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A new type of boronic acid fluorescent reporter compound for sugar recognition

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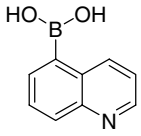
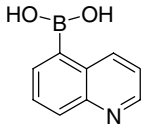
Abstract—Fluorescent boronic acids that change fluorescent properties upon carbohydrate binding are very useful for the preparation of fluorescent sensors for sugars. Herein we report 5-quinolineboronic acid (5-QBA) that shows significant fluorescent property changes through a unique pK_a-switching mechanism upon binding a diol in aqueous solution.



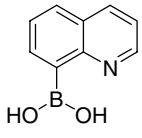
5-quinolineboronic acid (5-QBA)

Carbohydrates play a critical role in a variety of biological processes.[1] Lectin[2] mimetics that can recognize certain carbohydrates with high affinity and specificity under near physiological conditions can be used in sensing, diagnostic, and therapeutic applications. In preparing compounds for carbohydrate recognition, the boronic acid moiety holds a special place because of the ability for boronic acid to form tight and reversible complexes with diols,[3] which are commonly found on carbohydrates. Several laboratories have devoted much effort in this area to developing boronic acid-base fluorescent sensors for carbohydrates.[4,5] Our laboratory and others have been especially interested in the development of boronic acid compounds that can recognize complex carbohydrate biomarkers.[6] These compounds are essentially lectin mimetics and are therefore referred to as boronolectins.[3d,4e,h] Our aim is to develop these boronolectins for diagnostic and therapeutic applications. Along this line, we have studied fluorescent labeling of mammalian cells based on their cell-surface carbohydrate biomarkers using small molecule chemosensors.[6a,b] In our continuing effort to search for fluorescent boronolectins for various biologically important carbohydrates, we are interested in developing a combinatorial approach. In order to increase the structural and spectroscopic diversity in our libraries, we are in need of boronic acids of different structural classes that (1) change fluorescent properties upon binding and (2) are water soluble.[3d,4g,7]

Herein we report 5-quinolineboronic acid (5-QBA) as a fluorescent reporter for carbohydrates, which shows large fluorescence intensity changes upon binding with carbohydrates in aqueous solution at physiological pH. More important, 5-QBA also shows an unique pK_a-switching between the quinolinium and boronic acid groups upon binding with a diol and has much stronger binding with each individual sugar compared with 8-QBA.



5-QBA



8-QBA

5-QBA itself is essentially non-fluorescent at pH above 5 and weakly fluorescent at lower pH in aqueous solution. However, upon addition of D-fructose, the fluorescence intensity increased dramatically in a concentration-dependent manner (Fig. 1). In an effort to examine the

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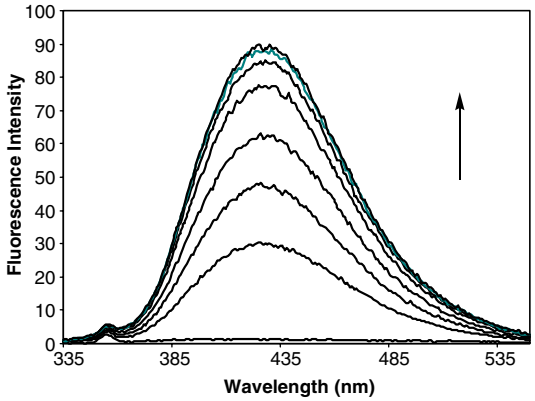


Figure 1. Fluorescence spectra of 5-QBA (5.8×10^{-5} M) upon addition of D-fructose (0, 0.5, 1.0, 2.0, 5.0, 10, 20, 50 mM) in 0.1 M phosphate buffer at pH 7.4: $\lambda_{ex} = 315$ nm.

