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Improving the Strategy and Performance of Molecularly Imprinted Polymers Using Cross-Linking Functional Monomers

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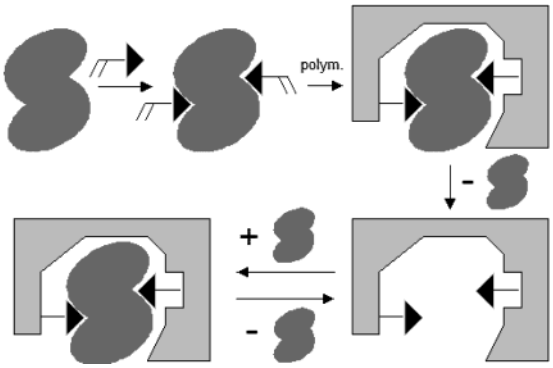
A new strategy for monomer design has been investigated that combines interactive monomer functionality with a cross-linking format, giving as a result noncovalent molecularly imprinted polymers (MIPs) with improved performance. This strategy was explored under the premise that more functionality could be introduced without suffering performance losses due to reduced cross-linking. While this proved to be correct, equally important contributions to selectivity enhancement at the molecular level by conformation control and diastereomeric complexation were also discovered. Monomers derived from L-serine and L-aspartic acid were synthesized and used to prepare MIPs, with the best performance obtained for the MIP formulated with the serine-based cross-linker (*N*,*O*-bis-methacryloyl L-serine, **3**), versus the aspartic-acid-based cross-linkers and the traditional methacrylic acid/ethylene glycol dimethacrylate (MAA/EGDMA) formulation. Quantitative structure−selectivity relationship (QSSR) studies revealed that the improved performance of **3** was due to three key factors: (1) the cross-linking nature of this monomer; (2) control of conformational flexibility; (3) a strong influence of monomer chirality on enantioselectivity in MIPs.

Introduction

Molecular imprinting is a maturing method of producing polymeric materials with specific recognition sites tailored for targeted compounds.[1−5] These have found use as chromatographic and solid-phase extraction media, and offer tremendous potential as the recognition element for immunoassays and sensors.[4,5] However, advanced applications of MIPs such as in microanalytical systems and microsensors will depend on the development of higher performance MIP materials. Although the practice of molecular imprinting is well established, new strategies for the rational design of new and better materials based on the underlying mechanisms will continue to improve the process. This paper presents an important example of this rational approach to improving the performance of molecularly imprinted polymers (MIPs), and the important discoveries that will guide the design of monomers in the future.

An outline of the generally accepted mechanism for MIP binding site formation is illustrated in Scheme 1. Pre-organization of functional monomers in the vicinity of the template molecule, referred to as the pre-polymer complex (PPC), is followed by copolymerization with cross-linking monomers to form a polymer network around the template molecule. After removal of the template, the resulting polymer is postulated to contain binding cavities that are complementary in shape and functionality to the template molecule. A seminal study by Wulff and co-workers illustrated an important fact that increasing the amount of cross-linking matrix increases the specific recognition by covalently imprinted polymers.[6] However, imprinted polymers formed using a noncovalent strategy rely on the solution concentration of functional monomer to form the PPC. By Le Chatlier's principle, increasing concentration of functional monomer should increase the concentration of PPC, which is in turn postulated to play a large role in the formation of specific sites in the polymer.

SCHEME 1



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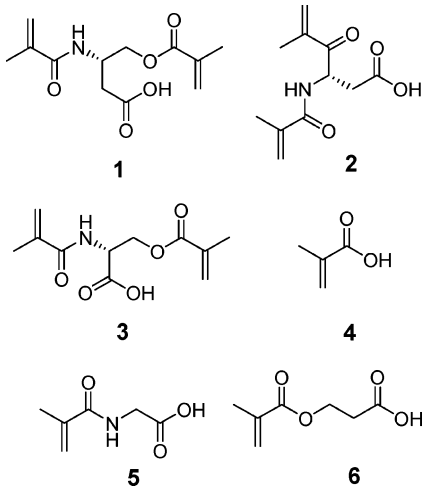

FIGURE 1. Cross-linking functional monomers and non-cross-linking controls.

One problem with increasing the functional monomer is that the amount of cross-linker becomes too low, and the MIP loses its recognition properties from random motion of non-cross-linked polymer domains.[7] Thus, a balance must be struck for optimum imprinting conditions which never allows the optimum concentration of cross-linker or functional monomer. In an effort to design the most effective monomers for molecular imprinting, we decided to combine the interactive monomer functionality with the cross-linking requirements, thus eliminating the need to limit the ratio cross-linking monomer in favor of functional group monomers and vice versa. While this strategy proved successful, quantitative structure-selectivity relationship (QSSR) experiments also elucidated two other important design principles that will guide future designs of monomers for molecular imprinting.

Results and Discussion

For this study, functional cross-linkers incorporating carboxylic acids were developed for comparison to the most widely used MIP formulation, which utilizes the combination of methacrylic acid (MAA) as a functional monomer with ethylene glycol dimethacrylate (EGDMA) as the cross-linker. We designed monomers **1** − **3** based on amino acids incorporating carboxylate functionality, while providing end groups that could be transformed into cross-linking polymerizable groups. As non-crosslinking controls, monomers **4**−**6** were included which incorporate the same interactive functionality to determine the contributions the cross-linking in functional monomers might have toward improved binding (Figure 1).

