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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3789–3792
Bioorganic & Medicinal Chemistry Letters

Ortho-azo substituted phenylboronic acids for colorimetric sugar sensors

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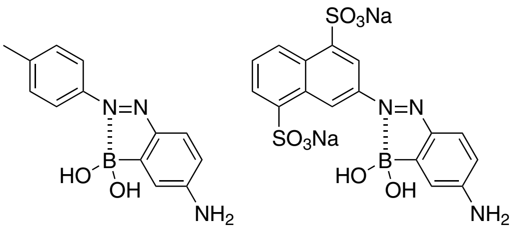
Received 26 December 2006; revised 31 January 2007; accepted 27 February 2007
Available online 3 March 2007

Abstract—The phenylboronic acids substituted with an azo group on the *ortho*-position show a significant change in UV–vis spectra upon sugar binding. A new mechanism for the spectral change of the dyes is proposed based on the formation and cleavage of B–N dative bond between boronic acid group and azo group.

Sugar sensors have been extensively developed based on enzymes such as glucose oxidase.[1] The enzyme-based methods appear to be successful in practical glucose monitorings, though high costs and low stability of glucose sensors are still problems. The development of synthetic probes has been expected to overcome these problems. One of the most promising probes is phenylboronic acid derivatives that form a complex with vicinal diols of sugar.[2–18]

There are many reports on fluorescent boronic acid derivatives that show a significant change in emission spectra upon sugar binding.[3–14] On the other hand, reports on colorimetric sugar sensors using boronic acid are still limited.[15–18] DiCesare and Lakowicz developed boronic acid sugar sensors based on azo dye, in which a boronic acid group was attached on the *para*-position to azo group and linked directly in resonance with the aromatic ring.[17] Although the azo dyes showed a visible color change upon sugar binding, the spectral changes of the dyes were too small to put in practical use. We report here a synthesis and spectroscopic properties of a novel class of boronic acid-appended azo dyes (**1** and **2** in Fig. 1), in which boronic acid group is linked to the aromatic ring on the *ortho*-position to azo group. It has been found that these dyes show a significant change in UV–vis absorption spectra upon sugar binding, which can be clearly recognized by the naked eyes.



1 **2**

Figure 1. Structures of *ortho*-azo substituted phenylboronic acids.

Dyes **1** and **2** were successfully synthesized in diazo-coupling reactions. In the first step, the diazonium ion of corresponding aniline derivative was generated with sodium nitrite in HCl and then coupled with 3-aminophenylboronic acid. Synthetic details and characterization data can be found in the reference and notes section.[28,29]

Figure 2 shows UV–vis absorption spectra of **1** (10 μM) in a methanol/water mixture (1/1, v/v) as a function of pH. Although the measurements were carried out in a methanol/water mixture rather than simply water, the pH value was monitored directly with a pH meter using a standard electrode. It has been shown that for solutions in 50% methanol the pH is changed by only 0.1 pH unit compared to a 100% water solution.[19] The dye **1** exhibited an absorption maximum at 502 nm in the neutral media. The absorbance at 502 nm decreased as the pH increased, and a new absorption maximum appeared at 384 nm in pH 12. An isosbestic point was observed at 442 nm, suggesting an equilibrium between two kinds of species. The pH-induced spectral changes

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Keywords: Azo dye; Boronic acid; Colorimetric sensor; Dative bond; Sugar recognition.

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doi:10.1016/j.bmcl.2007.02.073

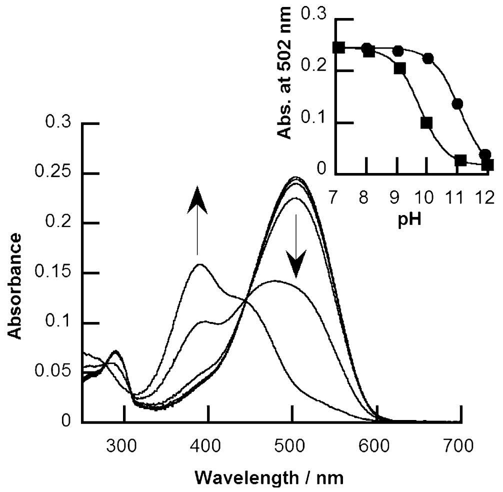
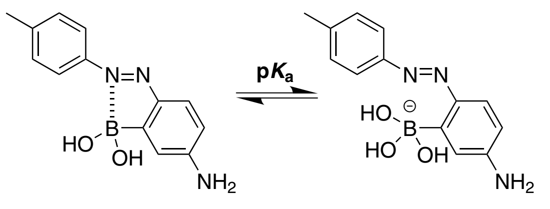


