A dual role of phenylboronic acid as a receptor for carbohydrates as well as a quencher for neighboring pyrene fluorophore

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**A B S T R A C T**

A simple amino acid based compound (1) containing a phenyl boronic group and pyrene fluorophore showed an enhanced fluorescence in aqueous solutions at physiological pH through suppression of the photoinduced electron transfer from pyrene to boronic acid on carbohydrate binding. The compound exhibited an interesting fluorescence change depending on pH with decreased emission intensity at acidic pH but enhanced emission intensity at basic pH unlike the fluorescent carbohydrate chemosensors using a PET process with amine and aryl-boronic acid. We have characterized a dual role of phenylboronic acid as a receptor for carbohydrates as well as a quencher for the fluorescence of pyrene fluorophore.

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1. Introduction

Detection methods for carbohydrates have been of great interest because carbohydrates play important roles in many biological processes such as nutrition, metabolism, and cell structure. During the last decade, there have been many advances in the development of fluorescent chemosensors for carbohydrate using aryl-boronic acid as a receptor part because the aryl-boronic acid rapidly formed reversible covalent bonding with carbohydrates in aqueous solutions. Aryl-boronic acid has been also used as a receptor part in the chemosensor for anions, diol compounds, serine, and glycoproteins as well as a building block of self-assembled molecules.

As it was considered that the covalent interactions of arylboronic acid with carbohydrates did not considerably change the fluorescence of the neighboring fluorophore, the fluorescent chemosensors based on the aryl-boronic acid have required certain processes for the change of fluorescence depending on the boronic acid—carbohydrate interactions such as an internal charge-transfer (ICT) process and a photoinduced electron transfer (PET) process. Fluorescent chemosensors for carbohydrates with ICT process have received attention because of their sensitive response and ratiometric detection of sugars with fluorescent shift of the complex. However, this kind of chemosensors required a sophisticated design and difficult synthesis of the fluorophores containing both electron-donating group and boronic acid group for carbohydrate binding.

Shinkai et al. have reported pioneer works for fluorescent carbohydrate chemosensors using a PET process with tertiary amine and aryl-boronic acid. In general, this type of fluorescent chemosensor consists of an electron-rich group (e.g., tertiary amine and tetraphiafulvalene group), a fluorophore, and an aryl-boronic acid group (Scheme 1). Before carbohydrate binding, electron–rich amine group quenches fluorescence from the adjacent fluorophore by PET (fluorophore as the acceptor of ET). When the boronic acid group forms cyclic boronate esters with carbohydrates, the acidity of the boronic acid increases and therefore the acid–base interaction between the boronic acid and the amine group increases. As a result, PET from amine to the fluorophore is inhibited and the fluorescence from the fluorophore increases. This kind of PET process with amine and aryl-boronic acid has been widely used for detecting carbohydrates and diol compounds because of sensitive change of emission intensity upon formation of cyclic boronate esters between boronic acid and carbohydrates.

In recent years, there have been many efforts to synthesize chemosensors based on the scaffold of amino acids and peptides because of their compatibility with aqueous media and biological compatibility, and high binding affinity for specific metal ions and biomolecules. These chemosensors based on the scaffold of amino acids and peptides have shown highly sensitive responses to specific analytes in aqueous solutions. In accordance with this trend, as an effort to search for sensitive and water-soluble fluorescent chemosensor for carbohydrates, we synthesized compounds by...
incorporating fluorophores and phenylboronic acid into an ornithine amino acid (see compound 1 in Scheme 2). Surprisingly, although compound 1 did not contain amine as an electron-rich group unlike the PET process proposed by Shinkai et al., it showed turn-on response (ca. 5.8-fold enhancement to fructose) to carbohydrate in aqueous solutions at physiological pH. The emission intensity of 1 increased as the boronate anion form of 1 increased. The pH titration experiment revealed that 1 showed weak fluorescence at neutral pH but strong fluorescence at basic pH. From further experiments, we confirmed that phenylboronic acid served as a quencher for the neighboring pyrene fluorophore, (fluorophore as the donor of the ET). When fructose interacted with the boronic acid to form boronate ester, the \( pK_a \) value of the boronic acid decreased. As a result, PET from pyrene to boronic acid might be inhibited and the fluorescence intensity from the pyrene fluorophore increased. Therefore, boronic acid uniquely serves as a carbohydrate receptor as well as a quencher for the pyrene fluorophore in aqueous solutions. This indicates that the electronic change of phenylboronic acid by covalent interaction with carbohydrates is enough to change the fluorescence of the adjacent pyrene fluorophore. In addition, the new role of phenylboronic acid can make it possible to develop new carbohydrate chemosensor with new fluorescence mechanism using the quenching effect of phenylboronic acid.

