Molecular Imprinting of Carboxylic Acids Employing Novel Functional Macroporous Polymers

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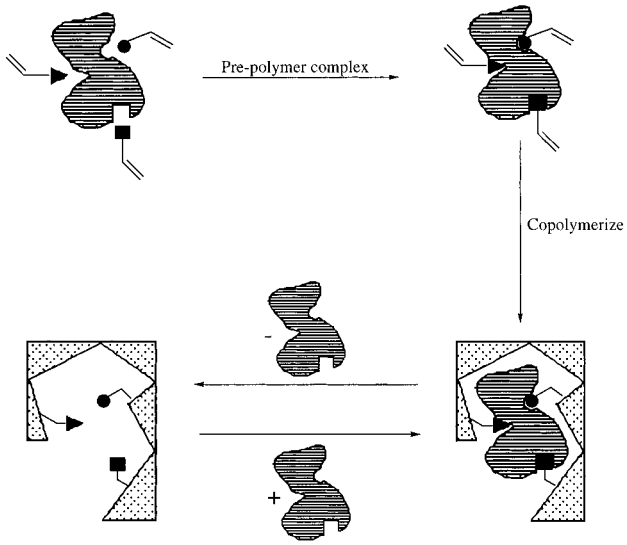
Imprinted network polymers incorporating basic functional groups were developed to assess the binding and specificity of carboxylic acids. The binding affinities were determined using *t*-BOC-phenylalanine and 2-phenylbutyric acid as templates and substrates. Chiral selectivity for the enantiomers of *t*-BOC-phenylalanine was found for polymers incorporating adenine or 2-aminopyridine functionality. Chiral selectivity in the case of (*R*)-(−)-2-phenylbutyric acid was found for the polymer utilizing *N*-(2-aminoethyl) methacrylamide as the functional monomer. Optimization of binding was achieved by changing polymerization conditions (thermal versus photochemical polymerization, monomer:template ratio) for *t*-BOC-phenylalanine imprinted polymers employing the *N*-(2-aminopyridine) methacrylamide monomer.

Introduction

Biological macromolecules, such as antibodies and enzymes, benefit from a variety of functional groups to effect recognition and catalytic properties. The rapidly developing technique of molecular imprinting,[1] which provides polymeric artificial receptors and catalysts, would also benefit from an expanded repertoire of functional groups. The concept of molecular imprinting is illustrated in Scheme 1. Functional monomers are bound either covalently or noncovalently to a print molecule or template. The resulting pre-polymer complex is copolymerized with an excess of cross-linking monomer in the presence of an equal volume of inert solvent (porogen) and a free radical initiator. Thermal or photochemical polymerization results in a highly cross-linked insoluble polymer.[2] Removal of the template leaves cavities in the polymer that are complementary in size, shape, and chemical functionality to the template molecule.

Two methods are available for formation of the pre-polymer complex; one uses covalently bound templates, and the other utilizes noncovalent interactions. Molecular imprinting using noncovalent interactions has been dominated by the use of the functional monomer methacrylic acid.[3,4] Despite its utility, this limits the choice of templates to those that bind to carboxylate functionality. To imprint templates that do not bind carboxylic acids, new monomers needed to be synthesized. The goal of this project was to design, synthesize, and survey the binding potential of methacrylamide-based monomers incorporating various carboxylate binding functional groups. Some weakly basic monomers such as *N*-vinylimidazole, 4-vinylpyridine, and 2-vinylpyridine have been used as functional monomers for molecular imprinting.[5] Reports of methacrylamide-based monomers containing amine functionality primarily dealt with catalysis rather than binding by imprinted polymers.[6] Six novel monomers to create selective binding sites in polymers for carboxylic acids by molecular imprinting are reported in this paper.

Scheme 1. Outline of the Molecular Imprinting Strategy



Monomer Design. Candidates for functional monomers were generated from three areas: amino acids and

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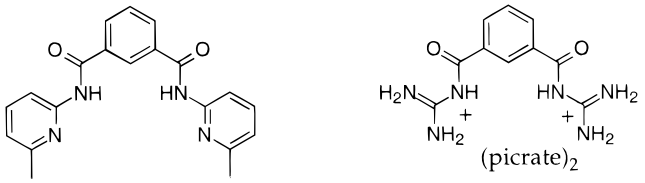

Figure 1. Selective receptors using the 2-aminopyridine[9] or guanidinium[10] functionality to bind dicarboxylic acids.

proteins, nucleic acids, and designed small molecule carboxylate receptors. The amino acids capable of binding carboxylic acids are essentially those with ionizable side chains.[7] The guanidinium group of arginine in proteins is known to bind strongly to carboxylate, as well as to phosphate groups.[8−13] Other carboxylate-binding amino acid subgroups are the primary amine of lysine, the secondary amine of imidazole on histidine, and finally, heterodimers with the carboxylate group of aspartic and glutamic acid. The DNA bases guanine and adenine form the strongest association of all of the nucleotide bases with butyric acid in chloroform.[14] Previous molecular imprinting experiments have shown that adenine binds well to imprinted polymers containing carboxylate groups.[4] Guanine, however, does not bind as well to these templated polymers. Designed synthetic small molecules that bind carboxylic acids have been developed in a number of laboratories including those of Hamilton,[8−10] Lehn,[11] Schmidtchen,[12] and Anslyn.[13] The majority of these incorporate either a 2-aminopyridine substructure or a guanidinium moiety embedded in their structures (Figure 1).