Cross-linking monomers employing both natural and nonnatural amino acids, have previously been developed for molecular imprinting. Phenylalanine-based *N*,*O*-bisacryloyl-L-phenylalaninol and a similar cross-linker *N*,*O*-dimethacryloyl phenylglycinol, were synthesized and tested for chiral separations.[8] Use of these cross-linking monomers for MIPs showed modest enantioselectivity for binding the template molecule. Nontemplated polymers showed no chiral separation, even though the polymers had a chiral backbone. An interesting cross-linker has been synthesized, 2,6-bis(acrylamido)pyridine (BAAP), which uses a donor−acceptor-donor motif for forming hydrogen bonds to a template, that mimics interactions found in DNA.[9] This example showed that the cross-linker can also serve as the complexing functionality needed to form the pre-polymer complex, and provide affinity for the template upon rebinding.

Monomer and Cross-Linker Syntheses. Compound **1** was synthesized following the protocol reported in the literature,[10] while syntheses of compounds **2** and **3** were developed for this project. The syntheses of monomers **2** and **3** were constructed from commercially available protected amino acids as the starting materials for the corresponding compounds. The first step, acylation of the amine and hydroxyl functionalities for introducing the methacrylamide and methacrylate polymerizable groups was carried out using traditional esterification procedures. Introduction of the vinyl ketone polymerizable group in **2** was accomplished by the use of a Weinreb amide,[11] which also uniquely served as a protecting group for the carboxylate side chain as well as a latent electrophile for the nucleophilic addition of isopropenylmagnesium bromide in the penultimate step. Deprotection of the amine group in the aspartic acid derivative was achieved by acidic hydrolysis with HCl/Et_2O, which provided a cleaner product versus TFA treatment. Final deprotection of the α-carboxylic group of **8** was unsuccessful using chemical procedures such as nucleophilic dealkylation[12] or basic hydrolysis,[13] which hydrolyzed both esters rather than the desired methyl ester saponification. Therefore, an enzymatic approach for this step was investigated.[14−16] Three enzymes were screened for selective hydrolysis of the methyl ester: porcine pancreatic lipase, porcine liver esterase, and carbonic anhydrase (bovine erythrocytes). In the case of monomer **3**, porcine pancreas lipase (PPL) gave the highest yield of the product at 62% yield, while in the case of the aspartic acid derivative **2**, porcine liver esterase gave the highest yield (55%). The overall syntheses for compounds **2** and **3** are shown in Schemes 2 and 3. Last, syntheses of non-cross-linking functional monomers **5** and **6** as shown in Schemes 4 and 5 were performed using known procedures.[17−19]

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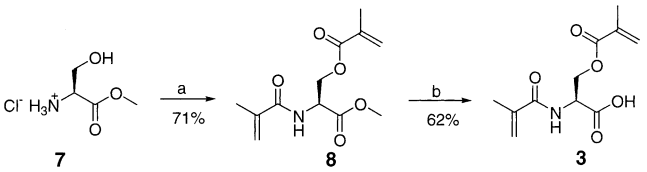
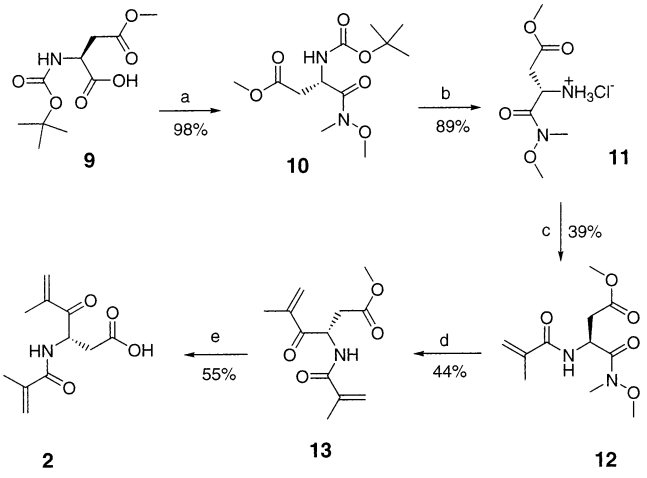
(19) Curci, M.; Mieloszynski, J. L.; Paquer, D. *Org. Prep. Proced. Int.* **1993**, *25* (6), 649.

SCHEME 2[a]



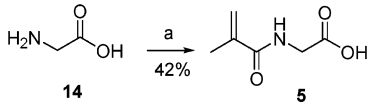
[a] Reaction conditions: (a) MAA/Et_3N/DMAP/DCC/CH_2Cl_2, rt/5 d; (b) PPL (EC 3.1.1.3)/pH = 7.5, rt/72 h.

SCHEME 3[a]



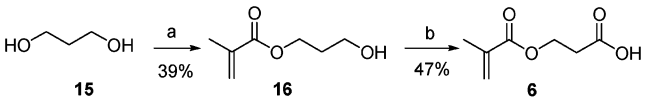
[a] Reaction conditions: (a) NMM/*i*-$BuCO_2Cl$/HCl·$HN(CH_3)(OCH_3)$/THF, −15 °C/1 h, rt/24 h; (b) HCl/Et_2O, 0 °C/6 h, rt/18 h; (c) $H_2C{=}C(CH_3)COCl/Et_3N/CH_2Cl_2$, rt/48 h; (d) C_3H_5MgBr/THF, −15 °C/30 min, rt/30 min; (e) PLE (EC 3.1.1.1), pH = 8, rt/72 h.

SCHEME 4[a]



[a] Reaction conditions: (a) $H_2C{=}C(CH_3)COCl$/NaOH, rt/48 h.

SCHEME 5[a]



[a] Reaction conditions: (a) $H_2C{=}C(CH_3)COCl/Et_3N$, 50 °C/2 h; (b) Cr_2O_3/H_2SO_4/acetone, reflux.