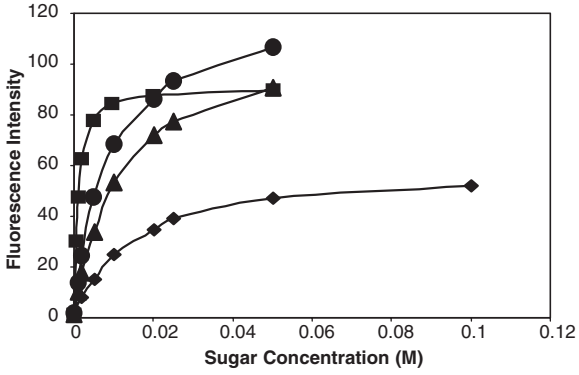


Figure 2. Fluorescence intensity of 5-QBA (5.8×10^{-5} M) in 0.10 M phosphate buffer at pH 7.4 in the presence of D-fructose (■), D-galactose (▲), D-mannose (♦), and L-arabinose (●): $\lambda_{ex} = 315$ nm, $\lambda_{em} = 425$ nm.

generality of this phenomenon, a few other sugars were tested. Figure 2 shows the concentration profiles of fructose, arabinose, galactose, and mannose with binding constants of 924 ± 88, 121 ± 16, 90 ± 2, and $60 \pm 2\ M^{-1}$, respectively. It is interesting to point out that the binding constants of 5-QBA with different monosaccharides are significantly higher compared to 8-QBA.[7b] For example, the binding constant of 5-QBA with D-fructose is 8 times that of 8-QBA.

Since the increase in fluorescence intensity upon binding with a sugar seems to be a general phenomenon, next we were interested in examining how 5-QBA functions as a fluorescent probe for diols. Therefore, we studied the fluorescence pH profiles of both 5-QBA alone and 5-QBA in the presence of D-fructose (0.5 M). Based on the fluorescence intensity changes, only one apparent pK_a at 5.2 was observed for 5-QBA in the absence of sugar and two apparent pK_a values at 3.5 and 6.6 were observed in the presence of fructose (Fig. 3). The UV pH titration of 5-QBA and its ester did not provide useful information for the pK_a determination (data not shown). In order to assign each pK_a, we recorded the ^{11}B NMR spectra of 5-QBA and its ester in a mixed

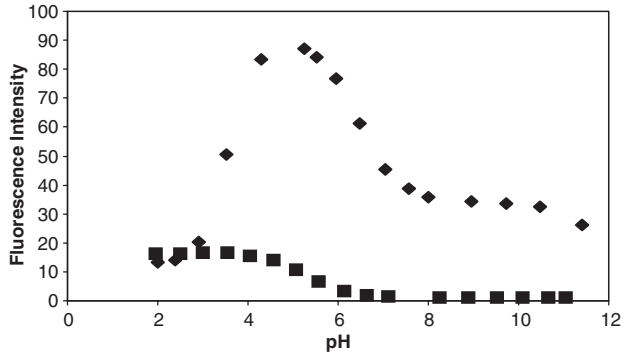
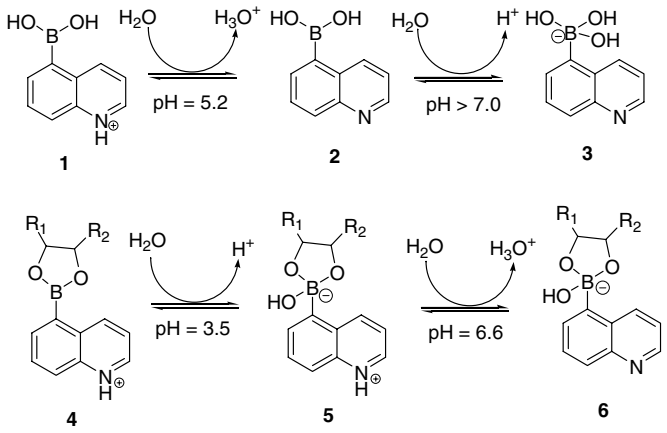


Figure 3. Fluorescence intensity pH profile of 5-QBA (5.8×10^{-5} M) in 0.10 M phosphate buffer: $\lambda_{ex} = 315$ nm, $\lambda_{em} = 425$ nm. (■) 5-QBA, (♦) 5-QBA + 0.5 M D-fructose.

