Novel fluorescence sensor for 'small' saccharides

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The small binding pocket of compound 2 can exclude 'large' saccharides such as D-glucose, whereas 'small' saccharides such as D-sorbitol are strongly bound as 1 : 1 complexes; the binding events can be sensitively monitored by changes in the fluorescence intensity.

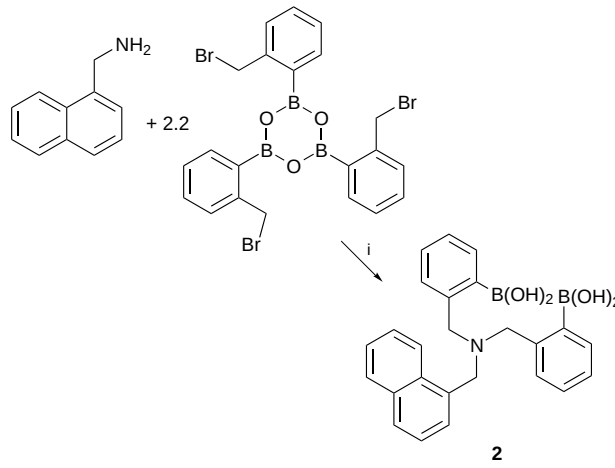
The development of boronic acid receptors for saccharides has recently gained much attention.[1] One problem with many of these systems is that they only function in basic aqueous media. Wulff was the first to report that a neighbouring nitrogen enhances the formation of boronate esters even under neutral pH conditions.[2] In a series of recent papers we employed the interaction between boronic acid and amine[3–7] to create photoinduced electron transfer (PET)[8,9] sensory systems for saccharides. When saccharides form cyclic boronate esters with boronic acids, the acidity of the boronic acid is enhanced[10] and therefore the Lewis acid–base interaction between the boronic acid and the tertiary amine is strengthened. The strength of this acid-base interaction modulates the PET from the amine (acting as a quencher) to anthracene (acting as a fluorophore). These compounds show increased fluorescence at pH 7.77 through suppression of the photoinduced electron transfer from nitrogen to anthracene on saccharide binding; a direct result of the stronger boron–nitrogen interaction.[3–7]

With diboronic acid **1** glucose selectivity was predicted from CPK models and then observed experimentally.[4] With other diboronic acids chiral discrimination[5] and metal ion allosterism[6] have been achieved. In these systems the formation of a 1 : 1 intramolecular complex which is classified as a saccharide-containing macrocycle was shown to be the important fluorescent species and the origin of the circular dichroism (CD) activity. With this work we have reduced the size of the cleft in order to modulate the selectivity of the boronic acid unit towards 'small' saccharides. From CPK models glucose cannot easily bind as a cyclic 1 : 1 complex with diboronic acid **2** and should be excluded from the binding pocket.

Compound **2** was synthesised from 1-aminomethylnaphthalene according to Scheme 1 and characterised using IR and ^{1}H NMR spectral evidence and elemental analysis.

The absorption spectrum (25 °C, H_2O–MeOH, 300 : 1 v/v) and λ_{max} were not affected over a large pH range (pH 1.7–11.8). The pK_a values were determined using fluorescence spectroscopy (excitation 296 nm). The pH dependence of the relative fluorescence intensity (I/I_0; emission maximum 347 nm) is illustrated in Fig. 1. In the absence of saccharides, the I/I_0 is nearly constant from pH 1.5 to 8.0. Judging from the pH dependence of **1** a change from $\mathbf{2H}^+$ to **2** (*cf.* Scheme 2) should cause a large decrease in the fluorescence intensity. This means that deprotonation of $\mathbf{2H}^+$ occurs in a more acidic region. On the other hand, the I/I_0 gradually decreases above pH 8.0. This reflects the dissociation of boronic acid groups to $\mathbf{2}^-$ and $\mathbf{2}^{2-}$.

