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Molecular Imprinting Made Easy

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Abstract: A simple method of molecular imprinting is presented that uses a single cross-linking monomer *N*,*O*-bismethacryloyl ethanolamine (NOBE) along with template, initiator, and solvent. This formulation eliminates the need for additional functional monomers and empirical optimization of relative ratios of functional monomers, cross-linkers, and template. In fact, utilization of NOBE alone often provides molecularly imprinted polymers (MIPs) with higher performance than MIPs incorporating functional monomer (e.g., methacrylic acid).

Introduction

There is a tremendous interest in the analytical applications of molecularly imprinted polymers (MIPs), which can serve as the molecular recognition element of biosensors, immunoassays, separation media, and affinity supports for screening libraries of bioactive compounds.[1−5] The generally accepted mechanism for the formation of specific binding sites in noncovalently formed MIPs is outlined in Scheme 1. In the scheme, preorganization of functional monomers in the vicinity of the template molecule 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.

Although the methodology of MIP formation is relatively easy, optimization of MIP formulation components is complicated by variables such as which functional monomers (M) to use, how many functional monomers to use, what type of cross-linker (X) to use, the optimum ratio of functional monomer/cross-linker (M/X), and the optimum ratio of functional monomer/template (M/T). The best results for MIP formulation can only be determined by empirical optimization via synthesis and evaluation of several polymers. This process is very time-consuming and not yet generalized for any substrate of interest, which has inhibited the widespread use of MIPs by the general scientific community and limited the broader impact of MIP technology on society.

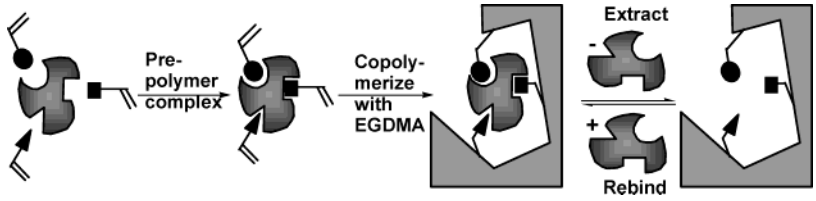
While investigating new cross-linkers for molecular imprinting, we have recently discovered a much simpler approach to MIP formation which utilizes a single cross-linking monomer, *N*,*O*-bismethacryloyl ethanolamine (NOBE, **1**),[6] in addition to template, solvent, and initiator (Scheme 2). We have coined the term "OMNiMIPs" (one monomer molecularly imprinted polymers) to describe this approach, which eliminates variables such as choice of functional monomer and cross-linker, the ratio of functional monomer/cross-linker, and the ratio of functional monomer/template which normally complicate MIP design.

Results and Discussion

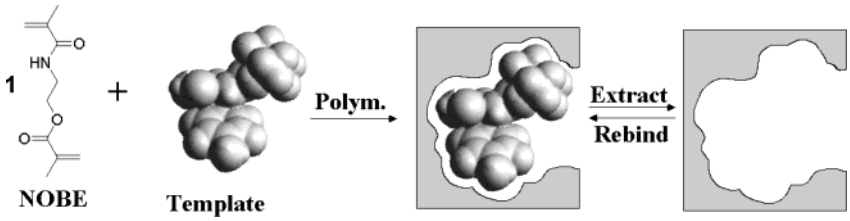
Scope and Selectivity of Different Templates. To investigate the scope of binding and selectivity using NOBE in the OMNiMIP approach, polymers were imprinted to an array of templates and compared with polymers imprinted with the traditionally used MIP formulation EGDMA/MAA. The enantioseparation factors for the survey compounds shown in Table 1 indicate selectivity is frequently better for the MIPs using NOBE versus the traditional EGDMA/MAA formulation (additional data including capacity factors and separation factors for polymers imprinted with only EGDMA are given in the Supporting Information). For this study, three different mobile phases were employed: acetonitrile, which provides nonpolar conditions, acetonitrile/acetic acid for polar-ionic conditions, and an acetonitrile/aqueous buffer system to test reverse phase conditions. The selectivities found for compounds using acetonitrile and acetonitrile/acetic acid mobile phases are roughly comparable, while selectivity is greatly reduced under reverse phase conditions. The lack of molecular recognition under reverse phase conditions appears to indicate that selectivity in the NOBE polymers is primarily due to hydrogen-bonding interactions. Furthermore, electrostatic interactions do not play a large role in the molecular recognition by the NOBE MIPs, except for templates/substrates incorporating amine functionality; this is indicated by the small changes in selectivity for substrate rebinding in polar-ionic versus nonpolar mobile phases. NOBE does not provide optimal selectivity for imprinting amine compounds, which are imprinted better using electrostatic

