Specific Complexation of Glucose with a Diphenylmethane-3,3'-diboronic Acid Derivative: Correlation between the Absolute Configuration of Mono- and Di-saccharides and the Circular Dichroic Activity of the complex

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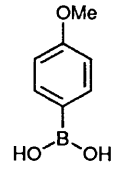
For the development of receptor molecules that can precisely recognize sugar molecules, we recently synthesized bis-(6-methoxyphenyl)methane-3,3'-diboronic acid **2**. This compound forms 1:1 complexes with mono- and di-saccharides and gives circular dichroism (CD) spectra specific to each saccharide. It was shown on the basis of ^{1}H NMR spectroscopy that the complex with D-glucose is a macrocyclic compound formed by the reaction of the two boronic acids with *cis*-1,2-diol and *trans*-4-OH, 5-CH_2OH moieties. Thus, compound **2** becomes CD-active because of asymmetric immobilization of the two chromophoric benzene rings by ring closure with chiral saccharides. The association constants were in the following order: D-glucose (19 000 $dm^3\ mol^{-1}$) $\gg$ D-talose > D-galactose > D-mannose > D-fructose (= 0 $dm^3\ mol^{-1}$) for monosaccharides, and D-maltose (100 $dm^3\ mol^{-1}$) > D-cellobiose > D-lactose > D-saccharose (= 0 $dm^3\ mol^{-1}$) for disaccharides. In particular, compound **2** showed a very high affinity toward D-glucose. D-Glucose gave a CD spectrum with positive exciton coupling whereas L-glucose gave a CD spectrum with negative exciton coupling. D-Galactose gave a CD spectrum with negative exciton coupling, whereas all other D-mono- and D-di-saccharides tested herein gave CD spectra with positive exciton coupling. The results indicate that the absolute configuration of saccharides can be conveniently predicted from the sign and the strength of the CD spectra of their complex with compound **2**. This means that the CD spectroscopic method using compound **2** as a receptor probe serves as a new sensory system for sugar molecules.

The development of receptor molecules that can precisely recognize and specifically bind guest molecules has been the focus of much recent attention.[1,2] In the design of such artificial receptor molecules hydrogen-bonding interactions play a central role.[3–8] For example, Rebek *et al.*[3] synthesized model receptors that have carboxylate functions in a molecular cleft. Hamilton *et al.*[4] synthesized macrocyclic receptors that feature a 2,6-diaminopyridine unit as a recognition site. It was recently demonstrated that recognition through hydrogen-bonding interactions is also effective for sugars and cyclodextrins.[9,10] One should note, however, that hydrogen-bonding interactions are useful only in aprotic solvents while sugars are soluble only in water or protic solvents such as alcohols. To overcome this dilemma, we considered that precise sugar recognition may be achieved through the formation of covalent bonds useful even in protic solvents rather than through non-covalent interactions. The disadvantages in the use of the covalent bonds would be, if any, the lack of reversibility and the slow reaction rate towards equilibrium. It is known that boronic acids form cyclic esters with saccharides, particularly with those including *cis*-diol groups, and that the reaction occurs reversibly and rapidly at ambient temperature. Therefore, the boronic acid–*cis*-diol bond formation is not hampered by the above mentioned disadvantages.[11] Wulff *et al.*[12] demonstrated that certain saccharide molecules are precisely recognized by two phenylboronic acids immobilized in polymer matrices. We therefore considered that two boronic acids located appropriately may selectively bind certain saccharides and that the binding event may be conveniently monitored by circular dichroism (CD) spectroscopy. In this paper we report the specific complexation of phenylboronic acid derivatives (**1** and **2**) with mono- and di-saccharides. Interestingly, we found that compound **2** showed high selectivity towards glucose and that the absolute configuration can be predicted from the sign of the exciton coupling in the CD spectrum.[13,†]



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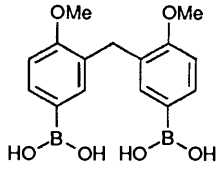

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Results and Discussion

Complexation with Glucose and its Derivatives.—Compound **2** gives three UV absorption maxima at 208, 239 and 273 nm at pH 7.0 which shift to 200, 220 and 274 nm, respectively, at pH 11.3 (Fig. 1). These two spectra are assigned to neutral $ArB(OH)_2$ and anionic $ArB(OH)_3^-$, respectively. When compound **2** was mixed with D-glucose at pH 11.3, the absorption spectrum was very similar to that of uncomplexed compound **2** at pH 11.3. These results indicate that the absorption spectra of compound **2** and its complex consist of three electronic transition bands and that the boron atoms in the **2**·D-glucose complex are sp^3-hybridized [*i.e.*, as $ArB(OH)(OR)_2^-$]. The analogous mixture of compound **1** and D-glucose did not give

† The method of using CD spectroscopy for the determination of the absolute configuration of saccharides has been demonstrated by Harada *et al.*[14a] and Nakanishi and co-workers.[14b] In these papers the chromophoric groups are attached to saccharides *via* ester or amide linkages. More recently, Aoyama and co-workers[14c] reported a new method, using a resorcinol–aldehyde cyclotetramer as a probe to predict the saccharide structure.

