Allosteric Interaction of Metal Ions with Saccharides in a Crowned Diboronic Acid

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Abstract: A diboronic acid saccharide (covalent) receptor site and a crown metal ion (electrostatic) receptor site are succesfully coupled in an allosteric system. The binding of monosaccharides with **2b** as intramolecular 1:1 complexes was monitored by circular dichroism (CD). Added calcium perchlorate reduces the binding of the 1:1 saccharide complexes as followed by a decrease in the CD activity. This is a novel allosteric system which mimics the action of the Na^+/D-glucose cotransport protein in nature.

Introduction

Nature relies on allosteric interactions to modulate modes of action and message transduction.[1] Simple synthetic models should allow for a greater understanding of the more complex allosteric interactions occurring in nature. Our group[2-7] and other groups[8-13] have started to exploit the interactions of boronic acids with saccharides.[14] Such interactions we believe can be exploited in the development of receptor sites for saccharide detectors. The remarkably strong interaction between the diphenylmethane-3,3′-diboronic acid[4-6] (**1**) and glucose has shown that the design of a glucose selective pocket is possible. With the success of this molecular design we were prompted to exploit our saccharide cleft in more complex systems such as allosteric devices. Such systems should mimic the modes of sugar recognition in nature more precisely.

The strong interaction exhibited by the diphenylmethane-3,3′-diboronic acid with saccharides allies itself as a suitable candidate for the main site in an allosteric device. Synthetic allosteric devices in the past have relied on metal ion coordination or lipophilic interactions.[15-25] Light has also been employed in allosteric devices to control the conformation and hence binding of diazo crowns.[26-30] Here, we report on the first allosteric receptor that couples covalent interactions (the formation of saccharide boronate esters) with metal ion complexation. In the design of an allosteric system binding at the first or main site should either activate (positive allostericity) or deactivate (negative allostericity) binding at the second site. To facilitate activation or deactivation, binding at the first site should induce a major conformational change in the molecule. From our previous work with the diphenylmethane-3,3′-diboronic acid, we know that binding of a saccharide immobilizes the two phenyls with a twist (Figure 1). This asymmetric immobilization can be readily "read out" as a change in circular dichroism (CD) of the benzene chromophore. If the second binding site requires a different disposition of the two aromatic rings, then negative cooperativity or negative allostericity will be observed; conversely if both sites align with the same disposition, positive cooperativity or positive allostericity will result. One possible secondary site is a metal-binding site; crown ethers are then the obvious first choice.[31] If a crown ether is employed in the molecular design, then the ideal starting structure **2a** or **2b** has the methylene bridge of **1** replaced with an oxygen. For these molecules metal binding should induce the classic "crown" of oxygens, since this will force the phenyls into the same plane; negative cooperativity or negative allosterism is predicted.

Results and Discussion

Synthesis. The syntheses of **2a** or **2b** and **7** (a model compound) were both simple and facile. The route employed is given in Scheme 1 (for details see the Experimental Section).

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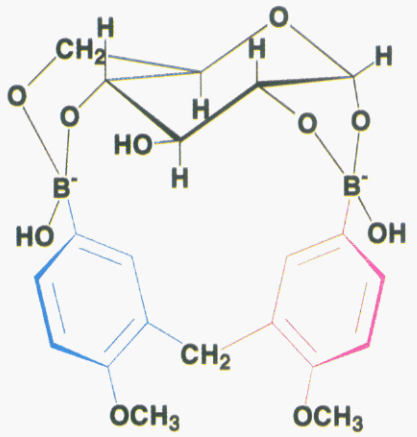


