Novel Saccharide-Photoinduced Electron Transfer Sensors Based on the Interaction of Boronic Acid and Amine

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Received November 4, 1994[⊗]

Abstract: Two boronic acid systems, monoboronic acid **3** and diboronic acid **8**, were synthesized. When saccharides form cyclic boronate esters with these boronic acids, the Lewis acid-base interaction between the boronic acid moiety and tertiary amine is strengthened; when saccharides form cyclic boronate esters with boronic acids the acidity of the boronic acid is enhanced. The strength of this acid-base interaction modulates the photoinduced electron transfer (PET) from the amine to anthracene. Both of these compounds show increased fluoresecence at pH 7.77 through supression of the photoinduced electron transfer from nitrogen to anthracene on saccharide binding, a direct result of the stronger boron-nitrogen bond. Compound **3** shows the typical selectivity of monoboronic acids towards saccharides. Compound **8** which has a cleftlike structure is particularly selective and sensitive for glucose due to the formation of an intramolecular 1:1 complex between the two boronic acids and the 1,2- and 4,6-hydroxyls of glucose. This is the first example in which ditopic recognition of monosaccharides is achieved in a PET sensor system.

Introduction

Saccharides are nature's conveyors of energy and therefore essential for cell survival.[1] The breakdown of glucose transport has been correlated with certain diseases: renal glycosuria,[2,3] cystic fibrosis,[4] diabetes,[5,6] and also human cancer.[7] The study of glucose and other monosaccharide gradients *in vivo* is therefore of seminal importance.

Photoinduced electron transfer (PET) has been employed as a stratagem of fluorescent sensor design for many analytes.[8] The remote nature of the binding site and the fluorophoric site in PET systems provides a way to preciswly design molecular sensory systems. The binding ability of the parent binding site is preserved in the PET system. Also, the switching of fluorescence intensity achieved by remote suppression of PET quenching preserves the properties of the fluorophore except for the fluorescence intensity, providing excellent detection ability. The design of fluorescent sensors for neutral organic species presents a harsher challenge due to the lack of sufficient electronic changes upon inclusion. Complexation of neutral saccharides with boronic acid via covalent interactions in aqueous media has drawn our attention[9-24] and that of others[25-31] due to its superiority over hydrogen bonding interactions found

[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1995.

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(31) From our work and that of others (references 9-30) it is known that the boronate ester is rapidly and reversibly formed under basic conditions. Noncovalent interactions are described using such terms as "recognition", "complex", and "binding constants". These terms will be used to describe the equilibrium between covalent boronate ester and free boronic acid at high pH.

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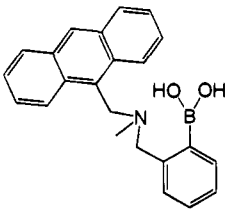
in other synthetic receptor systems in aqueous media.[32] Saccharides are only soluble in polar protic solvents, but such solvents are competitive with the guest in a hydrogen bonding receptor. However, the design of a fluorescent sensor based on the boronic acid–saccharide interaction has been difficult due to the lack of sufficient electronic changes found in either the boronic acid moiety or the saccharide moiety. These above-mentioned disadvantages of boronic acid saccharide interactions can be overcome by modifying the boronic acid binding site to create an electron rich center around the boronic acid moiety.[30,33] In the molecular sensor[24a] **3**, for example, the basic skeleton of a known PET sensor **9** has been preserved within the framework.[34] The intramolecular interaction of the amine with the boronic acid which creates a five-memberd ring is modulated by saccharide binding. The formation of a five-membered ring precludes the nitrogen lone pair of electrons from photoinduced electron transfer and consequent fluorescence quenching of the anthracene moiety.



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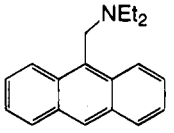

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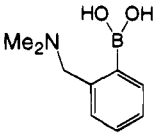

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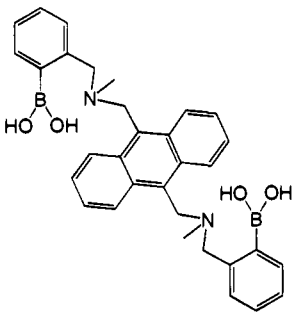
Czarnik[25] has observed that 2-anthrylboronic acid displays enhanced acidity upon binding to saccharides and consequent fluorescent suppression by the boronate anion. However, the suggested photoinduced electron transfer from the boronate anion was not efficient despite the fact that boronate anion is directly bound to the chromophore (I (in the presence of saccharide)/(I_0 in the absence of saccharide) = *ca.* 0.7).