(1) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812−1832.
(2) Mosbach, K. *Trends Biochem. Sci.* **1994**, *19*, 9−14.
(3) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166.
(4) (a) *Molecular and Ionic Recognition with Imprinted Polymers*; Bartsch, R. A., Maeda, M., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 1998; Vol. 703. (b) *Molecularly Imprinted Polymers-Man made Mimics of Antibodies and Their Application in Analytical Chemistry*; Sellergren, B., Ed.; Elsevier: Amsterdam, 2001. (c) *Drug Development Assay Approaches Including Molecular Imprinting and Biomarkers*; Reid, E., Hill, H. M., Wilson, I. D., Eds.; Athenaeum Press, Ltd.: Gateshead, Tyne and Wear, U.K., 1998.
(5) Haupt, K.; Mosbach, K. *Chem. Rev.* **2000**, *100*, 2495−2504.
(6) Sibrian-Vazquez, M.; Spivak, D. A. *Macromolecules* **2003**, *36*, 5105−5113.

Scheme 1. Outline of the Traditional Molecular Imprinting Process



Scheme 2. Outline of OMN:MIP Strategy



interactions with carboxylate functional monomers such as MAA.

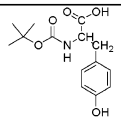
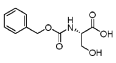
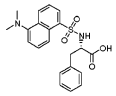
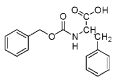
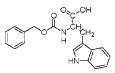
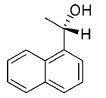
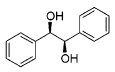
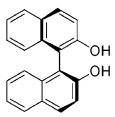
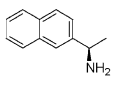
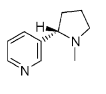
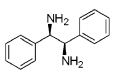
The compounds in Table 1 are roughly organized into three groups with compounds incorporating carboxylic acids in the first five entries, alcohols in the next three entries, and amine groups in the last three entries, showing that the effect is general for carboxylic acids and alcohols, with no improvement for the amine compounds tested. Template molecules containing more than one functional group capable of forming hydrogen bonds with the amide group in NOBE gave the best selectivity (entries 1, 3, 5, and 9, Table 1). The relatively low selectivity seen for amine templates by the NOBE MIPs is likely due to the relatively weak hydrogen-bonding interactions of amine groups with the amide group on NOBE, that is, R−OH- - -O═C−NHR > RR′NH- - -O═C−NHR.[7] To test this hypothesis, the association constants for the different templates with NOBE were determined by ^{1}H NMR titration studies in CD_3CN. Although a strict correlation between association constant and selectivity enhancement is not found, better selectivities are found for templates exhibiting stronger hydrogen-bonding interactions. Conversely, worse selectivities are found for the amine templates that exhibit poor hydrogen-bonding interactions with NOBE. Similarly, under reverse phase conditions, a general trend was found between log *P* values and selectivity enhancement.[8] Chromatographic results (Table 1) show that the selectivity observed in organic media for MIPs formulated with NOBE was greatly reduced in the reverse phase mode. However, slight selectivity was observed for templates having high log *P* values (entries 1, 3, 5, and 8, Table 1). Template molecules containing polar groups and low log *P* values (entries 2, 4, 7, 9, and 10, Table 1) gave low binding affinities, and essentially no selectivity was found. For EGDMA/MAA imprinted polymers, a similar behavior was observed with the exception of the amine templates (entries 9 and 11, Table 1) for which a higher selectivity was obtained. In summary, the OMNiMIP approach using NOBE is effective for creating highly selective MIPs. Hydrogen-bonding interactions appear to be the key for molecular recognition in this novel MIP approach; furthermore, increasing the number or strength of hydrogen-bonding interactions leads to an increase in selectivity. This is supported by the low selectivities found for templates that exhibit poor hydrogen bonding, especially in the case of amine-functionalized templates.

