Research article

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Received: 5 May 2014, Revised: 4 July 2014, Accepted: 25 August 2014 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/bio.2776

Fluorene-based boronic acids as fluorescent chemosensor for monosaccharides at physiological pH

Rahman Hosseinzadeh,[a]* Maryam Mohadjerani[b] and Mona Pooryousef[a]

ABSTRACT: Two fluorene-based boronic acids, 9,9-dimethyl-9H-fluoren-2-yl-2-boronic acid (1) and 9,9-dimethyl-9H-fluoren-2,7-diyl-2,7-diboronic acid (2), were synthesized and their sensing abilities for detection of D-monosaccharides were investigated by fluorescence at physiological pH. It was found that both boronic acids 1 and 2 have high selectivity and sensitivity for D-fructose with stability constant of 47.2 and 412.9, respectively. The sensor 2 showed a linear response toward D-fructose in the concentration range from 5×10^{-5} to 10^{-1} mol L^{-1} with the detection limit of 2×10^{-5} mol L^{-1}. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: fluorescence; boronic acid; fluorene; saccharide; chemosensor

Introduction

Fluorescent chemosensors have versatile applications in chemical, biological and environmental science because fluorescence can offer the advantages of high sensitivity, quick response, remote detection capabilities and multiple sensing modes (1–4). In addition to its high sensibility and selectivity, fluorescence spectroscopy is a non-invasive method that can be performed in whole blood (5) and through the skin (6). Monosaccharides not only play a significant role in the metabolic pathways of living organisms but also lead to some diseases such as renal glycosuria, cystic fibrosis, diabetes and cancers (7–10). Therefore, identifying and detecting concentration of monosaccharides in aqueous solution is desired by biologist and chemists. Among the fluorescent chemosensors, fluorescent boronic acid sensors have attracted considerable attention for selective detection of saccharides owing to their unique ability to bind guests in aqueous media (11). The boronic acid group interacts strongly and reversibly with *cis*-1,2- and 1,3-diols of saccharide to form five- or six- membered ring, respectively, leading to an increase in the Lewis acidity of the boron atom (12–15). In addition, boronic acids can also sensing α-hydroxy acids (16,17) and other Lewis bases (anions) (18).

Several interesting fluorescence probes functionalized with boronic acids have been developed over the past decade. The most popular fluorophores are anthracene (16,19), naphthalene (20,21), naphthalimide (22–24) and quinoline (25).

Fluorene shows interesting spectroscopic and photophysical properties. This fluorophore was extensively used as a building block for the development of dyes with good absorption properties (26–31). Therefore, in the research for new fluorescent monosaccharide sensors, we have prepared two boronic acid chemosensors with fluorene as the fluorophore (**1** and **2** shown in Fig. 1) and the effect of various saccharides on the fluorescent properties of these compounds were examined in phosphate buffer at pH 7.4. The fluorescence of chemosensors **1** and **2** were quenched by saccharides binding to the boronic acid moiety. The pK_a values are reduced on addition of saccharides. The photo-induced electron transfer (PET) from boronate anion is believed to be the source of the fluorescence quenching.

Experimental

Materials and general methods

All the solvents and chemicals were purchased from Aldrich and Merck and were used without further purification. Diethyl ether and tetrahydrofuran were dried under argon. They were refluxed with Na in the presence of benzophenone until a blue colour appeared and then distilled. 2-Bromofluorene (3) and 2,7-dibromofluorne (5) were synthesized according to the literature (32–34).

Apparatus

NMR spectra were recorded on a Bruker Avance III 400 MHz. Fluorescence spectra were recorded on a Jasco Fp-6200 spectrofluorometer. Absorption spectra were recorded on a Braic-2100 UV-VIS spectrophotometer. Fluorescence quantum yield were measured with phenol as the standard ($\Phi = 0.14$) (35). All pH measurements were recorded on 744-metrohm pH meter. The binding constants and pK_a were calculated using non-linear

* Correspondence to: R. Hosseinzadeh. Department of Organic Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran. E-mail: r.hosseinzadeh@umz.ac.ir

[a] Department of Organic Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran

[b] Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran

Abbreviations: DMSO, dimethyl sulphoxide; PET, photo induced electron transfer.

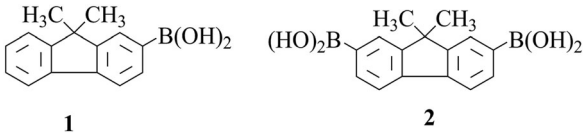


Figure 1. Structure of synthesized boronic acids.

curve-fitting programs implemented using Erithacus Software GraFit (version 5).

Synthesis

2-Bromo-9,9-dimethyl-9H fluorene (4). This compound was synthesized according to the literature procedure (36,37) from 2-bromofluorene and iodomethane in the presence of sodium hydroxide and triethylammonium chloride in dimethyl sulphoxide (DMSO). The crude product was purified by crystallization from methanol to give product **4** in 96% as yellow crystals. M.p.: 67–69 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.51 (s, 6H), 7.35–7.38 (m, 2H), 7.44–7.47 (m, 2H), 7.49–7.51 (m, 2H), 7.71–7.72 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 27.12, 47.12, 120.08, 121.04, 121.40, 122.66, 126.17, 126.23, 127.19, 127.69, 130.10, 138.16, 138.24, 135.25.

