Selective D-glucosamine hydrochloride fluorescence signalling based on ammonium cation and diol recognition

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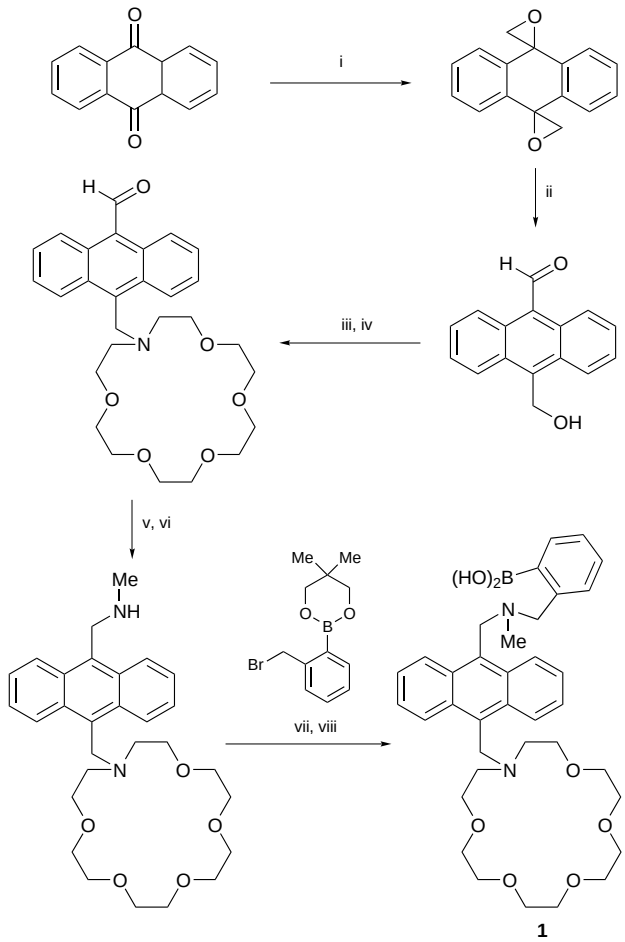
Fluorescent photoinduced electron transfer sensor 1 with monoaza-18-crown-6 ether and boronic acid receptor units shows selective fluorescent enhancement with D-glucosamine hydrochloride in aqueous solution at pH 7.18.

The development of fluorescent receptors for saccharides has recently gained much attention.[1] In a series of recent papers the interaction of boronic acid and amine[2–6] has been used to create photoinduced electron transfer (PET)[7,8] sensory systems for saccharides. When saccharides from cyclic boronate esters with boronic acids, the acidity of the boronic acid is enhanced[9] 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 neutral pH through suppression of the photoinduced electron transfer from nitrogen to anthracene on saccharide binding; a direct result of the stronger boron–nitrogen interaction.[2–7]



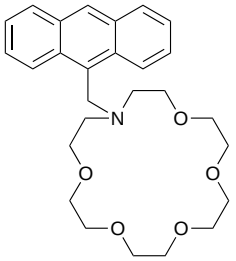
Scheme 1 *Reagents and conditions*: i, NaH, Me_3SI, DMSO, room temp., 80%; ii, LiBr, MeCN, 60 °C, quant.; iii, $MeSO_2Cl$, Et_3N, CH_2Cl_2, −10 °C, 93%; iv, 1-aza-18-crown-6, K_2CO_3, NaI, MeCN, reflux, 50%; v, MeOH, $MeNH_2$, room temp., quant.; vi, $NaBH_4$, MeOH, room temp., quant.; vii, K_2CO_3, MeCN, reflux, 30%; viii, EtOH–H_2O (1 : 2), pH 7.3, quant.

In a recent paper de Silva reports[10] on how two different binding sites can be used to develop a selective fluorescent sensor for γ-aminobutyric acid. The system consists of an azacrown ether (ammonium binding site) and guanadinium (carboxylate binding site). This work shows how two different receptor sites can be used to obtain high selectivities for molecules containing multiple functional groups. We have previously shown how two boronic acid binding units can be used in the selective recognition of glucose[3] and the chiral discrimination of monosaccharides.[4] Here we extend our work on boronic acid fluorescent sensors by the incorporation of a second and *different* binding site. The incorporation of a second binding site will allow the development of sensors for a variety of biologically important molecules containing saccharide fragments.

Sensor **1** consists of monoaza-18-crown-6 ether as a binding site for the ammonium terminal of D-glucosamine hydrochloride, while a boronic acid serves as a binding site for the diol (carbohydrate) part of D-glucosamine hydrochloride. The nitrogen of the azacrown ether unit can participate in PET with the anthracene fluorophore; ammonium ion binding can then cause fluorescence recovery. This recovery is due to hydrogen bonding from the ammonium ion to the nitrogen of the azacrown ether. The strength of this hydrogen bonding interaction modulates the PET from the amine to anthracene. As explained above, the boronic acid unit can also participate in PET with the anthracene fluorophore, and diol binding can also cause fluorescence recovery. The anthracene unit serves as a rigid spacer between the two receptor units, with the appropriate spacing for the glucose moiety.[3] This system is expected to behave like an AND logic gate[11] in that fluorescence recovery will only be observed when two chemical inputs are supplied; for this system the two chemical inputs are an ammonium cation and a diol group. Compound **1** was synthesised as outlined in Scheme 1. The fluorescence of compound **1** varies as a function of pH giving a pK_a of 7.32 (water, 0.05 mol^{-1} dm^{-3} tetramethylammonium chloride); under the same conditions the pK_a of D-glucosamine hydrochloride is 7.56. Binding studies were carried out in a 33.2% (w/w) ethanol–water buffer[12] at pH 7.18. This pH was chosen to balance the protonation of the azacrown ether and deprotonation of the D-glucosamine hydrochloride. At low pH the sensor is protonated and no signal is observed, conversely at higher pH the D-glucosamine hydrochloride is present predominantly as free amine and will not bind strongly with the azacrown ether.