Comparison of OMNiMIP Approach to Traditional Mixed Monomer/Cross-Linker MIP Formulations. The examples in Table 1 show that NOBE not only acts as a cross-linker, but also provides binding functionality that interacts with the template as well, presumably through hydrogen bonding via the amide group. In fact, it was surprising to find that adding the carboxylate functional monomer MAA to NOBE in the imprinting formula actually decreased the selectivity for representative templates (shown in entry 3 versus entry 1 in Table 2), implying a greater molecular recognition effect is afforded by the amide group of NOBE. Thus, a more thorough study comparing the performance of OMNiMIPs to traditional MIP formulations that utilize both a functional monomer and a cross-linking monomer was conducted (Table 2). In general, the examples in Table 2 of functional monomer/cross-linker combinations show that they do not exhibit selectivity behavior as high as equivalent MIPs made only with NOBE. For example, entries 3, 5, 7, and 9 in Table 2 correspond to MIPs formulated with NOBE as the cross-linker along with several typical functional monomers. Conversely, polymers imprinted with EGDMA and a functional monomer (entries 4, 6, 8, and 10) most often exhibited increased selectivity versus EGDMA alone (entry 2). The last entry in Table 2 demonstrates that the presence of the non-cross-linked amide group alone imparts little or no additional selectivity over MIPs made only with EGDMA. Therefore, a requirement for increased selectivity by NOBE polymers is that the amide functionality should be incorporated into a cross-linking format. This is a result of stabilization of the amide conformation, thereby reducing the entropy of monomer motion, and leading to a more stable interaction with the template.[6] These results show that the amide group, incorporated into the cross-linking format found in NOBE, becomes an effective functional group that rivals template binding interactions of most other combinations of functional monomers and cross-linkers.

Chromatographic Properties of NOBE-Based OMNiMIPs. The behavior of the OMNiMIPs appears to be similar to that of the more traditional formulated MIPs. For example, the sample load capacity of the OMNiMIPs was determined by successive injections of substrate at different concentrations onto the HPLC column. The binding (related to the capacity factor)

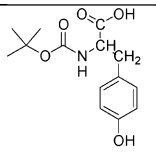
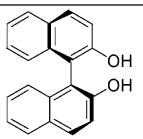
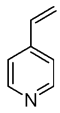
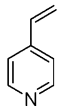
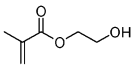
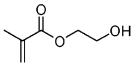
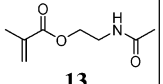
(7) *An Introduction to Hydrogen Bonding*; Jeffrey, G. A.; Oxford University Press: Oxford, New York, 1997; pp 15−78.
(8) The log *P* values were estimated using the program log P DB (ACD Labs).

Table 1. Separation Factors for MIPs[a]

