

# Building Fluorescent Sensors for Carbohydrates Using Template-Directed Polymerizations<sup>1</sup>

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*Received May 3, 2001*

The ability to custom-make fluorescent sensors for different analytes could have a tremendous impact in a variety of areas. Template-directed polymerization or molecular imprinting seems to be a promising approach for the preparation of high-affinity and specific binding sites for different template molecules. However, the application of molecular imprinting in the preparation of 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 (**1**) that allows for the preparation of fluorescent sensors of *cis* diols using molecular imprinting methods. This monomer has been used for the preparation of imprinted polymers as sensitive fluorescent sensors for D-fructose. The imprinted polymers prepared showed significant fluorescence intensity enhancement upon binding with the template carbohydrate. © 2001 Academic Press

**Key Words:** fluorescence; chemical sensor; boronic acid; molecular imprinting; polymerization; saccharides.

## INTRODUCTION

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. Recently, molecular imprinting or template polymerization, first demonstrated in the late 1940s by Dickey (5), 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 three-dimensional structure of the analyte and the *de novo* design of the complementary binding site. This technique has been used for the preparation of selective recognition sites for a wide variety of molecules (6–9). For example, molecular imprinting has been used for the preparation of HPLC stationary phases (10,11), antibody mimics (12–14), and sensors (15,16). The preparation of imprinted polymers usually involves: (i)

<sup>1</sup> Part of this work has been communicated: Wang, W., Gao, S., and Wang, B. (1999) *Org. Lett.* **1**, 1209–1212.

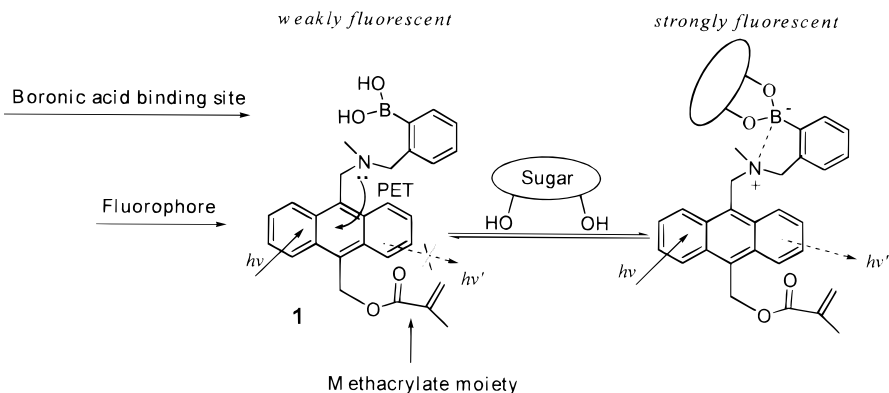
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prearrangement of the print molecule (template) and the functional monomers at low temperature so that complementary intermolecular interactions among functional groups can develop; (ii) polymerization of the monomers under conditions that cause minimal disturbance to the print molecule–monomer interactions; and (iii) 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 functional group orientations. Naturally, such polymeric receptors also have the potential to be developed as fluorescent sensors (17–29). 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 that could respond to the binding event with a significant fluorescence intensity change unless certain chromophores are present in the template molecule, which could quench the fluorescence of the fluorescent tag (26,27). 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 (22). Ideally, one would like to develop a fluorescent sensing system that is intrinsically sensitive to the binding event without the use of an external quencher. To this end, we have been interested in the synthesis of fluorescent sensors for carbohydrates because of their importance as biomarkers in different biological systems (30–34). Herein, we report our efforts in searching for ways to prepare fluorescent sensors for carbohydrates using molecular imprinting methods.

