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Tuning saccharide selectivity in modular fluorescent sensors

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Abstract—Five modular photoinduced electron-transfer (PET) sensors bearing two phenylboronic acid receptors with different fluorophores have been prepared. The sensors' interaction with saccharides was assessed via fluorescence spectroscopy. It was shown that monosaccharide selectivity is influenced by the choice of fluorescent moiety.

Receptors with the capacity to selectively bind saccharides and signal this event by altering their optical signature have attracted considerable interest in recent years.[1–13] Boronic acids are known to bind saccharides via covalent interactions in aqueous media. The most common interaction is with *cis*-1,2- or 1,3-diols of saccharides to form five- or six-membered rings respectively.[14] The interaction between boronic acids and amines has been used to create photoinduced electron-transfer (PET) sensory systems for saccharides.[15–17] The interaction of a boronic acid (Lewis acid) and neighbouring tertiary amine (Lewis base) is strengthened on saccharide binding. The strength of this boronic acid–tertiary amine interaction modulates the PET mechanism between nitrogen and the fluorophore. These compounds show increased fluorescence through suppression of PET on saccharide binding, a direct result of the stronger boron–nitrogen interaction.

Over the last few years we have been involved in the development of sensors with increased selectivity for saccharides.[18–22] We have constructed our sensors using a modular approach. The sensors consist of three components; receptor units, linker units, and 'read-out' units. The approach can be illustrated by describing the D-glucose selective fluorescent sensor **3a** which contains two boronic acid units (receptors), a hexamethylene unit (linker), and a pyrene unit (fluorophore-'read-out').[19,20] Sensor **3a** contains two boronic acid units because only through two point binding can saccharide selectivity be controlled. Sensor **3a** with a hexamethylene linker unit displays enhanced D-glucose selectivity, whilst systems with longer linker units display enhanced selectivity for D-galactose.[19,20] This sensor also contains a fluorescent pyrene 'read-out' unit to report saccharide binding events.

Having determined the effect of the linker on saccharide selectivity, we set out to probe the other factors affecting saccharide selectivity. The next logical component to vary is the fluorophore or 'read-out' unit. Although not directly involved in saccharide binding, the nature of this unit will directly influence both the solvation and steric crowding of the binding site. We now report our investigations into the effect of the fluorophore units on saccharide binding in our modular fluorescent sensors (Table 1).

Diboronic acid PET sensors **3a**–**e** and monoboronic acid PET sensors **5a**–**e** were synthesised according to Scheme 1 from readily available starting materials. The synthesis allows the facile preparation of diboronic acid PET sensors appended with a range of different fluorophores.

The fluorescence titrations of **3a**–**e** and **5a**–**e** with different saccharides were carried out in aqueous methanolic buffer solution [52.1 wt% methanol at pH 8.21 (KCl,

Table 1. Fluorescence spectra measurement conditions

Fluorophore	Concentration (mol dm^{-3})	λ_{ex} (nm)	λ_{em} (nm)
a	1.0×10^{-6}	342	397
b	5.0×10^{-6}	299	369
c	1.0×10^{-6}	370	420
d	2.5×10^{-6}	275	335
e	2.5×10^{-6}	274	335

