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Carbohydrate Recognition by Porphyrin-Based Molecularly Imprinted Polymers

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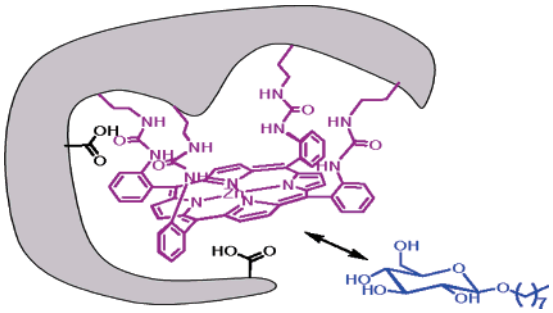
ABSTRACT



Porphyrin-based molecularly imprinted polymers (MIPs) were prepared for carbohydrate recognition. A urea-appended porphyrin functional monomer was utilized to provide complementary functionality and quality binding sites throughout the polymer. Each porphyrin-based polymer demonstrates high affinity and differential selectivity for closely related carbohydrates that correlate to the structure of the template used in the imprinting process.

Molecular imprinting is an attractive approach for mimicking molecular recognition in nature because it allows for the formation of specific recognition and catalytic sites in polymer matrixes without elaborate molecular designs and multistep synthesis.[1] Extensive efforts have been made to develop molecularly imprinted polymers (MIPs) by both covalent and noncovalent approaches, and a number of applications have been studied using MIPs for chromatography,[2] solid-phase extraction,[3] catalysis,[4] and sensing.[5]

The imprinting procedure involves formation of a self-assembled prepolymerization complex between template and

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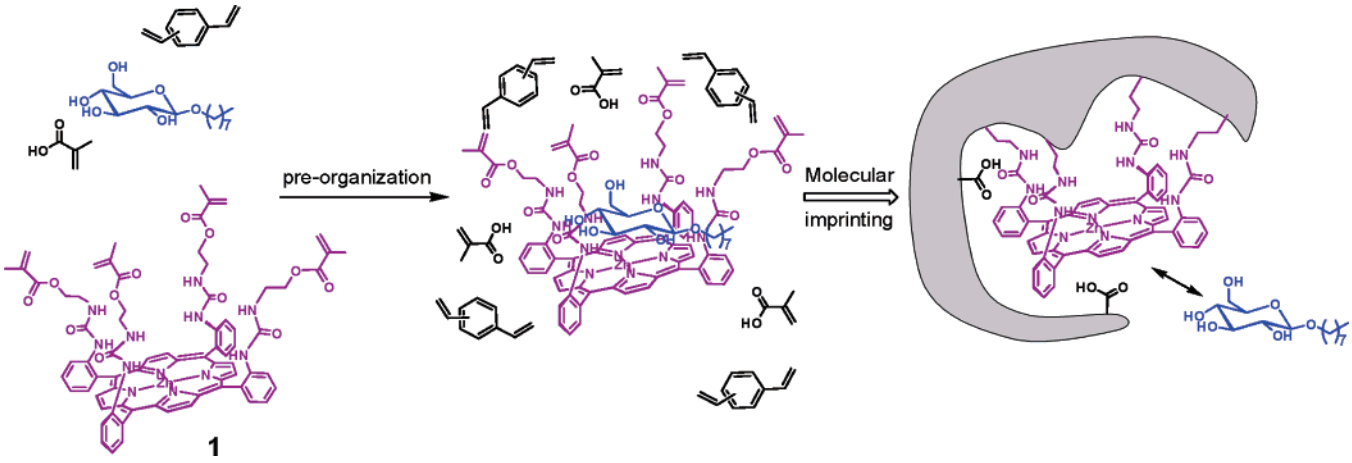
Scheme 1



functional monomer, followed by polymerization. After extraction of the template, complementary recognition sites remain in the polymer network. Commercially available monomers such as methacrylic acid (MAA) and vinylpyridine have been widely used, making polymer preparation a simple and facile process. In comparison, a more deliberate approach using synthetically designed functional monomers enables better control in rationally designing high-affinity binding sites for each corresponding template while at the same time minimizing the inherent nonspecific binding properties common in noncovalent imprinted polymers.[6]

Recently, we have reported a new class of carbohydrate receptors based on aspartate urea-appended porphyrins that were found to display the highest current levels of binding for pyranosides in chloroform.[7] Similar findings by Bonar−Law et al. report of carbohydrate recognition using an analogous urea-appended porphyrin.[8] Although the proposed porphyrin receptors show sufficient overall affinity for carbohydrates, it is difficult to rationally design a receptor for a particular carbohydrate by using this system. Herein, we report the preparation of porphyrin-based carbohydrate-imprinted polymer that incorporates the advantages of imprinting as a tool for selectivity control (Scheme 1).[9]

Monomer **1** was synthesized from the reaction between *meso*-αααα-tetrakis(*o*-aminophenyl)porphyrin and 2-isocyanatoethyl methacrylate, followed by metal insertion, in 38% overall yield (see Supporting Information).

