Metal-Coordination Interactions in the Template-Mediated Synthesis of Substrate-Selective Polymers: Recognition of Bis(imidazole) Substrates by Copper(II) Iminodiacetate Containing Polymers

Pradeep K. Dhal and Frances H. Arnold*

Division of Chemistry and Chemical Engineering, 210-41, California Institute of Technology, Pasadena, California 91125

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ABSTRACT: Metal-complexing polymer matrices capable of specific recognition and binding to metal-coordinating substrates have been prepared by template-directed polymerization. The synthesis of these materials involves preorganization of a copper-containing vinyl monomer, copper(II) [*N*-(4-vinylbenzyl)-imino]diacetic acid (1), with bifunctional bis(imidazole) templates (2–5) of varying geometry and subsequent polymerization with a large excess of ethylene glycol dimethacrylate as the cross-linking agent. Complexation of metal-chelating monomers with the template during polymerization directs the positioning of metal ions in the polymer matrices, while a high degree of cross-linking stabilizes the functional group arrangement. In equilibrium binding experiments with single substrates and selected substrate pairs, the polymers preferentially bind their own templates, with separation factors (α) of 1.17–1.35 and binding constants that range from 1800 to 3800 M^{-1}. The capacities and affinities of the polymers for different substrates and ESR spectral analyses of the polymers loaded with substrate suggest a defined arrangement of metal ion sites in the templated materials that is absent in nontemplated polymers. The substrate selectivity likely involves some cooperative two-site coordination of the bis(imidazoles) as well as steric interactions with the binding cavities ("cavity fitting"). This template polymerization strategy is discussed from the viewpoint of designing highly specific abiotic receptors for recognition of delicate and complex biomolecules.

Introduction

The rational design of materials possessing ordered and predictable molecular architecture is a central focus of current research in molecular recognition.[1] In addition to myriad potential technological applications, such designed materials enable studies of the mechanisms by which one molecule can selectively bind another, a critical component of important molecular events such as selective catalysis. Current strategies for the preparation of these materials involve directed preorganization of functional groups in the host system (receptor) corresponding to complementary functional groups of the guest molecule (substrate). Complementarity between the binding sites dictates the placement of functional groups in the synthetic receptor, and the multiple interactions and molecular rigidity contribute to the stability and specificity obtained in the complexes. Various strategies involving chemical synthesis[2] and molecular self-assembly processes[3] have been developed for preparing molecules or assemblies of molecules with functional groups carefully positioned on the scale of nanometers or less.

The technique of template polymerization, also known as molecular imprinting, has been used to prepare polymeric materials for applications in molecular recognition. Template polymerization produces cross-linked polymers containing functional groups strategically arranged in the polymer matrix.[4] The general approach to preparing such polymers involves interactive preorganization of a functional monomer around a substrate bearing complementary binding sites (the template), followed by polymerization in the presence of a large excess of cross-linking agent. Removal of the template yields a functional polymer matrix whose cavities and/or spatial arrangements of functional groups correspond to the template molecule, such that the polymer exhibits selectivity for rebinding the template with which it was prepared. Wulff and coworkers first demonstrated this approach to designing polymeric receptor molecules using reversible covalent bonds for monomer–template interactions.[5] The laboratories of Shea and of Mosbach have reported the preparation of a variety of templated polymers utilizing reversible covalent[6] and noncovalent (electrostatic and hydrogen bonding) interactions.[7] This polymeric approach to the synthesis of abiotic receptor molecules offers certain distinct advantages over low-molecular-weight analogs, the most significant of which is generality. Thus, for a given pair of interacting functional groups, similar synthetic strategies (using the same functional monomer) can be employed for preparing receptor polymers for a large variety of template molecules. In addition, since the template molecule itself directs the organization of the functional groups, specific knowledge of the template structure is not necessary. Further advantages of these polymeric systems are found in the vast potentials for materials engineering for specific applications such as stationary phases for chromatographic separations.[8]

Although this template polymerization approach is conceptually attractive, few practically useful materials have been reported. Problems encountered include low binding selectivities, loss of selectivity with time, and slow rebinding kinetics.[9] Ideally, templated polymers would exhibit fast rebinding kinetics and require mild conditions for desorption, considerations which are particularly critical in the design of new receptor polymers for recognition of complex and delicate substrates such as proteins. Preorganization of the template with the functional monomers during polymerization is a critical step in the preparation of these materials. Since random incorporation of functional monomers in the polymeric matrix reduces its specificity, interactions between monomer and template should be as strong as possible during polymerization—ideally they would be covalent. However, systems utilizing reversible covalent bonds exhibit slow

* To whom correspondence should be addressed.

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kinetics during rebinding and often necessitate severe conditions for desorption,[5,10] properties which render them unsuitable for many applications.

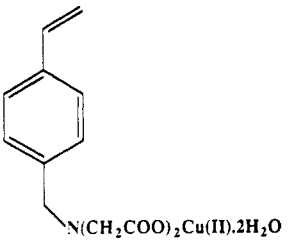
Analysis of the variety of interactions involved in biological recognition processes reveals that interactions between biological molecules and metal ions can be highly specific and are formed and broken under mild conditions.[11] Of particular interest to us is the interaction between the imidazole groups of surface-exposed histidine residues in proteins and the transition metal complexes used in the purification of proteins by metal-affinity chromatography. Polymeric supports containing, for example, Cu^{II} iminodiacetate (IDA) or Zn^{II} iminodiacetate are effective for fractionating proteins based on surface histidine content, and the kinetics for formation of the ternary histidine–Cu^{II}–IDA complex are sufficiently rapid for high-resolution chromatography.[12] The analogous technique for separating small metal-coordinating compounds is ligand-exchange chromatography.[13]

We are preparing metal-complexing polymers by template polymerization, with the goal of improving the selectivity of metal-affinity and ligand-exchange separations. The positioning of metal ions to match the arrangement of ligands on a target molecule (e.g., histidines on a protein surface) through preorganization with a metal-complexing monomer and subsequent cross-linking polymerization might offer a route to preparing receptor polymers for highly specific recognition and purification of proteins. Since the strengths and specificities of the metal–ligand coordination are amenable to regulation through the choice of the appropriate metal ion(s) and the corresponding ligand(s),[14] this strategy may be used to tailor-make polymeric receptors for substrates containing a variety of functional groups. Because this route does not require detailed knowledge of the template structure, it is particularly well-suited for recognition of complex biological molecules, the majority of whose structures are not known.

