Amplifying the Sensitivity of Zinc(II) Responsive MRI Contrast Agents by Altering Water Exchange Rates

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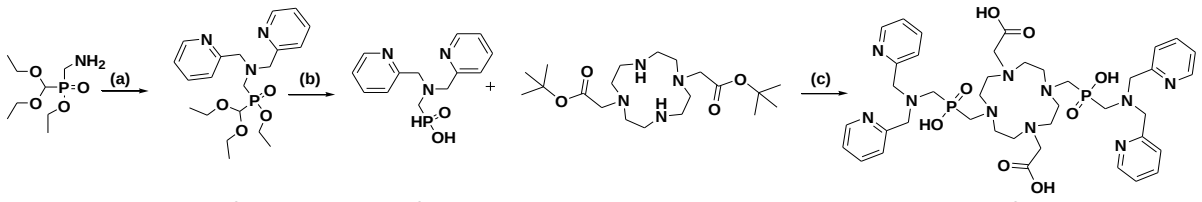
SUPPORTING INFORMATION

Contents

1. General

All reagents and solvents were purchased from commercial sources and used as received unless otherwise noted. Human Serum Albumin (HAS, fatty acid and globulin free) was purchased from Sigma-Aldrich. Silica gel (200-400 mesh, 60Å) for column chromatography was purchased from Sigma-Aldrich. TLC analyses were conducted using EMD Millipore precoated aluminum oxide or Whatman precoated silica gel on polyester plates. Lanthanide chloride stock solutions (0.035 M of $GdCl_3$ and 0.576 M of $EuCl_3$) were standardized with EDTA standard solution (0.005 M) using xylenol orange as the endpoint indicator in acetate buffer (pH = 5.8). All hydrogenation reactions were carried out using a Parr hydrogenation apparatus. ^{1}H, ^{13}C and ^{31}P NMR spectra of all synthetic intermediates, final products, ^{17}O temperature studies of all lanthanide complexes were recorded on a Bruker AVANCE III 400 MHz NMR spectrometer. Analytical HPLC was performed on an Agilent Technologies 1220 Infinity LC using a RESTEK Ultra C-18 IBD column (3 μm, 100 × 4.6 mm). Preparative HPLC was performed on a Waters Delta Prep HPLC system equipped with a Water® 2996 photodiode array detector and a Phenomenex Luna C18 column (5 μm, 30 mm x 250 mm) or an Atlantis Prep T3 OBD Column (5 μm, 30 mm x 250 mm). A Fisher Science Education pH-meter coupled with Thermo Scientific Orion Micro pH electrode was used for pH measurements. Milli-Q purified water was used for the preparation of all samples and for preparative and analytical HPLC. A VirTis Freeze Dryer (Benchtop-k) was used to lyophilize the samples. Mass spectra were obtained using either a HT Laboratories (San Diego, CA) instrument or a Waters Alliance e2695 Separations Module coupled with Xevo QTof MS using an Atlantis T3 Column (5 μm, 6 mm x 250 mm) at The Advanced Imaging Research Center (The University of Texas Southwestern Medical Center, Dallas). The metal concentrations were determined via Inductively Coupled Plasma (ICP) from Galbraith Laboratories (Knoxville, TN).

2. Synthesis and Characterization



Scheme s1. Synthesis of Ligand for Sensor Gd-**2**. Reagents and conditions: (a) 2-(chloromethyl)pyridine, K_2CO_3, CH_3CN, reflux, 10 hours (30%); (b) HCl (5 M), reflux, 12 hours (100%); (c) paraformaldehyde, concentrated HCl, reflux, 24 hours (60%).

Ethyl (aminomethyl)(diethoxymethyl)phosphinate (1)

To a solution of ethyl ((benzylamino)methyl)(diethoxymethyl)phosphinate (synthesized according to published procedures[1,2]) (8.0 g, 25.39 mmol) in ethanol (150 mL) was added palladium hydroxide on carbon (20%, 500 mg). The reaction mixture was shaken in a Parr high pressure hydrogenation apparatus and allowed to react at a hydrogen pressure of 60 psi at room temperature for 24 hours. The black reaction mixture was filtered through celite and the solution was evaporated *in vacuo* to afford a colorless oil (5.2 g, 23.11 mmol, 91%).

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.37 (6H, t, $^{3}J_{HH}$ = 7.2 Hz, CH_3), 1.46 (3H, t, $^{3}J_{HH}$ = 7.2 Hz, CH_3), 3.16 (2H, d, $^{2}J_{HP}$ = 8.0 Hz, $PC\underline{H_2}NH_2$), 3.83 (2H, qd, $^{3}J_{HH}$ = 7.2 Hz, $^{4}J_{HP}$ = 1.2 Hz, CH_2O), 4.00 (2H, qd, $^{3}J_{HH}$ = 7.2 Hz, $^{4}J_{HP}$ = 1.2 Hz, CH_2O), 4.33 (2H, qd, $^{3}J_{HH}$ = 7.2, Hz, $^{4}J_{HP}$ = 1.2 Hz, CH_2O), 5.05 (1H, d, $^{2}J_{Hp}$ = 7.2 Hz, O_2CHP).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 14.28 (CH_3), 15.60 (CH_3), 36.43 (NH_2CH_2P, d, $^{1}J_{CP}$ = 93 Hz), 62.17 (CH_2O), 65.85 (CH_2O, d, $^{2}J_{CP}$ = 6 Hz), 100.45 (O_2CP, d, J_{CP} = 141 Hz).

^{31}P NMR (161.9 MHz, CD_3OD): δ (ppm) 42.76 (95% purity).

Ethyl ((bis(pyridin-2-ylmethyl)amino)methyl)(diethoxymethyl)phosphinate ester (2)

Compound **1** (1.00 g, 4.44 mmol) and 2-(chloromethyl)pyridine (1.25 g, 9.80 mmol) was dissolved in anhydrous CH_3CN (20 mL) in the presence of 5 equivalents of K_2CO_3. The resulting reaction mixture was stirred at room temperature overnight and then at reflux for an additional 10 hours. The organic phase was filtered and evaporated *in vacuo*. Column chromatography (silica gel, 90% chloroform/10% methanol to elute column) afforded pure compound **2** as a yellow oil (0.53 g, 1.30 mmol, 30%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.15 (6H, td, $^3J_{HH}$ = 6.8 Hz, $^4J_{HP}$ = 0.8 Hz, CH_3), 1.26 (3H, td, $^3J_{HH}$ = 7.2 Hz, $^4J_{HP}$ = 0.8 Hz, CH_3), 3.20 (2H, m, PCH_2N), 3.61 (2H, qd, $^3J_{HH}$ = 7.2 Hz, $^3J_{HP}$ = 1.2 Hz, CH_2O), 3.72 (2H, qd, $^3J_{HH}$ = 7.2 Hz, $^3J_{HP}$ = 1.2 Hz, CH_2O), 3.85 (2H, qd, $^3J_{HH}$ = 7.2 Hz, $^3J_{HP}$ = 1.2 Hz, CH_2O), 4.07 (4H, dd, $^3J_{HH}$ = 14.4 Hz, $^4J_{HP}$ = 11.2 Hz, NCH_2Ph), 4.26 (2H, qd, $^3J_{HH}$ = 7.2 Hz, $^3J_{HP}$ = 1.2 Hz, CH_2O), 4.81 (1H, d, $^3J_{Hp}$ = 8.4 Hz, O_2CHP) 7.20 (2H, m, Ph), 7.64 (2H, d, $^3J_{HH}$ = 7.6 Hz, Ph), 7.71 (2H, t, $^3J_{HH}$ = 7.6 Hz, Ph), 8.57 (2H, d, $^3J_{HH}$ = 7.6 Hz, Ph).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.13 (CH_3), 16.59 (CH_3), 49.47 (d, $^1J_{CP}$ = 105 Hz, NCH_2P), 61.33 (CH_2), 61.61 (CH_2), 65.41 (d, $^2J_{CP}$ = 32 Hz, CH_2), 100.32 (d, J_{CP} = 138 Hz, O_2CP), 121.98 (Ph), 123.45 (Ph), 136.29 (Ph), 148.73 (Ph), 158.80 (Ph).

^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 40.31 (98% purity).

LCMS (ESI): 408.13 $(M+H)^+$.

((Bis(pyridin-2-ylmethyl)amino)methyl)phosphinic acid, hydrochloride (3)

Compound **2** (0.53 g, 1.30 mmol) was dissolved in HCl (1mL, 5 M) and refluxed for 12 hours. The HCl solution was removed in vacuo to afford compound **3** in virtually quantitative yield (0.50 g, 1.30 mmol, 100%).

^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.07 (2H, d, $^3J_{HP}$ = 8.4 Hz, CH_2P), 4.42 (4H, s, NCH_2Ph), 7.95 (2H, t, $^3J_{HH}$ = 7.6 Hz, Ph), 8.03 (2H, d, $^3J_{HH}$ = 8.0 Hz, Ph), 8.51 (2H, t, $^3J_{HH}$ = 7.6 Hz, Ph), 8.71 (2H, d, $^3J_{HH}$ = 6.0 Hz, Ph).

^{13}C NMR (100 MHz, D_2O): δ (ppm) 54.54 (d, $^1J_{CP}$ = 102 Hz, NCH_2P), 57.33 (d, $^3J_{CP}$ = 7 Hz, NCH_2Ph), 126.38 (Ph), 127.13 (Ph), 141.38 (Ph), 147.30 (Ph), 152.19 (Ph).

^{31}P NMR (161.9 MHz, D_2O): δ (ppm) 24.06 (98% purity).

LCMS (ESI): 278.16 $[M+H]^+$.

Di-tert-butyl 2,2'-(1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (4)

To a solution of dibenzyl 4,10-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate(10.00 g, 15.00 mmol) in ethanol (150 mL) was added palladium on carbon (10%, 500 mg). The reaction mixture was shaken in a Parr high pressure hydrogenation apparatus and allowed to react at a hydrogen pressure of 60 psi at room temperature for 24 hours. The black reaction mixture was filtered through celite and the resulting colorless solution was then evaporated *in vacuo* to afford **4** as a colorless oil (5.72 g, 14.26 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.56 (18H, s, CH_3), 2.90-2.97 (8H, m br, macrocycle CH_2), 3.06-3.12 (8H, m, macrocycle CH_2), 3.52 (4H, s, CH_2).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 28.19 (CH_3), 47.18 (macrocycle CH_2), 51.82 (macrocycle CH_2), 56.80 (CH_2), 81.53 ($\underline{C}(CH_3)_3$), 171.14 (C=O).

LCMS (ESI): 201.21 $[M/2+H)]^+$, 401.27 $[M+H]^+$, 801.45 $[2M+H]^+$.

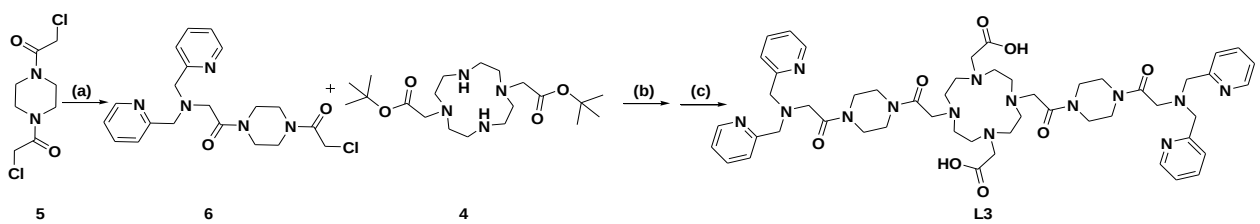
2,2'-(4,10-Bis((((bis(pyridin-2-ylmethyl)amino)methyl)(hydroxy)phosphoryl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L2)

The HCl salt of compound **3** (0.50 g, 1.30 mmol) and compound **4** (0.17 g, 0.43 mmol) were dissolved in concentrated HCl (1 mL) and stirred at reflux. For the next 24 hours, paraformaldehyde (0.39 g) was added for a total of four times. The resulting yellow solution was evaporated *in vacuo* and the resulting product was purified using preparative HPLC. The ligand was obtained as a colorless oil (225 mg, 0.26 mmol, 60%).

^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.11 (12H, s br, macrocycle CH_2), 3.22 (4H, d, $^3J_{HH}$ = 6.4 Hz, NCH_2P), 3.45 (4H, d, $^3J_{HH}$ = 6.8 Hz, NCH_2P), 3.57 (8H, s br, macrocycle CH_2), 3.76 (4H, s, NCH_2CO_2), 4.42 (8H, s, NCH_2Ph), 7.92 (4H, d, $^3J_{HH}$ = 6.4 Hz, Ph), 7.95 (4H, m, Ph), 8.48 (4H, t, $^3J_{HH}$ = 8.0 Hz, Ph), 8.71 (4H, d, $^3J_{HH}$ = 6.4 Hz, Ph).

^{13}C NMR (100 MHz, D_2O): δ (ppm) 48.58 (NCH_2P), 52.13 (macrocycle CH_2), 52.55 (macrocycle CH_2), 52.97 (macrocycle CH_2), 53.27 (macrocycle CH_2), 55.42 (d, $^1J_{CP}$ = 109 Hz, NCH_2P), 57.37 (d, $^3J_{CP}$ = 7 Hz, NCH_2Ph), 126.33 (Ph), 127.10 (Ph), 141.55 (Ph), 147.15 (Ph), 152.20 (Ph), 173.65 (CO_2H).

LCMS (ESI): 434.18 $[M/2+H)]^+$, 867.20 $[M+H]^+$.



5 6 4 L3

Scheme s2. Synthesis of Ligand for Sensor Gd-**3**. Reagents and conditions: (a) di-(2-picolyl)amine, $NaHCO_3$, CH_3CN, reflux, 8 hours (65%); (b) $NaHCO_3$, CH_3CN, reflux, 8 hours (90%); (c) TFA, RT, 12 hours (95%).

1,1'-(Piperazine-1,4-diyl)bis(2-chloroethan-1-one) (5)

To a solution of piperazine (5.00 g, 58.14 mmol) in $CHCl_3$ (200 mL) was added 100 mL of saturated K_2CO_3. The reaction mixture was stirred vigorously and cooled to 0 °C in an ice bath. 2-Chloroacetyl chloride (19.71 g, 174.44 mmol) in $CHCl_3$was added dropwise over a period of 1 hour. The reaction mixture was then allowed to warm to room temperature and stirred for 2 hours. Then the organic phase was washed with HCl (2 × 100 mL, 1 M) and water (2 × 100 mL). Finally, the $CHCl_3$ was dried and evaporated *in vacuo* to afford the product as a white solid (13.74 g, 57.49 mmol, 99%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.74 (8H, d, $^3J_{HH}$= 7.2 Hz, CH_2N), 4.20 (4H, s, CH_2Cl).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 40.64 (CH_2Cl), 42.02(br, CH_2N), 45.86 (br, CH_2N), 165.60 (NC=O).

