Supporting Information

Gadolinium Complexes of Diethylenetriamine-N-oxide Pentaacetic Acid-Bisamide: A New Class of Highly Stable MRI Contrast Agents with Hydration Number of 3

*Beibei Zhang,[a] Likun Cheng,[a] Bing Duan,[b] Weijun Tang,[c] Yuan Yuan,[b] Yun Ding,[a] Aiguo Hu[a]**

[a] Shanghai Key Laboratory of Advanced Polymeric Materials, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai, 200237, China. [b] The State Key Laboratory of Bioreactor Engineering East China University of Science and Technology, Shanghai, 200237, China.[c] Department of Radiology, Huashan Hospital Affiliated to Fudan University, Shanghai, 200040, China.

Corresponding author: Prof. Aiguo Hu (email address: hagmhsn@ecust.edu.cn)

Matrials and Methods

All the solvents were purchased from commercial resources and used as received except for N,N-dimethylformamide (DMF), which was dried over calcium hydride and distilled before used. Diethylenetriamine-pentaacetic-acid-dianhydride (DTPAA) was prepared according to literature procedures.[1] Human umbilical vein endothelial cells (HUVEC) and human cervical carcinoma cells (HeLa) were provided by China Centre for Type Culture Collection. All cell-culture related reagents were purchased from Gibco (Grand Island, NY, USA).

NMR spectrascopy.

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on an Ultra Shield 400 spectrometer (Bruker BioSpin AG, Magnet System 400 MHz/54 mm) in D_2O. ^{15}N NMR (600 MHz) spectra were recorded on a Bruker 600 MHz spectrometer and using the HMBC pulse sequence with a mixing time of 100 ms. The nucleus ^{15}N in

2D spectra is correlated with another nucleus ^{1}H. Chemical shifts are expressed in ppm and are referenced to TMS for ^{1}H and ^{13}C (δ = 0 ppm) or nitromethane for ^{15}N (δ = 375 ppm).

Mass spectrometry.

Electrospray ionization mass spectrometry (ESI-MS) were recorded on Xevo G2 TOF/MS spectrometer, Waters, USA. The mass spectra of the Gd(III) complexes were recorded on a LC/TOF MS spectrometer (Micromass Ltd, England).

Job plot.

The stoichiometries and the binding constants of the complexes were found by making use of Job plot. The contents of gadolinium were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, Agilent 725ES). A stock solution of ligand and $GdCl_3$ of 10 mM concentration was prepared in methanol. Solutions of different volumetric ratios (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0) were mixed by maintaining the total ligand and metal ion concentration (C), constant. (i.e., $C=C_M+C_L$ where C, C_M and C_L are the total concentration of metal ion Gd and ligand，the concentration of metal ion Gd and the concentration of the ligand, respectively). Then excessive amount of pyridine was added and excessive Gd^{3+} was removed by precipitation, following the general synthesis procedure of Gd(III) complexes in this work. Finally the contents of gadolinium were measured by ICP-AES and plotted against ratio of concentration of C_M/C.

Hydration number measurement.

Samples with same concentrations for europium complexes but different volumetric ratios of H_2O versus D_2O (2:8, 4:6, 5:5, 6:4, 8:2, 10:0) were prepared. Europium luminescence intensities were measured on a BioTek Synergy™ automatic microplate reader with a 50 μs interval at 616 nm for Eu (excitation at 395 nm). The decays of luminescence intensities followed systematically monoexponential laws and were analyzed as single-exponential decays to calculate the europium luminescence lifetimes (τ) which was plotted as $1/\tau$ versus χ. (i.e., $\chi=V_{H2O}/V$ where V, V_{H2O} and V_{D2O} are the total volume of H_2O and D_2O, the volume of H_2O and the volume of D_2O, respectively). Then by the method of extrapolation, $1/\tau$ of H_2O versus D_2O at 0:10 ($1/\tau_{D2O)}$ was founded and hydration number was calculated using the following equation:

$$q = 1.05\ (1/\tau_{H2O} - 1/\tau_{D2O})-0.25$$

T_1 relaxivity.

T_1 relaxivity measurements were performed using a GE SIGNA EXCITE at 1.5 T and ambient temperature. The samples were diluted to different concentrations in 1.5 mL centrifuge tubes. For T_1 measurement, the samples were imaged collectively with a high-resolution inversion recovery pulse sequence (repeat time (T_R) = 1600 ms; echo time (T_E) = 9 ms; inversion time (T_1) = 50, 100, 200, 300, 400, 600, 700, 900, 1200, 1500 ms; field of view (FOV) = 150 mm × 150 mm; matrix = 320 × 320). The resulting images were analyzed on a pixel-by-pixel basis to a single exponential. These T_1 values were averaged over at least 45 pixels in the centre of each sample and

plotted as $1/T_1$ versus $[Gd^{3+}]$. The relaxivity values for the CAs were calculated using the following equation:

$$\frac{1}{T_1} = \frac{1}{T_{1d}} + r_1[Gd^{3+}]$$

where the slope of the plot represents the relaxivity, r_1.

The proton $1/T_1$ NMRD profiles were measured at room temperature and neutral pH on a Stelar Spinmaster FFC-1T fast field cycling NMR relaxometer (provided by Stelar srl-Italy) over a continuum of magnetic field strengths from 0.006 to 80 MHz proton Larmor frequencies.

Potentiometric measurements.

The pH-potentiometric measurements were carried out using a Five Easy Plus pH meter (Mettler Toledo) equipped with a FE28-Standard combined glass electrode and temperature-controlled reaction vessel held at 298K. A standardized solution of 0.1 M NaOH was used as the titrant. Samples were purged with N_2 prior to measurement, and an inert atmosphere was maintained by constant N2 passage over the titration vessel. The electrode was calibrated prior to each titration with standard buffer solutions (pH 4.01, 7.00, and 9.21) at 25.0 (± 0.1) ºC. The pH of the titration mixture was adjusted initially by the addition of a known volume of standard aqueous HCl and pH data points were collected after each addition of 0.05 mL of the standardized solution. All titration samples were prepared in solution of 0.1M NaCl in distilled, de-ionized water. Ligand solutions were prepared by dissolving a weighed quantity into the electrolyte and concentration was calculated from the effective weight of the ligand. Titrations for metal-ligand complex stability determination were done at 1:1

metal-to-ligand concentration ratios over the range 2.0 < pH < 11.0. All titrations were performed in triplicate. The data was analyzed using the HYPERQUAD 2008 software, giving the values for the protonation constants and stability constants.[2]

Transmetalation kinetics

The kinetic inertness of Gd-HAO-2 against transmetallation was evaluated *in vitro* in comparison with Gd-DTPA, Gd-DOTA and Gd-DTPA-BMA. Firstly, 5 ml of 1 mM Gd complex in phosphate buffer and 20 μl of a 250 mM aqueous solution of $ZnCl_2$ were mixed, the mixture was stirred and samples (0.3 ml) were collected at 2 h, 4 h, 6 h, 8 h, 10 h, 24 h, 48 h and then filtered. The concentration of Gd(III) in the supernatant was measured by ICP-AES. The kinetic inertness was determined as the percentage of the bound Gd(III) post-incubation to that of before incubation.

***In vitro* cytotoxicity.**

The cytotoxicity of the Gd(III) complexes were individually tested with human cervical carcinoma cell (HeLa) as the model cell lines by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In brief, The cells were cultured in 25 mL flasks with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), and antibiotics (100 U mL^{-1} penicillin-G, and 100 μg mL^{-1} streptomycin) at 37 ºC in a humidified 5% CO_2-containing atmosphere. For *in vitro* cytotoxicity assay, the cells were plated into 96-wellplates at a density of 4.0×10^3 cells per well in 0.1 mL culture medium and allowed to attach for 24 h. Afterwards, the growth media was removed and the cells were washed with PBS (2×). Then, 200 mL DMEM medium was added to each well with the solutions at

different concentrations of 20, 40, 60, 80, 100, 150, 200 μM. The MTT assay was conducted following the standard protocol after incubation for 12, 24, and 48 h. The purple formazan in the supernatant was quantified by measuring the absorbance at 595 nm with a spectrophotometer (SPECTRAmax384, Molecular Devices, USA).

***In vivo* application**

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of East China University of Science and Technology and Experiments were approved by the Animal Ethics Committee of East China University of Science and Technology. To examine the MRI enhancement in animal model, three nude mice bearing subcutaneous human breast tumors (MDAMB-231) were injected Gd-HAO-2 (100 μL, 0.3×10^{-3} M). T_1-weighted MR images were acquired at 0, 15, 30, 45, 75, 110, 150, 200, 240 and 300 min post injection by the machine MesoMR23-060H-I produced by Niumag® analytical instrument Corporation. All methods were carried out in accordance with relevant guidelines and regulations.

Synthesis.

General procedure for the synthesis of diethylenetriamine pentaacetic acid-bisamides (b_1-b_8) [3]

DTPAA (1.0 mmol) was added to a 50 mL Schlenk flask. After charged with dry DMF (5.0 mL), the flask was sealed and heated at 50 ºC under N_2 atmosphere. Then amine (2.2 mmol) was added to the solution dropwise, followed by stirring for 24 h. After evaporation of the solvent under vacuum, the yellow oil was dissolved in water

before adjusting the pH to 12.00 to break the hydrogen-bonding interactions between the carboxyl groups and the free amine, thus the excessive amine could be excluded after the evaporation under vacuum. With the aim of changing the sodium carboxylates back into carboxyl groups, the provided solid was dissolved in water again to adjust the pH to 7.0. When the water was evaporated under vacuum, ethanol was added to the mixture and the precipitated NaCl was removed by filtration. Finally the solution was concentrated *in vacuo* and recrystallized from ethanol / diethyl ether(1:4) to afford a yellow fluffy solid.

Compound b_1.

The reaction was performed between DTPAA and diethylamine. ^{1}H NMR (D_2O, ppm): 4.33 (s, 4H), 3.86 (s, 4H), 3.48 (s, 2H), 3.45 (t, 4H), 3.37 (q, 4H), 3.26 (m, 4H), 3.06 (t, 4H), 1.14 (t, 6H), 1.08 (t, 6H). ^{13}C NMR (D_2O, ppm): 177.8, 173.1, 170.9, 58.5, 55.8, 52.3, 51.0, 42.9, 42.6, 41.7, 17.5, 13.8, 12.8, 11.2. Mass spectrum (ESI): M/Z calc. for $C_{22}H_{41}N_5O_8Na^+$: 526.2853; found: 526.2856. Anal. Calc. for $C_{22}H_{41}N_5O_8 \cdot 3H_2O$: C, 47.38; H, 8.50; N, 12.56. Found: C, 47.36; H, 8.48; N, 12.59.

Compound b_2.

The reaction was performed between DTPAA and isopropylamine. ^{1}H NMR (D_2O, ppm): 3.92 (m, 2H), 3.74 (s, 2H), 3.31 (m, 8H), 3.17 (s, 4H), 3.02 (t, 4H), 1.13 (d, 12H). ^{13}C NMR (D_2O, ppm): 179.7, 173.0, 171.7, 59.9, 59.6, 58.6, 55.1, 53.1, 51.2, 42.3, 22.0. Mass spectrum (ESI): M/Z calc. for $C_{20}H_{36}N_5O_8NaK^+$: 536.2099; found: 536.2101. Anal. Calc. for $C_{20}H_{37}N_5O_8 \cdot H_2O$: C, 48.67; H, 7.96; N, 14.19. Found: C, 48.42; H, 7.68; N, 14.30.

Compound b_3.

The reaction was performed between DTPAA and isobutylamine. ^{1}H NMR (D_2O, ppm): 3.77 (s, 2H), 3.37 (s, 4H), 3.33 (t, 4H), 3.21 (s, 4H), 3.08-3.02 (m, 8H), 1.80 (m, 2H), 0.86 (d, 2H). ^{13}C NMR (D_2O, ppm): 179.7, 174.1, 171.7, 171.1, 59.6, 58.6, 55.1, 53.4, 51.0, 47.2, 28.6, 20.0. Mass spectrum (ESI): M/Z calc. for $C_{22}H_{41}N_5O_8Na^+$: 526.2853; found: 526.2853. Anal. Calc. for $C_{22}H_{41}N_5O_8 \cdot 5H_2O$: C, 44.51; H, 8.66; N, 11.80. Found: C, 44.42; H, 8.51; N, 11.75.

Compound b_4.

The reaction was performed between DTPAA and diisobutylamine. ^{1}H NMR (D_2O, ppm): 3.84 (s, 4H), 3.67 (s, 2H), 3.39 (s, 4H), 3.28 (t, 4H), 3.20 (d, 4H), 3.15 (d, 4H), 3.14 (t, 4H), 1.97 (m, 2H), 0.87 (m, 24H), 0.88 (d, 12H), 0.85 (d, 12H). ^{13}C NMR (D_2O, ppm): 177.3, 173.2, 171.6, 58.2, 56.0, 55.1, 53.9, 52.2, 51.2, 27.6, 26.6, 20.0, 19.7. Mass spectrum (ESI): M/Z calc. for $C_{30}H_{57}N_5O_8K^+$: 654.3844; found: 654.3843. Anal. Calc. for $C_{30}H_{57}N_5O_8 \cdot 2H_2O$: C, 55.28; H, 9.43; N, 10.74. Found: C, 55.15; H, 9.32; N, 10.51.

Compound b_5.

