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SUPPORTING INFORMATION

A Multimeric MR-Optical Contrast Agent for Multimodal Imaging

Victoria S.R. Harrison, Christiane E. Carney, Keith W. Macrenaris and Thomas J. Meade

Department of Chemistry, Molecular Biosciences, Neurobiology, Biomedical

Engineering, and Radiology, Northwestern University, 2145 Sheridan Road, Evanston,

Illinois 60208-3113, United States

Email: tmeade@northwestern.edu

Supporting Information

Methods

General Synthesis Unless otherwise noted, materials and solvents were obtained from commercial suppliers and used without further purification. EMD 60F 254 silica gel plates were used for thin layer chromatography and visualized using UV light or ninhydrin stain. Column chromatography was performed using standard grade 60 Å 230 – 400 mesh silica gel (Sorbent Technologies). ^{1}H NMR and ^{13}C NMR spectra were obtained at room temperature on a Bruker Avance III 500 MHz NMR spectrometer. An Agilent 6210 LC-TOF spectrometer was used to acquire electrospray ionization mass spectra (ESI-MS). Matrix-Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry was carried out using a Bruker Autoflex III MALDI. Semi-preparative HPLC was performed on either a Waters 19 x 250 mm Xbridge C18 Column or a Waters 19 x 250 mm Sunfire C8 column. Analytical HPLC was performed using a Waters 4.6 X 250 mm 5 μM Xbridge C18 column using the Varian Prostar 500 system equipped with a Varian 363 fluorescence detector, and a Varian 335 UV/Vis Detector.

Synthesis of Gd(HPN$_3$DO3A)(H$_2$O) (Scheme S1)

Gd(HPN$_3$DO3A)(H$_2$O) was synthesized as described by Mastarone *et al.*[18] m/z observed = 597.1 [M$^+$], m/z calculated = 597.1[M$^+$]

Synthesis of 3-(2,4,6-triethynylphenoxy)-propan-1-amine (8) (Scheme S2)

Tert-butyl (3-chloropropyl)carbamate (4): To a solution of **1** (2.0 g, 15.4 mmol) in dichloromethane (10 mL), was added TEA (2.6 mL, 18.6 mmol) and di-tert-butyl dicarbonate

(3.36 g, 15.4 mmol). After 16 hours, TLC (20% ethyl acetate: hexanes) confirmed that the reaction was completed. The reaction mixture was filtered and extracted three times with water and once with brine. The organic layer was dried with Na_2SO_4, filtered, and the solvent evaporated to give 2.13 g of the product as a yellow oil (72% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.44 (s, 9H), 1.97 (d, *J*=12.8, 2H), 3.28 (q, *J*=6.2, 2H), 3.59 (t, *J*=6.4, 2H), 4.77 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.38, 32.56, 37.88, 42.40, 79.37, 155.98. MS (ESI-positive): m/z observed = 238.9 [M + $2Na^+$], 259.0 [M + Na^+ + K^+], m/z calculated = 239.1 [M + $2Na^+$], 257.1 [M + Na^+ + K^+]

Tert-butyl(3-(2,4,6-tribromophenoxy)propyl)carbamate (5): To a solution of tribromophenol (1.16 g, 3.5 mmol) in 30 mL DMF was added (0.98 g, 7.1 mmol) K_2CO_3, and (0.75 g, 3.9 mmol) **4**. The reaction was heated to 60°C and left to stir for 48 hours under nitrogen. Once completion of the reaction was confirmed by TLC (20% ethyl acetate: hexanes), the reaction was diluted with 50 mL of H_2O and extracted three times with 50 mL of diethyl ether. The combined organic layers were dried over $MgSO_4$, filtered and concentrated using a rotary evaporator. The residue was purified by column chromatography (9:1 hexanes: ethyl acetate) to give 0.89 g of the product as a white solid (52% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.45 (s, 9H), 2.04 (p, *J*=6.2, 2H), 3.44 (q, *J*=6.1, 2H), 4.04 (t, *J*=5.8, 2H), 4.90 (s, 1H), 7.65 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.61, 38.04, 71.38, 79.23, 117.70, 119.26, 134.94, 152.50, 156.26. MS (ESI-positive): m/z observed = 511.9 [M + Na^+], 998.4 [2M + Na^+], m/z calculated = 511.9 [M + Na^+], 998.8 [2M + Na^+]

Tert-butyl (3-(2,4,6-tris((trimethylsilyl)ethynl)phenoxy)propyl)carbamate (6): To a flame dried round bottom flask was added **5** (3.97 g, 8.0 mmol), bis(triphenylphosphine palladium (II) chloride (0.55 g, 0.8 mmol), copper (I) iodide (0.28 g, 1.43 mmol) and triphenylphosphine (0.58 g, 2.2 mmol). After applying vacuum to the materials for 2 hours, the flask was charged with 100 mL of dry TEA. Nitrogen was bubbled through the solution for 15 minutes, followed by the addition of TMS-acetylene (12.0 mL, 85.5 mmol). The flask was heated to 65°C and left to stir under nitrogen. Once completion of the reaction was confirmed by TLC (20% ethyl acetate: hexanes), the reaction was concentrated using a rotary evaporator. 50 mL of hexane was added to the flask and the remaining solids were filtered. The solvent was evaporated and the final residue was purified by silica gel chromatography (79:1 hexanes: ethyl acetate) to give 3.17 g of the product as a yellow oil (88% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ -0.00 (s, 20H), 1.20 (s, 9H), 1.73 (d, *J* = 5.8 Hz, 2H), 3.21 (d, *J* = 6.0 Hz, 2H), 4.03 (d, *J* = 5.6 Hz, 2H), 7.25 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 0.27, 28.66, 30.19, 38.44, 72.19, 79.59, 95.17, 99.90, 102.88, 117.78, 119.13, 137.99, 156.62, 161.23. MS (ESI-positive): m/z observed = 562.2 [M + Na^+], 1101.0 [2M + Na^+], m/z calculated = 562.3 [M + Na^+], 1101.5 [2M + Na^+]

***Tert*-butyl (3-(2,4,6-triethynylphenoxy)propyl)carbamate (7):** To a solution of **6** (3.17 g, 7.2 mmol) in 50 mL methanol was added potassium fluoride (6.30 g, 109.1 mmol). The reaction was heated to 45°C and allowed to stir overnight under nitrogen. After 16 hours, TLC (20% ethyl acetate: hexanes) confirmed completion of the reaction. The reaction mixture was filtered and the solvent evaporated. The residue was dissolved in 75 mL diethyl ether and washed with water and brine. The organic layer was dried with Na_2SO_4, filtered and the solvent evaporated. The residue was purified by column chromatography (19:1 hexanes: ethyl acetate) to give 1.13 g of the

product as a yellow oil (49% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.99 (m, 2H), 3.05 (s, 1H), 3.32 (s, 2H), 3.42 (d, *J* = 6.0 Hz, 2H), 4.30 (t, *J* = 5.8 Hz, 2H), 7.57 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.44, 30.30, 37.91, 72.57, 77.91, 78.50, 81.29, 82.67, 117.06, 117.90, 138.23, 156.41, 162.08. MS (ESI-positive): m/z observed = 345.9 [M + Na^+], m/z calculated = 346.1 [M + Na^+]

3-(2,4,6-triethynylphenoxy)propan-1-amine (8): 7 (1.13 g, 3.50 mmol) was dissolved in 25 mL of formic acid and stirred for 4 hours or until completion was determined by TLC (10% methanol: dichloromethane). The solvent was evaporated and the residue was purified by column chromatography (15% methanol: dichloromethane) to give 0.62 g of the product as a yellow oil (82% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (s, 2H), 2.16 (dt, *J* = 11.5, 5.7 Hz, 2H), 3.07 (s, 1H), 3.29 (t, *J* = 6.0 Hz, 2H), 3.50 (s, 2H), 4.37 (d, *J* = 5.4 Hz, 2H), 7.56 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 27.55, 38.74, 72.77, 78.11, 78.33, 81.06, 83.65, 116.77, 119.04, 138.01, 160.94. MS (ESI-positive): m/z observed = 224.0 [M + H^+], 446.82 [2M +H^+], m/z calculated = 224.1 [M + H^+], 447.2 [2M +H^+]

Synthesis of 1 and 2 (Scheme S3)

3-(2,4,6)-Tris(1-2(hydroxyl-3-(1H-1,2,3-triazol-1-yl(propyl)-3,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecylgadolinium(III)phenoxy)-propan-1-amine (2): To a solution of Gd(HPN_3DO3A)(H_2O) (1.02 g, 1.7 mmole) in a 40 mL mixture of 2:1 T-butanol:H_2O was added **6** (108 mg, 0.48 mmoles). Nitrogen was bubbled through the solution for 15 minutes, followed by the addition of $[Cu(MeCN)_4]PF_6$ (24.5 mg, 0.06 mmole) and tris[(1-benzyl-1-H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (26.0 mg, 0.05 mmole). The reaction was heated to 50°C and stirred

under a nitrogen atmosphere for 48 hours. The solvent was removed by lyophilization and the product was purified by reverse phase HPLC using a C18 column, held at 5% for 5 minutes and eluting with a gradient of 5%-18% acetonitrile in pH 10.38 buffered water over 15 min, $t_{r=}$ 11.9 minutes. This gave 450 mg of the product as a white solid (46.3% yield). The purity and identity of the product was confirmed using analytical HPLC-MS on a C18 column, held at 5% for 5 minutes and eluting with a gradient of 5%-25% acetonitrile in pH 10.38 buffered water over 20 min, t_r = 15.68 min. MS (MALDI-TOF): m/z observed = 2023.12 [M^+], m/z calculated = 2023.4 [M^+]. Anal. cald. (%) for $C_{66}H_{109}Cl_3Gd_3N_{22}Na_3O_{28}$: C, 34.38; H, 4.77; N, 13.36 Found: C, 33.89; H, 5.28; N, 13.01.

3-(2,4,6)-Tris(1-2(hydroxyl-3-(1H-1,2,3-triazol-1-yl(propyl)-3,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecylgadolinium(III)phenoxy)-propan-1-amino-fluoroscein-isothiocyanate (1): To a solution of **7** (74.0 mg, 0.04 mmole) in 20 mL H_2O was added fluorescein-isothiocyanate (38.0 mg, 0.10 mmole). The pH was adjusted to 9.0 and the reaction was left to stir for 24 hours under nitrogen. The solvent was removed by lyophilization, and after a sephadex G10 column, the product was purified by reverse phase HPLC, using a C8 column, held at 5% for 5 minutes and eluting with a gradient of 5%-30 % acetonitrile in water, over 15 min, t_r =14.6. This gave 51 mg of the product as an orange solid (56.7% yield). The purity and identity of the product was confirmed using analytical HPLC-MS on a C18 column, held at 5% for 5 minutes and eluting with a gradient of 5%-25% acetonitrile in pH 10.38 buffered water over 20 min, t_r = 15.35 min. MS (MALDI-TOF): m/z observed = 2411.3 [$M^+ + H^+$], m/z calculated = 2411.8 [$M^+ + H^+$]. Anal. cald. (%) for $C_{87}H_{125}Cl_8Gd_3N_{23}Na_8O_{37}S$: C, 34.19; H, 4.12; N, 10.54 Found: C, 34.77; H, 4.98; N, 9.40.

Relaxation Time Measurements at 1.41 T T_1 and T_2 relaxation times were measured on a Bruker mq60 NMR analyzer equipped with Minispec v. 2.51 Rev.00/NT software (Billerica, MA, USA) operating at 1.41 T (60 MHz) and 37 °C. T_1 relaxation times were measured using an inversion recovery pulse sequence (t1_ir_mb) with the following parameters: four scans per point, 10 data points for fitting, mono-exponential curve fitting, phase cycling, 10 ms first pulse separation, and a recycle delay and final pulse separation $\geq 5T_1$. T_2 relaxation times were measured using a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (t2_cp_mb) with the following parameters: four scans per point, mono-exponential curve fitting, phase cycling, 10 ms first pulse separation, 15 second recycle delay, 1 ms 90°–180° pulse separation (tau), while altering the number of data points to ensure accurate mono-exponential curve fitting (500–10000 data points for fitting). Relaxivities were determined by taking the slope of a plot of $1/T_1$ (s^{-1}) or $1/T_2$ (s^{-1}) vs. gadolinium concentration (mM) of each compound in 10 mM PBS buffer.

Photophysical Studies UV-visible absorption spectra were recorded on an Agilent 8453 spectrophotometer. The steady-state fluorescence emission and excitation spectra were obtained using a Hitachi F-45000 Fluorescence Spectrophotometer. The fluorescence quantum yield (ϕ_{exp}) was calculated from equation 1.

$$\phi_{exp} = \phi_{ref} \frac{F\{1-\exp(-A_{ref}\ln 10\}n^2}{F_{ref}\{1-\exp(-A_{exp}\ln 10\}n^2_{ref}}$$

Equation 1

Here, F denotes the integral of the corrected fluorescence spectrum, A is the absorbance at the excitation wavelength, and n is the refractive index of the medium. The reference system used was fluorescein (ϕ=.78 in .1 M NaOH, $\lambda_{excitation}$=490 nm)**.**

Octanol-Water partition coefficient measurements Approximately 1 mg of **1** or **2** was dissolved in 1 mL of a 1:1 mixture of water:octanol. After vortexing the sample tube vigorously for 30 s, the tube was placed on a rotator for gentle mixing over 8 hours. The tube was removed from the rotator and allowed to sit for 12 hours to ensure complete separation of the aqueous and organic phases. An aliquot was removed from each layer and analyzed by ICP-MS to determine the Gd(III) concentration in each layer. The partition coefficient was calculated from the following equation: $\log_{10} P = \log_{10}(C_o/C_w)$, where $\log_{10} P$ is the logarithm of the partition coefficient, C_o is the concentration of Gd(III) in the 1-octanol layer, and C_w is the concentration of Gd(III) in the water layer.