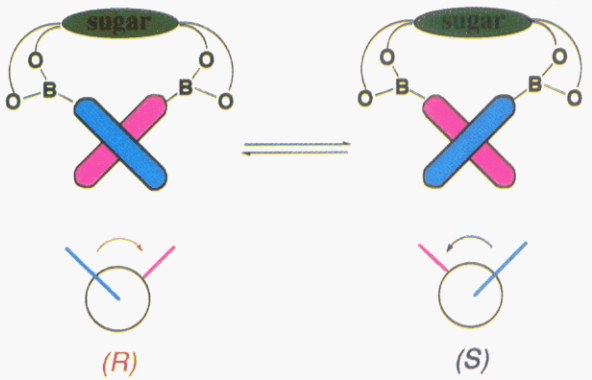
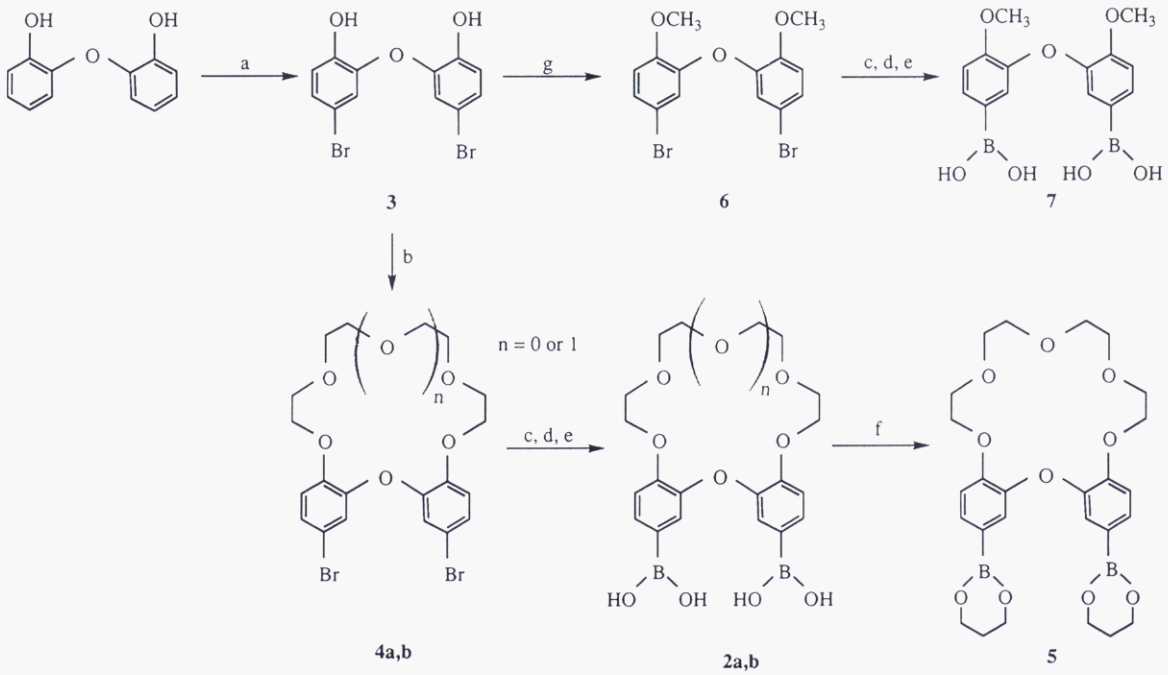
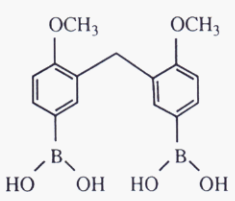


Figure 1. Proposed structure of the D-glucose complex[5,6] and a pair of altropisomers.

Scheme 1. Synthetic Routes to **2a,b** and **7**[a]



[a] (a) Br_2, –35 °C, 6 h, $CHCl_3$. (b) High dilute solution, tetraethylene glycol di-*p*-tosylate, NaH, reflux, 36 h, THF. (c) *n*-Butyllithium, –75 °C, 3 h, THF. (d) Trimethyl borate, –75 °C, 2 h, THF. (e) HCl, H_2O, 25 °C, 18 h. (f) 1,3-Propanediol, molecular sieves 4 Å, reflux, 6 h, toluene. (g) CH_3I, NaH, reflux, 36 h, THF.



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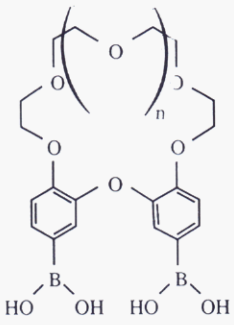

2a: n=0

2b: n=1

Saccharide Binding. The saccharide binding of **1** has been thoroughly characterized.[3-6] An intramolecular saccharide diboronic acid 1:1 complex has been shown to be the source of the induced circular dichroism (CD). The CD spectra of **2b** (Figure 2) with D- and L-glucose are almost identical to those obtained with **1**; conversely, **2a** is completely CD silent with both D- and L-glucose, implying that our structural modifications have not affected the binding site significantly with compound **2b** but have completely deformed the binding site in **2a**. Perhaps, with the shorter ethylene glycol linker of compound **2a**, the desired spatial disposition of the two boronic acid units required for 1:1 intramolecular saccharide binding cannot be achieved. This negative result with compound **2a** gave us great hope in our design concept, since such a small structural change could destroy the saccharide cleft in an allosteric manner.