The mechanisms by which selectivity is achieved in molecularly imprinted polymers are poorly understood, and this lack of understanding impedes the further development of these materials. For our initial studies of template polymerization using metal ion complexes, we have developed a set of model templates bearing imidazole functionalities with slightly different spatial arrangements. With their well-defined structural and chemical features, these small "protein analogs" are well-suited to evaluating the ability of the metal-coordination interaction to direct the positioning of metal ions in a polymer matrix. To elucidate some of the factors that influence the ability of the templated polymers to discriminate template from nontemplate molecules, we have investigated individual and competitive binding of these substrates that differ in size and placement of the metal-coordinating ligands. This indirect probe of the coordination complexes in the polymer is complemented by direct spectroscopic studies (electronic absorption and electron spin resonance (ESR)) of the complexes in solution and in the solid polymers. Early results of our template polymerization experiments have been reported in a brief communication.[15]

Results and Discussion

Synthesis and Preorganization of Cu^{II}-monomer and Bis(imidazole) Templates. The transition metal ions Cu^{II}, Zn^{II}, and Ni^{II} typically used in metal-affinity protein separations are kinetically labile and exchange their ligands rapidly. Of these, Cu^{II} has the highest affinity for imidazole and was therefore chosen for these studies. Iminodiacetate is an excellent choice to retain the metal ion because, as a tridentate chelator, it leaves coordination sites available for weaker interactions with target ligands and its complex with Cu^{II} is uncharged. We synthesized [*N*-(4-vinylbenzyl)imino]diacetic acid as the polymerizable metal chelator, which upon treatment with Cu^{II} salt gave the copper-containing monomer **1**. Four different bis-



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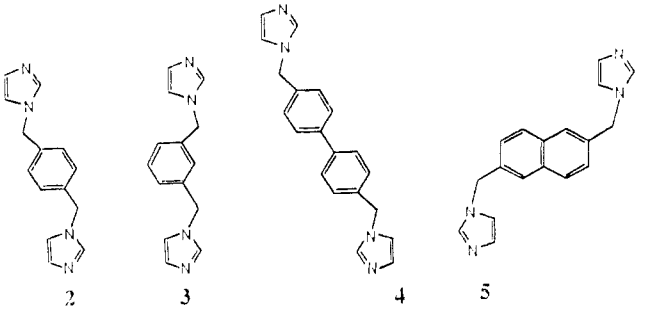
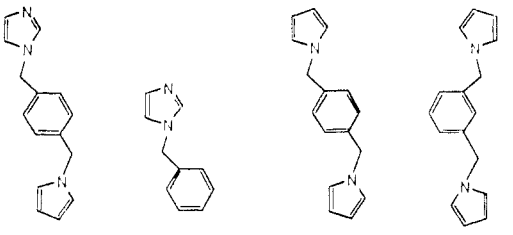
(imidazole) molecules (**2**–**5**) were synthesized for use as templates during polymerization and substrates in rebinding studies to assess the extent to which the templated polymers can discriminate very small differences in the arrangements of the ligands. Various mono(imidazole) compounds and imidazole analogs were also prepared for rebinding studies: **6** is a structural analog of bis(imidazole) **2** with only one imidazole metal-binding site. **8** and **9** are structural analogs of **2** and **3** that contain no imidazole ligands for coordination to the metal ions.



2 **3** **4** **5**



6 **7** **8** **9**

A polymerizable metal-coordinated template assembly is formed by combining monomer **1** with bis(imidazole) template in methanol. The electronic absorption spectrum of monomer **1** contains a broad band in the visible region with a maximum at 728 nm. Upon addition of an imidazole substrate this maximum is blue-shifted, a consequence of the ligand field transition of the Cu^{II} center upon coordination with the nitrogen atom of the imidazole.[16] Gradual addition of template increased the absorbance and shifted the Cu^{II}-monomer absorption maximum, as illustrated for template **2** in Table I (entries 2–5). Precipitation occurs at template concentrations above the stoichiometric composition (2:1 ~ 0.5:1). With further addition of template above a 2:1 ratio of ~0.6:1, the mixture again becomes soluble, and the absorption max-

Table I
Electronic and ESR Spectroscopic Characterization of the Polymerizable Monomer–Template Assemblies

entry	template (template:monomer)	λ_{max} (nm)	$g_\parallel$	$A_\parallel$ (G)
1	none	728	2.32	166
2	2 (0.1:1)	715	2.28	172
3	2 (0.2:1)	705	2.28	176
4	2 (0.3:1)	693	2.25	185
5	2 (0.5:1)	685	2.22	198
6	3 (0.5:1)	688	2.21	197
7	4 (0.5:1)	686	2.23	197
8	7 (1:1)	681	2.21	199

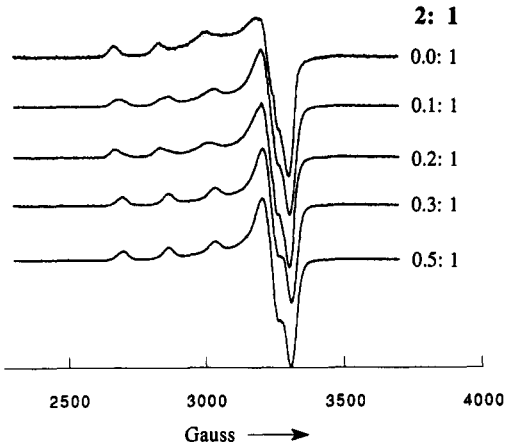


Figure 1. ESR spectra of monomer 1 (0.1 mM) with varying mole fractions of bis(imidazole) template 2.

imum undergoes a further large blue shift (λ_{max} = 628 nm), reflecting coordination of a second imidazole to the metal centers.

The polymerizable monomer–template assemblies were also characterized by ESR spectroscopy (Figure 1). The ESR spectral pattern of paramagnetic Cu^{II} is influenced by the nature and number of coordinating ligands as well as by the geometry of the complexes.[17,18] $A_\parallel$ and $g_\parallel$ values for the Cu^{II}-containing monomer in the presence and absence of different templates are given in Table I. The decrease in $g_\parallel$ and increase in $A_\parallel$ observed upon addition of template are characteristic of coordination of nitrogen-donating ligands.[18] As can be seen in Figure 1, peaks in the $g_\parallel$ region become broader as template is added to the pure monomer, indicating the presence of multiple Cu^{II} species (imidazole-coordinated and free Cu^{II}-IDA). At the stoichiometric monomer-to-imidazole concentration (0.5:1 template: monomer) the lines again become narrow, indicating that the majority of the Cu^{II} is coordinated by template imidazole groups and that formation of the template–monomer assemblies is essentially complete.