## RESULTS AND DISCUSSION

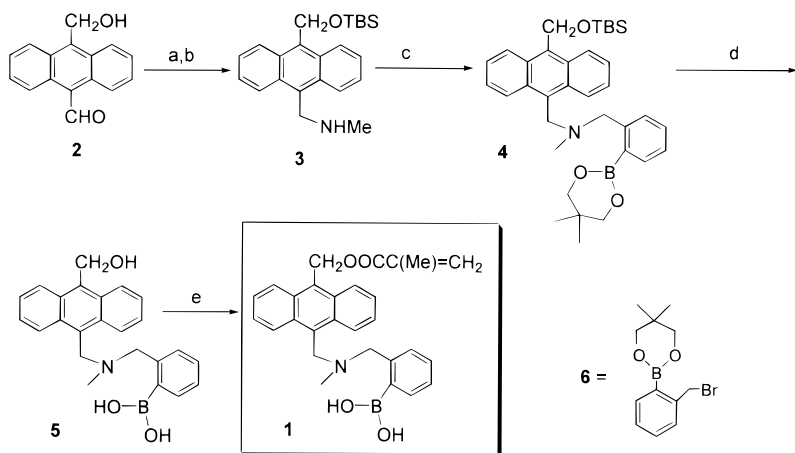
*The Design.* One of the key elements for the success of this project is the design of a functional monomer that has intrinsically strong intermolecular interactions with certain structural elements of carbohydrates and such interactions result in changes in fluorescence intensity. There are ample literature precedents showing that boronic acid derivatives can bind to *cis* diols tightly through ester formation (35–37) and boronic acid-containing monomers can be used for the preparation of selective binding sites for saccharides through molecular imprinting (38–40). 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 (41–43). One specific example is the conjugate of an anthracene moiety and a benzylamine moiety with boronic acid attached at the ortho position (44). 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 have designed 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 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 fluorescence of the anthracene moiety through photoelectron transfer (PET), this complex formation



**FIG. 1.** The binding of the carbohydrate with the boronic acid-containing monomer **1**.

will take away the quenching mechanism and, therefore, increase the fluorescence intensity (Fig. 1).

**Synthesis of the monomer.** The functional monomer (**1**) was synthesized in five steps starting from 10-(hydroxymethyl)-9-anthraldehyde (**2**) (**46**) (Scheme 1). Reaction of the hydroxy aldehyde (**2**) with *tert*-butyldimethylsilyl chloride (TBSCl) in the presence of imidazole in DMF at room temperature yielded the silyl ether aldehyde in 91% yield. Treatment of the silyl ether aldehyde with methylamine in methanol followed by reduction with sodium borohydride afforded the silyl ether amine **3** in 89% yield. The reaction of amine **3** with 2,2-dimethylpropane-1,3-diyl[*o*-(bromomethyl)phenyl]-boronate (**6**) (**44**) in the presence of potassium carbonate in acetonitrile

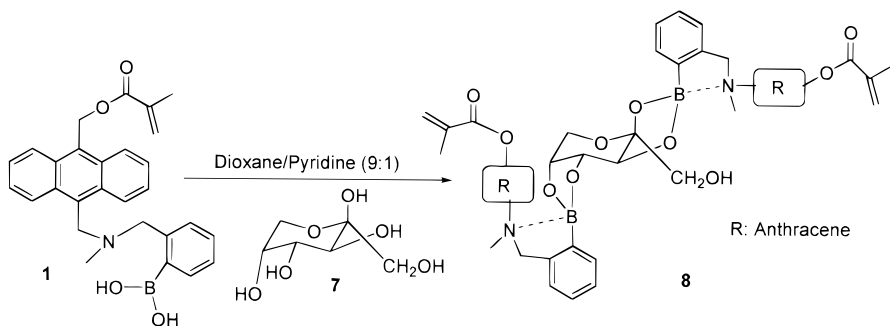


**SCHEME 1.** Synthesis of the functional monomer **1**. (a) TBSCl/imidazole/DMF, 91%; (b) (i)  $\text{NH}_2\text{Me}$ /MeOH, 6 h, (ii)  $\text{NaBH}_4$ /MeOH, 2 h, 89%; (c) **6**,  $\text{K}_2\text{CO}_3$ /MeCN, reflux, 42%; (d) TBAF/THF, 74%; (e) methacrylic anhydride, DMAP/TEA/ $\text{CH}_2\text{Cl}_2$ , 70%.

gave the 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 work-up gave **5** in 74% yield. Reaction of **5** with methacrylic anhydride in dry  $\text{CH}_2\text{Cl}_2$  in the presence of DMAP (4-dimethylaminopyridine) afforded the methacrylic ester (**1**) in 70% yield.