In the presence of saccharides, the I/I_0 increases from pH 4.0 and reaches a maximum at around pH 8.0. This change is ascribed to the intensified B–N interaction caused by the saccharide binding to the boronic acid group. Since the largest I/I_0 increase was observed at around pH 8.0, we determined the association constants (K) at pH 8.0 from plots of I/I_0 *vs.*



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Scheme 1 *Reagents*: i, K_2CO_3, MeCN, heat, 70%

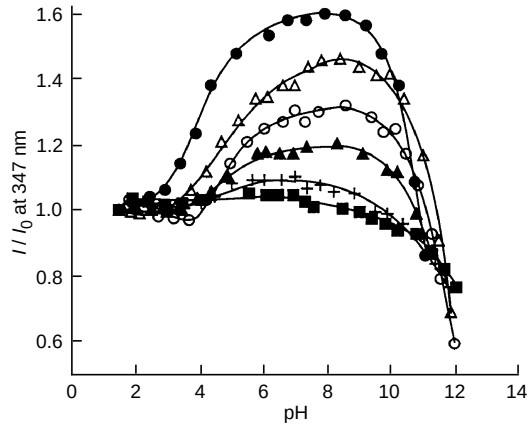
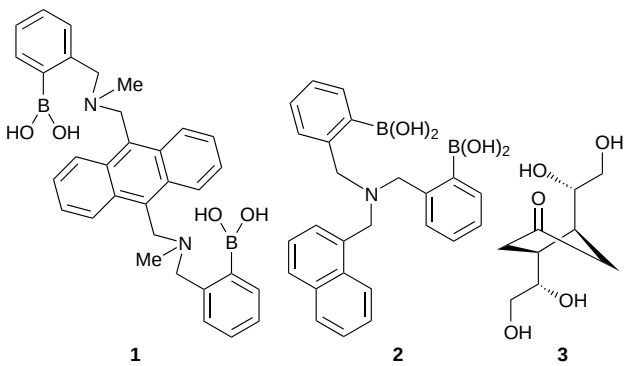
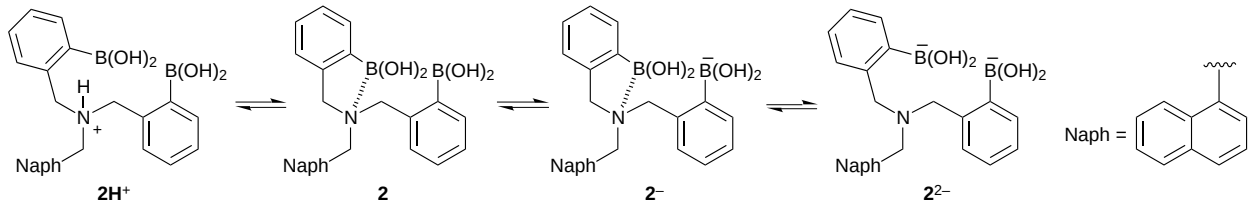


Fig. 1 pH Dependence of the relative fluorescence intensity (I/I_0 at 347 nm) in the absence and presence of saccharides: [**2**] = 1.00×10^{-5} mol dm^{-3}, [saccharide] = 0.10 mol dm^{-3}, 25 °C, H_2O–MeOH, 300 : 1 v/v buffered with HCl and NaOH, excitation 296 nm: (●) D-fructose, (△) pentaerythritol, (○) D-threitol, (▲) D-glucose, (+)-glycerine, (■) no saccharide



1 **2** **3**



Scheme 2

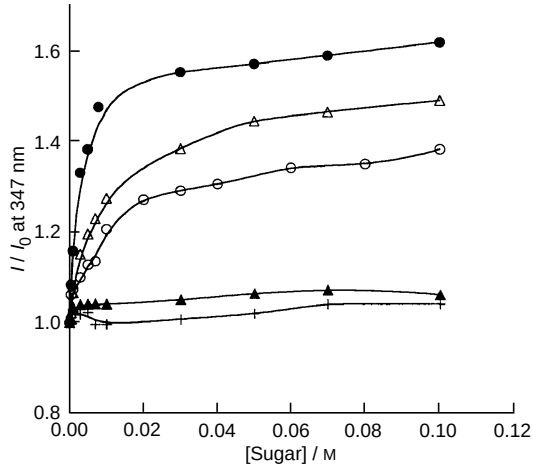


Fig. 2 Plots of I/I_0 *vs.* [saccharide] for selected saccharides: (●) d-fructose, (△) pentaerythritol, (○) d-threitol, (▲) d-glucose, (+)-glycerine, (■) no saccharide