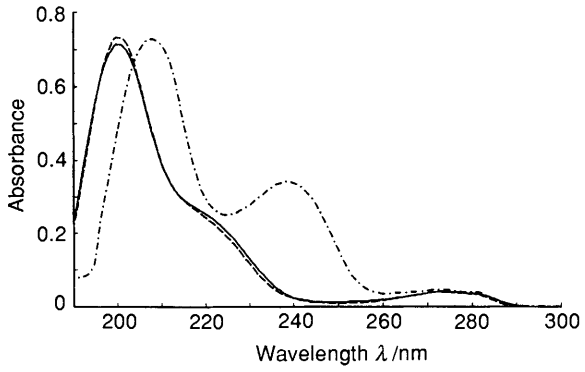


Fig. 1 Absorption spectra of compound **2**: -•---•- at pH 7.0, ---- at Ph 11.3, ——— at pH 11.3 in the presence of D-glucose (2.0×10^{-3} mol dm^{-3}), [**2**] = 1.00×10^{-3} mol dm^{-3}, 25 °C

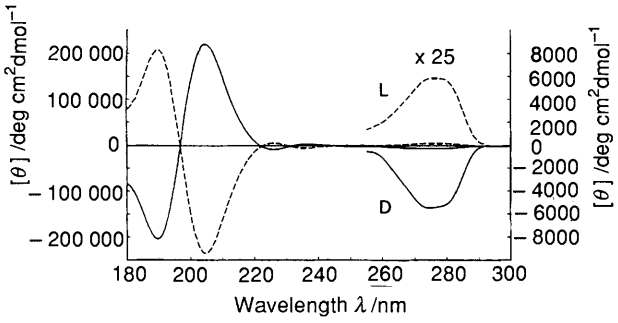
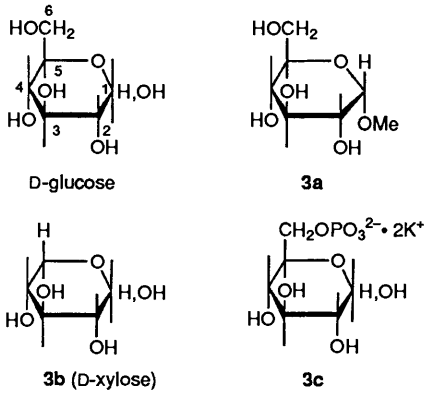


Fig. 2 CD spectra of compound **2** in the presence of D-glucose (solid line) and L-glucose (dashed line): [glucose] = 2.00×10^{-3} mol dm^{-3}, [**2**] = 1.00×10^{-3} mol dm^{-3}, pH 11.3 with 0.10 mol dm^{-3} carbonate buffer, 25 °C

any perceptible CD band in the UV region (λ > 220 nm). In the presence of compound **2**, in contrast, D-glucose gave rise to a negative, single-signed CD band at 275 nm associated with the 274 nm absorption maximum and a positive exciton-coupling band at λ_{max} 205 nm ($[\theta]_{max}$ +231 000 and −214 000 deg cm^2 dmol^{-1}), the cross-over wavelength ([θ] = 0 at 197 nm) correlating well with the λ_{max} (200 nm) of the absorption spectrum [hereafter, we designate the sign of the first (lower energy) band for the sign of the CD spectra].* As shown in Fig. 2, D- and L-isomer afforded mirror-image CD spectra ($[\theta]_{275}$ −5400 deg cm^2 dmol^{-1} for D-glucose and + 5800 deg cm^2 dmol^{-1} for L-glucose). Clearly, the sign of the CD spectrum reflects the absolute configuration of the glucose.



* When the complex between compound **2** and D-glucose was left in a buffered (pH 11.3) solution, the CD band disappeared gradually. The $[\theta]_{max}$-value was reduced to about half after one day and to one-third after three days. This is attributed to base-catalysed isomerization and decomposition of D-glucose. However, the CD spectra were reproducible within experimental error at least for 6 h after mixing.

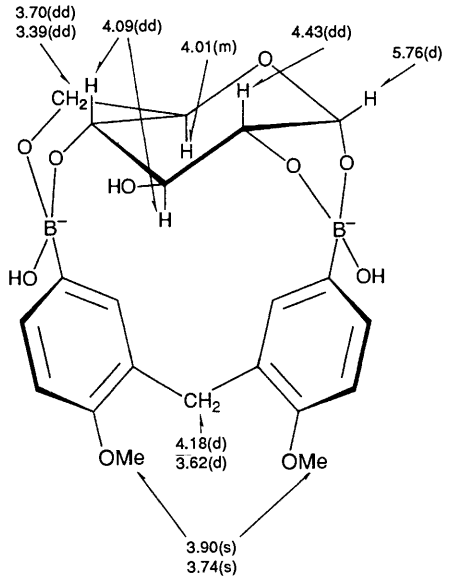


Fig. 3 Proposed structure for the **2**·D-glucose complex. The numbers indicate chemical shifts in the ^{1}H NMR spectrum.