**Imprinted polymer preparations.** The utility of the monomer (**1**) prepared was tested through template-directed polymerization using D-fructose (**7**) as the print molecule. D-Fructose was chosen as the test compound because it was known to bind tightly with a boronic acid moiety in a 1:2 ratio (40). The D-fructose-boronic acid complex (**8**) was preformed so as to maximize the complementary interactions at the binding sites. Therefore, the 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 the reaction mixture yielded the complex **8**, which was confirmed with FAB-MS (1015,  $\text{M}^+ + \text{H}$ ). 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.

**Polymerizations.** For the imprinted polymer preparation, 2-hydroxyethyl methacrylate (HEMA) was used as a comonomer to afford other possible interaction sites with the carbohydrate. For the imprinted polymers to maintain certain three-dimensional structures, it is important that the polymers have a high degree of cross-linking. Ethyleneglycol dimethacrylate (EGDM) was used as the cross linker. For the free-radical initiated polymerization reactions, we have tested both AIBN-initiated polymerization and transition metal-catalyzed atom transfer radical polymerization (ATRP). AIBN-initiated polymerization has traditionally been used for the preparation of imprinted polymers. However, such bulk polymerization methods often suffer from the drawbacks of poor control of the macromolecular structures and the lack of homogeneity in the polymers prepared. Therefore, there has been a great deal of interest in developing new methods that can be used for preparing imprinted polymers (47–51). In recent years, controlled/“living” radical polymerization, using transition metal-catalyzed atom transfer radical polymerization (ATRP), has been developed for making new polymeric materials (52). Several transition metal systems have been



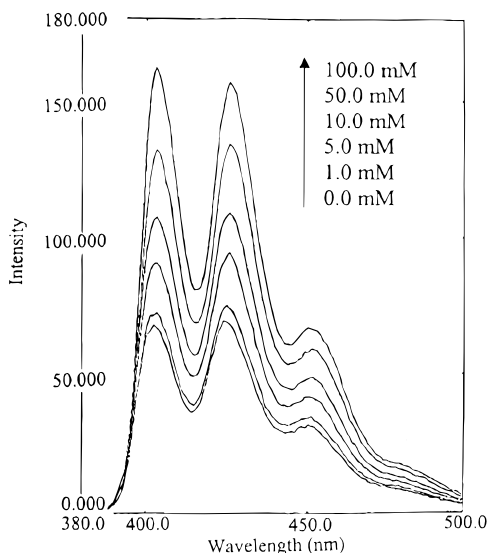
**SCHEME 2.** Synthesis of the boronate–D-fructose complex **8**.

used in ATRP (53–59). ATRP was said to have better control of the final average molecular weight of the polymer by varying the initial monomer to initiator ratio while maintaining a narrow molecular weight distribution. Therefore, we were also interested in examining how the use of ATRP would affect of the overall outcome of molecular imprinting.