The reaction was performed between DTPAA and butylamine. ^{1}H NMR (D_2O, ppm): 3.79 (s, 4H), 3.47 (t, 4H), 3.41 (t, 4H), 3.39 (s, 4H), 3.26 (t, 4H), 3.11 (t, 4H), 1.68-1.46 (m, 12H). ^{13}C NMR (D_2O, ppm): 177.9, 173.2, 169.7, 58.6, 56.1, 55.8, 52.3, 51.0, 47.0, 44.2, 26.5, 25.9, 24.3. Mass spectrum (ESI): M/Z c-alc. for $C_{22}H_{41}N_5O_8K^+$: 542.2592; found: 542.2591. Anal. Calc. for $C_{22}H_{41}N_5O_8 \cdot H_2O$: C, 50.66; H, 8.31; N, 13.43. Found: C, 50.82; H, 8.45; N, 13.51.

Compound b_6.

The reaction was performed between DTPAA and benzylamine. ^{1}H NMR (D_2O, ppm): 7.40-7.34 (m, 4H), 7.33-7.26 (m, 6H), 4.37 (s, 4H), 3.64 (s, 2H), 3.36 (s, 4H), 3.18 (m, 8H), 3.15 (s, 4H), 2.95 (t, 4H). ^{13}C NMR (D_2O, ppm): 179.0, 173.9, 171.1, 138.7, 129.5, 129.5, 128.2, 128.1, 127.9, 59.3, 59.2, 55.1, 53.1, 50.9, 43.4. Mass spectrum (ESI): M/Z calc. for $C_{28}H_{37}N_5O_8Na^+$: 594.2540; found: 594.2541. Anal. Calc. for $C_{28}H_{37}N_5O_8 \cdot 4H_2O$: C, 52.25; H, 7.05; N, 10.88. Found: C, 52.36; H, 7.13; N, 10.96.

Compound b_7.

The reaction was performed between DTPAA and piperidine. ^{1}H NMR (D_2O, ppm): 3.77 (s, 4H), 3.67 (s, 2H), 3.47 (t, 4H), 3.42 (t, 4H), 3.37 (s, 4H), 3.28 (t, 4H), 3.10 (t, 4H). 1.66-1.49 (m, 12H). ^{13}C NMR (D_2O, ppm): 178.1, 172.8, 169.9, 58.6, 56.2, 55.8, 52.4, 50.9, 47.1, 44.2, 26.5, 26.0, 24.3. Mass spectrum (ESI): M/Z calc. for $C_{24}H_{41}N_5O_8Na^+$: 550.2853; found: 550.2855. Anal. Calc. for $C_{24}H_{41}N_5O_8 \cdot 5H_2O$: C,46.67; H, 8.32; N, 11.34. Found: C, 46.52; H, 8.50; N, 11.42.

Compound b_8.

The reaction was performed between DTPAA and morpholine. ^{1}H NMR (D_2O, ppm): 3.76 (s, 2H), 3.72 (t, 8H), 3.70 (s, 4H), 3.58-3.53 (m, 8H), 3.32 (t, 4H), 3.31 (s, 4H), 3.07 (t, 4H). ^{13}C NMR (D_2O, ppm): 179.2, 171.8, 171.6, 67.0, 66.8, 58.6, 56.0, 55.6, 52.9, 50.3, 45.9, 42.8. Mass spectrum (ESI): M/Z calc. for $C_{22}H_{37}N_5O_{10}Na^+$: 554.2438; found: 554.2437. Anal. Calc. for $C_{22}H_{37}N_5O_{10} \cdot 3H_2O$: C, 45.12; H, 7.40; N, 11.96. Found: C, 45.24; H, 7.52; N, 11.71.

General procedure for the synthesis of diethylenetriamine-N-oxide pentaacetic acid-bisamides (c_1-c_8).

Compound **b** (1.5 mmol) was dissolved in methanol or acetic acid (3.0 mL) at room temperature before excessive hydrogen peroxide was added into the solution. After stirring for 72 h, Pd/C catalyst was used to consume the unreacted hydrogen peroxide. Then the mixture was filtered to remove Pd/C catalyst and the filtrate was evaporated to provide transparent oil, which was dissolved in ethanol. After adding diethyl ether, a white solid precipitated out. The mixture was placed in the refrigerator overnight at -20 ºC, and then filtered and dried *in vacuo* to afford compound c.

Compound c_1.

The reaction was performed between $\mathbf{b_1}$ and hydrogen peroxide in methanol. ^{1}H NMR (D_2O, ppm): 4.75-3.23 (m, 26H), 1.16 (t, 6H), 1.08 (t, 6H). ^{13}C NMR (D_2O, ppm): 170.2, 168.8, 164.6, 68.3, 68.0, 67.4, 66.7, 64.2, 63.9, 61.5, 60.9, 60.4, 43.6, 42.9, 42.0, 13.7, 12.5, 11.2. Mass spectrum (ESI): M/Z calc. for $C_{22}H_{40}N_5O_{11}Na_2^+$: 596.2520; found: 596.2520. Anal. Calc. for $C_{22}H_{41}N_5O_{11} \cdot 5H_2O$: C, 41.18; H, 8.01; N, 10.91; Found: C, 41.15; H, 7.99; N, 10.98.

Compound c_2.

The reaction was performed between $\mathbf{b_2}$ and hydrogen peroxide in methanol. ^{1}H NMR (D_2O, ppm): 4.69-4.11 (m, 18H), 3.95 (m, 2H), 3.46-3.16 (m, 2H), 1.15 (s, 12H). ^{13}C NMR (D_2O, ppm): 170.4, 169.4, 164.5, 69.0, 67.5, 67.2, 61.4, 60.9, 60.2, 43.0, 22.01. Mass spectrum (ESI): M/Z calc. for $C_{20}H_{36}N_5O_{11}Na_2^+$: 568.2207; found:

568.2209. Anal. Calc. for $C_{20}H_{37}N_5O_{11}$ • $5H_2O$: C, 39.15; H, 7.72; N, 11.41. Found: C, 39.31; H, 7.45; N, 11.46.

Compound c_3.

The reaction was performed between $\mathbf{b_3}$ and hydrogen peroxide in methanol. ^{1}H NMR (D_2O, ppm): 4.64-2.98 (m, 18H), 2.97 (d, 4H), 1.70 (m, 2H), 0.79 (d, 12H).^{13}C NMR (D_2O, ppm): 179.7, 174.1, 171.7, 171.1, 59.6, 58.6, 55.0, 53.4, 51.0, 47.2, 28.6, 20.0. Mass spectrum (ESI): M/Z calc. for $C_{22}H_{40}N_5O_{11}Na_2^+$: 596.2520; found: 596.2529. Anal. Calc. for $C_{22}H_{41}N_5O_{11}$ • $7H_2O$: C, 38.99; H, 8.18; N, 10.33. Found: C,39.10; H, 8.25; N, 10.42.

Compound c_4.

The reaction was performed between $\mathbf{b_4}$ and hydrogen peroxide in acetic acid. ^{1}H NMR (D_2O, ppm): 4.75-3.67 (m, 14H), 3.22 (m, 8H), 2.09-1.88 (m, 8H), 0.91-0.82 (m, 24H). ^{13}C NMR (D_2O, ppm): 170.9, 169.6, 165.0, 67.5, 64.0, 61.7, 55.9, 54.6, 50.6, 28.0, 26.7, 23.0, 19.9. Mass spectrum (ESI): M/Z calc. for $C_{30}H_{56}N_5O_{11}Na_2^+$: 708.3772; found: 708.3760. Anal. Calc. for $C_{30}H_{57}N_5O_{11}$ • $7H_2O$: C, 45.62; H, 9.06; N, 8.87. Found: C, 45.80; H, 9.15; N, 8.96.

Compound c_5.

The reaction was performed between $\mathbf{b_5}$ and hydrogen peroxide in methanol. ^{1}H NMR (D_2O, ppm): 4.30-3.88 (m, 4H), 3.54-2.87 (m, 20H), 1.69-1.45 (m, 12H). ^{13}C NMR (D_2O, ppm): 177.9, 171.0, 169.3, 164.7, 67.9, 66.5, 60.1, 57.4, 48.2, 46.9, 46.5, 27.9, 27.6, 19.3. Mass spectrum (ESI): M/Z calc. for $C_{22}H_{40}N_5O_{11}Na_2^+$: 596.2520;

found: 596.2517. Anal. Calc. for $C_{22}H_{41}N_5O_{11}$ • $11H_2O$: C, 35.24; H, 8.47; N, 9.34. Found: C, 35.11; H, 8.42; N, 9.41.

Compound c_6.

The reaction was performed between **b_6** **a**nd hydrogen peroxide in methanol. ^{1}H NMR (D_2O, ppm): 7.48-7.25 (m, 10H), 4.77-2.65 (m, 22H).^{13}C NMR (D_2O, ppm): 170.9, 168.9, 164.3, 137.3, 128.8, 127.6, 127.5, 127.4, 127.2, 127.1, 66.3, 63.2, 62.1, 60.1, 49.6, 43.2, 34.5. Mass spectrum (ESI): M/Z calc. for $C_{28}H_{36}N_5O_{11}Na_2^+$: 664.2207; found: 664.2210. Anal. Calc. for $C_{28}H_{37}N_5O_{11}$ • $9H_2O$: C, 43.02; H, 7.09; N, 8.96. Found: C, 42.98; H, 7.12; N, 9.00.

Compound c_7.

The reaction was performed between **b_7** and hydrogen peroxide in methanol. ^{1}H NMR (D_2O, ppm): 4.68-4.14 (m, 14H), 3.58-3.43 (m, 8H), 1.70-1.50 (m, 12H). ^{13}C NMR (D_2O, ppm): 171.7, 170.1, 169.0, 163.4, 84.6, 68.3, 66.7, 63.9, 61.2, 48.4, 44.4, 26.5, 25.7, 24.2. Mass spectrum (ESI): M/Z calc. for $C_{24}H_{40}N_5O_{11}Na_2^+$: 620.2520; found: 620.2521. Anal. Calc. for $C_{24}H_{41}N_5O_{11}$ • $11H_2O$: C, 37.25; H, 8.21; N, 9.05. Found: C, 37.11; H, 8.05; N, 9.16.

Compound c_8.

The reaction was performed between **b_8** and hydrogen peroxide in methanol. ^{1}H NMR (D_2O, ppm): 4.70-3.68 (m, 30H), 3.55 (t, 4H), 3.45 (t, 4H). ^{13}C NMR (D_2O, ppm): 171.5, 170.3, 168.6, 163.7, 67.09, 66.8, 64.3, 63.8, 61.3, 61.1, 47.0, 41.6. Mass spectrum (MALTI-TOF): M/Z calc. for $C_{22}H_{34}N_5O_{13}Na_3^+$: 645.1846; found: 645.1788.

Anal. Calc. for $C_{22}H_{37}N_5O_{13} \cdot 9H_2O$: C, 35.63; H, 7.47; N, 9.44. Found: C, 35.47; H, 7.25; N, 9.56.

General procedure for the synthesis of diethylenetriamine-N-oxide pentaacetic acid-bisamide-based Gd(III) complexes (d_1-d_8).

A solution of $GdCl_3 \cdot 6H_2O$ (1.05 mmole) in methanol (3.0 mL) was added to a stirred solution of diethylenetriamine-N-oxide pentaacetic acid-bisamide (1.00 mmol) in methanol (10.0 mL). An excess of pyridine (0.8 mL) was added and the suspension was heated at reflux for approximately 4 h. After evaporating the solvent, the mixture was dissolved in water and the pH was adjusted to 10 by adding 1 M NaOH in order to precipitate the excessive $GdCl_3$. The mixture was filtered through a 0.22 mm Millipore filter and recrystallized from ethanol/diethyl ether (1:5) to afford the final product.

Complex d_1

ESI-MS: M/Z calc. for C22H38N5O11Gd: 706.2; found:706.2.

Complex d_2

ESI-MS: M/Z calc. for C20H34N5O11Gd: 678.1; found:678.1.

Complex d_3

ESI-MS: M/Z calc. for $C22H38N5O11GdNa^+$: 729.2; found: 729.2.

Complex d_4

ESI-MS: M/Z calc. for C30H54N5O11Gd: 818.3; found: 818.3.

Complex d_5

ESI-MS: M/Z calc. for $C22H38N5O11GdNa^+$: 729.2; found: 729.2.

Complex d_6

ESI-MS: M/Z calc. for $C_{28}H_{34}N_5O_{11}GdNa^+$: 797.1; found: 797.1.

Complex d_7

ESI-MS: M/Z calc. for $C_{24}H_{38}N_5O_{11}Gd$: 730.2; found: 730.2.

Complex d_8

ESI-MS: M/Z calc. for $C_{22}H_{33}N_5O_{13}Gd$:733.1; found: 733.1.

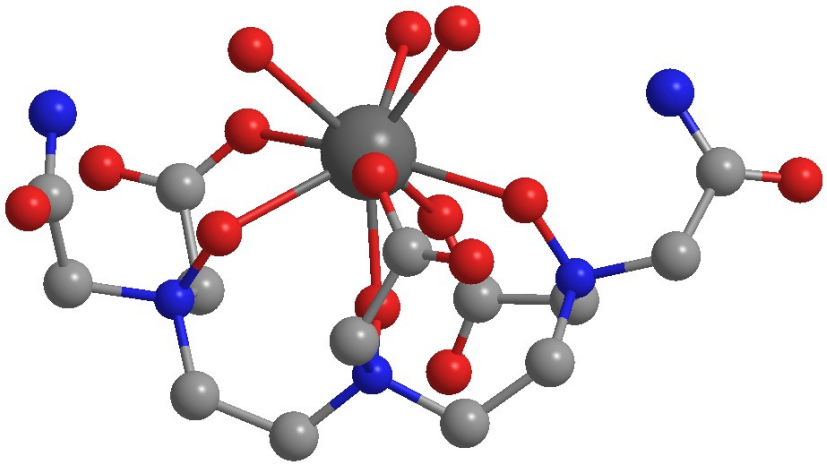


Figure S1. Computer simulated structure of Gd-HAO complex with Gaussian 09[4].

