Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 94–96
Bioorganic & Medicinal Chemistry Letters

Formation of the intermediate nitronyl nitroxide–anthracene dyad sensing saccharides

Yanxin Yu,[a,b] Deqing Zhang,[a,*] Wei Tan,[a] Zhiyong Wang[b] and Daoben Zhu[a,*]

[a]*Beijing National Laboratory for Molecular Sciences, Organic Solids Laboratory, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China*
[b]*Hefei National Laboratory for Physical Sciences at Microscale and Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, China*

Received 8 August 2006; revised 26 September 2006; accepted 28 September 2006
Available online 30 September 2006

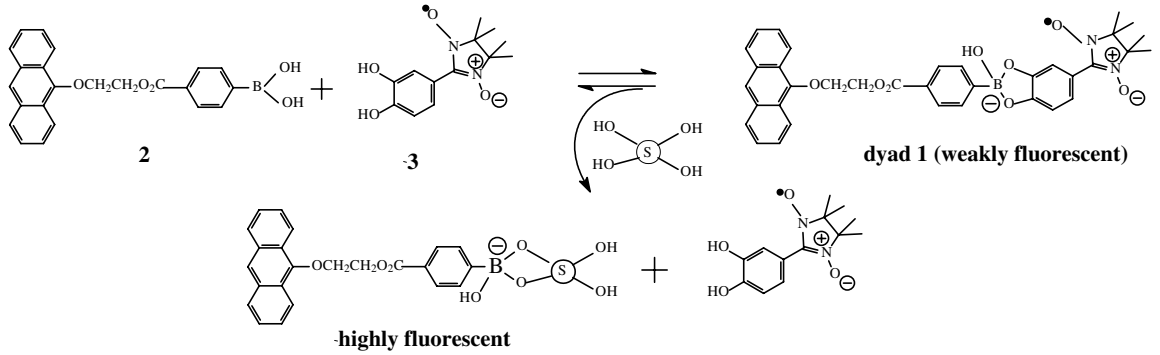
Abstract—We design a new saccharides sensor based on the ensemble of compound **2** with a boronic group and compound **3** with two phenolic –OH groups, taking advantage of the fluorescence quenching ability of nitronyl nitroxides and reversible boronate formation between boronic acid and diol. The results show that the fluorescence of compound **2** was largely quenched upon addition of compound **3** due to the formation of the intermediate nitronyl nitroxide–anthracene dyad **1**. Sequential addition of saccharides such as fructose to the ensemble of compounds **2** and **3** together with dyad **1** induced the fluorescence enhancement. These results clearly demonstrate the possibility to employ the ensemble of compounds **2** and **3** (with dyad **1**) to sense saccharides.

It is well known that saccharides play significant roles in biological processes. For better understanding of the effects of saccharides on the biological processes, development of efficient approaches to detect saccharides is highly desired. By taking advantage of the high affinity of boronic acids for diols through reversible boronate formation, various saccharides sensors containing boronic acid group have been described.[1] For instance, Shinkai and coworkers have studied extensively the saccharides sensors based on PET (photoinduced electron transfer) mechanism containing three components: a fluorophore, an amine, and a boronic acid group.[1a,2] Similarly, saccharides sensors featuring boronic acid groups based on ICT (internal charge-transfer) mechanism have been reported.[3] Besides, other saccharides sensors by making use of the formation of multiple hydrogen bonds have also received attention.[4] We have just recently reported a new saccharides sensor based on a tetrathiafulvalene–anthracene dyad with a boronic acid group.[5]

Nitronyl nitroxides, also referred to as Ullman radicals[6] as spin probes, have been intensively investigated as components of organic magnets since the first report of purely organic ferromagnet in 1991.[7] In fact, nitronyl nitroxides are electron donors and can function as fluorescence quenchers. For example, 2-nitronyl nitroxide pyrene was found to be rather weakly fluorescent.[8] We design a new saccharides sensor based on nitronyl nitroxide–anthracene dyad **1** generated in situ from the reaction between compound **2** with a boronic acid group and compound **3** with two phenolic –OH groups. The design rationale is illustrated in Scheme 1. Reaction of compound **2** with compound **3** would form the nitronyl nitroxide–anthracene dyad **1** leading to the fluorescence quenching of the anthracene unit. Addition of saccharides to the solution would shift the existing equilibrium between dyad **1** and compounds **2** and **3** due to the competitive binding of compound **2** with saccharides. As a result, the quantity of dyad **1** in the solution would be reduced, and accordingly the fluorescence intensity of the solution is expected to increase because boronate anion is a weak fluorescence quencher. Apparently our design employs both fluorescence quenching ability of the nitryonyl nitroxides and the reversible reaction of boronic acids with diols. We want to mention that Anslyn and coworkers have successfully employed similar competition assay for detection of saccharide derivatives

