

Supplementary Information

Tetracationic Macrocycles Form Highly Stable Inclusion Complexes with Uric Acid That Enables Solution-Phase and Paper-Strip Bicolor Colorimetric Detection of Hyperuricemia

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1. General methods and materials. ^1H NMR spectra were recorded with an AVANCE III HD 400 MHz spectrometer (Bruker) in the indicated solvents at 25°C. Chemical shifts were referenced to the residual solvent peaks. Fluorescent measurements were performed on a VARIAN CARY Eclipse Fluorescence Spectrophotometer. The crystal was measured using Bruker D8 Venture-Metaljet diffractometer equipped with a PHOTON II area detector and HELIOS multilayer optics monochrommated Cu-K and Mo-K alpha radiation ($\lambda = 1.54184$ and 0.71073 \AA) and the crystal structure was solved by direct method and refined by full-matrix least-squares methods based on F2 using SHELXL-2014 software. All the reagents and solvents were commercially available and used as received unless otherwise specified purification.

2. Calculation of binding constants (K_a).

The calculation of K_a by Origin software follows formula below (1:1 binding model, including titration experiments of UV, fluorescence, and NMR):

$$\Delta_A = \Delta_\epsilon \left[0.5 * \left([\text{Guest}] + [\text{Host}]^0 + \frac{1}{K_a} \right) - \sqrt{0.25 * [\text{Guest}] + [\text{Host}]^0 + \frac{1}{K_a} \right)^2 - [\text{Guest}] * [\text{Host}]^0} \right]$$

[Guest] is the concentration of the guest (purines), and $[\text{Host}]^0$ is the initial concentration of the host (macrocycles, unchanged during titration), Δ_A represents the difference in the y-axis after adding a given concentration of the guest (Intensity in fluorescence titration). Δ_ϵ is coefficient, and K_a is the binding constant. When $\Delta_A < 0$ in titration (Intensity decrease), taking the absolute value of Δ_A for non-linear fitting.

3. Testing of Real Urine Samples

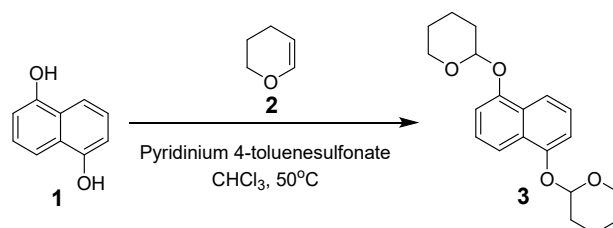
Prepare UA standard samples (0.2 mM, 0.4 mM, 0.8 mM, 1.2 mM, 1.6 mM, 2.0 mM) in 20 mM phosphate buffer (pH = 7.4). Written informed consent was signed by 6 volunteers (25 ± 2 years old). All urine specimens were collected from the midstream of morning urine with the corresponding supernatant collected after 15 min centrifugation (8000 rpm) prior to analysis and the supernatant was taken through 0.22 μm microporous filter membrane for later use. HPLC and enzymatic kit first constructed their standard curve using above standard samples, and then conducted urine sample testing. A 0.40 mM standard sample was also measured as an external standard at the same time for correction. The UA concentration of one urine sample that directly calculated by standard curve recorded as C1, and the UA concentration of 0.40 mM standard sample that directly calculated by standard curve recorded as C2. Corrected concentration $[\text{UA}] = 0.4 * (\text{C1}/\text{C2}) \text{ mM}$. The operations of three methods are as follows ($n = 3$):

- ① Enzymatic UA Test Kit (Nanjing Jiancheng Bioengineering Institute): The testing follows the protocol of the vendor.
- ② HPLC: The concentration analysis of UA formulation by HPLC on reverse C18 columns at 292 nm (5% MeCN/H₂O with 0.1% TFA as additive, 1 mL/min, 25°C).
- ③ FL-Probe (**D2**/RHB): Firstly, the 530 nm fluorescence intensity of probe solution ([RHB] = 1 μM, [**D2**] = 15 μM, 20 mM PB, pH = 7.4, 1980 μL) was tested and regarded as I₀. Then, 20 μL sample was added to the probe solution and 530 nm fluorescence intensity was tested again, regarded as I. Ignoring the deviation caused by volume changes.

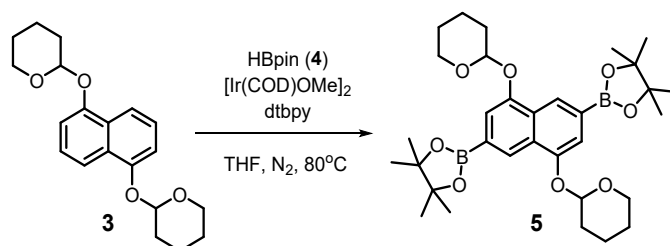
4. Preparation of fluorescent test papers

The fluorescent test papers were prepared by dropped 6 μM Rhodamine B in PB buffer (10 mM, pH 7.4) on the paper strips firstly and dried at 60 °C. Then, 90 μM **D2** in the same buffer above was dropped on the paper strips which was also dried at 60 °C subsequently.

5. Synthesis of **D2-D5**

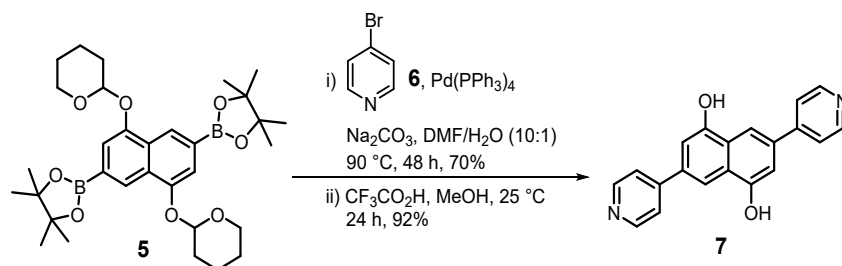


Compound 3. 1,5-Dihydroxy naphthalene **1** (10 g, 62 mmol), Pyridinium p-Toluenesulfonate (4.7 g, 19 mmol) and 3,4-Dihydro-2H-pyran **2** (15.7g, 0.19 mol) were added to CHCl₃ (200 mL). The mixture was stirred at 50°C for 12 h then cooled to rt and followed by extraction with 100 mL H₂O three times. The combined organic phase was dried over Na₂SO₄, filtered through a plug of celite and concentrated in vacuo. And then subjected to column chromatography on silica gel (1:1, hexanes/DCM) to afforded **3** as a white solid (17 g, 83%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.82 (d, *J* = 8.3 Hz, 2H), 7.41 (t, *J* = 8.1 Hz, 2H), 7.15 (d, *J* = 7.7 Hz, 2H), 5.70 (s, 2H), 3.81 – 3.72 (m, 2H), 3.62 – 3.54 (m, 2H), 2.10 – 1.98 (m, 2H), 1.96 – 1.88 (m, 4H), 1.78 – 1.68 (m, 2H), 1.66 – 1.58 (m, 4H).

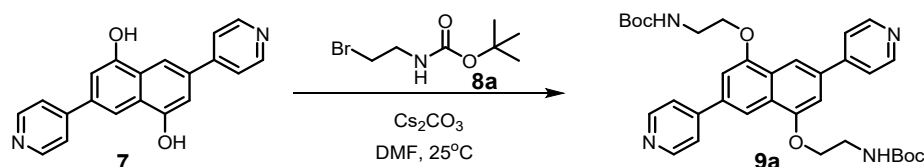


Compound 5. [Ir(COD)OMe]₂ (44 mg, 0.066 mmol), 4,4'-di-tert-butyl-2,2'-bipyridine

(46 mg, 0.168 mmol) and **2** were added to a three-necked flask. After degassing with N₂ for 15 min, THF (8 mL) and HBpin **4** (0.76 g, 7.5 mmol) were added to the reaction mixture and the solution was heated to 80°C under N₂ for 24 h. Then cooled to rt and filtered the solution by celite. The organic phase was concentrated in vacuo to get black crude product, which was further washed by CH₃OH (3 × 10 mL) and vacuum drying to afford **5** as a white solid (0.98 g, 55%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.24 (s, 2H), 7.35 (s, 2H), 5.74 (s, 2H), 3.78 – 3.70 (m, 2H), 2.01 – 1.88 (m, 4H), 1.81 – 1.69 (m, 4H), 1.67 – 1.56 (m, 2H), 1.34 (s, 18H).

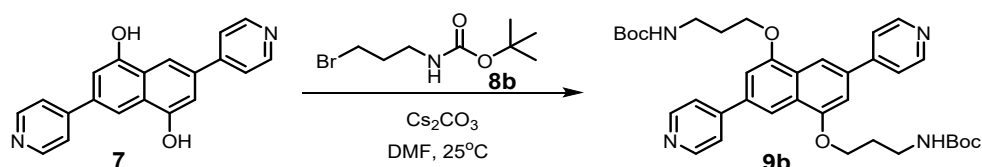


Compound 7. 4-Bromopyridine hydrochloride **6** (0.54 g, 3.4 mmol), Na₂CO₃ (1.3 g, 9.2 mmol) and **5** (1.0 g, 1.7 mmol) were added to a 10:1 mixture of DMF/H₂O (66 mL), which had been degassed with N₂ for 15 min. Next, Pd(PPh₃)₄ (0.20 g, 0.17 mmol) was added to the reaction mixture and the solution heated to 90°C under N₂ for 48 h. Then, the reaction mixture was cooled to room temperature and the palladium catalyst filtered off using celite. 300 mL H₂O was added to the organic phase and the precipitate was collected by filtration as a yellow solid (0.58 g, 70%) after vacuum drying. Then, CH₃OH (100 mL) and CF₃CO₂H (3 mL) were added to a round bottom flask containing the solid above (0.5 g, 1.3 mmol) and the mixture was stirred at 25°C for 24 h. Then, 1 M NaHCO₃(aq) was added dropwise to the solution until no bubbles were formed. The solution was concentrated in vacuo to get yellow crude product, which was further washed by H₂O (3 × 20 mL) and vacuum drying to afford **7** as a yellow solid (0.36 g, 92%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.69 (s, 2H), 8.75 (d, *J* = 5.4 Hz, 4H), 8.07 (s, 2H), 7.91 (d, *J* = 5.0 Hz, 4H), 7.30 (s, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 154.85, 149.54, 148.27, 134.37, 126.41, 121.95, 111.81, 107.25. HRMS calcd for C₂₀H₁₄N₂O₂ [M+H]⁺: 315.1129; found: 315.1142.

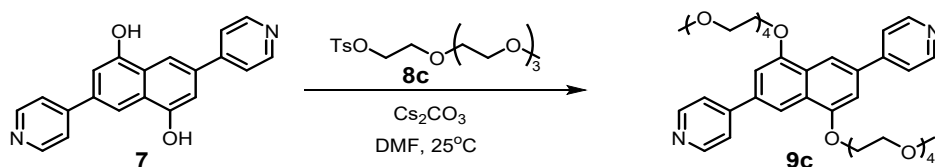


Compound 9a. Anhydrous DMF (10 mL) and Cs₂CO₃ (0.31 g, 0.96 mmol) were added to a round bottom flask containing **7** (0.1 g, 0.32 mmol) and the mixture was stirred at room temperature for 10 minutes. Then, tert-Butyl N-(2-bromoethyl) carbamate **8a** (0.22 g, 0.96 mmol) was added, and the reaction mixture was stirred at 25°C for 24 h. The reaction was stopped and added 50 mL H₂O. The deposit was filtered and washed with water and dried in vacuum to afford yellow solid **9a** (0.11 g, 57%). ¹H NMR (400

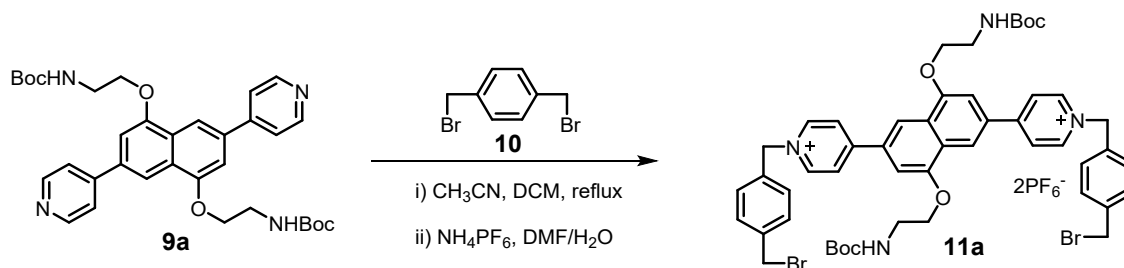
MHz, DMSO-*d*₆) δ 8.68 (d, *J* = 5.5 Hz, 4H), 8.28 (s, 2H), 7.92 (d, *J* = 5.2 Hz, 4H), 7.41 (s, 2H), 7.26 (t, *J* = 6.0 Hz, 2H), 4.28 (t, *J* = 5.1 Hz, 4H), 3.52 (q, *J* = 8.3, 6.4 Hz, 4H), 1.33 (s, 18H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.81, 155.27, 150.13, 147.03, 135.14, 126.13, 121.58, 112.46, 104.78, 77.73, 68.06, 28.18. HRMS calcd for C₃₄H₄₀N₄O₆ [M+H]⁺: 601.3021; found: 601.3028.



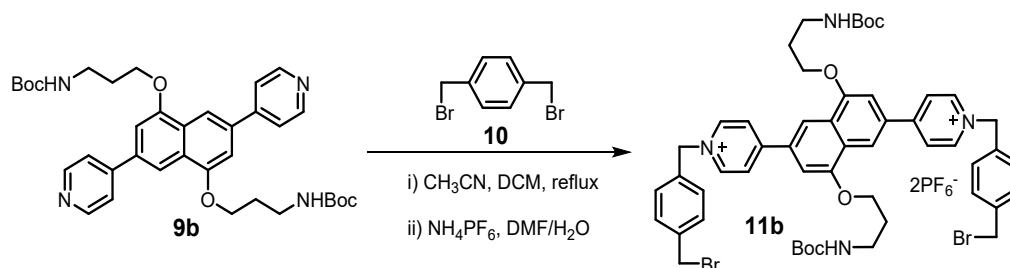
Compound 9b. Anhydrous DMF (10 mL) and Cs₂CO₃ (0.31 g, 0.96 mmol) were added to a round bottom flask containing **7** (0.1 g, 0.32 mmol) and the mixture was stirred at room temperature for 10 minutes. Then, tert-Butyl N-(2-bromopropyl) carbamate **8b** (0.22 g, 0.96 mmol) was added, and the reaction mixture was stirred at 25°C for 24 h. The reaction was stopped and added 50 mL H₂O. The deposit was filtered and washed with water and dried in vacuum to afford yellow solid **9b** (98 mg, 49%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.69 (d, *J* = 5.1 Hz, 4H), 8.15 (s, 2H), 7.88 (d, *J* = 5.1 Hz, 4H), 7.40 (s, 2H), 6.98 (t, *J* = 5.8 Hz, 2H), 4.36 (t, *J* = 6.2 Hz, 4H), 3.20 (q, *J* = 9.3, 7.2 Hz, 4H), 2.09 – 1.99 (m, 4H), 1.35 (s, 18H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.67, 155.28, 150.23, 147.18, 135.26, 126.18, 121.51, 111.92, 105.01, 77.46, 65.97, 36.99, 29.21, 28.17. HRMS calcd for C₃₆H₄₄N₄O₆ [M+H]⁺: 629.3334; found: 629.3338.



Compound 9c. Anhydrous DMF (10 mL) and Cs₂CO₃ (0.31 g, 0.96 mmol) were added to a round bottom flask containing **7** (0.1 g, 0.32 mmol) and the mixture was stirred at room temperature for 10 minutes. Then, m-PEG5-Tos **8c** (0.35 g, 0.96 mmol) was added, and the reaction mixture was stirred at 25°C for 24 h. Then cooled to rt and filtered the solution by celite. The organic phase was concentrated in vacuo and subjected to column chromatography on silica gel (1:20, CH₃OH/DCM) to afford **9c** as a yellow oil (54 mg, 24%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.70 (d, *J* = 5.0 Hz, 4H), 8.13 (s, 2H), 7.85 (d, *J* = 5.2 Hz, 4H), 7.46 (s, 2H), 4.48 (t, *J* = 2.8 Hz, 4H), 3.97 (t, *J* = 2.5 Hz, 4H), 3.71 (t, *J* = 2.7 Hz, 4H), 3.58 (t, *J* = 2.0 Hz, 4H), 3.53 – 3.47 (m, 8H), 3.44 – 3.39 (m, 8H), 3.17 (s, 6H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.21, 150.25, 147.18, 135.38, 126.15, 121.50, 112.09, 105.53, 71.15, 70.04, 69.82, 69.77, 69.70, 69.48, 68.88, 68.26, 57.93. HRMS calcd for C₃₈H₅₀N₂O₁₀ [M+H]⁺: 695.3539; found: 695.3541.

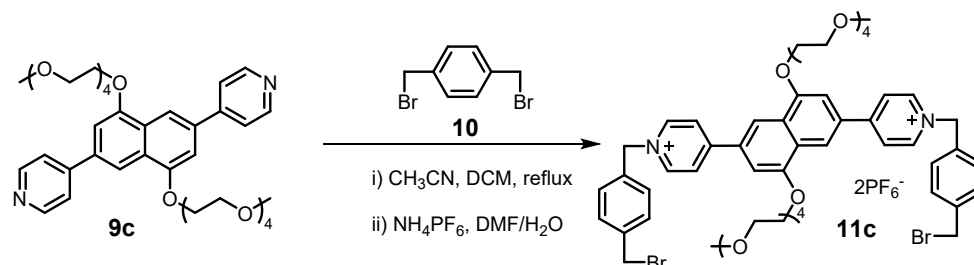


Compound 11a. α, α' -Dibromo-*p*-xylene **10** (3.0 g, 11 mmol) was added to MeCN (60 mL) in a 250 mL round-bottomed three-neck flask and the resulting mixture was refluxed while stirring until all of the solid material dissolved. Next, the temperature of the oil bath was raised to 80°C, allowing the reaction mixture to reflux while a suspension of **9a** (0.90 g, 1.5 mmol) in DCM (30 mL), was added slowly over 1 h. After heating under reflux for 48 h, the reaction mixture was cooled to room temperature and the orange precipitate was collected by filtration and washed with DCM (3 \times 10 mL). The solid was then dissolved in DMF (20 mL) and 100 mL saturated solution of NH_4PF_6 was added subsequently. Then the precipitate formed was filtered, washed with water (10 mL \times 3) and dried to afford **11a** as an orange solid (1.7 g, 90%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.30 (d, $J = 6.4$ Hz, 4H), 8.71 (d, $J = 6.4$ Hz, 4H), 8.50 (s, 2H), 7.63 (s, 2H), 7.56 (s, 8H), 7.25 (t, $J = 6.0$ Hz, 2H), 5.89 (s, 4H), 4.72 (s, 4H), 4.31 (t, $J = 5.1$ Hz, 4H), 3.53 (q, $J = 9.4, 7.3$ Hz, 4H), 1.26 (s, 18H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.97, 155.80, 155.02, 144.69, 139.25, 134.60, 133.22, 130.05, 128.92, 127.01, 125.63, 115.35, 105.27, 77.81, 68.69, 62.15, 33.53, 31.27, 28.11. HRMS calcd for $\text{C}_{50}\text{H}_{56}\text{Br}_2\text{N}_4\text{O}_6\text{PF}_6$ $[\text{M-PF}_6]^+$: 1113.2183; found: 1113.2195.

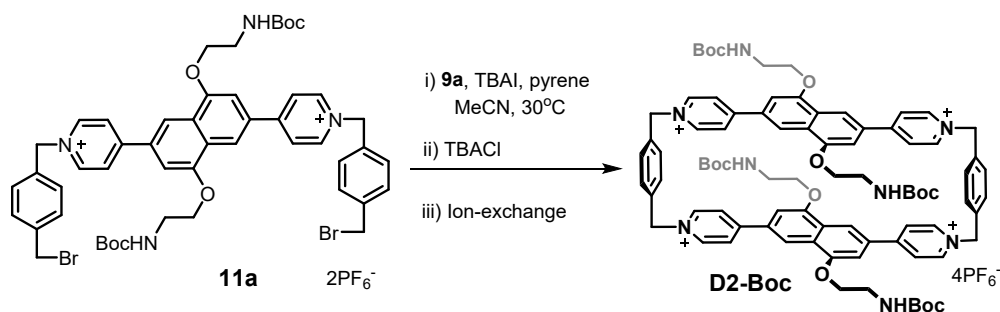


Compound 11b. α, α' -Dibromo-*p*-xylene (3.0 g, 11 mmol) **10** was added to MeCN (60 mL) in a 250 mL round-bottomed three-neck flask and the resulting mixture was refluxed while stirring until all of the solid material dissolved. Next, the temperature of the oil bath was raised to 80°C, allowing the reaction mixture to reflux while a suspension of **9b** (0.94 g, 1.5 mmol) in DCM (30 mL), was added slowly over 1 h. After heating under reflux for 48 h, the reaction mixture was cooled to room temperature and the orange precipitate was collected by filtration and washed with DCM (3 \times 10 mL). The solid was then dissolved in DMF (20 mL) and 100 mL saturated solution of NH_4PF_6 was added subsequently. Then the precipitate formed was filtered, washed with water (10 mL \times 3) and dried to afford **11b** as an orange solid (1.6 g, 83%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.25 (d, $J = 6.2$ Hz, 4H), 8.70 (d, $J = 6.3$ Hz, 4H), 8.39 (s, 2H), 7.63 (s, 2H), 7.56 (s, 8H), 6.95 (t, $J = 6.2$ Hz, 2H), 5.89 (s, 4H), 4.73 (s, 4H), 4.40 (t, $J = 6.4$

Hz, 4H), 3.25 (q, $J = 8.3, 6.1$ Hz, 4H), 2.11 – 1.99 (m, 4H), 1.29 (s, 18H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.99, 155.68, 155.12, 144.75, 139.24, 134.50, 133.28, 130.06, 128.96, 127.12, 125.67, 114.84, 105.53, 77.48, 66.39, 62.13, 36.92, 33.54, 28.88, 28.14. HRMS calcd for $\text{C}_{52}\text{H}_{58}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M-2PF}_6]^{2+}$: 498.1424; found: 498.1429.

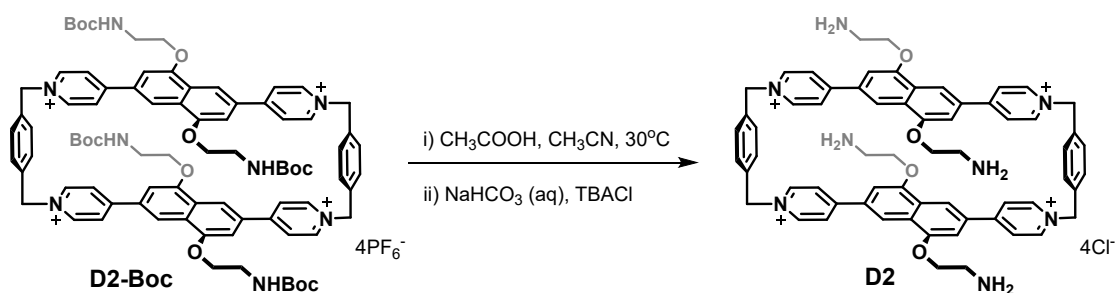


Compound 11c. α, α' -Dibromo-p-xylene (3.0 g, 11 mmol) was added to MeCN (60 mL) in a 250 mL round-bottomed three-neck flask and the resulting mixture was refluxed while stirring until all of the solid material dissolved. Next, the temperature of the oil bath was raised to 80°C , allowing the reaction mixture to reflux while **9c** (0.90 g, 1.5 mmol) in DCM (30 mL), was added slowly over 1 h. After heating under reflux for 48 h, the reaction mixture was cooled to room temperature. Then, Et_2O (200 mL) was added to the mixture and the orange precipitate was collected by filtration and washed with DCM (3×10 mL). The solid was then dissolved in DMF (10 mL) and 100 mL saturated solution of NH_4PF_6 was added subsequently. Then the precipitate formed was filtered, washed with water (10 mL $\times 3$) and dried to afford **11c** as an orange solid (1.0 g, 53%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.28 (d, $J = 6.4$ Hz, 4H), 8.68 (d, $J = 6.4$ Hz, 4H), 8.36 (s, 2H), 7.69 (s, 2H), 7.56 (s, 8H), 5.89 (s, 4H), 4.72 (s, 4H), 4.54 (t, $J = 2.8$ Hz, 4H), 3.98 (t, $J = 2.5$ Hz, 4H), 3.69 (t, $J = 2.7$ Hz, 4H), 3.56 (t, $J = 2.3$ Hz, 4H), 3.47 (t, $J = 2.2$ Hz, 4H), 3.43 – 3.33 (m, 12H), 3.15 (s, 6H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 156.14, 155.29, 144.94, 139.45, 133.56, 132.80, 130.25, 129.83, 129.16, 128.75, 127.30, 125.84, 115.09, 106.14, 71.30, 70.17, 69.95, 69.88, 69.83, 69.60, 68.89, 58.10, 33.68. HRMS calcd for $\text{C}_{54}\text{H}_{64}\text{Br}_2\text{N}_2\text{O}_{10}$ $[\text{M-2PF}_6]^{2+}$: 531.1527; found: 531.1529.

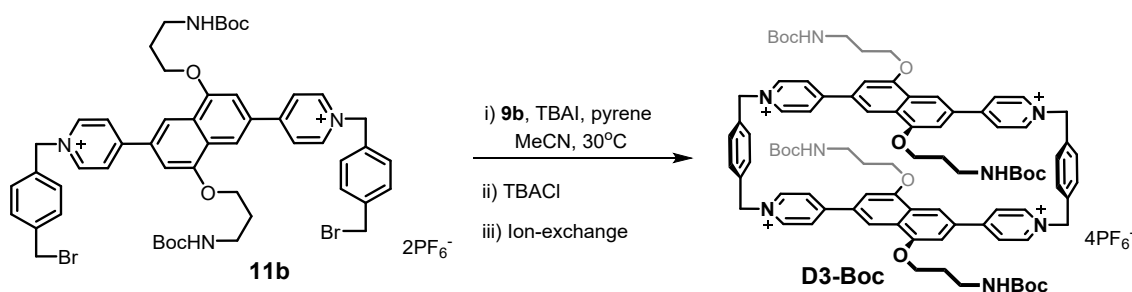


D2-Boc. Adding **11a** (0.55 g, 0.44 mmol), **9a** (0.26 g, 0.44 mmol), TBAI (32 mg, 0.087 mmol), and the template Pyrene (0.44 g, 2.2 mmol) to dry MeCN (400 mL) and stirring at 30°C . After 7 days, the reaction was stopped by adding $(t\text{-Bu})_4\text{NCl}$ (1M in CH_3CN , 3 mL), causing the crude product to precipitate from solution. The orange precipitate was collected by filtration and then to be subjected to column chromatography using

silica gel and CH₃OH : H₂O : NH₄Cl (aq, sat) = 6:3:1 as the eluents. After ion-exchange to yield the pure product (0.32 g, 37%) as an orange solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.29 (d, *J* = 6.3 Hz, 8H), 8.60 (d, *J* = 6.4 Hz, 8H), 8.34 (s, 4H), 7.78 (s, 8H), 7.48 (s, 4H), 7.17 (t, *J* = 6.1 Hz, 4H), 5.82 (s, 8H), 4.17 (t, *J* = 5.5 Hz, 8H), 3.44 (q, *J* = 8.4, 5.2 Hz, 8H), 1.22 (s, 36H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.81, 155.71, 154.02, 144.05, 136.42, 132.10, 129.82, 126.97, 125.01, 115.41, 104.84, 77.72, 68.45, 62.18, 28.11. HRMS calcd for C₈₄H₉₆N₈O₁₂PF₆ [M-3PF₆]³⁺: 517.8925; found: 517.8925.