Polymerization and Workup. In order to incorporate Cu^{II} into the polymer at positions corresponding to the imidazole ligands of the templates, individual template–monomer assemblies were polymerized in the presence of a large excess of ethylene glycol dimethacrylate acting as the cross-linker. A typical polymerization recipe is 5:95 molar ratio of functional monomer to cross-linker. A control polymer with a random distribution of copper ions (P-1) was also prepared using the monofunctional ligand 1-benzylimidazole (7) instead of a bifunctional template during polymerization. The blue, solid polymers were crushed and washed with methanol to remove soluble, unpolymerized residues and dried before further characterization.

Dimensional stability and matrix rigidity leading to preservation of the spatial arrangement of the metal ions demand the use of an excess of cross-linking agent. This high degree of cross-linking, however, might restrict access to the binding cavities in the polymer. The key to success in achieving accessible binding sites in highly cross-linked, rigid polymers is the formation of macroporous domains, which is accomplished by using an appropriate porogenic solvent during polymerization.[19] Methanol was found to be an effective porogen for these polymerizations. Specific surface areas and pore volumes were determined from adsorption isotherms for nitrogen (BET analysis) and are reported in Table II. The polymers have very high surface areas (200–300 m^2/g), and a typical electron micrograph of polymer P-2, shown in Figure 2a, clearly reveals macroporous domains.

The accessibility of the binding sites was evaluated by removal and reloading of the imidazole templates and metal ions. Results of these experiments are summarized in Table II. Washing with pure methanol or water or with mildly acidified aqueous methanol (pH ~ 4.0) did not remove measurable amounts of template. Treatment with acidified aqueous methanol (pH ~ 2.0) at 37 °C for 36 h, however, resulted in nearly quantitative removal of the imidazole templates without any bleeding of Cu^{II} ions. The templates, although relatively tightly bound, remain accessible and can be removed under appropriate conditions. Treating the template-free polymers with excess aqueous ethylenediaminetetraacetic acid (EDTA) for 36 h removed only 60% of the Cu^{II} ions. A two-step procedure involving treatment with 1,4,7-triazacyclononane (a chelating agent soluble in organic media) followed by aqueous EDTA significantly improved copper removal (>95%). It is possible that the less polar microenvironments of the polymer cavities are more accessible to triazacyclononane than to EDTA. The polymers can be reloaded with metal ion by treating the copper-free polymers with a metal salt such as $Cu^{II}Cl_2$. Electron micrographs of the polymer after metal ion removal and reloading show that the macroporous morphology is retained (Figure 2b).

Substrate Binding and Selectivity. The primary objective of the template polymerization methodology is to synthesize polymers which can selectively bind a particular substrate. Experiments with the nontemplated polymer (P-1) were used to separate the effects of nonspecific interactions and random metal ion coordination from the specific effects of templating with a multifunctional substrate. As shown in Table III, this random polymer binds nearly equal amounts of the four different bis(imidazole) substrates 2–5. P-1 also exhibits no selectivity in a competitive binding experiment with bis(imidazole) substrates 2 and 3 (Table IV, entry 1). In contrast, polymers prepared in the presence of various bis(imidazole) templates exhibit measurable preferences for their own templates in both saturation and competitive rebinding experiments. The larger the structural differences between the substrates, the more pronounced are the selectivities. Thus, polymer P-2 prepared using 2 as the template binds considerably more 2 than substrates 3, 4 or 5 (Table III, entries 5–8). In the competitive rebinding experiments, P-2 shows a small but measurable selectivity for its own template over the close structural analog 3, which has only a very minor variation in the placement of the imidazole groups (Table IV, entry 2). (2 and 3 are in fact so similar that we were unable to separate them by reverse phase HPLC). The separation factor increases to above 1.3 when the polymer is asked to distinguish template 2 from substrates 4 and 5 in which the imidazole groups are spaced further apart (Table IV, entries 3 and 4).

Table II
Polymerization and Characterization of Metal-Complexing Polymers[a]

polymer code	template used (mmol/g)	Cu(II) in polymn (mmol/g)	recovery of Cu(II) (mmol/g)	recovery of template (mmol/g)	surface area (m^2/g)	pore volume (cm^3/g)
P-1	**7** (0.51)	0.52	0.48	0.49	232	1.2
P-2	**2** (0.26)	0.52	0.47	0.26	263	1.4
P-3	**3** (0.27)	0.53	0.49	0.26	280	1.3
P-4[b]	**4** (0.25)	0.54	0.50	0.23	252	1.3

[a] Polymerizations were carried out using EGDMA at a molar ratio of monomer 1:EGDMA of 5:95 in methanol. [b] This template:monomer assembly precipitates in methanol. Therefore polymerization was carried out in 90:10 methanol:dioxane.

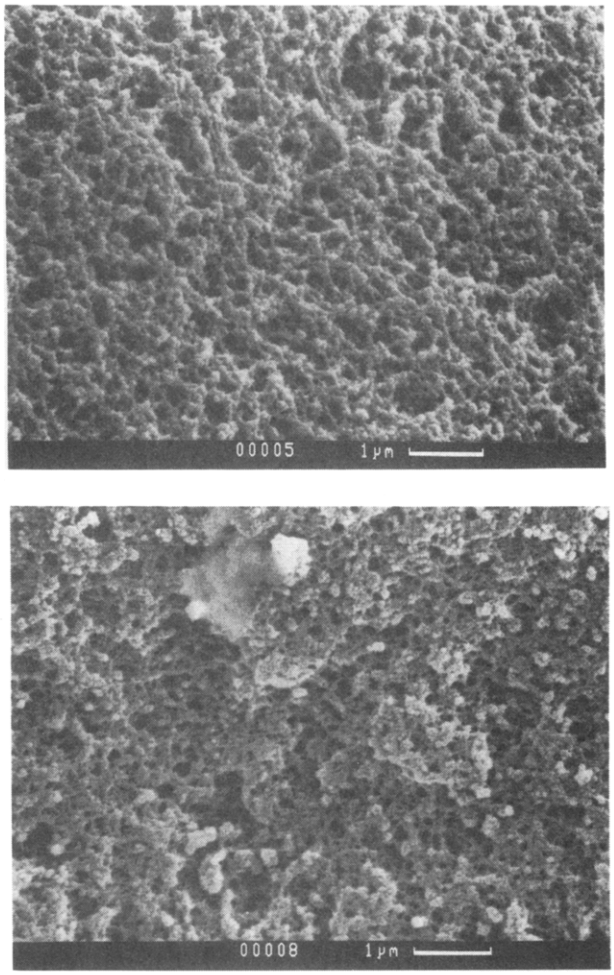


Figure 2. Scanning electron micrographs of macroporous metal-complexing polymer prepared using **2** as the template: (a) methanol-washed polymer; (b) polymer after removal and reloading of template and copper.