2, 7-Dibromo-9,9-dimethyl-9H-fluorene (6). This compound was prepared using the same procedure reported for **4**. The crude product was purified by column chromatography using hexane as eluent to yield a white crystalline solid in 76% yield (8.25 gr). M.p:175°C (38,39). ^{1}H NMR (400 MHz, $CDCl_3$). δ (ppm) = 1.48 (s, 6H), 7.48 (dd, *J* = 8.0, 2.0 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.58 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 26.88, 47.34, 121.48, 121.52, 126.22, 130.36, 137.18, 155.27.

9, 9-Dimethyl-9H-fluoren-2-yl-2-boronic acid (1). To a suspension of magnesium metal turnings (0.31 g, 12 mmol) in dry tetrahydrofuran (5 mL) under a nitrogen atmosphere, a few crystals of iodine were added. A solution of 2-bromofluorene **4** (3.00 g, 11 mmol) in dry tetrahydrofuran (5 mL) was added dropwise (20 min). After complete addition, the solution was kept at reflux for 1 h and then cooled to 0 °C (±5 °C). Then, the mixture was added to a cooled (–14°C) solution of trimethyl borate (3.42 mL, 33 mmol) in dry tetrahydrofuran (20 mL). After stirring for further 2 h, the reaction mixture was first quenched with water and then aqueous HCl (2 M, 50 ml) was added until the solution turned acidic. The resulting mixture was poured into water and extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate and concentrated with a rotavapour. The crude product was purified by column chromatography using EtOAc/hexane (30/70, v/v) as eluent to (1.82 g) a white powder in 70% yield. M.p.: 297–300 °C (40,41). ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm) = 1.42 (s, 6H), 7.31–7.33 (m, 2H), 7.52–7.55 (m, 1H), 7.76–7.83 (m, 3H), 7.96 (s, 1H), 8.06 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d6): δ (ppm) = 27.41, 46.69, 119.54, 120.80, 127.47, 128.08, 133.55, 138.99, 140.79, 152.58, 154.15.

9, 9-Dimethyl-9H-fluoren-2,7-diyl-2,7-diboronic acid (2). The solution of 2, 7-dibromo-9,9-dimethyl-9H-fluorene (6) (5.00 g, 14.2 mmol) in ether (50 mL) was cooled to –78 °C. To the solution was added dropwise n-BuLi (1.3 M) (27 mL, 35 mmol) and then allowed to slowly warm to room temperature. The reaction mixture was stirred 1 h and then cooled again to –78 °C. After addition of triisopropyl borate (13.5 mL, 58.5 mmol), the resulting mixture was stirred at –78 °C for 1 h, and overnight at room temperature. Then 2 N HCl (50 mL) was added to the solution and stirring was continued at room temperature for 1 h. The organic layer was separated and the water layer was extracted with 100 mL of diethyl ether. The combined ether layers were washed twice with 100 mL of water. The solvent was then removed under reduced pressure. Purification was carried out by column chromatography on silica gel using a hexane/ethylacetate mixture (4/6) to give a (2.31 g) white powder in 58% yield. M p: >300 °C. NMR spectra are in good accordance with the literature (41). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.44 (s, 6H), 7.39 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.96 (s, 2H), 8.06 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 27.52, 46.52, 119.78, 128.83, 133.52, 140.78, 153.01.

Fluorescence measurements

The fluorescence emission spectra's of the boronic acid **1** and **2** with or without the fructose and glucose were recorded as the pH was changed from pH 2 to 12. The pH of the solutions was controlled using minimum amount of sodium hydroxide and hydrochloric acid solutions. Titration curves against pH were measured in buffer solutions: acetate buffer for pH 3.0–5.5, phosphate buffer for pH 6.0–9.0, and carbonate buffer for pH 10.0–11.0 (42).

Titration curves were fitted and pK_a values (pK_a = –log K_a) were obtained using the equation:

$$I = \frac{10^{-pH} I_{acid} + K_a I_{base}}{K_a + 10^{-pH}} \quad (1)$$

Where I_{acid} and I_{base} are the fluorescence intensity limits in the acid and basic region, respectively (42,43).

For saccharides binding experimental distinct solutions of the boronic acid **1** and **2** (2×10^{-5} M) in 1:1 mixture of methanol /phosphate buffer and the sugars (various concentrations) were prepared in 0.1 M phosphate buffer at pH 7.4. Then, 2 mL of each boronic acid solution was mixed with 2 mL of a sugar solution. After stirring for 20 min, the mixture was transferred into a 1 cm quartz cell and the fluorescence intensity was recorded immediately (44).

Titration curves against saccharide were fitted, and binding constants (K) were obtained from global analysis of data obtained from two independent titrations using the equation:

$$I = \frac{I_{min} + I_{max} K[sugar]^n}{1 + K[sugar]^n} \quad (2)$$

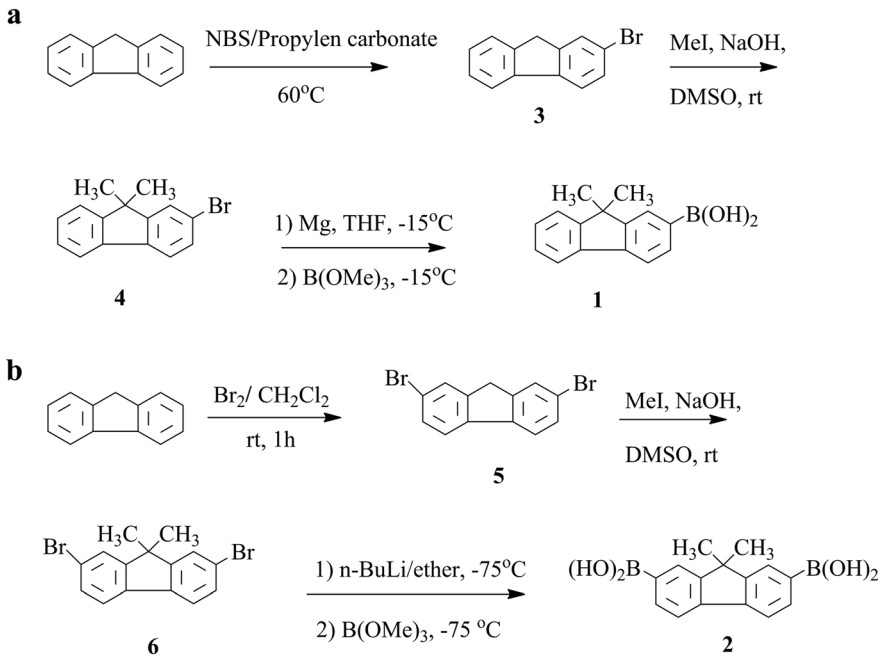
Where, I_{min} and I_{max} are the initial (no sugar) and final fluorescence intensity of the titration curves. In case of an apparent 1:1 complexation model, *n* = 1, and in the case of an apparent 1:2 complexation model, *n* = 2 (45,46).

Results and discussion

Synthesis

Herein we report the preparation of two boronic acid PET chemosensors based on fluorene as the fluorophore. Fluorene was used as the starting material for the synthesis of desired chemosensors (Scheme 1). First fluorene was brominated under suitable conditions and then methylated with

LUMINESCENCE
The Journal of Biological and Chemical Luminescence



Scheme 1. Synthesis of (a) monoboronic acid 1, and (b) diboronic acid 2.

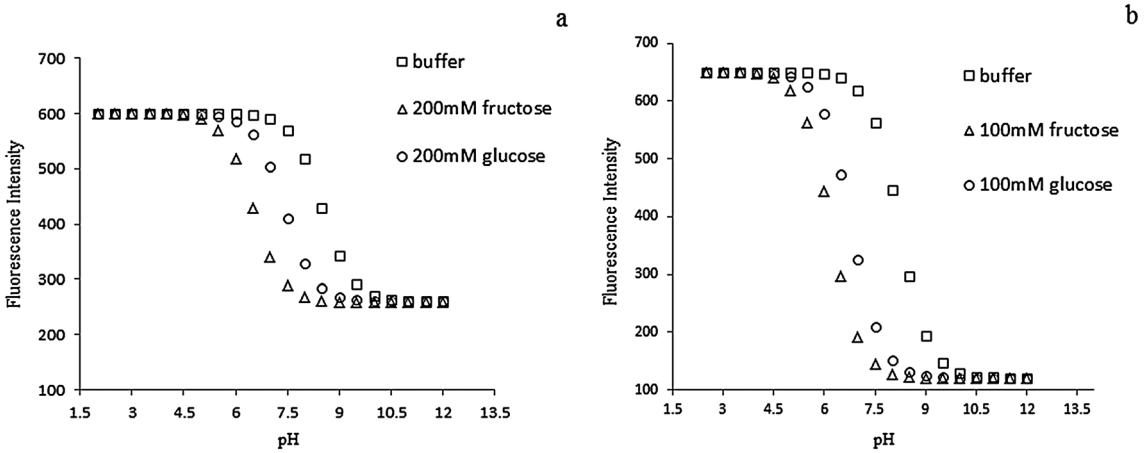


Figure 2. pH titration of the fluorescence intensity of (1 ×10^{-5} M) (a) monoboronic acid 1, and (b) diboronic acid 2 in the absence and presence of sugars.

iodomethane to give compound **4** and **6**. In the last step, the boronic acids were obtained from the reaction of corresponding organomagnesium (in the case of monoboronic acid **1**) and organolithium (in the case of diboronic acid **2**) with trimethylborate, followed by hydrolysis.

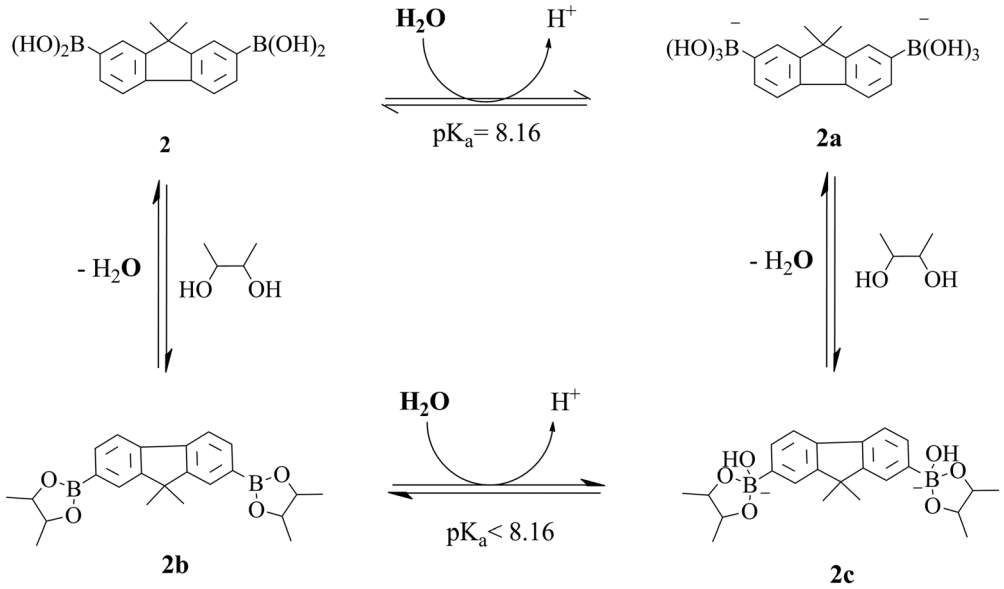
Fluorescence properties of chemosensor

Both compounds are fluorescent with emission maxima at about 325 nm and fluorescence quantum yields equal to 0.59 and 0.64 for **1** and **2**, respectively (phenol was used as the reference compound (35)).

