitative estimation of the unreacted double bonds by ^{13}C CP-MAS NMR (Table 4) was obtained from the integrated intensity of the carbonyl resonance assigned to the unsaturated carbonyl, relative to the total integrated intensity of the saturated carbonyl:[21] 167 and 175 ppm for methacrylate, 167 and 177 ppm for methacrylamide, and 190 and 210 ppm for vinyl ketone. The average number of unreacted double bonds was 20% with all polymers falling close to that value. The NOBE and NAG cross-linkers showed the highest number of free double bonds, which most likely corresponds to slow reactivity by the methacrylamide moiety. There are no correlations between residual double bonds and polymer performance in the literature. This is mainly due to the fact that there is one other published result on the solid-state NMR analysis of photopolymerized MAA/EGDMA imprinted

polymers, which reported residual double bond content on the order of 9%.[8] To test whether it is the residual unreacted methacrylamide functionality that is responsible for the increased molecular recognition (specificity and binding affinity) of the template molecules, compound **21** was synthesized, and substituted for NOBE to provide the desired pendant amide functionality. Table 1 shows the binding results for polymers where half of the EGDMA component was substituted with compound **21** (entry 6), or one-fourth of the EGDMA was substituted with compound **21** (entry 7). This was anticipated to give polymers with the same pendant amide functionality as the polymer in entry 2. The data in entries 6 and 7 show that good binding and selectivity (seen for entry 2) do not originate from pendant amide functionality; instead, it must be concluded that the amide functionality incorporated in the cross-linked backbone of the polymer is responsible for the enhanced binding properties of NOBE polymers (and NAG polymers). Last, it should be pointed out that the MIPs made with the new cross-linkers are similar with respect to residual double bond content vs standard EGDMA polymers.

Conclusions

Using EGDMA as the lead compound, three new cross-linking monomers were designed, synthesized, and evaluated as matrix elements for molecularly imprinted polymers. Polymers made using the new cross-linking monomers and imprinted with the template dansyl-L-phenylalanine were evaluated using enantioselectivity as the figure of merit for MIP performance. Chromatographic evaluation of the imprinted polymers revealed that MIPs made with amide-functionalized cross-linkers (NAG, NOBE) showed increased enantioselectivity for the template molecule vs MVK, EDVK, and the traditionally used EGDMA. The same trend was observed when Boc-L-tyrosine and Cbz-L-tryptophan were used as the template molecules. The origins of the enhanced selectivity could arise from both molecular and macromolecular contributions. At the molecular level, selectivity of template binding may be enhanced by cooperative 1,3-interactions of the amide and carbonyl group of NAG, vs the more distant 1,5-interaction of NOBE. A more general enhancement to molecular recognition in the MIPs employing the new cross-linkers may be simply attributed to a higher functional group density by the polymers in vicinity of the template, which may provide more positive binding interactions vs EGDMA cross-linked polymers.

From a materials point of view, the shorter length of NAG may provide a better resolved binding site by wrapping more snugly around the template molecule. However, this appears only to be effective in cooperation with a more rigid cross-linker to maintain the site fidelity, compared to MVK which has similar size but does not have the rigid amide linkage. Surface area and porosity measurements reveal similar characteristics for all materials. Solid state NMR measurements of residual double bonds in the new materials show values close in magnitude for all the polymers. Thus, the new materials can all be classified as macroporous, without drastic differences in the polymer materials vs the standard MAA/EGDMA polymer formulation. Overall, these results show that the cross-linking monomer is not merely an inert component of the MIPs, but also plays a role in promoting positive interactions with the template to afford molecular recognition.

Experimental Section

Materials. Unless otherwise indicated, chemicals were purchased from Aldrich and used without further purification. Solvents were obtained from commercial suppliers and used as is. THF was dried by refluxing over K, followed by distillation. CH_2Cl_2 was dried by refluxing over CaH_2, followed by distillation. Reactions under anhydrous conditions were performed in dry glassware under N_2 atmosphere. Reactions were monitored by thin-layer chromatography using 0.25 mm Macherey Nagel silica gel glass plates (60F-254), fractions being visualized by UV light, by iodine, or by staining with molybdophosphoric acid with subsequent heating. Column chromatography was carried out with flash silicagel, 32−63 μm from Science Adsorbents Inc.

