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

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Supporting Information

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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) spectorometer. Chemical shifts were reported in ppm (δ), 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_3OD (^{1}H, 3.31 ppm), $CDCl_3$ (^{13}C, 77.2 ppm) and CD_3OD (^{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 ν_{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.

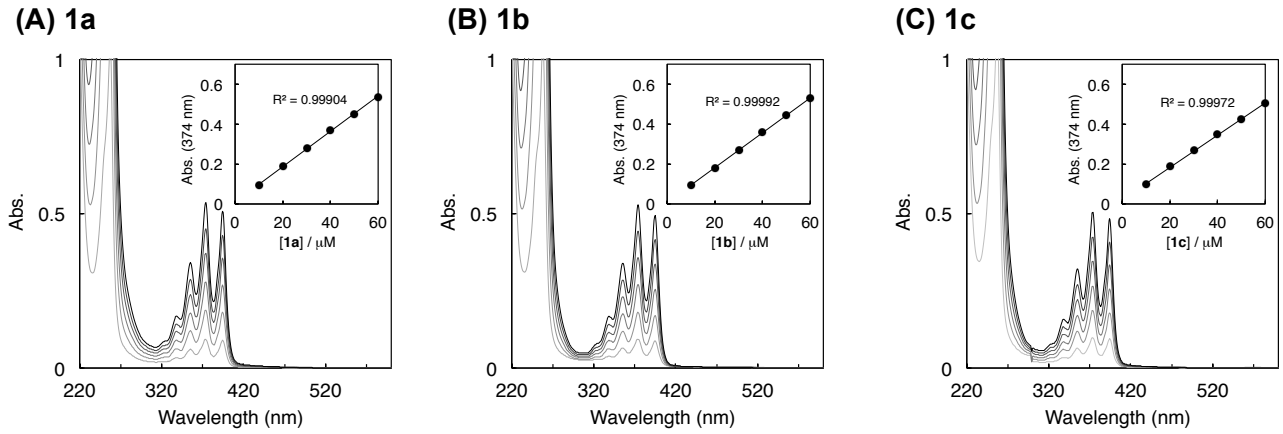
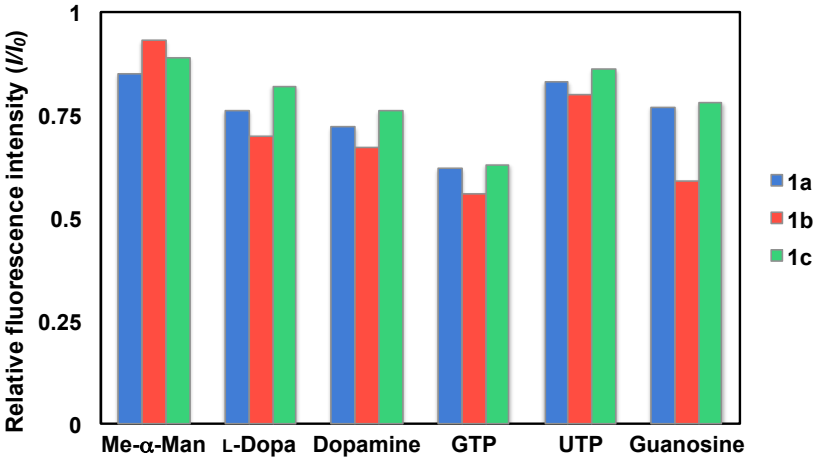


Fig. S1. UV-Vis spectra of **1a-c**, [**1**] = 10, 20, 30, 40, 50, 60 μM. In set: plot of abosorbance at 374 nm against the concentration of **1a-c**

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% CH_3OH), 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% CH_3OH/H_2O. 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% CH_3OH unless otherwise noted.



Relative large quenching of **1b** in the case of aromatic cis-1,2-diols may be originated from the increased flexibility of benzoxaborole moiety in **1b**. The longer spacer of **1b** likely allows the aromatic cis-1,2-diol to orient in close proximity to anthracene moiety through a hydrophobic interaction when forming the boronate adduct, and hence increasing the PeT efficiency. Actually, in DFT-calculated most stable structures of **1**-GTP adduct, the distance between anthracene and guanine base in **1b**-GTP adduct was shorter than that of **1a**-GTP adduct.

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_3OH), 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.

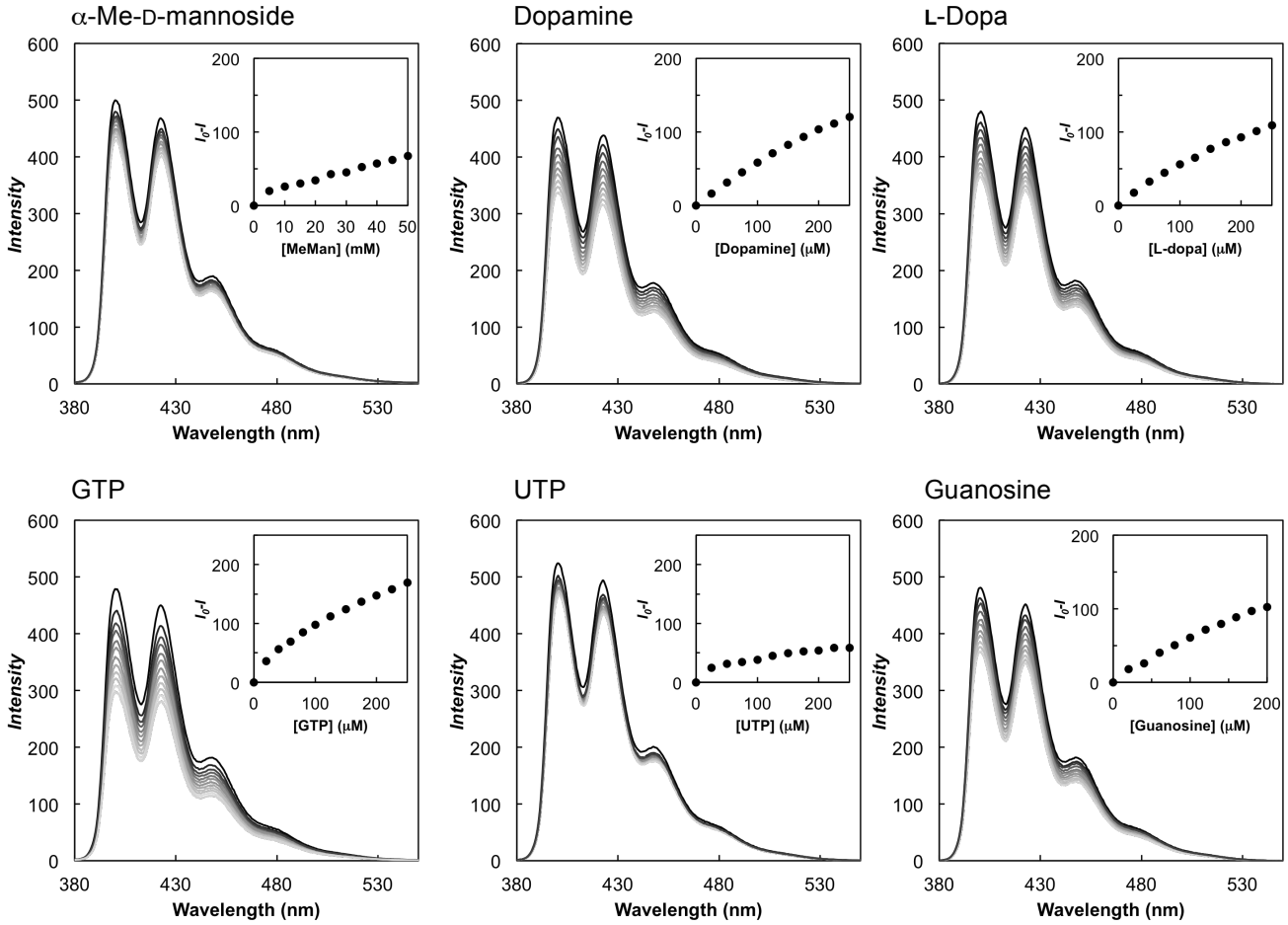


Fig. S3. Fluorescence spectra of **1a** up on the addition of cis-1,2-diols. [**1a**] = 2 μM. [carbohydrate] = 0 to 50 mM, [catechol] = 0 to 250 μM, [guanosine] = 0 to 200 μM, [NTP] = 0 to 250 μM, λex = 373 nm. Inset is a plot of *Δ*I* at 422 nm against [**1a**]

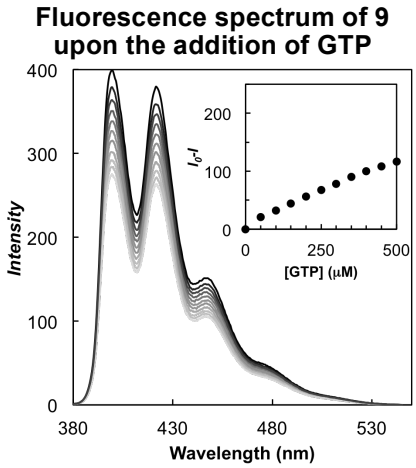
V. Binding study of control 9 toward cis-1,2-diols

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

cis-1,2-diol	K (M^{-1})
ATP	7.7×10^{2} a)
GTP	9.2×10^{2} b)
Dopamine	No interaction[a)]
Fructose	No interaction[a)]

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



[**9**] = 2 μM, [GTP] = 0 to 250 μM, λex = 373 nm. Inset is a plot of ΔI at 422 nm against [**9**].

VI. Fluorescence titration study of 1a in various CH_3OH/H_2O 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

MeOH (%)	GTP	Dopamine
0	6.6×10^{3}	3.7×10^{3}
5	5.5×10^{3}	1.5×10^{3}
9.5	–[a)]	2.1×10^{3} b)
10	5.7×10^{3}	2.7×10^{3}
20	4.8×10^{3}	2.4×10^{3}

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_3OD/deuterated phosphate buffer (50 mM, pD 7.4) (**Fig. S4-6**). The association constants were calculated from the equation K_a = [**1'**]/[**1**][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_3OD (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**.

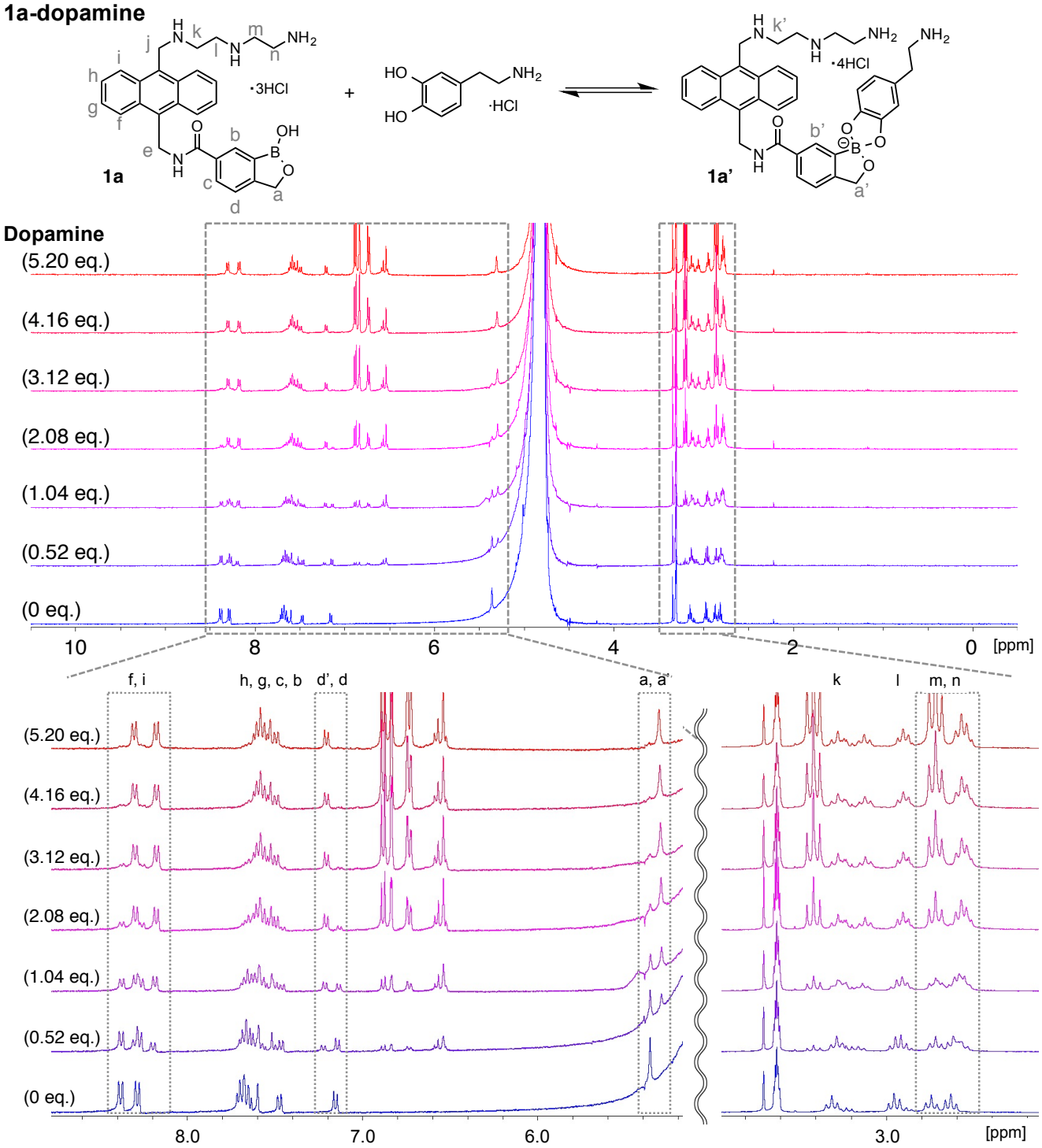
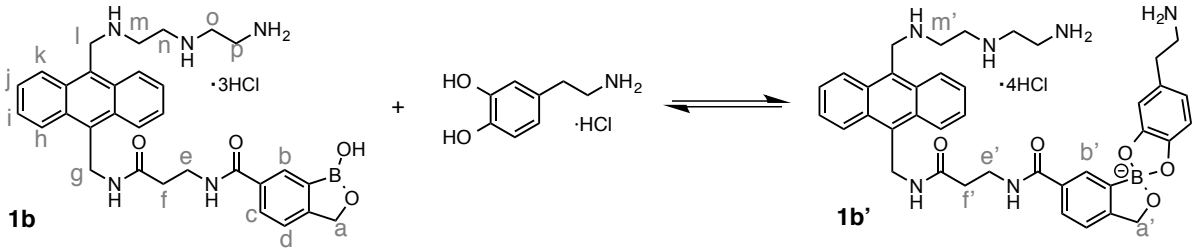


Fig. S4. ^{1}H NMR spectra of **1a** up on the addition of dopamine (0, 1.04, 2.08, 3.12, 4.16, 5.20 equivalent). [**1a**] = 2.0 mM, in 9.5% CD_3OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC.

