Synthesis of water-soluble anthracene-appended benzoaboroles and evaluation of their cis-1,2-diol recognition properties

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I. General

All air sensitive reactions were carried out under nitrogen in oven-dried glassware using standard syringe and septa techniques, unless otherwise noted.

The $^1$H, $^{13}$C, and $^{19}$F NMR were recorded on a Bruker 400 (400 MHz for $^1$H, 100 MHz for $^{13}$C, and 376 MHz for $^{19}$F) spectrometer. Chemical shifts were reported in ppm ($\delta$), and coupling constants were reported in Hz. $^1$H and $^{13}$C-resonances were referenced to solvent residual peaks for CDCl$_3$ ($^1$H, 7.26 ppm), CD$_3$OD ($^1$H, 3.31 ppm), CDCl$_3$ ($^{13}$C, 77.2 ppm) and CD$_3$OD ($^{13}$C, 49.0 ppm). $^{19}$F NMR was recorded using CFCl$_3$ ($^{19}$F, 0.00 ppm) as external standard. Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, m = multiplet, br = broad, doublet of doublets (dd), doublet of doublets of doublets. Spectra were processed by Bruker Top-spin.

High resolution mass analyses (HRSM) were recorded on JEOL JMS-T100CS spectrometer. UV-Vis spectra were recorded on Perkin Elmer UV/VIS spectrometer Lambda 35. Fluorescence spectra were recorded on JASCO FP-8200. Infrared spectra were recorded on Perkin Elmer Spectrum Two ATR/FT-IR spectrometer, and $\nu_{\text{max}}$ are partially reported in cm$^{-1}$.

Thin-layer chromatography was performed on Merck 60 F254 pre-coated silica gel plates. column chromatography was performed on silica gel (Silica Gel 60 N; 63–210 mesh, KANTO CHEMICAL CO., INC. or 40–50 mesh, KANTO CHEMICAL CO., INC.).

Commercial available reagents were obtained from Tokyo Kasei, Wako Pure Chemical Industries Ltd., KANTO CHEMICAL CO., INC. and Nacalai tesque, and used without further purification.
II. Solubility analysis of 1a-c in aqueous media

The aqueous solubility of 1a-c was analyzed by UV-Vis spectroscopy. All UV-Vis spectra of 1a-c were measured in pH 7.4, 10 mM HEPES, 150 mM NaCl, 25 ºC. Good linear relationships were observed between the concentration and the absorbance.

![Fluorescence spectra](image)

**Fig. S1.** UV-Vis spectra of 1a-c, [1] = 10, 20, 30, 40, 50, 60 µM. In set: plot of absorbance at 374 nm against the concentration of 1a-c

III. Fluorescence response of 1a-c toward cis-1,2-diols

Fluorescence spectra of 1a-c were measured in the presence of cis-1,2-diols including carbohydrate (α-methyl-D-mannoside), catechols (L-dopa and dopamine), nucleoside (guanosine), and NTPs (GTP and UTP) in pH 7.4, 10 mM HEPES (containing 5% CH3OH), 150 mM NaCl, 25 ºC. The relative fluorescence intensity of 1a-c is shown in **Fig. S2**.

*For fluorescence study, a stock solution of 1a-c was prepared as 1 mM in 5% CH3OH/H2O. To prevent the potential aggregation of 1a-c under the storage, methanol was added in the stock solution. All fluorescence spectra were measured in 10 mM HEPES containing 5% CH3OH unless otherwise noted.

![Fluorescence response](image)

**Fig. S2.** Relative fluorescence intensity at 422 nm of 1a-c in the presence of cis-1,2-diols. [1a-c] = 2 µM. [carbohydrate] = 50 mM, [catechol] = 250 µM, [guanosine] = 200 µM, [NTP] = 250 µM, λex = 373 nm, slit band = 2.5 nm. The relative intensities are the average of the two separates experiments.
IV. Fluorescence titration study of 1a-c

Fluorescence titrations of 1a-c were performed upon the addition of cis-1,2-diols including carbohydrate, catechol, nucleoside, and NTP in pH 7.4, 10 mM HEPES (containing 5% CH$_3$OH), 150 mM NaCl, 25 ºC. As a selected example, the titration results of 1a were shown in Fig. S3. The titration results were fitted with fitting program ‘Titration fit’.

