[Electronic Supplementary Information]

Pseudorotaxane-type fluorescent receptor exhibiting unique response to saccharides

Akiyo Yamauchi,[a] Yoshiko Sakashita,[a] Kazuaki Hirose,[a] Takashi Hayashita,[b] and Iwao Suzuki*[a]

[a]Graduate School of Pharmaceutical Sciences, Tohoku University

Aramaki, Aoba-ku, Sendai 980-8578, Japan.

Fax: +81-22-795-5916; Tel: +81-22-795-6802; E-mail: isuzuki@mail.pharm.tohoku.ac.jp

[b]Department of Chemistry, Faculty of Science and technology, Sophia University

7-1 Kioicho, Chiyoda-ku, Tokyo 102-8554, Japan.

Fax: +81-3-3238-3361; Tel: +81-3-3238-3372; E-mail: ta-hayas@sophia.ac.jp

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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)[S1] 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 ºC).

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 ($[M+TG-2H_2O+Na]^+$); δ_H (600 MHz, DMSO-d_6, referred to DMSO-d_5 (internal, 2.490 ppm)) 3.2-4.0 (m, overlapped with H_2O), 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. $C_{49}H_{76}BNO_{37}\bullet 3H_2O$ 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 N_2 atmosphere. After being left in ice bath, reddish precipitates formed were colleted, 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); δ_H (600 MHz, D_2O, referred to CH_3CN (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. $C_{22}H_{31}BrN_2$ 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 **1**.*

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 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 pK_a value of **C7SP** to be 3.27 ± 0.01.

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

Based on the pK_a 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.[S2] 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. ^{1}H 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 ^{1}H 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 K_1 and K_2 values for the **C7SP/1** complex were successfully determined (Fig S7). The obtained K_1 and K_2 values are listed in Table S_1. In summary, both the K_1 and K_2 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 K_1 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 K_1) 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.[S3] 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 π– π interaction between the aromatics of **C7SP** and **1** was more effective at pH 9.6. The increased π– π 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 π– π interaction between the aromatics of **C7SP** and **1**. The weakened π– π 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

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S3 T. Kuwabara, H. Nakajima, N. Nanasawa and A. Ueno, *Anal. Chem.*, 1999, **71**, 2844; Y. Liu, C. –C. You, S. He, G.. –G. Chen and Y. –L. Zhao, *J. Chem. Soc., Perkin Trans. 2*, 2002, 463.

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.

pH	K_1 / M^{-1}	R_1	K_2 / M^{-1}	R_2
5.7	5100 ± 720	25	350 ± 90	51
7.2	16200 ± 1500	128	1760 ± 190	241
9.7	23100 ± 2600	189	2320 ± 240	278

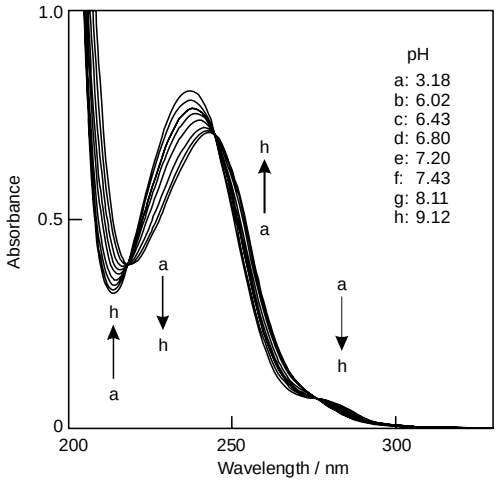

Fig. S1 UV-vis spectra of **1** (5.08×10^{-5} M) at various pH in water.

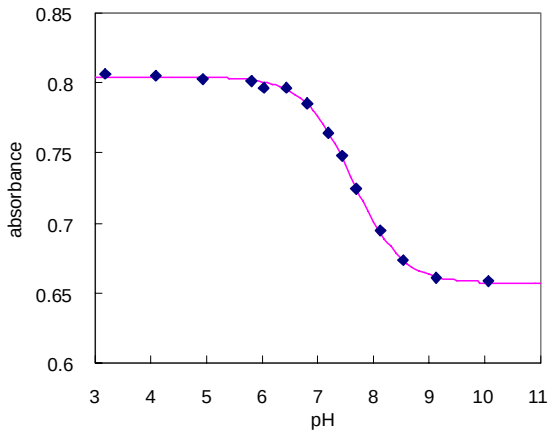

Fig. S2 Plots of absorbance at 237 nm of **1** as a function of pH. The solid line is the best fit pH titration curve with pK_a 7.63.

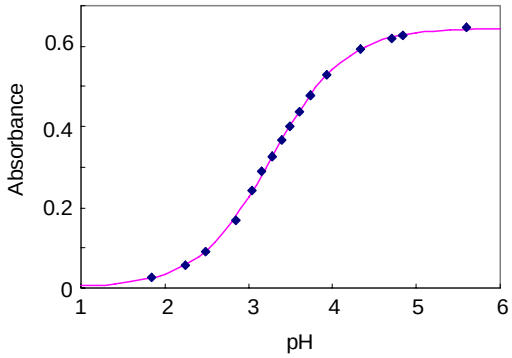

Fig. S3 Plots of absorbance at 444 nm of **C7SP** as a function of pH. The solid line is the best fit pH titration curve with pK_a 3.27.

