Modular design for fluorophore homodimer probes using diethylentriamine as a common spacer

Shuhei Kusano*, Keisuke Matsumoto, and Osamu Hayashida*

*e-mail: skusano@fukuoka-u.ac.jp
*e-mail: hayashida@fukuoka-u.ac.jp

Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma 8-19-1, Fukuoka 814-0180, Japan

Supporting Information

Table of Contents

I. General

The ^{1}H and ^{13}C NMR were recorded on a Bruker 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) 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), DMSO-d_6 (^{1}H, 2.50 ppm), CD_3OD (^{1}H, 3.31 ppm), $CDCl_3$ (^{13}C, 77.2 ppm), DMSO-d_6 (^{13}C, 39.5 ppm), and CD_3OD (^{13}C, 49.0 ppm). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, doublet of doublets (dd), doublet of triplet (dt). Spectra were processed by Bruker Top-spin.

High resolution mass analyses (HRSM) were 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}. Melting temperature of DNA was recoded on BECKMAN COULTER DU800.

Thin-layer chromatography was performed on Merck 60 F254 precoated 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.).

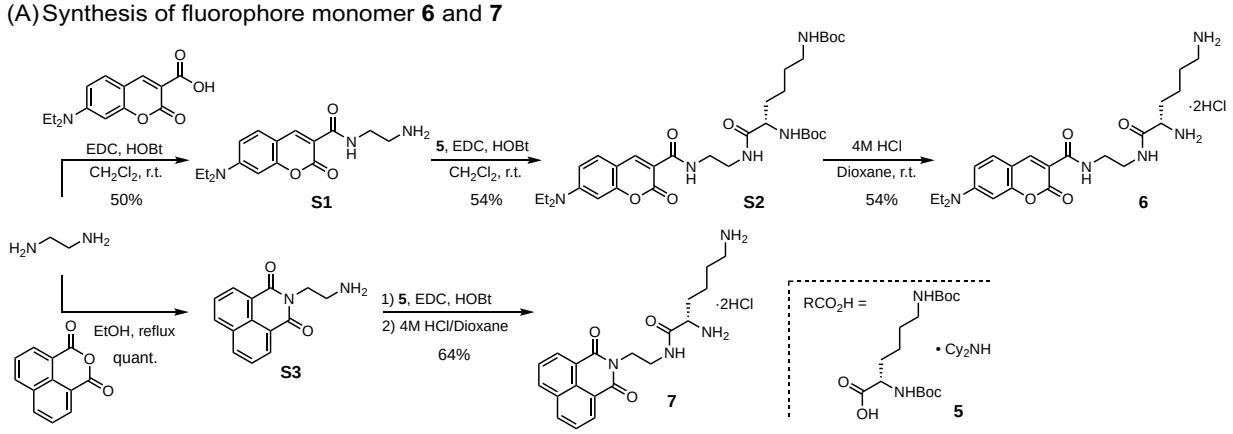
Commercially available reagents were obtained from Tokyo Kasei, Wako Pure Chemical Industries Ltd., KANTO CHEMICAL CO., INC. and Nacalai tesque, and used without further purification. Calf thymus DNA was obtained from TREVIGEN. Bovine serum albumin, lysozyme, trypsin, heparin, and chondroitine were obtained from Nacalai tesque. Hyaruonic acid was obtained from Wako Pure Chemical Industries Ltd. Oligo DNA was purchased from Bio Service CO., LTD. (Saitama, JAPAN).

II. Synthesis of control compounds 6–8

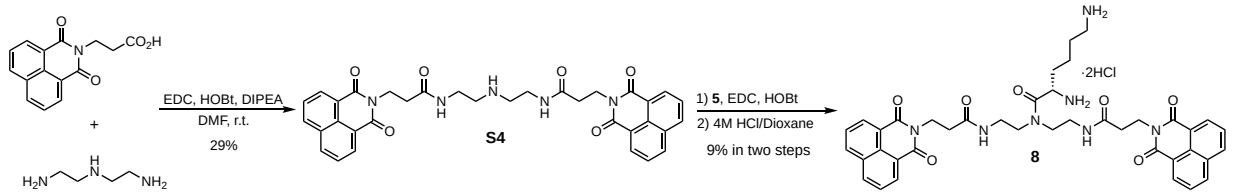
Control compounds **6**–**8** were synthesized as summarized in **Scheme S1**. Synthetic detail was described in the section V: Synthetic procedure.

Scheme S1. Synthesis of control compounds **6**–**8**.

(A) Synthesis of fluorophore monomer **6** and **7**



(B) Synthesis of long naphthalimide dimer **8**



III. Analysis of aqueous solubility

The aqueous solubility of **1**, **2** and **6-8** was analyzed by UV-Vis spectroscopy. All UV-Vis spectra were measured in pH 7.4, 10 mM HEPES, 150 mM NaCl, 25 ºC. Good linear relationships were observed between the concentration (0 to 20 μM) and absorbance.

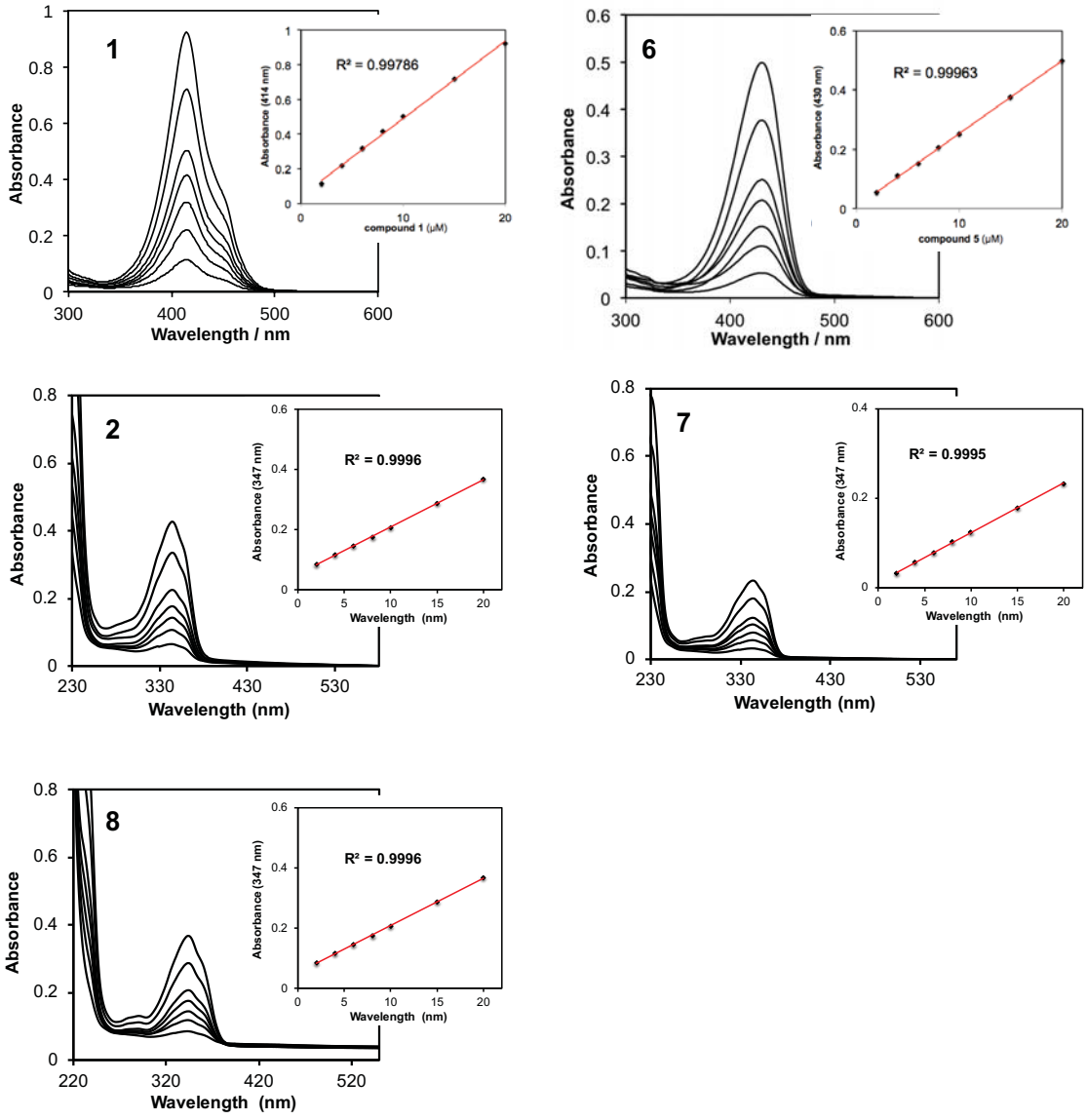
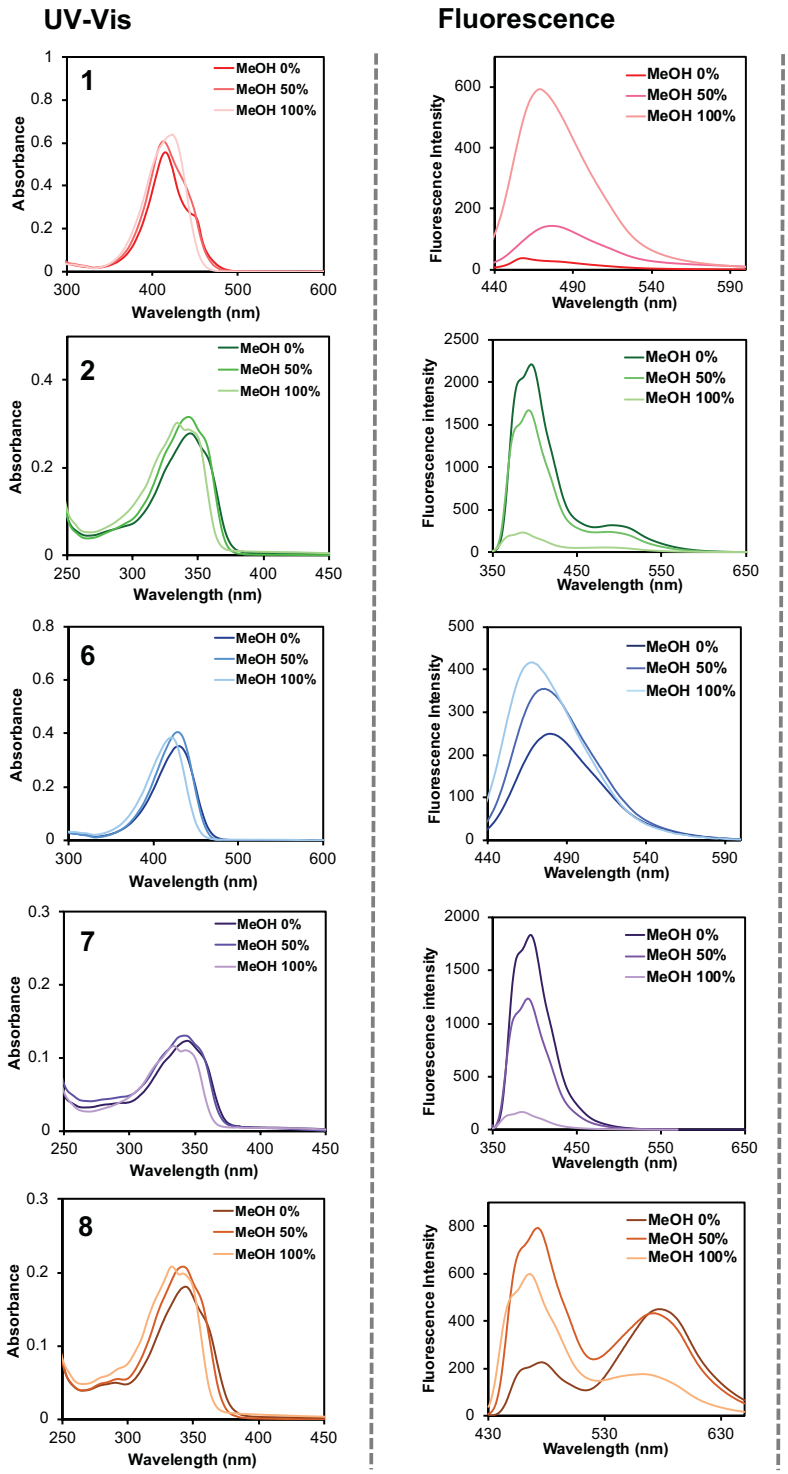


Fig. S1. UV-Vis spectra of probes and a plot of absorbance against the concentration of probe

IV. UV-Vis and fluorescence spectra of 1 and 5 in MeOH/H_2O and their quantum yields

UV-Vis and fluorescence spectra for all compounds were measured in the indicated ration of MeOH/H_2O at 25 ºC. The relative quantum yields of **1** and **6** in H_2O and MeOH were determined according to the following equation (1). The quantum yield of fluorescein (Φ = 0.97) in EtOH was used as a standard sample.



Quantum yield

$$\Phi_x = \Phi_{st} \cdot \frac{F_x}{F_{st}} \cdot \frac{A_{st}}{A_x} \cdot \left(\frac{n_x}{n_{st}}\right)^2 \quad (1)$$

- Φ_x and Φ_{st} are the fluorescence quantum yield of the probe and standard sample, respectively.
- F_x and F_{st} are the integrated value of the fluorescence spectra of the probe and standard sample, respectively.
- A_x and A_{st} are the absorbance of the probe and standard sample, respectively.
- n_x and n_{st} are the refractive index of the fluorescence spectra of the probe and standard sample, respectively.

	$\Phi_{(H2O)}$	$\Phi_{(MeOH)}$
1	< 0.01	0.33
6	0.10	0.26

Fig. S2. (A) UV-Vis and (B) fluorescence spectra of **1**, **2** and **6-8** in various ratio of MeOH/H_2O solution. [**probe**] = 10 μM for UV-Vis spectroscopy, [**probe**] = 5 μM for fluorescence spectroscopy. λex = 414 nm for **1**, λex = 344 nm for **2**, **7**, and **8**, λex = 430 nm for **6**. (C) Quantum yield of **1** and **6**.

V. ^{1}H NMR analysis for coumarin derivatives 1 and 6 in CD_3OD/D_2O

To validate the proposed dynamic conformational change of **1** depending on the surrounding polarity, we measured ^{1}H NMR spectra of **1** and **6** in various CD_3OD/D_2O ratios and compared their NMR spectra. In D_2O, the proton signals corresponding to coumairn moieties of **1** were observed in higher field relative to **6** because of π-π stacking in **1** (**Fig. S3**). Those proton signals of **1** were shifted into lower field when increasing CD_3OD content while **6** exhibiting no significant change, and eventually showed close chemical shift with **6** in 75% CD_3OD/D_2O. These NMR study strongly supported that the H-type dimer of **1** formed in aqueous media was eliminated in hydrophobic condition as observed in the fluorescence spectroscopy.

