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A Glucose-Selective Fluorescence Sensor Based on Boronic Acid-Diol Recognition

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Abstract—A glucose selective diphenylboronic acid fluorescent sensor (**10a**) with a K_a of 1472 M^{-1} has been synthesized and evaluated. This sensor shows a 43- and 49-fold selectivity for glucose over fructose and galactose, respectively. The binding affinity improvement is about 300-fold and the selectivity improvement for glucose over fructose is about 1400-fold compared with the monoboronic acid compound, phenylboronic acid. ^{1}H NMR studies indicate that sensor **10a** binds with α-D-glucofuranose in a bidentate manner (1:1 ratio).

One of the major challenges in the management of diabetes is the monitoring of glucose concentrations. The most commonly used technology for blood glucose concentration determination is an enzyme-based method, which requires frequent collection of blood samples. Although blood collection using a small needle to prick a finger is a relatively painless process, this approach of glucose monitoring does present some practical problems. The first one is inconvenience, which affects compliance by patients. Second, this is not a continuous monitoring method. Recently, there is a great deal of interest in the development of continuous glucose monitoring systems, which would be able to provide patients with instantaneous feedback and should help to improve the management of proper glucose concentration in diabetic patients.[1–5] It is also conceivable that devices capable of continuous glucose monitoring can be coupled to an insulin delivery device to achieve feed-back controlled delivery of insulin. A new method has been in the development for the monitoring of glucose concentrations without the conventional method of using a needle for sample collection. The method relies on the analysis of fluid obtained through microdialysis.[5,6] However, such a method is still not a continuous monitoring system. To develop a continuous monitoring system, it would be ideal to use an implantable device that is in constant contact with the biological fluid to give a continuous reading of glucose concentration. It is unlikely that the currently used enzyme-based method could be developed as an implantable device due to the instability issues associated with protein-based products.[2] Chemical sensors on the other hand do offer the advantage of higher stability and (relatively) easy manufacturing. Such a concept has already been put into test by companies such as Sensors for Medicine and Science.[3] To develop a chemical sensor-based continuous monitoring device, one needs to develop glucose sensors that show high selectivity and appropriate affinity. Along this line, we are interested in the development of fluorescent sensors for glucose.

As with the development of any sensors, there are three issues. The first one is the identification of functional groups that can afford strong intermolecular interactions. The second one is the construction of the appropriate three-dimensional scaffold as the 'receptor' site. The third one is the identification of a proper 'reporter.' The latter two issues will be addressed later in the paper. For the identification of proper functional interactions, boronic acid has been known to have high affinity for diol-containing compounds such as carbohydrates.[7–9] Such tight complexation has been used for the construction of carbohydrate sensors,[10–20] transporters,[21] and chromatographic materials.[22] Most noticeably, the Shinkai group has been leading the field in many regards. Recently, our group for the first time

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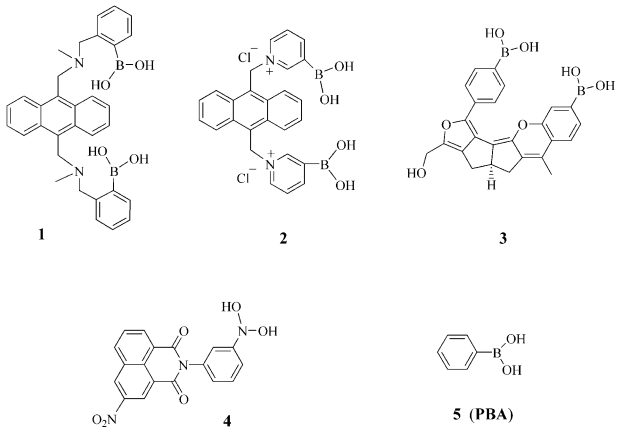
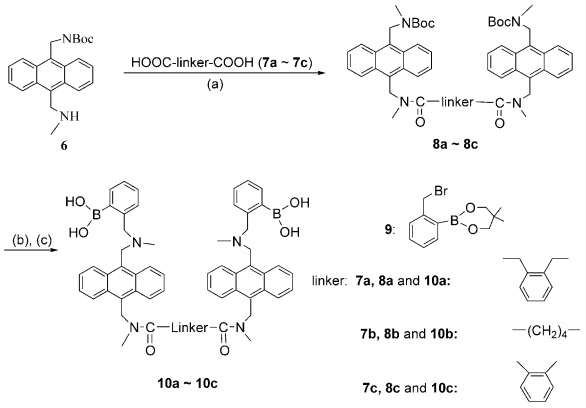


Figure 1. Phenyl boronic acid (**PBA**) and some glucose-selective sensors reported in the literature.



