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A molecular colour sensor for monosaccharides†

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Boronic acid colour sensor 2 undergoes a large visible colour change, from purple to red in aqueous solution on the addition of monosaccharides.

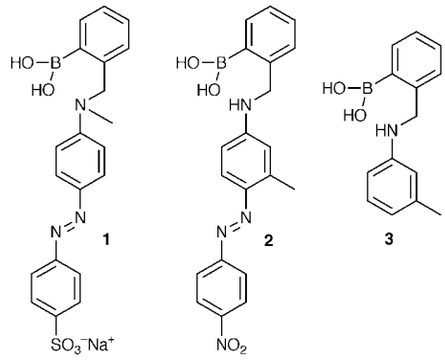
Much recent attention has been paid to the development of synthetic molecular receptors with the ability to recognise neutral organic species, including saccharides. A large majority of these systems have utilised hydrogen bonding interactions for the purposes of recognition and binding of guest species. However, there is still no designed, monomeric receptor which can compete effectively with bulk water for low concentrations of monosaccharide substrates.[1]

Boronic acids readily and reversibly form cyclic esters with diols in aqueous basic media. Saccharides contain a linked array of hydroxy groups ideal for binding to boronic acids. The most common interaction is with 1,2- and 1,3-diols of saccharides to form five- or six-membered rings respectively *via* two covalent bonds. Lorand and Edwards determined the selectivity and first stability trends of various polyols and saccharides towards phenylboronic acid.[2]

The complex stability increases from ethylene glycol to D-fructose, *i.e.* from the simple acyclic diols to the rigid, vicinal *cis*-diols of saccharides. This observed selectivity order is common to all monoboronic acids, not just to phenylboronic acid.[3] The suitability of the boronic acid functionality as a receptor for saccharides has been established in both circular dichroism and fluorescence detection studies. Indeed, a number of fluorescent sensors have been reported in the literature.[4–6]

A fairly recent development has been the study of the effect of saccharides on the colour of dyes containing boronic acid functionality. Russell[7] synthesised a boronic acid azo dye from *m*-aminophenylboronic acid which was found to be sensitive to saccharides. Nagasaki, Shinmori and Shinkai observed that chromophores containing boronic acid moieties and which aggregate in water, changed colour and deaggregated upon addition of saccharides.[8] This year Strongin[9] reported a system based on resorcinarenes for the visual sensing of saccharides. However, for a colour change to be observed the saccharide must be heated (90 °C) in DMSO.

In 1994 Sandanayake and Shinkai reported 'the first known synthetic molecular colour sensor for saccharides'.[10] This designed molecular internal charge transfer (ICT) sensor, dye molecule **1**, was based on the intramolecular interaction between the tertiary amine and the boronic acid group.[11,12] The electron-rich amine creates a basic environment around the electron-deficient boron centre, which has the effect of inducing the boronic acid–saccharide interaction and reducing the working pH of the sensor.[13] Electronic changes associated with this decrease in the pK_a of the boronic acid moiety on saccharide complexation were shown to be transmitted to the neighbouring amine. This creates a spectral change in the connected ICT chromophore, which can be detected spectrophotometrically. The pK_a value associated with the boron–nitrogen interaction shifted on saccharide addition.



The main drawback of this system is the relatively small shifts in the absorption bands of the chromophore upon saccharide binding. The aim with this research was to develop a molecular ICT sensor, which produces a large visible colour change on saccharide binding. If a system with a large colour change can be developed it could be incorporated into a diagnostic test paper for D-glucose, similar to universal indicator paper for pH. Such a system would make it possible to measure D-glucose concentrations without the need of specialist instrumentation. This would be of particular benefit to diabetics in developing countries.