Figure 2. UV–vis absorption spectra of **1** (10 μM) in different pH solutions (pH 7, 8, 9, 10, 11, 12), measured in a methanol/water mixture (1/1, v/v) containing KH_2PO_4 (5.0 mM). Inset shows titration curves against the pH in the absence (●) and in the presence (■) of 50 mM D-fructose for **1** (10 μM).

of **1** are strikingly large as compared to those reported for the azobenzene dye bearing a boronic acid residue at *para*-position to azo group.[17] This is probably due to a direct interaction between the boron atom and azo nitrogen as illustrated in Scheme 1 (**1a**), which is not possible for the azo benzene dye substituted with boronic acid residue at the *para*-position.

In fact, Kano et al. have recently found the formation of a B–N dative bond between azo group and boronic acid in *ortho*-(phenylazo)phenylboronic acid, although they have not studied a pH dependent absorption spectra.[20] In a similar manner, it is reasonable to assume that a B–N bond is formed in **1**, leading to a five-membered ring in neutral solutions (**1a**). The absorption maximum of **1a** is observed at a significantly longer wavelength than that of azobenzene derivatives such as 4-aminoazobenzene, whose absorption maximum is around 365 nm.[21] Thus, the formation of a B–N bond may be a reason for the red shift of the absorption maximum of **1a**. On the other hand, the B–N bond of **1a** is broken in the basic media as a result of addition of OH^- ion to the boron atom (**1b**). A new absorption maximum appears at 384 nm, which is close to the absorption maximum of 4-aminoazobenzene.

The absorption-pH titration was carried out to obtain pK_a of **1** in the absence and presence of sugar. The results are plotted in the inset in Figure 2. From the data, the pK_a values are estimated to be 11.1 and 9.8 in the absence and presence of 50 mM D-fructose, respectively. The decrease in pK_a upon sugar binding may allow the detection of sugar in a buffered solution. To study the response of **1** to sugars, we used pH 10.0 solutions in which the maximum optical changes can be expected upon sugar binding. The pK_a of **1** is higher than that of general phenylboronic acids. It is known that boron nitrogen interaction raises the boronic acid's pK_a.[22] The quasitetrahedral boron of **1a** is less electrophilic because it is more electron-rich compared with trigonal boron. Hence, relatively high pH is needed to break the B–N dative bond of **1a**. The high pK_a of **1** supports the acid-base equilibrium, as presented in Scheme 1.



1a **1b**

Scheme 1. Proposed acid-base equilibrium of **1**.

Figure 3(A) shows effects of D-fructose on the absorption spectrum of **1** in a methanol/water mixture (1/1, v/v) at pH 10.0. Similar spectral changes as for the pH effect, i.e., a decrease in the absorbance at 502 nm and an increase of 384 nm band were observed. **1** showed color change from reddish orange to yellow by sugar addition. Figure 3(B) is plots of absorbance at 502 nm as a function of the concentration of D-fructose and D-glucose. The binding constants are calculated from Figure 3(B) to be 36 and 2.8 M^{-1} for D-fructose and D-glucose, respectively. The pK_a and binding constants were calculated by a curve fitting analysis.[16]