2-(Bis(pyridin-2-ylmethyl)amino)-1-(4-(2-chloroacetyl)piperazin-1-yl)ethan-1-one (6)

To a solution of compound **5** (3.60 g, 15.06 mmol) in CH_3CN (50 mL) was added di-(2-picolyl) amine (3.00 g, 15.07 mmol) and $NaHCO_3$ (1.68 g, 20.00 mmol). The reaction mixture was heated at 65 °C for 8 hours while stirring, then cooled to room temperature and filtered. The filtrate was evaporated to afford a foam-like solid, which was purified by flash column chromatography on silica, eluting first with CH_2Cl_2 and then with CH_2Cl_2/methanol (95:5 *v/v*), to afford a pale yellow solid (3.64 g, 9.05 mmol, 65%). *Rf* = 0.85 (15% MeOH in chloroform, neutral Al_2O_3).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.46 (m, 8H, CH_2NC=O), 4.27 (s, 2H, CH_2Cl), 4.46 (4H, s, NCH_2Ph), 5.19 (s, 2H, NCH_2C=O), 7.29 (d, 2H, $^3J_{HH}$ = 8.0 Hz, Ph), 7.32-7.41 (m, 4H, Ph), 7.88 (d, 2H, $^3J_{HH}$ = 8.0 Hz, Ph).

^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 44.52 (CH_2Cl), 47.62 (br, CH_2NC=O), 48.94 (br, CH_2NC=O), 57.41 (NCH_2C=O), 61.20 (NCH_2Ph), 127.45 (Ph), 129.20 (Ph), 140.45 (Ph), 148.23 (Ph), 150.34 (Ph), 167.35 (C=O), 169.33 (C=O).

LCMS (ESI): 402.13 $[M+H]^+$, 825.15 $[2M+Na]^+$.

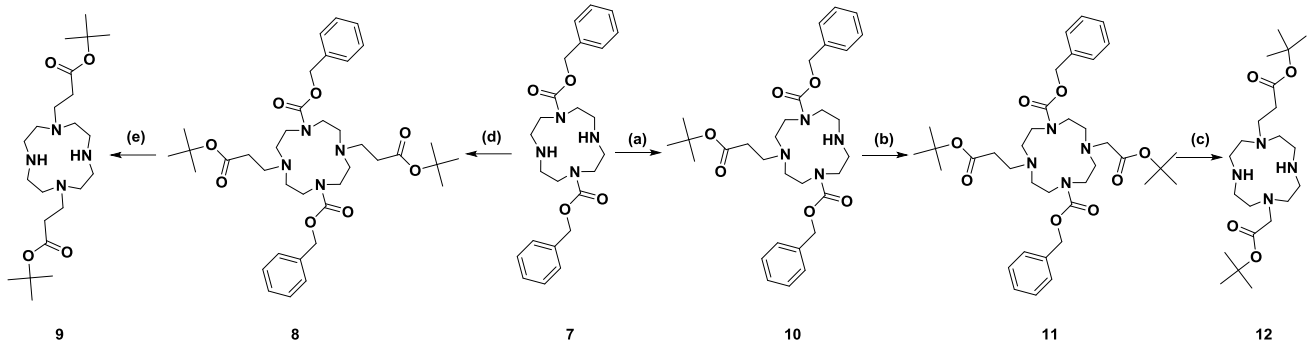
2,2'-(4,10-Bis(2-(4-(bis(pyridin-2-ylmethyl)glycyl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L3)

The reaction mixture of compound **6** (0.36 g, 0.89 mmol), compound **4** (0.18 g, 0.44 mmol) and $NaHCO_3$ (0.08 g, 1.00 mmol) in CH_3CN (5 mL) was heated at 65 °C for 24 hours while stirring. The mixture was then cooled to room temperature and filtered. The filtrate was evaporated to give a foam-like solid product which was dissolved in 2 mL trifluoroacetic acid and stirred at room temperature for 12 hours. The mixture was evaporated to dryness and purified using preparative HPLC to afford the pure ligand (377 mg, 0.37 mmol, 84%, two steps).

^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.15 (8H, s, br, macrocycle CH_2), 3.53 (8H, br, macrocycle CH_2), 3.58 (4H, s, br, NCH_2C=O), 3.65 (16H, s, NCH_2CH_2N), 4.02 (4H, s, NCH_2CO_2), 4.48 (12H, s, NCH_2Ph, NCH_2CON), 7.89 (4H, t, $^3J_{HH}$ = 8.0 Hz, Ph), 7.94 (4H, d, $^3J_{HH}$ = 8.0 Hz, Ph), 8.51 (4H, t, $^3J_{HH}$ = 8.0 Hz, Ph), 8.73 (4H, d, $^3J_{HH}$ = 8.0 Hz, Ph).

^{13}C NMR (100 MHz, D_2O): δ (ppm) 41.29 (piperazine CH_2), 43.69 (piperazin CH_2), 47.79 ($N\underline{C}H_2CON$), 51.79 (macrocycle CH_2), 53.04 (macrocycle CH_2), 55.02 ($N\underline{C}H_2CO_2H$), 55.68 ($N\underline{C}H_2CON$), 56.02 (NCH_2Ph), 126.28 (Ph), 126.75 (Ph), 141.25 (Ph), 147.19 (Ph), 152.85 (Ph), 164.12 (piperazine C=O), 170.71 (piperazine C=O), 173.79 (CO_2).

LCMS (ESI): 510.20 $[M/2+H)]^+$, 1019.26 $[M+H]^+$.



Scheme s3. Synthesis of key intermediates. Reagents and conditions: (a) tert-butyl 3-bromopropanoate, K_2CO_3, CH_3CN, reflux, 10 hours (62 %); (b)tert-butyl 2-bromoacetate, K_2CO_3, CH_3CN, RT, 12 hours (95 %); (c) H_2 (60 psi), Pd/C, EtOH, RT, 12hours (95%); (d) tert-butyl but-3-enoate, MeOH, RT, 3 days (100%); (e) H_2 (60 psi), Pd/C, EtOH, RT, 12 hours (95%).

Dibenzyl 1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (7)

1,4,7,10-tetraazacyclododecane (18.06 g, 105.00 mmol) was dissolved in CH_2Cl_2 (500 mL) and cooled to 0 °C in an ice bath. Benzyl chloroformate (35.83 g, 209.53 mmol) in CH_2Cl_2 (250 mL) was added dropwise over the course of 3 hours while keeping the reaction temperature at 0 °C. The light yellow solution was allowed to warm to room temperature and was stirred overnight. The solvent was evaporated to roughly 25% of its initial volume, to which diethyl ether (500 mL) was added to induce the precipitation of the product as a HCl salt. The white solid was filtered off and washed with diethyl ether (3 × 100 mL). After suspending the solid in 500 mL of water, NaOH solution (20%) was added slowly while vigoroulys stirring the mixture until a pH of 12.0 was reached. The milky solution was transferred into a separatory funnel and extracted with diethyl ether (3 × 200 mL). The organic phase was then washed with water (3 × 200 mL), dried and evaporated to dryness to afford the product as colorless oil (33.30 g, 75.51 mmol, 72%)

^{1}H NMR (400 MHz, CD_3OD): δ 2.75-2.91 (8H, m, br, macrocycle CH_2), 3.51 (8H, br, macrocycle CH_2), 5.23 (4H, s, CH_2Ph), 7.22-7.45 (10H, br, Ph).

^{13}C NMR (400 MHz, CD_3OD): δ 44.95 (macrocycle CH_2), 50.13 (macrocycle CH_2), 66.95 ($\underline{C}H_2Ph$), 127.51 (Ph), 127.75 (Ph), 128.22 (Ph), 136.65 (Ph), 157.06 (Ph).

LCMS (ESI): 441.55 $(M+H)^+$.

Dibenzyl 4,10-bis(3-(tert-butoxy)-3-oxopropyl)-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (8)

To a solution of compound **7** (2.92 g, 6.62 mmol) in MeOH (10 mL) was added tert-butyl acrylate (16.95 g, 132.40 mmol). The resulting reaction mixture was stirred at room temperature for 3 days. The completion of reaction was confirmed by TLC. Then, MeOH and excess tert-butyl acrylate were removed *in vacuo* to afford the product as a yellow oil in nearly wuantitative yield (4.62 g, 6.62 mmol, 100%).

^{1}H NMR (400 MHz, CD_3OD): δ 1.58 (18H, s, CH_3), 2.88 (4H, s br, $NCH_2\underline{C}H_2$), 3.26-3.85 (16H, m, br, macrocycle CH_2), 4.34 (4H, br, NCH_2), 5.32 (4H, s, OCH_2Ph), 7.51 (6H, m, Ph), 7.62 (4H, d, $^3J_{HH}$ = 8.0 Hz, Ph).

^{13}C NMR (100 MHz, CD_3OD): δ 26.91 (CH_3), 28.47 ($NCH_2\underline{C}H_2$), 45.38 (macrocycle CH_2), 49.79 (macrocycle CH_2), 52.35 ($N\underline{C}H_2CH_2$), 68.60 ($O\underline{C}H_2Ph$), 81.56 ($\underline{C}(CH_3)_3$), 128.24 (Ph), 128.37 (Ph), 128.65 (Ph), 135.72 (Ph), 157.95 (NC=O), 169.28 ($\underline{C}O_2$).

LCMS (ESI): 697.13 $(M+H)^+$.

Di-tert-butyl 3,3'-(1,4,7,10-tetraazacyclododecane-1,7-diyl)dipropionate (9)

To a solution of compound **8** (1.00 g, 1.43 mmol) in 50 mL EtOH was added palladium on carbon (10%, 100 mg). The reaction mixture was shaken in a Parr high pressure hydrogenation apparatus and allowed to react under a hydrogen pressure (60 psi) at room temperature for 24 hours. The black reaction mixtures was filtered through celite and the resulting colorless solution was evaporated *in vacuo* to afford compound **9** as a white solid (0.58 g, 1.36 mmol, 95%).

^{1}H NMR (400 MHz, CD_3OD): δ 1.59 (18H, s, CH_3), 2.66 (4H, t, $^3J_{HH}$ = 7.2 Hz, $C\underline{H}_2CO$), 2.95-3.08 (12H, br, macrocycle CH_2 and side arm $NC\underline{H}_2$), 3.39 (8H, br, macrocycle CH_2).

^{13}C NMR (100 MHz, CD_3OD): δ 27.10 (CH_3), 30.54 ($\underline{C}H_2C=O$), 42.63 (macrocycle CH_2), 47.33 (macrocycle CH_2), 60.84 (side arm NCH_2), 81.30 ($\underline{C}(CH_3)_3$), 173.48 ($\underline{C}O_2$).

ESI-MS: 429.43 $(M+H)^+$.

Dibenzyl 4-(3-(tert-butoxy)-3-oxopropyl)-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (10)

To a solution of compound **7** (2.92 g, 6.62 mmol) in CH_3CN (20 mL) was added tert-butyl bromopropionate (2.77 g, 13.24 mmol) and K_2CO_3 (1.83 g, 13.24 mmol). The reaction mixture was refluxed for 10 hours. The solvent was evaporated *in vacuo* to afford a white solid, to which 100 mL of ethyl acetate and 100 mL water were added. The organic phase was collected using a separatory funnel, dried and evaporated *in vacuo*. The crude product was purified via column chromatography, eluting first with CH_2Cl_2 and then with CH_2Cl_2/methanol (95:5 *v*/*v*), to afford compound **10** as a slightly yellow oil (2.33 g, 4.10 mmol, 62%).

^{1}H NMR (400 MHz, CD_3CN): δ 1.53 (9H, br, CH_3), 2.38 (2H, br, $C\underline{H}_2C=O$), 2.84-3.00 (8H, br, macrocycle CH_2), 3.09 (2H, br, side arm NCH_2), 3.49 (8H, s br, macrocycle CH_2), 5.26 (4H, s, CH_2Ph), 7.45 (10H, br, Ph).

^{13}C NMR (100 MHz, CD_3CN): δ 27.62 (CH_3) 29.83 ($\underline{C}H_2CO_2$), 44.34-52.59 (m, b, macrocycle CH_2), 53.20 (side arm NCH_2), 66.73 ($O\underline{C}H_2Ph$), 80.07 ($\underline{C}(CH_3)_3$), 127.83 (Ph), 128.08 (Ph), 128.76 (Ph), 137.63 (Ph), 156.52 (NC=O), 172.41 ($\underline{C}O_2$).

ESI-MS: 569.13 $(M+H)^+$.

Dibenzyl 4-(2-(tert-butoxy)-2-oxoethyl)-10-(3-(tert-butoxy)-3-oxopropyl)-1,4,7,10-tetraazacyclo-dodecane-1,7-dicarboxylate (11)

To a solution of compound **10** (1.00 g, 1.76 mmol) in CH_3CN (10 mL) was added tert-butyl bromoacetate (0.35 g, 1.80 mmol) and K_2CO_3 (0.28 g, 2.03 mmol). The reaction mixture was stirred at room temperature for 12 hours. The solvent was filtered and evaporated *in vacuo* to afford the product as a yellow oil (1.14 g, 1.67 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.54 (18H, d, br, CH_3), 2.44 (2H, br, C$\underline{H}_2CO_2$), 2.72 (2H, br, NC$\underline{H}_2CH_2$), 2.98 (8H, br, macrocycle CH_2), 3.43 (2H, br, NC$\underline{H}_2$C=O), 3.54 (8H, br, macrocycle CH_2), 5.23 (4H, s, CH_2Ph), 7.31-7.45 (10H, m, Ph).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.19 (CH_3), 32.95 ($\underline{C}H_2COO$), 46.84 (b, macrocycle CH_2), 50.15 (macrocycle CH_2), 54.14 (N$\underline{C}H_2CH_2$), 56.03 (N$\underline{C}H_2$C=O), 67.01 ($\underline{C}H_2$Ph), 80.52 ($\underline{C}(CH_3)_3$), 80.97 ($\underline{C}(CH_3)_3$), 127.91 (Ph), 128.50 (Ph), 128.61 (Ph), 136.85 (Ph), 156.46 (NC=O), 170.56 ($\underline{C}O_2$), 172.08 ($\underline{C}O_2$).

ESI-MS: 683.13 $(M+H)^+$.