Measurements. ^{1}H NMR and ^{13}C NMR were measured in $CDCl_3$ unless otherwise indicated on a Bruker DPX-250 spectrometer. Chemical shifts (δ) are given in ppm relative to $CDCl_3$ (7.24 ppm, ^{1}H; 77.00 ppm, ^{13}C) unless otherwise indicated. ^{13}C CP-MAS NMR analysis was performed in a Chemagnetics Infinity 400 spectrometer. IR spectra were obtained as neat samples on a Nicolet AVATAR 320 FT-IR unless otherwise indicated. High-resolution mass spectra (HRMS) were obtained on a Finnigan MAT900 double sector instrument, under fast atom bombardent (FAB, liquid sims) ionization or electrospray ionization (EI). Imprinted polymerization was performed in a photochemical turntable reactor (ACE Glass Inc.), which was immersed in a constant-temperature bath. A standard laboratory UV light source (a Canrad-Hanovia medium pressure 450 W mercury arc lamp) jacketed in a borosilicate double-walled immersion well was placed at the center of the turntable. HPLC columns were packed using a Beckman 1108 Solvent Delivery Module, into stainless steel columns (length 7.5 cm; i.d. 2.1 mm) to full volume for chromatographic experiments. HPLC analyses were performed isocratically at room temperature (21 °C) using a Hitachi L-7100 pump with a Hitachi L-7400 detector. Pore size measurementes were obtained in a Quantachrom AUTOSORB-1 AS-1.

2-(Methacryloylamino)ethyl 2-Methyl Acrylate (6). 2-Aminoethanol (**10**) (0.976 g, 16 mmol) was mixed with 15 mL of CH_2Cl_2, and then Et_3N (3.74 g, 5.15 mL, 37 mmol) was added in small portions to the initial mixture with stirring. The reaction mixture was cooled to 0 °C and then (3.867 g, 3.6 mL, 37 mmol) methacryloyl chloride was added dropwise with vigorous stirring and keeping the temperature at 0 °C. After complete addition of methacryloyl chloride, the temperature was increased to 40 °C and the reaction mixture was allowed to react 23 h at this temperature. The reaction mixture was filtered out and the precipitate (Et_3NHCl) discarded. The filtrate was extracted with 0.5 M $NaHCO_3$ (3 × 15 mL) and 0.5 M sodium citrate (3 × 15 mL). The solvent was evaporated under vacuum, and the compound was isolated by flash chromatography with EtOAc/hexanes 50/50, EtOAc 100%. The product (**6**) was isolated as a pale yellow oil in a 59% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 6.80 (1H, bs, NH), 5.99 (1H, m, $RO_2C(CH_3)C{=}C\boldsymbol{H_a}H_b$), 5.71 (1H, t, J = 0.905 Hz, $RHNCOC(CH_3)C{=}C\boldsymbol{H_c}H_d$), 5.60 (1H, t, J = 0.517 Hz, $RO_2C(CH_3)C{=}CH_a\boldsymbol{H_b}$), 5.38 (1H, t, J = 0.517 Hz, $RHNCOC(CH_3)C{=}CH_c\boldsymbol{H_d}$), 4.29 (2H, t, J = 5.69 Hz, $OC\boldsymbol{H_2}$), 3.60 (2H, quad, J = 5.69, Hz, $NHC\boldsymbol{H_2}$), 1.94 (3H, s, $C\boldsymbol{H_3}$), 1.8 (3H, s, $C\boldsymbol{H_3}$). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 169.08 ($RNH\boldsymbol{C}OR$), 167.82 ($R\boldsymbol{C}O_2R$), 140.11 [$RHNCO(CH_3)\boldsymbol{C}{=}CH_2$], 136.25 [$RCO_2(CH_3)\boldsymbol{C}{=}CH_2$], 126.40 [$RCO_2(CH_3)C{=}\boldsymbol{C}H_2$], 120.04 [$RHNCO(CH_3)C{=}\boldsymbol{C}H_2$] 63.50 ($O\boldsymbol{C}H_2$), 39.29 ($NH\boldsymbol{C}H_2$), 18.90 [$RHNCO(\boldsymbol{C}H_3)C{=}CH_2$], 18.60 [$RCO_2(\boldsymbol{C}H_3)C{=}CH_2$]. IR (cm^{-1}): 1722.55, 1660.17, 1628.55, 1453.10, 1336.68, 1161.81, 1083.65, 937.62. HRMS (EI) (M^+): calcd, 197.1052; found, 197.1063.

