ORGANIC LETTERS

2003
Vol. 5, No. 24
4615−4618

New Boronic Acid Fluorescent Reporter Compounds. 2. A Naphthalene-Based On–Off Sensor Functional at Physiological pH†

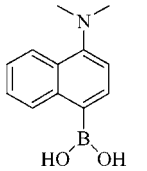
Xingming Gao, Yanling Zhang, and Binghe Wang*

Department of Chemistry, Georgia State University, 33 Gilmer Street S.E., Atlanta, Georgia 30303

wang@gsu.edu

Received September 15, 2003

ABSTRACT



DMANBA
1

A new boronic acid fluorescent on–off reporter compound (1) was synthesized. This fluorescent sensor shows a 41-fold emission intensity increase upon addition of 50 mM fructose in 0.1 M aqueous phosphate buffer at pH 7.4.

During the past decade, a great deal of effort has been directed toward the detection of saccharides by fluorescent chemosensors.[1] Critical to the design of sensors is the availability of fluorescent reporter moieties that respond to the saccharide recognition event with significant fluorescence intensity changes. Our laboratory has been interested in the preparation of fluorescent sensors for cell-surface polysaccharides for in vivo applications.[2]

Boronic acids have been known for decades to bind saccharides via covalent interactions.[3] During the past decade, there has been much important progress made in the construction of boronic acid-based sensors for carbohydrates.[4] Different mechanisms have been used to induce spectroscopic changes upon binding of the boronic acid moiety with a saccharide.[1,4i] Among the most important discoveries is an anthracene-based fluorescent reporter system developed by Shinkai and co-workers. This system has been widely used because of its fairly large fluorescence intensity increase upon ester formation due to the switching of a photoinduced electron transfer (PET) process.[4b,5] Our group has also applied the Shinkai system for the preparation of sensors for mono- and oligosaccharides.[2,6]

In our continuing effort to search for more efficient and water-soluble boronic acid fluorescent reporter compounds,[7]

† Part of this work was conducted at North Carolina State University. For part 1 of this series, see ref 7.

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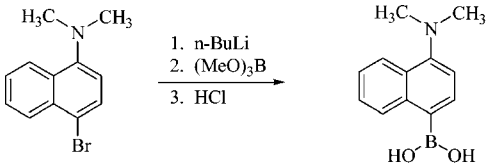
10.1021/ol035783i CCC: $25.00
Published on Web 10/28/2003

we are interested in taking advantage of the manipulation of the internal charge transfer (ICT) process for the construction of boronic acid-based sensors. The ICT process is well-known to be very sensitive to small perturbations that can induce spectroscopic changes and has been used in the past for the synthesis of fluorescent boronic acid reporter compounds with a maximum intensity change of about 5-fold.[8] Usually, an ICT system contains an electron donor group and an electron acceptor group in the same chromophore. Lakowicz[4i,8b,c] and James[8a] have reported a series of boronic acid-based sensors involving the ICT mechanism. In these sensors, the sp^2-hybridized boron atom of the boronic acid group linked directly to the fluorophore can form a conjugated system with the aromatic moiety and act as an electron acceptor group because of the empty p-orbital in the boron atom. Incorporation of a donor group on the same chromophore can result in excited-state charge transfer. However, conversion from an sp^2- to an sp^3-hybridization would leave the boron atom without the empty orbital, and consequently switch off the ICT process. Since it is known that conversion of a phenylboronic acid analogue to its ester with sugars commonly results in the lowering of the pK_a by about 2−3 pH units,[3b,c] boronic ester formation frequently means the conversion of the boron atom from the neutral sp^2 form to the anionic sp^3 at physiological pH. Consequently, addition of a sugar to a boronic acid solution could bring about a change in the ICT states.

In this work, we report a new water-soluble boronic acid-based fluorescent ICT saccharide sensor (DMANBA, **1**) that shows a large fluorescence intensity increase upon binding with a sugar in aqueous solution at physiological pH.

Compound **1** was readily synthesized from the commercially available 1-bromo-4-(dimethylamino)naphthalene through lithiation and reaction with trimethylborate (Scheme 1). Purification of the crude product by silica gel chromatography and recrystalization from dichloromethane−hexanes afforded **1** as a colorless crystal.[9] In this compound (**1**), the amino group can act as a donor and the boron atom in a sp^2 state can act as an acceptor in an ICT process. We reasoned that ester formation would change the hybridization of the boron to sp^3 and switch off the ICT process.