The binding constants for **2b** and **7** with D-glucose, D-talose, D-allose, and D-mannose are given in Tables 1 and 2, respectively. Interestingly, both **2b** and **7** are less promiscuous in their saccharide binding than **1**. Also, compound **7** and compound **2b** display different saccharide selectivities amongst this smaller

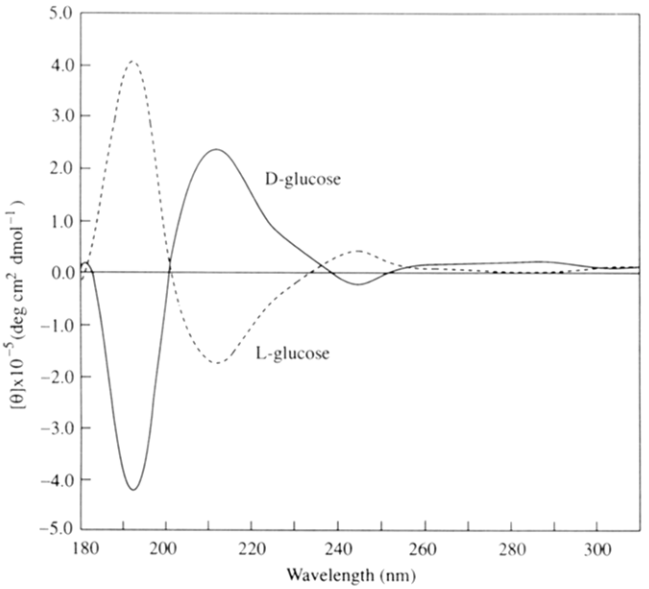

Figure 2. CD spectra of **2b**·D-glucose (solid line) and **2b**·L-glucose (dashed line) complexes (1.63×10^{-3} M) in 9:1 CH_3OH:H_2O with 0.2% choline, pH 11.6, 25 °C.

Table 1. Absorption and CD Spectra Parameters of **2b**·Saccharide Complexes[a]

	UV	CD			
saccharide	λ_{max} (nm)	λ_{max} (nm)	$[\theta]_{max}$[b] (deg cm² dmol⁻¹)	stoichiometry	K[c] (M^{-1})
D-glucose	287	289	+32 810	1:1	31 000
	200	212	+245 100		
		192	−432 000		
D-talose	288	288	+7298	1:1	14 000
	200	212	+126 000		
		196	−334 300		
D-allose	288	288	+3815	1:1	1180
	200	213	+107 400		
		193	−251 000		
D-mannose	287	silent	silent		
	200	silent	silent		

[a] [**2b**] = 1.63×10^{-3} M, pH = 11.6 with 0.2% choline hydroxide in 9:1 CH_3OH:H_2O at 25 °C. [b] $[\theta]_{max}$ values were calculated for 100% complexation. [c] Maximum error ±200 M^{-1}.

Table 2. Absorption and CD Spectra Parameters of **7**·Saccharide Complexes[a]

	UV	CD			
saccharide	λ_{max} (nm)	λ_{max} (nm)	$[\theta]_{max}$[b] (deg cm² dmol⁻¹)	stoichiometry	K[c] (M^{-1})
D-glucose	280	280	+6820	1:1	14 800
	200	205	+132 100		
		190	−210 000		
D-talose	281	283	+3298	1:1	6600
	200	205	+15 400		
		191	−35 200		
D-allose	280	281	+2775	1:1	3100
	200	203	+14 210		
		190	−27 000		
D-mannose	280	280	+800	1:1	850
	201	205	+8560		
		190	−17 500		

[a] [**7**] = 1.60×10^{-3} M, pH = 11.6 with 0.2% choline in 9:1 CH_3OH:H_2O at 25 °C. [b] $[\theta]_{max}$ values are calculated for 100% complexation. [c] Maximum error ±200 M^{-1}.

group of saccharides. Linking the two phenyls by an oxygen rather than a methylene has altered the spatial disposition of the two boronic acids in a way that disfavors binding with some saccharides, the notable example being D-galactose. Tethering the two phenyls as a macrocycle has also influenced the saccharide

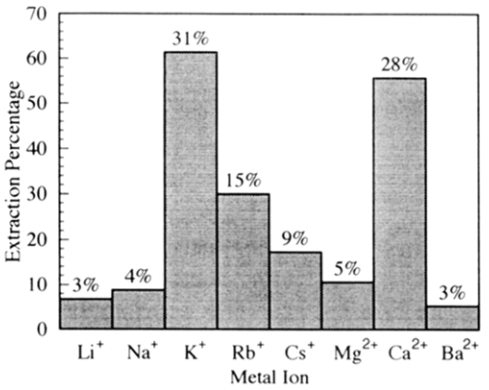

Figure 3. Percentage extraction of metal picrates into CH_2Cl_2 at 25 °C by compound **4a**.

binding. The increased binding observed for **2b** with D-glucose and D-talose and decreased binding with D-allose (no appreciable binding with D-mannose was observed) can be attributed to preorganization of the binding site. This preorganization enhances the selectivity for D-glucose and D-talose and lowers the binding with D-allose and D-mannose. This result gave us great hope, since the crown is enlarging the span in binding ability of **2b** for saccharides even prior to the addition of metal ions.