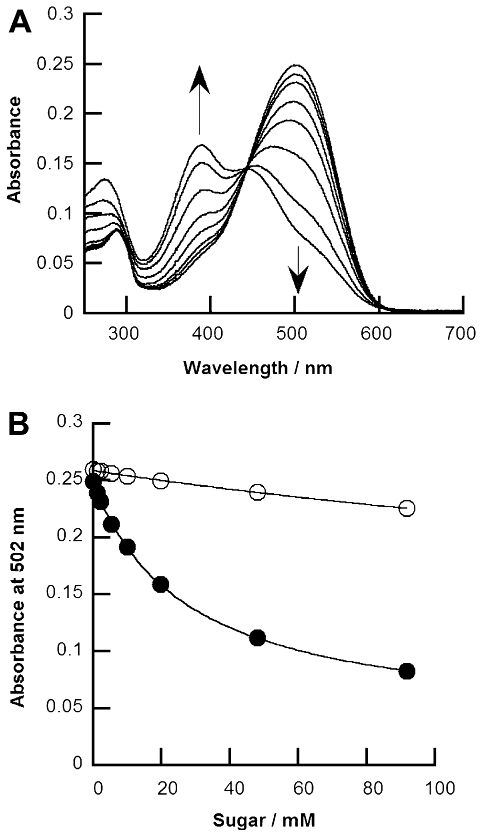
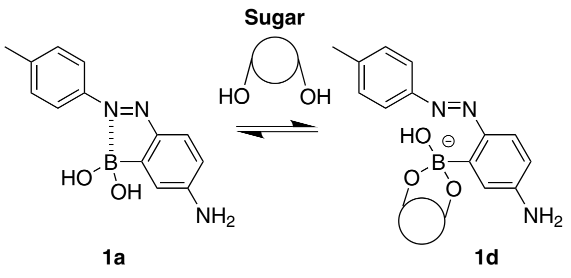


Figure 3. (A) UV–vis absorption spectra of **1** (10 μM) in the presence and absence of D-fructose (0, 1.1, 2.2, 5.3, 10, 20, 48, and 92 mM), measured in a methanol/water mixture (1/1, v/v) containing 2-(cyclohexylamino)ethanesulfonic acid (CHES, 5.0 mM), pH 10.0. (B) Absorbance at 502 nm of **1** as a function of the concentration of D-fructose (●) and D-glucose (○).

Scheme 2 shows a proposed mechanism for the spectral changes of **1** upon binding of sugar. In the absence of sugar, **1a** involves a B–N dative bond at pH 10.0 and shows an absorption maximum at 502 nm. Sugar addition decreases the pK_a of the boronic acid and OH^- coordinate to the boronic acid, which resulted in the cleavage of the B–N bond of **1a** and increase of the absorbance at 384 nm. This proposed mechanism for the spectral changes is based on the cleavage of the B–N bond. A similar mechanism for boronic acid-based fluorometric sensors has been reported by Ni et al.[23]

To our knowledge, such a large change in UV–vis spectra has never been reported for colorimetric boronic acid-based sugar sensors. Ward et al. suggested that B–N interactions play an important roll in spectral change of boronic acid-linked azobenzene dyes.[15,16] However, in their case, the B–N bond was formed between the boronic acid and nitrogen atom in the aniline side chain. Other groups also discussed the importance of B–N bonds in improving the response characteristics of boronic acid-based colorimetric and fluorometric sensors.[22–25] On the contrary, the B–N bond of **1** is linked directly to the nitrogen atom of the chromophoric azo group. The direct interaction of the chromophore group and boronic acid in **1** is a key to induce the significant change in UV–vis absorption spectra.

Experiments using **2** were carried out in an aqueous solution without methanol since **2** is soluble in water. The pK_a of **2** were calculated to be 10.5 and 9.0 in the absence and presence of 50 mM D-fructose, respectively, which are lower than that of **1**. The shift of pK_a is probably due to sulfonic acid groups. It is known that the introduction of electron withdrawing group onto the conjugated system stabilizes the boronate form of the acid and lowers the pK_a value.[8,26] Figure 4 shows the effect of sugar on the absorption spectrum of **2**. The absorption maximum of **2** was observed at 521 nm. Sugar addition induced the decrease of absorbance at 521 nm and the increase of 398 nm. An isosbestic point was observed at 453 nm. **2** showed color change from red to yellow by sugar addition (Fig. 5). The binding constants are calculated to be 110 and 6.2 M^{-1} for D-fructose and D-glucose, respectively. These results indicated that the appropriate introduction of functional group on the dyes can improve their property such as binding constants, solubility in water.



Scheme 2. Proposed mechanism for the sugar-induced spectral changes of **1**.

