Molecular design of sugar recognition systems by sugar–diboronic acid macrocyclization

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Abstract Boronic acid is known to complex with saccharides in basic aqueous media with an inherent selectivity order. Cooprative binding of diboronic acid which creates a sugar diboronic acid macrocycle has been utilized in design of molecular receptors with a diffrent selectivity order. Cooparative binding together with photoinduced electron transfer mechanism has been utilized in design of sensitive and selective saccharide sensors.

Boronic acids are known for a long time to form complexes with diols rapidly and reversibly in basic aqueous media (1). Saccharides, having prearranged cis-diols, form stronger complexes with boronic acids and therefore are useful as an interface for artificial sugar receptors. The complexing ability of the phenyl boronic acid **1** is inherently better for fructose relative to all other commonly available sugars including glucose (2). Glucose is one of the worst bound monosaccharides to phenyl boronic acid. Since glucose is one of our prime target species we need to achieve better selectivity and sensitivity for glucose and also for other common saccharides. As most saccharide species contain more than two hydroxy groups with or without sufficient reactivity difference between them, it may be appropriate to design diboronic acid or polyboronic acid species of which boronic acid moieties can cooperatively bind with hydroxy groups. We have demonstrated the feasibility of such cooperative binding and the specificity that can be achieve by arranging boronic acid moieties in a single molecular receptor system.
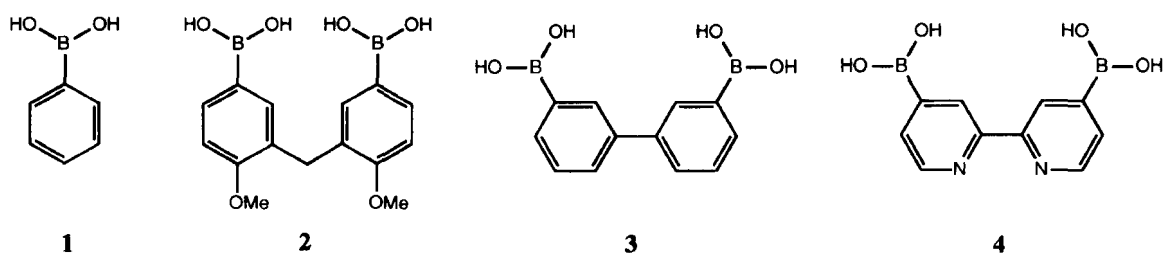
Simple boronic acids reversibly complex with saccharides in basic aqueous media creating five- or six-membered covalent esters. The quantitative evaluation of saccharide-boronic acid interaction was first reported by Lorand and Edwards (2) in 1959 and summarized in Table 1. The diboronic acid **2** has two independent boronic acid moieties which are attached by a single methylene bridge at the m-position. Two boronic acid moieties in **2** can cooperatively bind to monosaccharides creating a rigid, macrocyclic species with induced chirality. The formation of a rigid complex with induced chirality could be detected by circular dichroism (CD) spectroscopy. The sign of the exciton coupling of the CD band provides an excellent means of reading out the chirality of the saccharide. As an example D- and L-glucose providing opposite signs in exciton coupling (Figure 1). In this case very high selectivity against fructose has been achieved since fructose gave virtually no CD activity (3,4,5).

Table 1. Stability constants for saccharide: boronic acid complexes of **1** and **2** in basic aqueous media

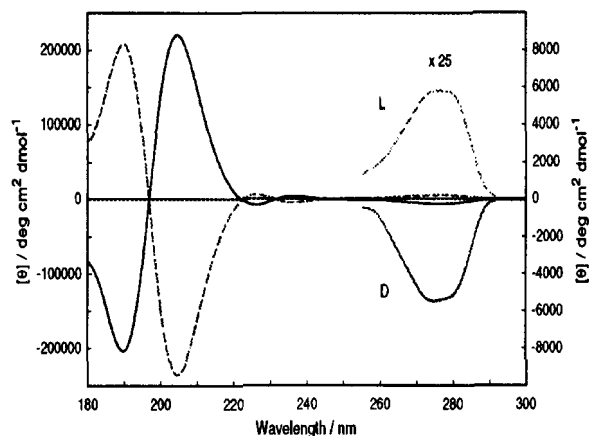| Saccharide	**1** K (dm^3 mol^{-1})	**2** K (dm^3 mol^{-1})
D-glucose	110	19000
D-fructose	4370	-
D-mannose	172	60
D-galactose	276	2200
D-talose	-	4600

Figure 1. CD spectra of **2** in the presence of D-glucose (D) and L-glucose (L): [glucose] = 2.00 x 10^{-3} mol dm^{-3}, [**1**] = 1.00 x 10^{-3} mol dm^{-3}, pH 11.3 with 0.10 mol dm^{-3} carbonate buffer, 25 °C.

