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Building Fluorescent Sensors by Template Polymerization: The Preparation of a Fluorescent Sensor for D-Fructose

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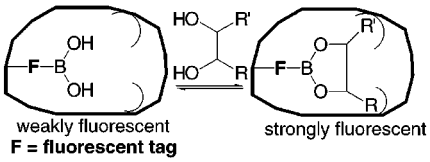
ABSTRACT



The application of molecular imprinting in making fluorescent sensors has been hampered by the lack of suitable fluorescent tags, which would respond to the binding event with significant fluorescence intensity changes. We have designed and synthesized a fluorescent monomer which allows for the preparation of fluorescent sensors of *cis* diols using molecular imprinting methods. This monomer was used for the preparation of sensitive fluorescent sensors for D-fructose.

Custom-made fluorescent sensors for organic molecules have a wide range of application potentials.[1−4] Traditionally, such sensors have been prepared through *de novo* design and synthesis. For example, many very sensitive fluorescent sensors have been designed for peptides,[5] metal ions,[6−10] saccharides,[11−13] and others.[12,14−18] Recently, molecular imprinting or template polymerization has gained much attention as a convenient method for the construction of binding sites for different analytes. Such an approach does not require the prior knowledge of the 3-dimensional structure of the analyte and the *de novo* construction of the complementary binding site.

Molecular imprinting is a technique first demonstrated in the late 1940s by Dickey.[19] The preparation of imprinted polymers involves (1) prearrangement of the print molecule (template) and the functional monomers at low temperature so that complementary intermolecular interactions among functional groups can develop, (2) polymerization of the monomers under conditions that cause minimal disturbance to the print molecule−monomer interactions, and (3) extraction of the print molecules from the polymers, which leaves behind "receptor sites" that are complementary to the templates or print molecules in terms of size, shape, and

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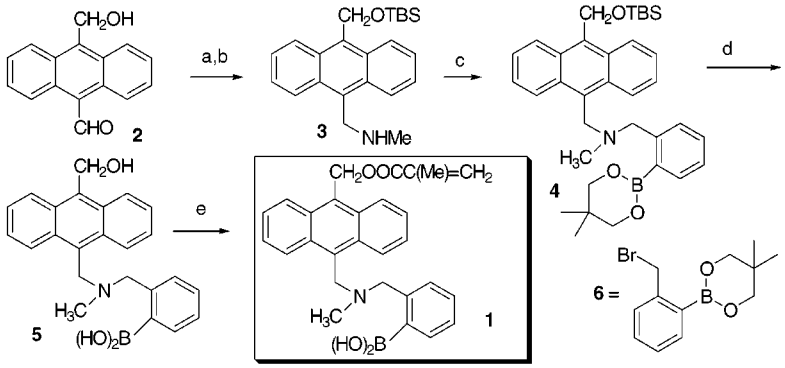
Scheme 1[a]



[a] (a) TBSCl/imidazole/DMF, 91%; (b) (i) NH_2Me/MeOH, 4−5 h, (ii) $NaBH_4$/MeOH, 2 h, 89%; (c) **6**, K_2CO_3/MeCN, reflux, 42%; (d) TBAF/THF, 74%; (e) methacrylic anhydride, DMAP/TEA/CH_2Cl_2, 70%.

functional group orientations. This technique has been used for the preparation of selective recognition sites for a wide variety of molecules.[20−23] Naturally, such polymeric receptors also have the potential to be developed as fluorescent sensors.[22,24−28] Conceivably, polymeric receptors could be constructed with a fluorescent tag built into them so that binding and dissociation of the analyte would change the fluorescence emission sufficiently for the binding event to be monitored conveniently. However, such efforts have been hampered by the lack of appropriate fluorescent tags which could respond to the binding event with a significant fluorescence intensity change unless certain chromophores are present in the template molecules, which could quench the fluorescence of the fluorescent tag. Recently, we have reported a method of making fluorescent sensors using molecular imprinting methods through the use of an external fluorescence quencher to manifest the fluorescence intensity change upon binding of the target compound.[29]

In this report, we describe our efforts in the development of polymeric sensors which respond to the binding event with a significant fluorescence intensity change without the use of an external quencher. The key to the project was the design and synthesis of a fluorescent tag which is intrinsically sensitive to the binding event and the incorporation of this fluorescent tag into the imprinted polymers. There are ample literature precedents showing that boronic acid derivatives can bind to *cis* diols tightly through ester formation[30−32] and boronic acid-containing monomers can be used for the preparation of selective binding sites for saccharides through molecular imprinting.[33−35] Furthermore, boronic acid moieties, when attached to certain fluorescent molecules, have been shown to significantly affect the fluorescence intensity of such fluorescent tags upon ester formation with *cis* diols.[12,15,36−38] One specific example is the conjugate of an anthracene moiety and a benzylamine moiety with boronic acid attached at the ortho position.[39] Such a conjugate was known to show significant fluorescence intensity changes upon ester formation with *cis* diols. By taking advantage of such known properties, we designed and synthesized an anthracene−boronic acid conjugate (**1**) with a methacrylate moiety attached to it to allow for its incorporation into imprinted polymers. The fluorescence intensity of the anthracene moiety of the functional monomer (**1**) is expected to increase with the formation of an ester with a *cis* diol structure (**8**, Scheme 2) because of the increased acidity of the boron atom after the ester formation, which increases the tendency for the nitrogen lone pair electrons to be donated to the open shell of boron. Because the lone pair electrons of the nitrogen can quench the fluoresence of the anthracene moiety through photoelectron transfer, this complex (**8**) formation will take away the quenching mechanism and, therefore, increase the fluorescence intensity. The utility of such a functional monomer (**1**) was tested by preparing a polymeric fluorescent sensor for D-fructose, which is known to bind to two boronic acid moieties.[40] As designed, the binding of fructose to the resulting fluorescent polymers showed significant fluorescence intensity changes (Figure 1).

