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A D-glucose selective fluorescent internal charge transfer (ICT) sensor

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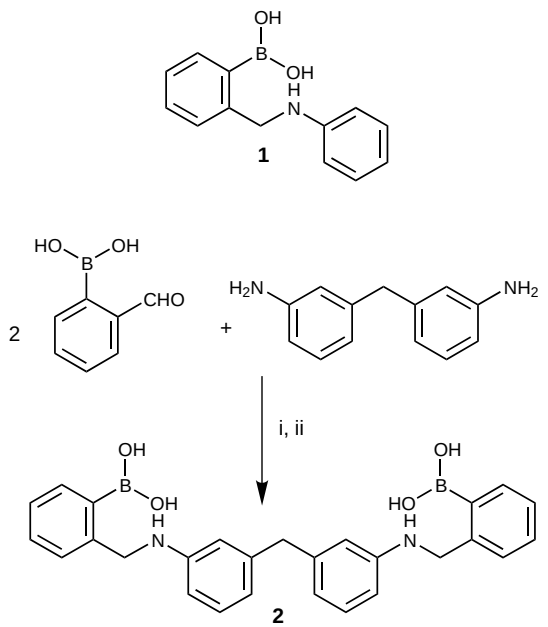
Abstract—An efficient D-glucose selective internal charge transfer (ICT) fluorescent sensor has been prepared. When D-glucose is added to sensor **2** in aqueous solution at pH 8.21 the emission maximum at 405 nm shifts to 360 nm. © 2002 Published by Elsevier Science Ltd.

Saccharide receptors based on the covalent interactions between phenylboronic acid and diols are becoming increasingly important.[1,2] The most common interactions are with *cis*-1,2- or 1,3-diols of saccharides to form five- or six-membered rings, respectively. These bonds are rapidly and reversibly formed between a diol and boronic acid (sp^3-hybridized) in aqueous solution. Formation of a sp^3-hybridized boronic acid is possible in a basic pH region (boronate anion) or by incorporation of a neighboring tertiary amine (B–N bond) at neutral pH.

The B–N bond has been successfully used in the development of saccharide sensors. The interaction of the neighboring amine with the boronic acid is strengthened on saccharide binding. The strength of this boronic acid–tertiary amine interaction can be used to signal the binding event. Many successful photoinduced electron transfer (PET) sensors have been developed based on this approach.[2]

Internal charge transfer (ICT) is an important mechanism for fluorescent signaling. However, there are still very few ICT sensors for neutral molecules.[3] The group of Shinkai prepared a monoboronic acid ICT fluorescent sensor for saccharides.[4] However, the compound showed only small shifts in emission wavelength and intensity on saccharide binding.

Our success with the development of ICT color sensors[5] led us to investigate the ICT fluorescent properties of a related system, compound **1**. We believed that if a working ICT system could be prepared the many advantages of fluorescence could be used to develop working saccharide sensors.[3] Compound **1** is a monoboronic acid ICT fluorescent sensor which shows a large shift in wavelength on saccharide binding.[6] Lakowicz has also recently reported his work with monoboronic acid ICT fluorescent sensors. The diphenoxazole system shows a five-fold fluorescence enhancement with D-fructose and smaller enhancements with other saccharides.[7] Our system compound **1** and that of



Scheme 1. *Reagents and conditions*: (i) MeOH, rt; (ii) MeOH, $NaBH_4$, 56% (two steps).

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Lacowitz both show D-fructose selectivity which is the inherent selectivity of all monoboronic acids.[2,8]

Having successfully designed an ICT fluorescent signaling unit compound **1**, our subsequent goal was to prepare a D-glucose selective receptor containing the same signaling unit. From our previous work with fluorescent PET sensors, D-glucose selectivity can be achieved through the correct spacing of two boronic acids.[2] Combining the requirements of the signaling unit with those of a D-glucose selective receptor we designed diboronic acid sensor **2**.

Diboronic acid sensor **2** was prepared according to Scheme 1 from readily available starting materials, 3,3′-methylenedianiline and 2-formylbenzeneboronic acid.†

The pK_a of compounds **1** and **2** were 10.21±0.01 and 10.88±0.02, respectively and in the presence of D-fructose (0.05 mol dm^{-3}) were 7.92±0.06 and 8.71±0.08, respectively, as calculated from the fluorescence intensity versus pH titrations.[9,10] The observed shift in pK_a to lower values on saccharide binding is in agreement with previous work.[2,8]

The fluorescence titration of **1** (2.0×10^{-5} mol dm^{-3}) and **2** (4.2×10^{-5} mol dm^{-3}) with different saccharides were carried out in a pH 8.21 buffer (52.1 wt% methanol in water with KCl, 0.01000; KH_2PO_4, 0.002752; Na_2HPO_4, 0.002757 mol dm^{-3}).[11] The fluorescence spectra of **2** in the presence of D-glucose (0–0.1 mol dm^{-3}) are shown in Fig. 1.