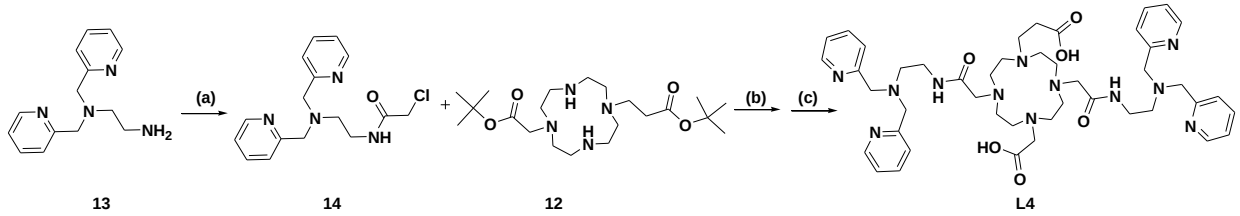
Tert-butyl 3-(7-(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)propanoate (12)

To a solution of compound **11** (1.14 g, 1.67 mmol) in ethanol (50 mL) was added palladium on carbon (10%, 100 mg). The reaction mixture was shaken in a Parr high pressure hydrogenation apparatus and allowed to react at a hydrogen pressure of 60 psi at room temperature for 24 hours. The black reaction mixture was filtered through celite and the resulting colorless solution was evaporated *in vacuo* to afford compound **12** as a colorless oil (0.66 g, 1.59 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53 (9H, s, CH_3), δ 1.54 (9H, s, CH_3), 2.51 (2H, t, $^3J_{HH}$ = 7.2 Hz, C$\underline{H}_2CO_2$), 2.72-2.85 (12H, br, macrocycle CH_2), 2.91 (2H, t, $^3J_{HH}$ = 7.2 Hz, NC$\underline{H}_2CH_2$), 2.97 (4H, br, macrocycle CH_2), 3.41 (2H, s, NC$\underline{H}_2$C=O).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.19 (CH_3), 33.55 ($\underline{C}H_2CO_2$), 46.18 (macrocycle CH_2), 46.66 (N$\underline{C}H_2CH_2$), 50.99 (macrocycle CH_2), 51.55 (macrocycle CH_2), 51.88 (macrocycle CH_2), 56.97 (N$\underline{C}H_2$C=O), 80.42 ($\underline{C}(CH_3)_3$), 81.29 ($\underline{C}(CH_3)_3$), 170.98 (C=O), 171.89 (C=O).

ESI-MS: 415.26 $(M+H)^+$.



Scheme s4. Synthesis of Ligand for Sensor Gd-**4**. Reagents and conditions: (a) chloroacetyl chloride, K_2CO_3, CH_2Cl_2, RT, 16 hours (93 %); (b) K_2CO_3, CH_3CN, reflux, 2 days (57%); (c) HCl (3 M), RT, 5 days (100 %).

N1,N1-bis(pyridin-2-ylmethyl)ethane-1,2-diamine (13)

Compound **13** was synthesized according to published procedures.[2] Only modification: The pure product was obtained upon chromatographic purification (neutral alumina, 100% ethyl acetate).

^{1}H NMR (400 MHz, CD_3CN): δ 2.06 (2H, m, $^3J_{HH}$ = 2.4 Hz, NH_2), 3.10 (2H, t, $^3J_{HH}$ = 5.6 Hz, NC$\underline{H}_2CH_2NH_2$), 3.35 (2H, t, $^3J_{HH}$ = 5.6 Hz, $NCH_2C\underline{H}_2NH_2$), 4.26 (4H, s, NC$\underline{H}_2$Ph), 8.02 (4H, t, $^3J_{HH}$ = 8.0 Hz, Ph), 8.54 (2H, t, $^3J_{HH}$ = 8.0 Hz, Ph), 8.96 (2H, d, $^3J_{HH}$ = 6.4 Hz, Ph).

^{13}C NMR (100 MHz, CD_3CN): δ 37.11 (H_2NCH_2), 52.42 (N$\underline{C}H_2CH_2NH_2$), 55.81 (N$\underline{C}H_2$Ph), 126.53 (Ph), 127.77 (Ph), 142.61 (Ph), 146.89 (Ph), 152.08 (Ph).

LCMS (ESI): 243.02 $(M+H)^+$.

N-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)-2-chloroacetamide(14)

Compound **13** (12.70 g, 52.41 mmol) was dissolved in 100 mL dichloromethane. The deep red solution was cooled to 0 °C using an ice bath. Chloroacetyl chloride (6.22g, 55.03 mmol) and potassium carbonate (7.61 g, 55.03 mmol) dissolved in 100 mL dichloromethane were added dropwise over the course of 3 hours. The reaction mixture was allowed to slowly warm up to room temperature and was stirred for an additional 16 hours. The solvent was evaporated *in vacuo* and 150 mL of diethyl ether was added to the brownish oily residue. This leads to the precipitation of product. Diethyl ether was decanted off and the grey pre-

cipitate was repeatedly washed with diethyl ether (5 × 100 mL). The product was further purified via column chromatography (silica, 95% chloroform/5% methanol to remove a polar impurities, the product fraction elutes upon gradual increase of eluent polarity with 40% chloroform/59% methanol/1% triethylamine (15.54 g,48.74mmol, 93%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.94 (t, 2H), 3.48 (t, 2H), 3.99 (s, 2H), 4.26 (s, 4H), 7.69 (m, 2H), 7.92 (m, 2H), 8.20 (m, 2H), 8.84 (d, 2H), 12.07 (br, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 36.80 ($N_{amide}\mathbf{C}H_2$), 42.44 ($CO\mathbf{C}H_2$), 54.10 ($N_{amine}\mathbf{C}H_2CH_2$), 56.51 ($N_{amine}(\mathbf{C}H_2)_2$), 125.34 (pyridyl-2**C**), 126.82 (pyridyl-2**C**), 143.60-144.40 (pyridyl-6**C**), 153.1 (**C**O).

LCMS (ESI): 319.8 $(M+H)^+$

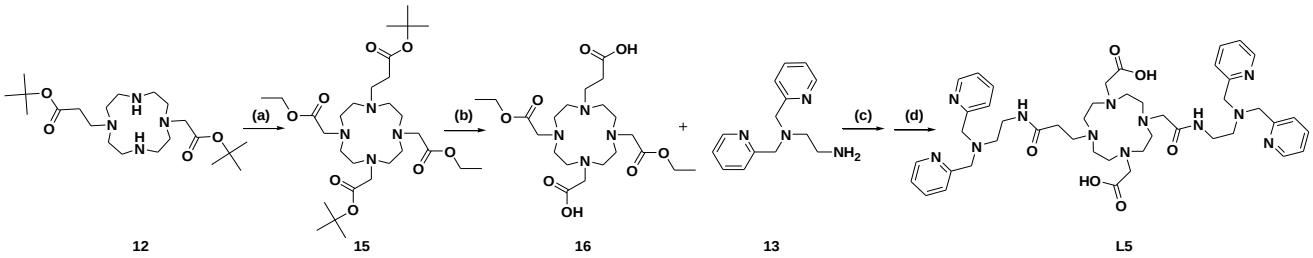
3-(4,10-Bis(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)amino)-2-oxoethyl)-7-(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)propanoic acid (L4)

Compound **12** (5.86 g, 14.07 mmol) was suspended in 100 mL CH_3CN. Compound **14** (11 g, 30.96 mmol) together with potassium carbonate (21.40 g, 154.80mmol) were added. The volume was increased to 250 mL and the suspension was refluxed at 63 °C for 48 hours. The solvent was removed *in vacuo* and the red-brown sludge was redissolved in dichloromethane, filtered and concentrated. Column chromatography (alumina, 97.8% chloroform/2% methanol/0.2% triethylamine) afforded the product as a yellow/orange fraction in 57% yield. After evaporation, the product was dissolved in 100 mL 3 M HCl. The deep red solution was stirred at room temperature for five days to afford **L4** in virtually quantitative yield (5.21 g, 5.1 mmol, 100%).

^{1}H NMR (400 MHz, D_2O): δ 3.20 (16H, m, br, macrocycle CH_2, $NHC\underline{H}_2C\underline{H}_2N$), 3.28 (4H, m, br, $CONHCH_2C\underline{H}_2N$), 3.44 (8H, m, $4NC\underline{H}_2$), 3.57 (2H, br, $NCH_2C\underline{H}_2COOH$), 3.94 (2H, s, $NC\underline{H}_2CONH$), 4.46 (8H, s, $C\underline{H}_2Ph$), 7.77 (8H, m, Ph), 8.28 (4H, m, Ph), 8.50 (2H, br, $CON\underline{H}$), 8.76 (4H, m, Ph).

^{13}C NMR (100 MHz, D_2O): δ 27.74 ($NCH_2\underline{C}H_2NCO$), 29.75 ($CH_2\underline{C}H_2COOH$), 36.10 ($\underline{C}H_2CH_2COOH$), 49.36 (macrocycle $\underline{C}H_2$), 50.37 ($N\underline{C}H_2CH_2NCO$), 50.78 (macrocycle CH_2), 53.05 ($NH\underline{C}H_2CH_2N$), 53.25 (macrocycle $\underline{C}H_2$), 56.07 (macrocycle $\underline{C}H_2$), 55.36 ($N\underline{C}H_2CO_2H$), 55.47 (CH_2Ph), 117.81 (Ph), 124.78 (Ph), 139.45 (Ph), 146.84 (Ph), 152.45 (Ph), 155.5 (CO), 160.2 ($\underline{C}OOH$), 161.1 ($\underline{C}OOH$).

LCMS (ESI): 868.1 $(M+H)^+$;



Scheme s5. Synthesis of Ligand for Sensor Gd-**5**. Reagents and conditions: (a) ethyl bromoacetate, $NaHCO_3$, CH_3CN, reflux, 12 hours (95 %); (b)HCl (1.0 M) in ether, RT, 12 hours (100 %); (c) HBTU, DIPEA, DMF, RT, 12 hours; (d) NaOH (1.0 N), RT, 12 hours.

Diethyl 2,2'-(4-(2-(tert-butoxy)-2-oxoethyl)-10-(3-(tert-butoxy)-3-oxopropyl)-1,4,7,10-tetraazacyclo-dodecane-1,7-diyl)diacetate (15)

To a solution of compound **12** (0.60 g, 1.45 mmol) in CH_3CN (20 mL) was added ethyl bromoacetate (0.49 g, 2.95 mmol) and $NaHCO_3$ (0.65 g, 7.68 mmol). The reaction mixture was refluxed for 12 hours and then cooled to room temperature. Excess $NaHCO_3$ was filtered off and the solution was evaporated *in vacuo* to afford the title compound (0.81 g, 1.38 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (6H, t, $^3J_{HH}$ = 7.2 Hz, $C\underline{H}_3CH_2O$), 1.41 (9H, s, $C(C\underline{H}_3)_3$), 1.47 (9H, s, $C(C\underline{H}_3)_3$), 2.40-3.51 (26H, m, br, macrocycle CH_2 and side arm CH_2), 4.15 (4H, br, $CH_3C\underline{H}_2O$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.16 ($\underline{C}H_3CH_2O$), 27.98 ($C(\underline{C}H_3)_3$), 31.12 ($NCH_2\underline{C}H_2CO_2$), 49.56 ($N\underline{C}H_2CH_2CO_2$), 51.88 (macrocycle CH_2), 55.98 (br, $N\underline{C}H_2CO_2$), 57.15 (br, $N\underline{C}H_2CO_2$), 61.03 ($CH_3\underline{C}H_2O$), 80.58 ($\underline{C}(CH_3)_3$), 82.10 ($\underline{C}(CH_3)_3$), 172.26 (C=O), 172.51 (C=O), 172.74 (C=O).

LCMS (ESI): 587.35 $[M+H]^+$, 1173.60 $[2M+H]^+$.

3-(7-(Carboxymethyl)-4,10-bis(2-ethoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)propanoic acid (16)

To a solution of compound **15** (0.81 g, 1.38 mmol)in CH_2Cl_2 (2 mL) was added HCl solution (5 ml, 1.0 M) in ether. The reaction mixture was stirred at room temperature overnight. The solvent was then evaporated to afford compound **16** as HCL salt. The material was used for the subsequent coupling reaction without further purification (0.75 g, 1.38 mmol, 100%).

^{1}H NMR (400 MHz, D_2O): δ 1.27 (6H, t, $^3J_{HH}$ = 7.2 Hz, C$\underline{H}_3$CH$_2$O), 3.01 (2H, t, $^3J_{HH}$ = 7.2 Hz, NCH$_2$C$\underline{H}_2$CO$_2$) 3.15-3.25 (8H, m, br, macrocycle CH_2), 3.47 (8H, m, br, macrocycle CH_2), 3.62 (6H, s, br, side arm NCH_2), 4.22 (4H, q, $^3J_{HH}$ = 7.2 Hz, CH$_3$C$\underline{H}_2$O).

^{13}C NMR (100 MHz, D_2O): δ 13.35 (C$\underline{H}_3$CH$_2$O), 47.88 (NCH$_2$C$\underline{H}_2$CO$_2$), 48.44 (macrocycle CH_2), 49.79 (macrocycle CH_2), 50.34 (macrocycle CH_2), 51.85 (macrocycle CH_2) 52.90 (side arm NCH_2), 54.79 (side arm NCH_2), 59.27 (side arm NCH_2), 62.28 (CH$_3$C$\underline{H}_2$O), 168.66 (C=O), 172.76 (C=O), 172.98 (C=O).

LCMS (ESI): 238.02 $[M/2+H)]^+$, 474.96 $[M+H]^+$, 948.97 $[2M+H]^+$.

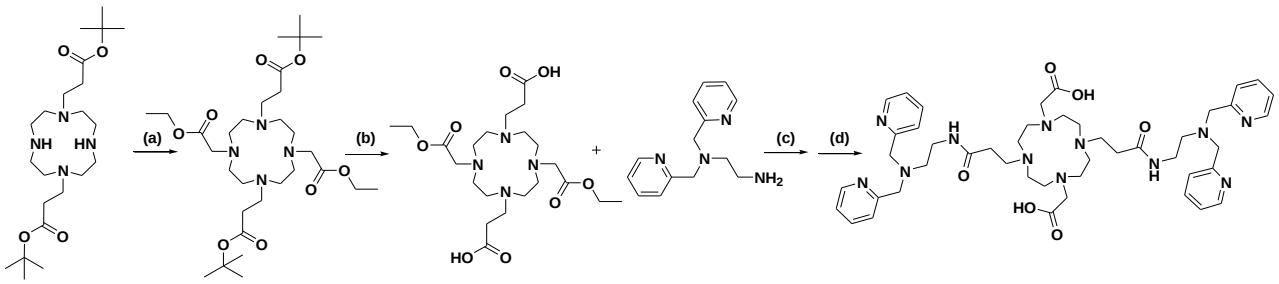
2,2'-(4-(2-((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)amino)-2-oxoethyl)-10-(3-((2-(bis(pyridin-2-ylmethyl)amino)-ethyl)amino)-3-oxopropyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L5)

To a solution of compound **16** (0.10 g, 0.18 mmol) in DMF (4 mL) was added DIPEA (0.12 g, 0.90 mmol) and HBTU (0.14 g, 0.37 mmol). The reaction mixture was stirred at room temperature for 5 minutes. Then, compound **13** (0.11 g, 0.45 mmol) was added to the above reaction mixture, which was stirred at room temperature overnight. The DMF and DIPEA were both evaporated off *in vacuo* and the resulting solid was dissolved in deionized water. The intermediate product was extracted with ethyl acetate (3 × 10 mL), the organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to afford the intermediate as a yellow oil, which was dissolved in a mixture of CH_3CN and water (2 mL: 2 mL). NaOH was added to maintain the pH of the solution at ~12.0 while stirring overnight. The pH of the solution was then adjusted to ~7.0 with HCl and the solution was evaporated to give the crude product, which was purified using preparative HPLC to afford the pure ligand as a yellow oil (75 mg, 0.09 mmol, 48%, two steps).

