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Highly Water-Soluble Monoboronic Acid Probes That Show Optical Sensitivity to Glucose Based on 4-Sulfo-1,8-naphthalic Anhydride

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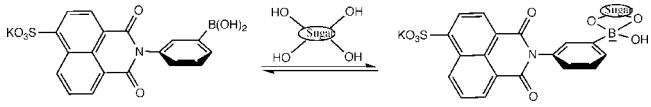

Two highly water-soluble monoboronic acid probes that display the more desirable off−on fluorescence response were synthesized based on 4-sulfo-1,8-naphthalic anhydride and a remarkable sensitivity for glucose rather than fructose and galactose was also observed.

With cases of diabetes reaching epidemic proportions, there continues to be a strong demand for methods of detecting saccharide concentration in blood for those patients who are suffering from this chronic disease.[1−6] Since the ability of recognition of saccharides, boronic acids with diol moiety have been widely investigated for their huge potential biomedical applications.[1,3,7−9] Unfortunately, most of the carbohydrate probes based on boronic acid moiety continue to have limited water solubility and those based on enzymes exhibit poor stability or consumption of substrate during the detection procedure which also limit further biosensing applications.[10,11] Because of their ability to bind to the diols of sugars, phenylboronic acid and its derivatives have been developed for saccharide sensing based on different measurements, such as fluorescence,[12,13] UV−vis absorption,[14,15] and other methods.[16−18] Some key challenges in this field that continue to limit the number of useful probes are the following: the design of synthesis of reporters that have (1) excitation and emission wavelength above 500 nm, (2) low molecular weight, (3) photostability, and (4) perhaps most important water solubility.[19,20]

As a highly photostable and fluorescent probe, naphthalic anhydrides and their derivatives have been widely used for fluorescent tags and receptor antagonists.[21−26] Of particular interest, when appropriately substituted at both the naphthalic and phenyl rings of *N*-aryl-1,8-naphthalimide, a clear dual-fluorescence was observed.[26,27] For instance, by introducing a nitro group into the naphthalic anhydride ring, two emission bands (430 nm/550 nm, respectively) of the dye molecule were reported by our group.[27]

A bis-boronic acid based probe was first synthesized by Shinkai et al.[28] when 3-aminophenylboronic acid was added into a protoporphyrin system. At pH 10.5, in the presence of fructose, the fluorescence signal of this probe could be increased 100-fold. In addition, other studies[23,29] have shown that bis-boronic acid probe designs exhibit higher binding affinity specific for glucose than monoboronic acid probes by comparing K_d values of both sensors, $10^{-5}-10^{-4}$ M for bis-boronic acid probe and $10^{-3}-10^{-2}$ M for monoboronic acid probe, respectively. Unlike changing distances between the boronic acids through synthetic modifications of bis-boronic acid probes, recent investigations on simple monoboronic acid probes showed that fluorophores and substituents on fluorophores also could contribute to saccharide selectivity.[23,27,29] Therefore, more complicated synthetic schemes of bis-boronic acid probes could be avoided by employing appropriate substituents of fluorophore, without losing saccharide binding efficiency.

In this work, we synthesized two different *N*-phenylnaphthalimide-based monoboronic acid probes. Upon consideration of the structurally related Lucifer yellow dye and its high water solubility,[30] we utilized the 4-sulfo potassium salt group of 1,8-naphthalic anhydride. By changing substituted $-B(OH)_2$ positions on the phenyl ring, we investigated the steric effect on

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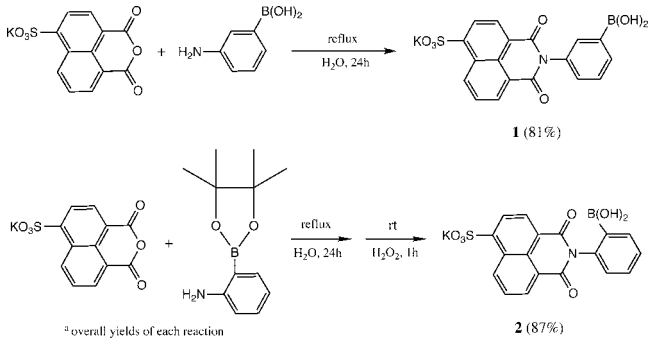
SCHEME 1. Synthetic Route of *o*- and *m*-Phenyl Monoboronic Acid Probes for Saccharides Based on 4-Sulfo-1,8-naphthalic Anhydride



saccharide binding of different probes and structural configuration during this procedure.