Scheme 1. Fluorescent carbohydrate chemosensors using a PET process with tertiary amine and aryl-boronic acid.5

2. Results and discussion

2.1. Solid-phase synthesis of compound 1

Compound 1 was easily synthesized in solid-phase synthesis (Supplementary data, Fig. S1). Fmoc-Orn(Alloc)-OH was attached to the Rink amide resin. After deprotection of alloc group, phenylboronic group was conjugated into the amine group of the side change of Orn acid on the resin. Fmoc group was removed in the basic condition and then, pyrene fluorophore was conjugated. The successful synthesis and high purity (>98%) of 1 was confirmed by using HPLC, ESI mass spectrum, and NMR spectrum (Supplementary data, Figs. S2–S7).

2.2. Fluorescence response of compound 1 to d-fructose

As 1 is highly soluble in water, all photochemical experiments were carried out in aqueous solution containing 1% DMSO. The fluorescent response of 1 was measured with varying amounts of fructose in 50 mM phosphate buffer solution at pH 7.4 (Fig. 1). Compound 1 exhibited a turn-on response to fructose in aqueous solution at physiological pH. About 8 mM of fructose was enough for the saturation of the emission intensity change. Upon the addition of fructose at a saturation amount, the maximum emission intensity of 1 exhibited a ca. 5.8-fold enhancement compared to the emission intensity without any fructose. The fluorescent chemosensors using a PET process with amine and aryl-boronic acid were reported to exhibit 3–7 fold enhancement in the presence of fructose.3,4,6

The UV–vis spectra of 1 are quite sharp both in the presence and absence of fructose and did not change in the presence of milli-

Scheme 2. PET process for carbohydrate sensing and the structure of 1.
absence of fructose at different pH (Fig. 2a). At pH lower than 7.4, 1 exhibited very weak emission intensities. However, the emission intensity increased as pH increased over 7.4 and the emission intensity change was saturated at the pH around 10.5.

![Fig. 1. Fluorescence emission spectra of 1 (2 μM) upon addition of α-fructose (0–8 mM) (λex=342 nm) in 50 mM phosphate buffer solution containing 1% DMSO at pH 7.4.](image)

The pH titration experiment revealed that the emission intensity change of 1 depending on pH seemed to be related to the pKₐ value of phenylboronic acid in water. As pH increased, the emission intensity of 1 increased due to the increase of phenylboronic acid formation. The quantum yield of 1 in acidic condition (pH<6.5) is close to 0, while the quantum yields at pH of 7.4 and 10.5 were 0.0413 and 0.386, respectively. This indicates the strong quenching effect of phenylboronic acid to the pyrene fluorophore. As pH became higher than the pKₐ value of the phenylboronic acid, the boronic acid with sp² hybridization was converted to the tetrahedral boronate form with sp³ hybridization, which induced the increase of fluorescence. This suggests that the tetrahedral boronate form showed a much weaker quenching effect rather than the corresponding boronic form. The pKₐ shift of 1 in the presence of fructose increased the amount of the tetrahedral boronate form with sp³ hybridization at neutral pH, which resulted in the enhanced emission intensity. The fluorescent carbohydrate chemo-sensors using a PET process with tertiary amine and aryl-boronic acid showed increased emission intensity at acidic pH and decreased emission intensity at basic pH due to the quenching effect of amine depending on pH whereas compound 1 showed a reversed emission intensity depending on pH. From the pH titration experiments, we confirmed that phenylboronic acid served as a quencher for the neighboring pyrene fluorophore, (fluorophore as the donor of the ET). The boronate formation between 1 and fructose was investigated by ESI mass spectrometry. When fructose was added to the solution containing 1, a new peak appeared at 688.19 (m/z), which corresponds to [1–fructose–2H₂O+Na⁺⁻]⁻ (Supplementary data, Fig. S9). This result confirms that 1 interacted with fructose and then a boronate ester was formed with fructose. Overall results suggest that the enhancement of emission intensity of 1 in the presence of fructose was due to the tetrahedral boronate form with sp³ hybridization, which suggest that quenching effect of the phenylboronic acid with sp² hybridization for the pyrene fluorophore and a weak quenching effect of the tetrahedral boronate form for the fluorophore.