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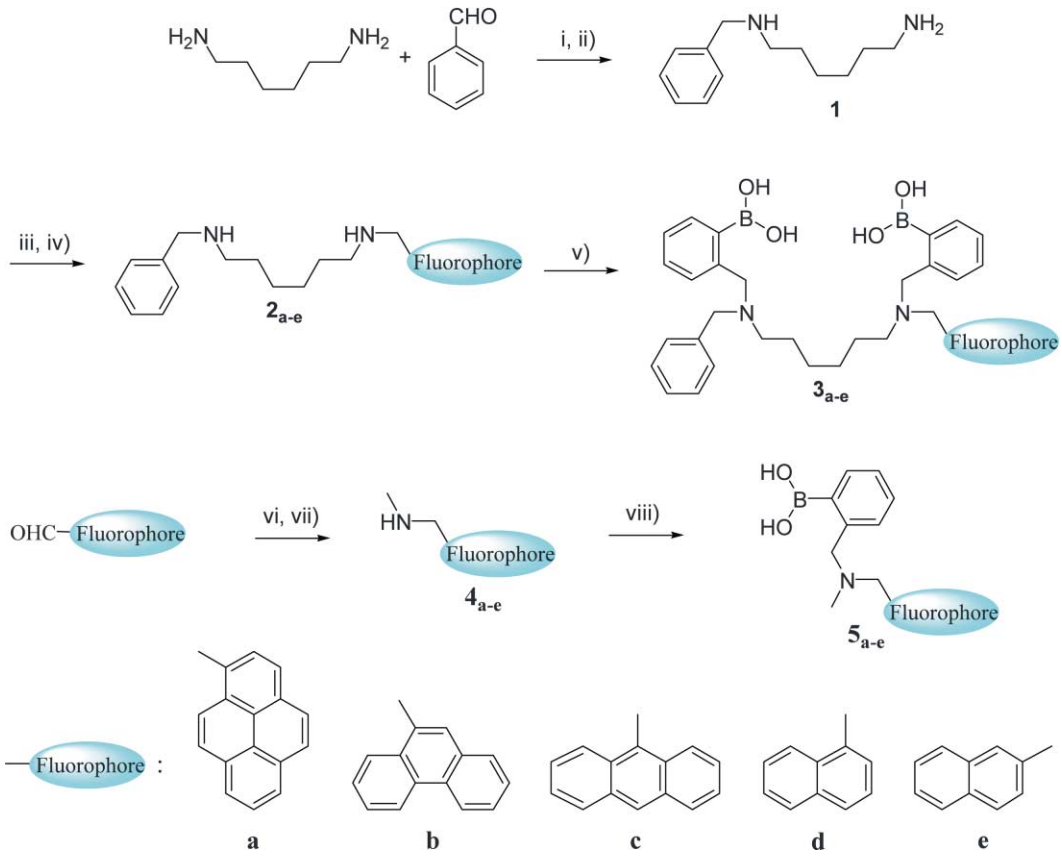

Scheme 1. *Reagents and conditions*: (i) *p*-Toluenesulfonic acid, THF/EtOH; (ii) $NaBH_4$ 79% (**1**); (iii) fluorophore-aldehyde, THF/MeOH; (iv) $NaBH_4$ 86% (**2a**), 73% (**2b**), 37% (**2c**), 77% (**2d**), 54% (**2e**); (v) 2-(2-bromobenzyl)-1,3,2-dioxaborinane, K_2CO_3, MeCN, 35% (**3a**), 53% (**3b**), 56% (**3c**), 49% (**3d**), 55% (**3e**); (vi) methylamine, THF/MeOH; (vii) $NaBH_4$, 91% (**4a**), 86% (**4b**), 86% (**4c**), 85% (**4d**), 90% (**4e**); (viii) 2-(2-bromobenzyl)-1,3,2-dioxaborinane, K_2CO_3, MeCN, 36% (**5a**), 44% (**5b**), 86% (**5c**), 39% (**5d**), 8% (**5e**).

0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3})].[23] The fluorescence intensity of **3a**–**e** increased with increasing saccharide concentration. The observed stability constants (K) of PET sensors **3a**–**e** and **5a**–**e** were calculated by the fitting of emission intensity versus saccharide concentration curves and are reported in Table 2.[24]

It is well known that D-glucose and D-galactose tend to form 1:1 cyclic complexes with diboronic acids whereas D-fructose and D-mannose prefer to form 2:1 acyclic complexes.[1]

To help visualise the trends in the observed stability constants from Table 2, the stability constants of the diboronic acid sensors **3n** are reported in Figure 1 relative to the stability constants of the equivalent monoboronic acid analogues **5n**. The relative stability clearly illustrates that an increase in selectivity is obtained by cooperative binding through the formation of 1:1 cyclic systems. The large enhancement of the relative stability observed for the 1:1 cyclic systems (D-glucose, D-galactose) are clearly contrasted with the small two-fold enhancement observed for the 2:1 acyclic systems (D-fructose, D-mannose).

The largest enhancements in stability in Figure 1 are by **3a** and **3e** the pyrene and 2-naphthalene appended sensors, with **3a** exhibiting enhanced D-glucose selectivity and **3e** exhibiting enhanced D-galactose selectivity.

In interpreting these values the size of the fluorophore's π-surface (solvation) coupled with the number of *peri*-hydrogens each fluorophore contains (steric crowding) must be considered.

Sensor **3a** contains a pyrene moiety which is the largest π-surface in this series. Sensor **3a** displays the greatest enhancement in selectivity for D-glucose over D-galactose. By reducing the size of the hydrophobic π-surface, as with **3d** and **3e**, (appended with 1- and 2-naphthalene), then the selectivity for the monosaccharides switches from D-glucose to D-galactose. Since, the size of the π-surface affects the solvation of the receptor, the larger the π-surface the more hydrophobic the receptor. These results indicate that the best match between receptor and guest is for **3a**, **3b** and **3c** with D-glucose and **3d** and **3e** with D-galactose.

