Available online at www.sciencedirect.com

Bioorganic & Medicinal Chemistry Letters 14 (2004) 25–27

Bioorganic & Medicinal Chemistry Letters

3-Methoxycarbonyl-5-nitrophenyl boronic acid: high affinity diol recognition at neutral pH

Hormuzd R. Mulla, Nicholas J. Agard and Amit Basu*

Chemistry Department, Brown University, Box H, Providence, RI 02912, USA

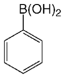
Received 13 August 2003; revised 29 September 2003; accepted 15 October 2003

Abstract—Several boronic acids were screened for their ability to bind to diols. 3-Methoxycarbonyl-5-nitrophenyl boronic acid bound to both a catechol dye as well as fructose with a comparable affinity to that of an *ortho*-methylamino substituted boronic acid. This work suggests a greater role for appropriately functionalized electron deficient boronic acids in diol and carbohydrate recognition.

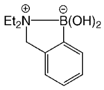
In this paper, we report that a phenyl boronic acid suitably substituted with a nitro group can bind to a sugar in aqueous solution at neutral pH with reasonable affinity. Boronic acids are commonly used as carbohydrate recognition motifs because they react with some 1,2 and 1,3 diols to form boronate esters.[1–4] Although the boronate esters can form in aqueous solution, the equilibrium constant for their formation is generally not very large at neutral pH. Higher association constants can be obtained under basic conditions (generally pH = 10), where the population of the tetrahedral boronate form is greater.[1,5]

In order to obtain higher association constants at neutral pH, an *ortho* aminomethyl substituent is often introduced, as seen in compound **2**. Introduction of the Lewis basic aminomethyl group facilitates boronate ester formation due to the donation of the nitrogen lone pair into the empty boron *p*-orbital.[6] The benzylic amine functionality is most often introduced by reductive amination of the corresponding benzaldehyde or by nucleophilic displacement of a benzylic bromide. However, once formed, the purification and subsequent synthetic manipulation of these types of compounds is often problematic due to the zwitterionic and amphoteric character of the B–N moiety. As part of an ongoing project for the development of oligosaccharide sensors, we sought an alternative boronic acid scaffold that would retain an appreciable affinity for carbohydrates at physiological pH, but without recourse to the prosthetic benzyl amino functionality.

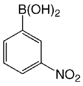
The introduction of electron withdrawing groups onto the ring of a phenyl boronic acid stabilizes the boronate form of the acid and lowers the pK_a value, which in turn favors ester formation at a lower pH.[7] We reasoned that an electron deficient aryl boronic acid may have an appreciable affinity for a carbohydrate at neutral pH.[8] Therefore, we screened several commercially available boronic acids (compounds **3**–**9**) which varied in the location and identity of electron withdrawing groups on the ring. Phenyl boronic acid (PBA) **1** and the *N*,*N*-diethyl aminomethyl boronic acid **2** were included for comparison.[9]



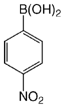
1



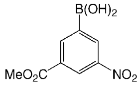
2



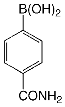
3



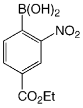
4



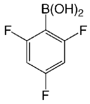
5



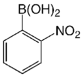
6



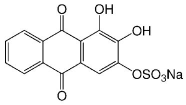
7



8



9



10

We used a fluorimetric assay recently reported by Springsteen and Wang to initially determine the binding constants of the boronic acids **1**–**9** with the catechol dye Alizarin Red S (ARS, **10**).[10] The fluorescence of ARS increases significantly upon binding, providing a con-

---

[*] Corresponding author. Tel.: +1-401-863-9897; fax: +1-253-540-0698; e-mail: amit_basu@brown.edu

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.10.017

Table 1. Binding constants of boronic acids with ARS in 0.1 M PBS buffer with 2.5–3.8% THF by volume (pH = 7.5)

Boronic Acid	K_a (M^{-1})
1	1500 ± 140
2	8110 ± 95
3	6110 ± 75
4	4660 ± 370
5	3910 ± 130
6	3510 ± 65
7	1130 ± 60
8	950 ± 260
9	615 ± 150

All studies were done in triplicate.

venient spectroscopic method for determining association constants. Thus, monitoring the fluorescence of ARS as a function of increasing boronic acid concentration allowed the calculation of the binding constant (K_a) upon Benesi–Hildebrand treatment of the data.[11] The measured K_a values are shown in Table 1.

