The first fluorescent sensor for boronic and boric acids with sensitivity at sub-micromolar concentrations

Wei Wang, Greg Springsteen, Shouhai Gao and Binghe Wang*

Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204, USA.
*E-mail: binghe_wang@ncsu.edu*

An anthracene-based PET sensor which uses a diethanolamine recognition site is selective for boronic and boric acids, showing up to a 19-fold fluorescent intensity enhancement upon binding.

Boronic acids and boric acid are ubiquitous compounds in chemistry and biology. Boronic acids are important organic intermediates that have been widely used in Suzuki cross-coupling reactions,[1] protection of diols,[2] Diels–Alder reactions[3] and asymmetric synthesis of amino acids.[4] In addition, this class of compounds has been used for the development of sensors for carbohydrates and amino acids;[5,6] selective transporters of nucleosides, saccharides and nucleotides;[7] inhibitors of proteases;[8] and therapeutic agents in boron neutron capture therapy (BNCT) of certain brain tumors.[9] Therefore, fluorescent sensors for boronic acids could be used for the analysis and detection of such compounds in a variety of applications. Boric acid plays an essential role in plant growth.[10] However, the cellular and molecular mechanisms through which boric acid functions in plant cells are far from clear. Appropriately designed sensors could be used for probing the detailed biological functions of boric acid at the cellular and molecular levels. To the best of our knowledge, no fluorescent sensors for either boronic or boric acids have been reported.

The design presented here takes advantage of the known high affinity binding of boronic and boric acid moieties with diethanolamine through boronate formation.[11] This boronate is stabilized by the donation of the nitrogen lone pair electrons to the open shell of the boron atom,[12] which allows for the formation of two five-membered rings (*e.g.* **2**, Scheme 1). Such binding has been used for the stabilization, purification and characterization of boronic acids.[13] Furthermore, it is known that the nitrogen lone pair electrons of 9-aminomethylanthracene can quench the fluorescence of the anthracene moiety through photoinduced electron transfer (PET).[5,6,14] Masking of the nitrogen lone pair electrons causes a suppression of this fluorescence quenching and, therefore, results in fluorescence intensity increases.[6,12] We envisioned that the diethanolamine recognition motif could be incorporated into an anthracene molecule so that its binding with boronic and boric acids would lead to the formation of boronate/borate **2**, which has a boron
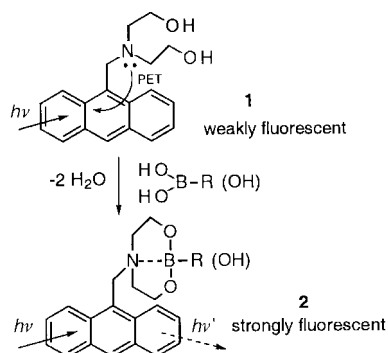
atom ideally positioned to accept, and therefore mask the nitrogen lone pair electrons. This masking of the nitrogen lone pair electrons could then lead to a fluorescence increase of the anthracene moiety. A fluorescent sensor, *N*-(9-anthrylmethyl)diethanolamine **1**, was designed, synthesized, and evaluated for its binding with boronic and boric acids (Scheme 1). In the absence of boronic and boric acids, the sensor displayed very weak fluorescence. However, upon addition of boronic or boric acid, the fluorescence intensity of the sensor increased by > 16-fold, at saturation concentrations (data not shown).

N-(9-Anthrylmethyl)diethanolamine **1** was readily synthesized from 9-(chloromethyl)anthracene through reaction with diethanolamine (6.0 equiv.) in the presence of K_2CO_3 (10.0 equiv.) and a catalytic amount of KI (0.3 equiv.) in a mixture of $CHCl_3$ and MeCN under reflux for 2.5 h (97% yield). In a typical binding experiment, the sensor was dissolved in methanol and was added to the solutions of different concentrations of boronic or boric acid in MeOH. The final concentration of the sensor was fixed at 10^{-5} M.