Metal Binding. The liquid–liquid extraction percentages of metal picrates with **4b** were measured (Figure 3). The selectivity observed amongst the metal ions is the characteristic size selectivity order for 18-crown-6: that is, ions larger than or smaller than K^+ are extracted less favorably. The findings imply that compound **4b** is a well-behaved crown ether.

Further binding information was obtained by the NMR titration of **4b** with calcium perchlorate (Figure 4). During the metal titration the internal ortho protons undergo a large downfield shift. As a metal ion complex is formed with **4b**, the structure is rigidified and the two phenyl groups are brought into the same plane. This forces the two internal ortho protons together. As the two internal ortho protons are brought closer, they are deshielded by each other and appear further downfield in the NMR spectra.

Saccharide and Metal Binding. Having confirmed that both the saccharide and metal ion binding site function, the next step is to confirm that they are indeed coupled and display allosterism. The change of the CD intensity of the D-allose 1:1 complex with **2b** upon addition of metal is given in Figure 5. With the monocations of lithium, sodium, rubidium, and cesium, no significant change was observed, but with potassium a significant perturbation of the D-allose binding with increasing metal concentration was observed. The equilibrium constant of 1180 M^{-1} decreases to a minimum of 910 M^{-1}. The dications of magnesium and calcium are both efficient in perturbing the binding of D-allose, with calcium being particularly efficient. The equilibrium constant of 1180 M^{-1} decreases to 300 M^{-1} with added calcium. In order to confirm that we are in fact observing allosterism and not some secondary effect, the CD spectra of **7** in the presence of added metal were recorded. As expected, no significant change was observed with any of the metal ions tested herein.

Why do dications induce a larger negative allosterism than monocations? This question has a very simple answer. The measurements were carried out at pH 11.6, and at this pH both boronic acid groups exist as boronate anions. Thus, the electrostatic stabilization with dications is stronger than with the monocations.

Further confirmation that it is negative allosterism we are observing is given by the change in the CD intensity of D-glucose, D-talose, and D-allose 1:1 complexes on addition of calcium perchlorate. The equilibrium constants change from 31 000 to 25 400, 14 000 to 8400, and 1180 to 300 M^{-1}, respectively (Figure

