Re-investigation of optical sensing properties of boronic-acid-appended Re^{I} complexes for saccharides

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A number of unanswered questions occurred to us upon reading a communication by Yam and Kai (ref. 16) which had reported optical sensing properties of a boronic-acid-appended Re^{I} complex for saccharides. Careful re-examination has disclosed that the pK_a-value proposed by them (5.9) is wrong and that the saccharide-binding mode at pH above the pK_a is totally different from that at pH below the pK_a. The absorption spectral change, which reflects an sp^2-to-sp^3 boron hybridisation change induced by the saccharide complexation, was observed only at pH below the pK_a, and the CD band, which reflects the formation of 1 : 1 cyclic complexes, appeared only at pH above the pK_a. The results imply that the optimum pH should be carefully selected for the precise optical sensing of saccharides.

Introduction

Recognition of neutral organic species by synthetic molecular receptors has been of great interest to many chemists, but some new methodology which is different from recognition of ionic species has to be exploited. Most of the known synthetic molecular receptors utilise hydrogen-bonding interactions in order to recognise and bind with neutral guest molecules.[1] However, these interactions are less effective in aqueous media where guest species are water-soluble and well hydrated. On the other hand, covalent interactions found in the binding between boronic acids and saccharides are stronger than such hydrogen-bonding interactions in aqueous media and, therefore, should be more effective. The usefulness of the boronic acid function as a saccharide receptor has been demonstrated in saccharide recognition in rigid matrices, CD detection, fluorescence detection, molecular assemblies, membrane transport, *etc.*[2–9] A potential breakthrough found through these studies is the finding that the acidity of boronic acids is intensified when they form cyclic boronate esters with diols.[2,7,9,10] Thus, the sp^2-hybridised boronic acid group can be converted to the sp^3-hybridised boronate anion by the saccharide complexation.[2,7,9,10] In addition, the tertiary amine bearing an intramolecular boronic acid group changes its basicity upon saccharide-binding through the boronic acid–nitrogen (B–N) interaction. Therefore, the spectroscopic properties of amine-containing chromophores are also changed by the saccharide binding.[9] These findings have enabled us to detect the saccharide-binding event with absorption and fluorescence spectroscopic methods.[6,7,9,11–14]

From a practical viewpoint for optical saccharide sensing, it is desirable to use a longer wavelength region in both absorption and fluorescence spectroscopic methods. One potential issue of this requirement is to utilise metal complexes as a chromophoric site.[8,15] More recently, Yam and Kai[16] reported a Re^{I} complex bearing two boronic acids (compound **1**) which changed electronic absorption characteristics in response to saccharide binding. Although this system is useful for the detection of saccharides in the visible wavelength region, some of their results are curious to our eyes and quite difficult to understand. First, the pK_a of the boronic acid groups was estimated to be 5.9, which is much lower than those for conventional boronic acids (≈9).[2,7,17] Secondly, it is known that the pK_a is lowered by ≈ 2 pK units upon complexation with saccharides.[2,7,9,10] Thus, the spectral change observed for the saccharide complexation is mainly due to a change in neutral boronic acids [R-B(OH)$_2$] to anionic boronate–saccharide complexes [R-(HO)B$^-$⟨O,O⟩(saccharide)].[2,6,7] If the pK_a for **1** is really 5.9 as reported by Yam and Kai,[16] it follows that the boronic acid groups should exist as anionic boronate groups [R-B$^-$(OH)$_3$] under the measurement conditions (pH 8.3) and the significant spectral change should not take place upon complexation with saccharides.[6,7] In fact, however, they observed large spectral changes for **1**–saccharide complexation. Thirdly, it is not clear how the association constants (K_{ass}) were determined. They suggested the formation of cyclic 1 : 1, noncyclic 1 : 1 and noncyclic 1 : 2 complexes on the basis of mass spectrometric data. Nevertheless, the complexation processes were expressed by a single K_{ass}-value. Fourthly, the interaction of saccharides and the deprotonated amide anions was proposed to occur at pH 12.1 on the basis of comparison with an N-methylated reference compound. To the best of our knowledge, however, it is quite rare for a significant contribution of hydrogen-bonding interactions to molecular recognition to be found in an aqueous host–guest system and, in fact, they did not present any constructive evidence for this interaction. Fifthly, they used glycine as a buffer, which is known to interact with boronic acids.[18] Because of these queries, we considered that it was worthwhile to carefully re-examine the content of the paper reported by Yam and Kai.[16] We thus synthesised compounds **1** and **2** and extensively studied their saccharide-binding properties. The results showed that they have pK_a-values of 8.4–8.9 and the association mode is totally different depending on the medium's pH.

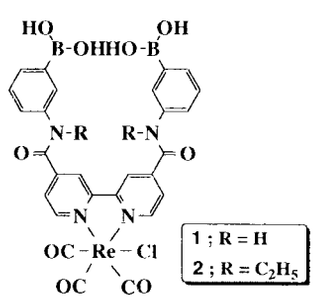

Chart 1

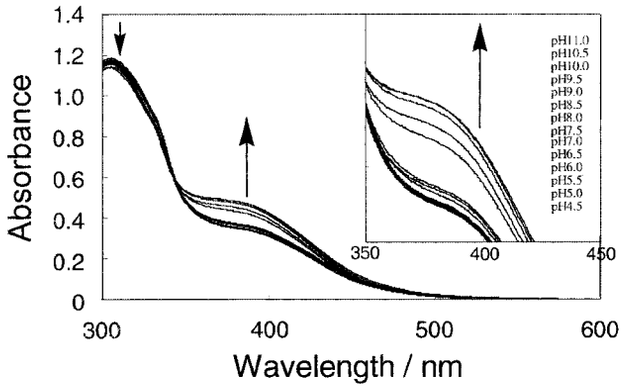

Fig. 1 pH Dependence of the absorption spectra for **1**: buffered water–Me_2SO = 1 : 1 v/v, [**1**] = 5.00×10^{-5} mol dm^{-3}, 25 °C.