To confirm the role of boronic acid in fluorescence quenching, the fluorescence spectrum of a pyrene-labeled alanine (2) was measured in the presence of 4-aminocarbonylphenylboronic acid (3) that mimic the moiety of phenylboronic acid of 1 (Supplementary data, Fig. S10). As shown in Fig. 3, the fluorescence of 2 decreased by increasing concentration of 3 (0–26 mM) without the change of UV–vis spectra (Supplementary data, Fig. S10). This result strongly supports that the decrease of emission intensity of 1 in neutral and acidic pH is due to the quenching effect of the phenylboronic acid moiety of 1.

![Fig. 2. (a) Fluorescence response of 1 (2 μM) in the absence of carbohydrate at different pH and (b) the emission intensity at 377 nm as a function of pH in the presence (●) and absence (■) of fructose (8 mM) in 50 mM phosphate buffer solution containing 1% DMSO.](image)

![Fig. 3. Fluorescence emission spectra of 2 (10 μM) upon addition of 3 (0–30 mM) in 50% DMSO/water (λex=342 nm).](image)

We investigated the fluorescent response of 1 to D-tagatose, D-galactose, D-mannose and D-glucose, respectively. As shown in Fig. 4, compound 1 showed an enhanced emission intensity with the addition of the carbohydrates. The higher enhancements of fluorescence were observed in ketohexoses (D-tagatose and D-fructose) rather than aldohexoses (D-mannose, D-galactose, and D-glucose). There are considerable differences of the fluorescence enhancement in aldohexoses, whereas there is a small difference of the fluorescence change between D-tagatose and D-fructose. The structural parameters such as the dihedral angles of the binding
diols of the aldohexoses showed a considerable influence on the fluorescence response and the structural parameters such as the dihedral angles of the binding diols of the ketohexoses also showed some effect on the fluorescence response. Assuming a 1:1 complex formation, the association constants ($K_a$) of compound 1 for the sugars were calculated based on the titration curve. The $K_a$ values of compound 1 for the sugars are summarized in Table 1. The order of $K_a$ values for the sugars is similar to those observed for the sugars with phenylboronic acid. The affinity trend of the sugars with compound 1 followed that of the reported carbohydrate chemosensors using boronic acid, and the order of $K_a$ values for the sugars is similar to those observed for the sugars with phenylboronic acid. The enhancement of the emission intensity of compound 1 correlates with the association constants for the sugars. This observation also suggests that the enhancement of emission intensity of compound 1 in the presence of the sugar is due to the boronic ester formation between compound 1 and the sugar.

**Table 1** Apparent association constants ($K_a$) and the detection limit of compound 1 for carbohydrates

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>$K_a$ [M$^{-1}$]</th>
<th>The detection limit [M]</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Fructose</td>
<td>1044.0</td>
<td>4.6 $\times 10^{-6}$</td>
</tr>
<tr>
<td>d-Tagatose</td>
<td>927.9</td>
<td>6.7 $\times 10^{-6}$</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>83.2</td>
<td>6.8 $\times 10^{-5}$</td>
</tr>
<tr>
<td>d-Mannose</td>
<td>70.3</td>
<td>10.5 $\times 10^{-5}$</td>
</tr>
<tr>
<td>d-Glucose</td>
<td>30.7</td>
<td>28.3 $\times 10^{-5}$</td>
</tr>
</tbody>
</table>

**2.4. The detection limit of compound 1 for the sugars**

Compound 1 showed a linear response of the fluorescence emission intensity toward fructose in the concentration range from 0 to 2.0 $\times 10^{-4}$ M, as shown in Fig. 5. The detection limit of compound 1 for fructose was calculated as 4.6 $\times 10^{-6}$ M based on 3σ/m, where σ is the standard deviation of the blank measurements, and m is the slope of the intensity versus sample concentration in Fig. 5.