1b-Dopamine



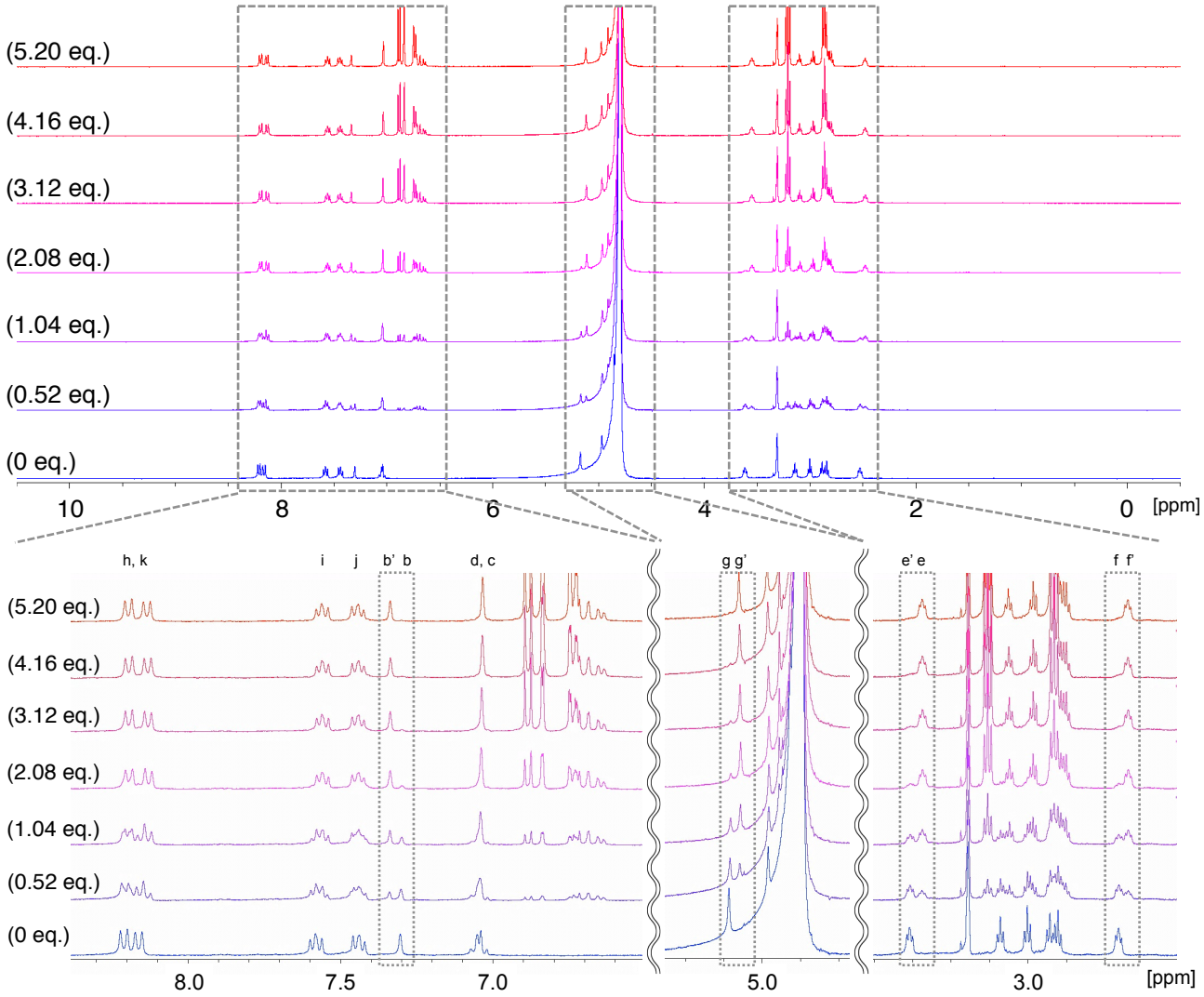
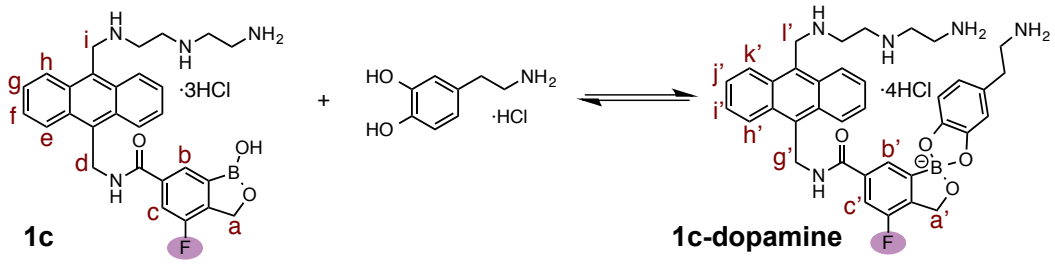
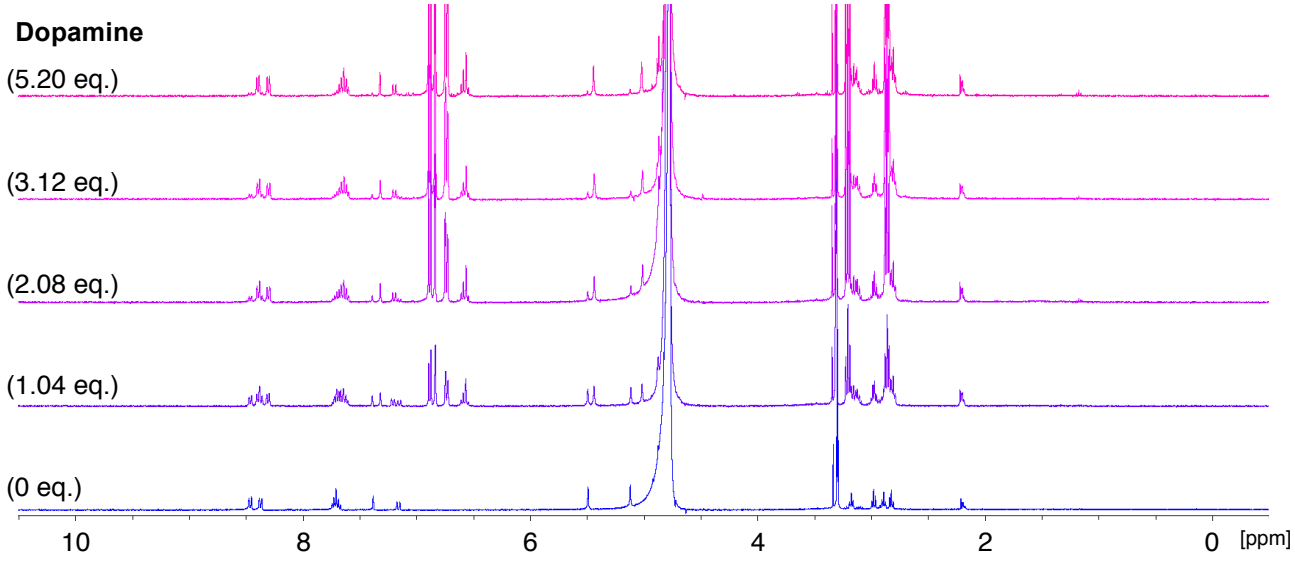


Fig. S5. ^{1}H NMR spectra of **1b** up on the addition of dopamine (0, 1.04, 2.08, 3.12, 4.16, 5.20 equivalent). [**1b**] = 2.4 mM, in 9.5% CD_3OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC.

1c-Dopamine



^{1}H NMR



^{19}F NMR

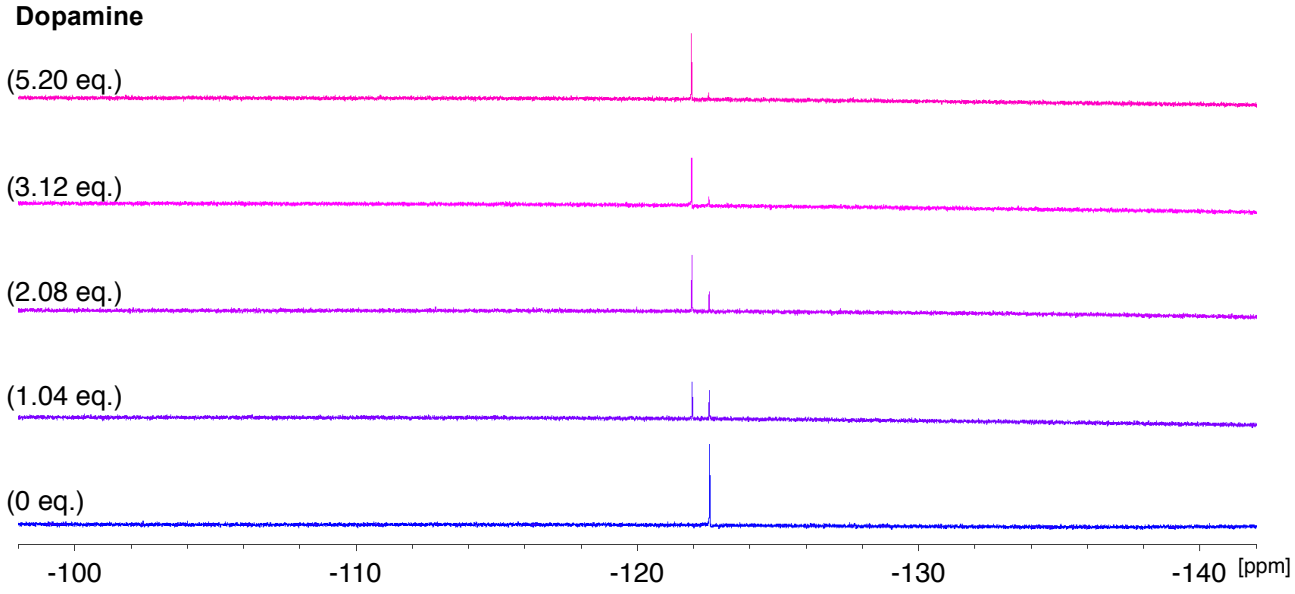


Fig. S6. ^{1}H and ^{19}F NMR spectra of **1c** up on the addition of dopamine (0, 1.04, 2.08, 3.12, 5.20 equivalent). [**1c**] = 1.0 mM, in 9.5% CD_3OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC.

1b-Fructose (50% CD_3OD/deuterated phosphate buffer)

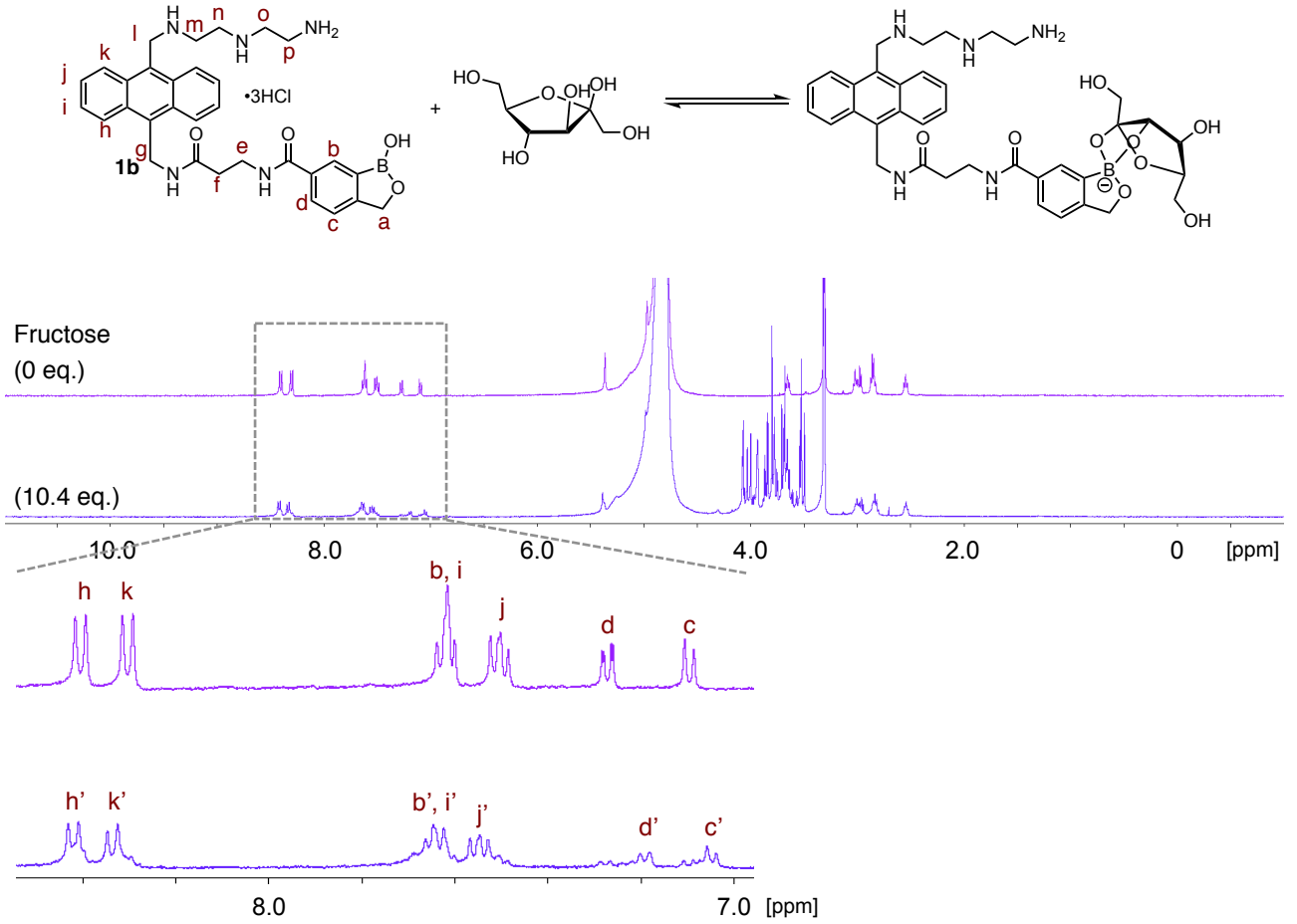


Fig. S7. ^{1}H NMR spectra of **1b** up on the addition of fructose (0, 10.4 equivalent). [**1b**] = 1.2 mM, in 50% CD_3OD/Deuterated phosphate buffer (25 mM, pD 7.4), 25 ºC.

9-ATP

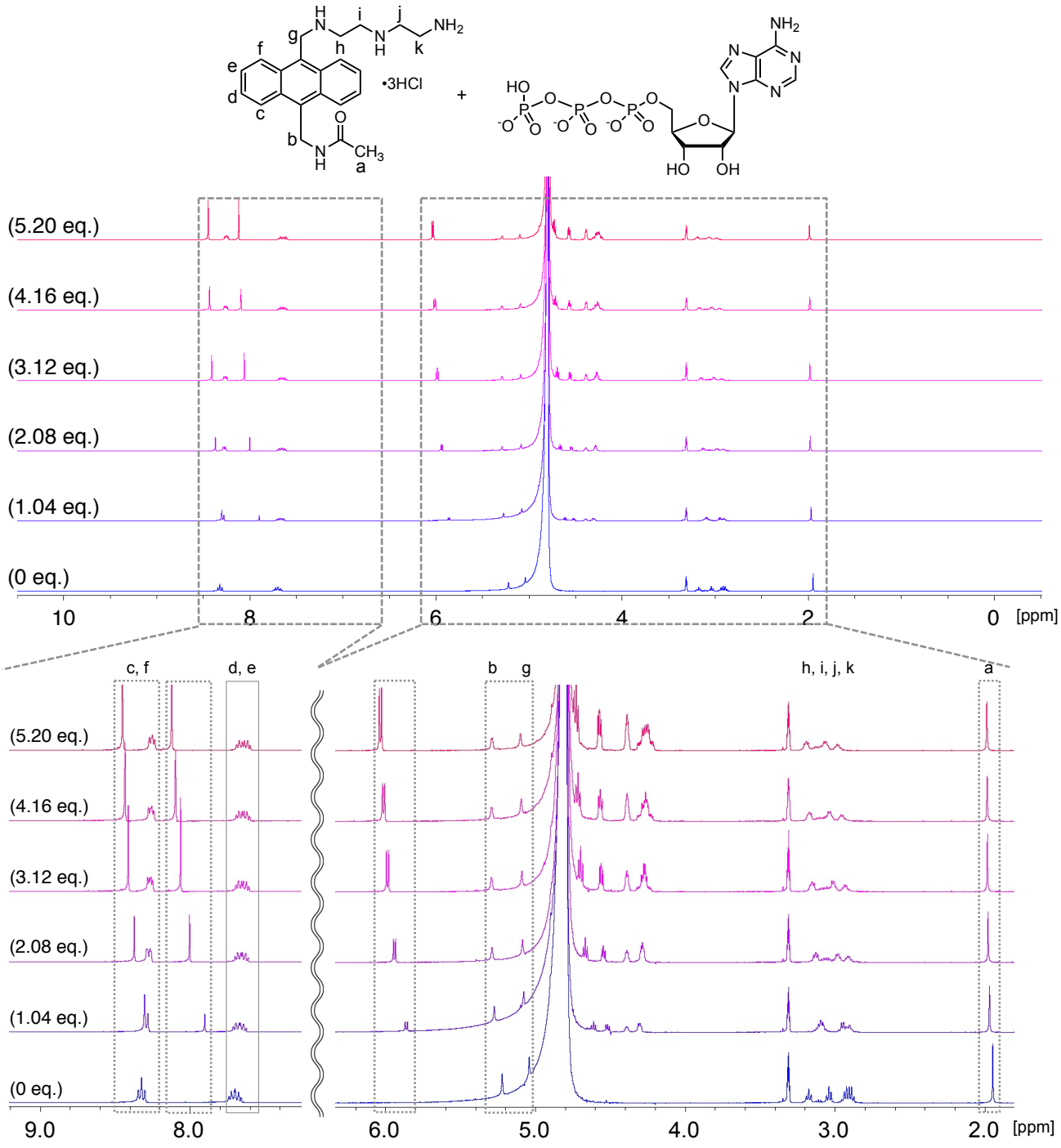


Fig. S8. ^{1}H NMR spectra of **9** up on the addition of ATP (0, 1.04, 2.08, 3.12, 4.16, 5.20 equivalent). [**9**] = 2.4 mM in in 9.5% CD_3OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC

9-Dopamine

No signal shift

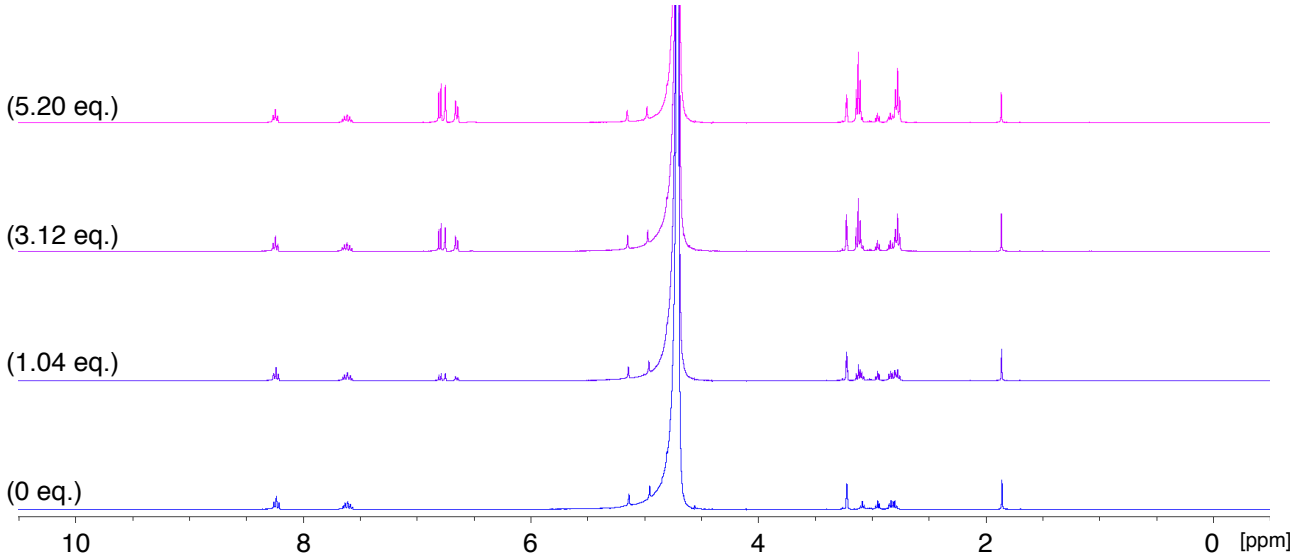


Fig. S9. ^{1}H NMR spectra of **9** up on the addition of dopamine (0, 1.04, 2.08, 3.12, 5.20 equivalent), [**9**] = 2.4 mM in in 9.5% CD_3OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC.