Results and discussion

Determination of pK_a-values

The absorption spectral measurements were carried out in a water–Me_2SO (1 : 1 v/v) mixed solvent. The medium pH was adjusted with 50 vol% water using AcOH–AcONa for pH 4.5–5.0, KH_2PO_4–Na_2HPO_4 for pH 5.5–8.5 and $NaHCO_3$–Na_2CO_3 for pH 9.0–11.0 (50 mmol dm^{-3} for each), buffer constituents which do not or scarcely interact with the boronic acid groups. To confirm that **1** and **2** are completely dissolved into these media we measured the absorption spectra as a function of [**1**] or [**2**]. Plots of A_{370} *vs.* [**1**] or [**2**] showed a good linear relationship over 0–1.50 mmol dm^{-3} (data not shown). The result supports the view that this is a "homogeneous" system satisfying the Lambert–Beer law.

Next, the pK_a-values were estimated by photometric titration in the absence and the presence of saccharide (D-fructose which shows the highest affinity with monoboronic acids[2,7,10] was chosen). The spectral change observed for **1** in the absence of saccharide is shown in Fig. 1. It is seen from Fig. 1 that there is a large difference in absorbance between pH 8.5 and 9.0. Plots of A_{370} *vs.* pH (Fig. 2A) establish that the titration curves are satisfactorily simulated with a single pK_a of 8.9 in the absence, and 5.3 in the presence, of D-fructose. Hence, one can speculate that the two boronic acid groups form anionic OH^- adducts simultaneously or nearly so in the same pH region. Since the affinity of D-fructose with boronic acids is very high,[2,7,10] one can assume that the boronic acid groups in **1** are totally converted to the D-fructose complexes in the presence of 100 mmol dm^{-3} D-fructose. This implies that the acidity of the boronic acid groups is intensified by 3.6 pK units by D-fructose complexation. From the similar A_{370} *vs.* pH plots the pK_a-values for **2** were estimated to be 8.4 in the absence, and 5.5 in the presence, of D-fructose (100 mmol dm^{-3}: Fig. 2B).

The foregoing results consistently indicate that the 'true' pK_a-values for **1** and **2** are much higher than those reported by Yam and Kai (*i.e.*, 5.9)[16] and rather closer to those of conventional boronic acids (*i.e.*, ≈9).[2,7,17]

Saccharide-binding properties at pH 10.0

First, the saccharide-binding properties were estimated at pH 10.0 (50 mmol dm^{-3} $NaHCO_3$–Na_2CO_3) and 25 °C where the boronic acid groups are converted to the OH^-adducts and the K_{ass}-values are apparently enhanced owing to the high OH^- concentration. In this medium, the absorption spectra were scarcely changed by the saccharide addition. This implies that a change in the boronic acid group from R-$B^-(OH)_3$ to R-$(HO)B^-\langle O \rangle$(saccharide) scarcely affects the absorbance of the chromophoric Re^I·bipyridine moiety. However, the saccharide binding could be conveniently monitored by a change in CD spectroscopy.

As shown in Fig. 3, a negative CD band appeared at 370 nm

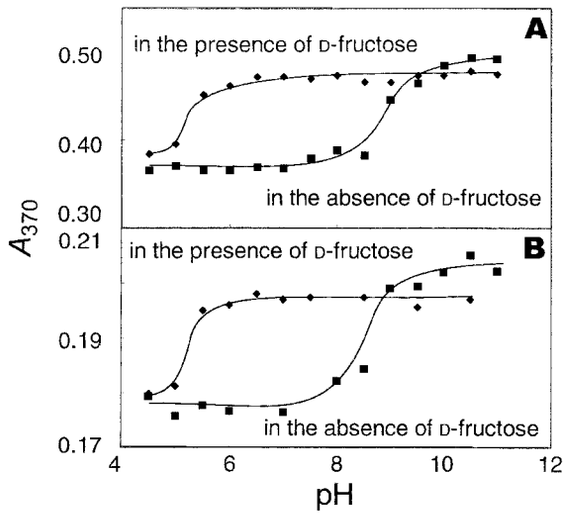

Fig. 2 Plots of A_{370} *vs.* pH for (A) **1** and (B) **2** in the absence and the presence of D-fructose (100 mmol dm^{-3}): buffered water–Me_2SO = 1 : 1 v/v, [**1**] or [**2**] = 5.00×10^{-5} mol dm^{-3}, 25 °C. -◆-, In the presence of D-fructose, -■-, in the absence of D-fructose.