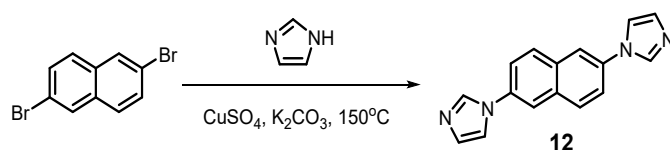


D2. CH₃CN (30 mL) and CF₃COOH (3 mL) were added to a round bottom flask containing **D2-Boc** (0.30 g, 0.15 mmol) and the mixture was stirred at 30°C for 24 h. Then, the solution was concentrated in vacuo to get yellow crude product, which was further dissolved in H₂O (30 mL). And then, 1 M NaHCO₃(aq) was added dropwise to the solution to adjust pH = 7. Saturated solution of NH₄PF₆ (10 mL) was added subsequently and the precipitate formed was filtered, washed with water (10 mL × 3) and dried to get yellow solid. Finally, the yellow solid was dissolved in CH₃CN (10 mL), (t-Bu)₄NCl (1M in CH₃CN, 1 mL) was added subsequently. The precipitate was collected by filtration and dried to afford **D2** as a yellow solid (0.17 g, 98%). ¹H NMR (400 MHz, D₂O) δ 8.95 (d, *J* = 6.3 Hz, 8H), 8.31 (d, *J* = 5.9 Hz, 8H), 8.30 (s, 4H), 7.69 (s, 8H), 7.27 (s, 4H), 5.83 (s, 8H), 4.42 (t, *J* = 7.5 Hz, 8H), 3.52 – 3.47 (m, 8H). ¹³C NMR (101 MHz, D₂O) δ 156.21, 155.13, 143.55, 135.93, 133.24, 130.02, 127.00, 125.85, 116.07, 104.99, 64.77, 63.75, 38.67. HRMS calcd for C₆₄H₆₄N₈O₄PF₆ [M-3PF₆]³⁺: 384.4892; found: 384.4891.

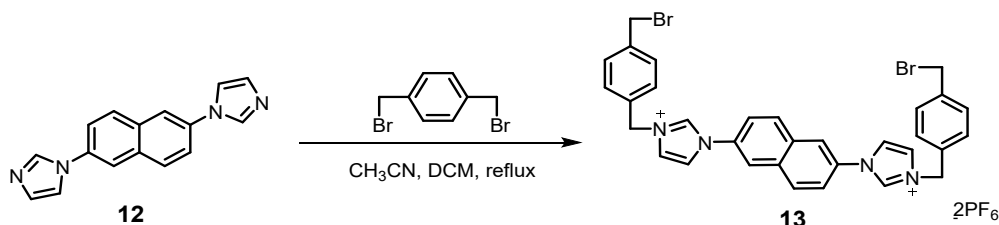


D3-Boc. Adding **11b** (0.56 g, 0.44 mmol), **9b** (0.27 g, 0.44 mmol), TBAI (32 mg, 0.087 mmol), and the template Pyrene (0.44 g, 2.2 mmol) to dry MeCN (400 mL) and stirring at 30°C. After 7 days, the reaction was stopped by adding (t-Bu)₄NCl (1M in CH₃CN, 3 mL), causing the crude product to precipitate from solution. The orange precipitate was collected by filtration and then to be subjected to column chromatography using

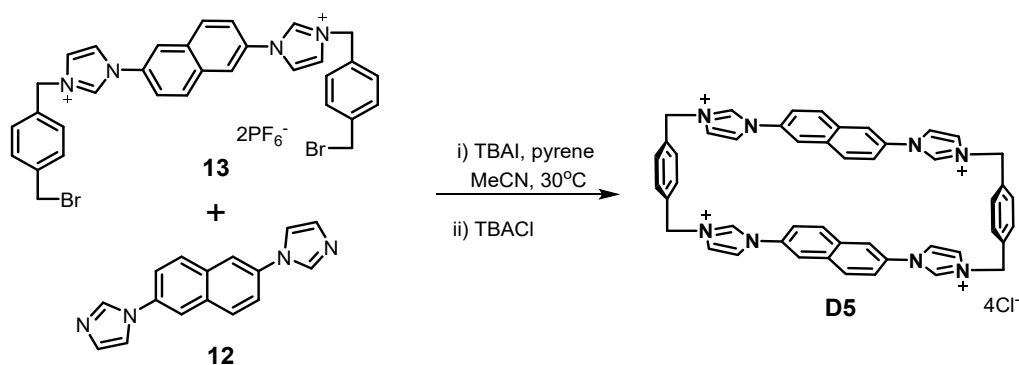
was collected by filtration and then to be subjected to column chromatography using silica gel and $\text{CH}_3\text{OH} : \text{H}_2\text{O} : \text{NH}_4\text{Cl}$ (aq, sat) = 6:3:1 as the eluents to yield the pure product **D4** (76 mg, 8%) as a yellow solid. ^1H NMR (400 MHz, D_2O) δ 9.04 (d, $J = 5.5$ Hz, 8H), 8.44 (d, $J = 6.6$ Hz, 8H), 8.40 (s, 4H), 7.74 (s, 8H), 7.41 (s, 4H), 5.83 (s, 8H), 4.43 (t, $J = 2.3$ Hz, 4H), 4.01 (t, $J = 2.1$ Hz, 4H), 3.74 (t, $J = 2.6$ Hz, 4H), 3.54 (t, $J = 2.2$ Hz, 4H), 3.25 (t, $J = 2.6$ Hz, 4H), 2.98 (t, $J = 2.1$ Hz, 4H), 2.85 (t, $J = 2.2$ Hz, 4H), 2.76 (t, $J = 2.7$ Hz, 4H), 2.64 (s, 12H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.75, 154.36, 144.06, 136.42, 132.57, 129.87, 126.96, 125.36, 115.05, 105.65, 70.94, 69.91, 69.73, 69.59, 69.52, 69.28, 68.57, 68.56, 57.73. HRMS calcd for $\text{C}_{92}\text{H}_{112}\text{N}_4\text{O}_{20}$ [M-4PF_6] $^{4+}$: 399.2040; found: 399.2042.



Compound 12. The mixture of 2,6-Dibromonaphthalene (1.0 g, 3.5 mmol), Imidazole (6.0 g, 88 mmol), K_2CO_3 (1.9 g, 14 mmol) and CuSO_4 (29 mg, 0.18 mmol) were stirred at 150°C . After 24 h, the reaction was cooled to rt and a large amount of white solid precipitated. Then, 15 mL of water was added to the system and stirred for 12 h. The deposit was filtered and washed with water and dried in vacuum to afford white solid **12** (0.76 g, 84%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.42 (s, 2H), 8.29 (d, $J = 2.2$ Hz, 2H), 8.12 (d, $J = 8.8$ Hz, 2H), 7.95 (dd, $J = 8.7, 2.2$ Hz, 2H), 7.91 (s, 2H), 7.18 (s, 2H).



Compound 13. α, α' -Dibromo-p-xylene (3.0 g, 11 mmol) was added to MeCN (60 mL) in a 250 mL round-bottomed three-neck flask and the resulting mixture was refluxed while stirring until all of the solid material dissolved. Next, the temperature of the oil bath was raised to 80°C , allowing the reaction mixture to reflux while a suspension of **12** (0.40 g, 1.5 mmol) in DCM (30 mL), was added slowly over 1 h. After heating under reflux for 48 h, the reaction mixture was cooled to room temperature and the orange precipitate was collected by filtration and washed with DCM (3×10 mL). The solid was then dissolved in DMF (20 mL) and 100 mL saturated solution of NH_4PF_6 was added subsequently. Then the precipitate formed was filtered, washed with water (10 mL \times 3) and dried to afford **13** as white solid (1.3 g, 92%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 10.21 (s, 2H), 8.59 (d, $J = 8.9$ Hz, 2H), 8.52 (s, 2H), 8.35 (d, $J = 8.8$ Hz, 2H), 8.14 (s, 2H), 8.10 (d, $J = 8.3$ Hz, 2H), 7.54 (s, 8H), 5.57 (s, 4H), 4.74 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 138.77, 136.08, 134.43, 133.41, 132.30, 130.58, 129.86, 128.81, 123.50, 121.86, 121.75, 120.68, 52.16, 33.72. HRMS calcd for $\text{C}_{32}\text{H}_{28}\text{Br}_2\text{N}_4\text{PF}_6$ [M-PF_6] $^+$: 773.0297; found: 773.0302.



D5. Adding **13** (0.40 g, 0.44 mmol), **12** (0.11 g, 0.44 mmol), TBAI (32 mg, 0.087 mmol), and the template Pyrene (0.44 g, 2.2 mmol) to dry MeCN (400 mL) and stirring at 30°C. After 7 days, the reaction was stopped by adding (t-Bu)₄NCl (1M in CH₃CN, 3 mL), causing the crude product to precipitate from solution. The orange precipitate was collected by filtration and then to be subjected to column chromatography using silica gel and CH₃OH : H₂O : NH₄Cl (aq, sat) = 6:3:1 as the eluents to yield the pure product (0.26 g, 68%) as an white solid. ¹H NMR (400 MHz, D₂O) δ 8.15 (d, *J* = 2.2 Hz, 4H), 8.12 (s, 2H), 8.10 (s, 2H), 8.06 (d, *J* = 2.1 Hz, 4H), 8.02 (d, *J* = 2.1 Hz, 4H), 7.72 (d, *J* = 2.2 Hz, 2H), 7.70 (d, *J* = 2.4 Hz, 2H), 7.69 (s, 8H), 5.61 (s, 8H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 135.34, 135.25, 133.07, 132.04, 130.40, 129.78, 124.36, 121.31, 120.86, 120.24, 52.54. HRMS calcd for C₄₈H₄₀N₈P₃F₁₈ [M-PF₆]⁺: 1163.2296; found: 1163.2301.

6. ¹H NMR Titrations

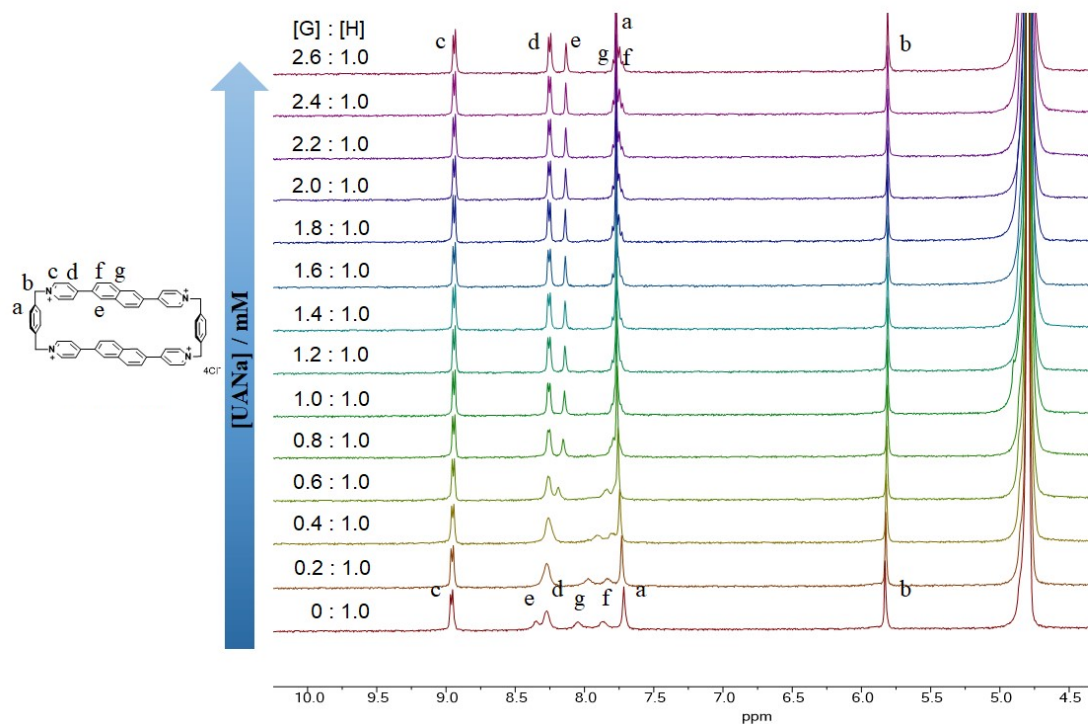


Figure S1. ¹H NMR spectra (400 MHz) of the mixtures of **D1** (0.2 mM) and UANa (0-0.52 mM) in D₂O at 25°C.

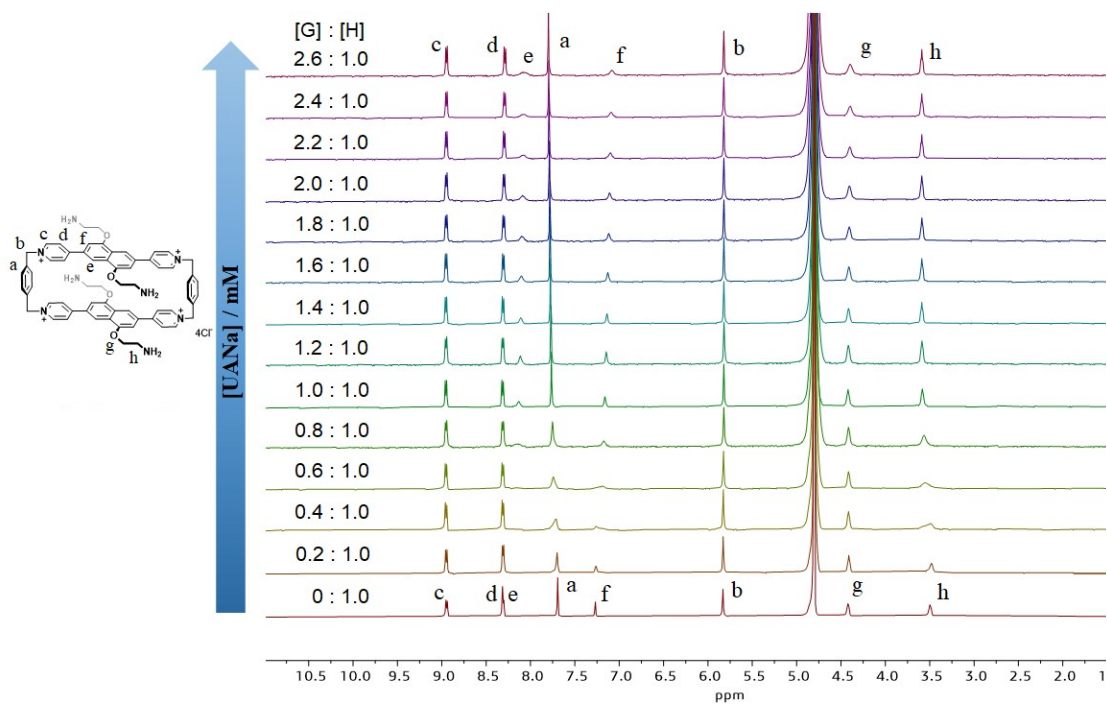


Figure S2. ^1H NMR spectra (400 MHz) of the mixtures of **D2** (0.2 mM) and UANa (0-0.52 mM) in D_2O at 25°C .

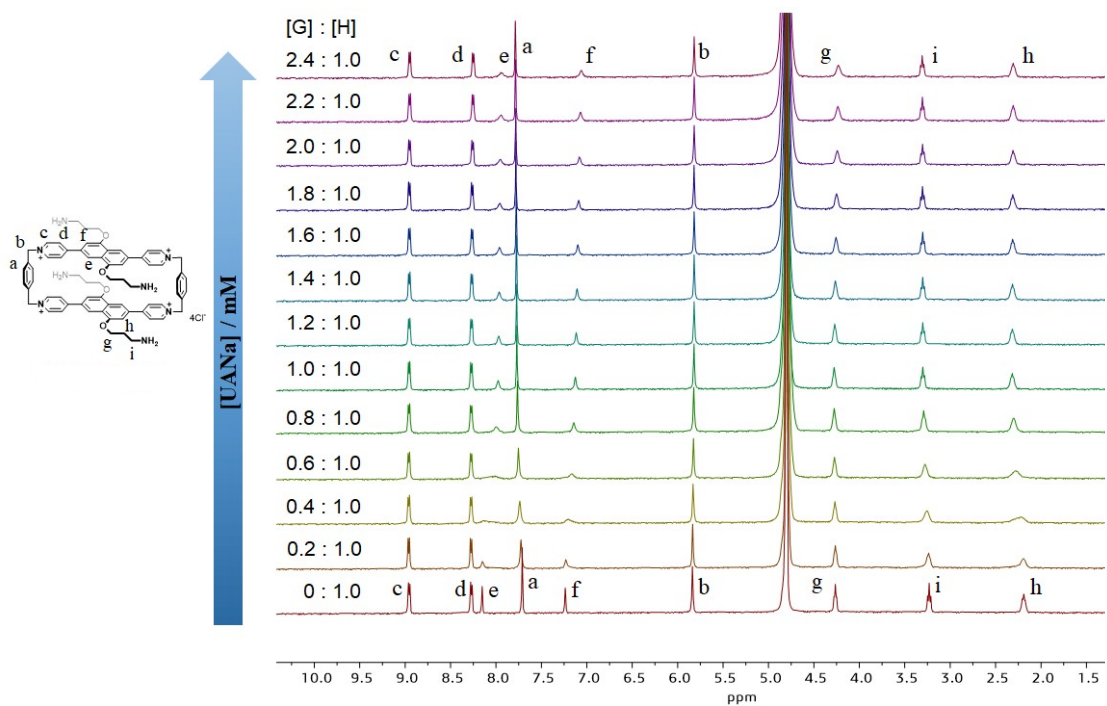


Figure S3. ^1H NMR spectra (400 MHz) of the mixtures of **D3** (0.2 mM) and UANa (0-0.52 mM) in D_2O at 25°C .

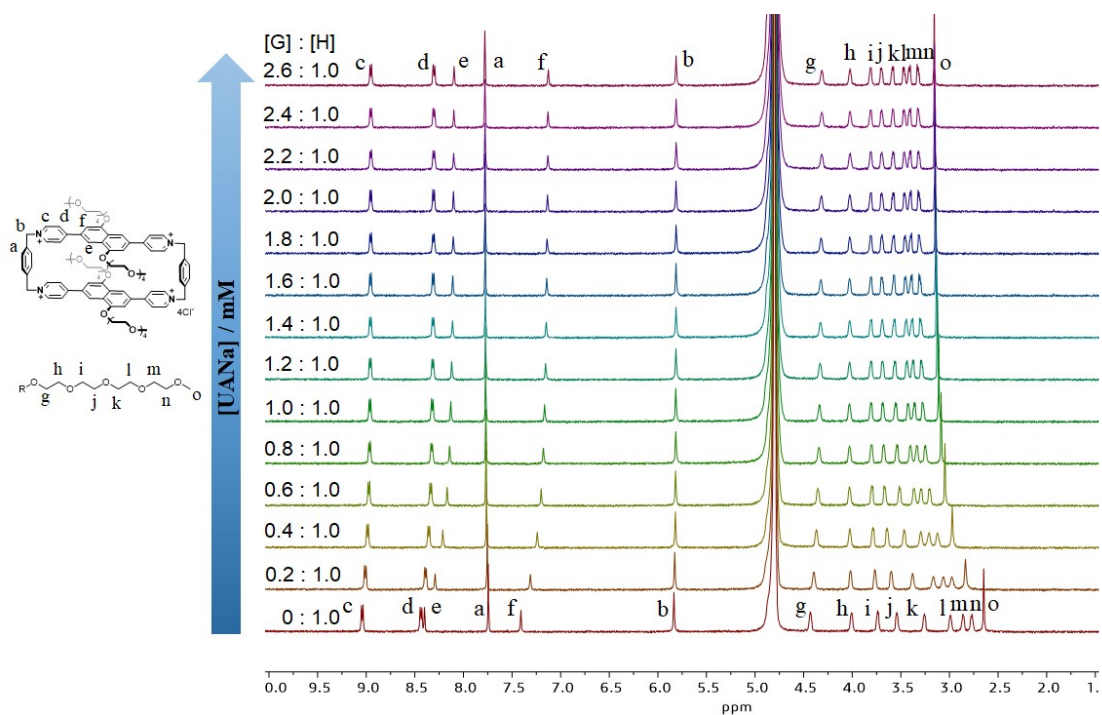


Figure S4. ^1H NMR spectra (400 MHz) of the mixtures of **D4** (0.2 mM) and UANa (0-0.52 mM) in D_2O at 25°C .

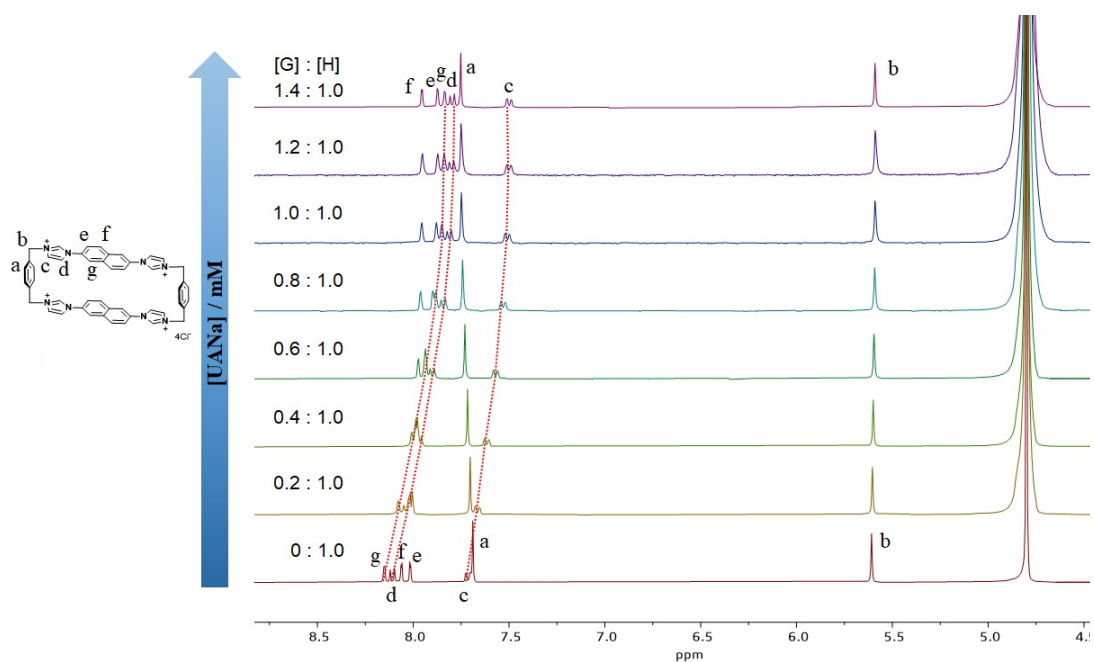


Figure S5. ^1H NMR spectra (400 MHz) of the mixtures of **D5** (0.2 mM) and UANa (0-0.52 mM) in D_2O at 25°C .

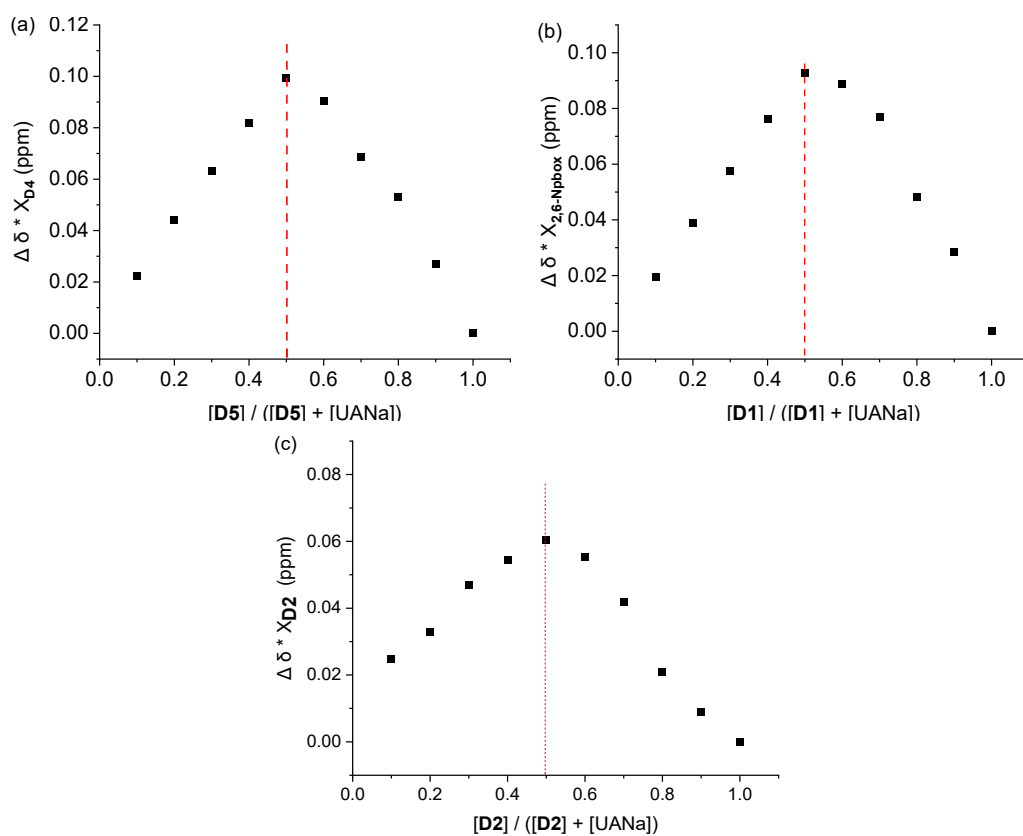


Figure S6. Job's plot of UANa and (a) **D5**; (b) **D1**; (c) **D2**.