Table S1. Cartesian coordinates of Gd chelate optimized with B3LYP functional. Stuttgart pseudopotential with quasi-relativistic effect (MWB53) and correspondent basis set are employed for Gd^{3+} ion, where 53 inner shell electrons are treated with pseudopotential while eight valence electrons are treated explicitly. For other atomic kinds, 6-31G* basis set is employed.

Atomic Symbol	Coordinates(Å)		
	X	Y	Z
Gd	-0.01847300	-0.95280900	-0.21210100
C	1.39866900	2.95658500	0.50700700
H	1.44248600	4.00992000	0.20338900
H	1.42301100	2.87420600	1.59524400
C	2.60880900	2.26007200	-0.12477700
H	2.55291500	2.28340100	-1.21228700
H	3.46797300	2.85523100	0.19945600
C	-1.05237600	3.01245600	0.92502700
H	-1.06855100	4.09273000	0.73484000
H	-0.75134700	2.82875000	1.95730500
C	-2.48089200	2.50556900	0.66273800
H	-2.85028900	2.84989100	-0.30400300
H	-3.09953200	2.95910400	1.44543900
N	-2.78767500	1.01192900	0.64578800
N	2.97747300	0.79628700	0.20474900
N	0.05592300	2.37799700	0.10732800

C	-0.22827400	2.49977800	-1.39649200
H	-1.28768800	2.30032400	-1.51912700
H	0.02818700	3.52343100	-1.67510700
C	0.51424900	1.49176600	-2.33408700
O	1.42050700	1.93126200	-3.03728800
O	0.03935200	0.29505600	-2.31831900
O	0.13476700	1.11583900	0.59825400
O	-2.16159200	0.43409200	-0.44016300
O	2.23055500	-0.09600500	-0.53334200
C	-4.31859300	0.87150100	0.44336600
H	-4.73533900	0.38625200	1.32520500
H	-4.74684800	1.86354400	0.31013400
C	-2.40057300	0.31231900	1.95092300
H	-3.02432900	0.72166200	2.74827900
H	-1.34624600	0.50982700	2.13224500
C	2.98633500	0.50374800	1.70793400
H	3.61246100	-0.38111400	1.82545500
H	3.46273300	1.35900600	2.19138400
C	4.41117600	0.64967300	-0.30324900
H	5.05469700	1.34594500	0.23670100
H	4.36560900	0.91061200	-1.36318400
C	-4.62701400	0.08753400	-0.84384900
C	-2.68517700	-1.20068300	1.74357600
C	1.61096400	0.20973300	2.36638600
C	5.05050300	-0.74500500	-0.14023600
O	1.21039700	1.00757800	3.21519400
O	6.19794900	-0.80869300	0.28333400
O	-3.82068500	-1.61295800	2.02784600
O	-4.93962200	0.67971100	-1.86319800

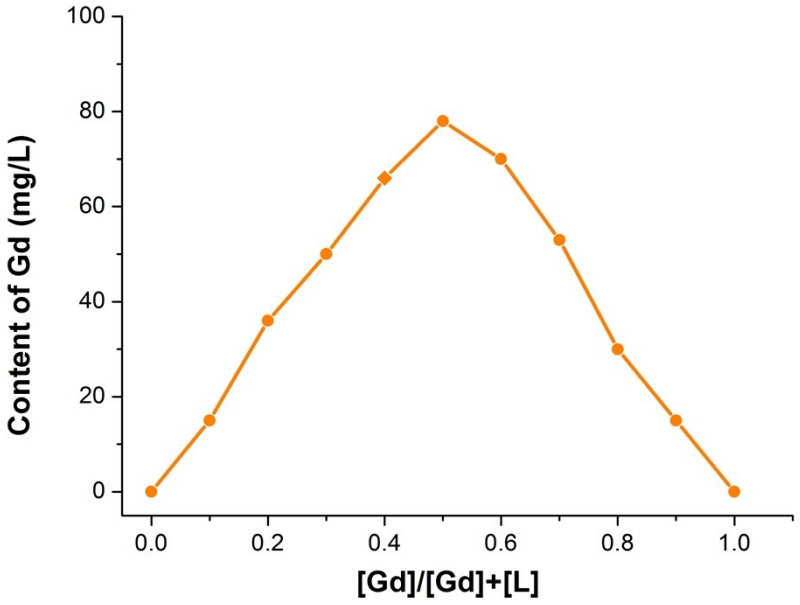
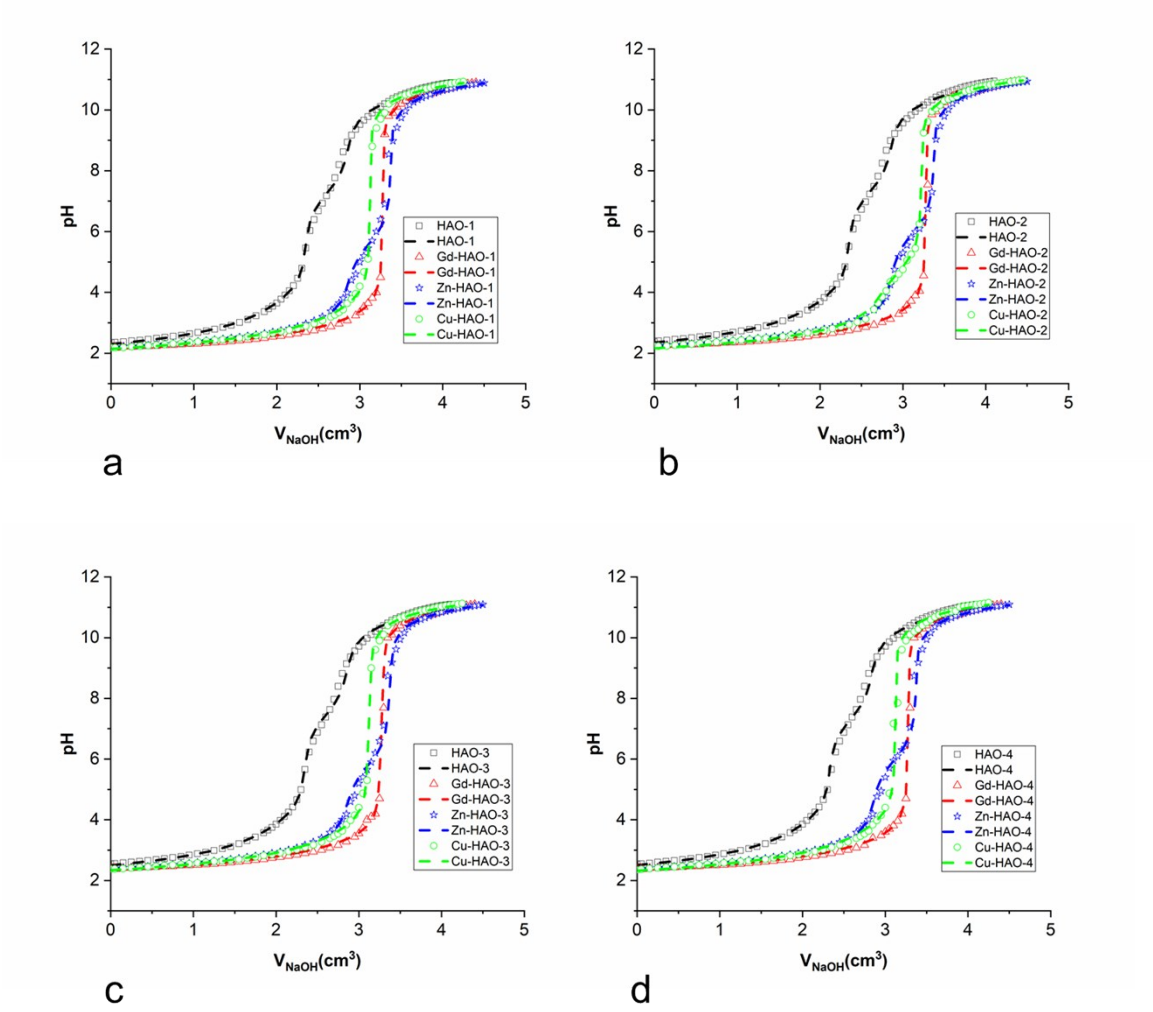


Figure S2. Job plots corresponding to the binary 1:1 combination of HAO-2 (L) and $GdCl_3$.



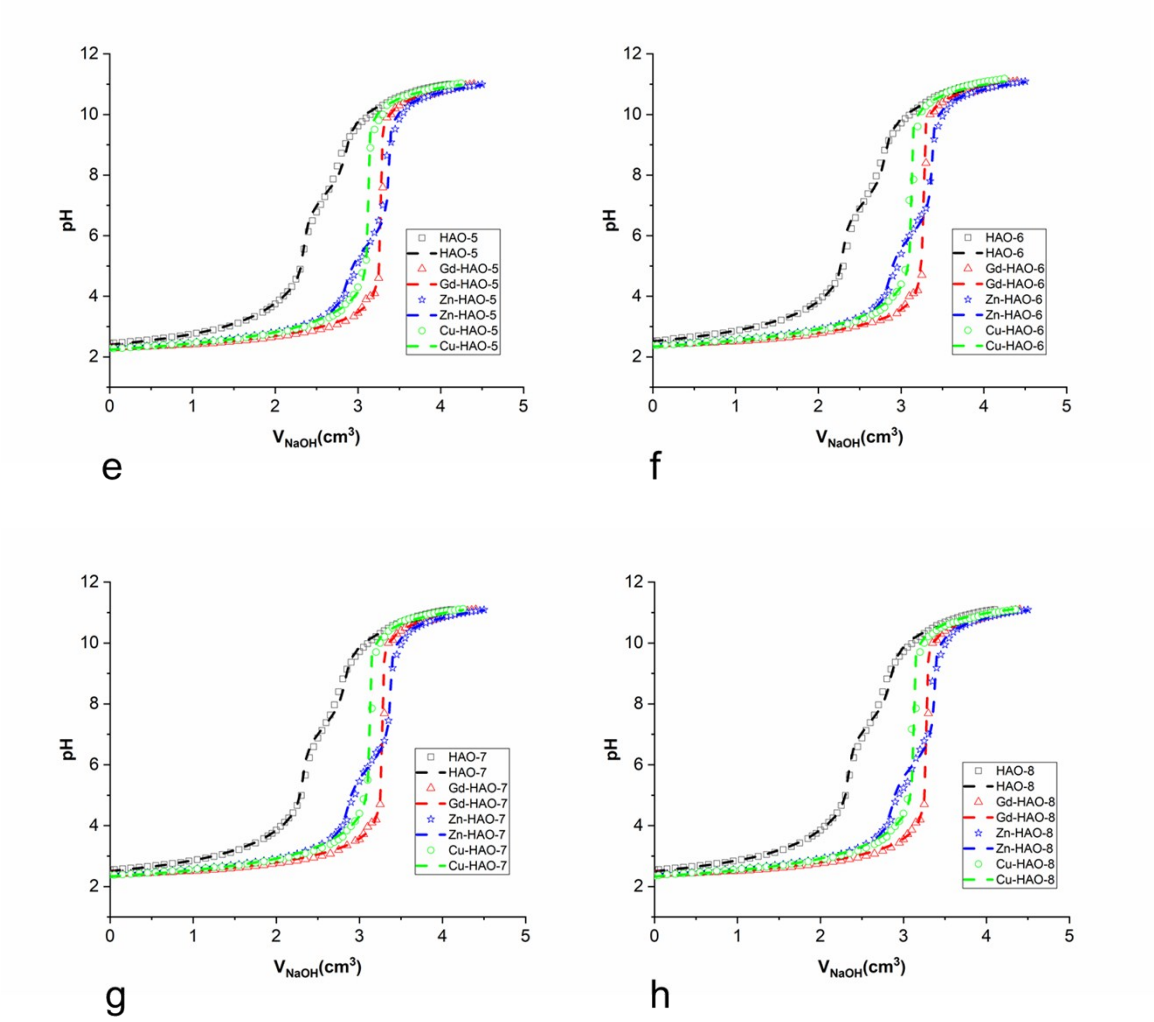


Figure S3. Potentiometric titration and fitting curves for the ligand HAO-1 to HAO-8 (a, b, c, d, e, f, g, h) in the absence and presence of Gd^{3+}, Zn^{2+}, Cu^{2+} (metal/ligand ratio 1:1; 0.1M NaCl).