***N*^{1}-Methoxy-*N*^{1}-Methylglycinamide Chloride (12).** *N*-Boc-glycine *N*-methoxy-*N*-methyl amide (**11**) (0.874 g, 4 mmol) was dissolved in 3 mL of dry $CHCl_3$ and cooled at 0 °C under N_2 atmosphere, and then 10 mL of 2 M HCl in ethyl ether

was added dropwise. The temperature was kept at 0 °C/6 h and increased to room temperature and the reaction stirred for 18 h. HCl and ether were purged under a current of N_2 with further removal under vacuum. The white sticky solid residue was washed with ethyl ether (3 × 20 mL) and dried under vacuum. Yield: 94%. ^{1}H NMR (DMSO-d_6, 250 MHz): δ/ppm = 8.5 (3H, bs, N***H*****$_3^+$**), 3.93 (2H, d, C***H*****$_2$**), 3.82 [3H, s, RN(OC***H*****$_3$**)(CH$_3$)], 3.25 [3H, s, RN(OCH$_3$)(C***H*****$_3$**)]. ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ/ppm = 167.60 [R***C***ON(OCH$_3$)(CH$_3$)], 62.32 [RCON(O***C***H$_3$)(CH$_3$)], 34.69 [N***C***H$_2$CON(OCH$_3$)(CH$_3$)], 32.74 [RCON(OCH$_3$)(***C***H$_3$)]. IR (cm^{-1}): 3418.68 (broad), 1671.57, 1494.94, 1183.14, 1130.96, 983.41, 907.01. HRMS(FAB) (M + H$^+$): calcd, 119.0821; found, 119.0825.

N*-Methacryloyl Glycine-*N*,*N*-Methoxymethyl Amide (13). 12** (0.706 g, 4 mmol) was suspended in 5 mL of CH_2Cl_2 and neutralized with Et_3N until pH 7−8. The mixture was cooled at 0 °C, and then the rest of the Et_3N was added. A total of 20 mmol (2.024 g, 2.78 mL) were added. To this mixture was added (0.122 g, 1 mmol) DMAP, followed by dropwise addittion of methacryloyl chloride (1.0454 g, 10 mmol). After complete addition of methacryloyl chloride, the mixture was kept at 0 °C for 30 min and then the temperature increased to room temperature, and the mixture was allowed to react for 48 h. The reaction mixture was filtered and the precipitate (Et_3N.HCl) discarded. The filtrate was washed with 0.5 M $NaHCO_3$ (3 × 15 mL) and 0.5 M sodium citrate (3 × 15 mL) and dried over $MgSO_4$. The solvent was evaporated under vacuum to leave a brown oil. **13** was isolated by flash chromatography with EtOAc giving a pale yellow oil in a 66% yield. ^{1}H NMR ($CDCl_3$): δ/ppm = 6.70 (1H, bs, NH***), 5.73 (1H, s, RHNCOC(CH$_3$)C=C***H*****$_a$**H$_b$), 5.30 (1H, s, RHNCOC(CH$_3$)C=CH$_a$***H*****$_b$**), 4.17 (2H, d, J = 4.42 Hz, C***H*****$_2$**), 3.67 [3H, s, RN(OC***H*****$_3$**)(CH$_3$)], 3.16 [3H, s, RN(OCH$_3$)(C***H*****$_3$**)], 1.93 (3H, s, C***H*****$_3$**). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 170.08 [R***C***ON(OC*H*$_3$)(CH$_3$)], 168.61 [RHN***C***OC(CH$_3$)C=CH$_2$], 139.68 [RHNCOC(CH$_3$)***C***=CH$_2$], 120.61 [RHNCOC(CH$_3$)C=***C***H$_2$], 61.93 [RN(O***C***H$_3$)(CH$_3$)], 41.24 (***C***H$_2$), 32.70 [RN(OCH$_3$)(***C***H$_3$)], 18.09 (***C***H$_3$). IR (cm^{-1}): 3349.22, 1658.85, 1621.44, 1533.06, 1313.81, 991.35, 931.64. HRMS(FAB) (M + H$^+$): calcd, 187.1082; found, 187.1078.