Imprinting and Analysis of Polymers. The best probe for MIP selectivity due to the "imprinting effect" is chiral differentiation, since all physical properties of enantiomers are the same except for the three-dimensional positioning of atoms in space. Thus, for our investigation we imprinted polymers using monomers **1** − **4** as the interactive functional component (10%), and EGDMA for the remainder of the cross-linked matrix (90%). The *S* enantiomer of nicotine was chosen as a readily available template with one chiral center for investigating enantioselectivity, two amine functional groups that provide binding interactions with the functional monomers, and a UV chromophore for HPLC detection. Polymerization was photochemically initiated at room temperature and allowed to proceed for 10 h. Once synthesized, the imprinted polymers were broken into coarse particles and the template removed by Soxhlet extraction with methanol. Subsequently, the polymers were ground by mortar and pestle and the fraction between 20 and 25 μm collected and packed into stainless steel columns for evaluation using HPLC methods. HPLC retention values were normalized to capacity factors (k') for elution of both enantiomers, and the enantioselectivity was evaluated by comparison of separation factors, α ($\alpha = k'_S/k'_R$).

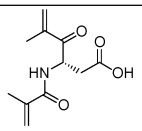
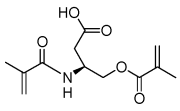
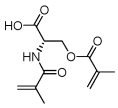
Table 1 shows the functional cross-linkers and their associated MIP separation factors, which are ordered by increasing α values. The results reveal improved retention on the MIP columns (i.e., larger k' values) made using each of the new cross-linkers, versus the traditionally formulated MIP incorporating MAA as the functional monomer. However, improved selectivity (i.e. a larger α value) is observed only for the MIP using the cross-linking functional monomer **3**. To test the hypothesis that combining fuctionality and cross-linking was a factor responsible for the improved behavior by the **3**, MIPs formulated with different ratios of monomer/cross-linking monomer (**3**/EGDMA) and *S*-nicotine as the template were synthesized and their performance compared to similar MIPs formulated with MAA.

The results shown in Table 2 (and Figure 2), indicate that the MIPs formulated with **3** (entries 3, 5, and 7) show improved binding affinity and selectivity as compared to those formulated with MAA (entries 2, 4, and 6). The effect on selectivity is most clearly seen in Figure 2 where both compounds reach their maximum selectivity initially at 10% functional monomer, after which **3** remains constant up to 25 mol % then decreases at a higher ratios of **3**/EGDMA. In contrast, MIPs formulated with MAA immediately lose selectivity after reaching the maximum at 10 mol % MAA. These results show that noncovalent imprinting is in agreement with covalent imprinting studies published by Wulff,[6] establishing that the selectivity increases with increase in the degree of cross-linking.

Structural Determinants for Enhanced Binding by Functional Cross-Linker 3. The improved performance by the functional cross-linker **3** over the aspartic-acid-based monomers prompted an investigation into the structural determinants responsible for the good binding and selective behavior. A quantitative structure-selectivity relationship (QSSR) study was carried out by synthesizing analogues of **3** to determine key elements of **3** structure leading to selective MIPs. Thus, substructures of **3**, shown in Figure 3 along with **3**, were synthesized to determine important interactions with the template. The polymers were imprinted as before using *S*-nicotine as the template with DMF as the porogen, which was needed in order to solubilize monomer **5**. Binding studies were carried out by HPLC for imprinted and nonimprinted polymers formulated with monomers in Figure 3 versus MAA. The polymers were evaluated for enantioselectivity using the mobile phase acetonitrile/HOAc 98/2, which gave the best results in all cases.

The results of the binding studies in Table 3 show that the non-crosslinking monomers as a whole exhibit poor selectivity, even if binding (k') values were strong. Therefore, cross-linking provides a structural design feature that improves selective molecular recognition by

TABLE 1. Binding Studies for MIPs Imprinted with (*S*)-Nicotine and Formulated Using the New Cross-Linking Functional Monomers[a]

Entry	Functional Monomer	Polymer	t_S (avg) min	t_R (avg) min	k'_S	k'_R	α
1	 **2**	Non Imprinted	0.63	0.63	1.03	1.03	1.00
		Imprinted	1.05	1.02	2.39	2.29	1.04
2	 **1**	Non Imprinted	0.88	0.88	1.75	1.75	1.00
		Imprinted	1.42	1.34	3.58	3.32	1.08
3	 **4**	Non Imprinted	0.51	0.51	0.28	0.28	1.00
		Imprinted	0.68	0.58	0.7	0.45	1.56
4	 **3**	Non Imprinted	1.88	1.86	3.7	3.65	1.01
		Imprinted	15.9	8.6	41.97	22.24	1.89

[a] HPLC conditions: particle size, 20−25 μm; column size, 75 mm length, 2.1 mm id.; mobile phase = MeCN/HOAc 97/3; flow rate = 1 mL/min; analytes: 1 mM (*S*)-nicotine, 1 mM (*R*)-nicotine, and acetone (used to determine dead volume); wavelength, 262 nm; injected volume, 5 μL.

TABLE 2. Chromatographic Results for MIPs Imprinted with (*S*)-Nicotine and Formulated with Different Functional Monomer/Cross-Linking Monomer Ratios[a]

entry	functional monomer	functional monomer/ cross-linker monomer ratio	t_S (avg) (min)	t_R (avg) (min)	k'_S	k'_R	α
1	none[b]	0	0.63	0.62	0.57	0.55	1.03
2	MAA[c]	1/9	0.68	0.58	0.70	0.45	1.56
3	**3**[c]	1/9	15.9	8.60	41.97	22.24	1.89
4	MAA[d]	1/3	1.78	1.35	3.45	2.40	1.44
5	**3**[d]	1/3	122.5	64.67	339.28	178.64	1.89
6	MAA[e]	1/1	0.66	0.61	0.57	0.45	1.26
7	**3**[e]	1/1	23.55	15.56	59.38	38.89	1.53

