Pseudorotaxane-type fluorescent receptor exhibiting unique response to saccharides

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Synthesis of 1.

4-Carboxyphenylboronic acid (266 mg, 1.6 mmol), DCC (330 mg, 1.6 mmol), and 1-hydroxybenzotriazole monohydrate (245 mg, 1.6 mmol) were dissolved with pyridine (20 mL), and stirred for 1 h in an ice bath. 3-Amino-3-deoxy-β-cyclodextrin (907 mg, 0.8 mmol)§1 was added to the solution at once, and the solution was stirred for 1 h in an ice bath and for 12 h at room temperature. Precipitates formed were removed with a cotton plug, and the filtrate was concentrated to approximately 5 mL. The concentrated mixture was poured into acetone (200 mL) with vigorous stirring. White precipitates formed were collected, washed with acetone followed by ether, and dried in vacuo (60 °C).
This material was dissolved with water (10 mL) and lead to a reverse phase column (Chromatorex DM-1020T, 2 × 10 cm, pre-equilibrated with 10% MeOH). Step gradient elution was made with 10% and 20% MeOH. The fractions of 20% MeOH were collected, and concentrated. Glassy solids obtained were re-dissolved with water to be lyophilized to afford white fluffy powders (808 mg, 71%). m/z (FAB, matrix thioglycerol (TG)) 1376 ([M+TG-2H2O+Na]+); δH (600 MHz, DMSO-d6, referred to DMSO-d5 (internal, 2.490 ppm)) 3.2-4.0 (m, overlapped with H2O), 4.34-4.52 (m, 6H), 4.66-5.02 (m, 8H), 5.30-5.90 (m, 15H), 7.7940 (d, J = 8.3 Hz, 2H), 7.8352 (d, J = 8.3 Hz, 2H), 8.0775 (d, J = 8.9 Hz, 1H), 8.1479 (s, 2H); Found C, 44.0; H, 6.1; N, 1.0. C49H76BNO37•3H2O requires C, 44.1; H, 6.2; N, 1.1%.

**Synthesis of C7SP.**

A mixture of 1-heptyl-4-methylpyridinium bromide (3.68 g, 13.5 mmol), which was prepared from 4-picoline and 1-bromoheptane, 4-dimethylaminobenzaldehyde (1.98 g, 13.3 mmol), and piperidine (1.3 mL) in EtOH (20 mL) was heated at reflux for 6 h under N2 atmosphere. After being left in ice bath, reddish precipitates formed were collected, and washed with a cold EtOH. The obtained solids were recrystallized from EtOH – n-hexane (1:3). Yield 2.68 g (50%). m/z (FAB) 323.2472 (M+ C22H31N2 requires 323.2486); δH (600 MHz, D2O, referred to CH3CN (internal, 1.930 ppm)) 0.7103 (t, J = 6.9 Hz, 3H), 1.12 (m, 4H), 1.19 (m, 4H), 1.8281 (quant, J = 7.6 Hz, 2H), 2.8714 (s, 3H), 4.2811 (t, J = 6.8 Hz, 3H), 6.8093 (d, J = 8.9 Hz, 2H), 6.9833 (d, J = 16.5 Hz, 1H), 7.5273 (d, J = 8.9 Hz, 2H), 7.61715 (d, J = 15.8 Hz, 1H), 7.7867 (d, J = 6.9 Hz, 2H), 8.3467 (d, J = 6.2 Hz, 2H); Found: C, 63.1; H, 7.5; N, 6.6. C22H31BrN2 requires C, 65.5; H, 7.8; N, 6.9%. Results of the elemental analyses indicate approximately 96% dye content.

**Determination of pK_a of I.**
The \( pK_a \) values of 1 alone and in the presence of D-fru, D-glc, D-gal, and D-man were determined from the pH-dependent UV-vis absorbance changes. Figure S1 shows the UV-vis spectra of 1 at various pH. A photometric method to determine \( pK_a \) of phenylboronic acid derivatives is straightforward. However, none of the researches have paid attention on the strong absorption band around 230 nm of the phenylboronic acid derivatives, rather many researches used the pH-dependent absorbance changes at the UV absorption band around 280 nm. Thus, we would like to brief description on the pH-dependent UV-vis spectral changes around the 230 nm band.