Scheme 1. (a) EDCI, HOBt, DMAP, DIEA; (b) TFA, DCM; (c) **9**, K_2CO_3, KI, CH_3CN.

reported the development of fluorescent boronic acid compounds that can selectively label cells over-expressing a target polysaccharide, sialyl Lewis X.[23] We have developed a general method for the examination of the binding between boronic acids and diols, and also systematically examined the binding of boronic acid compounds with twenty diol-containing compounds. Such studies allowed us to correct many mistakes that exist in the literature with regard to boronic acid-diol binding constants.[9]

Naturally, boronic acid compounds have been used for the synthesis of glucose sensors. Among the significant development in the field are the diboronic acid sensors by Shinkai (**1**, Fig. 1),[24,25] Norrild (**2**),[11,12,26] and Drueckhammer (**3**).[13] Sensors **1** and **2** showed enhanced fluorescence after binding with sugars with a modest selectivity for glucose over other carbohydrates. For example, sensor **1** exhibited a 12- and 25-fold selectivity for glucose over fructose and galactose, respectively. Sensor **3** exhibited much higher selectivity for glucose over fructose and galactose than that of sensor **1**. Recently, the Heagy group reported a very interesting monoboronic acid compound (**4**) that showed the greatest spectroscopic changes with glucose compared to other sugars such as fructose.[18] However, the binding constant with glucose is still lower than that of fructose.

Herein, we report the first dianthracene-boronic acid compound that showed high selectivity (over 43-fold over fructose and 49-fold over galactose) and the greatest fluorescence intensity changes within the range of physiological glucose concentration. Furthermore, the sensor showed about 300-fold improvement in binding affinity with glucose compared with simple phenylboronic acid.

Our overall design is based on the assumption that diboronic acid compounds (**10**, Scheme 1) with the proper distance and orientation that are complementary to the two pairs of diols[11,12,26,27] on glucose will be able to bind glucose with high affinity and selectivity. Such an idea is intuitive and has been demonstrated many times.[27,26,11–13,10,28] In our design the Shinkai fluorescent reporter[25] moiety was chosen due to its known property to increase fluorescence intensity upon binding with a diol through the regulation of excited state photoelectron transfer (PET). In this design the key is to find an appropriate linker that would give the proper distance and orientation of the two boronic acid moieties that are complementary to the two pairs of diols of glucose. We have synthesized diboronic acid compounds with the general structure of **10**. These compounds differ in linker length and flexibility. Scheme 1 shows the general synthetic approach. Reaction of anthracenyl methylamine (**6**)[23] with the appropriate diacid (**7**) using EDCI [1-(2-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] as a coupling reagent gave the Boc protected compounds **8**. After Boc-deprotection with TFA, the free amine was treated with **9**[25] in the presence of K_2CO_3 to give the diboronic acid compounds **10**.[29,30]

Fluorescence experiments were conducted to evaluate the affinity of the sensors (**10**) for glucose and their selectivity. Specifically, 2 mL of the sensor solution in methanol (2×10^{-6} M) was mixed with 2 mL of aqueous saccharide phosphate buffer solution (pH 7.4) at various concentrations, and then the fluorescence intensity was recorded (Fig. 2). Among the compounds prepared, sensor **10a** showed the highest selectivity for glucose

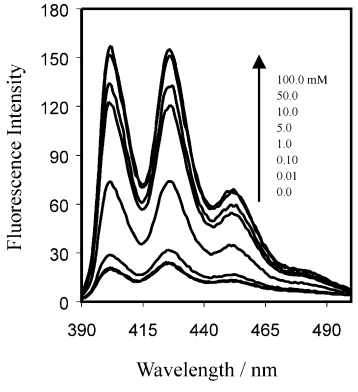


Figure 2. Fluorescence spectra of **10a** (1.0×10^{-6} M) upon addition of D-glucose (0–100 mM) at 25 °C in 50% MeOH/0.1 M aqueous phosphate buffer at pH 7.4; $\lambda_{ex}=370$ nm.