9-Glu

No signal shift

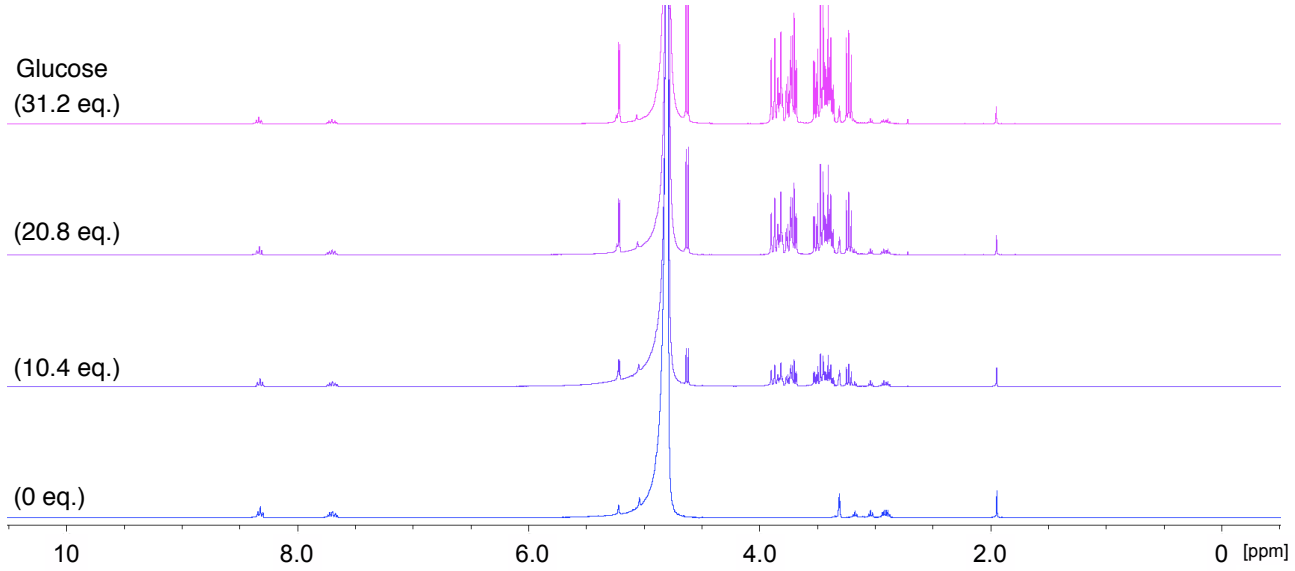


Fig. S10. ^{1}H NMR spectra of **9** up on the addition of glucose (0, 10.4, 20.8, 31.2 equivalent), [**9**] = 2.4 mM in 9.5% CD_3OD/Deuterated phosphate buffer (50 mM, pD 7.4), 25 ºC.

VIII. DFT-calculation study

All the density functional theory (DFT) calculations were performed utilizing the Gaussian 16 package.[1] 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_e) 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

1. M. J. Frisch, G. W. Trucks, H. B. Schlegel, *et al*., computer code *Gaussian 16*, *Revision A.03*, Gaussian, Inc., Wallingford, CT, 2016.
2. J.-D. Chai and M. Head-Gordon, "Long-range corrected hybrid density functionals with damped atom-atom dispersion corrections," *Phys. Chem. Chem. Phys.*, **10** (2008) 6615-6620.
3. J. Tomasi, B. Mennucci, and R. Cammi, "Quantum mechanical continuum solvation models," *Chem. Rev.*, **105** (2005) 2999-3093.

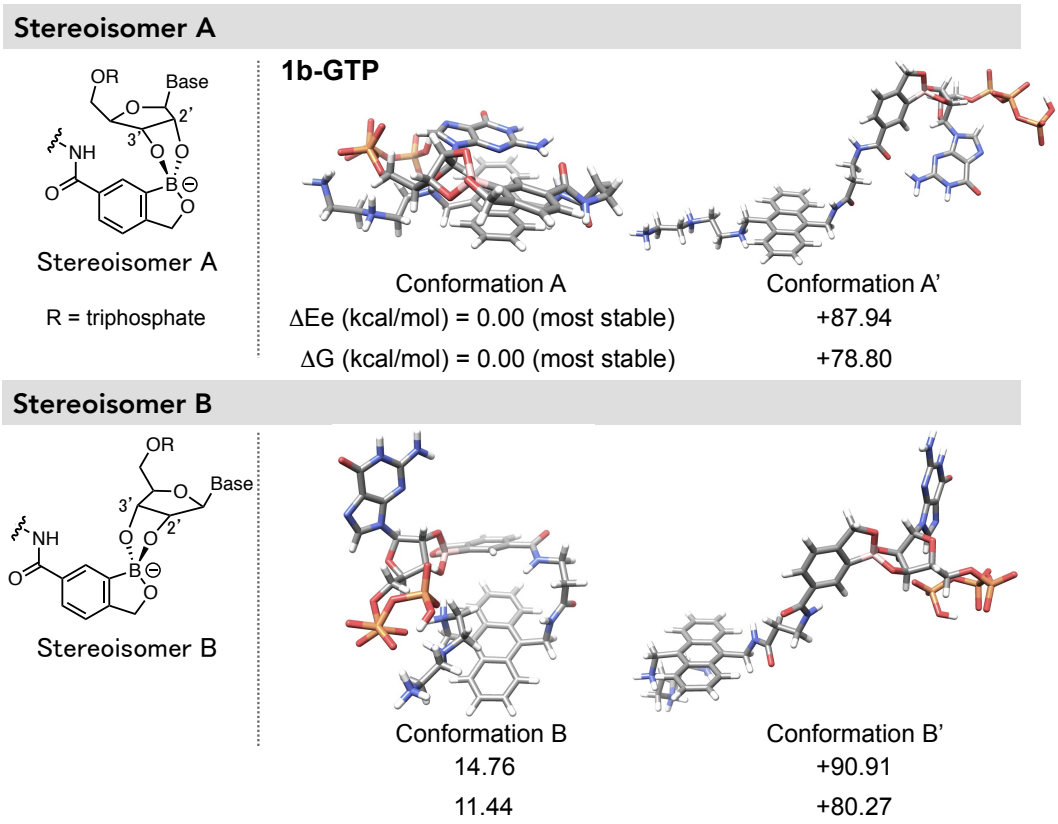
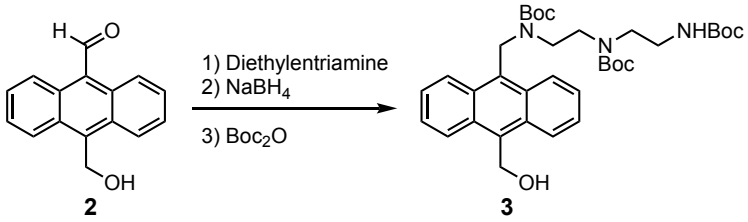


Fig. S11. DFT calculated optimized structure of **1b**-GTP complex

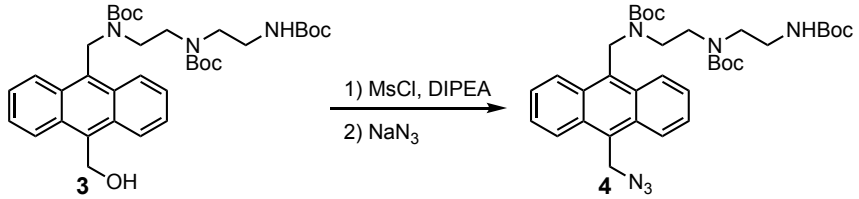
IX. Synthetic procedure of benzoxaborole 1a-c

*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.



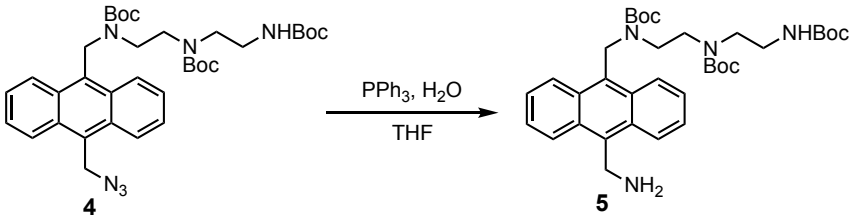
2 (2.26 g, 9.57 mmol) was reacted with diethylenetriamine (4.11 mL, 38.3 mmol) in EtOH (128 mL) and THF (63 mL) under a nitrogen atmosphere at room temperature. After stirring for 12 h, $NaBH_4$ (1.45 g, 38.3 mmol) was added to the reaction mixture at room temperature. After stirring for 18 h, the reaction mixture was quenched by the addition of H_2O. The mixture was extracted with $CHCl_3$ in three times, and the combined organic layer was washed with brine. The organic phase was dried with anhydrous Na_2SO_4, filtrated and concentrated. The resulting product was used in next step without any purification. A solution of Boc_2O (10.4 g, 47.9 mmol) in MeOH (96 mL) was added to the resulting triamine product at room temperature. After stirring for 12h, the mixture was concentrated. The crude product was purified by chromatography (silica gel,

$CHCl_3$/AcOEt/Hexane, 2:1:0.1) to afford **3** (2.17 g, 36% in three steps) as a pale yellow foam; ^{1}H NMR (400 MHz, CD_3OD) 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); ^{13}C NMR (100 MHz, CD_3OD) 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, 126.4, 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, 1682, 1451, 1413, 1365, 1244, 1157 cm^{-1}; HRMS (ESI): calcd. for $[C_{35}H_{29}N_3O_7+Na]^+$ 646.3463; found 646.3479.



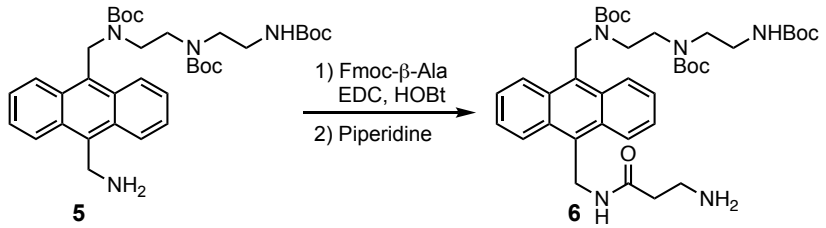
3 **4**

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 CH_2Cl_2 (68 mL) at 0 °C. After stirring at 0 ºC for two hours, the mixture was washed with sat. $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, 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 NaN_3 (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 H_2O and extracted with AcOEt. The organic phase was dried with Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography (silica gel, $CHCl_3$/AcOEt/Hexane, 9:1:0.1) to afford **4** (1.40 g, 59%) as a pale yellow foam; ^{1}H NMR (400 MHz, CD_3OD) 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); ^{13}C NMR (100 MHz, CD_3OD) 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 $[C_{35}H_{48}N_6O_6+Na]^+$ 671.3528; found 671.3492.

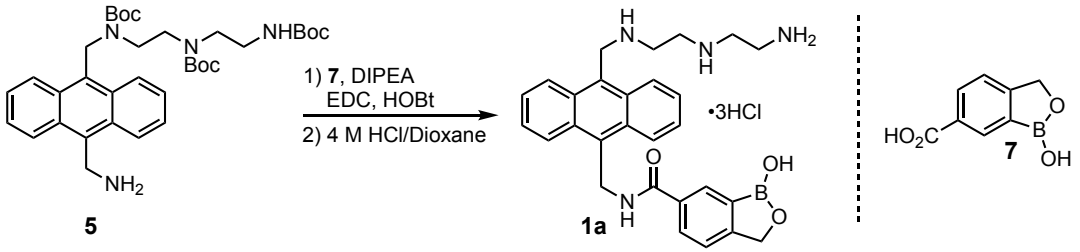


4 **5**

To a solution of **4** (1.30 g, 1.85 mmol) in THF (56 mL) and H_2O (6 mL) was added PPh_3 (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, $CHCl_3$/MeOH/Hexane, 50:1:5, 30:1:3, 20:1:2) to afford **5** (1.05g, 91%) as a pale yellow foam; ^{1}H NMR (400 MHz, CD_3OD) 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); ^{13}C NMR (100 MHz, CD_3OD) 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 $[C_{35}H_{50}N_4O_6+Na]^+$ 645.3623; found 623.3664.

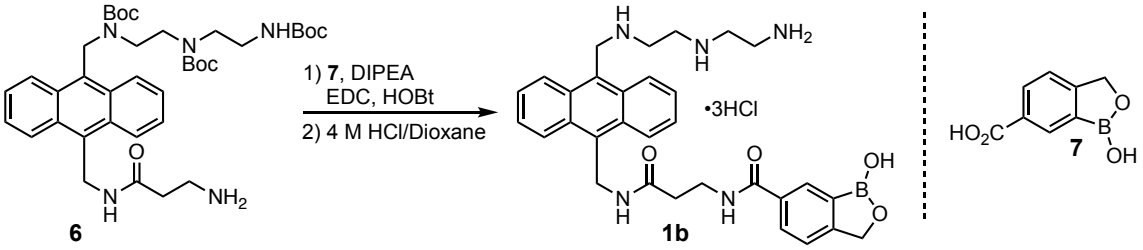


5 (441 mg, 0.707 mmol) was added to a solution of Fmoc-β-Ala-OH (242 mg, 0.778 mmol), HOBt (115 mg, 0.848 mmol), and EDC (230 mg, 0.848 mmol) in CH_2Cl_2 (25 mL). The mixture was stirred at room temperature for 24 h. The reaction mixture was washed with sat. $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, filtrated and concentrated. The residue was partially purified by column chlomatography (silica gel, $CHCl_3$/AcOEt, 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 solution (9:1, 15 mL). After stirring for 1 hour at room temperature, the solvent was removed. The residue was purified by chlomatography (silica gel, $CHCl_3$/MeOH/Hexane, 30:1:3, 20:1:2, 10:1:1) to afford **6** (361 mg, 74%) as a pale yellow foam; ^{1}H 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); ^{13}C NMR (100 MHz, CD_3OD) 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, 1527, 1454, 1412, 1364, 1245, 1158 cm^{-1}. HRMS (ESI): calcd. for $[C_{38}H_{55}N_5O_7+Na]^+$ 716.3994; found 717.3988.

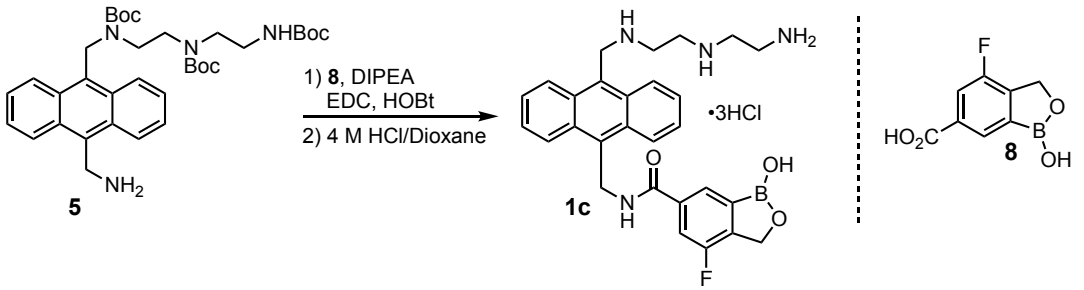


A solution of **5** (163 mg, 0.262 mmol) in DMF (8.7 mL) and DIPEA (53.8 μL, 0.314 mmol) was added to a solution of **7** (51.3 mg, 0. 288 mmol), HOBt (42.4 mg, 0. 314 mmol), and EDC (60.3 mg, 0.314 mmol). The mixture was stirred at room temperature for 18h. After that, the mixture was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, filtrated and concentrated. The resulting product was partially purified by column chromatography (silica gel, $CHCl_3$/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 $CHCl_3$ and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from $CHCl_3$ and MeOH to afford **1a** (70.0 mg, 39%) as a pale yellow solid; ^{1}H NMR (400 MHz, D_2O) 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),

3.38-3.24 (m, 6H); ^{13}C NMR (100 MHz, D_2O) 169.6, 156.2, 132.0, 131.7, 130.0, 129.5, 129.4, 128.8, 127.6, 126.6, 124.9, 123.4, 121.7, 121.5, 70.1, 44.6, 43.7, 43.5, 43.4, 36.6, 35.4; IR (FT-ATR): ν = 3289, 2719, 1622, 1435, 1270, 1054, 998 cm^{-1}; HRMS (ESI): calcd. for [$C_{28}H_{31}BN_4O_3$ – OH + CH_3OH + Na]$^+$ 519.2538; found 519.2486.