UV−visible titration of monomer **1** with octyl pyranosides was conducted in chloroform at 298 K, and then binding constants were calculated by fitting the curve of absorbance at λ_{max} as a function of change in carbohydrate concentration to a 1:1 binding isotherm. Binding constants for carbohydrates are comparable to those of other porphyrins reported before, and monomer **1** shows slight selectivity (Table 1).[8]

Table 1. Binding Constants (K_a, M^{-1}) and Free Energy Change ($\Delta G°$, kcal mol^{-1}) from UV−Visible Titration of Porphyrin Monomer **1** with Octyl Pyranosides in Chloroform at 298 K[a]

guest	K_a ($\times 10^4$ M^{-1})	$-\Delta G°$
n-octyl-β-D-glucopyranoside	6.2 (±0.40)	6.47
n-octyl-α-D-glucopyranoside	13 (±2.1)	6.90
n-octyl-β-D-galactopyranoside	9.9 (±2.6)	6.74

[a] Experimental conditions: host concentration, [**1**] = 2.3 μM for all guests; guest concentrations, 6.5−160 μM for *n*-octyl-β-D-glucopyranoside, 3.6−120 μM for *n*-octyl-α-D-glucopyranoside, and 2.7−41 μM for *n*-octyl-β-D-galactopyranoside.

Seven polymers were prepared via a polymerization method under UV irradiation at 20 °C (Table 2). MIP1 and NIP1 were prepared using designed monomer **1** and MAA as functional monomers. The incorporation of a nonpolar cross-linking monomer (divinylbenzene) and aprotic porogen solvent (chloroform) was chosen to best optimize hydrogen-bonding interactions between the template and the functional monomer. Controls included polymers MIP2 and NIP2, which were prepared without monomer **1**, followed by MIP3 and NIP3 prepared without MAA. For comparing binding selectivity for diastereomeric sugars, MIP4 was prepared using *n*-octyl-α-D-glucopyranoside as a template.

Batch rebinding studies were conducted by UV spectroscopy with *p*-nitrophenyl pyranosides in acetonitrile.[10] For

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(10) *p*-Nitrophenyl pyranosides were chosen because octyl pyranosides lack chromophores. Although polymers were prepared in chloroform, acetonitrile was utilized in the rebinding tests due to the poor solubility of *p*-nitrophenyl pyranosides in chloroform.

Table 2. Preparation of Imprinted and Nonimprinted Polymers[a]

polymer	template	monomer MAA (mmol)	monomer **1** (mmol)	monomer DVB (mL)
MIP1	*n*-octyl-β-D-glucopyranoside, 0.1 mmol	0.3	0.1	0.84
NIP1		0.3	0.1	0.84
MIP2	*n*-octyl-β-D-glucopyranoside, 0.1 mmol	0.3	0	0.84
NIP2		0.3	0	0.84
MIP3	*n*-octyl-β-D-glucopyranoside, 0.1 mmol	0	0.1	0.84
NIP3		0	0.1	0.84
MIP4	*n*-octyl-α-D-glucopyranoside, 0.1 mmol	0.3	0.1	0.84

[a] Reaction components and the initiator, azobisisobutyronitrile (5 mol % of the mixture), were dissolved in chloroform (2.0 mL). The solution was sonicated under nitrogen, and the polymerization reaction proceeded photochemically at 20 °C for 20 h. MIP = molecularly imprinted polymer; NIP = nonimprinted polymer; MAA = methacrylic acid; DVB = divinylbenzene. For details, see Supporting Information, p S4.

the rebinding study, 2.5 mL of a 0.05 mM solution of *p*-nitrophenyl pyranoside was shaken for 30 min in the presence of 20 mg of polymer, and the UV spectrum of the supernatant was subsequently obtained at 300 nm. The percent of *p*-nitrophenyl pyranoside bound was determined by the change in absorbance of the measured supernatant compared to a stock solution (0.05 mM) of *p*-nitrophenyl pyranoside.