2-Methyl-*N*-(3-methyl-2-oxobut-3-enyl)acrylamide (8). 13 (0.558 g, 3 mmol) was dissolved in 10 mL of dry THF and cooled to −15 °C under N_2. Isopropenylmagnesium bromide (15 mL of a 0.5 M solution/THF, 7.5 mmol) was added dropwise over a period of 5 min during which a pale yellow precipitate was formed. The mixture was stirred for other 5 min at −15 °C following complete addition of the Grignard reagent and then brought to room temperature and stirred for 25 min. After this period, 7.0 mL of saturated NH_4Cl solution was added to quench the reaction. The THF was removed by evaporation under vacuum and 30 mL of ethyl ether were added to recover the organic compounds. Phases were separated, and the organic layer was washed with H_2O (2 × 15 mL) and brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue, a pale yellow oil, was separated by flash chromatography with EtOAc/hexanes 50/50 giving 54% yield for the title product as a yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 6.75 (1H, bs, N***H***), 6.06 [1H, d, J = 0.948 Hz, RCOC(CH$_3$)C=C***H*****$_c$**H$_d$], 5.89 [1H, t, J = 1.580 Hz, RCOC(CH$_3$)C=CH$_c$***H*****$_d$**], 5.78 (1H, d, J = 0.948 Hz, RHNCOC(CH$_3$)C=C***H*****$_a$**H$_b$), 5.37 (1H, t, J = 1.580 Hz, RHNCOC(CH$_3$)C=CH$_a$***H*****$_b$**), 4.49 (2H, d, J = 4.423 Hz, C***H*****$_2$**), 1.97 (3H, t, J = 0.948 Hz, C***H*****$_3$**), 1.91 (3H, t, J = 0.948 Hz, C***H*****$_3$**). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ/ppm = 196.20 [R***C***OC(CH$_3$)C=CH$_2$], 168.52 [RHN***C***OC(CH$_3$)C=CH$_2$], 142.59 [RCOC(CH$_3$)***C***=CH$_2$], 139.71 [RHNCOC(CH$_3$)***C***=CH$_2$], 126.59 [RCOC(CH$_3$)C=***C***H$_2$], 120.76 [RHNCOC(CH$_3$)C=***C***H$_2$], 46.03 (***C***H$_2$), 18.93 (***C***H$_3$), 17.71 (***C***H$_3$). IR (cm^{-1}): 3347.10, 1692.50, 1660.19, 1621.08, 1531.16, 1453.91, 1058.59, 934.88. HRMS(FAB) (M + H$^+$): calcd, 168.0946; found, 168.1017.

2-[Methoxy(methyl)amino]-2-oxoethyl Acetate (15). To a solution of (acetyloxy)acetic acid (**14**) (2.40 g, 20 mmol) in dry CH_2Cl_2 (90 mL) under N_2 was added *N*-methylmorpholine (4.49 g, 44 mmol). The mixture was cooled at −15 °C, and isobutylchloroformate (3.06 g, 22 mmol) was added. The mixture was stirred at −15 °C/15 min followed by the addition of *N*,*O*-dimethyl hydroxylamine hydrochloride (2.21 g, 22 mmol). The mixture was stirred at −15 °C/1 h, allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was poured into water (60 mL), and the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$. The solvent was removed under vacuum giving a pale yellow oil. The product was isolated by flash chromatography using EtOAc/hexanes 60/40. Yield: 95%. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 4.53 (2H, s, C***H*****$_2$**), 3.46 [3H, s, RN(OC***H*****$_3$**)(CH$_3$)], 2.90 [3H, s RN(OCH$_3$)(C***H*****$_3$**)], 1.86 (3H, s, C***H*****$_3$**). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 170.56 (RO***C***OCH$_3$), 168.09 [R***C***ON(OC*H*$_3$)(CH$_3$)], 61.62 (***C***H$_2$), 61.19 [RN(O***C***H$_3$)(CH$_3$)], 32.32 [RN(OCH$_3$)(***C***H$_3$)], 20.64 (***C***H$_3$). IR (cm^{-1}): 3567.35, 1746.53, 1685.48, 1425.93, 1233.65, 1066.07, 982.08. HRMS(FAB) (M + H$^+$): calcd, 162.0688; found, 162.0774.

