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The Design of Boronic Acid Spectroscopic Reporter Compounds by Taking Advantage of the pK_a-Lowering Effect of Diol Binding: Nitrophenol-Based Color Reporters for Diols

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Received July 17, 2003

The complex that forms between a boronic acid and a diol is often much more acidic than the starting boronic acid. In conditions where the solution pH is between the two pK_a values, the boron atom will convert from a neutral trigonal form to an anionic tetrahedral form upon complexation. Such a change is likely to dramatically alter the electron density of neighboring groups. Utilizing this effect, we have designed and synthesized two nitrophenol-based boronic acid reporter compounds that change ionization states and therefore spectroscopic properties upon diol binding. Both compounds show significant UV changes upon addition of saccharides. For example, a blue shift of the absorption max from 373 to 332 nm was observed with the addition of D-fructose to 2-hydroxy-5-nitrophenylboronic acid at neutral pH. Such a reporter compound can be used as a recognition and signaling unit for the construction of polyboronic acid sensors for the selective and specific recognitions of saccharides of biological significance.

Introduction

Due to the unique strong interactions between boronic acid and diols through reversible ester formation, there has been a great deal of interest in using boronic acid as the recognition moiety for the development of fluorescent and color sensors,[1-16] carbohydrate transporters,[17-24] and chromatographic stationary materials.[25-30] Recently, our group for the first time showed that boronic acid-based fluorescent sensors for cell surface carbohydrate sialyl Lewis X (sLex) can be used for the fluorescent labeling of HEPG2 cells that are engineered to overexpress sLex.[15] Parallel with our sensor development effort, we have also examined systematically the binding between boronic acid with various diols.[31,32] Such studies allowed us to

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10.1021/jo0350357 CCC: $27.50 © 2004 American Chemical Society
Published on Web 02/13/2004

correct many literature mistakes[33] including the strength of the binding between phenylboronic acid and various sugars.

Critical to the sensor development effort is the availability of reporter compounds that can generate a detectable signal upon binding with the target molecules. Along this line, Czarnik,[16] Shinkai,[1,34,35] Lakowicz,[36–39] James,[40–43] and Heagy[44,45] have developed various fluorescent boronic acid compounds that show fluorescent intensity/wavelength changes upon binding with diols to varying degrees. The most prominent one is the Shinkai photoelectron transfer (PET) system using B–N bond strength to modulate the fluorescence quenching process and therefore the fluorescent intensity changes.[9,46,47] However, many aspects of these available reporters need to be improved. This includes water solubility, the magnitude of fluorescence intensity changes, and photostabilities and chemical stabilities of these compounds. Recently, our group has developed a novel type of fluorescent probe for carbohydrates, 8-quinolineboronic acid. This boronic acid responds to the binding of a carbohydrate with over 40-fold increases in fluorescence intensity and shows optimal fluorescence change at physiological pH in aqueous solution.[48]

There has also been a great deal of interest in developing colorimetric sensors for sugars with use of boronic acid compounds. Along this line, Strongin,[49] James,[50] Lakowicz,[51] and Shinkai[52] have reported several compounds that upon addition of sugar change colors. Most of these designs are based on the modulation of the electronic properties of the chromophores through B–N bond formation or inductive effects. The mechanism of action for the Strongin system, however, is not clear. The Anslyn group has reported several systems that use a reporter compound to form an ensemble for sugar and other diol detection.[11,12] Herein we report the design, synthesis, and evaluation of nitrophenol-based boronic acid reporter compounds that show significant spectroscopic changes upon addition of sugars at neutral pH in aqueous solution. Our design attempts to take advantage of the ability of a boronic acid functional group to modulate the pK_a and/or the electron density of a neighboring group upon addition of a diol, through both proximity effect as well as inductive effect. Such a spectroscopic reporter compound can be used for the construction of diboronic acid sensors for the specific recognition of various carbohydrates.

Results and Discussion

1. Design and Synthesis. It has long been recognized that the pK_a of a boronic ester is, most of the time, lower than that of the corresponding boronic acid.[32,33] For example, the pK_a of phenylboronic acid is about 8.8 and the pK_a values of its glucose and fructose esters are 6.8 and 4.5, respectively.[32] Such significant changes in pK_a upon ester formation have one major implication that can be taken advantage of in designing boronic acid-based fluorescent and color sensors. This is particularly important for sensors that have a desirable functional pH close to neutral. We have recently examined a series of about 30 phenylboronic acid compounds with varying substituents and found that the highest pK_a was about 9.0 when the substitute was a methoxy group. If binding to a sugar lowers its pK_a by 2–4 units, this would mean that at pH 7.4 the boronic acid would exist in the neutral form and addition of a sugar would change it to the anionic tetrahedral form.

This change in charge states can be used to modulate the electron density of the neighboring group through either proximity or inductive effect. There is also the possibility that the protonation state of a neighboring group that is either an acid or base can be affected by the ionization state change of the boronic acid. Both of these factors are known to affect the spectroscopic properties of a chromophore. *p*-Nitrophenol (PNP) is known to change its ionization states depending on the pH and such a change causes tremendous shifts in its spectroscopic properties.[53] Therefore, nitrophenol-based compounds have been widely used for the development of substrates for enzyme assays among other things.[54] We envisioned that if a boronic acid is positioned next to the phenol group of *p*-nitrophenol or its analogues, the ionization state changes of the boronic acid moiety upon binding with a diol would likely affect the pK_a and/or the electron density of the phenol group through either proximity or inductive effect. If this change straddles the neutral pH range, we should be able to develop a spectroscopic reporter compound functional at physiological pH. For this purpose, we designed (Scheme 1) 2-hydroxy-5-nitrophenylboronic acid (**1**) and, for comparison, 4,6-dinitrophenol-2-boronic acid (**2**).

