Carbohydrate sensing using a fluorescent molecular tweezer†

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A fluorescent molecular tweezer for carbohydrates has been prepared which utilises two boronic acid receptor groups.

Carbohydrates, or saccharides, are the most abundant of the four major classes of biomolecules, which also include proteins, lipids, and nucleic acids. Carbohydrates were originally thought of as food and building materials, however the realisation that oligosaccharides play an important role in biological regulation has attracted a great deal of interest.[1–3]

Receptors for carbohydrates have been designed based on hydrogen bonding interactions[4,5] and covalent ester formation with boronic acids.[6–8] Boronic acids can bind saccharides *via* covalent interactions in basic aqueous media through the formation of *cis*-1,2- or 1,3-diols which form five- or six-membered rings, respectively.[6–8]

Photoinduced electron transfer (PET) saccharide sensors based on boronic acids were first synthesised over 15 years ago[9–11] which exploit the interactions between *o*-methylphenylboronic acids (Lewis acids) and proximal tertiary amines (Lewis bases).

The boronic acid–amine (B–N) interaction provides two distinct advantages for saccharide binding. The first advantage of the B–N interaction is that it lowers the pK_a of the boronic acid and thus allows binding to occur at neutral, *i.e.* physiological pH.[12] The second is related to the contraction of the O–B–O bond angle upon association with a saccharide resulting in an increase of acidity at the boron centre. This increase in acidity of the boron centre strengthens the B–N interaction and thus disrupts PET. This has the effect of modulating fluorescence intensity *via* the amine group and introduces a digital "off–on" response from the fluorophore, indicative of the boronic acid being unbound or bound.[11,13]

Previous work within the group[14–16] has shown that the "inherent stability order" for simple boronic acids of D-fructose > D-galactose > D-mannose > D-glucose can be perturbed by the use of di-boronic acids. The most potent of these di-boronic acid receptors was receptor **1**, it had an association constant of 960 M^{-1} for D-glucose, 760 M^{-1} for D-fructose, 660 M^{-1} for D-galactose, and 70 M^{-1} for D-mannose.[15] Constructing a pair of molecular tweezers tipped with two large aromatic fluorophore units could be of potential benefit in enhancing the selectivity between specific saccharides by further augmenting the hydrophobicity with the binding pocket. With the results of receptor **1** and considering the potential advantages of allowing a degree of conformational flexibility to be designed into the recognition site, the structure of the di-boronic molecular tweezer **2** was postulated Fig. 1. It can be seen from the structure of molecular tweezer **2** that the *o*-phenylboronic acid units, tertiary amines, and pyrenyl fluorophores are positioned so as to permit fluorescence to be controlled *via* PET. The dual boronic acid units permit saccharide selectivity *via* two point binding with two pyrene units augmenting the hydrophobicity within the binding pocket. The two hexamethylene linkers will introduce a degree of conformational flexibility into the system and allow the binding cavity to accommodate different saccharides.

Synthesis of **2** was achieved according to Scheme 1 from readily available starting materials. The selective protection of diamine **3** gave the mono Boc-protected diamine **4** which was then reacted with isophthaloyl dichloride[17] to yield **5**. Cleavage of the Boc protecting groups was followed by reductive amination with pyrene-1-carboxaldehyde and a acidic work-up gave ammonium salt **6**. The boronic functionality was introduced by reacting the neopentyl protected 2-(bromomethyl)phenylboronic acid with **6** to give molecular tweezer **2**.

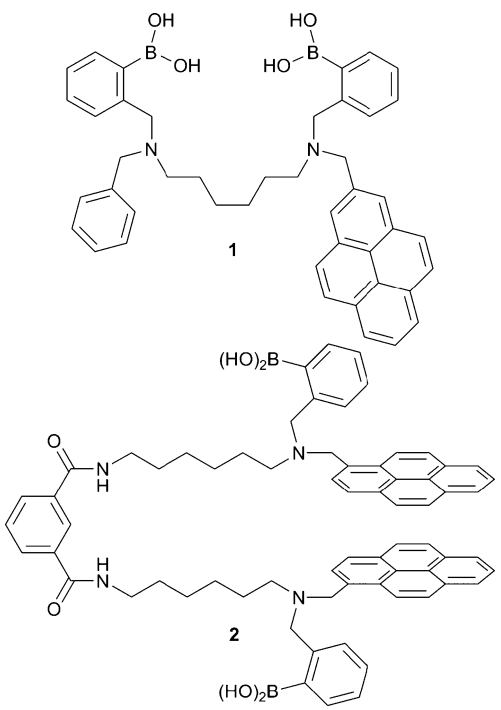

Fig. 1 Structure of a previously synthesised di-boronic acid receptor **1** and di-boronic molecular tweezer **2**.