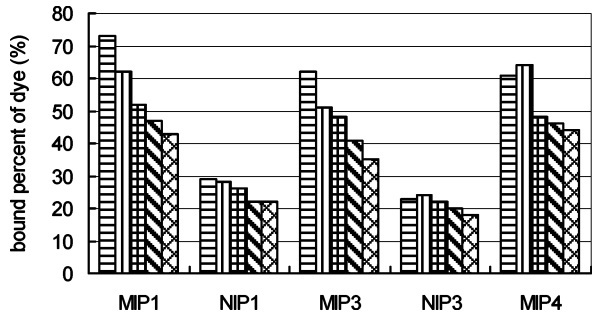

Figure 1. Binding percent of *p*-nitrophenyl pyranosides (dyes) on polymers (horizontally lined bar, *p*-nitrophenyl-β-D-glucopyranoside; vertically lined bar, *p*-nitrophenyl-α-D-glucopyranoside; checked bar, *p*-nitrophenyl-β-D-galactopyranoside; diagonally lined bar, *p*-nitrophenyl-α-D-galactopyranoside; diagonally checked bar, *p*-nitrophenyl-β-D-mannopyranoside). Data for MIP2 and NIP2 are omitted.[11]

As shown in Figure 1, polymers prepared with monomer **1** possess a heightened affinity for *p*-nitrophenyl pyranosides; in addition, a general trend can be seen that displays imprinted polymers having overall higher affinities than nonimprinted polymers. The fact that MIP2 and NIP2 showed no binding property supports that porphyrin monomer **1** is

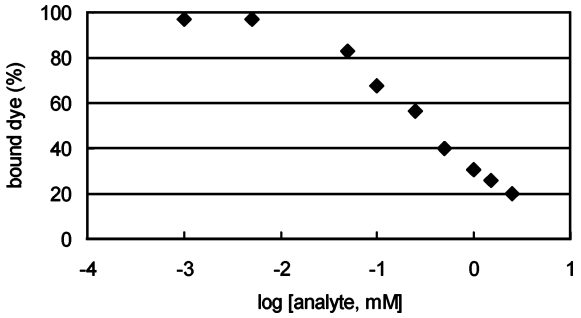

Figure 2. Normalized displacement isotherm of MIP1. Dye = *p*-nitrophenyl-α-D-glucopyranoside, analyte = *n*-octyl-α-D-glucopyranoside.

responsible for generating the effective binding site in the polymer matrix.[11] On the other hand, polymers prepared with both monomer **1** and MAA exhibit higher affinities compared to NIP3 and MIP3 prepared solely with monomer **1**, which means that MAA contributes to higher affinities, although MAA itself barely interacts with analytes. As for selectivity, MIPs have higher affinity for glucopyranoside over galactopyranoside and mannopyranoside. Besides, MIP1 and MIP3 showed a higher affinity for the *p*-nitrophenyl-β-D-glucopyranoside (*p*-nitrophenyl analogue of *n*-octyl-β-D-glucopyranoside).

In comparison, MIP4 showed a noticeably different binding pattern than the others. Although this difference is small, MIP4 is selective for *p*-nitrophenyl-α-D-glucopyranoside (*p*-nitrophenyl analogue of *n*-octyl-α-D-glucopyranoside) over *p*-nitrophenyl-β-D-glucopyranoside. Above all, it is important that the selectivity of MIP is different from that of monomer **1**. Such relevance alludes to the fact that during the imprinting process, the generation of selective binding sites specific for the template occurred throughout the polymer. This subtle reversal in selectivity is consistent with the templates used to make MIP1 and MIP4. MIP1 shows greater affinity for its template *n*-octyl-β-D-glucopyranoside than for *n*-octyl-α-D-glucopyranoside. MIP4, which was imprinted with *n*-octyl-α-D-glucopyranoside, reverses this selectivity pattern and shows greater affinity for *n*-octyl-α-D-glucopyranoside than for *n*-octyl-β-D-glucopyranoside.

Additional evidence for the ability of the imprinting process to rationally tailor the selectivity of molecular receptors was established from the displacement of *p*-nitrophenyl pyranosides from the imprinted polymers. As shown in Figure 2, it is clearly observed that as the concentration of *n*-octyl-α-D-glucopyranoside is increased, the corresponding bound *p*-nitrophenyl-α-D-glucopyranoside shows a direct rate of release in the imprinted polymer.[12] In

(11) MIP2 and NIP2 did not display any binding even with an increased amount of 370 mg and 400 mg per 2.5 mL, respectively. Although Mosbach et al.[9a] reported on *p*-nitrophenyl pyranoside imprinted polymers, MIP2 and NIP2 were prepared with much less MAA than that they had used.