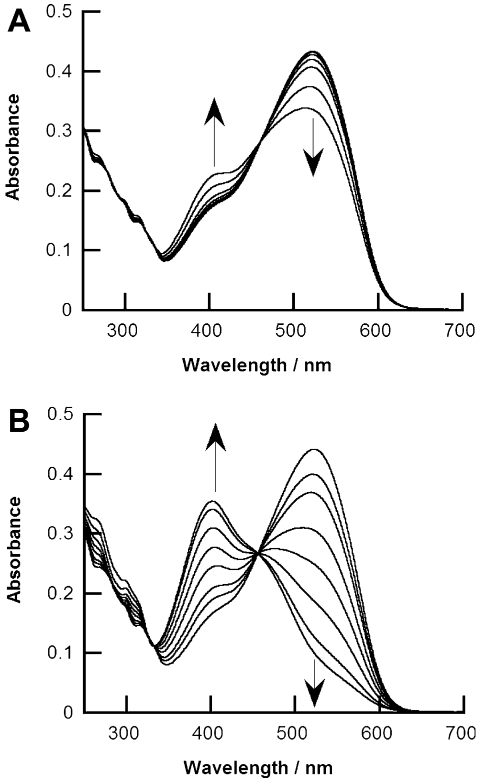


Figure 4. UV–vis absorption spectra of **2** (20 μM) with increasing concentration of D-glucose (A), D-fructose (B) (0, 1.1, 2.2, 5.3, 10, 20, 48, 92 mM), measured in CHES buffer solution (10 mM, pH 10.0).

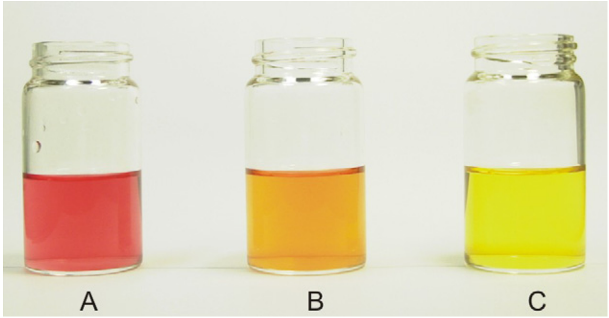


Figure 5. Solutions of **2** (20 μM) in CHES buffer (10 mM, pH 10.0), in the absence of sugar (A), in the presence of 100 mM of D-glucose (B), and in the presence of 100 mM of D-fructose (C).

The hybridizations of boron are reflected in its chemical shift in ^{11}B NMR.[20,22,25,27] When the boron is tetrahedral, its chemical shift is upfield from that of the trigonal planar geometry, where pure sp^3 and sp^2 are approximately 0 and 30 ppm, respectively. We measured ^{11}B NMR spectra of phenylboronic acid and **2** using $Et_2O{\cdot}BF_3$ in toluene-d_8 as an external standard. The ^{11}B NMR spectrum of phenylboronic acid in D_2O exhibited a sharp peak at 28.7 ppm. On the other hand, the ^{11}B NMR spectrum of **2** in D_2O exhibited a broad peak at 13.0 ppm, corresponding to a quasitetrahedral form like **1a** (Scheme 1). In the quasitetrahedral form, the boron center is not fully negatively charged, because a weakly coordinated intramolecular B–N interaction occurs between the boron and nitrogen of azo group. In D_2O containing 1 M NaOD, the peak of **2** was shifted in 2.4 ppm, which is almost the same as that of phenylboronic acid (2.1 ppm). The results indicate the tetrahedral form of **2** is generated in high pH solution because a strong nucleophilic reagent (OH^-) is able to break the weak intramolecular B–N bond. The data from ^{11}B NMR measurements strongly support the proposed mechanism of acid-base equilibrium of *ortho*-azo substituted phenylboronic acid, as presented in Scheme 1.

In conclusion, we have synthesized *ortho*-azo substituted phenylboronic acids and found that dyes **1** and **2** exhibit a significant change in UV–vis absorption spectra upon sugar addition. A new mechanism for the spectral change of the dyes is proposed based on the formation and cleavage of B–N dative bond. Highly sensitive sensor compounds would be developed based on the concept of the *ortho*-azo substituted phenylboronic acid because many kinds of starting materials can be used for the synthesis of azobenzene dyes bearing boronic acid moiety.

Acknowledgement

We thank Dr. Yoshiyuki Tanaka for valuable technical supports with ^{11}B NMR measurements.