To specify how compound **2** forms covalent bonds with D-glucose we examined monosaccharides **3a–3c** as reference compounds for D-glucose. In compound **3a** the 1-OH is methylated. In compound **3b** (*i.e.*, D-xylose) the 5-CH_2OH is removed. In compound **3c** 5-CH_2OH is protected by the phosphoric acid group. It was found that none of these substrates became CD-active. The result from compound **3a** indicates that 1-OH is essential for complexation and those from compounds **3b** and **3c** indicate that 5-CH_2OH is also essential. These finding support the view that, in the complexion with compound, **2**, a *cis*-diol (*i.e.*, as an α-anomer) and *trans*-4-OH, 5-CH_2OH moieties in D-glucose are used to form a five-membered and a six-membered ring, respectively, with the two boronic acids in compound **2**. This conclusion is in line with our previous work on the theoretical estimation of the heat of formation (ΔH_f) for 1 : 1 complexes formed from phenylboronic acid and D-glucose: the most stable complex includes the covalent bonds with *trans*-4-OH, 5-CH_2OH moieties (ΔH_f −417.1 kcal mol^{-1})† and the next includes the covalent bonds with the α-*cis*-1,2-diol (ΔH_f −413.9 kcal mol^{-1}). The complexes with covalent bonds including β-*trans*-1,2-diol, *trans*-2,3-diol and *trans*-3,4-diol moieties all had ΔH_f higher than these two values.[15] As described later, compound **2** forms a 1 : 1 complex with D-glucose. Therefore the foregoing results suggest that compound **2** and D-glucose form a cyclic complex through covalent bonds with *cis*-1,2-diol and *trans*-4-OH, 5-CH_2OH moieties (as shown in Fig. 3). In fact, we could construct the molecular model with little steric hindrance if the six-membered ring formed from *trans*-4-OH, 5-CH_2OH and a boronic acid adopted a boat conformation.

We tried to confirm the structure proposed in Fig. 3 by ^{1}H NMR spectroscopy. Generally, the ^{1}H NMR analysis of saccharides is very difficult because most signals appear at δ ~ 3–4 ppm. The ^{1}H NMR spectrum of the **2**·D-glucose complex is shown in Fig. 4. We employed an excess of compound **2** over D-glucose in order to eliminate the ^{1}H NMR signals arising from uncomplexed D-glucose. One of the 3-H or 4-H proton signals overlapped with that of HDO, so that the peak at δ 4.09 is only assignable either to 3-H or to 4-H. Except for these two protons, however, we could reasonably assign all peaks to the protons in the structure illustrated in Fig. 3. According to Karplus' rule, J_{HH} = 3.72 Hz for 1-H and 2-H indicates that

† 1 cal = 4.184 J.

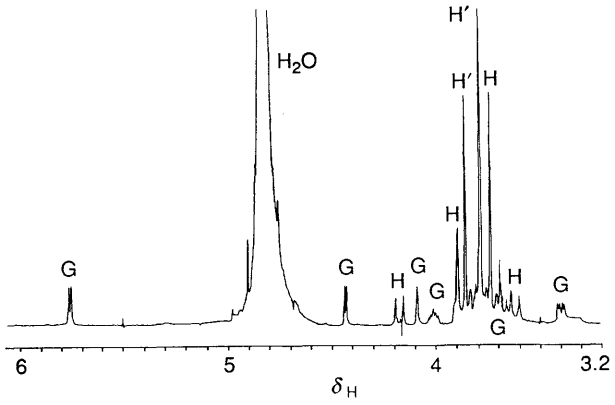
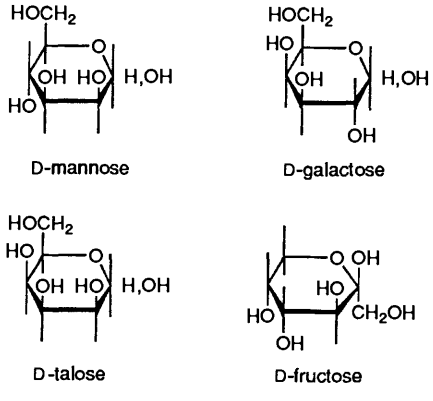


Fig. 4 Partial ^{1}H NMR spectrum of the **2**·D-glucose complex: [**2**] = 0.010 mol dm^{-3}, [D-glucose] = 5.6 × 10^{-3} mol dm^{-3}, D_2O (containing 2 vol% CD_3SOCD_3), pH 11.7 with NaOD, internal standard sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). H and G denote complexed host (*i.e.*, **2**) and guest (*i.e.*, D-glucose), respectively, and H' denotes uncomplexed host present in excess.

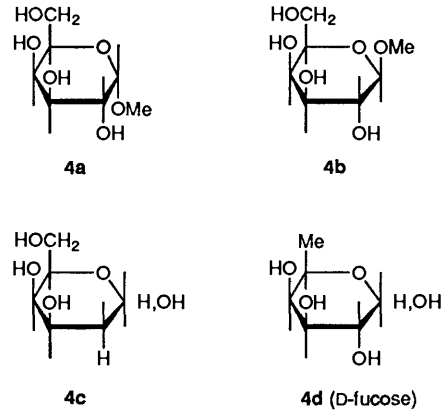
these two protons are fixed in a *gauche* conformation (*i.e.*, D-glucopyranose adopts the α-form; in the β-form 1-H and 2-H adopt an *anti* conformation).

The foregoing findings consistently support the view that the origin of the CD activity is associated with asymmetric immobilization of the two chromophoric benzene rings by the ring structure including a chiral D-glucose unit. The conclusion is compatible with the finding that the **1**·D-glucose complex, which cannot form the cyclic structure, is CD-silent.

CD Activity and Absolute Configuration of Monosaccharides.—We measured CD spectra of four monosaccharides (D-mannose, D-galactose, D-talose and D-fructose) in addition to D-glucose. When regarding D-glucose as a standard structure, 2-OH is inverted in D-mannose and 4-OH is inverted in D-galactose. In D-talose, both 2-OH and 4-OH are inverted.