Specific detection of saccharides is our aim, but to achieve this goal we must manipulate the characteristics of the boronic acid. Previously we found that two point interrogation by a diboronic acid is the most expeditious approach toward the selective detection of saccharides.[11–14] The spatial disposition of the two boronic acid moieties determines which saccharide is bound preferentially. With PET sensor **3** in mind and by reference to molecular models we discovered that the 9,10-bis-(aminomethyl)anthracene skeleton **8** is a perfect glucose cleft,[24b] and the structure of a known PET sensor **10** has also been preserved within the framework.[35] Compound **8** as drawn appears to have significant comformational flexibility. However, that flexibility is greatly reduced when a boron–nitrogen bond is formed, and a convergent binding cavity composed of two inwardly facing boronic acids results. The two convergent boronic acid moieties of compound **8** are also perfectly spaced and aligned for the 1,2- and 4,6-hydroxyls of glucose.



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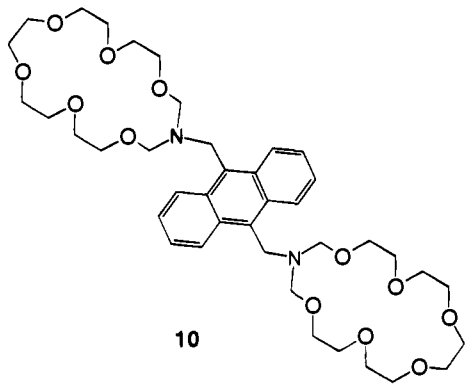

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Herein we report on our detailed investigations into the saccharide-binding properties of a monoboronic acid[24a] and diboronic acid[24b] PET system.

Results and Discussion

Synthesis. The syntheses of **3** and **8** were both simple and facile. The routes employed are given in Scheme 1 (for details see the Experimental Section).

Fluorescence Measurements. pH Titrations. The fluorescence pH profiles of **3** and **8**, in unbuffered aqueous media, are given in Figure 1. For **3** a large step at low pH (pK_a = 2.9) and a possible small step at high pH are observed; with **8** a large step at low pH (pK_a 4.8, from the curve two pK_a's are evident but only the average could be determined), and also a possible small step at high pH is observed. The pK_a values of **9** and **10** are known to be 9.3 and 7.1, respectively (fluorescence measurements in ethanolic aqueous media).[34,35] The large shift of the pK_a is due to the interaction found between the boronic acid moiety and the amine group. The boronic acid–amine interaction inhibits the photoinduced electron transfer quenching process in the complex **3b** (Scheme 2). Complete separation of the amine and the acid moiety at very high pH, as in **3c**, quenched the anthracene fluorescence further. However, the fluorescence decrease is not sufficient for the calculation of the pK_a. The introduction of D-glucose remarkably enhances the fluorescence of **3** over large pH (Figure 1). The enhanced interaction between boronic acid and amine, upon saccharide binding, inhibits the electron transfer process giving higher

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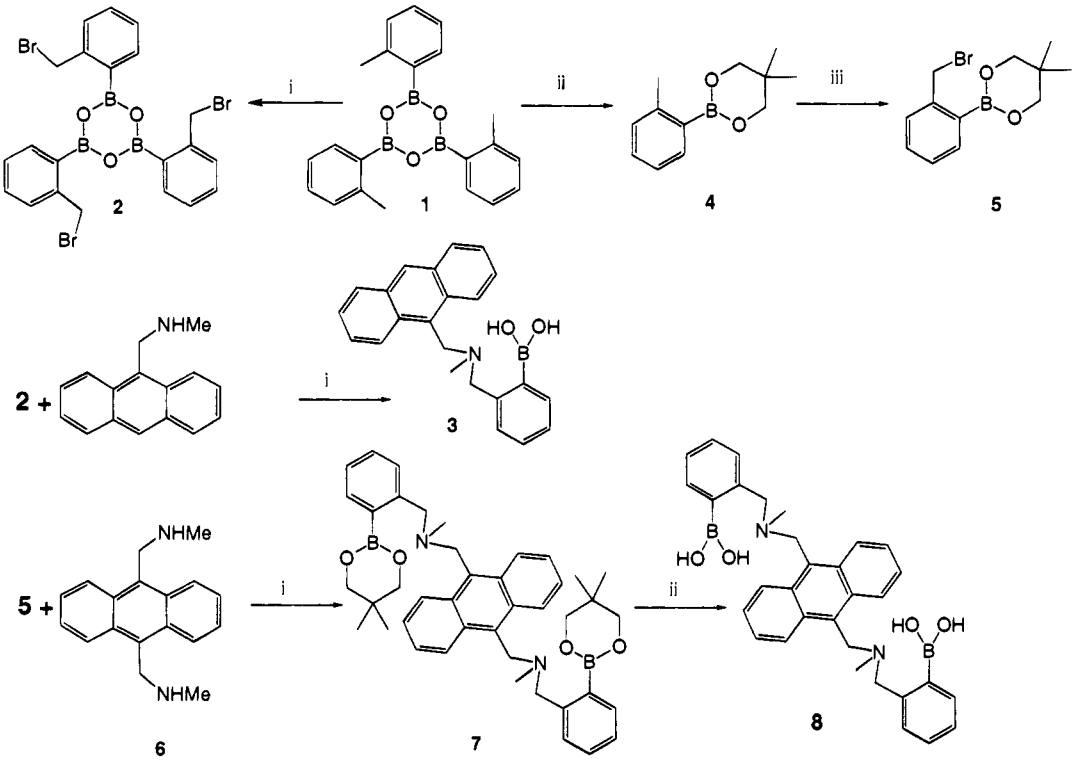
Scheme 1.[a] Synthesis of Boronic Acid Derivatives **3** and **8**