Scheme 1. Synthesis of DMANBA (**1**)



DMANBA (**1**)

The effect of various carbohydrates on the fluorescent properties of compound **1** was determined in phosphate buffer at pH 7.4. The emission spectral change of **1** with fructose at different concentrations in 0.1 M aqueous phosphate buffer (pH 7.4) is shown in Figure 1. A 41-fold

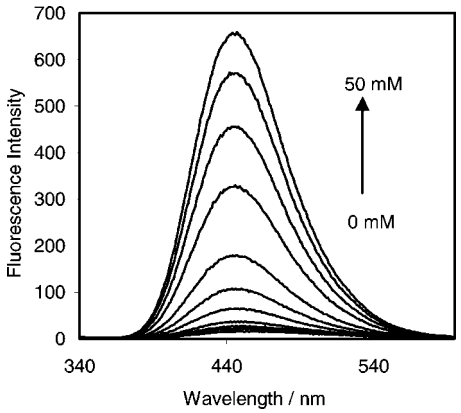


Figure 1. Fluorescence spectral change of **1** (1.0×10^{-5} M) with different concentrations of D-fructose (0−50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4, $\lambda_{ex} = 300$ nm.

emission intensity increase is observed in the presence of 50 mM fructose. To the best of our knowledge, such a large emission intensity increase has never been reported for boronic acid-based saccharide sensors involving the ICT mechanism. The quantum yields of **1** in the absence ($\phi_F = 0.010$) and in the presence of 50 mM fructose ($\phi_F = 0.42$) in 0.1 M phosphate buffer (pH 7.4) were obtained with use of 8-quinoline boronic acid ($\phi_F = 0.58$ in 12 M H_2SO_4) as a reference compound.[10] Such results indicate that indeed addition of a carbohydrate results in a very significant change in the spectroscopic properties of the sensor compound (**1**).

To examine the general applicability of this fluorescent reporter compound, we have also studied the effect of four other carbohydrates on its fluorescence intensity (Figure 2). These carbohydrates include sorbitol, tagatose, galactose, and glucose. From Figure 2, it is clear that all five carbohydrates tested caused significant fluorescence intensity increases at physiological pH with varying magnitude. Addition of sorbitol and fructose induced the largest fluorescence intensity changes, more than 40-fold, at concentrations above 50 mM. Glucose, on the other hand, induced a maximum of

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(9) Selected characterization data for 4-(dimethylamino)naphthalene boronic acid (**1**): colorless crystal, yield 42%; HRESI-MS calcd for $C_{12}H_{15}BNO_2$ 216.1196 $(M + H)^+$, found 216.1187; ^{1}H NMR (400 MHz, CD_3OD) δ 2.87 (s, 6H), 7.10 (d, $J = 7.2$ Hz, 1H), 7.45 (m, 3H), 7.76 (m, 1H), 8.21 (m, 1H); ^{13}C NMR (400 MHz, CD_3OD) δ 113.23, 124.39, 124.68, 125.71, 128.45, 128.52, 130.48. Anal. Calcd for $C_{12}H_{14}BNO_2 \cdot {}^3/_4H_2O$: C, 71.51, H, 6.25, N, 6.95. Found: C, 71.80, H, 6.42, N, 6.65.

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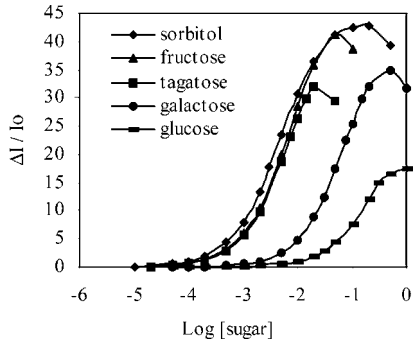

Figure 2. Fluorescence intensity changes ($\Delta I/I_0$) of **1** (1.0×10^{-5} M in 0.1 M aqueous phosphate buffer at pH 7.4) as a function of sugar concentration at 25 °C; $\lambda_{ex} = 300$ nm, $\lambda_{em} = 445$ nm.[11]

17-fold fluorescence intensity increase at a much higher concentration (1 M).

To examine the binding in a more quantitative fashion, the association constants (K_a) between **1** and the five carbohydrates were determined assuming the formation of a 1:1 complex.[4e,12] As expected, the affinity trend with **1** followed that of simple phenylboronic acid in the order of sorbitol > fructose > tagatose > galactose > glucose (Table 1). The absolute numbers are also similar to what was

Table 1. Association Constants (K_a) and Fluorescence Intensity Changes ($\Delta I/I_0$) of **1** with Different Sugars

sugar	K_a (M^{-1})	$\Delta I/I_0$ (sugar concn, M)
sorbitol	226 ± 5	42 (0.10)
fructose	207 ± 4	41 (0.05)
tagatose	116 ± 2	32 (0.02)
galactose	12.0 ± 0.2	35 (0.50)
glucose	4.0 ± 0.1	17 (1.0)

observed with phenylboronic acid.[3c] For example, phenylboronic acid (PBA) has a K_a of 162 M^{-1} for fructose and 5 M^{-1} for glucose.[3c]

To examine the relationship between the fluorescence intensity changes and the boron ionization states, we have also studied the pH profile of the fluorescence intensity in the absence and presence of fructose and glucose at a fixed concentration (50 mM) (Figure 3). The emission intensity