Steric crowding at the receptor site must also be considered. There is little difference between the size of 1-

Table 2. Stability constant K (coefficient of determination; r^2) and fluorescence enhancement for the saccharide complexes of molecular sensors **3** and **5**

Sensor	D-Glucose		D-Galactose		D-Fructose		D-Mannose	
	K (dm^3 mol^{-1})	Fluorescence enhancement	K (dm^3 mol^{-1})	Fluorescence enhancement	K (dm^3 mol^{-1})	Fluorescence enhancement	K (dm^3 mol^{-1})	Fluorescence enhancement
3a	962±70 (0.99)	2.8	657±39 (1.00)	3.1	784±44 (1.00)	3.2	74±3 (1.00)	2.8
5a	44±3 (1.00)	4.5	51±2 (1.00)	4.2	395±11 (1.00)	3.6	36±1 (1.00)	3.7
3b	325±58 (0.97)	1.5	611±101 (0.97)	1.4	1013±126 (0.98)	1.4	134±18 (0.98)	1.4
5b	30±7 (0.98)	1.5	77±12 (0.98)	1.4	548±55 (0.99)	1.4	58±8 (0.98)	1.4
3c	441±76 (0.98)	3.2	536±31 (1.00)	3.1	1000±69 (0.99)	3.0	111±6 (1.00)	2.8
5c	61±3 (1.00)	3.4	93±6 (1.00)	3.0	713±35 (1.00)	3.0	61±3 (1.00)	3.0
3d	417±60 (0.98)	6.1	1072±68 (0.99)	5.4	964±41 (1.00)	5.5	101±3 (1.00)	5.0
5d	52±1 (1.00)	5.7	78±5 (1.00)	5.0	529±45 (0.99)	5.4	46±1 (1.00)	5.2
3e	532±57 (0.99)	4.2	894±66 (0.99)	4.1	1068±63 (1.00)	3.8	98±4 (1.00)	3.5
5e	35±2 (1.00)	4.5	49±4 (1.00)	4.3	399±34 (0.99)	4.6	40±2 (1.00)	3.8

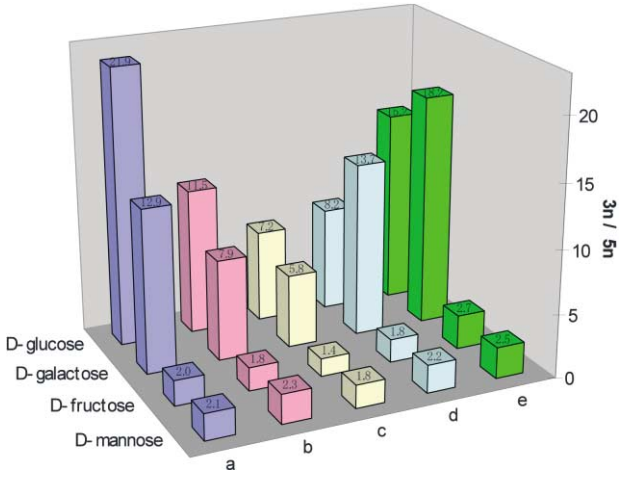

Figure 1. Relative stability with saccharides for **3n**/**5n**.

naphthalene's π-surface and that of 2-naphthalene. The same is true for phenanthrene and anthracene. In these cases the observed trends are probably due to increased steric crowding at the binding site. In both cases a decrease in the relative stability is coupled to an increase in the number of *peri*-hydrogens. Anthracene has 2 *peri*-hydrogens, more than any other fluorophore in the series and clearly displays the lowest relative stability. It is not surprising that increasing the steric crowding of the receptor site reduces binding efficiency.

These results demonstrate that in a PET saccharide sensor with two phenylboronic acid groups, a hexamethylene linker and a fluorophore, the choice of the fluorophore is crucial. Selectivity is fluorophore dependent and careful choice of the fluorophore, such that it complements the polarity of the chosen guest species, is imperative. As well as considering solubility, minimising the steric repulsions from *peri*-hydrogens not only increases the relative stability but can be used to fine tune sensitivity toward specific saccharides.

In conclusion, we have shown that it is possible to adopt a modular approach in the preparation of PET saccharide sensors **3a**–**e** and do so using simple building blocks. We have also shown that the sensitivity and selectivity displayed by fluorescent sensors is fluorophore dependent.

We believe that these observations will need to be considered in the construction of future fluorescent saccharide sensors. Our ongoing research is directed towards exploring these systems in detail and developing a theoretical model to explain these phenomena. Only by understanding these results can we rationally design new saccharide selective sensors.

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