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A D-glucose selective fluorescent assay

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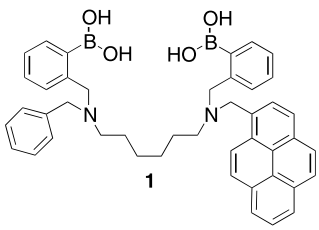
Abstract—A D-glucose selective competition assay has been devised using Alizarin Red S and diboronic acid **2** in buffered aqueous solution.

Over the last few years we have been interested in developing molecular sensors using boronic acids.[1–3] The systems we are developing contain a receptor and reporter (fluorophore or chromophore) as part of a discrete molecular unit. This is, however, not the only approach towards boronic acid based sensors. Anslyn has recently demonstrated that boronic acid receptors and a separate reporter unit can be used in competitive assays.[4–6] A competitive assay requires that the receptor and reporter (typically a commercial dye) associate under the measurement conditions. The receptor–reporter complex is then selectively dissociated by the addition of appropriate guests. When the reporter dissociates from the receptor a measurable response is produced.



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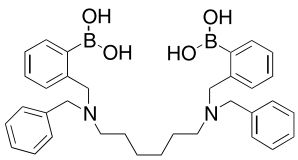


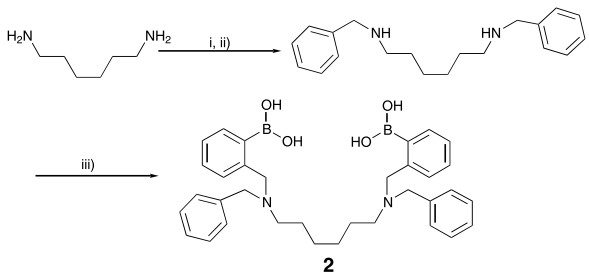
2

We have been exploring the use of coloured and fluorescent dyes to signal binding with boronic acids.[7] Our goal has been to develop effective dye molecules for competitive assays. We are interested in such competitive systems because they reduce the synthetic complexity of the receptor. Recently, a competitive assay for D-fructose has been developed using two commercial reagents, Alizarin Red S (ARS) and phenylboronic acid (PBA).[8] The system displays D-fructose selectivity, which is the inherent selectivity of all monoboronic acids.[3,9]

However, a D-glucose selective system is much more desirable. Detection and monitoring of D-glucose is particularly important for the rapidly increasing numbers of diabetics. Recent research provides clear evidence that a tight control of the blood glucose levels in diabetics reduces the risk of long terms complications.

From our previous work with fluorescent PET sensors, D-glucose selectivity can be achieved through the correct spacing of two boronic acids.[3]

In this communication, we report our work towards the development of a D-glucose fluorescent sensor using ARS in a competitive assay. We based our D-glucose selective receptor on the successful fluorescent PET sensor **1**.[2] The receptor components of **2** are identical to those of **1**, the difference is the lack of a fluorescent signalling unit (pyrene). Synthesis of **2** was achieved from readily available starting materials[10] (Scheme 1).



Scheme 1. *Reagent* (yield): (i) benzaldehyde, THF/MeOH, (ii) $NaBH_4$ (83%, two steps), (iii) 2-(2-bromobenzyl)-1,3,2-dioxaborinane, K_2CO_3, MeCN (35%).

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UV–vis absorbance titrations of Alizarin Red S (9.6×10^{-5} mol dm^{-3}) with PBA and **2** were performed in a pH 8.21 buffer (52.1 wt% methanol in water with KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3}).[11] As the concentration of boronic acid is increased, a visible colour change from burgundy to yellow was observed. Fig. 1 shows the absorption changes with added boronic acid **2**. The absorbance of the free dye at 530 nm decreases as boronic acid **2** is added, and a new absorbance at 464 nm appears. The stability constants (K) of ARS with the boronic acids were calculated by fitting the absorbance at 530 nm versus boronic acid concentration.[12] The calculated K values are 1719±22 for PBA and 8394±790 for **2**. The higher binding constant of **2** with ARS means that a lower concentration of receptor can be used to create the same colour or fluorescence response. This is particularly important if a competitive system is to be developed into a working sensor.

We then performed UV–vis absorbance titrations and fluorescence titrations with D-glucose and D-fructose in the presence of PBA (5.0×10^{-4} mol dm^{-3}) and **2** (5.0×10^{-5} mol dm^{-3}) with ARS (9.6×10^{-5} mol dm^{-3} for the UV–vis titrations and 1.0×10^{-4} mol dm^{-3} for the fluorescence titrations) in a pH 8.21 buffer (52.1 wt% methanol in water with KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3}).[11] As the concentration of D-glucose and D-fructose increased the colour changed from yellow (464 nm) to burgundy (530 nm) and the fluorescence emission intensity at 570 nm (excitation 495 nm) decreased. The absorption and fluorescence changes with added D-glucose are shown in Figs. 2 and 3. The observed change in absorbance and fluorescence represents release of free ARS as the saccharide competes for the boronic acid in solution (Scheme 2). The apparent stability constants (K) of D-glucose and D-fructose with the boronic acids were calculated by fitting the increase in absorbance at 530 nm and decrease in fluorescence intensity at 570 nm versus saccharide concentration.[12] The calculated K values are shown in Table 1.

