ORGANIC LETTERS

2003
Vol. 5, No. 19
3415−3418

An Orthogonal Approach to Multifunctional Molecularly Imprinted Polymers

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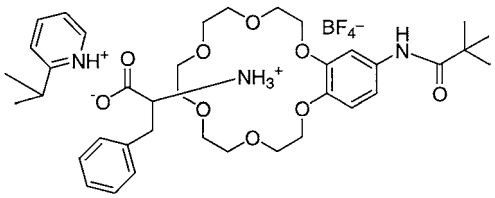
Received June 19, 2003

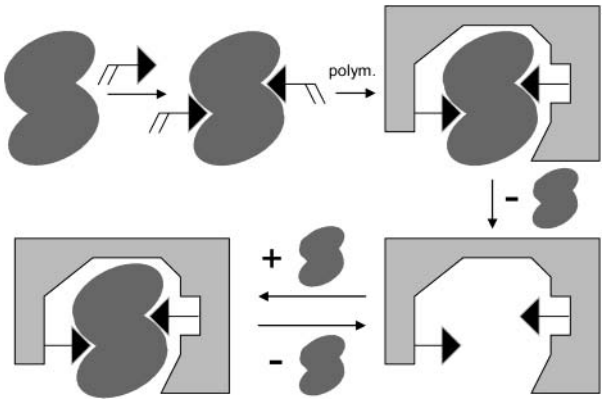
ABSTRACT



An "orthogonal" approach to molecularly imprinted polymers has been demonstrated using a crown ether derived monomer that does not exhibit cross-reactivity with other functional monomers. This strategy provides multiple functional groups in the binding site of molecularly imprinted polymers (MIPs) without unproductive interactions between functional monomers. The orthogonal functional group system was shown to act cooperatively in MIPs to bind a template with higher selectivity than any of the individual functional monomers alone.

Molecularly imprinted polymers (MIPs) are typically formulated using one functional monomer, usually methacrylic acid, for noncovalently formed MIPs. Noncovalent MIPs are formed by the strategy outlined in Scheme 1. Functional monomers are associated with a template via noncovalent interactions, and this complex is copolymerized with cross-linking monomer (usually ethylene glycol dimethacrylate). After polymerization, the template is removed simply by extraction, leaving binding sites in the polymer that have a complementary array of functional groups inside a shape selective cavity. Although this strategy has been very successful, there are many templates for which methacrylic acid (MAA) alone is not sufficient. For example, amino acids (and peptides) such as phenylalanine are zwitterionic and are able to interact with both acidic and basic functionality. In this case, it would be desirable to have polymer formulation incorporating a basic functional monomer simultaneously with methacrylic acid to complement both functional groups on the template.

Scheme 1



MIPs incorporating both MAA and a polymerizable zinc porphyrin have been shown to exhibit cooperative effects leading to improved binding and selectivity.[1,2] Similarly, combinations of MAA with the basic monomer 2-vinylpyridine (2-VPY) have also exhibited improved MIP performance in many cases.[3,4] There is a risk, however, of

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10.1021/ol035136r CCC: $25.00
Published on Web 08/28/2003

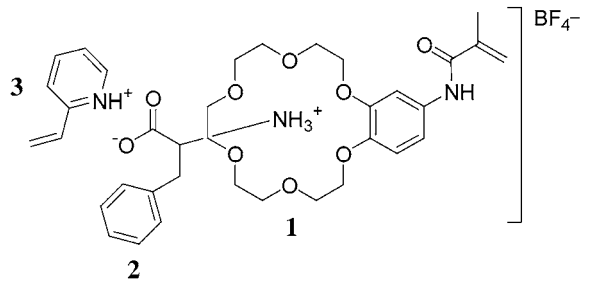

Figure 1.

nonproductive interactions between the functional monomers instead of with the template. A solution to this problem is to use an orthogonal imprinting strategy, that is, using two (or more) noninteractive functional monomers that act synergistically to optimize selectivity in MIPs. This strategy has been employed by Whitcombe and co-workers, who demonstrated the use of mutually exclusive monomers for binding to different parts of a template for aqueous imprinting.[5] Another report of aqueous imprinting by Nicholls and co-workers makes use of combining an electrostatic and a hydrophobic interaction to improve MIP selectivity.[6] However, all reports of orthogonal imprinting in organic solvents use a combination of covalent and noncovalent interactions.[7−11] The goal of this study was to devise the first orthogonal functional group system for noncovalent molecular imprinting in organic solvents. We adopted a similar strategy developed by de Mendoza and co-workers, who used the combination of guanidinium functionality along with a crown ether moiety for a small molecule receptor toward phenylalanine.[12] The key to this combination is that crown ethers bind well to primary ammonium groups (and metal ions) but do not interact other basic groups such as secondary and tertiary amines, quaternary ammonium compounds, and guanidinium groups. To carry out this strategy, the crown ether based functionalized monomer, 4′-aminobenzo-18-crown-6 methacrylate (**1**, 18C6-MA), shown in Figure 1, was synthesized according to literature.[13] This neutral monomer was anticipated to bind protonated primary amine groups orthogonally with a number of basic functional monomers for forming MIP pre-polymer complexes, and ultimately MIP binding sites, with multiple functionality as illustrated in Figure 1.