From a consideration of the examples above, functional monomers **1**−**7** (Figure 2) were chosen for molecular imprinting of carboxylates. *N*-(Diaminomethylene)-2-methylprop-2-enamide (**1**) incorporates the guanidine group; in addition, the rigid structure is expected to minimize conformational flexibility (inherent in monomer **2**) which will maintain the specific orientation of the guanidinium group(s) originally positioned by the imprinting process. This has been shown to positively influence selective binding by molecularly imprinted polymers.[15] The design of the monomer *N*-(3-guanidinopropyl) methacrylamide (**2**) mimics the amino acid arginine, which is responsible for binding and catalysis in many enzymes. Earlier work in designed polymer catalysts utilized *N*-propyl and *N*-ethyl methacrylamides for

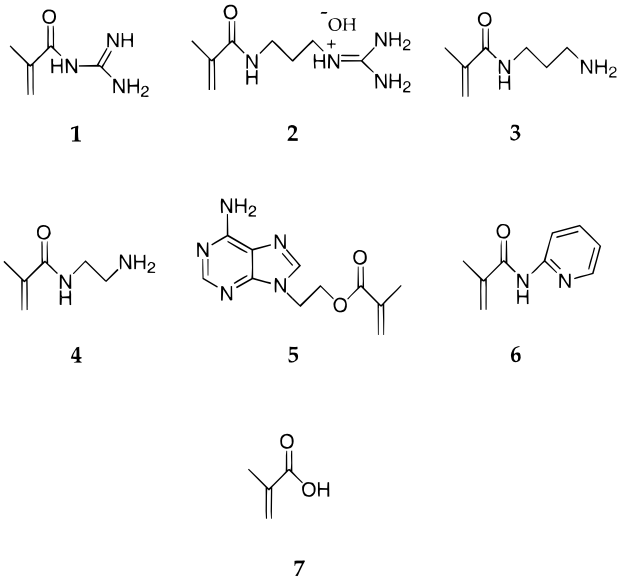

Figure 2. Chemical structures of functional monomers used for the carboxylic acid imprinting study.

imprinting dicarboxylic acid templates.[6] *N*-Propyl methacrylamide (**3**) was commercially available from Kodak. Use of the *N*-ethyl methacrylamide monomer (**4**) has been previously reported for use in a catalytic imprinted polymer; however, its carboxylate binding potential has not been evaluated.

The nucleotide base adenine binds strongly with carboxylate-functionalized imprinted polymers.[4] The methacrylate-based adenine monomer 9-(*β*-methacryloyloxyethyl) adenine (**5**) has been reported in the literature[16] and used for the synthesis polyadenine methacrylate. One of the major binding motifs of adenine is the 2-aminopyridine moiety, responsible for Watson−Crick binding in DNA duplexes.[4,17] As previously noted, Hamilton has used this moiety successfully for creating carboxylate receptors.[8−10] *N*-(2-Aminopyridine) methacrylamide (**6**) was predicted to take advantage of this binding. Finally, methacrylic acid (**7**) has the potential to create carboxylate heterodimers with target carboxylic acid templates.

Ethylene glycol dimethacrylate (EGDMA) was chosen as the principal cross-linking monomer. Although this monomer was not chosen by a systematic process, macroporous polymers prepared with EGDMA give stable, robust materials that were suitable for use as stationary phases in HPLC. In addition, they provide an average polarity that permits optimization of chromatographic retention with common organic elements.

Results and Discussion

Synthesis of Functional Monomers. *N*-(Diaminoethylene)-2-methylprop-2-enamide was synthesized by condensation of guanine and methyl methacrylate. Guanidinium hydrochloride was desalted by passing through ion-exchange resin prior to refluxing with methyl methacrylate to give **1** as a pale yellow solid (Scheme 2). Following the method of Lehn,[11] *S*-ethyl thiourea was reacted with *N*-(3-aminopropyl) methacrylamide to give

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(8) (a) Hamilton, A. D. *Advances in Supramolecular Chemistry, Vol. 1*; JAI Press Inc., 1990; pp 1−64. (b) Fan, E.; Van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369. (c) Vicent, C.; Fan, E.; Hamilton, A. D. *Tetrahedron Lett.* **1992**, 4269.
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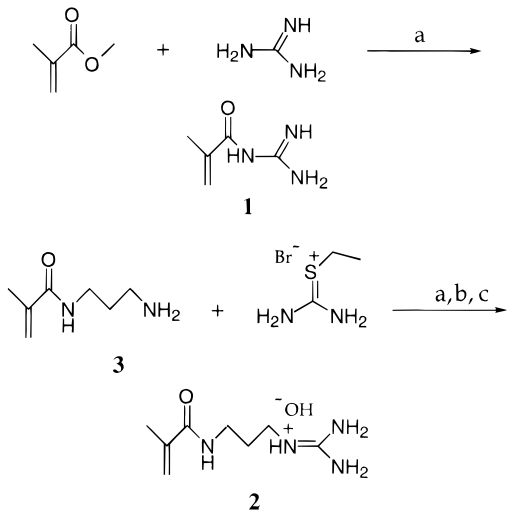
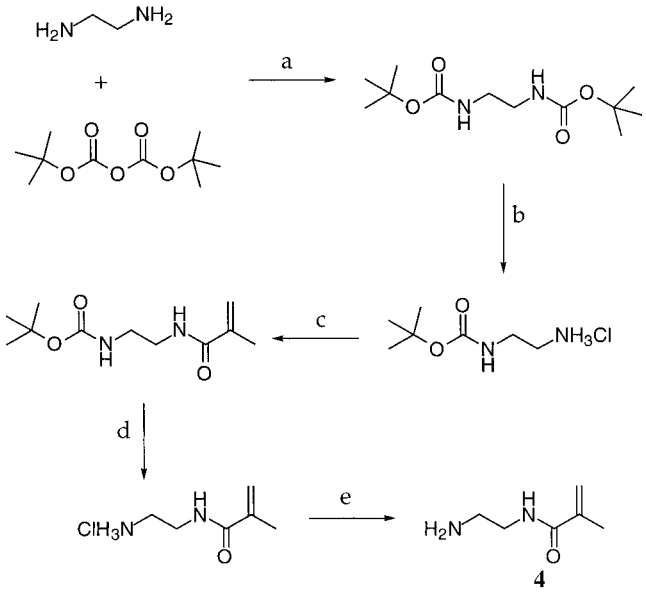
Scheme 2[a]



[a] (a) EtOH/100 °C. (b) NH_4OH (aq)/rt/16 h. (c) Dowex 1 × 8 anion exchange resin.

Scheme 3[a]



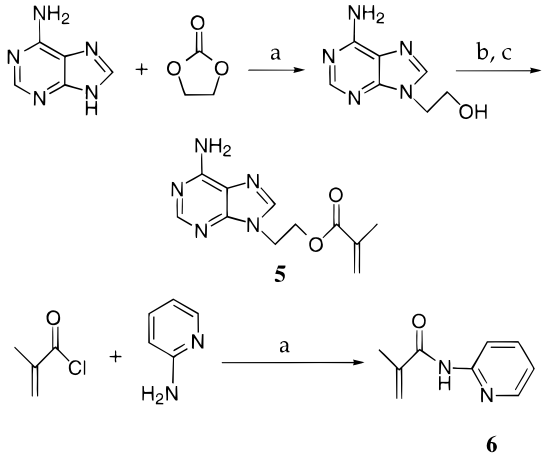
(a) THF/0 °C/12 h. (b) HCl/EtO_2/rt/3 h. (c) THF/Et_3N/0 °C to rt/24 h. (d) HCl/EtO_2/rt/24 h. (e) EtOH/NaOEt/rt/1 h.

