Stabilization of Sugar-Boronic Esters of Indolylboronic Acid in Water via Sugar–Indole Interaction: A Notable Selectivity in Oligosaccharides

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5-Indolylboronic acid (**1**) reversibly forms boronic esters with reducing sugars in water. The resulting boronates are stronger Lewis acids than the starting boronic acid in the deprotonation of water; $pK_a=10$ for **1** and <7 for its sugar-boronates. Thus, at pH 9.0, there exists an experimentally-evaluable sugar-binding equilibrium between neutral trivalent acid **1** and anionic tetravalent ester thereof, which can be conveniently monitored by fluorescence or ^{11}B NMR spectroscopy. A characteristic aspect of the present sugar-binding process is the notable selectivity of host **1** for oligosaccharides. The binding constants for maltodextrins (α-1,4-linked glucose oligomers) increase with increasing chain lengths or repetition numbers of the glucose unit. Such a chain length selectivity and those among stereoisomers and linkage isomers of disaccharides are discussed in terms of intracomplex oligosaccharide–indole interaction.

Selective binding of sugars is a growing area of host–guest complexation.[1,2] Molecular recognition of oligosaccharides in water is especially an intriguing subject in view of important roles of cell-surface oligosaccharides in intercellular recognition.[3] Multiple host–guest hydrogen bonding which provides the major driving force of sugar complexation in apolar organic media[1] becomes far less effective in aqueous media. Complexation of sugars in water must therefore rely on other forces. One of them is reversible boronic ester formation.[4,5] Phenylboronic acid, for example, forms more or less stable cyclic boronates with monosaccharides.[4] Oligosaccharides, however, show significantly lower affinities[4f] for the reason(s) which seem to be not well understood. Another potential binding strategy is to take advantage of the hydrophobicity of sugars. In fact, oligosaccharides and relatively hydrophobic monosaccharides in water can be bound to an aromatic cavity of a cyclic host[2] or even to simple hydrophobic fluorescence probes via apolar host–guest interaction.[6]

The object of the present work is to develop hydrophobic boronic acids and see how covalent (boronate formation) and noncovalent (apolar) host–guest interactions cooperate to give rise to a selectivity for oligosaccharides. The indolyl group was a hydrophobic moiety of particular choice here. There are a couple of reasons for this. First, survey of the X-ray crystal structures of sugar-protein complexes reveals that the C–H moieties of a bound sugar are often stacked to aromatic amino acid side chains, the indole ring of Trp in particular.[7] Second, the indolyl group is fluorescent.[6b] This allows the present boronic ester formation to be conveniently monitored by fluorescence spectroscopy. The fluorimetric titration has already been used by Yoon and Czarnik to monitor the complexation of fluorescent anthrylboronic acid.[8]

Experimental

Materials. Into an ether solution (20 ml) of 5-bromoindole (1.96 g, 10 mmol) was added dropwise under nitrogen 2 equivalents (20 mmol) of butyllithium in hexane (12.5 ml) at −20 °C. The mixture was stirred at room temperature for 4 h. The resulting solution containing 5-lithio derivative was added dropwise at −70 °C into a solution of trimethyl borate (1.1 ml, 10 mmol) in ether (10 ml). The mixture was allowed to warm up gradually to room temperature under stirring. After stirring for additional 1 h, an aliquot of aqueous 1 M HCl (20 ml) (1 M=1 mol dm^{-3}) was added to destroy excess organometallic derivatives. The mixture was extracted with ether after addition of more water. Usual workup and chromatography on silica gel with a 1 : 1 mixture of hexane and ethyl acetate as eluant gave 5-indolylboronic acid (**1**) as colorless crystals (0.26 g, 16%):[9] mp >290 °C; IR (KBr) 3410 (N–H), 1360 (B–O) cm^{-1}; ^{1}H NMR (D_2O) δ_H=6.62 (1H, d), 7.40 (1H, d), 7.57 (2H, q), and 8.12 (1H, s). 2-Naphthylboronic acid (**2**) was obtained in essentially the same porocedure: mp 253 °C; IR (KBr) 1360 cm^{-1} (B–O); ^{1}H NMR ($CDCl_3$) δ_H=7.50 (2H, m), 7.78 (1H, d), 7.87 (3H, m), and 8.29 (1H, s).