The synthesis of compound **1**, 2-hydroxy-5-nitrophenylboronic acid, started with commercially available 2-bromo-4-nitroanisole. Borylation of bromide **3** with Pd(dppf)-Cl_2 as the catalyst in the presence of potassium acetate

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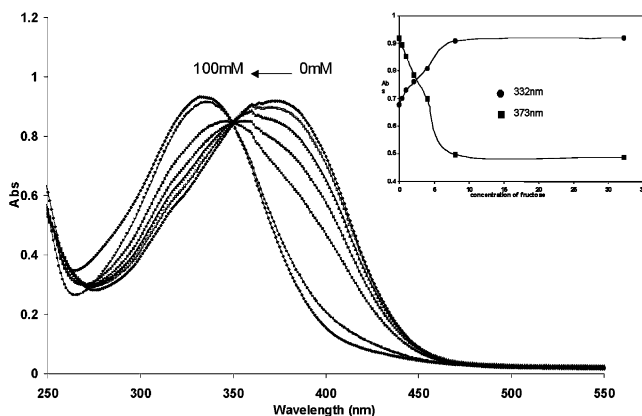
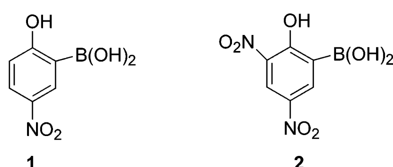


FIGURE 1. Absorption of compound **1** at 2×10^{-4} M in pH 7.4, 0.1 M phosphate buffer with increasing concentration of fructose (0, 0.4, 1.0, 2.1, 4.0, 16.0, 100 mM). Inset: Absorption increase ($\lambda = 332$ nm) and absorption decrease ($\lambda = 373$ nm) of **1** in the presence of fructose.

SCHEME 1. Designed Nitrophenol-Based Boronic Acids 1 and 2



furnished pinacolboronate **4** in 20% yield.[55] The oxidative deprotection of pinacolboronate ester **4** by sodium periodate[56] yielded 70% of methoxy phenylboronic acid **5**. Then, treatment of this substituted phenylboronic **5** with 1 M boron tribromide in methylene chloride deprotected the methoxyl group to give hydroxyl phenyl boronic acid **1** in 90% yield (Scheme 2).

The synthesis of 4,6-dinitrophenol-2-boronic acid (**2**) started with commercially available 2-methoxyphenylboronic acid (**6**). Nitration of this boronic acid with a mixture of nitric acid and sulfuric acid at −10 °C gave the desired dinitro product **7** in 50% yield with 10% of a side product, 1-methoxy-2,4-dinitrobenzene. There are literature precedents showing that nitration reactions can cause the cleavage of the C−B bond.[57] Treatment of boronic acid **7** with 1 M boron tribromide in methylene chloride yielded compound **2**, 4,6-dinitrophenol-2-boronic acid, in 90% yield (Scheme 3). Boronic acid compounds sometimes exhibit poor NMR spectra due to the possible formation of dimers or polymers. Therefore, for the full characterization of these compounds, boronic acids **7** and **2** were protected by reacting them with 2,2-dimethylpropane-1, 3-diol to give boronate **8** and **9**.

2. Binding Studies with Diol-Containing Compounds. To see whether our design works or not, the effect of fructose on the absorption of **1** was examined (Figure 1). Fructose was used because it was known to bind with monoboronic acid very well.[32] It should be noted that this does not mean that we are trying to develop a fructose-specific sensor. Monoboronic acid compounds are not expected to have intrinsic preference that is different from that of phenylboronic acid.[32] Such a reporter compound can be used for the preparation of specific sensors through the construction of di- or multiboronic acid compounds. The binding studies were carried out at a concentration of 2×10^{-4} M (4% methanol in pH 7.4, 0.1 M phosphate buffer).

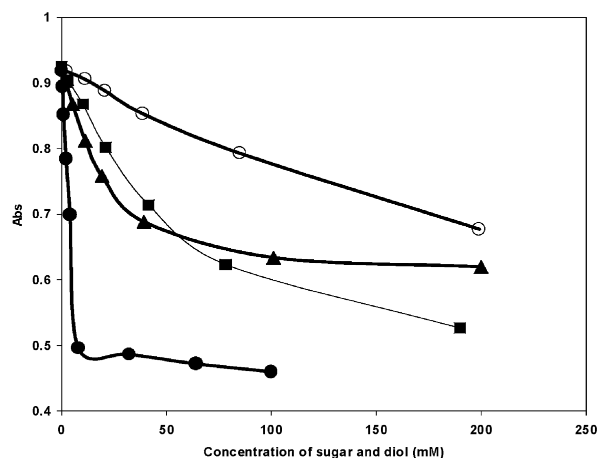


FIGURE 2. Absorption intensity changes of **1** (2×10^{-4} M) at $\lambda = 373$ nm with sugar addition: ○, glucose ($K_a = 8.0$ M^{-1}); ■, galactose ($K_a = 44.0$ M^{-1}); ▲, 1,2-*cis*-cyclopentanediol ($K_a = 34.7$ M^{-1}); ●, fructose ($K_a = 245$ M^{-1}). All studies were in 4% methanol in 0.1 M pH 7.4 phosphate buffer (v/v).

As can be seen from Figure 1, the spectroscopic properties of 2-hydroxy-5-nitrophenylboronic acid changed tremendously at pH 7.4 with the addition of fructose. Millimolar concentrations of fructose were sufficient to cause a significant blue shift of the absorption λ_{max} (from 373 to 332 nm). With increasing concentrations of fructose, the UV intensity decreased at 373 nm and increased at 332 nm. This essentially forms a ratiometric system with large λ_{max} shifts. The response seems to reach a plateau at 16 mM. An isosbestic point is observed at 350 nm showing the equilibrium of two species.

To further examine the detailed effect of sugars on the spectroscopic properties of 2-hydroxy-5-nitrophenylboronic acid (**1**), we studied the concentration effect of fructose, glucose, galactose, and 1,2-*cis*-cyclopentanediol on this compound (Figure 2).