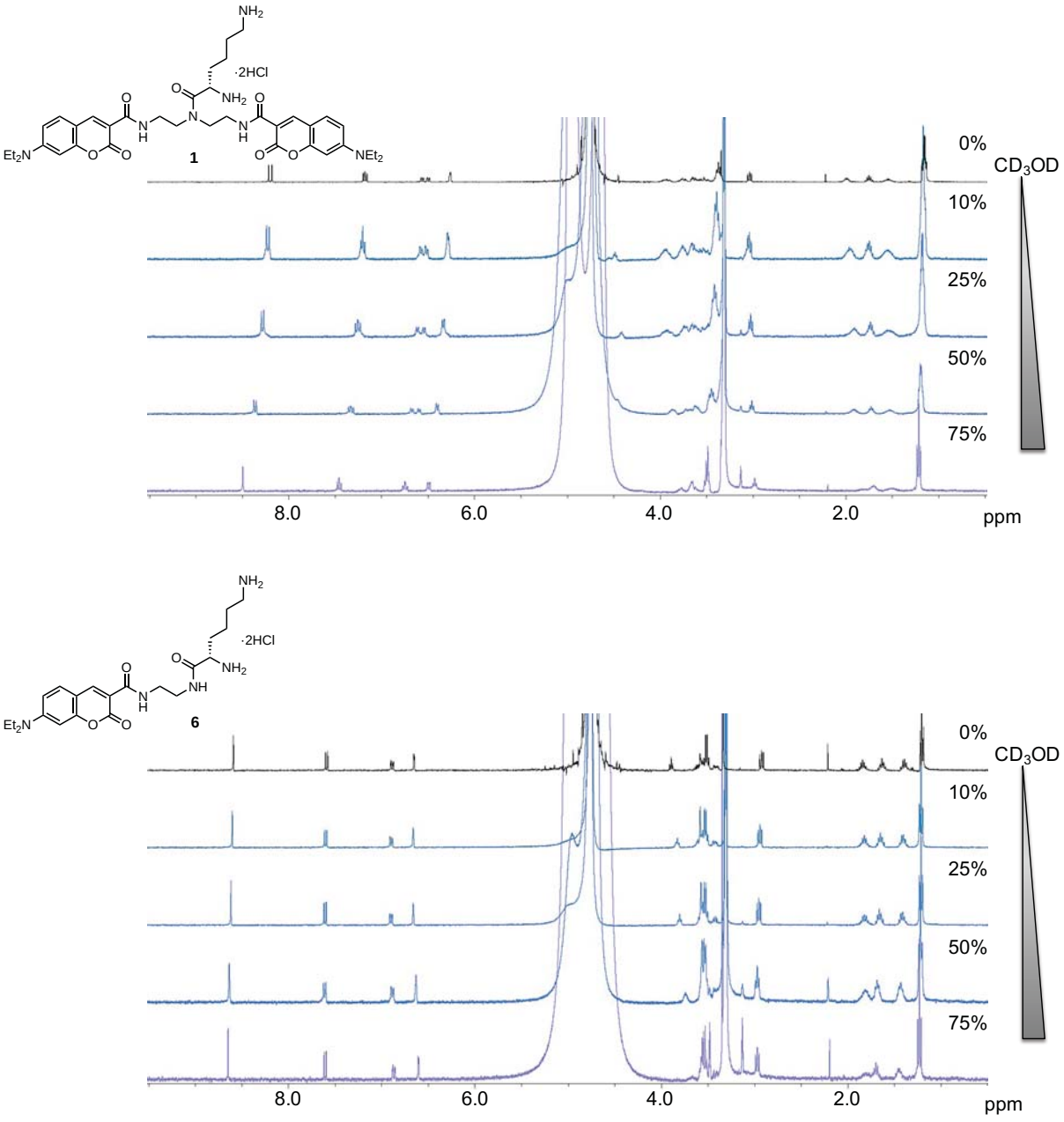


Fig. S3. ^{1}H NMR spectra of **1** (top) and **6** (bottom) in various CD_3OD/D_2O ratios at 25 ºC. [**1**] and [**6**] = 0.50 mM in 0 to 50% CD_3OD, [**1**] and [**6**] = 0.25 mM in 75% CD_3OD

VI. ^{1}H NMR analysis for naphthalimide derivatives 2, 7, and 8 in CD_3OD/D_2O

^{1}H NMR of naphthalimde dimers **2** was also measured in various ratio of CD_3OD/D_2O. Similar to coumarin homodimer **1**, the higher field shift of naphthalimide moiety in D_2O was canceled by increasing the content of CD_3OD, indicating the π-π stacking of their napthalimide rings in aqueous media. Again, ^{1}H signals of naphthalimide moiety of long dimer **8** was observed in higher field rather than those of short dimer **2**. This was probably originated from the subtle difference of the direction or the distance between two naphtalimide rings of **2** and **8** thereby leading their distinct fluorescence characteristic (**Fig. 2**).

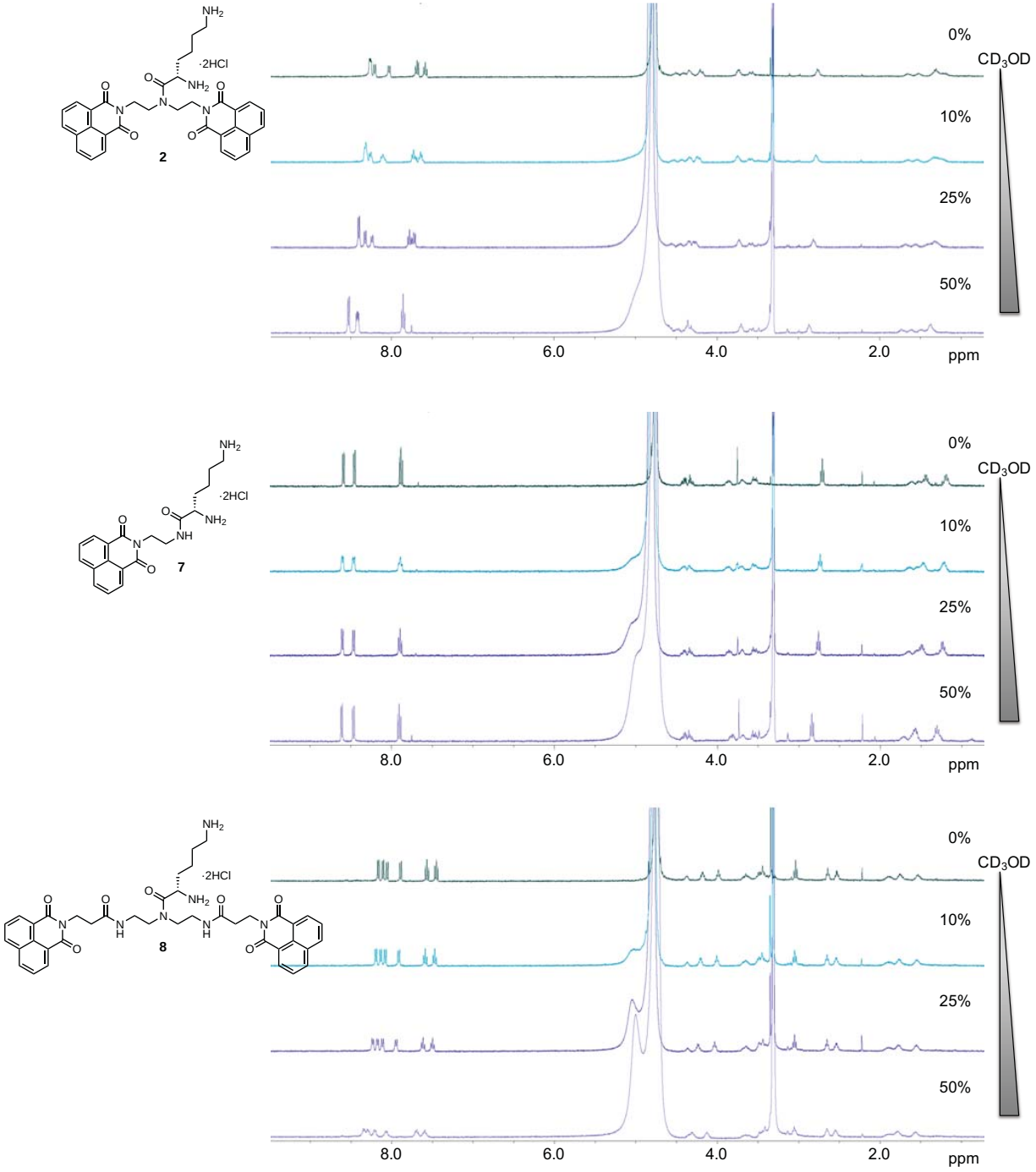


Fig. S4. ^{1}H NMR spectra of **2** (top), **7** (middle), and **8** (bottom) in various CD_3OD/D_2O ratios at 25 ºC, [**probe**] = 0.50 mM.

VII. Fluorescent titration study of 1 and 6 for ctDNA and oligo DNA

Fluorescence spectra of **1** and **6** were measured upon the addition of ctDNA or oligo DNA at pH 7.4, 10 mM HEPES, 150 mM NaCl, 25 ºC. The titration results of ctDNA were fitted with program software 'Titration fit'. The concentration of oligo DNA was calculated from the absorbance at 260 nm using its extinct molar ratio.

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

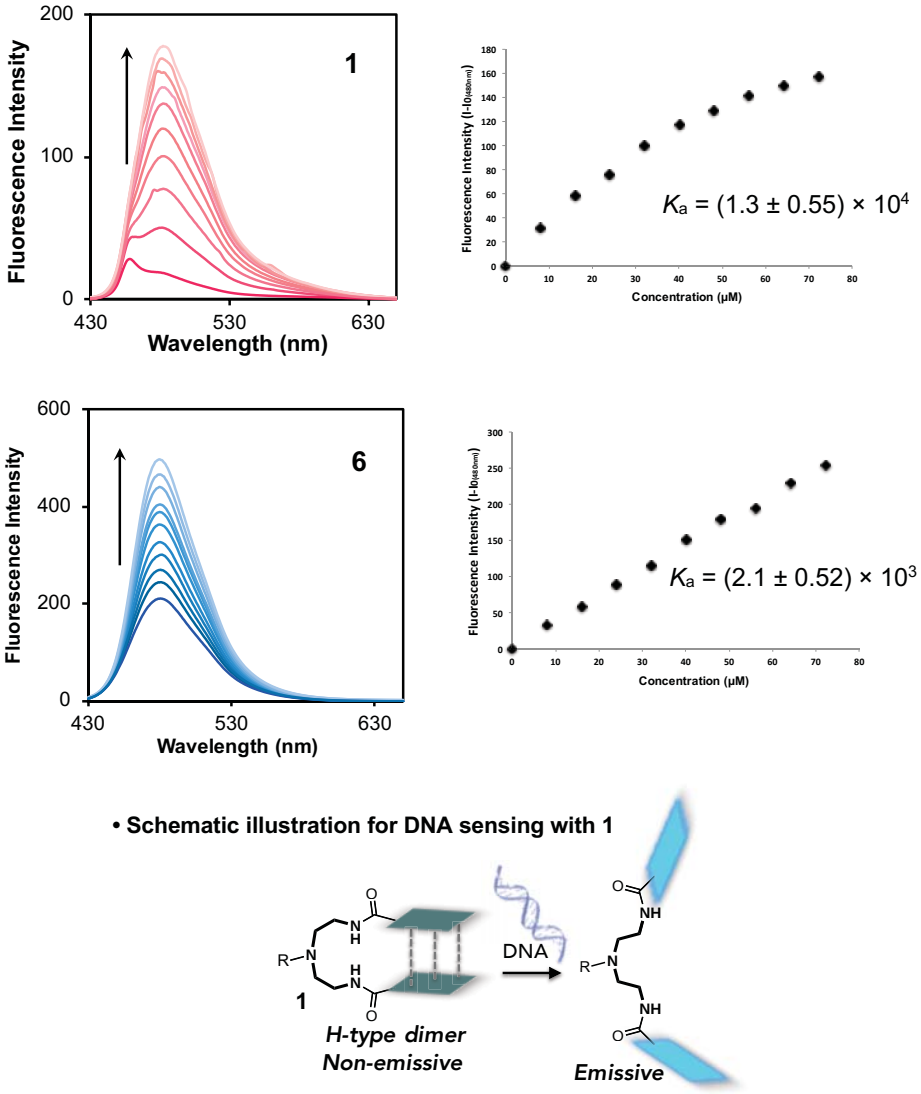


Fig. S5. Fluorescence spectra of **1** (left) and **6** (right) upon the addition of ctDNA (0 to 72 μM). All fluorescence spectra were measured at pH 7.4, 10 mM HEPES, 100 mM NaCl, 25 ºC

VIII. T_m analysis for probe-oligo DNA complexes

All samples for T_m experiments were consisted of 100 mM NaCl, 50 mM HEPES (pH 7.0), [**probe**] = 10 μM, and [**duplex**] = 1 μM. All measurements were performed with temperature controller. Both heating and cooling curves were measured over a temperature range of 25 ºC to 80 ºC at 0.5 ºC/min in three times. The absorbance at 260 nm was recorded at every 0.5 ºC.

Table S1. T_m analysis for oligo DNA in the presence or absence of **1** and **6**.

	T_m (ºC)
w/o	64.7±0.0
1	64.7±0.05
6	64.7±0.05

The sequence of oligo DNA

Oligo DNA 5′-CAG CGA TAA TTC GCG AGT-3′
3′-GTC GCT ATT AAG CGC TCA-5′

IX. Fluorescence sensing of anionic analytes with 7 and 8

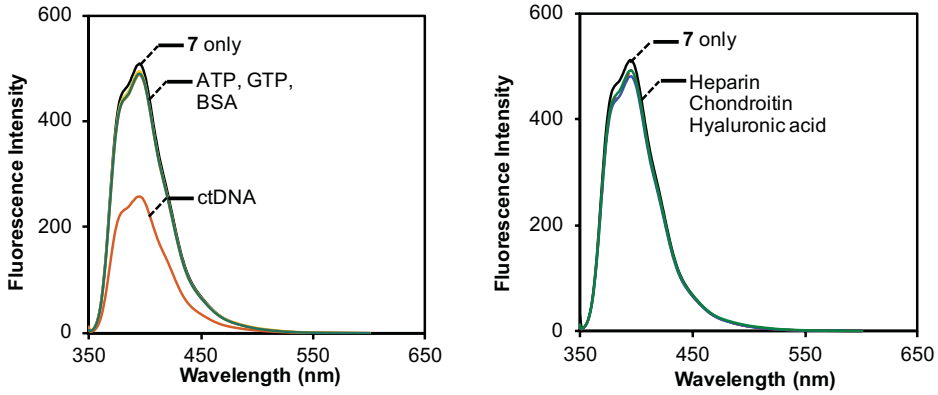


Fig. S6. Fluorescence spectra of **7** in the presence or absence of analyte (Left: for ATP, GTP, BSA, ctDNA, Right: heparin, chondroitin, hyaluronic acid). All fluorescence spectra were measured in pH 7.4, 10 mM HEPES, 100 mM NaCl at 25 ºC, [**7**] = 5 μM, [analyte] = 100 μM, [Heparin] = 3.8 μg/mL.