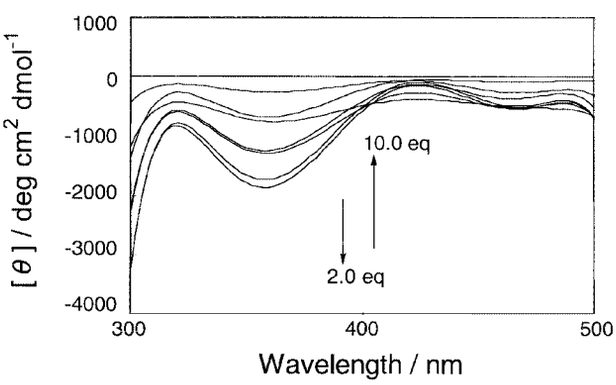

Fig. 3 CD spectral change in **1** (5.00×10^{-5} mol dm^{-3}) in the presence of D-fructose (0–5.00×10^{-4} mol dm^{-3}): water (pH 10.0 with 50 mmol dm^{-3} carbonate)–Me_2SO = 1 : 1 v/v, 25 °C.

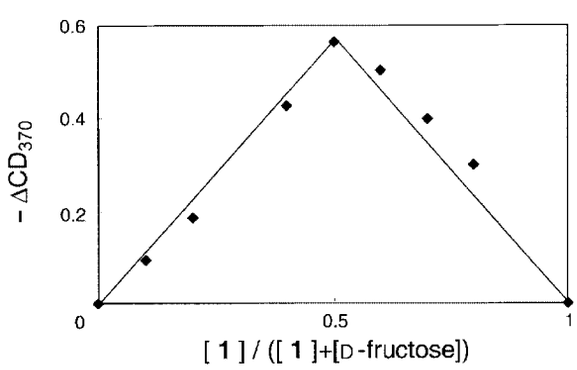

Fig. 4 Continuous-variation plot monitored at CD_{max} (370 nm): [**1**] + [D-fructose] = 1.00×10^{-4} mol dm^{-3} (constant): water (pH 10.0 with 50 mmol dm^{-3} carbonate)–Me_2SO = 1 : 1 v/v, 25 °C.

which is the same wavelength as the λ_{max} (shoulder) in absorption spectroscopy. It is known that the appearance of CD bands in diboronic acid receptors is due to the rigidification effect arising from the formation of 1 : 1 cyclic complexes.[6,7,9,13,14,19,20] In fact, a continuous-variation plot (Job's plot)[21] at [**1**] + [D-fructose] = 1.00×10^{-4} mol dm^{-3} (constant) gave a maximum at [**1**]/([**1**] + [D-fructose]) = 0.5 (Fig. 4), indicating that the CD-active species is the 1 : 1 cyclic complex.

It is seen from Fig. 3 that the CD intensity first increases up to 2 equivalents of [D-fructose]/[**1**] and then decreases with further increase in the D-fructose concentration. This biphasic

Table 1 $K_{ass\,(1:1)}$ and $K_{ass\,(1:2)}$ for **1** + saccharide systems

Saccharide	$K_{ass\,(1:1)}$/ $dm^3\ mol^{-1}$	$K_{ass\,(1:2)}$/ $dm^3\ mol^{-1}$
D-Fructose	2.0×10^5	1.5×10^3
D-Galactose	1.5×10^5	7.0×10^2
D-Arabinose	1.5×10^5	6.0×10^2
D-Mannose	1.0×10^5	2.5×10^2
D-Glucose	5.0×10^4	2.0×10^2

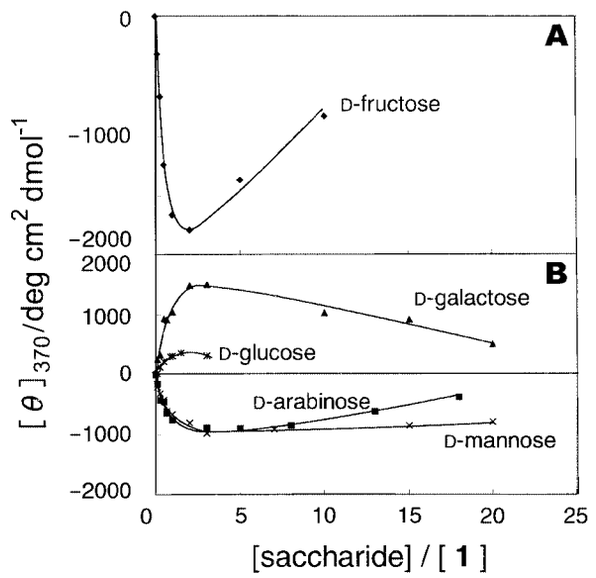

Fig. 5 Plots of $[\theta]_{370}$ *vs.* (A) [D-fructose]/[**1**] and (B) [other saccharides]/[**1**]: water (pH 10.0 with 50 mmol dm^{-3} carbonate)–Me_2SO = 1 : 1 v/v, [**1**] = 5.00×10^{-5} mol dm^{-3}, 25 °C.

binding manner (Fig. 5) is frequently observed for diboronic acid receptors and can be explained by the primary step to form CD-active 1 : 1 cyclic complexes at low saccharide concentration, followed by the secondary step to form CD-silent 1 : 2 receptor/saccharide noncyclic complexes at high saccharide concentration.[6,7,9,13,14,19,20] Fig. 5A shows that in the **1** + D-fructose system the maximum concentration for the 1 : 1 cyclic complex appears at [D-fructose]/[**1**] = 2.0. The biphasic dependence was analysed using a nonlinear least-squares method[22] to estimate the association constants for the 1 : 1 cyclic complex [$K_{ass\,(1:1)}$] and the 1 : 2 receptor/saccharide non-cyclic complexes [$K_{ass\,(1:2)}$]. The similar biphasic dependence was also observed for other saccharides such as D-arabinose, D-galactose, D-mannose and D-glucose (Fig. 5B). The $K_{ass\,(1:1)}$- and $K_{ass(\,1:2)}$-values for these saccharides were estimated by the same method and are summarised, together with those for D-fructose, in Table 1.