^{1}H NMR (400 MHz, D_2O): δ 2.73 (2H, t, $^3J_{HH}$ = 6.4 Hz, NCH$_2$C$\underline{H}_2$CO), 2.89 (4H, m, $^3J_{HH}$ = 6.4 Hz, NC$\underline{H}_2$CH$_2$CO), 3.29 (12H, m, br, macrocycle CH_2, NHCH$_2$C$\underline{H}_2$N), 3.40 (8H, m, br, macrocycle CH_2), 3.49 (2H, t, $^3J_{HH}$ = 6.4 Hz, NHC$\underline{H}_2$CH$_2$N), 3.67 (4H, s, NC$\underline{H}_2$CO$_2$H), 3.94 (2H, s, NC$\underline{H}_2$CONH), 4.34 (8H, s, C$\underline{H}_2$Ph), 7.99 (4H, m, Ph), 8.11 (4H, m, Ph), 8.57 (4H, m, Ph), 8.76 (4H, m, Ph).

^{13}C NMR (100 MHz, D_2O): δ 29.68 (NCH$_2$C$\underline{H}_2$CON), 36.60 (NH$\underline{C}$H$_2$CH$_2$N), 49.07 (macrocycle CH_2), 49.29 (macrocycle CH_2), 49.87 (N$\underline{C}$H$_2$CH$_2$CON), 50.78 (macrocycle CH_2), 53.46 (NHCH$_2$C$\underline{H}_2$N), 54.14 (N$\underline{C}$H$_2$CON), 55.24 (N$\underline{C}$H$_2$CO$_2$H), 55.42 (CH$_2$Ph), 126.38 (Ph), 127.14 (Ph), 141.45 (Ph), 147.24 (Ph), 152.54 (Ph).

LCMS (ESI): 434.20 $[M/2+H)]^+$, 867.28 $[M+H]^+$.



Scheme s6. Synthesis of Ligand for Sensor Gd-**6**. Reagents and conditions: (a) ethyl bromoacetate, $NaHCO_3$, CH_3CN, reflux, 12 hours (95 %); (b) HCl (1.0 M) in ether, RT, 12 hours (100 %); (c) HBTU, DIPEA, DMF, RT, 12 hours; (d) NaOH (1.0 N), RT, 12 hours.

Di-tert-butyl 3,3'-(4,10-bis(2-ethoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)dipropionate (17)

The title compound was obtained using similar procedures used to synthesize compound **15**. Yield: 0.40 g (0.66 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (6H, t, $^3J_{HH}$ = 7.2 Hz, C$\underline{H}_3$CH$_2$O), 1.42 (18H, s, C(C$\underline{H}_3$)$_3$), 2.39 (4H, s, NCH$_2$C$\underline{H}_2$CO$_2$), 2.60-3.20 (20H, br, macrocycle CH_2, NC$\underline{H}_2$CH$_2$CO$_2$), 3.39 (4H, s, br, NCH$_2$CO$_2$), 4.14 (4H, q, $^3J_{HH}$ = 7.2 Hz, CH$_3$C$\underline{H}_2$O).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.01 (C$\underline{H}_3$CH$_2$O), 27.97 (C(CH$_3$)$_3$), 51.62 (macrocycle CH_2), 55.97 (side arm NCH_2), 60.35 (CH$_3$C$\underline{H}_2$O), 171.3 (C=O), 173.5 (C=O).

LCMS (ESI): 301.24 $[M/2+H)]^+$, 601.37 $[M+H]^+$, 1201.67 $[2M+H]^+$.

3,3'-(4,10-Bis(2-ethoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)dipropionic acid (18)

The title compound was obtained using similar procedures used to synthesize compound **16**. Yield: 0.37 g (0.66 mmol, 100%).

^{1}H NMR (400 MHz, D_2O): δ 1.23 (6H, t, $^3J_{HH}$ = 7.2 Hz, $C\underline{H}_3CH_2O$), 2.93 (4H, t, $^3J_{HH}$ = 6.8 Hz, $NCH_2C\underline{H}_2CO_2$), 3.14-3.42 (8H, br, macrocycle CH_2), 3.44 (8H, br, macrocycle CH_2), 3.57 (4H, s, NCH_2CO_2), 3.61 (4H, t, $^3J_{HH}$ = 6.8 Hz, $NC\underline{H}_2CH_2CO_2$), 4.18 (4H, q, $^3J_{HH}$ = 7.2 Hz, $CH_3C\underline{H}_2O$).

^{13}C NMR (100 MHz, D_2O): δ 13.24 ($\underline{C}H_3CH_2O$), 27.99 ($NCH_2C\underline{H}_2CO_2$), 48.25 ($NC\underline{H}_2CH_2CO_2$), 50.03 (macrocycle CH_2), 50.57 (macrocycle CH_2), 53.30 (NCH_2CO_2), 62.40 ($CH_3\underline{C}H_2O$), 172.8 (C=O), 173.6 (C=O).

LCMS (ESI): 245.16 $[M/2+H)]^+$, 489.21 $[M+H]^+$

2,2'-(4,10-bis(3-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)amino)-3-oxopropyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L6)

The title compound was obtained using similar procedures that are used to synthesize compound **L5**. Yield: 80 mg (0.09 mmol, 45%, two steps).

^{1}H NMR (400 MHz, D_2O): δ 2.80 (4H, t, $^3J_{HH}$ = 6.8 Hz, $NCH_2C\underline{H}_2CO$), 2.89 (4H, t, $^3J_{HH}$ = 6.8 Hz, $NHCH_2C\underline{H}_2N$), 3.15-3.21 (20H, m, br, macrocycle CH_2, $NC\underline{H}_2CH_2CO$), 3.43 (4H, t, $^3J_{HH}$ = 6.8 Hz, $NHC\underline{H}_2CH_2N$), 3.53 (4H, s, NCH_2CO_2), 4.34 (8H, s, $C\underline{H}_2Ph$), 7.99 (4H, m, Ph), 8.11 (4H, d, $^3J_{HH}$ = 8.0 Hz, Ph), 8.57 (4H, m, Ph), 8.77 (4H, d, $^3J_{HH}$ = 5.2 Hz, Ph).

^{13}C NMR (100 MHz, D_2O): δ 28.82 ($NCH_2\underline{C}H_2CON$), 36.66 ($NH\underline{C}H_2CH_2N$), 48.81 ($N\underline{C}H_2CH_2CON$), 49.99 (macrocycle CH_2), 50.20 (macrocycle CH_2), 53.39 ($NHCH_2\underline{C}H_2N$), 54.21 ($N\underline{C}H_2CO_2H$), 55.37 (CH_2Ph), 126.37 (Ph), 127.11 (Ph), 141.4 (Ph), 147.2 (Ph), 152.5 (Ph), 171.4 (C=O), 174.8 (CO_2H).

LCMS (ESI): 441.23 $[M/2+H)]^+$, 881.35 $[M+H]^+$.

3. Lanthanide Complex Preparation and Characterization

Lanthanide complexes used in *ex vivo* experiments were prepared by mixing a ligand with 5-10% excess stoichiometic amounts of lanthanide chloride stock solutions. The reaction mixtures were adjusted and maintained to a pH 5.5-6.5 by adding NaOH. The solution was stirred at room temperature or was heated at 60 °C for 3 hours if necessary. After the reaction was completed, as judged by stabilization of the solution pH, the pH of the complex solution was adjusted to neutral. When excess lanthanide metal was detected, the mixture was adjusted to pH 8.5 and the precipitated $Ln(OH)_3$ was filtered off. The complexes were then lyophilized to a powdery solid and redissolved in water as necessary. Lanthanide complexes used in *in vivo* experiments were further purified and desalted by preparative HPLC. The purified complexes were characterized by LCMS (ESI) as shown below.

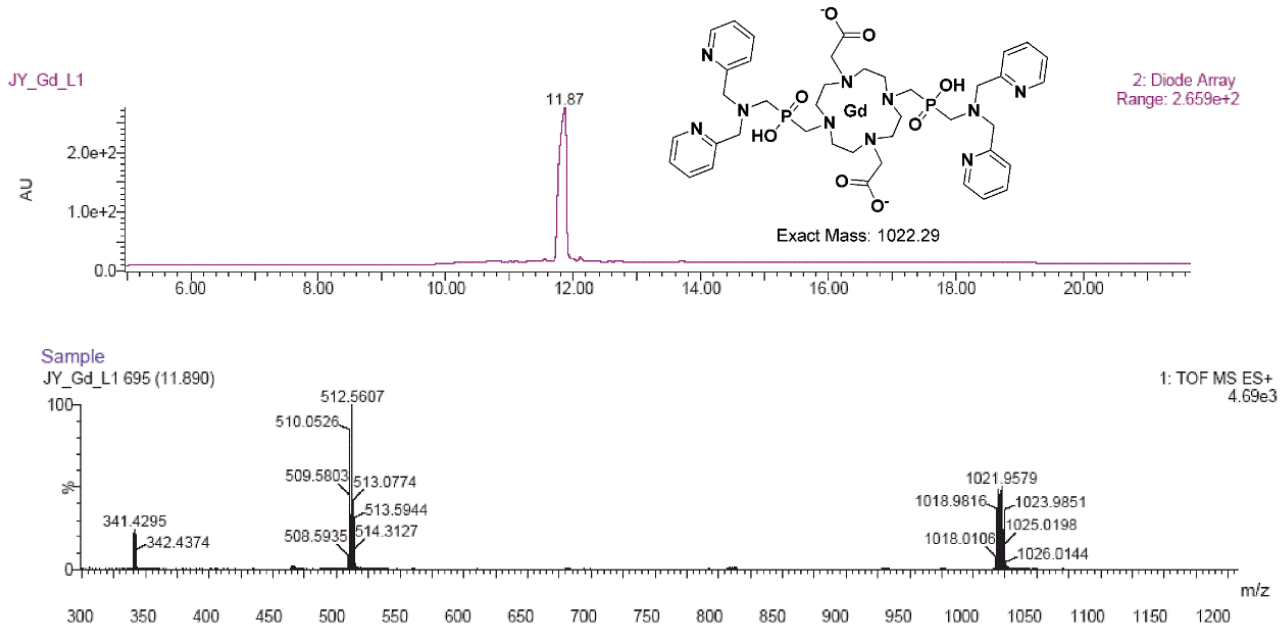


Figure S1.1. LC (top) and MS (below) spectra for Gd-**2**.

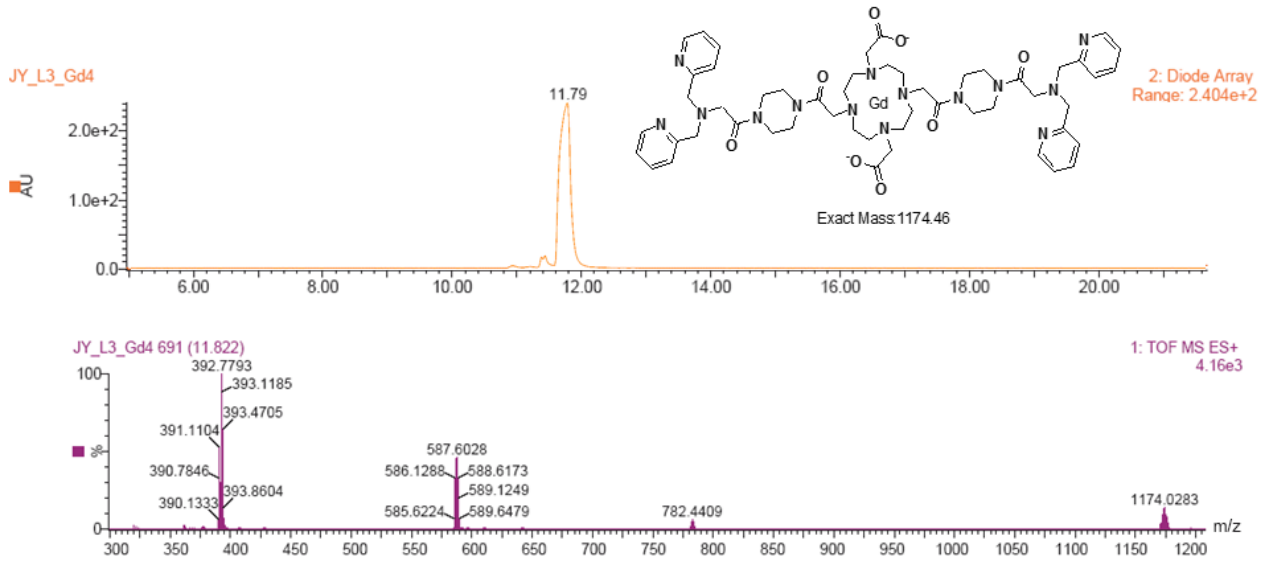


Figure S1.2. LC (top) and MS (below) spectra for Gd-**3**.

