General information

Deuterated solvents were purchased from Cambridge Isotope laboratories. All other solvents and reagents were of reagent grade and purchased from either Sigma-Aldrich Chemical Co. or Dieckmann and used without further purification. Thin-layer chromatography (TLC) was performed using pre-coated silica gel 60, F254 plates with a thickness of 0.2 mm. Column chromatography was conducted using silica gel and laboratory grade solvents. NMR spectra were recorded on Bruker Avance III 600 MHz spectrometers (^{1}H NMR on 600MHz, ^{13}C NMR on 151MHz) at 298 K. Chemical shifts δ were reported in parts per million (ppm) and referenced to corresponding solvent peak (2.50 for DMSO-d6 and 7.26 for $CDCl_3$). Coupling constants *J* were reported in Hertz (Hz) and multiplicities were abbreviated as: s = singlet, d = doublet, t = triplet, dd = doublets of doublet, td = triplets of doublet and m = multiplet. High-resolution mass spectra, reported as *m/z*, were conducted by University Research Facility in Life Science, Hong Kong Polytechnic University from Agilent 6540 liquid chromatography – electrospray ionisation quadrupole-TOF mass spectrometer or Bruker UltrafleXtreme MALDI-TOF-TOF mass spectrometer. Low-resolution mass spectra were conducted by Waters Acquity H-Class UPLC with QDa mass detector for monitoring reaction and determining correct fraction during purification of product. UV-vis absorption spectra were recorded on Agilent Cary 60 UV-Vis single beam spectrophotometer. Fluorescent spectra were recorded on Horiba FluoroMax 4 Spectrofluorometer.

HPLC methods

Analytical HPLC was carried out with Agilent 1260 Infinity II LC system equipped with a 1260 Infinity II Quaternary Pump and an inline diode array UV-Vis detector. Semi-preparative HPLC was carried out with Waters semi-preparative HPLC system equipped with 2535 Quaternary gradient solvent pump, 2707 Auotsampler, Fraction collector III and 2998 Photodiode array detector. Absorbance at 254 and 490 nm were monitored. The column used for analytical HPLC was Atlantis T3 Column, 5 μm, 4.6 x 250 mm C18 reverse phase column. The column used for semi-preparative HPLC was Atlantis T3 OBD Prep Column, 5 μm, 19 x 250 mm, C18 reverse phase column.

Table 1: Solvent gradient used for analytical HPLC.

Time (min)	% H_2O (+ 0.05% TFA)	% ACN (+ 0.05% TFA)	Flow (ml/min)
0	95	5	1.2
20	5	95	1.2
22	95	5	1.2
25	95	5	1.2

Table 2: Solvent gradient used for semi-preparative HPLC.

Time (min)	% H_2O (+ 0.05% TFA)	% ACN (+ 0.05% TFA)	Flow (ml/min)
0	95	5	8
30	5	95	8
31	95	5	8
35	95	5	8

Cell culture and cytotoxicity test

HK-2, HeLa and CT26 cells were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences. All cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin. The cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The cell viability by MTT assay. The cells were placed on a 96-well plate and maintained for twelve hours for adherence. The various concentrations of **NBD[Gd]** (0, 10, 50, 100, 200, 500, 1000 μM) in phosphate buffered saline (PBS) were added and incubated for 24 hours. After discarding media from cell cultures, 50 μL of serum-free media and 50 μL of MTT solution into each well and incubated the plate at 37°C for 3 hours. When the incubation finished 150 μL of MTT solvent was added into each well. The plate was wrapped in foil and shake on an orbital shaker for 15 minutes. Optical absorbance was measured at 590 nm on a BioTek Synergy 2 plate reader. Relative cell viability was determined by the equation $RV = (A_e - A_0)/(A_c - A_0) \times 100\%$, were A_e is the absorption of the experimental test; A_c is the absorption of the control test (DMSO only); A_0 is the absorption of the blank test (no cells).

***In vitro* fluorescence imaging**

HK-2, HeLa and CT26 cells were seeded on a 35 mm glass bottom petri dish. The cells were incubated with **NBD[Gd]** (10 μM) and co-staining with 4',6-diamidino-2-phenylindole (DAPI) for four hours. The cells were then washed twice with phosphate buffered saline (PBS) and was added with the fresh medium before imaging. Images were acquired using 405 and 488 nm laser excitation of ZEISS LSM 980 with Airyscan 2 confocal laser scanning microscope.