Dye molecule **2** was prepared in three steps in high yield.‡ The imine formed between comercial 2-formylphenylboronic acid and *m*-toluidine was reduced using $NaBH_4$ to give amine **3** in 75% yield. The final step is to couple **3** with the diazonium salt of 4-nitroaniline to give the boronic acid azo dye molecule **2** in 74% yield. The *m*-methyl group of **3** ensures that only the *p*-isomer is obtained.[14]

Absorption–pH titrations, from pH 2 to 12, of **2** in 0.05 mol dm^{-3} NaCl in MeOH–H_2O (1:2, w/w), were followed using a UV-VIS spectrometer. The experiments were then repeated with 0.05 mol dm^{-3} D-fructose also present. The NaCl present acts as an ionic buffer because small amounts of NaCl are formed on adjustment of the pH with NaOH and HCl. Because the titrations are carried out in a MeOH–H_2O mixture rather than simply water, the concept of pH is not strictly applicable to this situation. However, De Ligny and Rehbach have shown that for solutions in 50% MeOH the pH is only changed by 0.1 of a pH unit compared to a 100% water solution.[15]

The pK_a of compound **2** calculated from the absorption–pH titrations[16] was 10.2 and in the presence of 0.05 M fructose the value drops to 6.95. This shift on saccharide binding is in agreement with previous work[2,3,5,6,10] and can be explained by the decrease in oxygen–boron–oxygen bond angle upon saccharide binding, which increases the acidity of the boron centre.[3]

During these titrations it was noted that the UV–VIS absorption maxima of **2** does not move to as long a wavelength at high pH with D-fructose present. To investigate this phenomenon D-fructose, D-glucose and ethylene glycol titrations were performed at pH 11.32. The absorption spectra of the D-glucose titrations are shown in Fig 1.

† Details of the colour changes upon addition of D-glucose to **2** are available as supplementary data, see http://www.rsc.org/suppdata/cc/a9/a909204h/

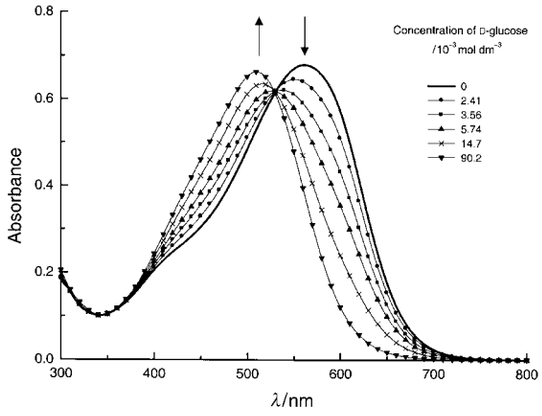
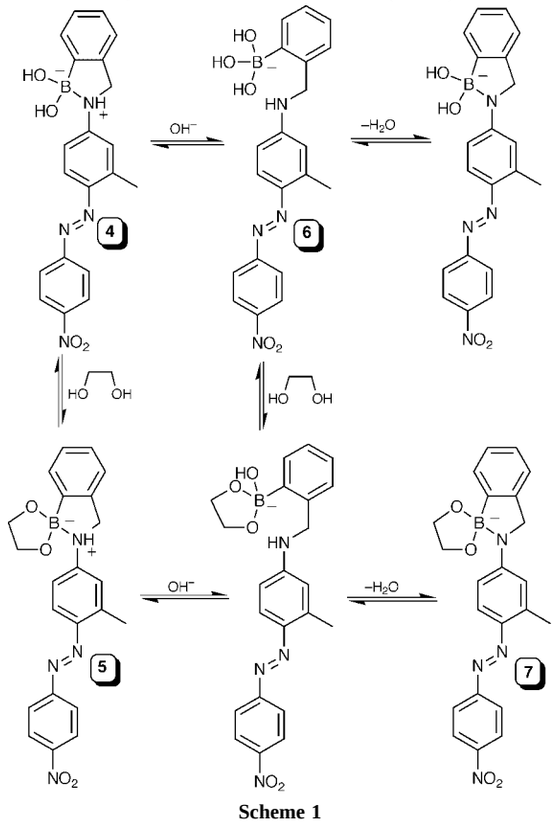


Fig. 1 Absorption spectral changes of dye molecule **2** (5.66×10^{-5} mol dm^{-3}) with increasing concentration of D-glucose at pH 11.32. pH 11.32 buffer: 0.01000 mol dm^{-3} KCl, 0.002771 mol dm^{-3} $NaHCO_3$, 0.002771 mol dm^{-3} Na_2CO_3 in 52.1% MeOH–47.9% H_2O (ref. 23).