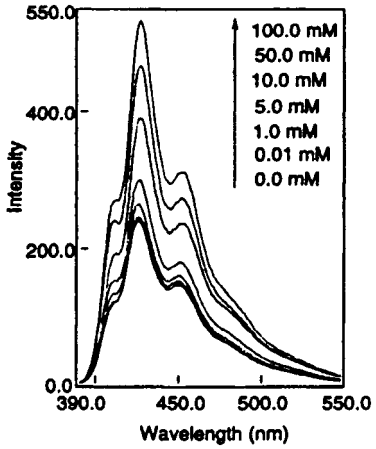


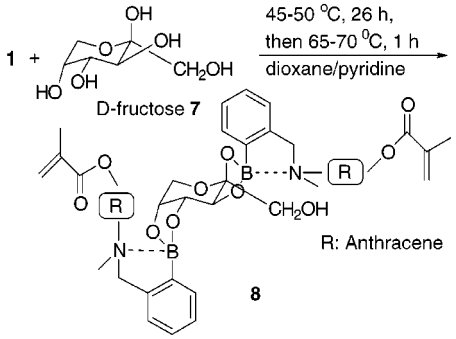
Figure 1. Typical set of fluorescence emission spectra of D-fructose imprinted polymer at different concentrations of D-fructose (λ_{ex} 370 nm).

Functional monomer **1** was synthesized in five steps starting from 10-(hydroxymethyl)-9-anthraldehyde (**2**)[41] (Scheme 1). Hydroxy aldehyde **2**, upon treatment with *tert*-

butyldimethylsilyl chloride (TBSCl) in the presence of imidazole in DMF at room temperature, yielded the silyl ether aldehyde in 91% yield.[42] Treatment of the silyl ether aldehyde with methylamine in methanol followed by reduction with sodium borohydride afforded silyl ether amine **3** in 89% yield. The reaction of amine **3** with 2,2-dimethylpropane-1,3-diyl[*o*-(bromomethyl)phenyl]boronate (**6**)[39] in the presence of potassium carbonate in acetonitrile gave amine boronate intermediate **4** in 42% yield. The cleavage of the silyl protecting group with tetrabutylammonium fluoride (TBAF) in THF at room temperature followed by an aqueous workup gave **5** in 74% yield. Reaction of **5** with methacrylic anhydride in anhydrous THF in the presence of DMAP (4-(dimethylamino)pyridine) afforded methacrylic ester **1** in 70% yield.

The template-directed polymerization was carried out using D-fructose (**7**) as the print molecule. D-Fructose was chosen as the test compound because it was known to bind tightly with the boronic acid moiety in a 1:2 ratio.[35] The D-fructose−boronic acid complex was preformed so as to maximize the complementary interactions at the binding sites. Therefore, D-fructose−boronic acid complex **8** was prepared by mixing D-fructose (**7**) with **1** in a 1:2 ratio in a mixture of dioxane and pyridine (9:1) (Scheme 2). Distillation of water from

Scheme 2



the reaction mixture yielded complex **8,** which was confirmed with FAB-MS (1015, M + 1). Due to the creation of four new chiral centers, **8** was expected to be a mixture of many diastereomers. This mixture of diastereomers **8** was used directly for the next step, polymerization, without purification. 2-Hydroxyethyl methacrylate was used as a comonomer and EGDM (ethyleneglycol dimethacrylate) was used as the cross linker. Then polymerization was carried out by following procedures previously reported with a ratio of complex **8**, 2-hydroxyethyl methacrylate, and EGDM of 1:4:50 at 65 °C under nitrogen using AIBN as the free radical initiator.[29] Then the polymer was ground and the template molecules were extracted by washing with MeOH twice for 1.5−2 h, a mixture of 0.1 N HCl and MeOH (1/1, v/v) three times for 3−5 h, and MeOH 10 times for a total of 22−24 h. After drying in a vacuum oven at 35 °C overnight, the polymer particles were sieved and then used for the binding studies.

Briefly, for the binding studies, about 10 mg of the polymer particles was suspended in 4.0 mL of D-fructose solutions at different concentrations in 50% MeOH/H_2O (v/v) with 0.05 M phosphate as the buffer at pH 7.4. The suspension was vortex-mixed for 3.0 h. Then about 3.5 mL of the suspension was transferred into a cuvette for fluorescence measurements. The emission spectra was recorded from 380 to 650 nm immediately after the solution was stirred with an INSTECT stirrer for 30 s. The excitation wavelength was set at 370 nm. A typical set of spectra is shown in Figure 1.