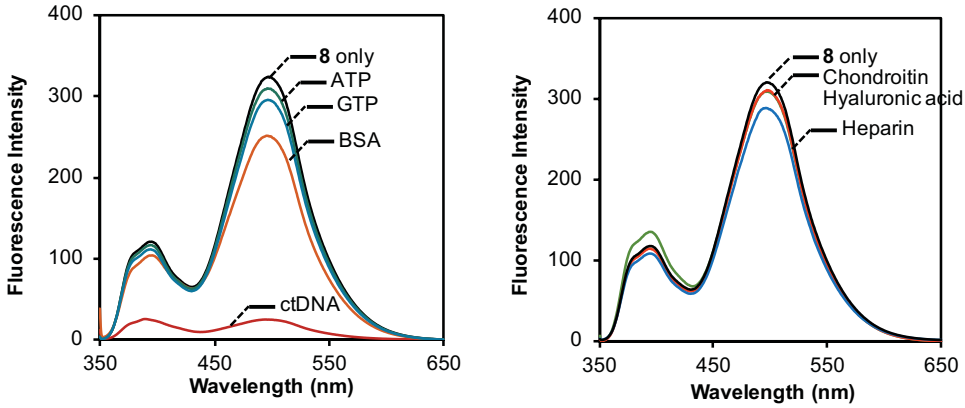
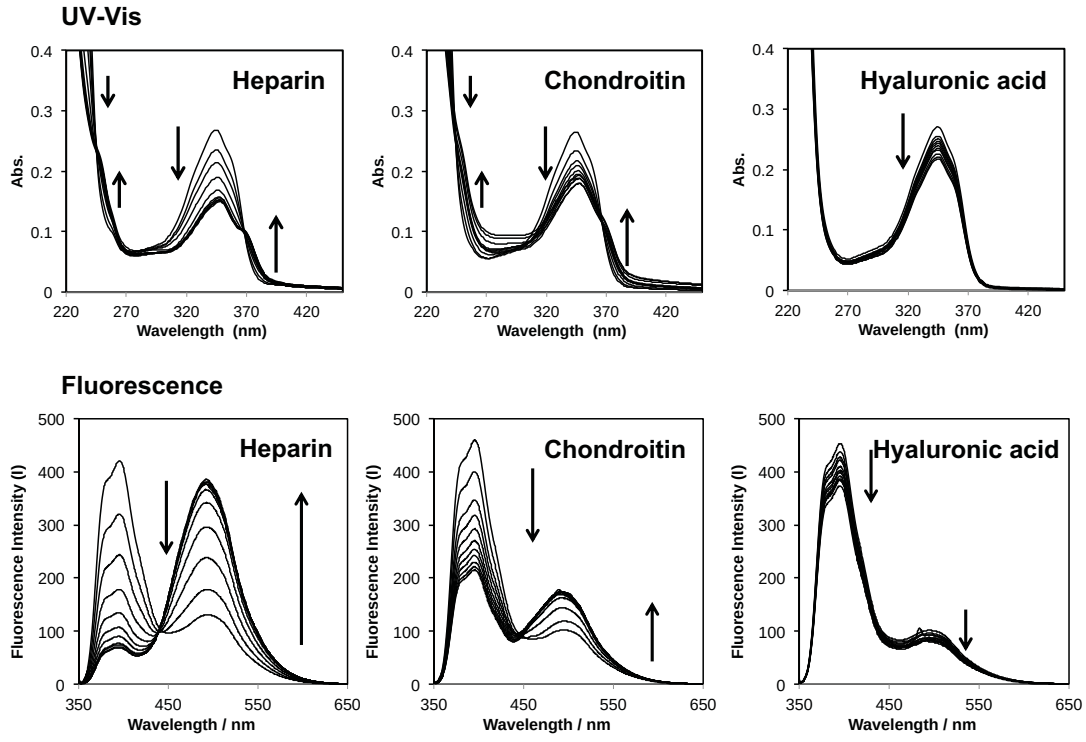


Fig. S7. Fluorescence spectra of **8** in the presence or absence of analyte (Left: for ATP, GTP, BSA, ctDNA, Right: heparin, chondroitin, hyaluronic acid). All fluorescence spectra were measured in pH 7.4, 10 mM HEPES, 100 mM NaCl at 25 ºC, [**8**] = 5 μM, [analyte] = 100 μM, [Heparin] = 3.8 μg/mL.

X. Polyanionic glycan sensing with naphthalimide dimer 2

- **Titration study of 2 for anionic glycans**



- **Schematic illustration for Heparin sensing with 2**

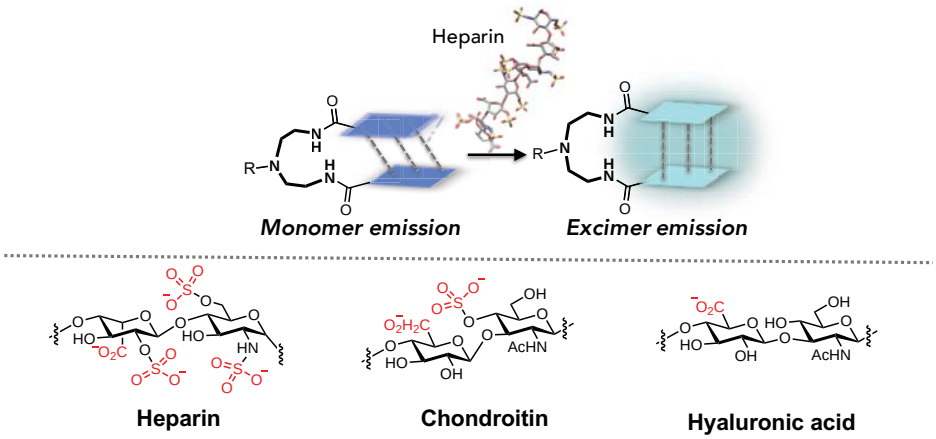


Fig. S8. UV-Vis (top) and fluorescence (bottom) titration study of **2** for anionic glycans. All UV-Vis and fluorescence spectra were measured in pH 7.4, 10 mM HEPES, 100 mM NaCl at 25 ºC. UV-Vis: [**2**] = 10 μM, [glycan] = 0, 0.75, 1.5, 2.25, 3.0, 3.75, 4.50, 5.25, 6.0, 6.75, 7.5 μg/mL. Fluorescence: [**2**] = 5 μM, [glycan] = 0, 0.38, 0.76. 1.14, 1.52, 1.90, 2.28, 2.66, 3.04, 3.42, 3.80 μg/mL.

• Concentration dependency of heparin sensing

In the concentration range of **2** from 2.5 to 10 μM, the ratiometric response for heparin was confirmed. Additionally, naphthalimide monomer **7** showed no excimer emission even over 10 μM of probe concentration (data not shown), strongly suggestive of the intramolecular excimer emission of **2**.

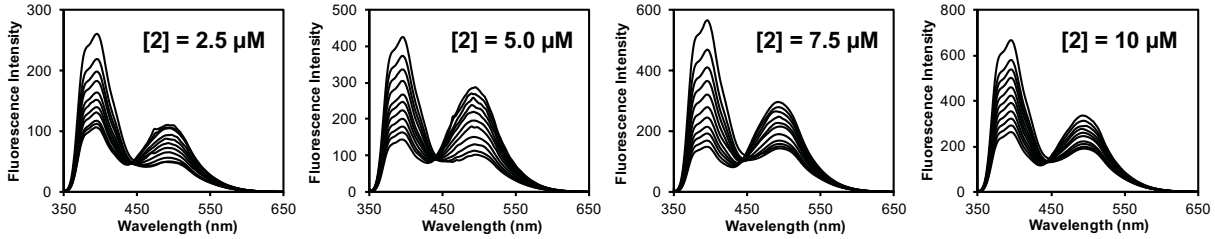


Fig. S9. Concentration dependency of heparin fluorescence sensing with 2. All spectra were measured at pH 7.4, 10 mM HEPES, 150 mM NaCl at 25 ºC, [**2**] = 5 μM, [Heparin] = 0, 0,15, 0,30, 0,45, 0,60, 0,75, 0,90, 1,05, 1,20, 1,30, 1,50 μg/mL.

• pH dependency

The increased monomer emission in acidic condition was likely due to the cancellation of phot-induced electron transfer quenching through the protonation on amino groups.[1)]

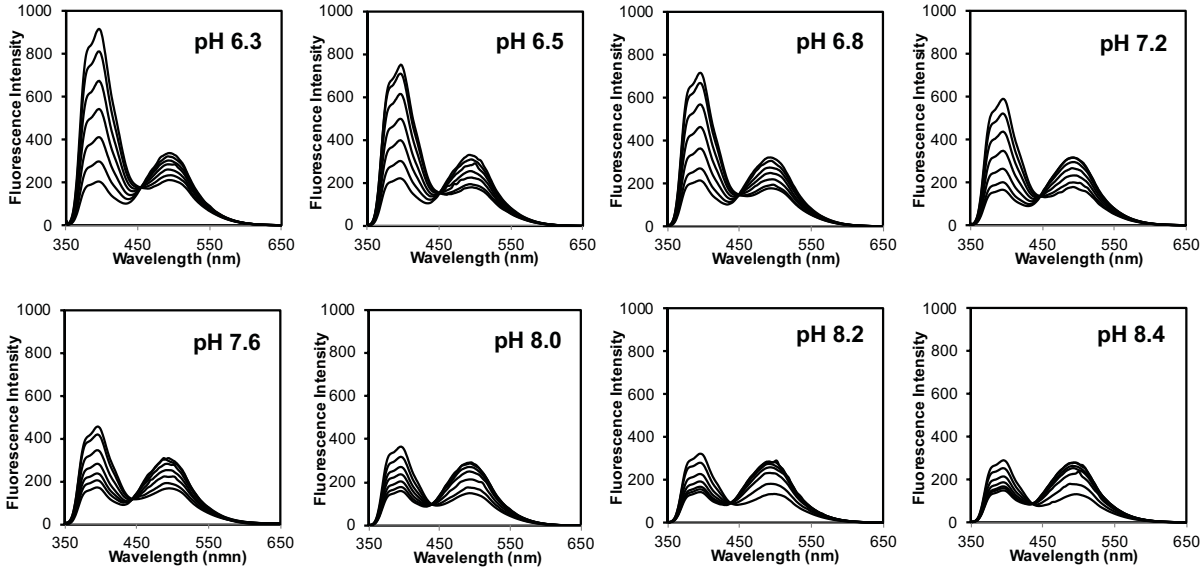


Fig. S10. pH dependency of heparin fluorescence sensing with **2**. All spectra were measured in the indicated of 50 mM phosphate buffer, 100 mM NaCl at 25 ºC, [**2**] = 5 μM, [Heparin] = 0, 0.38, 0.75, 1.13, 1.50, 1.88, 2.25 μg/mL.

• Lower detection limit

The lower detection limit was determined as (16 ± 0.8) ng/mL (0.023 U/mL) by following the equation; X = 3σ/m ('σ' is the ratio of signal and noise determined by the standard deviation of blank measurements (n = 10), 'm' is the slope of linear equation). Three separate experiments were carried out.

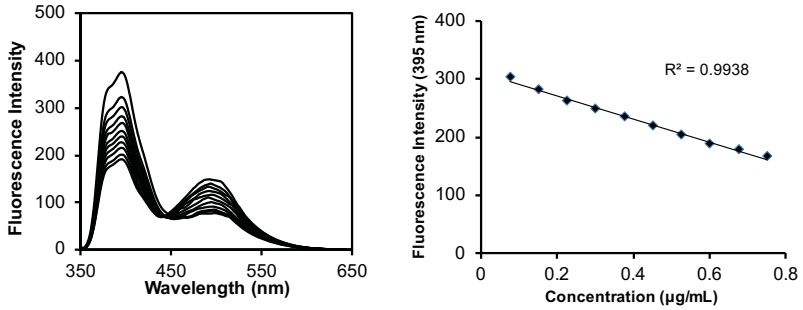


Fig. S11. Fluorescence titration of 2 for heparin. All spectra were measured in pH 7.4, 10 mM HEPES, 100 mM NaCl at 25 ºC, [**2**] = 5 μM, [Heparin] = 0, 0.075, 0.15, 0.225, 0.30, 0.375, 0.45, 0.525, 0.60, 0.675, 0.75 μg/mL.

XI. Ratiometric monitoring for the complex formation of heparin and protamine

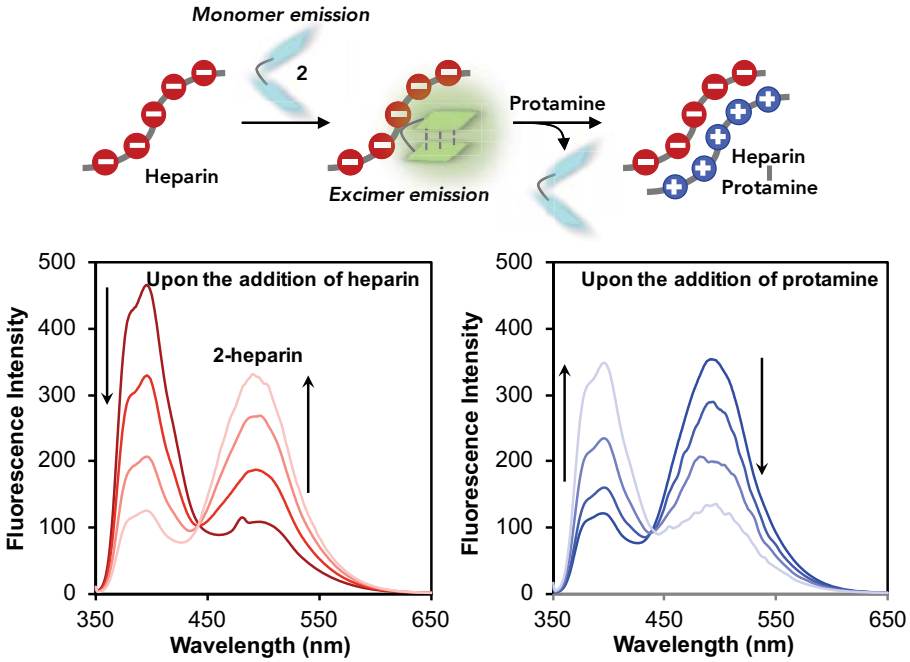
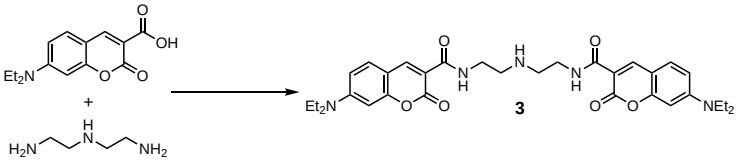


Fig. S12. Ratiometric monitoring for the complex formation of heparin and protamine. All spectra were measured at pH 7.4, 10 mM HEPES, 100 mM NaCl at 25 ºC, [**2**] = 5 μM, [Heparin] = 0, 0.75, 1.50, 2.25 μg/mL. Toward the complex of **2** (5 μM) and heparin (2.25 μg/mL), protamine (0, 30, 60, 150 μg/mL) was added.

XII. Synthetic procedure

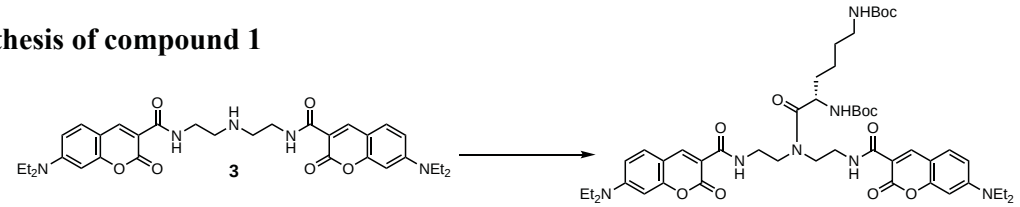
Compound **4** and **S3** were synthesized by following the reported procedure[1a, 2], and their structure were confirmed by ^{1}H NMR spectroscopy. For analytical use, all products were purified by recrystallization.

• Synthesis of compound 3



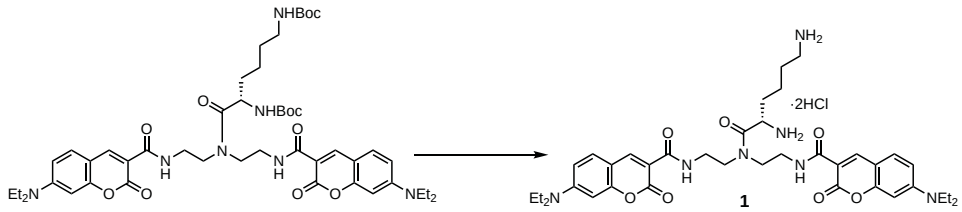
EDC (980 mg, 5.11 mmol) and HOBt (690 mg, 5.11 mmol) were added to a solution of 7-(diethylamino)coumarin-3-carboxylic acid (1.33 g, 5.09 mmol) in CH_2Cl_2 (30 mL). After 5 minutes, diethylenetriamine (323 μL, 2,98 mmol) was then added to the mixture. After stirring for 16 h at room temperature, the mixture was concentrated. The residue was applied for silica gel column chromatography (MeOH/$CHCl_3$, 1:10) to afford **1** (1.02 g, 57%) as a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, *J* = 7.2 Hz, 12H), 2.91 (t, *J* = 6.0 Hz 4H), 3.44 (q, *J* = 7.2 Hz, 8H), 3.47 (dt, *J* = 6.0, 6.0 Hz, 4H), 6.47 (d, *J* = 2.4 Hz, 2H), 6.62 (dd, *J* = 2.4 Hz 9.0 Hz, 2H), 7.39 (d, *J* = 9.0 Hz, 2H), 8.67 (s, 2H), 8.95 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 39.8, 45.2 48.7, 96.8, 108.5, 110.0, 110.7, 131.2, 148.1, 152.6, 157.75, 162.7, 163.5; IR (FT-ATR, cm^{-1}) 3320, 2925, 1692, 1616, 1582, 1506, 1415, 1349, 1190, 1133; HRMS-ESI: calculated for $[C_{32}H_{39}N_5O_6 + H]^+$ requires m/z = 590.2979, found 590.2982.