Spectroscopic changes were observed with the addition of all three sugars and *cis*-1,2-cyclopentanediol. As expected, compound **1** showed the same type of intrinsic preference for fructose over others as phenylboornic acid.[32] The binding constants between this boronic acid and fructose, glucose, galactose, and 1,2-*cis*-cyclopentanediol were determined as 245, 8.0, 44.0, and 34.7 M^{-1}, respectively.

3. Mechanistic Studies. Since compound **1** works very well for signaling sugar binding, it is very important to examine the mechanism. Since the design was based on the idea that the charge state changes of the boronic acid group upon ester formation may affect the charge state or density of a neighboring group, it is important to examine the possible ionization species existing in the reaction solution. Compound **1** may exist in different ionization states (Scheme 4) at neutral pH because of the presence of two ionizable functional groups, the phenol

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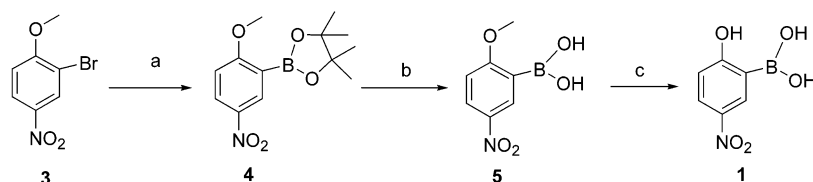
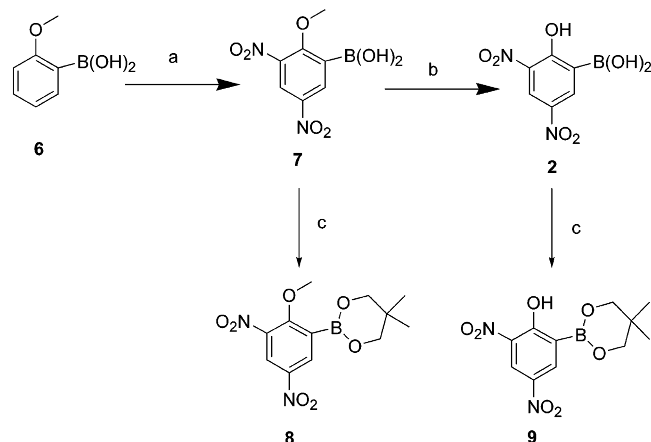
SCHEME 2. Synthesis of 2-Hydroxy-5-nitrophenylboronic Acid (1)[a]



[a] Reagents and conditions: (a) bis(pinacolato)diboron, Pd(dppf)Cl_2, KOAc, anhydrous DMSO, 20%; (b) $NaIO_4$, 2 N HCl, THF/H_2O, 70%; (c) 1 M BBr_3/DCM, 90%.

SCHEME 3. Synthesis of 4,6-Dinitrophenol-2-boronic Acid (2)[a]



[a] Reagents and conditions: (a) H_2SO_4/HNO_3, −10 °C, 50%; (b) 1 M BBr_3/DCM, 90%; (c) 2,2-dimethylpropane-1,3-diol, toluene, 115 °C, refluxing, 100%.

hydroxyl group and the boronic acid group. In evaluating the likelihood for the formation of different ionization species, one needs to analyze the pK_a of each functional group present (Scheme 4).

At physiological pH, if the hydroxyl group (of **1**, R = H) has a lower pK_a than the boronic acid, it would favor species **10a** and if the boronic acid has a lower pK_a it would favor the formation of **10c**. If these two groups have comparable pK_a values, both species may exist simultaneously in different ratios depending on the conditions. *p*-Nitrophenol has a pK_a of about 7.2. Since boronic acid is considered an electron-withdrawing functional group, it is most likely that the pK_a of the hydroxyl group of compound **1** is lower than 7.2. 2-Methoxy-5-nitrophenylboronic acid has a pK_a of about 7.1 (see below). This should be comparable to the boronic acid pK_a of **1**. Therefore, it is possible that the pK_a values of these two functional groups are so close that both **10a** and **10c** exist at the same time at physiological pH. The formation of the di-ionized species **10b** is unlikely at neutral pH. Upon ester formation, there are four possible species, **11a**−**d**. Since binding with a diol most of the time lowers the pK_a of the boron by 2−4 pK_a units,[32] we expect that species **11c** be predominant upon ester formation, which would lower the ratio of the hydroxyl ionized species **11a**. This shift in the concentration of the species with the hydroxyl group ionized should result in a change in the spectroscopic properties of the solution.

To understand the ionization state changes upon ester formation, it is also important to examine the spectroscopic properties of the different species existing in

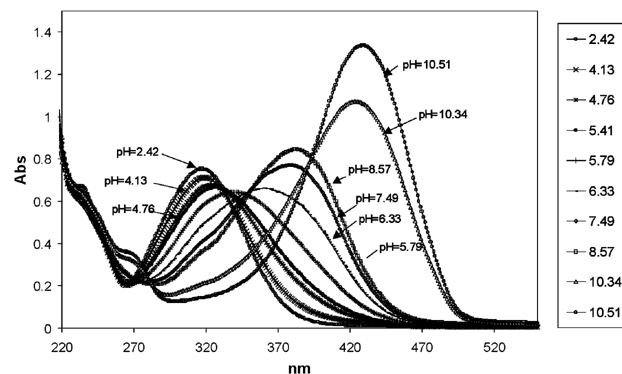


FIGURE 3. pH profile of compound **1** (initial concentration of **1** was set at 2×10^{-4} M in 4% methanol/96% water, and the ionic strength was initially fixed at 0.1 M NaCl).