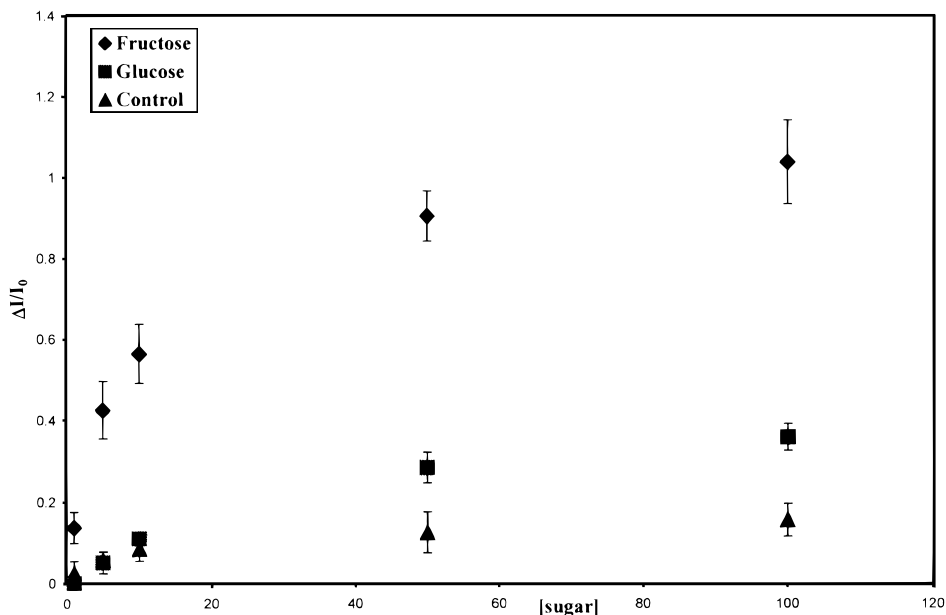
Again, for the polymerization HEMA was used as a comonomer and EGDM was used as the cross-linker. The AIBN-initiated polymerization was carried out using a procedure similar to one previously reported (22). The ATRP was carried out using copper chloride (CuCl) as the catalyst, 2,2'-bipyridine (bpy) as the ligand, and ethyl 2-bromoisobutyrate (BriB) as the initiator in DMF (60). The polymers prepared were ground, washed, and dried before they were used for the evaluation of their binding to the target carbohydrate.

*Fluorescent binding studies of the imprinted polymers.* 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<sub>2</sub>O (v/v) with 0.05 M of phosphate as the buffer at pH 7.4. The emission spectra were recorded from 380 nm to 550 nm with an excitation wavelength of 370 nm. The fluorescence binding studies were conducted in triplicate.

As designed, all imprinted polymers showed significant fluorescence intensity changes upon the addition of the print molecule, D-fructose, and the fluorescence intensity increase is concentration-dependent. A typical set of fluorescence spectra is shown in Fig. 2. Figure 3 shows the concentration-depended fluorescence intensity changes of the polymer prepared using AIBN-initiated polymerization (**P-a**) and Fig.



**FIG. 2.** A typical set of fluorescence spectra of D-fructose-imprinted polymer (**P-b**) at different concentrations of D-fructose ( $\lambda_{\text{ex}}$ , 370 nm;  $\lambda_{\text{em}}$ , 426 nm).



**FIG. 3.** Fluorescence intensity changes of D-fructose-imprinted polymer (**P-a**) vs concentration of sugars ( $\lambda_{\text{ex}}$ , 370 nm;  $\lambda_{\text{em}}$ , 426 nm).

4 shows the concentration-depended fluorescence intensity changes of the polymer prepared using atom transfer radical polymerization (**P-b**).

Because boronic acid was known to bind to any compounds with a *cis* diol structural moiety, the fluorescence intensity changes in response to D-fructose itself, as shown in Figs. 2–4, do not necessarily indicate that the imprinting process created specific polymeric receptors for D-fructose. Furthermore, very often the boronic acid moiety shows preferential binding for fructose (44,45,61) and additional structural constrains are needed to change this preference (62–67). Therefore, several control experiments were conducted. First, the binding of the polymeric receptors with D-glucose was also examined. It was found that the fluorescence intensity changes of the imprinted polymers upon addition of glucose at the same concentrations were far smaller than that of D-fructose (Figs. 3 and 4). Second, the effect of D-fructose and D-glucose 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 the fluorescent monomer **1** also exhibited preferential recognition of D-fructose (Table 1), however, to a lesser degree than that of the imprinted polymers (**P-a** and **P-b**). The ratio of  $(\Delta I/I_0)_{\text{fru}}/(\Delta I/I_0)_{\text{glu}}$ , indicating this preference, was found to be about two-fold greater for the AIBN-initiated polymer (**P-a**) than for the monomer **1** (Table 1 and Fig. 5). The polymer prepared using ATRP showed comparable or greater preference for D-fructose (Table 1 and Fig. 5), the template molecule. Furthermore, the control polymer prepared in the absence of D-fructose showed minimal fluorescence intensity changes upon addition of