Relaxometry at 1.41 T

T_1-weighed relaxation times were measured on a Magritek Benchtop NMR Spinsolve 60 MHz equipped with MestReNova software.

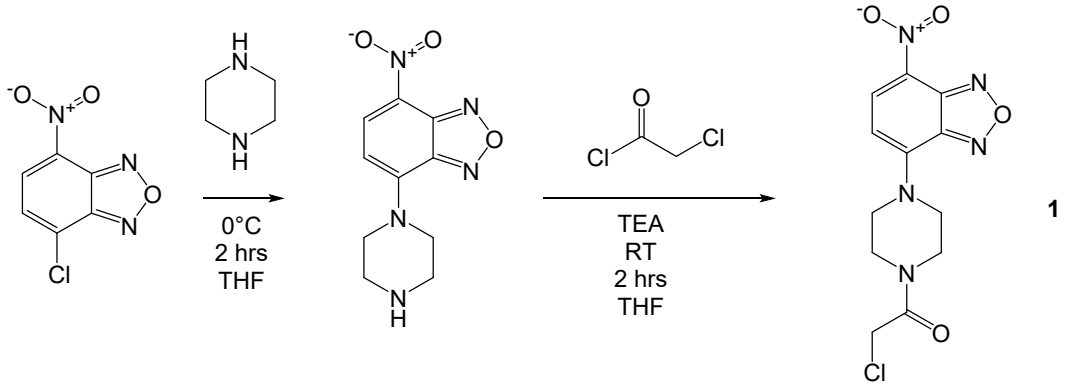
MR imaging of solution phantom at 7 T

Relaxivity measurement at 7 Tesla were performed on Bruker Biospec 70/20 USR Magnetic Resonance Imaging System with a 20 cm bore running on a ParaVision® 360 software. Samples were prepared and placed in glass capillary tube with approximate diameter of 1 mm. The capillary tube was then secured to a 50 ml centrifuge tube which was placed in a volume radiofrequency coil with 40 mm inner diameter and centered in the magnet bore. T_1 relaxation time were measured using variable repetition time accelerated spin echo sequence (RARE-VTR) with the parameter: TR = 300 ms; TE = 11ms, fields of view = 40mm x 40 mm, matrix size = 384 x 384, number of axial slices = 5, slice thickness = 1 mm.

Synthesis

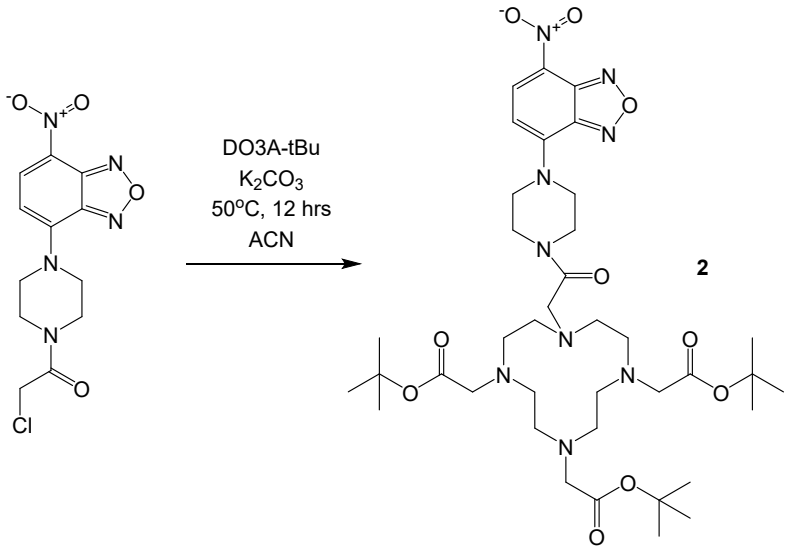
The synthesis towards **NBD[Gd]** started from the commercially available 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) and DO3A-tBu. The first two steps of reaction were carried out in a one-pot synthesis to improve the efficiency. NBD-Cl was first allowed to react with the excess piperazine to yield NBD-piperazine. The reaction was monitored by LRMS and NMR. Only <10% of over-reacted by-product was observed. Then, triethylamine and chloroacetyl chloride were directly added to the solution mixture to react with the second amine group on piperazine to obtain compound **1**, which was then reacted with the well-known thermodynamic stable Gd(III) chelator to produce compound **2**. After deprotection, lanthanide complexation was done by adding $GdCl_3 \cdot 6H_2O$ to the reaction mixture and the pH of reaction solution was maintained at 5 - 6 to yield the final product **NBD[Gd]**.