Fig. 6 shows the partial CD spectra in the presence of nine saccharides. The strongest CD spectra which are obtained at the maximum 1 : 1 cyclic complex concentration are recorded. Since the CD spectrum in the presence of D-glucose was so weak, the concentration was enhanced to 5.00×10^{-4} mol dm^{-3} (10-times higher than the standard concentration). Careful examination of the CD sign at around 370 nm reveals that there exists a close correlation between the CD sign and the monosaccharide configuration. When the 2-OH group is 'down', the CD sign is always positive (Group A). D-Xylose, D-glucose, D-galactose, D-ribose and D-allose are classified in this category. When the 2-OH (in D-fructose this OH group is counted as 3-OH) group is 'up', the CD sign is always negative (Group B). D-Mannose, D-talose and D-arabinose are classified in this category. Although the structure of D-fructose (ketose) is somewhat different from others, the 2-OH is 'up' and the CD sign is negative (Fig. 3). Hence, this saccharide can be also involved in this classification. Presumably, complexation of the first boronic acid group with the 1,2-*cis*-diol group governs the twisting direction of the Re^{I}·bipyridine moiety.

Saccharide-binding properties at pH 6.5

At pH 10.0, above the pK_a, the boronic acid groups show high affinity with saccharides and exist as sp^3-hybridised boronate anions both before and after the saccharide complexation. As a result, **1** could form a 1 : 1 cyclic complex (as evidenced by CD spectroscopy) whereas the absorption spectra scarcely changed. Next, we carried out spectral measurements at pH 6.5, which is higher than the pK_a in the absence of saccharides but lower than that in the presence of saccharides (*e.g.*, 8.9 in the presence of D-fructose: see Fig. 2A). Hence, the absorption spectra would be changed by the saccharide complexation but the formation of a 1 : 1 cyclic complex would become more difficult because of the low affinity with saccharides.

When saccharides were added to a solution of **1** adjusted to pH 6.5, the absorption spectral change was really induced. The spectral change was very similar to that induced by the pH change (Fig. 1), indicating that this change is attributable to a conversion of sp^2-hybridised R-$B(OH)_2$ to sp^3-hybridised R-$(OH)B^-\langle O \rangle$(saccharide). Plots of A_{370} *vs.* saccharide concentrations gave apparent saturation curves (Fig. 7). It is supposed that Yam and Kai[16] calculated the K_{ass}-values from an analysis of these plots, assuming the formation of 1 : 1 cyclic complexes.[16] However, this system is not so simple at pH 6.5. For example, it is not clear yet whether **1** forms only 1 : 1 cyclic complexes. The validity of this assumption may be confirmed by a continuous-variation plot. As shown in Fig. 8, the plot for D-fructose apparently gave a maximum at [**1**]/([**1**] + [D-fructose]) = 0.5. This implies that **1** tends to form a 1 : 1 complex with D-fructose, at least, at this concentration region (*i.e.*, [**1**] + [D-fructose] = 1.00×10^{-3} mol dm^{-3}). However, the solution was totally CD-silent even at [**1**]/([**1**] + [D-fructose]) = 0.5 where the macrocycle formation becomes most advantageous. The result supports the view that this 1 : 1 complex is not cyclic and D-fructose is appended only to one boronic acid group. This is due to the low saccharide affinity at pH 6.5. As described above, the phototitration data (Fig. 1) showed that the two boronic acid groups act independently, forming the OH^- adduct simultaneously or nearly so in the same pH region. This suggests that the saccharide complexation, which is similar to the OH^- adduct formation, should also occur independently. This implies that, in Fig. 7, not only 1 : 1 noncyclic complexes but also 1 : 2 noncyclic complexes can be formed (particularly at [saccharide] > [**1**]). In fact, the absorbance measured at pH 6.5 and [D-fructose] = 3.00×10^{-2} mol dm^{-3} was 0.47, which corresponds to the absorbance of two boronate anionic groups. The results imply, therefore, that at [D-fructose] = 1.00×10^{-3} mol dm^{-3} (Job's plot: Fig. 8) **1** tends to form the 1 : 1 complex whereas at [D-fructose] = 3.00×10^{-2} mol dm^{-3} (Fig. 7) **1** tends to form the 1 : 2 complex. Therefore, calculation of K_{ass} from the plots in Fig. 7, assuming the formation of 1 : 1 complexes, is meaningless.

Saccharide-binding properties of an *N*-ethylated reference compound (2)

The spectral properties of **2** for saccharide-binding at pH 6.5 were very similar to those of **1**. For example, plots of ΔA_{370} *vs.* saccharide concentration gave saturation curves as shown in Fig. 9. A continuous-variation plot for D-fructose resulted in a maximum at [**2**]/([**2**] + [D-fructose]) = 0.5, indicating that **2** also forms a 1 : 1 complex (Fig. 10). On the other hand, the CD band did not appear at any **2**/D-fructose ratio. The CD spectra were also measured for other saccharides but none of the **2** + saccharide systems became CD-active at pH 6.5. The foregoing results support the view that **2** also forms 1 : 1 noncyclic complexes with saccharides at pH 6.5.