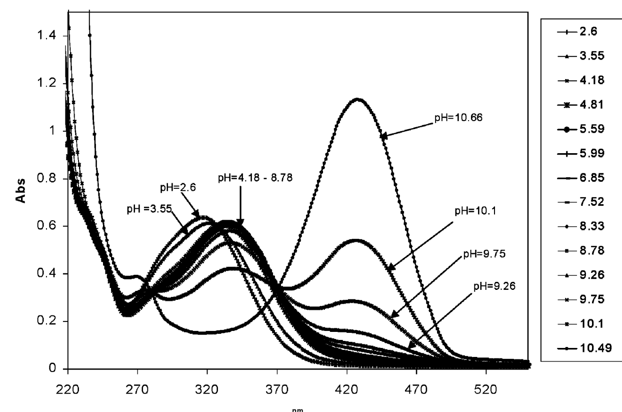
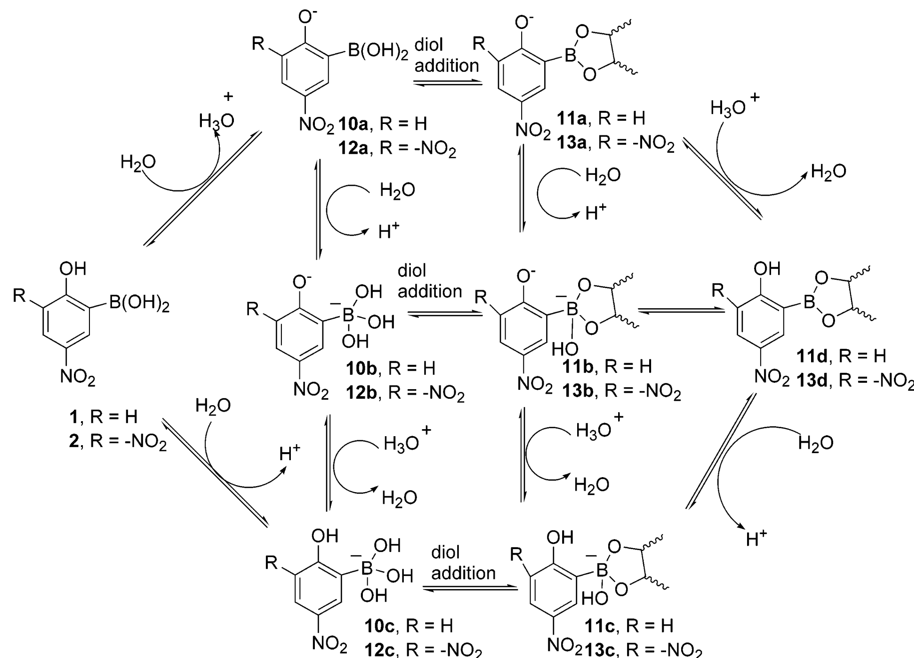


FIGURE 4. pH profile of compound **1** with 200 mM D-fructose (initial concentration of **1** was set at 2×10^{-4} M in 4% methanol/96% water, and the ionic strength was initially fixed at 0.1 M NaCl).

solution. Figure 3 shows the pH titration profile of **1** in the absence of any diols. One can readily see that there are three absorption maxima at 315, 380, and 427 nm. These three λ_{max} values represent three different ionization stages. At low pH (pH <4), the absorption λ_{max} is at 315 nm corresponding to the nonionized form of **1** (Scheme 4). The absorption peak at 380 nm represents the first ionization (first pK_a), which can be either the deprotonation of the hydroxyl group (**10a**) or the ionization of the boron (**10c**) or a combination of these two since their pK_a values are very close. The third peak at 427 nm represents the fully ionized form with two anions on the same molecule (**10b**).

Figure 4 shows the pH titration profile of **1** in the presence of D-fructose (200 mM). In this case, there also seems to be three ionization stages corresponding to λ_{max}

SCHEME 4. Equilibrium among Different Species of 1 and 2 in the Presence and Absence of D-Fructose



values of 315, 325, and 427 nm. The first λ_{max} is the same as that of **1** in the absence of any diol. This is understandable since at low pH little binding is expected[32] and the species present should be the free sensor **1** with no ionization. The last λ_{max} (427 nm) is also similar to that of **1** in the absence of any diol. It is known that the binding affinity of boronic acid with diols increases with increasing pH and at high pH one would expect that essentially all the boronic acid is converted to its ester. This would indicate that at pH 10.5, the species is also the dianion species (**11b**), which has the same spectroscopic properties as that of **10b**. This further indicates that forming an ester alone does not change the electronic properties of the complex enough to affect their spectroscopic properties. The major difference between the system with a diol (fructose) and without a diol (Scheme 4) is in the neutral pH region, which is ideal (and by design) for the sensing of diol compounds under near-physiological conditions. To understand the mechanism through which this spectroscopic property changes, one needs to analyze the predominant spectroscopic species in both situations (with and without fructose).

In the presence of fructose, there are two possible species at neutral pH, **11a** and **11c**. To see which the dominant species is, we also examined the spectroscopic changes of the methylated version of **1**, compound **5**, in the presence (Figure 6) and absence (Figure 5) of fructose. As expected, the pH titration curve of **5** shows two species (Figure 5), one with a λ_{max} of 315 nm and the other at 337 nm. It is easy to understand that the peak at 315 nm corresponds to the un-ionized form and the peak at 337 nm corresponds to the anionic tetrahedral boron species. It needs to be noted that the first λ_{max} is about the same as that of the un-ionized **1**, which indicates that methylation did not change the electronic properties of the molecules sufficiently to affect its UV properties. This would also suggest that **11c** should have about the same λ_{max} as the ionized form of **5**. If we compare the UV spectrum of **1** in the presence of fructose at around neutral pH, it is easy to see that the λ_{max} is about the same as that of the ionized (**5**). This would suggest that upon addition of fructose to **1**, the lowered pK_a of the boron in the ester makes **11c** the predominant species. Such a conclusion is also consistent with the pK_a-lowering effect of fructose on boronic acid.