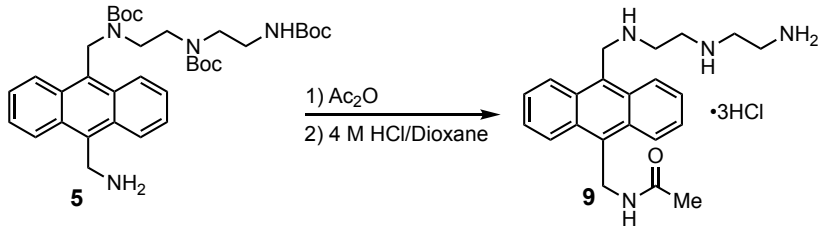


A solution of **6** (180 mg, 0.259 mmol) in DMF (8.5 mL) and DIPEA (53 μL, 0.311 mmol) was added to a solution of **7** (50.5 mg, 0.284 mmol), HOBt (42.0 mg, 0.311 mmol), and EDC (59.3 mg, 0.311 mmol). The mixture was stirred at room temperature for 12h. After that, the mixture was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, filtrated and concentrated. The resulting product was partially purified by column chromatography (silica gel, $CHCl_3$/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 $CHCl_3$ and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from $CHCl_3$ and MeOH to afford **1b** (89.2 mg, 53%) as a pale yellow solid; ^{1}H NMR (400 MHz, D_2O) 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); ^{13}C NMR (100 MHz, D_2O) 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 [$C_{31}H_{36}BN_5O_4$ – OH + CH_3OH + Na]$^+$ 590.2903; found 590.2970.



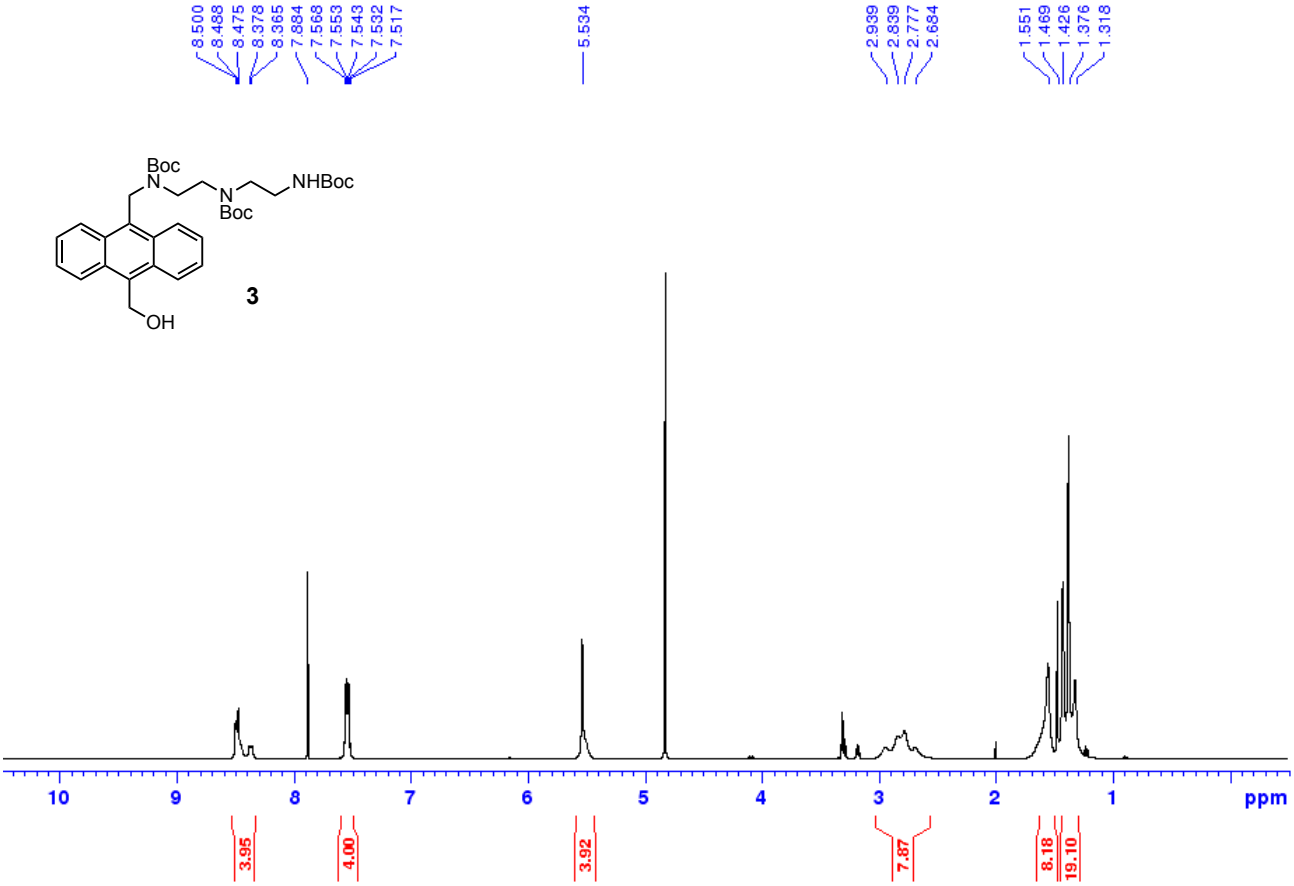
A solution of **5** (188 mg, 0.302 mmol) in DMF (10 mL) and DIPEA (62.1 mL, 0.362 mmol) was added to a solution of **8** (65.0 mg, 0.332 mmol), HOBt (48.9 mg, 0.362 mmol), and EDC (69.4 mg, 0.155 mmol). The mixture was stirred at room temperature for 18h. After that, the mixture was diluted with $CHCl_3$ and washed with sat. $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, filtrated and concentrated. The

resulting product was partially purified by column chromatography (silica gel, $CHCl_3$/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 $CHCl_3$, and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from $CHCl_3$ and MeOH to afford **1c** (66.0 mg, 36%) as a pale yellow solid; ^{1}H NMR (400 MHz, D_2O) 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), 3.45 (t, *J* = 6.4 Hz, 2H), 3.13-3.10 (m, 4H), 3.01 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, D_2O) 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; ^{19}F NMR (376 MHz, D_2O) δ -120.0; IR (FT-ATR): ν = 3317, 2718, 1616, 1526, 1445, 1313, 999 cm^{-1}; HRMS (ESI): calcd. for $[C_{28}H_{30}BFN_4O_3 - OH + CH_3OH + Na]^+$ 537.2444; found 537.2492.

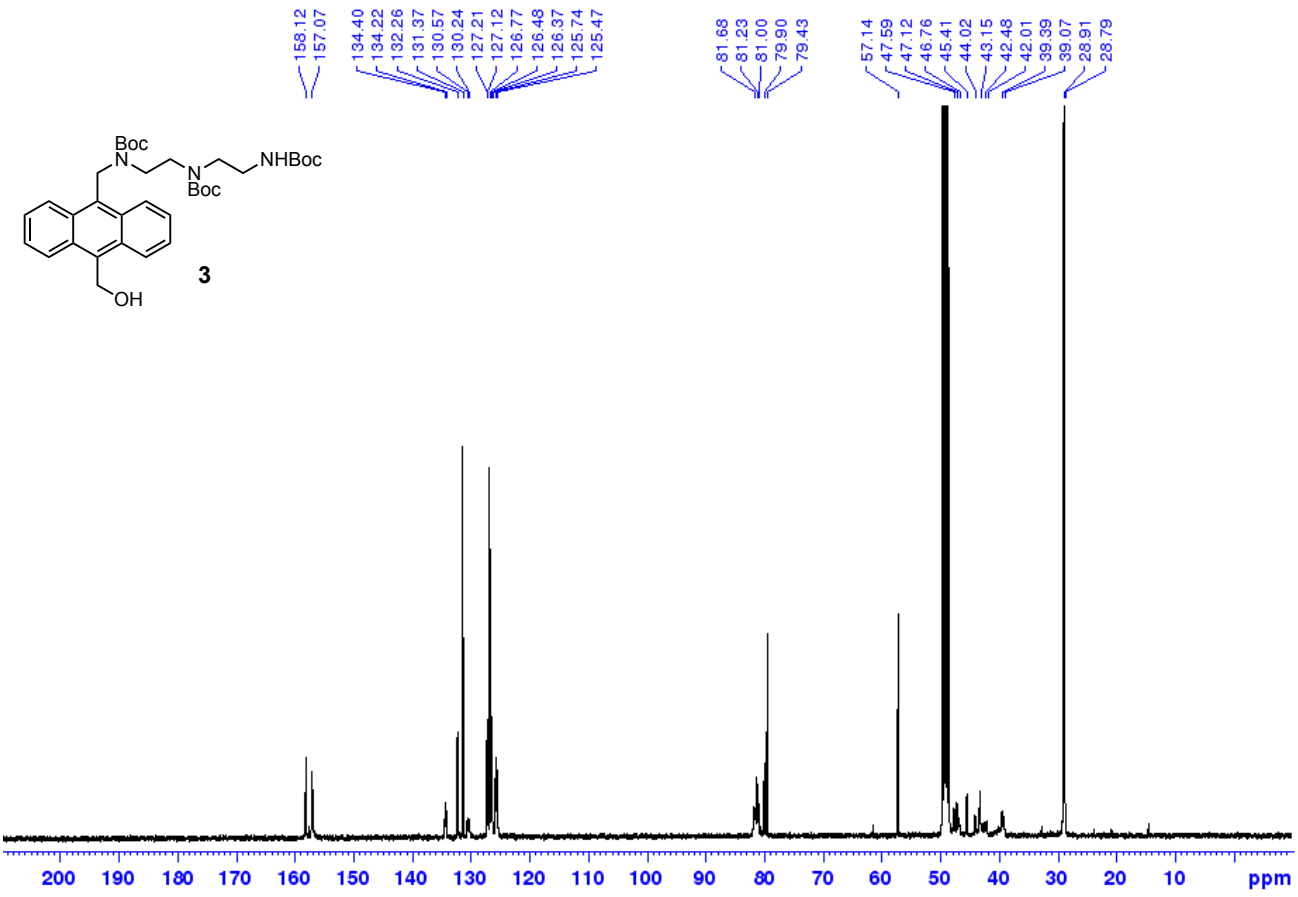


To a solution of **5** (364 mg, 0.584 mmol) in pyridine (6 mL) was added Ac_2O (1 mL) at room temperature. After stirring for 12h, the solvent was removed. The residue was partially purified by chromatography (silica gel, $CHCl_3$/AcOEt 1:0, 5:1). The resulting product was suspended to 4 M HCl/dioxane (2 mL), and the mixture was stirred for 1h at room temperature. After removing the solvent, the residue was filtrated and washed with $CHCl_3$ and cold MeOH. The resulting product was further purified for the fluorescence and NMR titration study by recrystallization from $CHCl_3$ and MeOH to afford **9** (63.7 mg, 23%) as a pale yellow solid; ^{1}H NMR (400 MHz, D_2O) 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); ^{13}C NMR (100 MHz, D_2O) 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 $[C_{22}H_{28}N_4O+H]^+$ 364.2336; found 364.2296.

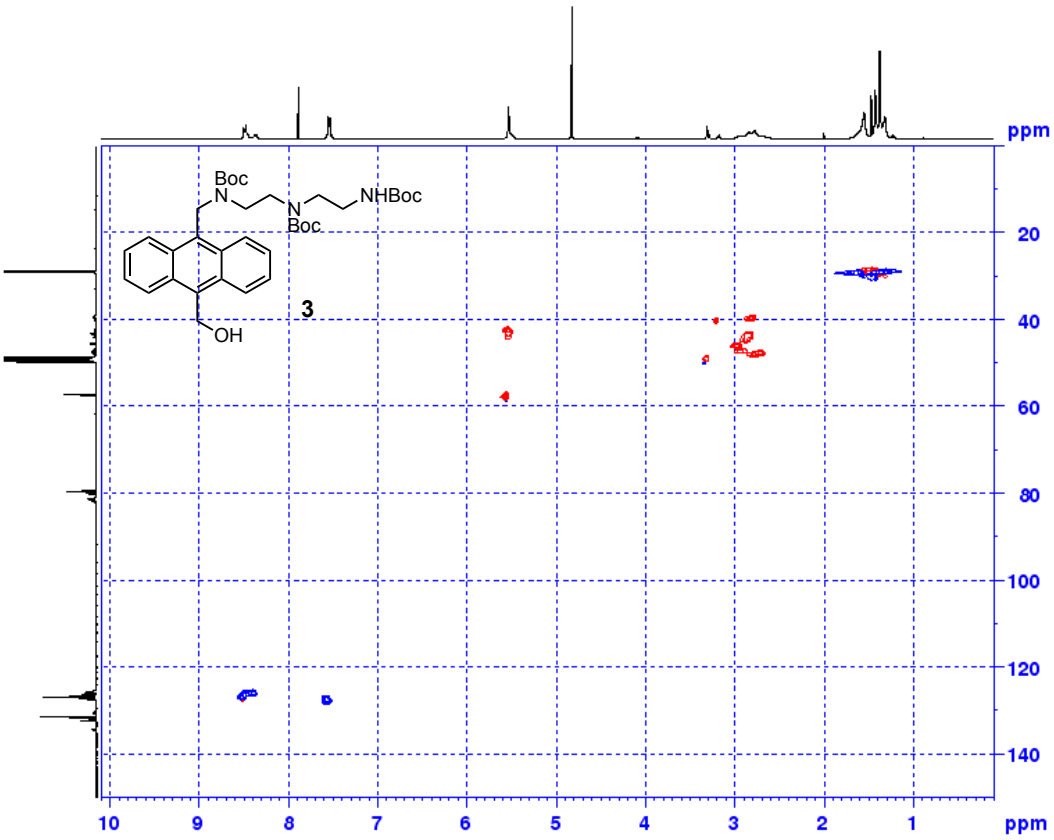
3: ^{1}H NMR (400 MHz, CD_3OD)



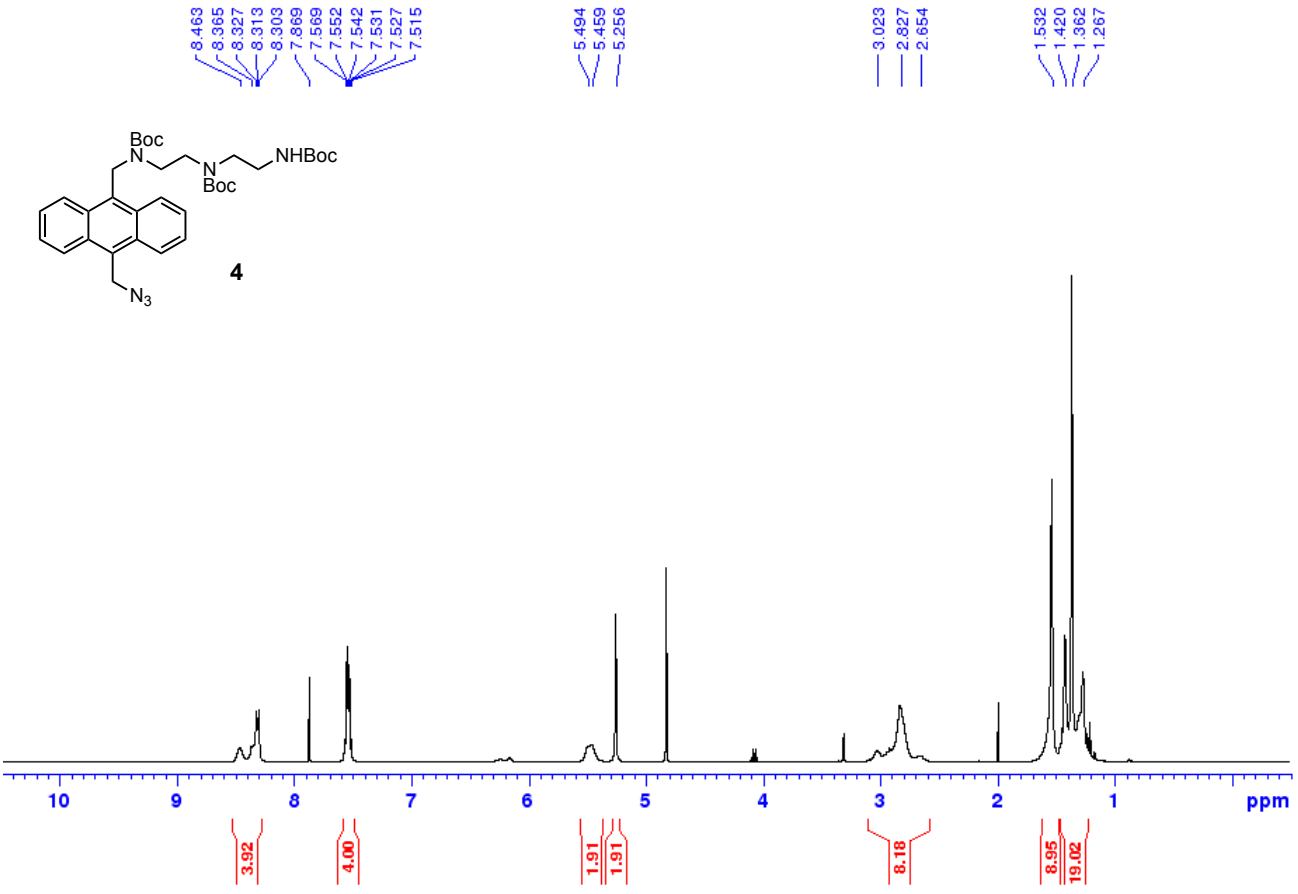
3: ^{13}C NMR (100 MHz, CD_3OD)