It is well known that the fluorescence response of the boronic acid sensors as well as the binding of the boronic acid with poly hydroxyl compounds is pH-dependent. Therefore, the fluorescence-pH profile of sensor **1** and **2** in the absent and presence of near- saturating fructose and glucose solution (100 and 200 mM) was investigated (47–51), since this would allow a rapid preview of the optimal pH region of the boronic acid sensors (52,53). In the absence of any sugar, the emission intensity of **1** and **2** decrease upon changing the pH from 2 to 12, with an apparent pK_a of 8.5 for **1** and 8.19 for **2.** The pH titration of both compounds in the presence of 100 and 200 mM of saccharide (fructose and glucose)

Table 1. The pKa values of 1 and 2 in the absence and presence of fructose or glucose

Chemosensor	pK_a
1	8.5
1 + (250 mM) fructose	6.5
1 + (250 mM) glucose	7.4
2	8.19
2 + (100 mM) fructose	6.2
2 + (100 mM) glucose	6.8



Scheme 2. Equilibria available to an aqueous solution of boronic acid 2 in the presence of sugar. Species 2 and 2b are higher fluorescent than species 2a and 2c.

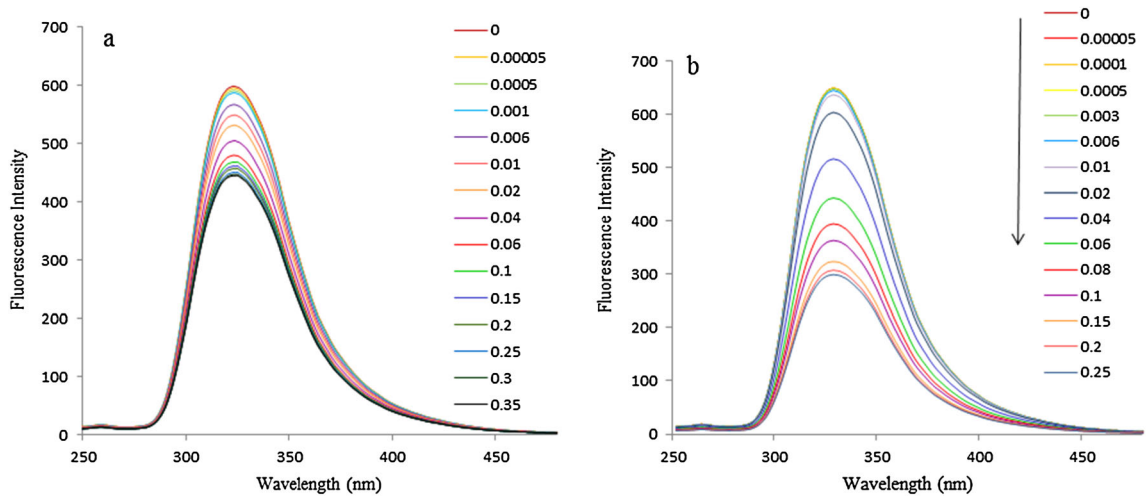


Figure 3. Fluorescence spectral changes of (a) 1 (1×10^{-5} M) upon addition of fructose (0–350 mM) in phosphate buffer (0.1 M) at pH 7.4, λ_{ex} = 287 nm, λ_{em} = 329 nm; (b) 2 (1×10^{-5} M) upon addition of fructose (0–250 mM) in phosphate buffer (0.1 M) at pH 7.4, λ_{ex} = 279 nm, λ_{em} = 323 nm.

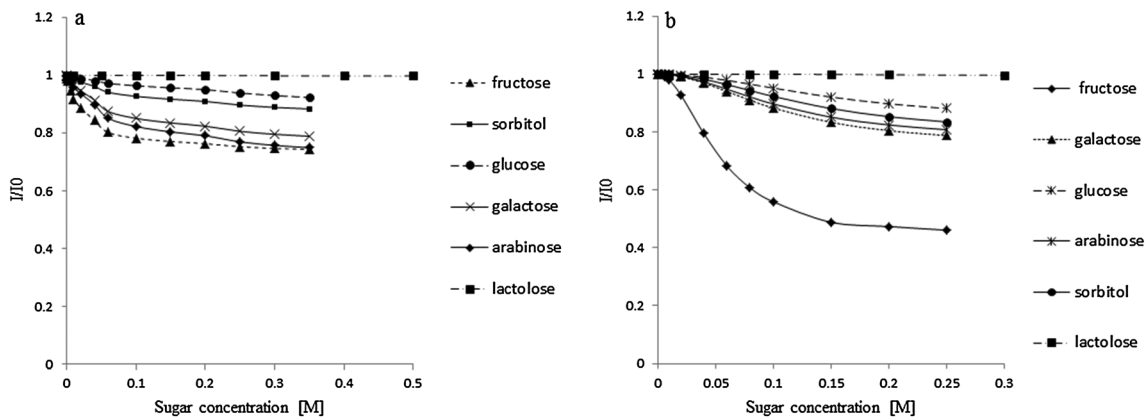


Figure 4. Relative fluorescence intensity versus sugars concentration profile of (a) monoboronic acid 1 and (b) diboronic acid 2.The measurement conditions are the same as those in Fig. 3.