[a] Reagents (yields) (top) (i) NBS, AIBN, CCl_4, Δ (60%); (ii) 1.1 equiv of 2,2-dimethyl-1,3-propanediol, $PhCH_3$, Dean–Stark (88%); (iii) NBS, AIBN, CCl_4, Δ (95%); (middle) (i) 2.1 equiv of amine, $CHCl_3$ (33%); (lower) (i) 0.4 equiv of diamine, 1.1 equiv of K_2CO_3, THF, Δ (25%); (ii) 33.3% MeOH/H_2O, pH 7.77 (quant).

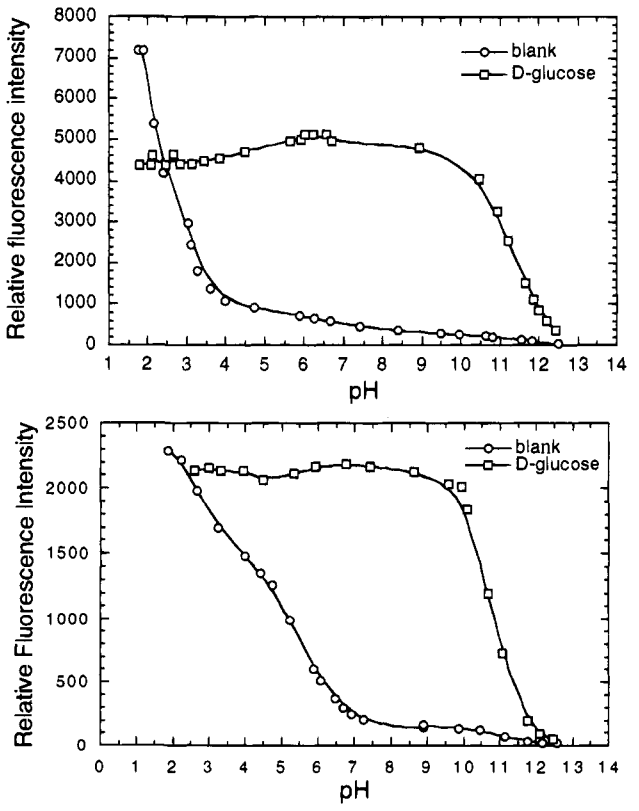


Figure 1. Fluorescence intensity pH profile of **3** (top) and **8** (lower) at 25 °C; 1.2×10^{-5} M of **3** or **8** in 0.05 M sodium chloride solution, [saccharide or ethylene glycol] = 0.05 M.

fluorescence (as **3d** in Scheme 2). This increased interaction would be expected since the saccharide binding to boronic acid increases its acidity[36] creating a more electron-deficient boron atomic center. With compound **8** similar equilibria to those observed with **3** exist. However for clarity, only species arising from neutral **8** and saccharide are shown (Scheme 3). The acidic and basic equilibria also exist but none of these species are fluorescent. (*cf.* compound **3** in Scheme 2). For compound **8** two possible saccharide binding modes can inhibit the electron transfer process so giving higher fluorescence; the 2:1 complex **8a** and 1:1 complex **8b**. The pK_a values for the saccharide complexes calculated from fluorescence measurements at high pH ($pK_a = 11.1$) are the same for **3** and **8** and are in line with the second pK_a of **11** in the absence of sugar ($pK_a = 11.8$).[30] From these fluorescent measurements we could not estimate the first pK_a of the saccharide–**3** or –**8** complex due to insufficient changes in fluorescence intensities.