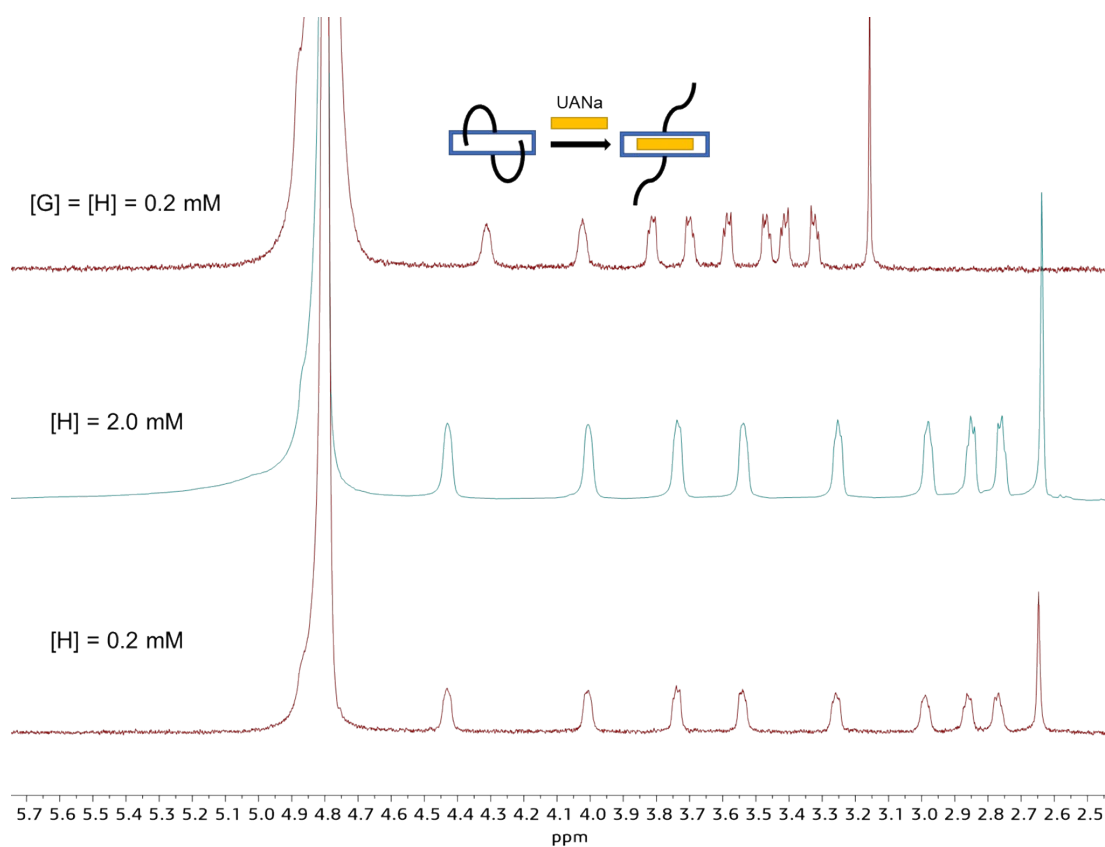


Figure S7. ^1H NMR spectra (400 MHz) of the side chains of **D4** (0.2 mM and 2.0 mM), and the 1:1 mixture of **D4** and UANa ($[\text{D4}] = [\text{UANa}] = 0.2 \text{ mM}$) in D_2O at 25°C .

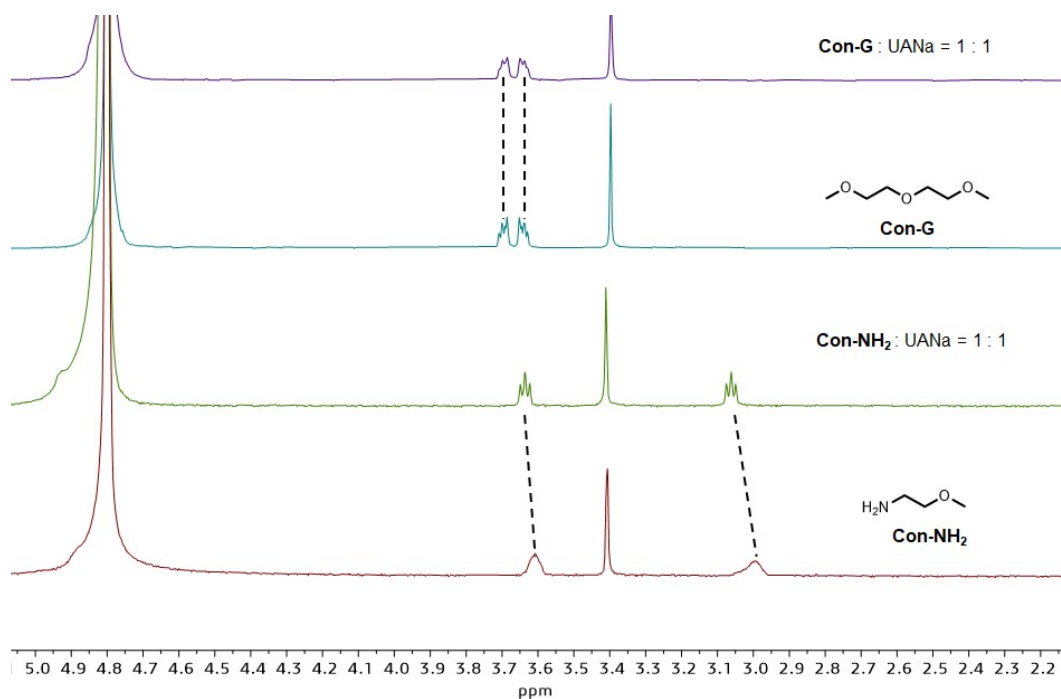


Figure S8. ^1H NMR spectra (400 MHz) of 2-Methoxyethylamine (Con-NH₂, 0.2mM), Diglyme (Con-G, 0.2mM) and their respective 1:1 mixture with UANa (0.2 mM) in D₂O at 25°C.

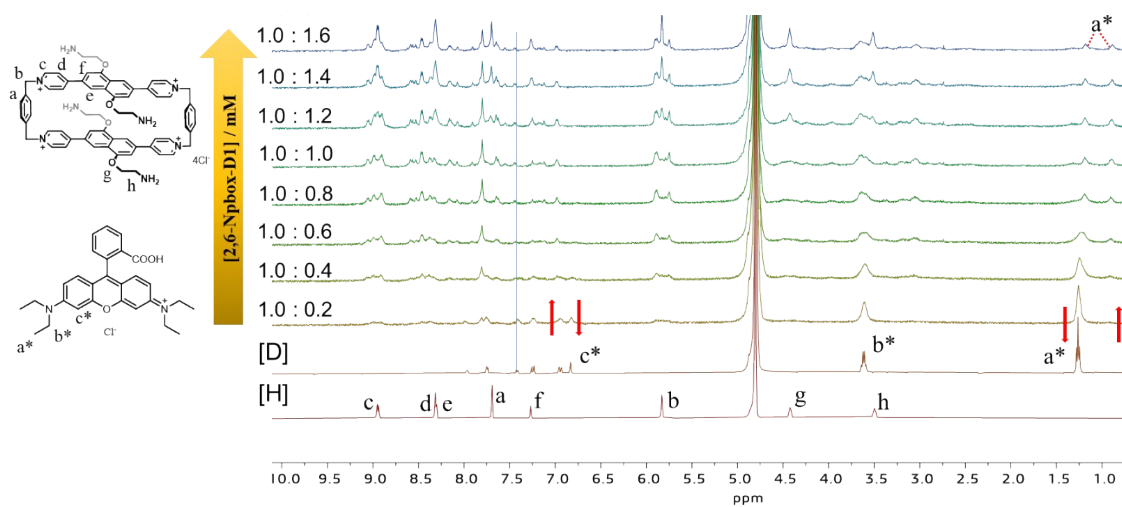


Figure S9. ^1H NMR spectra (400 MHz) of the mixtures of Rhodamine B [D] (0.2 mM) and D2 [H] (0-0.32 mM) in D₂O at 25°C.

7. UV/vis and Fluorescence Titrations

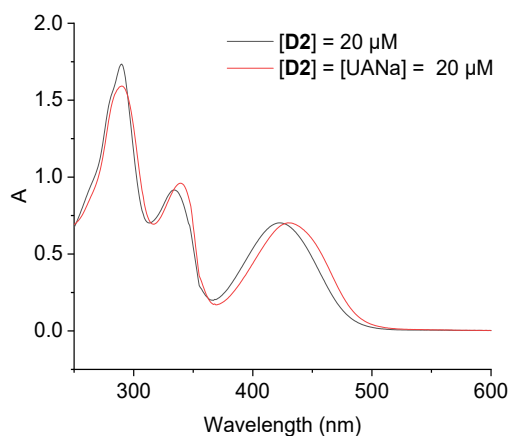


Figure S10. The UV-vis spectra of **D2** (20 μM) before and after the addition of UANa (20 μM) in 20 mM phosphate buffer (pH = 7.4).

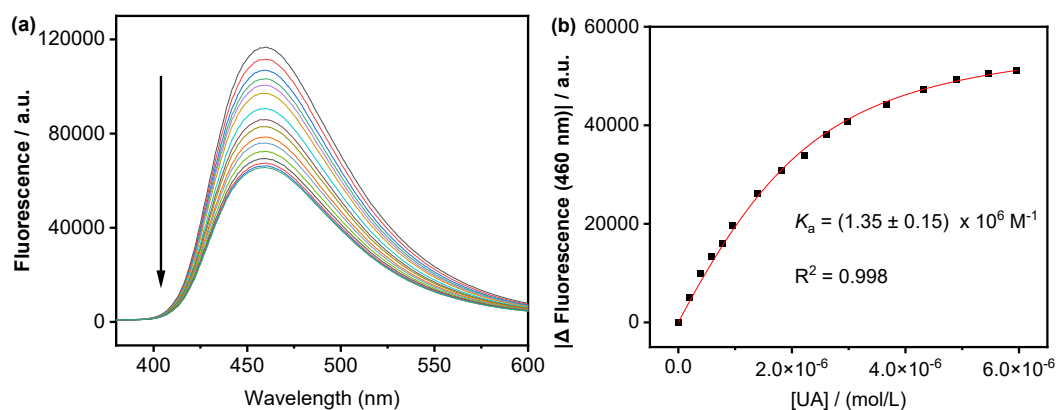


Figure S11. (a) The fluorescence spectra of **D1** (2 μM , $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of UA in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data to a 1:1 binding mode, the absolute value of fluorescence change in 460 nm was taken for K_a calculating (The same below).

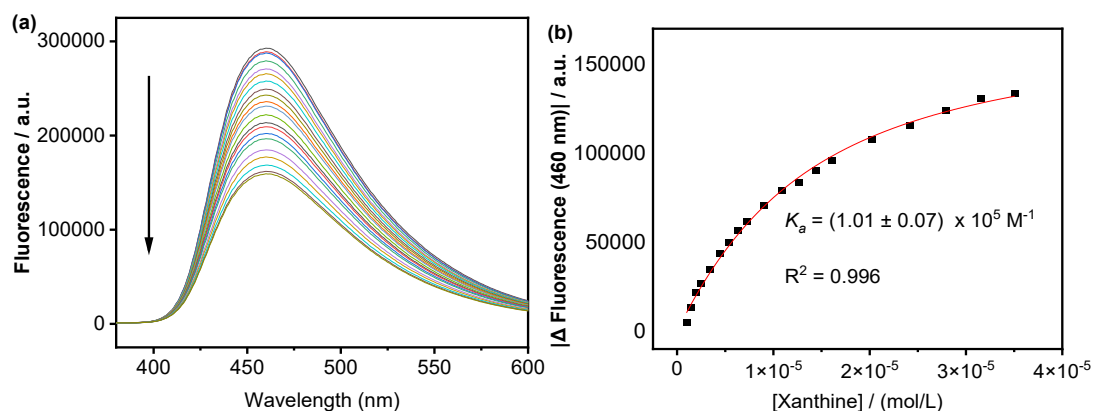


Figure S12. (a) The fluorescence spectra of **D1** (6 μM , $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of Xanthine in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

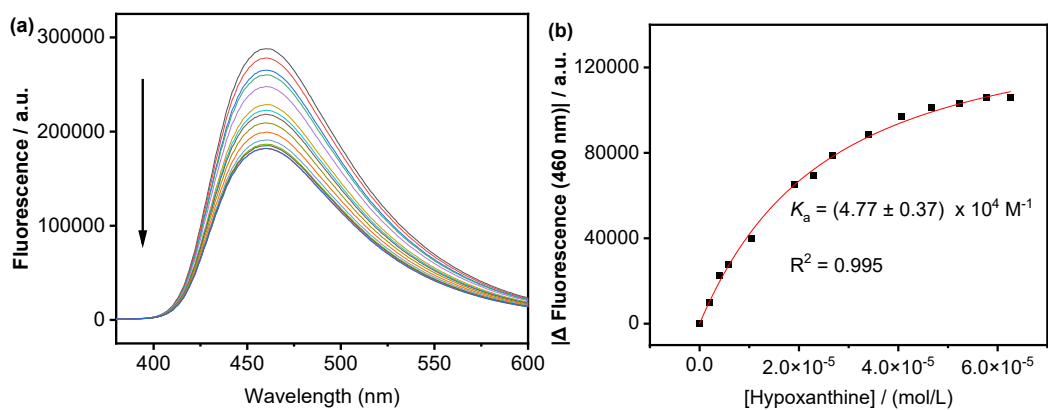


Figure S13. (a) The fluorescence spectra of **D1** ($6 \mu\text{M}$, $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of Hypoxanthine in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

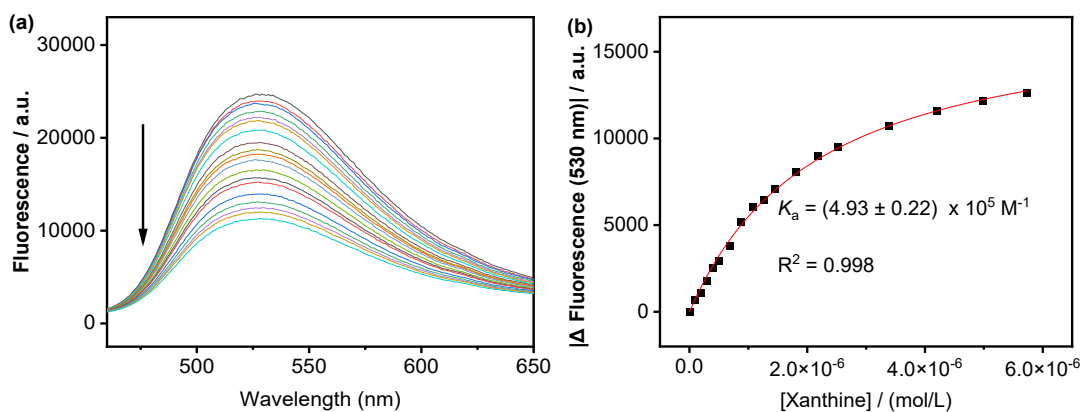


Figure S14. (a) The fluorescence spectra of **D2** ($0.5 \mu\text{M}$, $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of Xanthine in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

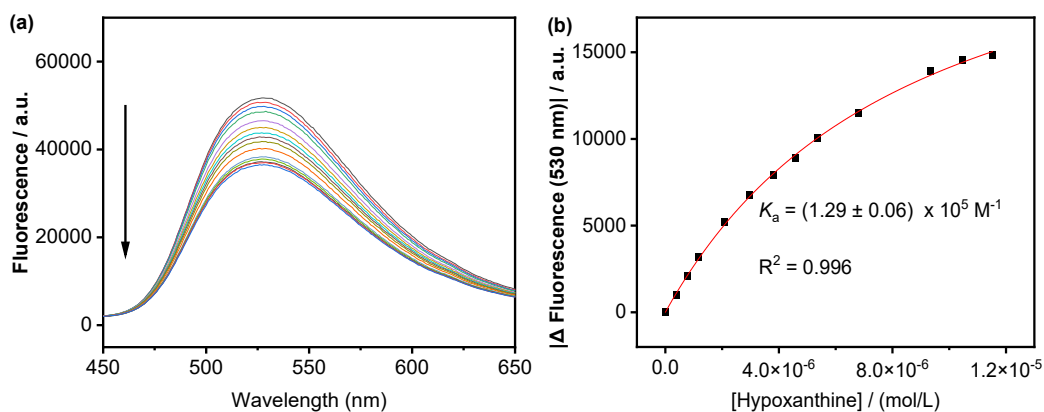


Figure S15. (a) The fluorescence spectra of **D2** ($1 \mu\text{M}$, $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of Hypoxanthine in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

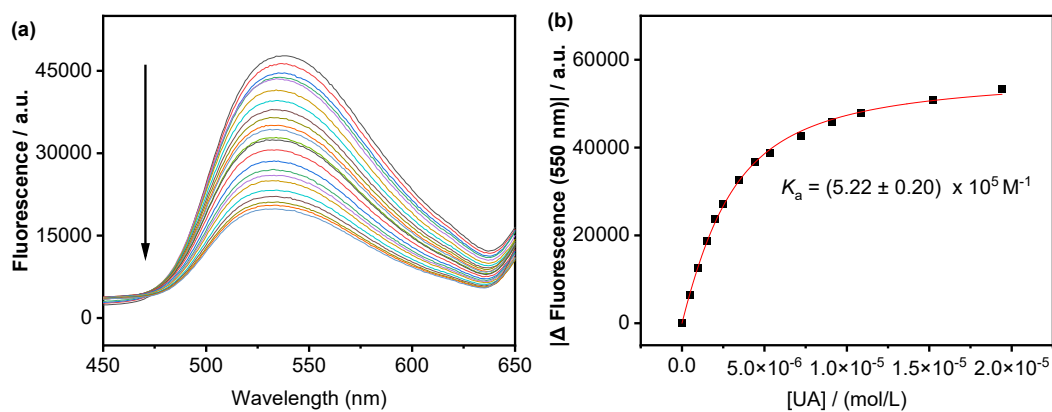


Figure S16. (a) The fluorescence spectra of **D3** ($1 \mu\text{M}$, $\lambda_{\text{ex}} = 330 \text{ nm}$) with the addition of UA in 20 mM phosphate buffer ($\text{pH} = 7.4$); (b) Fit of the titration data.

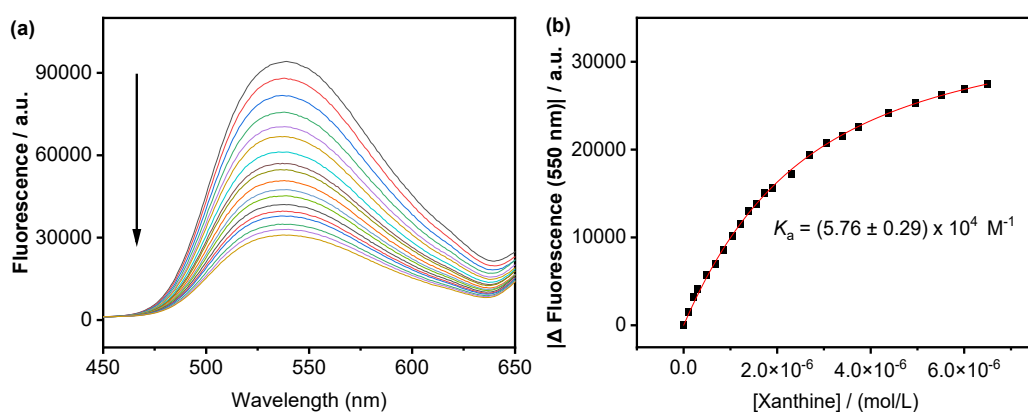


Figure S17. (a) The fluorescence spectra of **D3** ($2 \mu\text{M}$, $\lambda_{\text{ex}} = 330 \text{ nm}$) with the addition of Xanthine in 20 mM phosphate buffer ($\text{pH} = 7.4$); (b) Fit of the titration data.

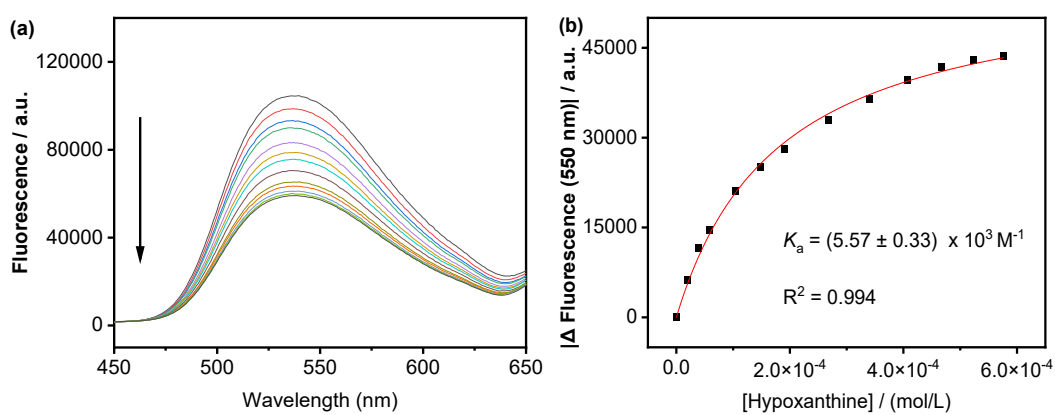


Figure S18. (a) The fluorescence spectra of **D3** ($2 \mu\text{M}$, $\lambda_{\text{ex}} = 330 \text{ nm}$) with the addition of Hypoxanthine in 20 mM phosphate buffer ($\text{pH} = 7.4$); (b) Fit of the titration data.

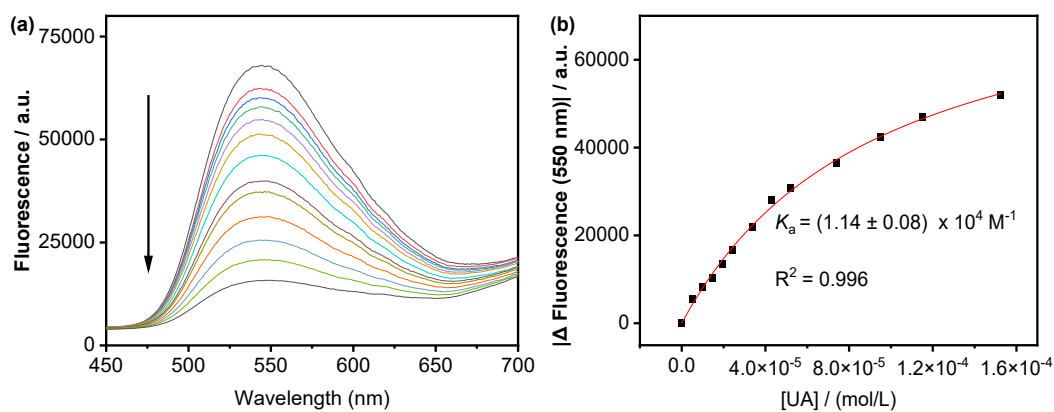


Figure S19. (a) The fluorescence spectra of **D4** ($6 \mu\text{M}$, $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of UA in 20 mM phosphate buffer ($\text{pH} = 7.4$); (b) Fit of the titration data.

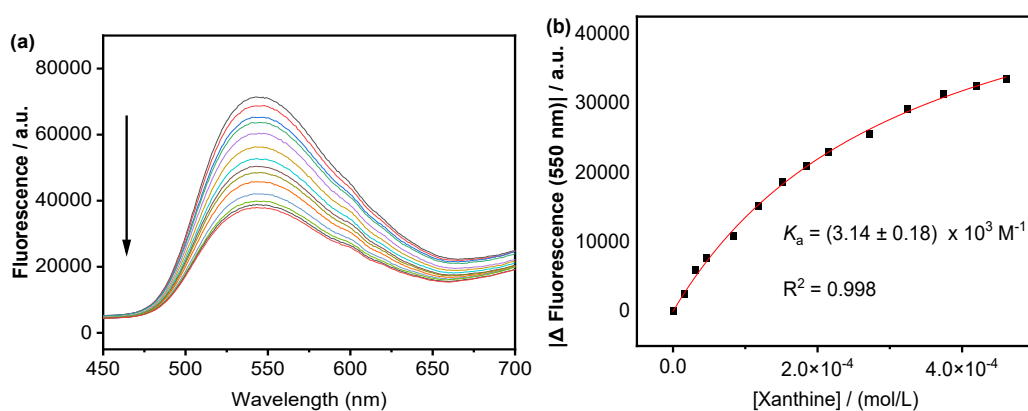


Figure S20. (a) The fluorescence spectra of **D4** ($6 \mu\text{M}$, $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of Xanthine in 20 mM phosphate buffer ($\text{pH} = 7.4$); (b) Fit of the titration data.

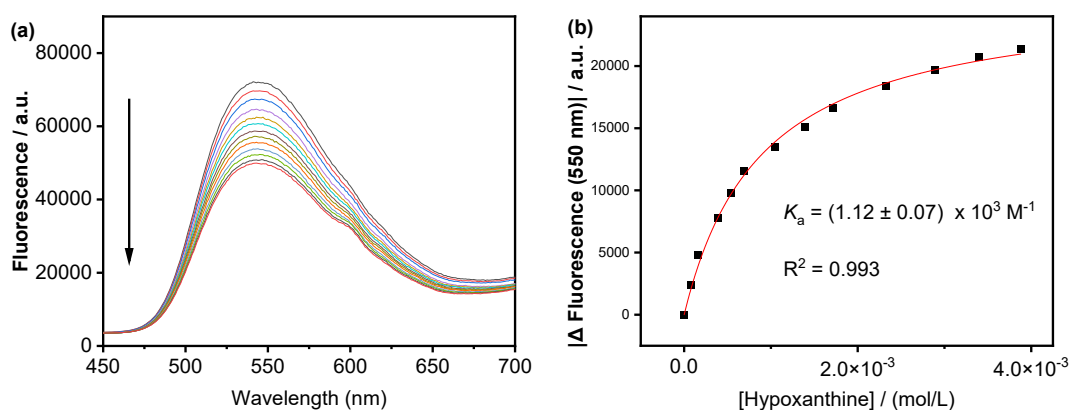


Figure S21. (a) The fluorescence spectra of **D4** ($6 \mu\text{M}$, $\lambda_{\text{ex}} = 360 \text{ nm}$) with the addition of Hypoxanthine in 20 mM phosphate buffer ($\text{pH} = 7.4$); (b) Fit of the titration data.