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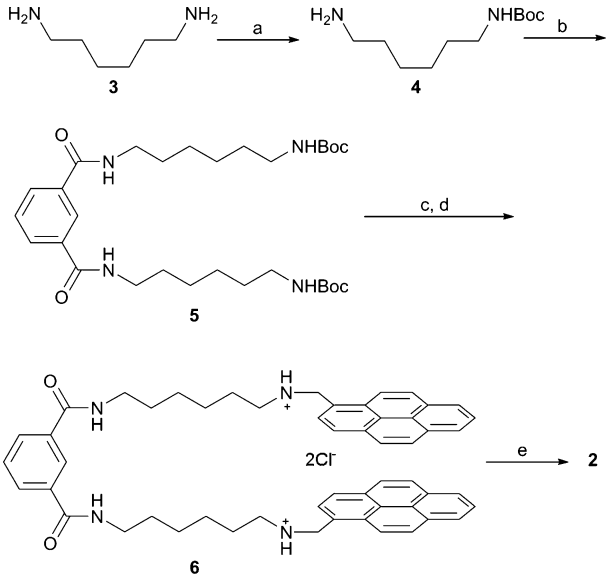

Scheme 1 Synthesis of **2**. *Reagents* (yields): (a) *tert*-butyl phenyl carbonate, EtOH, 78 °C, 45%; (b) isophthaloyl dichloride, triethylamine, DCM, 96%; (c) HCl, MeOH, EtOAc, 100%; (d) (i) pyrene-1-carboxaldehyde, caesium carbonate, THF–methanol, reflux; (ii) $NaBH_4$; (iii) HCl, MeOH, DCM, 68%; (e) 2-(2-(bromomethyl)phenyl)-5,5-dimethyl-1,3,2-dioxaborinane, potassium carbonate, acetonitrile, reflux, 14%.

The binding behaviour of boronic acids and its fluorescence are pH dependent.[9,18] The change in the fluorescence of tweezers **2**, with and without D-fructose, *versus* pH was investigated to determine the pH with the maximum fluorescence response.[19] As a result fluorescence titrations of **2** (0.1 μM) were carried out in an aqueous methanolic buffer at pH 8.21 with various saccharides. Fig. 2 shows the fluorescence spectra of **2** in the presence of increasing concentrations of either D-glucose or D-fructose. Spectra obtained by adding increasing amounts of D-galactose and D-mannose show the same spectroscopic features as those shown when adding D-glucose, although the initial quenching of the excimer band at 470 nm is partially reversed at high galactose concentrations.[20]

The fluorescence intensity increases with increasing concentration of all four carbohydrates at 377 nm resulting from PET as found with other aminomethyl boronate complexes. The fluorescence intensity changes at 470 nm differ among the four carbohydrates. With increasing concentrations of D-glucose and D-mannose, the 470 nm band decreases with increasing carbohydrate concentration. As shown in Fig. 2B, the intensity of the 470 nm band is apparently invariant with added D-fructose. Finally, D-galactose shows an initial quenching of the 470 nm band at low concentrations that is reversed as the concentration increases (Fig. 2D).

We interpret these diverse observations as follows. All carbohydrates form a 1 : 1 complex Fig. 3. In the case of D-glucose, D-galactose, and D-mannose (Fig. 4) this complex has the cyclic structure envisaged in Fig. 1. This would explain the quenching of the excimer emission since the binding of the carbohydrate forces a separation between the pyrene units. We interpret the more complex behaviour of D-galactose as indicating the subsequent formation of a 2 : 1 complex.

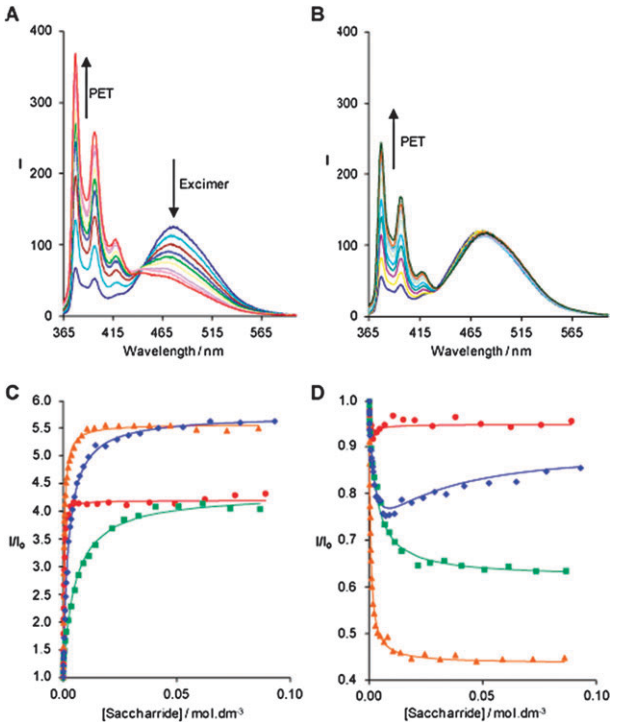

Fig. 2 Fluorescence emission spectra of **2** (0.1 μM) with increasing amounts of (A) D-glucose (0 to 0.1 M) or (B) D-fructose (0 to 0.1 M) in an aqueous methanolic buffer [52.1 wt% methanol, KCl (10.0 mM), KH_2PO_4 (2.75 mM) and Na_2HPO_4 (2.75 mM)] at a pH of 8.21. Relative fluorescence intensity *versus* carbohydrate concentration profile of **2** (0.1 μM, λ_{ex} = 342 nm, (C) λ_{em} = 377 nm, (D) λ_{em} = 470 nm) displaying PET with (orange triangle) D-glucose, (red circle) D-fructose, (blue diamond) D-galactose and (green square) D-mannose.

