ORGANIC LETTERS

2002
Vol. 4, No. 4
477−479

Molecular Color Sensors for Monosaccharides

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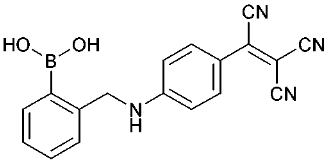
Received October 17, 2001

ABSTRACT



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Boronic acid color sensor 2 undergoes a visible color change, from purple to pink, on the addition of monosaccharides in aqueous methanol at pH 8.21.

The ability to recognize neutral organic species, including saccharides, is a major challenge in synthetic receptor design. Hydrogen bonding has been a particularly useful interaction for the purposes of recognition and binding of guest species. However, there is still no designed, monomeric hydrogen bonding receptor that can compete effectively with bulk water for low concentrations of monosaccharide substrates.[1]

Simple boronic acids readily and reversibly form cyclic esters with diols in aqueous basic media. Saccharides contain a linked array of hydroxyl groups ideal for binding to boronic acids. The most common interaction is with 1,2- and 1,3-diols of saccharides to form five- and six-membered rings, respectively, via two covalent bonds. Lorand and Edwards determined the selectivity and first stability trends of various polyols and saccharides toward 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.[3] Indeed, a number of fluorescent sensors have been reported in the literature.[3−6]

A fairly recent development has been the study of the effect of saccharides on the color of dyes containing the boronic acid functionality. Boronic acid azo dyes have been known for over forty years for their use in investigations into the treatment of cancer by the technique of boron neutron capture therapy (BNCT).[7,8] However, it has only been in the 1990s that related dyes and their interaction with saccharides have been studied. Russell has synthesized a boronic acid azo dye from *m*-aminophenylboronic acid, which was found to be sensitive to a selection of saccharides.[9] Shinkai observed that chromophores that contain boronic acid moieties and aggregate in water changed color and deaggregated upon addition of saccharides.[10] Strongin has also reported a system based on resorcinarenes for the visual

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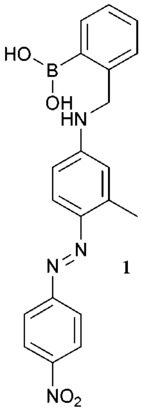
10.1021/ol016923w CCC: $22.00

sensing of saccharides.[11,12] Shinkai has also developed several interesting *intra*-[13,14] and *inter*molecular[15] color sensors for saccharides utilizing the boronic acid–amine interaction.

Previously, we reported our findings with azo dye molecule compound **1**. This compound shows a large color change, from purple to red.[16] However, the color change was only observed at pH 11.32, so the system will not be useful for the detection of saccharides in biological systems.

Since we reported our work with compound **1**, we have been searching for systems capable of producing a large visible color change with saccharides at neutral pH. We screened a number of azo dyes without success; then by serendipity, we discovered the tricyanovinylarylamine dyes.[17,18]



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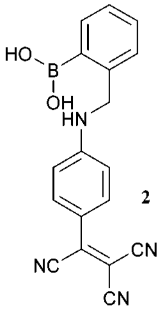

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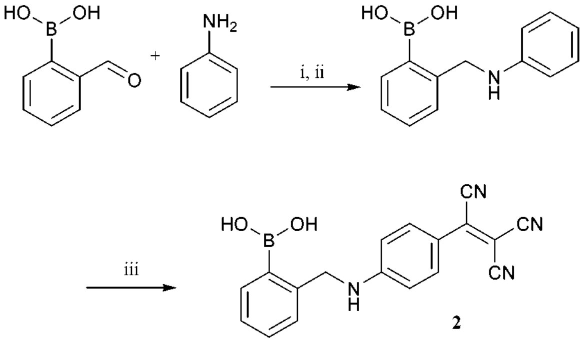
Compound **2** was synthesized from readily available starting materials as outlined in Scheme 1.

Scheme 1[a]



[a] Reagents and conditions: (i) EtOH–PhMe, Δ; (ii) MeOH, $NaBH_4$, 83% (2 steps); (iii) tetracyanoethylene, DMF, 55 °C, 63%.

Absorption–pH titrations, from pH 2 to 12, of **1** (5.66×10^{-5} mol/dm^3) in 0.05 mol/dm^3 NaCl 33:67 methanol/water (w/w) and **2** (1.57×10^{-5} mol/dm^3) in 0.05 mol/dm^3 NaCl 52.1:47.9 methanol/water (w/w) were followed using a UV–vis spectrometer. The experiments were then repeated with 0.05 mol/dm^3 D-fructose also present. Because the titrations are carried out in a methanol–water mixture rather than simply water, the measurement of pH using a standard electrode is not strictly applicable.[19] However, De Ligny and Rehbach have shown that for solutions in 50% methanol the pH is changed by only 0.1 pH unit compared to a 100% water solution.[20] Therefore, the pH values used are those recorded using a Hanna Instruments HI 9321 Microprocessor pH meter calibrated using Fisher Chemicals standard buffer solutions (pH 4.0 phthalate, 7.0 phosphate, and 10.0 borate).