Under acidic conditions, 1 shows the absorption maximum at 237 nm, whereas the peak position is shifted to 243 nm under alkaline conditions with the isosbestic point at 245 nm. The bathochromic shift of 1 at high pH may be related to the generation of a negative charge on the boron atom. The observed bathochromic shift accompanies the hypochromic effect. Figure S2 shows the pH dependence of absorbance at 237 nm. From this titration curve, the \( pK_a \) value of 1 was determined to be 7.63 ± 0.01. Apparent \( pK_a \) values of 1 in the presence of 30 mM of each saccharide were also determined. The obtained \( pK_a \) values are described in the text. In the \(^1\)H NMR spectra, a single peak at 7.60 ppm (referred to HOD at 4.67 ppm) was observed at pH(D) above 10. As pH(D) decreased, the single peak became split into two equivalent doublet peaks. Below pH(D) 6, the two peaks existed at 7.73 and 7.80 ppm. This \(^1\)H NMR titration results also yielded the \( pK_a \) value of 1 to be 8.06 ± 0.09, which agreed well with the \( pK_a \) value of 1 determined from the UV-vis titration experiments when the well-known relationship of \( pD = pH + 0.4 \) was taken into account.

**Determination of \( pK_a \) of C7SP.**

Since C7SP has an \( N,N \)-dimethylamino moiety on the benzene ring, it would act as a weak base in an aqueous solution. The \( pK_a \) of C7SP was determined by pH-dependent UV-vis spectral changes. Figure S3 shows plots of absorbance at 444 nm as a function of pH. This sigmoidal plot allowed us to
determine the pKₐ value of C7SP to be 3.27 ± 0.01.

*pH dependence of pseudorotaxane-type complexation of C7SP with 1.*

Based on the pKₐ values of 1 and C7SP, the neutral form of C7SP prevailed, whereas both anionic and neutral forms of 1 are present, when they exist in a neutral aqueous solution. It is known that both *intra-* and *intermolecular* guest binding properties of modified CDs are affected by the acid-base equilibrium of a pendant moiety. From this viewpoint, we investigated the complexation of C7SP with 1 at pH 5.6 and 9.6 in addition to pH 7.2. At pH 5.6, both 1 and C7SP exist as their neutral forms, whereas the anionic form is predominant species for 1 at pH 9.6. ¹H NMR titration experiments revealed that the C7SP formed both 1:1 and 1:2 host-guest complexes with 1 at pH(D) 5.6 and 9.6 (Figs S4 and S5) as in the case of pH(D)7.2. It is noteworthy that the titration plots of Figs. 2, S4, and S5 look similar. This similarity indicates that the binding conformations of the C7SP/1 complex are scarcely affected by the negative charge of 1. From the ¹H NMR titration results, we propose the conformations of the 1:1 and 2:1 complexes as Fig. S6.

Although the binding conformations of the C7SP/1 complex assumed to be independent of pH, the stabilities of the complexes are affected by pH. This was confirmed by fluorescence intensity changes (472 nm) of C7SP caused by 1 from which K₁ and K₂ values for the C7SP/1 complex were successfully determined (Fig S7). The obtained K₁ and K₂ values are listed in Table S1. In summary, both the K₁ and K₂ values become larger as pH increases. This pH dependence associated with the complex formation indicates that the anionic form of 1 is a better host for cationic C7SP. Contribution of the electrostatic interaction between 1 and C7SP may be a good reason for the increased stability of the C7SP/1 complex under alkaline conditions.

The largest K₁ value at pH 9.6 is acceptable, because the chemical shift changes (Figs. 2, S4, and S5) indicate that the first step complexation (resulting in K₁) occurs at the aromatic moiety of C7SP
where the positive charge exists. The $K_2$ value at pH 9.6 also increased. We consider that this increase in the $K_2$ value is due to the CH–π interaction between the anionic phenylboronate residue of 1 and the methylene (and methyl) groups of C7SP. The negative charge generated at the boron atom of 1 may increase the π basicity of the phenylboronic acid residue, leading to the increased stability of the 1:2 complexes. In addition, the anionic character of the phenylboronic acid residue makes it difficult to insert itself into the hydrophobic cavity of 1. Insertion of a pendant group of modified CDs (intramolecular complex formation) generally hinders guest accommodation. When the phenylboronic acid residue of 1 becomes its anionic form, intramolecular complex formation may be suppressed, and the intermolecular complex formation with C7SP may be facilitated.

On the mechanism of fluorescence enhancement.

