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The B–N bond controls the balance between locally excited (LE) and twisted internal charge transfer (TICT) states observed for aniline based fluorescent saccharide sensors

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Abstract—Three boronic acid based saccharide sensors with an aniline fluorophore have been prepared. One of the systems (**1a**) contains an intramolecular boron–nitrogen (B–N) bond and displays fluorescence due to both LE and TICT states. The other two systems (**1b** and **c**) have no B–N bond and only show fluorescence due to the LE state.
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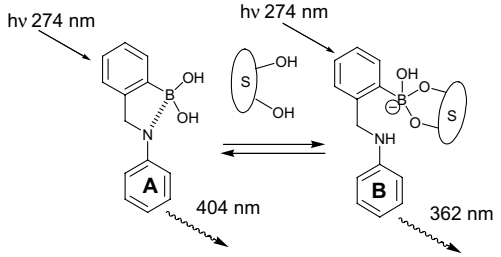
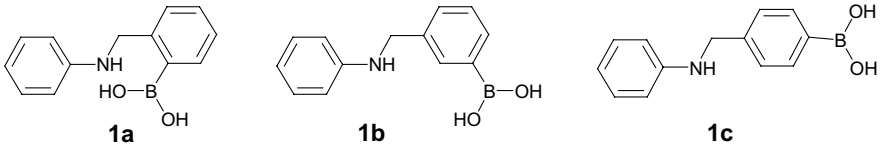
Boronic acid receptors with the capacity to bind saccharides selectively and signal this event by altering their optical signature have attracted considerable interest in recent years.[1–9] Boronic acids are known to bind saccharides via covalent interactions in aqueous media. The most common interaction is with the *cis*-1,2- or -1,3-diols of saccharides to form five- or six-membered rings, respectively.[10]

We recently published our findings on compound **1a** a monoboronic fluorescent sensor, this system shows large shifts in the emission wavelength on saccharide binding.[11] When saccharides interact with sensor **1a** in aqueous solution at pH 8.21 the emission maxima at 404 nm shifts to 362 nm ($\lambda_{ex} = 274$ nm).

The species responsible for the observed fluorescence properties were previously assigned (Scheme 1). Species **A** contains a B–N bond and when excited at 274 nm emits at 404 nm. On addition of a saccharide the B–N bond is broken to form boronate species **B**, which emits at 362 nm when excited at 274 nm (Scheme 1). The different fluorescent properties of species **A** and **B** can be ascribed to locally excited (LE) and twisted internal charge transfer (TICT) states of the aniline fluorophore.[12–15] Species **B** only shows the normal band associated with the LE state since the nitrogen lone pair is free to conjugate with the π-system while species **A** shows the anomalous band associated with the TICT



Scheme 1. Proposed fluorescent species.



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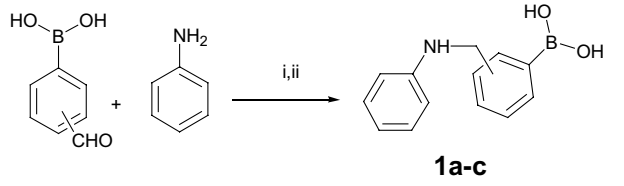
state since the lone pair is coordinated with the boron and perpendicular to the π-system.

From these investigations we realised that the species **B** emitting at 362 nm does not contain a B–N bond, but does shown fluorescent enhancement. Therefore, we decided to investigate systems where formation of a B–N bond was not possible. Compounds **1a–c** were prepared according to Scheme 2 from readily available starting materials, aniline and 2-, 3- or 4-formylbenzeneboronic acid.[16] For compound **1a** we were also able to obtain crystals suitable for X-ray analysis.[17] The structure is shown in Figure 1. It is clear from Figure 1 that the boronic acid forms a cyclic trimeric anhydride, where only one of the units contains a B–N bond (1.747(2) Å). This value is similar to those found in similar cyclic boroxins[18] and within the 1.5–1.8 Å range expected for a strong B–N bond.[19] The structure supports Scheme 1 in that it illustrates that a subtle balance exists for formation of a B–N bond.