N-(3-guanidinopropyl) methacrylamide **2** (Scheme 2). Because of sensitivity toward polymerization, the reaction with *N*-(3-aminopropyl) methacrylamide (**3**) was carried out at room temperature.

Synthesis of *N*-(aminoethyl) methacrylamide (Scheme 3) employed bis-protection of ethylenediamine and subsequent mono-deprotection to give the intermediate *N*-(2-aminoethyl) carbamic acid *tert*-butylester hydrochloride.[18] Coupling of this intermediate with methacryloyl chloride and deprotection give monomer **4**. Synthesis of 9-(*β*-methacryloyloxyethyl) adenine[16] was accomplished by alkylation with ethylene carbonate to give 9-(*β*-hydroxyethyl) adenine. This was followed by treatment with methacryloyl chloride to give monomer **5** (Scheme 4). *N*-(2-Aminopyridine) methacrylamide was synthesized by

(18) Futao Wakui, Ph.D. Thesis, University of California at Irvine, 1991.

Scheme 4[a]



[a] (a) DMF/NaOH/reflux/3 h. (b) NaH/DMF/rt/1 h. (c) 0 °C/methacryloyl chloride/14 h. (d) CH_2Cl_2/Et_3N/0 °C.

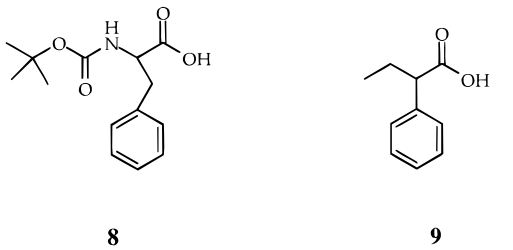


Figure 3. Carboxylic acid templates used for imprinting using new monomers: *t*-BOC-phenylalanine (**8**) and 2-phenylbutyric acid (**9**).

condensation of methacryloyl chloride with 2-aminopyridine (Scheme 4).

Polymers Imprinted with *t*-BOC-Phenylalanine. The monomers were first tested for their ability to bind a carboxylic acid in a molecularly imprinted polymer using the template *t*-BOC-L-phenylalanine (**8**, Figure 3). This template was chosen for the following reasons: (1) the chiral center allows for evaluation of stereochemical differentiation (one of the most difficult tasks in separation science); (2) there is a chromophore for UV detection; (3) both enantiomers are commercially available; and (4) *t*-BOC-L-phenylalanine is soluble in organic solvents and may assist in solubilizing the functional monomers. These results will also permit comparison with the performance of imprinted polymers using methacrylic acid and C-protected phenylalanine derivatives.[20] The polymerization mixtures were formulated using template (2.9 mol %), functional monomer (2.9 mol %), ethylene glycol dimethacrylate (92.7 mol %), and AIBN (1.5 mol %) in porogen (40/60% v/v monomers/porogen). The mixtures were polymerized photochemically to give a highly cross-linked network polymer.[2] The template, *t*-BOC-L-Phe, was removed by Soxhlet extraction and quantified by proton NMR using an internal standard (the fraction of template removed is called the split ratio).

The conditions for preparation of the imprinted polymers are shown in Table 1. Initially, DMF was used as porogen for all polymers. Additional porogens were used in subsequent studies based on binding studies described in the following sections. As a result of the insolubility of the functional monomer *N*-(3-guanidinopropyl) methacrylamide, a modified formulation utilizing a 10-fold

Table 1. Conditions for the Preparation of Each Imprinted Polymer

polymer	functional monomer	porogen	temp[a]	time[b]	split ratio
P(DOMPE)	**1** *N*-(diaminoethylene)-2-methylprop-2-enamide	$CHCl_3$	4.9 °C	10 h	>99%
P(GPM)	**2** *N*-(3-guanidinopropyl) methacrylamide	DMF	5.8 °C	8 h	85%
P(APM)	**3** *N*-(3-aminopropyl)methacrylamide	DMF	6.6 °C	6 h	53%
P(AEM)	**4** *N*-(2-aminoethyl)methacrylamide	DMF	5.8 °C	8 h	61%
P(MAOA)	**5** 9-(*β*-methacryloyloxyethyl) adenine	DMF	6.6 °C	6 h	57%
P(APYM)	**6** *N*-(2-aminopyridine)methacrylamide	$CHCl_3$	5.7 °C	8 h	>99%
P(MAA)	**7** methacrylic acid	MeCN	6.6 °C	6 h	83%

[a] Temperature of polymerization. [b] Time of polymerization.

Table 2. Chromatographic Conditions, Capacity Factors, and Separation Factors for L and D *t*-BOC-phenylalanine on Columns Packed with the Indicated Template Polymers[a]

polymer	functional monomer	mobile phase	k'_L	k'_D	*a*
P(DOMPE)	**1** *N*-(diaminoethylene)-2-methylprop-2-enamide	MeCN	0.0	0.0	1.0
P(GPM)	**2** *N*-(3-guanidinopropyl) methacrylamide	96/4 MeCN/AcOH	0.5	0.5	1.0
P(APM)	**3** *N*-(3-aminopropyl)methacrylamide	$CHCl_3$	0.0	0.0	1.0
P(AEM)	**4** *N*-(2-aminoethyl)methacrylamide	96/4 MeCN/AcOH	2.2	2.2	1.0
P(MAOA)	**5** 9-(*β*-methacryloyloxyethyl) adenine	MeCN	13.3	9.1	1.5
P(APYM)	**6** *N*-(2-aminopyridine)methacrylamide	$CHCl_3$	0.7	0.6	1.2
P(MAA)	**7** methacrylic acid	MeCN	0.4	0.4	1.0

[a] Flow rate = 1 mL/min, injections were 5.0 mL of a 1.0 mM solution of L or D *t*-BOC-phenylalanine, UV detection at $\lambda = 257$ nm. The one exception was P(MAOA), which required a 10.0 mL injection of 10.0 mM substrates.

excess of *t*-BOC-L-phenylalanine was used (10/1 *t*-BOC-L-phenylalanine/monomer) to dissolve the monomer in the polymerization mixture.