All sugars used in this study were commercial products; glucose, fructose, lactose, cellobiose, and sucrose from Nacalai, maltose, maltotriose, maltotetraose, maltopentaose, palatinose, and isomaltose from Hayashibara, gentiobiose and raffinose from Fluka, trehalose from Wako, and melibiose from TCI.

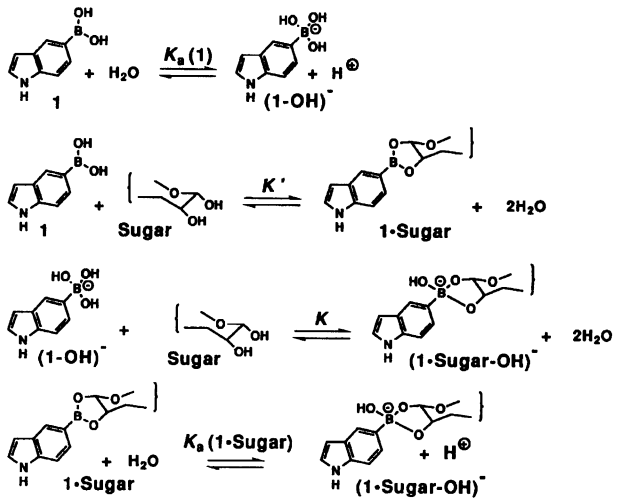
Instruments and Measurements. Fluorescence spectra were obtained with a Hitachi F-4000 fluorescence spectrophotometer at 25 °C. Sample solutions contained a fixed amount (5.0×10^{-5} M) of boronic acid **1** or reference **2** and varying amounts of a sugar. The excitation wavelength was 290 nm, where the sugars used were nearly transparent. A solution of maltopentaose at 0.05 M gave the maximal absorbance of 0.01, as compared with that (0.16) of host **1** at the concentration indicated. Emission intensities at either 361 (for **1**) or 346 nm (for **2**) were measured 5 minutes after sample solutions were prepared. The linearity of the Benesi–Hildebrand plots for the titration data was always excellent,

the correlation coefficients being >0.98. The reproducibility of the binding constants was within 25% (within 15% in most cases), provided that fresh stock solutions of host and guest were always used. ^{1}H and ^{11}B NMR spectra were taken also at 25 °C for D_2O and H_2O solutions, respectively. A JEOL JNM EX-400 spectrometer was used at 400 (^{1}H) or 128 MHz (^{11}B). Internal HDO (δ_H=4.80) or external $NaBF_4$ (δ_B=−20.4) was used as reference. The pH's of aqueous phosphate buffer solutions were measured with a pH meter TPX-90 (Tokyo Chemical Laboratories Co., Ltd.). The binding constants of phenylboronic acid (**3**) were determined by spectrophotometric titration in a similar manner as reported.[4]

Results and Discussion

Boronic Ester Formation. The reaction of 5-indolyllithium with trimethyl borate, followed by hydrolysis, afforded 5-indolylboronic acid (**1**). 2-Naphthylboronic acid (**2**) and phenylboronic acid (**3**)[4] were also used as references (Chart 1). Arylboronic acids are Lewis acids, as trivalent boron derivatives usualy are, and enhance the acidity of water (Scheme 1). The pK_a(**1**) value of this **1**-promoted acid dissociation of water can be readily determined by either fluorescence or ^{11}B NMR spectroscopy. A significant reduction in the fluorescence intensity as well as an upfield shift of the ^{11}B NMR signal accompany with the conversion of sp^2-hybridized neutral species **1** into sp^3-hybridized anion (**1**–OH)$^-$. The actual titration data are shown in Fig. 1. Both methods give essentially the same pK_a of 10, where K_a(**1**) is defined by Eq. 1 in Scheme 1.