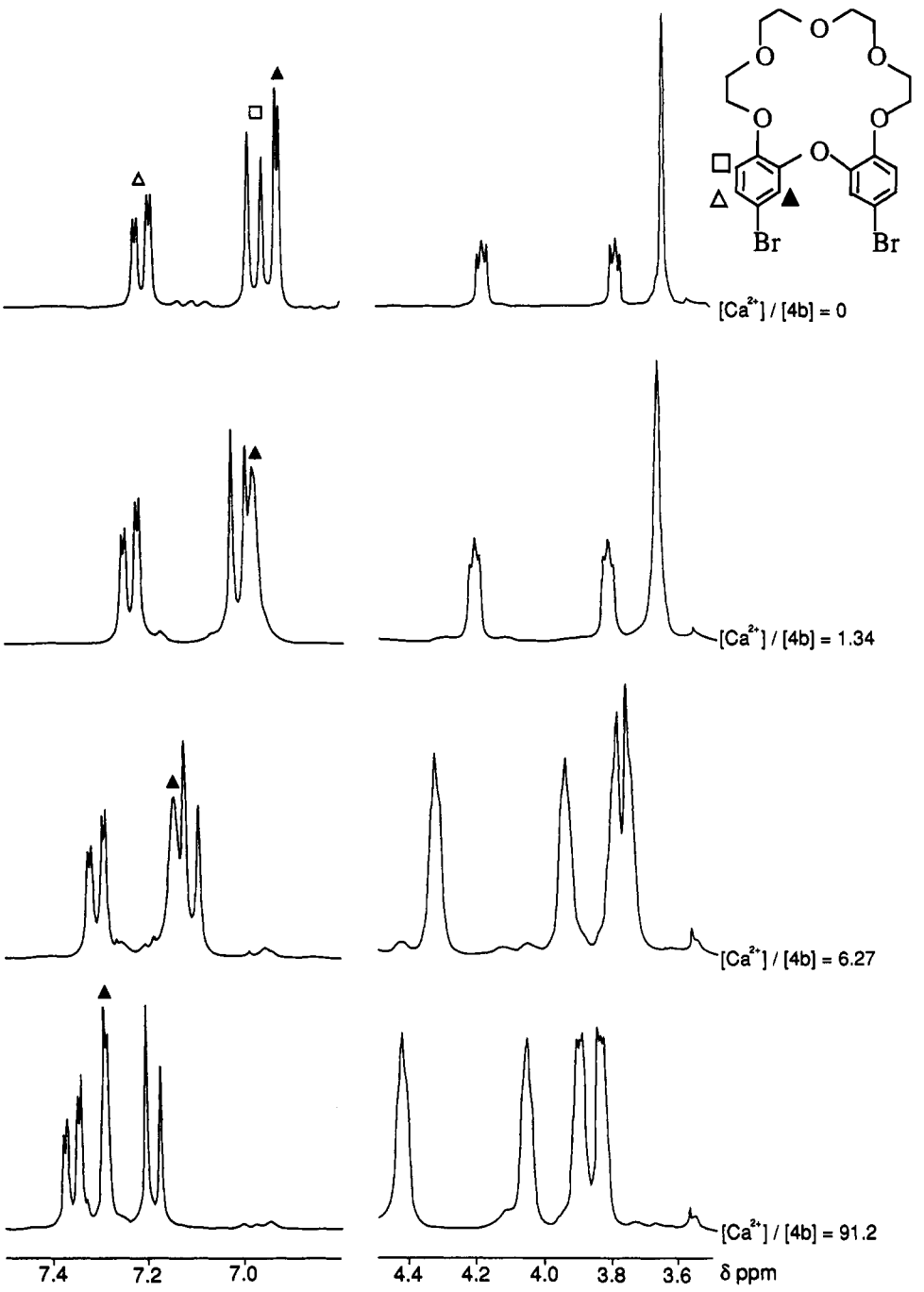

Figure 4. Titration of the ^1H NMR (300 MHz) spectra of **4b** (1.45×10^{-2} M) with $Ca(ClO_4)_2 \cdot 4H_2O$ in 2:1 CD_3OD:$CDCl_3$ at 25 °C.

6). From Table 1 the order of decreasing stability for the 1:1 complex is D-glucose, D-talose, and D-allose. The effect of calcium on the binding of these saccharides reflects this order, since the most stable complex is the least affected by added calcium.

Conclusion

With this work we present the first example in which a saccharide (covalent) binding site and a metal (electrostatic) binding site are allosterically coupled. Conformational reorganization of the host (**2b**) concomitant with metal ion complexation causes a reduction in the amount of 1:1 saccharide–diboronic acid complex. Presumably, the reorganization produces a disposition of boronic acids which is unsuitable for 1:1 binding with saccharides. The adroit combination of saccharide and metal ion recognition opens the way for a chiral allosteric device in which the binding of chiral ammonium ion to the crown moiety dictates the binding of chiral saccharides.

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). ^1H NMR spectra were recorded on either a Bruker ARX-300 (300 MHz) or a Hitachi R-1900 (90 MHz) spectrometer. The chemical shifts are reported in parts per million on the δ scale using tetramethylsilane as a reference, and the coupling constants are reported in hertz. Mass spectrometry was performed on a Hitachi M-2500 instrument. IR spectra were obtained as KBr disks using a Shimadzu FT-IR 8100 spectrometer. UV spectra were measured on a Shimadzu UV-2200 spectrometer. Circular dichroism (CD) spectra were measured on a Jasco J-720 spectrometer. pH values were determined on a Horiba pH meter. Melting points were determined on a Yanaco (MP-500D) micro melting point apparatus and are uncorrected.