General Cell Culture Dulbecco's modified phosphate buffered saline (DPBS), media, and dissociation reagents were purchased from Life Technologies (Carlsbad, CA, USA). CorningBrand® cell culture consumables (flasks, plates, etc.) and sera were purchased from VWR Scientific (Radnor, PA, USA). HeLa cells (ATCC® CCL-2™) and B16-F10 cells (ATCC® CRL-6475™) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). MDA-MB-231-mcherry cells were a gift from Northwestern University Developmental Therapeutics Core. HeLa and MDA-MB-mcherry cells were cultured in phenol red free minimum essential media (MEM) supplemented with 10% fetal bovine serum (FBS). B16-F10 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented

with 10% FBS. Prior to all experiments, cells were plated and allowed to incubate for 24 hours before dosing. Cells were harvested with 0.25% TrypLE for 10 min at 37 °C in a 5.0% CO_2 incubator. All doses were filtered with 0.2 μm sterile filters prior to administration. Cells were grown in a humidified incubator operating at 37 °C and 5.0% CO_2 unless otherwise specified.

Confocal 25**,**000 HeLa cells were plated on a 35 mm FluoroDish (World Precision Instruments, Sarasota, FL, USA). Cells were incubated with 100 μM of **1** (500 μL) for 4 hours. Cells were rinsed eight times with 500 μL of DPBS followed by addition of 1 mL fresh media and 1 μM DAPI (Life Technologies, Carlsbad, CA, USA) for 10 minutes. The media was aspirated, cells were washed twice with 500 μL DPBS, and 500 μL of fresh media were added. Images were acquired on a computer-controlled (via Zen 2009 software) Zeiss LSM 510 confocal laser scanning inverted microscope equipped with a mode-locked Mai Tai DeepSee® Ti:sapphire two-photon laser (Spectra Physics, Mountain View, CA, USA). For all images a Plan-Appochromat 40x water immersion objective lens with a numerical aperture of 1.2 was used. For DAPI and complex **1** excitation, a multi-track acquisition was used to minimize the promiscuity of the two-photon excitation for fluorescein. Excitation of complex **1** was accomplished using the 488 nm wavelength of an argon ion laser operating at 2.6% laser power and 6 Amps, through a HFT 488/543/633/KP725 beamsplitter. The emission from complex **1** was passed through a NFT 545 dichroic beamsplitter and imaged with a PMT through a 500-550 nm IR bandpass filter (72 μm pinhole, 1 Airy Unit). Excitation of DAPI was accomplished using a software tunable two-photon laser tuned to 780 nm operating at 7.5% laser power, through a HFT 488/543/633/KP725 beamsplitter. The emission from DAPI was passed through a NFT 545 dichroic beamsplitter and imaged with a PMT through a 435-485 nm IR bandpass filter (896 μm open). All images were

acquired at 1024 x 1024 resolution and 4 averages. Additionally, z-stacks were acquired using the same parameters with a slice thickness of 1.056 μm and 15 slices. Image processing and analysis was done using Image J (NIH, Bethesda, MD, USA).

Flow Cytometry Cell labeling efficiency was assessed with flow cytometry using a BD LSRII (BD Biosciences, San Jose, CA, USA). HeLa cells were incubated with 180 μL of 21 to 170 μM of **1** for 24 hours. Cells were washed twice with 500 μL DPBS, pelleted at 1000 x g for 5 min at 4 °C, suspended in 500 μL of DPBS, and assessed with flow cytometry. Dot plots were gated based upon FSC/SSC properties of unlabeled HeLa cells. Data was analyzed with FlowJo software.

Cell Counting Cell counting was accomplished using a Countess® Automated Cell Counter (Life Technologies, Carlsbad, CA, USA). After cell harvesting, an aliquot (10 μL) of the cell suspensions was mixed with 0.4% Trypan Blue (10 μL) and counted following the manufacturer's suggested protocol.

Cell Uptake Studies Cell uptake studies were performed with HeLa B16-F10, or MDA-MB-231-mcherry cells. Specifically, 20,000-25,000 cells were plated in each well of a 24-well plate. For concentration-dependent labeling studies, **1**, **2**, or ProHance® were dissolved in media at concentrations of approximately 240, 120, 60, and 30 μM (exact concentrations determined by ICP-MS) and incubated with cells for 24 hrs (180 μL dose). For time-dependent labeling studies, HeLa cells were incubated with 90 μM of **1** or **2** for 1, 2, 4, or 24 hours. For all studies, cells were rinsed twice with 500 μL DPBS and trypsinized following contrast agent incubation. Cells

were pelleted at 1000 x g for 5 min at 4 °C to reduce non-specific binding. The media was aspirated off and the cell pellets were resuspended in 200 μL of media. 10 μL of the cell suspension was used for cell counting and 130 μL was used for Gd(III) content analysis via ICP-MS.

Cytotoxicity The CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) was used to measure cell viability. Specifically, 5,000 cells were plated in each well of a 96-well plate. **1** and **2** were dissolved in media at various concentrations and incubated with cells (50 μL doses) for 24 hours. After incubation, cells were washed twice with 50 μL DPBS and 50 μL of fresh media was added to reduce fluorescent background. The assay was run according to the manufacturer's suggested protocol. Absorbance was measured using a Biotek Synergy4 microplate reader (Winooski, VT, USA) at 535 nm for **1** and 490 nm for **2**.

ICP-MS Sample Preparation and Instrument Parameters For relaxivity and cell studies Gd(III) content was measured via ICP-MS. Specifically, samples were prepared by adding ACS reagent grade nitric acid (70%) to solutions of contrast agent or cell suspensions (1:1 v/v sample:nitric acid) in 15-mL conical tubes. Samples were incubated at 70 °C for at least 4.0 hours to allow for complete sample digestion. Following sample digestion, multi-element internal standard (containing Bi, Ho, In, Li, Sc, Tb, and Y, Inorganic Ventures, Christiansburg, VA, USA) and filtered de-ionized H_2O (18.2 MΩ·cm) were added producing a final ICP-MS sample of 3% (v/v) nitric acid and 5 ng/mL internal standard.

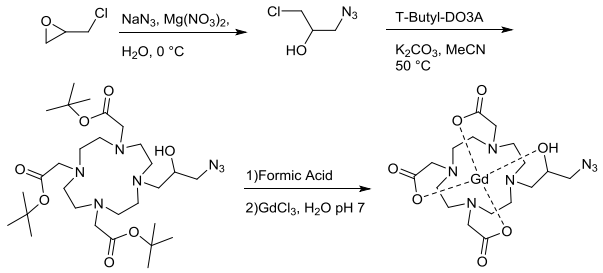
ICP-MS was performed on a computer-controlled (Plasmalab software) Thermo X series II ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA) operating in standard mode

equipped with an ESI SC-2 autosampler (Omaha, NE, USA). Each sample was acquired using 1 survey run (10 sweeps) and 3 main (peak jumping) runs (100 sweeps). The isotopes selected for analysis were ^{154,157,158}Gd with ^{115}In and ^{165}Ho isotopes selected as internal standards for data interpolation. Instrument performance is optimized daily through an autotune followed by verification via a performance report (passing manufacturer specifications). Instrument calibration was accomplished by preparing individual-element Gd(III) standards (Inorganic Ventures, Christiansburg, VA, USA) using concentrations of 0.78125, 1.5625, 3.125, 6.25, 12.50, 25.00, 50.00, 100.0, and 200.0 ng/mL containing 3.0% nitric acid (v/v) and 5.0 ng/mL of multi-element internal standard.

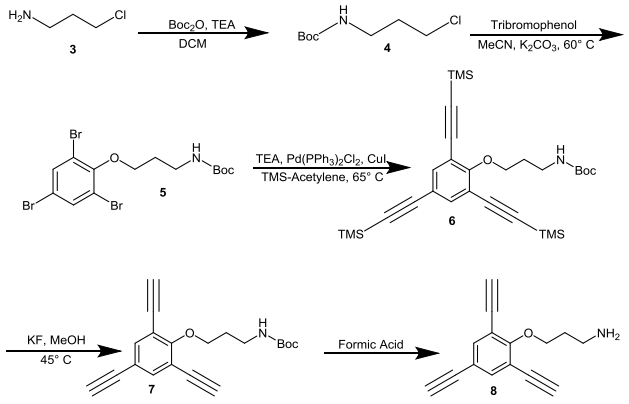
Cell Pellet MR Imaging 7 T MR imaging and T_1 measurements were performed on a Bruker Pharmscan 7 T imaging spectrometer fitted with shielded gradient coils at 25 °C. For cell pellet images, ~ 5 × 10^5 HeLa cells were incubated in 25-cm^2 T-flasks with **1**, **2**, or Prohance for 24 hours, rinsed with DPBS (2 × 1 mL/flask), and harvested with 500 μL of trypsin. After addition of 500 μL of fresh complete media, cells were transferred to 1.5-mL microcentrifuge tubes and centrifuged at 1000 x *g* at 4.0 °C for 5 minutes. The supernatant was removed; the cell pellets were re-suspended in 1 mL of complete media, added to 5¾″ flame-sealed Pasteur pipets, and centrifuged at 100 x *g* at 4.0 °C for 5 minutes. The bottom sections of the flame-sealed pipets were then scored with a glass scribe, broken into small capillaries, and imaged using a RF RES 300 1H 089/023 quadrature transmit receive 23-mm volume coil (Bruker BioSpin, Billerica, MA, USA). Solution phantoms were prepared, imaged and analyzed the same as above using serially diluted solutions.

Spin-lattice relaxation times (T_1) were measured using a rapid-acquisition rapid-echo (RARE-VTR) T_1-map pulse sequence, with static TE (11 ms) and variable TR (150, 250, 500, 750, 1000, 2000, 4000, 6000, 8000, and 10000 ms) values. Imaging parameters were as follows: field of view (FOV) = 25 × 25 mm^2, matrix size (MTX) = 256 × 256, number of axial slices = 4, slice thickness (SI) = 1.0 mm, and averages (NEX) = 3 (total scan time = 2 hrs 36 min). T_1 analysis was carried out using the image sequence analysis tool in Paravision 5.0 pl3 software (Bruker, Billerica, MA, USA) with mono-exponential curve-fitting of image intensities of selected regions of interest (ROIs) for each axial slice.

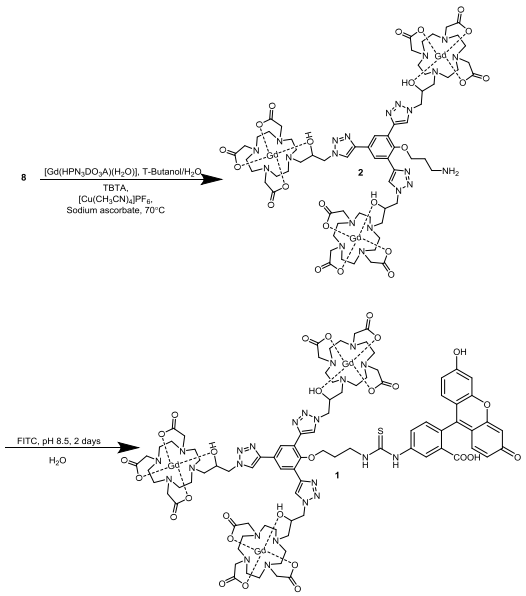
Synthetic Schemes



Scheme S1. Synthetic scheme of Gd-DOTA azide derivative



Scheme S2. Synthetic scheme of 3-(2, 4 ,6-triethynylphenoxy)propan-1-amine (**8**)



Scheme S3. Synthesis of **1** from 3-(2, 4 ,6-triethynylphenoxy)propan-1-amine (**8**)