The K_a value for **1** was found to be 1500 M^{-1}, in close agreement with the reported value.[6] As expected, the presence of the benzylic amine in **2** afforded a large increase in the association constant. The introduction of electron withdrawing substituents such as a carboxamide (**6**) or nitro (**3**–**5**) enhanced the association significantly relative to **1**. However, substitution *ortho* to the boronic acid (**7**–**9**) resulted in K_a values lower than that for PBA.[12] These primary results show that the presence of a nitro group *meta* or *para* to the boronic acid results in high affinities for ARS.

Although boronic acids **5** and **6** have K_a values for ARS slightly less than half that of the benzylamine substituted boronic acid **2**, they both contain functional groups which can serve as sites for further synthetic elaboration. The carboxyl group can be used to covalently attach the boronic acid to a variety of moieties, allowing its use with diverse sensor scaffolds. Since compound **5** has a slightly higher K_a value than **6**, we examined it in greater detail.

We determined the pK_a of **5** by monitoring the decrease in absorbance at 223 nm as a function of increasing pH, as shown below (Fig. 1). The measured pK_a value of 6.9 is lower than that for **1** (8.8),[5] and higher than that for an *ortho*-benzylamine substituted boronic acid similar to **2** (5.8), which exists almost exclusively as the boronate at neutral pH.[13] In contrast, although a neutral solution of **5** still contains appreciable amounts of trigonal boronic acid, the majority exists as the boronate, which should facilitate binding to a sugar.

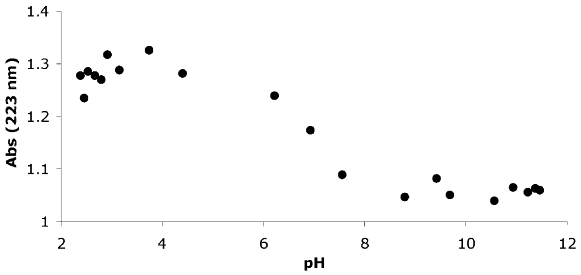


Figure 1. Determination of pK_a of **5** by monitoring change in absorbance at 223 nm as function of pH. (0.3 mM solution of **5** in H_2O with 1.5% THF by volume, pH adjusted with HCl/NaOH).

While binding to ARS provides a quick method for screening boronic acid–diol interactions, the electronic and structural properties of a catechol are very different from that of the aliphatic diols found in a carbohydrate.[14] In order to study the complexation of **5** with a sugar, we chose to examine its binding to fructose. Of all the readily available monosaccharides, fructose generally has the highest binding constants for most mono-boronic acid receptors, and provides a useful comparison for the efficacy of **5** compared to other boronic acids.[15]

The binding of **5** and **2** with fructose was determined by a competition assay using ARS as the indicator.[5] Addition of increasing concentrations of fructose to a solution of the highly fluorescent boronic acid·ARS complex displaces the bound ARS and decreases the observed fluorescence. The binding constant between the boronic acid and fructose can then be calculated using standard methods.[11] The K_a value for the binding of fructose to **5** was found to be 1350 ± 270 M^{-1}, and the K_a for **2** was 1640 ± 330 M^{-1}. In contrast, the unsubstituted boronic acid **1** binds fructose under identical conditions with a K_a of 160 M^{-1}.[5] This result indicates that the appropriate introduction of electron withdrawing groups on an arylboronic acid can enhance sugar-binding almost as effectively as the *ortho* benzylamine functionality.

In conclusion, we have found that the nitro substituted boronic acid **5** binds to fructose with an association constant comparable to that obtained with a boronic acid stabilized by a neighboring Lewis base.[16] Additionally, the presence of a carboxy functionality in **5** provides a useful synthetic handle for attachment of the boronic acid recognition module to a variety of receptor scaffolds for the synthesis of sugar specific sensors.[17,18] We are presently incorporating boronic acid **5** into polymers in order to achieve oligosaccharide recognition, and the results of these studies will be reported in due course.

Acknowledgements

We gratefully acknowledge financial support from Brown University (startup funds) and the NSF Faculty Early CAREER award program. N.J.A. was supported in part by an Undergraduate Teaching and Research Assistantship from Brown University. We thank Professors Seto and Zimmt for generous access to their UV–vis spectrophotometer and spectrofluorimeter, respectively.