Materials. All chemicals were of reagent grade and were used without further purification unless otherwise noted: lithium hydroxide monohydrate, sodium hydroxide, potassium hydroxide, magnesium hydroxide,

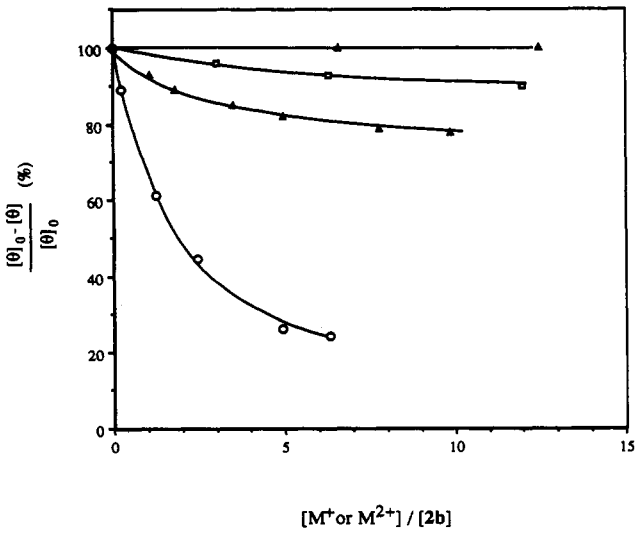

Figure 5. Decrease of the CD intensity of D-allose·**2b** complex in the presence of metal perchlorate: Ca^{2+} (○), K^+ (△), Mg^{2+} (□), [Li^+, Na^+, Rb^+, Cs^+] (▲). [**2b**] = 1.63×10^{-3} M. pH = 11.6 with 0.2% choline hydroxide as the base in 9:1 CH_3OH:H_2O at 25 °C. $[\theta]_0$ and $[\theta]$ are the CD intensity in the absence or in the presence of metal salt.

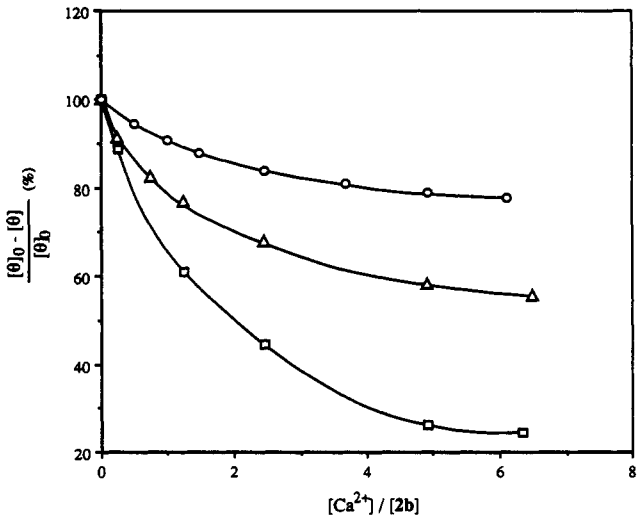

Figure 6. Decrease of the CD intensity of monosaccharide·**2b** complexes: D-glucose (○), D-talose (△), D-allose (□). [**2b**] = 1.63×10^{-3} M. pH = 11.6 with 0.2% choline hydroxide as the base in 9:1 CH_3OH:H_2O at 25 °C. $[\theta]_0$ and $[\theta]$ are the CD intensity in the absence or in the presence of calcium perchlorate.

lithium perchlorate, sodium perchlorate, potassium perchlorate, sodium sulfate anhydrous, and picric acid were obtained from Wako Pure Chemical Industries (Osaka). Choline hydroxide solution (50% in water), sodium hydride (60% in mineral oil), 2,2′-dihydroxydiphenyl ether, and bromine were received from Tokyo Kasei Kogyo Co. (Tokyo). Magnesium perchlorate, calcium perchlorate monohydrate, rubidium hydroxide monohydrate, and cesium hydroxide monohydrate were obtained from Kishida Chemical Co. (Fukuoka). Butyllithium, trimethyl borate, triethylene glycol di-*p*-tosylate, and triethylene glycol di-*p*-tosylate were purchased from Aldrich. The following saccharides, D- or L-allose, D-arabinose, D-fucose, D-galactose, D- or L-glucose, D-mannose, D-ribose, and D-talose, were purchased from Sigma Chemical Co. D-Xylose, D-glucose-6-phosphate disodium salt, and 3-*o*-methyl-D-glucose were obtained from Wako. D-Lactose and methyl-α-D-glucoside were received from Tokyo Kasei Kogyo Co. Tetrahydrofuran (THF) was dried under a nitrogen atmosphere from Na/benzophenone ketyl according to a literature procedure.

