Two Dimensional Photoinduced Electron Transfer (PET) Fluorescence Sensor for Saccharides

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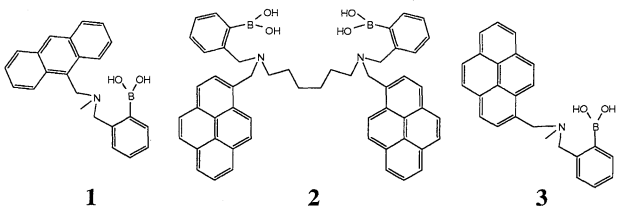
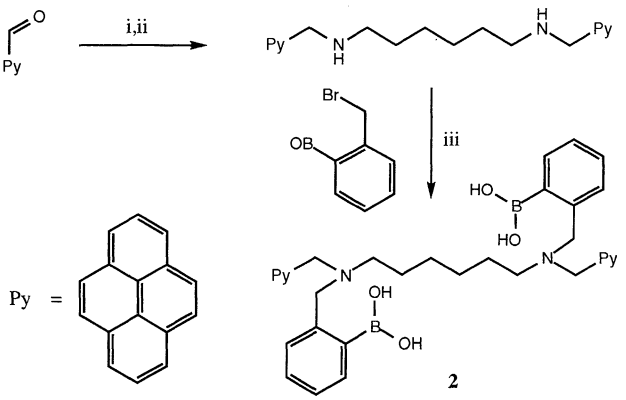
The cooperative binding of two boronic acid moieties has been utilized to create a two dimensional fluorescence sensor for saccharides. The photoinduced electron transfer switching mechanism has been used to provide information of saccharide concentration while the excimer emission changes of the pyrene moieties provide the nature of the complex.

Recognition and transduction of biologically important molecular species is our current interest. Conventional design of molecular receptors for the detection of molecular species has been made on the basis of hydrogen bonding interactions. The inefficient binding of such synthetic receptors in aqueous media prompted us to search for an alternative binding force in designing molecular sensors. Boronic acid is known to bind saccharides *via* covalent interactions in aqueous media.[1] However, the use of this interaction has not been realized until recently. Wulff *et al.* utilized this type of interaction in solid matrices to recognize and separate saccharides.[2,3] Czarnik *et al.* have demonstrated the use of anthracene boronic acid derivatives as fluorescence molecular sensors.[4]



1 2 3

We have recently demonstrated the use of neighboring group participation of an amine group to the boronic acid moiety which modifies the saccharide-boronic acid interaction.[5-7] The boron-nitrogen interaction has been utilized in photoinduced electron transfer (PET) fluorescence sensor[5,7] and colour sensor[8] design and found to be extremely useful. The large fluorescence switch-on factors observed for the molecular fluorescence sensor **1** is due to the strengthening of the boron-nitrogen interaction and subsequent cease of PET from the tertiary amine to the excited anthracene fluorophore. In order to achieve cooperative binding hence higher detection limits we have designed a molecular cleft with two boronic acid moieties. Molecular fluorescent sensor **2** which contains two pyrene units can interact at the excited state to give excimer emission.[8] The rigidification of the structure by cyclic 1:1 binding with sugar can restrict this excimer formation hence increase the monomer emission. Synthesis of **2** and the reference compound **3** was readily achieved according to the Scheme 1 from readily available starting materials.



Reagents (yields) : i, 1,6-hexanediamine, MeOH (100%); ii, $NaBH_4$, MeOH, THF (95%) ; iii, K_2CO_3, THF (95%)

Scheme 1. Synthesis of boronic acid derivative **2**.

Fluorescence titration of **2** with different saccharides in aqueous methanolic solutions (1:3) gave high fluorescence switch-on factors together with high stability constants (Figures

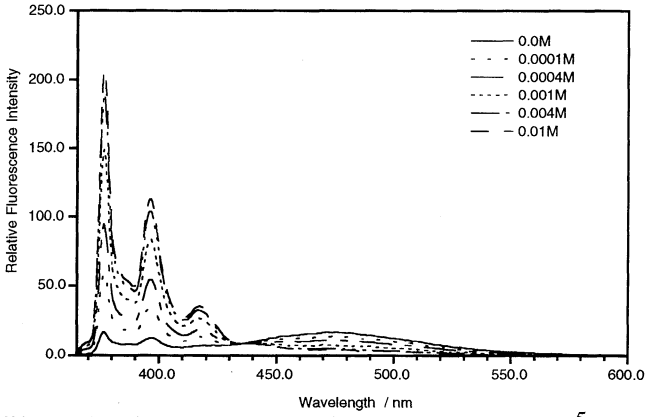


Figure 1. Fluorescence spectral changes of **2** (1.5×10^{-5} mol dm^{-3}) with different concentrations of glucose in a methanol:water (3:1) mixture. excitation at 343 nm.