				MeCN		MeCN/HOAc 99/1		MeCN/10 mM Glycine Buffer, pH =3, 30/70	
				EGDMA/ MAA	NOBE	EGDMA/ MAA	NOBE	EGDMA/ MAA	NOBE
Entry	**Template**	K_{ass} (M^{-1})	*Log P*	α	α	α	α	α	α
1	 **2**	3.82 (± 0.23)	2.23 (± 0.36)	1.99 (± 0.05)	3.89 (± 0.05)	1.78 (± 0.03)	4.12 (± 0.26)	1.05 (± 0.02)	1.14 (±0.01)
2	 **3**	1.22 (± 0.16)	0.83 (± 0.57)	1.99 (± 0.47)	2.33 (± 0.10)	1.23 (± 0.03)	1.99 (± 0.02)	1.05 (± 0.02)	1.08 (± 0.00)
3	 **4**	2.24 (± 0.27)	4.50 (± 0.42)	1.65 (± 0.01)	3.58 (± 0.01)	1.7 (± 0.01)	3.16 (± 0.01)	1.08 (± 0.01)	1.29 (± 0.03)
4	 **5**	0.87 (± 0.21)	3.58 (± 0.56)	1.67 (± 0.01)	1.96 (± 0.14)	1.52 (± 0.01)	2.04 (± 0.06)	1.10 (± 0.03)	1.10 (± 0.01)
5	 **6**	5.50 (± 0.17)	3.50 (± 0.56)	2.29 (± 0.13)	4.15 (± 0.10)	2.36 (± 0.01)	3.23 (± 0.16)	1.16 (± 0.03)	1.32 (± 0.03)
6	 **7**	0.60 (± 0.13)	2.61 (± 0.21)	1.35 (± 0.01)	1.23 (± 0.01)	1.33 (± 0.02)	1.18 (± 0.02)	1.05 (± 0.01)	1.00 (± 0.03)
7	 **8**	0.45 (± 0.13)	1.86 (± 0.33)	1.89 (± 0.08)	1.89 (± 0.03)	1.84 (± 0.15)	1.75 (± 0.01)	1.19 (± 0.01)	1.06 (± 0.02)
8	 **9**	1.44 (± 0.23)	4.36 (± 0.26)	3.59 (± 0.17)	13.05 (± 0.49)	5.2 (± 0.01)	9.47 (± 0.56)	2.08 (± 0.01)	2.11 (± 0.08)
9	 **10**	1.94 (± 0.34)	2.67 (± 0.22)	[b]	[b]	2.54 (± 0.54)	1.35 (± 0.03)	2.14 (± 0.19)	1.10 (± 0.03)
10	 **11**	0.32 (± 0.06)	0.72 (± 0.26)	[b]	1.00 (± 0.03)	1.8 (± 0.13)	1.12 (± 0.19)	[c]	[c]
11	 **12**	0.55 (± 0.14)	1.60 (± 0.50)	[b]	1.03 (± 0.00)	1.62 (± 0.46)	1.05 (± 0.32)	4.11 (± 0.37)	1.16 (± 0.03)

[a] HPLC conditions: particle size, 20−25 μm; column size, 100 × 2.1 mm; flow rate: 0.1 mL/min; injected volume: 5 μL; analytes/wavelength detection: 0.1 mM DLP, 0.1 mM DLP/330 nm, 1 mM Boc-L-tyrosine/270 nm, 1 mM Cbz-L-phenylalanine/250 nm, 0.2 mM Cbz-L-tryptophan/260 nm, 1 mM Cbz-L-serine/260 nm, 0.2 mM (*S*)-(−)-α-methyl-1-naphthalenmethanol/260 nm, 1 mM (*S*,*S*)-(−)-hydrobenzoin/254 nm, 0.2 mM (*S*)-(−)-1,1′-bi-2-naphthol/300 nm, 1 mM (*S*)-(−)-(1)-(1-naphthyl)ethylamine/260, 1 mM *S*-nicotine/262 nm, 10 mM (1*S*,2*S*)-(−)-1,2-diphenylethylenediamine/260 nm. [b] Retention time > 400 min for both enantiomers in MIP formulated with NOBE. [c] Both enantiomers eluted at the void time.

Table 2. Separation Factors for MIPs Imprinted with Different Monomer Combinations[a]

			 **2**	 **9**
Entry	**Cross-linker**	**Functional Monomer**	**α**	**α**
1	NOBE	No Functional Monomer	4.12 ± 0.26	9.47 ± 0.56
2	EGDMA	No Functional Monomer	1.23 ± 0.05	2.64 ± 0.02
3	NOBE	 **14**	1.80 ± 0.04	6.33 ± 0.29
4	EGDMA	 **14**	1.78 ± 0.03	5.2 ± 0.00
5	NOBE	 **15**	2.39 ± 0.09	8.92 ± 0.40
6	EGDMA	 **15**	2.26 ± 0.06	17.35 ± 0.91
7	NOBE	 **16**	3.82 ± 0.05	9.65 ± 0.35
8	EGDMA	 **16**	2.45 ± 0.03	6.56 ± 0.41
9	NOBE	 **17**	2.25 ± 0.04	7.34 ± 0.56
10	EGDMA	 **17**	1.25 ± 0.05	2.87 ± 0.04
11	EGDMA	 **13**	1.24 ± 0.01	3.24 ± 0.09