In the presence of a sugar (0.2 M), the fluorescence intensity vs. pH plot underwent a shift to low pH region. The sugars investigated include monosaccharides [glucose (Glc, $\mathbf{4}_1$) and fructose (Fru, **5**)], disaccharides [maltose (Glcα1→4Glc, $\mathbf{4}_2$), cellobiose (Glcβ1→4Glc, **6**), isomaltose (Glcα1→6Glc, **7**), gentiobiose (Glcβ1→6Glc **8**), trehalose (Glcα1↔1αGlc, **9**), lactose (Galβ1→4Glc, **10**; Gal is galactose), melibiose (Galα1→6Glc, **11**), palatinose (Glcβ1→6Fru, **12**), and sucrose (Fruβ2↔1αGlc, **13**)], and higher oligosaccharides [maltotriose ($\mathbf{4}_3$), maltotetraose ($\mathbf{4}_4$), maltopentaose ($\mathbf{4}_5$), and raffinose (Galα1→6Glcα1↔2βFru, **14**)]. All of the Glc and Gal residue in oligosaccharides are of the six-membered pyranose form. Monosaccharide fructose preferentially exists as a pyranose, while that in disaccharides sucrose and palatinose and trisaccharide raffinose is a five-membered furanose. The extents of apparent pK_a lowering depend on the sugars used, as typically shown in Fig. 2 for the systems of **5**, $\mathbf{4}_2$, and $\mathbf{4}_4$. A similar observation has already been reported for anthrylboronic acid.[8] The results are interpreted as indicating that (1) both neutral **1** and anionic (**1**–OH)$^-$ reversibly form cyclic boronic esters with a sugar with equilibrium constants K' and K (Eqs. 2 and 3 in Scheme 1), respectively, and (2) **1**·sugar boronic ester thus formed is a stronger acid than **1** and further lowers the pK_a of water (Scheme 1). The apparent pK_a in the presence of fructose (**5**) (0.2 M), for example, is 7.0 (Fig. 2). The true pK_a defined by Eq. 4 in Scheme 1 for boronate **1**·fructose, pK_a(**1**·fructose), must be lower than this. Thus, at pH 9.0, for example, most of free host exists as trivalent neutral species **1**



$$K_a(1) = \frac{[(1\text{-OH})^-][H^+]}{[1]} \quad (1)$$

$$K' = \frac{[1\cdot\text{Sugar}]}{[1][\text{Sugar}]} \quad (2)$$

$$K = \frac{[(1\cdot\text{Sugar-OH})^-]}{[(1\text{-OH})^-][\text{Sugar}]} \quad (3)$$

$$K_a(1\cdot\text{Sugar}) = \frac{[(1\cdot\text{Sugar-OH})^-][H^+]}{[1\cdot\text{Sugar}]} \quad (4)$$

Scheme 1.

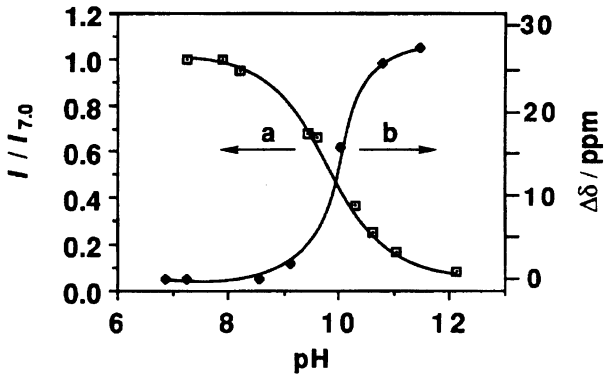


Fig. 1. pH titrations of (a) fluorescence intensity and (b) ^{11}B NMR chemical shift of **1** in H_2O at 25 °C; fluorescence intensity (I) is normalized with that at pH 7.0 ($I_{7.0}$) and ^{11}B NMR chemical shift is ppm upfield of that at pH 7.0. For fluorimetric titration, [**1**]=5.0×10^{-5} M and excitation and emission wavelengths are 290 and 361 nm, respectively. For NMR titration, [**1**]=1.0×10^{-2} M.