^{1}H and ^{13}C NMRs of 4-8

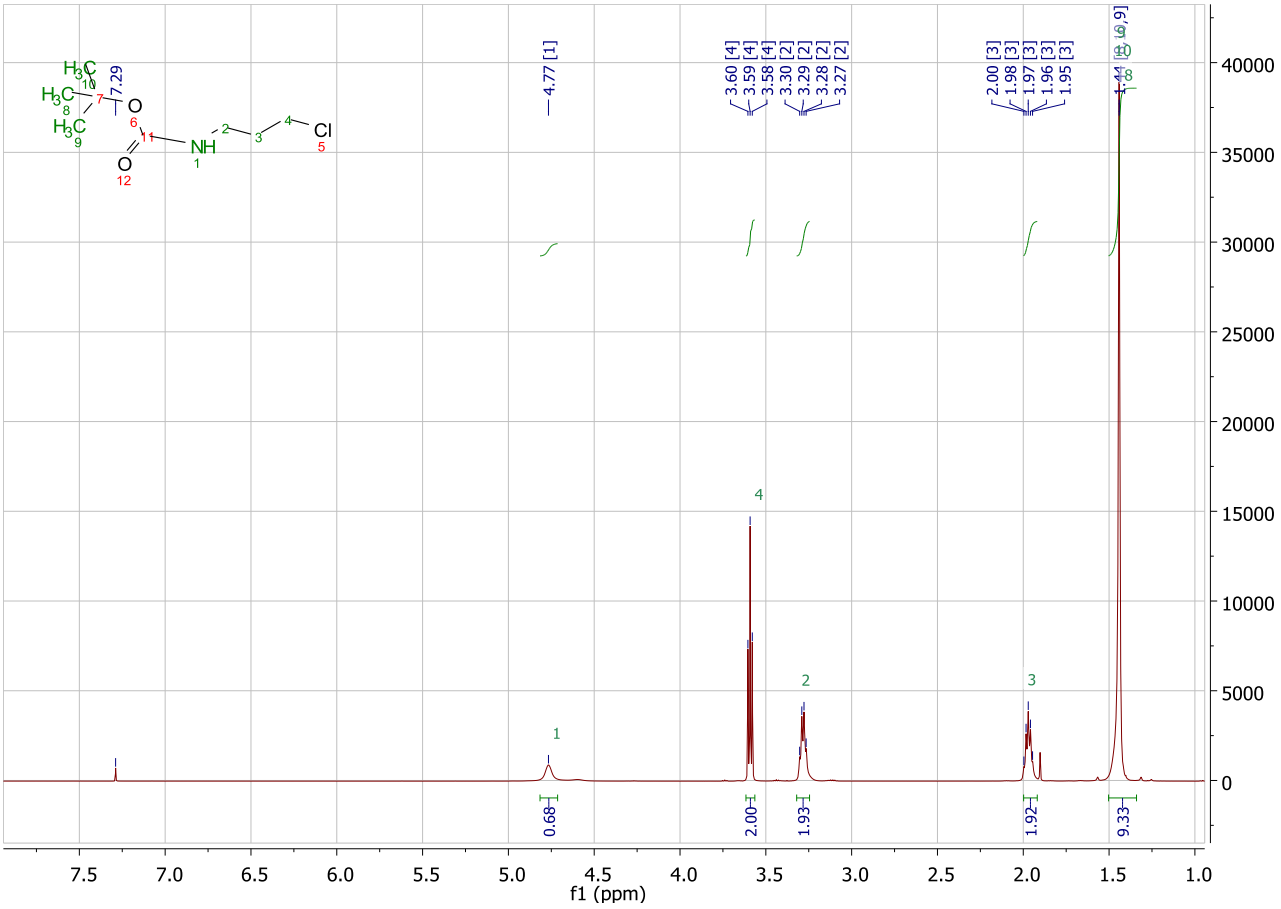


Figure S1. ^{1}H NMR of **4**

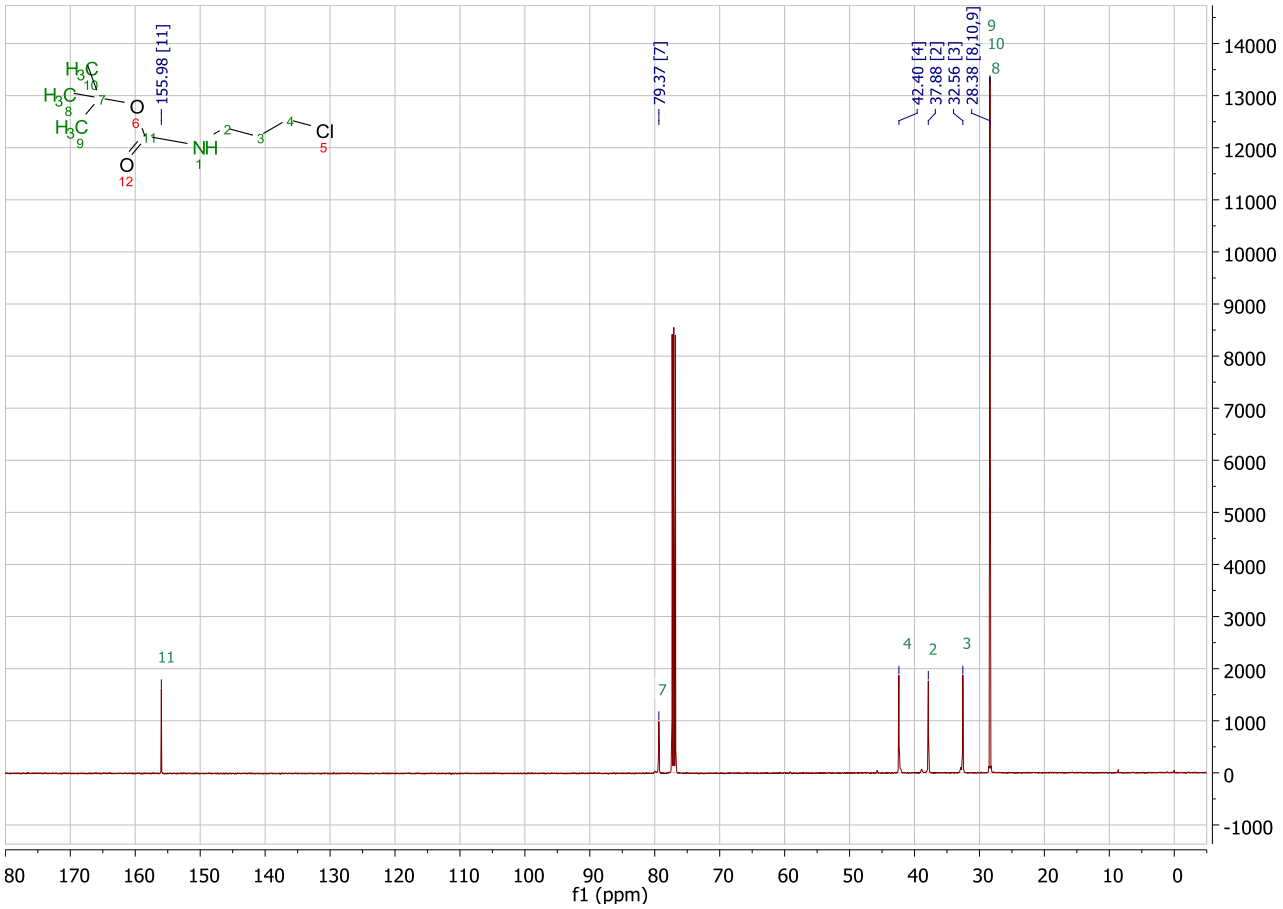


Figure S2. ^{13}C NMR of **4**

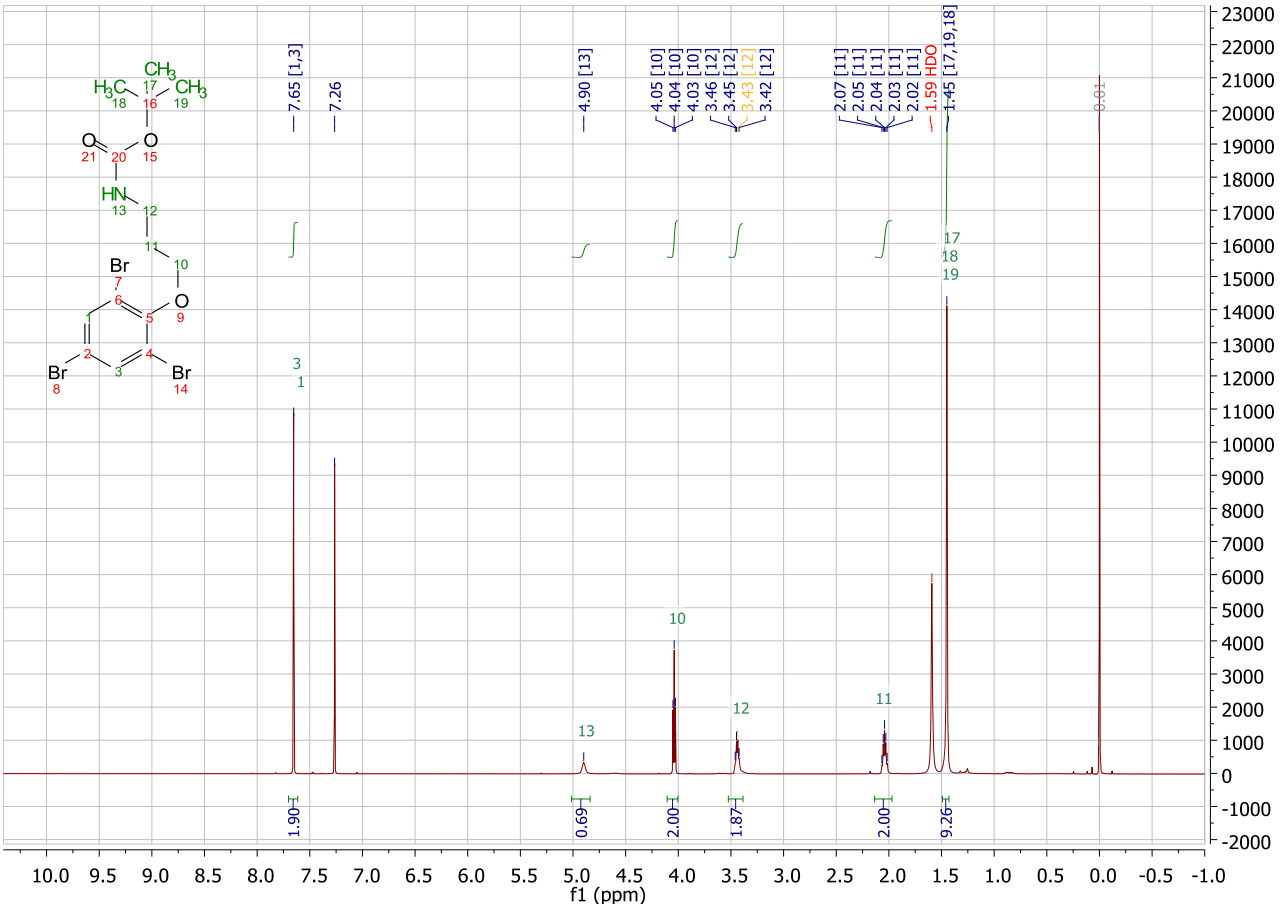


Figure S3. ^{1}H NMR of **5**

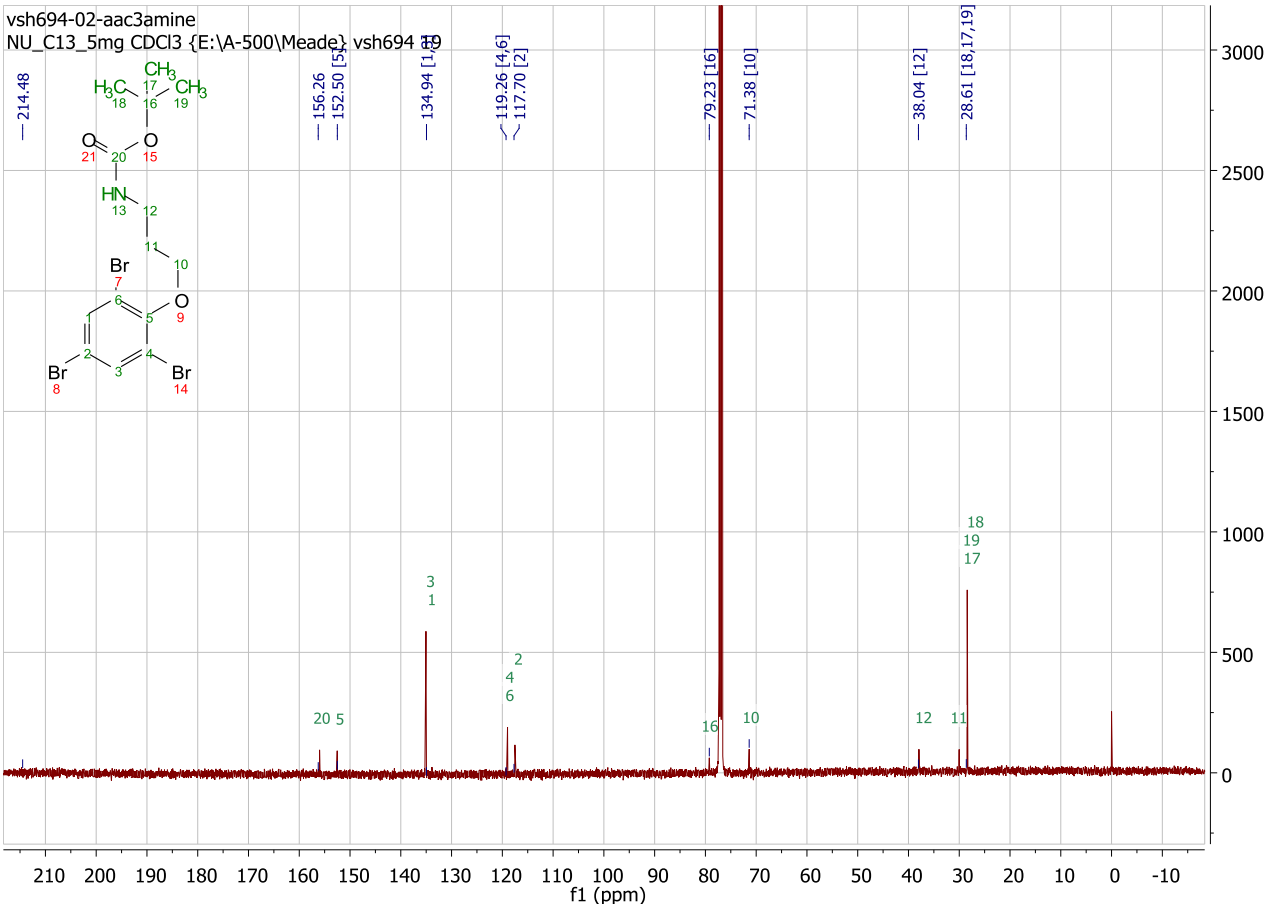


Figure S4. ^{13}C NMR of **5**

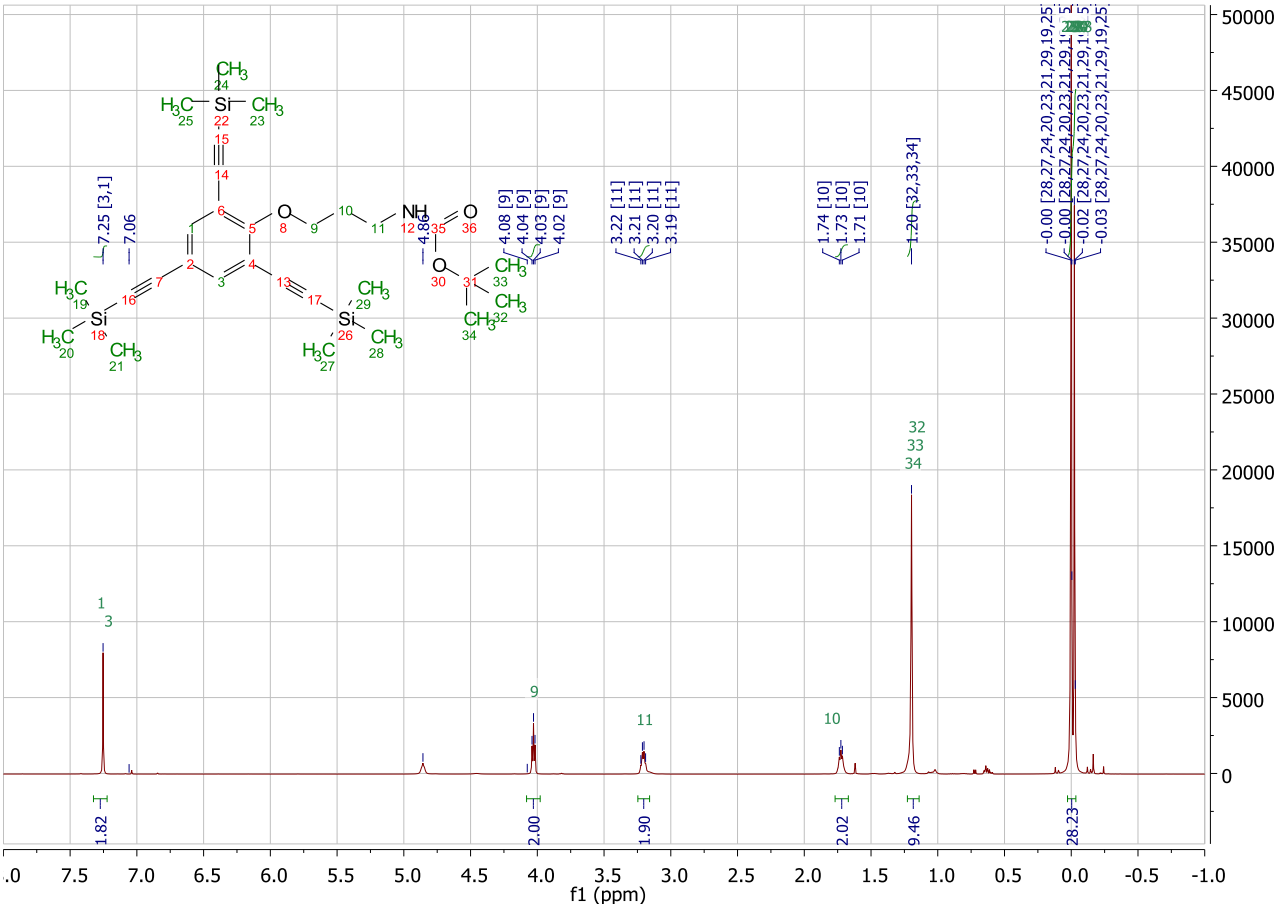


Figure S5: ^{1}H NMR of **6**

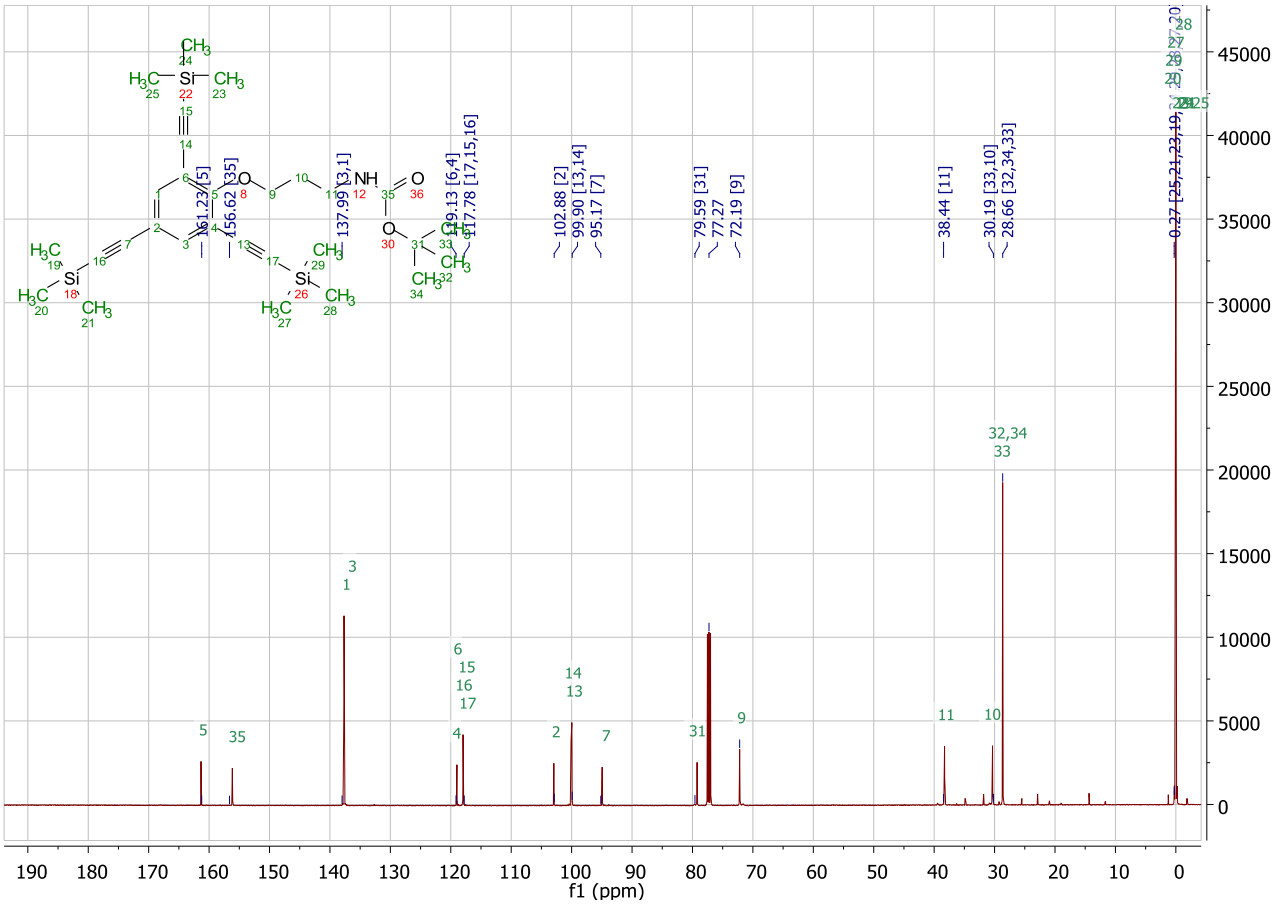


Figure S6. ^{13}C NMR of **6**

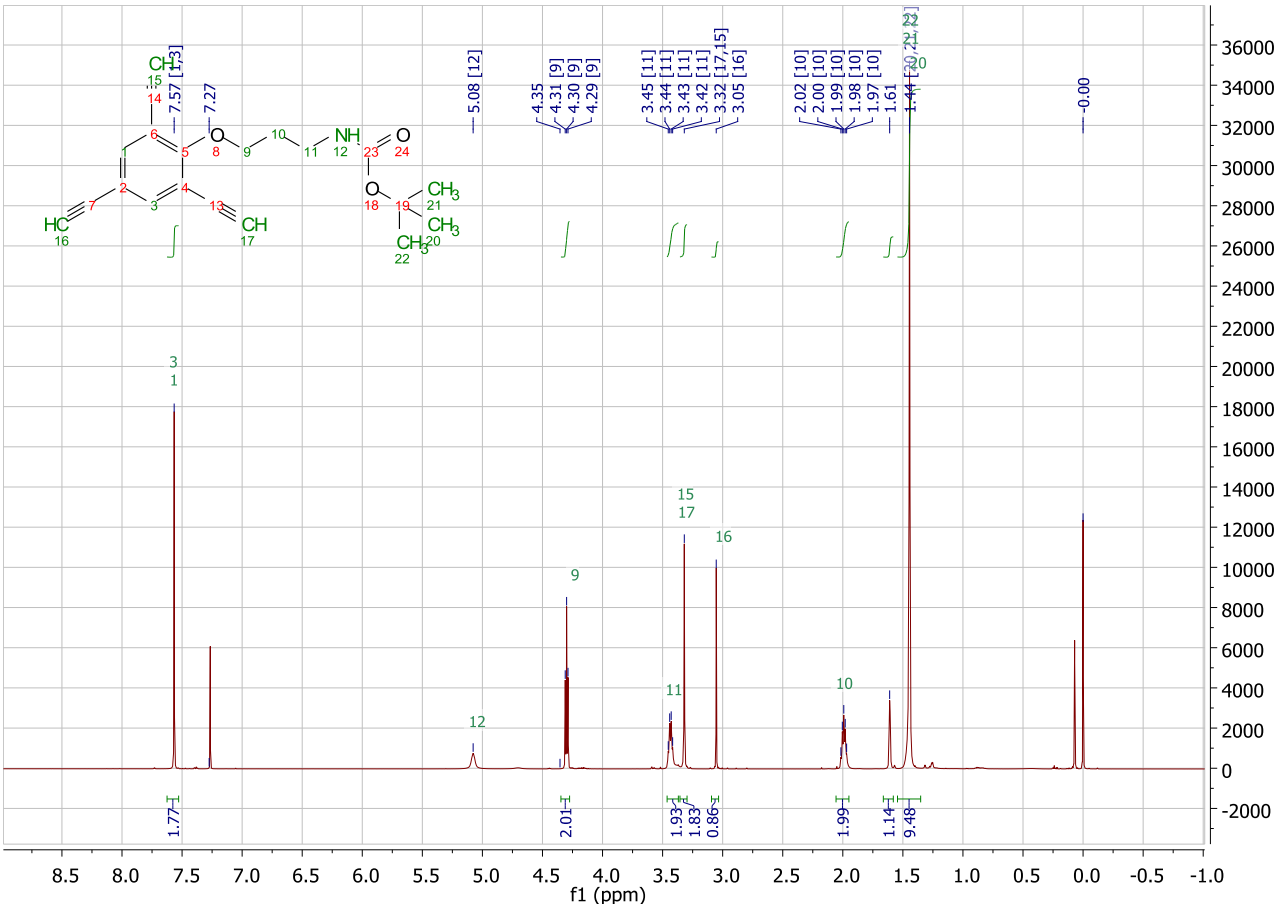


Figure S7. ^{1}H NMR of **7**