It is widely accepted that the 1,2-hydroxy groups of monosaccharides act as the primary binding site. There are number of possibilities for the second binding site since glucose, as an example, may exist and may bind as either furanose or pyranose form. The absolute structure of **2** and glucose is still in dispute despite detailed NMR (^{1}H, ^{13}C and ^{11}B) investigations into the structure of such complexes (6).

Diboronic acid **3**, which has a larger spacing between the two boronic acid moieties, was expected to give strong association with disaccharides. However, due to mismatching of the spacer, association constants for disaccharides were much less than those for the complex of monosaccharides and **2**. The CPK model revealed that the distance between the two boronic acids in **3** is shorter than the distance between the prospective hydroxy groups of the disaccharides. Accordingly, the CD activity was much weaker and was not adequate for the estimation of association constants (7). The related diboronic acid **4** and also its Fe^{2+} (as FeCl$_3$) complex gave CD active saccharide complexes. The CD activity of **4** was also derived from the asymmetric immobilization of the two pyridine units. Fe^{2+}.**4** gave a CD band in the region of the metal to ligand charge transfer band. Δ or Λ complexes were found depending on the saccharide it complexes with. For example, D-maltose complex adopted the Λ chirality whereas D-cellobiose complex adopted the Δ chirality. However, the formation of a 1:1 cyclic complex with the diboronic acid was not a prerequisite for the CD activity in the Fe^{2+}:boronic acid complex. The formation of 1:2 complexes of diboronic acid:saccharide also gave CD activity (8).

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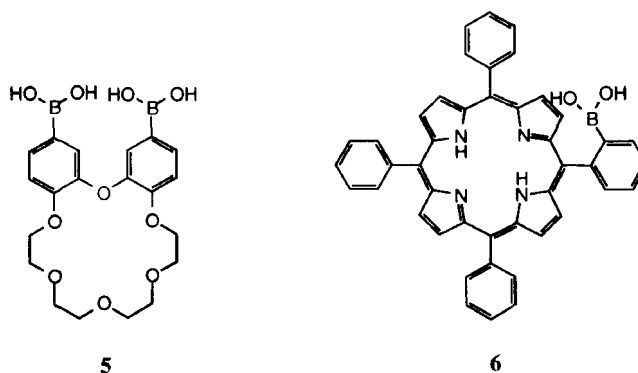
Allosteric Interactions

Nature relies on allosteric interactions in many biological functions. The transport of sugars across a cell membrane into the cell is controlled by such interactions. In an attempt to mimic such systems, a diboronic acid saccharide binding unit has been incorporated into a simple crown ether derivative. The control of the angle between two phenyl rings of **2** by a secondary effect could result in changes of sugar binding ability to **2**. Having a very similar binding site to **2** and lower flexibility in rotation of the phenyl rings, **5** exhibits even larger association constants for glucose (31000 dm^{3}mol^{-1}) in basic methanolic aqueous solutions (CD measurements). The binding of metal ions to the crown ether has been monitored by ^{1}H NMR spectroscopy. Metal ion binding to the crown ether induces a twist in the crown ether resulting in negative allosterism (9).

Two Point Binding of Glucose Phosphates

Glucose phosphates are important molecular species in biological energy storage processes. It has been selectively recognized against glucose-1-phosphates by the boronic acid appended metalloporphyrin derivative **6**. The two point binding of glucose-6-phosphate creates a rigid complex which gives a strong exciton coupling signal in its CD spectrum. It is believed that the strong binding by the primary binding site of glucose (1,2-diol) to the boronic acid followed by the secondary interaction of phosphate-metal center creates the strong binding ability of glucose-6-phosphate. The replacement of 1-hydroxy group, which is a part of the primary binding site of glucose, by phosphate unit in glucose-1-phosphate weakens the saccharide-boronic acid interaction and hence the overall strength of the complex. Binding of phosphate into the metal center is displayed by the ^{31}P NMR peak shifts. CD exciton coupling peaks were found neither for metal ion free **6** with glucose derivatives nor for the **6** with glucose, indicating the importance of two point binding (10).