Akine, S. TitrationFit, Program for Analyses of Host–guest Complexation; Kanazawa University: Kanazawa, Japan, 2013.

![Fig. S3](image_url)

**Fig. S3.** Fluorescence spectra of 1a up on the addition of cis-1,2-diols. [1a] = 2 µM. [carbohydrate] = 0 to 50 mM, [catechol] = 0 to 250 µM, [guanosine] = 0 to 200 µM, [NTP] = 0 to 250 µM, λ$_{ex}$ = 373 nm. Inset is a plot of ΔI at 422 nm against [1a].
V. Binding study of control 9 toward cis-1,2-diols

Fluorescence titration of 9 for GTP was carried out with same method as described in page S3. The details of NMR study for the complex formation between 9 and 1,2-diols were described in page S5-10. The association constant for 9-ATP adduct was calculated by fitting the shifts of proton signal to 1:1 isotherm with ‘Titration fit’

Table S1. Association constants of 9 for ATP and GTP

<table>
<thead>
<tr>
<th>cis-1,2-diol</th>
<th>ATP</th>
<th>GTP</th>
<th>Dopamine</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$7.7 \times 10^2$ a)</td>
<td>$9.2 \times 10^2$ b)</td>
<td>No interaction a)</td>
<td>No interaction b)</td>
</tr>
</tbody>
</table>

These association constants are the average of the two separates experiments. a) Determined by $^1$H NMR. b) Determined by the fluorescence titration.

Fluorescence spectrum of 9 upon the addition of GTP

![Fluorescence spectrum of 9](image)

[9] = 2 $\mu$M, [GTP] = 0 to 250 $\mu$M, $\lambda_{ex}$ = 373 nm. Inset is a plot of $\Delta I$ at 422 nm against [9].

VI. Fluorescence titration study of 1a in various CH$_3$OH/H$_2$O ratios

To assess the solvent effect for the boronate formation of 1a, the fluorescence titrations for GTP and dopamine were carried out in various MeOH/HEPES buffer ratios (Table S1). We found no significant effect of MeOH for $K_a$ in the range of 0 to 20% MeOH contents.

Table S2. Association constants of 1a for GTP and dopamine

<table>
<thead>
<tr>
<th>MeOH (%)</th>
<th>GTP</th>
<th>Dopamine</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>$6.6 \times 10^3$</td>
<td>$3.7 \times 10^3$</td>
</tr>
<tr>
<td>5</td>
<td>$5.5 \times 10^3$</td>
<td>$1.5 \times 10^3$</td>
</tr>
<tr>
<td>9.5</td>
<td>$2.1 \times 10^3$ b)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>$5.7 \times 10^3$</td>
<td>$2.7 \times 10^3$</td>
</tr>
<tr>
<td>20</td>
<td>$4.8 \times 10^3$</td>
<td>$2.4 \times 10^3$</td>
</tr>
</tbody>
</table>

These association constants are the average of the two separates experiments. a) Not determined. b) Determined by $^1$H NMR.
VII. NMR study for 1-diol and 9-diol complexes

We successfully obtained the NMR spectra of 1-dopamine complexes in 9.5% CD$_3$OD/deuterated phosphate buffer (50 mM, pD 7.4) (Fig. S4-6). The association constants were calculated from the equation $K_a = [1']/[1][\text{Diol}]$. The concentration of 1, 1’ and cis-1,2-diol was acquired from the integrated value of $^1$H NMR. Other cis-1,2-diols such as carbohydrate could be applicable for the NMR analysis (Fig. S7), although a large amount of CD$_3$OD (50%) was required due to the limited solubility of the boronate adduct. The NMR spectra of 9 in the presence of cis-1,2-diols (fructose, dopamine, and ATP) were also measured and summarized in Fig. S8-S10.