An important problem in template-mediated synthesis of molecular recognition polymers is the elucidation of the mechanism(s) underlying selectivity in binding. Selectivity most likely results from a combination of factors which include multisite binding, one-site binding coupled with "shape selectivity", or even selectivity that is based on shape recognition alone.[20] Some heterogeneity in the structures and microenvironments of the sites must be expected, and this heterogeneity will affect the equilibrium and kinetics of rebinding. Shea and co-workers[10] have carried out a systematic ^{13}C CP/MAS NMR and FT-IR study to analyze rebinding events in templated polymers bearing two 1,3-diol groups for selective binding of diketones by reversible covalent interaction. The fraction of two-site binding was relatively high after 13 h of reketalization (55–64% of the occupied sites were bisketals), but dropped significantly at longer times. It was found that bisketal formation (two-site binding) is not necessary for rebinding selectivity and that only one covalent bond is formed at the probable rate-determining step for rebinding. These authors concluded that selectivity involved a combination of some (1) two-site binding along with (2) single-site binding and weak noncovalent interactions between the remaining carbonyl and diol groups and/or fitting into a favorably-shaped microenvironment formed during the templating process.

Table III
Substrate Binding by Templated and Nontemplated Polymers under Saturation Rebinding Conditions

entry	polymer	substrate	substrate bound[a] (mmol/g)
1	P-1	**2**	0.46
2	P-1	**3**	0.44
3	P-1	**4**	0.45
4	P-1	**5**	0.44
5	P-2	**2**	0.33
6	P-2	**3**	0.22
7	P-2	**4**	0.19
8	P-2	**5**	0.18
9	P-3	**2**	0.17
10	P-3	**3**	0.24
11	P-4	**2**	0.20
12	P-4	**4**	0.24

[a] The polymers were equilibrated with a twofold molar excess of substrate over available copper sites.

Table IV
Selectivity of Templated and Nontemplated Polymers during Competitive Rebinding of Bis(imidazole) Substrates

entry	polymer	substrates	rel selectivity during rebinding[a]
1	P-1	**2** + **3**	$\alpha_{2/3} = 1.02$
2	P-2	**2** + **3**	$\alpha_{2/3} = 1.17$
3	P-2	**2** + **4**	$\alpha_{2/4} = 1.35$
4	P-2	**2** + **5**	$\alpha_{2/5} = 1.32$
5	P-3	**2** + **3**	$\alpha_{3/2} = 1.15$
6	P-4	**2** + **4**	$\alpha_{4/2} = 1.22$

[a] The polymers were equilibrated with a fivefold excess of an equimolar mixture of the two substrates.

Several lines of evidence support the contention that substrate binding by the current metal-complexing polymers involves coordination to the metal ion; that is, binding and selectivity do not arise through nonspecific interactions and simple shape recognition alone. Our preliminary study showed, for example, that the copper-free polymers exhibit very low binding capacities and essentially no substrate selectivity.[15] The fact that relatively acidic conditions (~pH 2.5) are required to remove the templates also implies coordination of the imidazole-containing substrates to the polymer-bound metal ions. Furthermore, the polymers bind only very small amounts of pseudotemplates **8** and **9** that are structurally similar to the bis(imidazoles) but lack the nitrogen ligands. P-2 binds only 0.016 mmol of **8** per gram of polymer, while P-3 binds 0.014 mmol of **9** per gram, 30–50 times less than the imidazole-containing substrates.

To what extent, then, does the binding selectivity of the templated polymers result from the simultaneous coordination of a bis(imidazole) by two Cu^{II} centers (two-site

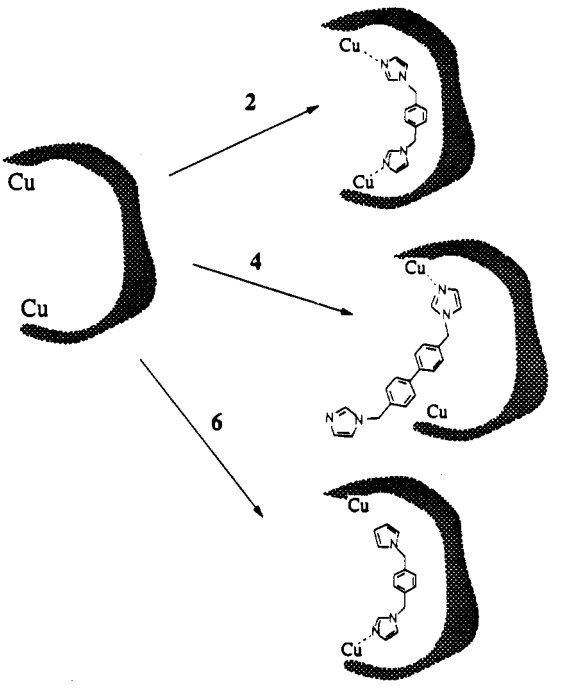
Table V
Maximum Capacities (Q_{max}) and Apparent Binding Constants (K_L) for Substrate Binding by Random (P-1) and Templated (P-2) Polymers

polymer	substrate used	Q_{max} (mmol/g)	K_L (M^{-1})
P-1	2	0.49 ± 0.027	2900 ± 203
P-1	4	0.47 ± 0.032	2700 ± 145
P-1	7	0.64 ± 0.025	2600 ± 135
P-2	2	0.33 ± 0.012	3800 ± 286
P-2	3	0.29 ± 0.015	2950 ± 206
P-2	4	0.24 ± 0.013	1900 ± 133
P-2	5	0.23 ± 0.012	1800 ± 128
P-2	6	0.30 ± 0.015	1700 ± 102
P-2	7	0.55 ± 0.028	2800 ± 144