Similarly, the detection limits of compound 1 for the other sugars were calculated as shown in Fig. S11 and were summarized in Table 1. The detection limits for the sugars are in agreement with their binding constants for the sugars. For example, compound 1 showed the highest binding constant for fructose and the lowest detection limit for fructose.

**2.5. DFT calculation**

To examine the molecular-level origin of the fluorescence quenching occurring in compound 1, we performed quantum chemical calculation using Gaussian03 program. First, we optimized the ground-state structure of an isolated molecule of compound 1 using density functional theory (DFT) method with B3LYP/6-31G(d) functional. Then, using one of the optimized structures, we performed time-dependent DFT (TD-DFT) calculation to obtain the electronic structure of the excited states and associated electronic transitions. From the calculation, we found that the $\pi$ $\rightarrow$ $\pi^*$ transition between HOMO and LUMO is a major electronic transition of compound 1. In both HOMO and LUMO, electron density is highly localized in pyrene and the $\pi$ $\rightarrow$ $\pi^*$ transition occurs only around the pyrene fluorophore. In addition, we found an electronic transition that induces PET from pyrene to boronic acid. The molecular orbitals involved in the PET transition are shown in Fig. 6 (Supplementary data, Fig. S12). The orbital with charge-transfer (CT) character, MO$_{CT}$, has highly localized electron density around the boronic acid moiety. As a result of the electronic transition between HOMO and MO$_{CT}$, the charges are transferred from pyrene to the boronic acid moiety, resulting in the quenching of fluorescence from the pyrene fluorophore. Thus, we confirmed that PET between the pyrene fluorophore and boronic acid moiety might give rise to fluorescence quenching of compound 1.

The process of phenylboronic acid for monitoring carbohydrate presented in this work is a unique PET process because an electronically rich group (e.g., tertiary amine and tetrathiafulvalene group) is absent between fluorophore and boronic acid and the boronic acid itself acting as the acceptor of ET directly quenches the pyrene fluorophore. A chemosensor with similar working mechanism was previously reported by using a complex between $\beta$-cyclodextrin ($\beta$-CD) and pyrene-appended chemosensor using boronic acid. However, an additional chemical component of $\beta$-CD, which is known to inhibit PET as well to change the fluorescent property in the complexation with CD, is involved in the complex of the sensor and, therefore, the synthesis and possibly analysis of carbohydrate detection can be more complicated.

In contrast, as the carbohydrate sensing compound presented in this work was a simpler system that contains only fluorophore and.
boronic acid separated by non-functional chemical backbone, the role of phenylboronic acid for quenching the pyrene fluorophore was much clearly characterized.

3. Conclusion

We report that the phenylboronic acid moiety of 1 acts as a carbohydrate receptor as well as a fluorescence quencher for the pyrene. The boronate ester formation of 1 with carbohydrates results in the large enhancement of emission intensity in aqueous solution at physiological pH by preventing PET process from the pyrene fluorophore to boronic acid. As pH increased, the emission intensity of 1 increased due to the increase of phenyl boronate formation, whereas as pH decreased, the emission intensity of 1 decreased due to the increase of phenylboronic acid formation. The unique role of phenylboronic acid elucidate in this study will provide a new sensing mechanism and readily synthesizable system for sensing carbohydrates by turn-on response in aqueous solutions.