Fluorescence sensors based on Molecular rigidification

The 1:1 binding creates a rigid molecular complex. This rigidification effect can be utilized in designing fluorescence sensors for disaccharides. Diboronic acid species **7** selectively complexes with disaccharides in basic aqueous media to create cyclic complexes which alter the fluorescence properties of the molecule. It is known that excited stilbene is quenched by radiationless decay via rotation of the ethylene double bond. Obstruction to such rotation leads to increased fluorescence (Scheme 1). The rigidification of **7** by disaccharide binding increases the stilbene fluorescence (Figure 2). In particular, the disaccharide melibiose shows high selectivity for **7** against other common disaccharides and some trisaccharides (11).

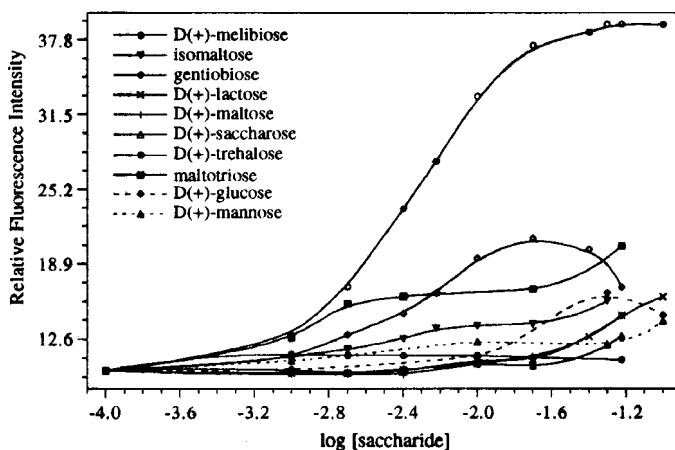**Non Fluorescent**

$+ H_2O$ ‖ $- H_2O$
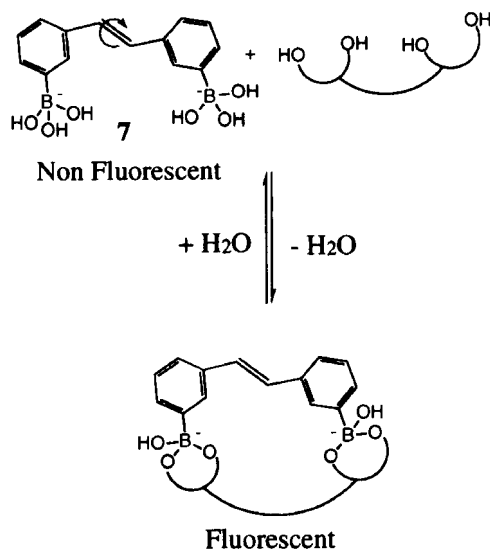
Fluorescent

Scheme 1

Figure 2. Fluorescence titration of stilbene diboronic acid **7** (1.0×10^{-5} mol dm^{-3}) at pH 10.6 (0.1 mol dm^{-3} sodium carbonate / sodium bicarbonate buffer) as a function of log of disaccharide concentration (λ_{ex} 310 nm, λ_{em} 358 nm)

Fluorescnece Sensors Based on Photoinduced Electron Transfer

The main disadvantage of boronic acid is its requirement of basic conditions for saccharide binding. Facile saccharide binding occurs upon formation of sp^3 boron center in basic media. The introduction of an amine group as a neighboring group participant (NGP) to the boron center facilitates the sp^3 conversion hence providing a receptor which works at neutral bulk pH. The boron nitrogen interaction also provides a better electron center around the boron center which helps in designing photoinduced electron transfer (PET) sensors. The fluorescent PET sensor **8** was designed based on such electron rich center. The saccharide binding to the boronic acid at neural pH strengthens the boron-nitrogen bond suppressing PET process from the nitrogen to excited anthracene. When the boronic acid is free from saccharide, the weak interaction does not suppresses or very weakly suppress the PET process providing a large fluorescence increase upon saccharide binding. Interestingly, NGP does not alter the inherent selectivity order of boronic acid towards simple monosaccharides. The arrangement of such binding units together with fluorescent signaling moieties has provided sensitive and selective molecular sensors for saccharides (12).