We found that, in the presence of compound **2**, D-mannose and D-galactose afford very weak CD bands whereas D-talose affords a strong CD band comparable with that of D-glucose. Also interesting is the finding that D-mannose and D-talose give a positive exciton-coupling bond as D-glucose does, whereas D-galactose gives a negative exciton-coupling bond as L-glucose does. In contrast, D-fructose was CD-silent in the presence of compound **2**. In order to specify the OH groups which form the covalent bonds with compound **2**, we measured the CD spectra of compound **2** in the presence of several D-galactose derivatives (**4a**–**4d**). In compounds **4a**–**4c** one of the 1,2-diol OH groups is eliminated or protected. In compound **4d** 5-CH_2OH is dehydroxylated. We found that in the presence of compound **2** none of these D-galactose derivatives shows CD activity. The results again support the concept that the CD activity can be rationalized on the basis of the formation of covalent bonds with the 1,2-*cis*-diol and the 4-OH, 5-CH_2OH-diol.



What is the origin of the sign and the strength of these CD spectra? From the above conclusions, one can readily explain why D-fructose is CD-silent. D-Fructose has a 1,2-*cis*-diol pointing upwards and a 3,4-*cis*-diol pointing downwards. These *cis*-diols may react with boronic acids but the phenyl rings cannot be linked as illustrated in Fig. 3: that is, D-fructose and compound **2** cannot form a cyclic structure. As recorded in Table 1, D-glucose and D-talose give large $[\theta]_{max}$-values whereas D-mannose and D-galactose give small $[\theta]_{max}$-values. When carefully examining the absolute configuration of these four monosaccharides, we noticed a basic structural difference between the former two and the latter two monosaccharides. In the former two monosaccharides (large $[\theta]_{max}$-values) 2-OH and 4-OH adopt the same configuration. In the latter two monosaccharides (small $[\theta]_{max}$-values) 2-OH and 4-OH adopt opposite configurations. Examinations with CPK molecular models and by theoretical calculations[15] suggested that when phenylboronic acid (PB) forms a six-membered ring with 4-OH, 5-CH_2OH, the spatial position of the phenyl ring is governed by the configuration of 4-OH: for example, the phenyl ring in the PB·D-glucose complex occupies the α-face. The phenyl ring in the second PB, which forms a complex with α-D-glucopyranose *via* the *cis*-1,2-diol, also occupies the α-face. As a result, the two phenyl rings can be easily linked with a methylene bridge to afford a cyclic structure below the saccharide skeleton (as shown in Fig. 3). This explanation is readily applied to D-talose: when it adopts the β-D-talopyranose structure with the 1,2-*cis*-diol moiety, the two phenyl rings occupy the β-face and should easily form a cyclic structure with compound **2** above the saccharide skeleton. In contrast, it is difficult for D-mannose and D-galactose to form such a cyclic structure with compound **2**: in β-D-mannopyranose the phenyl ring in PB complexed with the *cis*-1,2-diol occupies the β-face while that complexed with 4-OH, 5-CH_2OH occupies the α-face, and in α-D-galactopyranose the phenyl ring in PB complexed with the *cis*-1,2-diol occupies the α-face while that complexed with 4-OH, 5-CH_2OH occupies the β-face. It is conceivable, therefore, that cyclization of compound **2** with these monosaccharides should inevitably lead to serious steric distortion. The foregoing considerations reasonably explain why the $[\theta]_{max}$-values are sensitively affected by the absolute configuration of the respective monosaccharide.

It is more difficult to explain the sign of the exciton coupling band. D-Galactose and L-glucose gave a negative first exciton coupling whereas all other monosaccharides tested herein gave a positive first exciton coupling. The results imply that two dipoles in the chromophoric benzene rings of compound **2**

Table 1 Absorption and CD maxima of **2**•monosaccharide complexes[a]

	UV	CD			*K*
Saccharide	λ_{max}(nm)	λ_{max} (nm)	$[\theta]_{max}$[b] (deg cm^2 dmol^{-1})	Stoichiometry	(dm^3 mol^{-1})
D-Glucose	274	275	−5 300	1:1	19 000
	200	205	+231 000		
		190	−214 000		
D-Mannose	272	274	−400	1:1	60
	200	205	+69 000		
		191	−23 000		
D-Galactose	273	276	+410	1:1	2 200
	200	205	−22 000		
		191	+19 000		
D-Talose	272	275	−3 700	1:1	4 600
	200	205	+247 000		
		190	−196 000		
D-Fructose	274	Nd[c]	Nd		
	200	Nd	Nd		

[a] 25 °C, [**2**] = 1.00 or 2.00 × 10^{-3} mol dm^{-3}, pH 11.3 with 0.10 mol dm^{-3} carbonate buffer. [b] $[\theta]_{max}$-Values are calculated, assuming 100% complex, from the concentration dependence for the 1:1 complex region. [c] Nd denotes that the perceptible CD band does not appear.