Following removal of the template, the polymers were ground to 25−38 μm particle size and slurry packed into strainless steel chromatographic columns. Binding data was recorded in the form of capacity factors (k')[20] for L and D *t*-BOC-Phe as substrates to determine binding affinities and enantiomeric discrimination. This measure of binding is superior to the use retention time because it is independent of column size or the amount of stationary phase in the column. Chiral selectivity was measured by the separation factor (α), taken as the ratio of capacity factors for each enantiomer of *t*-BOC-Phe (i.e., $\alpha = k'_L/k'_D$). Table 2 summarizes the mobile phase conditions and chromatographic results for each polymer.

The chromatographic data was used to evaluate both the carboxylate binding potential and the molecular specificity of the polymers. Two criteria were used to evaluate binding strength: first, the elutropic strength of the mobile phase and, second, the magnitude of the capacity factor found in each mobile phase. Three mobile phases with different degrees of elutropic strength were employed to probe binding affinities. If the retention volume of *t*-BOC-L-Phe using the mobile phase 96/4 MeCN/AcOH was not significantly above dead volume (i.e., $k' = 0$), a less elutropic mobile phase, acetonitrile, was evaluated next. Finally, chloroform was employed as the mobile phase if no significant binding was found in acetonitrile. The magnitude of the capacity factors within each mobile phase group was used to fine tune the order of binding affinities. Using these criteria, the order of binding strength by the polymers for *t*-BOC-L-phenylalanine was found to be P(AEM) > P(GPM) > P(MAOA) > P(MAA) > P(APYM), P(DOMPE), P(APM).

The highest binding affinity for *t*-BOC-phenylalanine was obtained by polymer P(AEM). This polymer incorporates the *N*-(2-aminoethyl) methacrylamide monomer (**4**). An interesting result is that polymers incorporating the *N*-(3-aminopropyl) methacrylamide monomer (**3**) showed no affinity for the template, despite differing from monomer **4** by only a single methylene group. This is unusual because monomers **3** and **4** should provide the same electrostatic interactions. It may be that the diminished affinity of monomer **3** is due to intramolecular chelation preventing interaction with the substrate. The second largest binding affinity was observed with monomer **2**, which contains the guanidium moiety. *N*-(Diaminoethylene)-2-methylprop-2-enamide (monomer **1**) also incorporates the guanidinium functionality; however, *t*-BOC-phe imprinted polymers made using monomer **1** show little affinity for **8**. The reason may be due to a decrease in the inherent binding strength of the guanidinium moiety of monomer **1** from direct acylation to the methacrylate moiety. P(MAOA) made with monomer **5** exhibited moderate binding affinity; P(MAA) and P(APYM) provided minimal binding affinities.

Evidence of chiral selectivity, indicated by separation factors (α) greater than 1.0, was found for P(MAOA) and, to a lesser extent, P(APYM). These two polymers have in common the 2-aminopyridine substructure. A possible reason for the selectivity observed may be the directional hydrogen bonding afforded by the 2-aminopyridine moiety demonstrated by Hamilton.[8,9] Directed hydrogen bonding groups in a cleft or cavity have been shown to lead to good orientation of a substrate with complementary functional groups.[8,21] The selectivity of the polymers may also benefit from this type of directional hydrogen bonding. Specific binding by P(MAA) is not seen even though directional bonds are anticipated to exist between carboxylate groups. It is possible that the functional groups of P(APYM) and P(MAOA) interact with the carbamate moiety, as well as the carboxylate group, to aid in discrimination of enantiomers, whereas P(MAA) may not. P(AEM) showed very good binding potential;

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(20) The capacity factor (*kl*) is defined as: $k' = [V(t) - V(0)]/V(0)]$ where V(t) is the retention volume and V(0) is the dead volume or the retention volume of a nonbinding substrate.

(21) (a) Zimmerman, S. C.; Wu, W.; Zeng, Z. *J. Am. Chem. Soc.* **1991**, *113*, 196. (b) Rebek, J.; Askew, B.; Ballester, P.; Buhr, C.; Costero, A.; Jones, S.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 6866.

Table 3. Conditions for Each (*R*)-(−)-2-Phenylbutyric Acid Imprinted Polymer

polymer	monomer	porogen	temp[a]	time[b]	split ratio
P2(GPM)	**2** *N*-(3-guanidinopropyl) methacrylamide	DMF	5.4 °C	8 h	91%
P2(AEM)	**4** *N*-(2-aminoethyl)methacrylamide	DMF	5.4 °C	8 h	65.%
P2(MAOA)	**5** 9-(β-methacryloyloxyethyl) adenine	DMF	5.4 °C	8 h	>99%

[a] Temperature of polymerization. [b] Time of polymerization.

Table 4. Chromatographic Conditions, Capacity Factors, and Separation Factors for (*R*)- or (*S*)-2-Phenylbutyric Acid on Columns Packed with the Indicated Polymers[a]

polymer	monomer	mobile phase	k'_R	k'_S	*a*
P2(GPM)	**2** *N*-(3-guanidinopropyl) methacrylamide	MeCN	0.1	0.1	1.0
P2(AEM)	**4** *N*-(2-aminoethyl)methacrylamide	MeCN	0.8	0.7	1.1
P2(MAOA)	**5** 9-(β-methacryloyloxyethyl) adenine	MeCN	0.1	0.1	1.0

[a] Flow rate = 1 mL/min, and injections were 5.0 mL of a 1.0 mM solution of *R* or *S* 2-phenylbutyric acid, UV detection at $\lambda = 208$ nm.

however, under the protic chromatographic conditions, the primary amine probably interacts via electrostatic interactions that may be considered point charges and lack directionality. P(GPM) can interact both by electrostatic interaction and by hydrogen bonding; however, the latter can have multiple points of interaction that may preclude any directional binding. Lastly, polymers P(DOMPE) and P(APM) simply do not bind substrates well enough to evaluate selectivity.