binding) and to what extent from steric (or other) interactions superimposed upon coordination to a single imidazole (one-site binding)? Useful information regarding the distribution of functional groups in the polymer and insights into the origins of selectivity can be obtained from carefully-designed rebinding studies. A polymer's capacity to bind different substrates should reflect steric constraints on the binding sites that have been imparted by polymerization in the presence of templates of different molecular dimensions. The strength of binding, on the other hand, should reflect the recognition mode (one-site versus two-site binding). Adsorption isotherms for the bis(imidazole) substrates and 1-benzylimidazole were measured for the random polymer P-1 and the templated polymer P-2. Maximum binding capacities (Q_{max}) and apparent binding constants (K_L) obtained from the adsorption isotherms are summarized in Table V. The maximum capacities of both P-1 and P-2 for 1-benzylimidazole are higher than their recoverable copper contents, which suggests that the majority of copper centers in these macroporous polymers are indeed accessible to this small, monofunctional substrate and also that some of the centers can coordinate additional imidazoles. Interestingly, P-1 binds more 1-benzylimidazole than does the templated polymer P-2. When sterically allowed, the Cu^{II}-IDA complex can coordinate two imidazole donors.[21] Close proximity of the metal centers may hinder multiple imidazole coordination in the templated polymer. Conversely, the greater capacity of the random polymer to bind 1-benzylimidazole may reflect a higher degree of isolation of the Cu^{II} centers that allows access to additional ligands.

Table III reports the amounts of individual substrates taken up by the random and various templated polymers in the presence of excess substrate. The uptake of all four bis(imidazoles) by P-1 is nearly equal to the amount of recoverable copper ions in this polymer (0.48 mm/g, Table II). In contrast, the templated polymers P-2, P-3, and P-4 bind considerably less bis(imidazole) than the amount of removable copper. As can be seen in Tables III and V, P-2, P-3, and P-4 bind only about half as much bis(imidazole) as the random polymer, even though all the polymers contain essentially the same amount of removable copper. This reduced capacity for bis(imidazole) substrates relative to the random polymer once again indicates that templating can significantly affect the distribution of copper ions in the polymer. In the templated polymer, it appears that a bis(imidazole) can either bind to two copper ions simultaneously, or it can bind to one and block copper sites nearby in the binding cavity. The ability of substrate to block access to adjacent copper sites is apparent from the templated polymer's ability to bind **6**, an analog of **2** containing a single potential ligand. Consequently, substrate **6** can fit into any cavities that accept substrate **2**, but it cannot coordinate two Cu^{II} centers simultaneously. As shown in Table V, the maximum capacity of P-2 for **6** is approximately the same as that for **2**, and this capacity is once again much less than the removable copper content and much less than the capacity for the smaller, monofunctional substrate **7**.

Scheme I



Further information on the nature of substrate binding to the functional polymer matrix can be obtained by comparing the polymer–substrate binding constants summarized in Table V for polymers P-1 and P-2. While the random polymer binds all the substrates with essentially the same affinity, P-2 has a higher affinity for its own template **2** than for any of the nontemplate bis(imidazoles), 1-benzylimidazole, or **6**. The lowest affinities are for substrates **4** and **5**, which are significantly larger than the template, and for **6**, which contains only one binding site. The similarity of the binding constants for **4**, **5**, and **6** suggests that these substrates are bound in a similar manner, via a single imidazole. The higher affinity for **2** indicates that it (and possibly **3**) may experience some degree of two-point attachment. A mechanism for this discrimination is illustrated in Scheme I. A cavity formed around template **2** can accommodate **2** with two-site coordination to Cu^{II}, but not the bigger substrate **4**. **6** can be accommodated into the cavity but will not experience two-site binding.

The difference between the binding constants for template and nontemplate are in general not large (1900 versus 3800 M^{-1}), however, and do not by themselves justify assigning predominant two-site binding for one versus one-site binding for another. As seen for the functional polymers of Shea and co-workers[10] (vide supra), there is most likely a mixture of mechanisms involved in interactions with the substrates that include two-site binding in some cavities along with single-site binding and steric interactions with the sites' microenvironments in others. A role for cavity size and steric interactions becomes apparent when templated polymers prepared using the larger bis(imidazoles) are used to distinguish their template from a smaller bis(imidazole). As can be seen in Table IV, the polymer prepared using **4** as the template is less able to discriminate **4** from the smaller substrate **2** ($\alpha_{4/2} = 1.22$) than is P-2 ($\alpha_{2/4} = 1.35$).

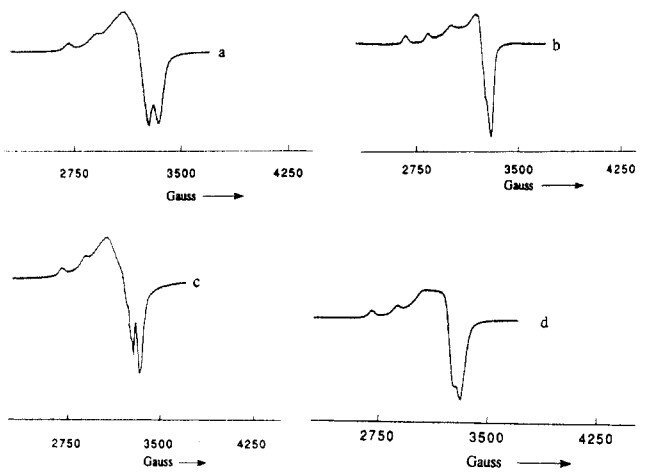


Figure 3. ESR spectra of the metal-complexing templated polymer P-2; (a) washed with methanol; (b) after removal of template and Cu^{II}, followed by reloading with Cu^{II}; (c) Cu^{II}-containing polymer after treatment with substrate **2**: (d) Cu^{II}-containing polymer after treatment with substrate **4**.

Table VI
ESR Spectral Parameters for Polymers P-1 and P-2 Loaded with Different Substrates[a]

entry	polymer	substrate	$g_\parallel$	$A_\parallel$ (G)
1	P-1	substrate-free	2.30	166
2	P-1	**2**	2.22	197
3	P-1	**4**	2.23	199
4	P-1	**7**	2.21	199
5	P-2	methanol-washed	2.23	198
6	P-2	substrate-free	2.31	169
7	P-2	**2**	2.21	201
8	P-2	**4**	2.27	186
9	P-2	**7**	2.23	199

[a] Measurements were carried out after equilibration with a twofold molar excess of substrate.