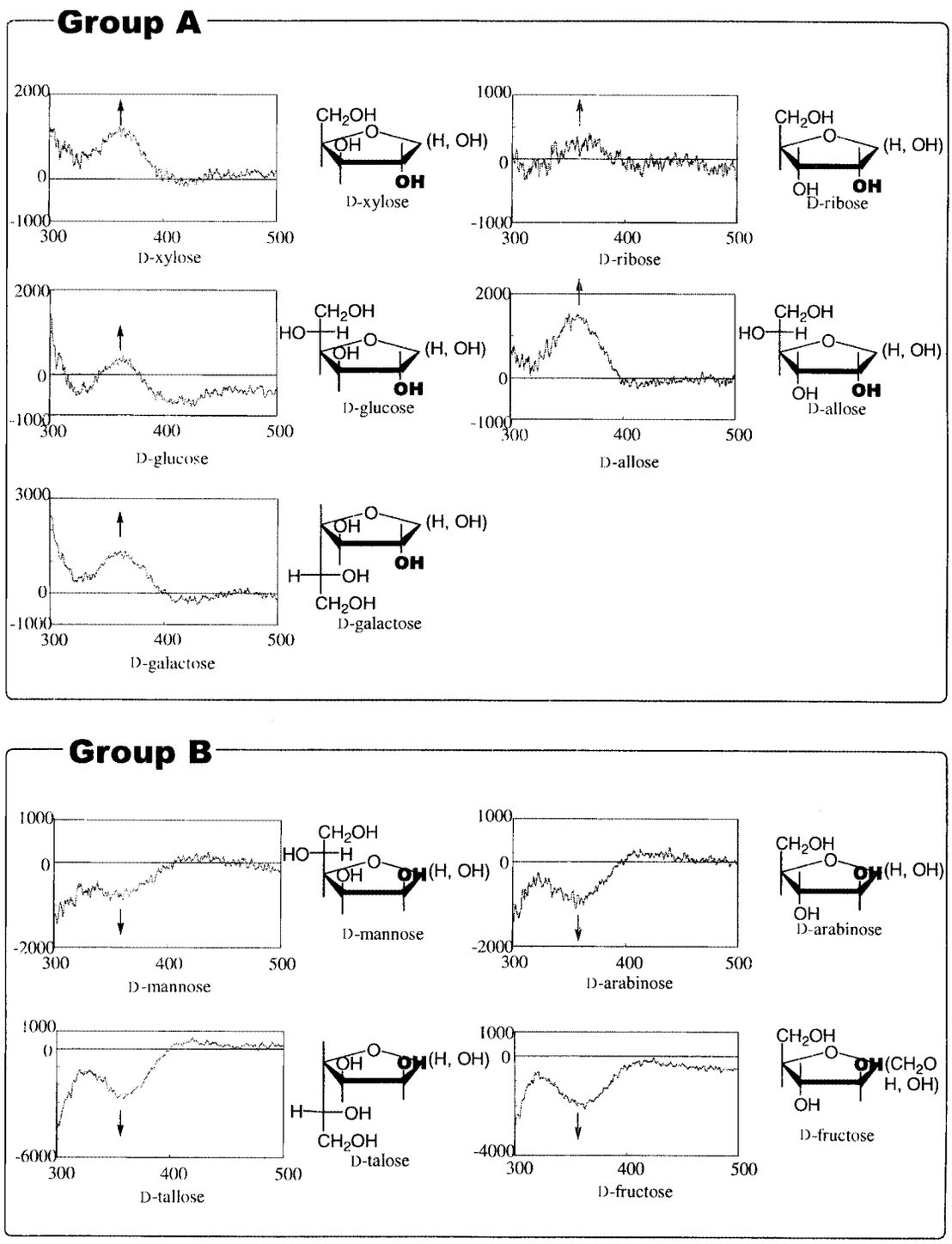

Fig. 6 Partial CD spectra of **1** (5.00×10^{-5} mol dm^{-3}; 5.00×10^{-4} mol dm^{-3} for D-glucose) obtained in the presence of various saccharides. The CD spectra are those around the maximum 1:1 cyclic complex concentration (*i.e.*, [saccharide]/[**1**] = 2.0 for D-galactose, 5.0 for D-arabinose and D-mannose, and 1.5 for D-glucose). Saccharide structures are illustrated with their furanose forms.

The amide protons in **1** might participate in the binding of saccharides through a hydrogen-bonding interaction even in aqueous solution. It is seen, however, that the curves for **2** in Fig. 9 are very similar to those for **1** in Fig. 7. This implies that the association properties for **2** to form 1:1 noncyclic complexes are not much different from those for **1** (although it is not correct to estimate the K_{ass} directly from these curves). Hence, it seems to be unnecessary to take the additional participation of the hydrogen-bonding interaction into consideration.

In high-pH region, Yam and Kai considered that the amide groups are dissociated into their anionic groups, which participate in saccharide binding through hydrogen-bonding interactions.[16] We observed that with increasing medium pH up to 12.5 the absorption spectrum of **1** changes whereas that of **2** does not change, supporting the fact that deprotonation occurs from **1**. However, this significant difference was not seen between **1** and **2** when D-fructose (1.00×10^{-3} mol dm^{-3}) was added. We consider, therefore, that there exists no strong evidence for the interaction between the amide anions and the saccharide guest.