1a-dopamine

![Diagram of 1a-dopamine reaction](attachment:image.png)

Dopamine

<table>
<thead>
<tr>
<th>(5.20 eq.)</th>
<th>(4.16 eq.)</th>
<th>(3.12 eq.)</th>
<th>(2.08 eq.)</th>
<th>(1.04 eq.)</th>
<th>(0.52 eq.)</th>
<th>(0 eq.)</th>
</tr>
</thead>
</table>

![NMR spectra of 1a up on the addition of dopamine](attachment:image.png)

**Fig. S4.** $^1$H NMR spectra of 1a up on the addition of dopamine (0, 1.04, 2.08, 3.12, 4.16, 5.20 equivalent). [1a] = 2.0 mM, in 9.5% CD$_3$OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25°C.
**Fig. S5.** $^1$H NMR spectra of 1b up on the addition of dopamine (0, 1.04, 2.08, 3.12, 4.16, 5.20 equivalent). [1b] = 2.4 mM, in 9.5% CD$_3$OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 °C.
Fig. S6. $^1$H and $^{19}$F NMR spectra of 1c up on the addition of dopamine (0, 1.04, 2.08, 3.12, 5.20 equivalent). [1c] = 1.0 mM, in 9.5% CD$_3$OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 °C.
Fig. S7. $^1$H NMR spectra of 1b up on the addition of fructose (0, 10.4 equivalent). [1b] = 1.2 mM, in 50% CD$_3$OD/Deuterated phosphate buffer (25 mM, pD 7.4), 25 °C.
**Fig. S8.** $^1$H NMR spectra of 9 up on the addition of ATP (0, 1.04, 2.08, 3.12, 4.16, 5.20 equivalent). [9] = 2.4 mM in in 9.5% CD$_3$OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 °C
**9-Dopamine**

No signal shift

![NMR spectra of 9 up on the addition of dopamine](image)

*Fig. S9.* ^1^H NMR spectra of 9 up on the addition of dopamine (0, 1.04, 2.08, 3.12, 5.20 equivalent), [9] = 2.4 mM in 9.5% CD$_3$OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC.

**9-Glu**

No signal shift

![NMR spectra of 9 up on the addition of glucose](image)

*Fig. S10.* ^1^H NMR spectra of 9 up on the addition of glucose (0, 10.4, 20.8, 31.2 equivalent), [9] = 2.4 mM in 9.5% CD$_3$OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC.
VIII. DFT-calculation study

All the density functional theory (DFT) calculations were performed utilizing the Gaussian 16 package. The Head-Gordon and coworker’s Long-range corrected hybrid density functional including Grimme's D2 dispersion term (ωB97X-D)² was employed with a standard split valence-type basis sets, 6-31G(d), for phosphorous atom and 6-31G for others. The geometry optimization and subsequent vibrational frequency analysis at each local minimum for the check of the nature of stationary points were performed at the same criteria without symmetry restriction. Though specific solvation effects such as hydrogen bonding were absent, the bulk solvent effects of water might be covered adequately by using polarizable continuum model (PCM).³ Note that all the structures of boronate complex anions relaxed to coiled structures within gas-phase geometry optimizations without PCM due to strong Coulombic attractive force between tri-cationic and tri-anionic side chains, while use of a PCM model provided not only coiled structures but also extended local minima where Coulombic attraction between tri-cationic and tri-anionic side chains is sufficiently shielded by bulk solvent effects. Since it was expected that there are a large number of local minima with respect to side chain conformation, we performed geometry optimization starting from ten conformers of each extended and coiled structure. Furthermore, they were divided into two types; one is named as “Stereoisomer B” structure where the oxygen atom in a furan ring points to the same direction as one in an internal coordinating hydroxyl group at ortho position, while the other has opposite direction, named as “Stereoisomer A”. Fig.4 and Fig. S11 show the most stable structures in each type and their relative adiabatic energies (ΔE_a) estimated without vibrational zero-point energy correction. The Gibbs free energies (ΔG) were calculated at the condition of 298.15 K and 1 atm. All computation were carried out using the computer facilities at Research Institute for Information Technology, Kyushu University.