Table 1. The complexation properties for monosaccharides with **10a**, **10b**, **10c**, and the sensors reported in the literature

Sensors	K_a with Glucose	K_a with Fructose	K_a with Galactose	Selectivity $K_{glu}/K_{fructose}$	Fluorescence intensity changes[a]
1	3981	316	158	13	Increased by 6.5-fold
2	2512	—[b]	—[b]	—[b]	Increased by 0.9-fold
3	4000	—[c]	100	—[c]	Decreased by 0.5-fold
4	38	476	—[b]	0.08	Decreased by 0.4-fold
5	5	162	15	0.03	—[d]
10a	1472	34	30	43	Increased by 7.0-fold
10b	638	77	105	8	Increased by 4.3-fold
10c	178	283	33	0.6	Increased by 5.5-fold

[a]Fluorescence intensity changes ($\Delta I/I_o$) upon binding of glucose (100 mM).
[b]The authors did not give K_a for fructose and galactose.
[c]Fluorescence intensity change upon binding with fructose was too small for an accurate K_a determination.
[d]PBA is not fluorescent.

over fructose and galactose with a K_a of 1472, 34, and 30 M^{-1}, respectively (Fig. 3, Table 1). This represents a 43- and 49-fold selectivity for glucose over fructose and galactose, respectively. In addition, fluorescence enhancement was very large (about 7-fold).

It needs to be noted that the 'natural tendency' for boronic acid is to favor the binding with fructose over glucose.[8,9] For example, phenylboronic acid (PBA, **5**) has a K_a of 162 M^{-1} for fructose and 5 M^{-1} for glucose.[9] Sensor **10a** represents an improvement of about 300-fold in affinity and about 1400-fold improvement in selectivity for glucose over fructose compared with simple PBA (**5**). It should also be noted that sensor **10a** showed the most sensitive fluorescence intensity changes to glucose in the mM region (Fig. 3), which is the most physiologically relevant concentration range in terms of blood glucose detection. Compared with the sensors reported in the literature, sensor **10a** showed higher selectivity for glucose over fructose than sensor **1**,[24,25] but lower selectivity than the sensor **3** (Table 1).[13] However, sensor **3**[13] responds to the binding event by a decrease in fluorescence instead of an increase (Table 1).

Based on the structure of **10a**, it seems that two acetamides attached to a phenyl ring in an *ortho* relationship offer the appropriate diboronic acid orientation and distance for binding with glucose. In an effort to examine the effect of structurally similar linkers on the selectivity for glucose, sensors **10b** and **10c** were also synthesized and examined. Sensor **10b** has the same number of carbons for the linker moiety, however, in a linear arrangement. Sensor **10c** has two fewer carbons for the linker moiety, but still has an *ortho* substituted phenyl ring as the key component. Neither **10b** nor **10c** showed the kind of selectivity and affinity for glucose as did **10a** (Table 1). The binding constants of sensor **10b** for glucose, fructose, and galactose were determined as 638, 77, and 105 M^{-1}, respectively. For **10c**, the binding constants were determined as 178, 283, and 33 M^{-1} for glucose, fructose, and galactose, respectively.

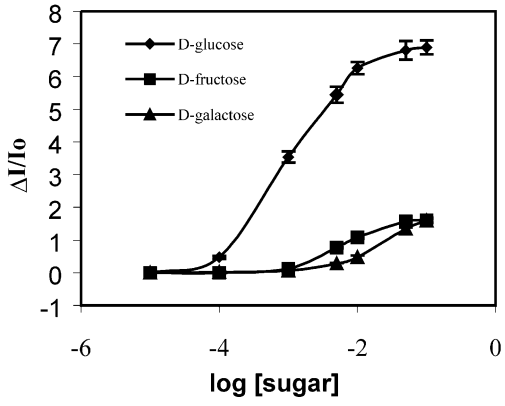


Figure 3. Fluorescence intensity changes ($\Delta I/I_0$) of **10a** as a function of the saccharide concentrations at 25 °C; 1.0×10^{-6} M in 50% MeOH/0.1 M aqueous phosphate buffer at pH 7.4; $\lambda_{ex}=370$ nm, $\lambda_{em}=423$ nm.

It is reasonable to expect that only if glucose forms a bidentate complex with sensor **10a**, do we expect to see the observed selectivity.[11,12,26,27] Such reasoning is also in line with what has been observed by Shinkai, Norrild, and Drueckhammer. In order to confirm this, we examined the formation of the complex between **10a** and glucose using ^{1}H NMR. Specifically, **10a** and D-glucose in a 1:1 ratio were dissolved in methanol-d_4 containing 1% of D_2O. Norrild and co-workers have conducted a detailed examination of D-glucose binding with a diboronic acid compound using ^{1}H NMR, and found that in the initial complex, glucose was in the α-D-glucopyranose form.[11,12,26,27] However, with time this complex was converted to the thermodynamically more stable α-D-glucofuranose form. It is known that water facilitates this mutarotation of the α-D-glucopyranose to α-D-glucofuranose. Therefore, in our study, a small amount of D_2O (1%) was used to aid the mutarotation to the more thermodynamically stable form.

