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A new boronic acid fluorescent reporter that changes emission intensities at three wavelengths upon sugar binding

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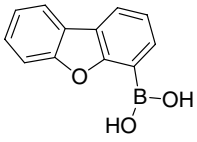
Abstract—The boronic acid moiety is a very useful functional group for the preparation of sugar sensors. Along this line, water-soluble boronic acids that change fluorescent properties upon sugar binding are especially useful as reporter units in fluorescent sensors for carbohydrates. Herein, we report the discovery of a new water-soluble boronic acid (**1**, dibenzofuran-4-boronic acid) that exhibits unique fluorescence changes at three wavelengths upon binding with sugars under near physiological conditions.



Dibenzofuran-4-boronic acid (DBFBA)

The boronic acid functional group is known to form tight complexes with compounds that contain two adjacent nucleophiles. Such compounds may include diols,[1–4] α-hydroxyacids,[5,6] α-amino acids,[7] and most likely amino alcohols. Among these interactions, the binding with diols have been studied the most, in developing fluorescent carbohydrate sensors[8–34] and lectin mimics.[9,20,35–40] Boronic acids are also known to interact with cyanide[28] and fluoride,[41,42] which has been explored for sensor development. In developing sensors for various analytes, the availability of reporter compounds, especially water-soluble ones, that change spectroscopic properties upon binding, is a critical component.[35,43] In our effort to develop combinatorial libraries for the synthesis of boronic acid-based sensors and boronolectins for biological applications,[35,36] we are in need of a large number of water-soluble boronic acid reporters with structural and spectral diversity. Along this line, we are interested in the development of water-soluble boronic acid reporter compounds[44–47] for combinatorial application. Herein, we report a new water-soluble boronic acid, dibenzofuran-4-boronic acid (**1**, DBFBA), that is in a new structural class and has unique spectroscopic properties. DBFBA, at as low as 10^{-7} M, shows significant triplex band fluorescence changes upon binding with a sugar.

The effect of various carbohydrates on the fluorescent properties of DBFBA was examined in phosphate buffer at pH 7.4. DBFBA itself shows an emission λ_{max} of 327 nm. Upon addition of a model sugar, fructose, the emission at 327 nm showed a slight dip with a concomitant significant increase in intensity of emission at two other wavelengths, 301 and 318 nm (Fig. 1).

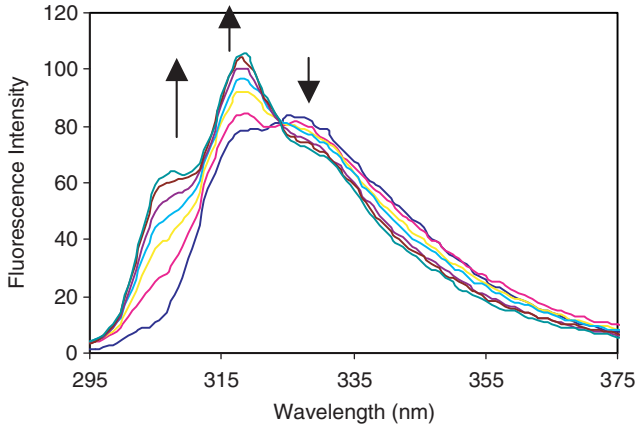


Figure 1. Fluorescence spectra of **1** (2×10^{-7} M) upon addition of D-fructose (0–20 mM) in 0.1 M phosphate buffer at pH 7.4, $\lambda_{ex} = 286$ nm.

Keywords: Boronic acid; Fluorescence.
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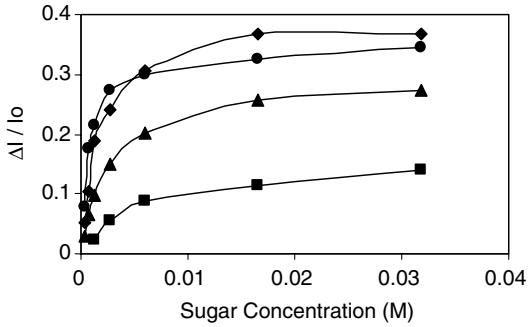


Figure 2. Relative fluorescence intensity of **1** (2×10^{-7} M) in 0.1 M phosphate buffer at pH 7.4 in the presence of D-fructose (♦), D-arabinose (■), D-mannitol (▲), and D-sorbitol (●): $\lambda_{ex} = 286$ nm, $\lambda_{em} = 318$ nm.

To examine whether such changes are a general phenomena for binding with diols of different compounds, we also studied the binding between DBFBA and sorbitol, arabinose, mannitol, and glucose (Fig. 2). All the sugars tested were found to give similar emission spectral changes as that of fructose, except for glucose, which gave a much smaller fluorescent intensity change and required a much high concentration to 'saturate'.

The largest fluorescent intensity changes were observed with sorbitol, which is 7-fold at 307 and 40% at 318 nm. Because, the fluorescence changes at three wavelengths, this allows for DBFBA to be used as a ratiometric sensor. For example, the intensity ratio between 307 and 327 nm is 0.24 before the addition of any sugar and 0.79 after the addition of 0.016 M of fructose (Fig. 3), resulting in a 3-fold change. Figure 3 shows the pattern of such ratiometric changes for three other sugars, sorbitol, mannitol, and arabinose.