deuterated methanol–water (1:1) solvent at different pH. Methanol was used so that the concentration of 5-QBA can be increased to 57 mM so as to allow for a meaningful NMR determination within a reasonable period of time. It should be noted that the addition of 50% methanol to water solution results in minimal changes of the solution pH.[8] The boron signal of 5-QBA appeared at 30.0 ppm at both pH 2.0 and 7.0, consistent with the neutral trigonal boron (**1** or **2**). At an increased pH (7.5), two boron signals at 30.0 and 10.0 ppm were observed indicating a mixture of both the neutral (**1** and **2**) and anionic (**3**) species. It should be noted that in Anslyn's work with a different boronic acid compound, a single boron peak was observed at different pH reflecting an average of two species.[9] In this case, the appearance of two peaks at pH 7.5 indicates that the inter-conversion between the anionic and neutral species must be sufficiently slow with 5-QBA so that the two ionization states can be observed individually. At pH 12, a single peak at 5.7 ppm was found indicating the presence of only the anionic tetrahedral state (**3**). These results indicated that the boron of the free acid changed hybridization from sp^2 to sp^3 between pH 7.0 and 12.[5k] Thus, we assigned the first pK_a of 5-QBA at 5.2 to the quinolinium nitrogen, and the second pK_a corresponding to boronic acid ionization must be between 7.0 and 12. This pK_a assignment is opposite to that of 8-QBA. For the fructose ester of 5-QBA, two pK_a values were observed: 3.5 and 6.6. In the B NMR, we observed the same chemical shifts (at about 10.5 ppm) at pH 6.0 and 11. This clearly indicated that the boron in the ester did not change hybridization state between pH 6.0 and 11. Therefore, it is reasonable to assign the first pK_a at 3.5 to the boronic ester group, and the second pK_a at 6.6 to the quinolinium nitrogen. Such results indicate that the pK_a of the boronic acid is higher in the absence of a sugar, but lower in the presence of a sugar than that of the quinolinium nitrogen. Such a pK_a-switching seems to correspond to the highest fluorescence intensity change at pH 5.8 (Fig. 3), which suggest that the zwitterionic species **5** is the more fluorescent one (Scheme 1).

Past work in searching for new boronic acid-based fluorescent reporter compounds has been focused on exploring the inductive effect of boronic ester formation on a



Scheme 1. The ionization steps of 5-QBA and its esters.

conjugated π chromophoric system[4g,3d] and/or the utilization of B–N interactions.[10] We recently reported that 8-QBA could show large fluorescence intensity change due to diols binding.[7b] In this study, the fluorescence intensity increase of 5-QBA upon binding with sugars is observable in the whole range of pH above 3.5, suggesting that 5-QBA can be used for monitoring sugar within a large pH range. Of course, measuring saccharide concentrations at physiological pH is most useful. At pH 7.4, 5-QBA ester exists predominantly in the boronate form (**6**) and 5-QBA itself exists as a mixture of the boronic acid form (**2**) and the boronate form (**3**). Both species **2** and **3** are non-fluorescent, and yet the boronate ester form **6** is fluorescent. It seems that the fluorescence increase of 5-QBA at pH 7.4 is also due to the diol binding as that of 8-QBA.[7b] However, it should be pointed out that the zwitterionic quinolinium boronate form (**5**) is more fluorescent than the boronate **6**. This does provide room for further improvement in sensitivity for this sensor system by moving the fluorescence intensity to maximum range of the corresponding ester beyond pH 7.4. It is conceivable that such manipulation can be achieved through modulating the nitrogen pK_a of 5-QBA with the introduction of different substituents.

The availability of 5-QBA-based fluorescent reporter compounds will be very useful to our combinatorial effort in search of fluorescent sensor for cell-surface carbohydrates. In our earlier efforts of making such cell-surface carbohydrate sensors using an anthracene-based fluorophore,[6a,b] it was always necessary to add some organic co-solvent (commonly methanol) for the cell-labeling studies due to their poor water solubility. The need for organic co-solvent can be tolerated in an in vitro experiment, but not in an in vivo experiment. The availability of water-soluble fluorescent reporter compounds such as 5-QBA will significantly help the effort of making biocompatible fluorescent sensors for cell-surface carbohydrates biomarkers.

