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Modular fluorescence sensors for saccharides

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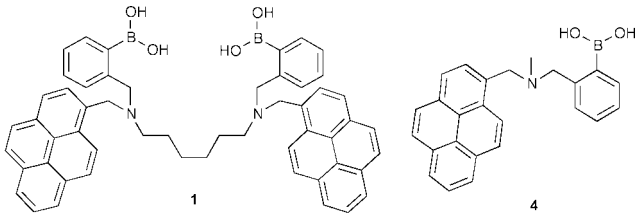
Modular and modular polymer supported fluorescence photoinduced electron transfer (PET) sensors 2 and 3 with two boronic acid receptor units, a pyren-1-yl fluorophore, and hexamethylene linker show selective saccharide binding in aqueous methanolic solution at pH 8.21.

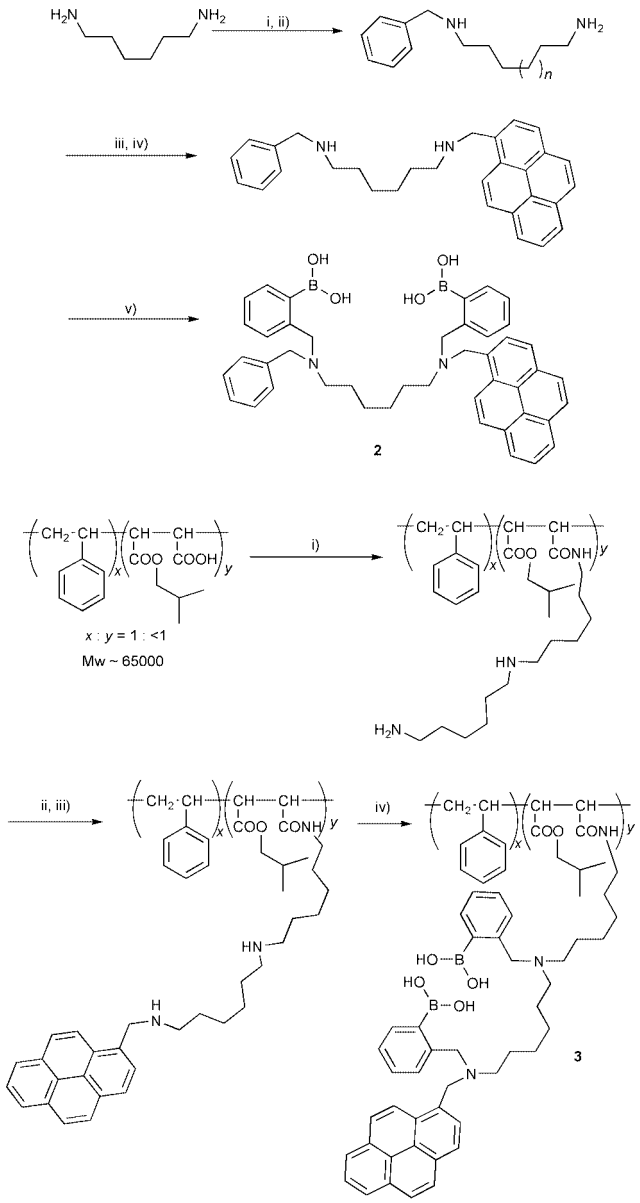
Fluorescent receptors for saccharides have recently received considerable attention. Boronic acids are known to bind saccharides *via* covalent interactions in aqueous basic media. The most common interaction is with *cis*-1,2- or 1,3-diols of saccharides to form five- or six-membered rings respectively. The interaction between boronic acids and amines has been used to create photoinduced electron transfer (PET) sensory system for saccharides.[1–10] 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 from the amine to the fluorophore. These compounds show increased fluorescence at neutral pH through suppression of the PET from nitrogen to the fluorophore on saccharide binding, a direct result of the stronger boron–nitrogen interaction.

Over the last few years we have been interested in developing new fluorescence sensors selective for saccharides employing a modular approach. The basic idea was to break a sensor into three components; receptor units, linker units, and fluorophore units. The approach requires the selection and synthesis of a set of molecular building blocks from which the selective fluorescent sensors can be easily constructed. The quick assembly of a diverse selection of fluorescent sensors will require that the receptor and fluorophore units are linked to a core unit using the minimum of synthetic linkage reactions. The use of common reactions means the synthetic routes towards the new sensors will be convergent. The anthracene core unit has been used for sugars such as D-glucose[3,7] and D-glucosamine hydrochloride.[11,12] However, using anthracene as both the core and fluorophore units will limit the further development of the system. Therefore, it is important to use different core and fluorophore units. This will result in systems where the selectivity (core unit) and emission wavelength (fluorophore) can be varied independently, which hitherto has not been possible. Sensors with different emission wavelengths will allow the simultaneous detection of several analytes.

Shinkai has demonstrated with compound **1** the value of a hexamethylene linker (core unit) in obtaining D-glucose selectivity.[13] However, two fluorophores as with sensor **1** are not required and may in fact be detrimental to an operational sensor since the fluorescence spectra of sensor **1** are complicated by excimer emission due to stacking of the two-pyrene units.