[a] HPLC conditions: particle size, 20−25 μm; column size, 100 × 2.1 mm; mobile phase, MeCN/acetic acid 99/1; analytes/wavelength detection, 1 mM Boc-L-tyrosine/270 nm, 0.2 mM (*S*)-(−)-1,1′-bi-2-naphthol/300 nm, and acetone (used to determine void volume); flow rate, 0.1 mL/min; injected volume, 5 μL.

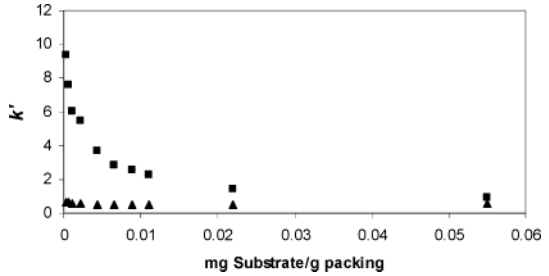


Figure 1. Binding affinity k' versus the amount of applied racemic 1,1′-bi-2-naphthol per gram of the (*S*)-(−)-1,1′-bi-2-naphthol/NOBE-based OMNiMIP. ▲ = k_R', ■ = k_S'. HPLC conditions: particle size, 20−25 μm; column size, 100 × 2.1 mm; mobile phase, MeCN/acetic acid 99/1; flow rate, 0.1 mL/min; injected volume, 5 μL; wavelength detection, 300 nm.

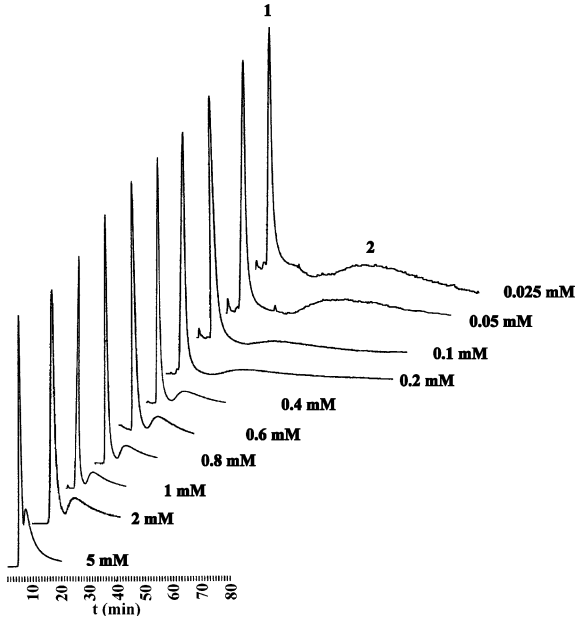


Figure 2. Elution profile of a racemic mixture of 1,1′-bi-2-naphthol at different concentrations on the (*S*)-(−)-1,1′-bi-2-naphthol NOBE-based OMNiMIP. (1) (*R*)-(+)-1,1′-bi-2-naphthol, (2) (*S*)-(−)-1,1′-bi-2-naphthol. HPLC conditions: particle size, 20−25 μm; column size, 100 × 2.1 mm; mobile phase, MeCN/acetic acid 99/1; flow rate, 0.1 mL/min; injected volume, 5 μL; wavelength detection, 300 nm. (Note: attenuation was adjusted for 0.05 and 0.025 concentrations to visualize broad peaks.)

of the substrate is shown to decrease as the concentration increases (Figure 1), which is typically seen for MIP columns[9] due to sample overloading as seen for ordinary HPLC columns. A significant factor to point out is that only the concentration of the "*S*" enantiomer imprinted shows any changes in capacity factor, while the capacity factors for the nonimprinted "*R*" enantiomer remain largely unchanged as the substrate concentration is increased. A second similarity of OMNiMIPs to traditionally formulated MIP columns is that peak broadening increases as the concentration of substrate is lowered (Figure 2). Although the phenomenon of peak broadening due to diffusion increases as capacity factor increases exists for any chromatographic phase, this is particularly severe for MIPs and has been attributed to the distribution of binding sites calculated to exist to a large extent in imprinted polymers.[10] The influence