Keywords: Saccharides sensor; Nitronyl nitroxide; Anthracene; Boronic acid; Fluorescence.
* Corresponding authors. Fax: +86 10 62569564; e-mail: dqzhang@iccas.ac.cn

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.09.081



Scheme 1. Chemical structures of compounds **2** and **3** and the competitive binding of saccharides with compound **2** in the presence of compound **3**.

based on the absorption spectral changes.[9] In our case, we are monitoring fluorescence property of anthracene.

Compounds **2** and **3** were synthesized and characterized according to the reported procedures.[5,10] Figure 1 shows the fluorescence spectrum of **2** (1.0×10^{-5} M at pH 7.3 adjusted by 0.033 phosphate buffer in THF/H_2O (1:1 v/v)) and those upon addition of compound **3**. Obviously, the fluorescence intensity decreased gradually by introducing compound **3** to the solution. When 70 equiv of **3** (7.0×10^{-4} M) was added, the fluorescence intensity was reduced to 6.0% of that of the initial solution in the absence of compound **3**. The inset of Figure 1 shows the plot of $I_o/(I_o - I)$ versus the reciprocal of the concentration of compound **3**, where I_o and I are the fluorescence intensities at 419 nm of the solution in the absence and presence of compound **3**. The titration data were analyzed with Benesi-Hildebrand equation leading to the corresponding binding constant $K = 2410$ ($r = 0.9981$), which was comparable to those of boronic acids with two phenolic –OH groups.[11] The reduction of the fluorescence intensity of compound **2** after reaction with compound **3** should be due to the formation of the nitronyl nitroxide–anthracene dyad **1** as shown in Scheme 1. It is understandable that dyad **1** shows weak fluorescence due to the quenching effect of nitronyl nitroxide as reported previously.[8]

In the following, we will show that the fluorescence intensity of the ensemble of compounds **2** and **3** together with dyad **1** increases gradually after introducing saccharides to the ensemble. For instance, Figure 2 displays the fluorescence enhancement observed for the ensemble after addition of different amounts of fructose. But, if less than 100 equiv of fructose (<1.0×10^{-3} M) was present the fluorescence intensity was only slightly enhanced. Only after more than 100 equiv of fructose (>1.0×10^{-3} M) were introduced large fluorescence enhancement was realized. These results can be well explained by the fact that the binding constant of the boronic acid with fructose (around 100 M^{-1}) was smaller than that of the boronic acid group of compound **2** with two phenolic –OH groups of compound **3** (2410 M^{-1}). The inset of Figure 2 shows the increase of the fluorescence intensity with the amount of fructose added to the ensemble; the maximum fluorescence enhancement was achieved when 2.5×10^4 equiv of fructose (0.25 M) versus compound **2** was introduced to the ensemble. Therefore, the ensemble can be used to detect fructose in the concentration range of 1.0×10^{-3}–0.25 M.

Similarly, fluorescence enhancement was also detected after adding mannose, glucose and galactose to the ensemble of compounds **2** and **3** (with dyad **1**) as shown