[a] HPLC conditions: particle size, 20−25 μm; column size, 100 mm length, 2.1 mm i.d.; flow rate, 1 mL/min; analytes, 1 mM (*S*)-nicotine, 1 mM (*R*)-nicotine, and acetone (used to determine dead volume); wavelength, 262 nm; injected volume, 5 μL. [b] Mobile phase = MeCN. [c] Mobile phase =MeCN/HOAc 97/3. [d] Mobile phase = MeCN/HOAc 95/5. [e] Mobile phase = MeCN/HOAc 85/15.

functional groups in MIPs. One reason for this is that cross-linking tethers the functional group covalently to the matrix in two places, greatly reducing conformational entropy of monomer motion. Consequently, this reduces the conformational flexibility of the pendant functional group, which creates a more stable interaction with the template (i.e., reduction in the entropy of association). However, recall that cross-linking did not help monomers based on aspartic-acid, which showed lower degrees of selectivity versus MAA-functionalized MIPs. This would reason that there is more than one conformational factor responsible for the improved performance by **3**. The other factor appears to be proximity of the pendant carboxylate group with respect to the cross-linker backbone. From a design point of view, it appears that providing closer proximity of the pendant functional group to the cross-linking group improves selectivity, again due to reducing the conformational flexibility of the pendant group. The effects of binding group flexibility have been shown to take an active role in the imprinting process by Wulff and co-workers, who examined the influence of conformational flexibility of functional monomers on selective behavior by MIPs.[21] In this study, it was determined that

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(21) Wulff, G.; Gimpel, J. *Makromol. Chem.* **1982**, *183*, 2469.

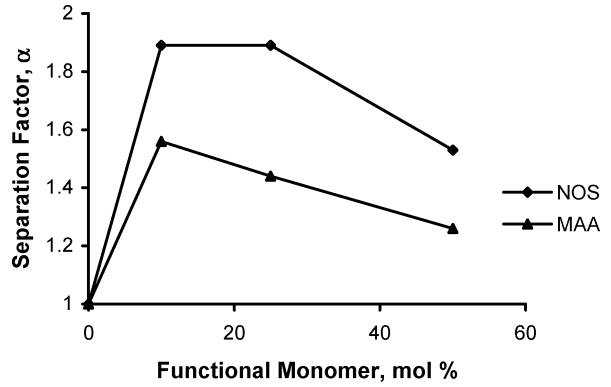


FIGURE 2. Enantioselectivity of MIPs imprinted with (*S*)-nicotine as a function of the amount of the functional monomer.

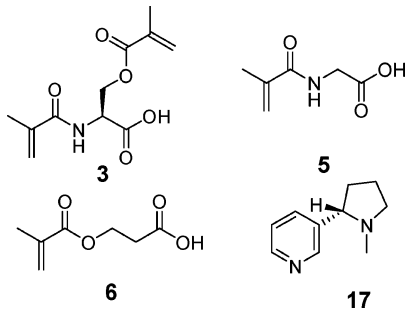
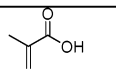
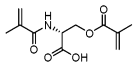
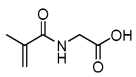
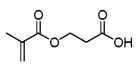


FIGURE 3.

TABLE 3. Chromatographic Results for MIPs Formulated with 3 and Functional Monomer Analogues and Imprinted with (*S*)-Nicotine[a]

Entry	Functional Monomer	Polymer	k'_S	k'_R	α
1	 **4**	Non-imprinted	0.25	0.23	1.09
		Imprinted	0.30	0.27	1.11
2	 **3**	Non-imprinted	0.77	0.70	1.10
		Imprinted	8.05	3.14	2.56
3	 **5**	Non-imprinted	3.13	2.95	1.06
		Imprinted	1.95	1.72	1.13
4	 **6**	Non-imprinted	3.21	3.03	1.06
		Imprinted	2.08	1.73	1.2

[a] HPLC conditions: particle size, 20−25 μm; column size, 100 mm length, 2.1 mm i.d.; mobile phase, MeCN/HOAc = 98/2; flow rate = 1 mL/min; analytes, 0.1 mM (*S*)-nicotine, 0.1 mM (*R*)-nicotine, and acetone (used to determine dead volume); wavelength, 262 nm; injected volume, 5 μL.

decreasing binding group flexibility resulted in greater selectivity; however, if the binding group becomes too rigid specificity was diminished.

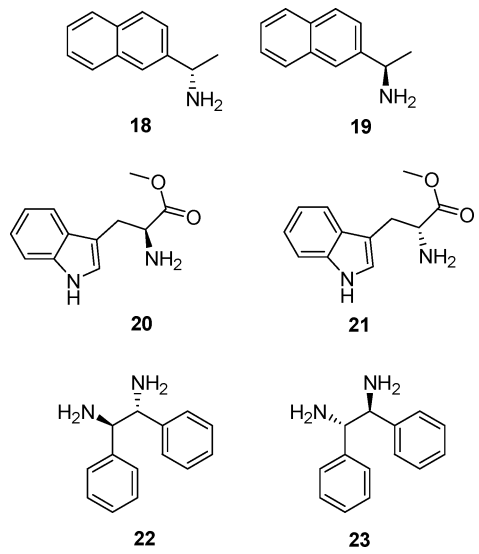
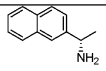
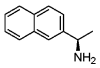
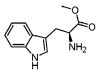
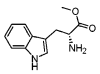
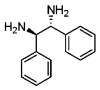
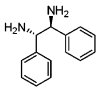


FIGURE 4.