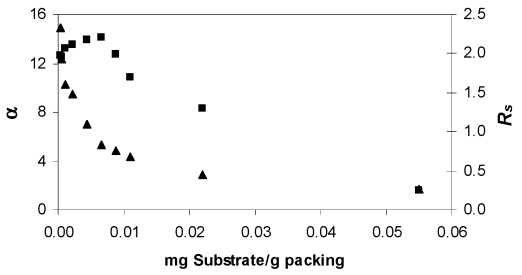


Figure 3. Separation factor α and resolution R_S versus the amount of applied racemic 1,1′-bi-2-naphthol per gram of the (*S*)-(−)-1,1′-bi-2-naphthol/NOBE-based OMNiMIP. ▲ = α, ■ = R_S. HPLC conditions: particle size, 20−25 μm; column size, 100 × 2.1 mm; mobile phase, MeCN/acetic acid 99/1; flow rate, 0.1 mL/min; injected volume, 5 μL; wavelength detection, 300 nm.

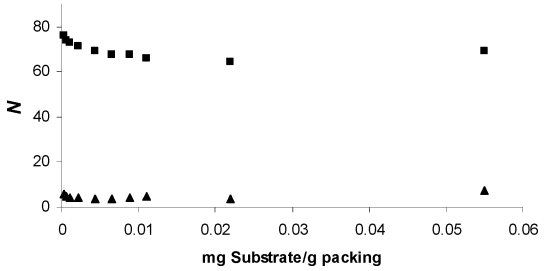


Figure 4. Number of plates versus the amount of applied racemic 1,1′-bi-2-naphthol per gram of the (*S*)-(−)-1,1′-bi-2-naphthol/NOBE-based OMNiMIP. ▲ = N_S, ■ = N_R. HPLC conditions: particle size, 20−25 μm; column size, 100 × 2.1 mm; mobile phase, MeCN/acetic acid 99/1; flow rate, 0.1 mL/min; injected volume, 5 μL; wavelength detection, 300 nm.

of the amount of template on the resolution (R_S)[11] and selectivity of enantioseparation is shown in Figure 3. At low substrate loading, the selectivity drops rapidly with increasing substrate concentration, followed by a slow decrease at an α value around 5 corresponding to a loading of 0.01 mg/g of packing material. The effective loading of 0.01 mg/g of packing material is fairly low as compared to traditionally formulated MIPs,[9] but still suitable for analytical applications. Even at higher sample loads the peaks are partly resolved (Figure 2), implying that OMNiMIPs can still be valuable for preparative purposes as well. On the other hand, resolution initially appears to increase as the sample load increases until the substrate concentration 0.01 mg/g of packing is reached, after which resolution steadily falls as substrate loading is increased. The small initial rise in resolution is due to the sharpening of the peaks as substrate concentration is increased, which is quickly overcome by the reduction in peak separation at higher substrate concentrations. The number of plates calculated[12] for a typical OMNiMIP is shown below at different loadings of substrate, which remained stable around 2 for the imprinted template and 70 for the nonimprinted template (Figure 4). While the values are low, they are of the same order of magnitude of traditionally formed MIPs.[9] The especially low values for the imprinted substrate reflect the peak broadening from the distribution of sites, as well as the use of relatively large and irregular particles. Broadening can be reduced somewhat by the use of small, well-packed, spherical particles; however, column chromatography by imprinted polymers should generally be regarded mechanistically as an affinity separation process. In the affinity process,

(9) Sellergren, B. *Chirality* **1989**, *1*, 63−68.
(10) (a) Umpleby, R. J., II; Baxter, S. C.; Bode, M.; Berch, J. K., Jr.; Shah, R. N.; Shimizu, K. D. *Anal. Chim. Acta* **2001**, *435*, 35−42. (b) Chen, Y.; Kele, M.; Quiñones, I.; Sellergren, B.; Guichon, G. *J. Chromatogr., A* **2001**, *927*, 1−17.
(11) Wulff, G.; Poll, H.-G.; Minárik, M. *J. Liq. Chromatogr.* **1986**, *9*, 385−405.
(12) Meyer, V. R. *Practical High-Performance Liquid Chromatography*; John Wiley & Sons Ltd.: NewYork, 1997; pp 15−43.