For compounds **1b** and **1c** when excited at 240 and 244 nm, respectively, an emission at 350 nm was observed (excitation at 274 nm resulted in no emission). Also, when compound **1a** was excited at 244 nm only an emission at 360 nm was observed. In all these cases only the LE state is formed.

The pK_a of compounds **1a–c** (1.0×10^{-5} mol dm^{-3}, 33.3 wt% (**1a**) and 52.1 wt% (**1b** and **c**) methanolic aqueous solution in the presence of 0.05 mol dm^{-3} sodium chloride osmotic buffer) were determined from the fluorescence intensity versus pH profiles in the absence and presence of D-fructose (0.05 mol dm^{-3}). The pK_a of compounds **1a–c** were: 10.20 ± 0.01, 9.30 ± 0.03 and 9.58 ± 0.02, respectively, and in the presence of D-fructose (0.05 mol dm^{-3}) were 7.96 ± 0.06, 6.95 ± 0.04 and 7.22 ± 0.02, respectively. The observed shift in pK_a to lower values on saccharide binding is in agreement with previous work.[2]



Scheme 2. Reagents and conditions: (i) EtOH/$PhCH_3$ reflux (**1a**) or MeOH, rt (**1b–c**); (ii) $NaBH_4$, **1a** 40% (two steps), **1b** and **1c** both 45% (two steps).

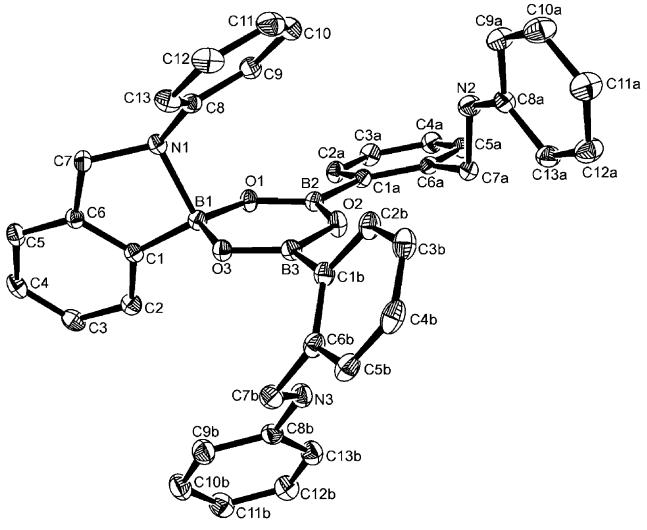


Figure 1. Crystal structure of **1a**. Ellipsoids are illustrated at the 30% probability level. Hydrogen atoms omitted for clarity.

The fluorescence titration of **1a** (2.0×10^{-5} mol dm^{-3}), **1b** (1.0×10^{-5} mol dm^{-3}) and **1c** (1.0×10^{-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 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3}).[20] The fluorescence spectra of **1c** in the presence of D-fructose (0–0.1 mol dm^{-3}) are shown in Figure 2.

The stability constants (K) of fluorescence sensors **1a–c** with D-fructose, D-glucose, D-galactose and D-mannose were calculated by fitting the emission intensity at 350 or 360 nm ($\lambda_{ex}=240$ and 244 nm) versus concentration of saccharides (Fig. 3).[21] The stability constants for sensors **1a–c** calculated from these titrations are given in Table 1.

The fluorescence enhancements obtained for **1a–c** on the addition of D-fructose are 15-, 18- and 25-fold, respectively. We believe that these large fluorescence enhancements can be attributed to fluorescence recovery of the aniline fluorophore. With these systems in the absence of saccharides the normal fluorescence of the LE state of the aniline donor is quenched by energy transfer to the phenylboronic acid acceptor. When saccharides are added, a negatively charged boronate anion is formed (cf. species **B** from Scheme 1), under these conditions energy transfer from the aniline donor is unfavourable and fluorescence recovery of the LE state of the aniline donor is observed.