To explore fluorescent properties through sugar binding, we synthesized two probes, **1** and **2**, by using a common fluorophore 4-sulfo-1,8-naphthalic anhydride as the starting material. 3-aminophenylboronic acid and 2-aminophenylboronic pinacol ester were selected for investigation of isomeric effects on different boronic acid binding pathways with sugar. Sensor **1** was prepared in a single-step through reaction of the commercial potassium 4-sulfo-1,8-naphthalic anhydride and 3-aminophenylboronic acid hemisulfate. The other probe was prepared through a two-step reaction. After reaction between naphthalic anhydride and aminophenylboronic pinacol ester, the product was treated with 33% aqueous H_2O_2 for 1 h and probe **2** was obtained.[31] A synthetic scheme for construction of those isomers is given in Scheme 1. High water solubility and significant emission signal change are observed in our experiments.

Beyond our expectations, both saccharide probes displayed a greater optical sensitivity to glucose than fructose. Numerous papers indicate that most of the monoboronic saccharide chemsensors favor fructose more than glucose. Some reports[3,21,23,29] indicated that the affinity of probe to fructose is approximately 100 times greater than that of glucose for monoboronic acid sensors. Probes **1** and **2** show large fluorescence intensity changes through chelation-enhanced fluorescence (CHEF) via three most abundant monosaccharides in human blood and receptor interactions, as shown in panels a, b and cin Figure 1. Currently, we attribute the increased fluorescence in the presence of analyte to rigidification of the biaryl probe.[32] Next, we examined the selectivity of these probes to common monosaccharides at pH 7.4. Figure 1d shows the relative fluorescence of **1** at 400 and 474 nm as a function of carbohydrate concentration. Photophysical properties of monoboronic acid probes **1** and **2** are listed in Table 1. The increase in fluorescence intensity ratios (I in the presence of saccharide, I_0 in the absence of saccharide) for this series shows an increase of about 2−5 times, respectively. Probe **1** displays an increase in the ratio of intensities for three common monosaccharides, showing the largest increase in fluorescence for glucose. Similar observations were made by our group previously in which the largest quenching effect took place on glucose.[23] The dissociation constant K_d for fructose was found to be 6.5 mM, while a higher K_d of 28.5 mM was obtained by calculation for glucose

