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4-*N*-Methyl-*N*′-(2-dihydroxyboryl-benzyl)amino benzonitrile and its boronate analogue sensing saccharides and fluoride ion

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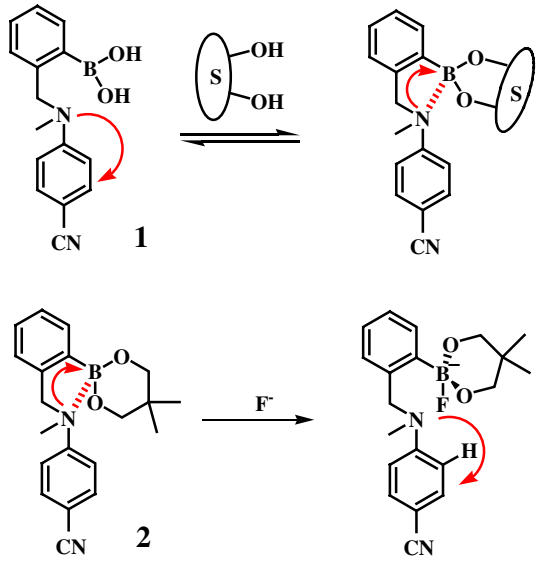
Abstract—DMABN (4-*N*,*N*-dimethylaminobenzonitrile) derivatives **1** and **2** were designed as new ratiometric fluorescent sensors for saccharides and fluoride ion (F^-), respectively, based on the TICT (twisted intramolecular charge transfer) mechanism.

For better understanding the biological roles of saccharides and diagnostic purposes for some diseases, development of efficient methods to detect saccharides is highly desired. Due to the interdisciplinary efforts, a number of saccharides sensors have been developed.[1] Most of the saccharides sensors reported so far contain the boronic acid groups which can bind saccharides leading to either absorption or fluorescence changes. For instance, Shinkai et al.[2] and James et al.[3] have reported a series of saccharides sensors containing three components: a fluorophore, an amine group, and a boronic acid group based on the photoinduced electron transfer (PET) mechanism. In recent years, Wang et al. have designed saccharides sensors also featuring boronic acid groups by employing intramolecular charge-transfer (ICT) mechanism.[4] We have recently reported a new saccharide sensor based on the tetrathiafulvalene-anthracene dyad with a boronic acid group.[5] Besides, other saccharides sensors by making use of the multiple H-bonding formation have also received attention.[6]

To the best of our knowledge, however, no saccharides sensors using typical 4-*N*,*N*-dimethylaminobenzonitrile (DMABN)-based TICT mechanism have been reported, although James et al. have reported an aniline-based ICT sensor showing no dual fluorescence but emission wavelength shift upon saccharide binding.[3g,h] Herein we report a saccharide sensor based on 4-*N*-methyl-*N*′-(2-dihydroxyborylbenzyl)-benzonitrile (**1**, Scheme 1), which shows ratiometric fluorescence change upon binding with saccharides. Besides, the results show that compound **2**, the boronate analogue of **1**, can be a selective sensor for F^-.

The design rationale is schematically illustrated in Scheme 1. Compound **1** can be regarded as the close derivative of DMABN. It is well known that DMABN



Scheme 1. Illustration of the design rationale for new saccharides and F^- sensors based on TICT mechanism.

Keywords: Saccharides sensor; 4-*N*,*N*-dimethylaminobenzonitrile; Boronic acid; Fluoride ion; Fluorescence.
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shows a dual fluorescence consisting of two bands, one being related to the local excited state ('B' band) and the other being ascribed to the twisted intramolecular charge-transfer (TICT) state ('A' band).[7] Moreover, the fluorescence intensity ratio between the 'A' band and 'B' band (I_A/I_B) can be affected by the electron donating ability of the amino group and the substituted groups on the N atom of amino group.[8] Compared to DMABN, one of the methyl groups is replaced by 2-dihydroxyborylbenzyl in compound **1**. It is reported that the boronate shows enhanced Lewis acidity compared to boronic acid.[9] As a result, the B–N interaction would be strengthened after the binding of boronic acid with saccharides. Accordingly, the corresponding charge transfer extent from the amino group to the benzonitrile unit would be reduced. Therefore, the corresponding fluorescence intensity ratio I_A/I_B for compound **1** is expected to be reduced after binding saccharides. Furthermore, the amino group of **1** would be surrounded by a bulky substituent after binding with saccharides. This structural alteration would also affect the dual fluorescence behavior. Therefore, compound **1** is designed as a potential saccharide sensor by combining the features of boronic acid and TICT phenomenon.[7]

Alternatively, compound **2**, the boronate analogue of compound **1**, was prepared as a potential sensor for F^- because of the following consideration: boronate is able to bind with F^- strongly to form the anion adduct and concomitantly the boron electronic configuration will be changed from sp2 to sp3.[10] Thus, compound **2** would be transformed into the anion adduct in the presence of F^-. As a result, the B–N interaction would be weakened, and the extent of charge transfer would be enhanced (Scheme 1). Accordingly, the dual fluorescence behavior of compound **2** is expected to be altered. But, as to be discussed below the fluorescent spectral variation of **2** in the presence of F^- behaves in an unexpected way.