Synthesis of compound **1**:



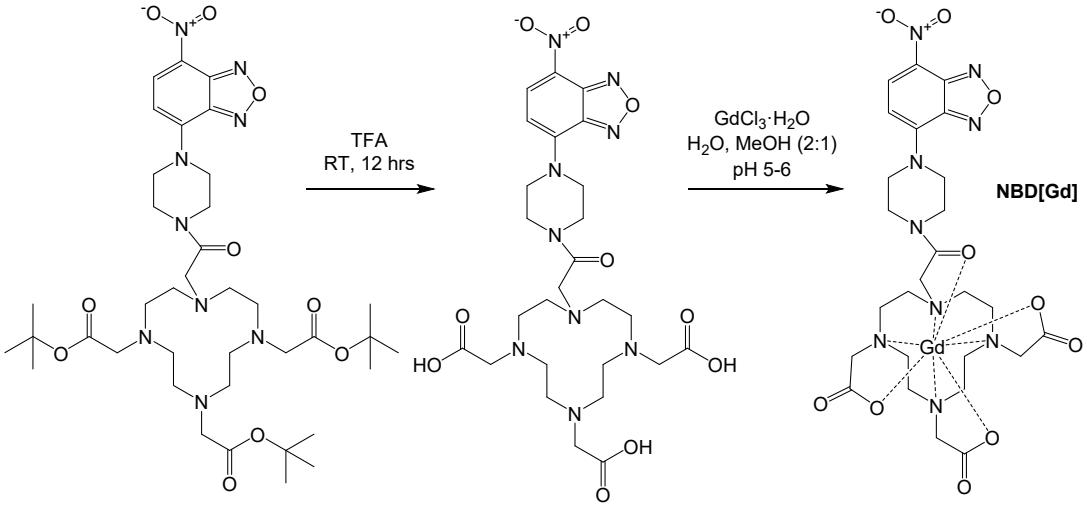
To a stirred solution of 4-Chloro-7-nitro-2,1,3-benzoxadiazole (0.5 mmol) in THF (5 mL) at 0°C were added piperazine (0.75 mmol). The resulting mixture was stirred for 4 hours. The progress of the reaction was checked by TLC. After the starting materials, 4-Chloro-7-nitro-2,1,3-benzoxadiazole, were used up, chloroacetyl chloride and triethylamine was dropwisely added to the reaction mixture and is allowed to return to room temperature for two hours. Then, the reaction mixture was evaporated to dryness under reduced pressure. The residue mixture was purified by flash column chromatography (dichloromethane/methanol = 100:1 v/v) on silica gel to give yellow solid compound **1** (Yield: 84%).^{1}H NMR (600 MHz, DMSO-d_6) δ 8.53 (dd, J = 9.1, 1.1 Hz, 1H), 6.64 (d, J = 9.1 Hz, 1H), 4.49 (s, 2H), 4.22 (d, J = 26.5 Hz, 4H), 3.79 (dt, J = 24.2, 5.4 Hz, 4H). ^{13}C NMR (151 MHz, DMSO) δ 165.55, 145.93, 145.29, 145.24, 136.82, 121.76, 103.78, 49.25, 48.79, 44.25, 42.53, 41.53. HRMS (ESI-TOF): m/z calcd. $[M+Na]^+$ = 348.0476, found 348.0475.

Synthesis of compound **2**:



Compound 1 (0.418 mmol), DO3A-tBu (0.4598 mmol) and K_2CO_3 (1.254 mmol) was suspended in anhydrous ACN under nitrogen environment and the suspension was stirred for 12 hours at 50°C. When the reaction completed, the reaction mixture was allowed to return to room temperature and filter off the inorganic salts. It was then evaporated to dryness under reduced pressure. The residue mixture was purified by flash column chromatography (dichloromethane/methanol = 15:1 v/v) on silica gel to give yellow solid compound **2** (Yield: 56%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.54 (d, J = 9.1 Hz, 1H), 6.65 (d, J = 9.2 Hz, 1H), 4.38 – 3.97 (m, 4H), 3.80 – 3.69 (m, 4H), 3.67 – 3.41 (m, 4H), 3.27 – 2.98 (m, 6H), 2.88 – 2.54 (m, 6H), 2.27 – 1.86 (m, 8H), 1.44 (s, 27H). ^{13}C NMR (151 MHz, DMSO) δ 173.06, 172.97, 171.32, 145.87, 145.27, 145.18, 136.78, 121.99, 103.87, 81.48, 55.72, 55.67, 55.06, 49.05, 48.65, 42.87, 40.82, 28.09, 28.00. HRMS (ESI-TOF): m/z calcd. $[M+Na]^+$ = 826.4439, found 826.4474.

Synthesis of **NBD[Gd]**:



Compound **2** was dissolved in trifluoroacetic acid and stirred at room temperature for 12 hours. The reaction mixture was evaporated to dryness under reduced pressure. The residue was then dissolved in MeOH/H_2O (1:2), followed by the addition of gadolinium(III) chloride hexahydrate. The mixture was maintained in a pH range of 5-6 with the addition of NaOH solution and stirred at 50°C for 12 hours. When the metal coordination completed, the crude product was purified by semi-preparative High-Performance Liquid Chromatography to yield yellow product **NBD[Gd]** (Yield: 86%). HRMS (ESI-TOF): m/z calcd. $[M+Na]^+$ = 813.1562, found 813.1739.

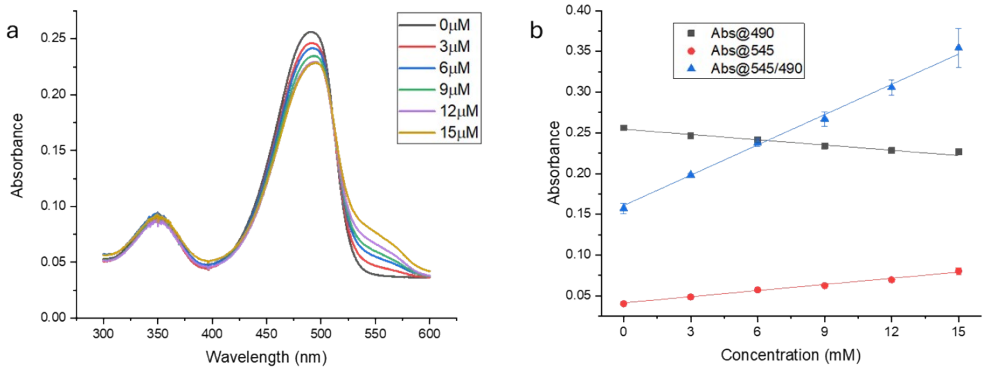


Figure S1: a) Dose-dependent absorption titration of **NBD[Gd]** to 0 – 15 μM of H_2S. b) Plot of absorbance against concentration graph shows the absorption at 490 nm, 545 nm and 545/490 nm at the timepoint of 60 min.

Characterization:

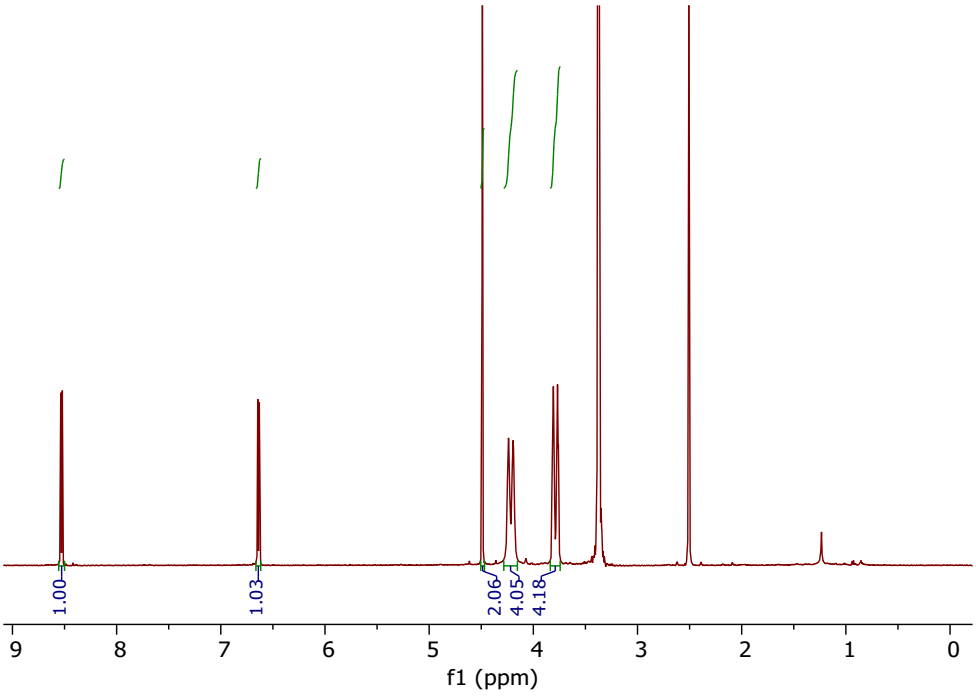


Figure S2: ^{1}H NMR spectrum of compound **1** (600 MHz, DMSO-d_6).

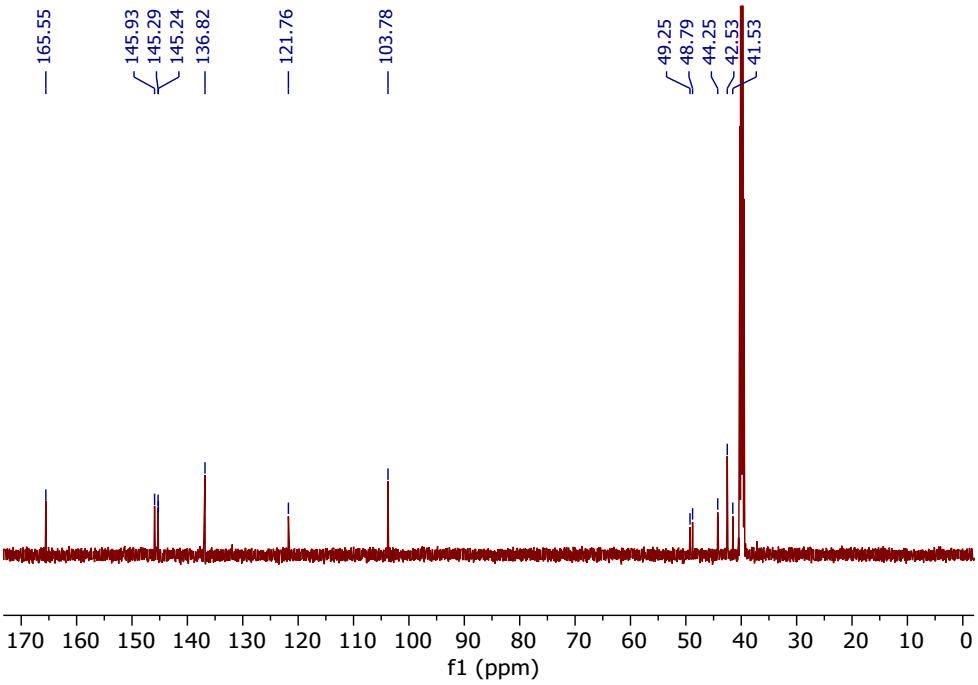


Figure S3: ^{13}C NMR spectrum of compound **1** (151 MHz, DMSO-d_6).