References for DFT calculation

**Fig. S11.** DFT calculated optimized structure of 1b-GTP complex

**IX. Synthetic procedure of benzoxaborole 1a-c**

*The $^1$H and $^{13}$C NMR of 3-6 showed partially broadening spectra due to the presence of tertiary carbonate groups. Regarding to 1a-c, the $^{13}$C signal of the carbon atom adjacent to boron atom was not observed. The purity of 1a-c and 9 was confirmed by $^1$H NMR using dimethylsulfoxide as an internal standard.*

2 (2.26 g, 9.57 mmol) was reacted with diethylenetriamine (4.11 mL, 38.3 mmol) in EtOH (128 mL) and THF (63 mL) under a nitrogen atmosphere at room temperature. After stirring for 12 h, NaBH₄ (1.45 g, 38.3 mmol) was added to the reaction mixture at room temperature. After stirring for 18 h, the reaction mixture was quenched by the addition of H₂O. The mixture was extracted with CHCl₃ in three times, and the combined organic layer was washed with brine. The organic phase was dried with anhydrous Na₂SO₄, filtrated and concentrated. The resulting product was used in next step without any purification. A solution of Boc₂O (10.4 g, 47.9 mmol) in MeOH (96 mL) was added to the resulting triamine product at room temperature. After stirring for 12 h, the mixture was concentrated. The crude product was purified by chromatography (silica gel,
CHCl₃/AcOEt/Hexane, 2:1:0.1) to afford 3 (2.17 g, 36% in three steps) as a pale yellow foam; ¹H NMR (400 MHz, CD₃OD) δ 8.50-8.36 (m, 4H), 7.51-7.57 (m, 4H), 5.53-5.49 (m, 4H), 2.68-2.94 (m, 8H), 1.55-1.32 (m, 27H); ¹³C NMR (100 MHz, CD₃OD) δ 158.1, 157.1, 134.4, 134.2, 132.3, 131.4, 130.6, 130.2, 127.2, 127.1, 126.8, 126.5, 125.7, 125.5, 81.7, 81.2, 81.0, 79.9, 57.1, 47.6, 47.1, 46.8, 45.4, 44.0, 43.1, 42.5, 42.0, 39.4, 39.1, 28.9, 28.8; IR (FT-ATR): ν = 3357, 2975, 1683, 1412, 1365 cm⁻¹; HRMS (ESI): calcd. for [C₁₃H₁₉N₂O₆+Na]^+ 646.3463; found 646.3479.

MsCl (0.52 mL, 6.76 mmol) was added to the mixture of 3 (2.11 g, 3.38 mmol) and DIPEA (1.27 mL, 7.44 mmol) in CH₂Cl₂ (68 mL) at 0 °C. After stirring at 0 °C for two hours, the mixture was washed with sat. NaHCO₃ and brine. The organic phase was dried with Na₂SO₄, filtrated, and concentrated. The resulting product was used in next step without any purification. To a solution of the mesylated product in DMF (17 mL) was added NaN₃ (989 mg, 15.2 mmol) at room temperature. The reaction mixture was stirred at 90 °C for 12h. After cooling to room temperature, the solvent was removed. The residue was dissolved in H₂O and extracted with AcOEt. The organic phase was dried with Na₂SO₄, filtrated and concentrated. The crude product was purified by chromatography (silica gel, CHCl₃/AcOEt/Hexane, 9:1:0.1) to afford 4 (1.40 g, 59%) as a pale yellow foam; ¹H NMR (400 MHz, CD₃OD) δ 8.46-8.30 (m, 4H), 7.57-7.51 (m, 4H), 5.49-5.46 (m, 2H), 5.25, (s, 2H), 3.02-2.65 (m, 8H), 1.53 (s, 9H), 1.42-1.27 (m, 18H); ¹³C NMR (100 MHz, CD₃OD) δ 158.1, 157.0, 132.1, 131.8, 131.6, 131.4, 129.1, 128.9, 127.4, 127.2, 126.1, 125.7, 81.7, 81.5, 81.1, 81.0, 79.9, 47.7, 47.1, 46.7, 45.3, 44.0, 43.2, 42.5, 42.0, 39.5, 39.2, 28.8, 28.7; IR (FT-ATR): ν = 3357, 2976, 2095, 1682, 1365, 1244, 1158 cm⁻¹; HRMS (ESI): calcd. for [C₁₃H₁₆N₆O₆+Na]^+ 671.3528; found 671.3492.