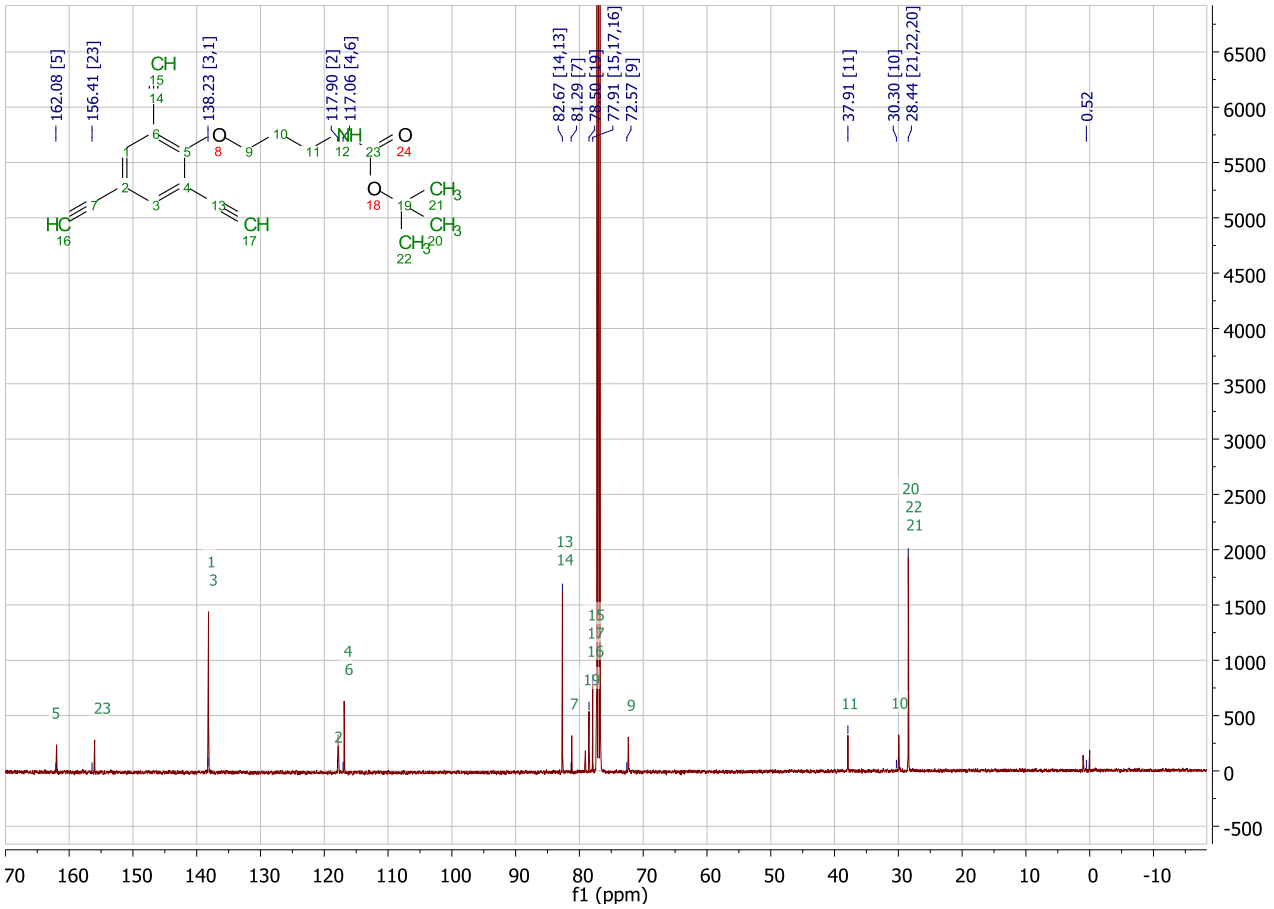


Figure S8. ^{13}C NMR of **7**

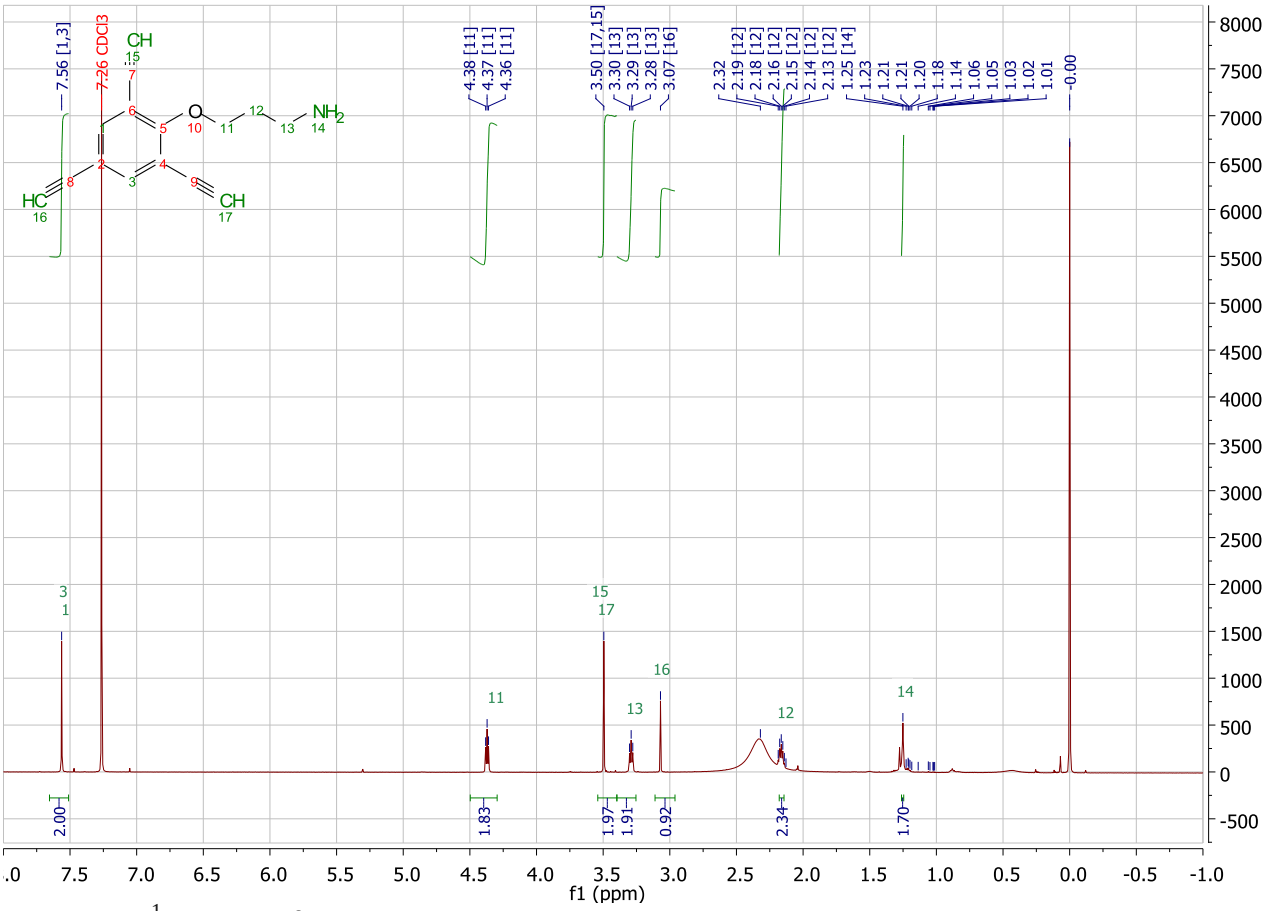


Figure S9. ^{1}H NMR of **8**

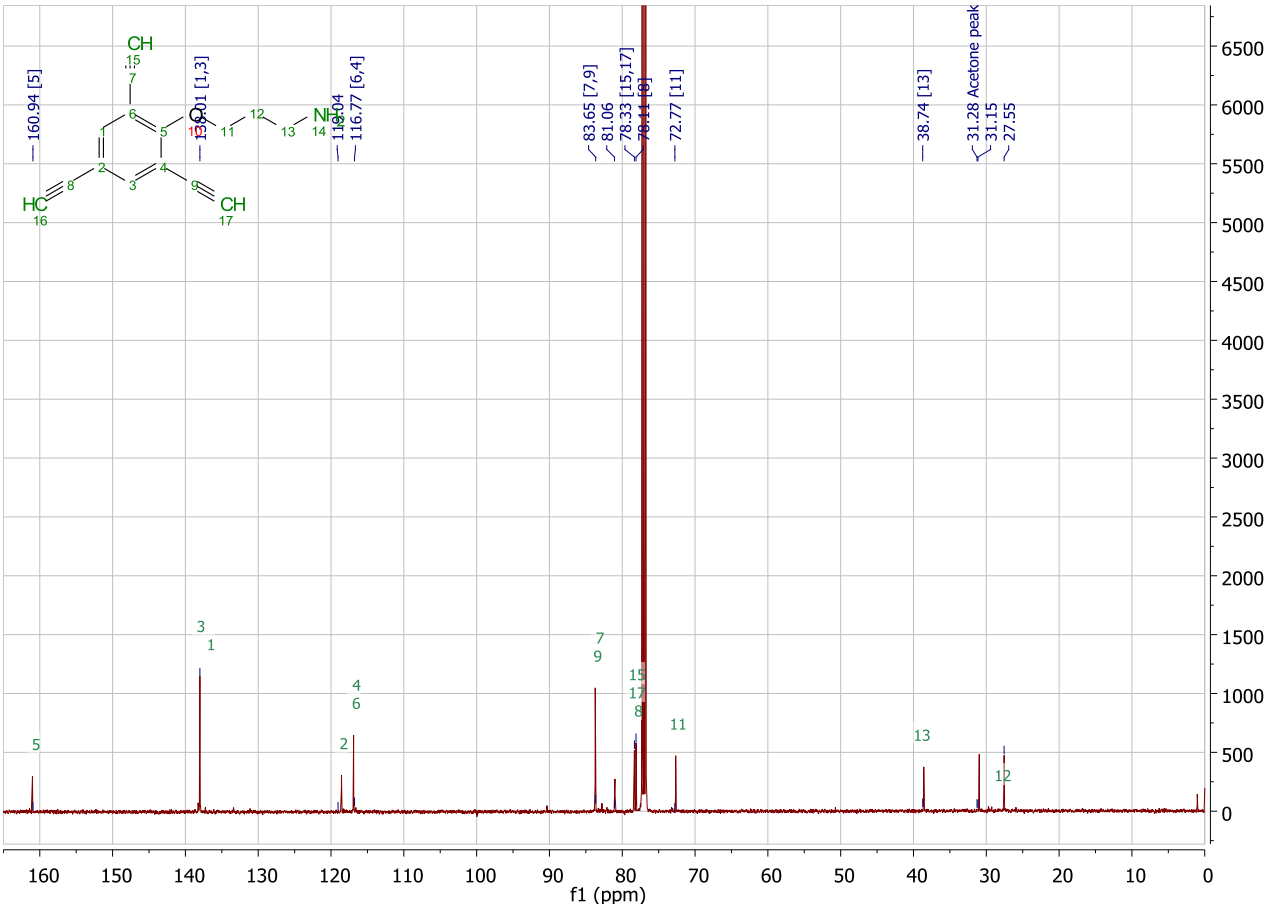


Figure S10. ^{13}C NMR of **8**

MS Spectra of 1 - 8

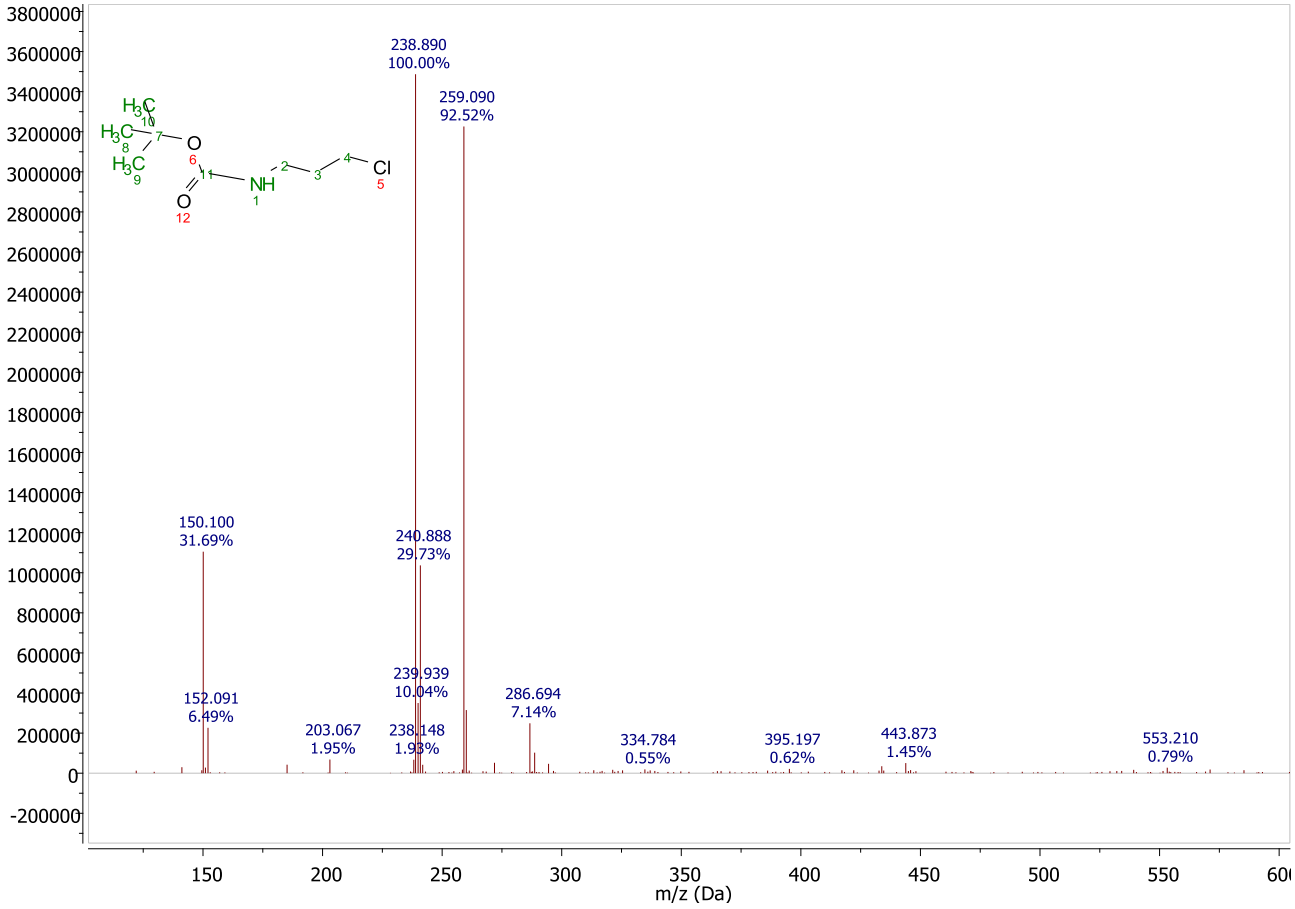


Figure S11. MS (ESI-positive) of **4**

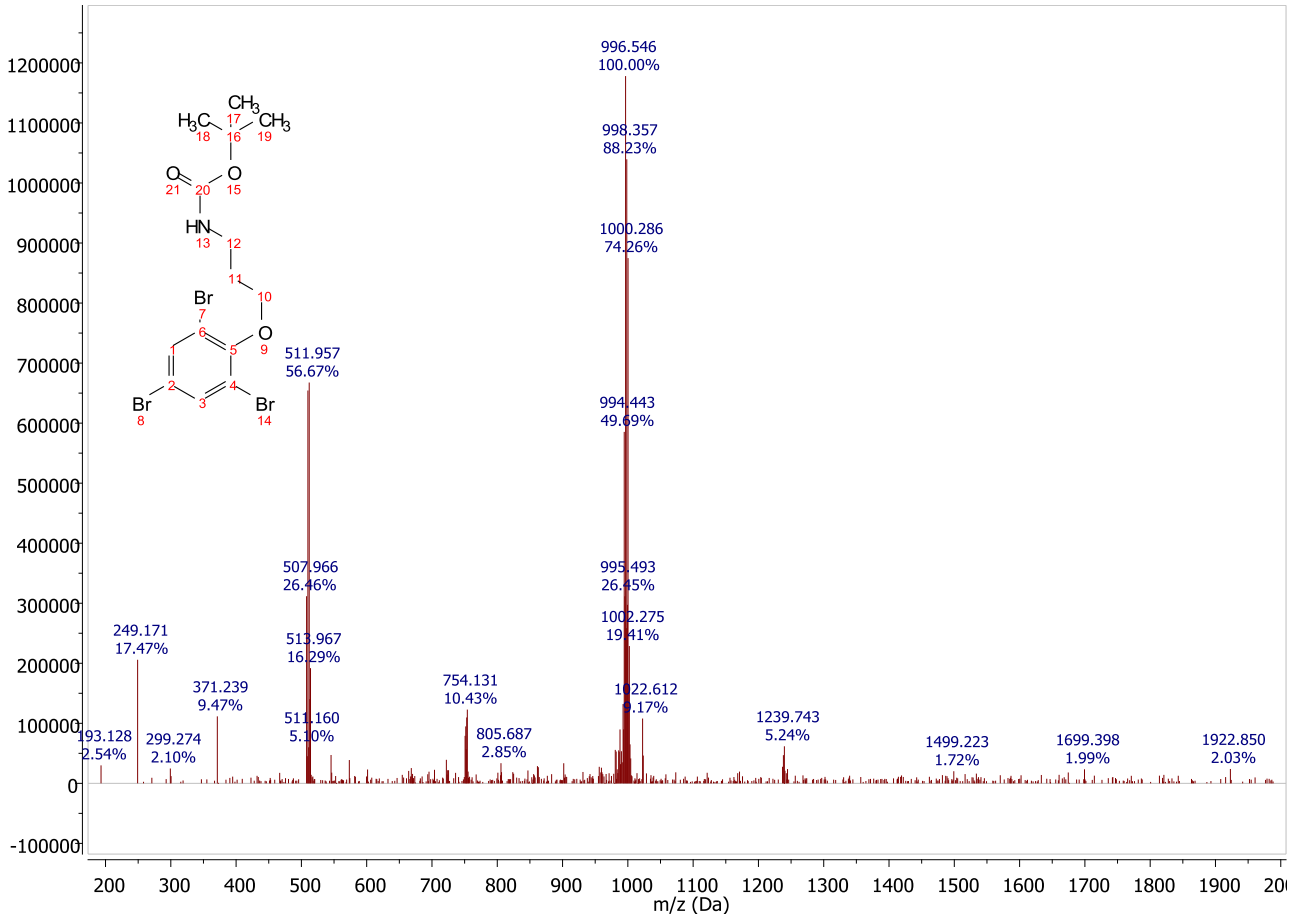


Figure S12. MS (ESI-positive) of **5**

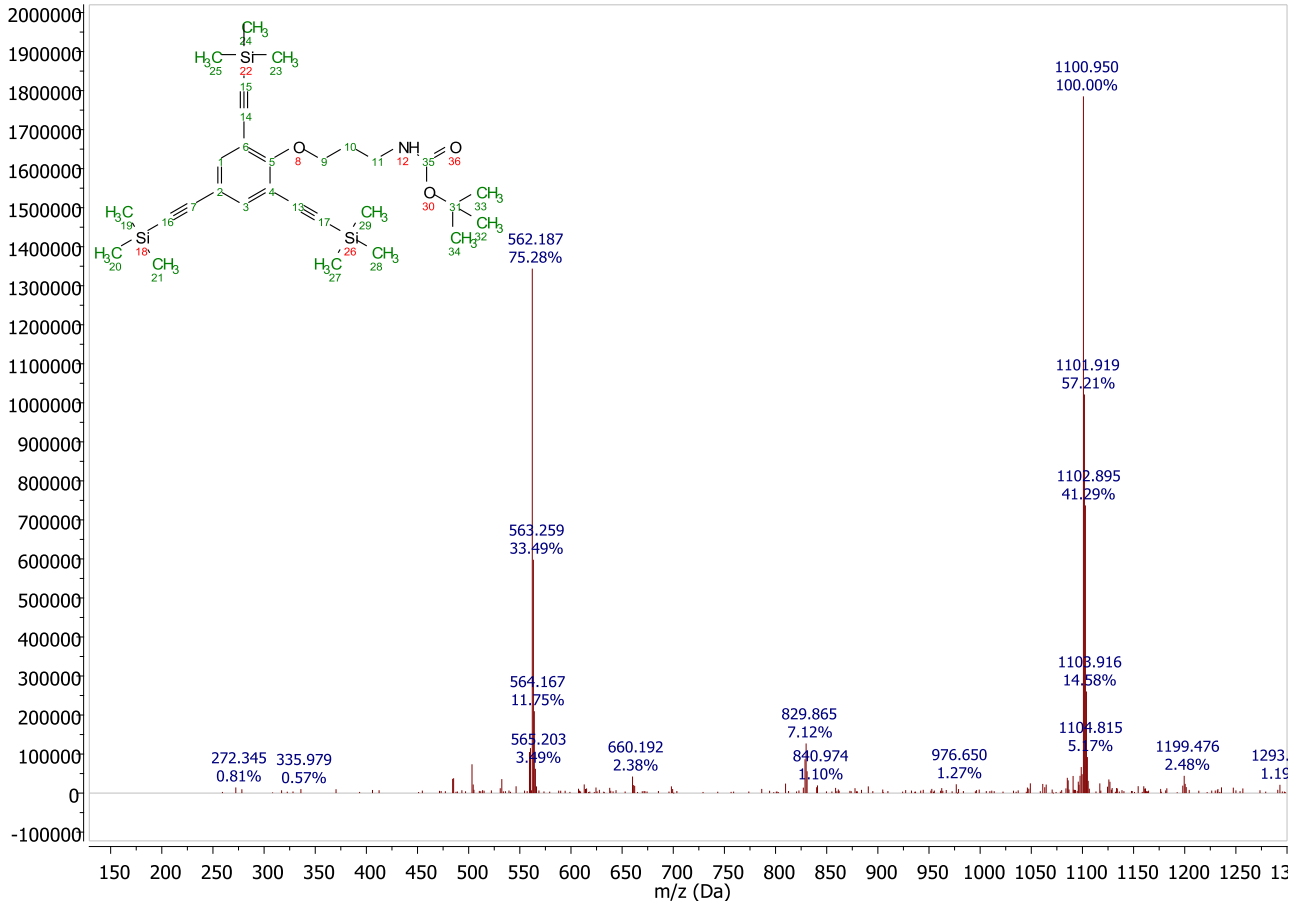


Figure S13. MS (ESI-positive) of **6**

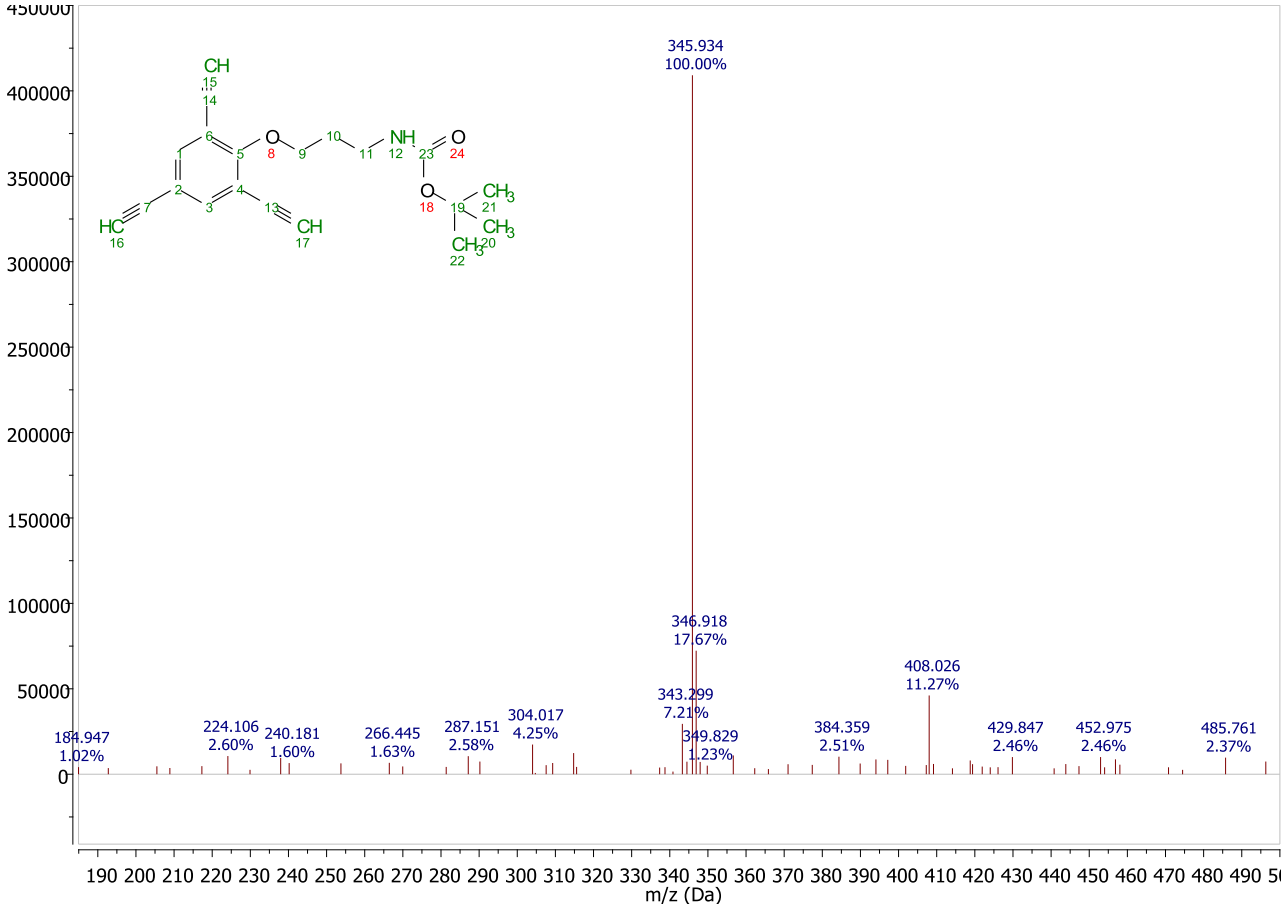


Figure S14: MS (ESI-positive) of **7**