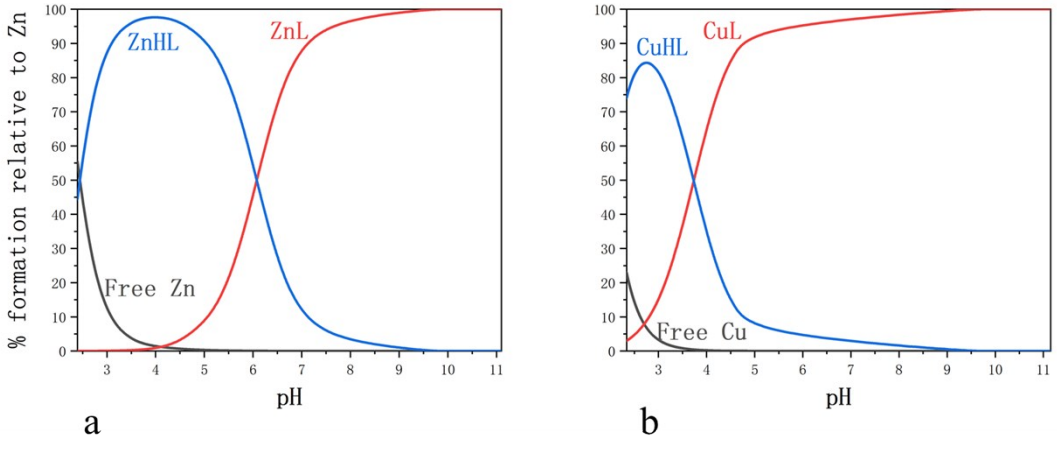
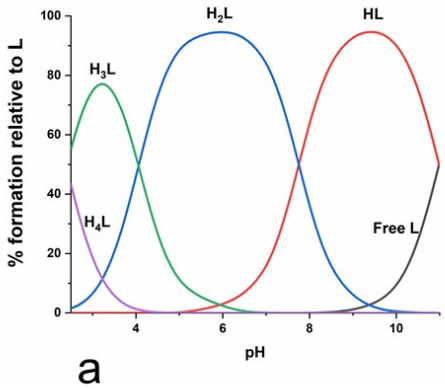
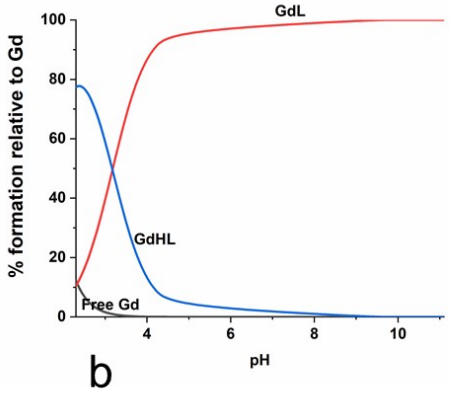


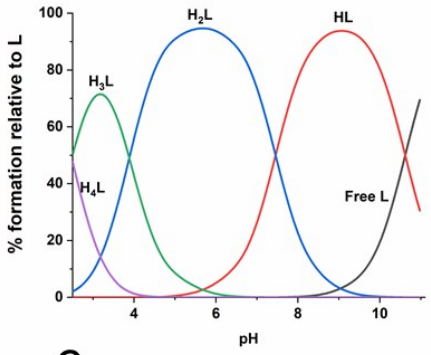
Figure S4. Species distribution curves for Zn-HAO-2 (a) and Cu-HAO-2 (b).


% formation relative to L
pH
H3L
H2L
HL
H4L
Free L

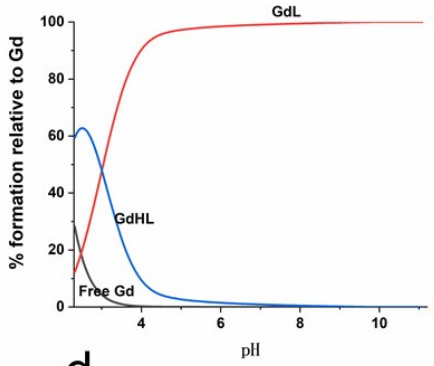
a


% formation relative to Gd
pH
GdL
GdHL
Free Gd

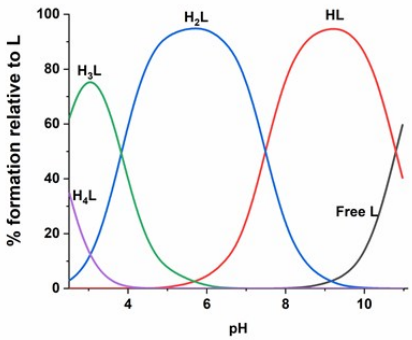
b


% formation relative to L
pH
H3L
H2L
HL
H4L
Free L

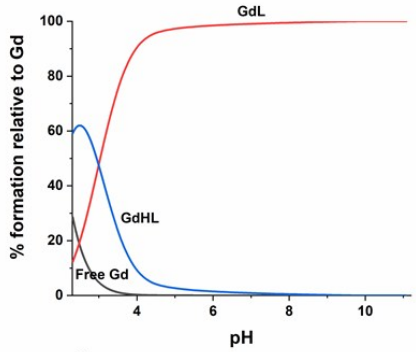
c


% formation relative to Gd
pH
GdL
GdHL
Free Gd

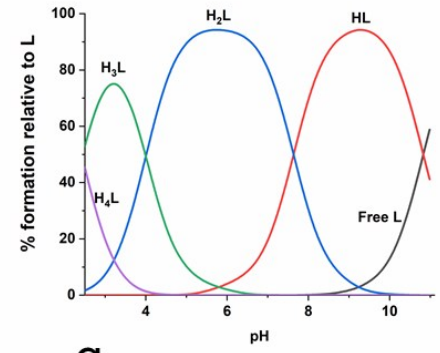
d


% formation relative to L
pH
H3L
H2L
HL
H4L
Free L

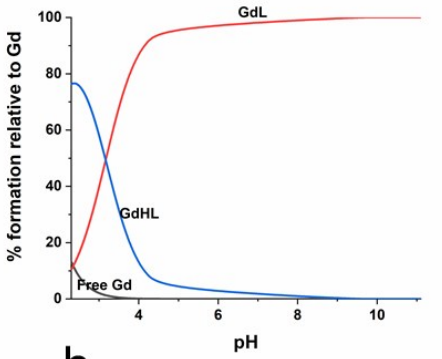
e


% formation relative to Gd
pH
GdL
GdHL
Free Gd

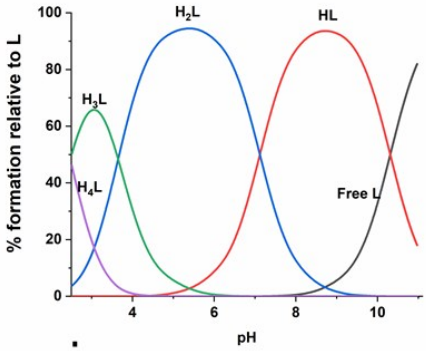
f


% formation relative to L
pH
H3L
H2L
HL
H4L
Free L

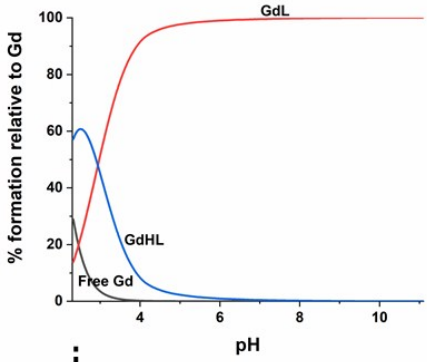
g


% formation relative to Gd
pH
GdL
GdHL
Free Gd

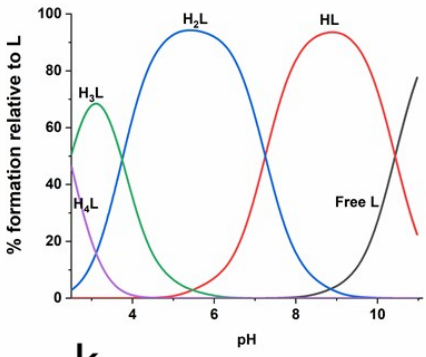
h


% formation relative to L
pH
H3L
H2L
HL
H4L
Free L

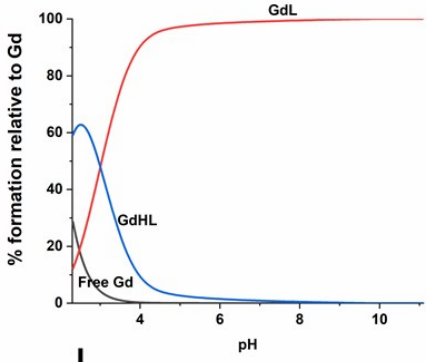
i


% formation relative to Gd
pH
GdL
GdHL
Free Gd

j


% formation relative to L
pH
H3L
H2L
HL
H4L
Free L

k


% formation relative to Gd
pH
GdL
GdHL
Free Gd

l

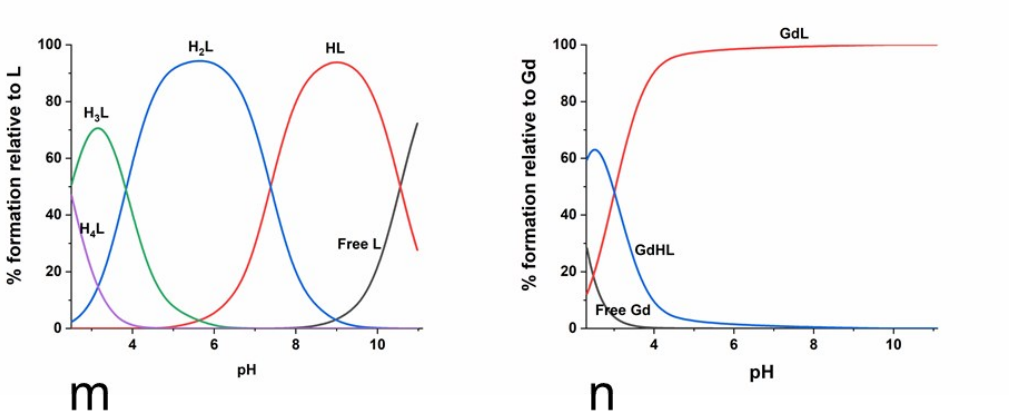


Figure S5. Species distribution curves for HAO-1 to HAO-8 (a, c, e, g, i, k, m) except HAO-2 and Gd-HAO-1 to Gd-HAO-8 (b, d, f, h, j, l, n) except Gd-HAO-2, where L represents HAO ligand.

Figure S6. Color photographs of xylenol orange solution in the presence of 100 μM (a), 50 μM (b), 25 μM (c), 15 μM (d), 10 μM (e), 0 μM (f) of Gd(III) ion and 1 mM Gd-HAO-2 (g), respectively. The concentration of xylenol orange solution is 10 mg/L.

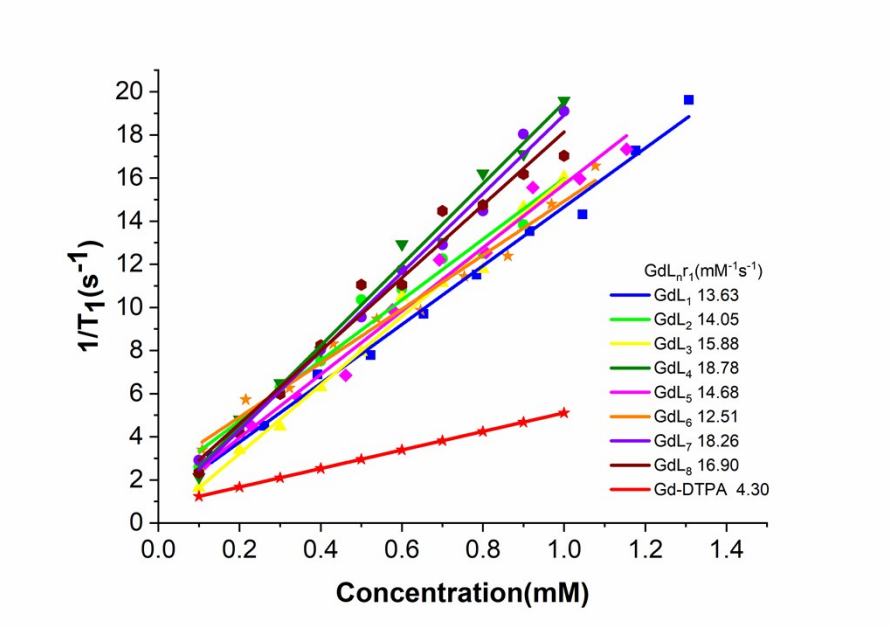


Figure S7. r_1 curves of HAO-based Gd(III) complexes and Gd-DTPA.

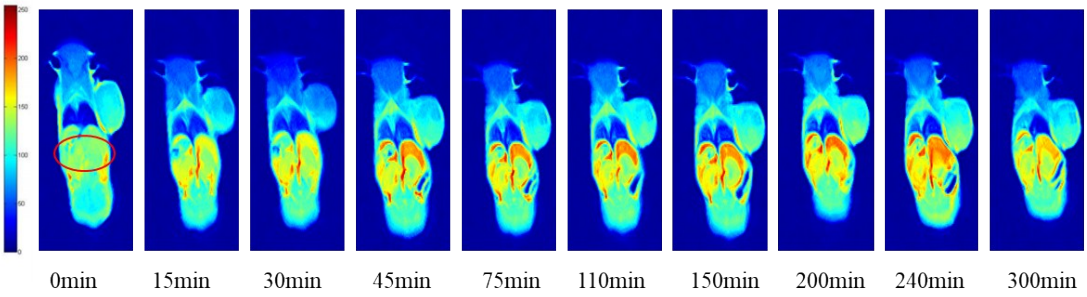


Figure S8. T1-wieghted MR images (color-coded by intensity), acquired at different time points after injection of Gd-HAO-2 into tumor-bearing nude mice. Kidney region is circled with red.