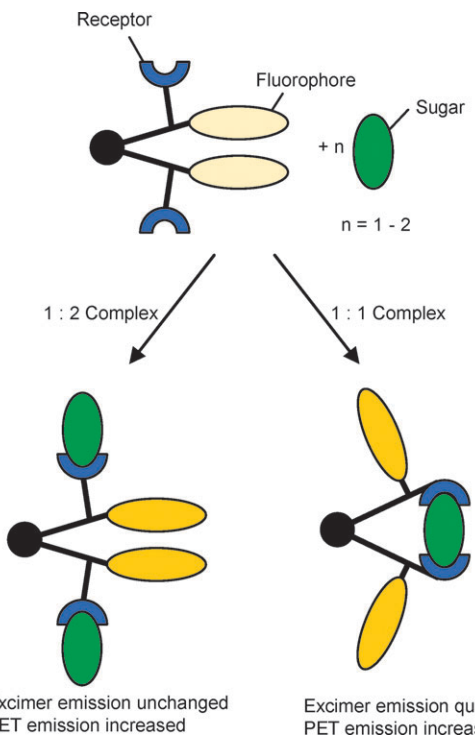

Fig. 3 Schematic representation showing the two possible ways of binding for the molecular tweezers with sugars.

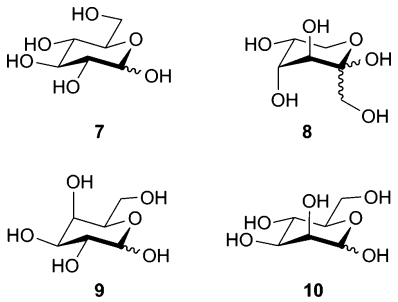

Fig. 4 Saccharides used in the study, D-glucose **7**, D-fructose **8**, D-galactose **9**, and D-mannose **10**.

Table 1 Observed 1 : 1 stability constants (K_{obs}), determination coefficient (r^2), and fluorescence enhancement for **2** (0.1 μM) with D-glucose, D-fructose, D-galactose, and D-mannose at pH 8.21 in an aqueous methanolic buffer [52.1 wt% methanol, KCl (10.0 mM), KH_2PO_4 (2.75 mM) and Na_2HPO_4 (2.75 mM)][a]

	K_{obs}/M^{-1}	r^2	I/I_0 at 377 nm	I/I_0 at 460 nm
D-Glucose **7**	2660 ± 200	0.999	6.5	0.43
D-Fructose **8**	2850 ± 140	0.999	5.0	0.96
D-Galactose[b] **9**	440 ± 10	0.999	4.9	0.66
D-Mannose **10**	200 ± 15	0.998	3.9	0.54

[a] The K values were analysed in Scientist using non-linear curve fitting. The errors reported are the standard errors obtained from the best fit, relative fluorescent enhancements (I/I_0) are also reported. [b] Stepwise formation of a 2 : 1 complex is required for the fit: K_{21}(stepwise from 1 : 1 complex) = 50 ± 20; I/I_0 at 377 nm = 5.2; I/I_0 at 470 nm = 0.91.

In such a structure the pyrene excimer can form since the carbohydrate is no longer forcing the rings apart.

In the case of D-fructose the 1 : 1 complex must form without disruption of the pyrene excimer, *i.e.* without formation of the chelated structure indicated in Fig. 1. The "inherent stability order" favoring D-fructose is based in part on its ability to coordinate *via* three hydroxyl groups. This does not leave any vicinal diol units for a second boronic acid binding location. It is also possible that 2 : 1 complexes form. This is known in other systems; carbohydrates such as D-glucose easily form 1 : 1 cyclic complexes with di-boronic acids while D-fructose resists forming cyclic complexes and tends to form 2 : 1 acyclic complexes instead.[11,21,22]

The stability constants (K_{obs}) of tweezer **2** were calculated by simultaneous fitting of the emission spectra at two wavelengths *versus* carbohydrate concentration and are shown in Table 1. As noted above, all carbohydrates form 1 : 1 complexes and, with the exception of D-galactose, the data could be adequately fit assuming only 1 : 1 complex formation. A sequential 1 : 1–2 : 1 binding model is required to fit the D-galactose data. The D-fructose data will also accommodate a 2 : 1 complex, but the fit statistics are insignificantly different from the simpler 1 : 1 binding isotherm. The magnitude of the binding constants reveal why the D-galactose system uniquely shows the stepwise behaviour; the 1 : 1 complex is moderately strong allowing its formation and destruction to be observed within the concentration range of the experiments. The other carbohydrates either form complexes that are too weak or too strong to be manipulated by experimental concentrations.

In conclusion we have developed a molecular tweezer **2** that selectively opens for carbohydrates with the stability order of D-glucose ≫ D-galactose > D-mannose. Although **2** forms a complex with D-fructose with a higher stability constant than D-glucose, it fails to open the tweezer **2**.

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