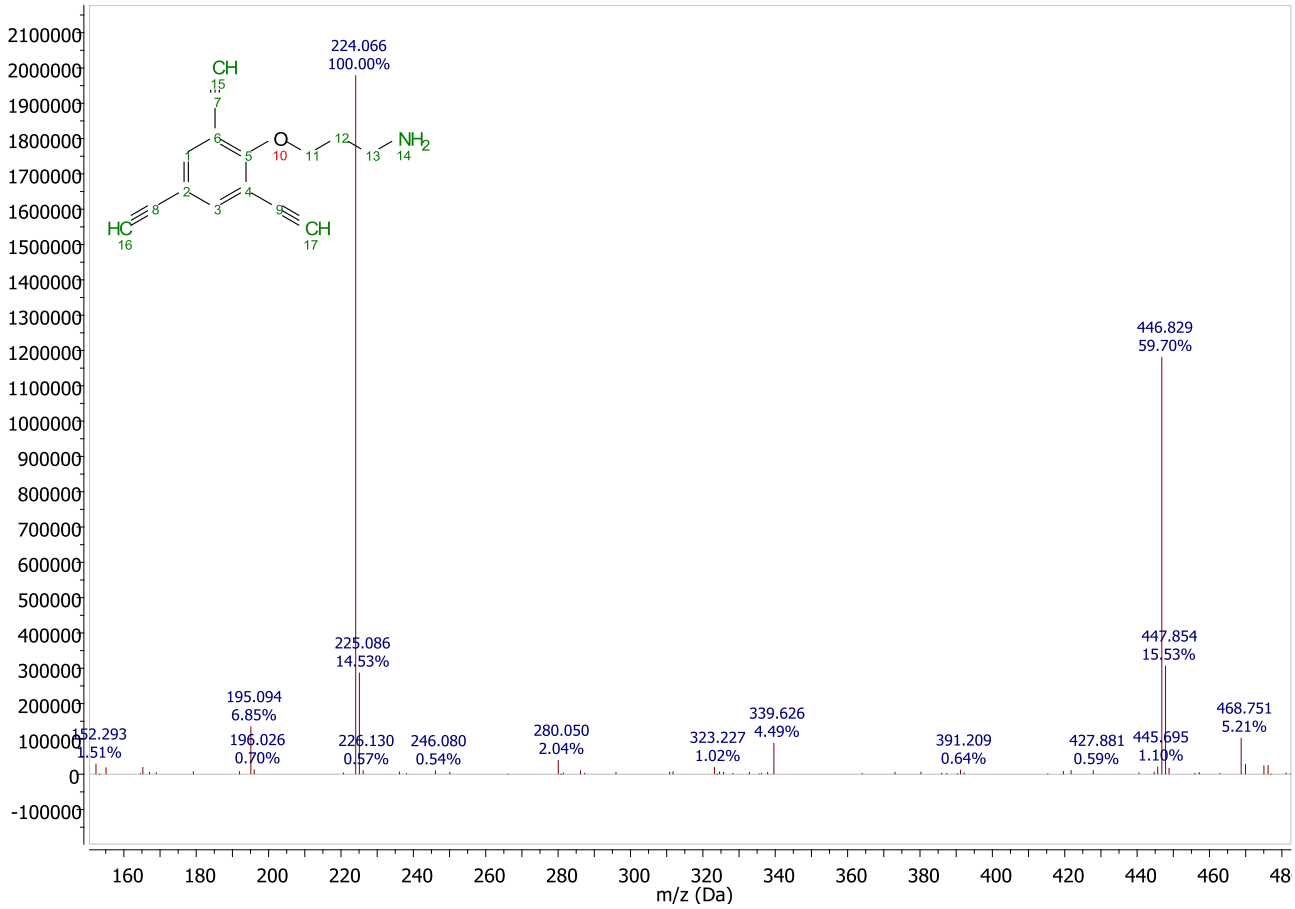


Figure S15. MS (ESI-positive) of **8**

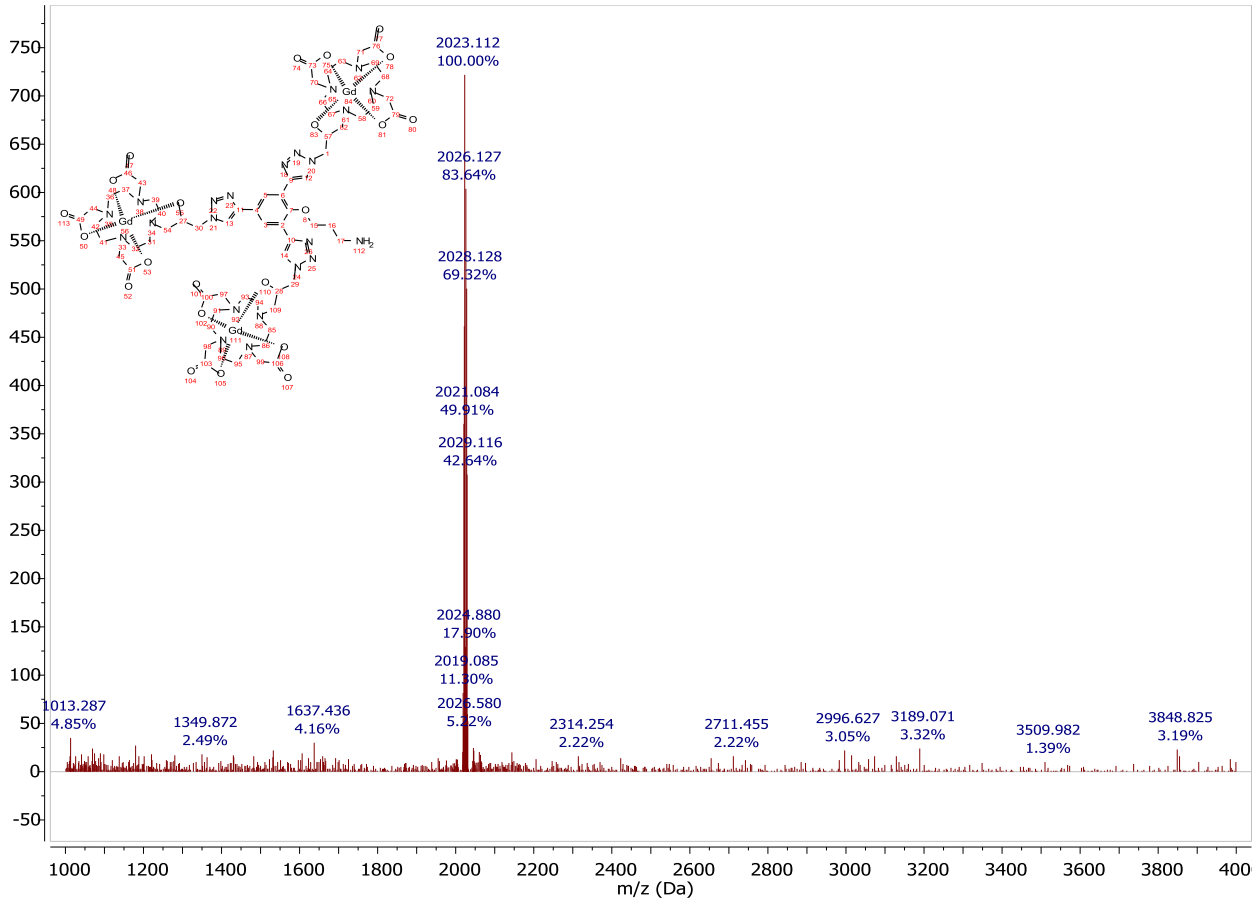


Figure S16. MS(MALDI-TOF) of **2**

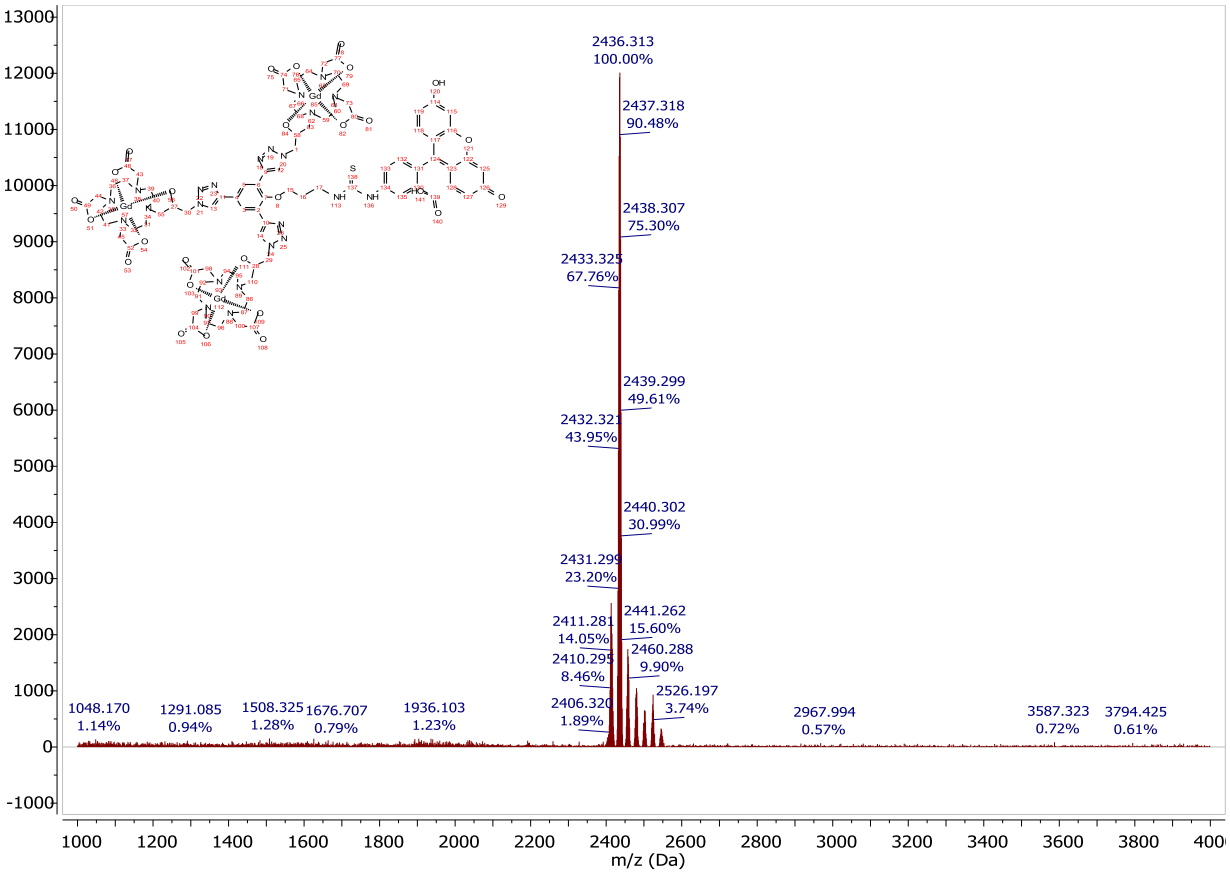


Figure S17. MS (MALDI-TOF) of **1**

Analytical HPLC Traces of 1 and 2

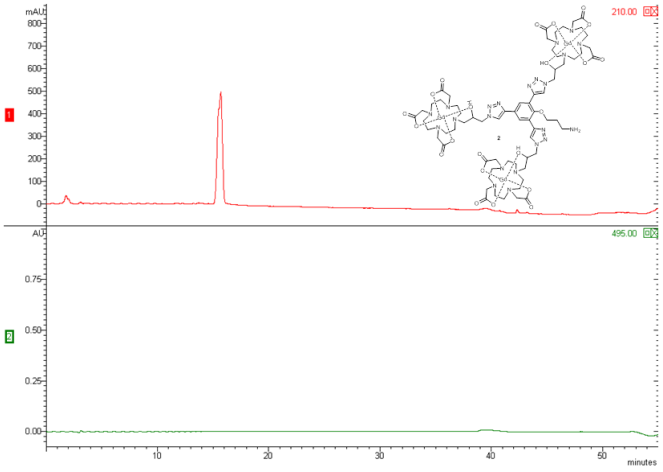


Figure S18. Analytical HPLC Trace, monitored at 210 and 495 nm, of **2**

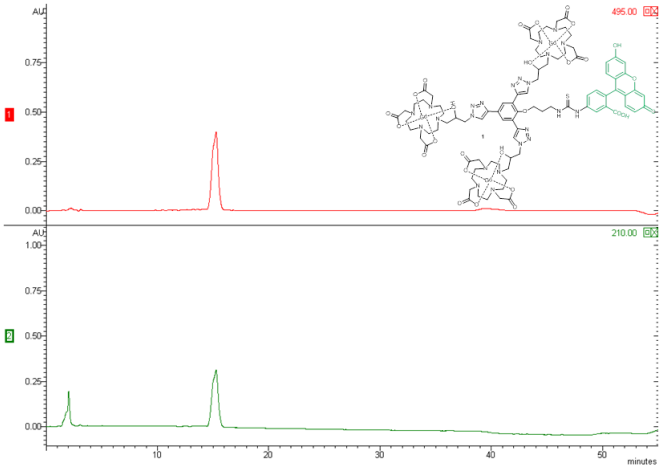


Figure S19. Analytical HPLC Trace, monitored at 210 and 495 nm, of **1**

Table S1. r_2 of **1** and **2** at 1.41 and 7 T at 37° C in 10 mM PBS buffer, pH 7.4