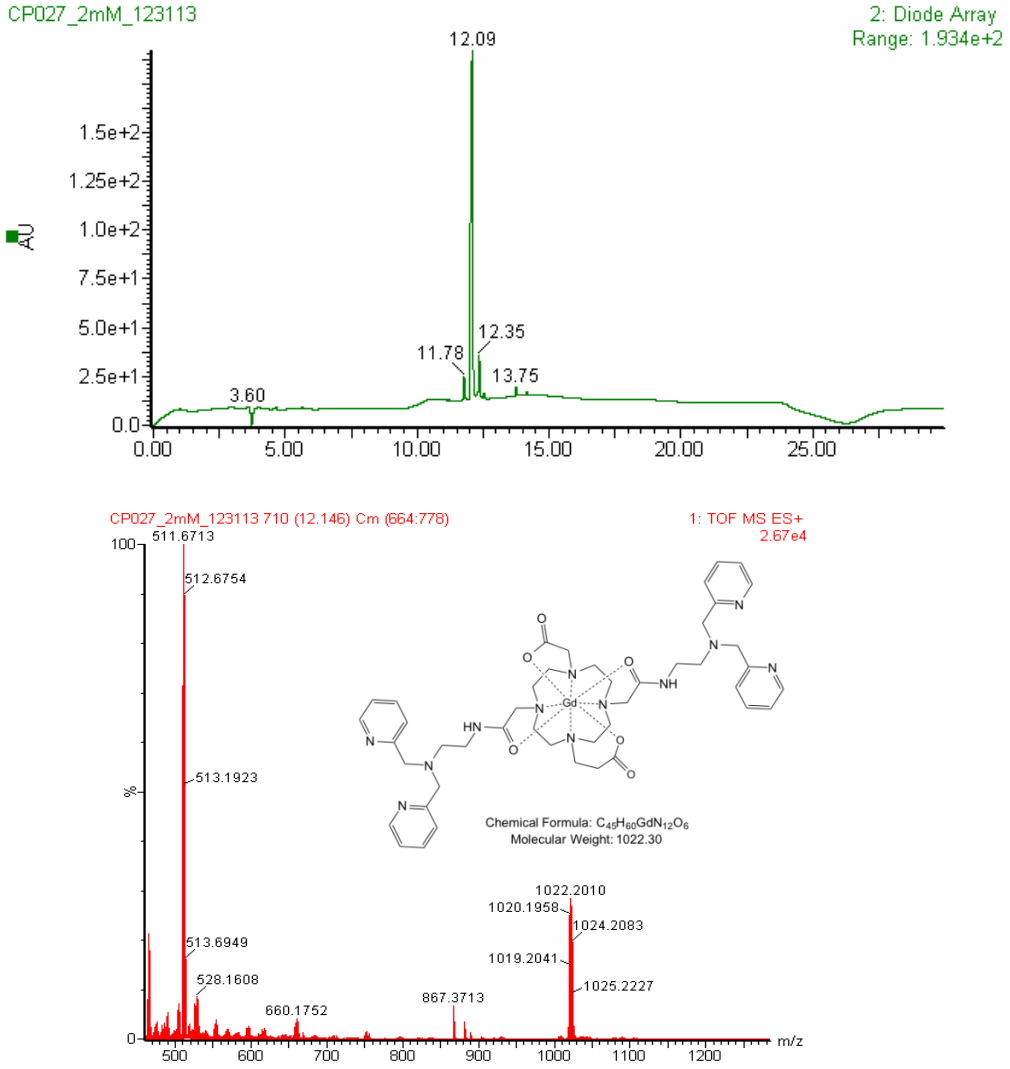


Figure S1.3. LC (top) and MS (below) spectra for Gd-**4**.

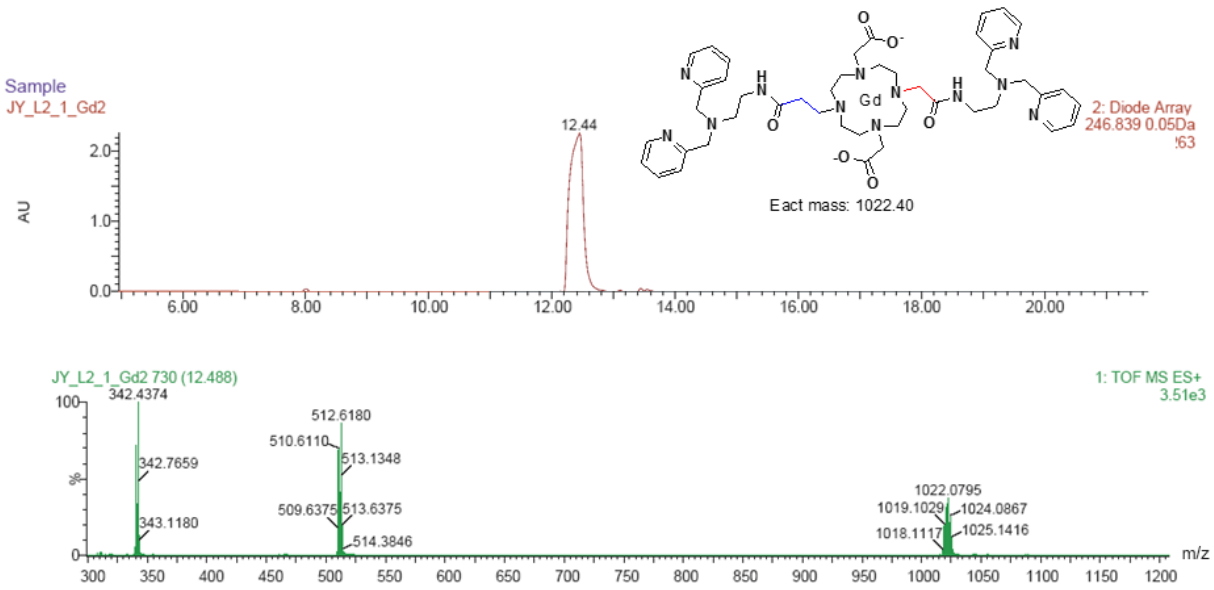


Figure S1.4. LC (top)-MS (below) spectra for Gd-**5**.

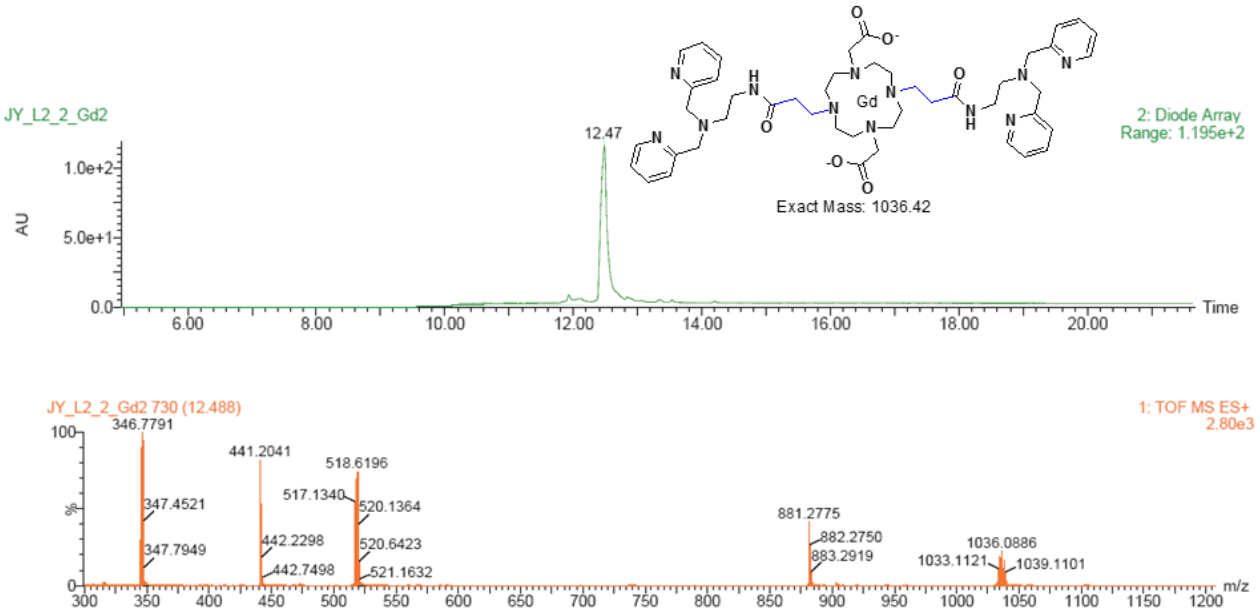


Figure S1.5. LC (top) and MS (below) spectra for Gd-**6**.

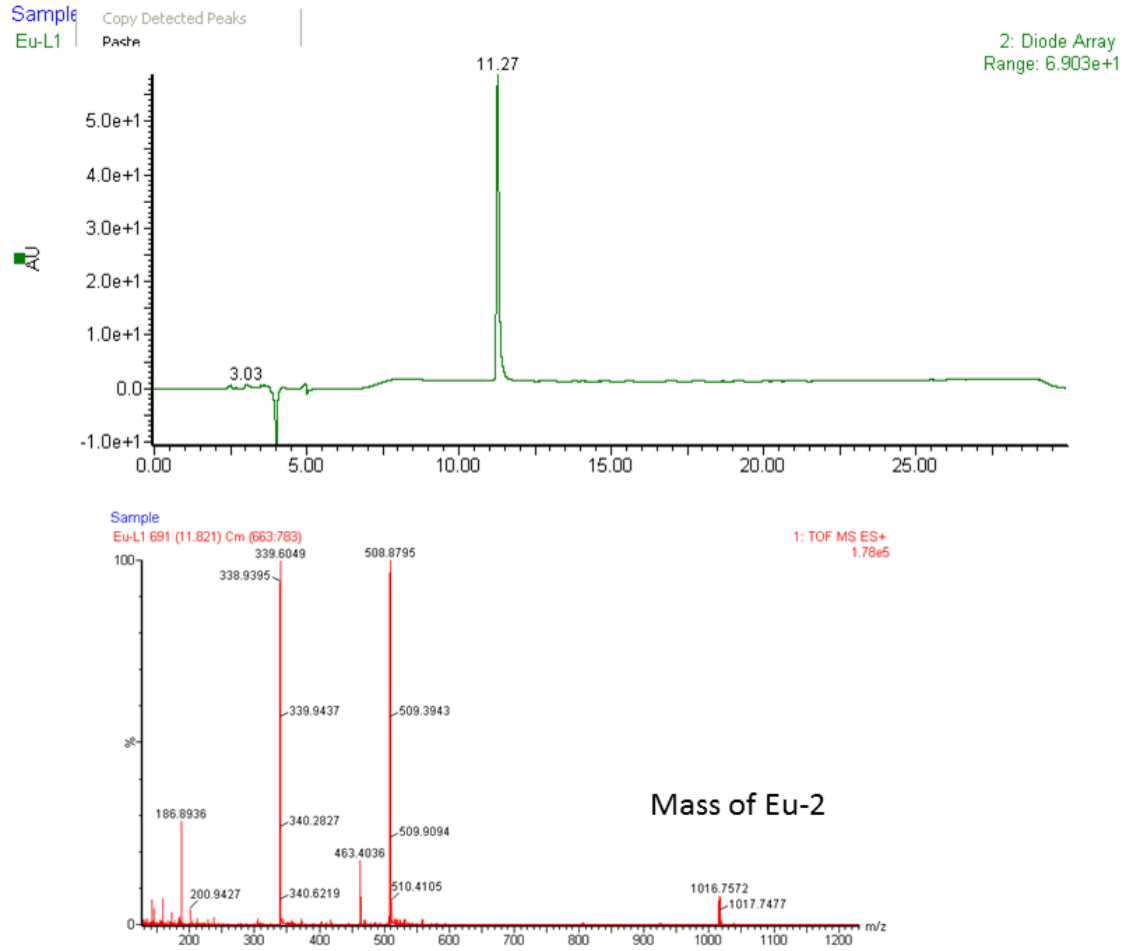


Figure S1.6. LC (top) and MS (below) spectra for Eu-**2**.

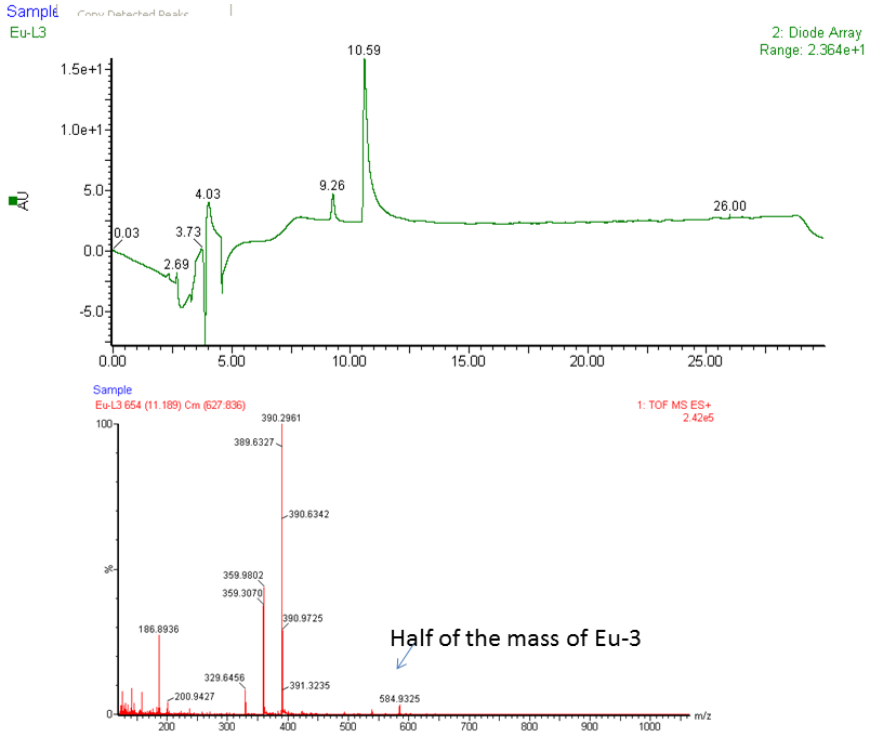


Figure S1.7. LC (top) and MS (below) spectra for Eu-**3**.