Even if the ability to distinguish template from non-template arises from two-site binding to the template, flexibility in the polymer networks and the substrates might tend to reduce selectivity, especially for very similar substrates, and binding affinity might be reduced by the associated entropy cost of forming what is essentially a large chelate complex. On the other hand, some degree of flexibility may be necessary to provide favorable two-site interactions without introducing strain into the resulting complex. A further possible cause for loss of selectivity is that the Cu^{II}-monomer–template assembly partially dissociates during polymerization and that the monomers then become randomly incorporated in the polymer. ESR studies of the solid polymers still containing template indicate, however, that the copper ions are still largely coordinated to imidazole following polymerization (vide infra) and therefore cannot be incorporated randomly to any great extent.

ESR of Cu^{II}-polymers. Interactions between the paramagnetic copper centers in the solid polymers and different substrates can be visualized more directly by ESR spectroscopy. ESR spectra are shown in Figure 3 for (a) freshly-prepared P-2 still containing the template, (b) template-free polymer, (c) polymer loaded with **2**, and (d) polymer loaded with **4**. Values of $g_\parallel$ and $A_\parallel$ are summarized in Table VI. The similarity between the spectral parameters of the polymer that still contains the original template (entry 5, Table VI) and those of the polymerizable monomer–template assemblies in solution (entry 2, Table I) indicates that the geometry of the preassembled coordination complex is largely preserved in the solid polymer. These data indicate that the polymer does not contain a large number of randomly incorporated copper ions, which would no longer have nitrogen ligands. This is also the case for the templated polymer that has been reloaded with its substrate: the $g_\parallel$ and $A_\parallel$ values (entry 7, Table VI) once again indicate that there are few or no copper centers not bound to imidazole. Since the binding capacity for **2** is much less than the amount of copper ions in the polymer, some significant fraction of the bis-(imidazole) must be coordinated to two metal ions in the polymer cavities.

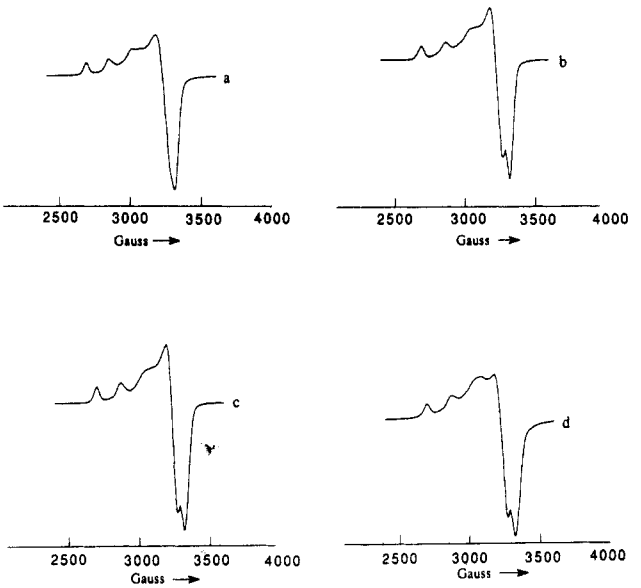


Figure 4. ESR spectra of the metal-complexing random polymer P-1: (a) in the absence of imidazole substrate; (b) loaded with substrate **2**; (c) loaded with substrate **4**; (d) loaded with substrate **7**.

Comparison of the ESR spectra for the templated polymer P-2 treated with substrates **2** and **4** (Figure 3c,d, Table VI) shows small but significant differences between the two, reflecting differences in the polymer's ability to bind substrate that were also seen in the binding experiments (Tables III and V). The appearance of the spectra in the $g_\perp$ region are different. Both the washed, template-containing polymer (Figure 3a) and the polymer reloaded with template (Figure 3c) show two components with similar peak shapes in the $g_\perp$ region. When Cu^{II} coordinates nitrogen ligands, superhyperfine splittings can appear in this region.[17] This splitting is lost in the template-free polymer (Figure 3b). When the polymer is treated with **4** (Figure 3d), the ESR spectral pattern shows a broad line exhibiting a very weak splitting pattern. The loss of resolution in this case may be due to overlap of the spectra of two (or more) different Cu^{II} species present in the polymer matrix: a significant population of Cu^{II} ions bound to imidazole as well as unbound Cu^{II}-IDA. Similar ESR experiments were carried out on the random polymer P-1. These spectra are presented in Figure 4, while the $g_\parallel$ and $A_\parallel$ values are also summarized in Table VI. The splitting patterns in the $g_\perp$ region and the spectral parameters (entries 5–8, Table VI) show no indication of discriminatory substrate binding for the random polymer.

Conclusions

From the ESR spectral patterns and substrate binding behavior of these materials, it appears that the microstructures of the binding cavities and the distribution of copper ions are significantly different in the templated and random polymers. For the templated polymer P-2, binding of its own template results in a more homogeneous

coordination environment for the copper centers, which is manifested in the higher resolution of the ESR spectra. On the other hand, a lack of complementary binding site interactions between polymer P-2 and substrate 4 leads to residual noncoordinating Cu^{II} and a heterogeneous coordination environment also reflected in the ESR spectrum. The higher affinity exhibited by the polymer P-2 for its template, the inability of the templated materials to bind as much bis(imidazole) as the random polymer, and the fact that the copper ions in the templated polymer are all coordinated in the presence of significantly less substrate than the copper ion content also indicate that the metal ions are distributed in the binding cavities as illustrated in Scheme I and that two-site binding provides a mechanism for distinguishing template from nontemplate.

Using the strategy of template polymerization, specific metal-complexing polymers with controlled and predetermined arrangements of metal coordination sites have been prepared. These polymers are able to distinguish bis(imidazole) substrates that differ very slightly in the placement of imidazole ligands. In terms of strength, specificity, and directionality, the metal coordination interaction is more like a covalent interaction than hydrogen bonding or electrostatic interactions, yet formation and breakage can occur rapidly and under relatively mild conditions. These features make metal coordination a promising binding mode for preparing highly specific templated polymers for recognition of biological molecules, such as proteins or viruses, via the arrangements of metal-coordinating ligands on their surfaces. We are currently developing the experimental conditions for template polymerization in aqueous media and the appropriate polymerizable metal ion complexes in order to realize this goal.

Experimental Section

Materials. All chemicals were of reagent grade and were obtained from Aldrich unless stated otherwise. Whenever required, reagents were purified by either recrystallization or distillation prior to use. The solvents were purified following standard purification methods.