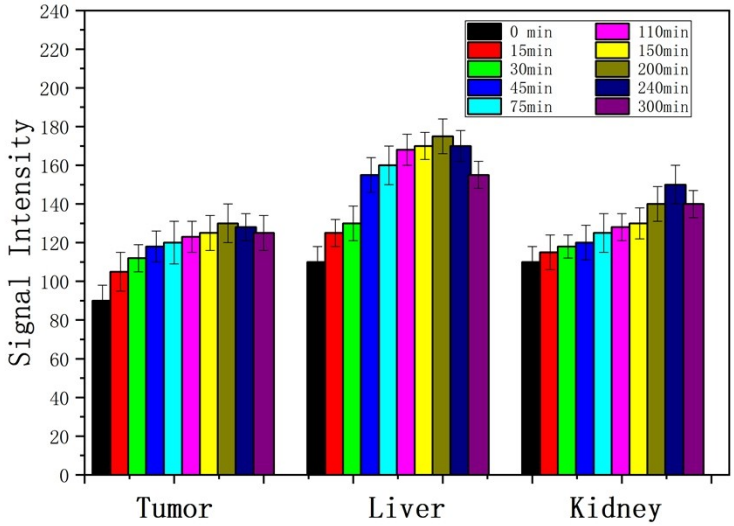


Figure S9. Signal intensity of tumor, liver, and kidney regions after administration.

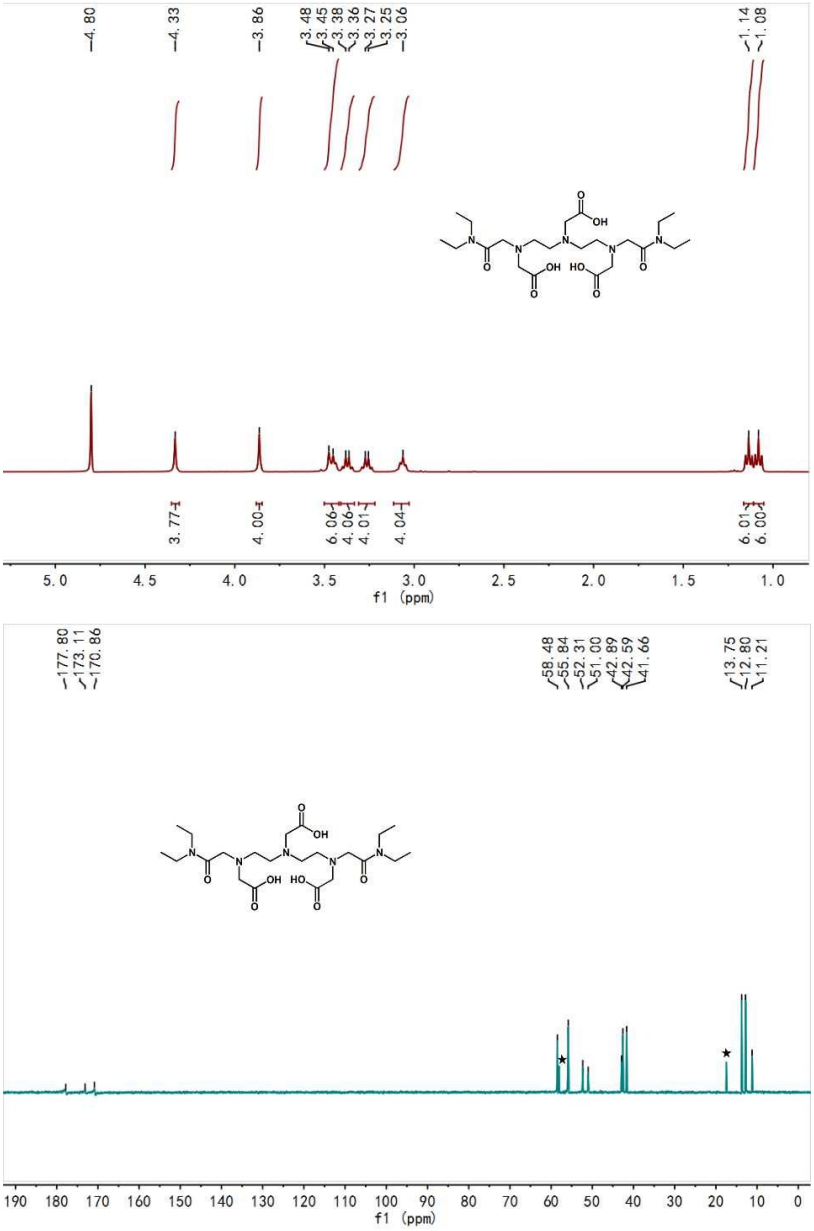


Figure S10. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_1}$ in D_2O (★ indicates ethanol).

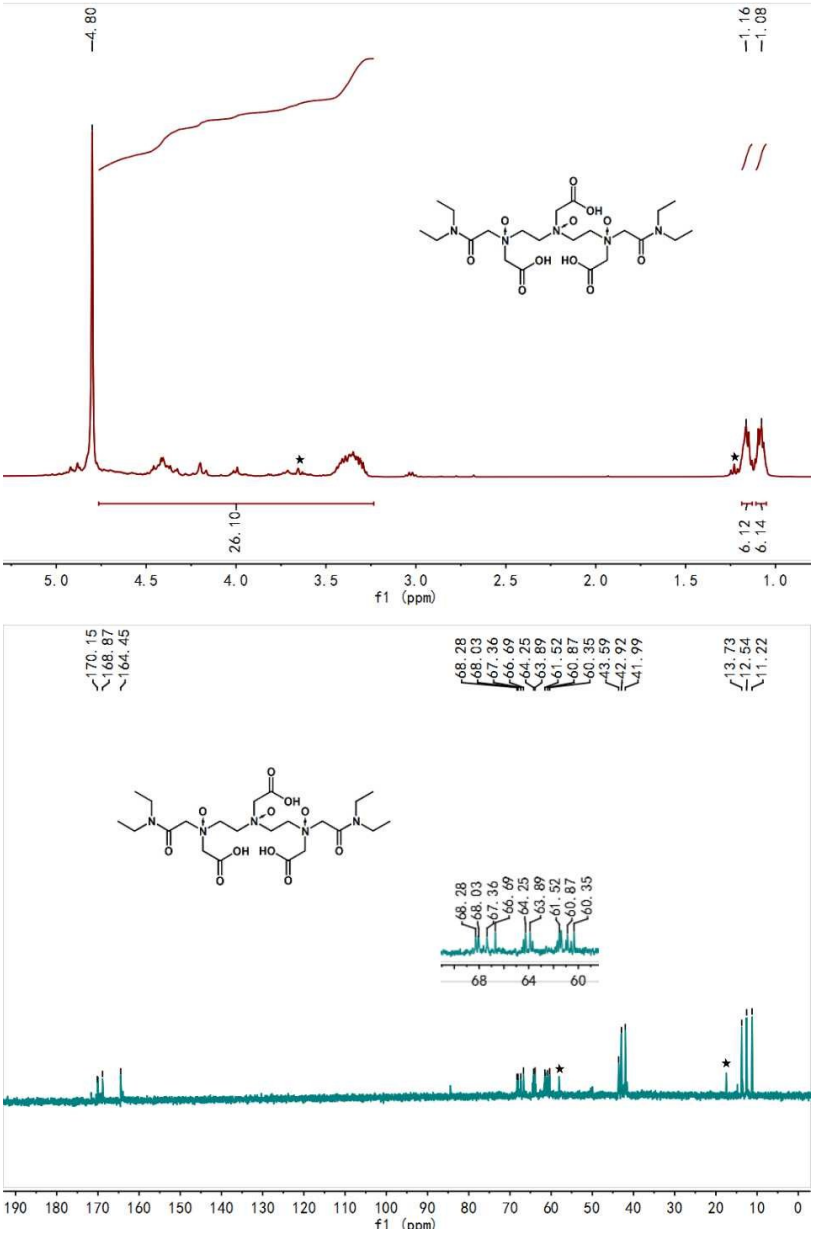


Figure S11. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_1}$ in D_2O (★ indicates ethanol).

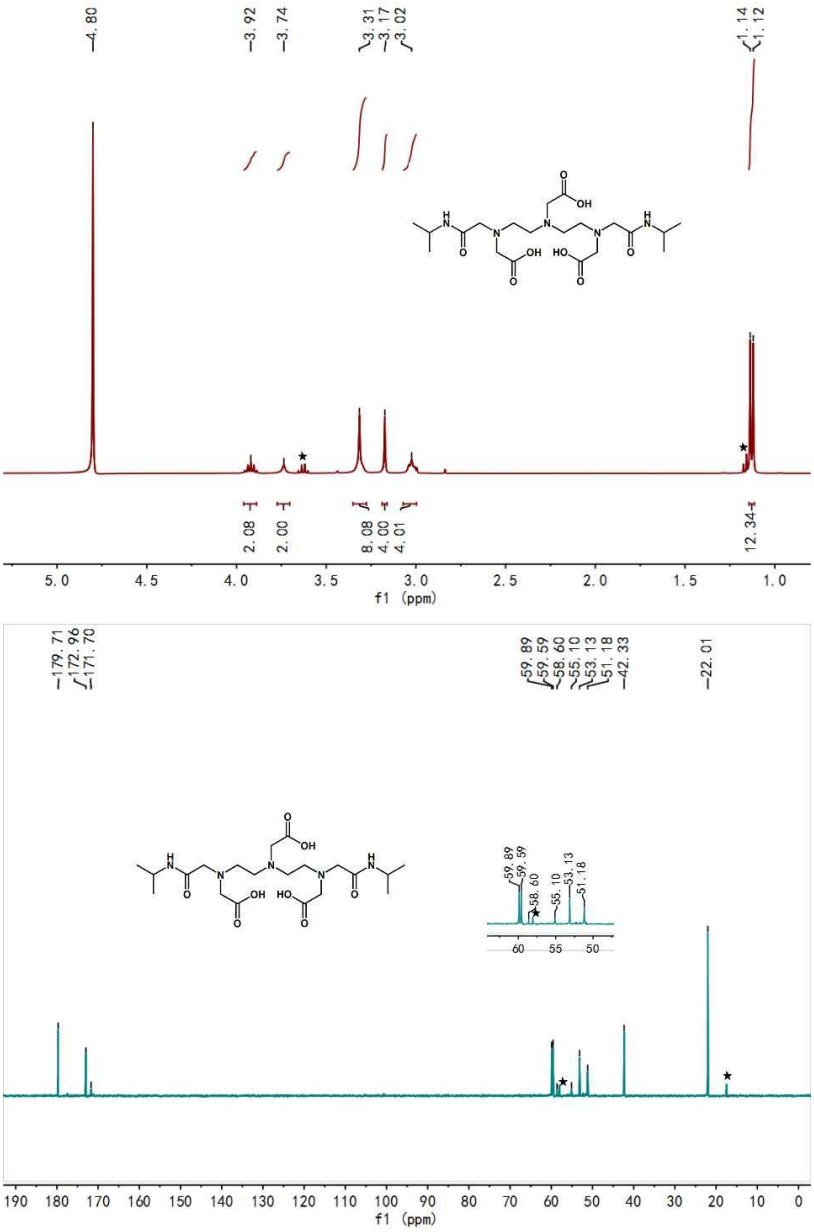


Figure S12. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_2}$ in D_2O (★ indicates ethanol).

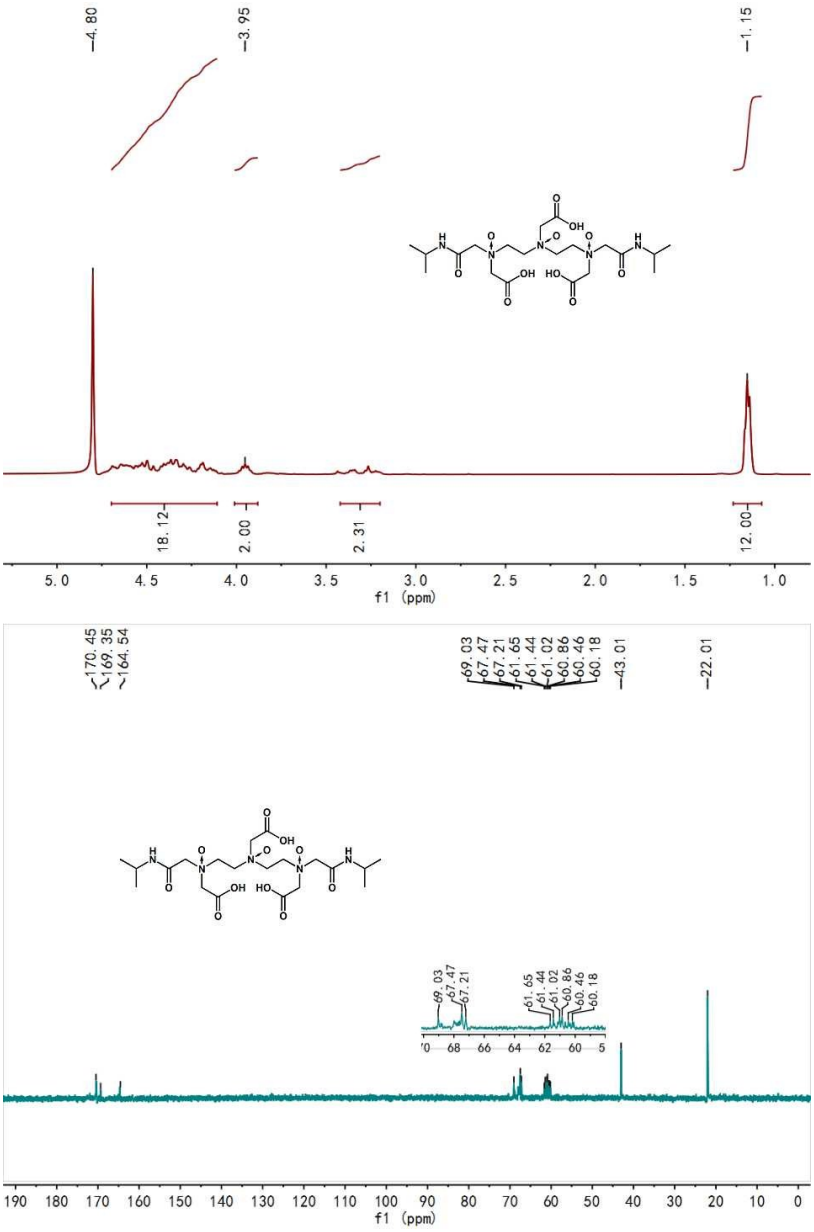


Figure S13. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_2}$ in D_2O.