(12) Amounts of solution and polymer for these experiments were 2.0 mL and 15 mg, respectively. The nonimprinted polymer shows the same pattern of displacement isotherm.

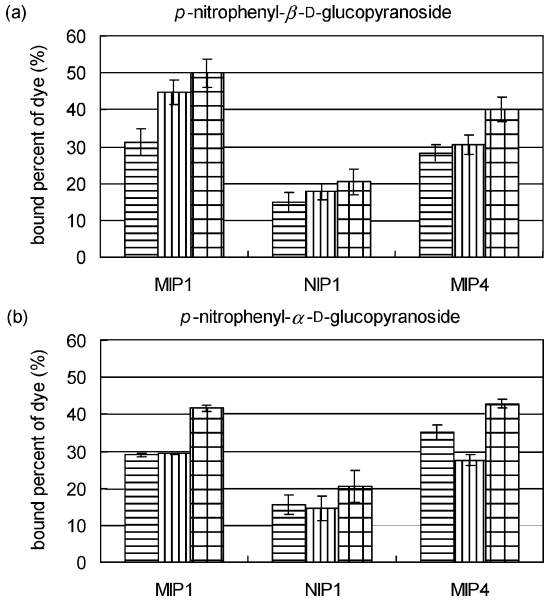

Figure 3. Percent of dye (0.05 mM) bound in the presence of analytes (0.25 mM). Data are averages of four measurements (horizontally lined bar, *n*-octyl-β-D-glucopyranoside; vertically lined bar, *n*-octyl-α-D-glucopyranoside; checked bar, *n*-octyl-β-D-galactopyranoside). (a) Dye = *p*-nitrophenyl-β-D-glucopyranoside; (b) dye = *p*-nitrophenyl-α-D-glucopyranoside.

other words, via displacement, the binding of octyl pyranoside (analyte) to the polymers can be monitored through the release of a bound indicator, *p*-nitrophenyl pyranoside.

Next, the selectivities of each polymer for octyl pyranosides were measured at conditions where about 50% of the actual bound *p*-nitrophenyl pyranoside was displaced by octyl pyranosides (Figure 2). Dye and analyte concentrations were 0.05 and 0.25 mM, respectively.[12]

As shown in Figure 3, it is clearly observed that MIPs have greater affinity for glucopyranoside than for galactopyranoside, as they showed the same selectivity for *p*-nitrophenyl pyranosides in Figure 1. In comparing MIP1 and MIP4 that were prepared via *n*-octyl-β-D-glucopyranoside and *n*-octyl-α-D-glucopyranoside, respectively, we can observe that when the *p*-nitrophenyl-β-D-glucopyranoside is placed in a competition study (Figure 3a), MIP1 demonstrates a greater selectivity for its template, *n*-octyl-β-D-glucopyranoside. On the other hand, MIP4 also shows selectivity for its template (*n*-octyl-α-D-glucopyranoside) in Figure 3b, where *p*-nitrophenyl-α-D-glucopyranoside was introduced. In both cases where *p*-nitrophenyl-β-D-glucopyranoside was tested for MIP4 (Figure 3a) and *p*-nitrophenyl-α-D-glucopyranoside for MIP1 (Figure 3b), minimal binding differences between *n*-octyl-β-D-glucopyranoside and *n*-octyl-α-D-glucopyranoside resulted. Although individually these minimal differences for a single analyte compared to a variety of imprinted polymers may not validate each imprinted polymer as a single highly selective receptor, applications of pattern recognition may later be an avenue to further promote the monomers' differential selectivity.

In conclusion, we have prepared carbohydrate-imprinted polymers using a porphyrin-based functional monomer that generate good overall affinities and possess differential selectivity for carbohydrates similar in structure. This study shows that by taking a predesigned receptor with fairly high affinity, one can rationally tailor the selectivity of a molecular receptor simply by polymerizing it in a highly cross-linked matrix in the presence of different guest molecules. There are many advantages to this in addition to the synthetic efficiency of this strategy. In particular, the resulting polymer is well-suited for different applications, as the receptor is now immobilized in the polymer matrix, especially for applications in sensing and separations.

With these promising results for easily constructed, highly efficient, and selective recognition sites for a range of carbohydrates via molecular imprinting, new investigations are now being directed toward the development of MIP-based sensor arrays for carbohydrate discrimination.

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Supporting Information Available: Synthesis and UV−visible titration data for monomer **1** and preparation of polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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