Compounds **1** and **2** were synthesized according to Scheme 2.[11] Briefly, the condensation of 4-methylamino-benzonitrile (**3**) and 2-formyl phenylboronic acid (**4**) followed by reduction yielded compound **1** in 30% yield. Compound **2** was prepared by the reaction of **3** and 2-(2-(bromomethyl)phenyl)-5,5-dimethyl-1,3,2-dioxaborinane (**5**) in 27% yield.

Figure 1 shows the fluorescence spectrum of compound **1** and those in the presence of different amounts of fructose. As expected, compound **1** displayed two bands at 350 and 472 nm. These two fluorescent bands can be regarded as the corresponding 'B' band (from the local excited state) and 'A' band (from the TICT state), respectively. After reaction with fructose, the fluorescence intensity of 'A' band decreased gradually, while that of 'B' band was only slightly reduced. The inset of Figure 1 displays the variation of the fluorescence

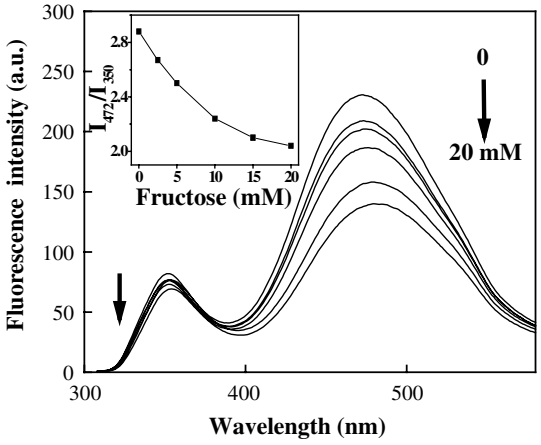
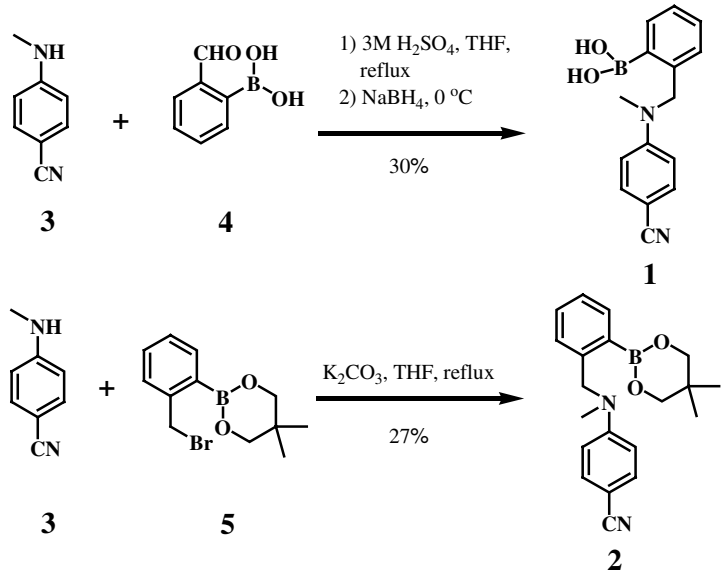


Figure 1. The fluorescence spectra of **1** (5.0×10^{-5} M) in the presence of different concentrations of fructose (0–20 mM) at pH 7.3 adjusted by 0.033 M phosphate buffer in THF/H_2O (1:2, V/V); $\lambda_{ex.}$ = 298 nm.



Scheme 2. Synthesis of compounds **1** and **2**.

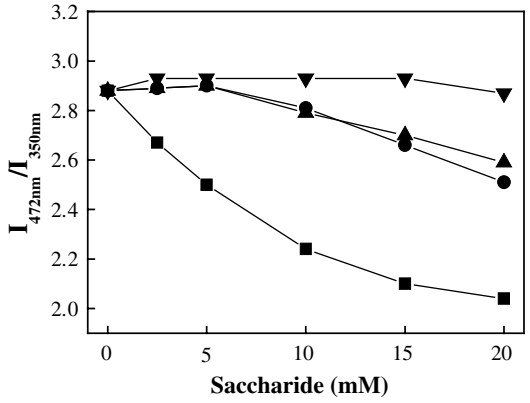


Figure 2. The plot of the fluorescence intensity ratio I_{472}/I_{350} versus the concentrations of glucose (▼), mannose (▲), galactose (●), fructose (■); pH 7.3, $\lambda_{ex.}$ = 298 nm.

