Fluorescence Sensing of Dopamine

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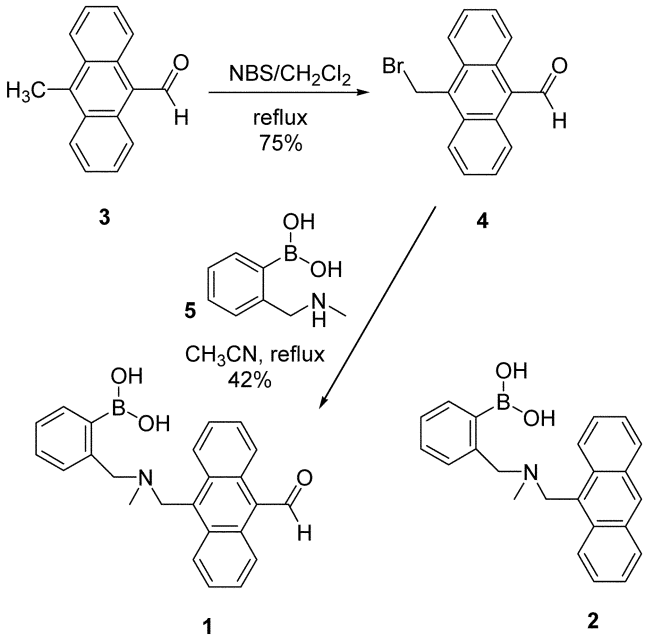
Even though boronic acid has been known for almost 50 years to have high affinity for diol-containing compounds such as carbohydrates,[1] Czarnik and his coworker reported[2] 2-anthrylboronic acid as the first example of fluorescent chemosensor in 1992, which displayed chelation-enhanced fluorescent quenching (CHEQ) effects upon the addition of polyols. Most noticeably, the Sinkai group[3] and James group[4] have been leading the field in many regards. Sinkai and his coworkers have reported new series of PET sensors for saccharides bearing boronic acid unite as well as benzyl amine unit.[3] Recently, James group also reported many noticeable results regarding boronic acid-based fluorescent receptors for saccharides.[4] Norrild,[5] Drueckhammer,[6] Wang,[7] Heagy,[8] and Yoon[9] groups have been actively participating in this field.

Catecholamines, including dopamines, are involved in a number of biological processes, most of them directly related to central nerve diseases, such as Parkinson's disease and hypertension.[10] There have been only few reports regarding fluorescence sensing of dopamine and L-DOPA,[11,12,13] which utilized the interaction between boronic acid and catechol group. Especially, Glass and his coworker reported coumarin aldehyde as a selective chemosensor for dopamine and norepinephrine.[13] The sensor binds to catecholamines by forming an iminium bond with the amine moiety as well as a boronate ester with the catechol moiety.

Herein, we report a new anthracene fluorophore bearing boronic acid and aldehyde group as a fluorescent chemosensor for dopamine. The title compound displayed large fluorescence quenching effects with dopamine, epinephrine and catechol at pH 7.4. Compound **1** containing aldehyde group bound to dopamine in methanol about two times tighter than compound **2** did.

Results and Discussion

Our synthesis began with 10-methylanthracene-9-carboxaldehyde **3**, which was then transformed to 10-(bromomethyl)anthracene-9-carboxaldehyde **4** *via N*-bromosuccinimide reaction (Scheme 1). Treatment of **4** with 2-methylaminomethylphenylboronic acid **5**[9] in refluxing acetonitrile gave the title compound 1 in 42% yield after the column chromatography using $CHCl_3$-MeOH (9 : 1, v/v). Compound **2** was synthesized following the published procedure.[3a]



Scheme 1. The structure and synthesis of compound **1** and **2**.

Dopamine, epinephrine and catechol were used to evaluate the binding properties of **1** and **2**. Compound **2** was reported previously, however, the binding studies with various sacchrides including glucose and fructose were reported.[3a] All titration studies were conducted either in 50% MeOH/0.05 M HEPES buffer at pH 7.4 or in MeOH. Compound **1** and **2** displayed large CHEQ effects with dopamine, epinephrine and catechol. Overall emission change was almost 10-fold in all cases. Fluorescent emission changes of compound **1** and **2** upon the addition of dopamine are shown in Figure 1 and Figure 2. The excitation wavelength for **1** and **2** were 397 nm and 367 nm, respectively. Epinephrine and catechol displayed similar fluorescent changes. From the fluorescent titrations in 50% MeOH/0.05 M HEPES buffer at pH 7.4, the association constants of **1** with dopamine, epinephrine and catechol were calculated as 5720, 5050 and 2010 M^{-1} (errors < 10%), respectively (Table 1).[14] Under the same conditions, the association constants of **2** with dopamine, epinephrine and catechol were calculated as 7300, 5750 and 2030 M^{-1} (errors < 10%), respectively (Table 1).[14] On the other hand, from the