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28. *5-Amino-2-(p-tolylazo)phenylboronic acid* **1**. *p*-Toluidine (0.54 g, 5.0 mmol) was dissolved in 20 ml of 1 M HCl, and the solution was cooled to 0 °C. Sodium nitrite (0.35 g, 5.1 mmol) in 10 ml of water was added to the *p*-toluidine solution keeping the temperature below 5 °C to prepare the diazonium salt. After 15 min, 0.68 g (5.0 mmol) of 3-aminophenylboronic acid in 10 ml of 1 M NaOH was added to the diazonium salt solution at 0 °C. The mixture was neutralized by adding a small amount of 1 M NaOH and stirred for 2 h at 0 °C and additional 2 h at room temperature. The resulting aqueous solution was extracted with AcOEt (3 × 50 ml). The organic extracts were combined, washed with brine, and dried over $MgSO_4$. The solvent was removed in vacuo. The crude was purified by silica gel chromatography using $CHCl_3$:MeOH:AcOH (80:20:1) as an eluent. The obtained black solid was dissolved in 1 M NaOH and filtered, and the filtrate was neutralized with 1 M HCl. The **1** was precipitated as an orange solid (0.12 g, 9.4%). Anal. Calcd for $C_{13}H_{14}BN_3O_2$: C, 61.21; H, 5.53; N, 16.47, found: C, 61.13; H, 5.66; N, 16.07. ^1H NMR (600 MHz; CD_3OD) 2.41 (s, 3H), 6.65 (dd, $J = 8.9$, 2.0 Hz, 1H,), 6.74 (d, $J = 2.0$ Hz, 1H,), 7.30 (d, $J = 8.2$ Hz, 2H,), 7.73 (d, $J = 8.9$ Hz, 1H,), 7.97 (d, $J = 8.2$ Hz, 2H,); ^{13}C NMR (150 MHz; CD_3OD) 21.5, 114.6, 117.5, 121.9, 131.1, 132.7, 141.5, 144.1, 149.4, 157.9. A signal due to the carbon atom directly attached to the boron atom could not be detected. MS (FAB, negative mode, matrix: glycerol) *m*/*z*: 338 (M-1+glycerol–$2H_2O$ requires 338). The compound **1** was detected as a complex composed of **1** and glycerol.
29. *3-(4-Amino-2-boronophenylazo)-1,5-naphthalenedisulfonic acid disodium salt* **2**. 3-Amino-1,5-naphthalenedisulfonic acid monosodium salt (0.81 g, 2.5 mmol) was dissolved in 20 ml of 1 M HCl, and the solution was cooled to 0 °C. Sodium nitrite (0.18 g, 2.6 mmol) in 10 ml of water was added to 3-amino-1,5-naphthalenedisulfonic acid solution keeping the temperature below 5 °C to prepare the diazonium salt. After 15 min, 0.34 g (2.5 mmol) of 3-aminophenylboronic acid in 10 ml of 1 M NaOH was added at 0 °C. The mixture was neutralized by adding a small amount of 1 M NaOH and stirred for 2 h at 0 °C and additional 2 h at room temperature. The water of the mixture was removed in vacuo. The crude product was recrystallized from the mixture of water and acetone. The compound **2** was obtained as a black solid. (0.13 g, 10%). Anal. Calcd for $C_{13}H_{14}BN_3O_2$+1.5 H_2O: C, 36.80; H, 2.90; N, 8.05, found: C, 36.73; H, 2.83; N, 8.09. ^1H NMR (600 MHz; CD_3OD); 6.70 (dd, $J = 8.9$, 2.0 Hz, 1H,), 6.76 (d, $J = 2.1$ Hz, 1H,), 7.63 (t, $J = 7.9$ Hz, 1H,), 7.83 (d, $J = 8.2$ Hz, 1H,), 8.25 (d, $J = 6.8$ Hz, 1H), 8.85 (s, 1H), 9.02 (d, $J = 8.9$ Hz, 1H,), 9.69 (s, 1H); ^{13}C NMR (150 MHz; CD_3OD) 115.0, 117.9, 118.5, 124.0, 127.0, 127.8, 131.0, 131.2, 131.7, 133.8, 142.8, 143.3, 144.5, 150.0, 158.7. A signal due to the carbon atom directly attached to the boron atom could not be detected.