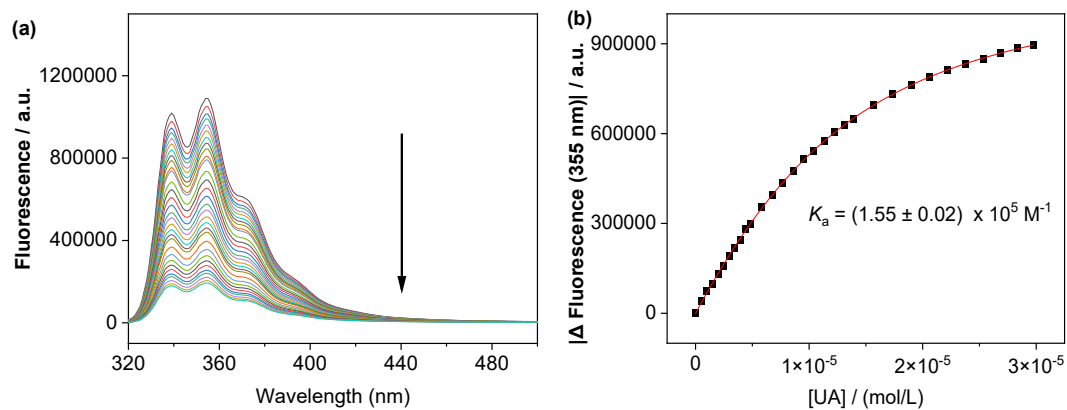


Figure S22. (a) The fluorescence spectra of **D5** ($10\ \mu\text{M}$, $\lambda_{\text{ex}} = 280\ \text{nm}$) with the addition of UA in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

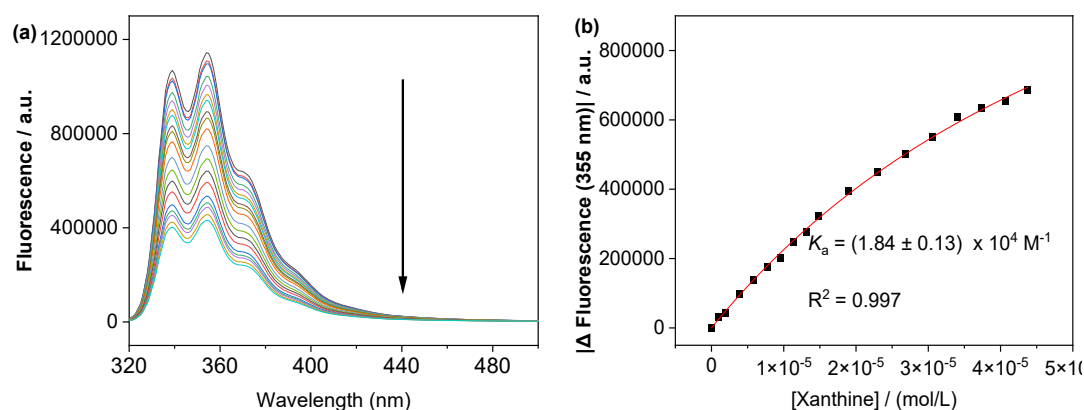


Figure S23. (a) The fluorescence spectra of **D5** ($10\ \mu\text{M}$, $\lambda_{\text{ex}} = 280\ \text{nm}$) with the addition of Xanthine in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

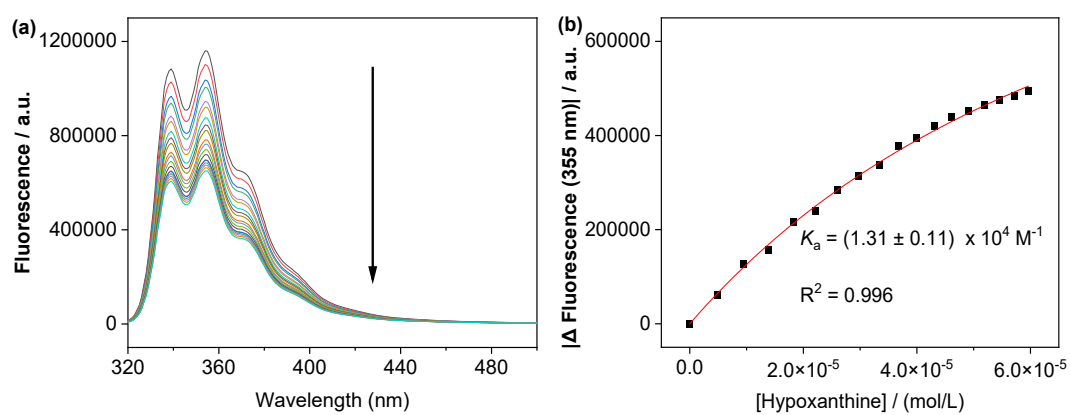


Figure S24. (a) The fluorescence spectra of **D5** ($10\ \mu\text{M}$, $\lambda_{\text{ex}} = 280\ \text{nm}$) with the addition of Hypoxanthine in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

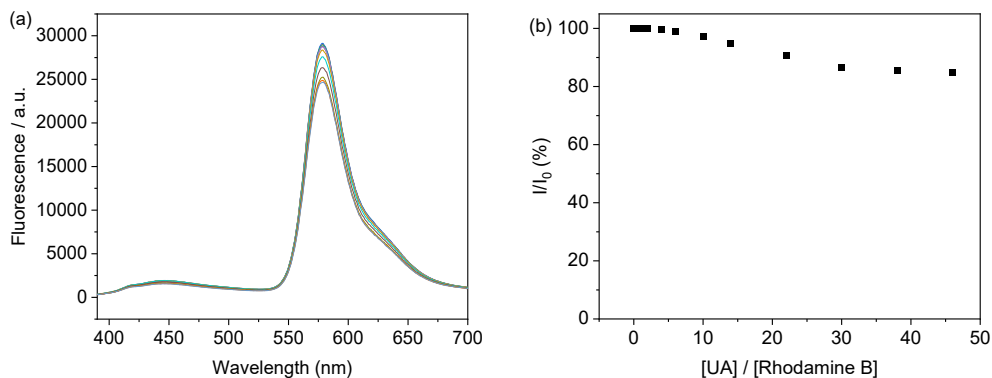


Figure S25. The relationship of I/I_0 to $[UA] / [\text{Rhodamine B}]$. I_0 and I are the fluorescence intensities (580 nm) of the Rhodamine B ($1 \mu\text{M}$) in the presence of increasing concentration of UA ($\lambda_{\text{ex}} = 365 \text{ nm}$, 20 mM phosphate buffer, pH = 7.4).

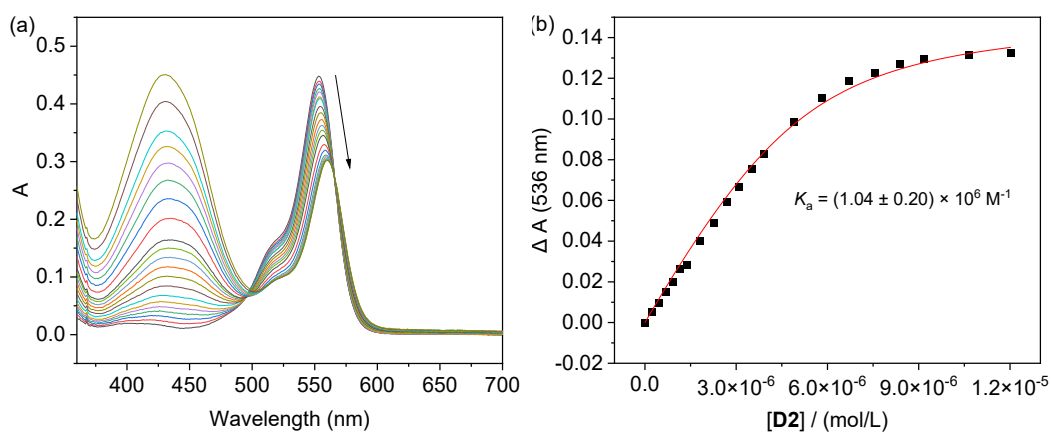


Figure S26. (a) The UV-vis spectra of Rhodamine B ($5 \mu\text{M}$) with the addition of **D2** in 20 mM phosphate buffer (pH = 7.4); (b) Fit of the titration data.

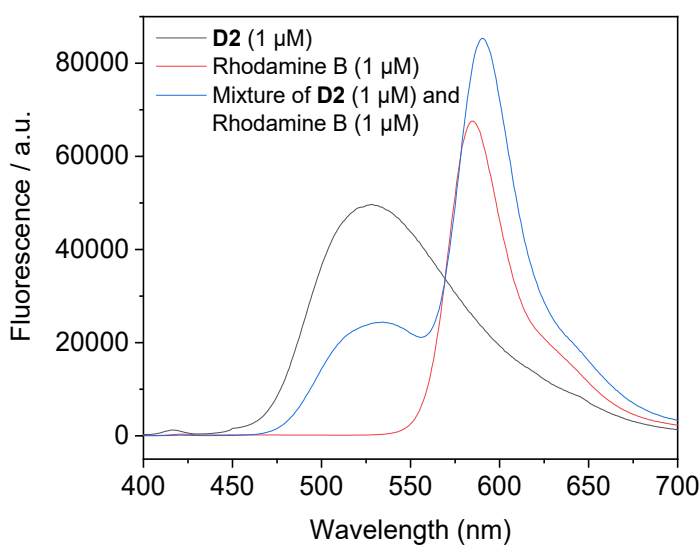


Figure S27. The fluorescence spectra of Rhodamine B ($1 \mu\text{M}$, $\lambda_{\text{ex}} = 365 \text{ nm}$) with the addition of **D2** ($1 \mu\text{M}$) in 20 mM phosphate buffer (pH = 7.4).

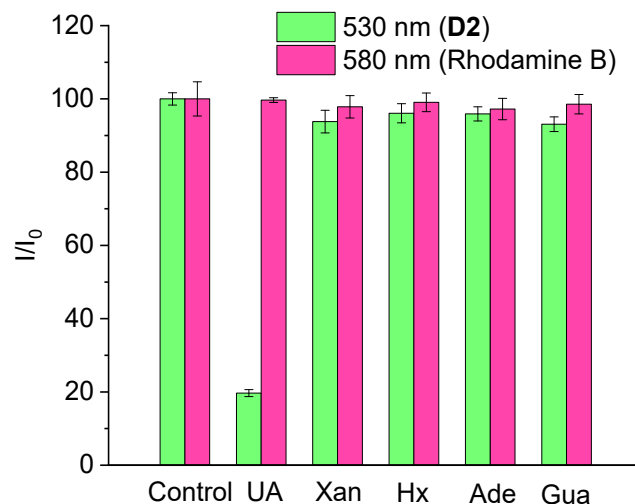
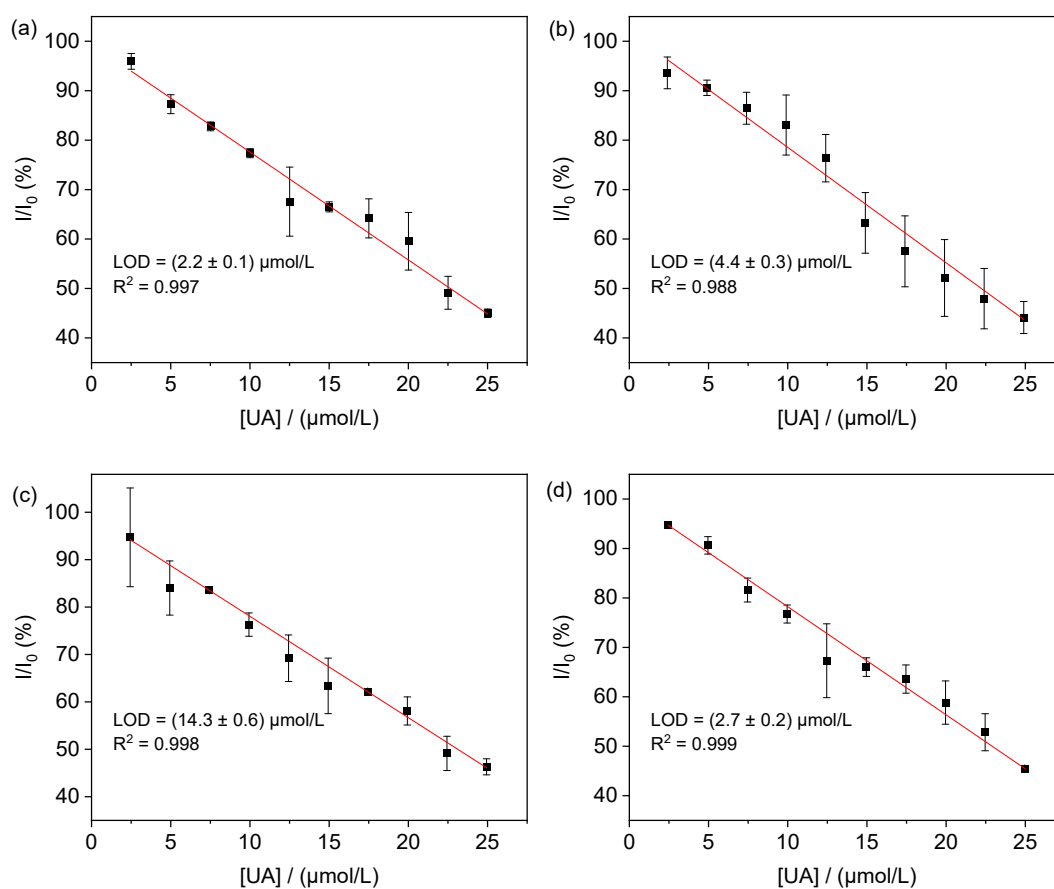


Figure S28. Fluorescence changes of the **D2**-RHB solution in PBS upon addition of UA or endogenous substances ($[D2] = 15 \mu\text{M}$, $[RHB] = 1 \mu\text{M}$, $\lambda_{\text{ex}} = 365 \text{ nm}$). I_0 : control group (**D2**/RHB). I : Experimental groups, adding UA ($25 \mu\text{M}$) or purine analogues ($2.5 \mu\text{M}$), including xanthine (Xan), hypoxanthine (HX), adenine (Ade) and guanine (Gua) ($n = 3$).



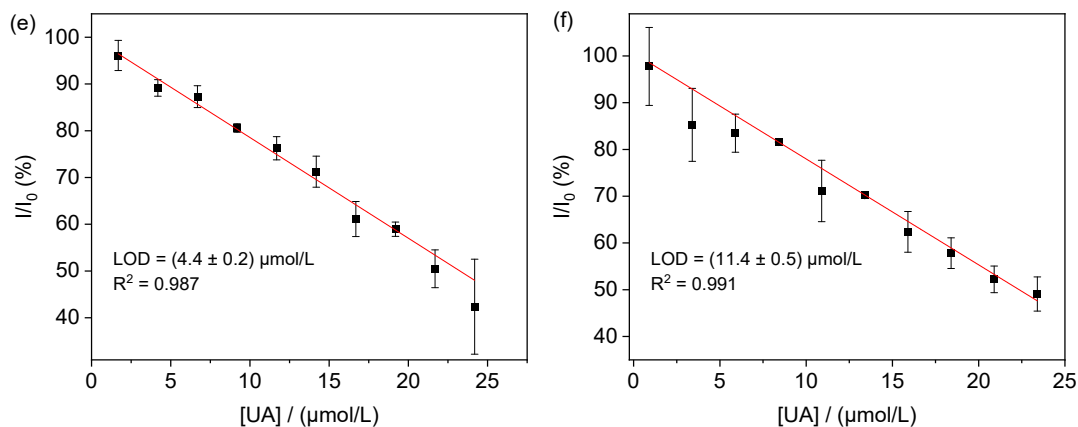


Figure S29. The calibration curves and LOD of UA acquired in the 4-fold diluted urine from different volunteers (a-f) at 25°C ($n = 3$). I_0 and I are the fluorescence intensities of the **D2**/RHB (15/1 μM) before and after addition of UA. The mixture of 10 μL 4-fold diluted urine sample and 10 μL UA with different concentration (0-6 mM) was added in 1980 μL probe solution ($[\text{RHB}] = 1 \mu\text{M}$, $[\text{D2}] = 15 \mu\text{M}$, 20 mM PB, pH = 7.4, $\lambda_{\text{ex}} = 365 \text{ nm}$).

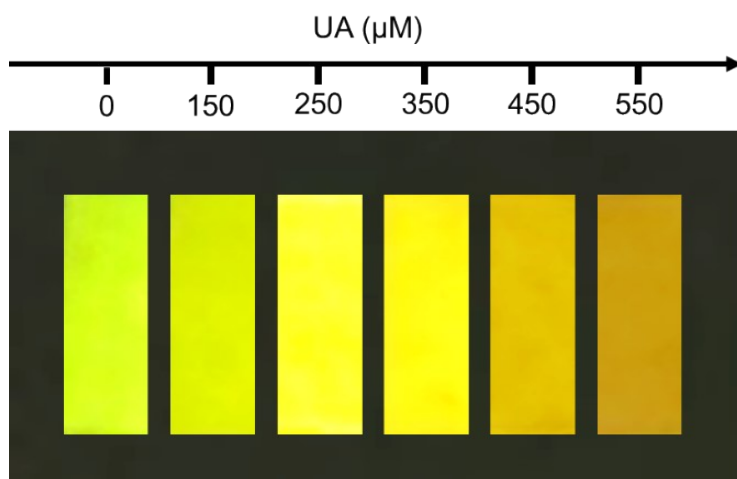


Figure S30. Visualization of standard solutions of UA using the **D2**@RHB loaded fluorescent test papers. The photos were taken under 365 nm UV lamp.

8. NMR Spectra of Compounds

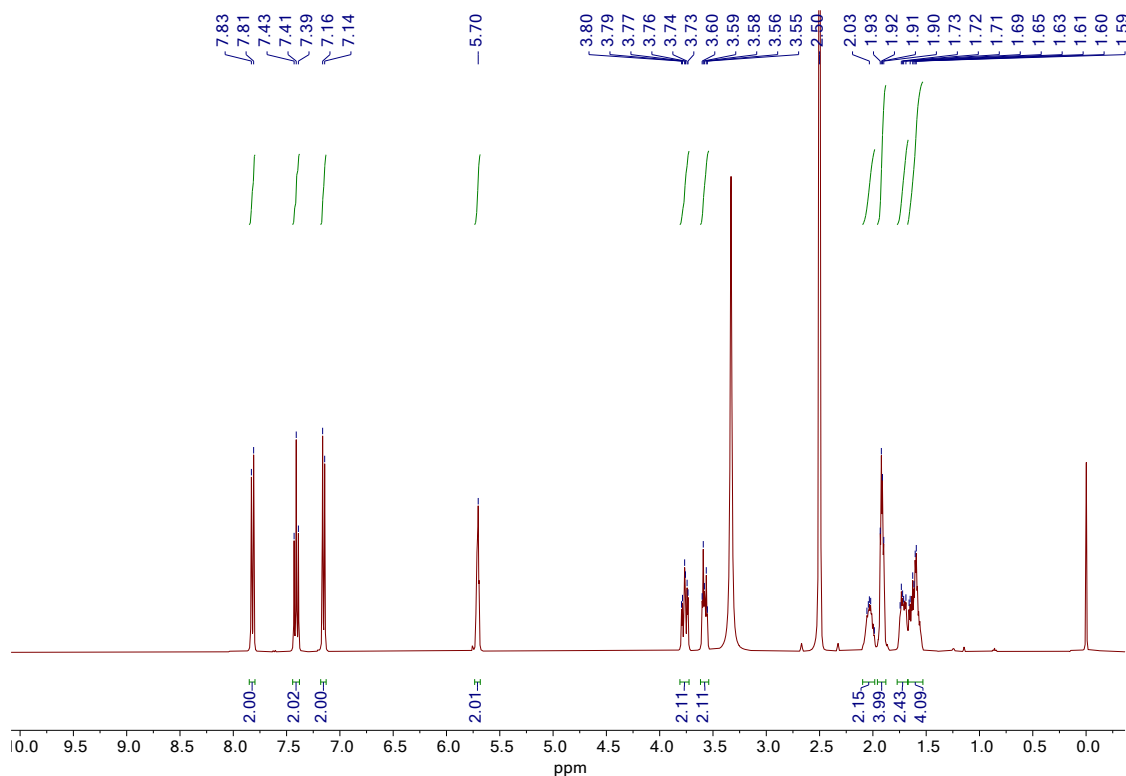


Figure S31. ¹H NMR spectrum (400 MHz) of **3** in DMSO-*d*₆ at 25°C.

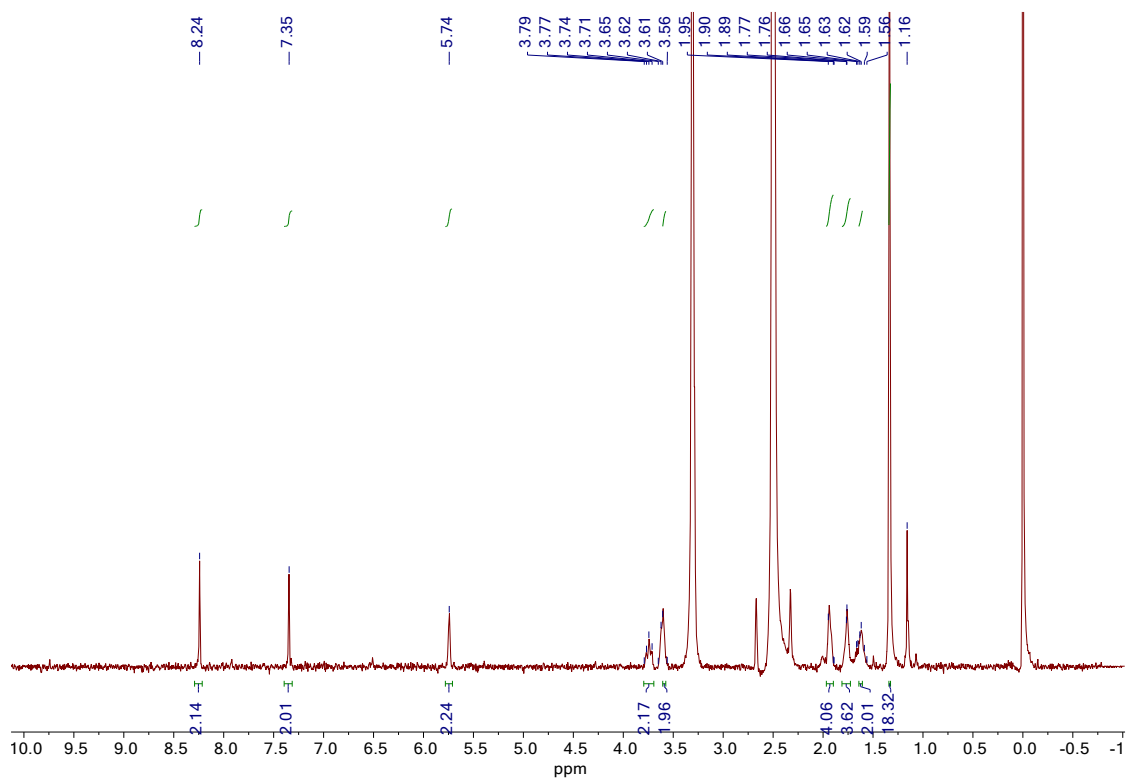


Figure S32. ¹H NMR spectrum (400 MHz) of **5** in DMSO-*d*₆ at 25°C.

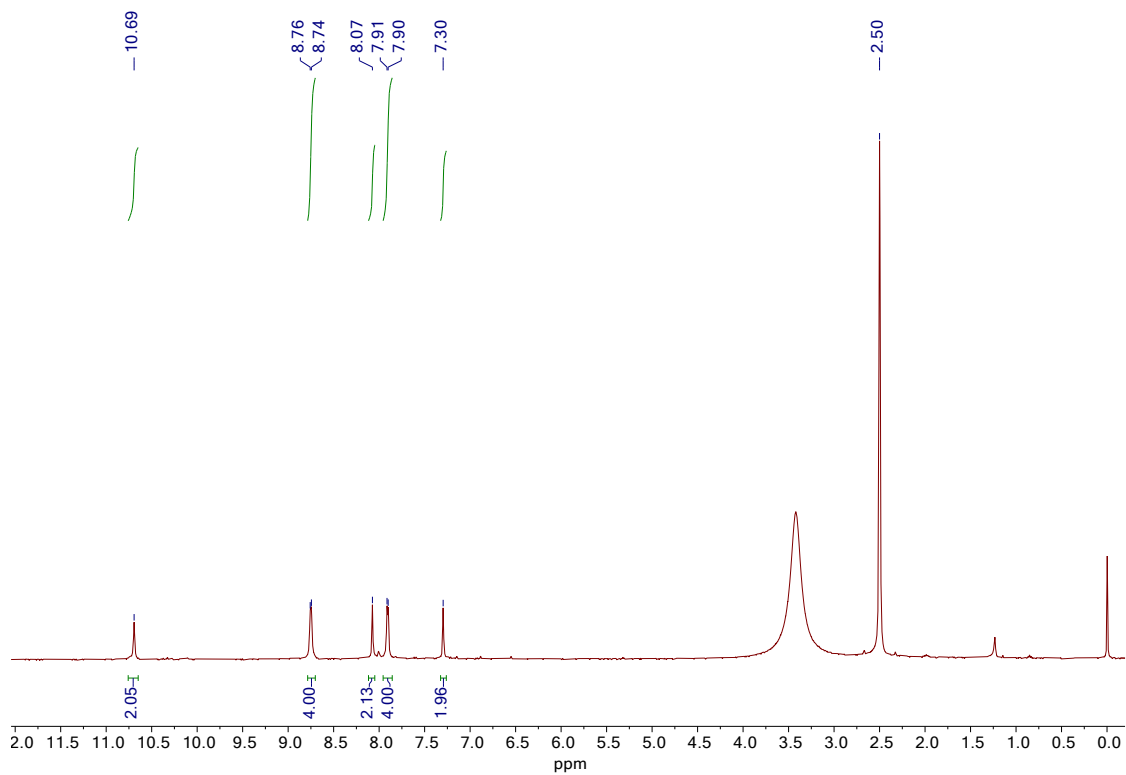


Figure S33. ^1H NMR spectrum (400 MHz) of **7** in $\text{DMSO-}d_6$ at 25°C .