intensity ratio I_A/I_B in the presence of different amounts of fructose. Obviously, the fluorescence intensity ratio I_A/I_B decreased by increasing the amounts of fructose added to the solution, reaching the minimum when the concentration of fructose became larger than 20 mM. In addition, the fluorescence spectra of compound **1** in the presence of fructose (20 mM) were also measured at different pH values. The results (see Fig. S1 of SI) showed that more dramatic fluorescence spectral variation occurred at about pH 7. This was in agreement with the previous results.

The fluorescence spectral change observed for compound **1** in the presence of fructose can be understood as follows: as reported early, binding of boronic acid group of **1** with fructose would strengthen the B–N interaction (see Scheme 1),[9] and consequently the charge transfer extent between the benzonitrile and amino groups of **1** would be reduced. The decrease of the charge transfer extent is expected to result in the reduction of the fluorescence intensity ratio I_A/I_B.[8]

Under identical conditions, the fluorescence spectra of compound **1** were also measured in the presence of other saccharides such as galactose, mannose, and glucose. Figure 2 displays the plot of the fluorescence intensity ratio I_A/I_B versus the concentrations of the four saccharides. After reaction of compound **1** with galactose, mannose, and glucose, the fluorescence intensity ratio I_A/I_B decreases. But, compared to fructose, the decrease extent is relatively small for galactose, mannose, and glucose.

The binding constants K_a between **1** and the four saccharides were estimated based on the fluorescence intensity variation at 472 nm in the presence of different amounts of saccharides at pH 7.3 adjusted by 0.033 M phosphate buffer in THF/H_2O (1:2, V/V). The corresponding binding constants of compound **1** with four saccharides were obtained as listed in Table 1, indicating that compound **1** binds more strongly with fructose.

In the following, we will describe the fluorescent spectral variation of compound **2** in the presence of F^- in DMF.

Table 1. The binding constants (K_a) of **1** with saccharides based on the fluorescence changes measured in THF/H_2O (1:2, V/V) at room temperature

Saccharides	K_a (M^{-1})
Fructose	175.1 ± 2.8
Galactose	42.1 ± 0.9
Mannose	26.5 ± 0.3
Glucose	14.3 ± 0.1

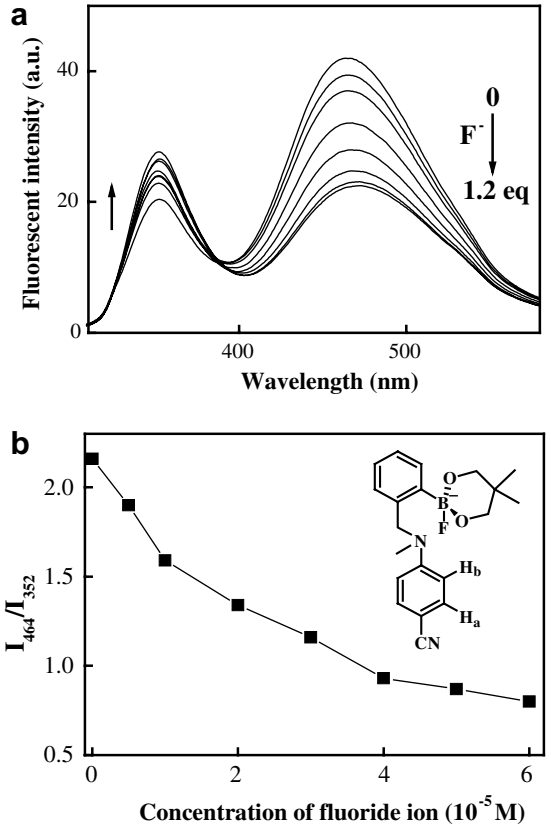


Figure 3. (a) The fluorescence spectra of **2** (5.0×10^{-5} M) in the presence of different concentrations of fluoride ion (0–1.2 equiv) in DMF; $\lambda_{ex.}$ = 298 nm; (b) The plot of the fluorescence intensity ratio I_{464}/I_{352} versus the concentration of fluoride ion in DMF; $\lambda_{ex.}$ = 298 nm.