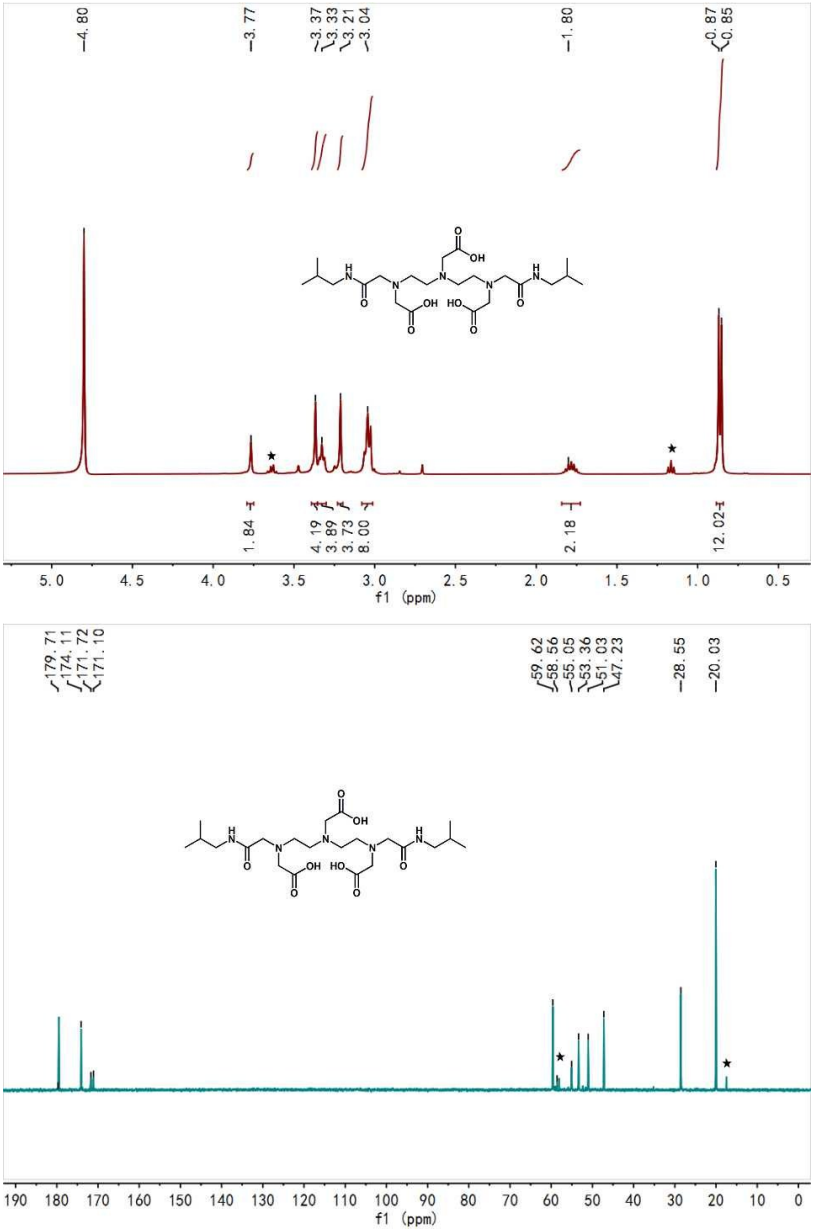


Figure S14. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_3}$ in D_2O (★ indicates ethanol).

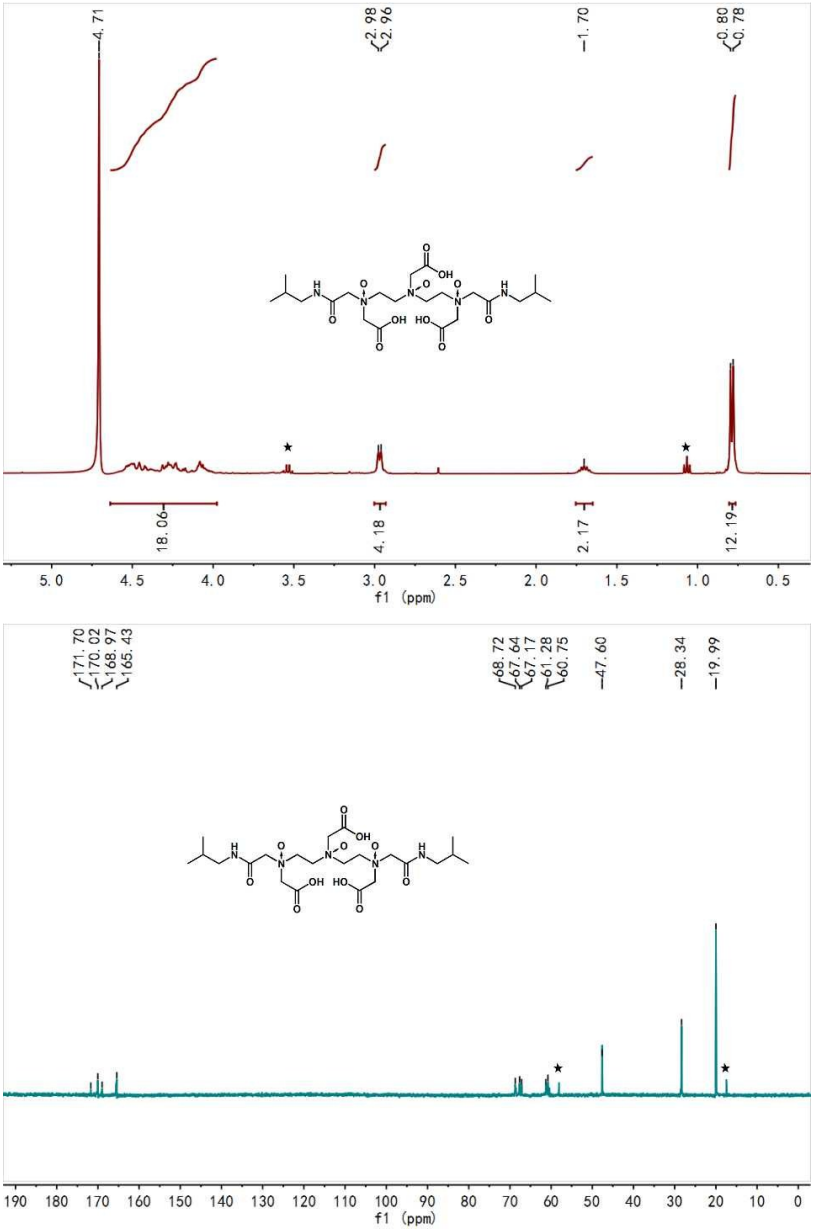


Figure S15. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_3}$ in D_2O (★ indicates ethanol).

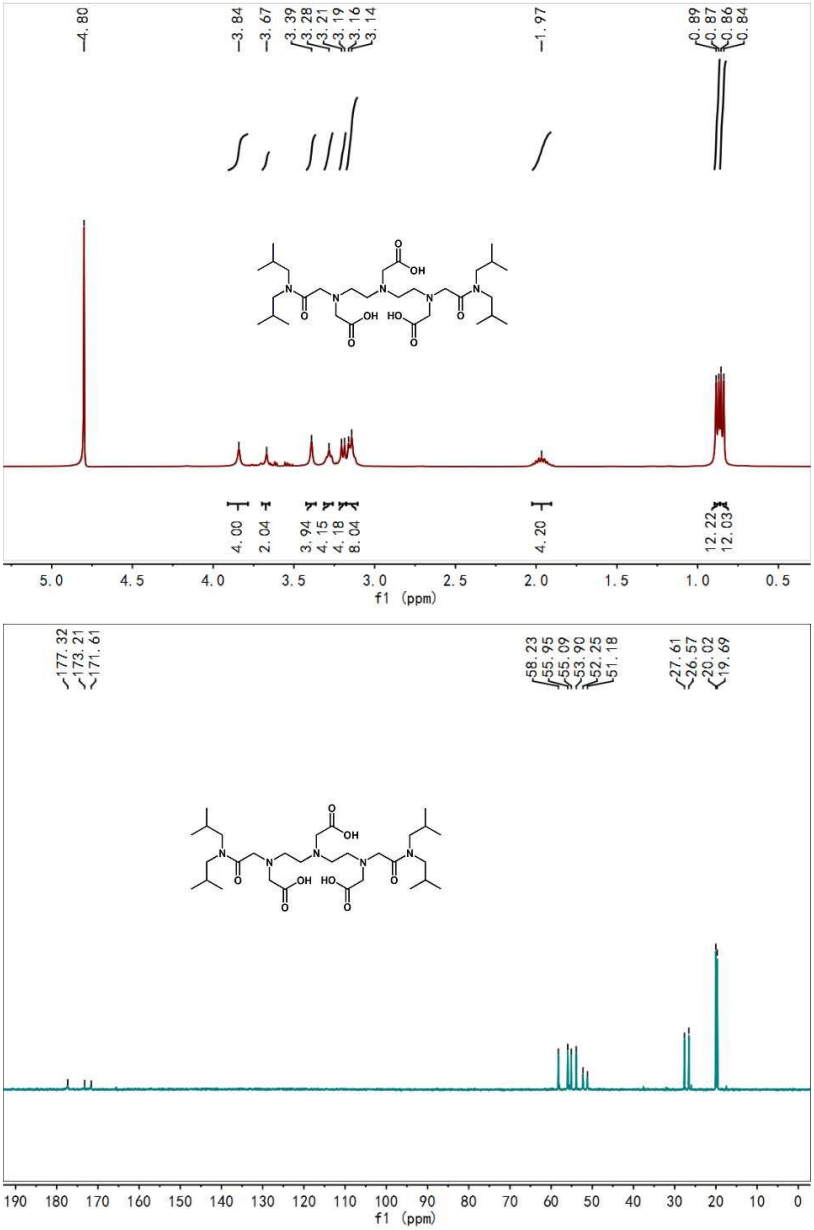


Figure S16. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_4}$ in D_2O.

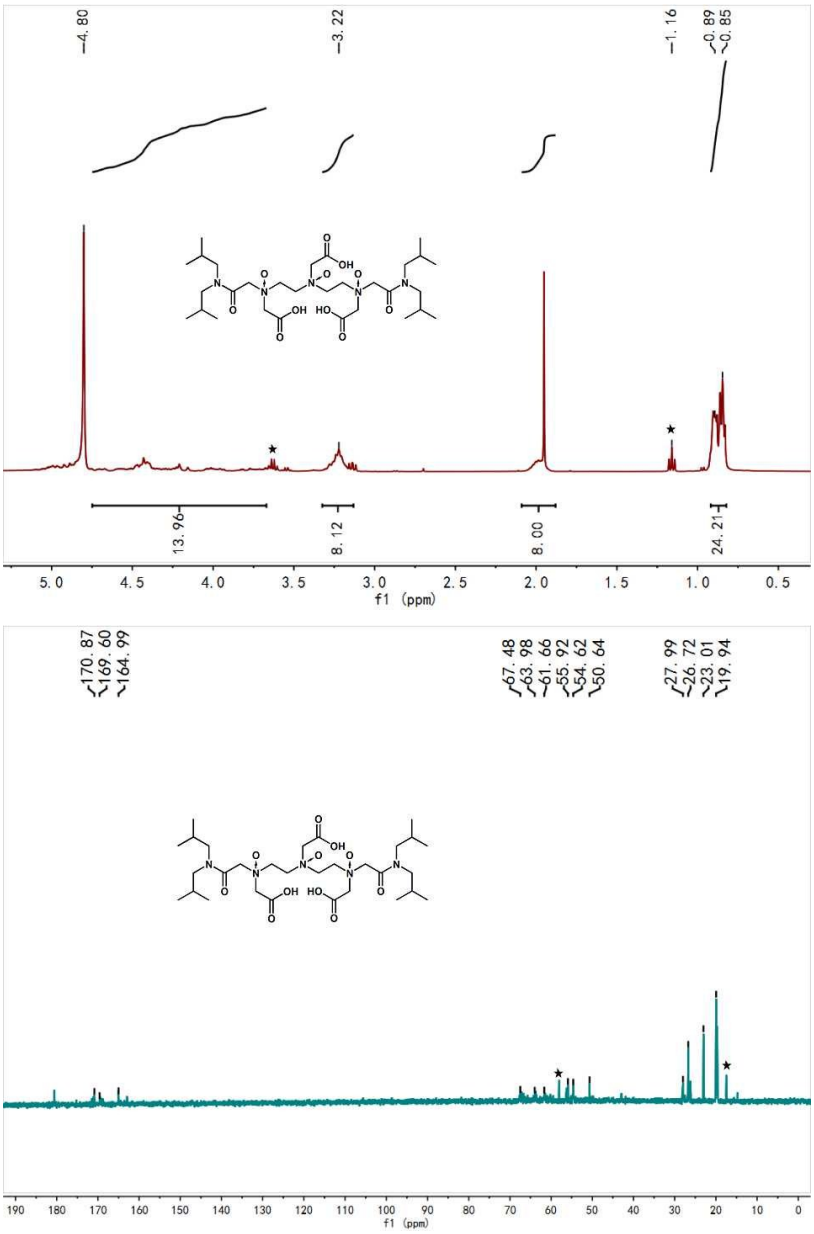


Figure S17. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_4}$ in D_2O (★ indicates ethanol).