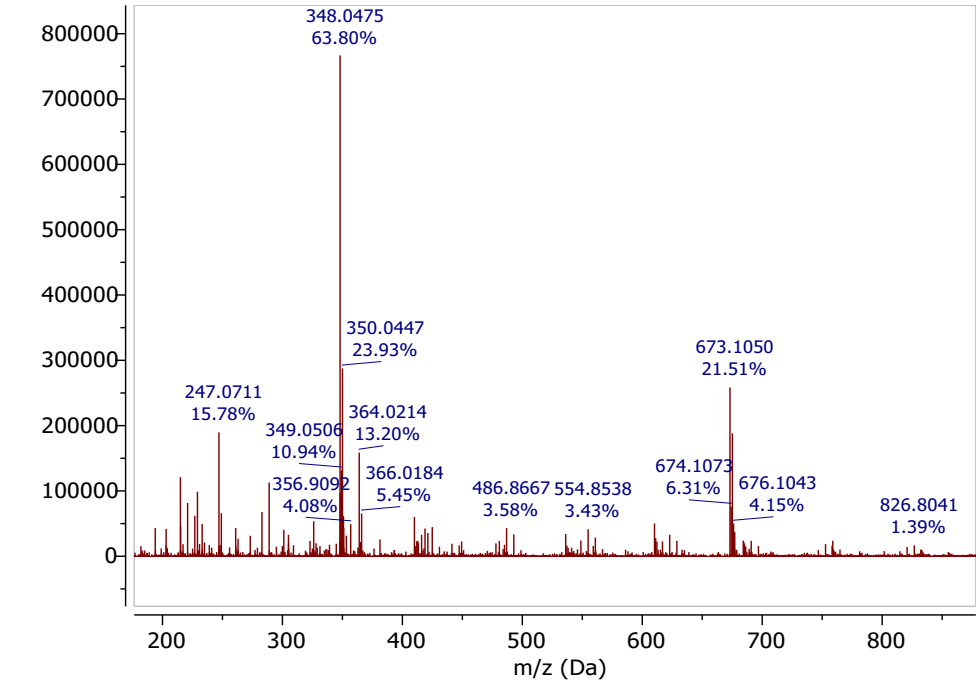


Figure S4: High-resolution mass spectrum of compound **1**.

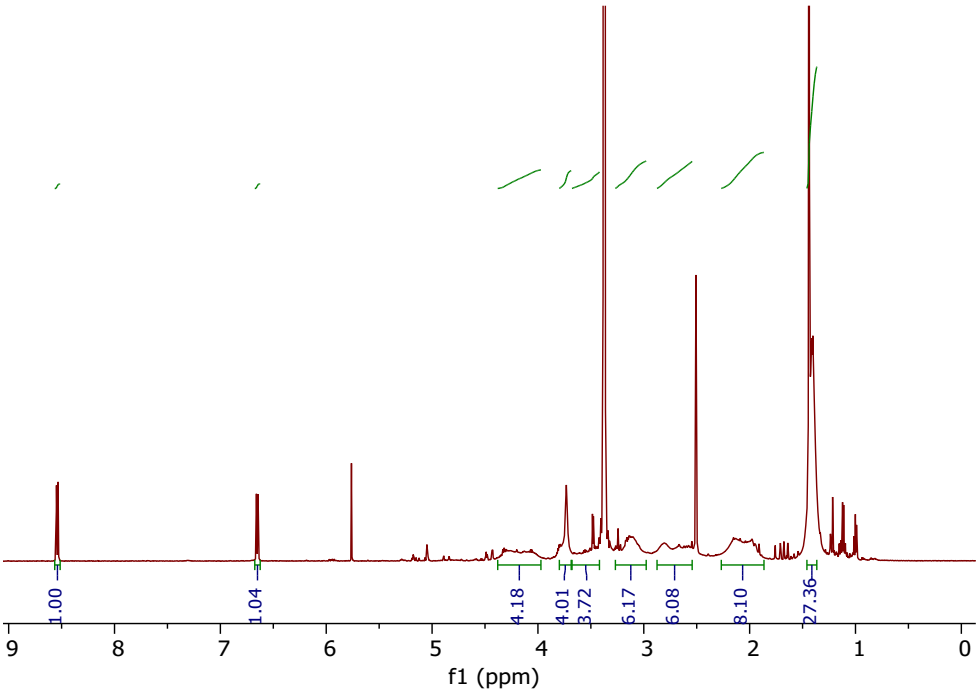


Figure S5: ^{1}H NMR spectrum of compound **2** (600 MHz, DMSO-d_6).