Table 1. Composition of MIP Formulations[a]

	functional monomers				
entry[b]	2-VPY[c]	2-DEMA[d]	18C6-MA[e]	18-crown-6[f]	template
1			0.35		0.23
2		0.39		0.39	0.26
3	0.38			0.38	0.25
4		0.38	0.38		0.23
5	0.38		0.38		0.25

[a] All quantities are in mmol. All formulations contained 5.0 mmol of EGDMA, 0.11 mmol of AIBN, and 1.7 mL of $CHCl_3$. [b] For each imprinted polymer, a separate control polymer was made using the same formulation without template. [c] 2-VPY = 2-vinylpyridine. [d] 2-DEMA = 2-(dimethylamino)ethyl methacrylate. [e] 18C6-MA = 4′-aminobenzo-18-crown-6 methacrylate. [f] 18-Crown-6 was purchased from Aldrich Chemical Co. and used without purification.

In this study, we set out to determine whether crown ether monomer **1** was effective for orthogonal imprinting of phenylalanine (L-phe). L-phe has both amine and carboxylate groups that are in a zwitterionic state that require different functional monomers for complexing each. 18C6-MA was anticipated to complex with the ammonium group of L-phe, which was employed as the tetrafluoroborate ammonium salt (**2**, L-phe-BF_4). For complexing the carboxylate moiety of L-phe, 2-vinylpyridine (**3**, 2-VPY) or 2-(dimethylamino)ethyl methacrylate (**4**, 2-DEMA) was used and compared for optimal selectivity. L-phe-BF_4 alone was not soluble in chloroform (a good solvent for noncovalent imprinting), nor was it solubilized by addition of 2-DEMA or 2-VPY. However, the presence of the crown ether monomer 18C6-MA was able to solubilize L-phe-BF_4 both alone and in the presence of 2-DEMA or 2-VPY, forming a soluble organic salt complex with the ammonium group. This extends the concept of crown ether-assisted molecular imprinting introduced for solubilizing MIP pre-polymer complexes.[14] Table 1 shows the formulations employed for formation of MIPs compared in this study. Entry 1 imprints L-phe-BF_4 using only 18C6-MA; entries 2 and 3 imprint the same template using 2-VPY or 2-DEMA, respectively, in the presence of nonpolymerizable crown ether 18-crown-6. Entries 4 and 5 give the formulations for MIPs incorporating 18C6-MA combined with 2-VPY or 2-DEMA, respectively, used to elucidate whether cooperative interactions exist between 18C6-MA and the other two basic monomers.

Each of the MIPs was evaluated by HPLC for enantioselective binding of L-phe-BF_4 to determine which formulation works best and whether a combination of monomers improves molecular recognition (Table 2). The polymers were ground into powders and slurry packed into stainless steel HPLC columns, and retention of L- or D-phe-BF_4 was determined separately using the mobile phase 96/4 MeCN/K_2HPO_4−H_2PO_4 buffer, pH = 3.0, 100 mM. Entry 1 shows

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Table 2. Capacity Factors (k') and Separation Factors (α) for L- and D-Phe-BF_4 Substrates Obtained from the Indicated Polymer HPLC Stationary Phases[a]

entry	polymer[b]	k'_L	k'_D	α
1	MIP(1)	2.95	2.85	1.04
	non-MIP(1)	1.88	1.82	1.03
2	MIP(2)	2.76 (5.20)[c]	2.59 (5.20)	1.07 (1.00)
	non-MIP(2)	2.33 (4.60)	2.25 (4.60)	1.04 (1.00)
3	MIP(3)	0.74 (1.90)	0.68 (1.90)	1.09 (1.00)
	non-MIP(3)	0.41 (1.40)	0.41 (1.40)	1.00 (1.00)
4	MIP(4)	11.71	10.21	1.15
	non-MIP(4)	10.0	9.8	1.02
5	MIP(5)	5.19	4.05	1.28
	non-MIP(5)	2.78	2.70	1.03

[a] Flow rate = 1.0 mL/min, injections were 5.0 μL of a 10 mM solution of L- or D-Phe-BF_4, mobile phase = acetonitrile/$K_2HPO_4-H_2PO_4$ buffer (PH = 3.0, 100 mM) 96/4; UV detection at 250 nm. [b] MIP represents imprinted polymer, non-MIP represents nonimprinted polymer; numbers in parentheses refer to entries in Table 1 defining polymer compositions. [c] Capacity factors (k') and separation factors (α) in parentheses were evaluated using the mobile-phase acetonitrile/$K_2HPO_4-H_2PO_4$ buffer (PH = 3.0, 100 mM) 98/2.