The fluorescence of **2** shifted from 405 to 360 nm with increasing saccharide concentration. The stability constants (K) of fluorescence sensors **1** and **2** with D-fructose, D-glucose, D-galactose and D-mannose were calculated by fitting the emission wavelength at 360 nm versus concentration of saccharide.[9,10] The stability constants for sensors **1** and **2** calculated from these titrations are given in Table 1.

The observed order of stability constants (K) for sensor **1** is D-fructose>D-galactose>D-glucose>D-mannose, and is the expected order for all monoboronic acids.[2,8] However, the observed order of stability constants (K) for sensor **2** is D-glucose>D-fructose>D-galactose>D-mannose, indicating our molecular design has been successful in achieving D-glucose selectivity.

The relative stability constants of the diboronic acid **2** to the monoboronic acid **1** are also given in Table 1.

† *Selected data for* **2**: mp: 130°C. Found: C, 70.20; H, 6.08; N, 5.99. $C_{27}H_{28}B_2N_2O_4$+0.15 C_6H_{12} requires C, 70.12; H, 6.34; N, 5.80%. (HRMS found: [M+4(3–$HOCH_2C_6H_4NO_2$)–$4H_2O$]$^+$, 1006.357040 (100%) $C_{55}H_{48}B_2N_6O_{12}$ requires m/z 1006.351632); δ_H (300 MHz; CD_3OD) 3.70 (2H, s, Ph-CH_2-Ph), 4.28 (4H, s, PhCH_2-NH-Ph), 6.45–6.73 (6H, m, ArH), 6.90–7.10 (2H, m, ArH), 7.15–7.33 (8H, m, ArH), δ_C (75 MHz; CD_3OD; Me_4Si) 43.8, 51.6, 115.0, 118.0, 121.7, 127.7, 128.0, 129.1, 130.3, 134.0, 144.0, 145.8, 149.8; m/z (FAB) 1006 ([M+4(3–$HOCH_2C_6H_4NO_2$)–$4H_2O$]$^+$, 100%).

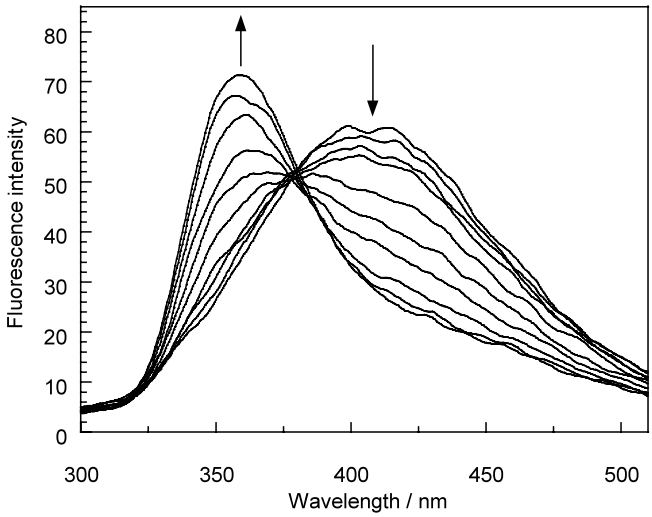


Figure 1. Fluorescence spectra change of **2** (4.2×10^{-5} mol dm^{-3}) with different concentration of D-glucose (0–0.1 mol dm^{-3}) in pH 8.21 buffer, λ_{ex} 265 nm.

Table 1. Stability constant K (coefficient of determination; r^2) for saccharide complexes of fluorescent sensor **1** and **2**, in pH 8.21 buffer at λ_{ex} 263 nm (sensor **1**) and 265 nm (sensor **2**)