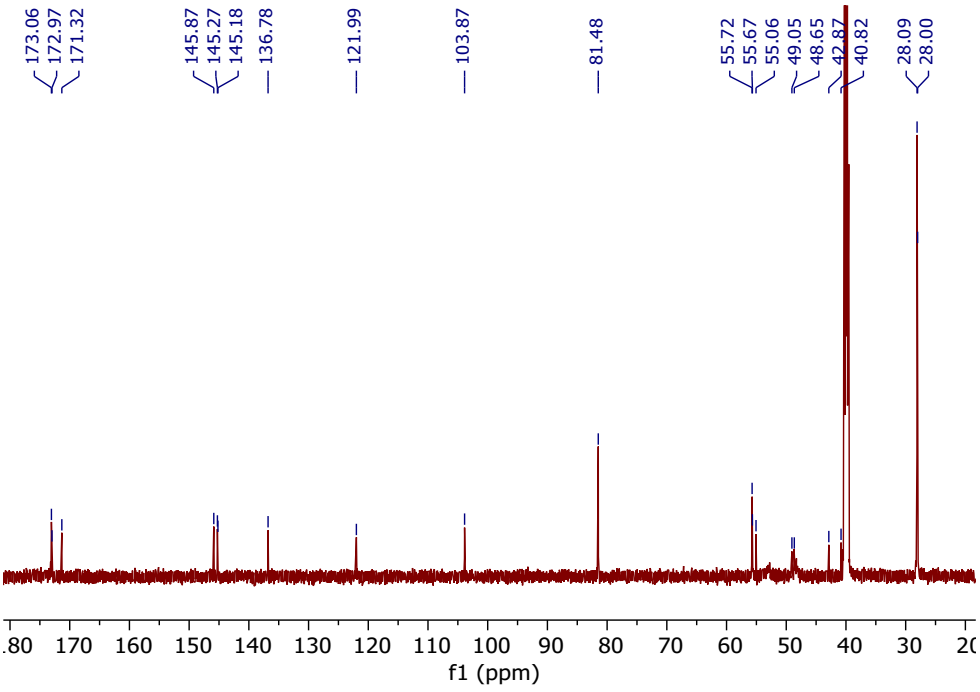


Figure S6: ^{13}C NMR spectrum of compound **2** (151 MHz, DMSO-d6).

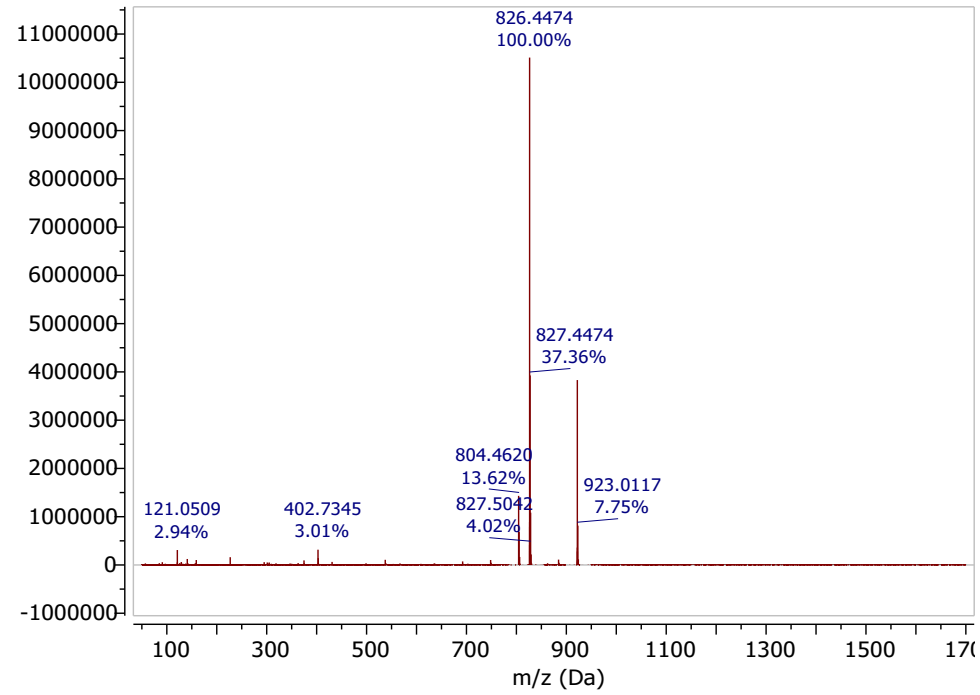


Figure S7: High-resolution mass spectrum of compound **2**.