the results for the MIP using only the crown ether monomer **1**, which displayed only a slight enantioselectivity for the template as indicated by the α value close to one. This is similar to entries 2 and 3 which correspond to MIPs made using 2-VPY or 2-DEMA, respectively, in the presence of crown ether 18-crown-6 (in nonpolymerizable form) to aid solubility. These polymers were further investigated using a less polar mobile phase (acetonitrile/$K_2HPO_4-H_2PO_4$ buffer, pH = 3.0, 100mM) 98/2); however, there was no improvement in selectivity. In contrast, when MIPs are synthesized using both the *monomeric* crown ether (18C6-MA) in conjunction with 2-VPY or 2-DMA, good enantioselectivity is achieved as shown in entries 4 and 5, respectively. These data support a cooperative binding model where selectivity depends on the simultaneous interaction of the template with both the amine-based functional monomer and the crown ether-based functional monomer (Figure 1) positioned complementarily within the polymer-binding site. An interesting observation is that the MIP using the 2-VPY/18C6-MAA combination shows higher enantioselectivity (i.e., higher α value) than the MIP made with 2-DEMA/18C6-MAA. On the other hand, the phenylalanine substrate binds stronger to the imprinted polymer with 2-DEMA/18C6-MAA than to the polymer imprinted with the monomer combination 2-VPY/18C6-MAA, as seen by the greater values in capacity factor (i.e., higher k' value). It appears that the 2-VPY provides a more structurally defined site using directional binding interactions versus 2-DEMA which acts as a point charge with no directional binding interactions.[15]

To verify the 18C6-MA participates orthogonally in MIP formulations along with 2-DEMA or 2-VPY, we carried NMR titration experiments with 18C6-MA/2-DEMA and 18C6-MA/2-VPY in chloroform. This was compared to

Table 3. Association Constants for Binary Functional Monomer Combinations[a]

entry	monomer 1	monomer 2	template	association constant (K_a) (M^{-1})
1	18C6-MA	2-VPY	no	0.0
2	18C6-MA	2-DEMA	no	0.0
3	18C6-MA	2-VPY	yes	150 (140)[b]
4	18C6-MA	2-DEMA	yes	130 (70)[b]
5	2-VPY	MAA	no	9.9
6	2-DEMA	MAA	no	11.0

[a] Measurements obtained by ^1H NMR titration using $CDCl_3$ provided in the Supporting Information. [b] Values not in parentheses are based on 18C6-MA; values in parentheses are based on monomer 2

formulations of MAA and 2-DEMA or 2-VPY, which suffer strong intermolecular interactions. The association constants shown in Table 3 show no association between the crown ether monomer 18C6-MA and either 2-VPY or 2-DEMA (entries 1 and 2). Thus, there is no interference of 18C6-MA with any of the other monomers, eliminating any risk of nonproductive interactions between functional monomers. Therefore, the productive interactions between monomers and template indicated by NMR titrations in the presence of template (entries 3 and 4) are maximized for optimal performance. On the other hand, there is a considerable association of basic monomers 2-VPY and 2-DEMA with methacrylic acid (entries 5 and 6). If the functional monomers bind each other in the MIP formulation, the amount of complex between the template and these functional monomers will be lowered significantly leading to nonoptimal MIP performance.

The performance of molecularly imprinted polymers is generally limited by use of a single functional group to achieve binding or catalysis. Future development of MIPs will benefit from employing several different functional groups that can act cooperatively to obtain binding, selectivity, or catalysis in the same way enzymes and antibodies are known to do. Thus MIP formulations using several different functional monomers will continue to be vigorously investigated to achieve the capabilities of proteins. However, the challenge for MIP formulations is to limit nonproductive interactions between functional monomers that will interfere with the desired template complex. Based on known small-molecule receptor designs, we have developed an orthogonal binary functional group system that acts in concert in MIPs to bind a template without forming random complexes. This system provides simultaneous pre-polymer complexing interactions with acidic and primary ammonium groups on a template, which should have general application to many other templates. In addition, for templates such as zwitterionic phenylalanine normally only soluble in water, this binary functional group system is able to form complexes that are soluble in organic media for facilitating the imprinting process.

Acknowledgment. This research was supported in part by Research Corporation, through Cottrell Scholar Award

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CS0801, and an NSF CAREER Program award CHE-0134290 (D.A.S.).

Supporting Information Available: Experimental details for the preparation of 4′-aminobenzo-18-crown-6 methacrylate (**1**, 18C6-MA) and L-phenylalanine tetrafluoroborate, polymer preparation, HPLC binding analysis, and ^{1}H NMR titration data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035136R