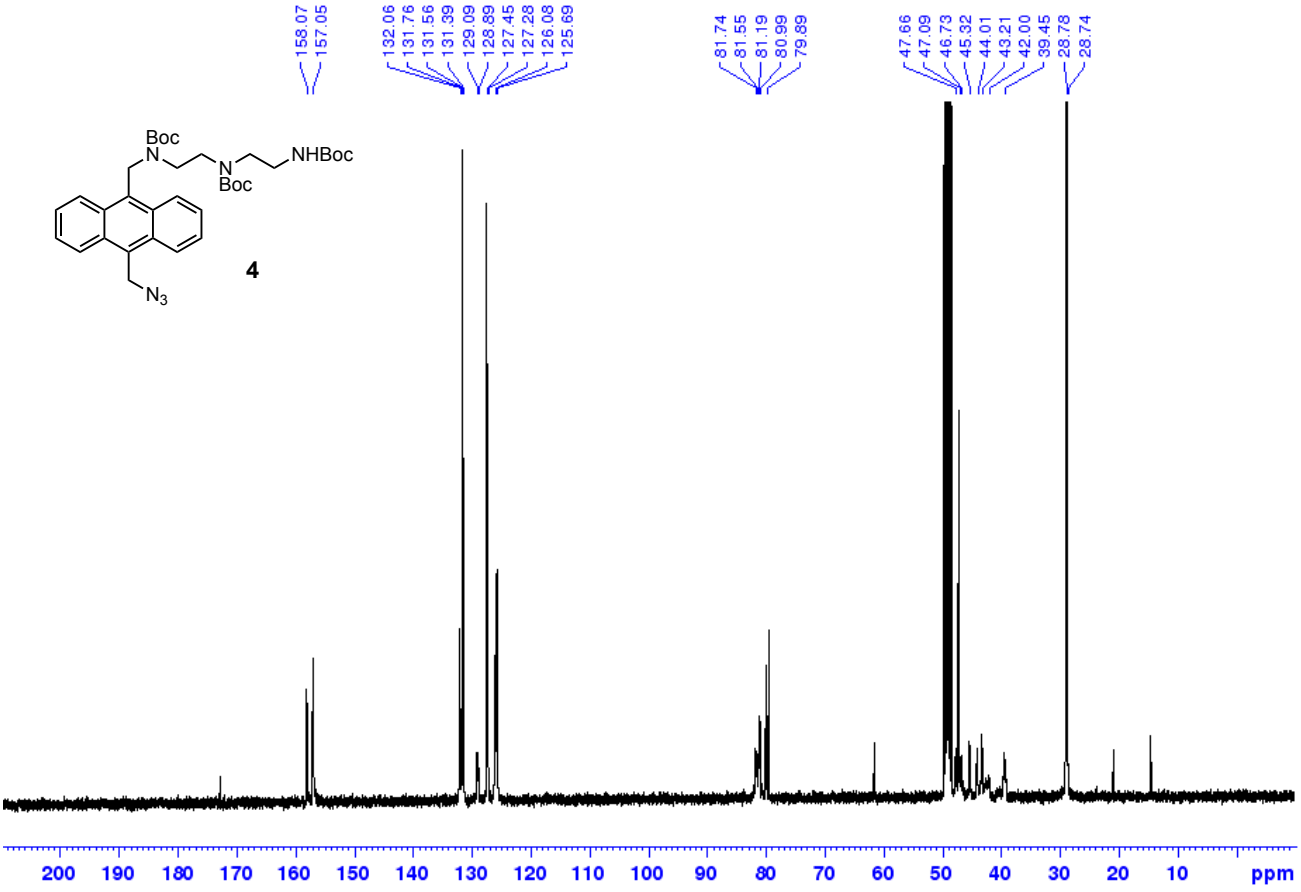
HSQC spectrum of **3** (CD_3OD)



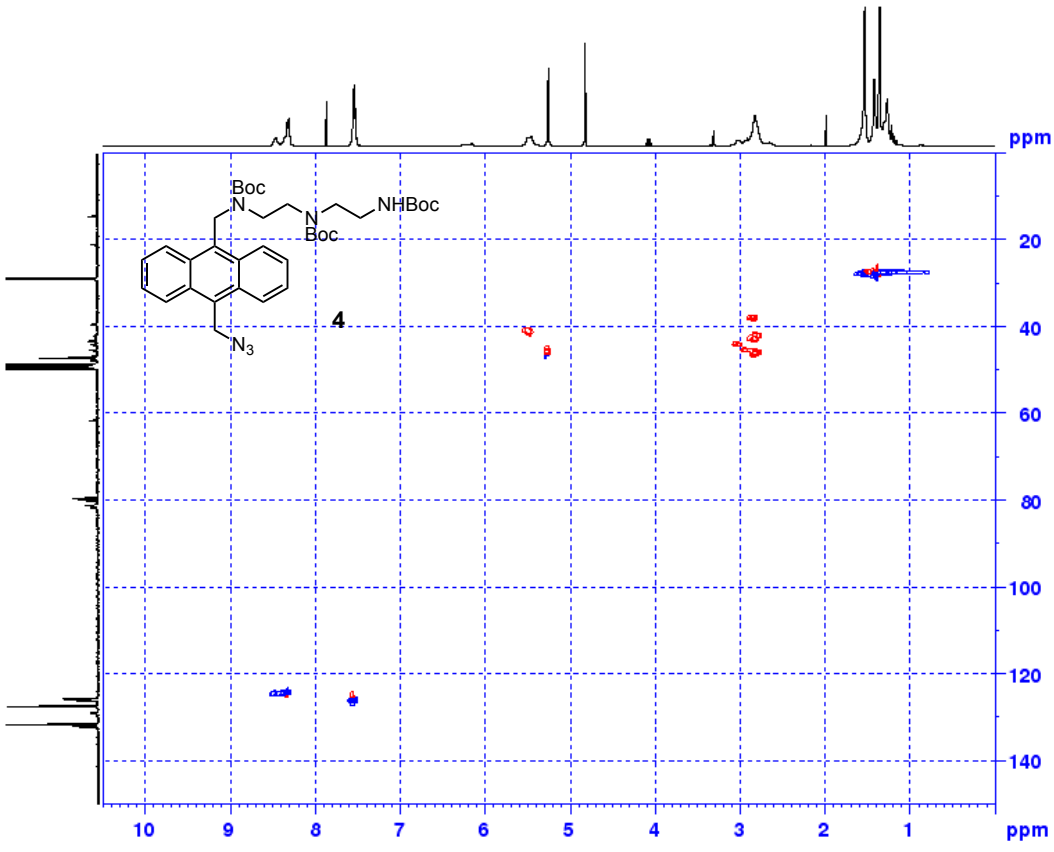
4: ^1H NMR (400 MHz, CD_3OD)



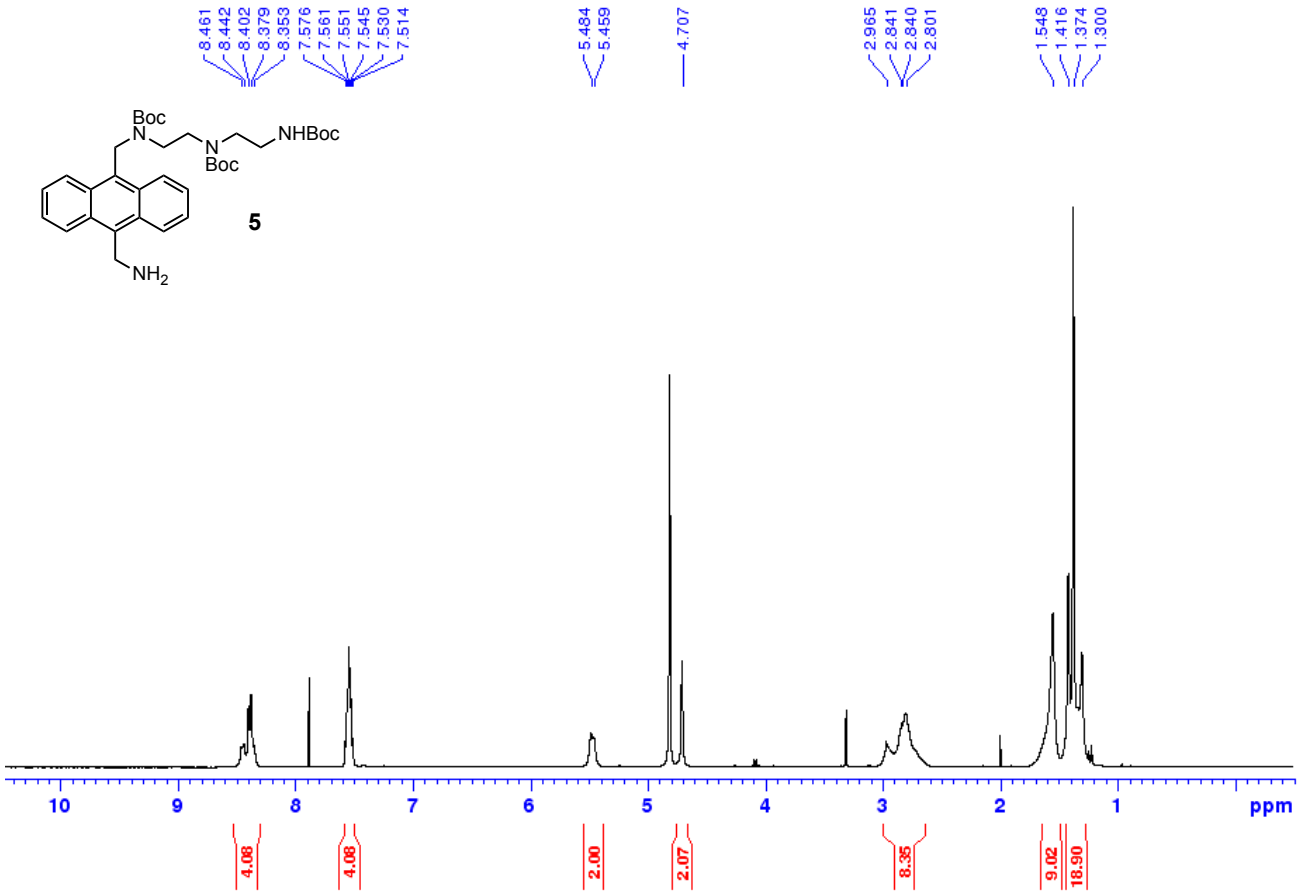
4: ^{13}C NMR (100 MHz, CD_3OD)



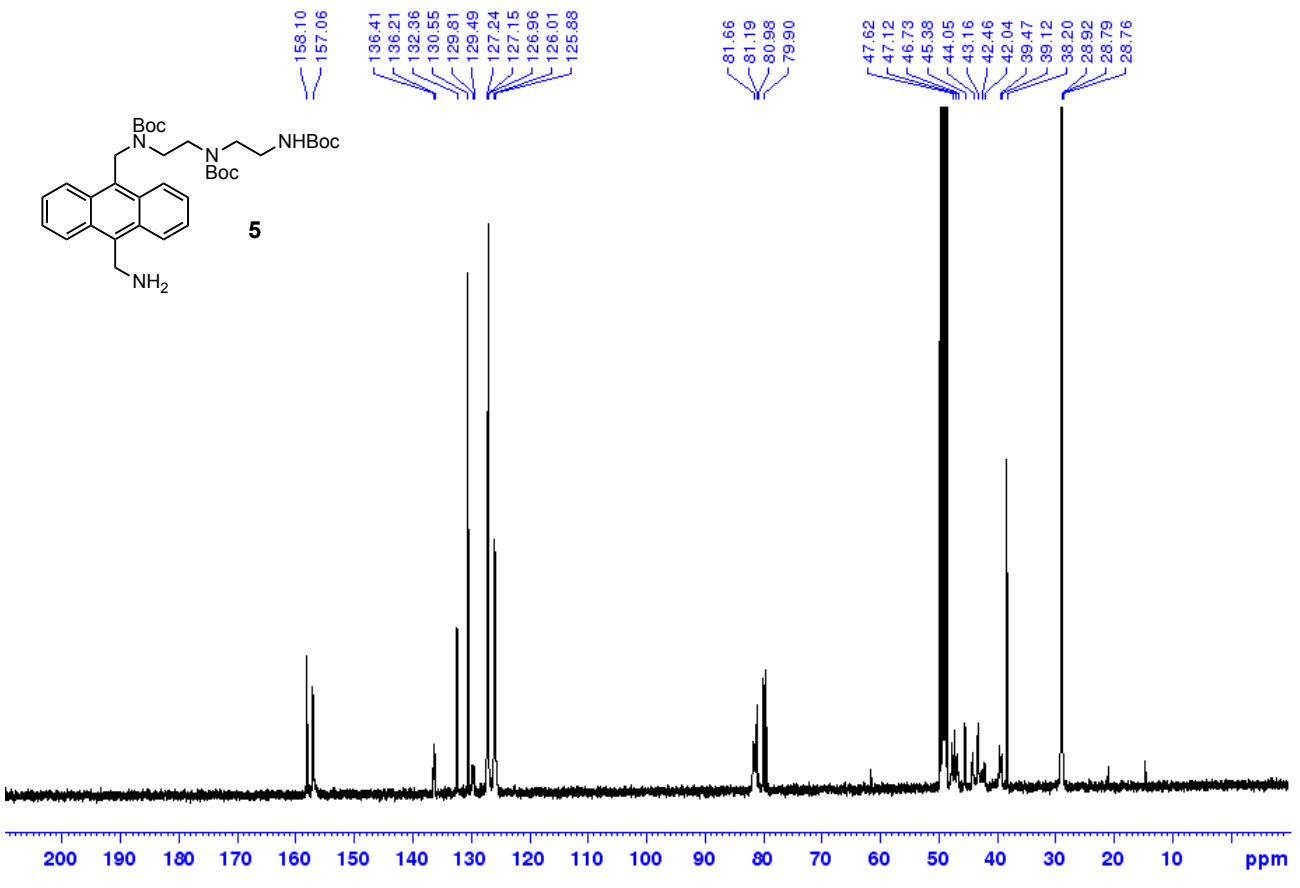
HSQC spectrum of **4** (CD_3OD)



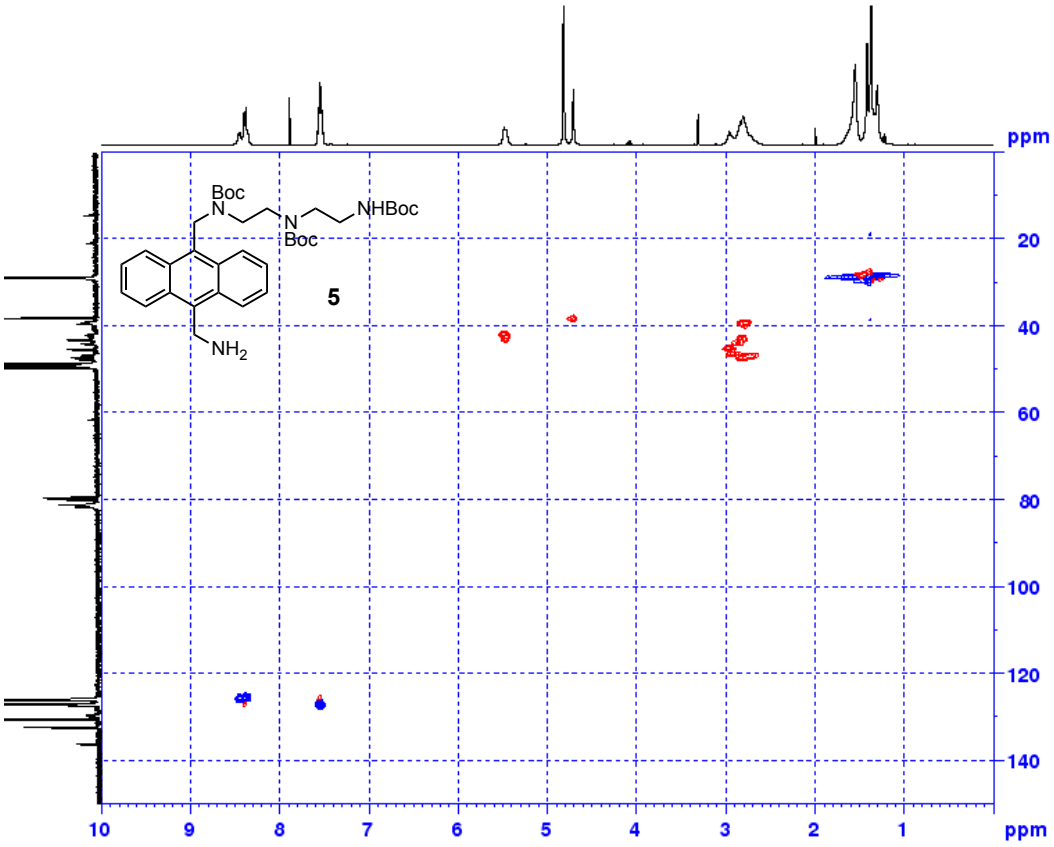
5: ^{1}H NMR (400 MHz, CD_3OD)