To a solution of 4 (1.30 g, 1.85 mmol) in THF (56 mL) and H₂O (6 mL) was added PPh₃ (1.36 g, 5.19 mmol). The solution was stirred for 12h at room temperature. After removing the solvent, the residue was purified by chromatography (silica gel, CHCl₃/MeOH/Hexane, 50:1.5, 30:1.3, 20:1:2) to afford 5 (1.05g, 91%) as a pale yellow foam; ¹H NMR (400 MHz, CD₂OD) δ 8.46-8.35 (m, 4H), 7.58-7.51 (m, 4H), 5.48-5.46 (m, 2H), 4.70 (s, 2H), 2.96-2.69 (m, 8H), 1.55 (s, 9H), 1.42-1.30 (s, 18H); ¹³C NMR (100 MHz, CD₂OD) δ 158.1, 157.1, 126.4, 136.2, 132.4, 130.6, 129.8, 129.5, 127.2, 127.1, 127.0, 126.0, 125.9, 81.7, 81.2, 81.0, 79.9, 47.6, 47.1, 46.7, 45.4, 44.0, 43.2, 42.5, 42.0, 39.5, 39.1, 38.2, 28.9, 28.8, 28.7; IR (FT-ATR): ν = 2975, 1683, 1412, 1365, 1243, 1157 cm⁻¹; HRMS (ESI): calcd. for [C₁₃H₁₈N₆O₆+Na]^+ 645.3623; found 623.3664.
5 (441 mg, 0.707 mmol) was added to a solution of Fmoc-β-Ala-OH (242 mg, 0.778 mmol), HOBr (115 mg, 0.848 mmol), and EDC (230 mg, 0.848 mmol) in CH₂Cl₂ (25 mL). The mixture was stirred at room temperature for 24 h. The reaction mixture was washed with sat. NaHCO₃ and brine. The organic phase was dried with Na₂SO₄, filtrated and concentrated. The residue was partially purified by column chromatography (silica gel, CHCl₃/MeOH/Hexane, 5:1, 3:1, 1:1) to afford the Fmoc-protected product. The resulting product was applied for the deprotection reaction. The resulting product was dissolved in DMF/piperidine (9:1, 15 mL). After stirring for 1 hour at room temperature, the solvent was removed. The residue was purified by chromatography (silica gel, CHCl₃/MeOH/Hexane, 30:1:3, 20:1:2, 10:1:1) to afford 6 (361 mg, 74%) as a pale yellow foam; ¹H NMR δ 8.46-8.33 (m, 4H), 7.56-7.53 (m, 4H), 5.48-5.46 (m, 2H), 5.28 (s, 2H), 3.02-2.80 (m, 8H), 2.90 (t, J = 6.3 Hz, 2H), 2.33 (t, J = 6.3 Hz, 2H), 1.55 (s, 9H), 1.43-1.29 (s, 18H); ¹³C NMR (100 MHz, CDCl₃) 173.8, 158.0, 156.9, 132.2, 131.6, 131.5, 131.4, 130.5, 128.5, 128.4, 127.2, 127.1, 126.2, 126.0, 125.7, 122.2, 81.6, 81.4, 81.1, 47.6, 47.1, 46.8, 45.2, 44.3, 43.1, 42.5, 41.9, 39.4, 39.1, 39.0, 36.8, 28.9, 28.8, 28.7; IR (FT-ATR): ν = 3302, 2973, 1682, 1524, 1454, 1412, 1364, 1245, 1158 cm⁻¹. HRMS (ESI): calcd. for [C₃₈H₅₃N₅O₇+Na]⁺ 716.3994; found 717.3988.