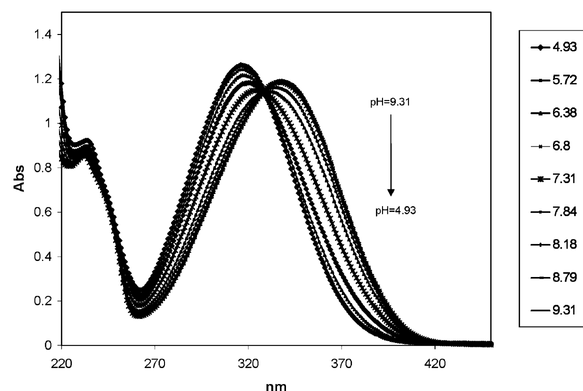


FIGURE 5. pH profile of compound **5** (initial concentration of **5** is set at 2×10^{-4} M in 4% methanol/96% water, and the ionic strength was initially fixed at 0.1 M NaCl).

Now we can go back to analyze the situation of **1** in the absence of fructose. A detailed examination of the spectral set with **1** in the absence of any sugar reveals a "shifting" λ_{max} for the middle peak (380 nm, Figure 3). The lack of an isosbestic point transitioning from the first to the second "ionization state" indicates the formation of more than one "intermediate". This could only mean that both **10a** and **10c** were formed in the pH range of 5.79 and 8.57. The shifting of the λ_{max} from 339 to 380 nm in this region indicates the formation of a species with a shorter λ_{max} wavelength at low pH and a species with a longer λ_{max} wavelength at high pH. Intuitively, it is reasonable to assign **10a** as the species with a longer

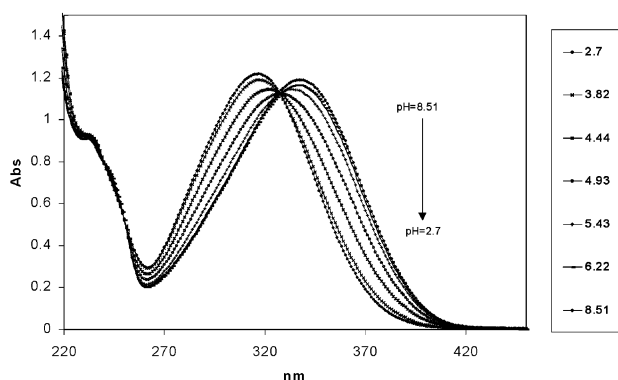
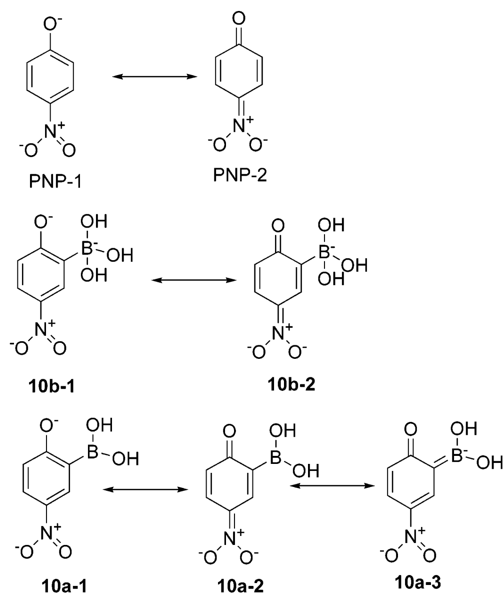


FIGURE 6. pH profile of compound **5** in the presence of 200 mM D-fructose (initial concentration of **5** was set at 2×10^{-4} M in 4% methanol/96% water, and the ionic strength was initially fixed at 0.1 M NaCl).

SCHEME 5. Resonance Structures of Deprotonated *p*-Nitrophenol and Some Species of Compound 1



wavelength, and **10c** as the one with a shorter wavelength. Such an assumption is also consistent with experimental data. For example, **11c** and the ionized form of **5** all have λ_{max} values around 325 nm, and they are structurally analogous to **10c**. Therefore, it is logical to assign the species with an approximate λ_{max} of 339 nm (please note that at this point, it is already a mixture of more than one species, therefore it may not reflect the λ_{max} of a single species), not 380 nm. Furthermore, the ionization of the hydroxyl group in a *p*-nitrophenol type of structure is known to significantly increase its λ_{max} value. For example, the ionized form *p*-nitrophenol itself has a λ_{max} of about 425 nm, which is similar to that of **10b** and **11b**.

One might ask if the deprotonated form of *p*-nitrophenol (PNP) and **10b** and **11b** all have similar λ_{max} values, why should the λ_{max} of **10a** be very different if it also has the hydroxyl group ionized. This can be explained by examining the resonance structures of these different species (Scheme 5). For PNP, there are two resonance structures, PNP-1 and PNP-2, with PNP-2 being the species contributing most to the long-wavelength UV absorption properties. For **10b**, the situation is similar; there are only two analogous resonance forms. Therefore, it is reasonable to expect that PNP and **10b** have similar λ_{max} values. Since **11b** should behave similarly to **10b**, its resonant forms are not shown. For **10a**, the situation is very different. Because the boron open shell is not occupied, it is resonantly electron-withdrawing. One can write a third resonance structure, **10a-3**. Therefore, the UV absorption of **10a** can be expected to be quite different from that of PNP, **10b**, and **11b**.

Overall, the pH titration (Figure 3) of **1** in the absence of any sugar can be interpreted as follows. At low pH, there is no ionization, and free **1** is the only species (Scheme 4). With increasing pH, the first ionization occurs to generate **10c**, which has a λ_{max} of around 330 nm. With pH increasing, **10a** starts to appear. Since **10a** has a longer λ_{max}, the UV spectra shift to longer wavelength. Because the transition from free **1** to the species with one ionized functional group involves two "products", no isosbestic point was observed.