2,2′-Dihydroxy-5,5′-dibromodiphenyl Ether (3). Bromine (18.0 g, 0.11 mol) was added over 6 h to a solution of 2,2′-dihydroxydiphenyl ether (10.0 g, 0.05 mol) in $CHCl_3$ (300 mL) at −35 °C. After the solution was stirred at room temperature over 12 h until the color of bromine completely disappeared, the solvent was removed in vacuo, and the solid residue was dissolved in $CHCl_3$ (300 mL) and washed with H_2O (5 × 50 mL). The organic phase was dried (Na_2SO_4), and the solvent was evaporated in vacuo. Recrystallization two times from chloroform–hexane yielded **3** as a white solid (14.04 g, 83%): mp 128–129 °C; MS (EI) 360 (M^+); IR (KBr) 3505 (OH st), 1595, 1491 (arom ring vib), 1259 (C-O st), 1120 (C-Br st), 821 cm^{-1} (C_6H_3 wagg); ^1H NMR ($CDCl_3$) δ 6.93 (d, J = 8.64, 2 H), 6.97 (d, J = 2.22, 2 H), 7.19 (dd, J = 8.64, J' = 2.22, 2 H). Anal. Calcd for $C_{12}H_8O_3Br_2$: C, 40.04; H, 2.24. Found: C, 40.04; H, 2.31.

1,2,4,5-Dibenzo-21,24,-dibromo-18-crown-6 (4b). A solution of **3** (3.60 g, 10 mmol) and tetraethylene glycol di-*p*-tosylate (6.03 g 12 mmol) in 450 mL of dry THF was added over 18 h to a stirred suspension of NaH (8.00 g, 60% in mineral oil, 0.2 mol) in refluxing dry THF (500 mL) under a nitrogen atmosphere. The mixture was refluxed for 36 h before being cooled down to room temperature. Excess NaH was quenched by the addition of methanol, and then the solvent was removed in vacuo and the residue was partitioned between $CHCl_3$ (300 mL) and H_2O (100 mL). The organic phase was washed with 1 N HCl (50 mL) and H_2O (3 × 50 mL), dried (Na_2SO_4), and then concentrated in vacuo. Column chromatography (SiO_2, $CHCl_3$, then 10% EtOEt–$CHCl_3$) afforded **4b** as a white solid (3.21 g, 62%): mp 149–150 °C; MS (EI) 518 (M^+); IR (KBr) 2930 (C-H st), 1578, 1499 (arom ring vib), 1130 (C-O-C st); ^1H NMR ($CDCl_3$) δ 3.66 (s, 8 H), 3.79 (t, J = 7.05, 4 H), 4.17 (t, J = 7.05, 4 H), 6.87 (d, J = 8.72, 2 H), 6.97 (d, J = 2.34, 2 H), 7.18 (dd, J = 8.72 and J' = 2.34, 2 H). Anal. Calcd for $C_{20}H_{22}O_6Br_2$: C, 46.37; H, 4.28. Found: C, 46.40; H, 4.25.

1,2,4,5-Dibenzo-21,24,-dihydroxyboro-18-crown-6 (2b). *n*-Butyllithium solution (10.0 mL, 1.6 M in hexane, 16 mmol) was added over 15 min to a stirred solution of **4b** (2.00 g, 3.86 mmol) in 100 mL of anhydrous THF at −75 °C under nitrogen. After the mixture was maintained for 2 h at the same temperature, it was dropped over 2 h into a solution of trimethyl borate (20 mL, 0.176 mol) in 100 mL of dry THF through a needle bridge at −75 °C. The temperature was gradually raised over 3 h to room temperature after maintaining at low temperature for 1 h. An excess of trimethyl borate was decomposed by the addition of 2 N HCl (40 mL) at 0 °C, and then the mixture was stirred at room temperature for 18 h. The solvent was removed, and the residue was washed with H_2O (5 × 80 mL) and hexane (5 × 50 mL). Finally it was recrystallized from CH_3OH–H_2O to afford **2b** as white powder (1.31 g, 76%): mp 210–213 °C; IR (KBr) 3300 (OH st), 2919 (C-H st), 1605, 1515 (arom ring vib), 1352 (B-O st), 1135 (C-O-C st); ^1H NMR (CD_3OD) δ 3.63 (s, 8 H), 3.76 (s, 4 H), 4.19 (s, 4 H), 6.96–7.50 (m, 6 H). Anal. Calcd for $C_{20}H_{26}O_{10}B_2$: C, 53.61; H, 5.85. Found: C, 53.37; H, 6.03. MS (EI) gave no detectable M^+ (448); however, after protection of the boronic acid with 1,3-propanediol, M^+ (528) of compound **5** was obtained.

Compounds **2a** and **4a** were synthesized and characterized according to the similar procedure for compounds **2b** and **4b**.