The pK_a of compounds **1** and **2** calculated from the absorption–pH titration[4,21] were 10.19 and 7.81, respectively, and in the presence of 0.05 M fructose, the values drop to 7.04 and 6.46, respectively. The observed shift in pK_a on saccharide binding is in agreement with previous work and can be explained by the decrease in oxygen–boron–oxygen bond angle upon saccharide binding, which increases the acidity of the boron center.[3]

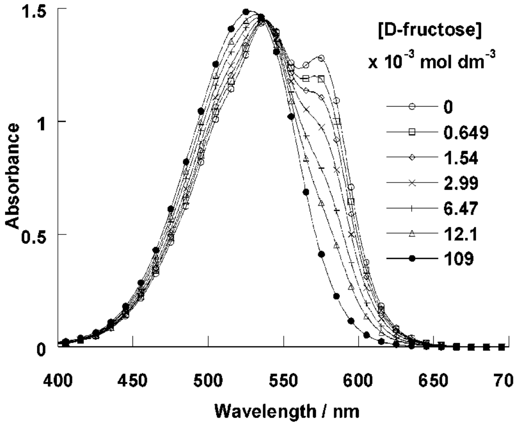


Figure 1. Absorption spectral changes of dye molecule **2** (3.14×10^{-5} mol/dm^3) with increasing concentration of D-fructose at pH 8.21.

With compound **1** we proposed that deprotonation of the anilinic nitrogen was the cause of the observed color change from purple to red with added saccharides at pH 11.32. With compound **2** the pK_a without saccharide was 7.81, which is significantly less than the value of 10.2 with compound **1**. Therefore, with compound **2** we were able to observe a color change with added saccharide from purple to pink at a pH

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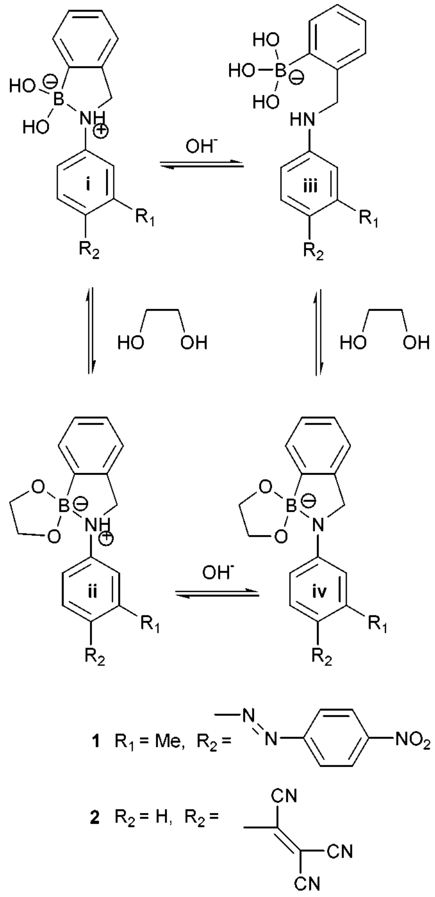
(21) The pK_a and K values were analyzed in KaleidaGraph using nonlinear (Levenberg–Marquardt algorithm) curve fitting. The errors reported are the standard errors obtained from the best fit.

Scheme 2. Proposed Equilibria for Dye Molecules **1** and **2**



of 8.21 (0.01000 mol/dm^3 KCl; 0.002752 mol/dm^3 KH_2PO_4; 0.002757 mol/dm^3 Na_2HPO_4 in 52.1:47.9 methanol/water (w/w)).[22] pH 8.21 was chosen to give the maximum response; however, we have also confirmed that a color change is also observed at lower pH values. The absorption spectral changes observed for the addition of D-fructose to **2** are shown in Figure 1.

The stability constants K of the boronic acid dye–saccharide complexes for compound **2** were calculated from the UV–vis absorption–concentration profiles at 573 nm and a pH of 8.21.[4,21] The calculated stability constants K are D-fructose (170 ± 6 dm^3/mol^1) and D-glucose (8.3 ± 0.3 dm^3/mol^1); a value for ethylene glycol could not be determined because the system became saturated with ethylene glycol before a shift in the absorption spectra could be observed.

Scheme 2 shows the equilibria species responsible for the observed color changes with compounds **1** and **2**.

As previously mentioned, 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] The stronger B–N interaction promotes the formation of a “new” colored species **iv**. The reason for this can be understood by considering species **i** and **ii** from Scheme 2. In the presence of saccharide, the B–N interaction in species **ii** is stronger than that in species **i**. The increased B–N interaction of species **ii** will make the N–H proton of species **ii** more acidic than the N–H proton in species **i**. Therefore, species **ii** will deprotonate to form species **iv**, whereas the weaker B–N bond in species **i** is broken to form species **iii**.

We are currently working on systems that not only function in a biologically acceptable pH range but also are selective among the monosaccharides. Our previous work demonstrates that selectivity can be achieved by the introduction of another appropriately spaced boronic acid.[3]

Acknowledgment. T.D.J. acknowledges the Royal Society for support through the award of a University Fellowship. C.J.W. wishes to acknowledge the EPSRC and Avecia Limited for support through the award of a Studentship.

Supporting Information Available: Experimental procedures and characterization data for dye molecules **1** and **2**, and observed color changes upon addition of D-fructose to dye molecule **2** (2.0×10^{-5} mol/dm^3) in pH 8.21 buffer. This material is available free of charge via the Internet at http://pubs.acs.org.

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