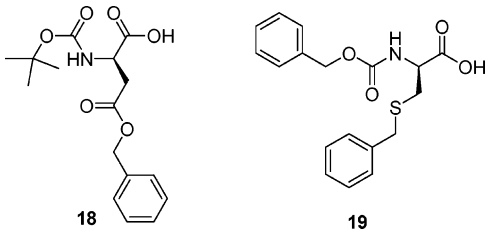


Figure 5. Other amino acids used to evaluate cross-reactivity of the NOBE-based OMNiMIP imprinted with Cbz-L-tryptophan **6** as the template.

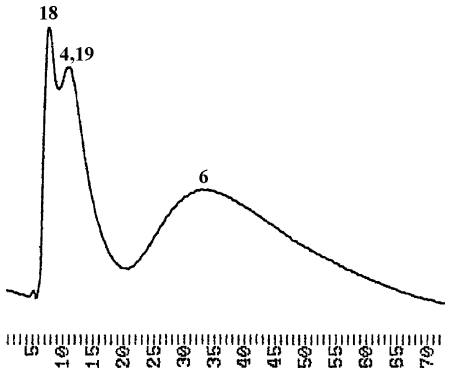


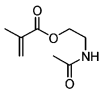
Figure 6. Chromatographic separation of a mixture of (**18**) Boc-L-aspartic acid β-benzyl ester (0.25 mM), (**4**) Cbz-L-phenylalanine (0.25 mM), (**19**) Cbz-*S*-Bz-L-cysteine (0.25 mM), and (**6**) Cbz-L-tryptophan (0.10 mM) obtained in NOBE-based OMNiMIP imprinted with Cbz-L-tryptophan as the template. Mobile phase, MeCN/MeOH 98/2; column size, 2.1 × 100 mm; flow rate, 0.1 mL/min; λ = 260 nm; injected volume, 5 μL.

the target molecule is bound while all other molecules pass through the stationary phase; thus, a high partitioning efficiency is not necessary to significantly purify or detect target species. For evaluating the "imprinting effect" in this study, the best probe is chiral differentiation, because all physical properties of enantiomers are the same except for the three-dimensional positioning of atoms in space. Therefore, the most important factor to evaluate the "imprinting effect" is enantioselectivity using the magnitude of the separation factor, α, as the figure of merit. It is important to note that enantioselective α values are constant for any particular polymer and are not dependent on column size, particle size, particle shape, or the amount of polymer packed into the column.

The ability of the OMNiMIP system to separate the imprinted molecule from a mixture of compounds similar in structure was also evaluated. For this purpose, a mixture of templates **4** and **6** from Table 1, along with Boc-L-aspartic acid β-benzyl ester **18** and *N*-CBz-*S*-Bz-cysteine **19** (shown in Figure 5), was prepared and resolved under isocratic conditions using the NOBE-based OMNiMIP imprinted with Cbz-L-tryptophan **6**. As shown in Figure 6, the imprinted compound Cbz-L-tryptophan **6** was the most retained, indicating that efficient substrate selectivity between compounds similar in structure is possible. The low cross-reactivity of the OMNiMIP for binding the template compound can be useful for applications in chromatography and solid-phase extraction[4b] and may prove useful as the molecular recognition phase for immunoaffinity techniques.[4c]

Characterization of NOBE Materials. In addition to the imprinting effect of the template, it was important to determine if the macroscopic properties of the new MIP materials contributed in any way to the selectivity behavior observed. MIPs are a class of network materials known as macroporous

Table 3. Surface Area and Pore Analysis on the MIPs

XL	FM	BET[a] Surf. Area (m^2/g)	Pore vol.[b] (mL/g)	Pore diam.[c] (Å)
NOBE	none	45.8	0.191	166
NOBE	 **14**	51	0.510	402
EGDMA	 **14**	165	0.486	118
EDGMA	 **13**	106	0.416	156

[a] Determined using the BET model on a seven-point linear plot. [b] BJH cumulative adsorption pore volume. [c] BJH average pore diameter.