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Table 2. Stability constant (K_s of fluorescent sensor 1 and 2 with D-saccharides in pH 7.4 buffer at λ_{ex} = 279 nm (sensor 1) and 287 nm (sensor 2)

Saccharides	1 K(M^{-1})	K(M^{-1})	K_2/K_1
Fructose	47.21±3.37	412.89±15	8.78
Glucose	11.38±1.5	84.75±9	7.40
Galactose	23.37±2.05	132.48±10.43	5.66
Sorbitol	17.91±1.76	100.7±9.94	5.62
Arabinose	23.19±2.04	125.79±10	5.42
Lactolose	3.37±0.6	8.82±1.29	2.61

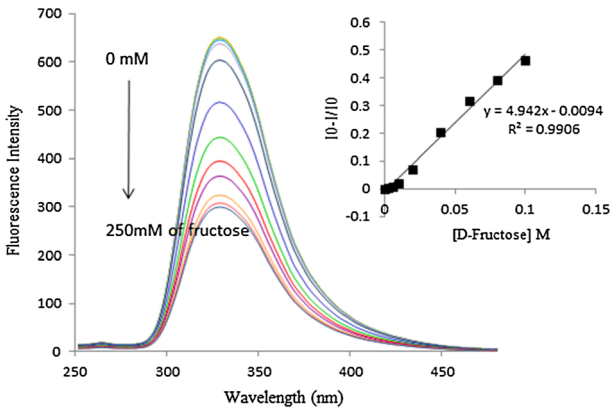


Figure 5. Fluorescence spectra of 2 (1×10^{-5} M) upon addition of fructose. The inset shows the changes of $(I_0 - I)/I_0$ versus the concentration of D-fructose.

reveals that increase of pH causes a decrease in fluorescence intensity (Fig. 2).

The pK_a values are listed in Table 1. Upon addition of fructose and glucose, the apparent pK_a value decrease. The explanation for this observation lies in the fact that, the formation of a boronic acid–saccharide complex acidifies the boron atom, making the resultant boronic ester more acidic than the initial uncomplexed boronic acid (50). In this example, as determined in the presence of a near-saturating amount of D-fructose (100 mM) the pKa of compound **1** decrease from 8.5 to 6.5. The greatest signal range available is therefore at a pH that is the average of corresponding pK_a of boronic acid and pK_a of boronic ester or 7.4 (47). Interestingly, a high-fluorescence emission intensity for the uncomplexed boronic acid (**2** in Scheme 2) ($pH < pK_a$) was observed. However, under these buffered conditions, addition of a saccharide to the solution formed the boronic ester (**2c**), lowering the acidity of the boronic species below the pH of the solution (pK_a ester $<$ pH). As a direct result, the boronate anion was generated inducing the decrease in fluorescence observed on addition of saccharides.

The fluorescence spectra of **1** and **2** (1×10^{-5} M) in the presence of fructose at different concentration in 0.1 M aqueous phosphate buffer (pH 7.4) are shown in Fig. 3.

The presence of fructose in both cases result in decrease in the fluorescence intensity. The larger fluorescence intensity changes are observed for compound **2** (about 54% decreases for 2 and 25% for **1**, Fig. 4).

To examine whether such changes are a general phenomenon for binding with other saccharides, we also studied the binding between **1** and **2** with sorbitol, galactose, arabinose and lactolose and the results are shown in Fig. 4.