Saccharide Tirations. Having proven the validity of our design stratergy, we had to determine the selectivity and sensitivity of our saccharide PET sensors. Aqueous solution at physiological pH is the best testing ground for any saccharide sensor. Therefore, selectivity studies were carried out in 33% methanol/water buffered at pH 7.77. A water-only buffer was suitable at low saccharide concentrations, but at higher concentrations precipitation becomes a problem. A mixed solvent was therefore chosen to avoid any complications arising from precipitation.

The stability constants of **3** and **8** with the monosaccharides (D-glucose, D-fructose, D-allose, D-galactose) and ethylene glycol are given in Table 1. The curves from which the stability constants were calculated are shown in Figures 2 and 3, respectively.

The order of selectivity for monoboronic acid **1** (Table 1) is: D-fructose > D-allose ≈ D-galactose > D-glucose > ethylene glycol. This order is indicative of the inherent selectivity of all monoboronic acids.[9,18,19,21,24a,25] The order of monosaccharide selectivity for the diboronic acid **8** (Table 1) is D-glucose

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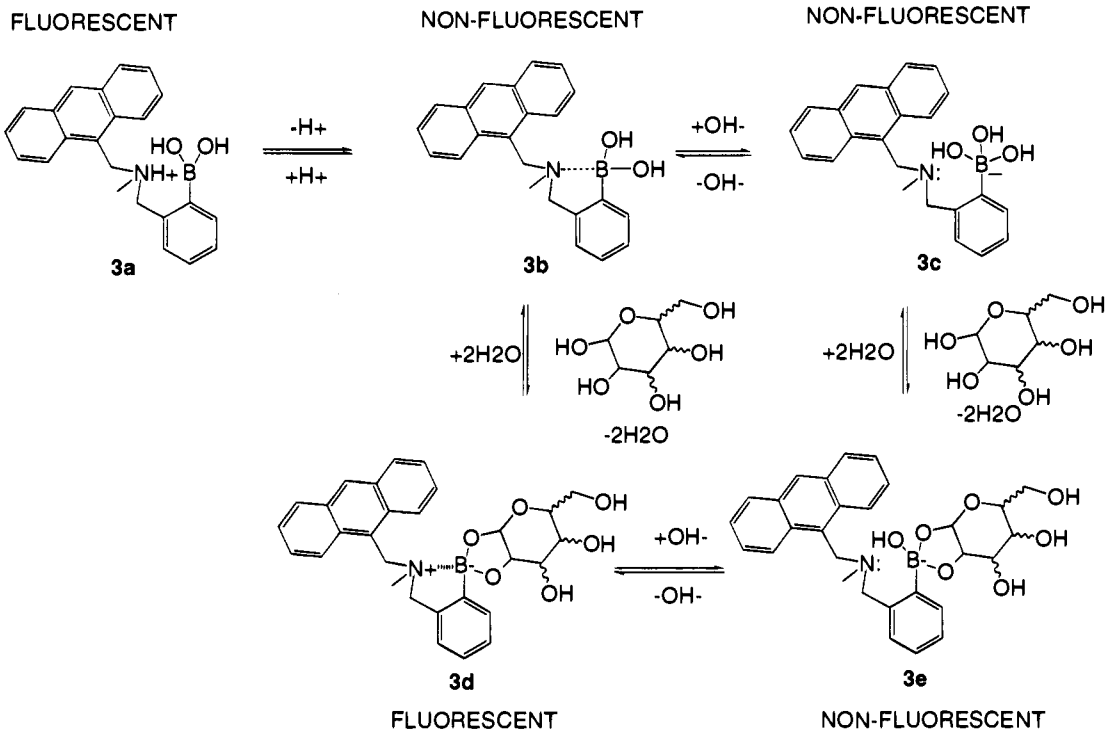
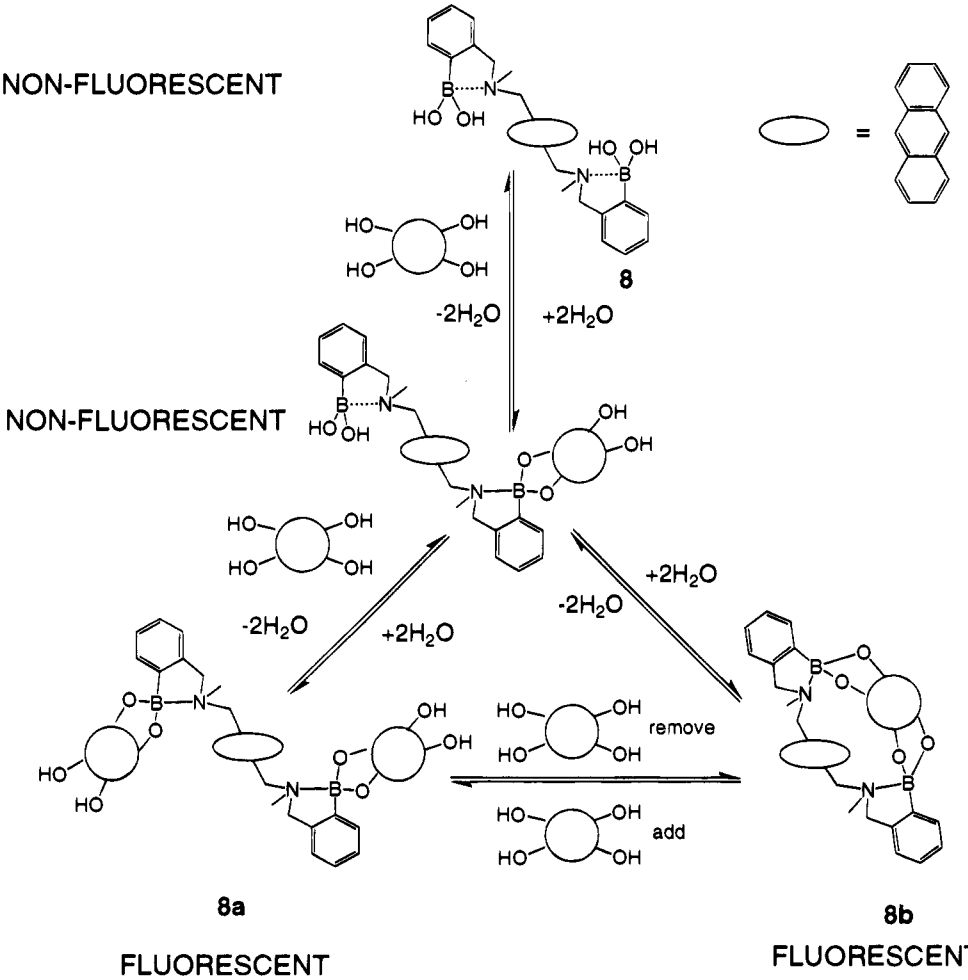
Scheme 2



