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A colorimetric titration method for quantification of millimolar glucose in a pH 7.4 aqueous phosphate buffer

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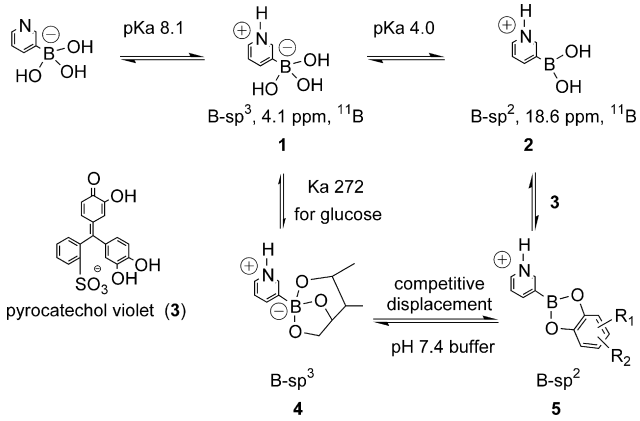
Abstract—3-Pyridinylboronic acid is identified as a key sensing element for reversible sugar complexation in an aqueous solution at the physiological pH. The utility of a sensing element has been demonstrated through a simple colorimetric titration of glucose using absorption spectroscopy in the visible region. The mechanism of the high diol/triol binding affinity of pyridine boronic acid in the neutral pH is discussed based on the ^{1}H and ^{11}B NMR spectroscopic studies.

The equilibriums between arylboronic acids and the corresponding boronic esters of monosaccharides in aqueous solutions have been studied extensively for over the past two decades for affinity sensing of glucose in biological fluids.[1] The principle of the reversible covalent binding has also been applied to the development of membrane transporters,[2] molecular imaging probes,[3] and medicines.[4] A boronic acid moiety with high complexation affinity for diols or polyols in aqueous solutions at the physiological pH is highly desirable for the above applications.[5]

In search of a surface-enhanced Raman molecular sensor for sugars, we observed that 3-pyridinylboronic acid displayed an unusually high glucose binding affinity in an aqueous phosphate buffer at pH 7.4. Raman spectroscopic detection is potentially selective of isomeric sugars and is also compatible with biological samples.[6] Pyridinylboronic acid, instead of the corresponding salts previously used by Czarnik and Norrild,[2a,7] was chosen because of the well-known tendency of pyridine to self-assemble on gold surfaces.[8] In addition, pyridinylboronic acids display relatively low fluorescence that is desirable for Raman detections.[9] Subsequent examination of the binding constant using Anslyn's indicator-displacement assay,[10] vide infra, revealed that 3-pyridinylboronic acid has an apparent association constant with a glucose of $272 \pm 25\ M^{-1}$ (Scheme 1). The high binding affinity of this boronic acid has also allowed us to investigate the supramolecular interactions between the boronic acid and glucose using NMR spectroscopy at the physiological pH in water. Because affinities of most arylboronic acids for glucose are quite moderate, it is usually difficult to observe any arylboronate–glucose interaction directly with NMR spectroscopy under these conditions.[7]

In a typical experiment, a solution of 3-pyridinylboronic acid (**1**, 6.60×10^{-4} M) and pyrocatechol violet (**3**, 7.54×10^{-5} M) in phosphate buffer (3.0 ml, 0.01 M, pH



Scheme 1.

Keywords: Arylboronic acid; Sugar sensing; Dynamic covalent complexation; Colorimetric.
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7.40) in a quartz cuvette (10.0 mm) was titrated using an aqueous solution of glucose (2.81 M in 5.00 μL portions using a Fisher Science microdispenser). The titration was monitored with an OceanOptics® S2000 spectrometer at 25 °C. The pH of the solution was checked to be steady by a pH meter (Oakton 510). The apparent binding constant was determined by using the competitive essay binding algorithm[11] and the method reported by Tobey and Anslyn.[12] As shown in Figure 1, the addition of glucose solution led to absorbance peak shifts and intensity changes of the solution that can be easily quantified with a low profile fiber-optic spectrometer. The dye displayed two absorption peaks in the buffer solution due to the acid–base equilibrium. When 3-pyridinylboronic acid was added, complex **5** was formed as the predominant boronic ester with a λ_{max} of 503 nm, redshifted from the conjugated acid form of the dye (λ_{max} = 463 nm, Scheme 1).[13] It can be estimated from the data that glucose of a few millimolar in neutral aqueous solutions can be reliably quantified using this assay (Fig. 2).