Table 2. Stability constants ($\log K_a$) for the saccharide complex with boronic acid **8**, **9** and **10**. pH 7.77 (0.05 mol dm^{-3} phosphate buffer in 33% aqueous methanol)

saccharide	**8** $\log K$ [a]	**9** $\log K$ [a]	**10** $\log K$ [b]
D-glucose	1.8	3.6	3.3
D-fructose	3.0	2.5	_[c]
D-allose	2.5	2.8	2.9
D-galactose	2.2	2.2	2.9
ethylene glycol	0.4	0.2	-0.5

[a] Measurements were done in 33% methanolic aqueous solutions. [b] Measurements were done in 67% methanolic aqueous solutions. [c] The plot of relative fluorescence intensity *vs.* saccharide concentration could not be analysed precisely by a simple Benesi-Hildebrand type equation assuming the formation of a 1:1 complex.
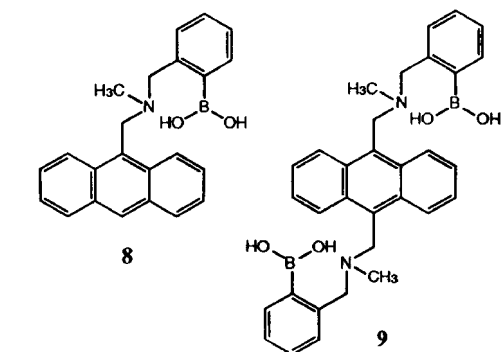
The arrangement of two boronic acids in "a cleft like" structure in **9**, using anthracenyl moiety as both the rigid spacer and the fluorescent moiety was found to be excellent in the selective recognition of glucose (13). Selectivity for glucose has more than doubled in **9** in comparison to **8** while conserving the high "switch-on" factors (Figure 3 & Table 2). The formation of a large macrocyclic structure on the 1:1 binding of glucose by **9** holds glucose close to the anthracene aromatic face. The C3 proton (δ = -0.3 ppm) of D-glucose, in particular, points towards the π-electrons of the anthracene moiety giving very large paramagnetic shift in ^{1}H NMR (Figure 4). The existence of a 1:1 complex of **9** and D-glucose was further assisted by mass spectral data of the complex. The cooperative binding of saccharides, specifically glucose, occurs in very low saccharide concentrations. Due to the PET design non-cyclic 1:1 bound species could not be detected by fluorescence spectroscopy; only the 1:1 cyclic and 1:2 complexes give fluorescence signal. In human blood three main monosaccharides are present: D-glucose(0.3 - 1.0 mM), D-fructose ($\leq$ 0.1 mM) and D-galactose ($\leq$ 0.1 mM). Competitive binding studies show that **8** is very suitable for the detection of glucose at physiological levels.
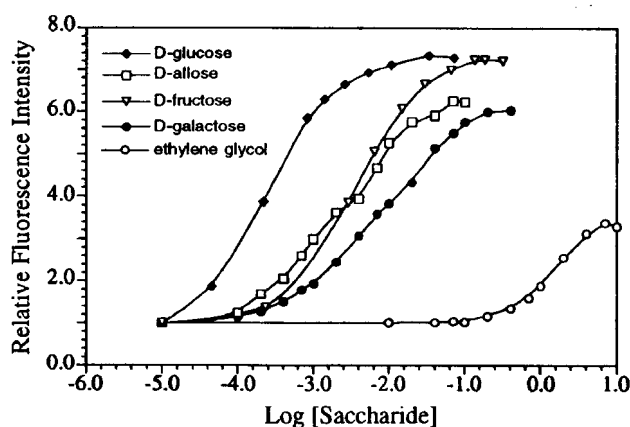

Figure 3. Fluorescence intensity versus log [saccharide] profile of **9** at 25 °C; 1.2x10^{-5} mol dm^{-3} of **9** in 0.05 mol dm^{-3} sodium chloride solution, [saccharide or ethylene glycol] = 0.05 mol dm^{-3}.