Scheme 3



> D-allose > D-fructose ≈ D-galactose > ethylene glycol. This order is only indicative of the inherent selectivity of diboronic saccharide cleft **8**.

From Table 1 it can be seen that the relative stability constants for the monosaccharides (D-glucose, D-allose, and D-galactose) vary between compound **3** and **8**. The order of relative stability,

Table 1. Stability Constant (log K_a) for the Monosaccharide Complex with Boronic Acid **3** or **8**

saccharide or diol	boronic acid **3**: log K (r^2; data points)	boronic acid **8**: log K (r^2; data points)
D-glucose	1.8 (0.998; 9)	3.6 (0.998; 7)
D-fructose	3.0 (0.998; 9)	2.5 (0.999; 6)
D-allose	2.5 (0.995; 6)	2.8 (0.997; 9)
D-galactose	2.2 (0.998; 7)	2.2 (0.998; 11)
ethylene glycol	<0.4[a] (0.995; 4)	<0.2[a] (0.998;7)

[a] Upper limit calculated assuming that the observed $(I/I_0)_{max}$ is the saturation value.

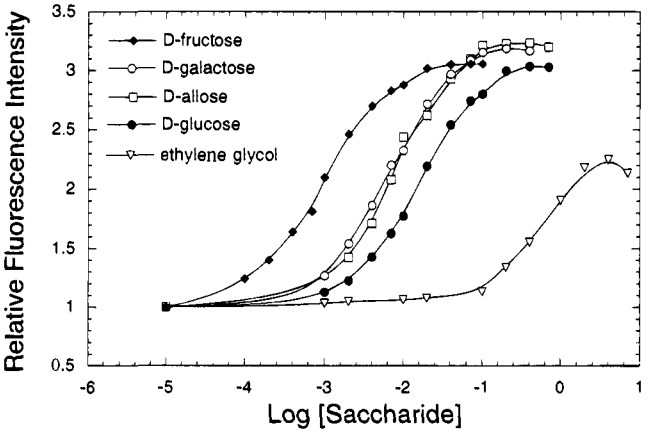


Figure 2. Fluorescence intensity log[saccharide or ethylene glycol] profile of **3** at 25 °C; 1.0×10^{-5} M of **3** in 33.3% MeOH/H_2O buffer at pH 7.77, λ_{ex} 370 nm, λ_{em} 423 nm.