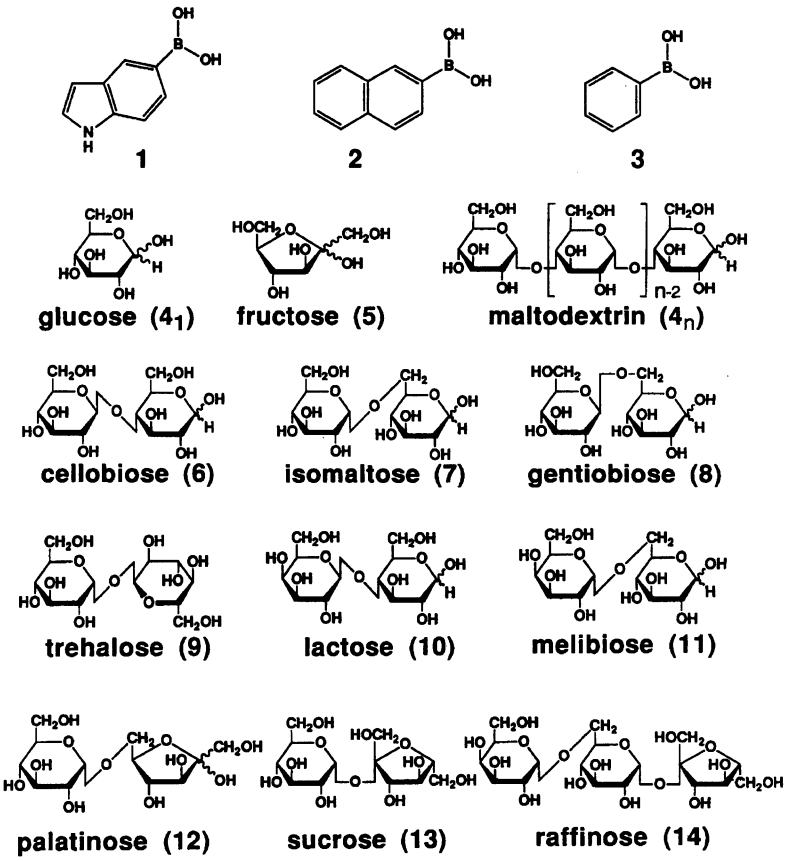


Chart 1.

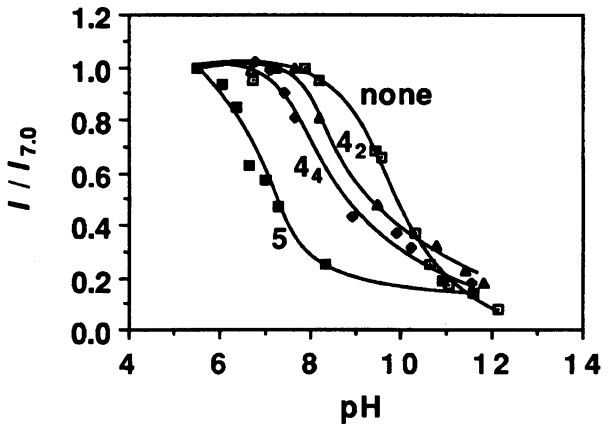
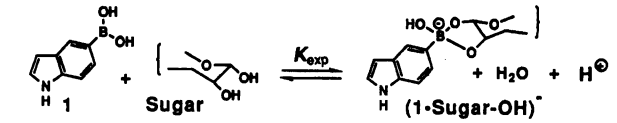


Fig. 2. pH titration of fluorescence intensity of **1** in H_2O at 25 °C in the absence (none) and presence (0.2 M) of fructose (**5**), maltose ($\mathbf{4}_2$), or maltotetraose ($\mathbf{4}_4$); fluorescence intensity (I) is normalized with that at pH 7.0 ($I_{7.0}$).

rather than tetravalent anion $(\mathbf{1}\text{–OH})^-$ ($pK_a(\mathbf{1})=10$), while most of boronate complex exists as tetravalent anion $(\mathbf{1}\cdot\text{sugar–OH})^-$ rather than trivalent neutral species **1**·sugar ($pK_a(\mathbf{1}\cdot\text{sugar})<7$). This results in an experimentally-evaluable, pH-dependent, sugar-complexation equilibrium, as shown in Scheme 2. The equilibrium constants (K_{exp}) or apparent equilibrium constants at



$$K_{exp} = \frac{[(1\cdot\text{Sugar-OH})^-][H^+]}{[1][\text{Sugar}]} = K_{exp}(\text{app})\cdot[H^+] \quad (5)$$

$$\frac{1}{I_0 - I} = \frac{1}{I_0 - I_\infty} + \frac{1}{I_0 - I_\infty}\cdot\frac{1}{K_{exp}(\text{app})}\cdot\frac{1}{[\text{Sugar}]} \quad (6)$$

Scheme 2.

fixed pH (K_{exp}(app)), defined in Eq. 5 in Scheme 2, were determined by fluorescence titration using a fixed amount of **1** (5.0×10^{-5} M) and varying amounts of sugar at pH 9.0. It again takes advantage of the fact that anionic boronic esters $(\mathbf{1}\cdot\text{sugar–OH})^-$ less strongly fluoresce than parent **1**. In every case, the titration data exhibited a saturation behavior and were satisfactorily analyzed by the Benesi–Hildebrand treatment (Eq. 6 in Scheme 2) assuming a 1 : 1 boronic ester formation (Scheme 2); I_0 and I are fluorescence intensities at 361 nm of host **1** excited at 290 nm in the absence and presence of a sugar and I_∞ is that of complex **1**·sugar. Typical examples of titration data and Benesi–Hildebrand plots thereof according to Eq. 6 in Scheme 2

for selected sugars are shown in Figs. 3 and 4.