As shown in Figure 3a, compound **2** also shows a dual fluorescence with two emission bands at 464 nm ('A' band) and 352 nm ('B' band). After introducing F^- to the solution of compound **2**, the fluorescence intensity at 464 nm ('A' band) decreased, while that at 352 nm ('B' band) increased. The fluorescence intensity ratio I_A/I_B varied almost linearly with the concentration of F^- when less than 1.0 equiv of F^- was added as shown in Figure 3b. Almost no further fluorescent spectral changes were observed after more than 1.0 equiv of F^- was introduced. Similar fluorescent spectral variation was also detected for compound **2** after addition of F^- in THF and CH_2Cl_2. The corresponding binding constants of compound **2** with F^- were estimated to be 1.47×10^4, 2.37×10^3, and 2.10×10^3 in DMF, THF, and CH_2Cl_2, respectively. Under the same conditions no fluorescent spectral variation was observed for

compound **2** in the presence of Cl^-, Br^-, I^-, AcO^-, and $HPO_4^{2-}/H_2PO_4^-$. These results show that compound **2** can be a selective sensor for F^-.

As discussed above, due to the binding of F^-, the extent of charge transfer between the amino and benzonitrile groups of **2** would be enhanced. Based on this consideration, it was expected that the fluorescence intensity ratio I_A/I_B would increase for **2** in the presence of F^-. But, as shown in Figure 3, the fluorescence intensity ratio I_A/I_B decreased after binding of **2** with F^-. This unexpected variation of the fluorescence intensity ratio I_A/I_B of **2** in the presence of F^- was tentatively explained as follows: before binding with F^- the 2-dihydroxyboryl-benzyl group of **2** was more rigid due to the 'B–N' interaction. In comparison, after binding with F^- the 'B–N' interaction would be weakened and as a result the 2-dihydroxyboryl-benzyl group of **2** would become more flexible; this may result in reducing the rotational freedom of the amino group,[12] and thus the formation of TICT state would be prohibited to some extent leading to the decrease of the fluorescence intensity of 'A' band.

In summary, by coupling the features of boronic acid and the TICT phenomenon, compound **1** was designed and synthesized as a new saccharide sensor. Ratiometric fluorescence change was observed upon its binding with saccharides. The results also indicated that compound **1** can bind with fructose more strongly than other saccharides tested. In addition, compound **2**, the boronate analogue of compound **1**, was found to be a potentially selective sensor for F^-. We also suggested possible explanation for the unexpected variation of the fluorescence intensity ratio I_A/I_B of **2** in the presence of F^-.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.099.

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11. Compound **1**. 4-(methylamino)benzonitrile (**3**, 88 mg, 0.67 mmol), 2-formylbenzeneboronic acid (**4**, 130 mg, 0.90 mmol), and sulfuric acid (3 M, 0.2 ml) were mixed in THF (5 ml). The mixture was stirred for 1 h at room temperature. Sodium borohydride (30 mg, 0.79 mmol) was added slowly to the mixture after cooling to 0 °C and the resultant solution was stirred overnight at room temperature. After removing THF under reduced pressure, the mixture was dissolved in ethyl acetate (20 ml). The solution was then washed with saturated $NaHCO_3$ (2×10 ml) and water (2×10 ml), and dried over Na_2SO_4. The resultant gum was purified by column chromatography to give **1** as a white solid. (53 mg, 30%). Mp 198–200 °C ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.06 (s, 3H), 4.56 (s, 2H), 6.81 (d, 3H), 6.88 (t, 1H), 6.99 (d, 1H), 7.18 (t, 1H); 7.48 (d, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.7, 53.0, 99.5, 113.3, 115.7, 120.2, 120.8, 122.6, 127.9, 128.7, 133.5, 152.6, 154.2; HRMS (ESI): calcd for ($C_{15}H_{15}BN_2O_2$·2NBA-2H_2O): 536.1867; found: 536.1845. Compound **2**. 4-(methylamino)benzonitrile (**3**, 350 mg, 2.6 mmol), 2-(2-(bromomethyl)phenyl)-5,5-dimethyl-1,3,2-dioxaborinane (**5**, 1.0 g, 3.5 mmol), and potassium carbonate (475 mg, 3.5 mmol) in anhydrous THF (50 ml) were refluxed for 12 h. The mixture was filtered, and the solvent was removed under reduced pressure. The crude material was purified by column chromatography to give **2** as a white solid (239 mg, 27%). Mp 159–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (s, 6H), 3.09 (s, 3H), 3.74 (s, 4H), 4.86 (s, 2H), 6.65 (d, 2H), 6.99 (d, 1H), 7.29 (m, 2H), 7.43 (d, 2H), 7.84 (d, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.8, 31.6, 38.5, 56.1, 72.3, 97.4, 111.6, 111.7, 120.8, 125.2, 126.3, 130.6, 133.4, 135.7, 142.8, 152.3; HRMS (ESI): *m*/*z* calcd for $C_{20}H_{23}BN_2O_2$: 334.1853 [M]; found: 334.1848.
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