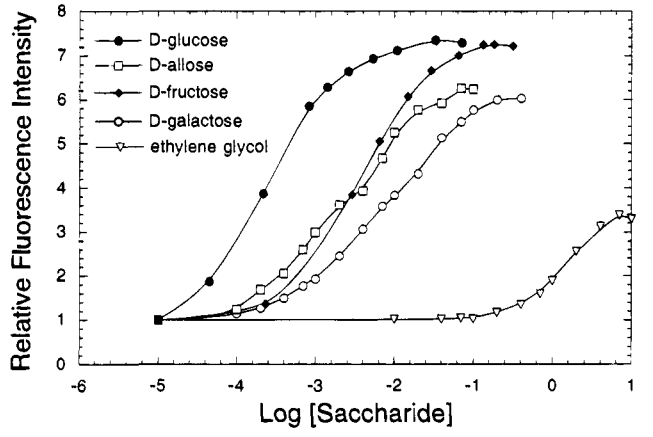
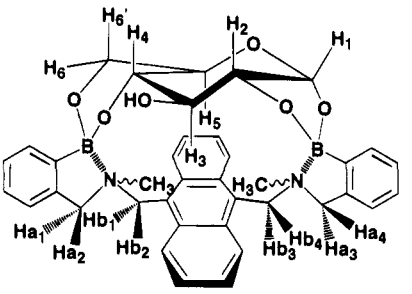


Figure 3. Fluorescence intensity log[saccharide or ethylene glycol] profile of **8** at 25 °C; 1.0×10^{-5} M of **8** in 33.3% MeOH/H_2O buffer at pH 7.77, λ_{ex} 370 nm, λ_{em} 423 nm.

where the relative stability is defined as (log K_2 − log K_1) is D-glucose (+1.8) > D-allose (+0.3) > D-galactose (0) > ethylene glycol (−0.2) > D-fructose (−0.5). This order is indicative of a switch in the active fluorescence species between **8a** and **8b**. With ethylene glycol and **8** the only fluorescent species that can exist is **8a**; conversely, the fluorescence molecular sensor **8** was designed to selectively bind glucose as a 1:1 complex **8b**. The order is therefore indicative of the relative stability of the 1:1 complex **8b**. Much to our satisfaction this selectivity order is in line with that predicted on the basis of CPK molecular models; from these molecular models glucose was the best fit with our saccharide cleft and fructose the worst.

^{1}H NMR and MS Analysis of Saccharide/8b Complex Stoichiometry. The existence of the 1:1 complex **8b** with D-glucose was substantiated by ^{1}H NMR spectroscopy. Figure 4 shows the ^{1}H NMR spectrum of the D-glucose 1:1 complex **8b**; the assignment is given in Table 2. The large upfield shift



8b

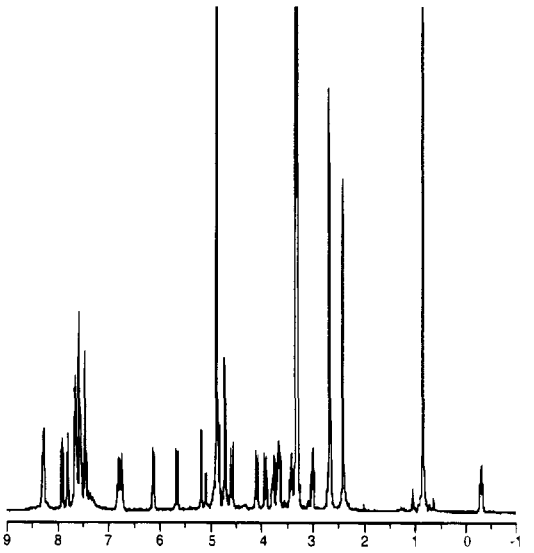


Figure 4. Structural assignment of 1:1 complex **8b** by ^{1}H NMR. The pyranose form of D-glucose is depicted, but the furanose form has not been ruled out.