• Synthesis of compound 1



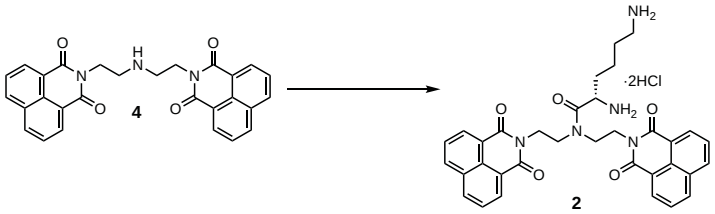
EDC (195 mg, 1.02 mmol) and HOBt (137 mg, 1.02 mmol) were added to a solution of N^{α}, N^{ε}-bis(tert-butoxycarbonyl)-L-lysine **5** (491 mg, 0.930 mmol) in CH_2Cl_2 (10 mL). After 5 minutes, **1** (550mg, 0.930 mmol) was then added to the mixture. After stirring for 15 hours at room temperature, the mixture was diluted with $CHCl_3$ and washed with $NaHCO_3$ and H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was applied for silica gel column chromatography (MeOH/$CHCl_3$ 1:20) to afford a condensation product (761 mg, 98%) as a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, *J* = 7.2 Hz, 12H), 1.35-1.60 (m, 23H), 1.72-1.73 (m, 1H), 3.07-3.09 (m, 4H), 3.36-3.46 (m, 10H), 3.56-3.71 (m, 6H), 3.87-3.92 (m, 2H), 4.61-4.62 (m, 1H), 4.97 (brs, 1H), 5.32-5.24 (m, 1H), 6.46-6.47 (m, 2H), 6.63 (dd, *J* = 2.3 Hz 9.0 Hz, 2H), 7.39-7.41 (m, 2H), 8.65 (s, 2H), 8.89 (t, *J* = 5.7 Hz, 1H), 8.95 (brs, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 12.5, 22.7, 28.4, 28.6, 29.4, 33.6, 37.6, 38.7, 40.4, 45.2, 46.1, 47.6, 50.2, 77.4, 78.8, 79.4, 96.71, 97.74, 108.5, 108.6, 110.0, 110.3, 131.3, 131.4, 148.2, 148.4, 152.7, 155.6, 156.2, 157.8, 162.7, 162.8, 163.7, 163.9, 173.3; IR (FT-ATR,

cm^{-1}) 3327, 2978, 1688, 1615, 1581, 1510, 1354, 1135; MS-ESI: calculated for $[C_{48}H_{67}N_7O_{11} + Na]^+$ requires m/z = 940.48, found 940.47.



The resulting condensation product (60 mg, 65.4 μmol) was suspended in 4 M HCl/dioxane (1 mL) and stirred for 30 minutes at room temperature. The mixture was concentrated, and the resulting product was recrystallized from EtOH to afford **3** (29.2 mg, 56%) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ1.14 (t, J = 7.0, 12H), 1.42-1.74 (m, 6H), 2.76 (t, J = 7.3, 2H), 3.20-3.24 (m, 2H), 3.51-3.57 (m, 12H), 3.87-3.94 (m, 1H), 4.10-4.11 (m, 1H), 4.31-4.34 (m, 1H), 6.69 (d, 12H), 6.59 (s, 1H), 6.60 (s, 1H), 6.80 (d, J = 9.0, 2H), 7.67 (d, J = 9.1 Hz, 1H), 7.71 (d, J = 9.1 Hz, 1H), 8.27 (brs, 6H), 8.63 (s, 1H), 8.64 (s, 1H), 8.71 (t, J = 6.0, 1H), 8.83 (t, J = 6.0, 1H), ^{13}C NMR (100 MHz, DMSO-d_6); δ12.3, 21.0, 26.5, 30.1, 36.6, 37.7, 38.1, 44.3, 45.9, 48.6, 49.5, 95.8, 107.6, 107.7, 109.2, 109.2, 110.2, 131.7, 147.7, 147.8, 152.5, 157.2, 157.3, 161.6, 161.6, 162.5, 163.0, 169.2; IR (FT-ATR, cm^{-1}) 2971, 1691, 1614, 1504, 1416, 1350, 1186, 1130; HRMS-ESI: calculated for $[C_{38}H_{51}N_7O_7 + H]^+$ requires m/z = 718.3928, found 718.3913.

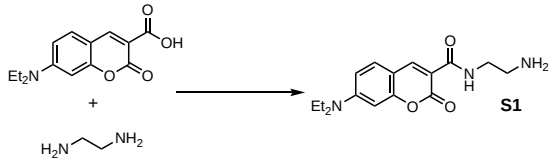
• Synthesis of compound 2



EDC (60 mg, 0.313 mmol) and HOBt (42.2 mg, 0.313 mmol) were added to a solution of N^{α}, N^{ε}-bis(tert-butoxycarbonyl)-L-lysine **5** (150 mg, 0.284 mmol) in DMF (4 mL). After five minutes, **4** (132 mg, 0.285 mmol) was added to the mixture. After stirring for 15 hours at room temperature, the mixture was diluted with $CHCl_3$ and washed with $NaHCO_3$ and H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was applied for silica gel chromatography (EtOAc/$CHCl_3$ 1:4) to give a condensation product. The resulting product was suspended in 4 M HCl/dioxane (2 mL) and stirred for 1 hour at room temperature. The mixture was concentrated, the residue was filtered and washed with $CHCl_3$ and EtOAc. The resulting product was further purified by recrystallization from MeOH and $CHCl_3$ to afford 2 (121 mg, 64%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16-1.23 (m, 1H), 1.31-1.42 (m, 3H), 1.54-1.64 (m, 2H), 2.60-2.67 (m, 2H), 3.59-3.64 (m, 2H), 4.17-4.26 (m, 3H), 4.36-4.56 (m, 3H), 7.89-7.93 (m, 4H), 8.01 (brs, 3H), 8.14 (brs, 3H), 8.47−8.55 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.4, 26.5, 29.9, 37.4, 37.8, 38.1, 43.2, 43.5, 49.7, 121.8, 121.4, 127.5,

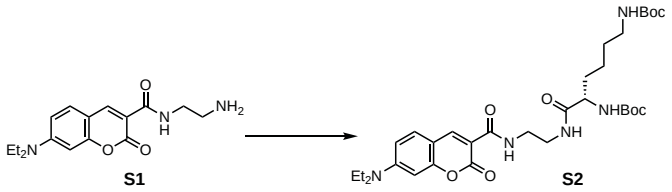
130.8, 131.3, 134.5, 134.5, 163.7, 169.6; IR (FT-ATR, cm^{-1}); 3710, 2973, 1692, 1652, 1585, 1345, 1234. 1055, 1033; HRMS-ESI: calculated for $[C_{34}H_{33}N_5O_5 + H]^+$ requires m/z = 592.2560, found 592.2548.

• Synthesis of compound S1



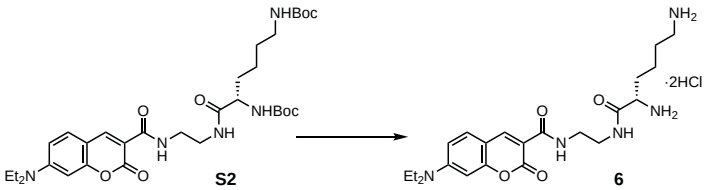
EDC (76.7 mg, 0.398 mmol) and HOBt (54.1 mg, 0.398 mmol) were added to a solution of 7-(diethylamino)coumarin-3-carboxylic acid (104 mg, 0.398 mmol) in CH_2Cl_2 (5 mL). After 5 minutes, ethylenediamine (80.1 μL, 1.19 mmol) was then added to the mixture. The mixture was stirred for 16 hours at room temperature. The mixture was concentrated, the residue was applied for silica gel column chromatography (MeOH/$CHCl_3$ 1:5) to afford **4** (60.6 mg, 50%) as a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, *J* = 7.2 Hz 6H), 3.05 (t, *J* = 5.6 Hz, 2H), 3.45 (dt, *J* = 7.2 Hz, 4H), 3.57-3.72 (m, 2H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.62 (dd, *J* = 2.4 Hz 8.8 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 8.67 (s, 1H), 9.02 (t, *J* = 5.6 Hz, 1H).

• Synthesis of compound S2



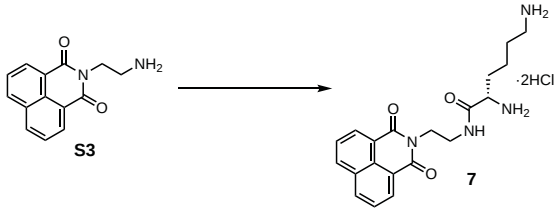
EDC (42.2 mg, 0.220 mmol) and HOBt (29.7 mg, 0.220 mmol) were added to a solution of N^{α}, N^{ε}-bis(tert-butoxycarbonyl)-L-lysine **5** (116 mg, 0.220 mmol) in CH_2Cl_2 (3 mL). After 5 minutes, **4** (60.6mg, 0.200 mmol) was then added to the mixture. After stirring for 19 hours at room temperature, the mixture was concentrated. The residue was applied for silica gel column chromatography (MeOH/$CHCl_3$ 1:30) to afford **5** (69 mg, 54%) as a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, *J* = 7.1 Hz, 6H), 1.34-1.50 (m, 2H), 1.41 (s, 18H), 1.57-1.66 (m, 1H), 1.78-1.87 (m, 2H), 3.07-3.09 (m, 2H), 3.42-3.46 (m, 6H), 3.58-3.61 (m, 2H), 4.08-4.09 (m, 1H), 4.73 (brs, 1H), 5.20 (brs, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.66 (d, *J* = 2.4, 9.0 Hz, 1H), 7.02 (brs, 1H), 7.44 (d, *J* = 9.0 Hz, 1H), 8.68 (s, 1H), 9.04 (d, *J* = 5.8 Hz, 1H);^{13}C NMR (100MHz, $CDCl_3$) δ 12.6, 22.7, 28.5, 28.6, 29.7, 32.8, 39.3, 40.2, 41.1, 45.2, 54.4, 96.7, 108.5, 109.8, 110.2, 131.4, 148.5, 152.9, 156.2, 157.9, 162.8, 164.8, 172.4; IR (FT-ATR, cm^{-1}) 2972, 1700, 1617, 1582, 1512, 1352, 1231, 1166; HRMS-ESI: calculated for $[C_{32}H_{49}N_5O_8 + Na^+$ requires m/z = 654.3479, found 654.3508.

• **Synthesis of compound 6**



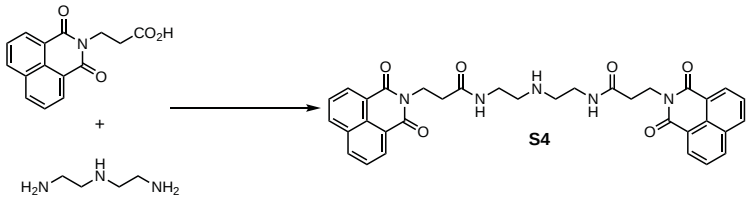
5 (44.7 mg, 70.8 µmol) in 4mM dioxane/HCl (1mL) was stirred for 30 minutes at room temperature. The mixture was concentrated, the residue was filtered by $CHCl_3$ and AcOEt. The resulting solid was recrystallized from EtOAc and MeOH to afford **6** (18.7 mg, 61%) as a yellow solid; ^{1}H NMR (400 MHz, D_2O) δ 1.21 (t, *J* = 7.6 Hz, 6H), 1.38-1.46 (m, 2H), 1.62-1.70 (m, 2H), 1.82-1.91 (m, 2H), 2.94 (dd, *J* = 7.8, 7.8 Hz, 2H), 3.42-3.61 (m, 2H), 3.62-3.66 (m, 12H), 3.95 (dd, *J* = 6.7, 6.7 Hz, 1H), 6.64 (s, 1H), 6.89 (dd, *J* = 2.4, 9.1 Hz, 1H), 7.58 (d, *J* = 9.1 Hz 1H), 8.57 (s, 1H); ^{13}C NMR (100MHz, D_2O) δ 11.6, 21.3, 26.4, 30.4, 38.7, 38.8, 38.9, 45.0, 53.2, 96.0, 106.6, 108.0, 111.2, 131.8, 148.7, 153.8, 157.5, 163.9, 165.7, 169.9; IR (FT-ATR, cm^{-1}) 3317, 2867, 1684, 1659, 1610, 1573, 1508, 1425, 1350, 1228, 1191, 1134; HRMS-ESI: calculated for $[C_{22}H_{33}N_5O_4 + Na]^+$ requires m/z = 454.2430, found 454.2444

• **Synthesis of 7**



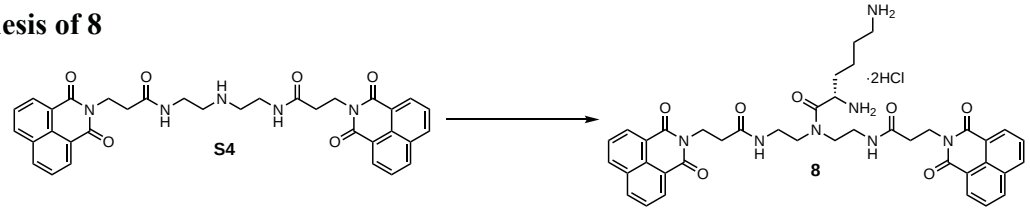
To a solution of N^{α}, N^{ε}-bis(tert-butoxycarbonyl)-L-lysine **5** (1.04 g, 2.9 mmol) in DMF (40 mL) was added EDC (548 mg, 2.9 mmol) and HOBt (387 mg, 2.9 mmol) and DIPEA (544 µL, 3.1 mmol). After five minutes, **6** (625 mg, 2.6 mmol) was added to mixture. After stirring for 15 h at room temperature, the mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was applied for silica gel chromatography (MeOH/$CHCl_3$ 1:80). The compound in 4 M HCl/dioxane (2.5 mL) was stirred for 1 h at room temperature. The mixture was concentrated, the residue was filtered by $CHCl_3$ and AcOEt. The resulting solid was recrystallized from EtOAc and MeOH to afford **7** (556 mg, 82%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29-1.32 (m, 2H), 1.51-1.58 (m, 3H), 1.64-1.73 (m, 1H), 2.66-2.74 (m, 2H), 3,28-3,35 (m, 2H, overlapped with solvent peak), 3.61-3.62 (m, 1H), 3.69-3.77 (m, 1H), 4.18-4.21 (m, 2H), 7.91 (dd, J = 8.2 Hz, 2H), 8.11 (brs, 3H), 8.22 (brs, 3H), 8.48 (dd, *J* = 1.0, 8.2 Hz, 2H), 8.51 (dd, *J* = 1.0, 8.2, 2H), 8.73 (t, *J* = 6.5, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ21.1, 26.3, 30.0, 36.5, 38.2, 51.9, 66.3, 122.1, 127.3, 127.5, 130.7, 131.3, 134.3, 163.7, 168.7; IR (FT-ATR, cm^{-1}) 3330, 2923, 2316, 1691, 1647, 15867, 1439, 1342, 1237, 1033; HRMS-ESI: calculated for $[C_{34}H_{33}N_5O_5 + Na]^+$ requires m/z = 391.1746, found 391.1757