When indole was used in place of its boronic acid derivative **1**, no reduction of the fluorescence intensity was observed in the presence of maltotetraose ($\mathbf{4}_4$) under otherwise identical conditions (Fig. 5A, where the titration curve for **1** is also shown for comparison). Figure 5B shows the fluorescence intensity vs. pH plots for indole in the presence (0.2 M) or absence of fructose (**5**). Clearly, there is no effect of added sugar.[10] These results further confirm that the boronic ester formation is responsible for the fluorescence quenching of **1**.

Another evidence came from ^{11}B NMR spectroscopy. A solution of host **1** (1.0×10^{-2} M) in H_2O at pH 9.0 (**1** $\rightleftarrows$ (**1**–OH)$^-$) showed a weighed-averaged single ^{11}B resonance at $\delta_B=9.5$. In the presence of sugar **12**, another resonance appeared at -10.5, which could be assigned to anionic boronate ester (**1**·**12**–OH)$^-$. ^{1}H NMR result was also consistent with this. The spectrum for **1** (1.0×10^{-2} M) in D_2O gave a set of aromatic proton resonances at δ_H 6.62, 7.40, 7.57, and 8.12. In the presence of sugar **12**, another set of resonances assigned to anionic boronate (**1**·**12**–OH)$^-$ appeared at δ_H 6.52, 7.30, 7.42, and 7.78 as a result of 0.1—0.34 ppm upfield shifts. The observation of distinct resonances for host **1** and complex (**1**·**12**–OH)$^-$ indicates that the exchange between these is slow as compared with NMR time scale. The integration ratio in the ^{11}B NMR spectrum was **1** : (**1**·**12**–OH)$^-$ = 0.23 : 1. This corresponds to $K_{exp(app)}=3.7\times10^2$ M^{-1}, which is in agreement with $K_{exp(app)}=3.4\times10^2$ M^{-1} obtained by fluorimetry.

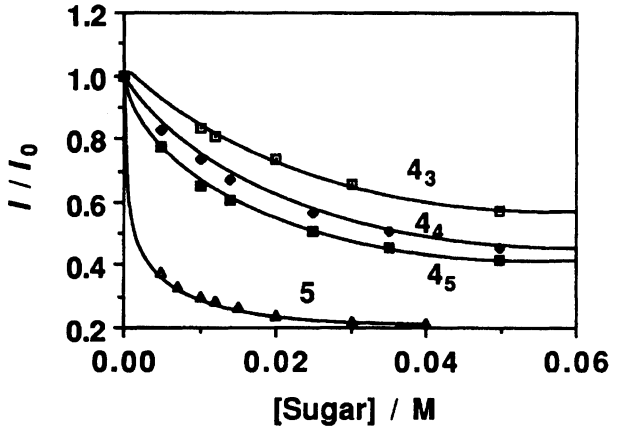


Fig. 3. Correlation between normalized fluorescence intensity of **1** and [sugar] in H_2O at pH 9.0 and at 25 °C; [**1**]$=5.0\times10^{-5}$ M and I_0 is the intensity in the absence of sugar. Sugar is fructose (**5**), maltotriose ($\mathbf{4}_3$), maltotetraose ($\mathbf{4}_4$), or maltopentaose ($\mathbf{4}_5$).

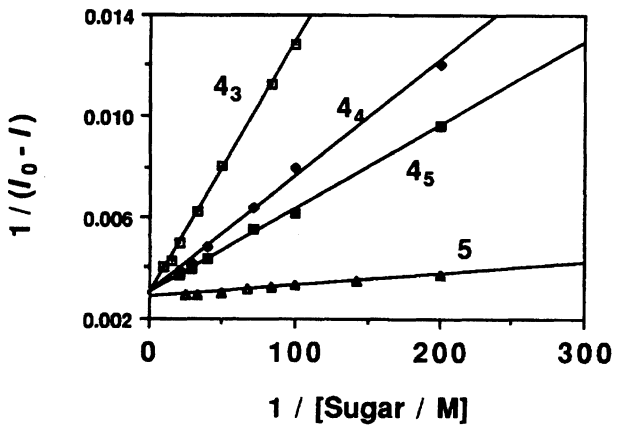


Fig. 4. Analysis of the titration data in Fig. 3 according to Benesi–Hildebrand (Eq. 6).