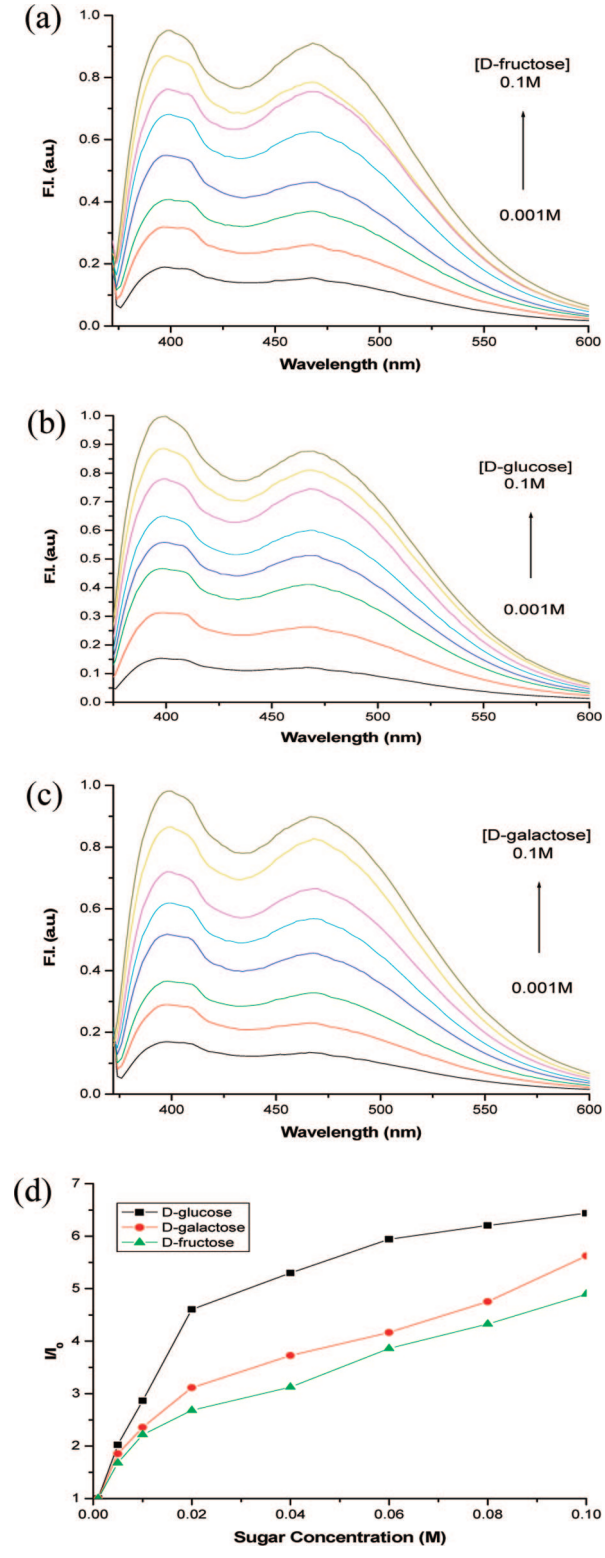


FIGURE 1. Fluorescent spectral changes of boronic acid probe **1** upon addition of different saccharides in phosphate buffer (0.1 M) at pH 7.4 (λex = 360 nm, $\lambda_1$em = 400 nm, and $\lambda_2$em = 474 nm): (a) fructose, (b) glucose, and (c) galactose. (d) Plots of fluorescent intensity changes of **1** as a function of sugar concentration at 474 nm.

at pH 7.4. Though several bisboronic acid sensors have been synthesized to favor glucose over fructose among saccharide bindings, their limitations to complex glucose make them less efficient as glucose probes at high blood glucose levels. Consequently, probe **1** shows an advantage at pH 7.4 by

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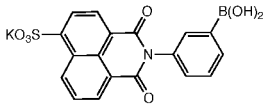
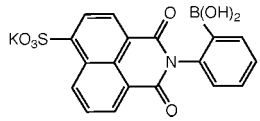
TABLE 1. Photophysical Properties of 1 and 2 Monoboronic Acid Probes

entry	sensor	λ_{ex} (nm)	λ_{1em} (nm)	λ_{2em} (nm)	Φ_F
1		360	400	474	0.092
2		340	391	/	0.112

displaying the largest fluorescence increase to glucose while maintaining an affinity within physiological limits, similar to our probe synthesized from 3-nitronaphthalic anhydride and 3-aminophenylboronic acid.[21] The incorporation of the boronic acid group in the meta position does not lead to significant spectroscopic and photophysical changes in comparison with the ortho derivative. A relatively smaller dissociation constant was observed for the meta derivative (probe **1**) in the presence of saccharides, which could be explained by the effect of less steric hindrance (Figure 2).[24] Quantum yields for both probes were within approximately the same order of magnitude. In addition, the apparent dissociation constants for meta (probe **1**) and ortho (probe **2**) derivatives are listed in Table 2. Fluorescence spectra as well as graphical analyses to detail K_d values are also provided in the Supporting Information.

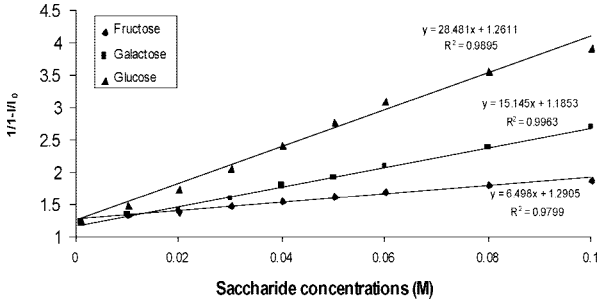


FIGURE 2. Prediction of apparent dissociation constants of probe **1** among different saccharides.