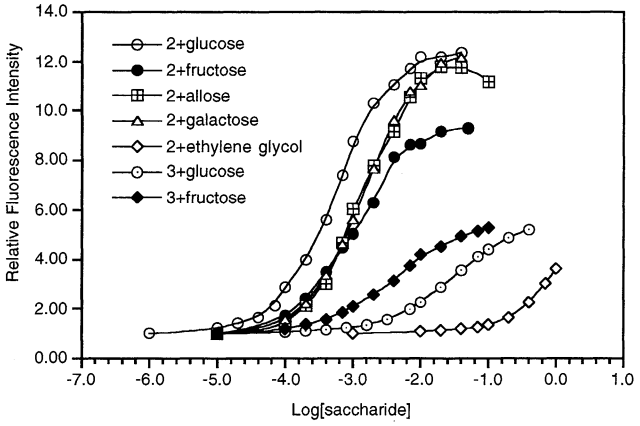


Figure 2. Saccharide concentration *vs* relative monomer fluorescence intensity profile for **2** and **3** in a methanol:water (3:1) mixture: excitation at 343 nm, emission at 376.5 nm.

Table 1. Stability constants for the saccharide complexes of molecular sensors **1**, **2** and **3**

	1 log K^a	**2** log K	**3** log K
glucose	1.8	3.3	1.7
fructose	3.0	-[b]	2.5
allose	2.5	2.9	-
galactose	2.2	2.9	-
ethylene glycol	0.4	-0.5	-

[a] Measurements were done in 33% methanolic aqueous solutions.[6] [b] The plot of relative fluorescence intensity *vs.* saccharide concentration could not be analysed precisely by a simple Benesi-Hildebrand type equation assuming the formation of a 1:1 complex.

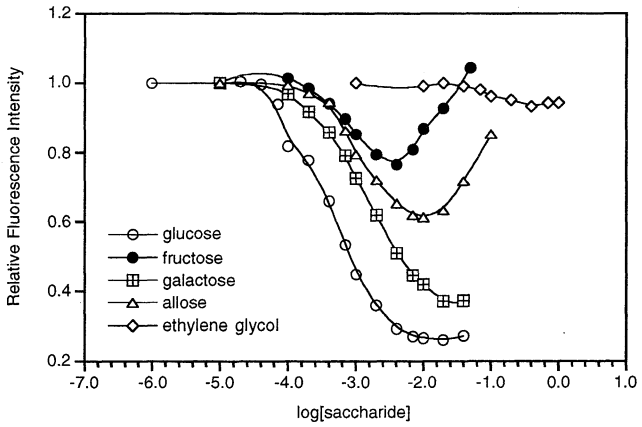


Figure 3. Saccharide concentration *vs* relative excimer fluorescence intensity profile for **2** in a methanol:water (3:1) mixture: excitation at 343 nm, emission at 475 nm.

1 and 2 and Table 1). The monomeric molecular sensor **3** gave low switch-on factors together with low stability constants (Figure 2 and Table 1). The cooperative binding of two boronic acid groups can be clearly recognized from the stability constant differences of mono- and di-boronic acid compounds (Table 1). The titration of sugars with **2** gave decrease of excimer and increase of monomer with a sharp isosbestic point (Figure 1) which indicates the presence of only two fluorescent species. For some saccharides, however, this isosbestic point drifted due to competitive 1:2 complex formation at higher concentration of saccharides (Figure 3). The plot of excimer emission for saccharides clearly shows the break-away point from 1:1 complexes to 1:2 complexes. This behaviour is best seen with fructose and allose which are the best binding saccharides for **1** and **3** (Table 1). The competitive 1:2 binding starts at lower saccharide concentrations for these saccharides. This type of competitive binding has been seen in other fluorescent molecular sensors with multiple binding sites.[10] Mass spectral investigation of this compound with different saccharides provided firm evidence of cyclic ester formation[11].

In conclusion we have shown that the cooperative binding of saccharide in **2** provides information on both saccharide concentration and the type of complex formed in one simple system. We believe that the cooperative binding of boronic acid could be tuned to a particular saccharide to create precise practical saccharide sensors.

References and Notes

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11 All saccharides used in this study gave mass spectral peaks of 1:1 and 1:2 complexes with **2**. Strong binders like glucose gave higher peak ratio for 1:1 complex whereas poor 1:1 binders such as fructose gave higher peak ratio for 1:2 complex.