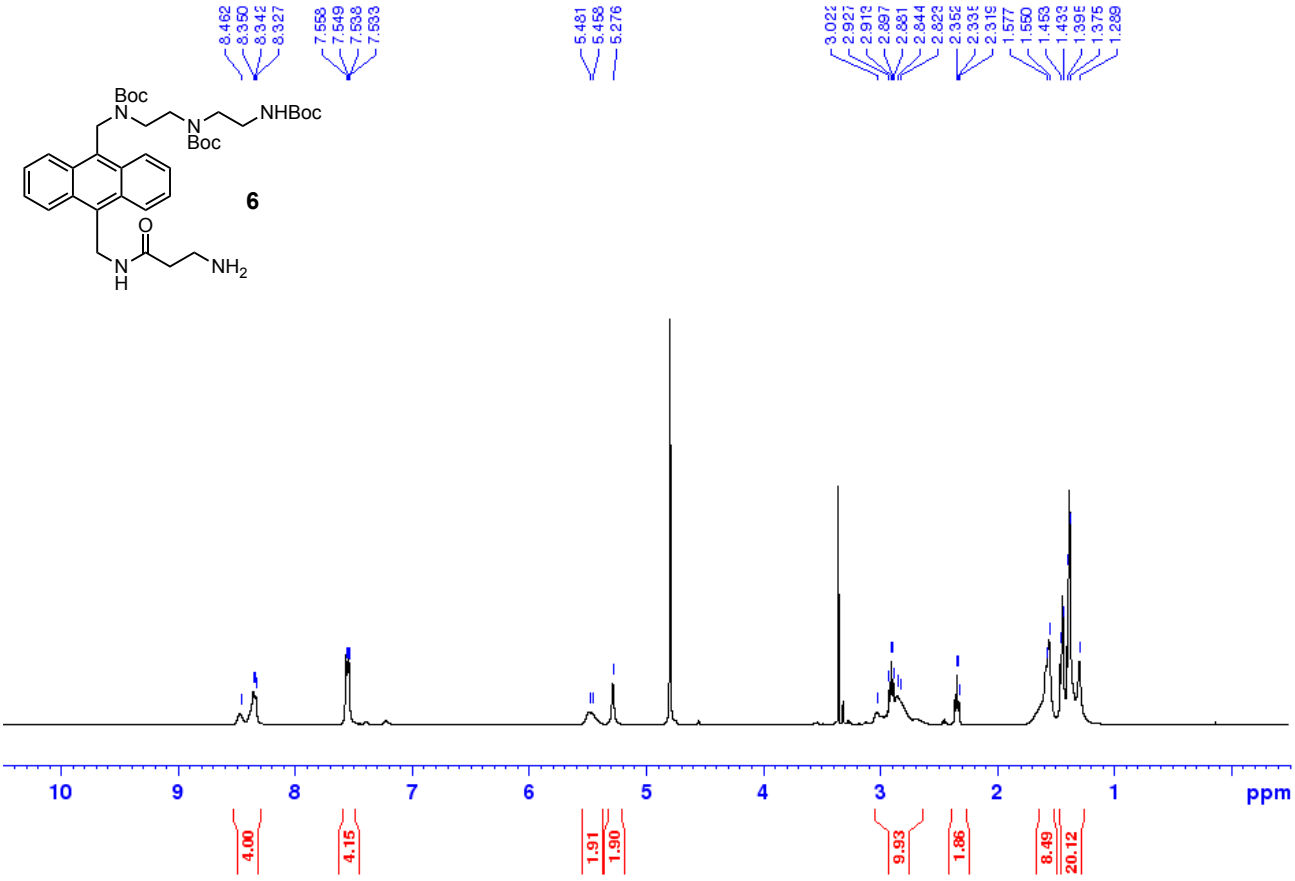
5: ^{13}C NMR (100 MHz, CD_3OD)



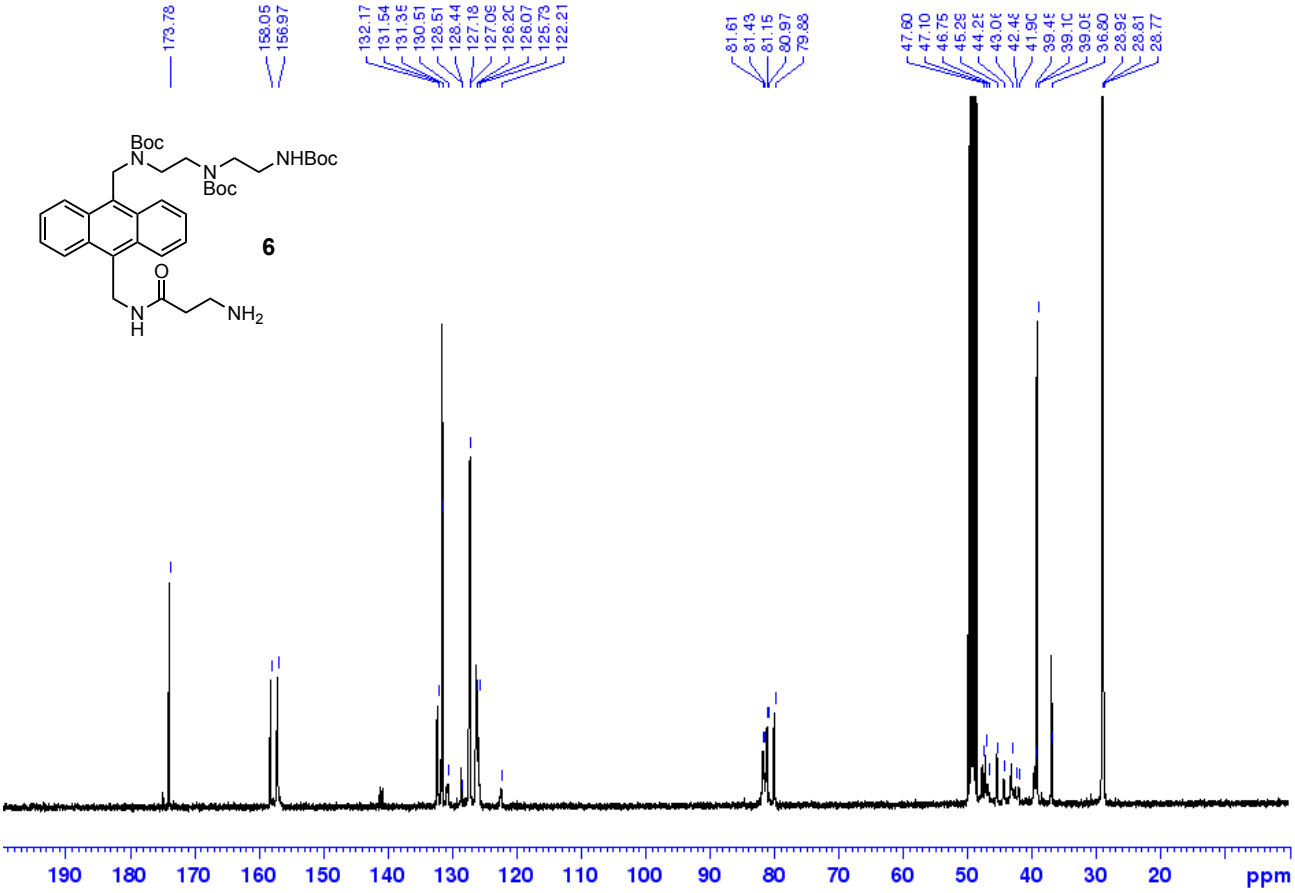
HSQC spectrum of **5** (CD_3OD)



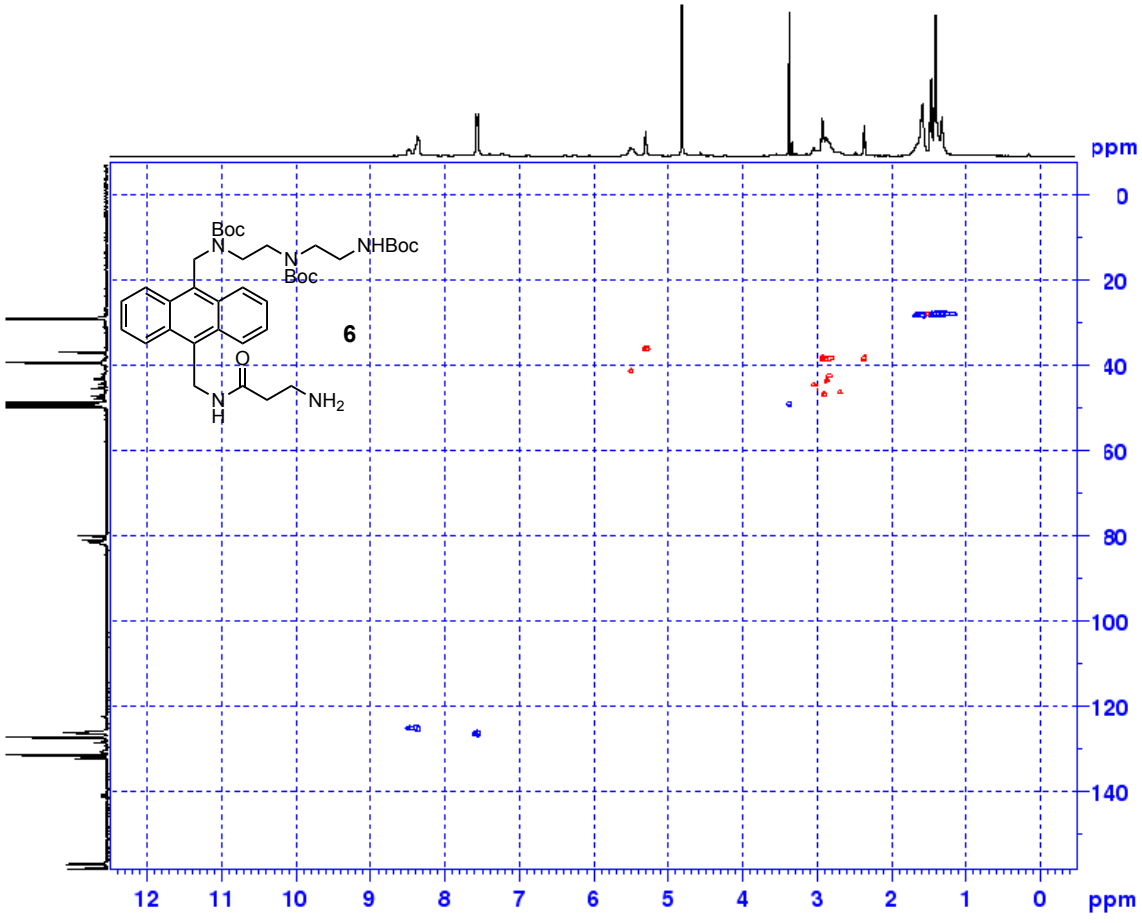
6: ^1H NMR (400 MHz, CD_3OD)



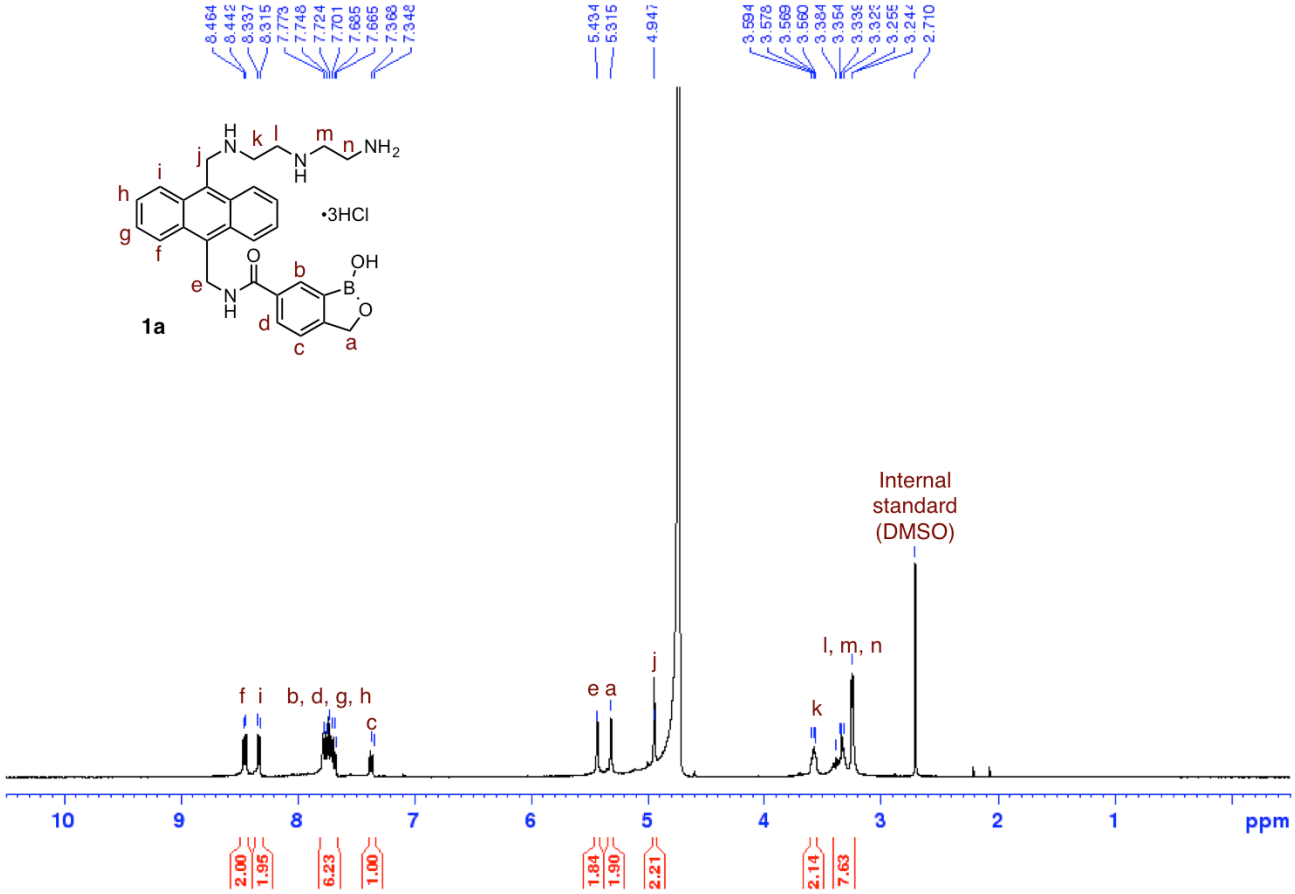
6: ^{13}C NMR (100 MHz, CD_3OD)



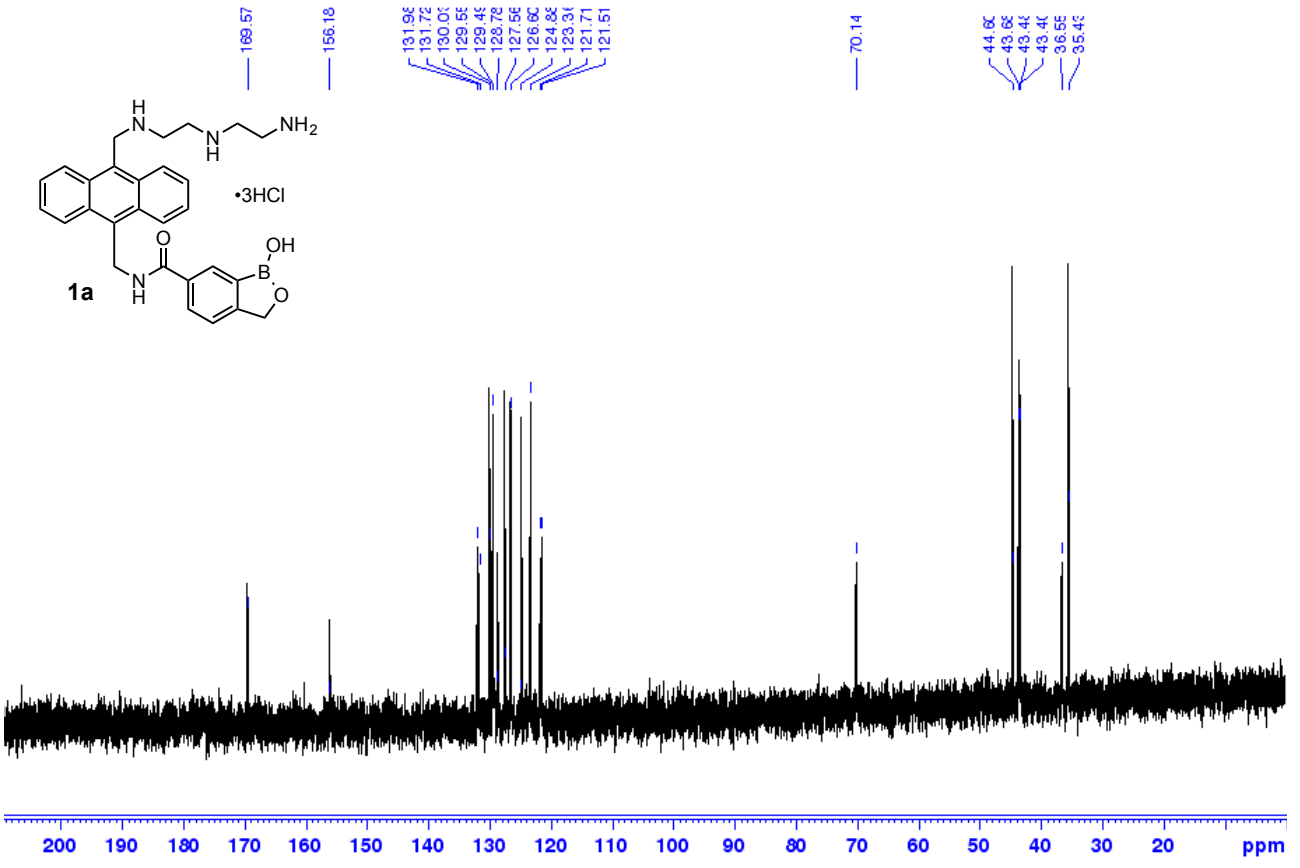
HSQC spectrum of **5** (CD_3OD)



