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A New Fluorescein Derivative Bearing a Boronic Acid Group as a Fluorescent Chemosensor for Fluoride Ion

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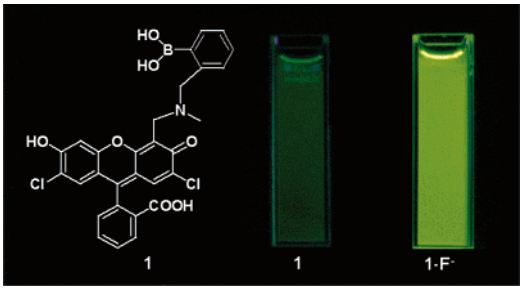

A new fluorescein derivative **1** bearing a boronic acid group was investigated as a fluorescent chemosensor for F^-. An off−on type fluorescence enhancement was observed by the blocking of the photoinduced electron transfer mechanism, which was induced by the interaction between fluoride and boronic acid moiety.

Anions play a fundamental role in a wide range of chemical and biological processes, and numerous efforts have been devoted to the development of abiotic receptors for anionic species.[1] Sensors based on anion-induced changes in fluorescence appear to be particularly attractive due to the simplicity and high detection limit of fluorescence.[1,2] Particularly, fluoride ions are biologically important anions because of their important role in dental care[3] and the treatment of osteoporosis,[4] etc.

In this regard, the fluorescent sensing of a fluoride ion has attracted growing attention.[5] In most cases, hydrogen bonding between the N−H of a urea or pyrrole group and fluoride was used for the recognition. On the other hand, there have been a few reports regarding fluoride ion detection utilizing a unique fluoride−boron interaction. Shinkai et al. reported ferrocenylboronic acids as fluoride selective receptors, whose binding affinity can be monitored electrochemically.[6] James et al. reported selective fluorescence detections of fluoride using boronic acids.[7] Davidson et al. utilized a bis(bora)calixarene as a selective fluorescent fluoride sensor.[8] On the other hand, Smith et al. reported that the tetrahedral fluoroboronate anion afforded by the reaction of phenyl boronic acid and fluoride ion enhances the formation of cyclic esters with diols.[9] Liu's group[10] and Yamaguchi's group[11] also reported organo borane derivatives as chemosensors for fluoride ion.

Here, we report a new fluorescein derivative that displays a selective fluorescent changes with fluoride ion among the halide ions. As far as we are aware of, this is the first example of a fluorescein derivative that bears a boronic acid group as a binding site. A unique boronate formation between the boronic acid and adjacent phenolic oxygen as well as the interaction between the boron and nitrogen were confirmed by X-ray crystallography. Since the fluorescein moiety was used as the fluorescent source, the emission changes can be monitored over 500 nm. Furthermore, off−on type fluorescence enhancement was observed by the blocking of the photoinduced electron transfer (PET) mechanism, which was induced by the interaction between fluoride and the boronic acid moiety. While anion chemosensors that function in water will clearly bring the greatest utility, the discovery of new mechanisms in organic solvents remains an important prelude to such future studies.

Our synthesis began with 2-methylaminomethyl boronic acid **3**, which was synthesized following the reported procedure.[12]

(1) For recent reviews for anion receptors, see; (a) Yoon, J.; Kim, S. K.; Singh, N. J.; Kim, K. S. *Chem. Soc. Rev.* **2006**, *35*, 355. (b) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 486. (c) Snowden, T. S.; Anslyn, E. V. *Chem. Biol.* **1999**, *3*, 740. (d) Antonisse, M. M. G.; Reinhoudt, D. N. *Chem. Commun.* **1998**, 143. (e) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609. (f) Rudkevich, D. M.; Brzozka, Z.; Palys, M.; Visser, H. C.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 467.

(2) (a) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551. (b) Martínez-Máñez, R.; Sancanón, F. *Chem. Rev.* **2003**, *103*, 4419. (c) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. A.; Huxley, T. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (d) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302. (e) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1994**, 197.

(3) Kirk, K. L. *Biochemistry of the Halogens and Inorganic Halides*; Plenum Press: New York, 1991; p 58.

(4) Kleerekoper, M. *Endocrinol. Metab. Clin. North Am.* **1998**, *27*, 441.