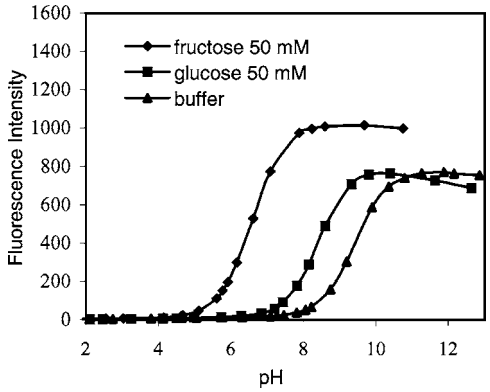

Figure 3. pH titration of the fluorescence intensity of **1** (1×10^{-5} M) in the absence and presence of sugars in 0.1 M aqueous phosphate buffer, $\lambda_{ex} = 300$ nm, $\lambda_{em} = 445$ nm.

of **1** in the absence of any carbohydrate increased by 168-fold at 445 nm upon changing the pH from 3 to 12. It is worth mentioning that the pK_a of the protonated dimethyl amino group of **1** is about 4.6 and the emission wavelength of **1** with the protonated dimethyl amino group at low pH is about 338 nm. Therefore, the fluorescence intensity of **1** at 445 nm is not affected by the protonated dimethylamino group. The fluorescence intensity changes correspond to the boronic acid pK_a (9.4) and therefore hybridization changes, which is responsible for the fluorescence intensity changes observed.

The pH titration curves of **1** in the presence of fructose and glucose showed over 140-fold increases in fluorescence intensity when pH increased from 3 to 10 (Figure 3). It is well-known that the binding of a diol to boronic acid most of the time lowers the pK_a of the boron species.[3b,c] In our case, an apparent pK_a of 6.4 was observed in the presence of 50 mM of fructose. As described earlier, the boronic acid moiety of **1** has a pK_a of about 9.4. Therefore, at physiological pH, it exists in the neutral, non-ionized form. However, upon addition of fructose, the apparent pK_a of the solution drops to about 6.4, which ensures that most of the boron species are in the anionic tetrahedral state at physiological pH. It should be noted that the pK_a here is the apparent pK_a because of the tridentate binding mode of fructose with a boronic acid.[13]

The apparent pK_a of the mixture of **1** and glucose (50 mM) was much higher (8.3) (Figure 3). However, it should be noted that in this mixture only a small portion of the boronic acid is expected to be in the ester state because of the low association constant (4 M^{-1}), which gives about 20% complexation at pH 7.4. Therefore, this pK_a is a reflection of a mixture of mostly the free boronic acid and a small portion of the ester.

To confirm the correlation of the fluorescence intensity increase of **1** with the ionization state change of the boronic acid, we recorded the ^{11}B NMR spectra of **1** in the absence and presence of fructose. The ^{11}B chemical shift of **1** changed from 32.5 ppm in the absence of fructose, which is

(11) **Procedures for the Binding Studies:** The sensor (**1**) was dissolved in 0.1 M phosphate buffer (pH 7.40) (2×10^{-5} M) and the sugar was dissolved in 0.1 M phosphate buffer (pH 7.40) at various concentrations. For fluorescent binding studies, 2 mL of the sensor solution was mixed with 2 mL of the sugar solution. After being stirred for 20 min, the mixture was transferred into a cuvette, and the fluorescence intensity was recorded immediately.

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characteristic of the neutral trigonal form of boronic acid,[13b] to 11.1 ppm in the presence of fructose (20 equiv), which is characteristic of the anionic tetrahedron form of boronic acid (Figure 4). Such ^{11}B NMR spectra results are consistent with the pH−fluorescence intensity titration results.

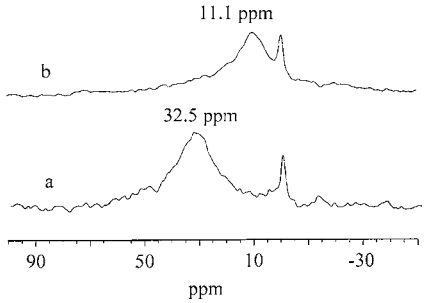

Figure 4. ^{11}B NMR in DMSO/0.1 M aqueous phosphate buffer (1:3, pH 7.4): (a) **1** (9 mM) alone; (b) the mixture of **1** (9 mM) and fructose (20 equiv, 0.18 M). BF_3 was used as an external reference.

In conclusion, a new water-soluble fluorescent saccharide sensor that shows large fluorescence intensity increases upon binding with a diol was conveniently synthesized from commercially available starting material through a one-step conversion. We assume that an ICT process is responsible for the low fluorescence intensity before addition of a sugar and the removal of an ICT process upon addition of a sugar is the reason for the increased fluorescence of compound **1**. This is essentially an on−off system. Work is underway to use this fluorescent reporter compound for the construction of di- and multiboronic acid sensors for the high-specificity identification and detection of biologically important carbohydrates.

Acknowledgment. Financial support from National Institutes of Health (CA88343 and NO1-CO-27184) and the Georgia Cancer Coalition through a GCC Distinguished Cancer Scientist Award is gratefully acknowledged. We would also like to thank Dr. Sabapathy Sankar for his help with the ^{11}B NMR work. We also acknowledge Frontier Scientific Corporation for providing certain boronic acid samples in some of our related studies.

Supporting Information Available: Synthetic procedures and ^{1}H and ^{13}C NMR of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035783I

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