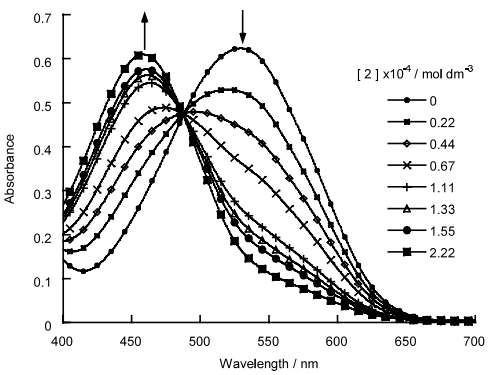


Figure 1. Absorption spectral changes of ARS with increasing concentration of **2**.

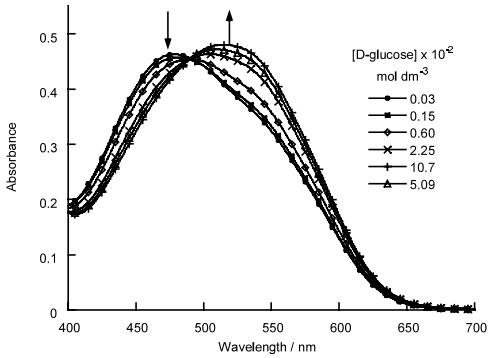


Figure 2. Absorption spectral changes of ARS and **2** with increasing concentration of D-glucose.

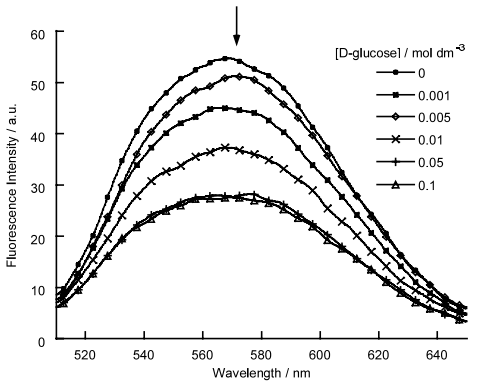
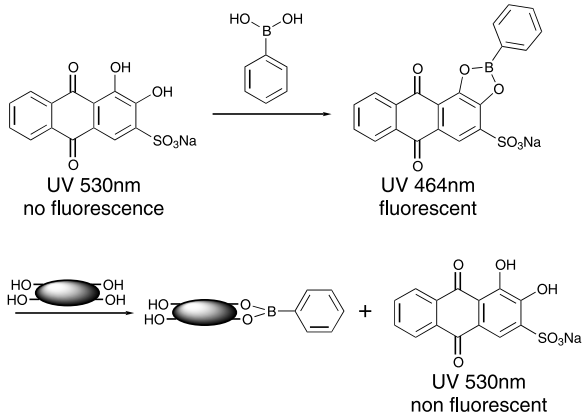


Figure 3. Fluorescence spectral changes of ARS and **2** with increasing concentration of D-glucose.



Scheme 2.

Table 1. Apparent stability constant K (coefficient of determination; r^2) for saccharide complexes of PBA and **2**, in pH 8.21 buffer

Saccharide	PBA	**2**	**2**/PBA
	Fluorescence (λ_{ex} 495 nm, λ_{em} 570 nm)		
D-Glucose	26±6 (0.96)	99±11 (0.98)	4
D-Fructose	78±4 (1.00)	95±8 (0.99)	1
	UV–vis (530 nm)		
D-Glucose	11±1 (0.99)	66±8 (0.99)	6
D-Fructose	73±4 (1.00)	141±8 (1.00)	2

Cooperative binding of the two boronic acid groups is clearly observed as illustrated by the stability constant differences between PBA and **2**. The stability constants K for diboronic acid sensor **2** with D-glucose are four (from fluorescence) and six (from UV–vis) times greater than with PBA. Whereas the stability constants K of **2** with D-fructose are only one (from fluorescence) and two (from UV–vis) times stronger than PBA. These results are not surprising since it is well known that D-glucose easily forms 1:1 cyclic complexes with diboronic acids, whereas D-fructose tends to form 2:1 acyclic complexes with diboronic acids.[3]

In conclusion sensor **2** and ARS produces a very efficient D-glucose assay. Sensor **2** and ARS show an enhanced response to D-glucose when compared to simple PBA (six-fold enhancement). Sensor **2** can can also be used at much lower concentrations (ten times) than PBA.

Acknowledgements

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10. Selected data for **2**: mp 110–113°C; m/z (FAB) 1106 ($[M+H+4(3\text{-}HOCH_2C_6H_4NO_2)-4(H_2O)]^+$, 100%); HRSMS found: 1105.47177. $C_{62}H_{62}B_2N_6O_{12}$ requires: 1105.464538; δ_H (300 MHz, $CDCl_3$+CD_3OD (a few drops), Me_4Si): 1.04 (4H, bs, NCCCH_2), 1.30 (4H, bs, NCCH_2), 2.62 (4H, t, NCH_2), 4.09 (4H, s, PhCH_2N), 4.21 (4H, s, PhB(OH)CH_2N), 7.18 (4H, d, $J=3.96$ Hz, Ar-H), 7.25–7.34 (2H, m, Ar-H), 7.43 (10H, m, Ar-H), 7.68 (2H, d, $J=7.14$ Hz, Ar-H); δ_C (75 MHz, $CDCl_3$+CD_3OD (a few drops), Me_4Si): 22.7, 26.1, 31.8, 55.3, 64.2, 126.5, 126.9, 127.2, 127.8, 128.3, 129.1, 129.3, 130.5, 130.7, 135.0; δ_B (96 MHz, $CDCl_3$+CD_3OD (a few drops)): 9.64.
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12. The K values were analysed in KaleidaGraph using non-linear (Levenberg–Marquardt algorithm) curve fitting. The errors reported are the standard errors obtained from the best fit.