It is important to note that at pH 7.4, the λ_{max} for the solution of **1** is at about 373 nm in the absence of any sugar and 325 nm in the presence of fructose, which forms the basis for the sugar sensing at physiological pH in aqueous solution. The mechanism responsible for this sensing is that the addition of fructose shifted the UV absorption of the solution from that of **10a** to that of **11c**. This was the direct result of the pK_a-lowering effect of fructose addition. Because the ratio of **11a** over **11c** (and **10a** over **10c)** is directly related to the relative pK_a of these two species and these two species are mutually exclusive, the lowered boron pK_a of the ester relative to the hydroxyl group would result in a shift in the acid−base equilibrium by lowering the percentage of the hydroxyl-ionized species (**11a**). Consequently, addition of fructose to the solution of **1** results in a shift of the UV absorption of the solution from that of **10a** to that of **11c**.

As discussed earlier, ^{11}B NMR is known to indicate the electronic and ionization state of the boron atom. For example, the ^{11}B NMR chemical shift for the neutral and trigonal form (such as **10a**) is about 30 ppm and that for the anion tetrahedral form (such as **10b**) it is about10 ppm.[58] At pH 7.4 (phosphate buffer), the ^{11}B NMR of compound **1** in the presence of excessive *cis*-1,2-cyclopentanediol was a fairly sharp peak at about 7.7 ppm (see the Supporting Information), indicative of anionic species consistent with either **11b** or **11c**. However, it is unlikely that at neutral pH, it can be doubly ionized. Therefore, the NMR results are consistent with the formation of **11c** upon addition of a diol to the solution of **1** at neutral pH. Cyclopentanediol was used in the NMR experiments to reduce the complexity of the NMR spectrum because fructose can bind to a boronic acid in several forms, which would complicate the interpretation. When the ^{11}B NMR of **1** in the absence of any diol was determined at pH 7.4, a broad peak center around 6.3 ppm was observed (see the Supporting Information), an

(58) Nöth, H.; Wrackmeyer, B. Nuclear Magnetic Properties of Boron. In *Nuclear Magnetic Resonance Spectroscopy of Boron Compounds*; Diehl, P., Fluck, E., Kosfeld, R., Eds.; NMR Basic Principles and Progress Series 14; Springer-Verlag: Berlin, Germany, 1978; pp 5−14 and 287.

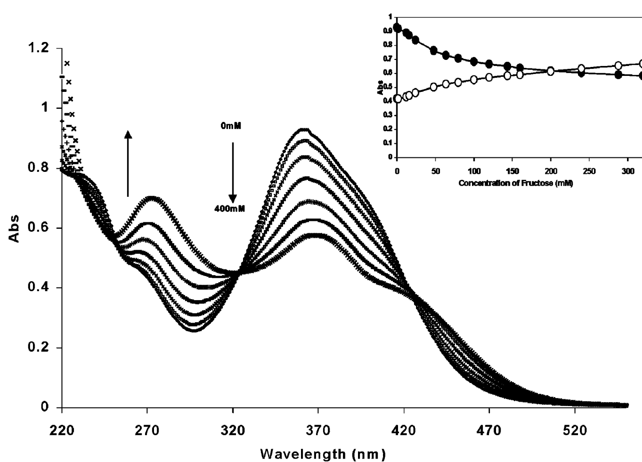


FIGURE 7. Absorption spectra of **2** (5×10^{-6} M) (4% methanol in water (v/v), 0.1 M phosphate buffer, pH 7.4) in the presence of fructose (0, 12, 24, 48, 100, 200, 400 mM). Inset: Absorption change of **2** at 361 (●) and 272 nm (○) upon addition of fructose.

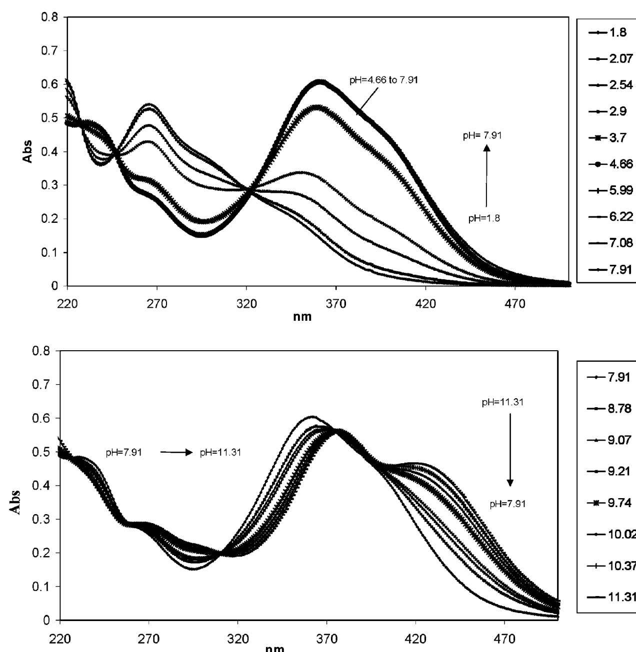


FIGURE 8. pH profile of **2** (initial concentration: 1×10^{-5} M in 4% methanol/96% water, and the ionic strength was initially fixed at 0.1 M NaCl).

indication that the boron is NOT in the trigonal neutral state. There are two things that need to be noted. First, the broad peak is an indication that there is most likely more than one species in the solution, which is consistent with the earlier proposal that it was a mixture of **10a** and **10c**. Second, the chemical shift is consistent with the boron being more shielded than in the trigonal neutral form. However, much more work will be needed to understand exactly what the composition is for all the different species.

For comparison, we also studied the binding of 4,6-dinitrophenol-2-boronic acid (compound **2**) with fructose, glucose, and galactose. The studies were carried out at a concentration of 5×10^{-6} M for **2** (4% methanol/96% 0.1 M phosphate buffer, pH = 7.4). Spectroscopic changes were observed with the addition of all three sugars, with binding constants of 13.5, 1.2, and 0.7 M^{-1} for fructose, glucose, and galactose, respectively. It is very interesting to see that the binding constants for **2** with various sugars are smaller than that of **1**. This contradicts the conventional notion that boronic acids with lower pK_a values bind more tightly to diols. Our group has also generated other data that indicate that boronic acid pK_a values are not directly proportional to the binding constants.[59]

As an example, the spectroscopic changes of **2** with the addition of fructose are shown in Figure 7. It can be seen that such spectroscopic changes at physiological pH are also significant with a pattern similar to that of **1**. With increasing fructose concentrations, the UV intensity decreased at 361 nm and increased at 272 nm. The presence of fructose also results in the appearance of a new peak at 369 nm. Two isosbestic points were observed at 323 and 423 nm, indicative of two equilibria of three species (Scheme 4).