Table 2. ^{1}H NMR Assignment of D-Glucose Complex **8b**

assignment	chemical shift (ppm)	coupling constant (Hz)
	Complex	
H1	5.18	$J_{1,2} = 5.7$
H2	3.01	$J_{1,2} = 5.7$, $J_{2,3} = 7.5$
H3	−0.30	$J_{2,3} = 7.5$, $J_{3,4} = 7.5$
H4	2.68 (masked)	
H5	3.43	$J_{4,5} = 10.5$, $J_{5,6} = 9.3$ or 0, $J_{5,6'} = 9.3$ or 0
H6 and H6′	3.73	
Ha1 and Ha2 or Ha3 and Ha4	3.93 and 4.85 (masked)	$J_{1a,2a}$ or $J_{3a,4a} = 11.7$
Ha1 and Ha2 or Ha3 and Ha4	4.10 and 4.60	$J_{1a,2a}$ or $J_{3a,4a} = 11.7$
Hb1 and Hb2 or Hb3 and Hb4	5.66 and 6.80	$J_{1b,2b}$ or $J_{3b,4b} = 8.7$
Hb1 and Hb2 or Hb3 and Hb4	6.13 and 6.78	$J_{1b,2b}$ or $J_{3b,4b} = 8.7$
CH_3 or H_3C	2.42	
CH_3 or H_3C	2.68	
	Solvent	
CH_3OH	3.30	
CH_3OH	4.89	
	Protecting Group[a]	
$(CH_3)_2C(CH_2OH)_2$	0.85	
$(CH_3)_2C(CH_2OH)_2$	3.40	
$(CH_3)_2C(CH_2OH)_2$	4.89	

[a] The protecting group is removed under the conditions of the NMR experiment.

of H-3 to −0.3 ppm is indicative of a proton pointing directly at the aromatic π-electrons of anthracene; the splitting of the anthrylic and benzylic protons is also characteristic of structural rigidification in a 1:1 complex. With D-allose, D-fructose, and D-galactose no 1:1 complex could be detected in the ^{1}H NMR

spectra. The mass (SIMS positive) spectra of a 1:1 mixture of **8** with D-glucose, D-allose, D-fructose, and D-galctose all contained the M^+ ion of **8b** but with D-fructose this signal was weak and the spectrum also contained the M^+ ion of **8a**.

Conclusions

With this work we have shown that the inherent selectivity of boronic acids toward monosaccharides can be modulated by molecular design. Molecular complementarity is the important underlying conclusion. When a diboronic acid is constructed with the appropriate convergence of functionallity, selectivity toward a monosaccharide with complementarity divergence of functionality is observed. With this work we have shown that the selectivity of a diboronic acid can be tuned for *glucose*. With compound **8** the maximum fluorescence changes observed are over a concentration range suitable for the detection of physiological glucose levels (0.3−1.0 mM).

We have shown that physiological saccharide *sensitivity* is possible in synthetic saccharide receptors based on boronic acids. We have also shown that it is possible to tailor saccharide *selectivity* using two boronic acids with the correct spatial disposition.

Experimental Section

General Procedures. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 (Merck 5554). Column chromatography was performed on silica gel 60 (Merck 9385, 230−400 mesh). ^{1}H NMR spectra were recorded on a Bruker ARX-300 (300 MHz) spectrometer. The chemical shifts are reported in ppm on the δ scale using tetramethylsilane as a reference. Mass spectrometry was performed on a Hitachi M-2500 instrument. Fluorescence spectroscopy was performed on a Hitachi F-4500 instrument.

Materials. All chemicals were of reagent grade and were used without further purification unless otherwise noted. *o*-Tolylmagnesium bromide, 9-[(methylamino)methyl]anthracene, trimethyl borate, and ethylene glycol were purchased from Aldrich. Saccharides: D-allose, D-galactose, D-glucose, and D-fructose were purchased from Sigma Chemical Co. 9,10-Bis(chloromethyl)anthracene and methylamine (40% water solution) were purchased from Tokyo Kasei Kogyo Co. Anhydrous diethyl ether was obtained from Dojin Kagaku (Kumamoto). Tetrahydrofuran (THF) was dried under a nitrogen atmosphere from Na/benzophenone ketyl.

2,4,6-*o*-Tolylboroxin (1) and 2,4,6-[*o*-(bromomethyl)phenyl]-boroxin (2): prepared according to literature procedure.[33]

9-[[*N*-Methyl-*N*-(*o*-boronobenzyl)amino]methyl]anthracene (3). 2,4,6-[*o*-(Bromomethyl)phenyl]boroxin (100 mg, 0.18 mmol) and 9-[(methylamino)methyl]anthracene (254 mg, 1.1 mmol, 2.1 equiv) were refluxed in chloroform (50 mL) for 2 h. The mixture was filtered and the solvent removed under reduced pressure. The crude material was purified by trituration with ethyl acetate to give **3** as a pale yellow powder: 107 mg (33%); mp 147.1−151.9 °C; IR (KBr) 3352 (OH, st), 1365 (BO, st) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.23 (3 H, s), 3.94 (2 H, s), 4.50 (2 H, s), 7.46 (4 H, m), 8.01 (4 H, m), 8.44 (1 H, s); MS (SIMS negative) M + (glycerol − $2H_2O$) − H^+ 410. Anal. Calcd for $C_{23}H_{22}NO_2B$: C, 77.75; H, 6.20; N, 3.94. Found: C, 77.68; H, 6.27; N, 3.97.