The fluorescence titration experiments at different pH confer important information for the prominent fluorescence of the C7SP/1 and C7SP/1/saccharide complexes. As described in the text, the presence of the phenylboronic acid residue was critical to the marked fluorescence enhancement of C7SP when it complexed with 1. The increased stabilities of the C7SP/1 complex under the alkaline condition demonstrate that the negative charge of the phenylboronic acid residue of 1 actually stabilizes the 1:1 and 1:2 complexes. We consider that the electrostatic and CH–π interactions are operative to gain the stabilities. From the viewpoint of the fluorescence enhancements, the first step complexation is more important, because this complexation step involves the π–π interaction attributable to a modulator of fluorescence in many complexes. In the case of C7SP, the weak fluorescence in aqueous solution is partly due to the hydrophilicity around C7SP molecules. However, the limited fluorescence enhancement caused by β-CD expects that other mechanisms are operative in the further enhancement of the fluorescence of C7SP.

The fluorescence enhancement factors for the first and second steps of the complexation ($R_1$ and $R_2$,
respectively, Table S1) were larger when pH increased. As described above, the \( \pi-\pi \) interaction between the aromatics of C7SP and 1 was more effective at pH 9.6. The increased \( \pi-\pi \) interaction restricts the molecular motion of C7SP, promoting the fluorescent decay pathway. When the phenylboronic acid residue of 1 is in its neutral form, the electrostatic interaction is absent to weaken the \( \pi-\pi \) interaction between the aromatics of C7SP and 1. The weakened \( \pi-\pi \) interaction renders the molecular motion of C7SP free in some extent, resulting in the observed smaller \( R_1 \) and \( R_2 \) values at pH 5.7. In the presence of the monosaccharides, the phenylboronic acid residue of 1 forms cyclic boronate ester in which a negative charge is appended at the boron atom. Owing to the generated negative charge, the fluorescence of C7SP would be enhanced further when saccharides to bind 1 are present in a solution. The smaller and larger fluorescence enhancements caused by D-fru and D-glc, respectively, may be due to the difference in the binding conformations, because D-fru /1 and D-glc/1 are different hosts for C7SP.

Since the above discussion is still speculative, we need more concrete evidence to clarify the mechanism for the observed fluorescence enhancement. Based on the above proposed mechanism, we are now undertaking several experiments to get insight into the fluorescence enhancements.

References

Table S1. Binding Constants ($K_1$ and $K_2$) and Fluorescence Enhancement Factors ($R_1$ and $R_2$) of 1 ($pK_a = 7.63$) with C7SP ($pK_a = 3.27$) at Different pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>$K_1$ / M$^{-1}$</th>
<th>$R_1$</th>
<th>$K_2$ / M$^{-1}$</th>
<th>$R_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>5100 ± 720</td>
<td>25</td>
<td>350 ± 90</td>
<td>51</td>
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<tr>
<td>7.2</td>
<td>16200 ± 1500</td>
<td>128</td>
<td>1760 ± 190</td>
<td>241</td>
</tr>
<tr>
<td>9.7</td>
<td>23100 ± 2600</td>
<td>189</td>
<td>2320 ± 240</td>
<td>278</td>
</tr>
</tbody>
</table>

Fig. S1  UV-vis spectra of 1 (5.08 × 10$^{-5}$ M) at various pH in water.

Fig. S2  Plots of absorbance at 237 nm of 1 as a function of pH. The solid line is the best fit pH titration curve with $pK_a$ 7.63.
Fig. S3  Plots of absorbance at 444 nm of C7SP as a function of pH. The solid line is the best fit pH titration curve with $pK_a$ 3.27.

Fig. S4  Chemical shift changes of C7SP (0.65 mM) upon the addition of 1 in D$_2$O buffered by acetate ($I = 0.1$ M, pH(D) 5.7). Negative and positive $\Delta\delta$ values correspond to high- and low-field shifts, respectively. Ha and Hb signals were severely broadened to disappear at 0.2 mM 1.

Fig. S5 Chemical shift changes of C7SP (0.35 mM) upon the addition of 1 in D$_2$O buffered by carbonates ($I = 0.1$ M, pH(D) 9.7). Negative and positive $\Delta\delta$ values correspond to high- and low-field shifts, respectively. Ha and Hb signals were severely broadened to disappear at 0.1 mM 1.
Fig. S6 Cartoons for plausible 1:1 (top) and 1:2 (bottom) complexes of C7SP with 1, deduced from the $^1$H NMR titration data.

Fig. S7 Fluorescence intensity variations of C7SP (36 μM) induced by 1 in pH 5.6 (acetate buffer, $I = 0.07$ M; blue circle) or pH 9.6 (carbonate buffer, $I = 0.07$ M; red circle). The solid and dashed lines are the best-fit curves with $K_1 = 5100$ M$^{-1}$ and $K_2 = 350$ M$^{-1}$ (pH 5.6, solid line) and with $K_1 = 23100$ M$^{-1}$ and $K_2 = 2320$ M$^{-1}$ (pH 9.6, dashed line).