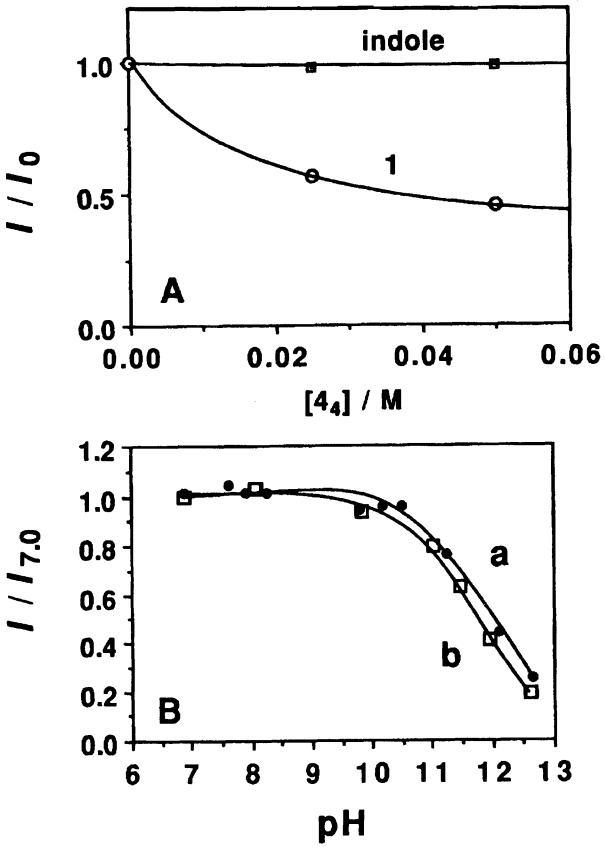


Fig. 5. A: Correlation between normalized fluorescence intensity of indole or **1** and [$\mathbf{4}_4$] in H_2O at pH 9.0 and at 25 °C; [indole]=[**1**]$=5.0\times10^{-5}$ M and I_0 is the intensity in the absence of $\mathbf{4}_4$. B: pH titration of fluorescence intensity of indole in H_2O at 25 °C; in the absence (a) and presence (0.2 M) of **5** (b); [indole]$=5.0\times10^{-5}$ M and the fluorescence intensity (I) is normalized with that at pH 7.0 ($I_{7.0}$).

Selectivity. The apparent experimental binding constants (K_{exp}(app)) (Eq. 5 in Scheme 2) obtained were converted to the true binding constants (K), as summarized in Table 1, by the relationship $K=K_{exp}/K_a=K_{exp}(app)[H^+]/K_a=10\ K_{exp}(app)$; under

Table 1. Binding Constants (K) for the Boronic Ester Formation between 5-Indolylboronic Acid (**1**) and Various Sugars in Water at 25 °C[a)]

Guest	$\mathbf{4_1}$	**5**	$\mathbf{4_2}$	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	$\mathbf{4_3}$	$\mathbf{4_4}$	$\mathbf{4_5}$	**14**
K/M^{-1}	71	6300	72	30	310	250	ca. 0	90	580	3400	ca. 0	290	670	930	ca. 0

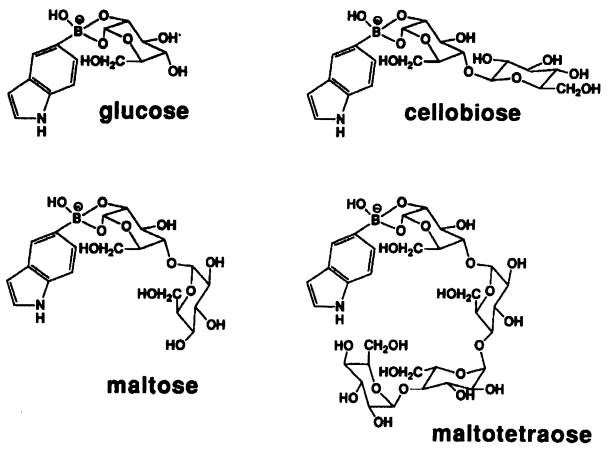
a) $[\mathbf{1}]=5.0\times10^{-5}$ M. The binding constants are reproducible within ±25% (±15% in most cases).

the experimental conditions (pH=9.0), $[H^+]=10^{-9}$ M and $K_a=10^{-10}$ M).