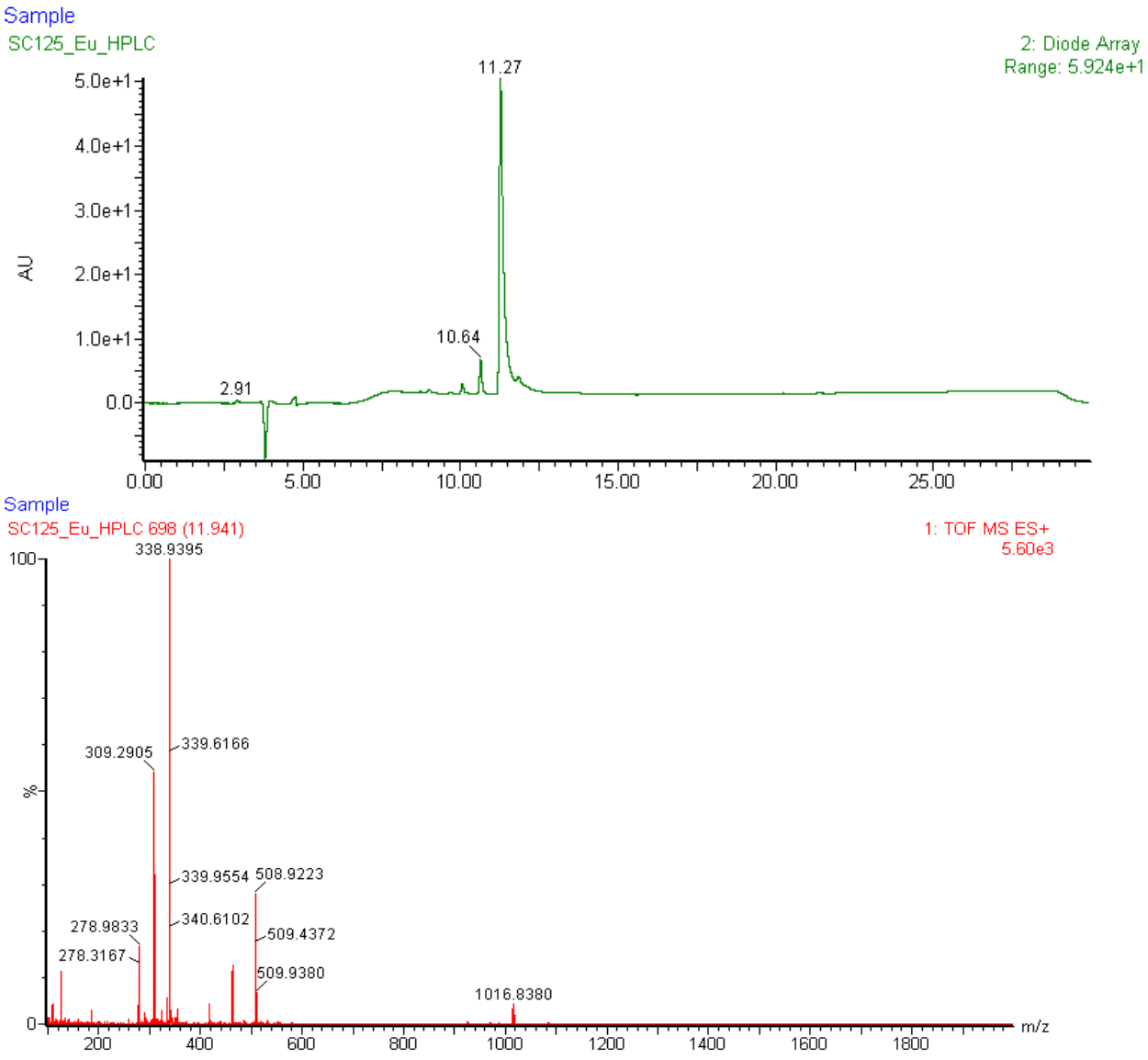


Figure S1.8. LC (top) and MS (below) spectra for Eu-**4**.

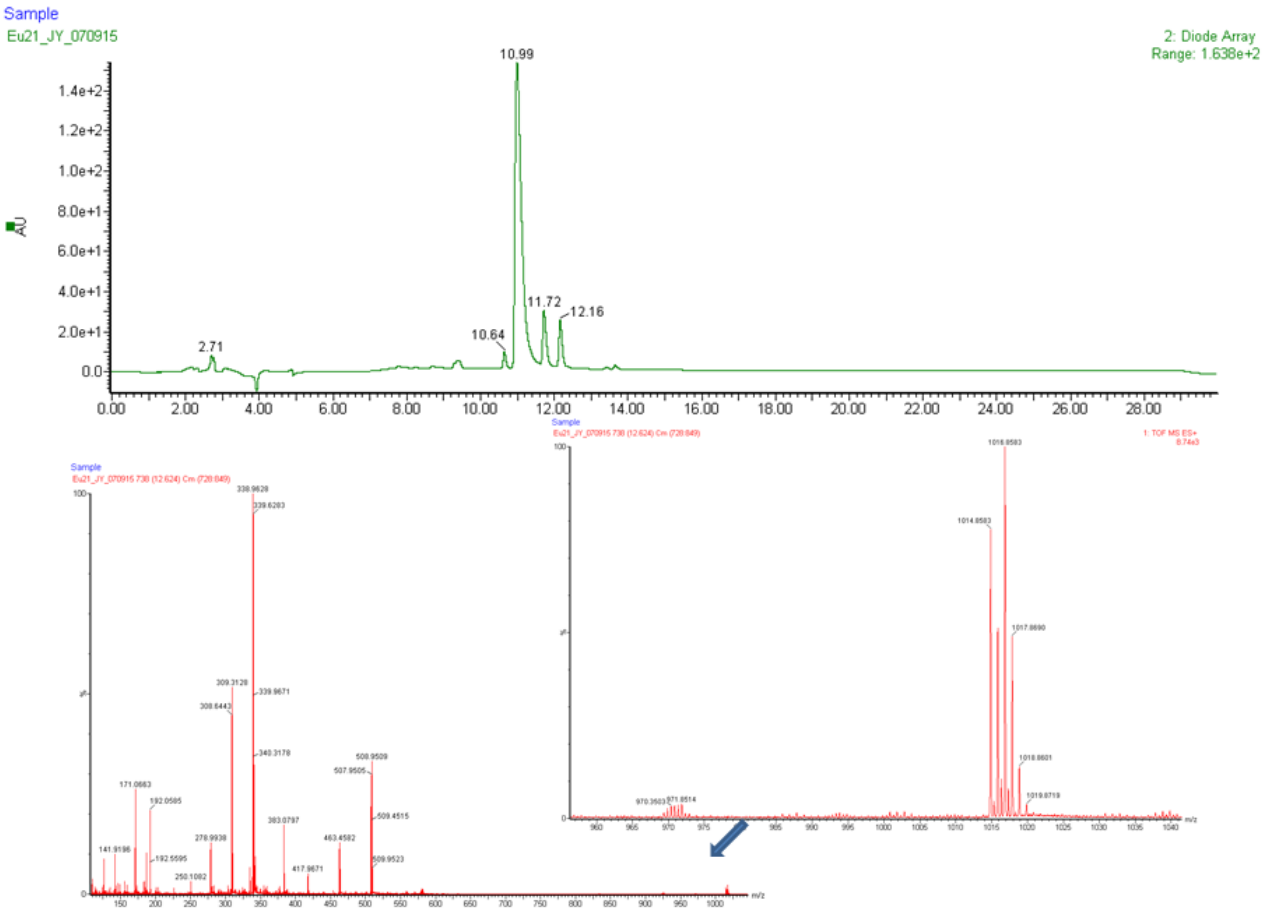


Figure S1.9. LC (top) and MS (below) spectra for Eu-**5**.

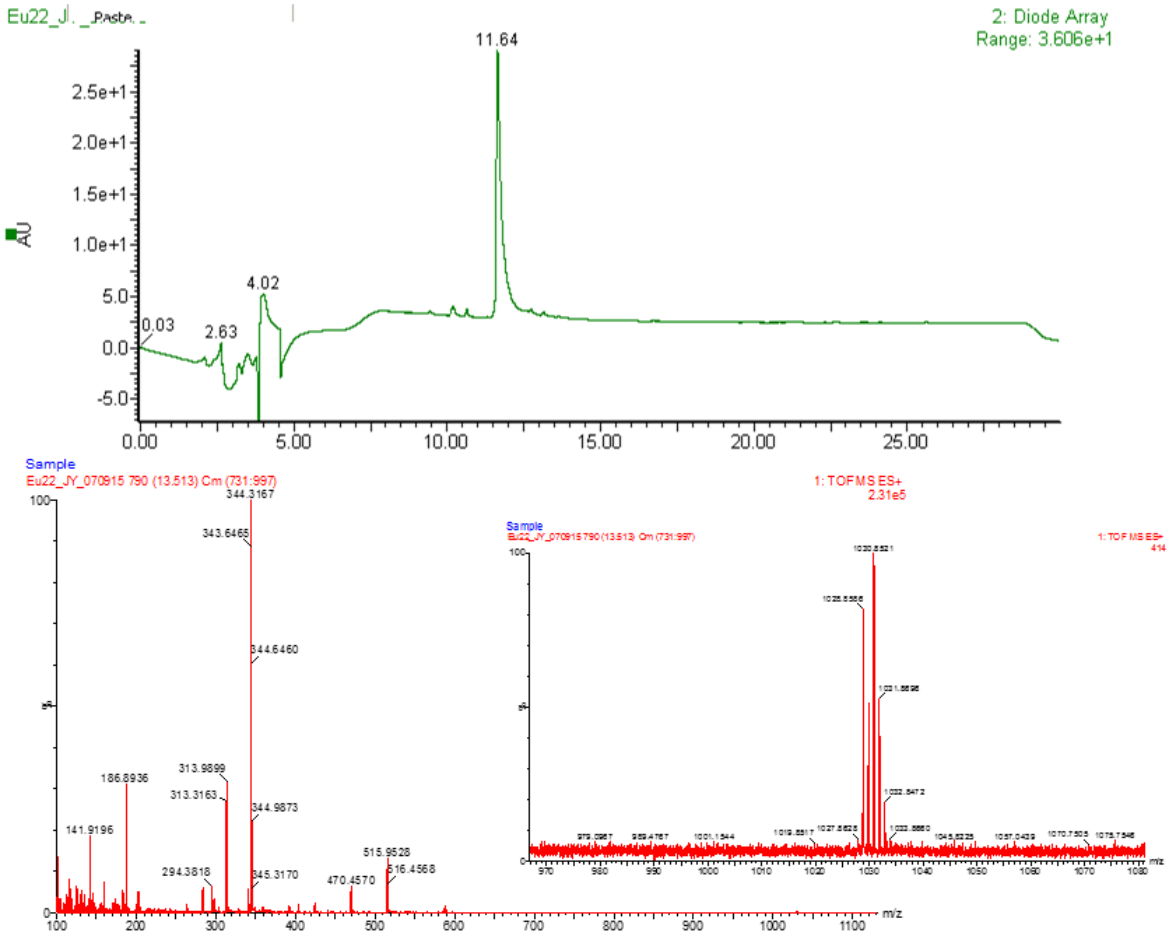


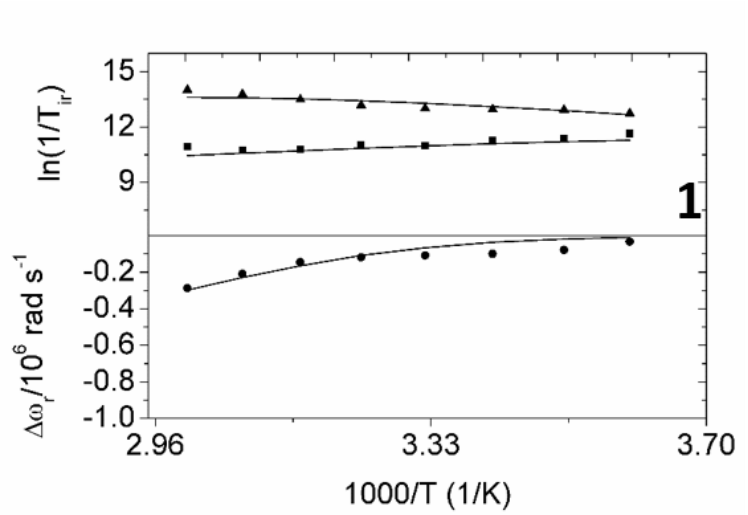
Figure S1.10. LC (top) and MS (below) spectra for Eu-**6**.

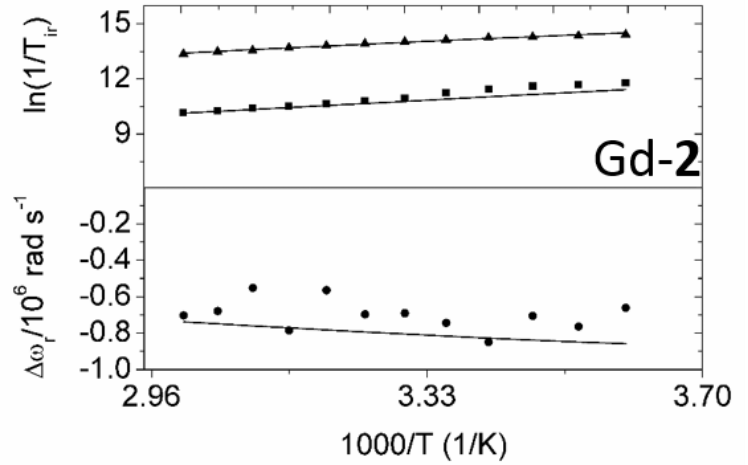
4. Relaxivity Measurements

Longitudinal relaxation times were measured using inversion recovery method on a MRS-6 NMR analyzer from the Institute Jožef Stefan (Ljubljana, Slovenija) operating at 20 MHz. Relaxivity was determined by linear regression analysis of the relaxation rates of five solutions (0 – 1.0 mM). All samples were measured at 310 K using a warm air blower. Five different sample concentrations (0, 0.25, 0.5, 0.75, 1.0 mM) of gadolinium complex were made up in Tris buffer at 0.1 M and pH 7.4 to generate the relaxivity value for the free complex. $ZnCl_2$ was then added to each of the above sample to produce a [Gd]:[Zn] ratio of 1:2. After 30 min of incubation at 310 K, T_1 measurements were performed, from which the relaxivity of sensor-Zn^{2+} was calculated. Finally, HSA was added to each of above sample to achieve HSA concentration of 0.6 mM. After further incubation at 310 K for 30 min, T_1 of each sensor-Zn^{2+}-HSA adduct was measured, from which the relaxivity of sensor-Zn^{2+}-HSA adduct was calculated.

5. ^{17}O NMR to Determine τ_M

^{17}O NMR experiments were performed at 9.4 T on a Bruker AVANCE III NMR spectrometer. The temperature was regulated by air flow controlled by a Bruker VT unit. The samples ([Gd^{3+}] = 25 mM) were prepared in ^{17}O enriched water (10%) with the pH being maintained at 7.4 with 100 mM Tris buffer. The sample was loaded into a 18 µL spherical bulb (Wilmad-Lab Glass, Vineland, NJ) and placed inside a 5 mm NMR tube containing 400 µL of water to eliminate any susceptibility effects. Longitudinal relaxation rates ($1/T_1$) were obtained by the inversion recovery method and transverse relaxation rates ($1/T_2$) were obtained by the Carr–Purcell–Meiboom–Gill spin echo technique. The acidified water (pH = 3.0) containing 10% enriched ^{17}O water was used as a reference for the measurements. The corresponding fittings were performed with the Scientist 3.0 software (Micromath®).