In conclusion, 5-QBA was found to be a fluorescent reporter compound with many desirable properties for biosensor preparation. Such properties include (1) large fluorescence intensity changes upon binding and (2) being functional in aqueous solution at physiological pH. Furthermore, 5-QBA shows a pK_a-switching between the quinolinium and boronic acid groups upon binding a diol in aqueous solution. The same principle can be used for the design of other new boronic acid-based sensors. Work is underway to use this new type of fluorescent boronic acid compounds in our combinatorial synthesis of diboronic acid compounds for high selectivity and affinity recognition of carbohydrates of biological interest.[6a,b]

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References and notes

1. (a) Fukuda, M.; Hindsgaul, O. *Molecular Glycobiology*; Oxford University Press: New York, 1994; pp 1–52; (b) Alavi, A.; Axford, J. S. Glycoimmunology In *Adv. Exp. Med. Biol.*; Plenum Press: New York, 1995; Vol. 376, (c) Brodesser, S.; Sawatzki, P.; Kolter, T. *Euro. J. Org. Chem.* **2003**, *11*, 2021–2034.
2. (a) Gabius, H.-J.; Gabius, S. *Lectins and Glycobiology*; Springer: New York, 1993; (b) Danguy, A.; Camby, I.; Kiss, R. *Biochem. Biophys. Acta-Gen. Subj.* **2002**, *1572*, 285–293; (c) Bidon-Wagner, N.; Le Pennec, J. P. *Glycoconjug. J.* **2004**, *19*, 557–563.
3. For general references on various factors that affect the binding affinity and boronic acid-based sensor design, see: (a) Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769; (b) Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291–5300; (c) Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Tetrahedron* **2004**, *60*, 11205–11209; (d) Fang, H.; Yan, J.; Wang, B. *Med. Res. Rev.* **2005**, *25*, 490–520.
4. For reviews, see: (a) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159–200; (b) Wiskur, S. L.; Lavigne, J. J.; Metzger, A.; Tobey, S. L.; Lynch, V.; Anslyn, E. V. *Chem.-A Eur. J.* **2004**, *10*, 3792–3804; (c) Hartley, J. H.;

James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155–3184; (d) Wang, W.; Gao, X.; Wang, B. *Curr. Org. Chem.* **2002**, *6*, 1285–1317; (e) Yang, W.; Gao, X.; Wang, B. *Med. Res. Rev.* **2003**, *23*, 346–368; (f) Striegler, S. *Curr. Org. Chem.* **2003**, *7*, 81–102; (g) Cao, H. S.; Heagy, M. D. *J. Fluorescence* **2004**, *14*, 569–584; (h) Yang, W.; Gao, S.; Wang, B. In *Organoboronic Acids*; Hall, D., Ed.; John Wiley and Sons: New York, 2005; pp 481–512; (i) Shinkai, S.; Takeuchi, M. *Trends Anal. Chem.* **1996**, *15*, 188–193.