A solution of 5 (163 mg, 0.262 mmol) in DMF (8.7 mL) and DIPEA (53.8 µL, 0.314 mmol) was added to a solution of 7 (51.3 mg, 0.288 mmol), HOBr (42.4 mg, 0.314 mmol), and EDC (60.3 mg, 0.314 mmol). The mixture was stirred at room temperature for 18 h. After that, the mixture was diluted with CHCl₃ and washed with sat. NaHCO₃ and brine. The organic phase was dried with Na₂SO₄, filtrated and concentrated. The resulting product was partially purified by column chromatography (silica gel, CHCl₃/MeOH/Hexane, 50:1:5, 30:1:3, 20:1:2) to afford the condensation product. The resulting product was suspended to 4 M HCl/dioxane (2 mL), and the mixture was stirred for 1 h at room temperature. After removing the solvent, the residue was filtrated and washed with CHCl₃ and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from CHCl₃ and MeOH to afford 1a (70.0 mg, 39%) as a pale yellow solid; ¹H NMR (400 MHz, D₂O) δ 8.45 (d, J = 8.4 Hz, 2H), 8.32 (d, J = 8.8 Hz, 2H), 7.77-7.66 (m, 6H), 7.35 (d, J = 8.4 Hz, 1H), 5.43 (s, 2H), 5.31 (s, 2H), 4.95 (s, 2H), 3.59-3.56 (m, 2H),
A solution of 6 (180 mg, 0.259 mmol) in DMF (8.5 mL) and DIPEA (53 µL, 0.311 mmol) was added to a solution of 7 (50.5 mg, 0.284 mmol), HOBt (42.0 mg, 0.311 mmol), and EDC (59.3 mg, 0.311 mmol). The mixture was stirred at room temperature for 12h. After that, the mixture was diluted with CHCl₃ and washed with sat. NaHCO₃ and brine. The organic phase was dried with Na₂SO₄, filtrated and concentrated. The resulting product was partially purified by column chromatography (silica gel, CHCl₃/MeOH/Hexane, 50:1:5, 30:1:3, 20:1:2) to afford the condensation product. The resulting product was suspended to 4 M HCl/dioxane (2 mL), and the mixture was stirred for 1h at room temperature. After removing the solvent, the residue was filtrated and washed with CHCl₃ and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from CHCl₃ and MeOH to afford 1b (89.2 mg, 53%) as a pale yellow solid; ¹H NMR (400 MHz, D₂O) δ 8.28 (d, J = 8.8 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H), 7.57 (dd, J = 8.8, 8.8 Hz, 2H), 7.47 (dd, J = 8.8, 8.8 Hz, 2H), 7.26 (dd, J = 1.6, 8.0 Hz, 1H), 7.17 (d, J = 8.0 Hz, 1H), 6.84 (s, 1H), 5.35 (s, 2H), 5.26 (s, 2H), 5.10 (s, 2H), 3.64 (t, J = 6.0 Hz, 2H), 3.46 (t, J = 6.8 Hz, 2H), 3.19-3.11 (m, 6H), 2.61 (t, J = 6.0 Hz, 2H); ¹³C NMR (100 MHz, D₂O) δ 173.4, 169.6, 156.1, 132.0, 131.5, 130.0, 129.4, 129.3, 127.8, 127.5, 126.5, 124.7, 123.0, 121.5, 121.4, 70.4, 44.6, 43.7, 43.5, 43.4, 36.4, 36.0, 35.6, 35.4; IR (FT-ATR): ν = 3289, 2716, 1634, 1543, 1433, 1362, 1055, 998 cm⁻¹; HRMS (ESI): calcd. for [C₃H₅BN₃O₄–OH + CH₃OH + Na⁺] 519.2538; found 519.2486.

A solution of 5 (188 mg, 0.302 mmol) in DMF (10 mL) and DIPEA (62.1 mL, 0.362 mmol) was added to a solution of 8 (65.0 mg, 0.332 mmol), HOBt (48.9 mg, 0.362 mmol), and EDC (69.4 mg, 0.155 mmol). The mixture was stirred at room temperature for 18h. After that, the mixture was diluted with CHCl₃ and washed with sat. NaHCO₃ and brine. The organic phase was dried with Na₂SO₄, filtrated and concentrated. The
resulting product was partially purified by column chromatography (silica gel, CHCl₃/MeOH/Hexane, 50:1:5, 30:1:3, 20:1:2) to afford the condensation product. The resulting product was suspended to 4 M HCl/dioxane (2 mL), and the mixture was stirred for 1h at room temperature. After removing the solvent, the residue was filtrated and washed with CHCl₃, and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from CHCl₃ and MeOH to afford 1c (66.0 mg, 36%) as a pale yellow solid; ¹H NMR (400 MHz, D₂O) δ 8.51 (d, J = 8.4 Hz, 2H), 8.37 (d, J = 8.4 Hz, 2H), 7.77 (dd, J = 8.4, 8.4 Hz, 2H), 7.72 (dd, J = 8.4, 8.4 Hz, 2H), 7.62 (s, 1H), 7.40 (d, J = 10.0 Hz, 1H), 5.54 (s, 2H), 5.36 (s, 2H), 5.01 (s, 2H), 3.5 (t, J = 6.4 Hz, 2H), 3.13-3.10 (m, 4H), 3.01 (t, J = 6.0 Hz, 2H); ¹³C NMR (100 MHz, D₂O) 167.1, 155.9 (d, J = 248 Hz), 154.6 141.2 (d, J = 15.6 Hz), 134.1, 131.6, 129.9, 129.5, 127.5, 126.5, 124.8, 124.5, 123.4, 121.5, 115.4 (d, J = 20.5 Hz), 66.5, 44.6, 43.7, 43.5, 43.4, 36.5, 35.4; ¹⁹F NMR (376 MHz, D₂O) δ -120.0; IR (FT-ATR): ν = 3317, 2718, 1616, 1526, 1445, 1313, 999 cm⁻¹; HRMS (ESI): calcd. for [C₂₈H₃₀BF₅N₄O₃–OH + CH₃OH + Na⁺] 537.244; found 537.2492.