	1.41 T (60 MHz)		**7 T (300 MHz)**	
	Ionic ($mM^{-1}s^{-1}$)	Molecular ($mM^{-1}s^{-1}$)	Ionic ($mM^{-1}s^{-1}$)	Molecular ($mM^{-1}s^{-1}$)
1	20.1 ± 1.0	60.3 ± 3.0	17.2 ± 0.2	51.6 ± 0.6
2	17.8 ± 0.4	53.4 ± 1.0	16.2 ± 0.3	48.6 ± 0.9

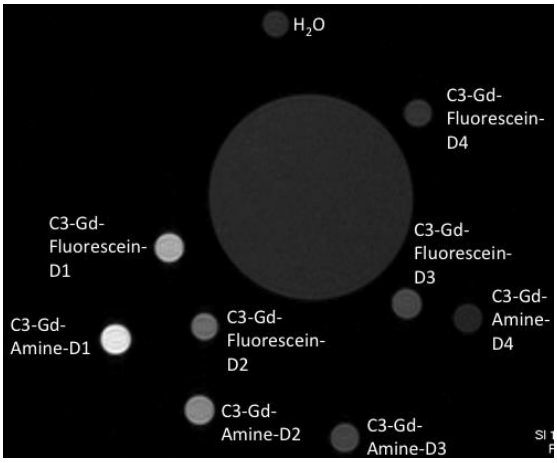


Figure S20. T_1 solution phantom image at 7 T and 25°C of complexes **1-2.** TE = 11 ms, TR = 500 ms, MTX = 256 x 256, and slice thickness is 1.0 mm. The inverse of the longitudinal relaxation time ($1/T_1$, s^{-1}) was plotted against Gd(III) concentration (mM) and