Figure 4. ^{1}H NMR of the D-glucose 1:1 complex of **9** in methanol-d$_4$.
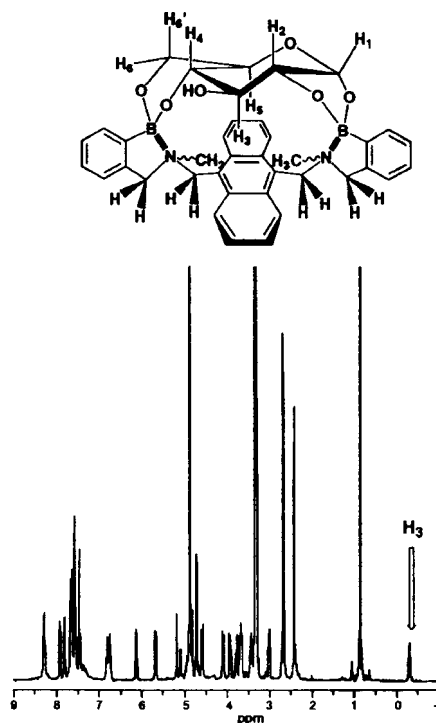
Figure 4

Diboronic acid derivative **10** which has a flexible spacer between the boronic acids behaves similar to **8**. Pyrene fluorophores which are capable of forming excimers give two dimensional information on both the saccharide concentration and the structure of the complex. The 1:1 binding of saccharide to **10** leads to increasing monomer fluorescence intensity. Monomer fluorescence increase was partially produced by the decrease in excimer formation and the over all fluorescence quantum yield is chiefly increased via the suppression of the PET process (Figure 5). The 1:2 binding of **10** to saccharides, on the other hand, increased the excimer:monomer fluorescence intensity ratio. In all cases the formation of a 1:1 complex was seen at low concentrations of saccharides, and as the saccharide concentration increased the predominant complex changes to 1:2. The stability constants for saccharides are given in the Table 2. The selectivity of **10** was found to be similar to that of **9** (14).
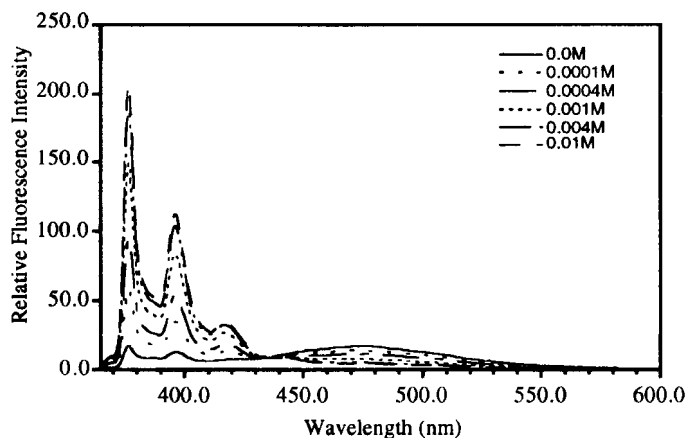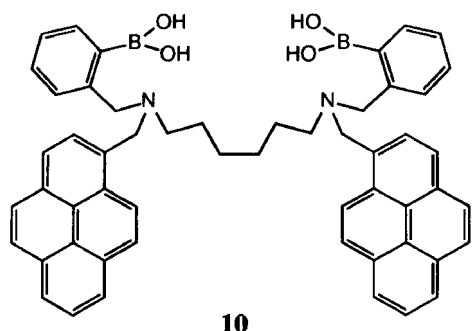

10

Figure 5. Fluorescence spectral changes of **10** $(1.5 \times 10^{-5}$ mol dm$^{-3})$ with different concentrations of glucose in a methanol:water (3:1) mixture. excitation at 343 nm.

Chiral Recognition of Saccharides

The success in molecular design of PET systems has prompted the design of **10** (R and S) for chiral recognition of monosaccharides. Work by Irie *et .al.* on the control of intermolecular chiral 1,1'-binapthyl fluorescence quenching by chiral amines (15) and the use of 1,1'-binaphthyl in the recognition of chiral amines by Cram *et. al.* (16) have been the inspiration in the design of **11**. Chiral recognition of saccharides by **11** utilizes both steric and electronic factors. The asymmetric immobilization of the amine groups relative to the binaphthyl moiety upon 1:1 complexation of saccharides by D- or L-isomers creates a difference in PET. This difference is represented in the highest fluorescence intensity of the complex. Steric factors arising from the chiral binaphthyl building block are chiefly represented by the stability constant of the complex. However, the inter-dependency of electronic and steric factors upon each other is not ruled out. Figure 6 shows the saccharide titration of R and S isomers with D- and L-saccharides. This new molecular cleft with a longer spacer unit compared to the anthracene based diboronic acid **9**, gave best recognition for fructose. D-fructose was best bound by (R)-**11** with a large fluorescence increase. Table 3 shows the binding constants for some D- and L-monosaccharides.
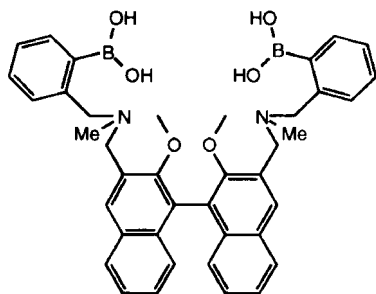