Scheme 1

The wavelength shifts by *ca.* 55 nm to shorter wavelength upon guest complexation. The concentration of the guest required to produce the change is different in each case, which is due to the different stability constants of the binding species, as mentioned earlier.[2] The wavelength shift obtained with **2** on addition of diols is the largest observed to date. The stability constants (log*K*) of the boronic acid dye–saccharide complexes were calculated from UV–VIS absorption–concentration profiles. The log*K* vales are D-fructose (3.75), D-glucose (1.85) and ethylene glycol (0.66) respectively.

Scheme 1 shows the species in equilibria responsible for the observed colour change, consistent with the experimental results. With dye molecule **1** Shinkai proposes that at intermediate pH a boron–nitrogen interaction is prevalent, whereas at high and low pH this bond is broken.[10] What makes the equilibria of dye molecule **2** more interesting is the presence of the *anilinic hydrogen*, which can give rise to different species at high pH. This apparently simple modification in the molecular structure is responsible for the enhanced response of these dyes relative to those previously reported.

In the absence of saccharide, at pH 11.32, the observed colour is purple and in the presence of saccharide the colour is red.† From previous work it is known that when saccharides form cyclic boronate esters with boronic acids, the Lewis acidity of the boronic acid is enhanced and therefore the Lewis acid–base interaction between the boronic acid and the amine is strengthened.[3,12] This stronger B–N interaction will favour the red species over the equivalent saccharide bound purple species. The reason for this can be understood by considering species **4** and **5** from Scheme 1. In the presence of saccharide the B–N interaction in species **5** is stronger than that in species **4**. The increased B–N interaction of species **5** will make the N–H proton of species **5** more acidic than the corresponding proton in species **4**. Therefore at higher pH, species **5** will deprotonate to form the red species **7**, whereas the weaker B–N bond in species **4** is broken by hydroxide ion to form the purple coloured species **6**.§

The colour change arises from the different electronic environment of the anilinic nitrogen. The anilinic nitrogen is conjugated to the azo chromophore. A change in the environment of this nitrogen leads to changes in the energy levels of the n and π* orbitals of the azo chromophore and hence to a change in the absorption energy and wavelength. The proposal of these equilibrium species may also explain why dye molecule **1** did not give a visible spectral shift on saccharide binding. Because the anilinic nitrogen is tertiary in nature rather than secondary, there is no possibility of deprotonation, so the high pH boron–nitrogen bond cannot be formed. Hence there is no differentiation between the equilibrium species at high pH and consequently no spectral shift is observed.

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Notes and references

‡ *Selected data* for **2**: mp 120–122 °C (decomp.) (HRMS: Found: $[M]^+$, 372.1381, $C_{20}H_{17}BN_4O_3$ requires 372.1394); ν_{max}(KBr)/cm^{-1} 1602s, 1518s and 1333s; δ_H(300 MHz; CD_3OD; Me_4Si) 2.63 (3 H, m), 4.45 (2 H, br s), 6.52–6.58 (1 H, m), 6.99–7.04 (1 H, m), 7.18–7.40 (5 H, m), 7.63–7.72 (1 H, m), 7.87–7.93 (1 H, m), 8.21–8.36 (2 H, m); δ_C(125 MHz; CD_3OD; Me_4Si) 18.1, 49.7, 113.2, 114.7, 118.3, 122.7, 123.6, 125.7, 127.6, 128.1, 129.8, 130.2, 132.8, 144.3; *m/z* (EI) 373 ($[MH - H_2O]^+$, 66%), 222 ($[M - H_2O–N_2C_6H_4NO_2]^+$, 100).

§ Negative ion electrospray ionisation (ESI) mass spectrometry using a Micromass LCT spectrometer confirmed the presence of the red species **7** (*m/z* 533).

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