The ~50 nm redshift of the absorbance peak and the ~20% increase in molar absorbance of the dye used in titration were attributed to an extension of the π-conjugation of the dye to the arylboronic acid moiety in the boronic ester (Fig. 1). ^{11}B NMR of the complex displayed a single peak at 18.8 ppm, indicating that the boron in the ester is predominantly trivalent (**5**, sp^2). This significant increase in the absorbance of the dye improves the sensitivity (S/N ratio) of colorimetric detection.[14]

The high binding affinity was confirmed from NMR studies at 25 °C. For example, the ^{1}H NMR spectrum of a mixture of **1** and glucose (15 mmol **1** and 10 mmol glucose, 0.70 ml D_2O) showed two broadened anomeric hydrogen peaks at 6.1 and 5.9 ppm, which were assigned to (α-D-glucofuranose)·**1** (**6**) and (α-D-glucofuranose)·$\mathbf{1}_2$ (**7** and **8**), respectively (Fig. 3 and Scheme 2). When 4 equiv of **1** were used, we observed that the peak at 6.1 ppm disappeared and the 5.9 ppm resonance became dominant. The peaks at 5.3 and 4.7 ppm are assigned to the anomeric hydrogen signals of uncomplexed glucose. From the integrations (Fig. 3), the concentrations of free glucose (**1**), the 1:1 complex (**6**), and the 1:2 complex (**7** and **8**) can be determined to be 4.2, 7.1, and 3.8 mM, respectively. With an error in NMR integration estimated to be less than 5%, it can be calculated from the integrations of the anomeric proton peaks that the apparent binding constant for the formation of **6** is about 130, consistent with the colorimetric titration described above. In comparison, the ^{1}H NMR spectrum of a mixture of *N*-methylpyridinium-3-boronic acid (28 mM) and glucose (7 mM) showed the complexed furanose anomeric hydrogen peak at 5.8 ppm. Less than 5% of the glucose in the sample is estimated to be complexed from the NMR integration in pH 7.4 phosphate buffer in D_2O at 20 °C.

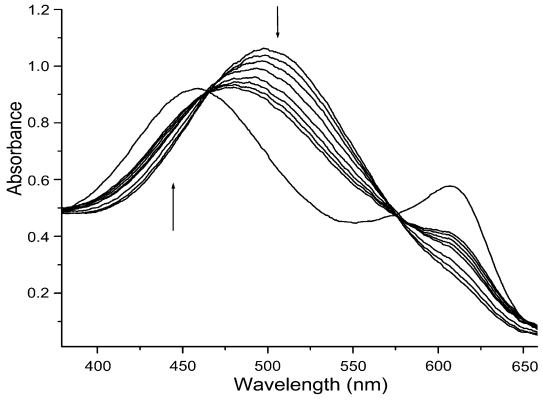


Figure 1. Colorimetric titration of glucose in a pH 7.4 aqueous phosphate buffer at 25 °C monitored by UV–vis spectroscopy.

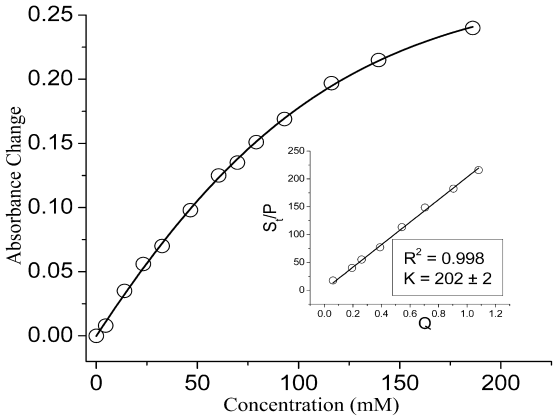


Figure 2. Competitive colorimetric titration curve and the calibration line determined at 503 nm.

3-Pyridinylboronic acid (20 mM, pH 7.4 phosphate buffer, and 0.7 ml D_2O) displayed two peaks at 4.1 (87.1%) and 18.6 (12.9%) ppm in ^{11}B NMR. They were assigned to the zwitterion **1** and its corresponding conjugate acid **2**, respectively (Scheme 1 and Fig. 4a).[15] When glucose (10 mM) was added to the above sample, two resolved new peaks at 5.1 and 6.1 ppm emerged in the ^{11}B NMR spectrum (Fig. 4b). Considering the ^{1}H NMR assignment above and qualitative intensity of the peaks, we assigned the resonance at 5.1 ppm (^{11}B NMR) to the glucose 3,5,6-OH complexed boron in **6**, **7**, and **8**, and the resonance at 6.1 ppm (^{11}B NMR) to the glucose 1,2-OH complexed boron in **7** and **8**. From the ^{11}B NMR integrations, we found that the relative ratio of the total sp^2 and total sp^3 boron changed only slightly before and after the complexation in the aqueous phosphate buffer at pH 7.4. The difference in colorimetric and NMR measurements is tentatively attributed to the well-known isotopic effect of D_2O solvent in the NMR study and other systematic errors.