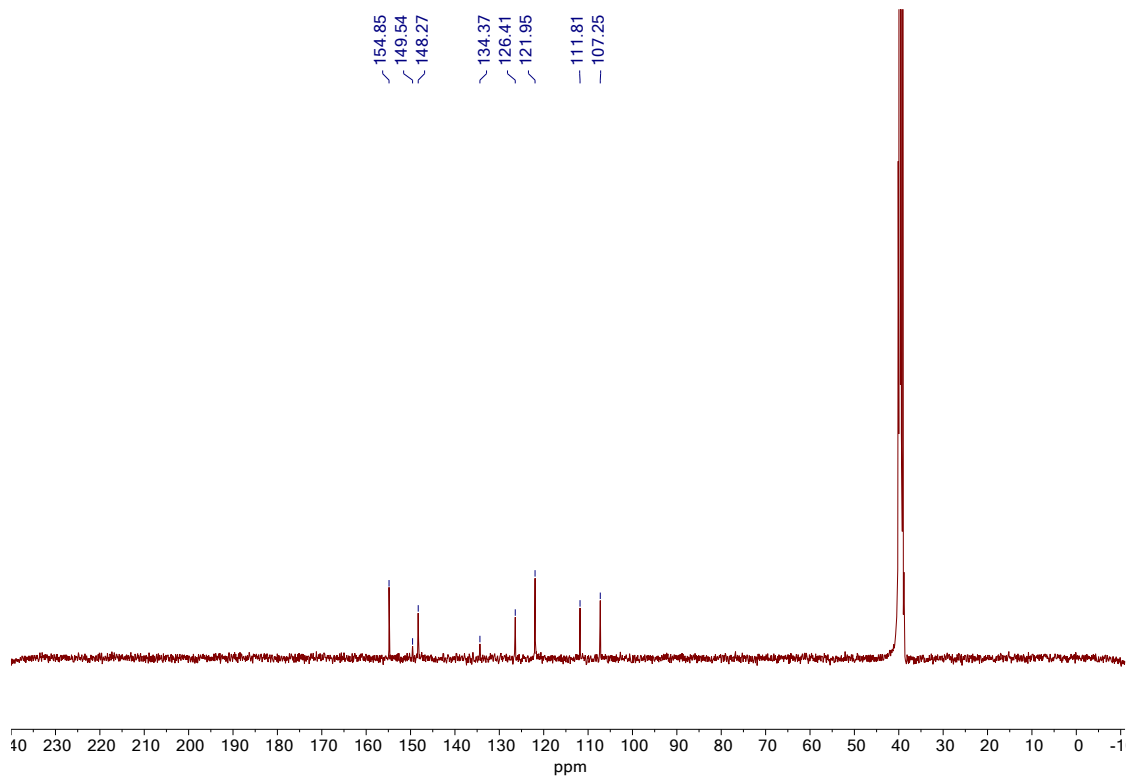


Figure S34. ^{13}C NMR spectrum (101 MHz) of **7** in $\text{DMSO-}d_6$ at 25°C .

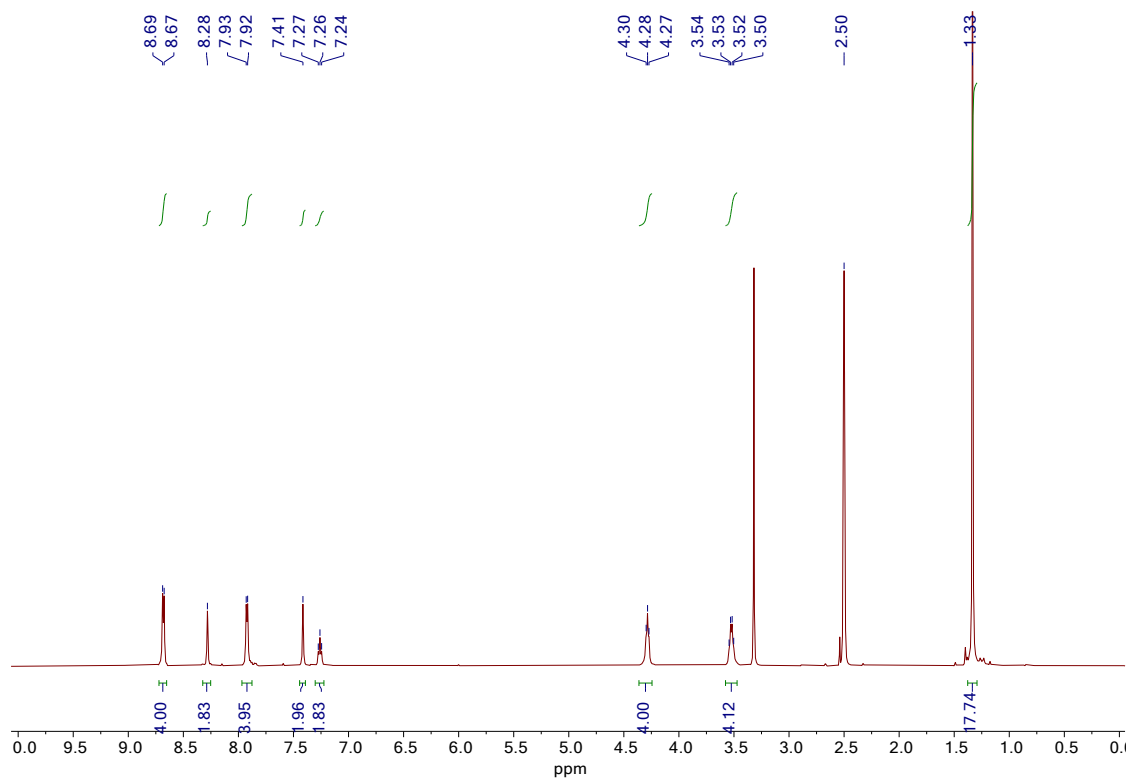


Figure S35. ^1H NMR spectrum (400 MHz) of **9a** in $\text{DMSO-}d_6$ at 25°C .

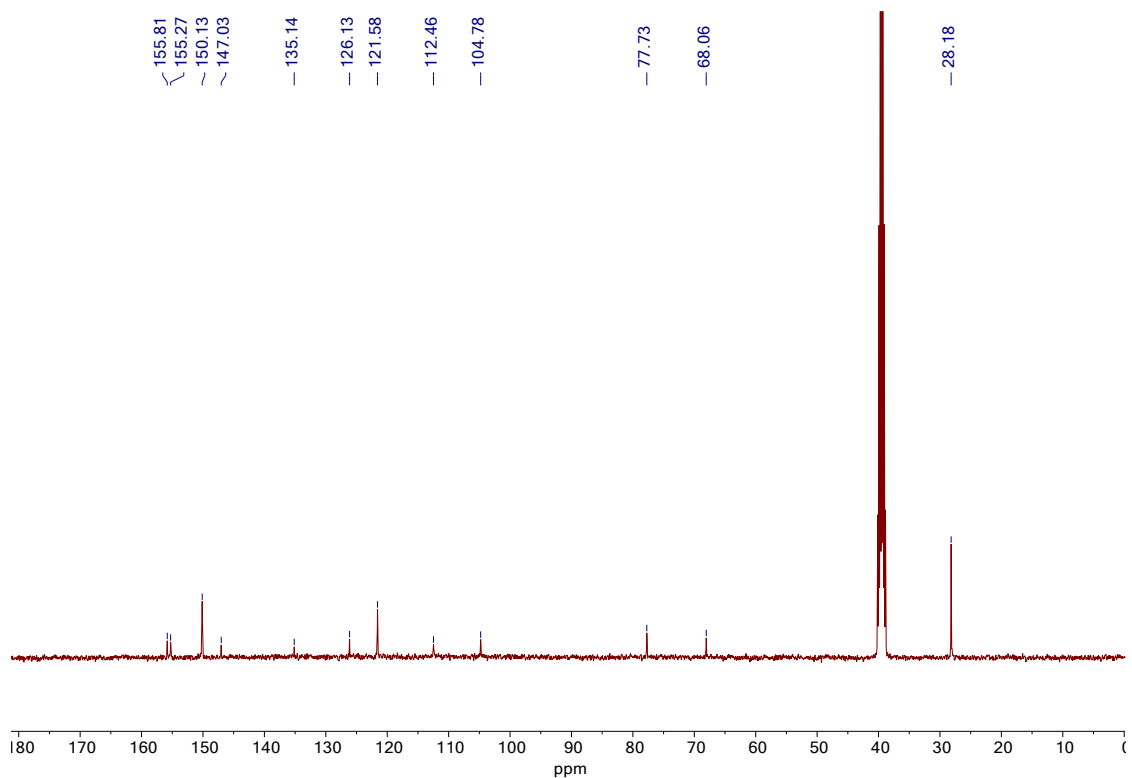


Figure S36. ^{13}C NMR spectrum (101 MHz) of **9a** in $\text{DMSO-}d_6$ at 25°C .

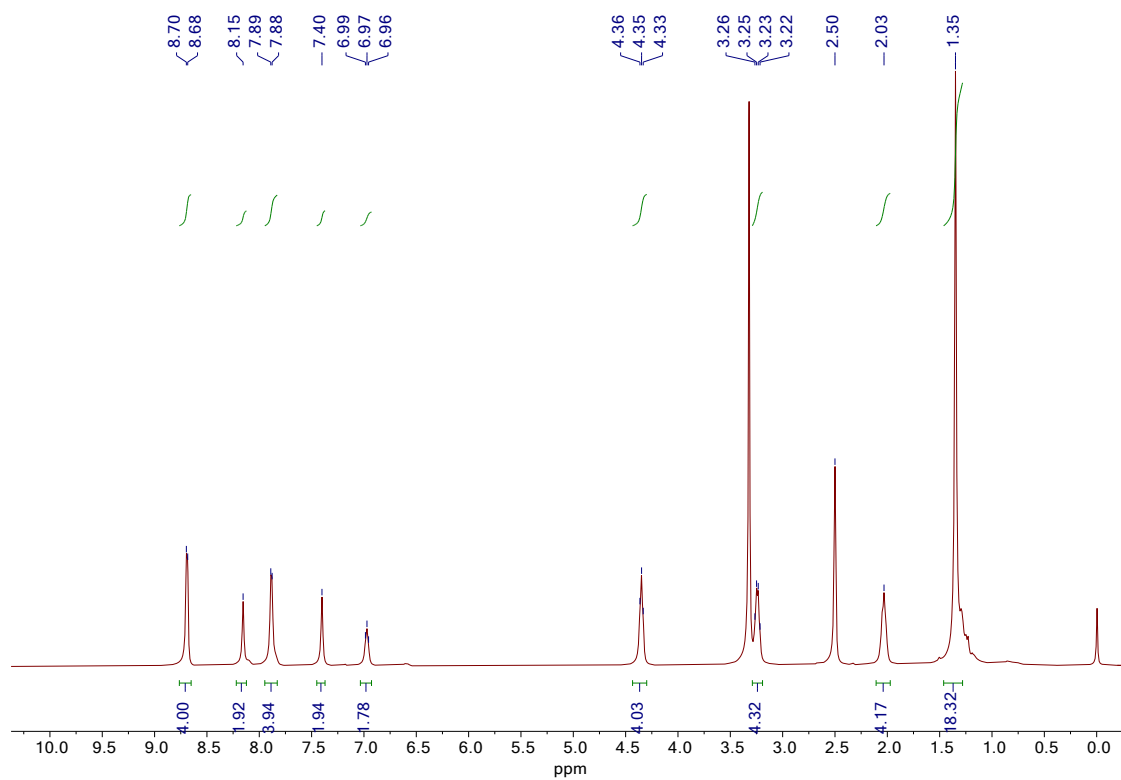


Figure S37. ^1H NMR spectrum (400 MHz) of **9b** in $\text{DMSO-}d_6$ at 25°C .

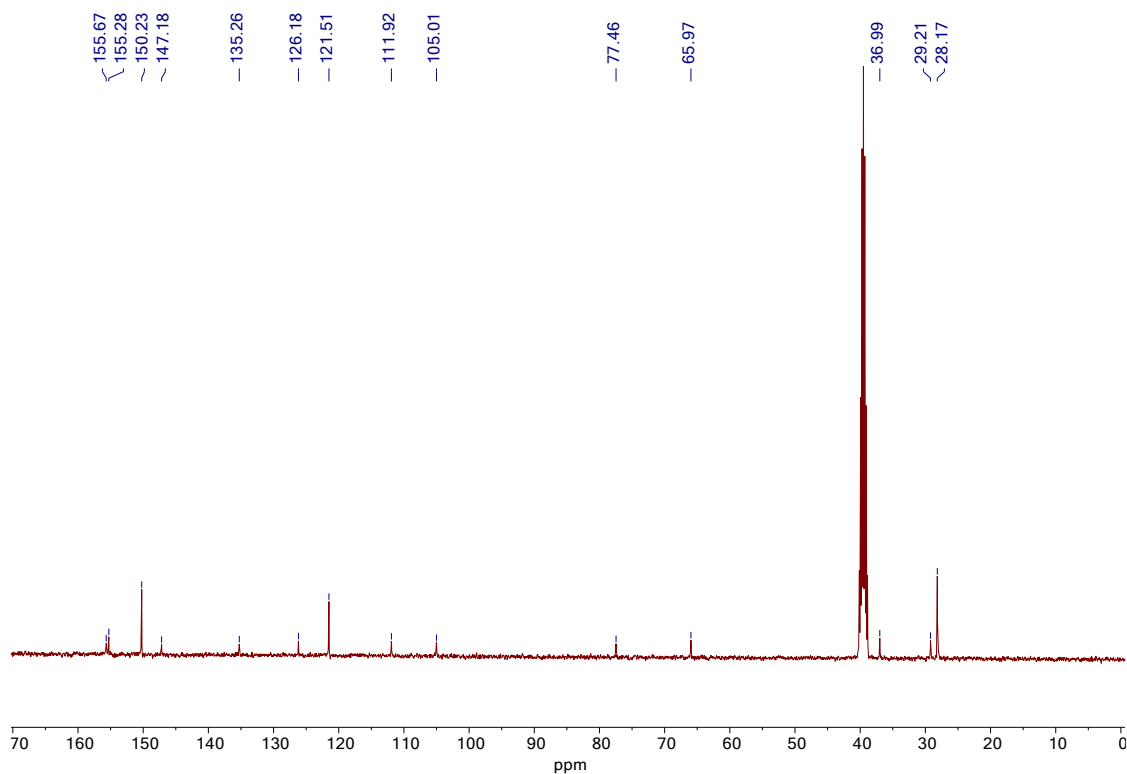


Figure S38. ^{13}C NMR spectrum (101 MHz) of **9b** in $\text{DMSO-}d_6$ at 25°C .

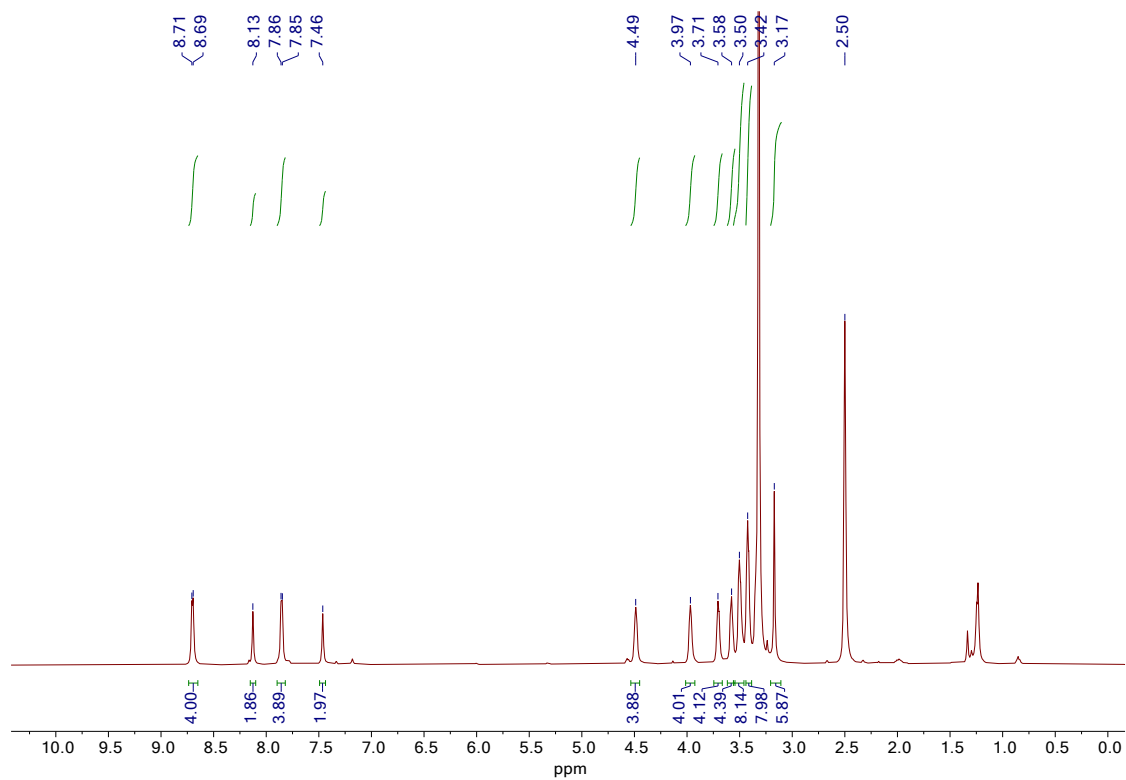


Figure S39. ^1H NMR spectrum (400 MHz) of **9c** in $\text{DMSO-}d_6$ at 25°C .

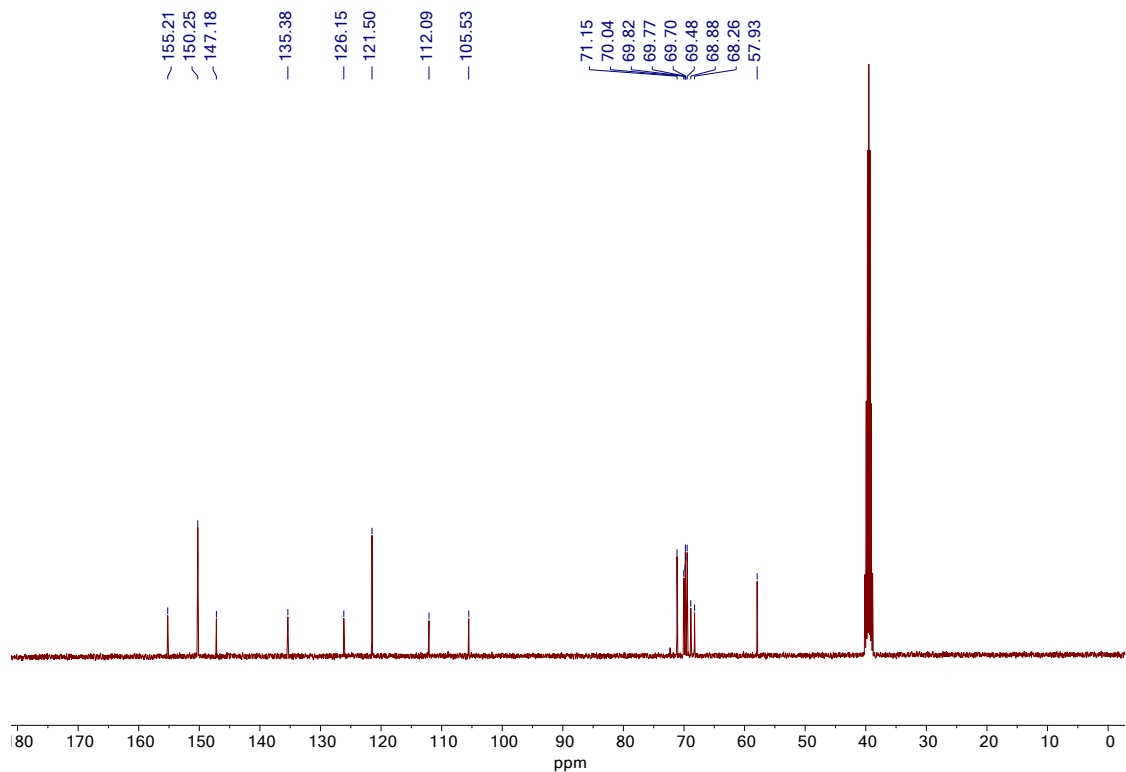


Figure S40. ^{13}C NMR spectrum (101 MHz) of **9c** in $\text{DMSO-}d_6$ at 25°C .

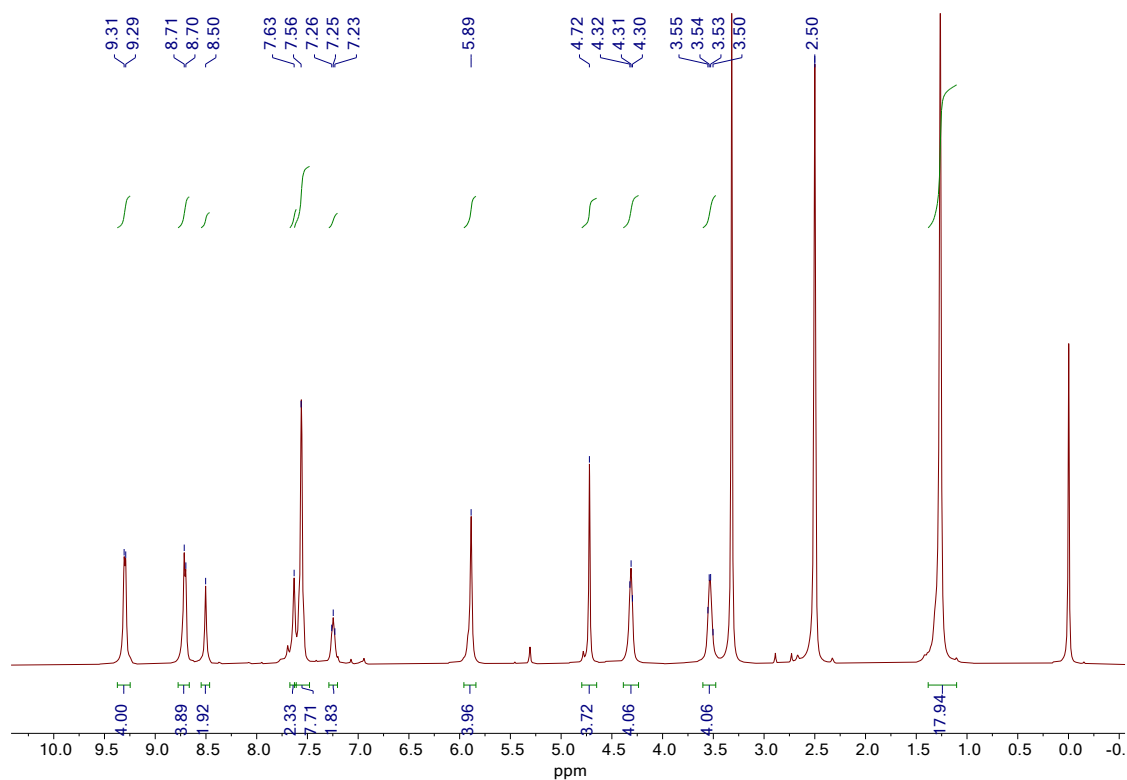


Figure S41. ^1H NMR spectrum (400 MHz) of **11a** in $\text{DMSO-}d_6$ at 25°C .

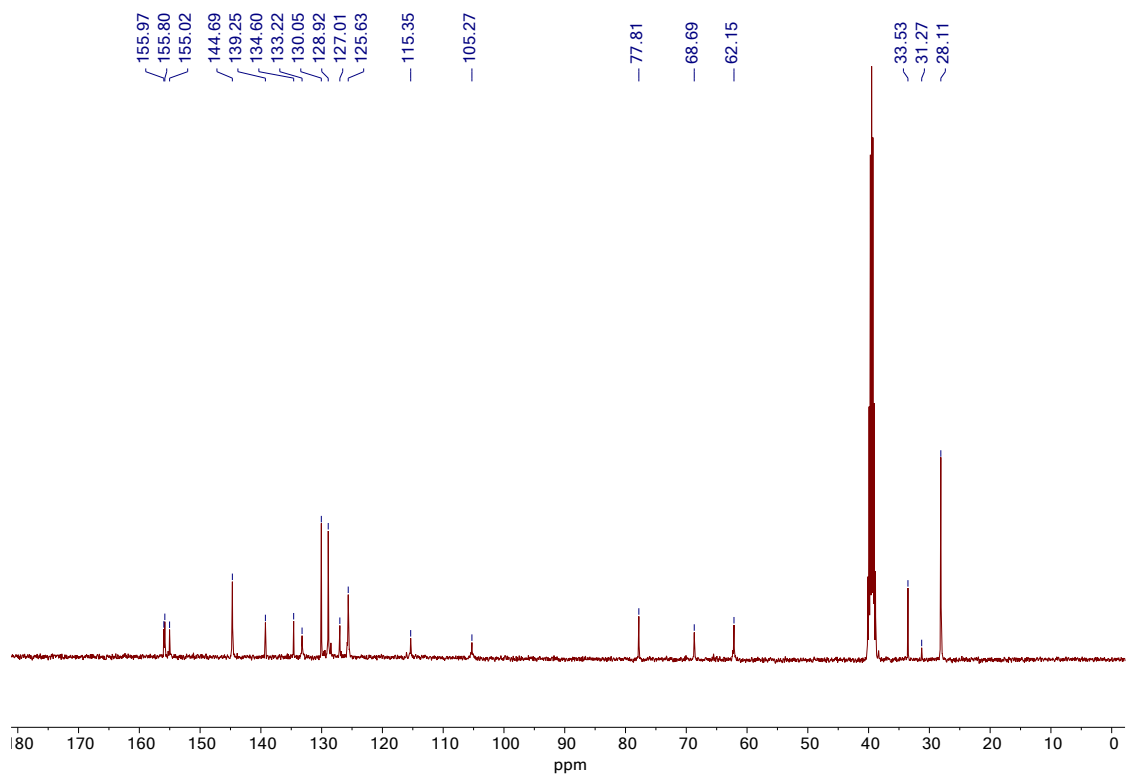


Figure S42. ^{13}C NMR spectrum (101 MHz) of **11a** in $\text{DMSO-}d_6$ at 25°C .

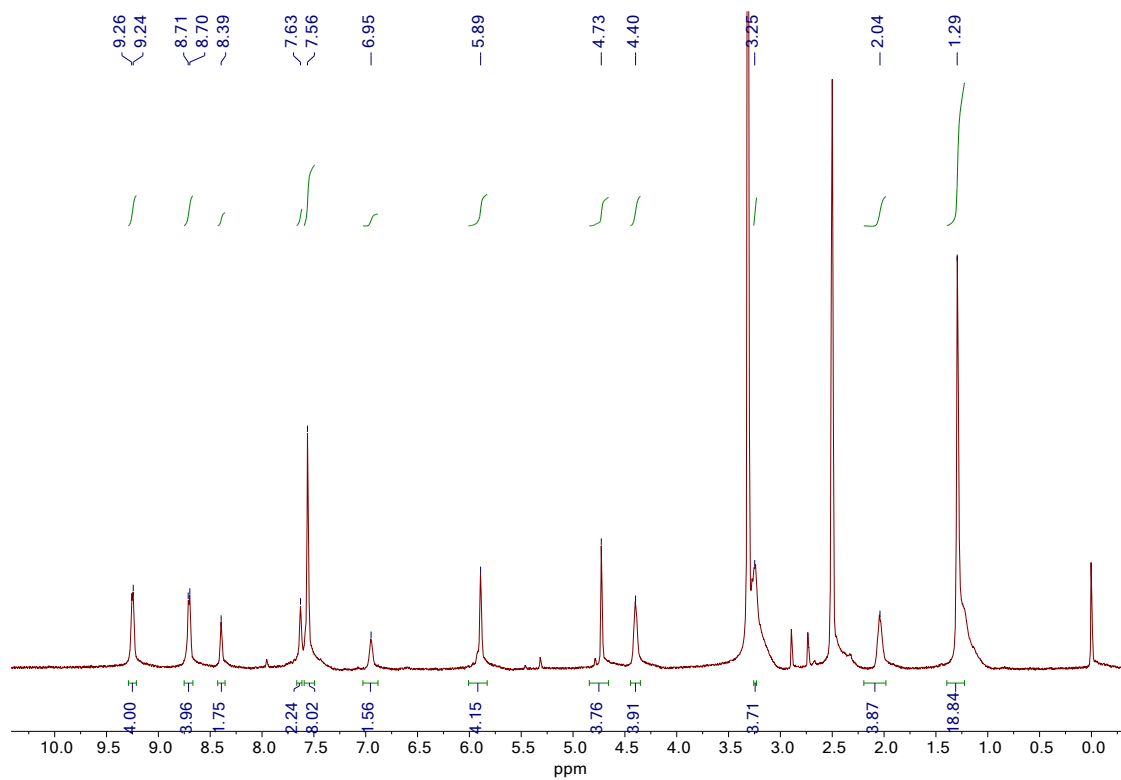


Figure S43. ^1H NMR spectrum (400 MHz) of **11b** in $\text{DMSO-}d_6$ at 25°C .