5. For examples see: (a) Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874–5875; (b) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Chem. Commun.* **1994**, 477–478; (c) Eggert, E.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem.* **1999**, *64*, 3846–3852; (d) Yang, W.; Yan, J.; Fang, H.; Wang, B. *Chem. Commun.* **2003**, 792–793; (e) Wang, W.; Gao, S.; Wang, B. *Org. Lett.* **1999**, *1*, 1209–1212; (f) Yang, W.; He, H.; Drueckhammer, D. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 1714–1718; (g) Cao, H.; Diaz, D. I.; DiCesare, D.; Lakowicz, J. R.; Heagy, M. D. *Org. Lett.* **2002**, *4*, 1503–1506; (h) DiCesare, N.; Lakowicz, J. R. *Anal. Biochem.* **2001**, *294*, 154–160; (i) Ward, C. J.; Patel, P.; Ashton, P. R.; James, T. D. *Chem. Commun.* **2000**, 229–230; (j) Alexeev, V. L.; Sharma, A. C.; Goponenko, A. V.; Das, S.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N.; Asher, S. A. *Anal. Chem.* **2003**, *75*, 2316–2323; (k) Davis, C. J.; Lewis, P. T.; McCarroll, M. E.; Read, M. W.; Cueto, R.; Strongin, R. M. *Org. Lett.* **1999**, *1*, 331–334; (l) Westmark, P. R.; Gardiner, S. J.; Smith, B. D. *J. Am. Chem. Soc.* **1996**, *118*, 11093–11100; (m) Draffin, S. P.; Duggan, P. J.; Duggan, S. A. M. *Org. Lett.* **2001**, *3*, 917–920; (n) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666–3669; (o) Stones, D.; Manku, S.; Lu, X.; Hall, D. G. *Chem. Eur. J.* **2004**, *10*, 92–100; (p) Irving, A. M.; Vogels, C. M.; Nikolcheva, L. G.; Edwards, J. P.; He, X. F.; Hamilton, M. G.; Baerlocher, M. O.; Baerlocher, F. J.; Decken, A.; Westcott, S. A. *New J. Chem.* **2003**, *27*, 1419–1424; (q) Mulla, H. R.; Agard, N. J.; Basu, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 25–27; (r) Karnati, V. R. R.; Gao, X.; Gao, S.; Yang, W.; Ni, W.; Wang, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3373–3377; (s) Rusin, O.; Alpturk, O.; He, M.; Escobedo, J. O.; Jiang, S.; Dawan, F.; Lian, K.; McCarroll, M. E.; Warner, I. M.; Strongin, R. M. *J. Fluorescence* **2004**, *14*, 611–615; (t) Kramp, K. L.; DeWitt, K.; Flora, J. W.; Muddiman, D. C.; Slunt, K. M.; Houston, T. A. *Tetrahedron Lett.* **2005**, *46*, 695–698.

6. (a) Yang, W.; Fan, H.; Gao, S.; Gao, X.; Ni, W.; Karnati, V.; Hooks, W. B.; Carson, J.; Weston, B.; Wang, B. *Chem. Biol.* **2004**, *11*, 439–448; (b) Yang, W.; Gao, S.; Gao, X.; Karnati, V. V. R.; Ni, W.; Wang, B.; Hooks, W. B.; Carson, J.; Weston, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2175; (c) Burnett, T. J.; Peebles, H. C.; Hageman, J. H. *Biochem. Biophy. Res. Comm.* **1980**, *96*, 157–162; (d) Patterson, S.; Smith, B. D.; Taylor, R. E. *Tetrahedron Lett.* **1998**, *39*, 3111–3114; (e) Zhang, Z. Y.; Smith, B. D. *J. Am. Chem. Soc.* **1998**, *120*, 7141–7142; (f) Kramp, K. L.; DeWitt, K.; Flora, J. W.; Muddiman, D. C.; Slunt, K. M.; Houston, T. A. *Tetrahedron Lett.* **2005**, *46*, 695–698.

7. (a) Gao, X.; Zhang, Y.; Wang, B. *Tetrahedron* **2005**, *61*, 9111–9117; (b) Yang, W.; Springsteen, G.; Yan, J.; Deeter, S.; Wang, B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1019–1022; (c) Gao, X.; Zhang, Y.; Wang, B. *Org. Lett.* **2003**, *5*, 4615–4618; (d) Yang, W.; Lin, L.; Wang, B. *Heterocycl. Commun.* **2004**, *10*, 383–388.

8. Bates, R. G. *Determination of pH*; John Wiley & Sons, Inc.: London, 1964.

9. Wiskur, S. L.; Lavigne, J. L.; Ait-Haddou, H.; Lynch, V.; Chiu, Y. H.; Canary, J. W.; Anslyn, E. V. *Org. Lett.* **2001**, *3*, 1311–1314.

10. See Ref. 5b; (a) Ni, W.; Kaur, G.; Springsteen, G.; Wang, B.; Franzen, S. *Bioorg. Chem.* **2004**, 32; (b) Franzen, S.; Ni, W.; Wang, B. *J. Phys. Chem. B* **2003**, *107*, 12942–12948.