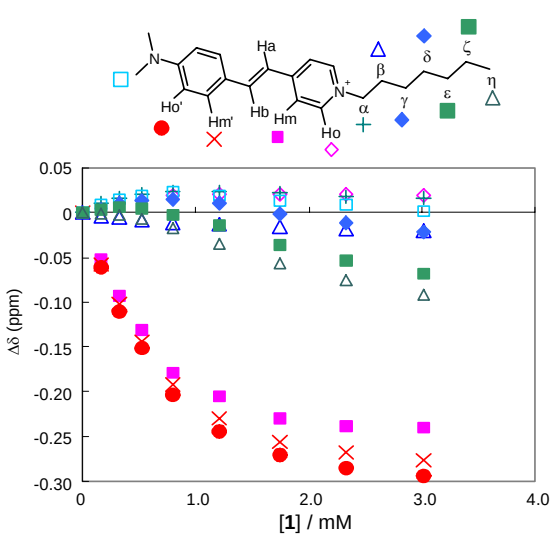

Fig. S4 Chemical shift changes of **C7SP** (0.65 mM) upon the addition of **1** in D_2O buffered by acetate (I = 0.1 M, pH(D) 5.7). Negative and positive Δδ values correspond to high- and low-field shifts, respectively. Ha and Hb signals were severely broadened to disappear at 0.2 mM **1**.

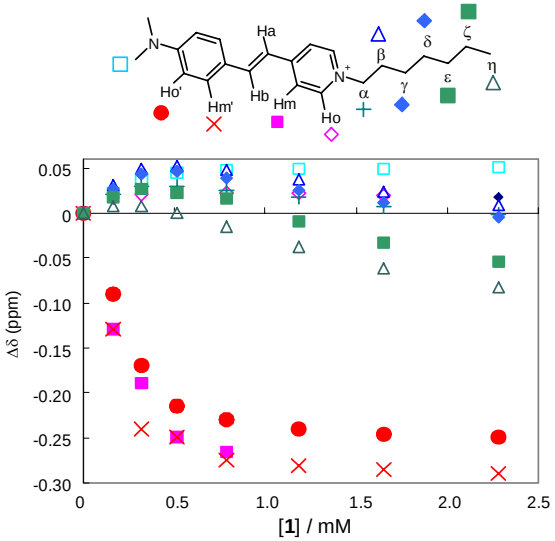

Fig. S5 Chemical shift changes of **C7SP** (0.35 mM) upon the addition of **1** in D_2O buffered by carbonates (I = 0.1 M, pH(D) 9.7). Negative and positive Δδ values correspond to high- and low-field shifts, respectively. Ha and Hb signals were severely broadened to disappear at 0.1 mM **1**.

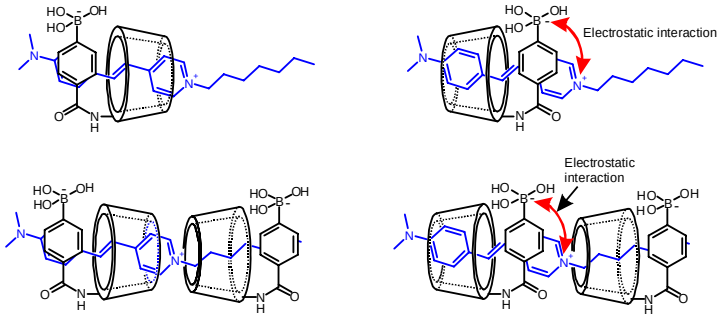

Fig. S6 Cartoons for plausible 1:1 (top) and 1:2 (bottom) complexes of **C7SP** with **1**, deduced from the ^{1}H NMR titration data.

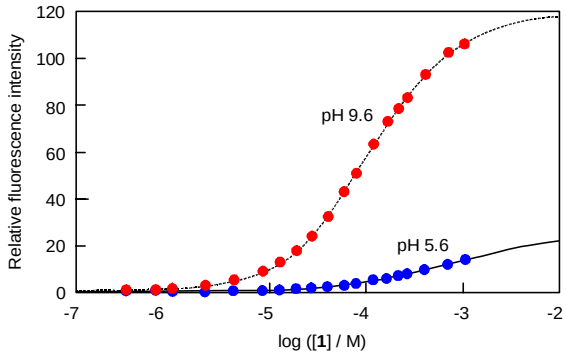

Fig. S7 Fluorescence intensity variations of **C7SP** (36 μM) induced by **1** in pH 5.6 (acetate buffer, I = 0.07 M; blue circle) or pH 9.6 (carbonate buffer, I = 0.07 M; red circle). The solid and dashed lines are the best-fit curves with K_1 = 5100 M^{-1} and K_2 = 350 M^{-1} (pH 5.6, solid line)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