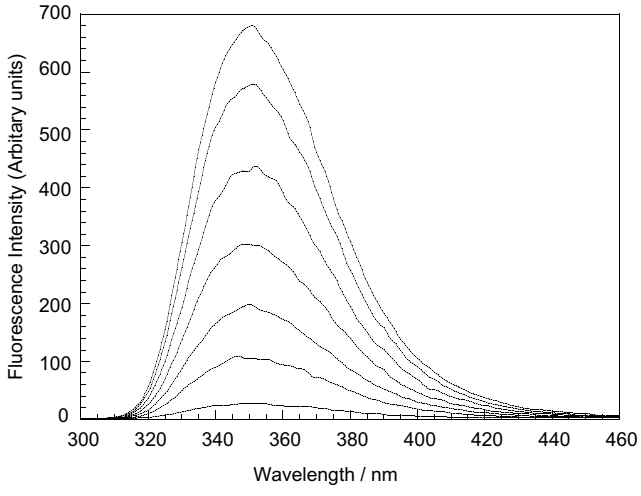


Figure 2. Fluorescence spectra change of **1c** (1.0×10^{-5} mol dm^{-3}) with different concentrations of D-fructose (0–0.1 mol dm^{-3}) in 52.1 wt% MeOH pH 8.21 phosphate buffer. $\lambda_{ex}=244$ nm.

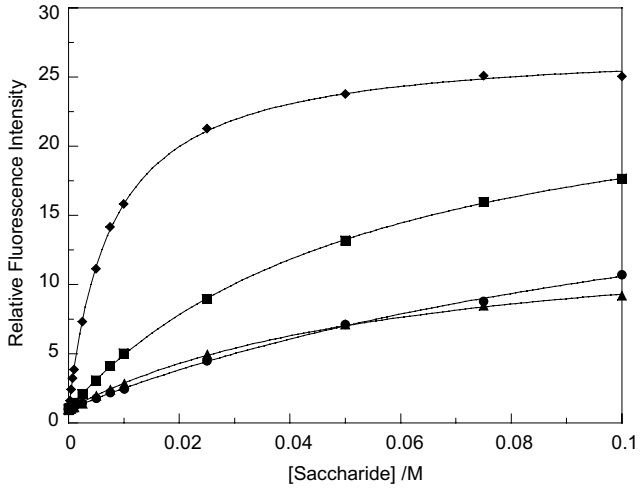


Figure 3. Relative fluorescence intensity versus saccharide concentration profile of **1c** with (◆) D-fructose, (●) D-glucose, (■) D-galactose, (▲) D-mannose. The measurement conditions are the same as those in Figure 2. $\lambda_{ex} = 244$ nm, $\lambda_{em} = 350$ nm.

Table 1. Stability constant K (coefficient of determination; r^2) for saccharide complexes of fluorescent sensors **1a**–**c**, in pH 8.21 buffer at $\lambda_{ex} = 244$ nm (sensors **1a** and **c**) and 240 nm (sensor **1b**)

	K mol^{-1} dm^3		
Saccharides	**1a**	**1b**	**1c**
D-Fructose	79.2 ± 1.7 (0.99)	212.1 ± 6.9 (0.99)	128.6 ± 2.6 (0.99)
D-Glucose	6.4 ± 0.4 (0.99)	8.7 ± 1 (0.99)	6.7 ± 0.5 (0.99)
D-Galactose	14.2 ± 0.6 (0.99)	26.6 ± 1.3 (0.99)	17.7 ± 0.3 (0.99)
D-Mannose	7.9 ± 0.3 (0.99)	13.9 ± 1.4 (0.99)	16.2 ± 0.8 (0.99)

This was confirmed by the quantum yield measurements of compounds **1a**–**c**. The quantum yield Φ of aniline is 0.09[22] (in methanol), and the measured quantum yield Φ of **1a** is 0.0082, **1b** is 0.0087 and **1c** is 0.0070 (in methanol).[23,24]

In conclusion, we have prepared two new systems (**1b** and **c**), which display large fluorescence enhancements on saccharide binding. The fluorescence changes observed for the LE state at 350 or 360 nm for all three systems (**1a**–**c**) has been ascribed to the fluorescence recovery of the aniline fluorophore. We are confident that these discoveries will lead to the development of improved boronic acid based fluorescent saccharide sensors. Our ongoing research is directed towards exploiting these findings in other saccharide selective systems.