To help understand the mechanism of the spectroscopic change of the binding of compound **2** with sugar, the apparent pK_a value of compound **2** was determined. This was achieved by observing the spectroscopic changes from pH 1.8 to pH 13. From Figure 8, it can be seen that in the absence of any sugar the first pK_a value (the ionization of only one functional group that can be either the boronic acid or the hydroxyl group) of compound **2** is at about 3.3 and the second pK_a value (the ionization of two functional groups) is at about 9.2. With the addition of fructose (200 mM) (Figure 9), the first pK_a value remained essentially the same and the second one was lowered to about 7.2.

One can assume a similar mechanism in operation for **2** as for **1** (Scheme 4). In the absence of any sugar, the peak at 369 nm is attributed to species **12a.** However, upon addition of a sugar and the lowering of the boronic pK_a value,[32] the concentration of **12a** decreased with increasing concentration of **13c**, which contributed to the decrease in absorption at 369 nm and increase in absorption at 323 nm. The major difference between **2** and **1** is that the second pK_a value with the addition of fructose is much lower (7.2). This means that at physiological pH, addition of fructose can also result in the formation of the di-ionized species **13b**, which is responsible for the peak at 425 nm (Scheme 4).

Conclusions

We have designed and synthesized two nitrophenol-boronic acids (**1** and **2**). Binding of sugars to these compounds resulted in very significant spectroscopic changes in both intensity and wavelength. The mechanism for the changes is through the manipulation of the pK_a values of the boronic acid, which affect the equilibrium among different chromophoric species. Such compounds can be used as colorimetric reporters functioning as both a recognition and a signaling unit for the construction of polyboronic acid spectroscopic sensors for

(59) Yan, J.; Springteen, G.; Wang, B. Unpublished results.

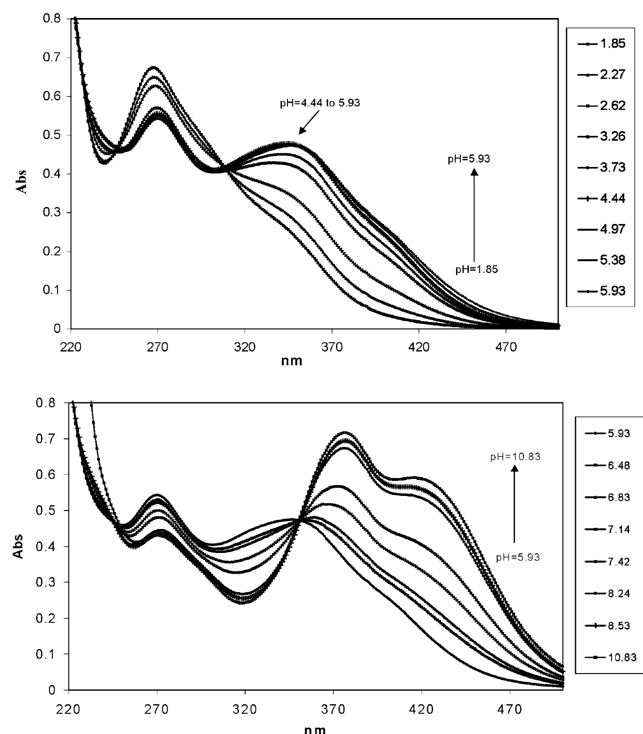


FIGURE 9. pH profile of **2** with 200 mM D-fructose (initial concentration of **2** was 1×10^{-5} M in 4% methanol/96% water, and the ionic strength was initially fixed at 0.1 M).

monocarbohydrates and complex carbohydrates, many of which are biomarkers for important biological and pathological events.

Experimental Section

General Procedures. Commercially available reagents were used without additional purification unless otherwise indicated. All glassware was oven-dried (>120 °C, >4 h) and cooled under vacuum. Dichloromethane (DCM) was distilled from CaH_2. THF was distilled from sodium and benzophenone. Analytical thin-layer chromatography (TLC) was performed with plastic-backed TLC silica gel 60 F hard layer plates. Flash chromatography was performed with silica gel (flash, 32−63 μm). Mass spectrometry (MS) analyses were performed by the Mass Spectrometry Laboratories of North Carolina State University and the University of Kansas. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz (for proton). ^{11}B NMR spectra were measured with $Et_2O{\cdot}BF_3$ in deuterated chloroform as the external standard. Chemical shifts (δ) are given in ppm relative to TMS for ^{1}H spectra and relative to residual solvent for ^{13}C spectra. Combustion analyses were performed by Atlantic Micro labs, Inc., Norcross, GA.

2-(2-Methoxy-5-nitrophenyl)-4,4,5,5-tetramethyl[1,3,2]-dioxaborolane (4). The mixture of 2-bromo-4-nitroanisole (360 mg, 1.56 mmol), pinacolboronate (360 mg, 1.41 mmol), $Pd(dppf)_2Cl_2$ (32 mg, 0.04 mmol), and potassium acetate (360 mg, 3.7 mmol) was dried under N_2. To this mixture was added 10 mL of anhydrous DMSO. The reaction mixture was stirred at 85 °C under N_2 for 5 h and then cooled to room temperature and poured into 20 mL of ice-cold water. Then the mixture was extracted with 2 × 30 mL of ethyl acetate and the combined organic extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Column chromatography of the residue [silica gel, EtOAc/DCM] afforded the boronate **4** (20%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.56 (1H, d, J = 3 Hz), 8.30−8.26 (1H, dd, J = 3, 9 Hz), 6.91 (1H, d, J = 9 Hz), 3.92 (3H, s), 1.36 (12H, s) ppm. ^{13}C NMR ($CDCl_3$) δ 168.9, 141.3, 132.9, 128.7, 110.3, 84.4, 56.6, 25.0 ppm. Anal. Calcd for $C_{13}H_{18}BNO_5{\cdot}0.5H_2O$: C, 54.19; H, 6.65; N, 4.86. Found: C, 54.52; H, 6.41; N, 4.64. ESI-MS for $C_{13}H_{18}BNO_5$ calcd 279.1, found 279.0.