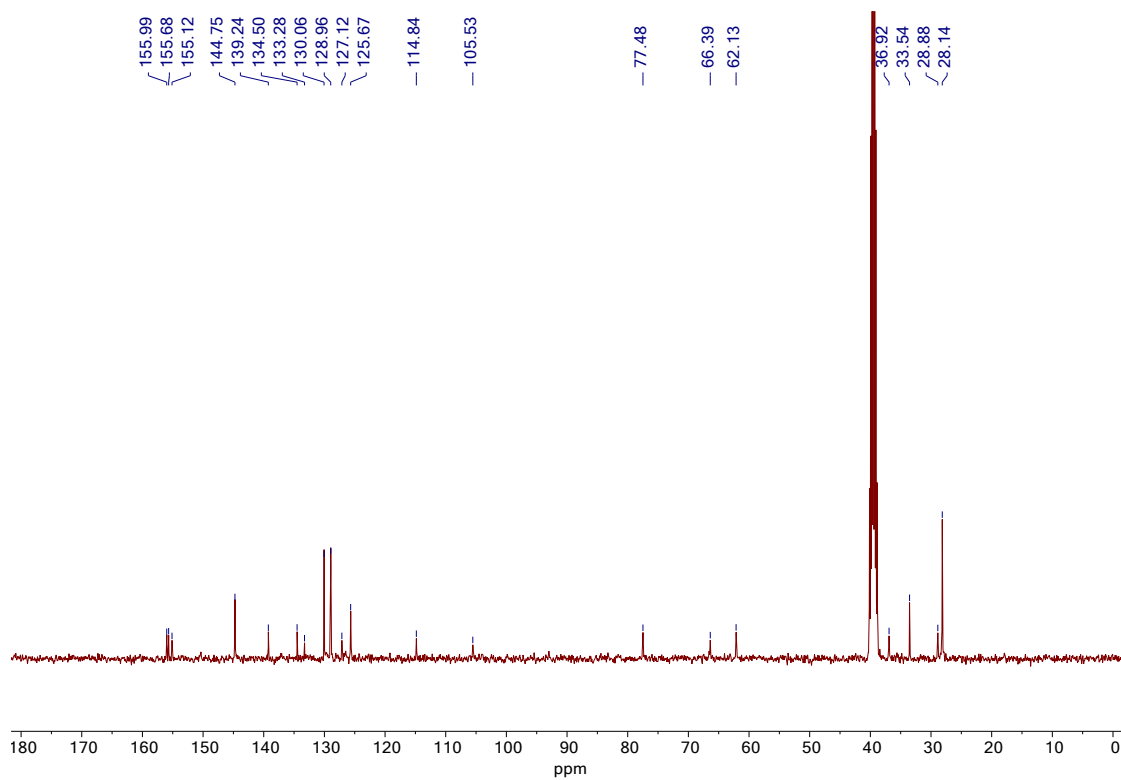


Figure S44. ^{13}C NMR spectrum (101 MHz) of **11b** in $\text{DMSO-}d_6$ at 25°C .

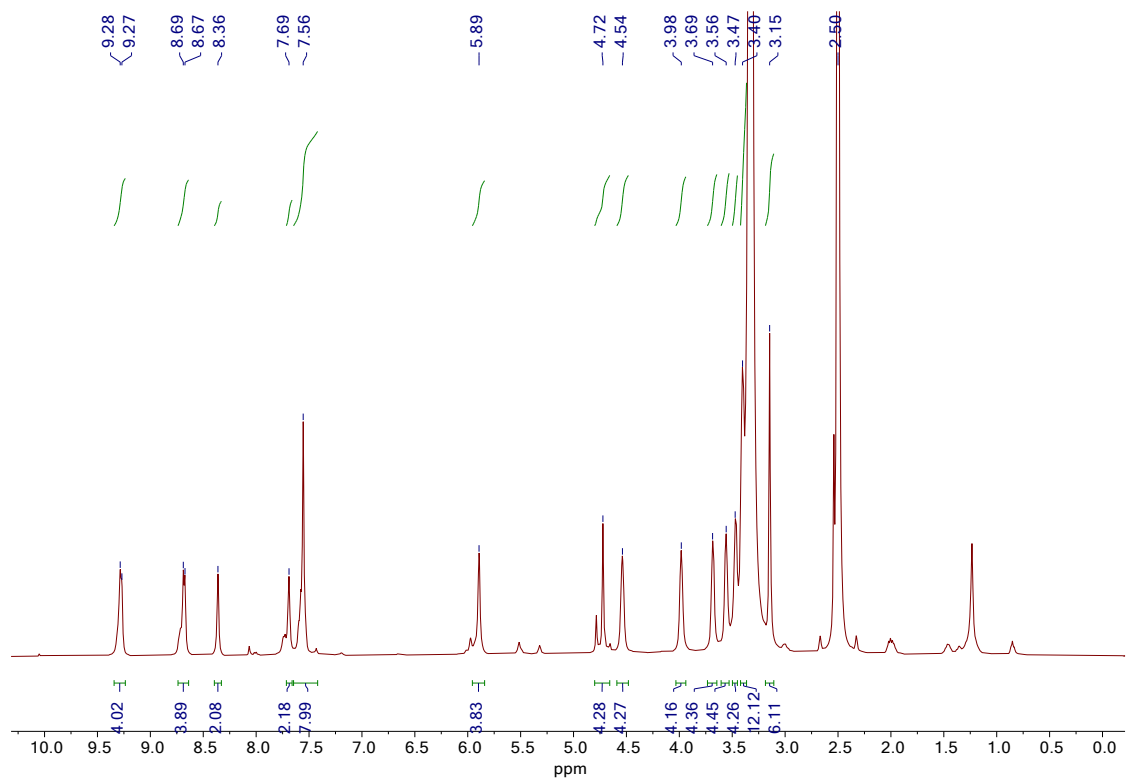


Figure S45. ^1H NMR spectrum (400 MHz) of **11c** in $\text{DMSO-}d_6$ at 25°C .

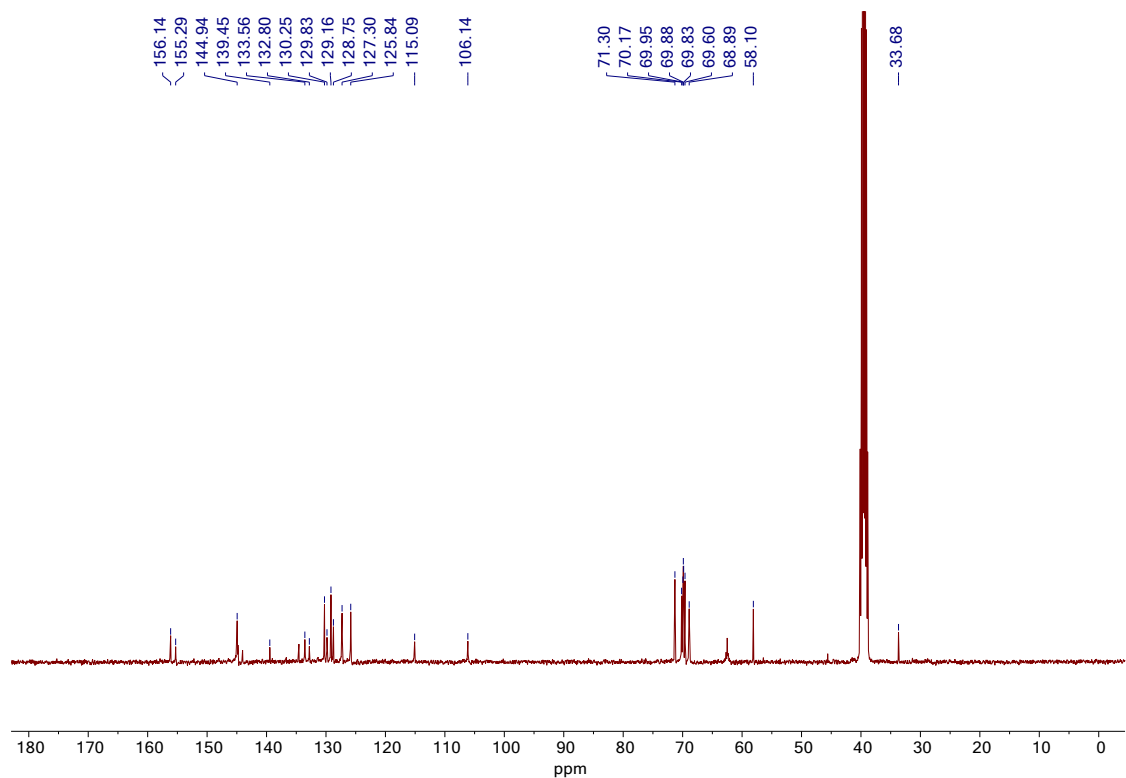


Figure S46. ^{13}C NMR spectrum (101 MHz) of **11c** in $\text{DMSO-}d_6$ at 25°C .

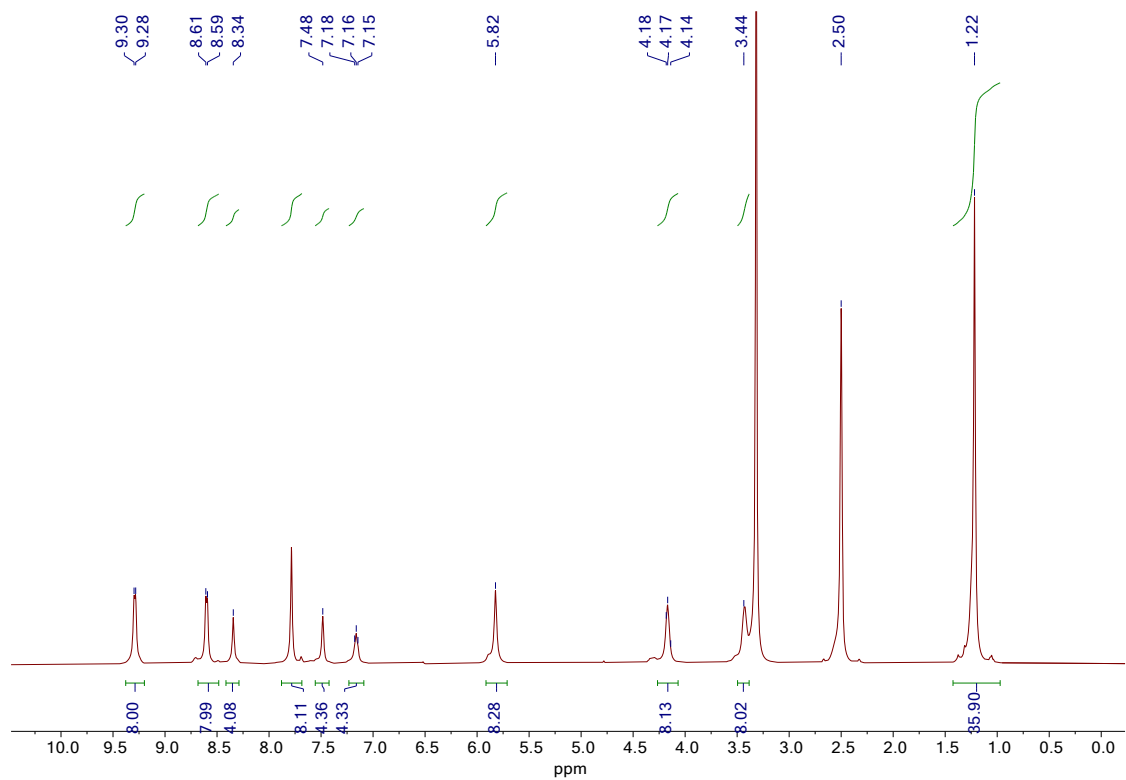


Figure S47. ^1H NMR spectrum (400 MHz) of **D2-Boc** in $\text{DMSO-}d_6$ at 25°C .

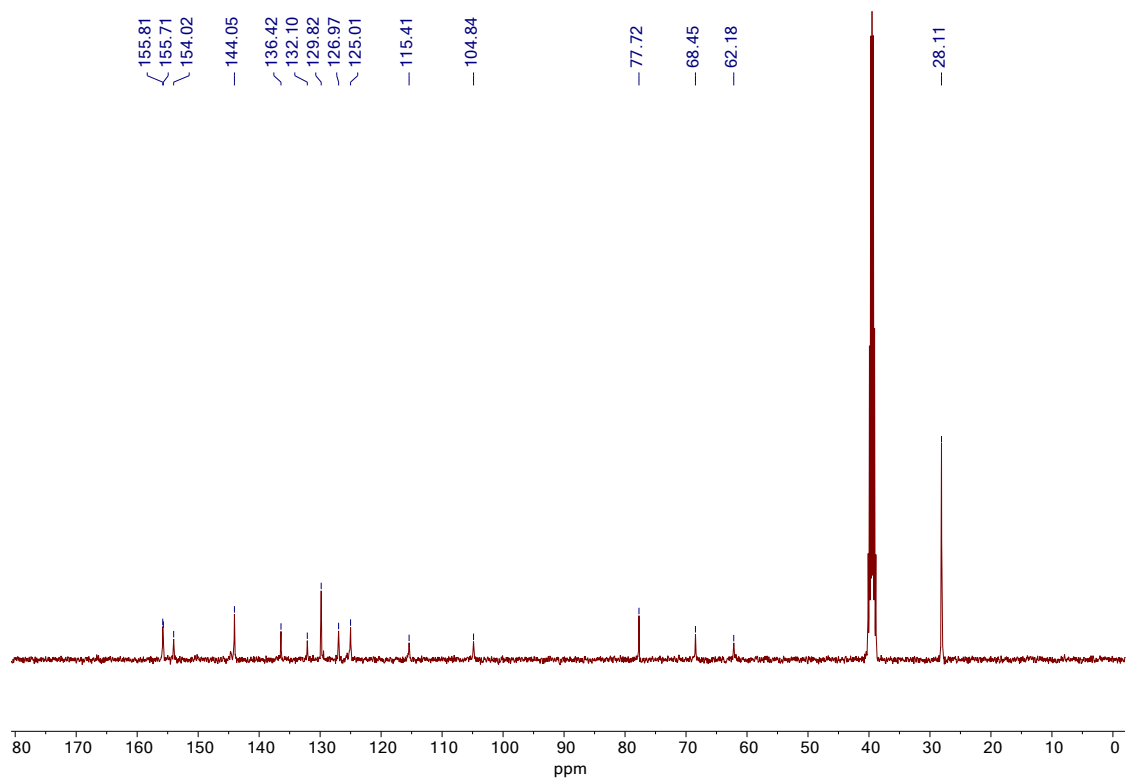


Figure S48. ^{13}C NMR spectrum (101 MHz) of **D2-Boc** in $\text{DMSO-}d_6$ at 25°C .

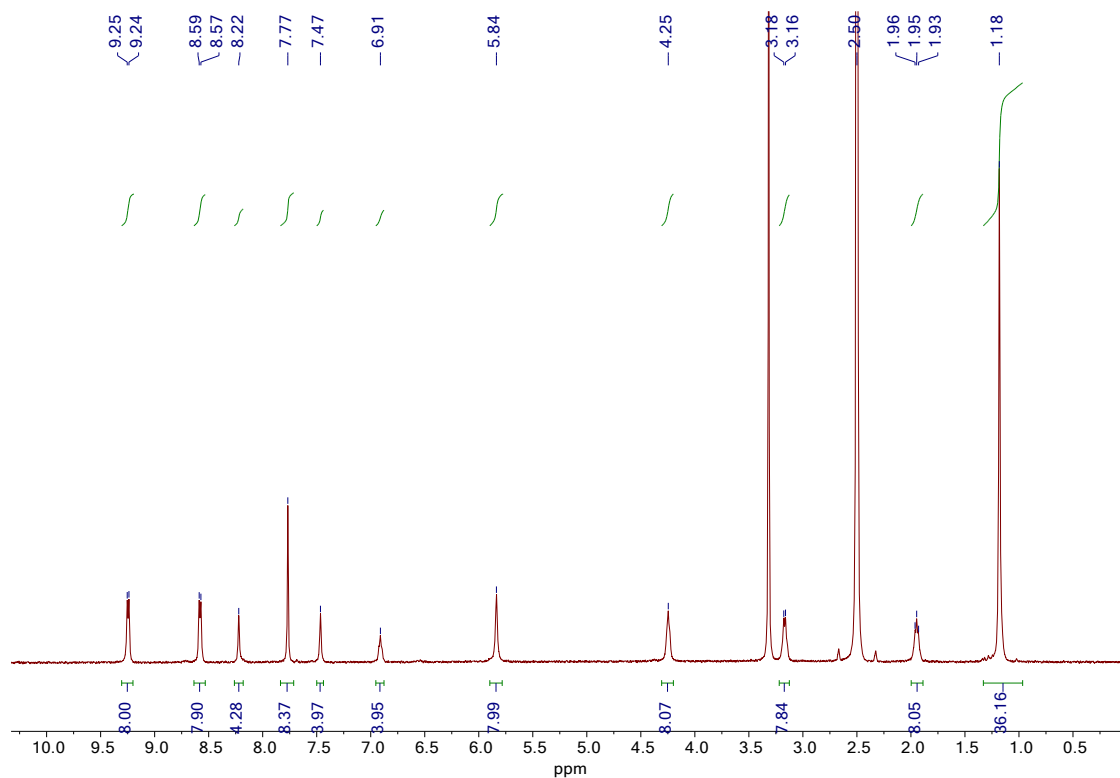


Figure S49. ^1H NMR spectrum (400 MHz) of **D3-Boc** in $\text{DMSO-}d_6$ at 25°C .

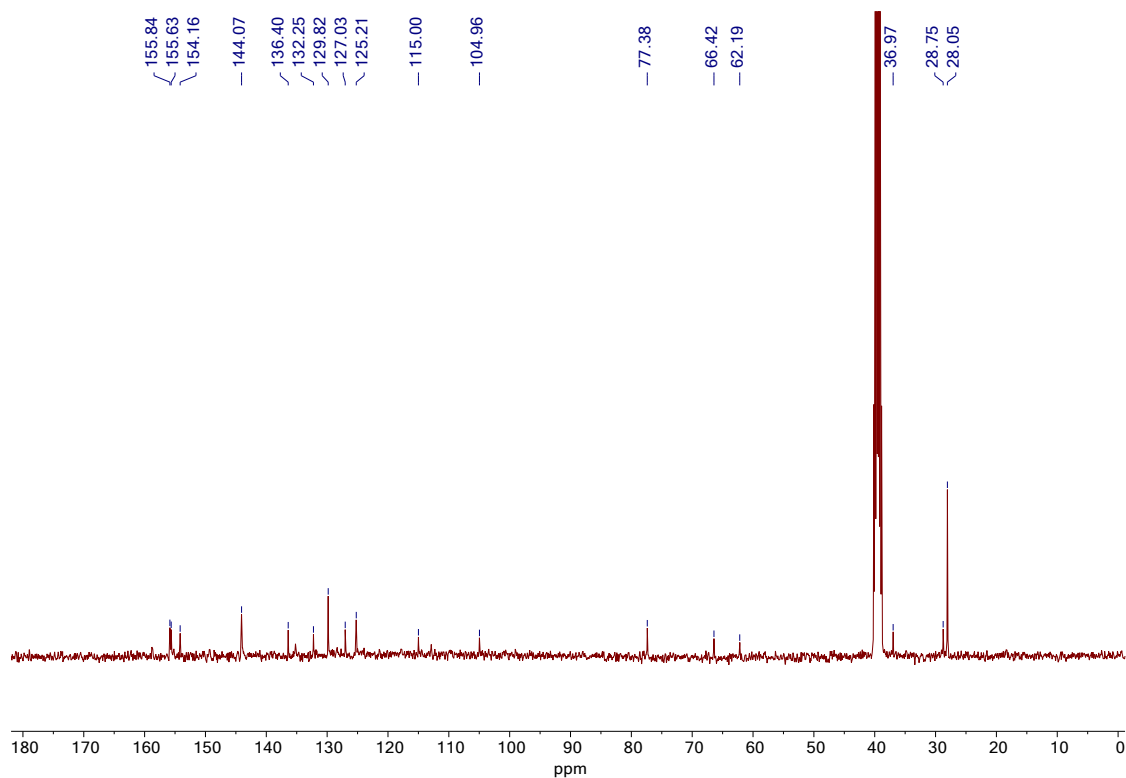


Figure S50. ^{13}C NMR spectrum (101 MHz) of **D3-Boc** in $\text{DMSO-}d_6$ at 25°C .

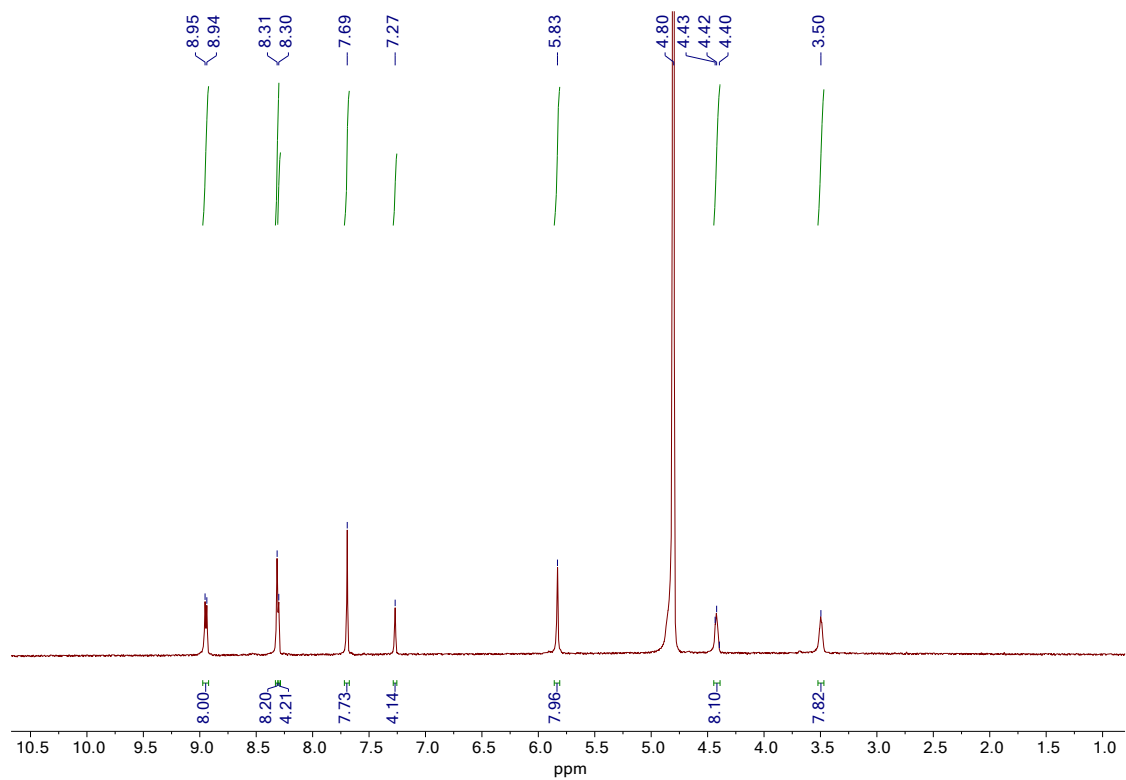


Figure S51. ^1H NMR spectrum (400 MHz) of **D2** in D_2O at 25°C .

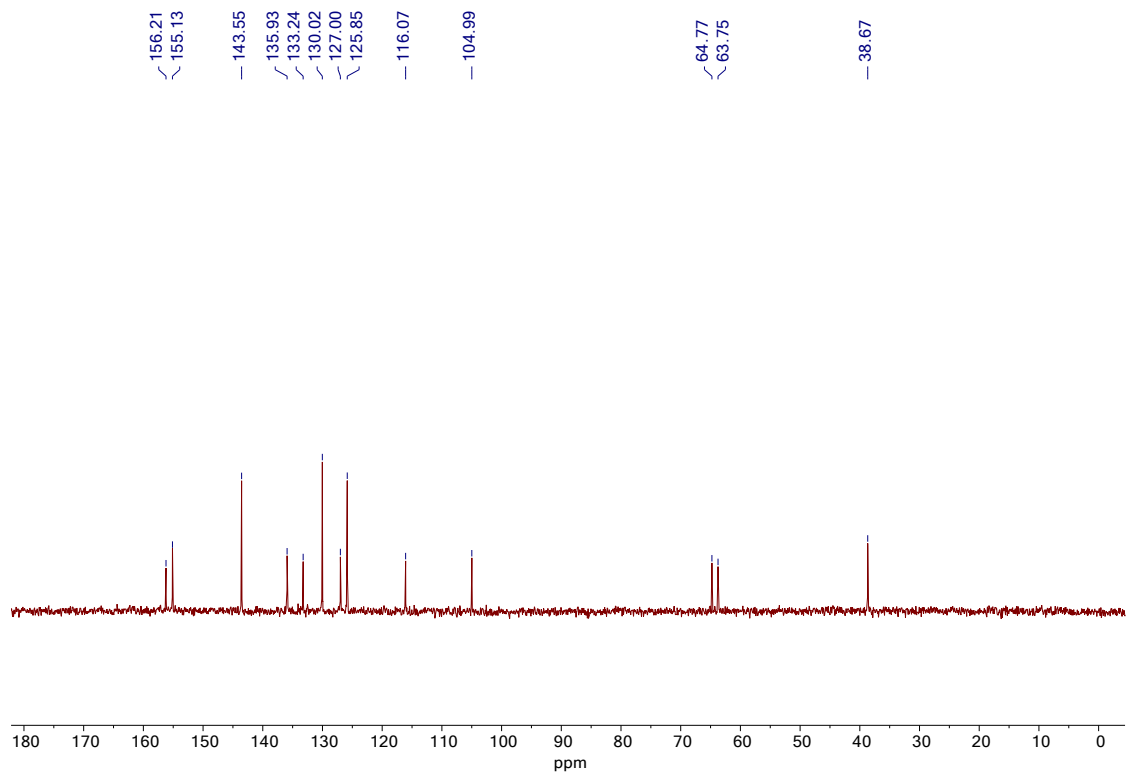


Figure S52. ^{13}C NMR spectrum (101 MHz) of **D2** in D_2O at 25°C .