Scope of Selectivity Enhancement and Chiral Influence by Crosslinker 3. The generality of improved selectivity found for MIPs using the functional cross-linker **3** was explored by imprinting several other templates. Six polymers were imprinted using each enantiomer of 1-naphthylethylamine (**18** and **19**), tryptophan methyl ester (**20** and **21**), and 1,2-diphenylethylenediamine (**22** and **23**), shown in Figure 4. Each of these polymers was compared to a traditional MIP imprinted with the same templates, but using MAA as the functional monomer. Binding (k') and selectivity (α) data were again evaluated and the results are shown in Table 4. Following the order in Table 4, entries 1 and 2 show improved enantioselectivity for both enantiomers of 1-naphthylethylamine over the traditional EGDMA/MAA formulated polymers. An interesting observation from these examples is that the polymer in entry 1 shows higher enantioselectivity than the polymer in entry 2. It appears that diastereomeric complexes "imprint" differently than complexes formed via nonstereogenic monomers.[15] The origin of this effect is either that one diastereomeric complex is preferred in the pre-polymer complex solution (i.e., chiral matching), or one of the diastereomeric complexes has properties that affords a structurally more defined binding site in the MIP. Preferential imprinting of one diastereomeric complex could occur from a more distinguishable geometry (e.g., twisted versus spherical), or by a complex having physical properties more compatible with the polymer (e.g. a more hydrophobic complex for the hydrophobic polymer).

Different behavior originating from diastereomeric complexes is seen again for tryptophan methyl ester imprinted polymers, entries 3 and 4 in Table 4, where the "L" enantiomer imprints well, while the "D" enantiomer provides MIPs with lower selectivity (even lower than the MAA functionalized MIP). This observation indicates that diastereomeric complexation (e.g. a chiral mismatch of functional monomer and template) effects can dominate any improvements to selectivity by the use of a cross-linking functional monomer. Entries 5 and 6 in Table 4 show the results for imprinting enantiomers

TABLE 4. Chromatographic Evaluation of MIPs Using 3 as the Functional Monomer and Imprinted with Different Templates[a]

Entry	Template	Mobile Phase	Polymer	Functional Monomer	
				MAA	3
				α	α
1		MeCN/HOAc	Non Imprinted	1.00	0.99
		97/3	Imprinted	1.08	2.69
	18				
2		MeCN/HOAc	Non Imprinted	1.00	0.99
		97/3	Imprinted	1.00	2.26
	19				
3		MeCN/HOAc	Non Imprinted	1.00	1.00
		97/3	Imprinted	1.65	2.78
	20				
4		MeCN/HOAc	Non Imprinted	1.00	1.00
		97/3	Imprinted	1.56	1.31
	21				
5		MeCN/HOAc	Non Imprinted	1.00	1.00
		90/10	Imprinted	1.84	4.65
	22				
6		MeCN/HOAc	Non Imprinted	1.00	1.00
		90/10	Imprinted	1.95	2.07
	23				

[a] HPLC conditions: particle size, 20−25 μm; column size, 100 mm length, 2.1 mm id.; flow rate, 1 mL/min.; injected volume, 5 μL; wavelength detection, 260 nm for naphthylethylamine, 250 nm for tryptophan methyl ester, and 260 nm for diphenylethylamine.

of 1,2-diphenylethylenediamine; this is a more complex system that involves two stereocenters on the template complexed with the chiral functional cross-linker **3**. The functionalized monomer **3** imprinted (1*R*,2*R*)-(+)-1,2-diphenylethylenediamine as the template, showed the highest enhancement in selectivity of roughly *2.5-fold* over the traditional MAA MIP, while the opposite enantiomer gave results roughly equal in terms of α to the MAA functionalized MIP for separately injected enantiomers. However, an important observation here is that when the racemic mixture was analyzed, nearly complete resolution was still obtained with the polymer imprinted with the S,S derivative using **3** (Figure 5b), while the MAA functionalized MIP (Figure 5a) did not afford any resolution at all; even though the two different MIPs have comparable α values. In general, polymers incorporating the methacrylamide moiety provided greater resolution efficiency with sharper and more well-defined peaks in the HPLC chromatograms, whereas the methacrylate derivatives gave broad peaks. This may be due to

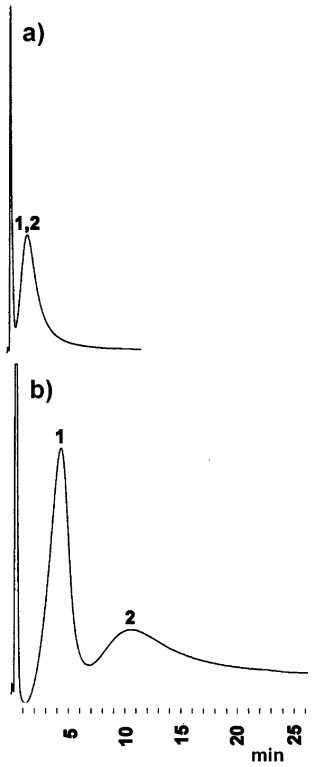


FIGURE 5. Elution profile for imprinted polymers with (1*S*,2*S*)-(−)-1,2-diphenylethylendiamine: (a) Imprinted polymer with MAA as the functional monomer, (b) Imprinted polymer with **3** as the functional monomer. Peak 1: (1*R*,2*R*)-(−)1,2-diphenylethylendiamine. Peak 2: (1*S*,2*S*)-(−)-1,2-diphenylethylendiamine. Condition: mobile phase, acetonitrile/HOAc 90/10, flow rate, 1 mL/min, wavelength detection, 260 nm, injected volume, 5 μL.

improved mass-transfer kinetics as a result of favorable morphology and properties afforded by acrylamide polymers. The three examples presented show that the chiral nature of the monomer **3** often improves the performance of imprinted polymers; although there is no general trend. Investigation into the underlying mechanism(s) for diastereoselective effects in MIPs will be reported in due course.