Polymers Imprinted with Phenylbutyric Acid. To investigate whether the binding and selectivity results for *t*-BOC-L-phenylalanine were general or template-specific, three of the functional monomers were used to imprint (*R*)-(−)-2-phenylbutyric acid (**9**, Figure 3). This template incorporates a single carboxylate functionality to test binding potential, one chiral center as a diagnostic for stereoselectivity, and a UV chromophore for detection in an HPLC assay. Polymers incorporating *N*-(aminoethyl) methacrylamide and *N*-(3-guanidinopropyl) methacrylamide were chosen to test the binding potential with the new template because of their high binding affinity in the case of *t*-BOC-L-Phe, and functional monomer 9-(β-ethacryloyloxyethyl) adenine was chosen to test selectivity. The three new polymers, P2(AEM), P2(GPM), and P2(MAOA), employing the functional monomers *N*-(2-aminoethyl) methacrylamide, *N*-(3-guanidinopropyl) methacrylamide, and 9-(β-methacryloyloxyethyl) adenine, respectively, were prepared in a fashion similar to that for the *t*-BOC-L-Phe imprinted polymers. The specific polymerization conditions and split ratios for each of the new imprinted polymers are shown in Table 3. It should be noted in the case of P2(MAOA) that 20% of unreacted functional monomer was recovered.

Columns were made with the polymers as in the previous experiments and evaluated by HPLC. The mobile phase for all columns was acetonitrile, which is less elutropic than the 96/4 acetonitrile/acetic acid employed for the chromatography of *t*-BOC-phenylalanine substrates on P(AEM) and P(GPM). No binding was observed for (*R*)-(−)-2-phenylbutyric acid employing mobile phases containing any portion of acetic acid for columns containing the polymers P2(AEM), P2(GPM), and P2(MAOA). Qualitatively, this indicates that binding in the case of phenylbutyric acid is not as good as for *t*-BOC-L-phenylalanine. The stronger binding affinities observed for *t*-BOC-L-Phe imprinted polymers must be due to binding contributions of the carbamate group in addition to the carboxylic acid. Table 4 summarizes the chromatographic data for the (*R*)-(−)-2-phenylbutyric acid imprinted polymers.

Chromatographic investigation of the polymers revealed the following important observations. First, the capacity factors in Table 4 show that the order of binding strength for these copolymers is P2(AEM) > P2(GPM), P2(MAOA). This is consistent with the order found for the *t*-BOC-L-phenylalanine imprinted polymers. Thus the binding potential of these polymers does appear to follow a general trend. Next, the chiral selectivity of the polymers was evaluated by comparing separation factor (α) values. Both P2(MAOA) and P2(GPM) gave α values of 1.0, indicating nonstereoselective binding. This result is the same as for P(GPM); however, the previous α value for P(MAOA) was 1.5. The chiral selectivity by P2(MAOA) may not be reflected in the α values as a result of its low binding affinity in this case. Without significant binding, the imprinted polymers cannot display their potential chiral selectivity. Thus, selectivity is to some extent a function of binding strength.

Effects of Changing the Polymerization Conditions on Binding and Selectivity. Although the *N*-(2-aminopyridine) methacrylamide monomer did not create the highest affinity polymer for *t*-BOC-L-phenylalanine, it did show positive results for chiral resolution. Further investigation was done to determine if selectivity could be enhanced and controlled by changing the polymerization parameters. Five imprinted polymers were prepared, using the *N*-(2-aminopyridine) methacrylamide monomer, with three objectives. First, the D enantiomer of *t*-BOC-phe was imprinted to verify that chiral selectivity is not specific to the L enantiomer. Second, the binding behavior of a thermally initiated polymer was compared to a photochemically initiated polymer. Third, the ratio of functionalized monomer to template was increased to probe the effect on binding and selectivity. Nonspecific binding was elucidated by control polymers made using benzoic acid as a "generic" template to randomly distribute the 2-aminopyridine functionality in the polymer.

The preparation of the polymers with a 1:1 ratio of monomer to template followed the general formulation and procedures outlined previously (and in the Experimental Section). The preparation of polymers with a 2:1 ratio of monomer to template followed the general formulation and procedures except that 5.8 mol % template, 86.9 mol % EGDMA, and 5.8 mol % *N*-(2-aminopyridine) methacrylamide were used. The porogen was $CHCl_3$, and the polymerization was carried out at 5.7 °C for 8 h for photopolymerized polymers and at 80 °C for 8 h for thermally initiated polymers. The split ratio

Table 5. Chromatographic Conditions, Capacity Factors, and Separation Factors for L and D *t*-BOC-Phe Substrates on Columns Packed with the Indicated P(APYM) Polymers[a]

entry	template	initiation method	ratio of 2apym/template	k'_L	k'_D	a (k'_L/k'_D)	a (k'_D/k'_L)
1	*t*-BOC-L-Phe	photo	1:1	0.7	0.6	1.2	
2	*t*-BOC-L-Phe	thermal	1:1	0.7	0.7	1.0	
3	benzoic acid	photo	1:1	0.5	0.5	1.0	1.0
4	benzoic acid	photo	2:1	0.7	0.7	1.0	1.0
5	*t*-BOC-D-Phe	photo	1:1	0.7	0.8		1.2
6	*t*-BOC-D-Phe	photo	2:1	1.0	1.3		1.4

[a] Flow rate = 1 mL/min; injections were 5.0 mL of a 1.0 mM solution of L or D *t*-BOC-phe, UV detection at $\lambda = 257$ nm.

of all polymers was >99%. Chromatographic data was obtained as before, and the results are shown in Table 5.

In Table 5, entries 1 and 5 show that both the L and D *t*-BOC-phe imprinted polymers are selective for their own enantiomer, and the magnitude of the α value for both is the same (1.2). Thus, chiral selectivity is equally obtained by either enantiomer. Comparison of entries 1 and 2 shows that photoinitiation results in polymers with higher α values than thermal initiation (1.2 vs 1.0). This is expected because there should be a stronger prepolymer complex at 5 °C compared to that found at 80 °C,[22] which is ultimately responsible for formation of the specific binding sites within the polymer. Increasing the ratio of functionalized monomer to template (*t*-BOC-D-phe) from 1:1 to 2:1 for entries 5 and 6 reveals an increase in both binding affinity (k') and selectivity (α). This may be an indication that the increase in k' may be responsible, at least in part, for the increase in α. For the control polymers (entries 3 and 4), doubling the ratio of functional monomer to template (benzoic acid) showed only an increase in binding affinity (k'); however, there was no increase in stereochemical bias imparted to these polymers ($\alpha = 1.0$), as expected. For previous work with methacrylic acid functionalized imprinted polymers, a ratio of 12/1 functional monomer/template is generally used with good results.[4] Thus there remains the potential to achieve even higher selectivity by using larger ratios of functional monomer to template.