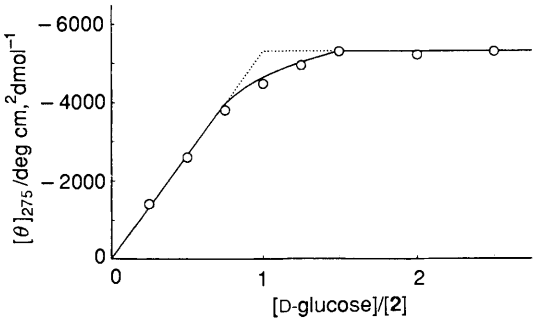
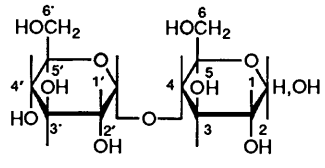


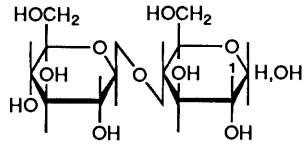
Fig. 5 Molar ratio plot of $[\theta]_{275}$ against D-glucose concentration: [**2**] = 2.00 × 10^{-3} mol dm^{-3}, pH 11.3 with 0.10 mol dm^{-3} carbonate buffer, 25 °C.

complexed with the former two monosaccharides are oriented in an *anti* clockwise direction [(*S*)-chirality] whereas those of compound **2** complexed with other monosaccharides are oriented in a clockwise direction [(*R*)-chirality]. As described above, the two benzene rings in compound **2** complexed with D-glucose occupy the α-face because 2-OH and 4-OH point downwards. In a similar manner, the two benzene rings in compound **2** complexed with D-talose should occupy the β-face because 2-OH and 4-OH point upwards. Nevertheless, these two monosaccharides gave the same positive exciton coupling. This means that the α- *vs.* β-face for the benzene rings is not a crucial factor the sign of exciton coupling.* At present, we cannot find any reasonable rationale for the relation between the absolute configuration and the sign of exciton coupling. We are going to continue further investigations into this problem.

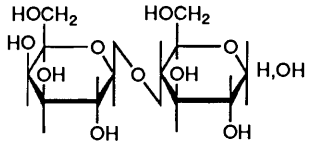
CD Activity of Disaccharides.—We have measured the CD spectra of four disaccharides in the presence of boronic acids **1** and **2**. Again, monoacid **1** was ineffective. In the presence of diacid **2**, on the other hand, D-maltose, D-cellobiose and D-lactose showed a negative CD band at 275 nm and an exciton-coupling band with a positive first Cotton effect (Table 2). The CD activity for D-maltose and D-cellobiose is readily explained on the basis of the prerequisites proposed for D-glucose: the molecules have *cis*-1,2-diol and *trans*-4′-OH, 5′-CH_2OH moieties required for the ring formation with compound **2**. We could construct molecular models for the cyclic complexes including covalent bonds between these diols and boronic acids. These $[\theta]_{max}$-values, smaller than that for D-glucose, may be attributed to the increased flexibility in the macrocycles formed from compound **2** and disaccharides. D-Lactose, which showed a relatively weak CD band, is exceptional. The configurations of its 1,2-diol and 3′-OH, 4′-OH, 5′-CH_2OH moieties are similar to those of the 1,2-diol and 3-OH, 4-OH, 5-CH_2OH of D-galactose, respectively. As mentioned above, the **2**•D-galactose complex is not so stable as the others because the configuration of 2-OH is opposite to that of 4-OH. This is also the case in the **2**•D-lactose complex. The small $[\theta]_{max}$ and *K* for D-lactose (see Table 2) may be related to the steric difficulty in forming a cyclic structure with compound **2**. On the other hand, D-saccharose (D-sucrose) was CD-silent. As expected from the absolute configuration, there is no *cis*-1,2-diol which is indispensable for the formation of a macrocyclic structure with compound **2**.



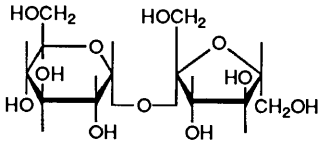
D-maltose



D-cellobiose



D-lactose



D- saccharose (D-sucrose)

Stoichiometry and Association Constants (K).—We estimated the stoichiometry of the complexes either by a molar-ratio method or by a continuous-variation method. Compound **2** showed a particularly high affinity for D-glucose, so that the stoichiometry is readily determined to be 1:1 from the molar-ratio plot (Fig. 5). From this plot the value of *K* was estimated to be 19 000 dm^3 mol^{-1}. Since the *K*-values for other mono- and di-saccharides are not large enough for us to use the molar ratio method, we applied the continuous-variation method. The

* We tried to estimate the relative stability between (*R*)- and (*S*)-chirality for the **2**-D-glucose complex on the basis of a computational method (MOPAC ver. 6.0, AM1 Hamiltonian). However, we could not find any significant difference in the heat of formation (ΔH_f) between these two chiralities.

Table 2 Absorption and CD maxima of **2**·disaccharide complexes[a]

	UV	CD			*K*
Disaccharide	λ_{max}(nm)	λ_{max}(nm)	$[\theta]_{max}$[b] (deg cm^2 dmol^{-1})	Stoichiometry	(dm^3 mol^{-1})
D-Maltose	274	275	−2 400	1:1	100
	200	204	+84 000		
		190	−71 000		
D-Cellobiose	274	275	−2 000	1:1	80
	200	205	+39 000		
		190	−36 000		
D-Lactose	274	275	−1 200	1:1	15
	200	205	+101 000		
		190	−60 000		
D-Saccharose	274	Nd[c]	Nd		
	200	Nd	Nd		

[a] Measurement conditions are recorded in footnote *a* to Table 1. [b] $[\theta]_{max}$-Values are calculated, assuming 100% complex, from the concentration dependence for the 1:1 complex region. [c] Nd denotes that the perceptible CD band does not appear.