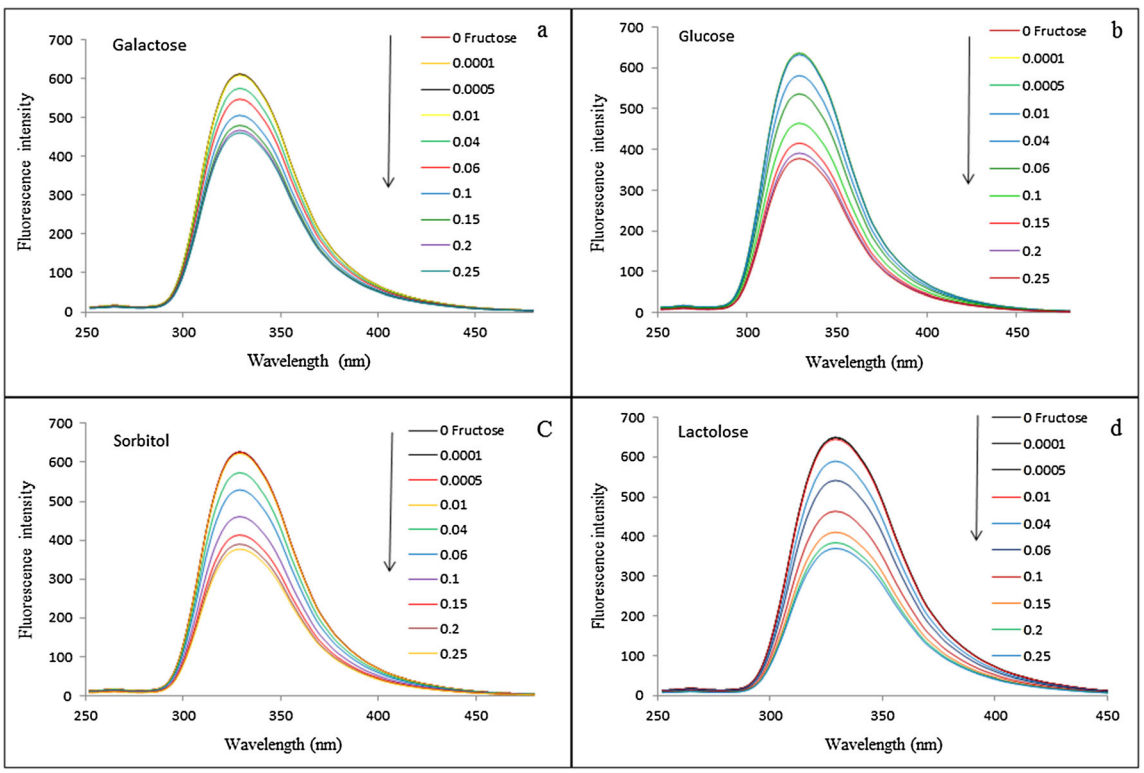


Figure 6. Fluorescence spectra of mixture of sensor 2 (1×10^{-5} M) in the 50% MeOH/phosphate buffer in the presence of different amount of fructose and 100 mM of (a) galactose, (b) glucose, (c) sorbitol and (d) lactolose.

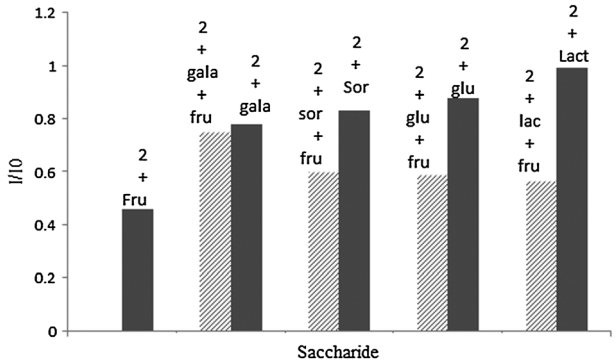


Figure 7. Relative fluorescence intensity of 2 to saccharides (solid bar) or to fructose in the presence of other saccharide interference (patterned bar) in phosphate buffer at pH 7.4.

Table 3. Selectivity coefficients of D-fructose over other sugar from 2

Saccharide	Selectivity coefficient of D fructose
Glucose	8.87
Galactose	6.33
Sorbitol	6.06
Arabinose	6.23
Lactolose	5.6

The stability constant (K_s) of fluorescence sensors **1** and **2** with fructose, D-glucose, galactose, sorbitol, arabinose and lactolose were calculated by fitting the emission wavelength at 323 and 329 nm versus concentration of saccharides (45,46). The stability constants for compound **1** and **2** are given in Table 2.

The observed order of stability constants (K_s) for sensor **2** is fructose$>$ galactose $>$ arabinose $\approx$ sorbitol $>$ glucose $>$ lactolose, and the calculated stability constant value is higher for **2** than **1** indicating a stronger interaction of this compound with sugar. The relative stability constants of the diboronic **2** to the monoboronic acid **1** are also given in Table 2.

As shown in Fig. 5 there is a good linear relationship between fluorescence intensity of **2** and the concentration of D-fructose in the range of from 5×10^{-5} to 10^{-1} mol L^{-1} with a correlation coefficient of R = 0.99. The detection limit based on the definition by IUPAC (CDL = 3S b/m) from 11 blank solutions was found to be 2×10^{-5} mol L^{-1}.

In the presence of glucose, galactose and sorbitol the fluorescence spectrum of sensor **2** was gradually intensified by the addition of fructose (Fig. 6), but the rate of decrease in intensity was lower (Fig. 7) because of the competition between fructose and other saccharides to bind with sensor **2**. The results shown in Fig. 7 indicate that diboronic acid **2** works well as a fructose-specific fluorescence sensor in both the absence and presence of saccharide interferent (54). In addition, the selectivity of **2** for D-fructose evaluated by the selectivity coefficient ($K_{D\text{-fructose}} = S_{D\text{-fructose}}/S_0$), where $S_{D\text{-fructose}}$ is the response to D-fructose and S_0 is the response to other sugar. The selectivity coefficients of D-fructose against other sugar are listed in Table 3. Clearly, the selectivity coefficient data indicated that the sensor **2** had a good selectivity for D-fructose over other sugars.

Conclusion

We have studied the interaction of two fluorene boronic acids with different saccharides. Our study shows that the **2** has a high affinity (412.89 M^{-1}) for D-fructose and can used in the sensing of this monosaccharide at physiological pH. In comparison with other similar boronic acid sensors, such as 2- and 9-anthrylboronic acid (47), 3-quinolineboronic acid (55) and stilbeneboronic acid (56), sensor **2** has a higher stability constant with D-fructose. Work is underway to synthesize new fluorene-based fluorescent boronic acid that fluoresce beyond 500 nm to sense important carbohydrates such as glucose in real samples.

Acknowledgements

Financial support from the research council of University of Mazandaran is gratefully acknowledged.