Summary and Conclusion

Functionalized methacrylamide monomers for imprinting carboxylic acids were designed, synthesized, and copolymerized with a large excess of ethylene glycol dimethacrylate in the presence of carboxylic acid templates. The binding and selectivity of the resulting imprinted polymers were evaluated chromatographically. The highest binding affinities were found for polymers incorporating primary amine monomer **4**, followed by polymers incorporating guanidine-functionalized monomer **2**. The proximity of the functional group to the polymerizable group of monomers **2** and **4** proved to be important for monomer design. For example, monomer **4** exhibited the best binding affinity of all the monomers, whereas monomer **3** showed worst binding affinity, even though these two differ by only one methylene unit. Similarly, of the two guanidinium functionalized polymers investigated, monomer **2** showed the second highest binding affinity, whereas monomer **1** did not exhibit any binding potential at all. Monomer **1** has the guanidinium group directly acylated to the methacrylate group, a significant difference from monomer **2**, which has a three-carbon linker in between. The adenine-functionalized polymer P(MAOA) exhibited good binding affinity, and significant but reduced binding was observed for polymers incorporating *N*-(2-aminopyridine) methacrylamide (e.g., P(APYM)), a monomer containing a carboxylate-binding substructure of adenine.

Possibly more important than the binding potential of these polymers is their ability to stereodifferentiate enantiomers of *t*-BOC-phenylalanine. Only two polymers exhibited enantioselective binding, P(MAOA) and P(APYM). The order of enantioselectivity was P(MAOA) > P(APYM), which might be attributed to a correlation with binding affinity. Furthermore, these two polymers have in common the 2-aminopyridine moiety, for which directional hydrogen-bond interactions have been demonstrated for synthetic carboxylate receptors.[8,21] This may serve to fine tune selectivity in the polymer binding sites; however, the origins of chiral selectivity are postulated to arise from the specific three-dimensional positioning of complementary functional groups within the binding site. Such is the case for chiral selectivity for (*R*)-(−)-2-phenylbutyric acid by P2(AEM), which contains a primary amine functional group that can be considered a nondirectional point charge.

The effects of changing the polymerization conditions on binding selectivity were explored for polymers made using the *N*-(2-aminopyridine) methacrylamide monomer. Polymers imprinted with the template *t*-BOC-L-phenylalanine were both photochemically and thermally initiated. Binding affinity was found to be the same for both photochemically and thermally initiated polymers. Stereoselectivity, however, was only observed for photochemically initiated polymers and not for thermally initiated polymers. This is in agreement with previous studies that suggest molecular recognition in imprinted polymers is enhanced at lower temperatures. It was also found that an increase in selectivity was found for polymer formulated with an increased monomer-to-template ratio. Doubling the ratio of monomer to template increased the nonspecific background binding of both imprinted and control polymers; however, there was a 14% net increase in selectivity by *t*-BOC-D-phenylalanine imprinted polymer for its own template. Future improvements for separation using these and other imprinted polymers should consider optimization of the monomer to template ratio.

Experimental Section

General. UV irradiation experiments utilized a Hanovia medium-pressure mercury arc lamp. Thin-layer chromatography separations were conducted on precoated plates of silica with a 0.025 mm thickness containing PF 254 indicator. Flash columns were packed with 230−400 mesh silica gel. Ethylene

(22) (a) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31. (b) Sellergren, B.; Lepisto, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853. (c) O'Shannessy, D. J.; Ekberg, B.; Andersson, L. I.; Mosbach, K. *J. Chromatogr.* **1989**, *470*, 391.

glycol dimethacrylate (EGDMA, Polysciences) was first washed twice with aqueous 1 M NaOH and once with aqueous saturated NaCl solution to remove inhibitor and further dried with anhydrous $MgSO_4$. The monomer was distilled under reduced pressure (10 mmHg, 60 °C). Methacrylic acid (MAA, Aldrich) was distilled over $CaCl_2$ (10 mmHg, 80 °C). AIBN (Fluka) was recrystallized from methanol.

All solvents were obtained from commercial suppliers and purified prior to use. THF and diethyl ether were dried over Na−benzophenone ketyl and distilled. Benzene and acetonitrile were dried by refluxing over CaH_2 and then distilled. DMF was dried over 4 Å molecular sieves and distilled under reduced pressure. Unless otherwise stated, all reactions were conducted in oven-dried (160 °C) glassware under a positive nitrogen atmosphere.

Chromatographic Experiments. The polymers were ground by mortar and pestle or by mechanical mill (Janke & Kunkel IKA WERK grinding mill type A 10 S, 20 000 rpm with water circulating temperature control) and sized using U.S.A. Standard Testing Sieves (ASTME.-11 specification). The particles (25−38 μm size range, unless otherwise indicated) were slurry packed in stainless steel chromatographic columns (length 100 mm, i.d. 4.6 mm). Approximately 0.5−0.8 g of material is necessary to pack a column of this size. Once slurry packed, the columns were then washed on line (in addition to previous Soxhlet extraction) with acetonitrile or 7/3 acetonitrile/water until a stable baseline was obtained. HPLC analyses were performed isocratically at room temperature; flow rate, mobile phase, and substrate conditions are indicated in the text for each experiment. The void volume was determined by injecting a small amount of an inert substance, namely acetone, acetonitrile or sodium nitrate; of the three, the void volume marker utilized was that with the smallest retention volume.