As shown in Fig. 11, a **2** + D-fructose system gave only a very weak CD band.[23] Molecular modelling suggests that when the distance between the two boronic acid groups becomes the shortest possible (that is, the conformation illustrated in Chart 1), the *N*-ethyl groups must be directed toward the inward-position. This conformation is seriously hampered by steric crowding between the ethyl groups. This should cause some rearrangement in the *N*-ethyl groups and lessen the K_{ass}. Assum-

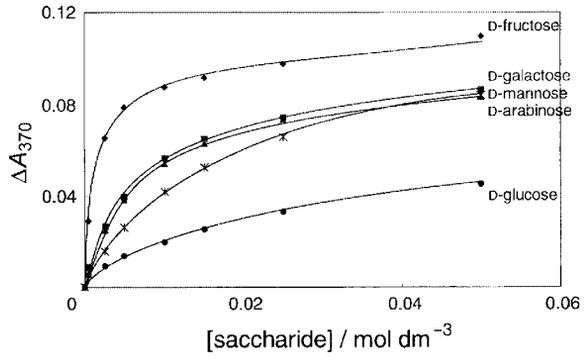

Fig. 7 Plots of absorbance difference at 370 nm (ΔA_{370}) *vs.* saccharide concentration at pH 6.5: water (pH 6.5 with 50 mmol dm^{-3} phosphate)–Me_2SO = 1 : 1 v/v, [**1**] = 5.00×10^{-5} mol dm^{-3}, 25 °C.

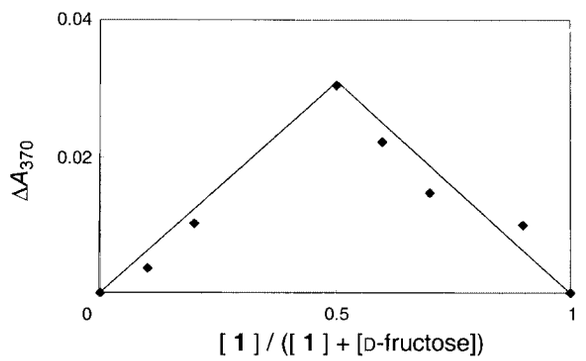

Fig. 8 Continuous-variation plot for a **1** + D-fructose system at pH 6.5: water (pH 6.5 with 50 mmol dm^{-3} phosphate)–Me_2SO = 1 : 1 v/v, [**1**] + [D-fructose] = 1.00×10^{-3} mol dm^{-3} (constant), 25 °C.

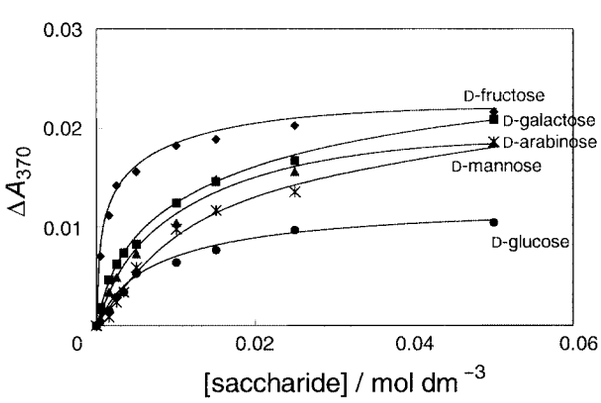

Fig. 9 Plots of absorbance difference at 370 nm (ΔA_{370}) *vs.* saccharide concentration at pH 6.5: water (pH 6.5 with 50 mmol dm^{-3} phosphate)–Me_2SO = 1 : 1 v/v, [**2**] = 5.00×10^{-5} mol dm^{-3}, [saccharide] = 0–50 mmol dm^{-3}, 25 °C.

ing that the major species is still a 1 : 1 cyclic complex, the $K_{ass\,(1:1)}$ was estimated to be roughly 4×10^3 dm^3 mol^{-1} from the CD spectra.

Concluding remarks

From the foregoing findings based on our careful re-examination, we can now derive the following conclusions which are not necessarily compatible with those proposed by Yam and Kai.[16] First, the pK_a is not so low as proposed by them[16] but, rather, is close to the conventional value for boronic acids. Secondly, **1** does not form 1 : 1 cyclic complexes at pH-values below the pK_a. Thirdly, the K_{ass}-values should not be estimated directly from the plot of ΔA_{370} *vs.* [saccharide]. Fourthly, neither the CONH groups nor the CON^- groups significantly contribute to the saccharide binding in aqueous solution. We consider that although the saccharide-binding

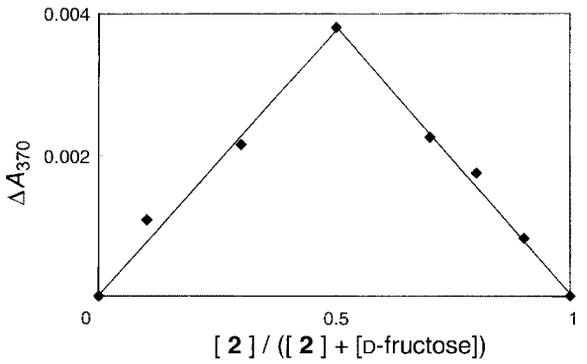

Fig. 10 Continuous-variation plot for a **2** + D-fructose system at pH 6.5: water (pH 6.5 with 50 mmol dm^{-3} phosphate)–Me_2SO = 1 : 1 v/v, [**2**] + [D-fructose] = 1.00×10^{-3} mol dm^{-3} (constant), 25 °C.