In the absence of boric or boronic acids, the sensor exhibited very low fluorescence owing to the quenching of the anthracene fluorescence through PET. For the boronic acid binding studies, phenylboronic acid was used as a model compound. Upon addition of phenylboronic acid, the sensor solution showed concentration-dependent fluorescence intensity increases from 34% at 10^{-6} M (Table 1, Fig. 1) to 16-fold at saturation (50 mM, data not shown). The sensor responded to boric acid with a 107% intensity increase at 10^{-7} M (Table 1, Fig. 1) and a 19-fold increase at saturation (500 mM, data not shown).
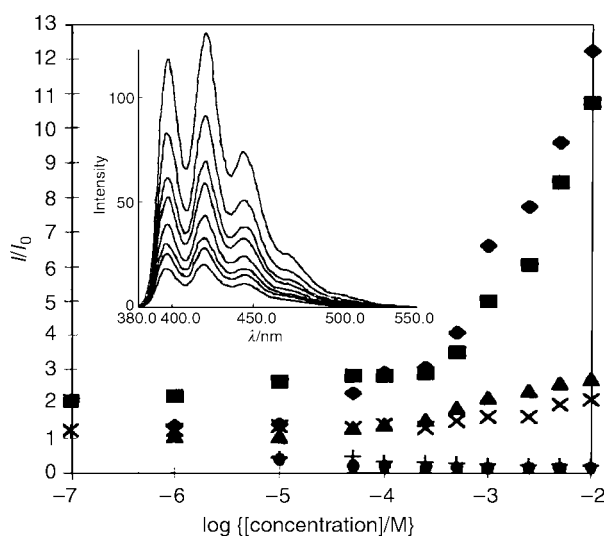

Scheme 1 The tight binding of boronic acid/boric acid with sensor **1**.

Fig. 1 Relative fluorescence intensity changes (I/I_0) as a function of log[boronic or boric acid] with **1** or **3** (1.0×10^{-5} M) in MeOH at room temperature, λ_{ex} = 370 nm, λ_{em} = 419 nm. $PhB(OH)_2$ with sensor **1** (◆); $B(OH)_3$ with sensor **1** (■); $PhB(OH)_2$ with control **3** (▲); $B(OH)_3$ with control **3** (×); $PhCO_2Na$ with sensor **1** (●); $PhOP(O)(ONa)_2$ with sensor **1** (+). *Inset:* a typical set of fluorescence emission spectra of sensor **1** (1.0×10^{-5} M) with $PhB(OH)_2$ in MeOH from 0 M to 1×10^{-3} M.

Table 1 The effect of $PhB(OH)_2/B(OH)_3$ on the fluorescent intensity of **1** and **3**

	$I/I_0{}^a$			
	$PhB(OH)_2{}^b$		$B(OH)_3{}^b$	
Conc(M)	**1**	**3**	**1**	**3**
1.0×10^{-7}	—	—	2.07 ± 0.17	1.22 ± 0.10
1.0×10^{-6}	1.34 ± 0.17	1.05 ± 0.07	2.20 ± 0.29	1.23 ± 0.16
1.0×10^{-5}	1.38 ± 0.01	1.04 ± 0.02	2.63 ± 0.38	1.32 ± 0.06
5.0×10^{-5}	2.28 ± 0.22	1.28 ± 0.04	2.79 ± 0.37	1.28 ± 0.10
1.0×10^{-4}	2.88 ± 0.43	1.37 ± 0.14	2.79 ± 0.40	1.36 ± 0.13
2.5×10^{-4}	3.03 ± 0.31	1.50 ± 0.15	2.86 ± 0.33	1.27 ± 0.08
5.0×10^{-4}	4.04 ± 0.37	1.85 ± 0.07	3.48 ± 1.18	1.47 ± 0.11
1.0×10^{-3}	6.58 ± 0.21	2.15 ± 0.08	4.96 ± 0.24	1.59 ± 0.25

^{a} I_0: the intensity of **1** or **3** (1.0×10^{-5} M) in the absence of phenylboronic/boric acid; I the intensity of **1** or **3** (1.0×10^{-5} M) in the presence of phenylboronic/boric acid. ^{b} The ratio is listed as the average of three runs with the standard deviation.

