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Fluorescent internal charge transfer (ICT) saccharide sensor

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Abstract—An efficient internal charge transfer (ICT) fluorescent saccharide sensor 3 has been prepared from 2-formyl benzeneboronic acid and aniline. When saccharides interact with sensor 3 in aqueous solution at pH 8.21 the emission maxima at 404 nm shifts to 362 nm. \bigcirc 2001 Elsevier Science Ltd. All rights reserved.

Saccharide receptors based on the covalent interactions between phenylboronic acid and diols are becoming increasingly important.¹ The most common interactions are with *cis*-1,2- or 1,3-diols of saccharides to form fiveor 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 an *sp*³-hybridized boronic acid is possible in a basic pH region (boronate anion) or by incorporation of a neighbouring 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 neighbouring 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.²⁻⁴ Internal charge transfer (ICT) is an important mechanism for fluorescent signalling. However, there are still very few ICT sensors for neutral molecules.⁵ The Shinkai group have prepared an ICT fluorescent sensor for saccharides.⁶ However, with compound 1 only a small shift in emission wavelength and intensity of emission on saccharide binding is observed. We were convinced that the failure of the coumarin system did not mean that an efficient fluorescent ICT system could not be found. What the Shinkai result had shown was that the coumarin ICT system compound 1 was insensitive to saccharide binding.

We recently reported⁷ our work on a saccharide selective ICT colour sensor, compound **2**. The wavelength of the azo dye **2** shifted by ca. 55 nm on binding with D-fructose; the observed colour changed from purple to red. The colour change was due to a change in the environment of the anilinic nitrogen which leads to changes in the energy levels of the n and π^* orbitals of the azo chromophore.



Scheme 1. Synthesis of fluorescence saccharide sensor 3. Reagent (yields): (i) EtOH/toluene; (ii) NaBH₄, MeOH (15%; two steps).

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Our success with the ICT colour sensor compound 2 led us to investigate the ICT fluorescent properties of a related system, compound 3. We believed that if a working ICT system could be prepared then the many advantages of fluorescence could be used to develop working saccharide sensors.⁵

The fluorescence sensor **3** was prepared according to Scheme 1 from readily available starting materials, aniline and 2-formylbenzeneboronic acid.⁸

The fluorescence intensity versus pH profile of compound 3 (1×10^{-5} mol dm⁻³) in the absence and presence of D-fructose (0.05 mol dm⁻³) are shown in Fig. 1. The pH was controlled using the minimum volumes of sodium hydroxide and hydrochloric acid solutions. The pK_a of compound 3 was 10.21±0.01 and 7.92±0.06 in the presence of D-fructose (0.05 mol dm⁻³), as calcu-



Figure 1. Fluorescence intensity versus pH profile of 3 at 25°C; $[3]=1\times10^{-5}$ mol dm⁻³, 33.3 wt% methanolic aqueous solution in the presence of [NaCl]=0.05 mol dm⁻³, (\blacksquare) without saccharide, (\bullet) in the presence of D-fructose (0.05 mol dm⁻³). $\lambda_{ex}=244$ nm, $\lambda_{em}=360$ nm.



Figure 2. Fluorescence spectra change of 3 $(2.0 \times 10^{-5} \text{ mol} \text{ dm}^{-3})$ with different concentration of D-fructose $(0-0.1 \text{ mol} \text{ dm}^{-3})$ in 52.1 wt% MeOH pH 8.21 phosphate buffer. $\lambda_{ex} = 274$ nm.

lated from the fluorescence intensity versus pH titration.⁹ The observed shift in pK_a to lower values on saccharide binding is in agreement with previous work.^{1,3,4,10}

The fluorescence titration of 3 (2.0×10^{-5} mol dm⁻³) with different saccharides was carried out in methanolic aqueous buffer solution [52.1 wt% methanol at pH 8.21 (KCl, 0.01000 mol dm⁻³; KH₂PO₄, 0.002752 mol dm⁻³; Na_2HPO_4 , 0.002757 mol dm⁻³)].¹¹ The fluorescence spectra of 3 in the presence of D-fructose (0-0.1 mol dm⁻³) are shown in Fig. 2. The fluorescence intensity of 3 increased and shifted from 404 to 362 nm with increasing saccharide concentration; an isosbestic point was observed at 377 nm. The observation of an isosbestic point indicates that fluorescence sensor 3 changes from a single unbound species to a single saccharide bound species. The stability constants (K) of fluorescence sensor 3 were calculated by fitting the emission wavelength at 360 nm versus concentration of saccharide.9 The curves are shown in Fig. 3 and the calculated values are given in Table 1.

The observed order of stability constants (*K*) with D-fructose>D-galactose>D-glucose, is the expected order for all monoboronic acids.^{1,3,4,10}

In conclusion, with this system we have shown that it is possible to prepare efficient ICT fluorescent sensors for saccharides which display both a significant change in



Figure 3. Relative fluorescence intensity versus saccharide concentration profile of 3 with (\bullet) D-fructose, (\blacksquare) D-glucose, (\blacklozenge) D-galactose, (\blacktriangle) D-mannose. The measurement conditions are the same as those in Fig. 2. $\lambda_{ex} = 274$ nm, $\lambda_{em} = 360$ nm.

Table 1. Stability constant K (coefficient of determination; r^2) for saccharide complexes of fluorescence sensor **3**

D-Fructose	D-Glucose	D-Galactose	D-Mannose
106±7 (0.99)	18±6 (0.98)	27±4 (0.99)	_a

^a The K could not be determined because of the small fluorescence change.

the intensity and wavelength of the emission maxima. We are currently developing other ICT fluorescent sensors selective for other saccharides.

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- 8. Selected data for **3**: 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; CD₃OD) 4.21 (2H, s, CH₂), 6.6–6.7 (3H, m, ArH), 6.95–7.25 (6H, m, ArH); δ_C (75 MHz; CD₃OD) 51.6, 117.4, 121.5, 126.9, 127.9, 129.8, 130.2, 132.7, 146.0, 149.1; m/z (FAB) 497.3 ([M+2(3-HOCH₂C₆H₄NO₂)–2H₂O]⁺, 100%).
- 9. The pK_a and K were analysed in KaleidaGraph using nonlinear (Levenberg–Marquardt algorithm) curve fitting.¹² The errors reported are the standard errors obtained from the best fit.
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