(5) (a) Zhao, Y.-P.; Zhao, C.-C.; Wu, L.-Z.; Zhang, L.-P.; Tung, C.-H.; Pan, Y.-J. *J. Org. Chem.* **2006**, *71*, 2143. (b) Jun, E. J.; Swamy, K. M. K.; Bang, H.; Kim, S.-J.; Yoon, J. *Tetrahedron Lett.* **2006**, *47*, 3103. (c) Kim, S. K.; Bok, J. H.; Bartsch, R. A.; Lee, J. Y.; Kim, J. S. *Org. Lett.* **2005**, *7*, 4839. (d) Peng, X.; Wu, Y.; Fan, J.; Tian, M.; Han, K. *J. Org. Chem.* **2005**, *70*, 10524. (e) Thiagarajan, V.; Ramamurthy, P.; Thirumalai, D.; Ramakrishnan, V. Y. *Org. Lett.* **2005**, *7*, 657. (f) Esteban-Gómez, D.; Fabbrizzi L.; Licchelli, M. *J. Org. Chem.* **2005**, *70*, 5717. (g) Liu, B.; Tian, H. *J. Mater. Chem.* **2005**, 2681. (h) Curiel, D.; Cowley, A.; Beer, P. D. *Chem. Commun.* **2005**, 236. (i) Kwon, J. Y.; Jang, Y. J.; Kim, S. K.; Lee, K.-H.; Kim, J. S.; Yoon, J. *J. Org. Chem.* **2004**, *69*, 5155. (j) Jose, D. A.; Kumar, D. K.; Ganguly, B.; Das, A. *Org. Lett.* **2004**, *6*, 3445. (k) Lee, J. Y.; Cho, E. J.; Mukamel, S.; Nam, K. C. *J. Org. Chem.* **2004**, *69*, 943. (l) Pohl, R.; Aldakov, D.; Kubát, P.; Jursíková, K.; Marquez, M.; Anzenbacher, Jr., P. *Chem Commun.* **2004**, 1282. (m) Xu, G.; Tarr, M. A. *Chem. Commun.* **2004**, 1050. (n) Cho, E. J.; Moon, J. W.; Ko, S. W.; Lee, J. Y.; Kim, S. K.; Yoon, J.; Nam, K. C. *J. Am. Chem. Soc.* **2003**, *125*, 12376. (o) Kubo, Y.; Yamamoto, M.; Ikeda, M.; Takeuchi, M.; Shinkai, S.; Yamamoto, S.; Tamao, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 2036. (p) Kim, S. K.; Yoon, J. *Chem. Commun.* **2002**, 770.

(6) (a) Yamamoto, H.; Ori, A.; Ueda, K.; Dusemund, C.; Shinkai, S. *Chem. Commun.* **1996**, 407. (b) Dusemund, C.; Sandanayake, K. R. A. S.; Shinkai, S. *Chem. Commun.* **1995**, 333.

(7) (a) Kubo, Y.; Kobayashi, A.; Ishida, T.; Misawa, Y.; James, T. D. *Chem. Commun.* **2005**, 2846. (b) Cooper, C. R.; Spencer, N.; James, T. D. *Chem. Commun.* **1998**, 1365.

(8) Arimori, S.; Davidson, M. G.; Fyles, T. M.; Hibbert, T. G.; James, T. D.; Kociok-Köhn, G. I. *Chem. Commun.* **2004**, 1640.

(9) M.-F. Paugam and B. D. Smith, *Tetrahedron Lett.* **1993**, *34*, 3723.

(10) Liu, Z.-Q.; Shi, M.; Li, F.-Y.; Fang, Q.; Chen, Z.-H.; Yi, T.; Huang, C.-H. *Org. Lett.* **2005**, *7*, 5481.

(11) Yamaguchi, S.; Akiyama, S.; Tamao, K. *J. Am. Chem. Soc.* **2001**, *123*, 11372.

(12) (a) Darrow, C. B.; Satcher Jr., J. H.; Lane, S. M.; Gable, J. H. **2001**, PCT Int. Appl. WO 2001-US11173 20010404. (b) Secor, K.; Glass, T. E. *Org. Lett.* **2004**, *6*, 3727.

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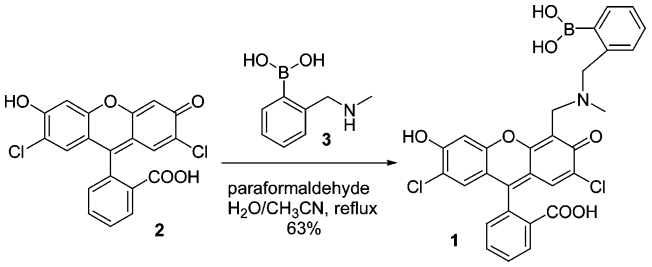
SCHEME 1. Syntheses of Compound 1



Compound **1** was synthesized using the Mannich reaction between 2′,7′-dichlorofluorescein (**2**) and the iminium ion condensation of the product of formaldehyde and 2-methylaminomethyl boronic acid **3** with a 63% yield after column chromatography using ethyl acetate−methanol (95:5, v/v) (Scheme 1). Under this reaction condition, the formation of bisboronic acid derivative was not observed.