In conclusion, two highly water-soluble monoboronic acid probes that exhibit large fluorescence increases in the presence of monosaccharides show remarkable sensitivity for glucose rather than fructose and galactose. To our knowledge, this is the first highly water-soluble monoboronic acid probe to display the more desirable off−on fluorescence response. While both the monoboronic acid probes displayed a greater change in fluorescence intensity for glucose, it should be emphasized that their binding affinities are still greater for fructose. By changing the position of the boronic acid group from ortho to meta on the phenyl ring, there are no significant spectroscopic and photophysical changes in comparison with the ortho derivative. Plans are currently underway to extend the absorption wavelength of theses dyes to longer wavelength.

TABLE 2. Dissociation Constants (K_d) of Probes 1 and 2 in the Presence of Monosaccharides

	K_d (mmol)		
probe	D-fructose	D-galactose	D-glucose
1	6.5 ± 0.2	15.2 ± 0.5	28.5 ± 1.2
2	13.2 ± 0.4	22 ± 0.6	35.4 ± 1.5

Experimental Section

***N*-(1,8-Naphthaloyl)-3-aminophenyl Boronic Acid (1).** 3-Aminophenylboronic acid hemisulfate (0.100 g, 0.54 mmol), 4-sulfo-1,8-naphthalic anhydride, potassium salt (0.143 g, 0.45 mmol), and 10 mL of pure water were placed in a 25 mL round-bottomed flask equipped with a Dean−Stark receiver and condenser. The reaction mixture was allowed to reflux for 24 h. Water was removed by drying overnight, and excess 3-aminophenylboronic acid was removed by crystallization in hot ethanol. The reaction offered a pink-white powder (0.162 g, 81%), mp 290−293 °C: ^{1}H NMR (D_2O) δ 9.04 (d, J = 9 Hz, 1H), 8.54 (d, J = 6 Hz, 2H), 8.30 (d, J = 7.7 Hz, 1H), 7.93 (t, J = 6.6 Hz, 1H), 7.65 (m, J = 8.8 Hz, 2H), 7.50 (m, 2H); ^{13}C NMR (DMSO) δ 164.5, 164.0, 150.5, 135.0, 134.8, 131.1, 131.0, 129.8, 128.3, 127.4, 125.6, 123.9, 123.0; IR 1217, 1366, 1730, 2970 cm^{-1}; HRMS for $C_{18}H_9KSBNO_7$, expected *m*/*z* 396.0349, found *m*/*z* 396.0351.

***N*-(1,8-Naphthaloyl)-2-aminophenyl Boronic Acid (2).** 2-Aminophenylboronic acid pinacol ester (0.165 g, 0.75 mmol), 4-sulfo-1,8-naphthalic anhydride, potassium salt (0.143 g, 0.45 mmol), and 10 mL of pure water were placed in a 25 mL round-bottomed flask equipped with a Dean−Stark receiver and condenser. The reaction mixture was allowed to reflux for 24 h. The mixture was allowed to reflux by adding 2 mL of 30% aqueous hydrogen peroxide at room temperature for 1 h. Water was removed by drying overnight, and excess 3-aminophenylboronic acid was removed by crystallization in hot ethanol. The reaction offered an orange-yellow powder (0.174 g, 87%), mp 355−358 °C: ^{1}H NMR (D_2O) δ 8.98 (d, J = 8.2 Hz, 2H), 8.50 (d, J = 6.6 Hz, 2H), 8.27 (d, J = 7.3 Hz, 1H), 7.89 (t, J = 7.2 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 7.35 (m, J = 7.2 Hz, 2H); ^{13}C NMR (DMSO) δ 164.6, 164.0, 150.7, 135.1, 131.0, 130.8, 129.0, 128.3, 127.2, 125.4, 124.1, 123.1, 123.0; IR 1209, 1368, 1732, 3011 cm^{-1}; HRMS for $C_{18}H_9KSBNO_7$, expected *m*/*z* 396.0349, found *m*/*z* 396.0348.

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Supporting Information Available: Fluorescent spectral study and apparent dissociation constants in the presence of monosaccharides of sensor **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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