Instrumentation and Analyses. Elemental analyses were carried out at the Caltech Microanalysis Facility. Melting points were determined on a Büchi melting point apparatus. ^1H and ^{13}C NMR spectra were recorded on a JEOL GX-400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C nuclei. Chemical shift values reported are relative to tetramethylsilane (TMS) as the internal reference. Infrared spectra were recorded using a Perkin-Elmer 1600 FTIR spectrophotometer. Electronic absorption spectroscopic measurements were carried out with a Milton Roy Array 3000 spectrophotometer. Specific surface areas and pore volumes of the polymers were determined from N_2 adsorption measurements using an Omnisorp 100 analyzer. Electron micrographs were taken with a Cam Scan Series 2 scanning electron microscope after vacuum coating with gold. X-band ESR spectra were measured at 100 K with an IBM ER 200 D-SRC ESR spectrometer operating at 9.42 GHz. The magnetic parameters were assigned using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the reference.

Synthesis. Copper(II) [*N*-(4-Vinylbenzyl)imino]diacetic Acid Dihydrate (1). [*N*-(4-Vinylbenzyl)imino]diacetic acid (5 g, 20 mmol), synthesized according to Morris et al.[22] using pure 4-vinylbenzyl chloride (Kodak), was suspended in 30 mL of distilled water. The suspension was treated with 0.1 N NaOH to achieve a pH of 7.0. $CuSO_4 \cdot 5H_2O$ (5 g, 20 mmol) dissolved in 50 mL of distilled water was added slowly to this solution with continuous stirring. The solution was allowed to stir for 3 h, and the solvent was removed under vacuum. The residue was treated with 30 mL of methanol and filtered to remove insoluble material. The methanol solution kept at −20 °C gave bright blue crystals of 1, which were further recrystallized from 80:20 ethanol:water: yield 65%; mp = 192–195 °C (dec). Anal. Calcd for $C_{13}H_{17}O_6NCu$: C, 45.02; N, 4.04; H, 4.94. Found: C, 44.85; N, 3.92; H, 5.12.

1,4-Bis(imidazol-1-ylmethyl)benzene (2). Sodium hydride (1.9 g, 48 mmol, 60% suspension in mineral oil) was washed with 10 mL of dry THF under nitrogen. Dry THF (30 mL) was added, followed by slow addition of 2.85 g (44 mmol) of imidazole in 15 mL of THF. The reaction mixture was allowed to stir for 30 min. α,α'-Dibromo-*p*-xylene (5.3 g, 20 mmol) in 20 mL of THF was added to the resulting suspension. (These dibromoxylene compounds are lachrymators, and appropriate precautions should be taken for their handling.) The temperature of the reaction was then raised to 50 °C for 4 h. After cooling, the reaction mixture was treated with 25 mL of ice-cold water and stirred for 20 min. The organic phase was extracted with chloroform (3 × 50 mL), and the combined organic phase was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was recrystallized twice from ethyl acetate to obtain 2: yield 55%; mp = 148–150 °C; ^1H NMR ($CDCl_3$) δ 5.2 (s, 4 H), 6.85 (s, 2 H), 7.05 (s, 2 H), 7.2 (m, 4 H), 7.55 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 49.6, 118.7, 127.3, 129.0, 135.8, 136.7. Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.56; N, 23.51; H, 5.92. Found: C, 70.11; N, 23.36; H, 6.02.

1,3-Bis(imidazol-1-ylmethyl)benzene (3). 3 was obtained in 60% yield starting with α,α'-dibromo-*m*-xylene and the experimental procedure described above for 2: mp = 146 °C; ^1H NMR ($CDCl_3$) δ 5.25 (s, 4 H), 6.85 (s, 2 H), 7.05 (s, 2 H), 7.25 (m, 4 H), 7.55 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 52.9, 121.0, 125.5, 126.2, 129.6, 138.7. Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.56; N, 23.51; H, 5.92. Found: C, 70.22; N, 23.28; H, 6.07.

4,4′-Bis(imidazol-1-ylmethyl)biphenyl (4). The dibromide bis(bromomethyl)biphenyl was prepared by *N*-bromosuccinimide bromination of 4,4′-dimethylbiphenyl,[23] which was used to synthesize 4 by the method described above. The product was obtained as a viscous liquid, which solidified after 2 days at −10 °C: yield 45%; ^1H NMR ($CDCl_3$) δ 5.2 (s, 4 H), 6.85 (s, 2 H), 7.05 (s, 2 H), 7.3–7.7 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 49.8, 119.2, 127.2, 127.8, 129.2, 136.2, 137.0, 139.8. Anal. Calcd for $C_{20}H_{18}N_4$: C, 76.41; N, 17.82; H, 5.77. Found: C, 76.23; N, 17.65; H, 5.70.

2,6-Bis(imidazol-1-ylmethyl)naphthalene (5). 5 was synthesized in an analogous manner by reacting 2,6-bis(bromomethyl)naphthalene[23] with imidazole: yield 50%; mp = 175–177 °C; ^1H NMR ($CDCl_3$) δ 5.3 (s, 4 H), 6.8 (s, 2 H), 7.06 (s, 2 H), 7.2–7.3 (m, 2 H), 7.4–7.8 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 50.7, 119.2, 125.5, 125.9, 128.7, 129.8, 132.7, 134.2, 137.4. Anal. Calcd for $C_{18}H_{16}N_4$: C, 74.97; N, 19.43; H, 5.59. Found: C, 74.65; N, 19.25; H, 5.49.

1-(Imidazol-1-ylmethyl)-4-(pyrrol-1-ylmethyl)benzene (6). Synthesis of this compound involves a three-step procedure as described below.

4-(Pyrrol-1-ylmethyl)benzyl Alcohol. Pyrrole (1.35 g, 20 mmol) in 20 mL of THF was added slowly to a solution of 2.7 g (24 mmol) of potassium *tert*-butoxide in 50 mL of THF under nitrogen. After stirring for 45 min, 4.2 g (18 mmol) of methyl 4-(bromomethyl)benzoate in 50 mL of THF was added to this suspension. The temperature of the resulting reaction mixture was raised to 50 °C for 5 h. After cooling, the reaction mixture was poured into 30 mL of ice-cold water and was extracted with dichloromethane (3 × 50 mL). The combined organic phase was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give 2.9 g of the product methyl 4-(pyrrol-1-ylmethyl)benzoate as a white solid.

Without any further purification this compound was reduced with 0.4 g (18 mmol) of lithium borohydride in refluxing THF for 2 h. After cooling to room temperature, the unreacted borohydride was quenched with 15 mL of ice-cold water and stirred for 30 min. The reaction mixture was filtered off, and the residue was washed with 25 mL of diethyl ether. The combined organic phase was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give 2 g of 4-(pyrrol-1-ylmethyl)benzyl alcohol.