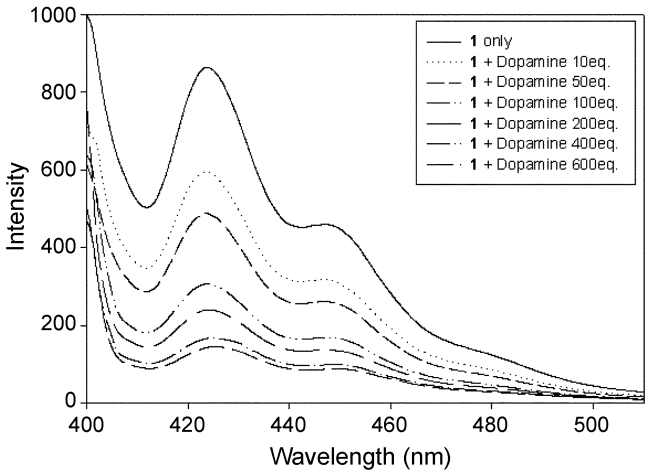


Figure 1. Fluorescence spectra of **1** (3 μM) upon the addition of Dopamine in MeOH (excitation at 397 nm).

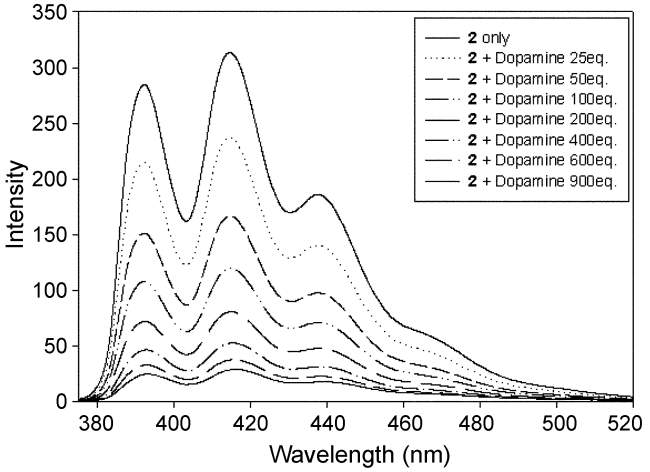


Figure 2. Fluorescence spectra of **2** (3 μM) upon the addition of Dopamine in 50% MeOH/0.05 M HEPES buffer at pH 7.4 (excitation at 367 nm).

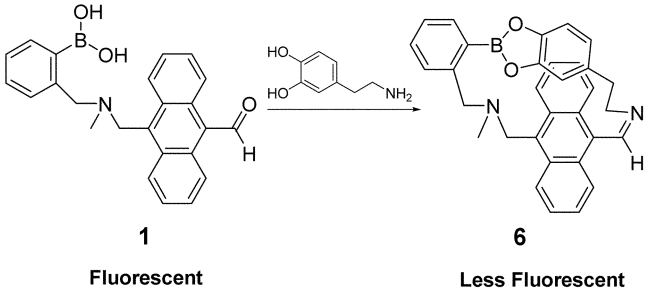
Table 1. Association constants (M^{-1}) of **1** and **2** for the binding with dopamine, epinephrine and catechol

	Epinephrine[a]	Dopamine[a]	Dopamine[b]	Catechol[a]
1	5050	5720	10780	2010
2	5750	7300	5960	2030

[a]in 50% MeOH/0.05 M HEPES buffer at pH 7.4. [b]in MeOH

fluorescent titrations in MeOH, the association constants of **1** and **2** with dopamine were calculated as 10780 and 5960 M^{-1} (errors < 10%), respectively (Table 1).[14] The job plot using the fluorescence changes indicated 1 : 1 binding for **1** with dopamine. Even though there were not significant differences between the association constants of **1** and **2** in 50% MeOH/0.05 M HEPES buffer at pH 7.4, the binding affinity of **1** with dopamine in methanol is almost 2 times that of **2**.

In general, for the hosts contain boronic acid and benzyl amine moieties, the interaction of boronic acid and benzyl amine moiety can only partially inhibit the photo-induced



Scheme 2. Proposed binding mode of compound **1** with dopamine.

electron transfer (PET), however, this inhibition of PET can be maximized upon the addition of saccharides because the complexed form of boronic acid-saccharide can lower the pK_a of boronic ester. Consequently, the anionic form of boronate can make a stronger interaction with adjacent benzlic amine moiety, which resulted in the fluorescent chelation enhanced fluorescent (CHEF) effect by blocking the PET efficiently.[3a] Indeed, our host **1** also displayed CHEF effects with glucose in 50% MeOH/0.05 M HEPES buffer at pH 7.4.