11 R and S

Table 3. Stability constants for D- and L- saccharides with (R)-**11** (or (S)-**11**) in pH 7.77 (0.05 mol dm^{-3} phosphate buffer , 33% methanolic aqueous solution)

Saccharide	D log$K$ (±0.05)	L log$K$ (±0.05)
Fructose	4.0 (3.7)	3.5 (4.0)
Glucose	3.3 (3.4)	3.1 (3.5)
Galactose	3.1	3.3
Mannose	<2.4	-
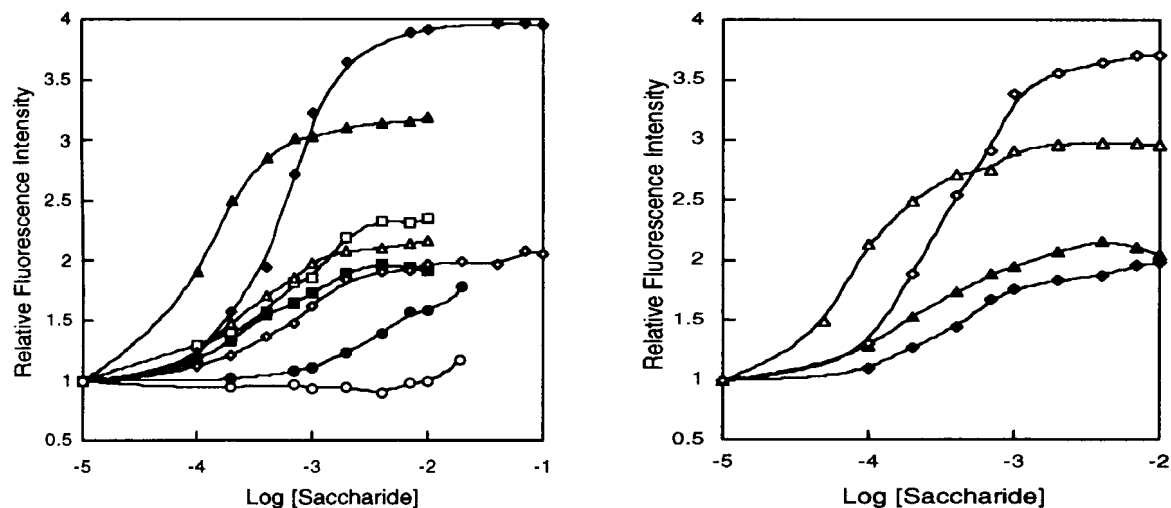
Figure 6. LEFT: Fluorescence intensity versus log [saccharide] profile of (R)-11 at 25 °C; 1.0×10^{-5} mol dm^{-3} of (R)-11 in 33.3% MeOH/ H$_2$O buffer at pH 7.77, λ_{ex} 289nm, λ_{em} 358nm. RIGHT: Fluorescence intensity versus log [saccharide] profile of (S)-11 at 25 °C; 1.0×10^{-5} mol dm^{-3} of (S)-11 in 33.3% MeOH/ H$_2$O buffer at pH 7.77, λ_{ex} 289nm, λ_{em} 358nm. ◆ D-glucose; ◇ L-glucose; ■ D-galactose; □ L-galactose; ▲ D-fructose; △ L-fructose; ● D-mannose; ○ L-mannose.

The chiral recognition of saccharides by polyhydroxy molecules in aprotic nonpolar media has been reported. However, discriminative detection of isomers in aqueous media, as far as we are aware, has not been achieved before. In this system steric factors and electronic factors bimodally discriminate the chirality of the saccharide. Competitive studies with D- and L-monosaccharides show the possibility of selective detection of saccharide isomers. The advantage of availability of the R and S isomers of this particular molecular sensor provides even wider possibilities since concomitant detection by two probes is possible (17).

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