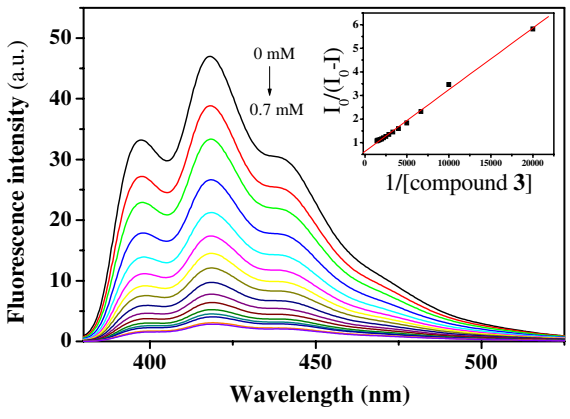


Figure 1. Fluorescence spectra of compound **2** (1.0×10^{-5} M) with different concentrations of compound **3** (0–0.7 mM) at pH 7.3 adjusted by 0.033 phosphate buffer in THF/H_2O (1:1 v/v); the excitation wavelength is 370 nm. The inset shows the plot of $I/(I - I_0)$ (at 419 nm) versus the reciprocal of the concentration of compound **3**.

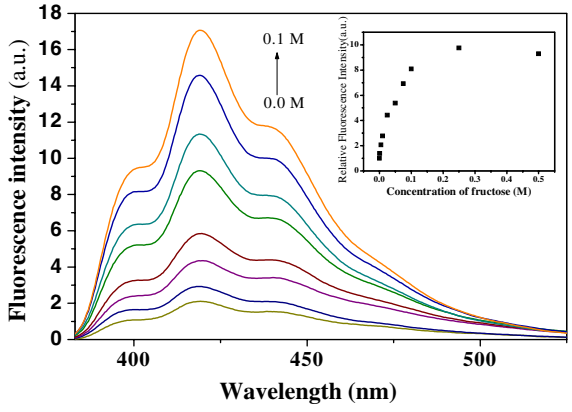


Figure 2. The fluorescence spectra of the ensemble of **2** (1.0×10^{-5} M) and compound **3** (7.0×10^{-4} M) (with dyad **1**) after addition of different amounts of fructose (0–0.10 M) at pH 7.3 adjusted by 0.033 phosphate buffer in THF/H_2O (1:1 v/v); the excitation wavelength is 370 nm; the inset shows the plot of the relative fluorescence intensity at 419 nm versus the fructose concentration.

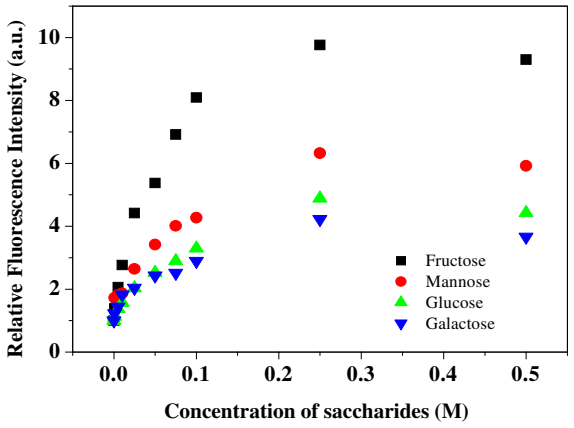


Figure 3. Relative fluorescence intensity at 419 nm for the ensemble of compound **2** (1.0×10^{-5} M) and compound **3** (7.0×10^{-4} M) after addition of saccharides (fructose, mannose, gluctose and galactose); the measurement conditions are the same as those listed in Figure 2 with excitation wavelength of 370 nm.

in Figure 3, where the plot of the relative fluorescence intensity (I/I_o, I and I_o at 419 nm are the fluorescence intensities of the ensemble in the absence and presence of saccharides, respectively) versus the concentrations of saccharides was displayed. Addition of fructose, mannose, glucose and galactose led to fluorescence enhancement in the following order: fructose, mannose, glucose and galactose. Among the four saccharides tested, the largest fluorescence enhancement was observed in the case of fructose under the present conditions. These results are indeed in agreement with the corresponding binding constants of boronic acid with these four saccharides.

In summary, we showed that the fluorescence of compound **2** was largely quenched upon addition of compound **3** due to the formation of the intermediate nitronyl nitroxide–anthracene dyad **1**. Addition of saccharides such as fructose reversed the reaction and induced the fluorescence enhancement. These results clearly demonstrate that a trimolecular system (compounds **2**, **3** and dyad **1**) can be employed to detect saccharides. Further studies include design of new analogues of compound **3**, the binding constants of which with compound **2** are comparable or even smaller than those of compound **2** with saccharides, and consequently it may result in development of new more sensitive ensemble for saccharides.