Conclusions

A new strategy for monomer design for noncovalently imprinted polymers has been shown to be successful for improving molecularly imprinted materials. Quantitative structure-selectivity relationship studies have verified a key improvement to monomer design in these studies was to provide the template-interactive functional group in a *cross-linking* monomer format. The origins of the improved selectivity were attributed to two factors of the cross-linking functional monomers. First, the degree of cross-linking is maximized without imposing restrictions on functional group concentrations. Second, covalently tethering of the functional group to the binding site matrix reduces conformational entropy that would otherwise interfere with specific binding. Last, a strong influence of diastereomeric complexes on MIP selectivity was discovered. However, more studies will be needed to determine whether the origins of this effect are to be

found in the solution-phase pre-polymer complex or the final polymeric binding site.

Experimental Section

***N,O*-Bismethacryloyl, L-Serine α-Methyl Ester (8).** L-Serine α-methyl ester hydrochloride (0.467 g, 3 mmol) was dissolved in DCM (15 mL) and cooled to 0 °C, followed by dropwise addition of Et_3N (0.607 g, 6 mmol). In another flask methacrylic acid (0.517 g, 6 mmol) and DMAP (0.0733 g, 0.6 mmol) were dissolved in DCM (30 mL), and the resulting solution was cooled at 0 °C. To this flask was added the hydrochloride solution in one portion. After 5 min, DCC (1.238 g, 6 mmol) was added to the cooled solution at 0 °C and stirred additional 30 min. After this period, the temperature was allowed to rise to room temperature and the reaction mixture was stirred 5 days. The DCU was filtered, the organic phase was extracted with 0.5 M $NaHCO_3$ (2 × 15 mL), 0.5 M sodium citrate (2 × 15 mL), dried over $MgSO_4$, and the solvent was evaporated under vacuum giving an orange oil. The product was isolated as a yellow oil by flash chromatography using EtOAc/hexanes 50/50 in 71% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 6.67−6.70 (1H, d, J = 7.58n Hz), 5.99 (1H, d, J = 0.95 Hz), 5.68 (1H, d, J = 0.95 Hz), 5.50 (1H, d, J = 1.58 Hz), 5.30 (1H, d, J = 0.95 Hz), 4.80−4.87 (1H, m), 4.41−4.43 (2H, dd, J = 4.10, 1.42 Hz), 3.69 (3H, s), 1.88 (3H, s), 1.82 (3H, s). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 170.4, 168.3, 167.2, 139.6, 135.9, 126.8, 120.9, 64.3, 53.1, 52.4, 18.7, 18.5. IR: 3337.66, 2956.58, 1724.14, 1663.26, 1625.90, 1522.48, 1162.74, 1018.98, 943.15. HRMS (FAB) (M^+): calcd 255.1107, found 255.1107.

***N,O*-Bismethacryloyl, L-Serine (3).** In a 100 mL amber bottle with cap was dissolved *N*,*O*-bismethacryloyl, L-serine α-methyl ester, **8** (0.334 g, 1.3 mmol), in acetone (5 mL) followed by the addition of 40 mL of 0.1 M phosphate buffer of pH 7.5. To this mixture was added porcine pancreatic lipase, EC 3.1.1.3 (100 mg). The mixture was sonicated for 1 min and then shaken for 72 h at room temperature. The reaction mixture was acidified to pH 3.0 with 1.0 M HCl. The aqueous phase was extracted with EtOAc (3 × 20 mL), and the combined organic extracts were washed with water (2 × 20 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated under vacuum to give a yellow oil. The product was isolated by flash chromatography using only EtOAc to give an isolated yield of 61.8%. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 10.68 (1H, s, broad), 6.87−6.90 (1H, d, J = 7.35 Hz), 6.04−6.05 (1H, d, J = 0.99 Hz), 5.74−5.75 (1H, d, J = 0.79 Hz), 5.54−5.55 (1H, d, J = 1.54 Hz), 5.37−5.38 (1H, d, J = 0.79 Hz), 4.85−4.91 (1H, m), 4.51−4.52 (2H, dd, J = 3.95, 1.26 Hz), 1.91 (3H, s), 1.85 (3H, s). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 172.5, 169.3, 167.7, 139.0, 135.9, 127.2, 122.1, 76.1, 64.3, 52.9, 30.7, 18.7, 18.6. IR: 3366.15, 2958.72, 1718.42, 1658.39, 1619.96, 1542.53, 1165.28, 1020.30, 943.78. HRMS (FAB) (M + H^+): calcd 242.1095, found 242.1036.

***N*-*t*-Boc-L-aspartic Acid β-Methyl Ester *N*-Methoxy-*N*-Methylamide (10).** To a solution of the protected amino acid (**9**, 0.742 g, 3 mmol) in DCM (11 mL) was added *N*-methylmorpholine (0.668 g, 6.6 mmol). The mixture was cooled to −15 °C, isobutylchloroformate (0.451 g, 3.3 mmol) was added, and then the mixture was stirred at −15 °C for 15 min, followed by addition of *N*,*O*-dimethylhydroxylamine hydrochloride (0.325 g, 3.3 mmol). The mixture was kept at −15 °C for an additional 1 h, allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was poured into water (11 mL) and the aqueous phase extracted with DCM (2 × 6 mL). The combined organic extracts were dried over $MgSO_4$ and filtered, and the solvent was removed under vacuum to give pale yellow oil. The product was isolated by flash chromatography using EtOAc/hexane 50/50 to give a final yield of 98.0%. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 5.36−5.39 (1H, d, J = 6.95 Hz), 4.96−4.98 (1H, m), 3.75 (3H, s), 3.62 (3H, s), 3.19 (3H, s), 2.55−2.78 (2H, dd, J = 6.79, 5.52 Hz), 1.49 (9H, s). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 171.1, 155.4, 80.2, 62.0, 55.3, 48.1, 37.5, 32.8, 28.6. IR: 3327.24, 2977.50, 1740.88, 1713.49, 1663.40, 1517.88, 1167.82, 1049.61, 989.21. HRMS (FAB) (M + H^+): calcd 291.1478, found 291.1556