15
12
9
ln(1/T1r)
1
-0.2
-0.4
-0.6
-0.8
-1.0
Δωr/10^6 rad s^-1
2.96
3.33
3.70
1000/T (1/K)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Δωr/10^6 rad s^-1
2.96
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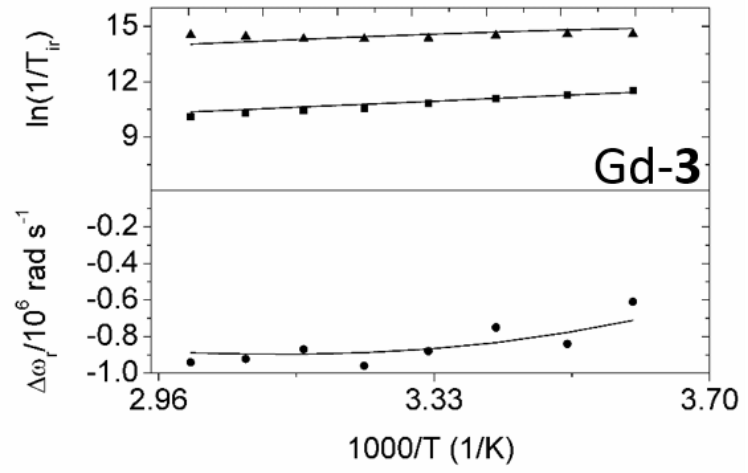
1000/T (1/K)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Gd-3
-0.2
-0.4
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-1.0
Δωr/10^6 rad s^-1
2.96
3.33
3.70
1000/T (1/K)

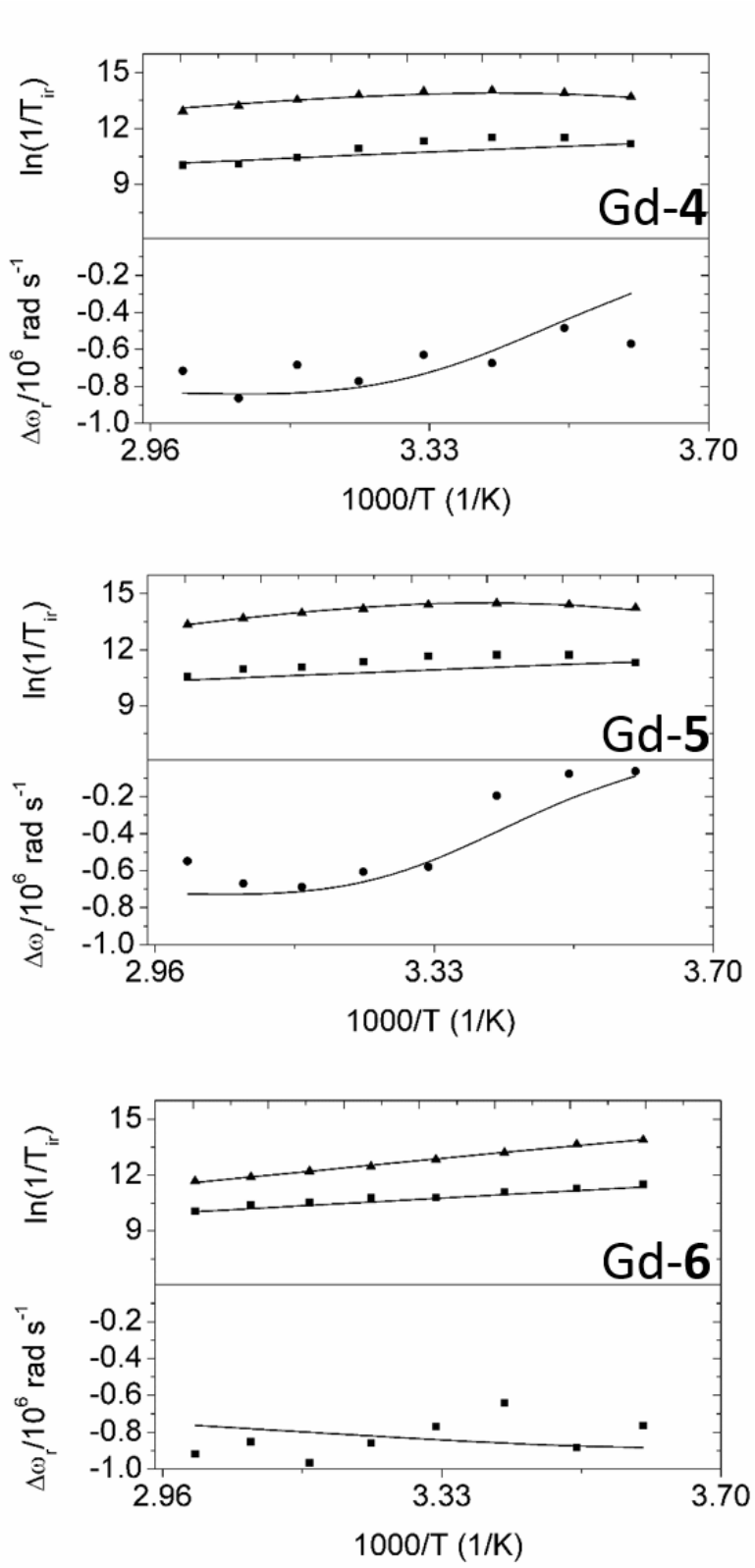

Figure s2. Temperature dependence of the reduced longitudinal (■) and transverse (▲) ^{17}O relaxation rates and reduced chemical shifts (•) of the Gd-complexes in aqueous solution (B_o = 9.4 T, [Gd^{3+}] = 25mM). The solid line represents the least-square fitting of the experimental data.

6. Determination of the Affinity Constants for the GdL Complexes binding t0 HSA

Affinity constants to HSA were assessed by proton relaxation enhancement (PRE) measurements according to published procedures.[3,4] The proton relaxation rates at increasing concentrations of protein were measured with a MRS-6 NMR analyzer (20 MHz, 310 K). For the E-titration, the concentrations of GdL complex (0.1 mM) and Zn(II) (0.2 mM) were kept constant, while the protein concentration was varied from 0 to 1 mM.

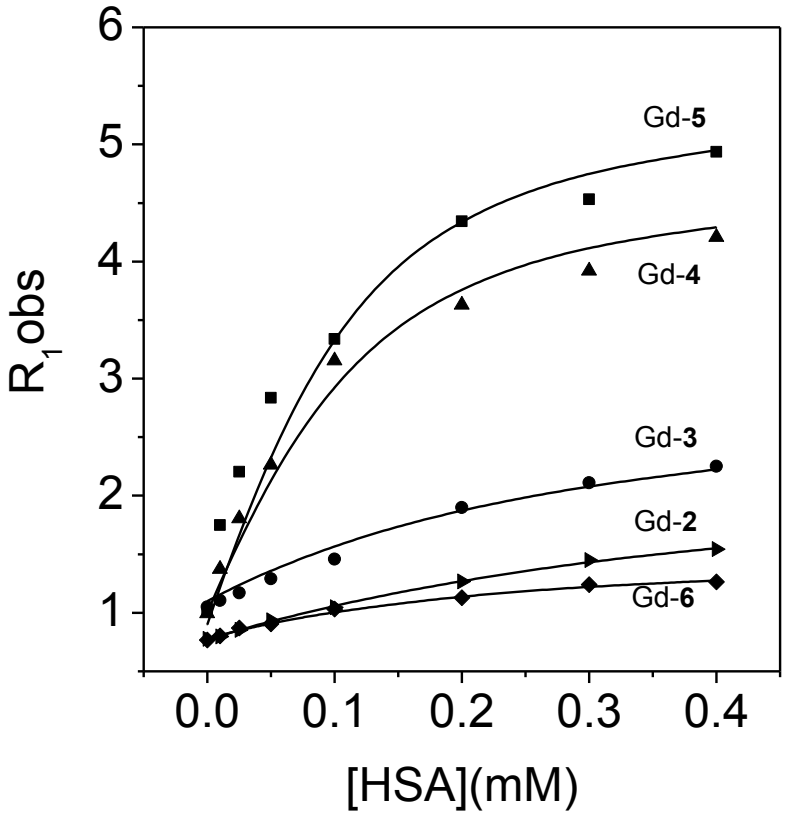

Figure s3. Proton relaxation enhancement of sensor-Zn(II) complexes with increasing concentration of HSA. All measurements were performed at 20 MHz and 310 K in 100 mM Tris buffer at pH 7.

7. Measurements to Determine Kinetic Inertness

Kinetic inertness was determined according to published procedures with minor modifications.[5,6] Stock solutions of phosphate buffer (67 mM, pH = 7.4), Gd(III) chelates (10 mM) and $ZnCl_2$ (231 mM) were prepared first. Calculated volumes of stock solutions or water were pipetted into small glass vials to obtain a test solution of 300 µL with 30 mM phosphate, 1.5 mM Gd(III) chelate, and 6.0 mM Zn(II). Samples were measured 15 minutes after the sample temperature stabilized at 310 K. During the entire experiment, the samples inside the NMR tubes were kept in an incubator at 310 K. The T_1 of the solutions was determined at 310 K and 0.47 T (20 MHz) at various time points within 7 days. As a reference, GdDTPA was analyzed under the same conditions. The R_1^P relaxation rate is obtained by subtracting the diamagnetic contribution of the water relaxation (0.28 s^{-1}) from the observed relaxation rate $R_1=(1/T_1)$.

8. Analysis of ^{17}O NMR Data

^{17}O NMR data have been analysed within the framework of Solomon-Bloembergen-Morgan theory.

^{17}O NMR spectroscopy

From the measured ^{17}O NMR relaxation rates and angular frequencies of the paramagnetic solutions, $1/T_1$, $1/T_2$ and ω, and of the acidified water reference, $1/T_{1A}$, $1/T_{2A}$ and ω_A, one can calculate the reduced relaxation rates and chemical shifts, $1/T_{2r}$ and $\Delta\omega_r$, which may be written in Equations (A1)-(A3), where, $1/T_{1m}$, $1/T_{2m}$ is the relaxation rate of the bound water and $\Delta\omega_m$ is the chemi-

cal shift difference between bound and bulk water molecules, τ_m is the mean residence time or the inverse of the water exchange rate k_{ex} and P_m is the mole fraction of the bound water. [7,8]

$$\frac{1}{T_{1r}} = \frac{1}{P_m}\left[\frac{1}{T_1} - \frac{1}{T_{1A}}\right] = \frac{1}{T_{1m} + \tau_m} + \frac{1}{T_{1os}} \quad \text{(A1)}$$

$$\frac{1}{T_{2r}} = \frac{1}{P_m}\left[\frac{1}{T_2} - \frac{1}{T_{2A}}\right] = \frac{1}{\tau_m}\frac{T_{2m}^{-2} + \tau_m^{-1}T_{2m^{-1}} + \Delta\omega_m^2}{(\tau_m^{-1} + T_{2m}^{-1})^2 + \Delta\omega_m^2} + \frac{1}{T_{2os}} \quad \text{(A2)}$$

$$\Delta\omega_r = \frac{1}{P_m}(\omega - \omega_A) = \frac{\Delta\omega_m}{(1 + \tau_m T_{2m}^{-1})^2 + \tau_m^2 \Delta\omega_m^2} + \Delta\omega_{os} \quad \text{(A3)}$$

The outer sphere contributions to the ^{17}O relaxation rates $1/T_{1OS}$ and $1/T_{2OS}$ are being neglected according to previous studies.[9] Therefore, Equations (A1-A2) can be further simplified to Equations (A4) and (A5):

$$\frac{1}{T_{1r}} = \frac{1}{T_{1m} + \tau_m} \quad \text{(A4)}$$

$$\frac{1}{T_{2r}} = \frac{1}{T_{2m} + \tau_m} \quad \text{(A5)}$$

The exchange rate is supposed to obey the Eyring Equation. In Equation (A6) $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are the entropy and enthalpy of activation for the water exchange process, and k_{ex}^{298} is the exchange rate at 298.15 K.

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_B T}{h}\exp\left\{\frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{RT}\right\} = \frac{k_{ex}^{298} T}{298.15}\exp\left\{\frac{\Delta H^{\ddagger}}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right\} \quad \text{(A6)}$$

In the transverse relaxation, the scalar contribution, $1/T_{2sc}$, is the most relevant [Equation (A7)]. $1/\tau_{s1}$ is the sum of the exchange rate constant and the electron spin relaxation rate [Equation (A8)].

$$\frac{1}{T_{2m}} \cong \frac{1}{T_{2sc}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left(\tau_{s1} + \frac{\tau_{s2}}{1 + \omega_S^2\tau_{s2}^2}\right) \quad \text{(A7)}$$

$$\frac{1}{\tau_{s1}} = \frac{1}{\tau_m} + \frac{1}{T_{1e}} \quad \text{(A8)}$$

The ^{17}O longitudinal relaxation rates in Gd^{3+} solutions are the sum of the contributions of the dipole-dipole (*dd*) and quadrupolar (*q*) mechanisms as expressed by Equations (A11-A13) for non-extreme narrowing conditions, where γ_S is the electron and γ_I is the nuclear gyromagnetic ratio ($\gamma_S = 1.76 \times 10^{11}$ rad s^{-1} T^{-1}, $\gamma_I = -3.626 \times 10^7$ rad s^{-1} T^{-1}), r_{GdO} is the effective distance between the electron charge and the ^{17}O nucleus, I is the nuclear spin (5/2 for ^{17}O), χ is the quadrupolar coupling constant and η is an asymmetry parameter :

$$\frac{1}{T_{1m}} = \frac{1}{T_{1dd}} + \frac{1}{T_{1q}} \quad \text{(A9)}$$

with:

$$\frac{1}{T_{1dd}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2\gamma_I^2\gamma_S^2}{r_{GdO}^6} S(S+1) \times \left[3J(\omega_I; \tau_{d1}) + 7J(\omega_S; \tau_{d2})\right] \quad \text{(A10)}$$

$$\frac{1}{T_{1q}} = \frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}\chi^2(1 + \eta^2/3) \times \left[0.2J_1(\omega_I) + 0.8J_2(\omega_I)\right] \quad \text{(A11)}$$

In Equation (A3) the chemical shift of the bound water molecule, $\Delta\omega_m$, depends on the hyperfine interaction between the Gd^{3+} electron spin and the ^{17}O nucleus and is directly proportional to the scalar coupling constant, $\frac{A}{\hbar}$, as expressed in Equation (A12).[10]

$$\Delta\omega_m = \frac{g_L\mu_B S(S+1)B}{3k_B T}\frac{A}{\hbar} \quad \text{(A12)}$$

The isotopic Landé g factor is equal to 2.0 for the Gd^{3+}, B represents the magnetic field, and k_B is the Boltzmann constant.