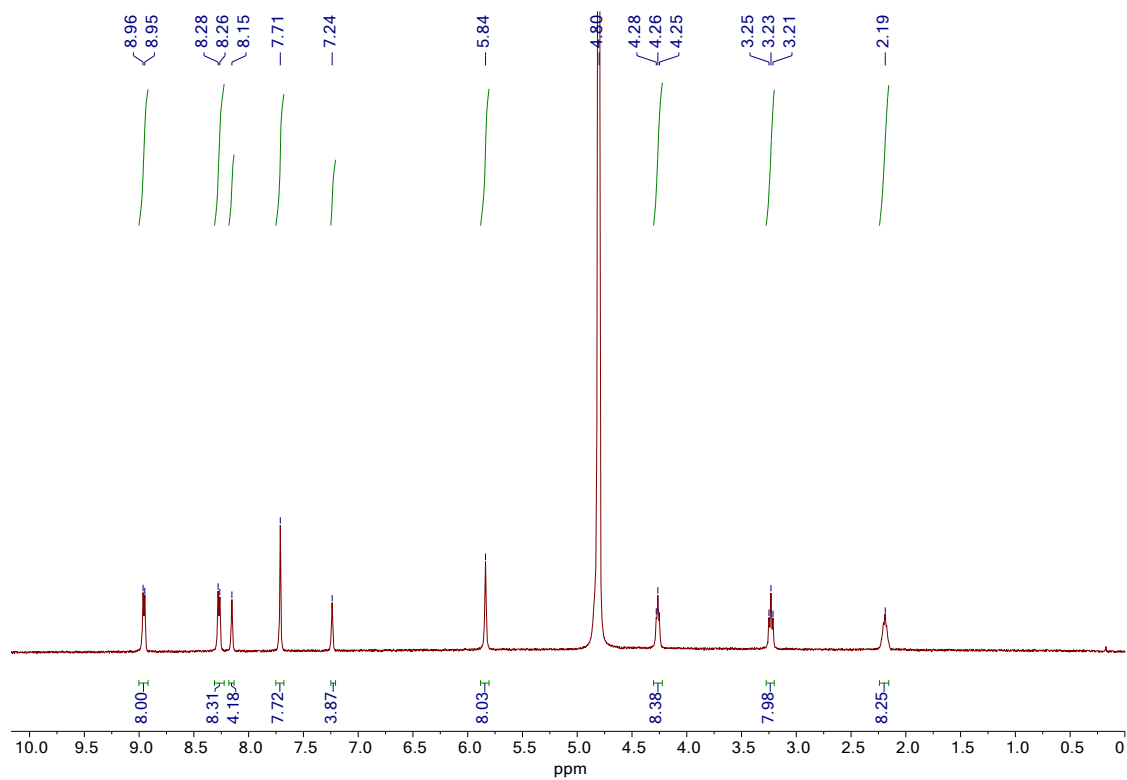


Figure S53. ^1H NMR spectrum (400 MHz) of **D3** in D_2O at 25°C .

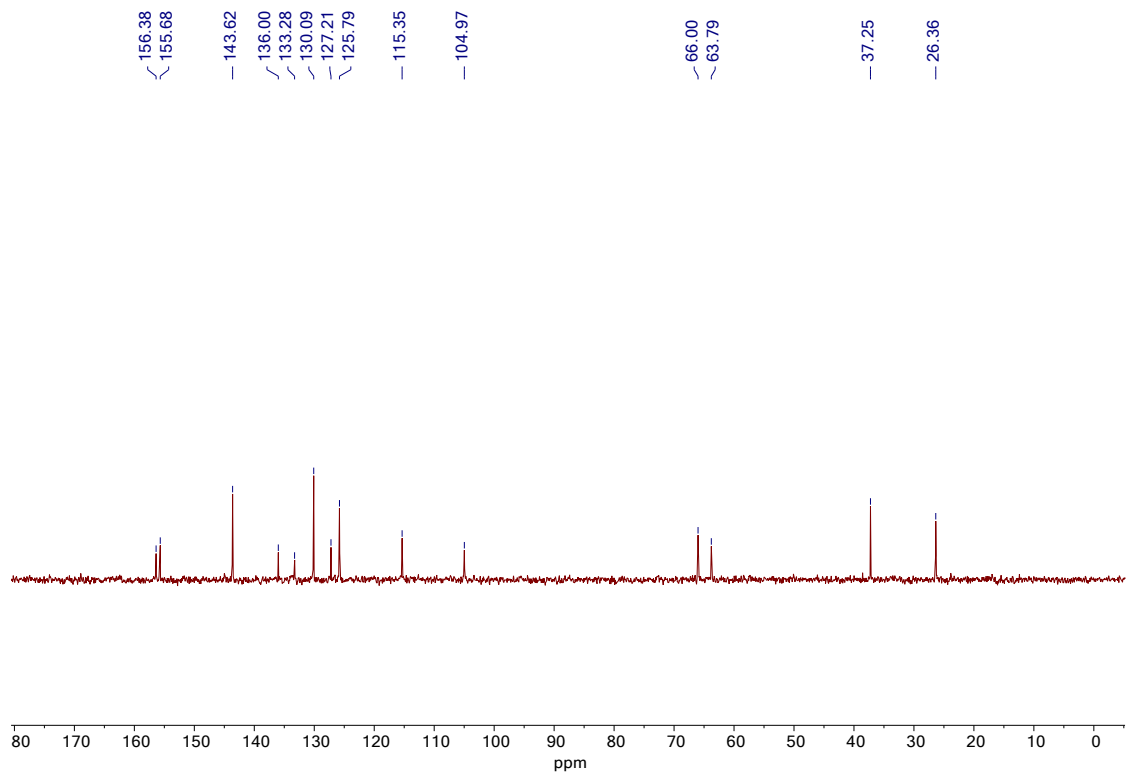


Figure S54. ^{13}C NMR spectrum (101 MHz) of **D3** in D_2O at 25°C .

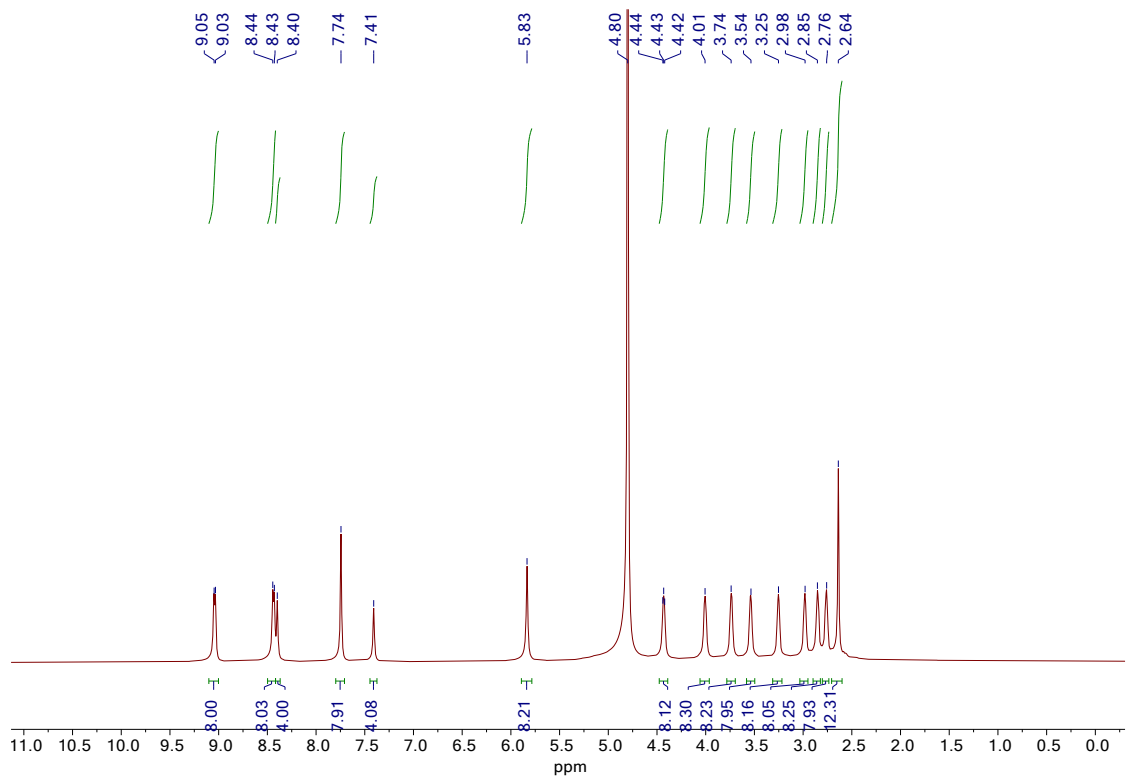


Figure S55. ^1H NMR spectrum (400 MHz) of **D4** in D_2O at 25°C .

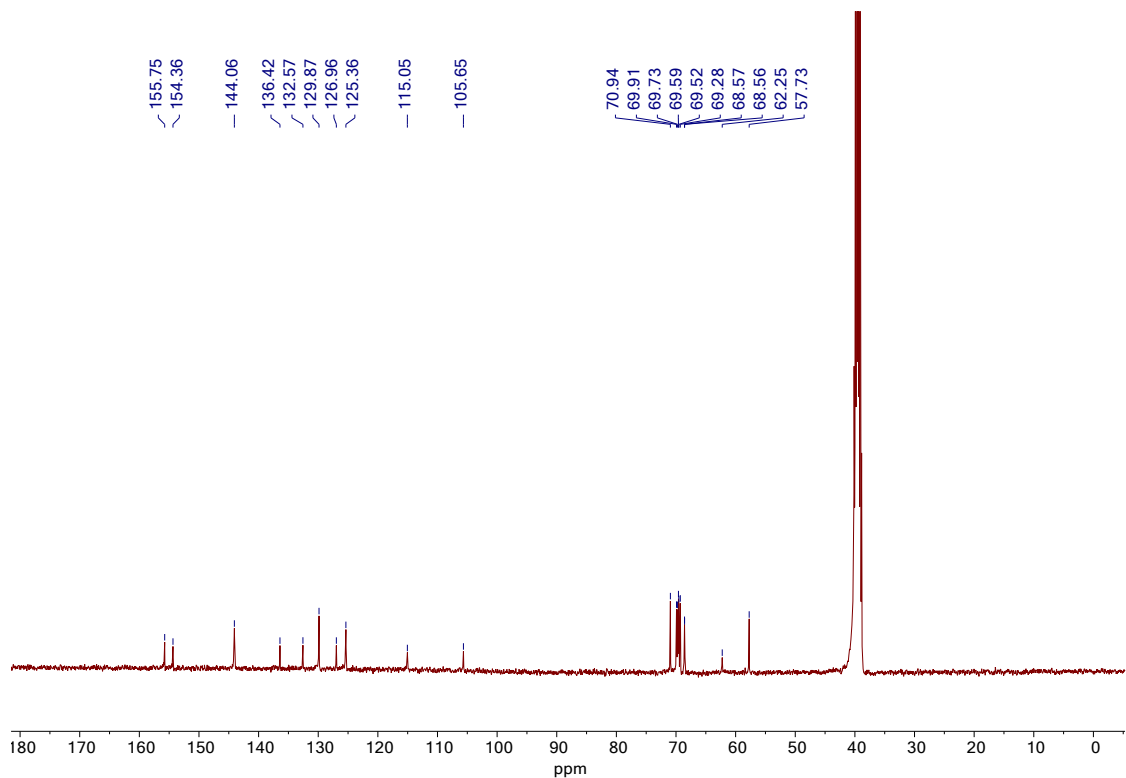


Figure S56. ^{13}C NMR spectrum (101 MHz) of **D4** in D_2O at 25°C .

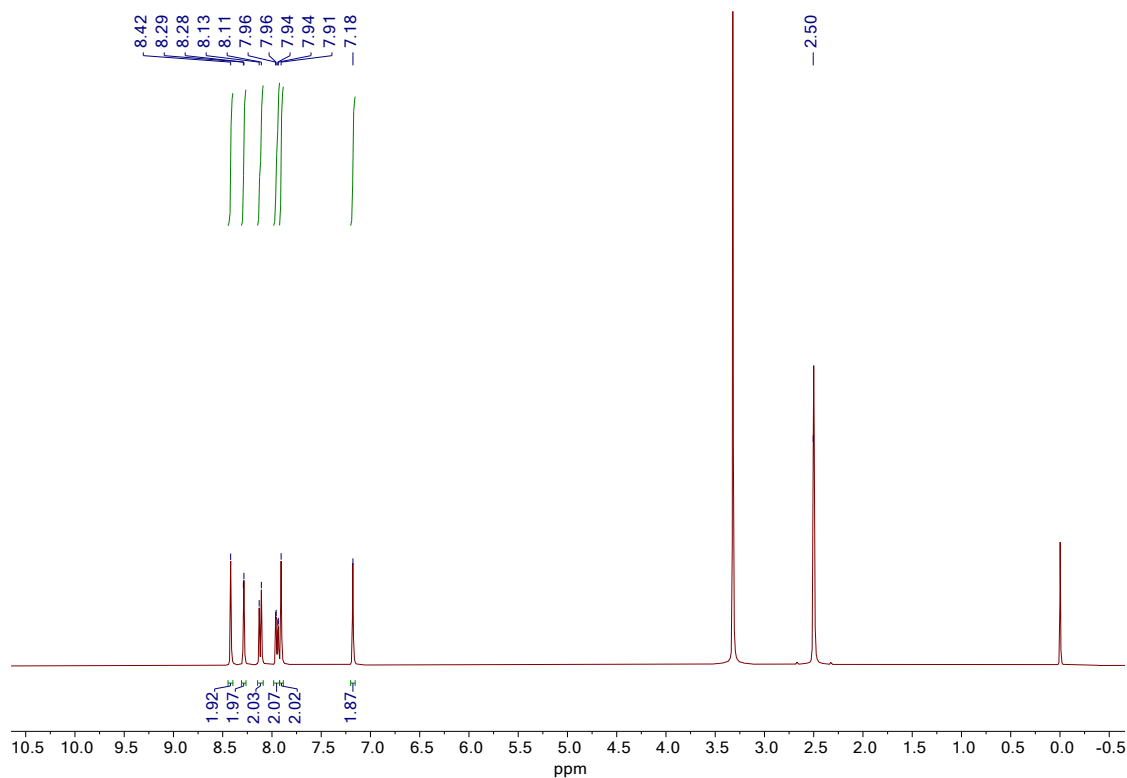


Figure S57. ^1H NMR spectrum (400 MHz) of **12** in $\text{DMSO-}d_6$ at 25°C .

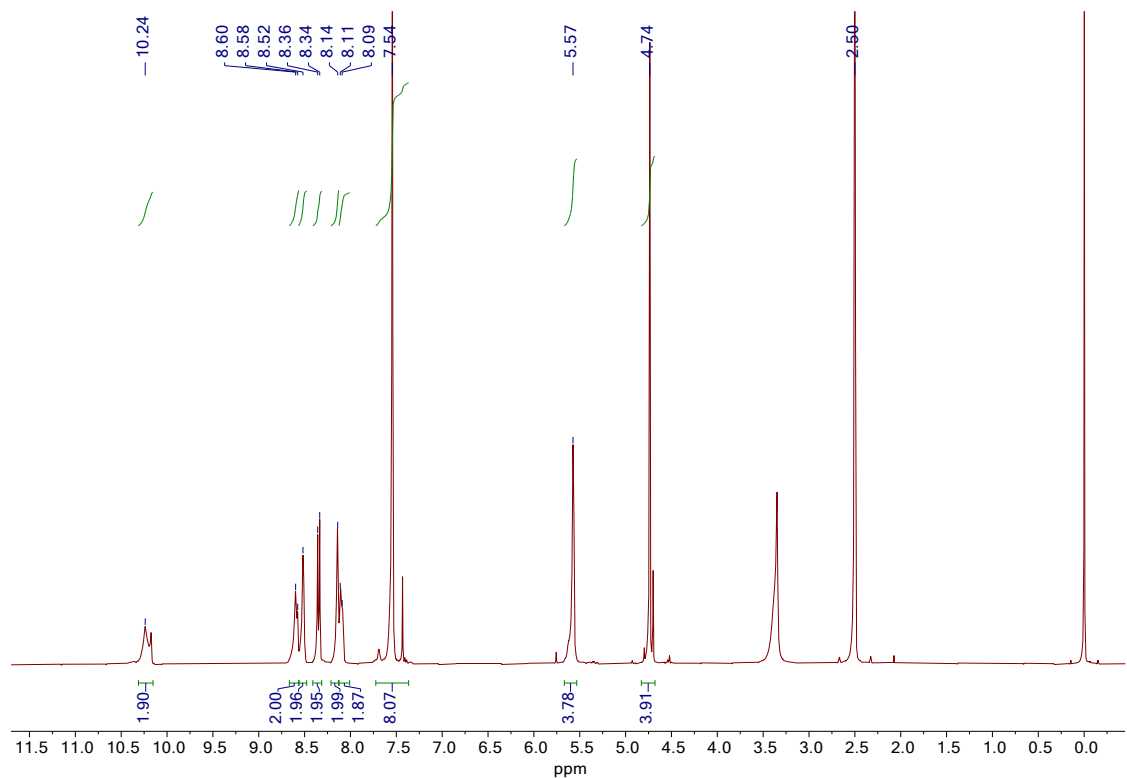


Figure S58. ^1H NMR spectrum (400 MHz) of **13** in $\text{DMSO-}d_6$ at 25°C .

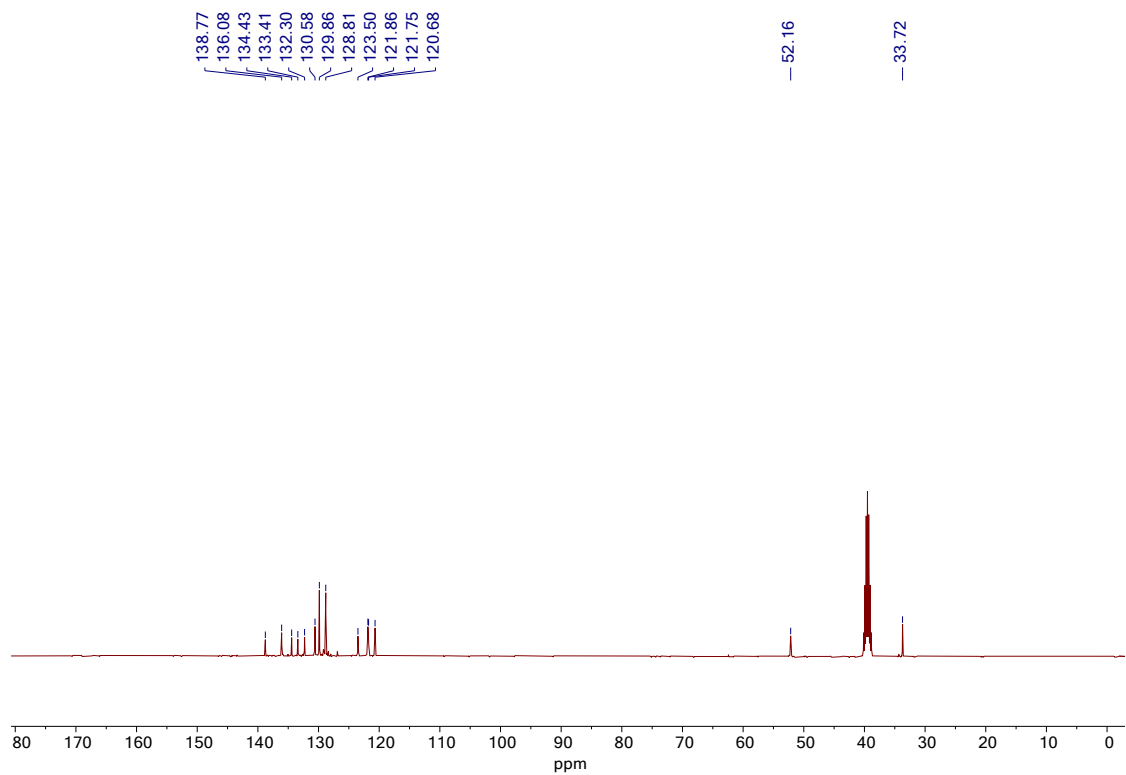


Figure S59. ^{13}C NMR spectrum (101 MHz) of **13** in $\text{DMSO-}d_6$ at 25°C .

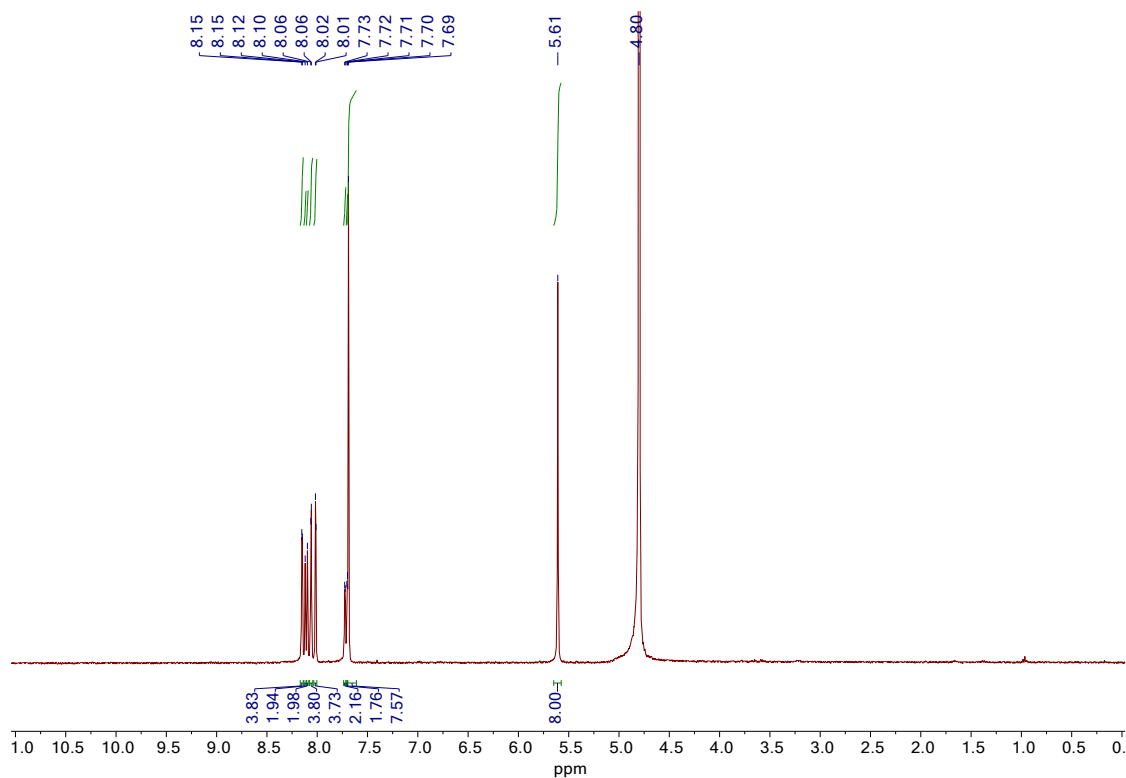


Figure S60. ^1H NMR spectrum (400 MHz) of **D5** in D_2O at 25°C .

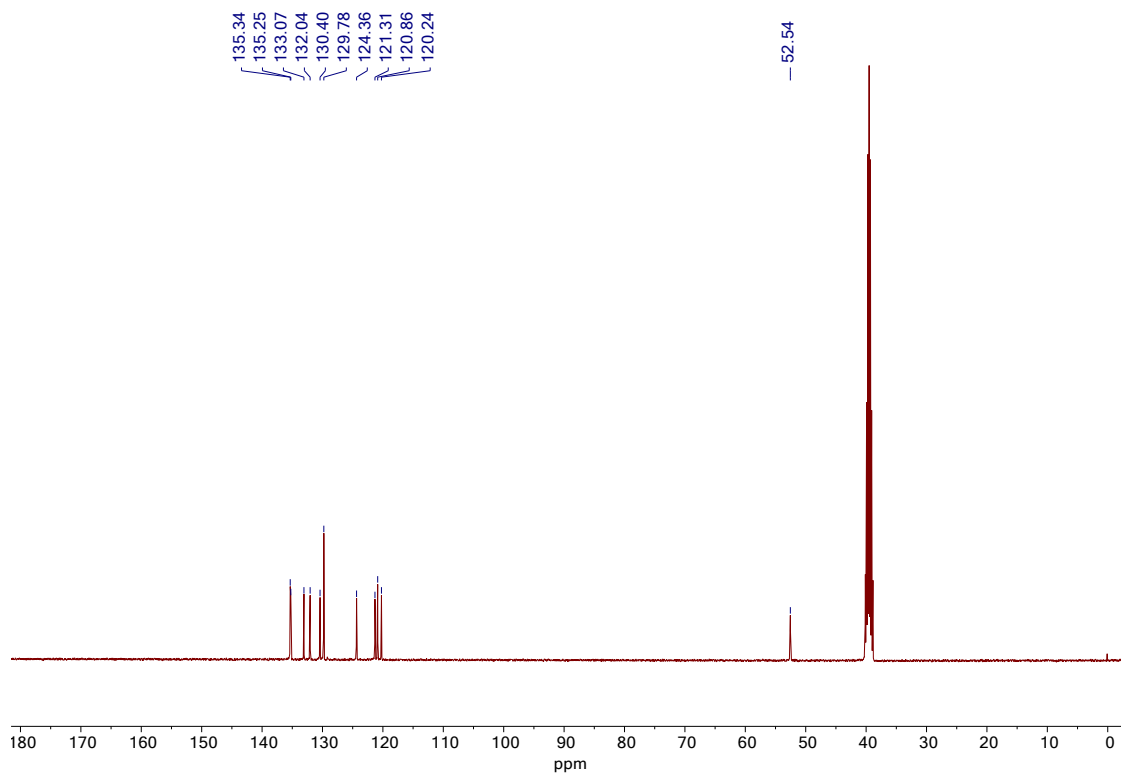


Figure S61. ^{13}C NMR spectrum (101 MHz) of **D5** in $\text{DMSO-}d_6$ at 25°C .