Inspection of Table 1 reveals a number of important aspects of the present sugar complexation. (1) Host **1** shows the highest affinity to fructose (**5**) and the lowest one to nonreducing sugars having no OH group on the anomeric carbon such as trehalose (**9**), sucrose (**13**), and raffinose (**14**). This is not surprising since reference host **3** exhibits the same trends. As is well-documented for arylboronic acids,[4,5)] the cis vicinal OH groups on the anomeric carbon and the adjacent one of a reducing sugar provide the primary site of boronic ester formation. (2) Except for cellobiose (**6**), the affinities of disaccharides having a glucose residue as the reducing terminus ($\mathbf{4_2}$, **7**, **8**, **10**, and **11**) are higher than or comparable to that of glucose ($\mathbf{4_1}$). This is not the case for reference host **3**, which shows significantly reduced affinities to disaccharides than to glucose.[15)] (3) There is a moderate α/β stereoselectivity between 1,4-linked glucose dimers, i.e., α-linked maltose ($\mathbf{4_2}$) and β-linked cellobiose (**6**), as well as between 1,6-linked glucose dimers, i.e., α-linked isomaltose (**7**) and β-linked gentiobiose (**8**). (4) There is a notable selectivity between linkage isomers of oligosaccharides. Thus, 1,6-linked dimers, gentiobiose (**8**), isomaltose (**7**), and melibiose (**11**), are bound significantly more strongly to host **1** than the corresponding 1,4-linked isomers, cellobiose (**6**), maltose ($\mathbf{4_2}$), and lactose (**10**), respectively. (5) As a result, there is a remarkable selectivity among disaccharides. The relative affinities increase in the order **9** (ca. 0)≈**13** (ca. 0)≪**6** (1, standard)< $\mathbf{4_2}$ (2.4)<**10** (3.0)<**8** (8.3)<**7** (10)<**11** (19)≪**12** (110). The high affinity of palatinose (**12**), although smaller than that of a component monosaccharide fructose (**5**), would be due to the presence of fructofuranose residue as the reducing terminus. (6) As another important fact, the affinities of maltodextrins increase with increasing chain lengths, i.e., in the order $\mathbf{4_1}\cong\mathbf{4_2}<\mathbf{4_3}<\mathbf{4_4}<\mathbf{4_5}$. This is not simply because of the availability of more vicinal OH pairs in higher homologs of maltodextrin, since reference host **3** binds $\mathbf{4_4}$ ($K=94$ M^{-1}) definitely less strongly than $\mathbf{4_1}$ ($K=390$ M^{-1}). Selectivities of sugars provide another significant comparison. Monosaccharides glucose ($\mathbf{4_1}$) forms a more stable boronate with phenylboronic acid (**3**) ($K=390$ M^{-1}) than with indolylboronic acid (**1**) ($K=71$ M^{-1}). On the other hand, oligosaccharides, maltotetraose ($\mathbf{4_4}$) for example, are more tightly bound to **1** ($K=670$ M^{-1}) than to **3** ($K=94$ M^{-1}). There must be some extra stabilization in the **1**-oligosaccharide interaction.

Sugars have both hydrophilic and hydrophobic characters.[2)] We have previously shown that hydrophobic fluorescence probes such as 8-anilino-1-naphthalenesulfonate and 6-(*p*-toluidino)-2-naphthalenesulfonate can be bound in water to higher homologs of maltodextrin, where the binding constants increase with increasing chain lengths (n).[6a)] The indole-sugar interaction has also been noted; maltodextrins were shown to be bound to L-tryptophanyl-L-tryptophane via a bis-indole-oligosaccharide intercalation.[6b)] A similar interaction involving the indole moiety probably gives the source of extra stabilization of the **1**·oligosaccharide boronates in the present case. Detailed nature of this interaction remains to be further investigated. It may either be the so-called hydrophobic effect or involve a guest–host CH–π interaction, or more probably a combination of these. It is interesting to refer here to the binding behavior of 2-naphthylboronic acid (**2**) as reference. The fluorescence intensity vs. pH titration for host **2** carried out as above gave a pK_a (**2**) value of 8.3, which is significantly lower than that for **1** ($pK_a(\mathbf{1})=10$). The pH-corrected, true binding constants of **2** for selected guest sugars were obtained from fluorimetric titration in a similar manner as above; $K=98$ and 200 M^{-1} for glucose ($\mathbf{4_1}$) and maltotetraose ($\mathbf{4_4}$), respectively. Thus, the less basic naphthalene derivative **2** forms less stable boronate with maltotetraose than the more basic indole derivative **1** ($K=71$ and 670 M^{-1} for $\mathbf{4_1}$ and $\mathbf{4_4}$, respectively).[16)]