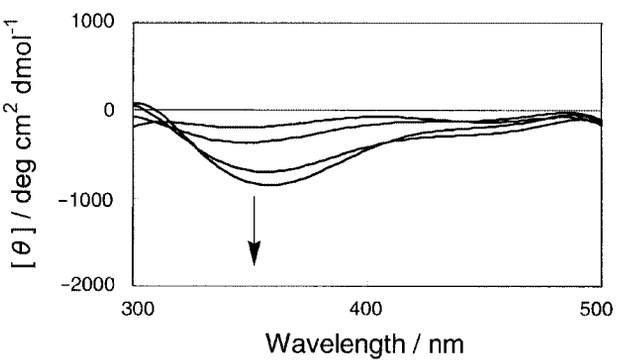

Fig. 11 CD spectrum of **2** in the presence of D-fructose at pH 10.0: water (pH 10.0 with 50 mmol dm^{-3} carbonate)–Me_2SO = 1 : 1 v/v, [**2**] = 5.00×10^{-4} mol dm^{-3}, [D-fructose] = 0–1.00×10^{-3} mol dm^{-3}, 25 °C.

properties are not so simple as proposed by Yam and Kai, the molecular design of **1** is still meaningful, particularly for saccharide sensing using longer-wavelength radiation. We believe that further modification of **1** based on skillful molecular design would enhance both the affinity and the selectivity for saccharides and enable us to read the saccharide-binding process directly in visible light region.

Experimental

Materials

2,2′-Bipyridine-4,4′-dicarboxylic acid was synthesised from 4,4′-dimethyl-2,2′-bipyridine according to the literature method.[24]

3-Acetamidophenylboronic acid 3. To a THF (50 ml) solution containing 3-aminophenylboronic acid (2.0 g, 12.9 mmol) and triethylamine (8.96 ml, 64.5 mmol) was gradually added acetyl bromide (1.9 ml, 25.8 mmol). The mixture was stirred for 14 h at room temperature. The precipitate was removed by filtration, the filtrate being washed with saturated aq. NaCl and dried over $MgSO_4$. Evaporation of this solution yielded a yellow powder, the purity of which was sufficient for its use in the next reaction without further purification: (2.1 g), mp 138–140 °C; δ_H (250 MHz; DMSO-d_6) 3.58 (3H, s, CH_3), 6.50 (2H, m, 4-, 5-ArH), 6.68 (1H, s, NH), 6.78 (1H, d, 6-ArH), 6.95 (1H, s, 2-ArH).

3-(*N*-Ethylamino)phenylboronic acid 4. Compound **3** (1.5 g, 7.6 mmol) was dissolved in dehydrated THF (100 ml) in a N_2-substituted flask. The solution was cooled in an ice-bath and $BH_3 \cdot Me_2S$ (7.2 ml, 76 mmol) was gradually added. The mixture was heated at 60 °C for 6 h and then concentrated to dryness. The residue was mixed with 6 mol dm^{-3} aq. HCl (70 ml) and the

mixture was refluxed for 2 h. It was neutralised by Na_2CO_3 and extracted with diethyl ether. The organic solution was dried over $MgSO_4$ and evaporated to dryness to give a slightly yellow powder, yield from 3-aminophenylboronic acid 71.6%, mp 48–50 °C; δ_H (250 MHz; DMSO-d_6) 1.15 (3H, t, CH_3), 3.03 (2H, q, CH_2), 5.64 (1H, br, NH), 6.60 (1H, s, 4-ArH), 6.98–7.07 (3H, m, 2-, 5-, 6-ArH), 7.81 (2H, s, BOH).

***N*-Ethyl-3-(1,3,2-dioxaborinan-2-yl)aniline 5.** To protect the boronic acid group, compound **4** (1.2 g, 6.56 mmol) was treated with propane-1,3-diol (0.95 ml, 13.1 mmol) in toluene (100 ml) using a Dean–Stark apparatus. After treatment at reflux for 14 h, the solution was evaporated to dryness. The product was purified by column chromatography (silica gel–ethyl acetate) to give a slightly yellow oil (1.5 g, quant.); δ_H (250 MHz; $CDCl_3$) 1.20 (3H, t, CH_3), 2.00 (2H, qv, $CH_2CH_2CH_2$), 3.14 (2H, q, CH_3CH_2), 4.21 (4H, t, OCH_2), 6.64 (1H, d, 6-ArH), 7.06–7.21 (3H, m, 2-, 4-, 5-ArH).

4,4′-Bis{*N*-[3-(1,3,2-dioxaborinan-2-yl)phenyl]-*N*-ethylcarbamoyl}-2,2′-bipyridine 6. 2,2′-Bipyridine-4,4′-dicarboxylic acid (2.0 g, 8.2 mmol) was converted to 4,4′-bis(chlorocarbonyl)-2,2′-bipyridine by treatment with $SOCl_2$. To a THF solution (50 ml) containing compound **5** (3.0 g, 18.1 mmol) and triethylamine (12.5 ml, excess) was added dropwise a THF solution containing 4,4′-bis(chlorocarbonyl)-2,2′-bipyridine prepared above. The mixture was stirred for 15 h at room temperature. The precipitate was removed by filtration, the filtrate being concentrated to dryness. The product **6** was isolated by GPC (Japan Industry Co., LC-908; JAIGEL-2H, chloroform). The title product was obtained as a white powder (130 mg, 6.8%), mp 194–195 °C; IR (KBr)/cm^{-1} 1647 (C=O), 1308 (B–O); δ_H (250 MHz; $CDCl_3$) 1.21 (6H, t, CH_3), 2.02 (4H, qv, $CH_2CH_2CH_2$), 3.97 (4H, q, CH_3CH_2), 4.11 (8H, t, OCH_2), 6.97–7.05 (4H, m, 4-, 6-ArH), 7.16 (2H, t, 5-ArH), 7.50–7.58 (4H, m, 2-ArH, 5-PyH), 8.24 (2H, s, 3-PyH), 8.34 (2H, d, 6-PyH).