Figure 1 explains the fluorescent emission changes of **1** (3 μM) upon the addition of halide ions (900 μM) in acetonitrile−methanol (9:1, v/v). Fluoride ion displayed a selective fluorescent enhancement among the halide ions. Fluorescence spectra were obtained by exciting of the fluorescein fluorophore at 483 nm. Both excitation and emission slit were 3 nm. Figure 2 shows the results of the fluorescence titration experiments of **1** (3 μM) with fluoride ion. The overall emission change upon the addition of fluoride ion was more than 3-fold. On the other hand, the addition of chloride ion induced only about 2-fold enhancement in its fluorescence emission (Figure 3). From the fluorescence titrations, the apparent association constant for fluoride was observed to be 9.2×10^{10} M^{-3}.[13] The ^{19}F NMR of compound **1** (2 mM) with fluoride ion (1.1 equiv) displayed a peak at −138.9 in CD_3CN-CD_3OD (9:1, v/v), which can be compared to the free fluoride signal at −150.1. In CD_3CN-CD_3OD (1:9, v/v), a new peak was observed at −139.3 and free fluoride signal appeared at −152.3 (Figure 4, Supporting Information).

The X-ray crystal structure of **1** is outlined in the Supporting Information. Even though the *R* value was not satisfied, since the X-ray diffraction data were not so good, we could obtain a well-refined structure of the main fluorescein moiety. The most interesting point in the structure is the boronate formation between boron and adjacent phenolic oxygen. Also, the molecular ion peak ($[M + H]^+ = 559.08$), which corresponds to this boronate ester, was observed as 560.20 in its FAB mass spectrum. Recently, James et al. reported the first X-ray crystal structure of the boronic acid−anthracene PET sensor and its complex form with tartaric acid.[14] In this report, the hydrogen atoms of the boronic acid participate in O−H···N hydrogen bonds in the presence and absence of guest compound. The interaction between boron and nitrogen was not observed in these crystal structures. As pointed out by the authors, these X-ray structures are quite unexpected results, since the fluorescent enhancement in a large number of boronic acid PET sensors have been explained by a strong B−N interaction.[15] However, Anslyn et al. reported B−N interaction in the arylboronate systems by X-ray crystal structure, ^{11}B NMR, as well as computational analysis.[16] We also observed a clear interaction between boron and nitrogen (B−N distance 1.686 Å) (Figure 1, Supporting Information).

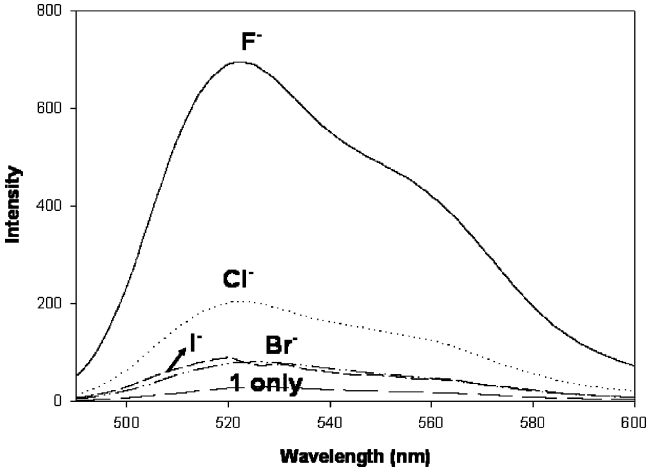


FIGURE 1. Fluorescent emission changes of **1** (3 μM) upon the addition of tetraethylammonium fluoride, bromide, chloride and iodide (300 equiv) in acetonitrile−MeOH (9:1, v/v) (excitation at 483 nm).