• **Synthesis of S4**



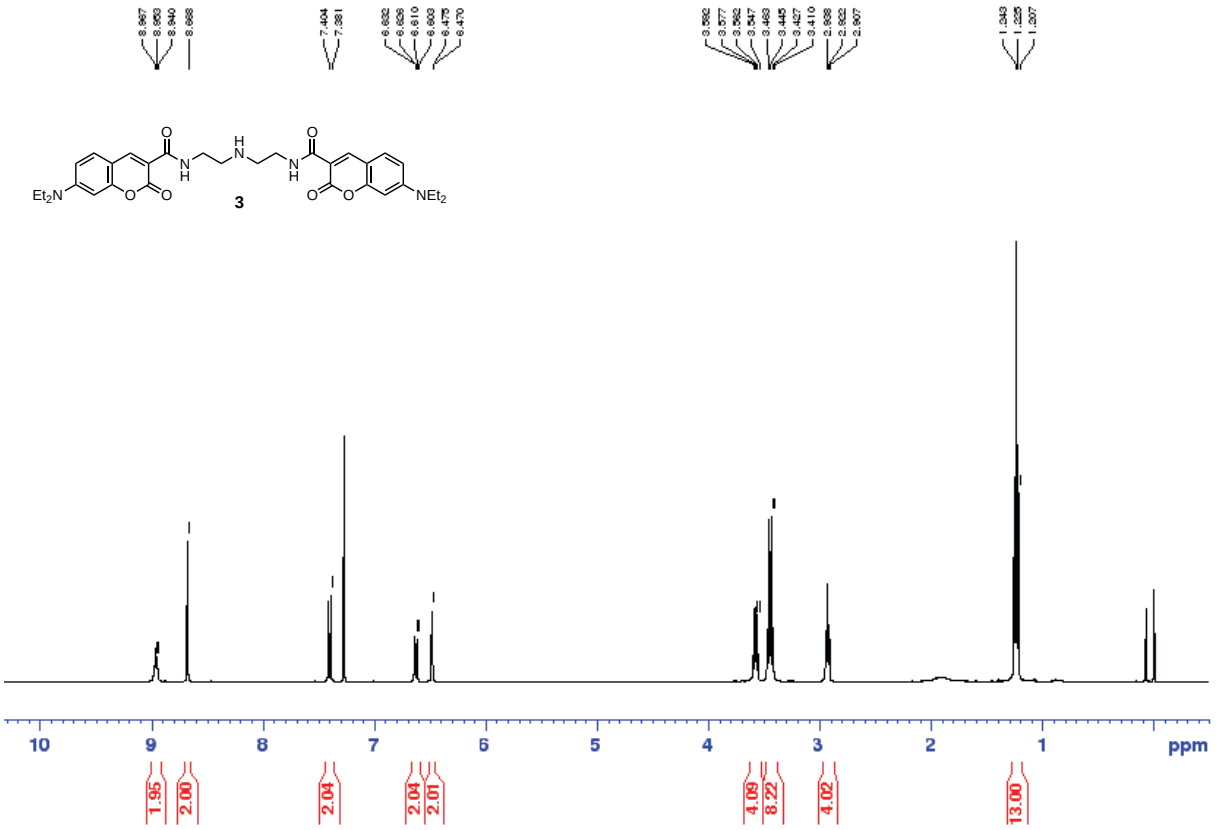
EDC (376 mg, 1.96 mmol), HOBt (265 mg, 1.96 mmol), and DIPEA (718 μL, 4.12 mmol) were added to a solution of naphthalimide derivative (528 mg, 1.96 mmol) in DMF (20 mL). After five minutes, diethylenetriamine (111 μL, 1.02 mmol) was added to the mixture. After stirring for 18 hours at room temperature, the mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was applied for silica gel column chromatography (MeOH /$CHCl_3$ 1:3) to afford **10** (181 mg, 29 %) as white solid; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.53 (m, 4H, overlapped with solvent peak), 2.91 (t, *J* = 5.6, 4H), 3.29 (m, 4H, overlapped with solvent peak), 4.27 (t, *J* = 7.6, 4H), 7.81 (t, *J* = 8.0, 4H), 8.19-8,21 (m, 2H), 8.40-8.44 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6); δ33.7, 35.8, 36.8, 46.8, 122.0, 127.1, 27.3, 130.6, 131.2, 134.3, 163.3, 170.9; IR (FT-ATR, cm^{-1}); 3710, 2923, 2316, 1691, 1647, 1587, 1439, 1342, 1237, 1033; HRMS-ESI: calculated for $[C_{34}H_{31}N_5O_6 + H]^+$ requires m/z = 606.2353, found 606.2339.

• **Synthesis of 8**

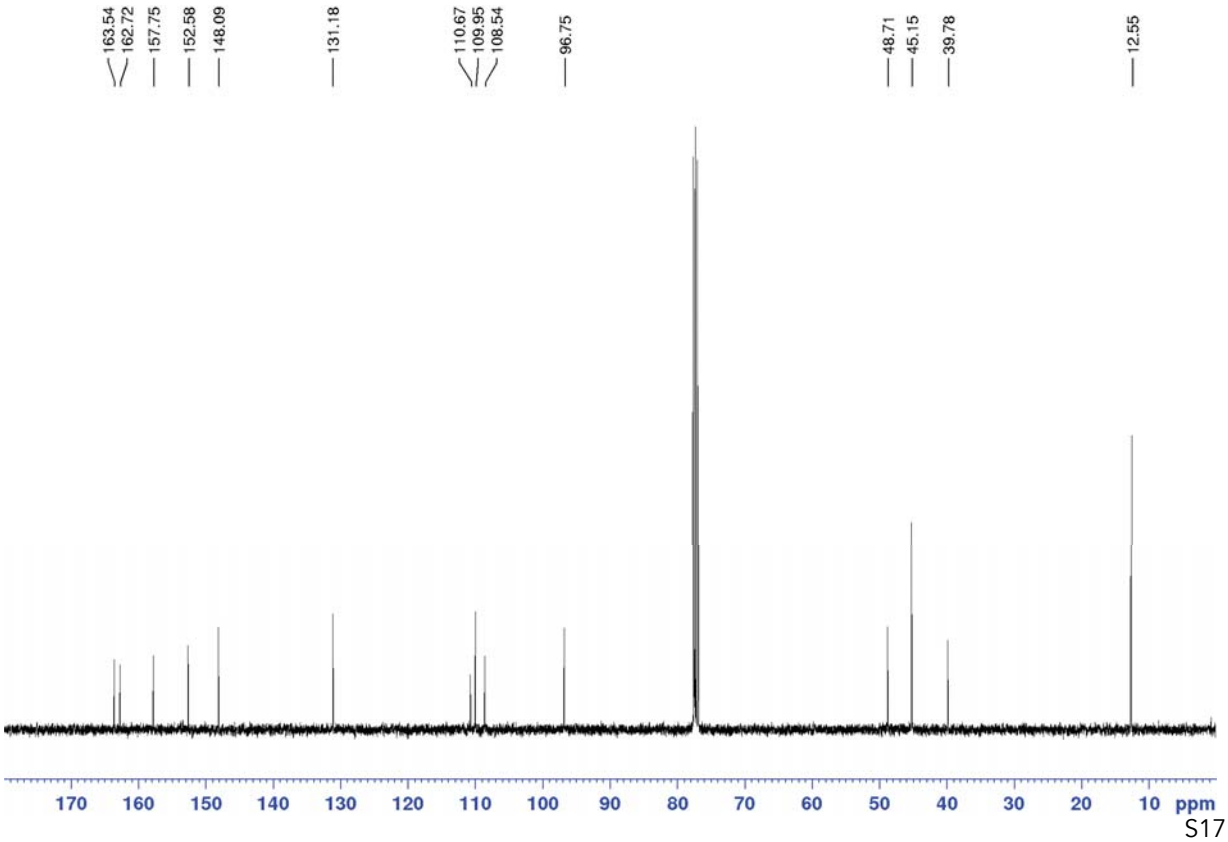


EDC (43.3 mg, 0.226 mmol), HOBt (30.5 mg, 0.226 mmol), and DIPEA (36 μL, 0.21 mmol) were added to a solution of N^{α}, N^{ε}-bis(tert-butoxycarbonyl)-L-lysine **5** (119 mg, 0.226 mmol) in DMF (20 mL). After stirring for 5 minutes, **S4** (160 mg, 2.64 mmol) was added to the mixture. After stirring for 15 hours at room temperature, the mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was applied for silica gel column chromatography (MeOH/$CHCl_3$ 1:20) to afford the condensation product. The resulting product was suspended in 4 M HCl/dioxane (2 mL). After stirring for 1 hour at room temperature, the mixture was concentrated. The residue was filtered and washed with $CHCl_3$ and EtOAc. The resulting solid was further purified by recrystallization from $CHCl_3$ and MeOH to afford **8** (9.3 mg, 5%) as a white solid; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.44-1.49 (m, 2H), 1.60-1.63 (m, 2H), 1.65-1.76 (m, 2H), 2.45-2.53 (m, 4H, overlapped with solvent peak), 2.77-2.80 (m, 2H), 3.33-3.39 (m, 6H), 3.41-3.46 (m, 2H), 3.54 (m, 2H, overlapped with solvent peak), 4.22-4.30 (m, 5H), 7.82-7.87 (m, 2H), 8.07 (brs, 3H), 8.22 (t, *J* = 5.6, 2H), 8.41-8.47 (m, 12H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$); 21.0, 26.4, 30.0, 33.5, 33.7, 34.1, 36.1, 36.4, 36.5, 37.5, 38.2, 45.4, 46.4, 49.4, 66.3, 79.2, 122.0, 122.0, 127.2, 127.3, 130.7, 131.3, 134.3, 163.3, 163.3, 169.0, 70.2, 170.8; IR (FT-ATR, cm^{-1}); 3711, 2923, 1695, 161, 1587, 136, 1243, 1055, 1033; HRMS-ESI: calculated for $[C_{40}H_{43}N_7O_7 + H]^+$ requires m/z = 734.3302, found 734.3261

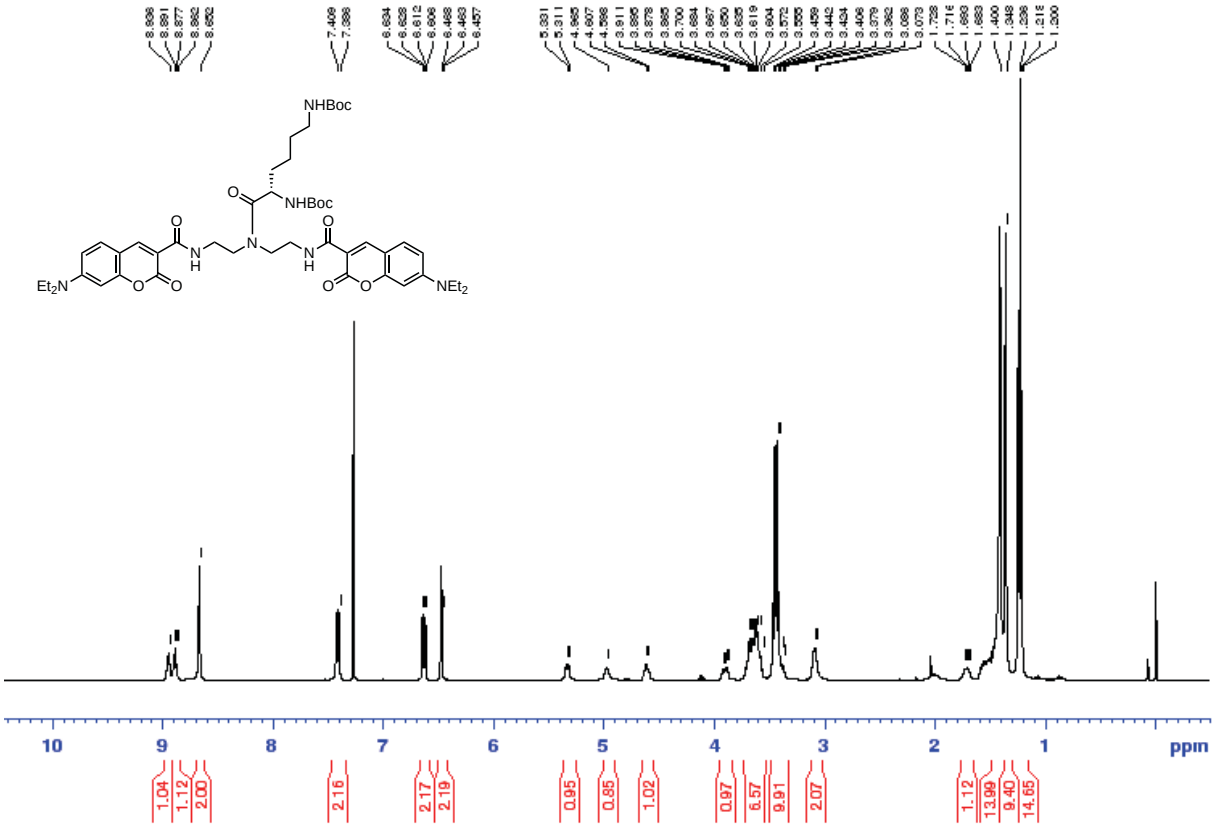
^{1}H NMR of **3** (400 MHz, $CDCl_3$)



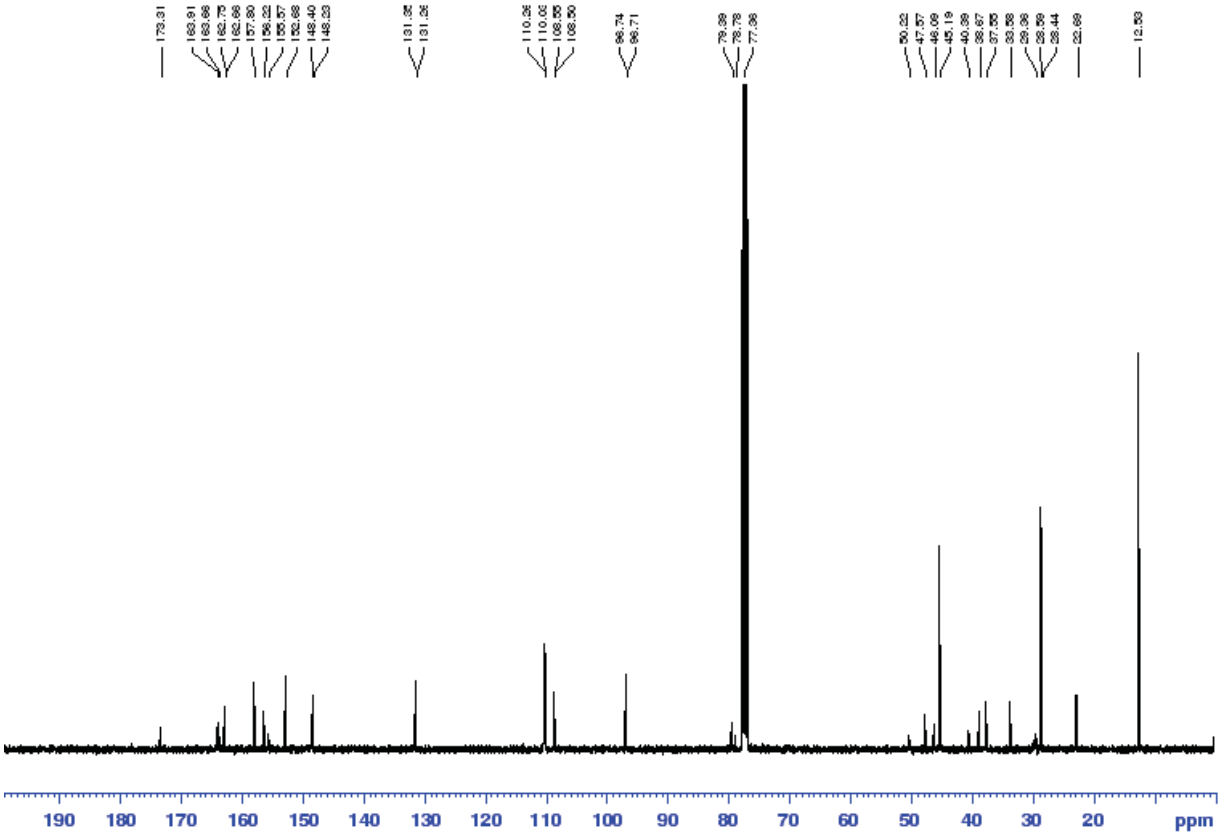
^{13}C NMR of **3** (100 MHz, $CDCl_3$)