The ^{1}H NMR spectrum obtained 20 min after mixing **10a** and glucose showed the appearance of new peaks compared with the two starting materials, the most obvious peaks of which were in the region from 2.5 to 2.8 ppm. After the mixture was kept at −20 °C for 2 weeks, the peaks at 2.08 and 2.25 ppm corresponding to the N–CH_3's of the free **10a** almost completely disappeared, and four new peaks in the range from 2.5 to 2.8 ppm corresponding to the N–CH_3's of the complex appeared. Such results indicate the formation of a 1:1 complex since the two starting materials were added in a 1:1 ratio. The chemical shifts for the sugar part of the complex were somewhat different from the data reported by Shinkai and Norrild, which are understandable

Table 2. ^{1}H NMR assignment for the glucose part of **10a**–D-glucose complex (1:1)

Assignment	Chemical shift (ppm)	Assignment	Chemical shift (ppm)
H1	6.50	H2	5.21
H3	3.66	H4	4.22
H5	2.95	H6a	3.46
		H6b	3.73

The solvent for NMR is methanol-d_4 containing 1% D_2O and the data are given relative to TMS. The assignments are in agree with the information obtained from ^{1}H–^{1}H-COSY, ^{1}H–^{13}C Heterocorrelated spectra, TOCSY, ROESY, and selected decoupling experiments.

since the complexing agents (sensor) are different, and at least the anisotropic effect of the aromatic groups is expected to be significantly different from that of the Norrild and Shinkai compounds. ^{1}H–^{1}H-COSY, ^{1}H–^{13}C Heterocorrelated spectra (HMQC and HMBC), TOCSY, and selective decoupling experiments were performed to give a reasonable assignment. In the proton spectrum, coupling constant between H2 and H3 is about zero ($J_{2,3}\sim 0$) and no cross peaks were found between these two protons in ^{1}H–^{1}H-COSY, TOCSY, and ROESY, indicating that the complex was in the form of α-D-glucofuranose as was found in similar boronic acid complexes with glucose reported by Norrild (Table 2).[11,26]

The electrospray ionization (ESI) mass spectrum showed a large peak at m/z 1063.5 $(M+1)^+$ for the corresponding 1:1 complex ($C_{66}H_{64}B_2N_4O_8$, M = 1062). No peak was observed at m/z 1243 $(M+1)^+$, which corresponded to the 1:2 complex ($C_{72}H_{76}B_2N_4O_{14}$, M = 1242). Therefore, MS data also confirmed the predicted formation of a 1:1 complex.

In conclusion, we have synthesized a glucose selective diboronic acid fluorescent sensor (**10a**). The sensor has a high affinity (K_a 1472 M^{-1}) and shows a 43- and 49-fold selectivity for glucose over fructose and galactose, respectively. The binding affinity improvement is about 300-fold and the selectivity improvement for glucose over fructose is about 1400-fold compared with the monoboronic acid compound, PBA. The fluorescence intensity change was also high, up to 7-fold. Compared with the Shinkai sensor (**1**), the magnitude of the fluorescence intensity changes were similar, but the sensor reported in this paper has a higher selectivity for glucose over fructose. Compared with the Drueckhammer sensor (**3**), sensor **10a** has a lower selectivity for glucose, but the advantage of showing a fluorescence intensity increase upon binding. ^{1}H NMR studies indicate that sensor **10a** binds with α-D-glucofuranose in a bidentate manner. The discovery of glucose-selective fluorescent sensors such as this one will aid the effort of developing an implantable glucose sensing device.