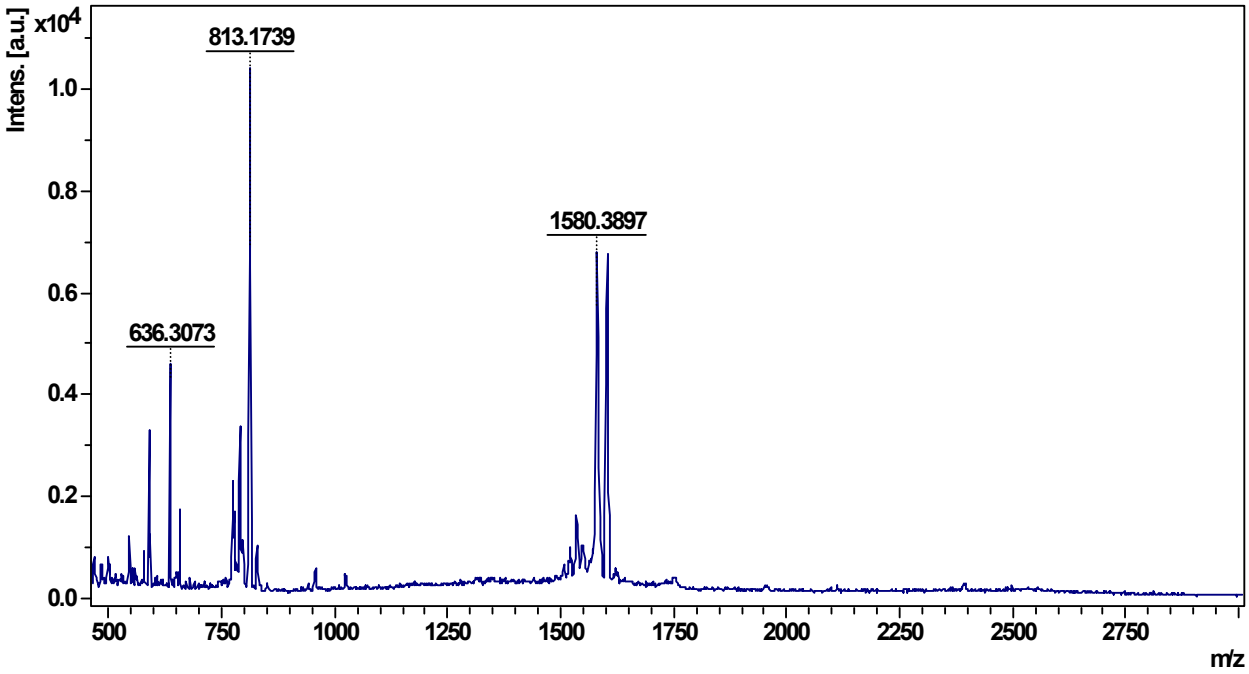


Figure S8: High-resolution mass spectrum of compound **NBD[Gd]**.

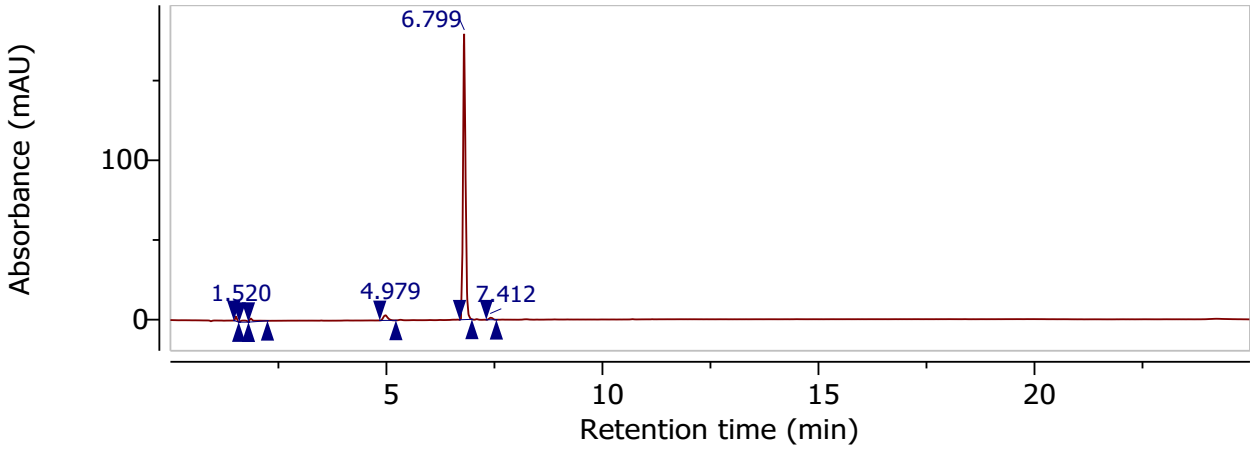


Figure S9: HPLC chromatogram of **NBD[Gd]**.