General Polymer Formulation and Procedures. The following procedure is general for all carboxylate-binding polymers unless otherwise indicated. The specific conditions (time of polymerization, temperature, etc.) for each polymer are indicated in the text. In a 20 mL scintillation vial is added a solution of template (0.6486 mmol, 2.9 mol %) dissolved in porogen (6.0 mL), functionalized monomer (0.6486 mmol, 2.9 mol %), ethylene glycol dimethacrylate (20.0 mmol, 92.7 mol %), and AIBN (0.3 mmol, 1.5 mol %). The solution was purged with nitrogen for 5 min at room temperature to remove oxygen, and the vial was sealed with a screw-on cap. Polymerization was initiated photochemically by a standard laboratory UV light source (Hanovia medium-pressure mercury arc lamp) at 5 °C and allowed to proceed for 6−10 h. The polymerization tubes were turned one-quarter turn every 15 min for the first hour of polymerization, then every hour for the duration. Afterward, the polymers were removed, crushed, and Soxhlet extracted in 80/20 methanol/triethylamine overnight. The splitting yield was determined by proton NMR of the extract using an exact amount of methylene chloride or benzene as an internal standard. Following preparation, the polymers were sized, packed into stainless steel chromatographic columns, and used for HPLC experiments as described in the previous section.

Synthesis of Functional Monomers. 1,2-Ethanebiscarbamic Acid Bis-*tert*-butylester. To a solution of 1,2-diaminoethane (6.11 g, 102 mmol) in THF (200 mL) at 0 °C was added dropwise a mixture of di-*tert*-butyl dicarbonate (44.6 g, 204 mmol) and THF (40 mL). The heterogeneous mixture was then heated at reflux for 12 h, after which time a clear solution was obtained. Removal of the solvent in vacuo provided a white solid (26.1 g, 99%) that could be recrystallized from benzene to give long needles (mp 139−140 °C). ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.11 (br s, 2H, CONH), 3.23 (M, 4H, CONH−CH_2), 1.44 (s, 18H, C(CH_3)$_3$). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 157.0, 79.9, 41.4, 29.0. IR (KBr pellet) 3373, 2983, 2937, 1685, 985, 871, 642 cm^{-1}. HRMS (CI) calcd for $C_{12}H_{24}N_2O_4$ 260.1736, found 260.1733.

(2-Aminoethyl)-carbamic Acid *tert*-Butylester Hydrochloride. Dry diethyl ether (500 mL) was saturated with HCl gas at 0 °C for 30 min. An aliquot of the solution (10 mL) was added to H_2O (50 mL) containing phenolphthalein (1.0 mg). The mixture was titrated with 1.0 M NaOH to establish HCl concentration. The HCl/Et_2O solution was diluted with enough dry diethyl ether to make a 2.0 M solution. To a portion of this solution (600 mL, 1.20 mole) was added 1,2-ethanebiscarbamic acid bis-*tert*-butylester (31.3 g, 0.12 mole). The heterogeneous mixture was stirred for 3 h. The precipitate was filtered and washed thoroughly with dry ether to provide a white solid. This solid was recrystallized from acetonitrile to give white, platelike crystals (8.28 g, 35% yield, mp 149−150 °C). ^{1}H NMR (250 MHz, CD_3OD) δ 3.24 (t, 2H, J = 6.1 Hz, CH_2 − NH_3^+), 2.94 (t, 2H, J = 6.0 Hz, CONH − CH_2), 1.36 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (125.8 MHz, CD_3OD) δ 159.0, 81.0, 41.3, 39.4, 29.0. IR (KBr pellet) 3375, 2978, 2902, 1697, 636 cm^{-1}.

{**[(2-Methyl-1-oxo-2-propenyl)amino]ethyl**}**-carbamic Acid *tert*-Butylester.** A solution containing (2-aminoethyl)-carbamic acid *tert*-butylester hydrochloride (5.96 g, 30.0 mmol), THF (200 mL), and triethylamine (12.7 mL, 90.0 mmol) was stirred at 0 °C for 1 h. To this solution (at 0 °C) was added dropwise a solution of methacryloyl chloride (2.96 mL, 30.3 mmol) in THF (50 mL). The mixture was stirred at room temperature for 24 h. The salts were filtered and washed thoroughly with THF, and the combined extracts were concentrated. The residue was triturated with hexane to provide a white powder (6.61 g, 96% yield). This product could be further purified by flash chromatography using hexane/diethyl ether as eluant (mp 82.5−83.5 °C). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.08 (br s, 1H, RCONH), 5.76 (s, 1H, vinyl), 5.52 (br s, 1H, OCONH), 5.32 (s, 1H, vinyl), 3.40 (m, 2H, RCONH−CH_2), 3.33 (m, 2H, OCONH−CH_2), 1.96 (s, 3H, C=C−CH_3), 1.43 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 169.5, 157.9, 140.1, 120.5, 80.2, 42.1, 40.5, 28.9, 19.1. FTIR (KBr pellet) 3361, 3327, 1685, 1651, 1618, 980, 667 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{20}N_2O_3$ 229.1551, found 229.1549.

***N*-(2-Aminoethyl) Methacrylamide.** A solution containing {[(2-methyl-1-oxo-2-propenyl)amino]ethyl}-carbamic acid *tert*-butylester (5.17 g, 22.6 mmol), CH_2Cl_2 (57 mL), and 4 M HCl/Et_2O (57 mL, 0.23 mole) was stirred at room temperature for 24 h. The hygroscopic salt was filtered and washed with diethyl ether to afford a white solid. This solid was added to 0.39 M ethanolic sodium ethoxide (59 mL, 23.0 mmol), and the mixture was stirred for 1 h. The salt was filtered and washed with ethanol. The combined filtrates were evaporated (keeping water bath below 25 °C), and the resulting oily residue was extracted with benzene or acetonitrile, dried over magnesium sulfate, and then concentrated to yield a clear, light yellow oil (2.58 g, 89% yield). Note, this compound is quite unstable with regard to polymerization. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.88 (br s, 1H, CONH), 5.72 (t, 1H, J = 0.9 Hz, vinyl), 5.32 (quin, 1H, J = 1.4 Hz, vinyl), 3.34 (q, 2H, J = 5.9 Hz, CONH−CH_2), 2.85 (t, 2H, J = 6.0 Hz, CH_2−NH_2), 1.97 (t, 3H, J = 1.2 Hz, NH_2). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 169.3, 140.4, 119.8, 42.7, 19.1. FTIR (neat) 2927, 2256 (br), 1655, 1612, 933 cm^{-1}.