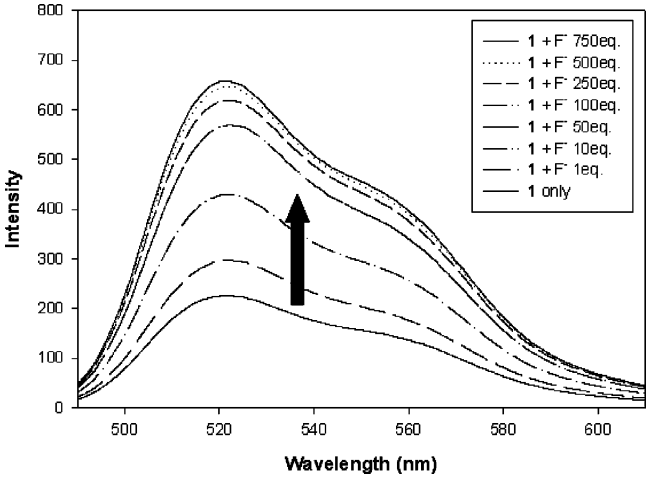


FIGURE 2. Fluorescent titrations of compound **1** (3 μM) with tetrabuthylammonium fluoride in acetonitrile−MeOH (9:1, v/v) (excitation at 483 nm).

The mechanism for the fluorescent changes upon the addition of fluoride ion is quite unique, which can be attributed to the combination of fluorescein core structure and the boron−fluoride interaction (Scheme 2). The fluorescent enhancement upon the addition of fluoride can be explained by blocking of the PET mechanism. The PET-quenching mechanism of the benzylic nitrogen is well described in many cases[2a,c,15] and also in the fluorescein systems by Lippard et al.[17] The relatively weak interaction between benzylic nitrogen and boron may be attributed to the moderate fluorescence emission of **1** before adding anions. Upon the addition of fluoride ion, the fluoride adduct of compound **1** can be stabilized by the additional hydrogen bonding with the proton in the phenol moiety. The phenolic hydrogen can make a strong hydrogen bond with

(13) (a) Conners, K. A. *Binding Constants*; Wiley: New York, 1987. (b) The association constants were determined as described in ref 7.
(14) Zhao, J.; Davidson, M. G.; Mahon, M. F.; Kociok-Köhn, G.; James, T. D. *J. Am. Chem. Soc.* **2004**, *126*, 16179.
(15) Cao, Haishi; Heagy, M. D. *J. Fluores.* **2004**, *14*, 569.
(16) Zhu, L.; Shabbir, S. H.; Gray, M.; Lynch, V. M.; Sorey, S.; Anslyn, E. V. *J. Am. Chem. Soc.* **2006**, *128*, 1222.
(17) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831.

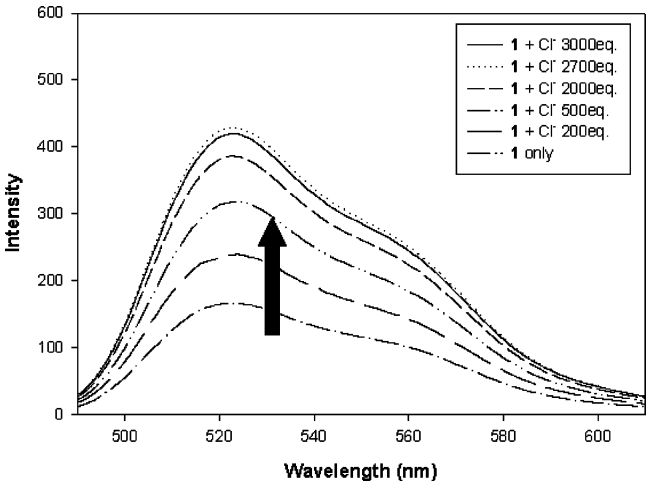
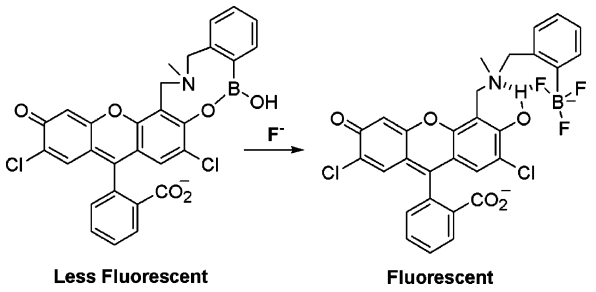


FIGURE 3. Fluorescent titrations of compound **1** (1 μM) with tetrabuthylammonium chloride in acetonitrile−MeOH (9:1, v/v) (excitation at 483 nm).