The apparent association constants (K_a) between DBFBA and four sugars were determined assuming the formation of a 1:1 complex (Table 1).[48] As expected, the affinity trend with **1** followed that of phenylboronic acid in the order of sorbitol > fructose > mannitol > arabinose > glucose. However, the actual affinities observed were quite different from that of phenylboronic acid. For example, phenylboronic acid (PBA) has a K_a of 162 M^{-1} for fructose and 5 M^{-1} for glucose,[3] while

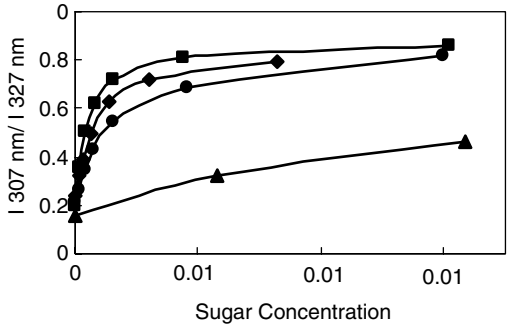


Figure 3. Fluorescence intensity changes ratio of **1** between 307 and 327 nm in 0.1 M phosphate buffer at pH 7.4 in the presence of D-fructose (♦), D-arabinose (▲), D-mannitol (●), and D-sorbitol (■).

Table 1. Apparent association constants (K_a) of **1** with different sugars

Sugar	K_a (M^{-1})
Sorbitol	705
Fructose	514
Mannitol	260
Arabinose	29
Glucose	0.6

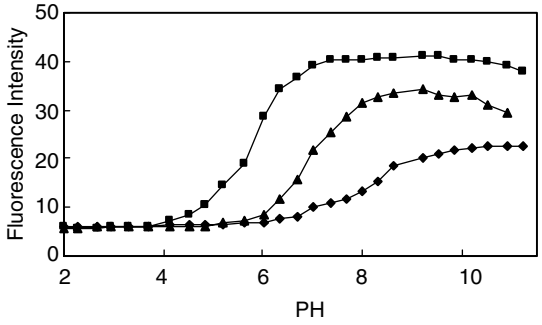


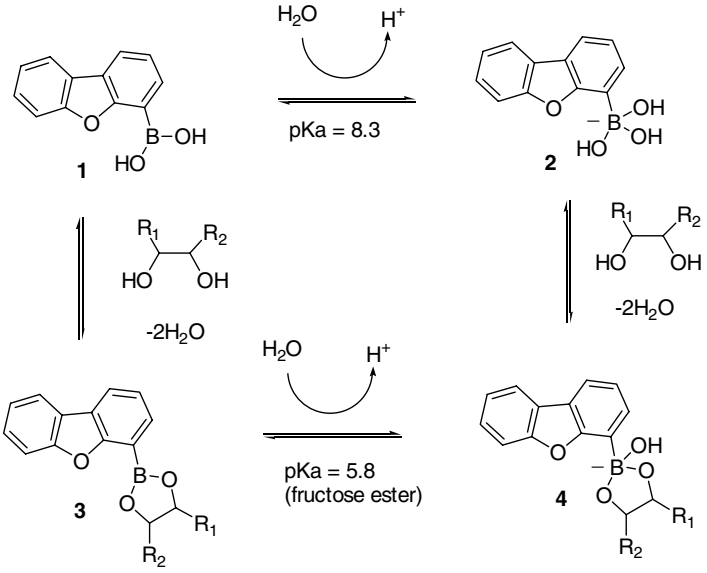
Figure 4. pH titration of the fluorescence intensity of DBFBA (2×10^{-7} M) in the absence and presence of sugars in 0.1 M aqueous phosphate buffer, [sugar] = 50 mM, $\lambda_{ex} = 286$ nm, $\lambda_{em} = 307$ nm. **1** alone (♦), in the presence of D-fructose (■), in the presence of D-glucose (▲).

DBFBA has a binding constant of 514 M^{-1} for fructose and 0.6 M^{-1} for glucose.

To examine the relationship between the fluorescence intensity changes and the boron ionization states, we have also studied the pH profiles of the fluorescence intensity in the absence and presence of fructose and glucose at a fixed concentration (50 mM) (Fig. 4). In the absence of any sugar, the emission intensity of **1** increased by 3.5-fold at 306 nm upon changing the pH from 3 to 12, with an apparent pK_a of 8.3, which was assigned to the boronic acid moiety. This pK_a is somewhat lower than that of PBA (8.8). It is not readily clear as to why compound **1** would have a lower apparent pK_a than PBA. One possible explanation is the presence of an *ortho* substitution, which helps to twist the boronic acid moiety out of the plane defined by the phenyl ring.[49,50] When out of conjugation with the phenyl ring, the boronic acid open shell becomes more available for reaction with a Lewis base, which would explain the increased acidity (and decreased pK_a). In the presence of a sugar, the fluorescence intensity of **1** at 307 nm increased by 7–8-fold upon changing the pH from 3 to 12, with an apparent pK_a of 7.2 and 5.8 for the glucose and fructose esters, respectively, as can be seen in Figure 4.

It is well-known that the binding of a diol to boronic acid often lowers the pK_a of the boron species.[1,3,4] Such results are consistent with the anionic tetrahedral species (**2** and **4**) being more fluorescent at 318 and 307 nm as shown in Scheme 1.

In conclusion, DBFBA was found to be a new type of water-soluble fluorescent reporter compound. It exhibits



Scheme 1. Equilibrium among different species with apparent pK_a for fructose ester indicated.

an unusual fluorescent change at three wavelengths. This compound will help to increase the diversity of structures available for combinatorial synthesis of boronic acid-based fluorescent sensors. The unique structural features of DBFBA could also serve as a lead for the search for other water-soluble boronic acid fluorescent reporters.

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