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16. Selected data for **1a**: mp: 125–129 °C; found: C, 74.70; H, 5.97; N, 6.63. $C_{13}H_{14}BNO_2$–H_2O requires C, 74.72; H, 5.80; N, 6.70%; δ_H (300 MHz; CD_3OD) 4.21 (2H, s, CH_2), 6.60–6.70 (3H, m, ArH), 6.95–7.25 (6H, m, ArH); δ_C (75 MHz; CD_3OD) 51.6, 117.4 (2C), 121.5, 126.9, 127.9, 129.8, 130.2 (2C), 132.7, 146.0, 149.1; m/z (FAB) 497.3 ([M+2(3-$HOCH_2C_6H_4NO_2$)−$2H_2O$]$^+$, 100%).
Selected data for **1b**: mp: 166 °C; found: C, 73.90; H, 5.56; N, 6.72. $C_{13}H_{14}BNO_2$–H_2O + 0.03 $CHCl_3$ requires C, 73.60; H, 5.70; N, 6.58%); (HRMS: found [M+2(3-$HOCH_2C_6H_4NO_2$)−$2H_2O$]$^+$, 497.1776. $C_{27}H_{24}BN_3O_6$ requires 497.1758); δ_H (300 MHz; CD_3OD) 4.31 (2H, s, CH_2), 6.55–6.65 (3H, m, ArH), 7.00–7.10 (2H, m, ArH), 7.30 (1H, m, ArH), 7.42 (1H, m, ArH), 7.60 (1H, m, ArH), 7.76 (1H, m, ArH); δ_C (75 MHz; CD_3OD) approximately 49.0 carbon masked by CD_3OD, 114.5, 118.3, 129.1, 130.3, 130.8, 133.8, 134.4, 140.8, 150.5; m/z (FAB) 498.1 ([M+H+2(3-$HOCH_2C_6H_4NO_2$)–$2H_2O$]$^+$, 100%).
Selected data for **1c**: mp: 164 °C; found: C, 73.90; H, 5.88; N, 6.15. $C_{13}H_{14}BNO_2$–H_2O+0.03$CHCl_3$ requires C, 73.60; H, 5.70; N, 6.58%); (HRMS: found [M+2 (3-$HOCH_2C_6H_4NO_2$)–$2H_2O$]$^+$, 497.1772. $C_{27}H_{24}BN_3O_6$ requires 497.1758); δ_H (300 MHz; CD_3OD) 4.30 (2H, s, CH_2–NH), 6.55–6.65 (3H, m, ArH), 7.00–7.10 (2H, m, ArH), 7.30–7.40 (2H, m, ArH), 7.56 (1H, m, ArH), 7.69 (1H, m, ArH); δ_C (75 MHz; CD_3OD) approximately 49.0 carbon masked by CD_3OD, 114.5, 118.4, 127.9, 130.3, 135.5, 144.1, 150.5; m/z (FAB) 497.1 ([M+2(3-$HOCH_2C_6H_4NO_2$)–$2H_2O$]$^+$, 100%).

17. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 228172. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: 144-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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24. The areas under the fluorescence spectra were calculated using KALEIDAGRAPH version 3.51 for PC, published by Synergy Software and developed by Abelbeck Software, 2457 Perikiomen Avenue, Reading, PA 19606. The quantum yields of sensors **1a**–**1c** were then calculated by comparison with aniline as standard using the following equation:[23]

$$\Phi_s = \Phi_{an} \times (FA_s/FA_{an}) \times (A_{an}/A_s)$$

Φ is quantum yield; FA is the area under the curve of the fluorescence peak; A is the absorbance of each sensor at 244 nm (for **1a** and **c**) and 240 nm for **1c**. The subscripts s and an are the abbreviations for sensors (**1a**–**c**) and the aniline reference, respectively. $\Phi = 0.09$ (in methanol)[22] is used as the reference quantum yield of aniline in methanol.