References and notes

1. Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769.
2. (a) For recent reviews on boronic acid mediated carbohydrate recognition, see: James, T. D.; Shinkai, S. *Top.*

Curr. Chem. **2002**, *218*, 159. (b) Wang, W.; Gao, X.; Wang, B. *Curr. Org. Chem.* **2002**, *6*, 1285.

3. Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 9014.
4. He, M.; Johnson, R. J.; Escobedo, J. O.; Beck, P. A.; Kim, K. K.; St Luce, N. N.; Davis, C. J.; Lewis, P. T.; Fronczek, F. R.; Melancon, B. J.; Mrse, A. A.; Treleaven, W. D.; Strongin, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 5000.
5. Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291.
6. Wulff, G. *Pure Appl. Chem.* **1982**, *54*, 2093.
7. (a) Soundararajan, S.; Badawi, M.; Kohlrust, C. M.; Hageman, J. H. *Anal. Biochem.* **1989**, *178*, 125. (b) DiCesare, N.; Lakowicz, J. R. *J. Phys. Chem. A* **2001**, *105*, 6834. (c) For additional examples of boronic acids with low pK_a values, see: Das, S.; Alexeev, A.; Sharma, A. C.; Geib, S. J.; Asher, S. A. *Tetrahedron Lett.* **2003**, *44*, 7719. (d) Westmark, P. R.; Gardiner, S. J.; Smith, B. D. *J. Am. Chem. Soc.* **1996**, *118*, 11093.
8. (a) The effect on electron withdrawing substituents on boronic acids has previously been examined for the use of nitroboronic acid modified polymers as affinity chromatography supports.[7a,b] Johnson, B. J. B. *Biochemistry* **1981**, *20*, 6103. (b) Singhal, R. P.; Ramamurthy, B.; Govindraj, N.; Sarwar, Y. *J. Chromat.* **1991**, *543*, 17.
9. All but one of the boronic acids used in this study were purchased from Aldrich Chemical Co. or Combiblocks (www.combi-blocks.com). Compound **2** was prepared from *o*-tolyl boronic acid by protection as the 1,3-propanediol ester, followed by benzylic bromination and reaction with diethylamine. ^1H NMR (methanol-d_4, 300 MHz): δ ppm 1.27 (t, 6H, $J = 7.31$ Hz, $C\underline{H}_3$–CH_2), 3.05, (q, 4H, $J = 7.32$ Hz, CH_3–$C\underline{H}_2$), 4.20, (s, 2H, Ar–$C\underline{H}_2$), 7.15–7.69 (m, 4H, Ar–$\underline{H}$); ^{13}C NMR (methanol-d_4, 75 MHz): δ ppm 7.6, 44.5, 58.9, 126.3, 127.8, 130.4, 134.4, 135.1.
10. Springsteen, G.; Wang, B. *Chem. Commun.* **2001**, 1608.
11. Connors, K. A. In *Binding Constants: The Measure of Molecular Complex Stability*; John Wiley and Sons: New York, 1987; pp 175–183.
12. Springsteen and Wang have found that 4-fluoro-2-methylphenyl boronic acid does not enhance the fluorescence of ARS as effectively as **1**.[5] This observation is consistent with steric inhibition of binding by the methyl group. See also: McDaniel, D. H.; Brown, H. C. *J. Am. Chem. Soc.* **1955**, *77*, 3756.
13. Wiskur, S. L.; Lavigne, J. J.; Aït-Haddou, H.; Lynch, V.; Chiu, Y. H.; Canary, J. W.; Anslyn, E. V. *Org. Lett.* **2001**, *3*, 1311.
14. Pizer, R.; Babcock, L. *Inorg. Chem.* **1977**, *16*, 1677.
15. Arimori, S.; Bell, M. L.; Oh, C. S.; Frimat, K. A.; James, T. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 803, and references therein..
16. For a recent report of a boronic acid which binds sugars at neutral pH without the aid of an aminomethyl group, see: Yang, W.; Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1019.
17. We have recently attached the boronic acid **5** to a fluorescein isothiocyanate derivative to serve as a fluorescent reporter. Preliminary studies indicate that this conjugate selectively labels polymer beads which contain a 1,2-diol. Further studies of this conjugate, along with cell labeling experiments, will be reported in due course.
18. While this manuscript was under review, the use of a low pK_a boronic acid in the preparation of glucose responsive polymers was reported. Matsumoto, A.; Ikeda, S.; Harada, A.; Kataoka, K. *Biomacromolecules* **2003**, *4*, 1410.