Table 1 Association constants (*K*), number of binding sites and number of carbon separating two binding sites (Δn) at 25 °C and pH 8.0 in H_2O–MeOH, 300 : 1 *v/v*

Sugar	log *K*	Binding site	Δn
d-Sorbitol	2.54	2	0–2
d-Fructose	2.52	2	0–1
Dulcitol	2.4	2	0–2
Xylitol	2.23	2	0–1
d-Mannitol	2.15	2	0–2
Pentaerythritol	2.11	2	0
Ribitol	1.97	2	0–1
d-Threitol	2.03	2	0
d-Arabitol	2.13	2	0–1
d-Ribose	1.91	2	0
d-Fucose	1.50	2	0
Erythritol	1.45	2	0
d-Glucose	*a*	2	1
Glycerine	*a*	1	—
Propane-1,3-diol	*a*	1	—
3[b]	*a*	2	2

[a] The *K* could not be determined because of the small fluorescence change. [b] H_2O–MeOH, 2 : 1 v/v (because of low solubility in water).

saccharide concentration (Fig. 2). The results are summarised in Table 1.

Examination of Table 1 reveals two important features of compound **2** as a PET sensor: that is, (i) **2** shows selectivity towards 'small' saccharides and (ii) only when they possess two binding sites (*i.e.* two diol units associative with a boronic acid) and they are not separated (*i.e.* the number of carbons separating the two diols, $\Delta n = 0$ in Table 1), do a large fluorescence increase and a large *K* value result. Glycerine and propane-1,3-diol, which have only one binding site, cannot enhance the fluorescence intensity. d-Glucose ($\Delta n = 1$) and mannitol derivative **3** ($\Delta n = 2$), in which two binding sites are separated by one or two carbons, cannot enhance the fluorescence either. Other compounds which can enhance the fluorescence intensity have two (or more than two) binding sites and $\Delta n = 0$ without exception. Even in those compounds with a choice of binding site, $\Delta n = 0$–2, the most stable complex should be the one with $\Delta n = 0$.

The formation of the cyclic structures is further supported by the fact that the complexes become CD active. For example, **2** (1×10^{-5} mol dm^{-3}) in the presence of sorbitol (0.10 mol dm^{-3}) gave $[\theta]_{max}$ -2.16×10^4 deg cm^2 $dmol^{-1}$ for d-sorbitol and 2.16×10^4 deg cm^2 $dmol^{-1}$ for l-sorbitol at 228 nm (25 °C, pH 8.0 with 50 mmol dm^{-3} phosphate buffer).

In conclusion we have clearly demonstrated that the selectivity of a diboronic acid cleft towards saccharides can be modified in a controlled manner by the correct spacing of two boronic acid units. This work illustrates the power of the synthetic saccharide sensor. Unlike enzymatic systems, selectivity can be designed for one particular saccharide. This offers the possibility of monitoring the concentrations of biologically important saccharides which are less abundant than d-glucose, in a variety of industrial and medicinal applications.

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References

1 G. Deng, T. D. James and S. Shinkai, *J. Am. Chem. Soc.*, 1994, **116**, 4567 and references cited therein; J. Yoon and A. W. Czarnik, *J. Am. Chem. Soc.*, 1992, **114**, 5874; G. T. Morin, M. P. Hughes, M.-F. Paugam and B. D. Smith, *J. Am. Chem. Soc.*, 1994, **116**, 8895 and references cited therein.
2 G. Wulff, *Pure Appl. Chem.*, 1982, **54**, 2093.
3 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1994, 477.
4 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2207.
5 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Nature*, 1995, **374**, 345.
6 T. D. James and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1995, 1483.
7 T. D. James, P. Linnane and S. Shinkai, *Chem. Commun.*, 1996, 281; (b) T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1996, **108**, 2038.
8 R. A. Bissel, A. P. de Silva, H. Q. N. Gunaratna, P. L. M. Lynch, G. E. M. Maguire, C. P. McCoy and K. R. A. S. Sandanayake, *Top. Curr. Chem.*, 1993, **168**, 223.
9 A. W. Czarnik, *Fluorescent Chemosensors for Ion and Molecular Recognition*, ACS Books, Washington, 1993.
10 J. P. Lorand and J. D. Edwards, *J. Org. Chem.*, 1959, **24**, 769.

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