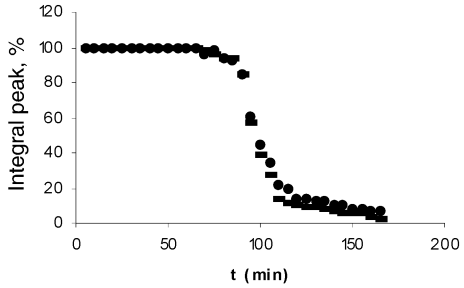


Figure 7. Conversion of NOBE double bonds as a function of polymerization time. (−) CH_2=$C(CH_3)$COOR, (●) CH_2=$C(CH_3)$CONHR).

polymers and are typically analyzed for surface area and porosity.[13] Using nitrogen adsorption porosimetry, we determined the surface area and porosity for NOBE and EGDMA polymers shown in Table 3, which indicates the polymers all have similar macroporous properties. Differences in swelling behavior were also minimal, with polymers exhibiting a low swelling constant of 1.05 in all cases.[14] Thus, the integrity of the polymer morphology is maintained whether in or not in the presence of solvent. A last consideration is that cross-linkers incorporating two different polymerizable groups (e.g., NOBE) can have differences in reactivity that could impact the structure and sequence morphology. This, in turn, can affect the molecular recognition properties of the imprinted polymers. However, Figure 7 indicates that both the methacrylate and the methacrylamide moieties have roughly the same reactivity. Therefore, the incorporation of polymerizable groups is equally statistical and will not influence polymer morphology, allowing the template to fully control binding site organization. Thus, the macroscopic

(13) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31−49.
(14) Data are provided in the Supporting Information.

properties of the NOBE polymer do not appear to affect the molecular recognition characteristics of the MIP over the imprinting effect of the template.

Conclusion

A simpler protocol for the synthesis of molecularly imprinted polymers has been developed that reduces most of the complicated formulation variables encountered. Only a single monomer is required that incorporates the template-binding functionality with the necessary cross-linking features for molecular recognition and network formation. The amide functionality in the cross-linker NOBE interacts sufficiently with most templates, with the only exception of amines, to afford molecular recognition without the need of introducing any other functional monomer. The OMNiMIP approach using NOBE exhibited improved or similiar results for enantioselective recognition in organic and aqueous media in comparison to the traditionally used MIPs prepared with EGDMA/MAA. Success in enantioselectivity indicates that simpler separations between different molecules or in sensor applications can be easily achieved. The origin of the improved molecular recognition observed in organic media was determined to be the result of hydrogen-bonding interactions, whereas in aqueous media moderate hydrophobic interactions were responsible for the selectivity observed. The chromatographic performance of the OMNiMIP materials is much the same as that of the traditional MIPs, with respect to peak broadening and loading capacity, showing that chromatographic resolution and efficiency is controlled mainly by the imprinting process and not the materials used.

Macromolecular contributions to molecular recognition in OMNiMIPs include incorporation of the amide group in the cross-linker backbone which not only reduces the conformational flexibility of the binding site, but also reduces the entropic effect associated with binding interactions. Incorporation of another functional monomer into the polymeric network of NOBE tends to interfere with good binding site interactions giving as a result MIPs with lower selectivity. The small differences in reactivity between the polymerizable groups in NOBE suggest that the morphology of these new materials may be similar to the traditional MIPs formulated with EGDMA. Overall, the ease of the OMNiMIP approach is anticipated to provide an accessible and dependable process for industry and academic research.

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Supporting Information Available: Experimental details, ^{1}H NMR binding data, and tables of binding data complete with k', α, and log P values. This material is available free of charge via the Internet at http://pubs.acs.org.

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