2-Hydroxy-*N*-methoxy-*N*-methylacetamide. (16). Compound **15** (2.576 g, 16 mmol) was dissolved in THF MeOH/THF 1/5 (90 mL), the solution was cooled to 0 °C, and then LiOH (0.3832 g, 16 mmol) was added in one portion. The reaction mixture was stirred for 20 min at 0 °C, and then water (40 mL) was added. The solvent was removed under vacuum, leaving the aqueous phase which was extracted with $CHCl_3$ and dried over $MgSO_4$, and the solvent was removed under vacuum to give a pale yellow oil. The product was isolated by flash chromatography using EtOAc. Yield: 60% ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 4.13 (2H, s, C***H*****$_2$**), 3.52 [3H, s, RN(OC***H*****$_3$**)(CH$_3$)], 3.48 (1H, s, broad, O***H***), 3.06 [3H, s, RN(OCH$_3$)(C***H*****$_3$**)]. ^{13}C NMR ($CDCl_3$, 62.5): δ/ppm = 173.54 [R***C***ON(OC*H*$_3$)(CH$_3$)], 61.75 (***C***H$_2$), 60.10 [RN(O***C***H$_3$)(CH$_3$)], 37.74 [RN(OCH$_3$)(***C***H$_3$)]. IR (cm^{-1}): 3421.61 (broad), 1660.35, 1442.80, 1182.55, 1071.84, 987.99. HRMS (FAB) (M + H$^+$): calcd, 120.0582; found, 120.0665.

2-[Methoxy(methyl)amino]-2-oxoethyl 2-Methyl Acrylate. (17). Compound **16** (0.87 g, 7 mmol) was dissolved in CH_2Cl_2 (50 mL). To this solution were added MAA (0.663 g, 7.7 mmol) and DMAP (0.094 g, 0.77 mmol) at room temperature. After 5 min, DCC (1.589 g, 7.7 mmol) was added and the reaction mixture was stirred 7 days. The DCU was filtered and the organic phase was extracted with 0.5 M $NaHCO_3$ (2 × 45 mL), 0.5 M sodium citrate (2 × 45 mL), dried over $MgSO_4$ and the solvent evaporated under vacuum to give a yellow-orange oil. The product was isolated by flash chromatography using EtOAc/hexanes 60/40. Yield: 89%. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 6.04 (1H, s, RO$_2$C(CH$_3$)C=C***H*****$_a$**H$_b$), 5.46 (1H, quin, J = 1.58 Hz, RO$_2$C(CH$_3$)C=CH$_a$***H*****$_b$**), 4.81 (2H, s, C***H*****$_2$**), 3.66 (3H, s, RN(OC***H*****$_3$**)(CH$_3$)], 3.11 [(3H, s, RN(OCH$_3$)(C***H*****$_3$**)], 1.90 (3H, s, C***H*****$_3$**). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 168.34 [R***C***ON(OCH$_3$)(CH$_3$)], 167.39 [RO$_2$***C***(CH$_3$)C=CH$_2$], 135.96 [RO$_2$C(CH$_3$)***C***=CH$_2$], 126.90 [RO$_2$C(CH$_3$)C=***C***H$_2$], 61.83 (***C***H$_2$), 61.65 [RN(O***C***H$_3$)(CH$_3$)], 32.61 [RN(OCH$_3$)(***C***H$_3$)], 18.57 (***C***H$_3$). IR (cm^{-1}): 1725.77, 1688.27, 1427.41, 1297.88, 1163.32, 986.46. HRMS (EI) (M + H$^+$): calcd, 188.0845; found, 188.0436.

2-Methylacrylic Acid 3-Methyl-2-oxo-but-3-enyl Ester (7). Compound **17** (0.748 g, 4.0 mmol) was dissolved in 45 mL of dry THF and cooled at −78 °C under N_2. Afterward, 0.5 M isopropenylmagnesium bromide (12.0 mL, 6.0 mmol) diluted with THF (9 mL), was added dropwise over a period of 5 min. The mixture was stirred for another 30 min at −78 °C after complete addition of the Grignard reagent; then the mixture was brought to 0 °C and stirred for 30 min. After this period, 15 mL of saturated NH_4Cl solution was added to quench the reaction. The THF was removed by evaporation under vacuum and then ethyl ether (30 mL) was added to recover the organic compounds. Phases were separated, and the organic layer was washed with H_2O (2 × 40 mL) and brine (1 × 40 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue, a pale yellow oil, was separated by flash chromatography with EtOAc/hexanes 20/80 giving 18% yield for the title product, as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 6.14 (1H, t, J = 0.948 Hz, RCOC(CH$_3$)C=C***H*****$_c$**H$_d$), 5.885 (1H, t, J = 0.948 Hz, RCOC(CH$_3$)C=CH$_c$***H*****$_d$**), 5.77 (1H, t, J = 1.58 Hz, RO$_2$C(CH$_3$)C=C***H*****$_a$**H$_b$), 5.57 (1H, t, 1.58 Hz, RO$_2$C(CH$_3$)C=CH$_a$***H*****$_b$**), 5.09 (2H, s, C***H*****$_2$**), 1.91 (3H, t,