For the CHEQ (chelation-enhanced quenching) effects with guests bearing catechol moiety can be explained by a photoinduced electron transfer (PET) mechanism. It is well documented by Czarnik[11] and Glass[13] that the electron-rich catechol is likely acting as a PET quencher of the anthracene when the tight complex was formed.

The boronate formation between boronic acid and catechol group is well known in the previous reports. In aqueous solutions, the association constants of **1** and **2** with the guests are about the same. On the other hand, in methanol, host **1** binds dopamine about two times better than host **2** does. These results suggest that there might be iminium bond forming between amine moiety of dopamine and aldehyde of host, which can provide additional binding site for dopamine. This was further confirmed by the electrospray ionization (ESI) mass spectrum in methanol. A peak at *m/z* 483.3 which corresponds to **6** ([**1** + **dopamine** – **3H$_2$O**]$^+$) (Scheme 2) was clearly observed. However, we could not observe the corresponding peak in 50% MeOH/0.05 M HEPES buffer. Figure 3 proposes the possible binding mode of compound with dopamine. The structure of compound **1** complexed with dopamine was fully optimised

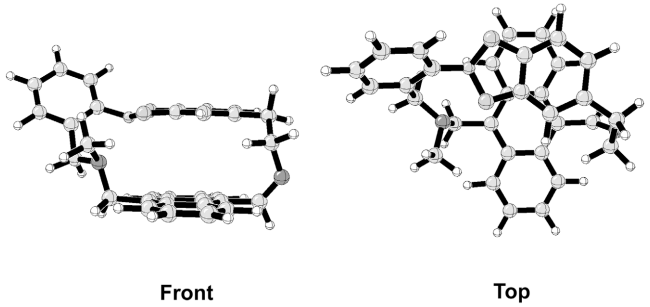


Figure 3. Proposed binding mode of compound **1** with dopamine (**6** in scheme 2) using *ab initio* molecular orbital calculations.

with *ab initio* MO calculations at the RHF/3-21G level using Gaussian94.[15]

In conclusion, we demonstrated that two anthracene derivatives bearing boronic acid group (**1** and **2**) could be used as a potential fluorescent chemosensors for dopamine in aqueous solution at pH 7.4. Furthermore, compound **1** containing an additional aldehyde group displays about 2 times better binding with dopamine in methanol than **2** does, which may be due to the imine bond formation between dopamine and host **1**.

Experimental Section

General methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel 60 (230-400 mesh ASTM; Merck). Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with a thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F_{254} plates with a thickness of 1 mm.

Melting points were measured using a Büchi 530 melting point apparatus, and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250 or Varian 500. Chemical shifts were expressed in ppm, using TMS as an internal standard. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).

10-(bromomethyl)anthracene-9-carboxaldehyde (4). To a solution of 10-methylanthracene-9-carboxaldehyde 3 (548 mg, 2.49 mmol) and NBS (*N*-bromosuccinimide, 487 mg, 2.71 mmol) in $CHCl_2$ (1.5 mL) and CCl_4 (7.5 mL), 23 mg of bezoyl peroxide (0.049 mmol) was added. The reaction mixture was stirred at 80 °C for 3 hours. After the evaporation of the solvent under vacuum, the crude product was purified by column chromatography using $CHCl_3$-MeOH (98 : 2, v/v) as an eluent. 558 mg of **4** (75%) was obtained as a yellow solid; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 11.42 (s, 1H), 8.79 (m, 2H), 8.29 (m, 2H), 7.60 (m, 4H), 5.39 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.9, 137.9, 137.4, 134.3, 128.3, 128.2, 127.5, 122.5, 122.0, 25.9; FAB mass spectrum m/e 299.0 $[M + H]^+$.

Compound 1. Compound **4** (160 mg, 0.53 mmol) and methylaminomethylphenylboronic acid **5** (130 mg, 0.78 mmol) were dissolved in acetonitrile (20 mL) under nitrogen. The reaction mixture was refluxed for 12 hours. A gummy crude product was obtained after filtering out the inorganics and evaporation of solvent. It was chromatographed over neutral alumina column using chloroform-methanol (99 : 1) as eluent. Evaporation of solvents under reduced pressure afforded compound **1** (85 mg, 42%) as pale yellow solid; m.p. 300 °C, dec; ^{1}H NMR (CD_3OD, 250 MHz) δ 11.78 (s, 1H), 8.83 (d, J = 8.6 Hz, 2H), 8.35 (d, J = 8.6 Hz, 2H), 7.64 (m, 5H), 7.33 (m, 3H), 5.08 (s, 2H), 4.17 (s, 2H), 2.34 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 194.9, 134.4, 131.2, 130.9, 130.4, 128.3, 128.1, 127.8, 127.0, 126.9, 126.7, 125.9, 125.2, 125.1, 124.3, 49.9, 48.9; ESI mass spectrum 384.2 $[M+H]^+$.

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