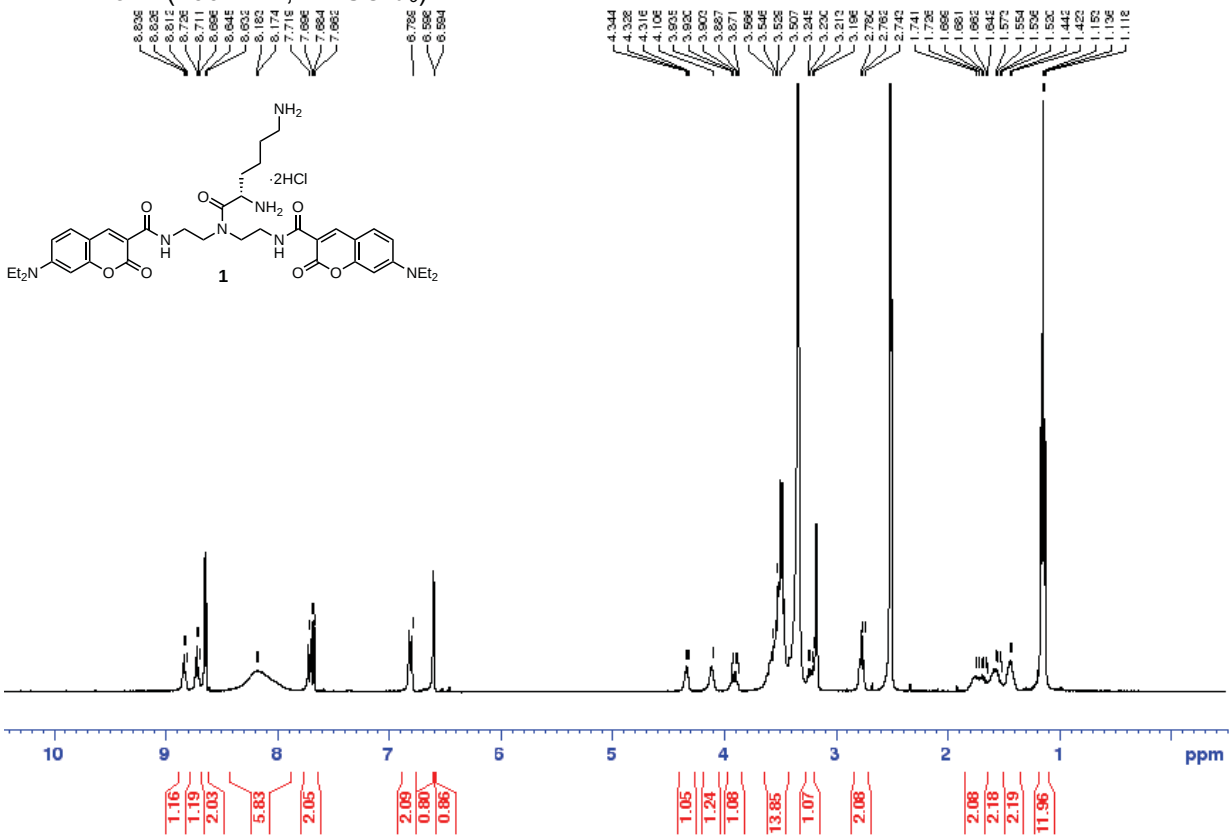
^{1}H NMR (400 MHz, $CDCl_3$)



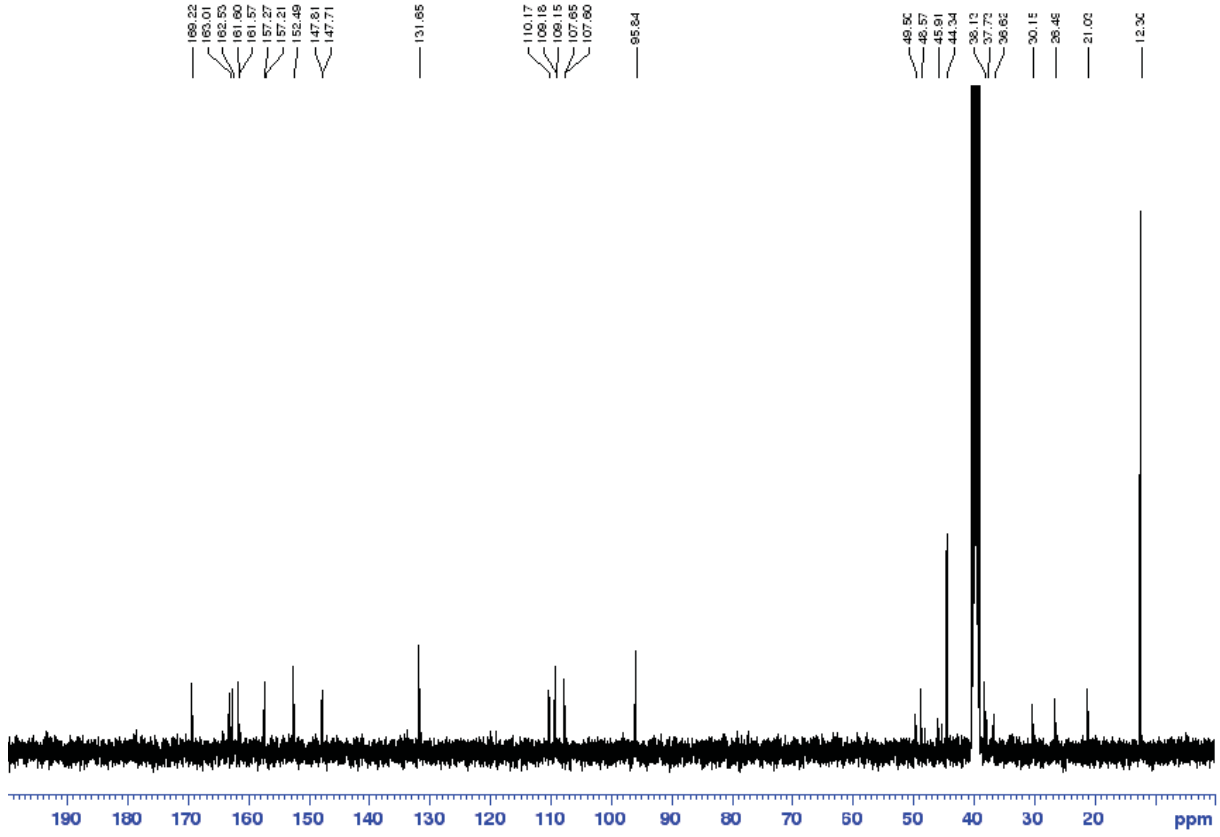
^{13}C NMR (100 MHz, $CDCl_3$)



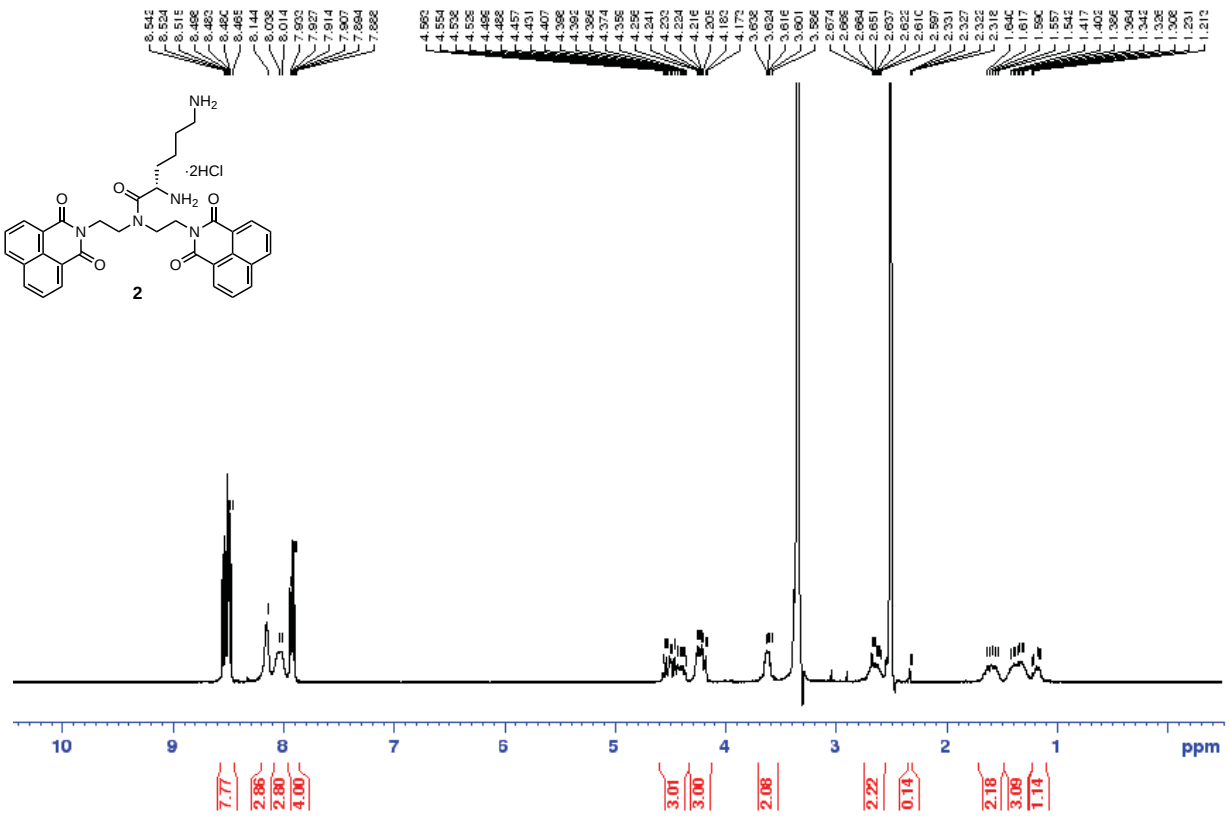
^{1}H NMR of **1** (400 MHz, DMSO-d_6)



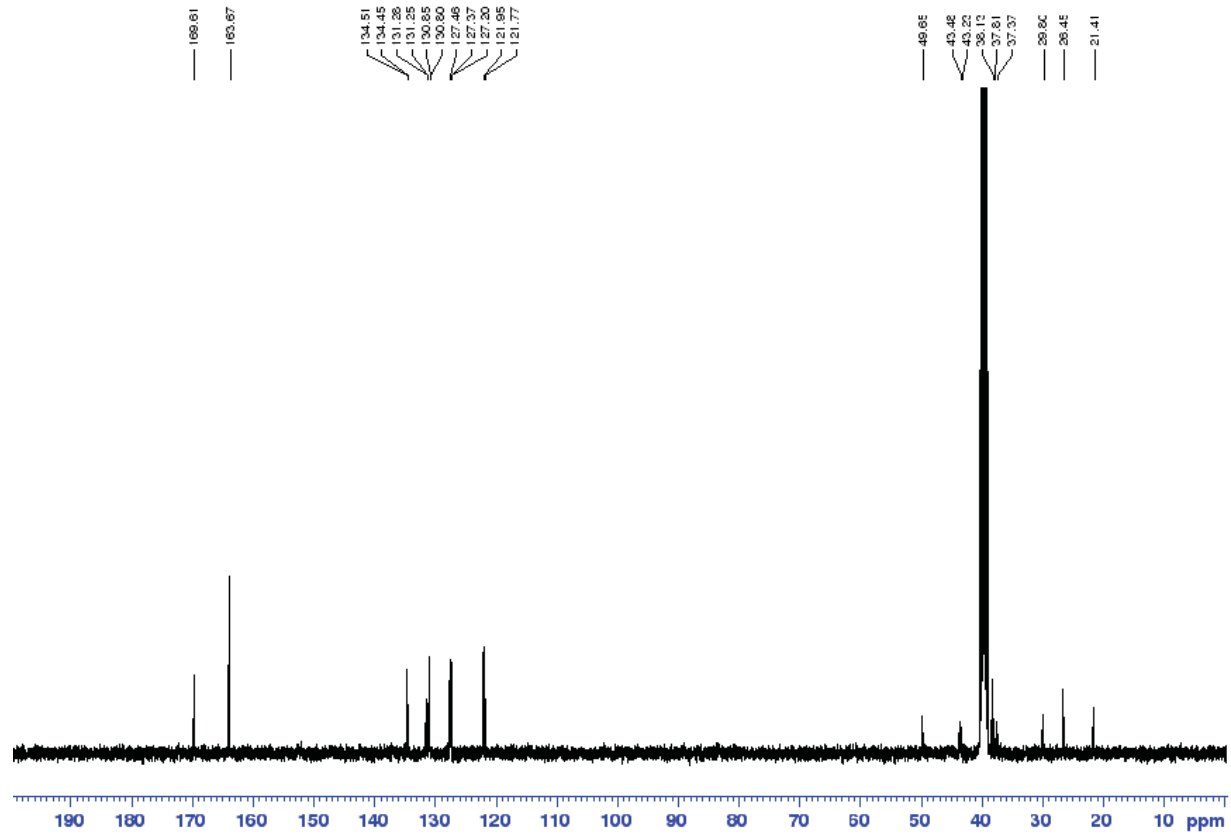
^{13}C NMR of **1** (100 MHz, DMSO-d_6)



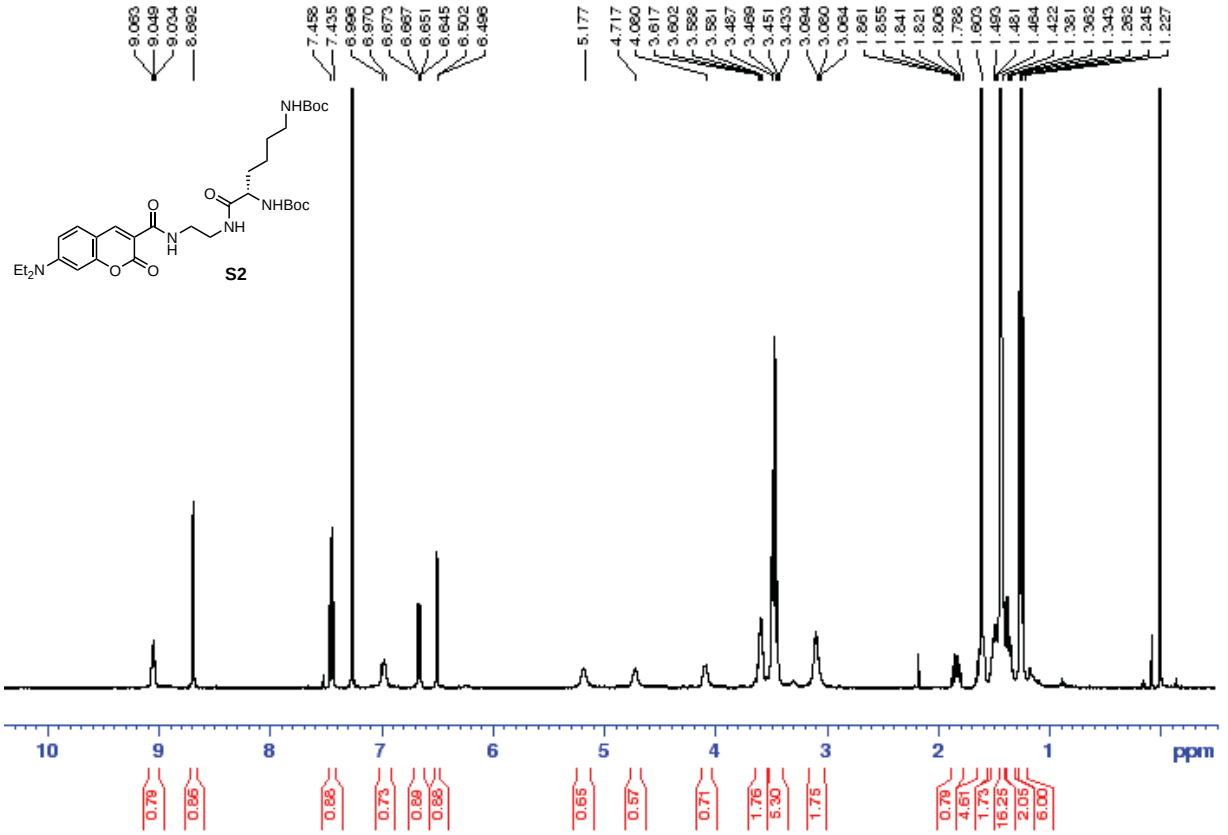
^{1}H NMR of **2** (400 MHz, DMSO-d_6)