The outer-sphere contribution to the chemical shift is assumed to be linearly related to $\Delta\omega_m$ by a constant C_{os} [Equation (A13)]. [11]

$$\Delta\omega_{os}=C_{os}\Delta\omega_m \qquad \text{(A13)}$$

Analysis Details

In the the ^{17}O NMR data fitting for the Gd^{3+} complexes, r_{GdO} has been fixed to 2.50 Å, based on available crystal structures and recent electron spin-echo envelope modulation (ESEEM) results [12]. The quadrupolar coupling constant, $\chi\,(1+\eta^2/3)^{1/2}$, has been set to the value for pure water, 7.58 MHzo The following parameters have been adjusted: the water exchange rate, k_{ex}^{298}, the activation enthalpy for water exchange, $\Delta H^{\ddagger}$, the scalar coupling constant, $A/\hbar$, the rotational correlation time (τ_R^{298}) and its activation energy, E_R. The parameters characterizing the electron spin relaxation, such as the correlation time for the modulation of the zero-field-splitting, τ_V^{298}, and its activation energy, E_v, and the mean-square zero-field-splitting energy, Δ^2 were fixed to 11 ps, 1 kJ mol^{-1} and 0.16 × 10^{20} s^{-1}, respectively, for simpler analogy as reported for various Gd-DOTA derivatives.[13] The empirical constant describing the outer sphere contribution to the ^{17}O chemical shift, C_{os}, was also fitted for complexes **3** (0.18) and **4** (0.15) otherwise small $A/\hbar$ values were obtained. C_{os} was fixed to 0 for the remaining complexes.[13] Inner sphere water molecules were adjusted to the values calculated by ^{17}O NMR for each complex.[14]

Table S1. Best-fit parameters obtained for [Gd(sensors)(H2O)] from the analysis of ^{17}O NMR data.

Parameters	GdDOTA-diBPEN **1**	Gd-**2**	Gd-**3**	Gd-**4**	Gd-**5**	Gd-**6**	$[GdDOTA(H_2O)]^-$
k_{ex}^{298} [10^6 s^{-1}]	0.7±0.1	222.2±2.3	114.4±1.6	5.3±0.2	7.8±0.1	269.4±3.2	4.1
τ_m^{298} [ns]	1362.0±97.2	4.5±0.1	8.7±0.1	188.7.0±7.1	128.2±1.6	3.7±0.1	243
$\Delta H^{\ddagger}$ [kJ/mol]	38.0±5.0	15.3±1.0	15.1±1.5	47.0±6.0	49.7±1.2	30.8±1.7	49.8
E_R [kJ/mol]	15.0	18.2±0.2	17.4±0.2	17.2±0.4	14.1±0.2	18.1±0.3	16.1
τ_{RO}^{298}[ps]	375±11	320±11	350±12	287±13	340±13	301±13	77
E_V [kJ/mol]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
τ_V^{298} [ps]	11	11	11	11	11	14±0.2	11
Δ^2 [10^{20} s^{-2}]	0.16	0.16	0.16	0.16	0.16	0.67±0.2	0.16
A/ħ [MHz/10^{-6}]	-3.7	-3.8	-4.0±0.4	-3.7	-3.7	-3.8	-3.7

Table S2. r_1 relaxivities ($mM^{-1}s^{-1}$) measured at 310 and 323 K at 0.47 T

Relaxivity ($mM^{-1}s^{-1}$) at 310 and 323 K	GdDOTA-diBPEN 1		Gd-**2**		Gd-**3**		Gd-**4**		Gd-**5**		Gd-**6**	
	310 K	323 K	310 K	323 K	310 K	323 K	310 K	323 K	310 K	323 K	310 K	323 K
$r_{1\ sensor}$	5.0±0.1	4.7±0.1	3.7±0.1	1.2±0.1	7.6±0.3	5.3±0.2	6.4±0.2	6.6±0.2	6.2±0.2	6.8±0.2	4.1± 0.1	3.0±0.1
$r_{1\ sensor+2Zn(II)+HSA}$	17.4±0.5	23.6±0.2	20.8±0.5	5.4±0.1	27.9±0.8	14.4±0.3	47.6±1.2	51.2±0.8	50.1±1.2	54.8±1.0	15.6±0.6	3.3±0.1

Table S3. r_1 relaxivities ($mM^{-1}s^{-1}$) measured at 0.47, 1.5 and 9.4 T at 310 K.

Relaxivity ($mM^{-1}s^{-1}$) at 0.47, 1.5, and 9.4T	GdDOTA-diBPEN 1			Gd-**4**			Gd-**5**		
	0.5T	1.5T	9.4T	0.5T	1.5T	9.4T	0.5T	1.5T	9.4T
$r_{1\ sensor}$	5.0 ± 0.1	4.9 ± 0.1	4.0	6.4 ± 0.2	5.9 ± 0.1	5.6 ± 0.1	6.2 ± 0.2	6.0 ± 0.1	5.8 ± 0.1
$r_{1\ sensor+2Zn(II)+HSA}$	17.4±0.5	15.1 ± 0.4	6.6	47.6±1.2	31.4 ± 0.4	9.4 ± 0.2	50.1±1.2	34.2 ± 0.3	12.0 ± 0.2
relaxivity enhancement ($r_{1\ sensor+2Zn(II)+HSA}$ / $r_{1\ sensor}$)	348	308	160	743	532	168	808	589	206

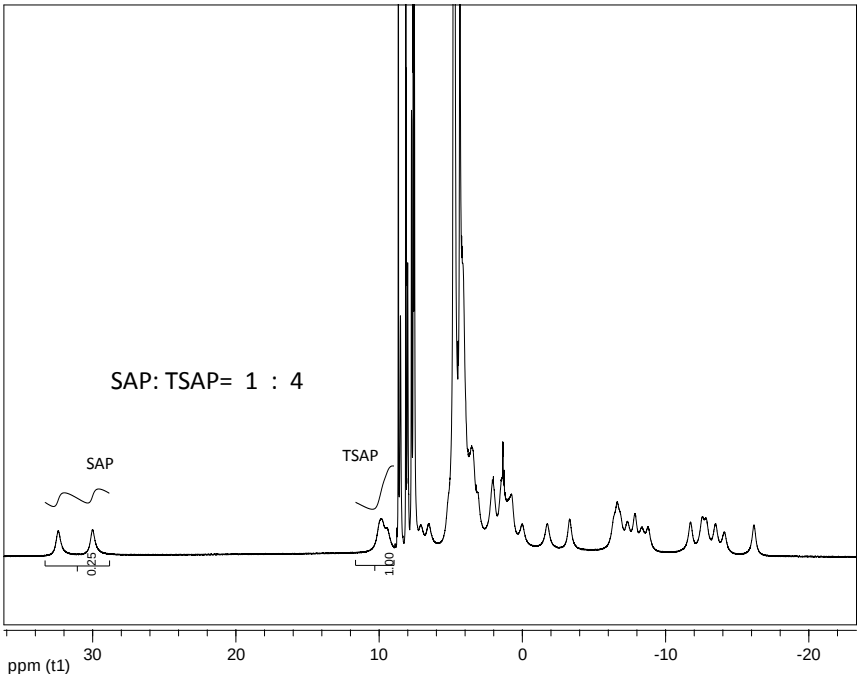

Figure S4.1. ^{1}H NMR of Eu-**3** in D_2O at 400 MHz showing square antiprismatic (SAP) and twisted square antiprismatic (TSAP) in an approximate 1:4 ratio.

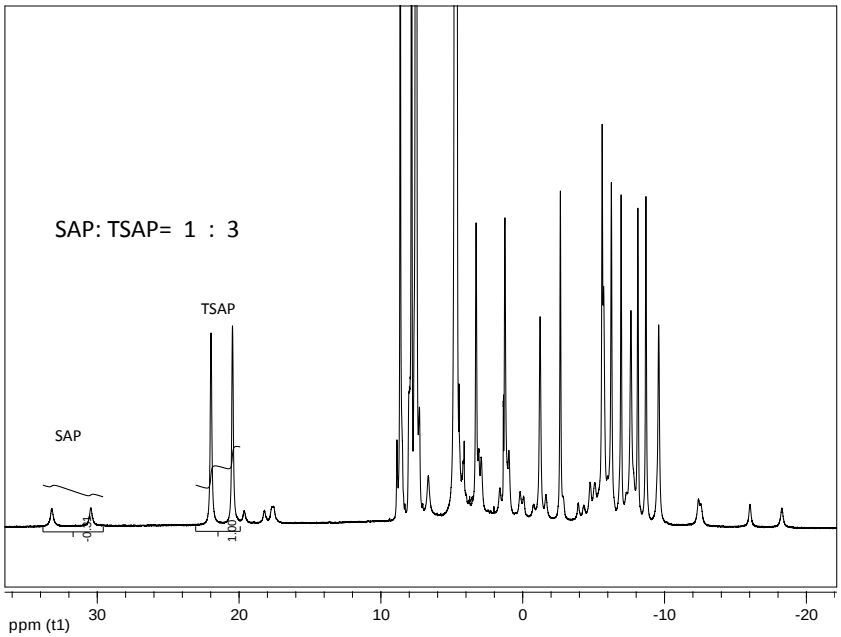

Figure S4.2. ^{1}H NMR of Eu-**2** in D_2O at 400 MHz showing square antiprismatic (SAP) and twisted square antiprismatic (TSAP) in an approximate 1:3 ratio.

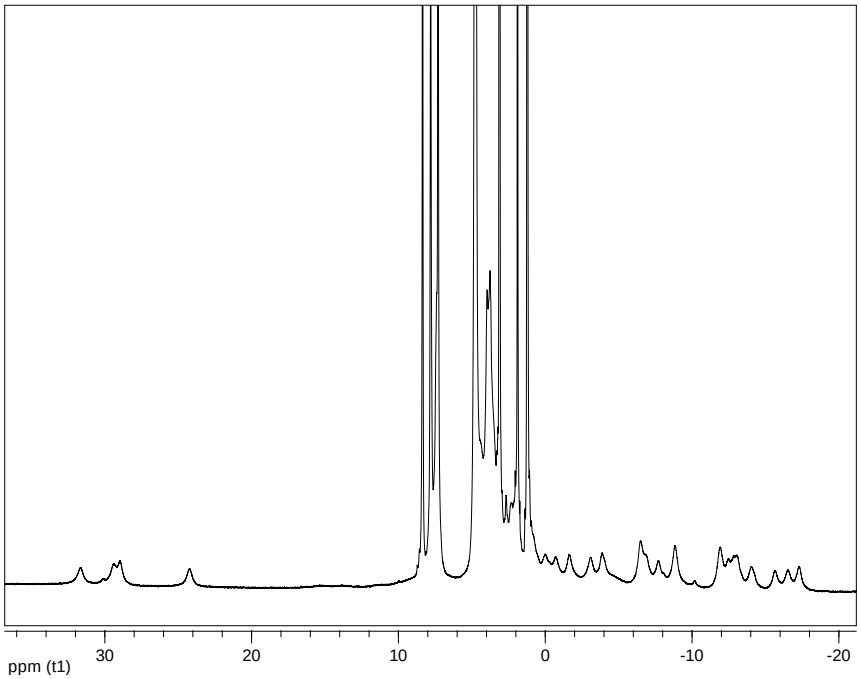

Figure S4.3. ^{1}H NMR of Eu-**4** in D_2O at 400 MHz showing TSAP/SAP less than 5%.

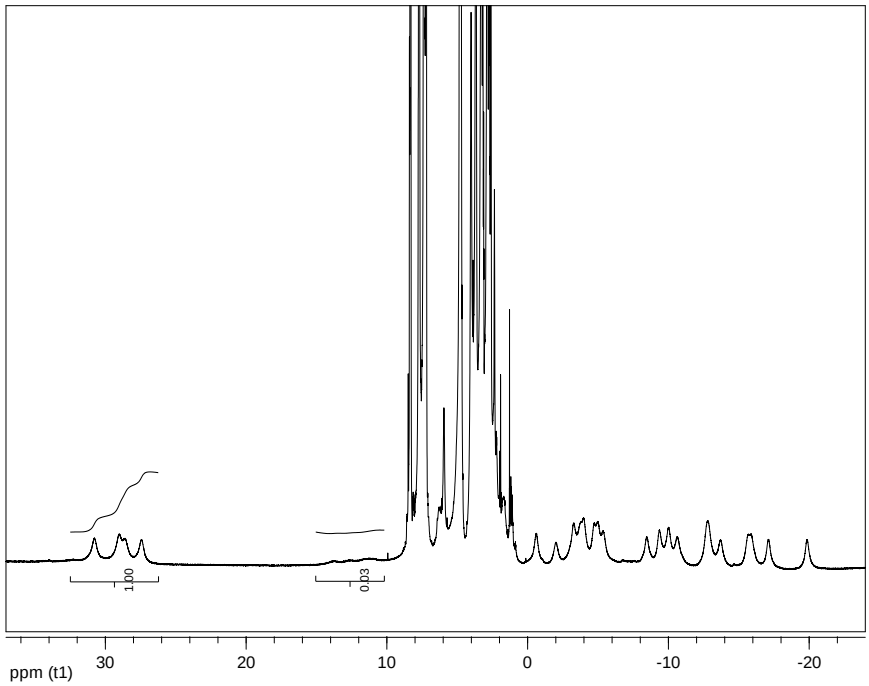

Figure S4.4. ^{1}H NMR of Eu-**5** in D_2O at 400 MHz showing TSAP/SAP less than 5%.

9. *In vivo* Imaging of the Pancreas in Mice

All animal experiments were performed in accordance with guidelines set by the UT Southwestern Institutional Animal Care and Use Committee (IACUC). Male C57bl/6 mice were fasted for at least 12 hours before imaging experiments.The animals were anaesthetized with isofluorane and catheterized via the tail vein. Once the animals were secured inside a 38 mm volume coil, the pancreas was positioned in the center of the 9.4 T Varian MRI scanner. Two 3D T1-weighted gradient echo pre-injection scans were obtained (TE/TR = 1.69/3.34 ms, NEX8, Matrix = 128 × 128 × 128). The catheter was connected to a syringe pump and 25 mM of contrast agent were injected at a rate of 5 µl/min for 30 minutes. Using a constant enhancement of the kidneys as an indicator of overall contrast agent distribution, 50 µl of 20% w/v D-glucose were injected intraperitoneally and consecutive 3D T1-weighted scans were obtained sequentially to monitor signal enhancement in the pancreas. Identification of the pancreas was accomplished by locating the tissues surrounded by the spleen, stomach, and liver. A total of 5 animals were scanned per group and images were quantified and analyzed using ImageJ (National Institutes of Health, Bethesda, MD). The signal intensities from ROIs of the tip and head of the pancreas were measured separately and averaged before, at 11, 15, and 19 minutes post D-glucose injection. The values were normalized to the signal intensity obtained from a water phantom placed on the mouse abdomen. Contrast enhancement was calculated using the formula $\left(\frac{S_{post-injection}}{S_{pre-injection}} - 1\right) \times 100\%$. Statistical analysis was performed by comparing the mean values using a two-tailed *t*-test. Statistical difference was evaluated using the *t*-statistic and p-values at a 95% confidence level.

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