4. Experimental section

4.1. Reagents

Fmoc·Orn(alloc)-OH was purchased from Bachem, Fmoc·Ala—OH, Rink Amide MBHA resin, 1-hydroxybenzotriazole (HOBt) and N,N′-diisopropylcarbodiimide (DIC) were purchased from Bead Tech. 1-pyreneacetic acid, 4-aminocarbonylphenylboronic acid, 4-carboxyphenylboronic acid pinacol ester, trifluoroacetic acid (TFA), N,N′-dimethylformamide (DMF), dichloromethane (DCM), tetraakis(triphenylphosphine) palladium(0) (Pd(PPh₃)₄), phenylsilane, piperidine were purchased from Sigma Aldrich.

4.2. Synthesis

4.2.1. Solid-phase synthesis of 1. Compound 1 was synthesized by solid-phase synthesis with Fmoc chemistry. Fmoc protected l-Ala—OH was assembled on Rink Amide MBHA resin. After deprotection of Fmoc group, 1-pyreneacetic acid (78 mg, 0.3 mmol, 3 equiv) was activated with DIC (47 µl, 0.3 mmol, 3 equiv) and HOBt (40 mg, 0.3 mmol, 3 equiv) and then was coupled with the resin bound amino acid. Cleavage of 1 from resin was achieved by treatment with a mixture of 3 ml TFA/H₂O (95:5, v/v) at room temperature for 3 h. The crude product was triturated with diethyl ether chilled at −20 °C and then centrifuged at 3000 rpm for 10 min at −10 °C. The crude product was purified by HPLC with a Vydac C18 column using a water (0.1% TFA)/acetonitrile (0.1% TFA) gradient to give 80% of final product. The successful synthesis was confirmed by ESI mass spectrometry (platform II, micromass, Manchester, UK) and its homogeneity (>95%) was confirmed by reversed phase analytical HPLC with C₁₈ column. Compound 1 was characterized by melting point, H NMR, ¹³C NMR, ESI mass data, IR and elemental analysis (Supplementary data, Figs. S2–S7).

White solid, M.P. = 265–266 °C, ¹³C NMR (400 MHz, DMSO-d₆) δ: 8.46 (1H, t, J = 8.4 Hz), 8.39 (1H, d, J = 8.4 Hz), 8.37 (1H, d, J = 8.4 Hz), 8.25 (1H, d, J = 8.4 Hz), 8.20 (1H, J = 8.6 Hz), 8.18–8.15 (3H, m), 8.13 (1H, d, J = 8.3 Hz), 8.12 (1H, s), 8.02 (1H, d, J = 8.4 Hz), 8.0 (1H, d, J = 8.4 Hz), 7.85 (2H, d, J = 8.6 Hz), 7.78 (2H, d, J = 8.6 Hz), 7.38 (br s, 1H), 7.0 (br s, 1H), 4.32–4.20 (m, 1H), 3.35 (s, 2H), 3.30–3.25 (2H, m), 1.78–1.70 (1H, m), 1.62–1.58 (2H, m), 1.47–1.43 (1H, m). ¹³C NMR (50 MHz, DMSO-d₆), δ: 174.07, 170.53, 166.76, 148.87, 137.37, 136.12, 134.19, 131.17, 131.01, 130.54, 129.95, 129.20, 129.02, 127.63, 127.43, 127.07, 126.41, 126.33, 125.33, 125.16, 124.98, 124.29, 124.26, 124.60, 56.83, 52.46, 30.05, 26.12. ESI Mass (m/z) calculated for C₁₀H₁₂BN₂O₅ [M + H]⁺, 522.21; found, 522.39; Elemental analysis (calcld, found for C₁₀H₁₂BN₂O₅): C (69.11, 68.98), H (5.41, 5.38), N (8.06, 7.99).