To a solution of 5 (364 mg, 0.584 mmol) in pyridine (6 mL) was added Ac₂O (1 mL) at room temperature. After stirring for 12h, the solvent was removed. The residue was partially purified by chromatography (silica gel, CHCl₃/AcOEt 1:0, 5:1). The resulting product was suspended to 4 M HCl/dioxane (2 mL), and the mixture was stirred for 1h at room temperature. After removing the solvent, the residue was filtrated and washed with CHCl₃ and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from CHCl₃ and MeOH to afford 9 (63.7 mg, 23%) as a pale yellow solid; ¹H NMR (400 MHz, D₂O) δ 8.43 (d, J = 8.4 Hz, 2H), 8.38 (d, J = 8.8 Hz, 2H), 7.78 (dd, J = 8.4, 8.8 Hz, 2H), 7.72 (dd, J = 8.4, 8.8 Hz, 2H), 5.39 (s, 2H), 5.37 (s, 2H), 3.52 (t, J = 6.8 Hz, 2H), 3.28-3.12 (m, 6H), 1.96 (s, 3H); ¹³C NMR (100 MHz, D₂O) δ 173.5, 131.9, 129.9, 129.2, 127.6, 126.6, 124.8, 123.3, 121.3, 44.6, 43.6, 43.4, 43.1, 35.9, 35.3, 35.3, 21.6; IR (FT-ATR): ν = 3316, 2907, 2679, 2436, 1614, 1531, 1442 cm⁻¹; HRMS (ESI): calcd. for [C₂₉H₂₈N₄O+H]⁺ 364.2336; found 364.2296.
3: $^1$H NMR (400 MHz, CD$_3$OD)

3: $^{13}$C NMR (100 MHz, CD$_3$OD)
HSQC spectrum of 3 (CD$_3$OD)

4: $^1$H NMR (400 MHz, CD$_3$OD)
4. $^{13}$C NMR (100 MHz, CD$_3$OD)

HSQC spectrum of 4 (CD$_3$OD)
5: $^1$H NMR (400 MHz, CD$_3$OD)

5: $^{13}$C NMR (100 MHz, CD$_3$OD)
HSQC spectrum of 5 (CD$_3$OD)

6: $^1$H NMR (400 MHz, CD$_3$OD)
6. $^{13}$C NMR (100 MHz, CD$_3$OD)
1a: $^1$H NMR (400 MHz, D$_2$O)

1a: $^{13}$C NMR (100 MHz, D$_2$O)
HSQC spectrum of $1b$ ($D_2O$)

$1b$: $^1H$ NMR (400 MHz, $D_2O$)
1b: $^{13}$C NMR (100 MHz, D$_2$O)

HSQC spectrum of 1b (D$_2$O)
**1c: $^1$H NMR (400 MHz, D$_2$O)**

![1H NMR spectrum](image)

**1c: $^{13}$C NMR (100 MHz, D$_2$O)**

![13C NMR spectrum](image)
$\textbf{1c}: ^{19}\text{F NMR (376 MHz, D}_2\text{O)}$

HSQC spectrum of $\textbf{1c}$ (D$_2$O)
9: $^1$H NMR (400 MHz, D$_2$O)

9: $^{13}$C NMR (100 MHz, D$_2$O)
HSQC spectrum of 9 (D₂O)