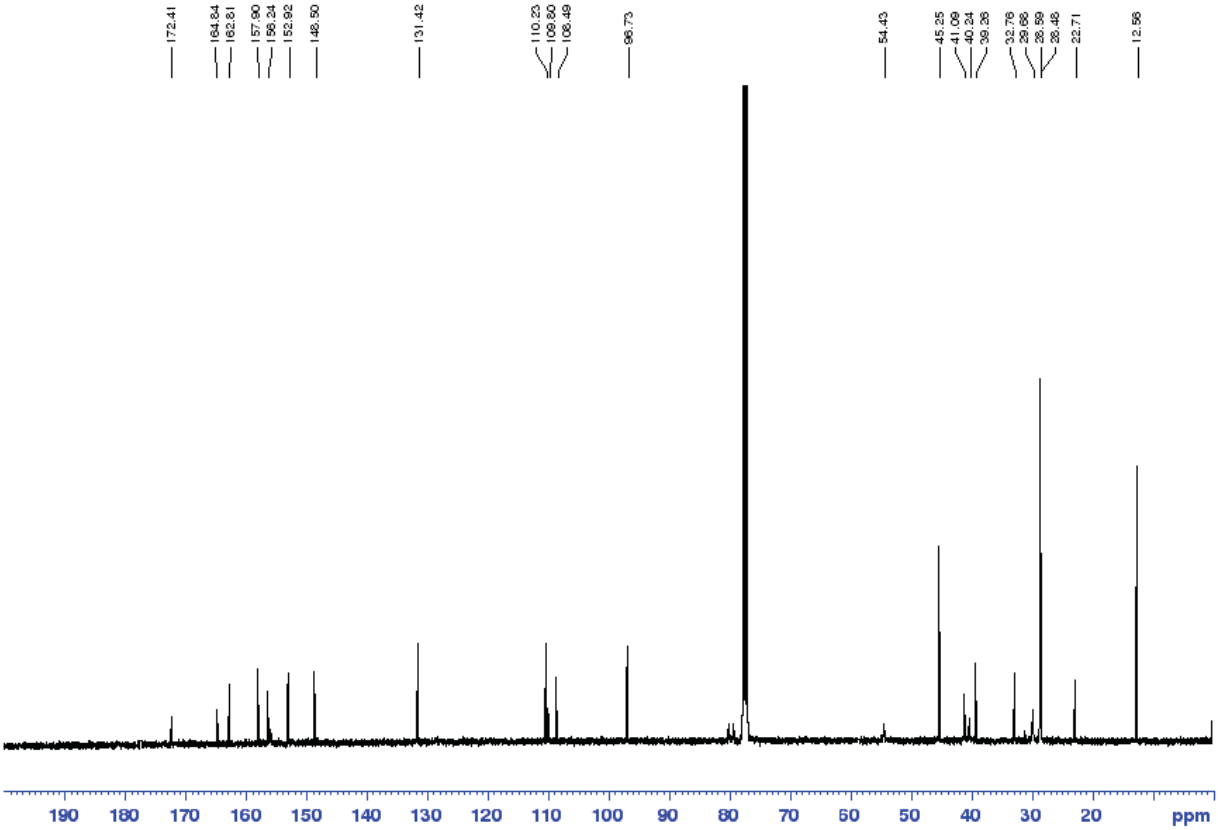
^{13}C NMR of **2** (100 MHz, DMSO-d_6)



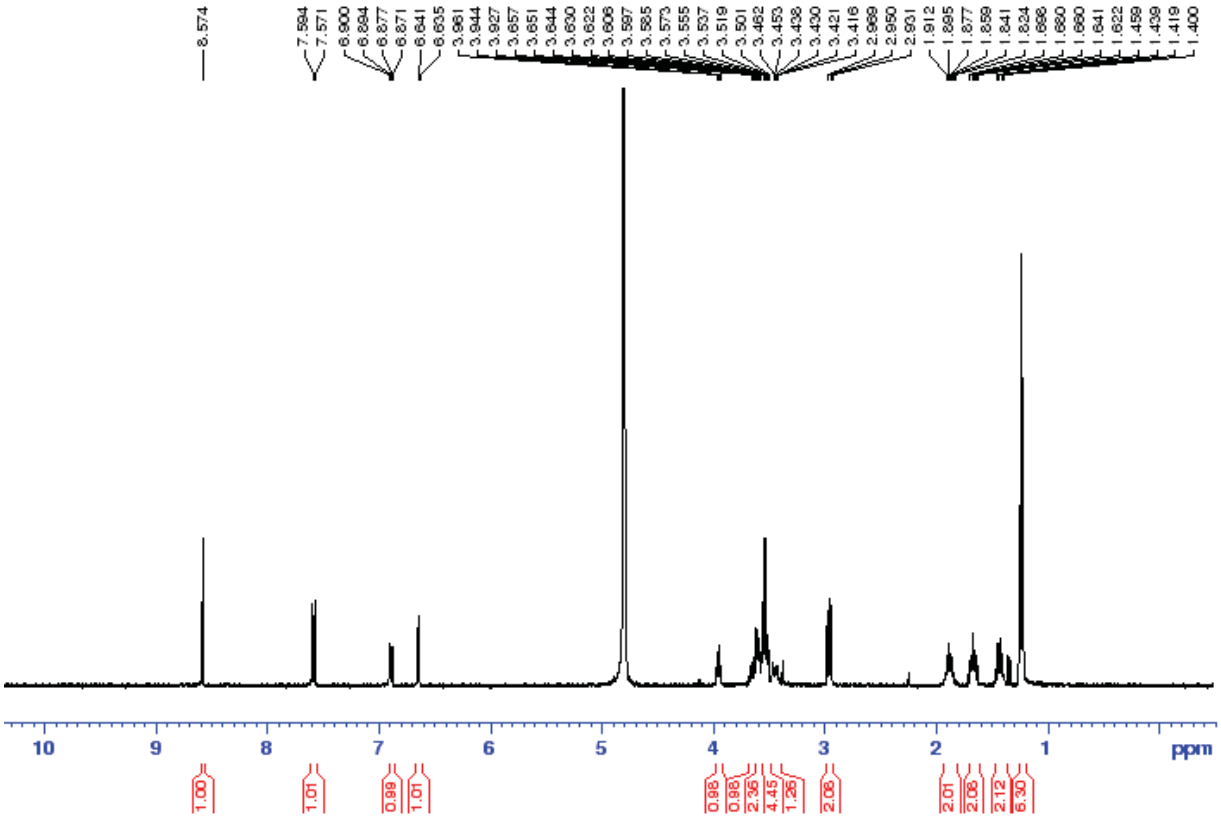
^{1}H NMR of **S1** (400 MHz, $CDCl_3$)



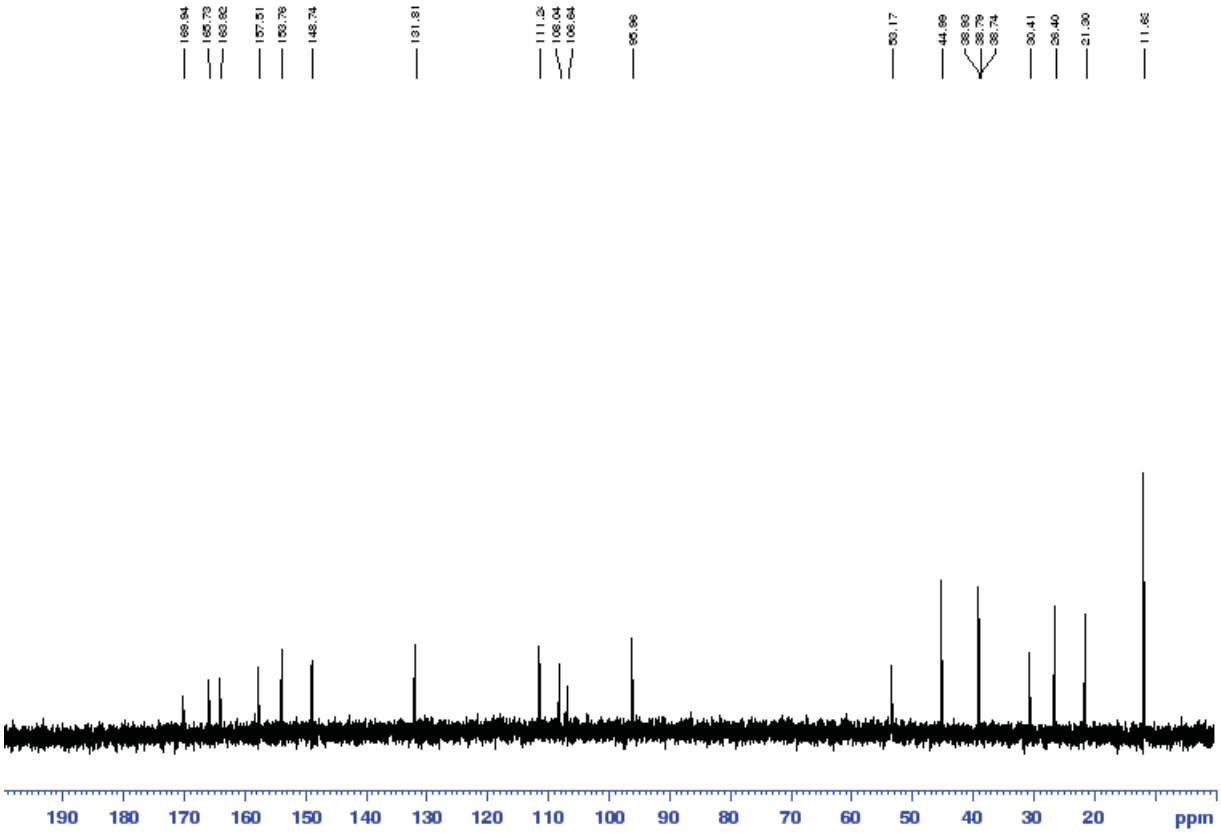
^{13}C NMR of **S1** (100 MHz, $CDCl_3$)



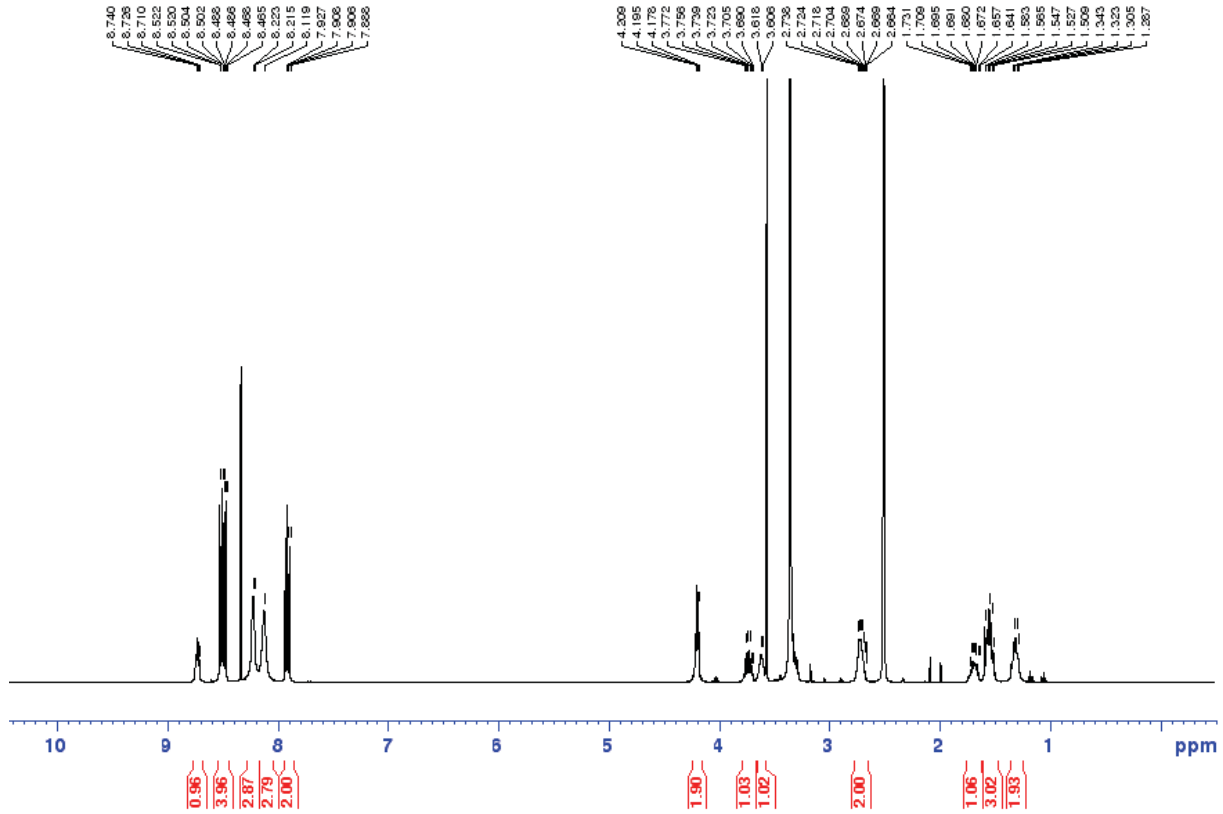
^{1}H NMR of **6** (400 MHz, D_2O)