J = 1.738, 0.79 Hz, C***H*****$_3$**), 1.86 (3H, t, J = 1.58, 0.79 Hz, C***H*****$_3$**). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 193.93 [R***C***O(CH$_3$)C=CH$_2$], 167.20 [RO$_2$***C***(CH$_3$)C=CH$_2$], 142.62 [RCO(CH$_3$)***C***=CH$_2$], 135.98 [RO$_2$C(CH$_3$)***C***=CH$_2$], 127.00 [RCO(CH$_3$)C=***C***H$_2$], 125.46 [RO$_2$C(CH$_3$)C=***C***H$_2$], 65.93 (***C***H$_2$), 18.69 (***C***H$_3$), 17.83 (***C***H$_3$). IR (cm^{-1}): 1728.99, 1696.33, 1637.07, 1430.18, 1294.80, 1167.64, 1093.52, 1034.89, 941.49, 818.01. HRMS (EI) (M + H$^+$): calcd, 169.0786; found, 169.0702.

N,N′*-Dimethoxy-*N,N′*-dimethylsuccinamide (19).** Compound **19** was prepared following a protocol literature,[19] providing product as dark brown needles in a 62% yield. Mp: 73−75 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 3.67 [3H, s, RN(OCH*****$_3$**)(CH$_3$)], 3.14 [3H, s, RN(OCH$_3$)(C***H*****$_3$**)], 2.72 (2H, s, C***H*****$_2$**). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 173.67 [R***C***ON(OC*H*$_3$)(CH$_3$)], 61.46 [RN(O***C***H$_3$)(CH$_3$)], 32.45 [RN(OCH$_3$)(***C***H$_3$)], 26.69 (***C***H$_2$). IR (cm^{-1}): 1660.58, 1454.93, 1392.19, 1194.01, 993.29, 933.47. HRMS (FAB) (M + H$^+$): calcd, 205.1110; found, 205.1194.

2,7-Dimethylocta-1,7-diene-3,6-dione (9). A solution of diamide **19** (2.02 g, 9.94 mmol) in 80 mL of dry THF under N_2 was cooled at −70 °C, followed by dropwise addition of 0.5 M isopropenylmagnesium bromide in THF (59.6 mL, 29.8 mmol) diluted in dry THF (30 mL) over 5 min. After complete addition, the reaction mixture was stirred additional 30 min at −70 °C, and then the temperature was left to rise to 0 °C and kept there for 2 h. Saturated NH_4Cl (20 mL) was added to quench the reaction. THF was removed under vacuum, and the aqueous phase was extracted with ethyl ether (3 × 50 mL). The organic phase was washed with water (2 × 50 mL) and brine (50 mL) and dried over $MgSO_4$, and the solvent was evaporated under vacuum, to leave an orange liquid. The target product, a pale yellow liquid, was isolated by flash chromatography on silica gel using EtOAc/hexanes 20/80. Yield: 27%. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 5.93 (1H, s, RCOC(CH$_3$)C=C***H*****$_a$**H$_b$), 5.66 (1H, t, J = 1.422, 0.632 Hz, RCOC(CH$_3$)C=CH$_a$***H*****$_b$**), 2.91 (2H, s, C***H*****$_2$**), 1.75 (3H, t, J = 1.58, 0.948 Hz, C***H*****$_3$**). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 200.56 [R***C***O(CH$_3$)C=CH$_2$], 144.5 [RCO(CH$_3$)***C***=CH$_2$], 124.98 [RCO(CH$_3$)C=***C***H$_2$], 31.74 (***C***H$_2$), 17.87 (***C***H$_3$). IR (cm^{-1}): 1674.51, 1453.00, 1368.02, 1070.75, 935.67. HRMS (EI) (M$^+$): calcd, 166.0993; found, 166.0990.