Examination of CPK molecular models reveals accessible conformation of **1**-sugar boronates (Scheme 3). In the case of monosaccharides, such as glucose, with their cis vicinal 1-OH and 2-OH groups boronated, the pyranose and indole rings are roughly perpendicular to each other; there must be no strong interaction between these. In maltose-derived boronate, the remote or the second pyranose ring and the indole moiety *can* take a nearly parallel orientation owing to the α-1,4-linkage, although the separation of these two rings seems to be significant. This is, however, not the case for cellobiose system, which has a β-1,4-linkage. The chance of such a sugar-indole contact seems to be significantly enhanced when the oligosaccharide chain is elongated in the maltodextrin series, as schematically shown for maltotetraose, or when a more flexible 1,6-linked disaccharide is used. The expectations from these model-building studies are thus consistent with the major experimental observations; (1) steady increase in K of maltodextrin in



Scheme 3.

the order $\mathbf{4}_2 < \mathbf{4}_3 < \mathbf{4}_4 < \mathbf{4}_5$, (2) a moderate stereoselectivity between α- and β-stereoisomers of oligosaccharides, and (3) a notable preference for 1,6-linked disaccharides over the corresponding 1,4-linked ones.

Conclusions

The formation of anionic boronates between host **1** and oligosaccharides in water actually involves a substantial contribution of sugar–indole interaction. This brings about a sizable selectivity for longer-chain oligosaccharides. Selectivities among stereoisomers and linkage isomers of disaccharides can also be achieved. Thus, there is a remarkable selectivity among oligosaccharides depending on the identity of component monosaccharides, chain length, and stereo- and regiochemistry of the glycoside linkage. The affinities of disaccharides having a glucose residue as the reducing terminus increase with respect to the nonreducing sugar moiety in the order Glcβ1→4 (**6**) < Glcα1→4 (**4**$_2$) < Galβ1→4 (**10**) < Glcβ1→6 (**8**) < Glcα1→6 (**7**) < Galα1→6 (**11**). The present study emphasizes potential contribution of aromatic moieties to the sugar-binding processes in water and the role of the indole ring in particular in this respect. This allows a deeper insight into the significance of close contact of aromatic amino acid side chains and C–H moieties of bound sugar in protein–sugar complexes and also provides a guide for designing more elaborate artificial sugar receptors.

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9) This compound was actually obtained as a nearly 1 : 1 mixture of compound **1** and its anhydride. Found: C, 62.38; H, 4.37; N, 8.90%. Calcd for a 1 : 1 mixture of $C_8H_8BNO_2$ and C_8H_6BNO: C, 63.20; H, 4.64; N, 9.26%.

10) The reduction in the fluorescence intensity at pH≈10.5 is due to deprotonation of indole.

11) Compound **1** also shows a blue shift of its electronic absorption band with concomitant reduction in the intensity (hypochromicity) upon binding of a sugar, in a similar manner as reference **3**. The extent of hypochromicity seems to be not enough to allow accurate determination of binding constants (K).

12) ^{11}B NMR spectroscopy always showed a single resonance for a five-membered cyclic boronate. In no case was detected a signal corresponding to a six-membered boronate involving 4- and 6-OH groups of a sugar moiety.[13] In fact, nonreducing oligosaccharides such as **9**, **13**, and **14** were not practically bound. These results, coupled with conjunction with the present experimental conditions using a large excess amount of sugar, can be taken as strong supporting evidence for the 1 : 1 (**1** to sugar) stoichiometry, if not convincing.[14]

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16) π-Basicity of the host is also an important factor in the sugar-binding process by the pyrogallol or resorcinol cyclic tetramer in water: K. Kobayashi, Y. Asakawa, Y. Kato, and Y. Aoyama, *J. Am. Chem. Soc.*, **114**, 10307 (1992).