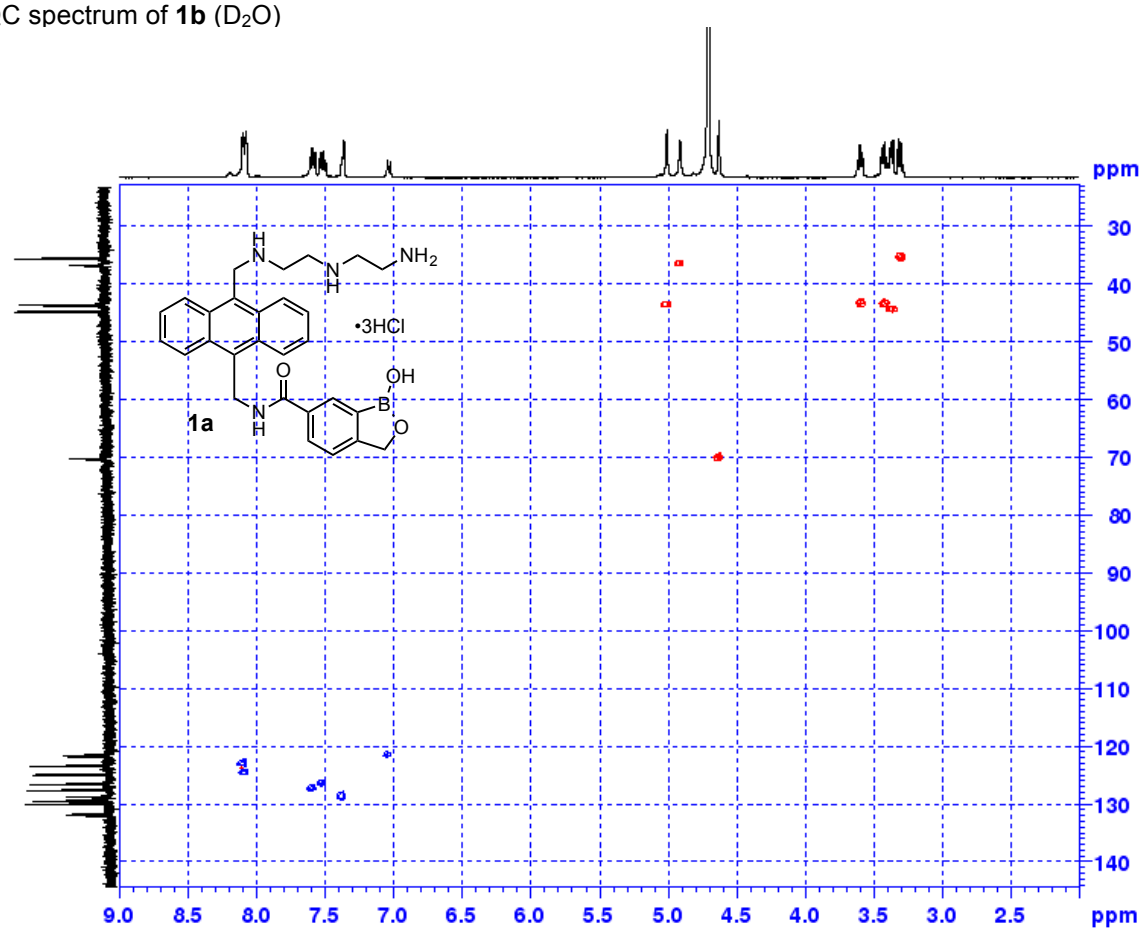
1a: ^{1}H NMR (400 MHz, D_2O)



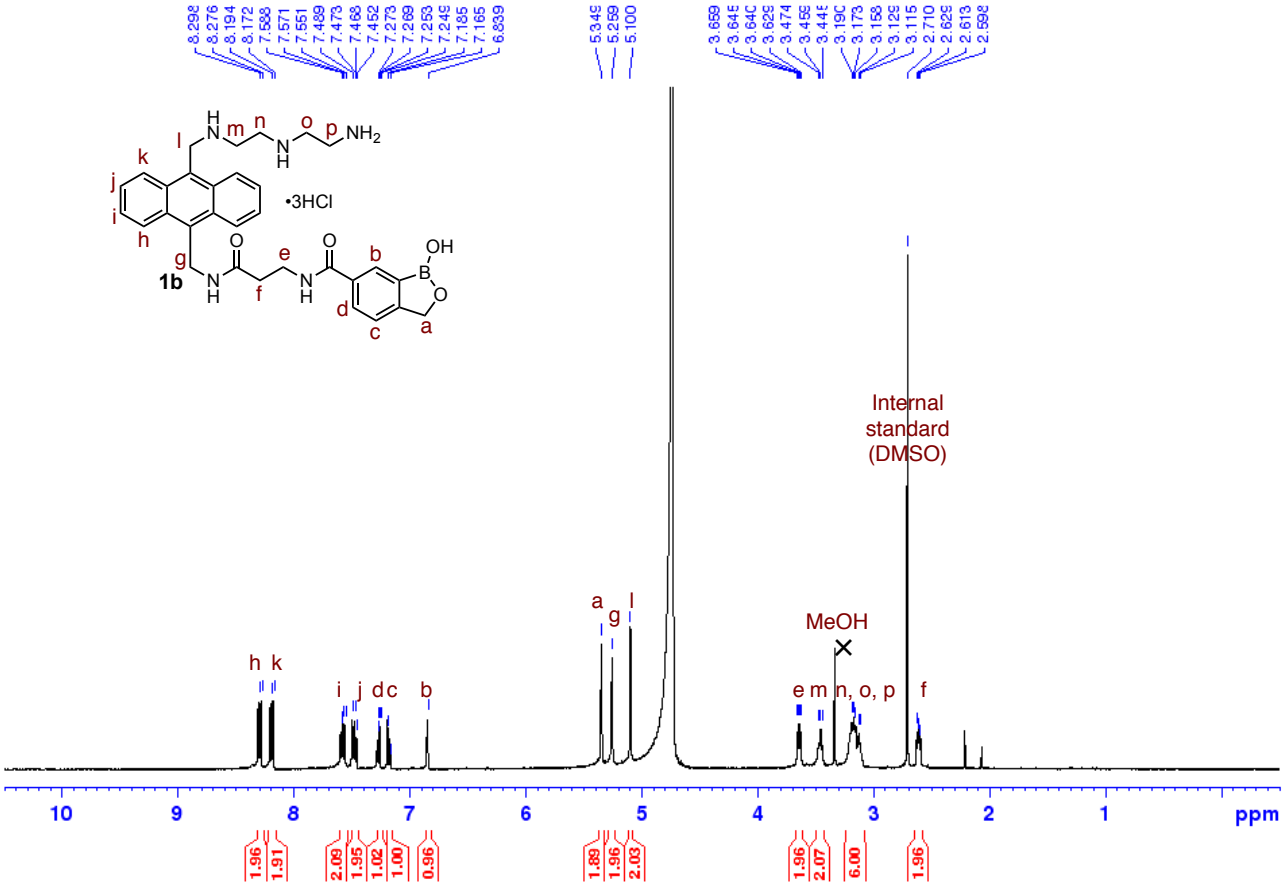
1a: ^{13}C NMR (100 MHz, D_2O)



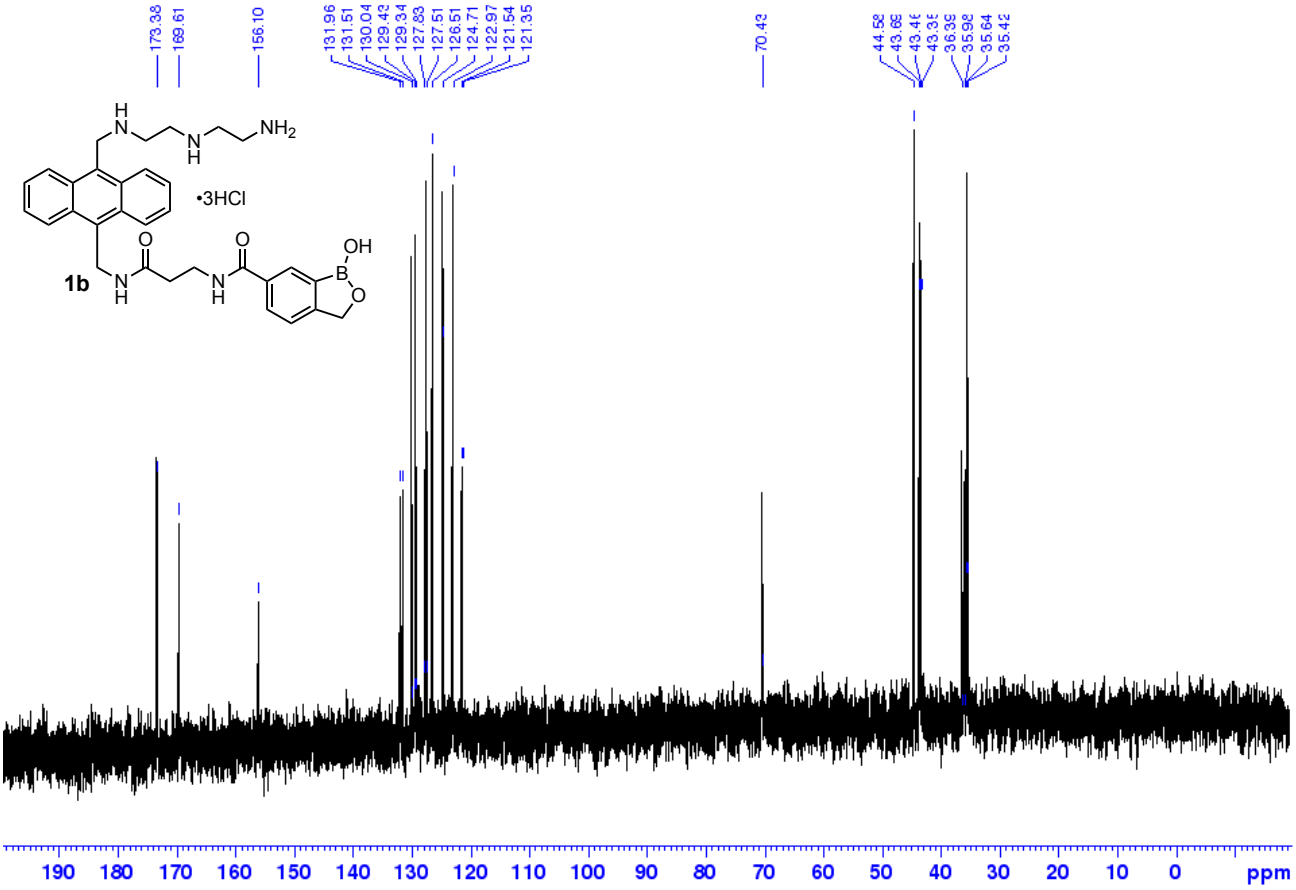
HSQC spectrum of **1b** (D_2O)



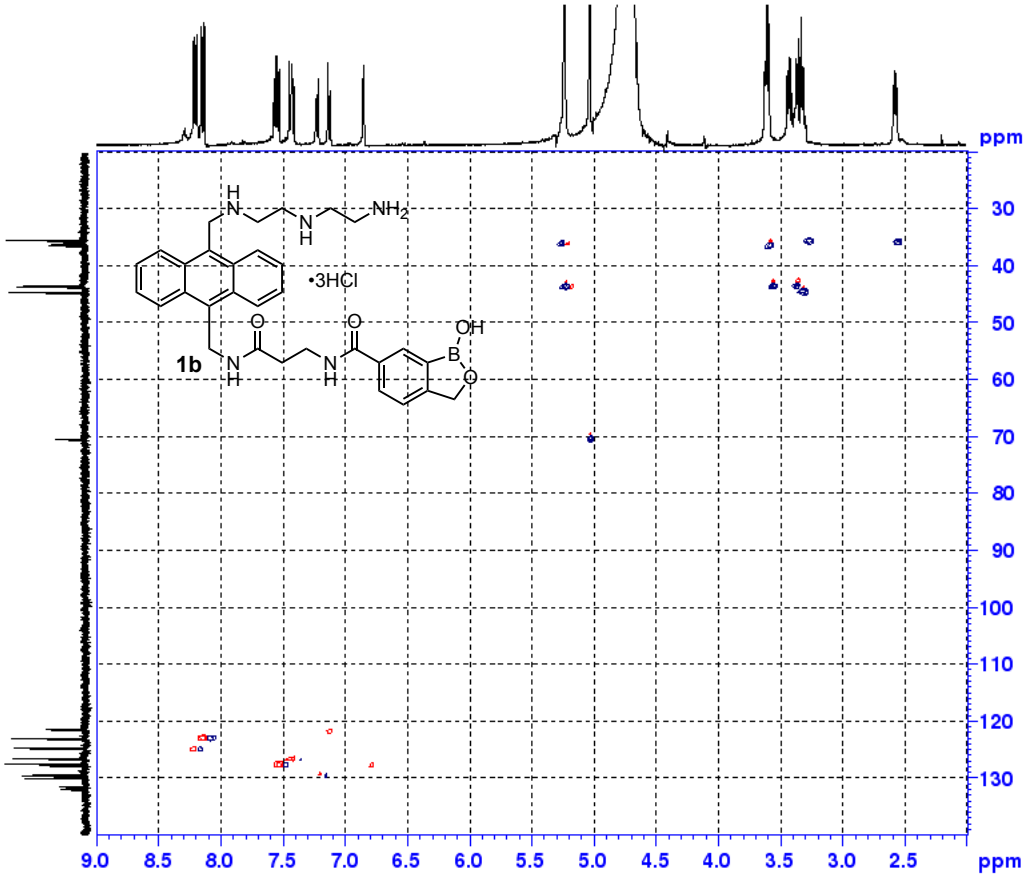
1b: ^{1}H NMR (400 MHz, D_2O)



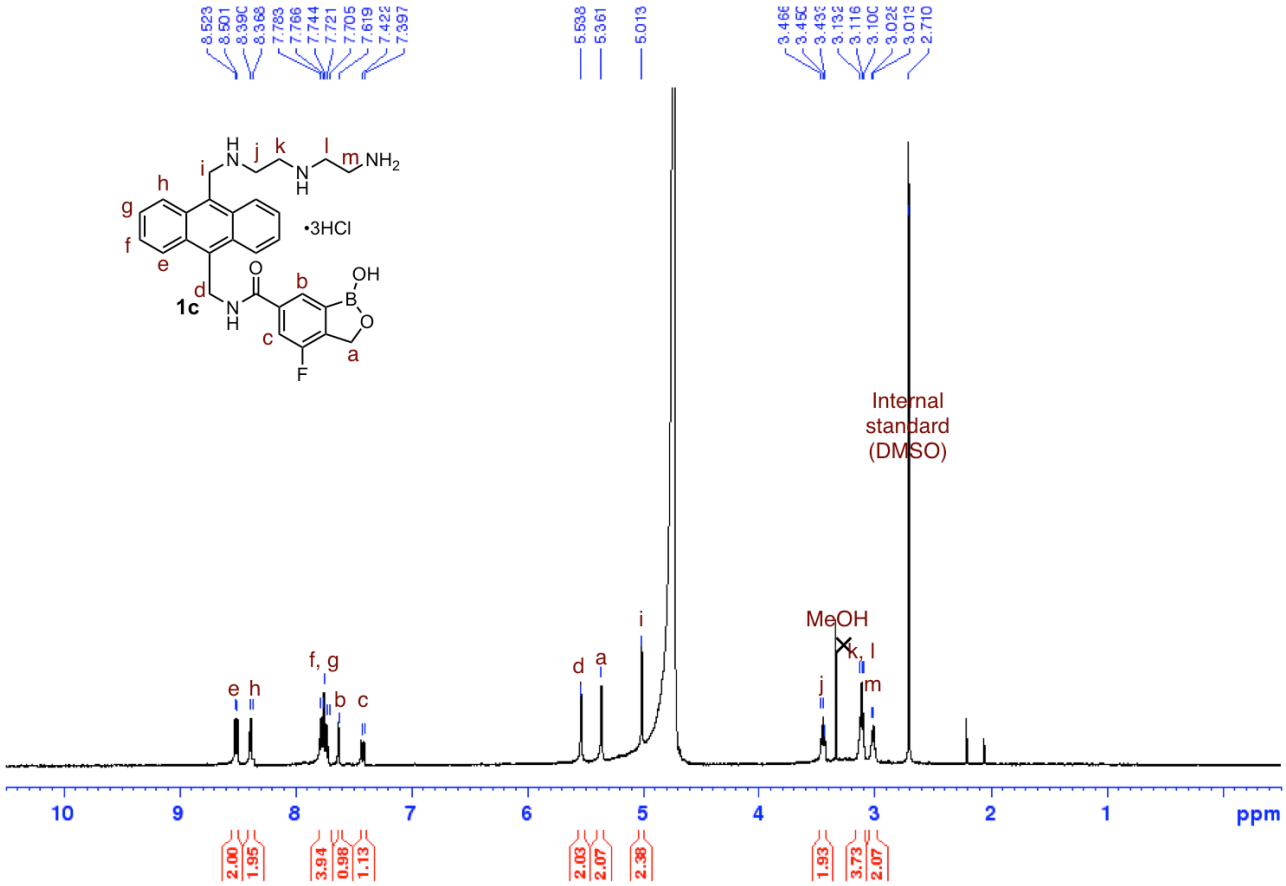
1b: ^{13}C NMR (100 MHz, D_2O)