The stability constants for compounds **1**, **2** and **3** with D-glucose and D-glucosamine hydrochloride are given in Table 1. The curves from which the constants were calculated are shown in Fig. 1 and 2.

Compound **2**[13] does not display any fluorescence enhancement or have a binding constant with D-glucose because it has no saccharide binding site. D-glucosamine hydrochloride binding with compound **2** is also not observed under the experimental conditions, because the buffer solution saturates with



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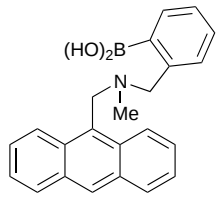

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Table 1 Stability constants for D-glucose and D-glucosamine hydrochloride with **1**, **2** and **3**

	log K_a (r^2; data points)		
	1	**2**	**3**
D-Glucose	—[a]	—[a]	2.92 (0.996; 8)
D-Glucosamine hydrochloride	3.31 (0.997; 8)	< 1[b]	2.70 (0.999; 7)

[a] No fluorescence switching is possible. [b] No fluorescence change observed up to the solubility limit (0.1 mol dm^{-3}).

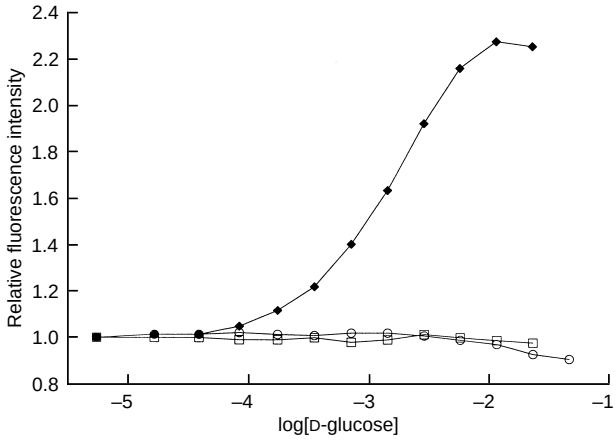

Fig. 1 Fluorescence intensity–log[D-glucose] profile of (○) **1**, (□) **2** and (◆) **3** at 25 °C; 3.33×10^{-6} M of **1**, **2** or **3** in EtOH–H_2O (1 : 2), pH 7.18, λ_{ex} 372 nm, λ_{em} 425 nm

D-glucosamine hydrochloride before the binding event can be observed, giving an upper limit on the logK_a for D-glucosamine hydrochloride of 1. As expected compound **3** shows a fluorescence enhancement with D-glucose (logK_a = 2.92) and D-glucosamine hydrochloride (logK_a = 2.70). With D-glucose the boronic acid has a choice of binding either the 1,2- or 4,6-diols, but with D-glucosamine hydrochloride binding with just the 4,6-diol is possible. The stability constant of **3** with D-glucose is higher than that observed with D-glucosamine hydrochloride, reflecting the known selectivity of boronic acids for the 1,2-diol of D-glucose.[14] Compound **1** shows a fluorescence increase with D-glucosamine hydrochloride (logK_a = 3.31), but no increase with D-glucose. The result clearly demonstrates that for a fluorescent output *both* a diol *and* ammonium group must be present in the guest. The stability of the D-glucosamine hydrochloride complex with compound **1** is greater than that with compound **3**. (logK_a = 3.31 *versus* 2.70) This increase in stability can be attributed to co-operative binding by the boronic acid and azacrown ether.

With this work we have demonstrated that the boronic acid PET system can be used in combination with other binding sites

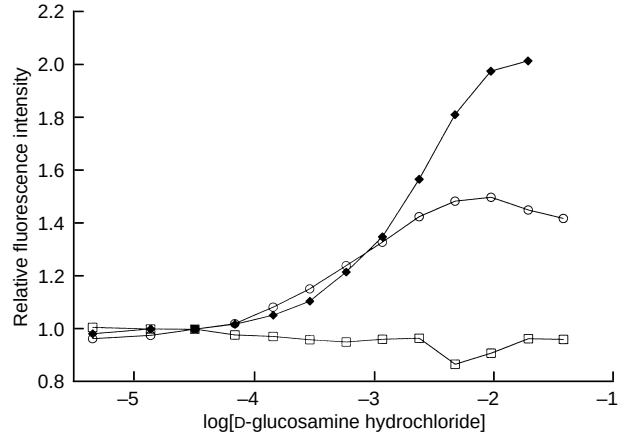

Fig. 2 Fluorescence intensity–log[D-glucosamine hydrochloride] profile of (○) **1**, (□) **2** and (◆) **3** at 25 °C; 3.33×10^{-6} M of **1**, **2** or **3** in EtOH–H_2O (1 : 2), pH 7.18, λ_{ex} 372 nm, λ_{em} 425 nm

to create new and selective receptors for important biological molecules. We believe that this unit will be increasingly used as a general building block in molecular receptor design.

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Footnote

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