2,2′-Dimethoxy-5,5′-dibromodiphenyl Ether (6). A mixture of **3** (1.80 g, 5 mmol), iodomethane (14.20 g, 0.1 mol), and NaH (2.0 g, 60% in mineral oil, 0.05 mol) in 300 mL of dry THF was heated to reflux for 36 h under nitrogen. After the mixture was cooled to room temperature, an excess of NaH was quenched by the addition of CH_3OH, and then the solvent was removed in vacuo and the residue was partitioned between $CHCl_3$ (300 mL) and H_2O (100 mL). The organic phase was washed with 1 N HCl (50 mL) and H_2O (3 × 50 mL), dried (Na_2SO_4), and then concentrated in vacuo. Recrystallization of the residue from chloroform–hexane gave **6** as a white solid (1.67 g, 86%): mp 126–127 °C; MS (EI) 388 (M^+); IR (KBr) 1576, 1496 (arom ring vib), 1265 (C-O-C st), 1134 (C-Br st); ^1H NMR ($CDCl_3$) δ 3.84 (s, 6 H), 6.85 (d, J = 8.70, 2 H), 6.94 (d, J = 2.37, 2 H), 7.21 (dd, J = 8.70, J' = 2.37, 2 H). Anal. Calcd for $C_{14}H_{12}O_3Br_2$: C, 43.33; H, 3.12. Found: C, 43.60; H, 3.08.

2,2′-Dimethoxy-5,5′-dihydroxyborodiphenyl Ether (7). This compound was prepared from **6** in 73% yield according to the procedure outlined above for **2b**: white powder, mp 163–165 °C; IR (KBr) 3227 (OH st), 1605, 1504 (arom ring vib), 1348 (B-O st), 1142 (C-O st); ^1H NMR (CD_3OD) δ 3.92 (s, 6 H), 6.85–7.80 (m, 6 H). Anal. Calcd for $C_{14}H_{16}B_2O_7$: C, 51.28; H, 4.92. Found: C, 51.20; H, 5.21.

Circular Dichroism Spectroscopy. A typical experiment was described as follows: 50 μL of the solution of host molecule **2b** in methanol (1.63×10^{-2} M), 50 μL of the solution of saccharide in water (0.1 M), 50 mL of 2% choline hydroxide solution in water, and 350 μL of the solvent (9:1 CH_3OH:H_2O) were mixed. CD spectra were measured with a 0.1-mm cell about the wavelength region 180–310 nm at 25 °C.

Stoichiometric Experiments. Certain amount solutions of **2b** (1.63×10^{-2} M), saccharide (0.1 M), and 50 μL of 2% choline hydroxide solution were mixed, and some solvent was added to make a total volume of 500 μL, keeping [**2b**] + [saccharide] = 1.63×10^{-3} M.

Estimation of Association Constants (K). The methods employed in this investigation were based on observing changes in CD densities of solutions where the relative concentration of one component (saccharide) is increased with respect to the other component (boronic acid host molecule). All association constants were determined in 9:1 CH_3OH:H_2O at 25 °C. The sample solutions were prepared by mixing 50 μL of the solution of host molecule **2b** or **7** (1.63×10^{-2} M), 50 μL of 2% choline hydroxide solution, 0–250 μL of saccharide solution (0.1 M), and some solvent to make a total volume of 500 μL. Since compounds **2b** and **7** form 1:1 complexes, the [θ]-saccharide plots were analyzed according to a Benesi–Hildebrand equation. The correlation coefficients were always better than 0.99.

Metal Ion Salt Effects. The sample solutions were made by mixing the following stock solutions to a total volume of 500 μL: 50 μL of the solution of **2b** (1.63×10^{-2} M), 50 μL of the solution of saccharide (0.1 M), 50 μL of 2% choline hydroxide solution, 0–350 μL of the metal perchlorate salt solution (0.1 M), and the solvent.

Picrate Extraction Measurements. Metal picrates (1.06×10^{-4} M) were prepared in situ by dissolving the metal hydroxide in 10 mL of 1.06×10^{-4} M picric acid solution, keeping [MOH] or $[M(OH)_2]$ = 0.1 M; twice deionized water was used for all aqueous solutions. A solution (2×10^{-3} M) of the crown ether derivative **4b** was prepared in dichloromethane. Equal volumes (5 mL) of the two solutions were shaken vigorously for 30 min in a 10-mL flask, and the solutions were left standing until separation was complete. The decrease of the concentration of picrate ion in the aqueous solution was then determined spectrometrically. Control experiments showed that no picrate extraction occurred in the absence of the crown ether derivatives.

Supplementary Material Available: Plots of $[\theta]_{193}$ against saccharide concentration (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.