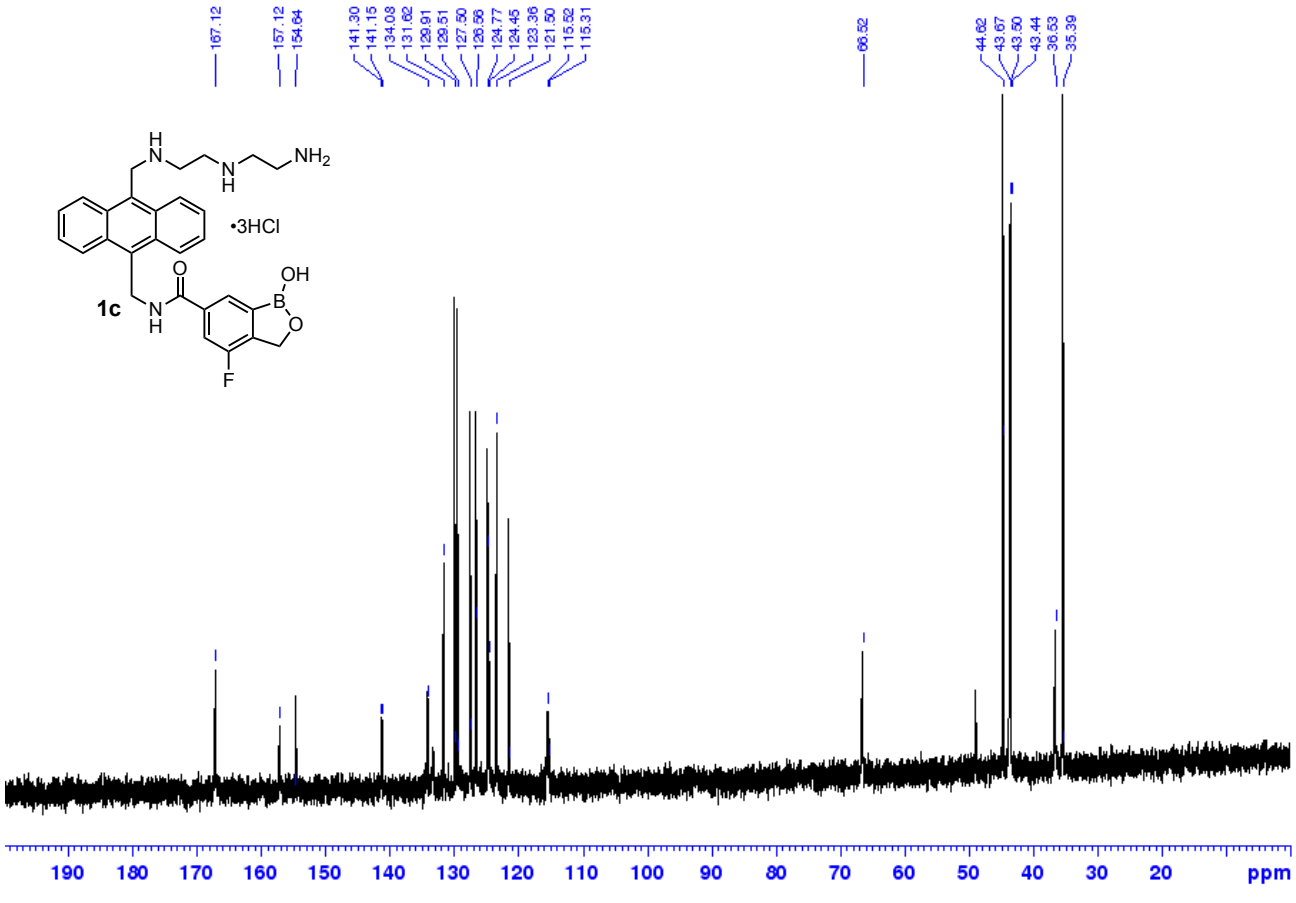
HSQC spectrum of **1b** (D_2O)



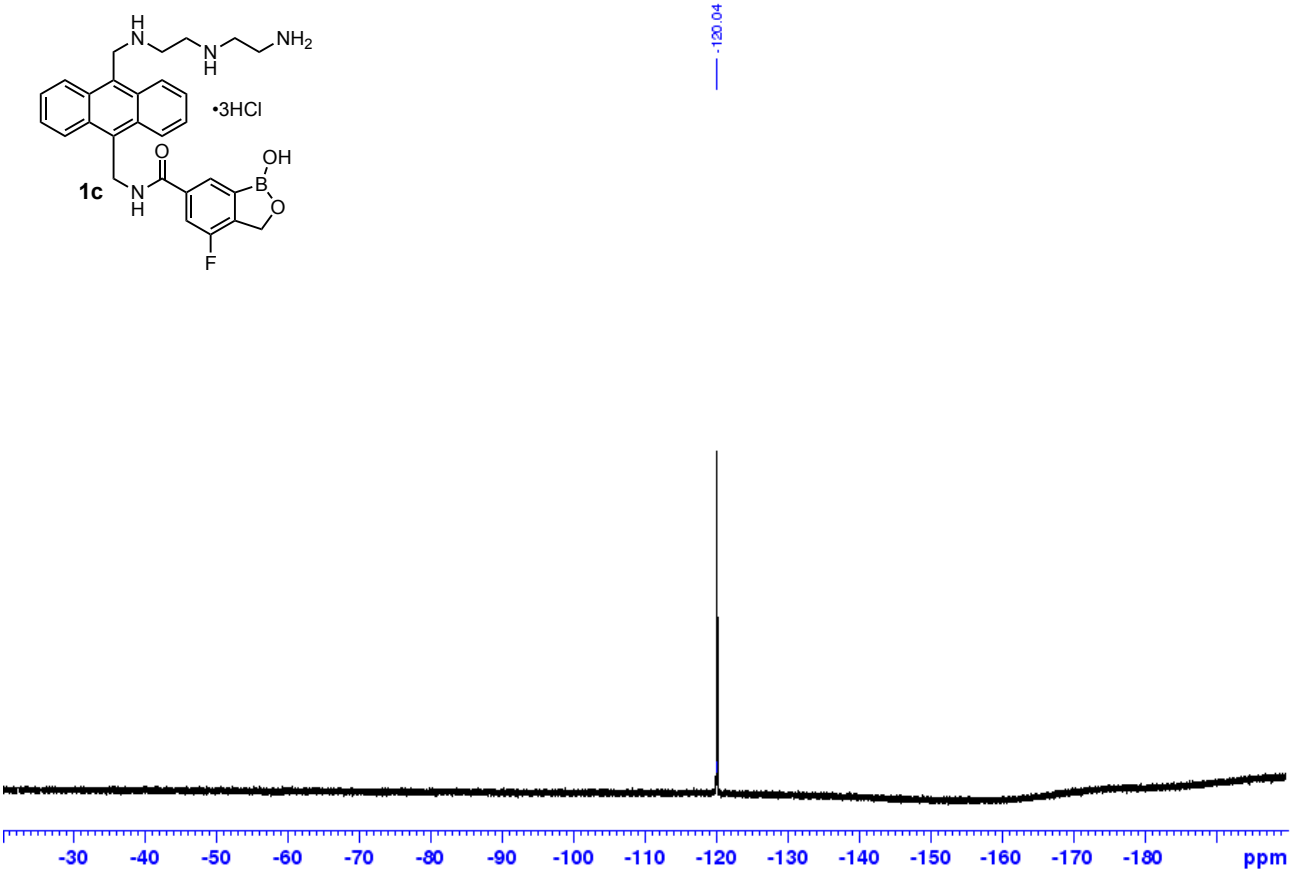
1c: ^{1}H NMR (400 MHz, D_2O)



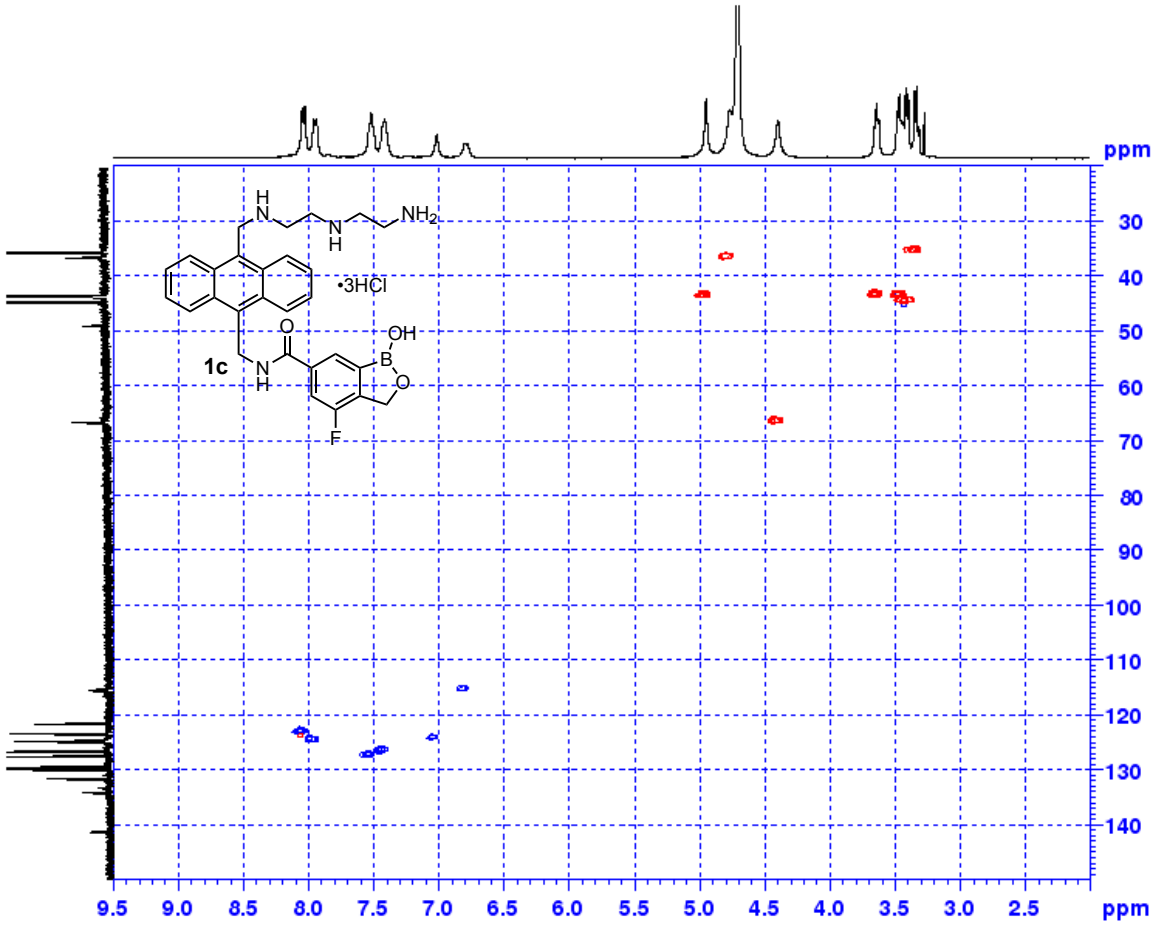
1c: ^{13}C NMR (100 MHz, D_2O)



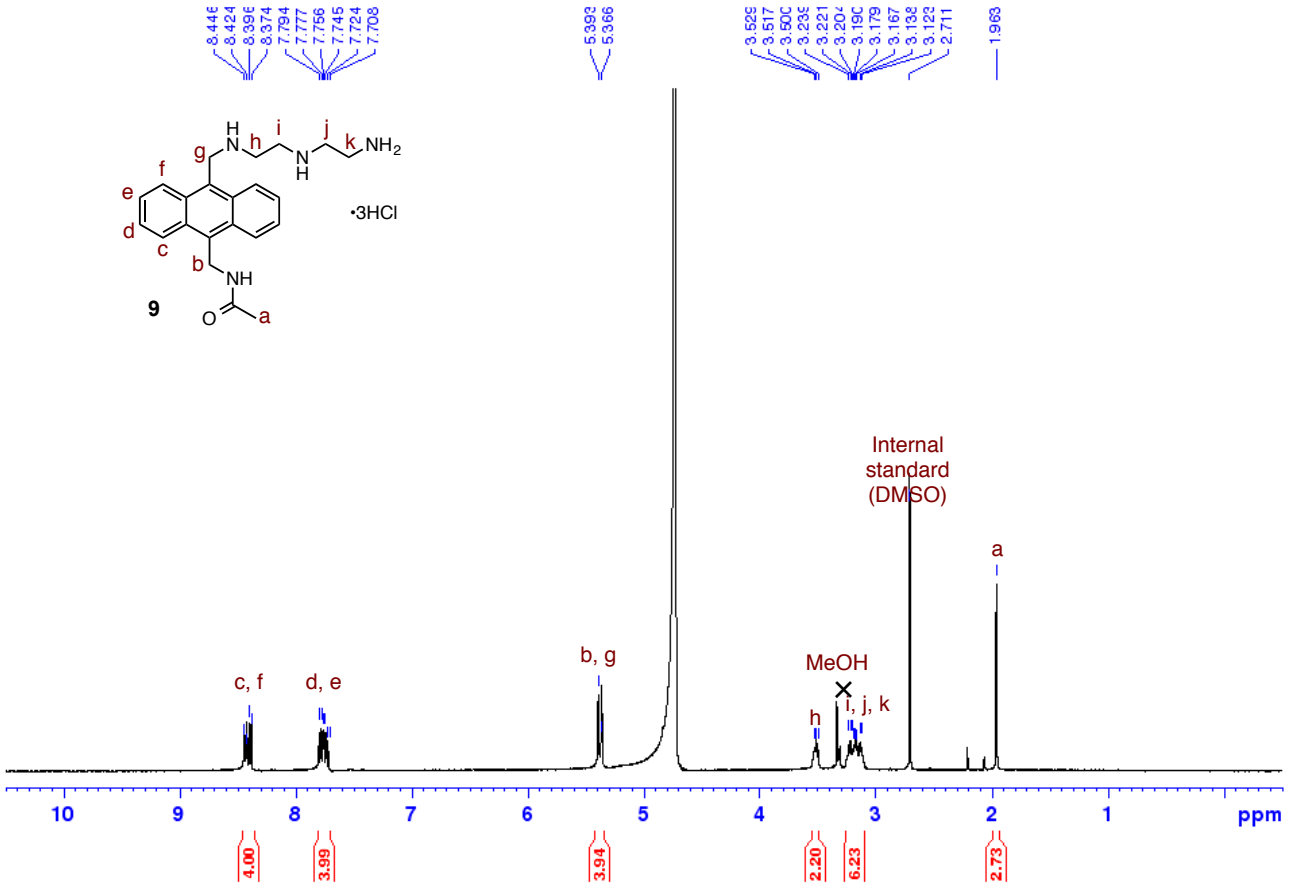
1c: ^{19}F NMR (376 MHz, D_2O)



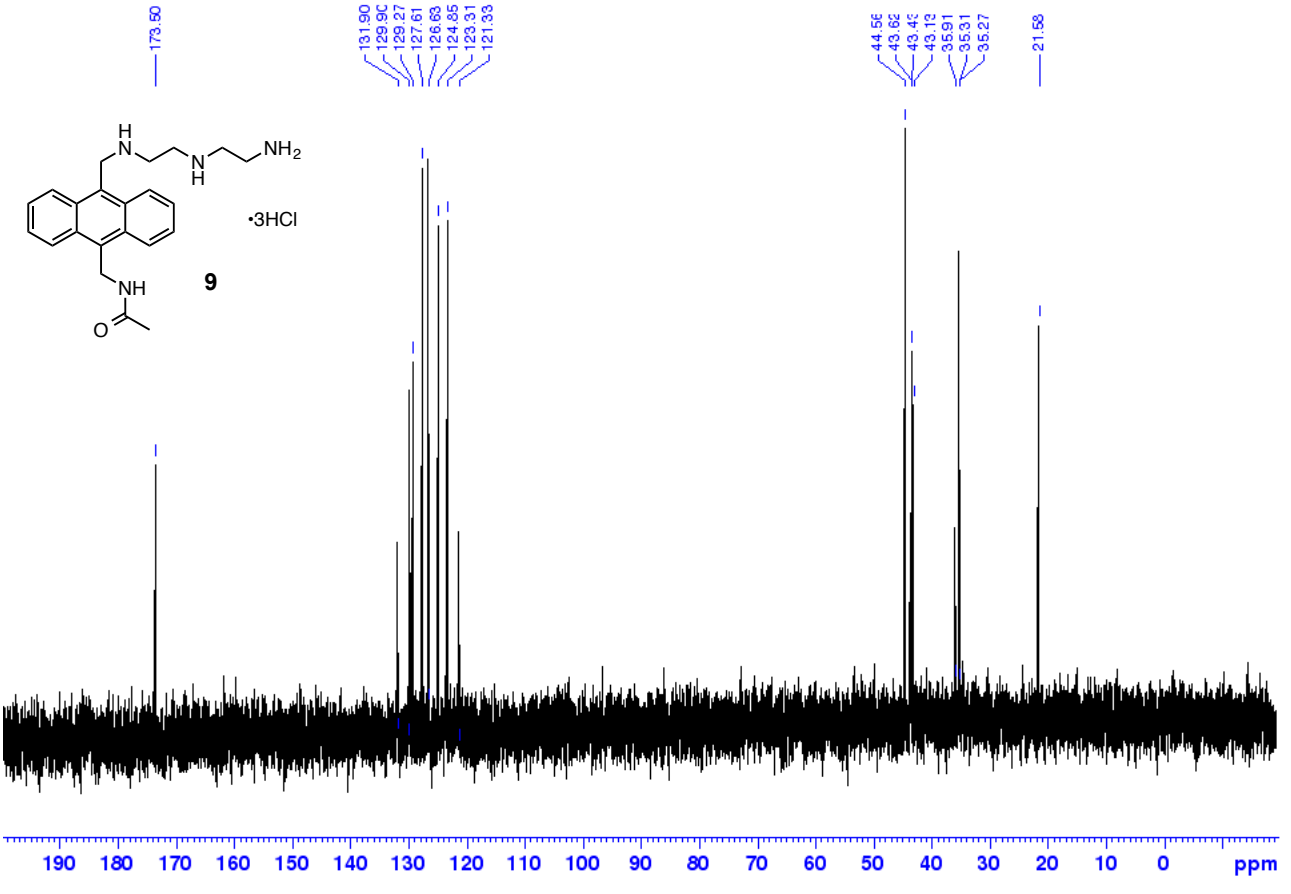
HSQC spectrum of **1c** (D_2O)



9: ^{1}H NMR (400 MHz, D_2O)



9: ^{13}C NMR (100 MHz, D_2O)



HSQC spectrum of **9** (D_2O)