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29. **A typical procedure for preparation of Boc-protected compounds (8a, 8b, and 8c)**: Compound **6** (146 mg, 0.40 mmol) and compound **7a** (39 mg, 0.20 mmol) were dissolved in dry DCM (20 mL). To this solution were added HOBt (65 mg, 0.48 mmol), DMAP (10 mg, 0.08 mmol) and DIEA (75 μL, 0.48 mmol). The mixture was cooled to 0 °C and EDCI (92 mg, 0.48 mmol) was added. The solution was stirred at 0 °C under nitrogen for 1 h, and at rt for another 2 h. After the addition of 30 mL of DCM, the solution was washed with 5% citric acid (2×20 mL), 5% Na_2CO_3 (2×20 mL) and brine (3×20 mL), and then dried over Na_2SO_4. Solvent evaporation gave a residue that was purified on a silica gel column, eluting with DCM/MeOH (100:1) to give the product **8a**. Yield 88%;

FAB-MS: m/z 887.4733 $(M+H)^+$; ^1H NMR ($CDCl_3$) 1.56 (s, 18H, $6\times CH_3$(Boc)). 2.45 (s, 6H, $2\times N–CH_3$), 2.62 (s, 6H, $2\times N–CH_3$), 3.81 (s, 4H,$–CH_2–CO–$), 5.53 (s, 4H, $2\times N–CH_2–$), 5.74 (s, 4H, $2\times N–CH_2–$), 7.26–8.49 (m, d, 20H, aromatic CH).

30. **A typical procedure for preparation of the boronic acid sensors 10a**, **10b** and **10c**: The Boc-protected compound **8a** (44 mg, 0.050 mmol) was dissolved in 4 mL dry DCM. Then 2 mL of trifluoroacetic acid (TFA) was added and the reaction was stirred at rt for 30 min. After solvent evaporation and drying in vacuum, the deprotected compound was dissolved in 15 mL of dry acetonitrile. To this solution were added compound **9** (57 mg, 0.20 mmol), K_2CO_3 (42 mg, 0.30 mmol) and KI (2 mg). The mixture was stirred at rt for 12 h, and then the solvent was removed in vacuum. To this residue was added 20 mL of DCM and 10 mL of 5% aq $NaHCO_3$. Then the mixture was stirred at rt for 1 h. The organic phase was separated, washed with water (4×10 mL), and dried over Na_2SO_4. After solvent removal, the residue was re-precipitated from DCM–hexane to give the product **10a**. Yield 81%; ESI-MS: m/z 478.4 1/2 $(M+2H)^+$; ^1H NMR (CD_3OD, 300 MHz) δ 2.23 (s, 6H, $2\times N–CH_3$), 2.35 (s, 6H, $2\times N–CH_3$), 3.69 (s, 4H,$–CH_2–CO–$), 4.23 (s, 4H, $2\times N–CH_2–$), 4.94 (s, 4H, $2\times N–CH_2–$), 5.64 (s, 4H, $2\times N–CH_2–$), 7.22–8.43 (d, d, m, 28H, aromatic CH). Anal. calcd for $C_{60}H_{60}B_2N_4O_6\cdot2.4H_2O$: C, 72.21; H, 6.49; N, 5.61. Found: C, 71.96; H, 6.19; N, 5.39.

10b: Yield 91%; ESI-MS: m/z 889.6 $(M–H_2O+H)^+$; ^1H NMR ($CDCl_3/CD_3OD$, 300 MHz) δ 1.77 (m, 4H,$–CH_2–$), 2.30 (s, 6H, $2\times N–CH_3$), 2.47 (t, 4H, $2\times CH_2–CO–$), 2.56 (s, 6H, $2\times N–CH_3$), 4.10 (s, 4H, $2\times N–CH_2–$), 4.70 (s, 4H, $2\times N–CH_2$), 5.63 (s, 4H, $2\times N–CH_2$), 7.40–8.40 (d, d, m, 24H, aromatic CH). Anal. calcd for $C_{56}H_{60}B_2N_4O_6$-1/$4H_2O$: C, 74.55; H, 6.65; N, 6.21. Found: C, 74.55; H, 7.00; N, 5.75.

10c: Yield 32%. MS-ESI: m/z 909.6 $(M-H_2O+H)^+$. ^1H NMR ($CD_3OD+CDCl_3$, 300 MHz) δ 2.44 (s, 6H, $2\times N–CH_3$), 2.61 (s, 6H, $2\times N–CH_3$), 4.36 (s, 4H, $2\times N–CH_2–$), 5.09 (s, 4H, $2\times N–CH_2$), 5.88 (s, 4H, $2\times N–CH_2$), 7.27–8.34 (d, d, m, 28H, aromatic CH). Anal. calcd for $C_{58}H_{56}B_2N_4O_6\cdot2H_2O$: C, 72.36; H, 6.23; N, 5.82. Found: C, 72.26; H, 5.75; N, 5.48.