***N*-(3-Aminopropyl) Methacrylamide**. *N*-(3-Aminopropyl) methacrylamide hydrochloride (Kodak, 2.91 g, 0.0163 mmol) was added to 0.324 M ethanolic sodium ethoxide (55 mL, 0.0178 mole). The mixture was allowed to stir for 30 min and then filtered. The filtrate was concentrated at room temperature, and the residue was triturated with 40 mL of benzene using a sonicator (Branson 2200) at room temperature. This was filtered through Whatman filter paper, and the supernatant was concentrated at room temperature, leaving a light brown oil (1.80 g, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (br s, 1H, CONH), 5.61 (s, 1 H, C=CH), 5.20 (s, 1H, C=CH), 3.31 (q, 2H, J = 6.2 Hz, CONH−CH_2), 2.72 (t, 2H, J = 6.2 Hz, NH_2−CH_2), 1.85 (s, 3H, CH_3), 1.56 (quin, 2H, J = 6.2, CH_2−CH_2−CH_2), 1.41 (s, 2H, NH_2). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 168.2, 139.8, 119.0, 40.1, 38.2, 31.6, 18.5. IR (neat) 3295 (br), 2931, 2867, 1658, 1612, 925 cm^{-1}.

***N*-(Diaminoethylene)-2-methylprop-2-enamide.** Guanidine hydrochloride (1.51 g, 15.8 mmol) was dissolved in methanol and converted into its free base via anion-exchange resin (Amberlite IRA-900, chloride form). The free base was

dried by rotary evaporation and redissolved in 10 mL of ethanol. Methyl methacrylate (1.60 g, 16.0 mmol) was added, and the mixture was refluxed at 100 °C for 4 h. The solvent was evaporated, and the residue was recrystallized from ethanol/diethyl ether to give a pale yellow solid (0.72 g, 5.7 mmol, 36% yield, mp 171−176 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.56 (d, 1H, C═CH), 5.01 (br s, 1H, C═CH), 3.38 (br s, 5H, −NH−), 1.71 (s, 3H, $-CH_3$). ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 173.03, 158.85, 144.51, 118.32, 20.33. FTIR (KBr pellet) 3401−3154 (br), 1654, 667, 597 cm^{-1}. HRMS (CI) calcd for $C_5H_{10}N_3O$ 128.0824, found 128.0818.

***N*-(2-Aminopyridine) Methacrylamide.** A solution of 2-aminopyridine (7.2 g, 81.9 mmol) and triethylamine (5.7 mL, 81.9 mmol) in methylene chloride (25 mL) in a 100 mL round-bottom flask was brought to 0 °C. Methacryloyl chloride (4.0 mL, 41.0 mmol) was added dropwise over 10 min, and the solution was allowed to come to room temperature and stirred for 40 min. Water (25 mL) was added, and the mixture was stirred for an additional 30 min. The organic layer was removed, and the aqueous layer was extracted twice with chloroform. The organics were combined, dried over sodium sulfate, and evaporated in vacuo. Column purification (eluant: methylene chloride/methanol 92.5/2.5), and recrystallization in hexanes gave 1.58 g (23.7%) of a white crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (br s, 1H, ═NH−), 8.29 (m, 1H, ═CH−N═), 8.26 (m, 1H, ═N−CN═CH−), 7.72 (t, 1H, N_2C═CH−C*H*═), 7.05 (t, 1H, ═N−CH−C*H*═), 5.88 (s, 1H, H_3C−CR═C*H*), 5.52 (s, 1H, H_3C−CR═C*H*), 2.07 (s, 3H, $-CH_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 166.92, 151.57, 147.68, 140.26, 138.35, 120.85, 119.69, 114.28, 18.60. FTIR (KBr pellet) 3235−2923 (br), 1674, 1627, 1600−1430 (br), 779, 736 cm^{-1}. HRMS (EI) calcd for $C_9H_{10}N_2O$ 162.0793, found 162.0790.

***S*-Ethylthiourea Hydrobromide.** A mixture of powdered thiourea (38.6 g, 0.5 mole), ethyl bromide (63.3 g, 0.58 mole), and 50 mL of absolute ethanol was placed in a 500 mL round-bottomed flask equipped with a condenser. The mixture was warmed on a water bath (55−65 °C) for 3 h, with occasional shaking. During this time, all of the thiourea dissolved. The reflux condenser was replaced by one set for downward distillation, and the ethanol and excess ethyl bromide were removed by vacuum distillation. The residual oil was placed on a high-vacuum line and allowed to crystallize. The solid was pulverized and dried in a desiccator to give 76.0 g (0.41 mol, 82% yield) of a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.06 (s, br, 2H, $-NH_2$), 8.94 (s, br, 2H, $-NH_2$), 3.13 (q, 2H, $-CH_2-$), 1.17 (t, 3H, $-CH_3$). ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 170.32, 25.26, 14.54.

***N*-(3-Guanidinopropyl) Methacrylamide.** In a 2 mL vial with lid were placed *N*-(3-aminopropyl) methacrylamide (0.89 g, 5.0 mmol), *S*-ethylthiourea hydrobromide (1.11 g, 6.0 mmol), and 1.0 mL of concentrated ammonium hydroxide solution. A stirbar was added, the vial was sealed tightly (stench!), and the mixture was shaken until all solids were dissolved. The mixture was then stirred for 16 h at room temperature. The vial was then opened, and the ethylmercaptan was removed by bubbling in nitrogen. The remaining solution was evaporated to dryness under high vacuum. Purification was accomplished in two parts. First, the mixed salts to be separated were converted to the free amines by passing down a column of Dowex 1 × 8 hydroxide form strongly basic anion-exchange resin. The base solution was evaporated to dryness under high vacuum, and purification was obtained by iterative flash chromatography using methanol/acetic acid as eluant. A final passing through the anion-exchange resin gave the hydroxide as counterion to form a white oily solid (0.201 g, 20% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.61 (s, 1H, −CR═CH), 5.37 (s, 1H, −CR═CH), 4.76 (s, 1H, −NH), 3.25 (t, 2H, $(H_2N)_2C$−NH−CH_2−), 3.14 (t, 2H, −CONH−CH_2−), 1.84 (s, 3H, $-CH_3$), 1.75 (t, 2H, $-CH_2-CH_2-CH_2-$). ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 171.97, 156.75, 139.04, 121.00, 38.62, 36.62, 27.52, 17.67. FTIR (KBr pellet) 3644−3178 (br), 1658, 863, 667 cm^{-1}. HRMS (FAB+) calcd for $C_8H_{17}N_4O$ 185.1402, found 185.1402.

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

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