4,4′-Bis{[3-(dihydroxyboryl)phenyl]carbamoyl}-2,2′-bipyridine 7. Compound **7** was synthesised from 3-(1,3,2-dioxaborinan-2-yl)aniline and 4,4′-bis(chlorocarbonyl)-2,2′-bipyridine. The precipitate was recovered by filtration and washed with water to remove triethylamine hydrochloride and unchanged compounds. The product was obtained as a white powder: 85% yield, mp 328–330 °C; IR (KBr)/cm^{-1} 3288 (O–H), 1643 (C=O), 1304 (B–O); δ_H (250 MHz; D_2O + NaOD) 7.06 (2H, d, 4-ArH), 7.30–7.33 (6H, m, 2-, 5-, 6-ArH), 7.94 (2H, d, 5-PyH), 8.45 (2H, s, 3-PyH), 8.80 (2H, d, 6-PyH).

Tricarbonylchloro(4,4′-bis{*N*-[3-(1,3,2-dioxaborinan-2-yl)-phenyl]-*N*-ethylcarbamoyl}-2,2′-bipyridine)rhenium 2′. Compound **6** (20 mg, 4.2×10^{-5} mol) and $Re(CO)_5Cl$ (15 mg, 4.2×10^{-5} mol) were dissolved in dry ethanol in a N_2-substituted flask. The solution was refluxed for 14 h. Evaporation resulted in a crude solid product, which was recrystallised from chloroform to give compound **2′** as an orange powder (52 mg, 50%), mp 132–134 °C; (Found: C, 45.79; H, 3.74; N, 5.70. $C_{37}H_{36}N_4B_2ClO_9Re$ + 0.5 $CHCl_3$ requires C, 45.88; H, 3.97; N, 5.50%); IR (KBr)/cm^{-1} 2021, 1917, 1890 (M–C=O), 1636 (C=O), 1312 (B–O); δ_H (250 MHz; DMSO-d_6) 1.28 (6H, t, CH_3), 2.06 (4H, qv, $CH_2CH_2CH_2$), 4.05 (4H, q, CH_3CH_2), 4.13 (8H, t, OCH_2), 7.05–7.10 (4H, m, 4-, 6-ArH), 7.33 (2H, t, 5-ArH), 7.57 (2H, t, 2-ArH), 7.71 (2H, t, 5-PyH), 8.15 (2H, s, 3-PyH), 8.65 (2H, d, 6-PyH).

Compound **2′** was directly used for spectral measurements. The results were basically identical with those of **2** (a small amount was obtained by deprotection).

Tricarbonylchloro(4,4′-bis{[3-(dihydroxyboryl)phenyl]carbamoyl}-2,2′-bipyridine)rhenium 1. Compound **1** was synthesised from **7** and $Re(CO)_5Cl$ in a manner similar to that used for **2**: (52 mg, 50%); mp > 320 °C (decomp.) (Found: C, 40.70; H, 2.66; N, 7.03. $C_{27}H_{20}N_4B_2ClO_9Re$ + 0.5 H_2O requires C, 40.83; H, 2.49; N, 6.91%); IR (KBr)/cm^{-1} 3420 (O–H), 2027, 1948, 1909 (M–C=O), 1647 (C=O), 1339 (B–O); δ_H (250 MHz; $CDCl_3$) 7.40 (2H, t, 5-ArH), 7.63 (2H, d, 6-ArH), 7.92 (2H, d, 4-ArH), 8.07 (2H, s, 2-ArH), 8.24 (2H, s, 5-PyH), 9.24–9.28 (4H, m, 3-, 6-PyH), 10.83 (2H, s, BOH).

Miscellaneous

For the phototitration of compounds **1** and **2**, the following buffer solutions were used: 50 mmol dm^{-3} acetate for pH 4.5–5.0, 50 mmol dm^{-3} phosphate for pH 6.5–9.0, 50 mmol dm^{-3} carbonate for pH 9.5–11.0. Absorption spectra, CD spectra and ^{1}H NMR spectra were measured with Shimadzu 2500PC, JASCO J-720 and Bruker AC-205P instruments, respectively.

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23 One referee raised an interesting comment on the known conformational preference of *N*-alkyl anilides to adopt a *cis* amide conformation: I. Azumaya, I. Okamoto, S. Nakayama, A. Tanatani, K. Yamaguchi, K. Shudo and H. Kagechika, *Tetrahedron*, 1999, **55**, 11237 and references cited therein. If this is the case in **2**, two interconvertible enantiomers can exist and the resultant D-fructose complexes become diastereomeric. The ^{1}H NMR spectrum (250 MHz) shows only one set of sharp triplet–quartet peaks. This indicates that *cis–trans* isomerism occurs faster than the ^{1}H NMR time-scale. This may be the reason why the CD intensity and the $K_{ass\,(1:1)}$ are smaller than those for **1.**
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