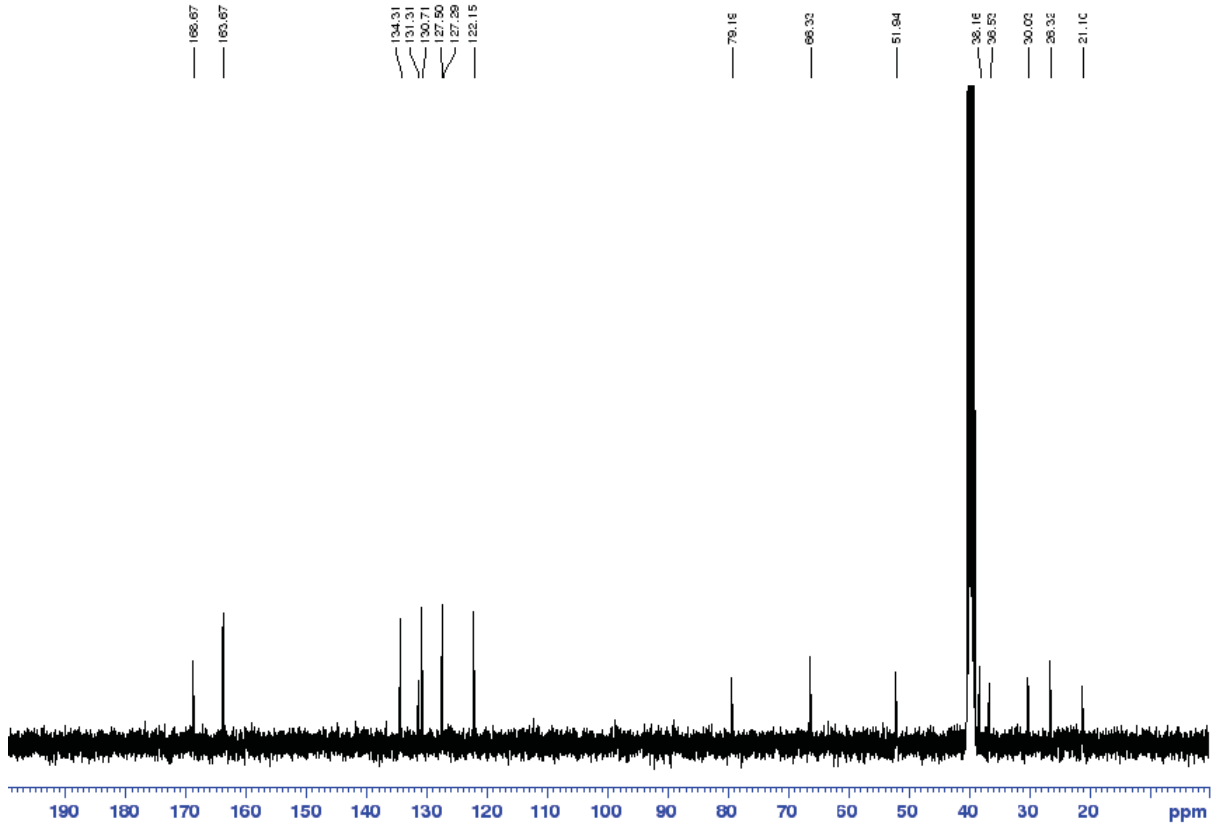
^{13}C NMR of **6** (100 MHz, D_2O)



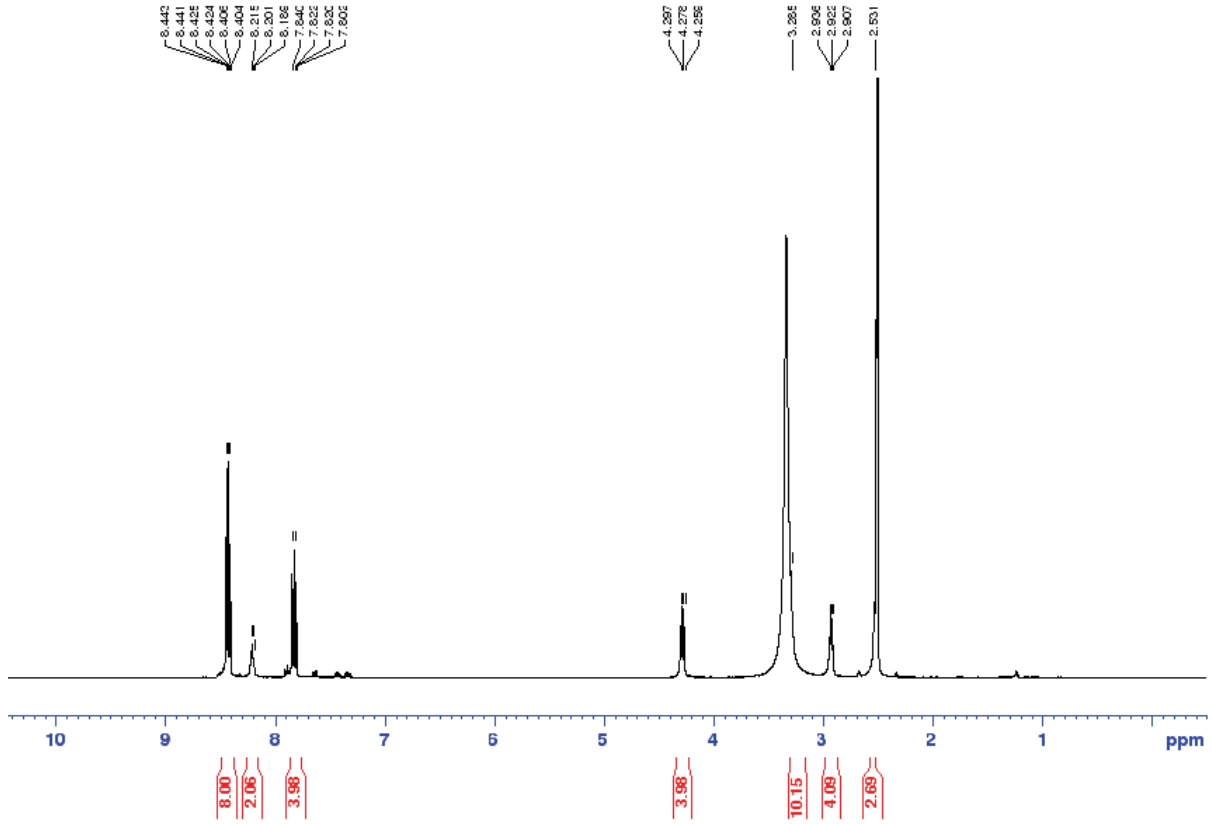
^{1}H NMR of **7** (400 MHz, DMSO-d_6)



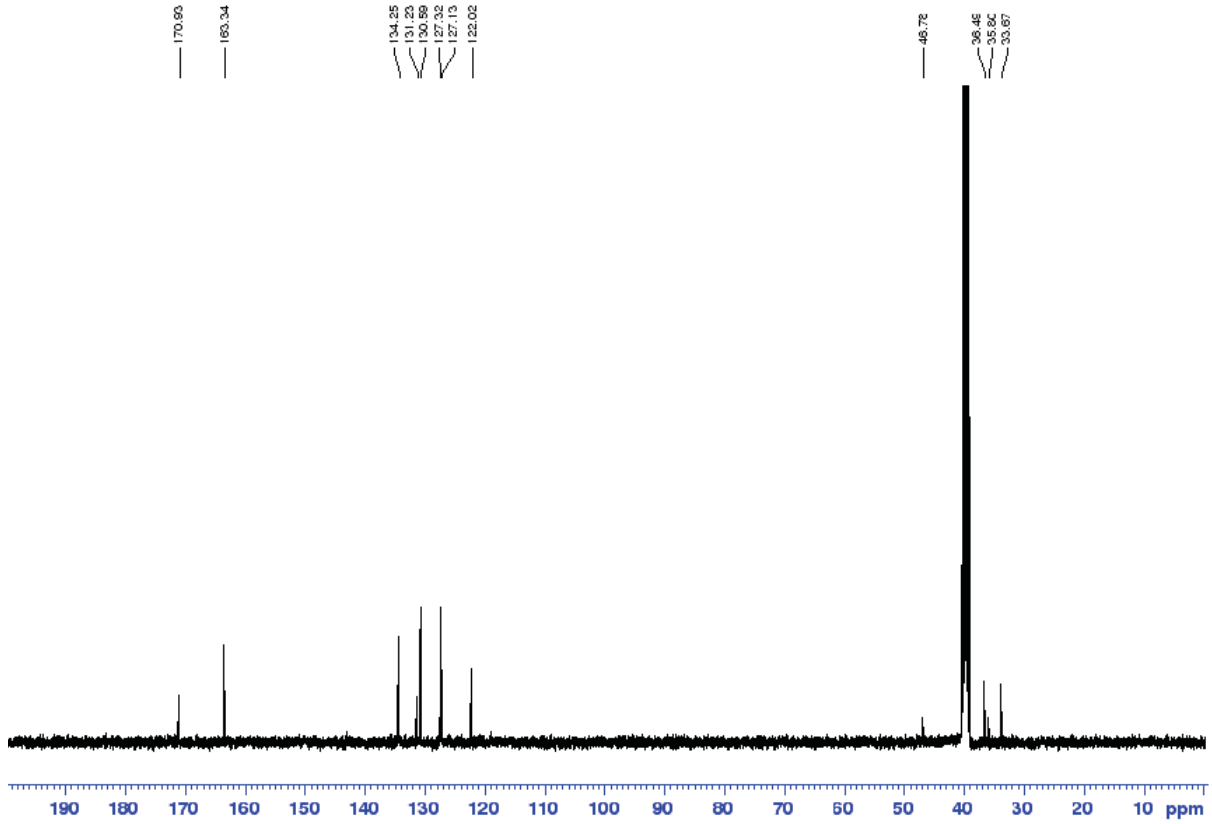
^{13}C NMR of **7** (100 MHz, DMSO-d_6)



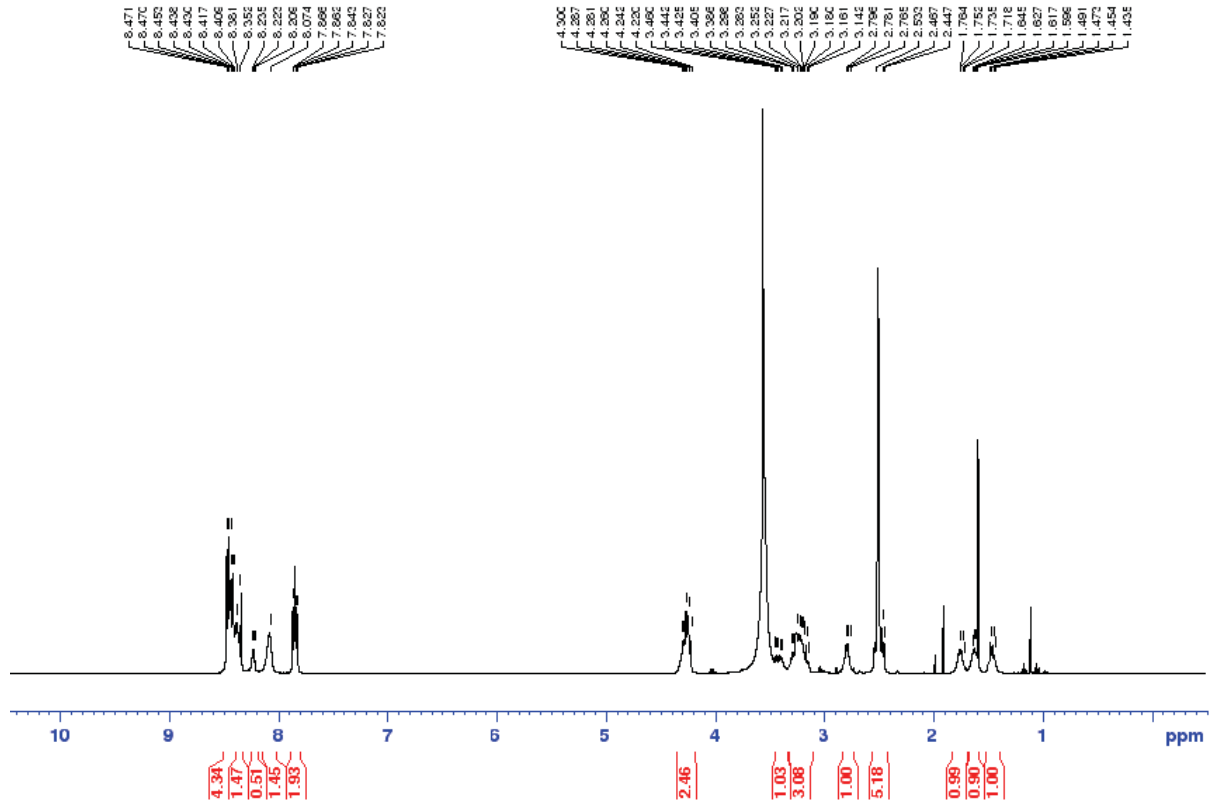
^{1}H NMR of **S4** (400 MHz, DMSO-d_6)



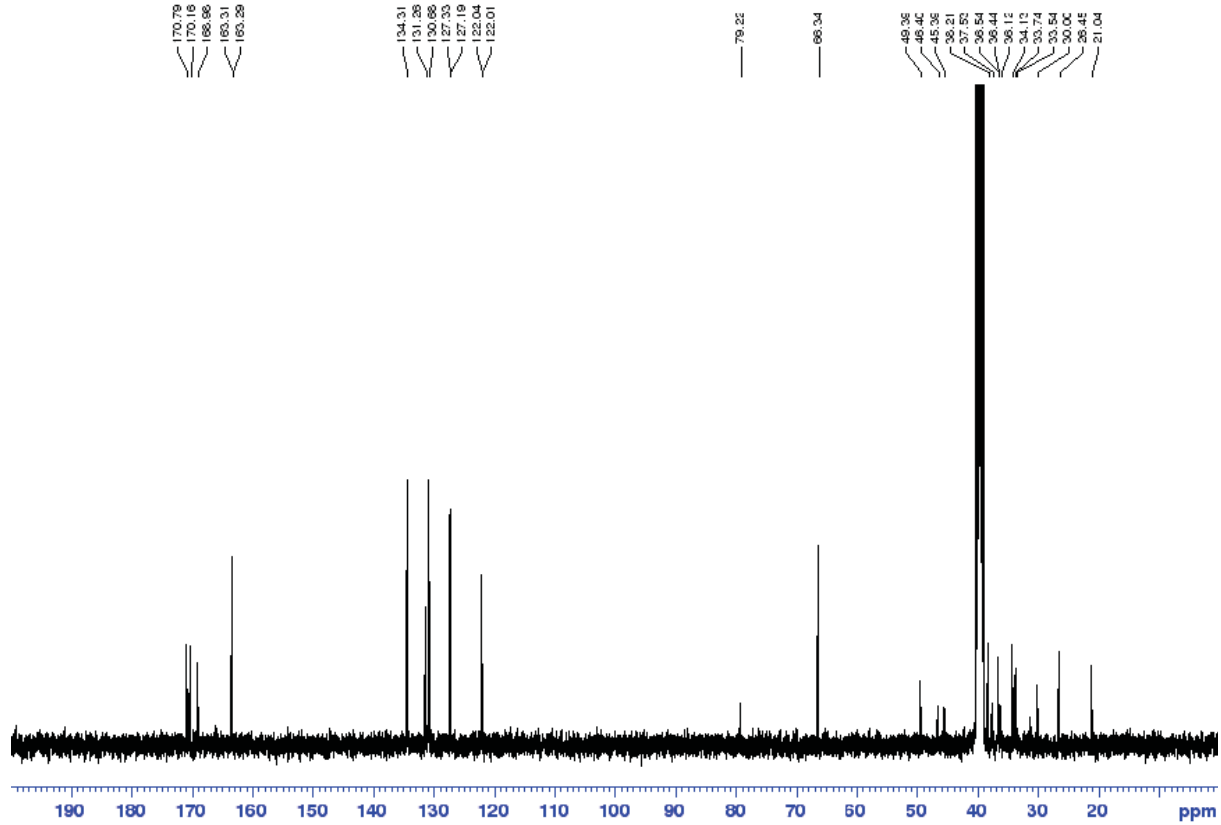
^{13}C NMR of **S4** (100 MHz, DMSO-d_6)



^{1}H NMR of **8** (400 MHz, DMSO-d_6)



^{13}C NMR of **8** (100 MHz, DMSO-d_6)



Reference

1. a) R. Ferreira, C. Baleizão, J. M. Muñoz-Molina, M. N. Berberan-Santos, U. Pischel, *J. Phys. Chem. A*, **2011**, *115*, 1092–1099; b) R. Parkesh, T. C. Leeb, T. Gunnlaugsson, *Org. Biomol. Chem.*, **2007**, *5*, 310–317.
2. P. Guo, Q. Chen, T. Liu, L. Xu, Q. Yang, X. Qian, *ACS Med. Chem. Lett.*, **2013**, *4*, 527–531.