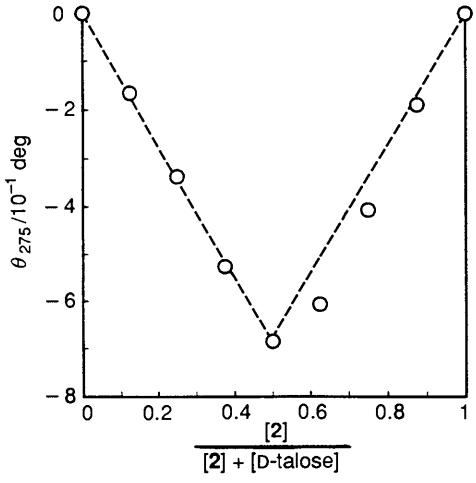


Fig. 6 Continuous-variation plot of $[\theta]_{275}$ against [2]/([2] + [D-talose]): [2] + [D-talose] = 4.00 × 10^{-3} mol dm^{-3}. Measurement conditions are recorded in the caption to Fig. 5.

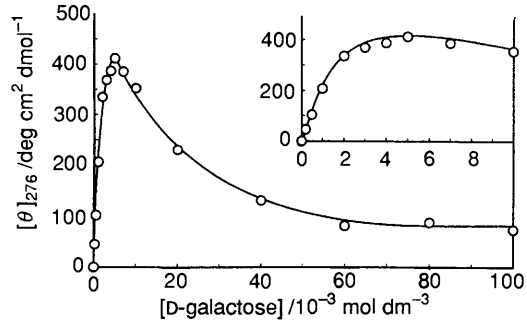
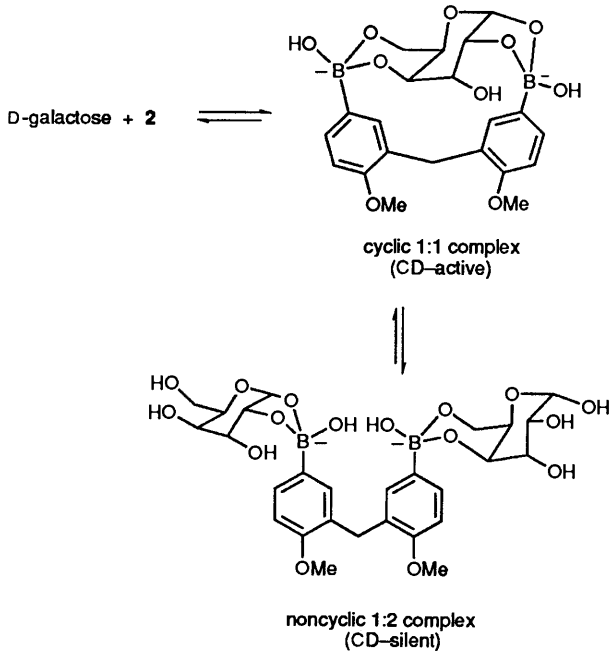


Fig. 7 $[\theta]_{276}$-Value for the **2**·D-galactose complex plotted against monosaccharide concentrations: [2] = 2.00 × 10^{-3} mol dm^{-3}. Measurement conditions are recorded in the caption to Fig. 5.

typical plot for D-talose is shown in Fig. 6. In conclusion, we found that all CD-active mono- and di-saccharides form 1:1 complexes with compound **2**.

We found that the concentration dependence of D-mannose, D-galactose and all disaccharides is more complicated, however. In the **2** (2 × 10^{-3} mol dm^{-3})·D-galactose complex, for example, $[\theta]_{276}$ first increases at [D-galactose] < 5 × 10^{-3} mol dm^{-3} and then decreases with increasing D-galactose concentration (Fig. 7). Similar behaviour was also observed for D-mannose. The biphasic dependence appeared clearly in the complex that accompanies some steric distortion upon formation of the macrocyclic complex. Such biphasic behaviour usually means that two different equilibria are occurring simultaneously in the concentration region concerned. We therefore considered that in these less stable complexes intermolecular 1:2 complexation to yield a CD-silent noncyclic complex can compete with intramolecular 1:1 cyclization to yield a CD-active cyclic complex (Scheme 1). Of course, the fraction of the



Scheme 1

intermolecular 1:2 complex increases with increasing saccharide concentration and the $[\theta]_{max}$ decreases. To estimate the *K*-values for these saccharides, we analysed the plots obtained at lower concentrations where the 1:1 complex should be predominantly formed. The results are summarized in Tables 1 and 2.