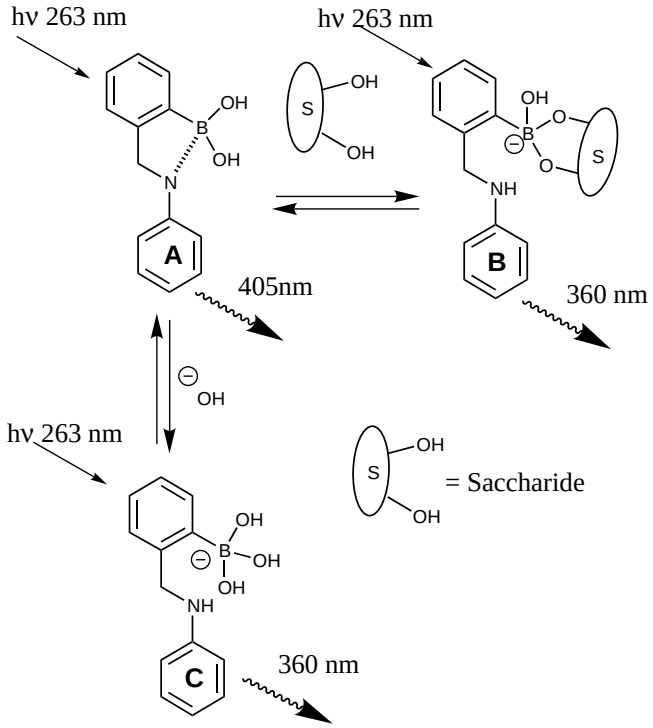
Saccharides	**1** K (mol^{-1} dm^3)	**2** K (mol^{-1} dm^3)	**2**/**1**
D-Glucose	10±2.6 (0.99)	140±13 (0.99)	14
D-Fructose	85±2.7 (0.99)	55±3.7 (0.99)	0.6
D-Galactose	16±1.2 (0.99)	26±1.8 (0.99)	1.6
D-Mannose	–[a]	7±0.9 (0.99)	–

[a] Change in fluorescence too small to calculate stability constant.

The ratio of stability constants shows how effective our molecular design is at enhancing the D-glucose binding. Cooperative binding of the two boronic acid groups is clearly observed as illustrated by the stability constant differences between the mono- and diboronic acid compounds. The stability constant K of diboronic acid sensor **2** with D-glucose is 14 times greater than with monoboronic sensor **1**. Whereas, the stability constant K of diboronic acid sensor **2** with D-fructose is 0.6 times weaker than monoboronic acid sensor **1**. This result can be explained since it is well known that D-glucose readily forms 1:1 cyclic complexes with diboronic acids, whereas D-fructose tends to form 2:1 acyclic complexes with diboronic acids.

From our results with compounds **1** and **2**, addition of saccharide produces the same fluorescence response to that observed when the pH is increased. This observation implies that in both cases a boronate anion must be formed.

Species **A** contains a B–N bond and when excited at 263 emits at 405 nm. On addition of saccharide or base the B–N bond is broken to form boronate species **B** or **C** which both emit at 360 when excited at 263 nm (Scheme 2).



Scheme 2. Proposed fluorescent species.

The equilibrium from **A** to **C** was expected and is well established. But, the equilibrium from **A** to **B** was not what we expected. From our work with fluorescent PET sensors the interaction of a neighboring amine with a boronic acid is strengthened on saccharide binding.[2] However, it should be noted that these systems contained more basic alkyl amines. Therefore, it is perhaps not surprising that **1** and **2** which contain less basic anilinic amines form boronate **B** and not a stronger B–N bond. This observation although surprising is not new, the breaking of a B–N bond has also been observed in a PET system where the receptor becomes crowded on saccharide binding.[12]

In conclusion, with this system we have shown that it is possible to prepare an efficient D-glucose selective ICT fluorescent sensor which displays a large change in the intensity and wavelength of the emission maxima. We are currently developing a range of ICT fluorescent sensors selective for other saccharides.

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References

1. Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2978–2996.
2. Hartley, J. H.; James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155–3184.
3. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
4. Sandanayake, K. R. A. S.; Imazu, S.; James, T. D.; Mikami, M.; Shinkai, S. *Chem. Lett.* **1995**, 139–140.
5. Ward, C. J.; Patel, P.; Ashton, P.; James, T. D. *Chem. Commun.* **2000**, 229–230.
6. Arimori, S.; Bosch, L. I.; Ward, C. J.; James, T. D. *Tetrahedron Lett.* **2001**, *42*, 4553–4555.
7. DiCesare, N.; Lakowicz, J. R. *Chem. Commun.* **2001**, 2022–2023.
8. Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769–774.
9. *The pK_a and K values were analysed in KaleidaGraph using nonlinear (Levenberg–Marquardt algorithm) curve fitting.*[10] *The errors reported are the standard errors obtained from the best fit.*
10. Cooper, C. R.; James, T. D. *J. Chem. Soc., Perkin Trans. 1* **2000**, 963–969.
11. Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ion Control*; Chapman and Hall: London, 1974.
12. Kijima, H.; Takeuchi, M.; Robertson, A.; Shinkai, S.; Cooper, C.; James, T. D. *Chem. Commun.* **1999**, 2011–2012.