The resolved NMR signals of the equilibrium species also provided new information on the rates of exchanges of the supramolecular interactions. For example, the differences in the resonance frequencies between sp^2–sp^3 boron signals allowed us to estimate that the exchange is slow compared to the NMR time scale of about 1500 Hz.[16] We did not observe any time-dependent spectroscopic changes during the colorimetric titrations and the color solutions reached equilibriums within seconds after mixing the aqueous buffers.

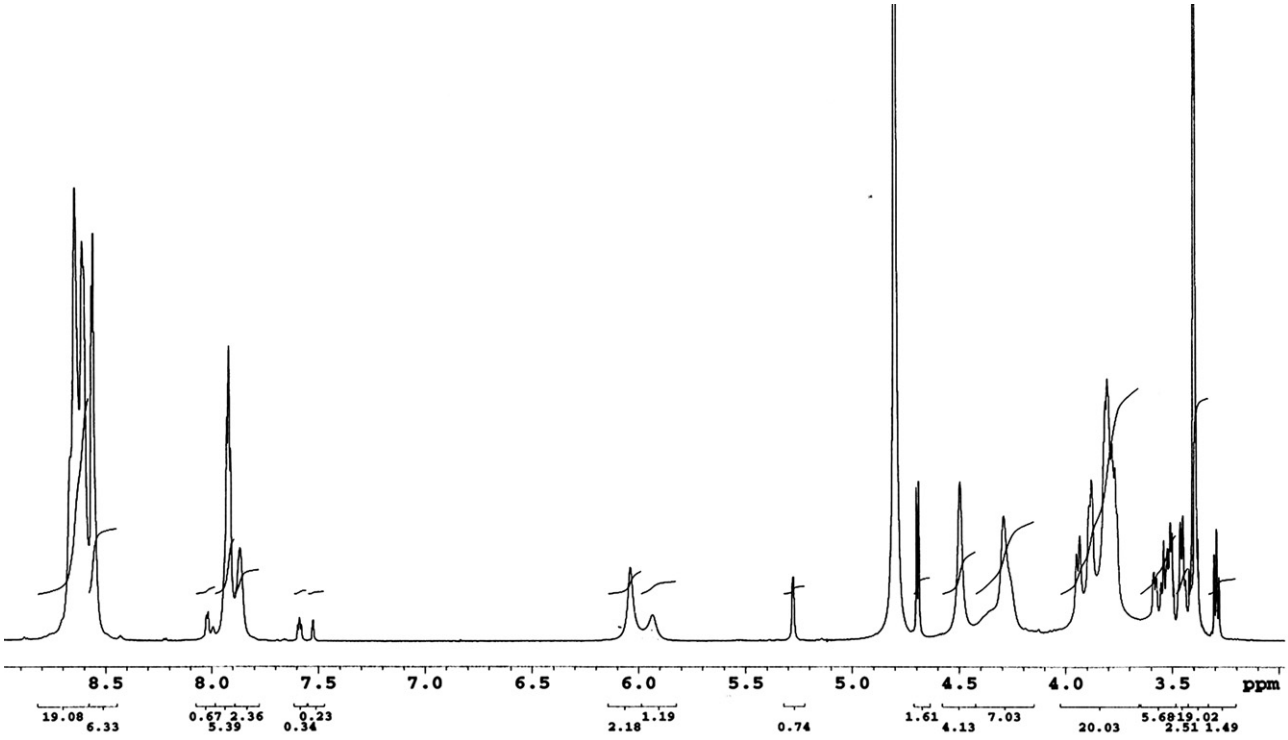
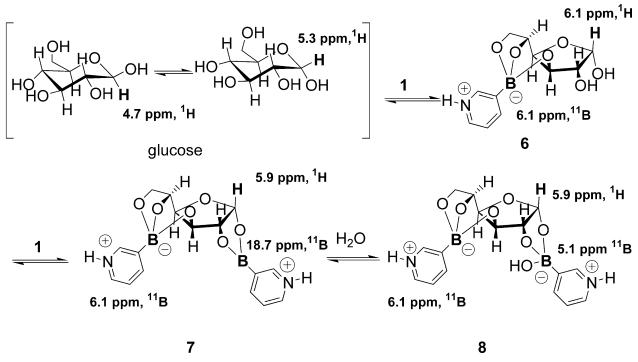


Figure 3. ^{1}H NMR (750 MHz) spectrum of a mixture of **1** (15 mmol) and glucose (10 mmol), in 0.70 ml D_2O at 25 °C.