2,2-Dimethylpropane-1,3-diyl *o*-Tolylboronate (4). 2,4,6-*o*-Tolylboroxin (6.13 g, 17 mmol) and 2,2-dimethyl-1,3-propanediol (5.7 g, 54 mmol, 3.3 equiv) were refluxed in toluene (200 mL) with azeotropic removal of water (Dean−Stark) for 12 h. The solvent was removed under reduced pressure. The 2,2-dimethyl-1,3-propanediol was removed by silica gel chromatography using methylene chloride as solvent to give 9.11 g (88%) of 2,2-dimethylpropane-1,3-diyl *o*-tolylboronate as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.02 (6H, s), 2.51 (3H, s), 3.75 (4H, s), 7.14 (2H, m), 7.27 (1H, m), 7.72 (1H, m).

2,2-Dimethylpropane-1,3-diyl [*o*-(Bromomethyl)phenyl]boronate (5). 2,2-Dimethylpropane-1,3-diyl *o*-tolylboronate (9.11 g, 44 mmol), recrystallized *N*-bromosuccinimide (8.6 g, 48 mmol, 1.1 equiv), and AIBN (0.1 g, cat.) in carbon tetrachloride (200 mL) were refluxed and irradiated with a 100 W lamp for 4 h. The succinimide was removed by filtration and the solvent removed. The crude product was then chromatographed on silica gel with methylene chloride as solvent to give 11.8 g (95%) of **5** as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.06 (6H, s), 3.80 (4H, s), 4.92 (2H, s), 7.25−7.36 (3H, m), 7.79 (1H, m).

9,10-Bis[(Methylamino)methyl]anthracene (6). 9,10-Bis(chloromethyl)anthracene (10 g, 36 mmol) in chloroform (100 mL) and methylamine 40% solution in water (100 mL) were stirred at room temperature for 1 h and then at gentle reflux for 3 h. The organic layer was separated and washed with water (3 × 100 mL) and dried over magnesium sulfate and the solvent removed under reduced pressure to give 9,10-bis[(methylamino)methyl]anthracene as a yellow/orange powder 9.5 g (quant): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.60 (2H, s), 2.67 (6H, s), 4.68 (4H, s), 7.52 (4H, m), 8.37 (4H, m); MS (SIMS positive) M^+, 264.

9,10-Bis[[*N*-[*o*-(5,5-dimethylborinan-2-yl)benzyl]-*N*-methylamino]-methyl]anthracene (7). 2,2-Dimethylpropane-1,3-diyl [*o*-(bromomethyl)phenyl]boronate (640 mg, 2.3 mmol, 2.7 equiv), 9,10-bis-[(methylamino)methyl]anthracene (224 mg, 0.85 mmol), and potassium carbonate (312 mg, 2.3 mmol) in anhydrous tetrahydrofuran (50 mL) were refluxed for 12 h. The mixture was filtered, and the solvent was removed under reduced pressure. The crude material was purified by trituration with ethyl acetate to give **7** as a yellow/orange powder 142 mg (25%): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.84 (12H, s), 2.39 (6H, s), 4.33 (4H, s), 4.99 (4H, s), 7.28−8.25 (16H, m); MS (SIMS positive) M + 668.

9,10-Bis[[*N*-methyl-*N*-(*o*-boronobenzyl)amino]methyl]anthracene (8). The protecting group was removed by silica gel chromatography in methanol to give **8** as a yellow/orange powder (quant): mp 183.1−239.6 °C; IR (KBr) 3352 (OH, st), 1385 (BO, st) cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 2.39 (6H, s), 4.32 (4H, s), 5.00 (4H, s), 7.26−8.27 (16H, m); HRMS (SIMS positive) 641.2990, calcd for $C_{38}H_{39}N_2O_6B_2$ [M + 109, 1:1 glucose−boronate ester (compound **8b**) + H] 641.2991.

Acknowledgment. Authors T.D.J. and K.R.A.S.S. wish to thank Dr. M. Mikami (JRDC Chemirecognics Project) for helpful advice and discussions. We would also like to thank Mr. K. Yamaguchi, Mr. S. Tsutsui, and Miss S. Imazu for technical assistance.

JA943594C