fitted to a straight line with R^2>0.99. The slope of the fitted line was recorded as the relaxivity, r_1 ($mM^{-1}s^{-1}$).

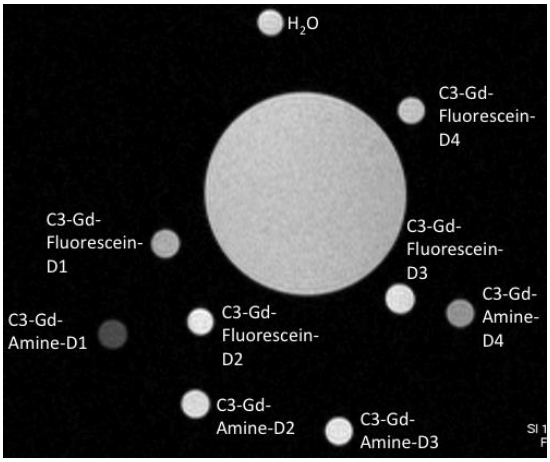


Figure S21. T_2 solution phantom image at 7 T and 25°C of complexes **1-2.** TE = 11 ms, TR = 500 ms, MTX = 256 x 256, and slice thickness is 1.0 mm. The inverse of the longitudinal relaxation time ($1/T_{2,}$ s^{-1}) was plotted against Gd(III) concentration (mM) and fitted to a straight line with R^2>0.99. The slope of the fitted line was recorded as the relaxivity, r_2 ($mM^{-1}s^{-1}$).