It is well known that aminomethylanthracene-based fluorescent sensors are sensitive to pH changes.[15] Therefore, incidental changes in pH due to the addition of the boric or boronic acids were a possible contributing factor in the fluorescence intensity changes observed with sensor **1**, which could complicate the interpretation of the results. To examine whether the fluorescence intensity changes were due to unintended changes in the pH of the solution, we also synthesized compound **3** as a control by following the same procedure for the preparation of **1**. If incidental pH changes were not the reason for the fluorescence intensity changes observed with **1**, we would not expect **3** to be sensitive to boronic and boric acids because compound **3** lacks the two hydroxy groups (Scheme 2), which are critical for the formation of the tight complex of **1** with boronic and boric acids (Scheme 1). Indeed, it was found that compound **3** showed minimal fluorescence intensity changes upon addition of boric or boronic acids at concentrations up to 10^{-5} M (Fig. 1, Table 1). Even at 10 mM, the fluorescence intensity changes were small [1.6- to 2.2-fold *cf.* 5.0- to 6.0-fold with **1**, (Table 1)]. Such results indicate that the fluorescence intensity changes observed with sensor **1** are not primarily due to unintended changes in pH. Furthermore, the minor changes in the fluorescence intensity of **3** may be due to the non-specific weak complexation of the nitrogen atom of **3** with boronic or boric acid (**4**, Scheme 2).

To examine the selectivity of the sensor for boronic and boric acids in the presence of other anions, we also studied the effect of sodium benzoate and disodium phenyl phosphate on the fluorescence intensity of sensor **1**. These anions were not able to increase the fluorescence intensity of the sensor and actually caused a slight lowering of the intensity (Fig. 1). A ^{1}H NMR
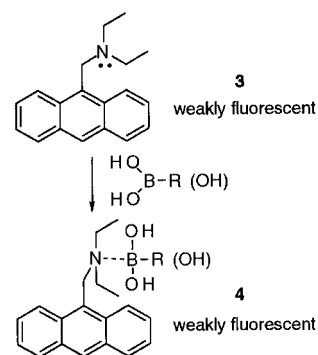
Scheme 2 The weak interaction of boronic acid/boric acid with control compound **3**.

spectral comparison of the complexed and uncomplexed forms of sensor **1** shows that the sensor/analyte binding is in a 1:1 molar ratio for both phenylboronic acid and boric acid.

In conclusion, the first fluorescent sensor **1** for boronic and boric acids shows high sensitivity and selectivity. Further work in this area may be beneficial to chemical process monitoring, impurity detection, examination of the intracellular functions of boric acid in plant cells, and biological analysis of medicinally useful boronic acid compounds.

Notes and references

1 N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457.
2 R. J. Ferrier, *Adv. Carbohydr. Chem. Biochem.*, 1978, **35**, 31.
3 K. Ishihara and H. Yamamoto, *Eur. J. Org. Chem.*, 1999, 527.
4 N. A. Petasis and I. A. Zavialov, *J. Am. Chem. Soc.*, 1997, **119**, 445.
5 T. D. James, P. Linnane and S. Shinkai, *Chem. Commun.*, 1996, 281.
6 J. Yoon and A. W. Czarnik, *J. Am. Chem. Soc.*, 1992, **114**, 5874.
7 P. R. Westmark, S. J. Gardiner and B. Smith, *J. Am. Chem. Soc.*, 1996, **118**, 11 093.
8 D. H. Bao, W. P. Huskey, C. A. Kettner and F. Jordan, *J. Am. Chem. Soc.*, 1999, **121**, 4684.
9 G. W. Kabalka, *Expert Opin. Ther. Pat.*, 1998, **8**, 545.
10 *Boron and Its Role in Crop Production*, ed. U. C. Gupta, CRC Press, Boca Raton, FL, 1993.
11 D. G. Hall, J. Tailor and M. Gravel, *Angew. Chem. Int. Ed.*, 1999, **38**, 3064.
12 T. Burgemeister, R. Grobe-Einsler, R. Grotstollen, A. Mannschreck and G. Wulff, *Chem. Ber.*, 1981, **114**, 3403.
13 P. B. Tripathy and D. S. Matteson, *Synthesis*, 1990, 200.
14 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1910.
15 R. S. Atkinson, D. R. G. Brimage, R. S. Davidson and E. Gray, *J. Chem. Soc., Perkin Trans. 1*, 1973, 960.