4.2.2. Solid-phase synthesis of 2. Compound 2 was synthesized by solid-phase synthesis with Fmoc chemistry. Fmoc protected l-Ala—OH was assembled on Rink Amide MBHA resin. After deprotection of Fmoc group, 1-pyreneacetic acid (78 mg, 0.3 mmol, 3 equiv) was activated with DIC (47 µl, 0.3 mmol, 3 equiv) and HOBt (40 mg, 0.3 mmol, 3 equiv) and then was coupled with the resin bound amino acid. Cleavage of 2 from resin was achieved by treatment with a mixture of 3 ml TFA/H₂O (95:5, v/v) at room temperature for 3 h. The crude product was triturated with diethyl ether chilled at −20 °C and then centrifuged at 3000 rpm for 10 min at −10 °C. The crude product was purified by HPLC with a Vydac C18 column using a water (0.1% TFA)/acetonitrile (0.1% TFA) gradient to give 85% of 2. Compound 2 was characterized by melting point, H NMR, ¹³C NMR, ESI mass data, IR, and elemental analysis (Supplementary data, Figs. S13–S18).

White solid, mp = 266–267 °C, ¹³C NMR (400 MHz, DMSO-d₆) δ: 8.39 (1H, d, J = 8.8 Hz), 8.36 (1H, d, J = 8.3 Hz), 8.27 (2H, d, J = 8.5 Hz), 8.22 (1H, J = 8.8 Hz), 8.19 (1H, d, J = 8.8 Hz), 8.14 (2H, m), 8.06 (1H, d, J = 8.5 Hz), 8.01 (1H, J = 8.5 Hz), 7.34 (1H, br s), 6.97 (1H, br s), 4.26–4.25 (1H, m), 3.35 (2H, s), 1.22 (3H, d, J = 7.6 Hz). ¹³C NMR (50 MHz, DMSO-d₆), δ: 174.28, 169.78, 169.78, 131.06, 130.83, 130.37, 129.70, 129.03, 128.70, 127.40, 127.17, 126.82, 126.17, 125.06, 124.74, 124.74, 124.15, 124.09, 123.93, 48.091, 40.13, 18.58, ESI mass (m/z) calculated for C₂₁H₁₈N₂O₂: [M − HCOO] −, 375.14; found, 374.93; Elemental analysis (calcld, found for C₂₁H₁₈N₂O₂): C (76.34, 76.01), H (5.49, 5.42), N (8.48, 8.23).

4.3. General fluorescence measurements

A stock solution of the compound 1 at the concentration of 1 × 10⁻³ M was prepared in DMSO/water (50:50 v/v) and stored in a cold and dark place. The concentration of stock solution was confirmed by UV absorbance at 342 nm for pyrene. For the convenience, the stock solution at the concentration of 0.1 × 10⁻³ M was used for the fluorescence measurements after appropriate dilution and fluorescence measurements were carried out in 50 mM phosphate buffer solution at pH 7.4 containing 1% DMSO. Fluorescence emission spectrum of the compound 1 in 10 nm path length quartz cuvette was measured using a Perkin–Elmer luminescence spectrometer (Model LS 55). Emission spectra of the sensor in the presence of different monosaccharides were measured by excitation with 342 nm for pyrene.

4.4. Determination of association constant

The association constant (Kₐ) was calculated based on the titration curve of the host with carbohydrate. The association constants were determined by a nonlinear least squares fitting of the data with the following equation.

\[
\text{Fluorescence} = \text{Fluorescence}_0 + \frac{\text{Fluorescence}_0 - \text{Fluorescence}_1}{1 + \left(\frac{[S]}{K_a}\right)}
\]

where \(\text{Fluorescence}_0\) is the fluorescence intensity in the absence of carbohydrate, \(\text{Fluorescence}_1\) is the fluorescence intensity in the presence of a large excess of carbohydrate, \([S]\) is the concentration of carbohydrate, and \(K_a\) is the association constant.
\[ I = \frac{I_{\text{min}} + I_{\text{max}}K_a |S|}{1 + K_a |S|} + K'_a |S| \]

Where \( I_{\text{min}} \) and \( I_{\text{max}} \) are the initial (no sugar) and final (plateau) fluorescence intensities of the titration curves and \( K'_a \) is slope of the linear equation for the fitting of the linearly increased emission intensity as function of the concentration of carbohydrate.