L-Aspartic Acid β-Methyl Ester *N*-Methoxy-*N*-methylamide Hydrochloride (11). Weinreb amide (**10**, 1.16 g, 4 mmol) was treated with 40 mL of 2 M ethereal HCl. The temperature was maintained at 0 °C for 6 h before being allowed to increase to room temperature and stirred for 18 h. The excess of ethereal HCl was evaporated under a stream of N_2 and then under vacuum. The white solid residue was filtered, washed with ethyl ether (3 × 20 mL), and dried at room temperature to give an 89% yield of product. ^{1}H NMR [$(CD_3)_2SO$, 250 MHz]: δ 8.59 (3H, s), 4.38−4.43 (1H, m), 3.72 (3H, s), 3.61 (3H, s), 3.13 (3H, s), 2.77−2.94 (2H, d, J = 6.9 Hz). ^{13}C NMR [$(CD_3)_2SO$, 62.5 MHz]: δ 170.2, 168.3, 62.4, 52.9, 49.4, 47.4, 35.4. IR: 3416.55, 2951.36, 1730.34, 1666.27, 1494.94, 1212.51, 988.08, 616.22. HRMS (FAB) (M + H^+): calcd 191.0954, found 191.1154.

L-Aspartic Acid β-Methyl Ester *N*-Methacryloyl-*N*-methoxy-*N*-methylamide (12). *N*-Methoxy-*N*-methylamide hydrochloride (**11**, 0.910 g, 4 mmol) was suspended in 5 mL of DCM, and the mixture was neutralized using Et_3N until pH 7−8 (a white precipitate was formed in this step). The mixture was cooled to 0 °C and another aliquot of Et_3N added for a total of 20 mmol of Et_3N in the reaction mixture, followed by dropwise addition of methacryloyl chloride (1.045 g, 10 mmol). The mixture was stirred at 0 °C for 30 min, and then the temperature was allowed to rise to room temperature and stirring continued for 48 h. The reaction mixture was filtered and the filtrate washed with 0.5 M $NaHCO_3$ (3 × 15 mL) and 0.5 M sodium citrate (3 × 15 mL), dried over magnesium sulfate, and evaporated under vacuum to leave a brown oil. The compound was isolated by flash chromatography using EtOAc/hexane 60/40, followed by EtOAc 100%, to give a yellow-orange oil in 39% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 6.89−6.92 (1H, d, J = 8.21 Hz). 5.66 (1H, s), 5.26 (1H, s), 5.15 (1H, m), 3.69 (3H, s), 3.54 (3H, s), 3.1 (3H, s), 2.63−2.69 (2H, dd, J = 6.02, 5.84 Hz), 1.85 (3H, s). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 171.1, 168.0, 139.6, 120.7, 62.0, 52.2, 46.8, 36.6, 32.6, 18.7. IR: 333.83, 2953.69, 1739.36, 1655.33, 1623.80, 1526.06, 1438.21, 1175.38, 988.38. HRMS (FAB) (M + H^+): calcd 259.2710, found 259.1287.

***N*,α-Bismethacryloyl, L-Aspartic Acid β-Methyl Ester (13).** L-Aspartic acid β-methyl ester *N*-methacryloyl-*N*-methoxy-*N*-methylamide (**13**, 2.58 g, 10 mmol) was dissolved in 10 mL of dry THF and cooled to −60 °C under N_2. A 30 mL portion of 0.5 M isopropenylmagnesium bromide (15 mmol) was added dropwise over a period of 5 min, during which time a pale yellow precipitate formed. The mixture was stirred for 30 min at −60 °C after complete addition of the Grignard reagent, and then the mixture was brought to 0 °C and stirred for another 30 min. After this period, 7 mL of saturated NH_4Cl solution cooled at 0 °C was added to quench the reaction. THF was removed under vacuum followed by addition of ethyl ether (30 mL), after which the phases were separated. 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/hexane 50/50 giving 44% yield of a yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 6.98−7.007 (1H, d, J = 6.79 Hz), 6.08 (1H, s), 5.86 (1H, t, J = 1.42), 5.70 (1H, d, J = 0.79 Hz), 5.47−5.54 (1H, m), 5.33 (1H, d, J = 0.95 Hz), 3.62 (3H, s), 2.66−2.87 (2H, dd, J = 5.37, 5.21 Hz), 1.91 (3H, s), 1.86 (3H, s). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 198.2, 171.7, 167.8, 142.1, 139.7, 126.8, 120.9, 52.3, 50.3 37.1, 18.8, 18.2. HRMS (FAB) (M^+): calcd 240.2677, found 240.1247.

***N*,α-Bismethacryloyl, L-Aspartic Acid (2).** In a 100 mL amber bottle with cap was dissolved *N*,α-bismethacryloyl, L-aspartic acid β-methyl ester (**13**, 0.406 g, 1.7 mmol) in 5 mL of acetone, followed by the addition of pH 8.0 (0.1 M) phosphate buffer (40 mL). To this mixture was added porcine liver

esterase, EC 3.1.1.1 (119 mg). The mixture was sonicated for 1 min and then shaken for 72 h at room temperature. The reaction mixture was acidified to pH 3.0 with 1 N HCl. The aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with water (2 × 20 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated under vacuum giving a yellow oil. The product was isolated by flash chromatography with 100% EtOAc to give a 55% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 9.27 (1H, s, broad), 7.08−7.24 (1H, d, J = 7.58 Hz), 6.10 (1H, d, J = 0.79 Hz), 5.89 (1H, d, J = 1.42 Hz), 5.75 (1H, d, J = 0.79 Hz), 5.50−5.57 (1H, m), 5.37 (1H, t, J = 0.95, 0.79 Hz), 2.70−2.91 (2H, dd, J = 5.21, 5.05 Hz), 1.93 (3H, s), 1.88 (3H, s). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 198.7, 175.2, 168.4, 141.9, 139.4 127.3, 121.7, 50.5, 37.7, 18.8, 18.3. IR: 3359.50, 2928.88, 1722.55, 1687.87. 1614.67, 1525.64, 1215.27, 940.79. HRMS (FAB) (M^+): calcd 226.1001, found 226.1090.