SCHEME 2. Proposed Binding Mechanism of Compound 1 with Fluoride Ion



fluoride as well as benzylic amine, which bolcks the PET mechanism, resulting in fluorescence enhancement as proposed in Scheme 2. The ^{11}B NMR experiments were performed to confirm the presence of the fluoride adduct proposed in Scheme 2. The ^{11}B NMR signal of compound **1** in CD_3CN-CD_3OD (9:1, v/v) shows a broad peak at 9.1 ppm when boron trifluoride diethyl etherate was used as an external reference (Figure 4). The signal shifted to 1.1 ppm upon the addition of 5 equiv of F^-. This chemical shift as well as a clear quartet at 1.1 ppm supports the formation of a ternary complex and a sp^3-hybridized boronate complex, as shown in Scheme 2.[7,18]

In conclusion, a fluorescein derivative bearing a boronic acid group has been synthesized for the first time. The title compound displayed a selective fluorescent enhancement with fluoride ion among the halide ions. Since the fluorescein moiety was used as the fluorescent source, the emission changes can be monitored over 500 nm. Compound **1** displayed a unique boronate formation between boronic acid and phenolate of fluorescein moiety, which was confirmed by the X-ray crystal structure. In addition, the interaction between boron and nitrogen was confirmed by the X-ray crystal structure. We believe this compound can be considered as a potential chemosensor for fluoride ion.

(18) (a) Reetz, M. T.; Niemeyer, C. M.; Harms, H. *Angew. Chem., Int. Ed.* **1991**, *30*, 1472. (b) Mesmer R. E.; Rutenberg, A. C. *Inorg. Chem.* **1972**, *12*, 699.

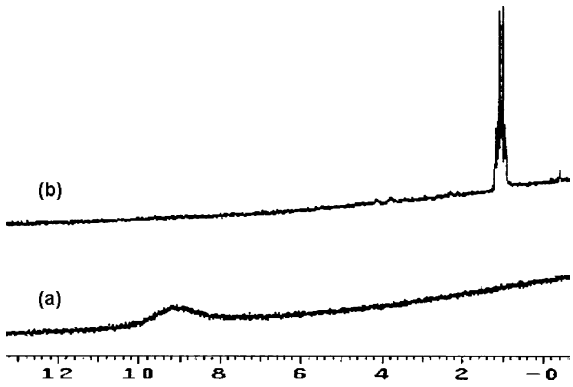


FIGURE 4. ^{11}B NMR spectra (CD_3CN-CD_3OD = 9:1, v/v) of compound **1** (a) and compound **1** upon adding 5 equiv of $(n\text{-Bu})_4NF$ (b).

Experimental Section

Compound 1. 2-Methylaminomethyl boronic acid[12] **3** (0.33 g, 2.0 mmol) and paraformaldehyde (0.05 g, 1.66 mmol) were dissolved in 10 mL of CH_3CN and refluxed for 30 min. 2′,7′-Dichlorofluoroscein (0.25 g, 0.62 mmol) in 20 mL of CH_3CN/H_2O (1:1) was added to the above solution and the reaction mixture was refluxed for 24 h. The CH_3CN was removed in a vacuum and purified the residue by column chromatography on silica gel (95:5 EtOAc:MeOH) to yield 63.8% of the pure product: mp 300 °C dec; ^{1}H NMR ($CDCl_3-CD_3OD$ = 8:2, v/v) δ 7.91 (d, 1H, J = 5.4), 7.58−7.62 (m, 3H), 7.01−7.15 (m, 4H), 6.70 (s, 1H), 6.52 (m, 2H), 4.01 (m, 4H), 2.73 (s, 3H); ^{13}C NMR (CD_3OD) δ170.8, 152.3, 149.2, 140.6, 136.7, 131.6, 131.4, 131.0, 129.5, 129.2, 128.9, 128.6, 128.4, 126.4, 125.5, 124.5, 124.3, 120.1, 119.3, 112.5, 111.3, 110.9, 108.5, 105.0, 65.9, 52.4, 44.3; FAB MS m/z = 560.20 (M + H)$^+$ calcd for $C_{29}H_{20}BCl_2NO_6$= 559.08.

Fluorometric Metal Ion Titrations. Stock solutions (1 mM) of the tetrabutylammonium salts of F^-, Cl^-, Br^-, and I^- in CH_3CN were prepared. Stock solutions of hosts (0.1 mM) were prepared in MeOH. Test solutions were prepared by placing 4−40 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 4 mL with CH_3CN−MeOH. For all measurements, excitation was at 483 nm; emission was measured at 522 nm. Both excitation and emission slit widths were 3 nm.

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Supporting Information Available: General experimental section and ^{1}H and ^{13}C NMR spectra of compound **1**. The material is available free of charge via the Internet at http://pubs.acs.org.

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