2-(Acetylamino)ethyl 2-Methacrylate (21). *N*-(2-Hydroxyetyl)acetamide (10.0 g, 96.97 mmol) was dissolved in $CHCl_3$ (250 mL). To this solution were added MAA (12.522 g, 145.45 mmol) and DMAP (1.777 g, 14.54 mmol) at room temperature. After 5 min, DCC (30.01 g, 145.55 mmol) was added, and the reaction mixture was stirred 4 days. The DCU was filtered, the organic phase was extracted with 0.5 M HCl (2 × 200 mL), H_2O (1 × 200 mL), 0.5 M $NaHCO_3$ (2 × 200 mL), dried over $MgSO_4$, and the solvent evaporated under vacuum to give a colorless oil. The product was isolated by flash chromatography using EtOAc/hexanes 50/50, EtOAc in a 35% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): δ/ppm = 6.65 (1H, s, ROCN***H***R), 6.02 (1H, s, ROCNHCH$_2$CH$_2$OCO(CH$_3$)C=CH$_a$***H*****$_b$**), 5.49 (1H, s, ROCNHCH$_2$-CH$_2$OCO(CH$_3$)C=C***H*****$_a$**H$_b$), 4.12 (2H, t, J = 5.42 Hz, RNHCH$_2$C***H*****$_2$**-OCOR), 3.45 (2H, quad, J = 11.15, 5.6 Hz, RNHC***H*****$_2$**CH$_2$-OCOR), 1.88 (3H, s, RCOC***H*****$_3$**), 1.84 (3H, s, ROCO(C***H*****$_3$**)C=CH$_a$H$_b$). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ/ppm = 171.07 (R***C***ONHR), 167.64 [RO$_2$***C***(CH$_3$)C=CH$_2$], 136.25 [RO$_2$C(CH$_3$)-***C***=CH$_2$], 126.26 [RO$_2$C(CH$_3$)C=***C***H$_2$], 63.61 (RO***C***H$_2$CH$_2$NHR), 38.87 (ROCH$_2$***C***H$_2$NHR), 23.23 (***C***H$_3$COR), 18.49 [RO$_2$C(***C***H$_3$)C=CH$_2$]. IR (cm^{-1}): 3288.27, 3083.16, 2958.86, 1713.91, 1658.95, 1555.36, 1165.83, 1041.19, 945.26. HRMS (FAB) M + H$^+$): calcd, 172.0895; found, 172.0975.

Polymer Preparation. The following procedure was used for imprinted polymers employing the new cross-linking monomers. In a 13 × 100 mm test tube, (0.062 g, 0.155 mmol) of dansyl-L-phenylalanine was dissolved in 1.5 mL of acetonitrile. To this solution was added **8** (1.0617 g, 6.35 mmol) (NAG), MAA (0.109 g, 1.27 mmol), and AIBN (0.020 g, 0.124 mmol). Similar imprinted polymers were prepared using **6** (NOBE), **7** (MVK), and **9** (EDVK) as the cross-linking monomers. For comparison to traditionally formulated imprinted polymers, another polymer was imprinted using the formulation above, substituting EGDMA as the cross-linking monomer. The solution was purged by bubbling nitrogen gas into the mixture for 5 min and then capped and sealed with Teflon tape and Parafilm. The samples were inserted into a photochemical turntable reactor, which was immersed in a constant-temperature bath. A standard laboratory UV light source (medium pressure 450 W mercury arc lamp) jacketed in a borosilicate double-walled immersion well was placed at the center of the turntable. The polymerization was initiated photochemically at 20 °C and the temperature maintained by both the cooling jacket surrounding the lamp and the constant-temperature bath holding the entire apparatus. The polymerization was allowed to proceed for 10 h and then used for chromatographic experiments.

Chromatographic Experiments. Removal of the template was achieved by Soxhlet extraction with methanol for 48 h. Then the polymers were ground using a mortar and pestle, the particles were sized using U.S.A. Standard Testing Sieves, and the fraction between 20 and 25 μm was collected. The particles were slurry packed, using a solvent delivery module, into stainless steel columns (length, 75 mm; i.d., 2.1 mm) to full volume for chromatographic experiments. The polymers were then washed on line for 12 h using acetonitrile/acetic acid, 98/2, at a flow rate of 0.1 mL/min to remove any residual template. HPLC analyses were performed isocratically at room temperature (21 °C). The flow rate in all cases was set at 0.1 mL/min using a mobile phase consisting of acetonitrile/acetic acid, 99/1, a substrate concentration of 0.1 mM dansyl−phenylalanine in acetonitrile, and a wavelength detection of 330 nm. The void volume was determined using acetone as an inert substrate. The separation factors (α) were measured as the ratio of capacity factors k'_L/k'_D. The capacity factors were determined by the relation $k' = (R_v - D_v)/D_v$, where R_v is the retention volume of the substrate and D_v is the void volume.

Pore Analysis. A sample of polymer (70−150 mg) was degassed at 150 °C/24 h under vacuum. The absorption and desorption isotherms were obtained using a 20 min equilibration time. Surface areas were determined according to the BET model; pore volumes and size distributions, according to the BJH model.