2-Methoxy-5-nitrophenylboronic Acid (5). Sodium periodate (320 mg, 1.50 mmol) was added to a solution of pinacolboronate ester **4** (133.7 mg, 0.48 mmol) in THF/H_2O (4:1, 4 mL) at room temperature. The mixture was stirred for 30 min, and then 2 N HCl (0.5 mL) was added and the solution stirred overnight. Then the reaction mixture was extracted with ethyl acetate (2 × 30 mL), and the combined organic extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Column chromatography of the residue [silica gel, EtOAc/DCM] afforded the boronic acid (70%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.29−8.26 (1H, dd, J = 2.8 Hz, 9 Hz), 8.13 (d, 1H, J = 2.8 Hz), 7.12 (1H, d, J = 9.2 Hz), 3.95 (3H, s) ppm. ^{13}C NMR (CD_3OD) δ 167.7, 142.801, 129.8, 127.9, 111.1, 56.7 ppm. Anal. Calcd for $C_7H_8BNO_5$: C, 42.69; H, 4.07; N, 7.11. Found: C, 43.07, H, 4.16, N, 6.90. EI-MS for $C_7H_8BNO_5$ calcd for (M$^+$) 197, found 197.

2-Hydroxy-5-nitrophenylboronic acid (1). General Procedure for the Deprotection of the Hydroxy Group. To the solution of methoxy phenylboronic acid (**5**) in dry DCM cooled with acetone/dry ice bath was added dropwise 3 equiv of 1.0 M boron triboromide in methylene choloride solution. The reaction solution was stirred for 1 h. Then the reaction mixture was warmed to rt and stirred for another 3 h under N_2. The organic solvent was removed and the residue was extracted with ether and washed with water. The organic layer was washed with 2 N sodium hydroxide solution and the resulting basic solution was neutralized with 2 N HCl. Then the product was obtained by extraction with DCM (50%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.31 (1H), 8.14 (1H, dd, J = 2.7, 9 Hz), 6.86 (1H, d, J = 9.2 Hz) ppm. ^{13}C NMR (CD_3OD) δ 142.0, 132.9, 128.6, 116.5, 103.0 ppm. ESI-MS for $C_6H_6BNO_5$ calcd for (2M − H_2O) 347, found 347.

2-Methoxy-3,5-nitrophenylboronic Acid (7). To the stirring mixture of 1.5 mL of nitric acid and 1.5 mL of sulfuric acid cooled in acetonitrile/dry ice bath was added slowly *o*-methoxyphenyl boronic acid **6** (0.1 g, 6.6 mmol) over 20 min. The reaction mixture was stirred for 3 h more in salt-ice solution while the temperature was kept below −10 °C. Brown solid was seen during the reaction. The reaction mixture was poured onto 5 g of ice and kept in an ice−water bath for 3 h. The brown solid precipitated. The solid was filter and washed with ice-cold water and dried under vacuum. Column chromatography of the residue [silica gel, EtOAc/DCM] afforded the boronic acid **7** (30%). ^{1}H NMR (CD_3OD) δ 8.64 (1H, d, J = 3.0 Hz), 8.46 (1H, d, J = 3.0 Hz), 4.02 (3H, s) ppm. ^{13}C NMR (CD_3OD) δ 160.6, 143.6, 143.2, 133.3, 122.5, 62.4 ppm. Anal. Calcd for $C_7H_7BN_2O_7$: C, 34.75; H, 2.92; N, 11.58. Found: C, 35.12, H, 2.94, N, 11.35. EI-MS for $C_7H_7BN_2O_7$ calcd for (M$^+$) 242, found 242.

2-(2-Methoxy-3,5-dinitrophenyl)-5,5-dimethyl[1,3,2]-dioxaborinane (8). The mixture of compound **7** (240 mg, 1.22 mmol) and pinacol (145.4 mg, 1.23 mmol) was refluxed in 20 mL of toluene with use of a Dean−Stark to remove water overnight. Then toluene was evaporated and the solid residue was extracted with DCM. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, concentrated, and dried in vacuo. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.94 (1H, d, J = 2.4 Hz), 8.63 (1H, d, J = 2.4 Hz), 4.05 (3H, s), 3.85 (4H, s), 1.09 (6H, s) ppm. Anal. Calcd for $C_{12}H_{15}BN_2O_9$: C, 46.48; H, 4.88; N, 9.03. Found: C, 46.40, H, 4.90, N, 8.89.

2-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)-4,6-dinitrophenol (9). The procedure was the same as the preparation of **8** from **7**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.80 (1H, s), 8.95 (1H, d J = 2.4 Hz), 8.77 (1H, d, J = 2.4 Hz), 3.89 (4H, s), 1.09 (6H, s) ppm. ^{13}C NMR (CD_3OD) δ 162.2, 139.8, 136.6, 124.6,

73.1, 32.3, 21.9 ppm. Anal. Calcd for $C_{11}H_{13}BN_2O_7$: C, 44.63; H, 4.43; N, 9.46. Found: C, 44.56; H, 4.47; N, 9.46.

Acknowledgment. Financial support from the National Institutes of Health (NO1-CO-27184 and CA88343), Georgia Cancer Coalition, and Georgia Research Alliance is greatly acknowledged.

Supporting Information Available: HPLC analysis for **1**, and ^{11}B NMR spectra for **1** in the presence and absence of *cis*-cyclopentanediol. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0350357