Without the addition of D-fructose, the fluorescence intensity (I) was low (Figures 1 and 2). However, with the addition of D-fructose, the fluorescence intensity was enhanced significantly in a concentration-dependent fashion. Fluorescence intensity changes were observed at high μM to high mM fructose concentrations (Figures 1 and 2). The fluorescence binding studies were conducted in triplicate.

Because boronic acid was known to bind to any compounds with a *cis* diol structural moiety, the fluorescence intensity change in response to D-fructose itself, as shown in Figures 1 and 2, did not necessarily indicate that the

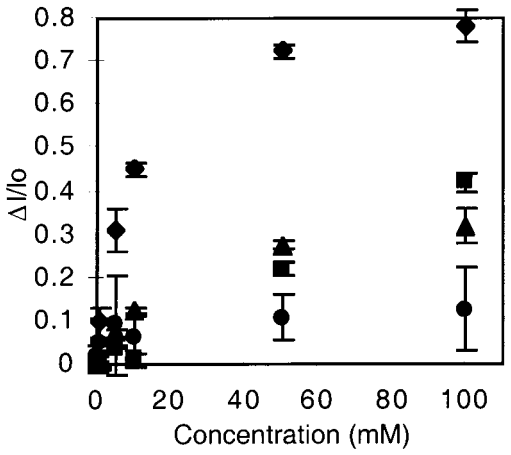


Figure 2. Fluorescence intensity changes of D-fructose imprinted polymers vs concentration of sugars (♦, D-fructose; ▲, D-mannose; ■, D-glucose; ●, control polymer) (λ_{ex} 370 nm, λ_{em} 426 nm).

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imprinting process created specific polymeric receptors for D-fructose. Furthermore, very often the boronic acid moiety shows preferential binding for fructose[12,39,40] and additional structural constrains are needed to change this preference.[43−46] Therefore, several control experiments were conducted. First, the binding of the polymeric receptors with D-glucose and D-mannose was also examined. It was found that the fluorescence intensity changes of the imprinted polymers upon addition of glucose and mannose at the same concentrations were far smaller than that of D-fructose (Figure 2). Second, the effect of D-fructose, D-glucose, and D-mannose on the fluorescence intensity of the fluorescent monomer **1** at a concentration of 10^{-5} M, a concentration comparable to that of the fluorescent moiety in studying the imprinted polymers, was also examined. It was found that fluorescent monomer **1** also exhibited preferential recognition of D-fructose (Figure 3), however, to a lesser degree than that of

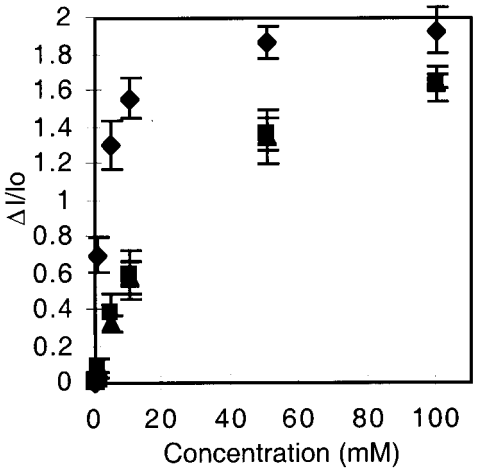

Figure 3. Fluorescence intensity changes of monomer **1** vs concentration of sugars (♦, D-fructose; ▲, D-mannose; ■, D-glucose) (λ_{ex} 370 nm, λ_{em} 426 nm).

the imprinted polymer. The ratio of $(\Delta I/I_0)_{fru}/(\Delta I/I_0)_{glu}$ was found to be about 2-fold greater for the imprinted polymer than for monomer **1** (Table 1). Furthermore, the control polymer prepared in the absence of D-fructose showed

Table 1. Comparison of Monomer **1** and the Imprinted Polymer in Their Preference for D-Fructose over D-Glucose.

	$(\Delta I/I_0)_{fru}/(\Delta I/I_0)$glu	
concn (mM)	monomer	polymer
1.0	7.7	∞[a]
5.0	4.1	8.8
10.0	2.7	5.7
50.0	1.4	2.7
100.0	1.2	3.0

[a] No intensity change was observed at 1.0 mM of D-glucose.

minimal fluorescence intensity changes upon addition of D-fructose (Figure 2). All of these indeed indicated that the imprinting process helped in the creation of binding sites which preferentially recognized the template molecules.

Our studies demonstrated that indeed sensitive fluorescent sensors can be prepared using molecular imprinting methods for compounds that do not necessarily have chromophores or quench fluorescence themselves. This can be achieved with properly designed recognition moieties. The fluorescent monomer **1** developed in this study could also be used for the preparation of fluorescent sensors of other sugars and biologically important catecholamines, which also have a *cis* diol structural moiety.

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