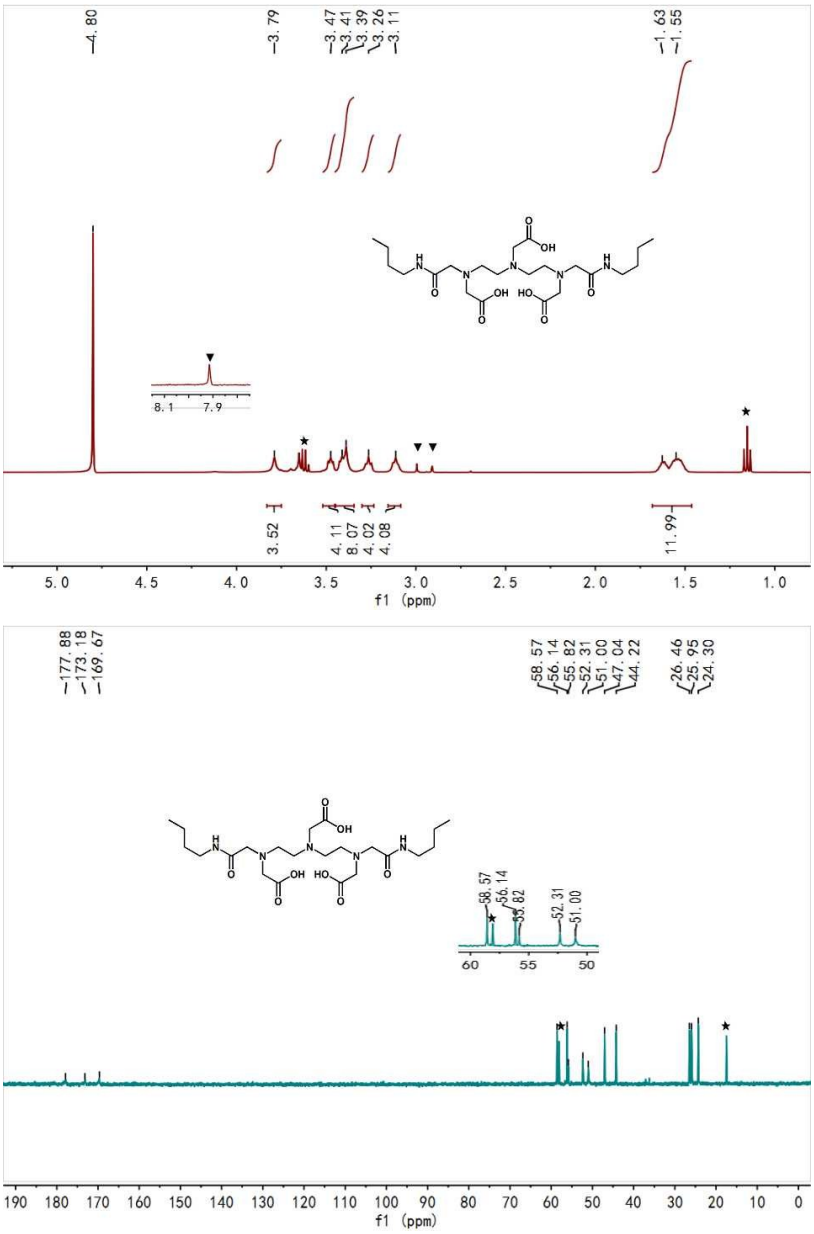


Figure S18. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_5}$ in D_2O (★ indicates ethanol and▼indicates DMF).

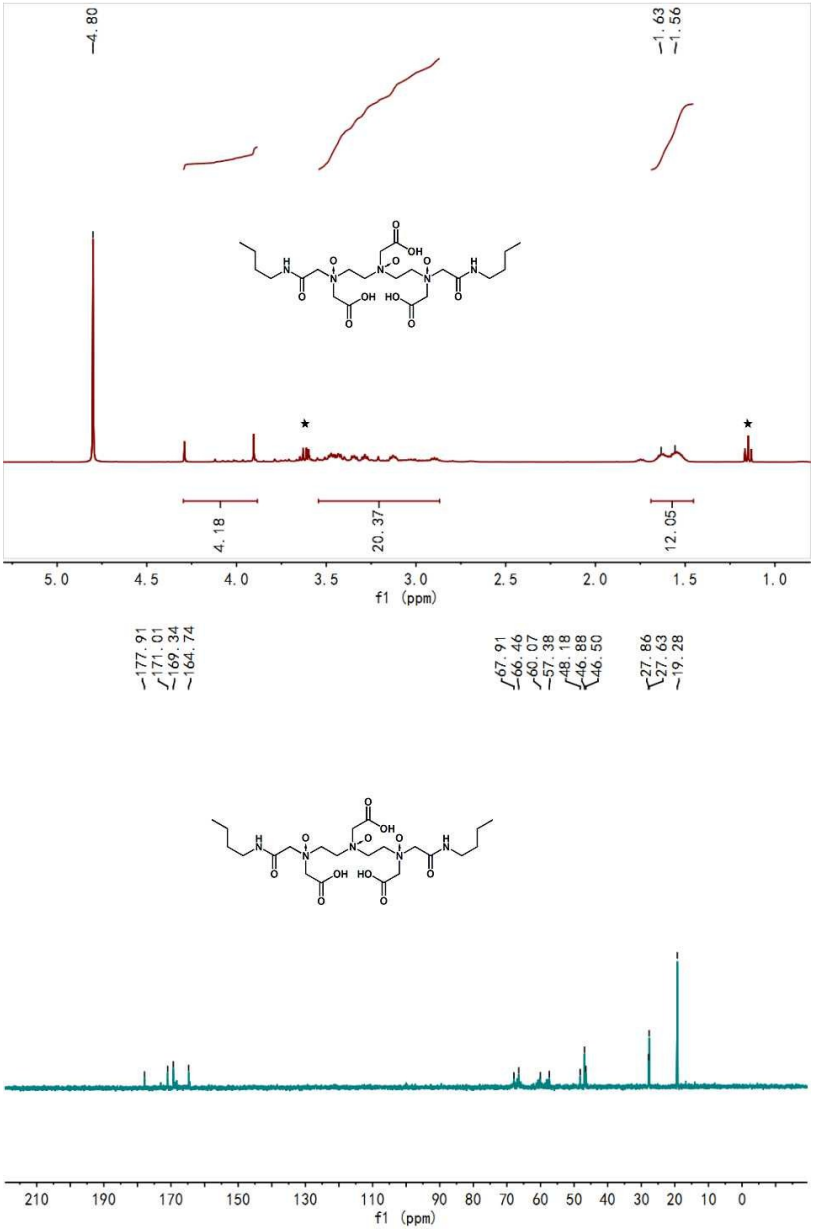


Figure S19. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_5}$ in D_2O (★ indicates ethanol).

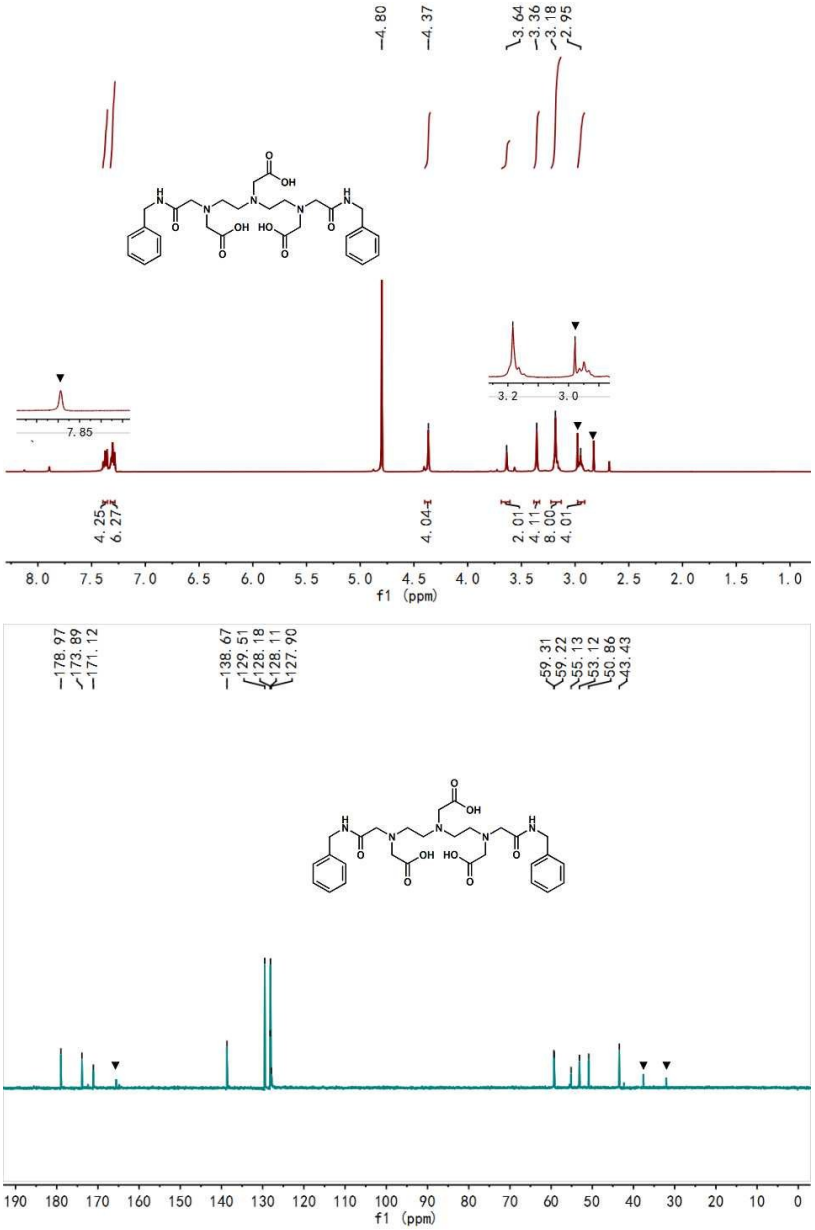


Figure S20. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_6}$ in D_2O (▼indicates DMF).

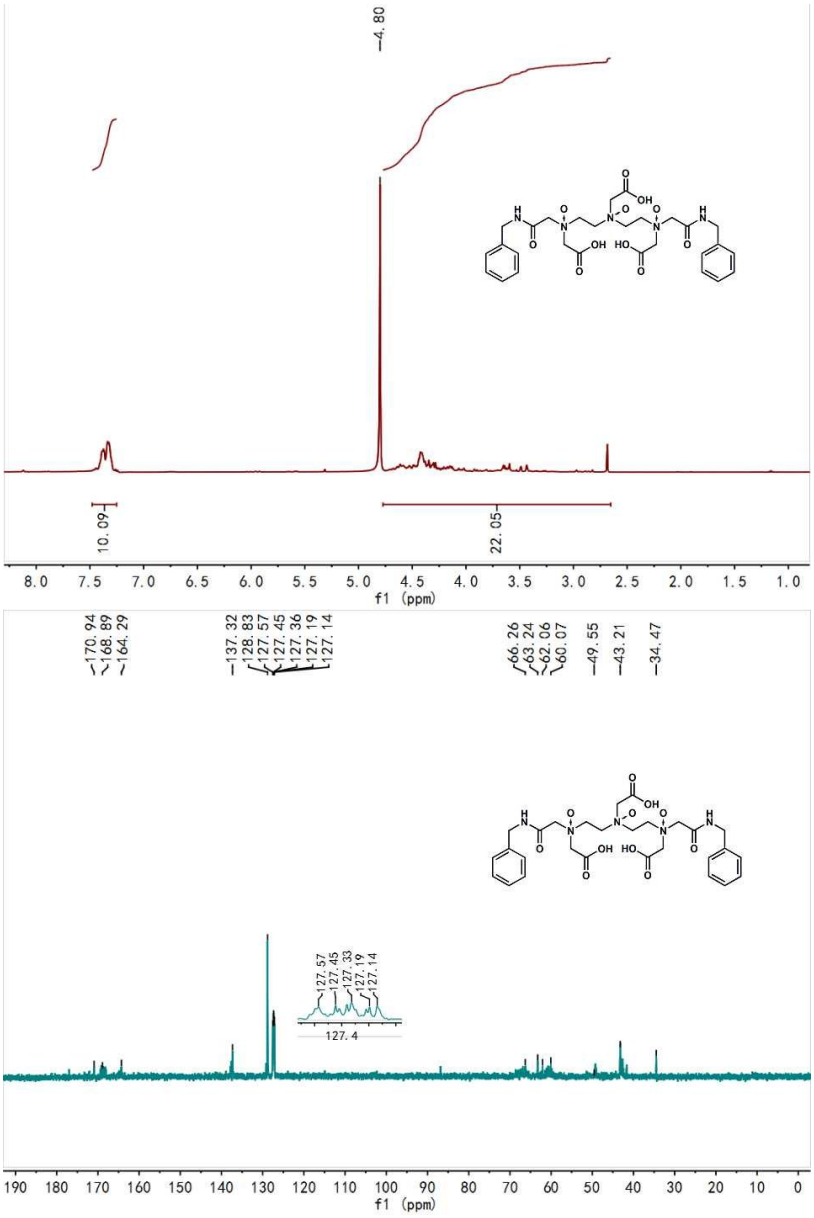


Figure S21. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_6}$ in D_2O.