References

1. Liu HL, Peng Q, Wu YD, Chen D, Hou XL, Sabat M, Pu L. Highly enantioselective recognition of structurally diverse α-hydroxycarboxylic acids by a fluorescent sensor. Angew Chem 2010;49:602–6.
2. McDonagh C, Burke CS, MacCraith BD. Optical chemical sensors. Chem Rev 2008;108:400–22.
3. McQuade DT, Pullen AE, Swager TM. Conjugated polymer based chemical sensors. Chem Rev 2000;100:2537–74.
4. Han F, Chi L, Liang X, Ji S, Liu S, Zhou F, Wu Y, Han K, Zhao J, James TD. 3,6-Disubstituted carbazole-based bisboronic acids with unusual fluorescence transduction as enantioselective fluorescent chemosensors for tartaric acid. J Org Chem 2009;74:1333–6.
5. Abugo O, Hermann P, Lakowicz JR. Fluorescent measurements in whole blood and plasma using red-emitting dyes. Proc SPIE 2000;3913:145–59.
6. Bambot SB, Rao G, Romauld M, Carter GM, Sipior J, Terpetching E, Lakowicz JR. Sensing oxygen through skin using a red diode laser and fluorescence lifetime. Biosens Bioelectron 1995;10:643–52.
7. Heller A, Feldman B. Electrochemical glucose sensors and their applications in diabetes management. Chem Rev 2008;108:2482–505.
8. Famulok M, Hartig JS, Mayer G. Functional aptamers and aptazymes in biotechnology, diagnostics and therapy. Chem Rev 2007;107:3715–43.
9. Jayakanthan K, Mohan S, Pinto BM. Glycosidase inhibitors isolated from an herbal remedy for the treatment of type-2 diabetes. J Am Chem Soc 2009;131:5621–6.
10. Geddes CD, Lakowicz JR. *Topics in fluorescence spectroscopy: Glucose sensing*. Springer, Science Business Center: New York, 2005.
11. Valeur B, Brochon J. *New trends in fluorescence spectroscopy: application to chemical and life sciences*. New York: Springer, 2001; pp. 173–86.
12. James TD, Sandanayake K, Shinkai. Saccharide sensing with molecular receptors based on boronic acid. Angew Chem 1996;35:1910–22.
13. Zhiqian G, Injae S, Juyoung Y. Recognition and sensing of various species using boronic acid derivatives. Chem Commun 2012;48:5956–67.
14. Pizer R, Tihal C. Equilibria and reaction mechanism of the complexation of methylboronic acid with polyols. Inorg Chem 1992;31:3243–7.
15. James TD, Shinkai S. Artificial receptors as chemosensors for carbohydrates. Top Curr Chem 2002;218:159–200.
16. Chi L, Zhao J, James TD. Chiral mono boronic acid as fluorescent enantioselective sensor for mono α-hydroxyl carboxylic acids. J Org Chem 2008;73;4684–7.
17. Zhang X, Chi L, Ji S, Wu Y, Song P, Keli Han J, Guo H, James TD, Zhao J. Rational design of d-PeT phenylethynylated-carbazole monoboronic acid fluorescent sensors for the selective detection of α-hydroxyl carboxylic acids and monosaccharides. J Am Chem Soc 2009;131:17452–63.