Acknowledgments

The present research was financially supported by NSFC and Chinese Academy of Sciences. D.-Q. Zhang thanks National Science Fund for Distinguished Young Scholars.

References and notes

1. (a) Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874; (b) James, T. D.; Linnane, P.; Shinkai, S. *Chem. Commun.* **1996**, 281; (b) Wang, W.; Gao, S.; Wang, B. *Org. Lett.* **1999**, *1*, 1209; (d) Gao, S.; Wang, W.; Wang, B. *Bioorg. Chem.* **2001**, *29*, 308; (e) 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; (f) Karnati, V. R.; Gao, X.; Gao, S.; Yang, W.; Ni, W.; Sankar, S.; Wang, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3373; (g) Wang, W.; Gao, X.; Wang, B. *Curr. Org. Chem.* **2002**, *6*, 1285; (h) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159; (i) Striegler, S. *Curr. Org. Chem.* **2003**, *7*, 81; (j) Hall, D. G. *Boronic Acides.* VCH, **2005**.
2. (a) James, T. D.; Sandanayake, K.; Iguchi, R.; Shinkai, S. *J. Am. Chem. Soc.* **1995**, *117*, 8982; (b) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem. Int. Ed.* **1996**, *35*, 1910; (c) Yamamoto, M.; Takeuchi, M.; Shinkai, S. *Tetrahedron* **1998**, *54*, 3215; (d) Kijima, H.; Takeuchi, M.; Robertson, A.; Shinkai, S.; Cooper, C.; James, T. D. *Chem. Commun.* **1999**, 2011; (e) Nagase, T.; Nakata, E.; Shinkai, S.; Hamachi, I. *Chem. Eur. J.* **2003**, *9*, 3660; (f) Nakata, E.; Nagase, T.; Shinkai, S.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 490; (g) Shinkai, S.; Takeuchi, M. *Biosens. Bioelectron.* **2004**, *20*, 1250.
3. (a) DiCesare, N.; Lakowicz, J. R. *J. Phys. Chem. A* **2001**, 6834; (b) Arimori, S.; Bosch, L. I.; Ward, C. J.; James, T. D. *Tetrahedron Lett.* **2001**, *42*, 4553; (c) DiCesare, N.; Lakowicz, J. R. *Tetrahedron Lett.* **2002**, *43*, 2615; (d) Gao, X.; Zhang, Y.; Wang, B. *Org. Lett.* **2003**, *5*, 4615; (e) Yang, W.; Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1019.
4. (a) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 2978, and further references therein; (b) Fang, J.; Selvi, S.; Liao, J.; Slanina, Z.; Chen, C.; Chou, P. *J. Am. Chem. Soc.* **2004**, *126*, 3559.
5. Wang, Z.; Zhang, D.; Zhu, D. *J. Org. Chem.* **2005**, *70*, 5729.
6. Ullman, E. F.; Osiecki, J. H.; Book, D. G. B.; Darcy, R. *J. Am. Chem. Soc.* **1972**, *94*, 7049.
7. (a) Awaga, K.; Maruyama, Y. *Chem. Phys. Lett.* **1989**, *158*, 556; (b) Tamura, M.; Nakazawa, Y.; Shiomi, D.; Nozawa, K.; Hosokoshi, Y.; Ishikawa, M.; Takahashi, M.; Kinoshita, M. *Chem. Phys. Lett.* **1991**, *186*, 401.
8. Wang, H.; Zhang, D.; Guo, X.; Zhu, L.; Shuai, Z.; Zhu, D. *Chem. Commun.* **2004**, 670.
9. (a) Cabell, L.; Monahan, M.; Anslyn, E. *Tetrahedron Lett.* **1999**, *40*, 7753; (b) Zhu, L.; Anslyn, E. *J. Am. Chem. Soc.* **2004**, *126*, 3676.
10. Cirujeda, J.; Ochando, L. E.; Amigo, J. M.; Rovira, C.; Rius, J.; Veciana. *J. Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 55.
11. Springsteen, G.; Wang, B. *Chem. Commun.* **2001**, 1608.