If a practically useful sensor is to be developed then the modular approach should also allow efficient solution based systems to be coupled to a polymer support. Polymeric boronic acid based sensors have been previously prepared[14–20] but these



Scheme 1 Synthesis of PET sensors **2** and **3**. *Reagent (yield)*: **Sensor 2**. i) Benzaldehyde, toluene-*p*-sulfonic acid, THF–EtOH; ii) $NaBH_4$, THF (78%) (2 steps); iii) pyrene-1-carbaldehyde, THF–MeOH (93%); iv) $NaBH_4$, MeOH (92%); v) 2-(2-bromobenzyl)-1,3,2-dioxaborinane, K_2CO_3, MeCN (35%); **Sensor 3**.[23] i) Bis(6-aminohexamethyl)amine, HOBt, DIPC, DMAP, DMF (88%); ii) pyrene-1-carbaldehyde, THF–MeOH; iii) $NaBH_4$ (33%); iv) 2-(2-bromobenzyl)-1,3,2-dioxaborinane, K_2CO_3, MeCN (65%).

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have not involved the linkage of selective solution based systems onto a polymer support.

Following the criteria laid out above we designed PET sensors **2** and **3** with two boronic acid units (saccharide selectivity), one pyrene fluorophore unit and a hexamethylene linker unit (D-glucose selectivity).[13]

Synthesis of compounds **2** and **3** was achieved according to Scheme 1 from readily available starting materials. Reference compound **4** was prepared as previously reported.[13]

Fluorescence titrations of **2** (1.0×10^{-7} mol dm^{-3}), **3** (pyrene concentration 1.0×10^{-7} mol dm^{-3}) and **4** (1.0×10^{-7} mol dm^{-3}) with different saccharides was carried out in aqueous methanolic buffer solution [52.1 wt% methanol at pH 8.21 (KCl, 0.01000 mol dm^{-3}, KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3})].[21] The fluorescence intensity increases with increasing saccharide concentration. The stability constants (K) of PET sensors **2**, **3** and **4** were calculated by fitting the emission wavelength at 397 nm *vs.* concentration curves[12,22] and are given in Table 1.

The relative stability constants of the diboronic acids **2** and **3** relative to the monoboronic acid **4** are given in Table 1. The ratio of stability constants shows how effective the molecular design is at enhancing the binding towards a specific saccharide.

From Table 1, in all cases, the stability constants with diboronic acid sensors **2** and **3** are higher than for monoboronic acid sensor **4**. Cooperative binding of the two boronic acid groups is clearly observed as illustrated by the stability constant differences between the mono- and di-boronic acid compounds. The stability constant K of diboronic acid sensor **2** with D-glucose and D-galactose are 22 and 13 times greater than with monoboronic sensor **4**. Whereas, the stability constant K of diboronic acid sensor **2** with D-fructose is only 2 times stronger than monoboronic acid sensor **4**. This result can be explained since it is well known that D-glucose readily forms 1 : 1 cyclic complexes with di-boronic acids, whereas D-fructose tends to form 2 : 1 acyclic complexes with di-boronic acids.[1–7,13]

Attachment of the solution-based receptor to a polymer support has altered the selectivity of the system towards D-glucose. The stability constant K of diboronic acid sensor **3** with D-glucose and D-galactose are 9 and 15 times greater than with monoboronic sensor **4**. Polymer bound system **3** shows the strongest binding with D-fructose however this value is only 3 times stronger than the monoboronic acid sensor **4**. The major difference between the polymer bound system **3** and solution based system **2** is the D-glucose selectivity. The D-glucose selectivity drops for compound **3** whereas the selectivity with other saccharides is similar to those observed for compound **2**. We believe the differences are due to the proximity of receptor to the polymer backbone. We are currently investigating these phenomena by the preparation of systems with longer linkers to the polymer.

Table 1 Stability constant K (coefficient of determination; r^2) for the saccharide complexes of molecular sensors **2, 3** and **4**

	2 K /dm^3 mol^{-1}	**2/4**	**3** K /dm^3 mol^{-1}	**3/4**	**4** K /dm^3 mol^{-1}
D-glucose	962 ± 70 (0.99)	22	385 ± 26 (0.99)	9	44 ± 3 (1.00)
D-galactose	657 ± 39 (1.00)	13	778 ± 58 (0.99)	15	51 ± 2 (1.00)
D-fructose	784 ± 44 (1.00)	2	1124 ± 109 (0.99)	3	395 ± 11 (1.00)
D-mannose	74 ± 3 (1.00)	2	79 ± 7 (0.99)	2	36 ± 1 (1.00)

In conclusion we have shown that it is possible to prepare saccharide selective sensors using simple building blocks and then attach the sensor to a polymer support. Although, the selectivity of the system has been modulated by attachment to the polymer support the system retains significantly enhanced selectivity for both D-glucose and D-galactose over monoboronic acid sensor **4**. Our ongoing research is directed towards new modular PET sensors with different linkers and fluorophore units.

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