***N*-Methacryloylglycine (5).** Compound **5** was synthesized as a white powder in 42% yield, following the protocol reported by Heilmann.[17] ^{1}H NMR (MeOD, 250 MHz): δ 5.60−5.61 (1H, t, J = 0.95 Hz), 5.24−5.25 (1H, t, J = 1.42 Hz), 4.77 (2H, s), 1.99 (3H, s), ^{13}C NMR (MeOD, 62.5 MHz): δ 172.1, 170.4, 139.8, 120.1, 41.0, 17.6. IR: 3359.85, 2983.28, 175.03, 1587.79, 1205.06, 1027.07, 960.75, 895.30. HRMS (FAB) (M^+): calcd 144.0582, found 144.0665.

Hydroxypropyl Methacrylate (16). A solution of 1,3-propanediol (**15,** 15.0 g, 197 mmol) and Et_3N (33.1 g, 45.6 mL, 327 mmol) in 200 mL of dry THF was cooled to 0 °C. Methacryloyl chloride (20.6 g, 197 mmol) was added dropwise over a period of 1 h with stirring, keeping the temperature between 0 and 3 °C. The reaction mixture was allowed to warm to 50 °C and kept at this temperature for 2 h. After this period, the reaction mixture was cooled and the precipitate filtered. The solvent was evaporated under vacuum and the residue redissolved in EtOAc (150 mL). The organic phase was washed with 0.5 M $NaHCO_3$ (2 × 100 mL) and 0.5 M sodium citrate (2 × 100 mL), dried over $MgSO_4$, and filtered and the solvent evaporated under vacuum. The residue was purified by flash chromatography on silica gel sequentially using the eluents EtOAc/hexanes 20/80, EtOAc/hexanes 50/50 to give a colorless liquid (39% yield). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 5.98−5.99 (1H, t, J = 1.60, 0.91 Hz), 5.44−5.46 (1H, t, J = 1.60 Hz), 4.14−4.19 (2H, t, J = 6.17 Hz), 3.51−3.61 (2H, t, J = 6.17 Hz), 3.03 (1H, broad), 1.77−1.85 (5H, m). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 168.1, 136.5, 126.0, 62.0, 59.2, 32.0, 18.4. IR: 3419.3, 2961.11, 2929.92, 2890.13, 1716.47, 1636.78, 1454.14, 1322.62, 1298.62, 1173.11, 1053, 944.66, 816.36.. HRMS (FAB) ($M + H^+$): calcd 145.0786, found 145.0871.

2-Methylacrylic Acid 2-Carboxyethyl Ester (6). Hydroxypropyl methacrylate (**16**, 1.15 g, 8 mmol) and 150 mg of BHT were dissolved in acetone (10 mL), and the solution was cooled to 5−10 °C. To the cooled solution was added Jones reagent (6.5 mL) dropwise over a period of 30 min, keeping the temperature between 5 and 10 °C. Afterward, the solution was allowed to warm to room temperature and stirred for additional 30 min. Then the reaction mixture was cooled at 10 °C, and excess Jones reagent was quenched by dropwise addition of 2 mL of 2-propanol. A 25 mL portion of H_2O was added to dissolve completely the solid formed, and the reaction mixture was extracted with EtOAc (3 × 25 mL). The organic phase was washed with water (1 × 30 mL), brine (3 × 25 mL), dried over $MgSO_4$, and the solvent was evaporated under vacuum. The target compound was isolated by flash chromatography on silica gel using EtOAc/hexanes 50/50 as eluent. The target compound was obtained in a 47% yield as a pale yellow oil that crystallized upon standing. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 10.49 (1H, broad), 6.04−6.05 (1H, t, J = 1.60, 0.91 Hz), 5.51−5.53 (1H, t, J = 1.60 Hz), 4.34−4.39 (2H, t, J = 6.17 Hz), 2.67−2.70 (2H, t, J = 6.17 Hz), 1.87−1.91 (3H, s). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 177.2, 167.6, 136.3, 126.5, 60.2, 34.0, 18.6. IR: 3450, 2963.3, 1718.2, 1700.1, 1634.97, 1454.15, 1404.79, 1325.07, 1300.43, 1166.68, 1023.63, 947.37, 814.56. HRMS (FAB) ($M + H^+$): calcd 159.0579, found 159.0485.

Polymer Preparation. Typical formulation: in a 13 × 100 mm test tube, 0.0698 g (0.43 mmol) of (*S*)-nicotine was dissolved in 2 mL of $CHCl_3$. To this solution was added 1.530 g (7.73 mmol) of EGDMA, 0.206 g (0.86 mmol) of **3**, and 0.028 g (0.172 mmol) of AIBN. The solution was purged by bubbling nitrogen gas into the mixture for 5 min, 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 or 100 mm; i.d., 2.1 mm) to full volume for chromatographic experiments. The polymers were then washed on line overnight using MeCN/HOAc 90/10, 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 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.

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Supporting Information Available: General experimental and copies of ^{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.

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