^{13}C CP-MAS NMR Analysis. ^{13}C CP-MAS NMR data were collected on a Chemagnetics spectrometer at ambient temperature at a frequency of 100.60 MHz. Samples were packed into 5 mm zirconia rotors (Varian, Palo Alto CA) and spun at 5 kHz. A total of 4096 data points were collected over a spectral window of 50 kHz: ^{1}H 90° pulse of 4.6 μs; acquisition time 81.92 ms; contact time 5 ms; repetition time 4.0 s.

Acknowledgment. This research was supported in part by the Research Corporation, through Cottrell Scholar Award CS0801, and an NSF CAREER Program award, CHE-0134290 (D.A.S.). We thank Dr. Dale Treleaven for assistance with ^{13}C CP-MAS NMR measurements.

Supporting Information Available: Figures showing ^{1}H NMR and ^{13}C NMR spectra of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Wade, H.; Scanlan, T. S. *J. Am. Chem. Soc.* **1999**, *121*, 1434. (b) Berisio, R.; Lamzin, V. S.; Sica, F.; Wilson, K. S.; Zagari, A.; Mazzarella, L. *J. Mol. Biol.* **1999**, *292*, 845.
(2) Wulff, G.; Sarhan, A. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 341.
(3) Wulff, G.; Vietmeier, J.; Poll, H.-G. *Makromol. Chem.* **1987**, *188*, 731.
(4) (a) Kempe, M.; Mosbach, K. *Tetrahedron Lett.* **1995**, *36*, 3563. (b) Schweitz, L.; Andersson, L. I.; Nilsson, S. *Anal. Chem.*, **1997**, *69*, 1179.
(5) Kempe, M. *Anal. Chem.* **1996**, *68*, 1948.
(6) (a) Shea, K. J.; Stoddard, G. J.; Shavelle, D. M.; Wakui, F.; Choate, R. M. *Macromolecules* **1990**, *23*, 4497. (b) Shea, K. J.; Spivak, D. A.; Sellergren, B. *J. Am. Chem. Soc.* **1993**, *115*, 3368.

(7) (a) Tanabe, K.; Takeuchi, T.; Matsui, J.; Ikebukuro, K.; Yano, K.; Karube, I. *J. Chem. Soc., Chem. Commun.* **1995**, *22*, 2303. (b) Yano, K.; Tanabe, K.; Takeuchi, T.; Matsui, J.; Ikebukuro, K.; Karube, I. *Anal. Chim. Acta* **1998**, *363*, 111.
(8) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31.
(9) Chan, G. Y. N.; Looney, M. G.; Solomon, D. H. *Aust. J. Chem.* **1998**, *51*, 31.
(10) Andersson, L.; Ekberg, B.; Mosbach, K. *Tetrahedron Lett.* **1985**, *26*, 3623.
(11) (a) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815. (b) Maugras, I.; Poncet, J.; Jouin, P. *Tetrahedron* **1990**, *46*, 2807.
(12) Sibi. M. P. *Org. Prep. Proc. Int.* **1993**, *25*, 15.
(13) Wipf, P.; Kim, H. Y. *J. Org. Chem.* **1993**, *58*, 5592.
(14) Heathcock, C. H.; von Geldern, T. W. *Heterocycles* **1987**, *25*, 75.
(15) Angelastro, M. R.; Peet, N. P.; Bey, P. *J. Org. Chem.* **1989**, *54*, 3913.
(16) Corey, E. J.; Szekely, I.; Shiner, C. S. *Tetrahedron Lett.* **1977**, *40*, 3529.
(17) Braun, M.; Waldmuller, D. *Synthesis* **1989**, 856.
(18) Sibi, M. P.; Sharma, R.; Paulson, K. L. *Tetrahedron Lett.* **1992**, *33*, 1941.
(19) Uchiyama, K.; Hayashi, Y.; Narasaka. K. *Tetrahedron*, **1999**, *55*, 8915.
(20) (a) Guyot, A. In *Synthesis and Separations using Functional Polymers*; Sherrington, D. C., Hodge, P., Eds.; John Wiley & Sons: New York, 1989; p 1. (b) Lloyd, L. L. *J. Chromatogr.* **1991**, *544*, 201. (c) Guyot, A.; Bartholin, M. *Prog. Polym. Sci.* **1982**, *8*, 277.
(21) Earnshaw, R. G.; Price, C. A.; O'Donnell, J. H.; Whittaker, A. K. *J. Appl. Polym. Sci.* **1986**, *32*, 5337.

MA025710Z