18. Bull SD, Davidson MG, Vandenelsen JMH, Fossey JS, Jenkins ATA, Jiang Y, Kubo Y, Marken F, Sakurai K, Zhao J, James TD. Exploiting the reversible covalent bonding of boronic acids: recognition, sensing, and assembly. Acc Chem Res 2013;46:312–26.
19. Gao X, Zhang Y, Wang B. New boronic acid fluorescent reporter compounds. 2. A naphthalene-based on–off sensor functional at physiological pH. Org Lett 2003;5:4616–8.
20. Wenqian Y, Li L, Wang B. A new type of boronic acid fluorescent reporter compound for sugar recognition. Tetrahedron Lett 2005;46:7981–4.
21. DiCesare N, Lakowicz JR. New color chemosensors for monosaccharides based on azo dyes. Org Lett 2001;3:3891–3.
22. Trupp S, Schweitzer A, Mohr GJ. A fluorescent water-soluble naphthalimide-based receptor for saccharides with highest sensitivity in the physiological pH range. Org Biomol Chem 2006;4:2965–8.
23. Cao HS, Diaz DI, DiCesare N, Lakowicz JR, Heagy MD. Monoboronic acid sensor that displays anomalous fluorescence sensitivity to glucose. Org Lett 2002;4:1503–5.
24. Jin S, Wang J, Li M, Wang B. Synthesis, evaluation and computational studies of naphthalimide-based long-wavelength fluorescent boronic acid reporters. Chem Eur J 2008;14:2795–804.
25. Wenqian Y, Jun Y, Springsteen G, Deeter S, Wang B. A novel type of fluorescent boronic acid that shows large fluorescence intensity changes upon binding with a carbohydrate in aqueous solution at physiological pH. Bioorg Med Chem Lett 2003;13:1019–22.
26. Belfield KD, Schafer KJ, Mourad W, Reinhardt BA. Synthesis of new two-photon absorbing fluorene derivatives via Cu-mediated ullmann condensations. J Org Chem 2006;5:4475–81.
27. Kannan R, He GS, Yuan L, Xu F, Prasad PN, Dombroskie AG, Reinhardt BA, Baur JW, Vaia RA, Tan LS. Diphenylamino fluorene-based two-photon-absorbing chromophores with various π-electron acceptors. Chem Mater 2001;13:1896–904.
28. Mongin O, Porres L, Katan C, Pons T, Mertz J, Blanchard-Desce M. Synthesis and two-photon absorption of highly soluble three-branched fluorenylene-vinylene derivatives. Tetrahedron Lett 2011;44:8121–5.
29. Droumaguet CL, Mongin O, Werts MHV, Blanchard-Desce M. Towards smart multiphoton fluorophores: strongly solvatochromic probes for two-photon sensing of micropolarity. Chem Commun 2005;2802–4.
30. Belfield KD, Bondar MV, Hernandez FE, Przhonska OV, Yao S. Two-photon absorption cross section determination for fluorene derivatives: analysis of the methodology and elucidation of the origin of the absorption. J Phys Chem B 2007;111:12723–9.
31. Nguyen KA, Rogers JE, Slagle JE, Day PN, Kannan R, Tan LS, Fleitz PA, Pachter R. Effects of conjugation in length and dimension on spectroscopic properties of fluorene based chromophores from experiment and theory. J Phys Chem A 2006;110:13172–82.
32. Lakowicz JR. *Principles of fluorescence spectroscopy*. Maryland, USA: Plenum Press, 2006.
33. Sidney DR, Finkelstein M, Peterson R. Solvent effects in the reactions of n-bromosuccinimide with toluene, fluorene and acenaphthene, evidence for a polar mechanism in propylene carbonate. J Am Chem Soc 1958;80:4327–30.
34. Wei W, Djurovich PI, Thompson EM. Properties of fluorenyl silanes in organic light emitting diodes. Chem Mat 2010;22:1724–31.
35. Xiaowei Z, Chad R, Fabrice A, Calvin C, Wei Z, Stephen B, Antoine K, Jean-Luc B, Seth RM. Electron Affinities of 1,1-diaryl-2,3,4,5-tetraphenylsiloles: direct measurements and comparison with experimental and theoretical estimates. J Am Chem Soc 2005;127:9021–9.
36. Louis AC. Convenient preparation of (9-fluoreny1)methanol and its 2,7-dihalo derivatives. J Org Chem 1980;45:4250–2.
37. Ding J, Day M, Robertson G, Roovers J. Synthesis and characterization of alternating copolymers of fluorene and oxadiazole. Macromolecules 2002;35:3474–83.
38. Saroja GS, Pingzhu Z, Ernsting N, Liebsche J. Synthesis of alkylated aminofluorenes by palladium-catalyzed substitution at halofluorenes. J Org Chem 2004;69:987–90.
39. Huang HF, Xu SH, He YB, Zhu CC, Fan HL, Zhou XH, Goa XC, Da YF. Synthesis and characterization of highly stable and efficient star-molecules. Dyes Pigments 2013;96:705–13.
40. Clandingboel DE. A large, laboratory-scale synthesis of [4-(2-(2H)-tetrahydropyranyloxy)phenyl]boronic acid. Org Process Res Dev 2000;4:153–5.
41. Suzuki K, Seno A, Tanabe H, Ueno K. New host materials for blue emitters. Synt Met 2004;143:89–96.
42. Badugu R, Lakowics JR, Geddes C. Boronic acid fluorescent sensors for monosaccharide signaling based on the 6-methoxyquinolinium heterocyclic nucleus: progress toward noninvasive and continuous glucose monitoring. Bioorg Med Chem 2005;13:113–9.
43. Dicesare N, Adhikari DP, Heynekamp J, Heagy M, Lakowicz JR. Spectroscopic and photophysical characterization of fluorescent chemosensors for monosaccharides based on N-phenylboronic acid derivatives of 1,8-naphthalimide. J Fluoresc 2002;12:147–54.
44. Xingming G, Yanling Z, Binghe W. A highly fluorescent water-soluble boronic acid reporter for saccharide sensing that shows ratiometric UV changes and significant fluorescence changes. Tetrahedron 2005;61:9111–7.
45. Connors K. *Binding constants: The measurement of molecular complex stability*. Wiley-Interscience publication: New York, 1987.
46. Liu SY, He YB, Chanb WH, Lee AWM. Cholic acid-based high sensitivity fluorescent sensor for α,ω-dicarboxylate: an intramolecular excimer emission quenched by complexation. Tetrahedron 2006;62:11687–96.
47. Yoon J, Czarnik AW. Fluorescent chemosensors of carbohydrates. a means of chemically communicating the binding of polyols in water based on chelation-enhanced quenching. J Am Chem Soc 1992;114:5874–5.
48. Wang J, Jin S, Wang B. A new boronic acid fluorescent reporter that changes emission intensities at three wavelengths upon sugar binding. Tetrahedron Lett 2005;46:7003–6.
49. Liang X, James TD, Zhao J. 6,6′-Bis-substituted BINOL boronic acids as enantioselective and chemoselective fluorescent chemosensors for D-sorbitol. Tetrahedron 2008;64:1309–15.
50. James TD, Phillips MD, Shinkai S. *Boronic acids in saccharide recognition*. Cambridge: RSC Publishing, 2006.
51. Yan J, Springsteen G, Deeter S, Wang B. The relationship among pKa, pH, and binding constants in the interactions between boronic acids and diols-it is not as simple as it appears. Tetrahedron 2004;60:11205–9.
52. Mader HS, Wolfbeis OS. Boronic acid based probes for microdetermination of saccharides and glycosylated biomolecules. Microchim Acta 2008;162:1–34.
53. Springsteen G, Wang B. A detailed examination of boronic acid-diol complexation. Tetrahedron 2002;52:5291–300.
54. Liu Y, Deng C, Tang L, Qin A, Hu R, Sun JZ, Zhong-Tang B. Specific detection of D-glucose by a tetraphenylethene-based fluorescent sensor. J Am Chem Soc 2011;13:660–3.
55. Qian JSh, Wei JJ. Chemical and photophysical mechanism of fluorescence enhancement of 3-quinolineboronic acid upon change of pH and binding with carbohydrates. Luminescence 2011;26:494–9.
56. DiCesare N, Lakowicz JR. Spectral properties of fluorophores combining the boronic acid group with electron donor or withdrawing groups. implication in the development of fluorescence probes for saccharides. J Phys Chem A 2001;105:6834–40.