4.5. Determination of \( pK_a \)

The \( pK_a \) value was calculated based on the fluorescence intensity of the host compound with and without D-fructose in 50 mM phosphate buffer solution containing 1% DMSO at different pH. The \( pK_a \) values were obtained by a nonlinear least squares fitting of the data with the Henderson-Hasselbalch equation.17

\[ I = I_{\text{max}} \times 10^{|pH-pK_a|} + I_{\text{min}} \]

where, \( I, I_{\text{min}}, \) and \( I_{\text{max}} \) are observed, minimum, and maximum fluorescence intensity, respectively.

4.6. Quantum yield measurement

Fluorescence quantum yields of 1 in different pH were calculated using anthracene as a standard (\( \Phi = 0.27 \)). The absorbance was recorded in buffer solutions containing 1% DMSO in 10 mm cell. The fluorescence spectra of the solutions were recorded with the excitation wavelength of 342 nm and the relative fluorescence was determined by weighing the area beneath the corrected fluorescence emission spectrum. Finally, the quantum yields of 1 in different pH were calculated as follows.19

\[ Q(\text{unknown}) = \frac{Q(\text{standard}) \\ F(\text{unknown}) \times A(\text{standard})}{F(\text{standard}) \times A(\text{unknown})} \]

where \( F \) is the fluorescence measured using a Perkin–Elmer luminescence spectrometer (Model LS 55) and \( A \) is the absorbance of Perkin–Elmer UV–vis spectrometer (Model L25).

4.7. Measurement of detection limit

The detection limit was calculated based on the fluorescence intensity of 1 in the absence of sugars was measured by 10 times and the standard deviation of blank measurements was determined. Three independent duplication measurements of emission intensity were performed in the presence of carbohydrates and each average value of the intensities was plotted as a concentration of carbohydrates for determining the slope. The detection limit is then calculated with the following equation.

Detection limit \( = 3\sigma / m \)

where \( \sigma \) is the standard deviation of blank measurements, \( m \) is the slope between intensity and sample concentration.

4.8. Quantum chemical calculation

To examine the molecular-level origin of fluorescence quenching occurring in 1, we performed quantum chemical calculation using Gaussian03 program. First, we optimized the ground-state structure of an isolated molecule of 1 using density functional theory (DFT) method with B3LYP/6-31G(d) functional. DFT method is most commonly used for optimizing the molecular structure and calculating the energy of a ground-state molecule at equilibrium because it requires less computational time than ab initio methods but still treats the electron correlation relevantly. Then, using one of the optimized structures, we performed time-dependent DFT (TD-DFT) calculation to obtain the electronic structure of the excited states and associated electronic transitions. TD-DFT method is appropriate for calculating the energies of excited states because it considers the effect of time-dependent potentials (e.g., electromagnetic fields) on the properties and dynamics of many-electron systems. From the TD-DFT calculation of 1, we found that the electronic transition at the wavelength \( \lambda = 345 \text{ nm} \) has the largest oscillator strength (\( f = 0.033 \)), and contains the \( \pi \rightarrow \pi^* \) transition between HOMO (MO #137) and LUMO (MO #138) as its major component (85%). As can be seen in Fig. S12, electron density is highly localized in pyrene for both HOMO and LUMO, and therefore the \( \pi \rightarrow \pi^* \) transition occurs only around the pyrene fluophore. Among other allowed transitions, an electronic transition at \( \lambda = 360 \text{ nm} \) (\( f = 0.002 \)) contains the transition between HOMO and MO #139 as its major component (92.3%). As can be seen in Fig. S12, MO #139 has highly localized electron density around boronic acid. Therefore, an electronic transition between HOMO and MO #139 will give rise to the photoinduced electron transfer (PET) from the pyrene fluorophore to boronic acid, and is responsible for the fluorescence quenching of 1. We term MO #139 as an orbital with charge-transfer (CT) character, MOCT.

Acknowledgements

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Supplementary data

Synthesis scheme, Copies of \(^1\text{H}\) NMR, \(^{13}\text{C}\) NMR, ESI mass and elemental analysis and additional spectroscopic data for compounds 1 and 2. This material is available free of charge via the internet at www.sciencedirect.com. Supplementary material associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2013.11.023.

References and notes