Examination of *K*-values in Tables 1 and 2 reveals that complexation with D-glucose has a particularly large *K*-value. This implies that, among monosaccharides, the *cis*-1,2-diol and *trans*-4-OH, 5-CH_2OH moieties in α-D-glucopyranose are in the best position to bind intramolecularly to the two boronic acids in compound **2**. Among disaccharides, D-maltose and D-cellobiose with *cis*-1,2-diol and *trans*-4′-OH, 5′-CH_2OH moieties basically satisfy the above requirement, but the *K*-values are rather small (100 and 80 dm^3 mol^{-1}, respectively). The difference can be ascribed either to the longer distance between *cis*-1,2-diol and *trans*-4′-OH, 5′-CH_2OH moieties or to

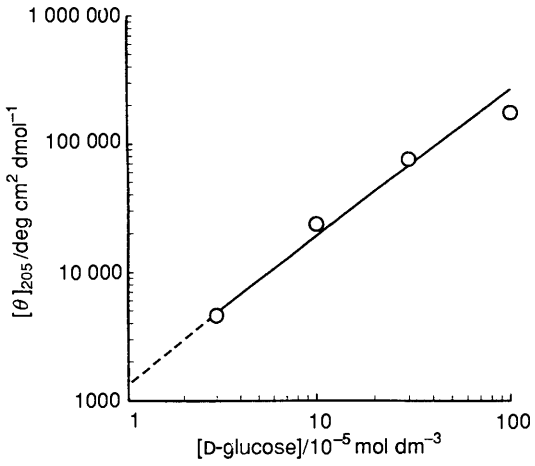


Fig. 8 Log–log plot of $[\theta]_{205}$ against D-glucose concentration: [**2**] = 1.00×10^{-3} mol dm^{-3}. Measurement conditions are recorded in the caption to Fig. 5.

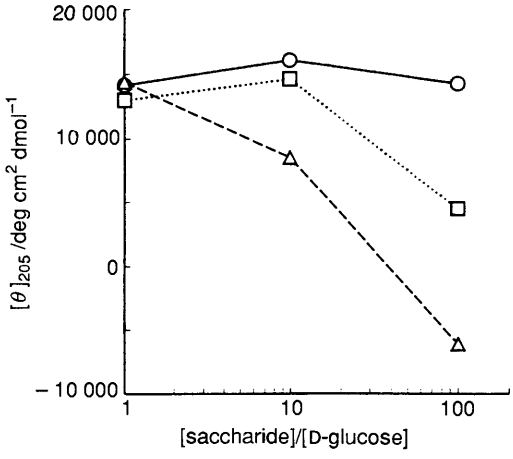
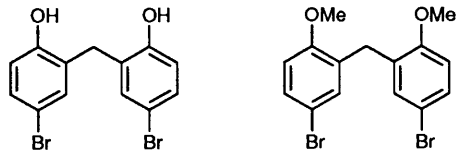


Fig. 9 Semilogarithmic plot of $[\theta]_{205}$ for **2**·D-glucose complex against [saccharide]/[D-glucose]: —○— D-mannose, – –△– – D-galactose, ···□··· D-fructose. [**2**] and [D-glucose] were maintained constant (1.00×10^{-3} and 5.00×10^{-5} mol dm^{-3}, respectively) while [saccharide] was varied. Measurement conditions are recorded in the caption to Fig. 5.

the increased flexibility of the macrocycles. We believe that if we can insert some suitable spacer longer than the CH_2 bridge, the new receptor will selectivity bind disaccharides.

Sensitive and Selective Detection of D-*Glucose.*—Since compound **2** shows high affinity and selectivity towards D-glucose, we estimated the capabilities of this compound as a reagent for detection of D-glucose. We used the strong exciton-coupling band at 205 nm. As shown in Fig. 8, the calibration plot of log [D-glucose] *vs.* log $[\theta]_{205}$ provides a good linear relationship down to 3×10^{-5} mol dm^{-3} glucose. In an enzymic method using D-glucose oxidase, the detection of 10^{-6} mol dm^{-3} D-glucose is possible. Hence, the present method using CD spectroscopy is inferior by one order of magnitude to the enzymic method. We believe, however, that the present method possesses several advantages that the enzymic method does not possess: *e.g.*, cheapness, stability for a long period, operational simplicity, *etc.*

Fig. 9 shows the influence of the coexistence of other saccharides. The presence of a 10-fold excess of other saccharides scarcely influenced the $[\theta]_{205}$-value of D-glucose. In the case of D-mannose, the spectral shape of the **2**·D-glucose complex was scarcely affected even in the presence of a 100-fold excess. In the case of D-galactose, on the other hand, the change in the $[\theta]_{205}$-value could not be neglected even in the presence of a 10-fold excess of D-galactose. These trends are mostly in line with those expected from the *K*-values.



5 **6**

Conclusions.—It is well known that boronic acids form stable complexes with diols.[11] The reaction occurs easily at room temperature in an aq. system. We have demonstrated that, by the combination of this 'classical' reaction with CD spectroscopy, sugar molecules can be easily detected and even the absolute configuration can be predicted. In particular, diphenylmethane-3,3′-diboronic acid behaves as an excellent probe for glucose. We believe that this concept is applicable not only to the detection of sugar molecules but also to the control of complex equilibria in sugar molecules and as a protecting method for selective modification of OH groups in sugar molecules.

Experimental

Miscellaneous.—^{1}H NMR spectra were measured with a JEOL GX-400 NMR spectrometer with DSS as reference. CD spectra were measured with a JASCO J-720 CD spectrometer. M.p.s were measured on a Yanako MP-500D micro melting point apparatus and are uncorrected.

Materials.—Compounds **1** and **2** were synthesized by the treatment of *p*-bromoanisole and bis(5-bromo-2-methoxyphenyl)methane **6**, respectively, with butyllithium followed by reaction with trimethyl borate.