Scheme 2.

The ^{11}B NMR study also revealed a unique stereoelectronic effect of pyridinylboronic acid that led to an unusually high sugar complexation affinity at the neutral pH. 3-Pyridinylboronic acid is predominantly sp^3 at the boron before and after complexation in phosphate buffer. There is no rehybridization energy to be expended during complexation. The 3,5,6-OH tridentate chelating interaction between arylboronic acid and glucose is the strongest among all the possible interactions. Most arylboronic acids at pH 7.4 predominantly display an sp^2 geometry, which requires rehybridization to achieve the same type of tridentate complexation. In comparison, *N*-methylpyridinyl-3-boronic acid (pK_a 4.4) has a similar pK_a as 3-pyridinylboronic acid (pK_a 4.0),[17] yet it displays a binding constant of 9.6 M^{-1} for glucose in the pH 7.4 buffer. ^{11}B NMR showed that it is predominantly sp^2 for the boronic acid group in the buffer, most likely due to a counterion effect, which is not present in **1**.

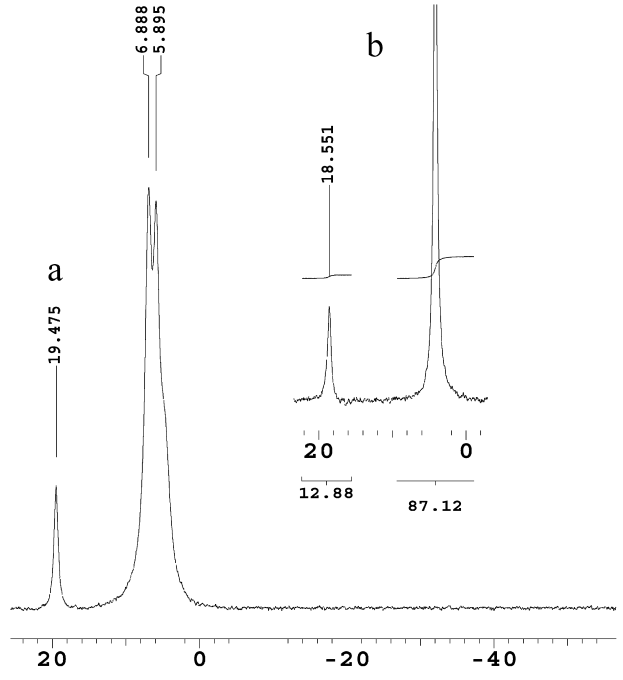


Figure 4. ^{11}B NMR of (a) 3-pyridinylboronic acid (20 mM) in a pH 7.4 phosphate buffer in D_2O (0.7 ml), and (b) a mixture of glucose (10 mM) and 3-pyridinylboronic acid (20 mM) in a pH 7.4 phosphate buffer in D_2O (0.7 ml).

Examination of the complexes of **1** and several other monosaccharides in the pH 7.4 aqueous phosphate buffer supported the above measurements and mechanistic propositions for glucose. For example, fructose displayed a complexation constant of 1.0×10^4 M^{-1} with

1 under the same experimental conditions as that of glucose. While fructose is predominantly in the furanose form, ready for tridentate complexation with **1**[7]; glucose is predominantly in the pyranose form in aqueous solutions. The concentration ratio of D-glucofuranose versus D-glucopyranose in water coincides with the ratio of the apparent binding constants of D-glucofuranose and D-fructopyranose with monoarylboronic acids in water. The smaller binding affinity for glucose can be attributed to the additional free energy requirement for it to rearrange to the furanose form during complexation.

The observed high binding affinity and reversibility of 3-pyridinylboronic acid to sugars and the mechanistic inquiries, particularly of the stereoelectronic effects in both the arylboronic acid receptor and the sugar ligands, should provide new insights into the development of the next generation arylboronic acid based sugar binding scaffolds and may be of general interest in sensing and other biomedical applications of this unique dynamic covalent supramolecular interaction.[18]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.05.075.

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