Toxicity

Toxicity was measured with MTS assays using HeLa cells that had been incubated with concentrations between 4 μM and 320 μM of either **1** or **2** (**Figure S22**). Higher concentrations were not tested due to insolubility of **1**. For all subsequent experiments, incubation concentrations were selected in the 90% or greater cell viability range.

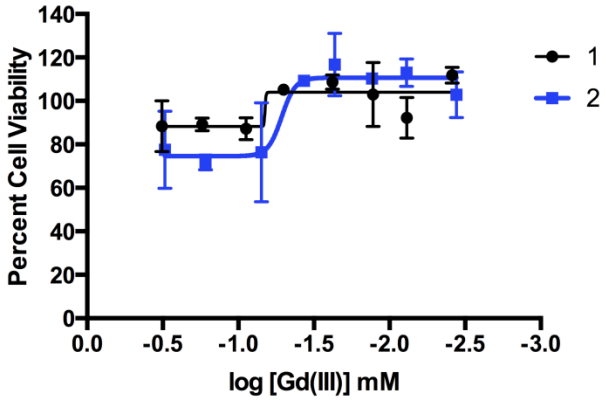


Figure S22. Toxicity of **1** and **2** was determined with MTS assays after a 24-hour incubation with HeLa cells. Based on these data, subsequent incubations were made at concentrations that maintain ≥ 90% cell viability.

Confocal Microscopy

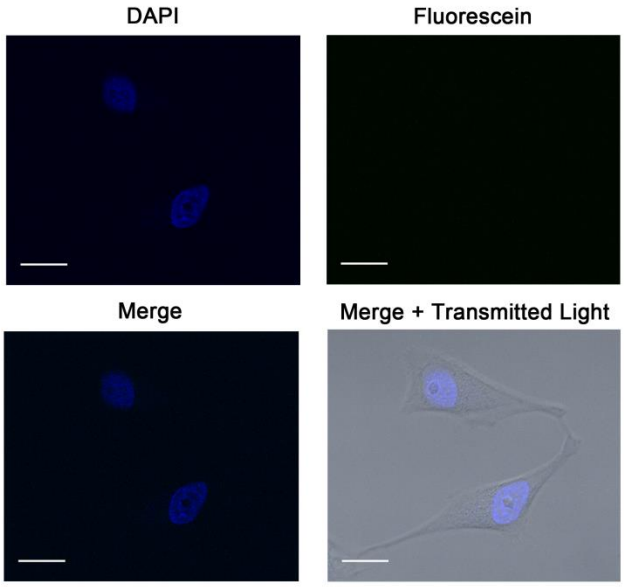


Figure S23. Confocal fluorescence micrographs of unlabeled HeLa cells incubated 1 μM DAPI for 10 minutes. Images show that fluorescein signal observed in labeled cells is not due to background fluorescence. Blue = DAPI, Green = Fluorescein. Scale bar = 20 μm.

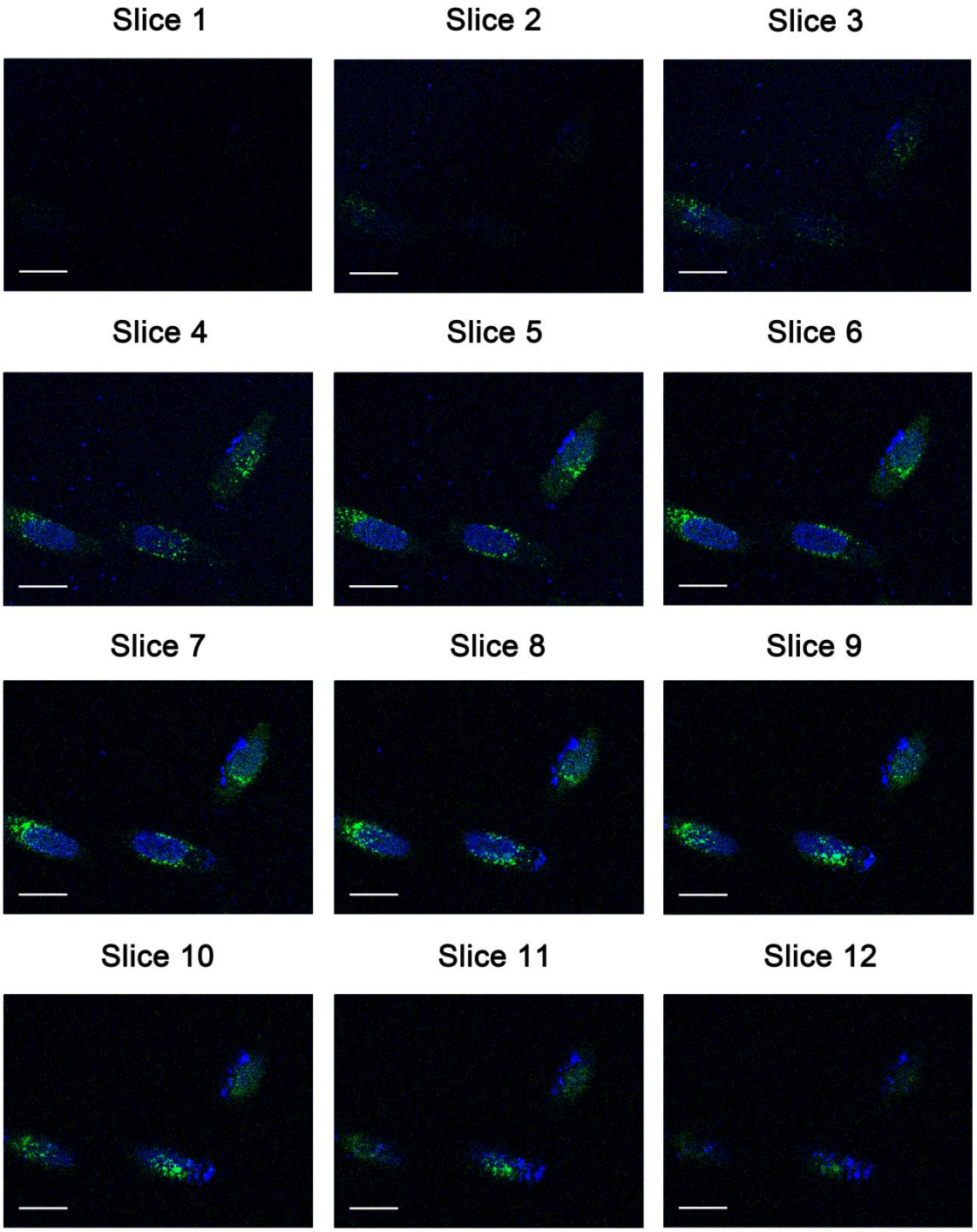


Figure S24. Z-stack confocal fluorescence micrographs of HeLa cells incubated with 85 μM of **1** for 4 hours and 1 μM DAPI for 10 minutes. Images show merge of blue (DAPI) and green

(fluorescein) channels and confirm intracellular accumulation of **1**. Blue = DAPI, Green = Fluorescein. Scale bar = 20 μm.

Flow Cytometry

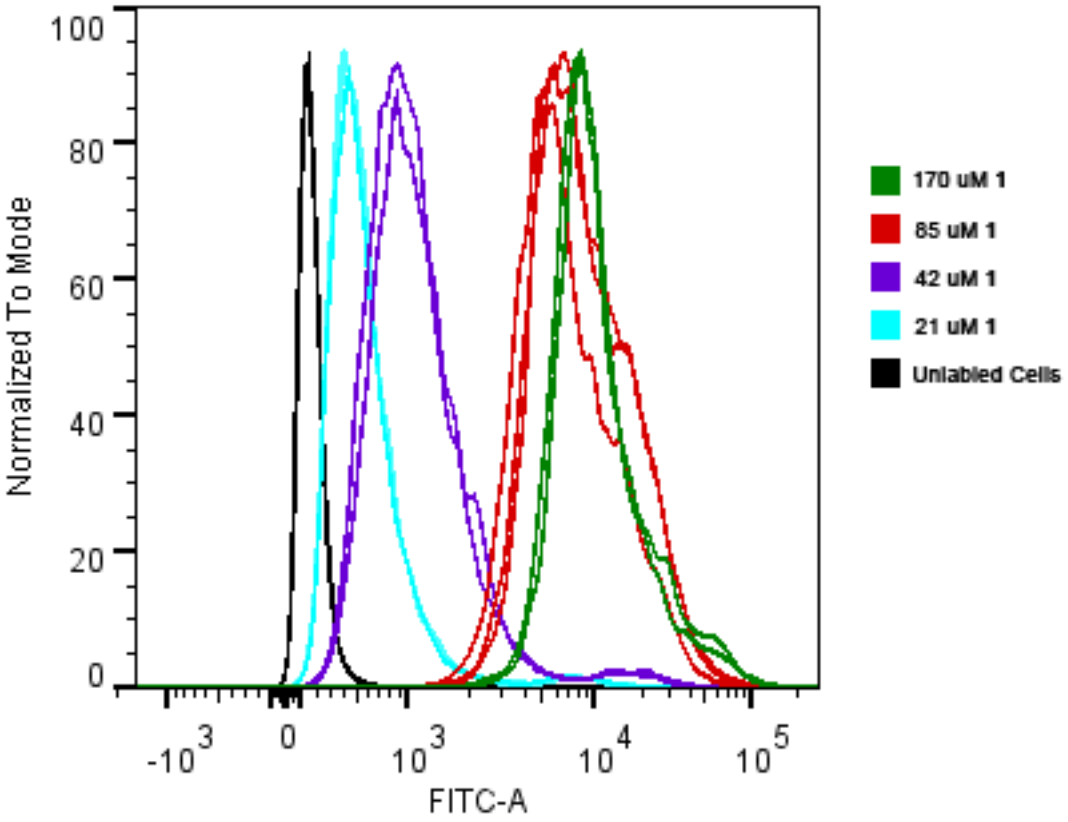


Figure S25. Histograms from analytical flow cytometry analysis showing fluorescein labeling of HeLa cells labeled with 21 to 170 μM of **1**. Each condition was performed in triplicate. Fluorescein labeling increases with increasing incubation concentration.

Time Dependence of Cell Uptake

The time dependence of cell uptake was investigated by incubating HeLa cells with 90 μM of either **1** or **2** for 1, 2, 4, or 24 hours. For both complexes, uptake increased with increasing time (**Figure S26**). A 24-hour incubation time was selected for all subsequent experiments.

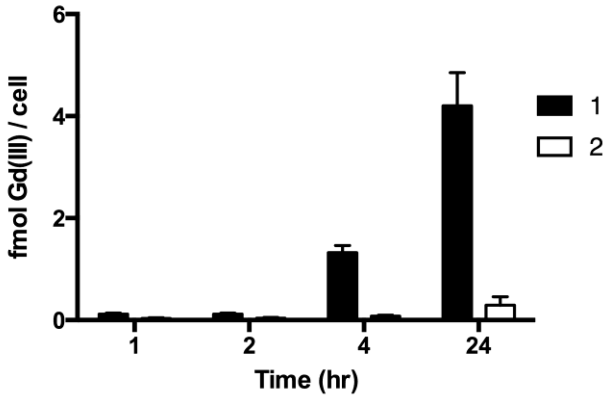


Figure S26. Time-dependent cell uptake was determined by incubating HeLa cells with 90 μM of **1** or **2**. For both complexes, uptake increases with increasing time. Error bars represent ± standard deviation.

Concentration Dependence of Cell Uptake

Cell uptake efficiency of **1** was compared to **2** and ProHance® by incubating HeLa cells with varying concentrations of each agent for 24 hours. At low incubation concentrations (below 0.3 mM Gd(III)), the uptake of **1** is up to 24-fold higher than **2**, however; at high incubation concentrations (greater than 0.3 mM Gd(III)), the uptake of the two probes is identical (**Figure S27**). Both probes deliver a 10-fold increase in cell uptake compared to clinically available ProHance®.

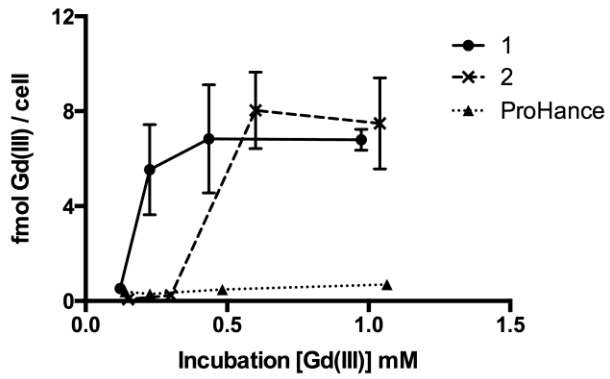


Figure S27. Concentration-dependent cell uptake was determined by incubating HeLa cells with varying concentrations of **1**, **2**, and ProHance® for 24 hours. At low incubation concentrations, **1** is the most effective at labeling cells, however at high incubation concentrations, the uptake of **1** and **2** is identical. Both probes label cells 10-fold more efficiently than ProHance®.