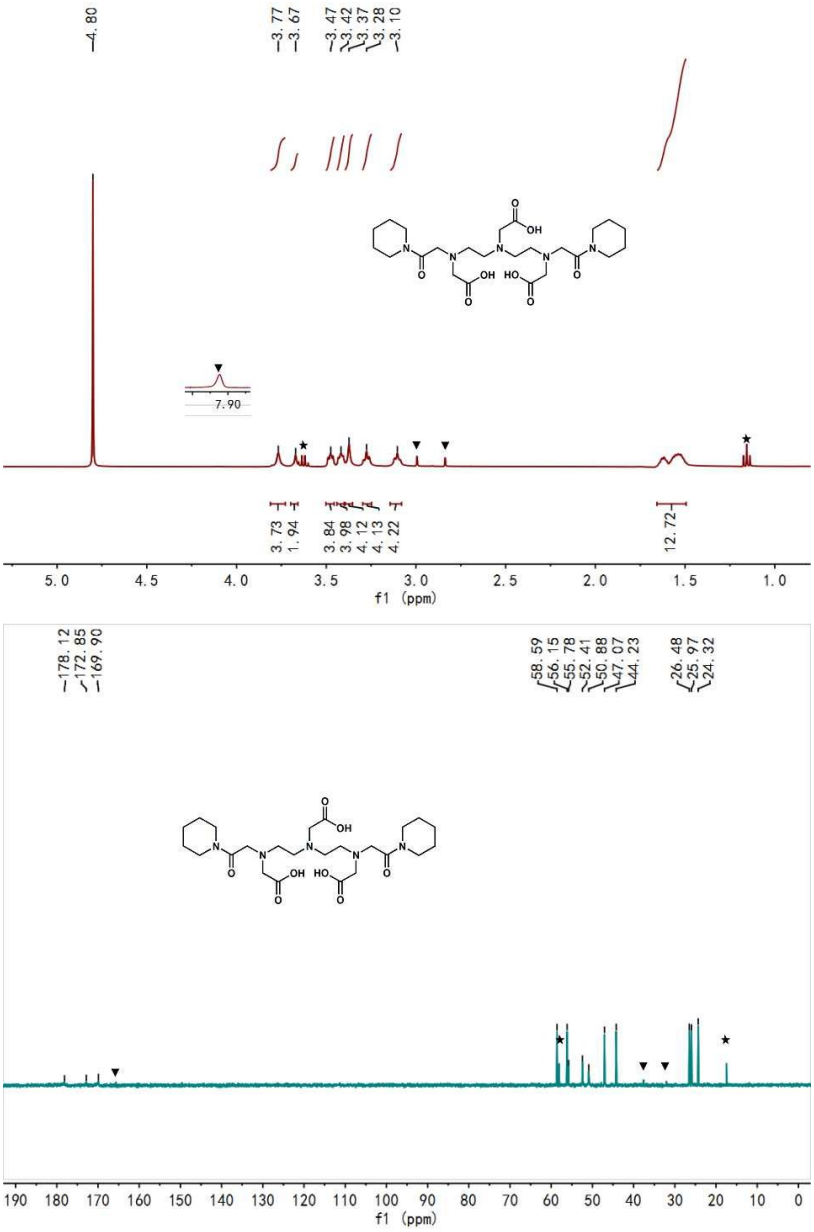


Figure S22. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_7}$ in D_2O (★ indicates ethanol and▼indicates DMF).

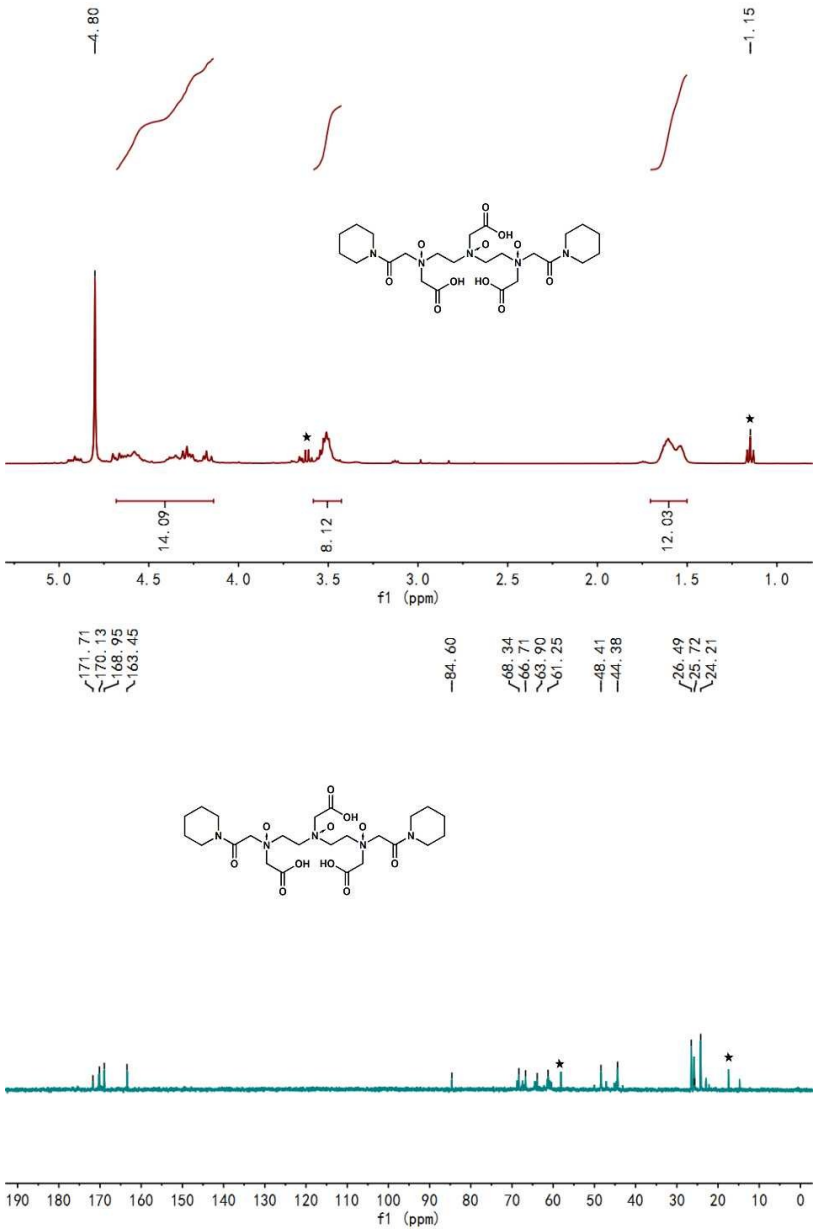


Figure S23. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_7}$ in D_2O (★ indicates ethanol).

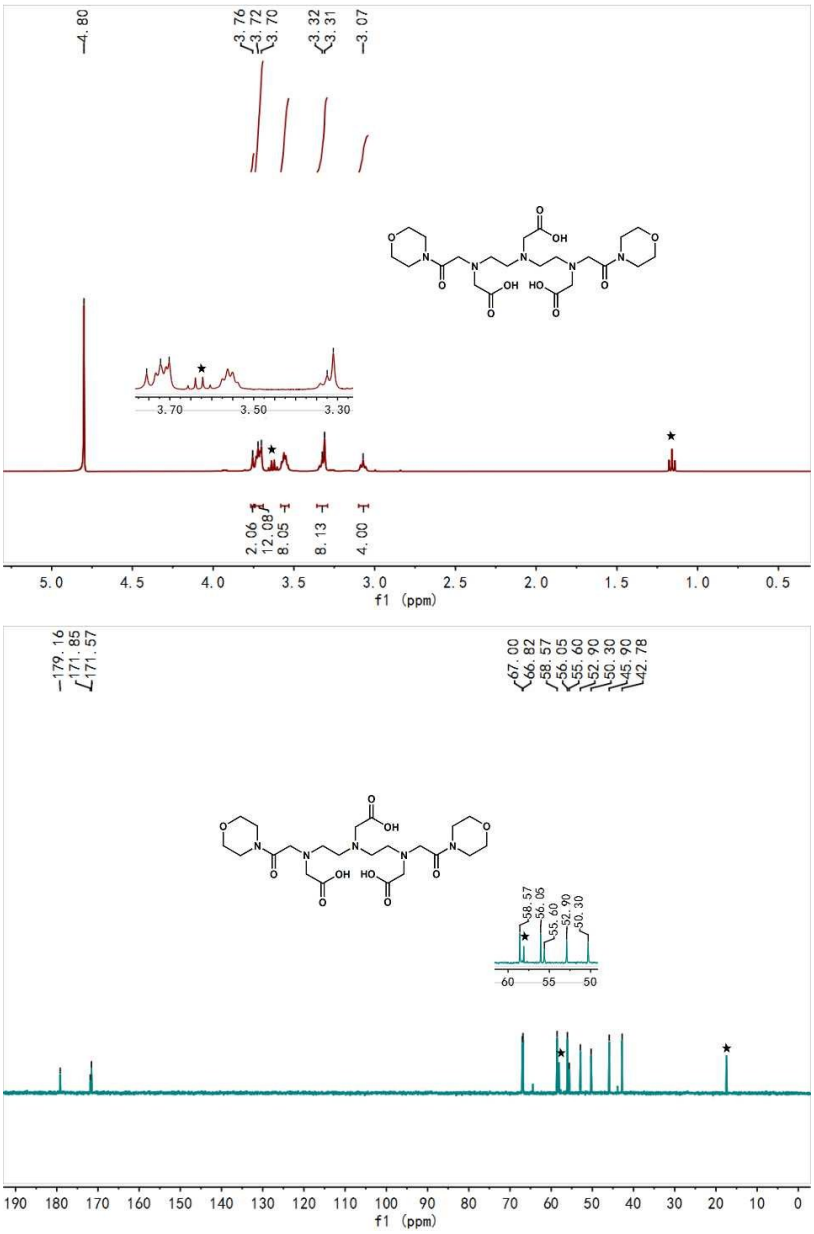


Figure S24. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{b_8}$ in D_2O (★ indicates ethanol).

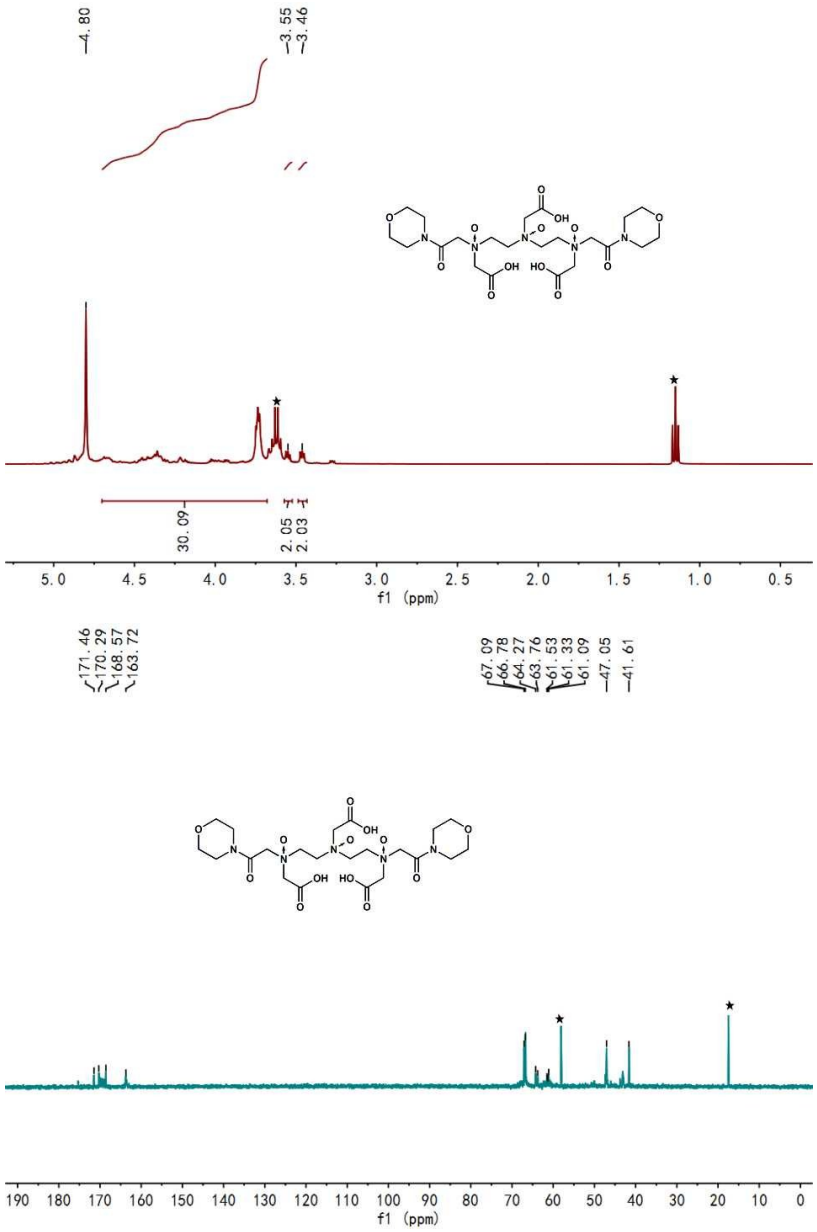


Figure S25. ^{1}H NMR and ^{13}C NMR spectra of $\mathbf{c_8}$ in D_2O (★ indicates ethanol).

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