4-(Pyrrol-1-ylmethyl)benzyl Toluene-*p*-sulfonate. 4-(Pyrrol-1-ylmethyl)benzyl alcohol (1.8 g, 10 mmol) in 10 mL of dry THF was added dropwise to a solution of 2 g (10.5 mmol) of toluene-*p*-sulfonyl chloride in 20 mL of dry pyridine at 0 °C. The

reaction mixture was slowly allowed to warm to room temperature and was stirred overnight. Ice-cold water (30 mL) was added, and the resulting mixture was extracted with diethyl ether (3 × 50 mL). The organic phase was dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, a viscous liquid was obtained which solidified at −20 °C.

6 was synthesized in a manner analogous to **2**, starting with 0.5 g (12.6 mmol) of sodium hydride and 2.8 g (8 mmol) of the above toluene-*p*-sulfonate ester in dry THF. The resulting product was purified by recrystallization from ethyl acetate: hexane (70:30 v/v): yield 55%; mp = 114 °C; ^{1}H NMR ($CDCl_3$) δ 5.1 (s, 2 H), 5.25 (s, 2 H), 6.20 (t, 2 H), 6.75 (t, 2 H), 6.85 (s, 1 H), 7.1 (s, 1 H), 7.25 (m, 4 H), 7.6 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 50.1, 52.8, 107.9, 119.0, 121.6, 127.4, 129.2, 136.0, 137.1, 138.3. Anal. Calcd for $C_{15}H_{15}N_3$: C, 75.92; N, 17.71; H, 6.37. Found: C, 75.72; N, 17.53; H, 6.32.

1,4-Bis(pyrrol-1-ylmethyl)benzene (8). Pyrrole (2.7 g, 40 mmol) in 25 mL of THF was added slowly under a nitrogen atmosphere to a solution of 5.6 g (50 mmol) of potassium *tert*-butoxide in 50 mL of THF. After stirring for 45 min, 5 g (19 mmol) of α,α′-dibromo-*p*-xylene in 50 mL of THF was added. The temperature of the resulting reaction mixture was raised to 50 °C for 5 h. After cooling, the mixture was poured into 40 mL of ice-cold water and was extracted with dichloromethane (3 × 50 mL). The combined organic phase was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was recrystallized from petroleum ether: yield 65%; mp = 102 °C; ^{1}H NMR ($CDCl_3$) δ 5.0 (s, 4 H), 6.15 (t, 4 H), 6.70 (t, 4 H), 7.25 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 52.7, 107.6, 121.5, 127.5, 138.2.

Polymerization and Workup. Typically, 0.5 g of monomer **1** was dissolved in 4 mL of methanol, and appropriate substrate (substrate concentration is ~0.5 molar ratio to that of monomer **1**) dissolved in 4 mL of methanol was added slowly with constant stirring. The resulting solution was allowed to stir for 6 h under a nitrogen atmosphere. Subsequently, an excess of ethylene glycol dimethacrylate (~95 mol %) with 2,2′-azobis(isobutyronitrile) (1 wt % with respect to total monomer) was added to this solution. The polymerization mixture was cooled to liquid nitrogen temperature, evacuated, thawed, and filled with nitrogen. This procedure was repeated three times to remove oxygen, and finally polymerization was carried out at 65 °C for 24 h under nitrogen. The solid mass thus obtained was cooled, ground, and extracted thoroughly with methanol. The resulting blue polymers were dried to constant weight under vacuum at 50 °C and were sieved to appropriate particle size.

Template Removal. The above polymers (typically, 2 g) were treated with 25 mL of acidified (pH ~ 2.0) aqueous methanol (60%) and were kept in a shaker bath at 37 °C for 24 h. The polymers were then filtered and washed thoroughly with the same solvent. For analysis, the combined filtrate was concentrated to approximately 10 mL. After neutralization with basic ion exchange resin, the resin was filtered and washed with 15 mL of methanol. The combined filtrate was evaporated to dryness. The quantity of template liberated was determined gravimetrically as well as by ^{1}H NMR using a known amount α,α′-dibromo-*p*-xylene as the internal standard.

Copper Removal. The template-free polymers were treated with 25 mL of 0.025 M 1,4,7-triazacyclononane (in 95:5 methanol: water) in a shaker bath at 37 °C for 24 h. The polymers were then filtered and washed thoroughly with methanol. The methanol-washed polymers were subsequently treated with 25 mL of 0.1 M aqueous EDTA (pH 7.0) in the shaker bath for another 24 h. The triazacyclononane-containing solution was evaporated to dryness, and the residue was combined with the EDTA wash. The copper contents of the EDTA solutions were determined spectrophotometrically.

Copper Reloading. The polymers (typically 2 g) were treated with 20 mL of 0.1 M aqueous copper sulfate solution and were allowed to equilibrate at 37 °C for 24 h. After filtration, the polymers were thoroughly washed with water. The combined washings were treated with EDTA solution, and the copper contents of these solutions were determined spectrophotometrically, from which the amounts of copper loaded were calculated.

Binding Experiments: Single Substrates. The copper-loaded polymers (typically 0.5 g) were treated with a 2-fold molar excess of the substrate in methanol at 37 °C for 24 h. The polymers were subsequently filtered and washed with methanol. The combined methanol solutions were evaporated to dryness, and the amount of substrate present in the wash was determined by ^{1}H NMR. To determine binding constants and maximum binding capacities, predetermined amounts of the polymers were equilibrated with different concentrations of the substrates (lower to higher concentration). The isotherms were fit to a Langmuir-type isotherm using the nonlinear least squares fitting routine Enzfitter[24] (with appropriate modifications).

Competitive Binding. The polymers (typically 0.5 g) were treated with a 2-fold molar excess of an equimolar mixture of the methanolic solutions of the appropriate substrates. After equilibration in the manner described above, the polymers were filtered and washed with methanol. After drying the combined filtrates, the residues were analyzed by ^{1}H NMR spectroscopy. The relative intensities of the benzylic protons of the different substrates gave the relative ratios of the free substrates from which the relative amounts of the substrates bound were estimated. For the substrate pair **2** and **4**, the benzylic protons are indistinguishable, and the ^{13}C NMR resonances of the benzylic carbons were used to determine relative concentrations (under quantitative conditions).

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