4-Methoxyphenylboronic Acid **1**.—To a diethyl ether solution (30 cm^3) containing *p*-bromoanisole (1.25 cm^3, 10 mmol) was added a hexane solution (7.3 cm^3) containing butyllithium (1.50 mol dm^{-3}; 11 mmol) and the mixture was stirred at −78 °C for 2 h under a stream of nitrogen. The reaction mixture was transferred to a dropping funnel and added to a diethyl ether solution containing trimethyl borate (19.1 cm^3, 160 mmol). The solution was stirred at −78 °C for 2 h and was then warmed to room temperature. After addition of 2 mol dm^{-3} HCl (48 cm^3), the mixture was stirred at room temperature for 12 h. After filtration, the filtrate was extracted with chloroform (70 cm^3 × 2) and the extracts were dried over $MgSO_4$. Concentration of the chloroform solution resulted in an oily product, which was crystallized from hexane. Finally, the solid was recrystallized from water to give *compound* **1** (21%), m.p. 210–212 °C; single spot (R_f 0.53) on TLC [silica gel; ethyl acetate–chloroform (1:1 v/v)]; ν_{max}(Nujol) no ν_{OH}; δ_H ($CDCl_3$) 3.86 (3 H, s, Me) and 6.97 and 8.16 (2 H each, d each, ArH), (Found: C, 62.8; H, 5.3. $C_7H_7BO_2$ requires C, 62.78; H, 5.23%). The analytical data indicated that compound **1** was isolated as its anhydride form.

Bis-(5-bromo-2-hydroxyphenyl)methane **5**.—In a three-necked flask equipped with a Dean–Stark trap, a condenser, and a dropping funnel were placed *p*-bromophenol (4.26 g, 24.6 mmol), benzene (100 cm^3) and a catalytic amount of toluene-*p*-sulfonic acid. The mixture was heated to reflux. From the dropping funnel was added a mixture of 2-hydroxymethyl-4-bromophenol (1.00 g, 4.9 mmol) in benzene (300 cm^3) dropwise for 6 h and then the mixture was refluxed for 15 h. Benzene was removed by evaporation, the oily product being crystallized

from hexane. The product was dispersed in chloroform and the insoluble material was removed by filtration. The chloroform solution was dried over $MgSO_4$ and concentrated to dryness. The residual solid was crystallized again from hexane to give the *bis-phenol* **5** (39%), m.p. 182–185 °C; single spot (R_f 0.35) on TLC [silica gel; ethyl acetate–hexane (1:3 v/v)]; δ_H[$(CD_3)_2$SO] 3.21 (2 H, s, ArCH_2Ar), 6.02–6.75 (6 H, m, ArH) and 8.05 (2 H, br s, OH). (Found: C, 43.4; H, 2.8. $C_{13}H_{10}Br_2O_2$ requires C, 43.60; H, 2.79%).

Bis-(5-bromo-2-methoxyphenyl)methane **6**.—Compound **5** (2.00 g, 5.59 mmol) and methyl iodide (13.9 cm^3, 224 mmol) were treated in dimethylformamide (130 cm^3) in the presence of K_2CO_3 (7.73 g, 55.9 mmol). The reaction was continued at 50 °C for 15 h. After filtration, the filtrate was concentrated to dryness under reduced pressure. The residual solid was dissolved in diethyl ether. The solution was washed twice with water and was then dried over $MgSO_4$. Concentration of the solution resulted in an oily product, which was crystallized from hexane. Finally, the *title product* **6** was recrystallized from methanol (59%), m.p. 107–109 °C; single spot (R_f 0.73) on TLC [silica gel; ethyl acetate–hexane (1:3 v/v)]; ν_{max}(Nujol) no ν_{OH}; δ_H($CDCl_3$) 3.76 (6 H, s, Me), 3.80 (2 H, s, CH_2) and 6.59–7.33 (6 H, m, ArH) (Found: C, 46.9; H, 3.7. $C_{15}H_{14}Br_2O_2$ requires C, 46.66; H, 3.90%).

4,4'-*Dimethoxy*-3,3'-*methylenedi*(*phenylboronic Acid*) **2**.—This *compound* was synthesized in 30% yield from dibromide **6** and trimethyl borate in the presence of butyllithium in a manner similar to that described for compound **1**, m.p. 177–180 °C; single spot (R_f 0.53) on TLC [silica gel; chloroform–ethyl acetate (1:3 v/v)]; ν_{max}(Nujol)/cm^{-1} 3100–3500 (OH); δ_H(CD_3OD) 3.80 (6 H, s, Me), 3.91 (2 H, s, CH_2) and 6.75–7.70 (6 H, m, ArH) (Found: C, 56.7; H, 5.8. $C_{15}H_{18}Br_2O_6 \cdot 0.1H_2O$ requires C, 56.71; H, 5.73%). The analytical data suggested that compound **2** was isolated as the diboronic acid.

Determination of Association Constants (K).—The *K*-values were determined from plots of [θ] *versus* [saccharide]. A typical experiment would consist of adding 10 separate 20 mm^3 aliquots of a 100 mmol dm^{-3} solution of compound **2** in methanol to 1000 mm^3 of a 100 mmol dm^{-3} solution of a saccharide in water (adjusted to pH 11.3 with 100 mmol dm^{-3} carbonate buffer). After 10 min, the CD spectrum were recorded at 25 °C. Since compound **2** and saccharides form 1:1 complexes, the [θ]–[saccharide] plots were analysed according to a Benesi–Hildebrand equation. The correlation coefficients were always better than 0.99.

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