9. Mass Spectra of New Compounds and Complex

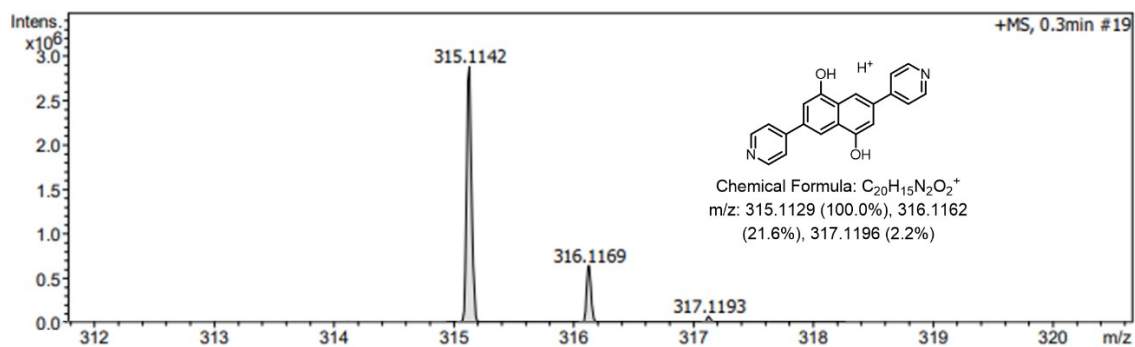


Figure S62. HRMS calcd for $\text{C}_{20}\text{H}_{14}\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 315.1129; found: 315.1142.

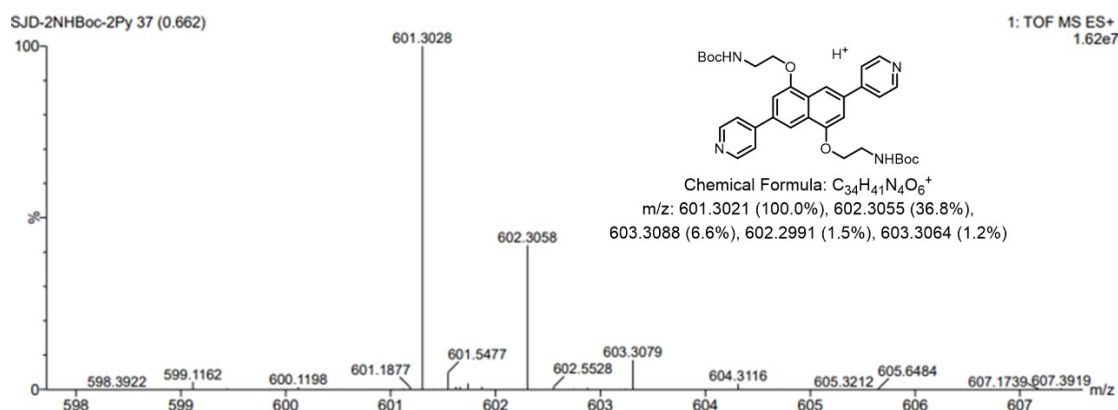


Figure S63. HRMS calcd for $\text{C}_{34}\text{H}_{40}\text{N}_4\text{O}_6$ $[\text{M}+\text{H}]^+$: 601.3021; found: 601.3028.

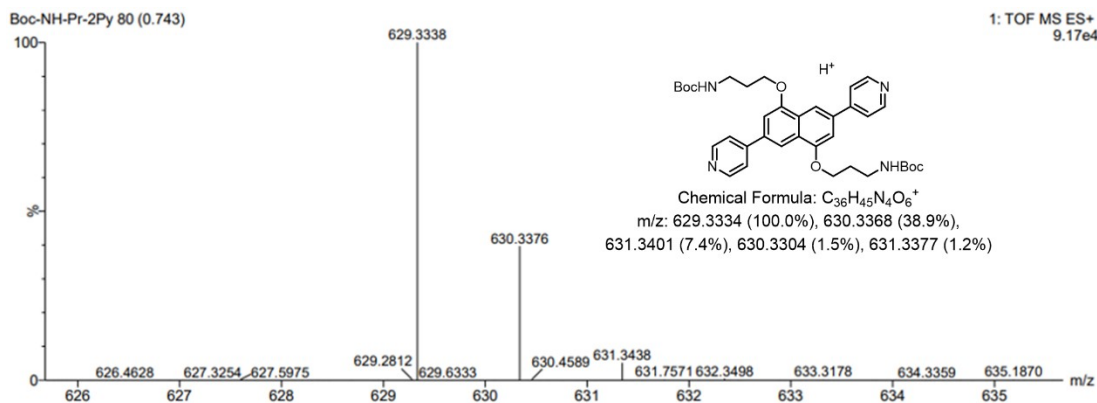


Figure S64. HRMS calcd for $C_{36}H_{44}N_4O_6 [M+H]^+$: 629.3334; found: 629.3338.

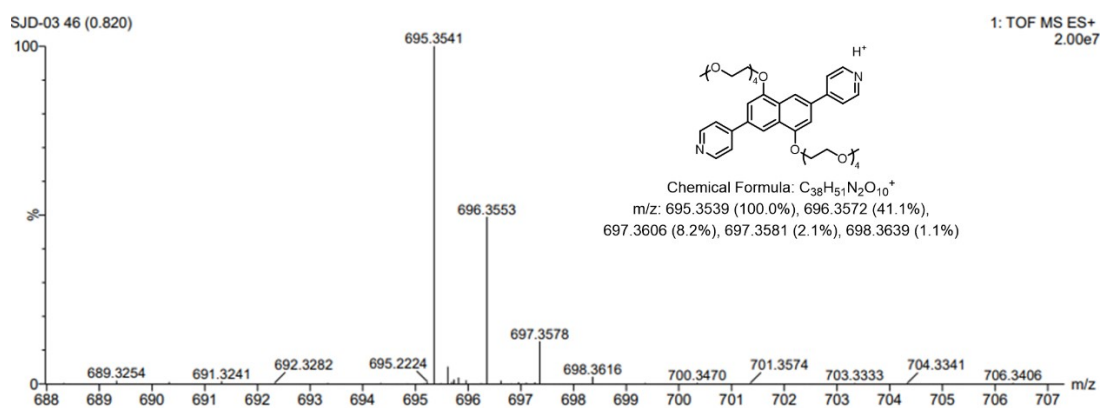


Figure S65. HRMS calcd for $C_{38}H_{50}N_2O_{10} [M+H]^+$: 695.3539; found: 695.3541.

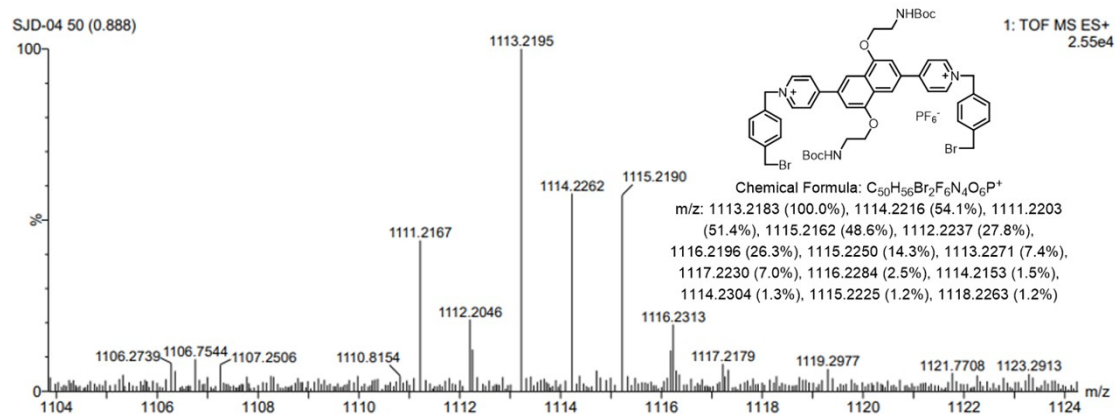


Figure S66. HRMS calcd for $C_{50}H_{56}Br_2N_4O_6PF_6 [M-PF_6]^+$: 1113.2183; found: 1113.2195.

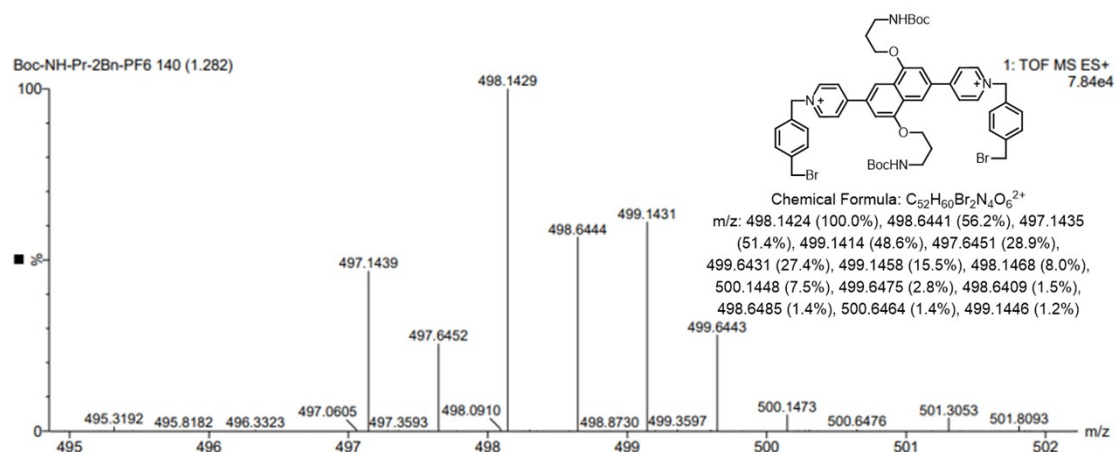


Figure S67. HRMS calcd for $C_{52}H_{58}Br_2N_4O_6 [M-2PF_6]^{2+}$: 498.1424; found: 498.1429.

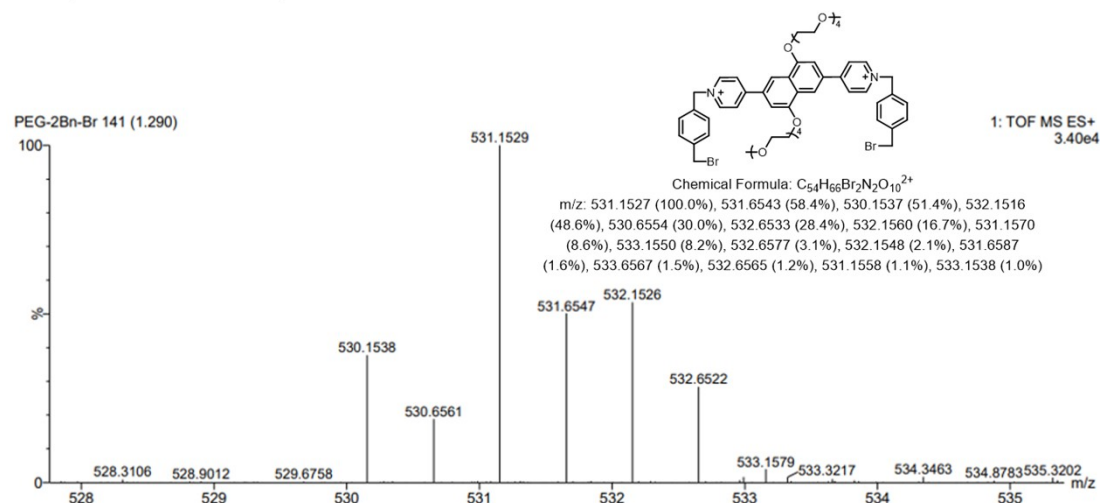


Figure S68. HRMS calcd for $C_{54}H_{64}Br_2N_2O_{10} [M-2PF_6]^{2+}$: 531.1527; found: 531.1529.

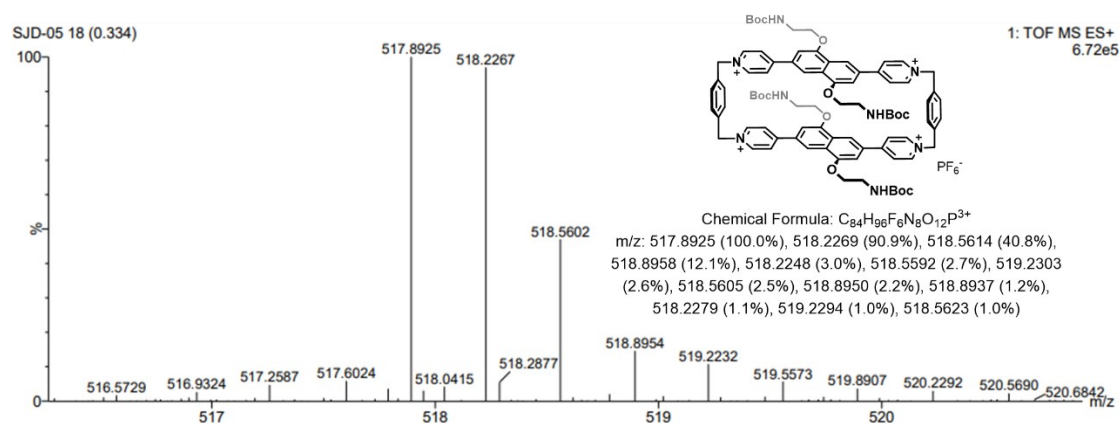


Figure S69. HRMS calcd for $C_{84}H_{96}N_8O_{12}PF_6 [M-3PF_6]^{3+}$: 517.8925; found: 517.8925.

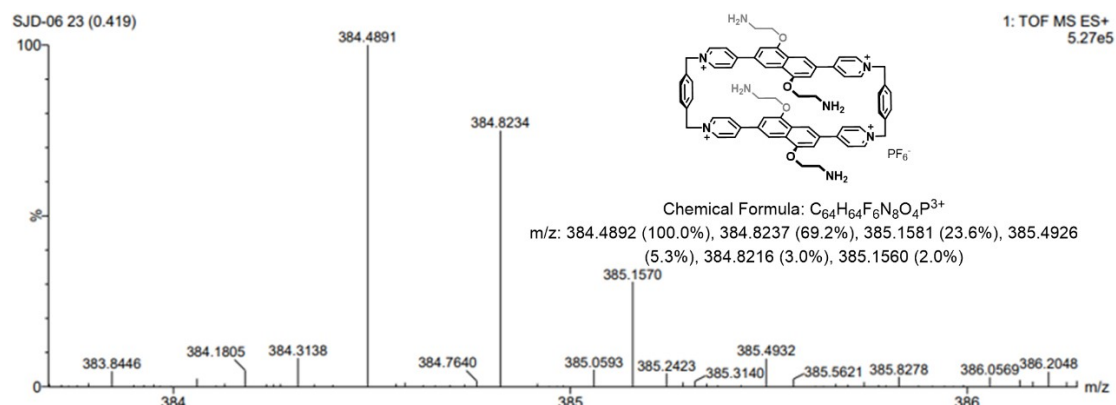


Figure S70. HRMS calcd for $C_{64}H_{64}N_8O_4PF_6 [M-3PF_6]^3+$: 384.4892; found: 384.4891.

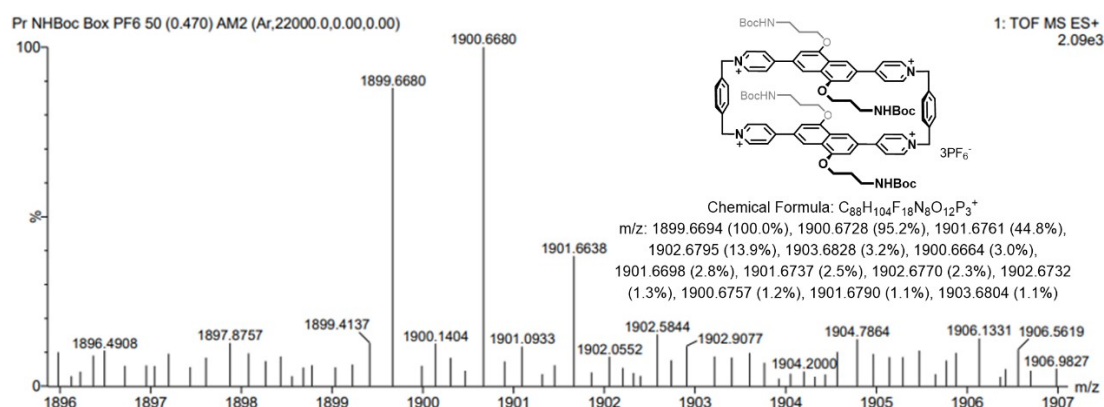


Figure S71. HRMS calcd for $C_{88}H_{104}N_8O_{12}P_3F_{18} [M-PF_6]^+$: 1899.6694; found: 1899.6680.

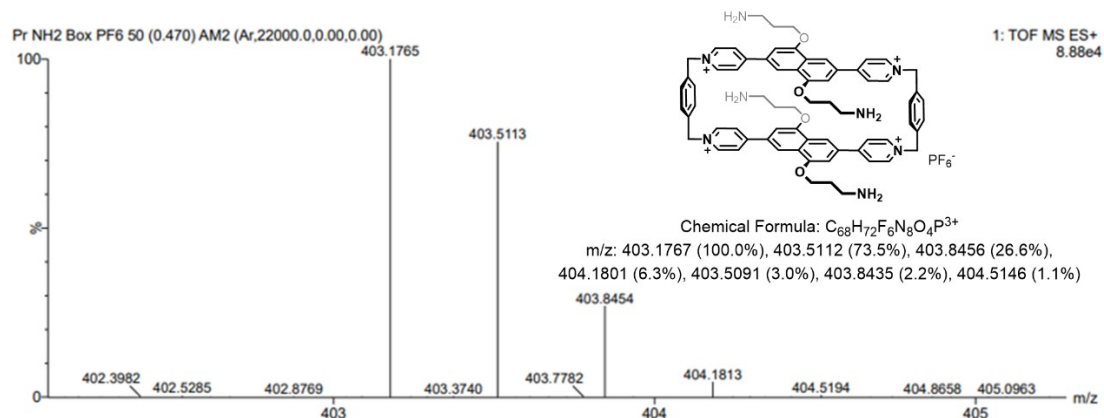


Figure S72. HRMS calcd for $C_{68}H_{72}N_8O_4PF_6 [M-3PF_6]^3+$: 403.1767; found: 403.1773.

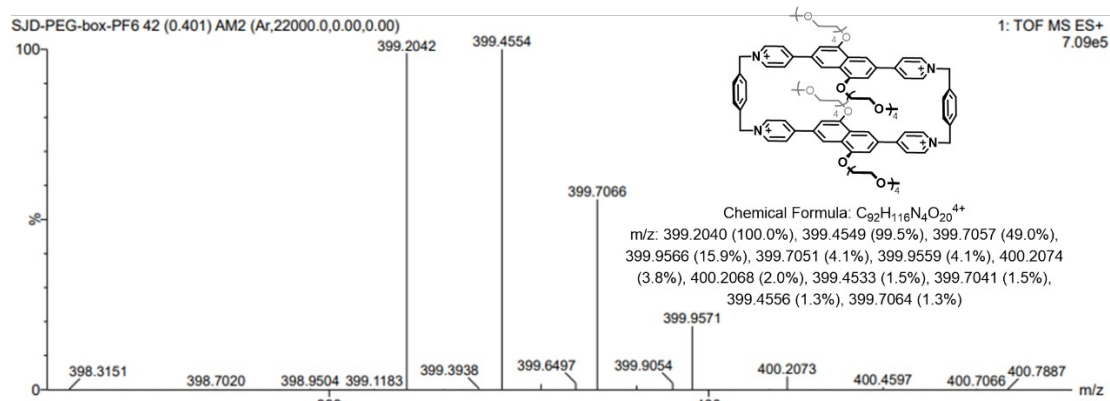


Figure S73. HRMS calcd for $C_{92}H_{112}N_4O_{20} [M-4PF_6]^{4+}$: 399.2040; found: 399.2042.

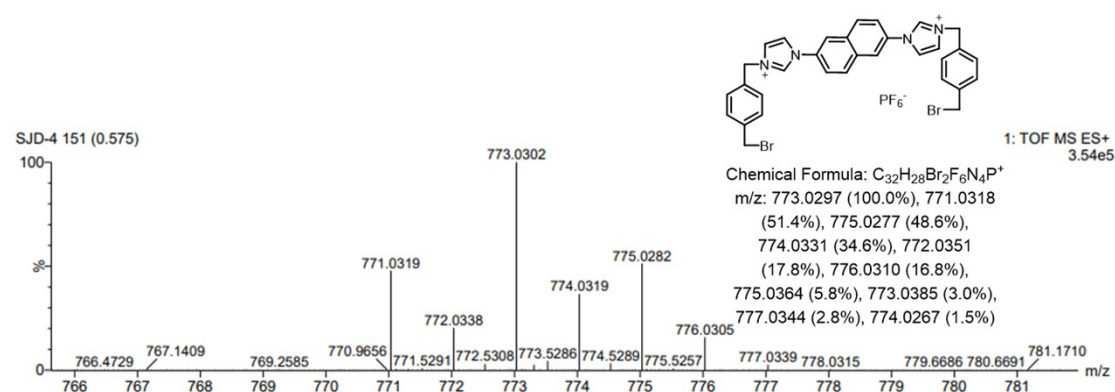


Figure S74. HRMS calcd for $C_{32}H_{28}Br_2N_4PF_6 [M-PF_6]^+$: 773.0297; found: 773.0302.

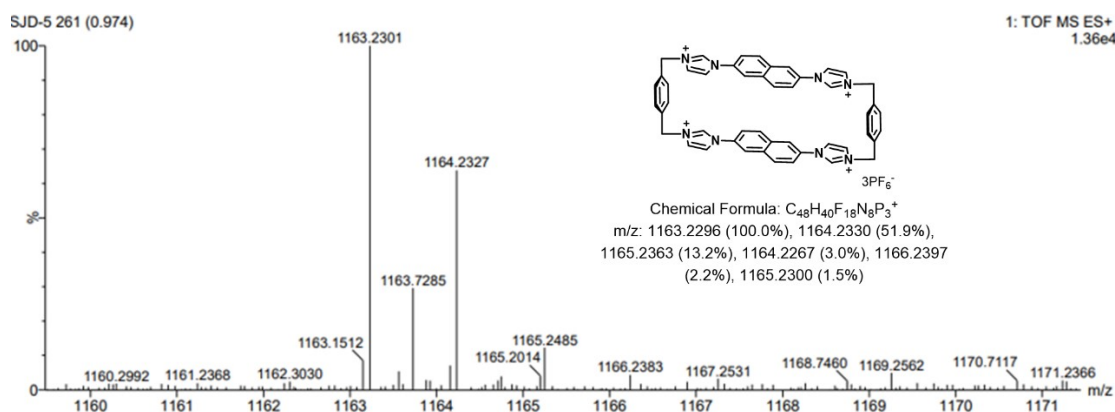


Figure S75. HRMS calcd for $C_{48}H_{40}N_8P_3F_{18} [M-PF_6]^+$: 1163.2296; found: 1163.2301.

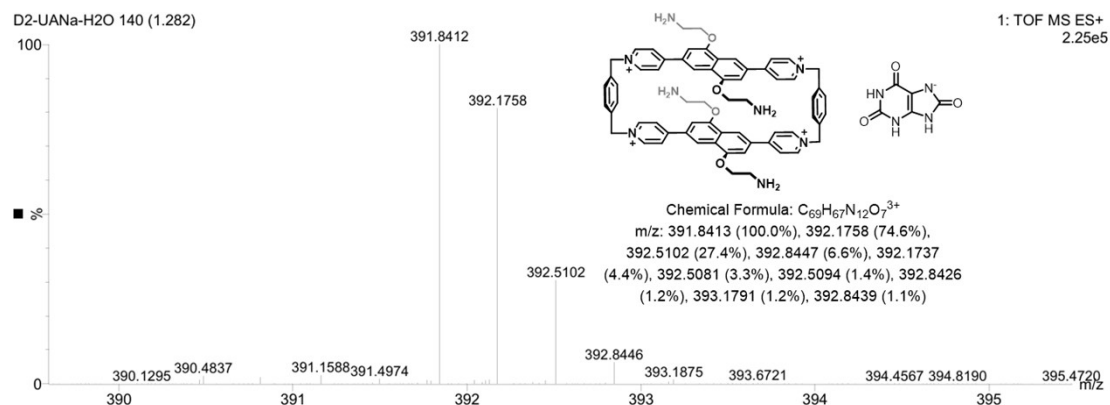


Figure S76. HRMS calcd for C₆₉H₆₇N₁₂O₇ [M-4Cl+UA-H]³⁺: 391.8413; found: 391.8412.

10. Crystal Data

Table. S1. Crystal data and structure refinement for **D2·4PF₆** and **D5·4PF₆**.

Compound	D2·4PF₆	D5·4PF₆
Empirical formula	C ₃₇ H ₄₁ F ₁₂ N ₅ O ₃ P ₂	C ₄₈ H ₄₀ F ₂₄ N ₈ P ₄
Formula weight	77.51	1418.22
Temperature	173(2) K	273.15 K
Crystal system	triclinic	orthorhombic
Space group	P-1	Pca21
a/Å	11.3852(10)	14.395(4)
b/Å	13.1780(11)	20.094(6)
c/Å	15.2657(12)	18.427(5)
α	91.744(3)°	90°
β	98.700(2)°	90°
γ	96.019(3)°	90°
Volume	2249.0(3) Å ³	5330(3) Å ³
Z	2	4
Density (calculated)	0.114 g/cm ³	1.767 g/cm ³
Absorption coefficient	0.174 mm ⁻¹	5.660 mm ⁻¹
F(000)	77	2806
Crystal size	0.05 × 0.05 × 0.02 mm ³	0.1 × 0.1 × 0.1 mm ³
Radiation	GaKα (λ = 1.34138)	CuKα (λ = 1.54178)
2θ/°	5.874~96.518	4.398~122.822
Index ranges	-12 ≤ h ≤ 12, -14 ≤ k ≤ 14, -16 ≤ l ≤ 16	-16 ≤ h ≤ 16, -22 ≤ k ≤ 22, -20 ≤ l ≤ 20

Reflections collected	20925	68545
Independent	6393 [R(int) = 0.0455]	7872 [R(int) = 0.0542]
Data/restraints/parameters	6396 / 15 / 547	7872 / 592 / 757
Goodness-of-fit on F2	1.046	1.061
Final R indexes [I>>=2σ (I)]	R1 = 0.1062, wR2 = 0.2888	R1 = 0.0369, wR2 = 0.1017
Final R indexes [all data]	R1 = 0.1396, wR2 = 0.3325	R1 = 0.0408, wR2 = 0.1062
peak/hole	0.98, -1.25 e.Å ⁻³	0.38, -0.31 e.Å ⁻³
