Substituent Effects on Gd(III)-based MRI Contrast Agents – Optimizing the Stability and Selectivity of the Complex and the Number of Coordinated Water Molecules

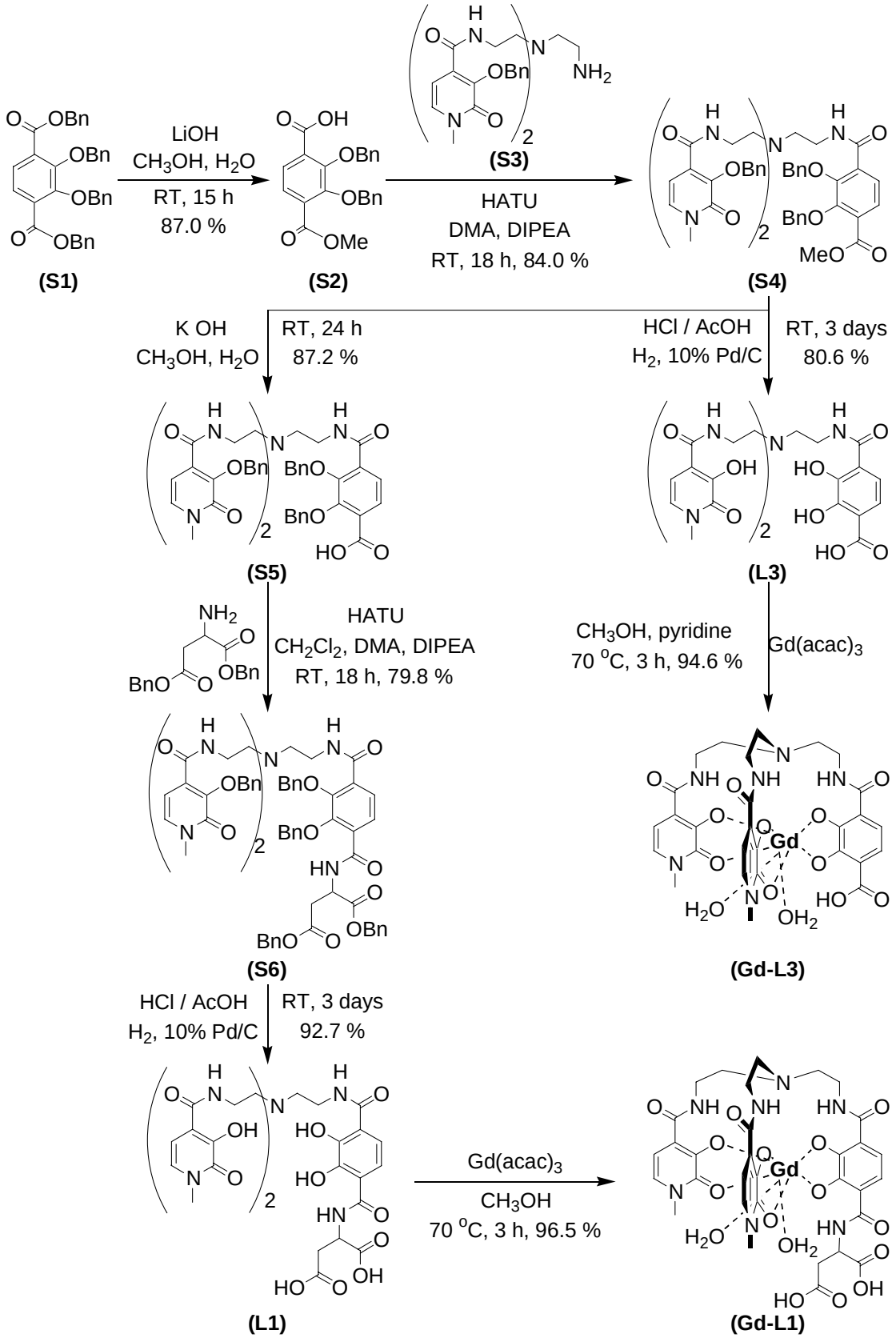
Valérie C Pierre[†], *Mauro Botta*[‡], *Silvio Aime*[§], *Kenneth N. Raymond*[†*]

[†]Department of Chemistry, University of California, Berkeley, California 94720-1460; [‡]Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "A. Avogadro", Via Bellini 25/G, I-15100 Alessandria, Italy and [§]Dipartimento di Chimica I.F.M., Università di Torino, Via P. Giuria 7, I-10125 Torino, Italy.

raymond@socrates.berkeley.edu

Supporting Information

General Considerations. Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. All solvents were dried over activated alumina and stored over 4 Å molecular sieves. Water was distilled and further purified by a Millipore cartridge system (resistivity 18 x 10^6 Ω). All organic extracts were dried over anhydrous $MgSO_4$ and solvents were removed with a rotary evaporator. Flash chromatography was performed on Merck Silica Gel (40-7 Mesh). ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 at 500 MHz and 125 MHz or a Bruker AVB 400 at 400 MHz and 100 MHz, respectively; the residual solvent peak was used as an internal reference. Elemental analysis and mass spectra (LR = low resolution; HR = high resolution; FAB MS = fast atom bombardment mass spectrometry; EI MS = electron ionization mass spectrometry; ES MS = electrospray mass spectrometry) were performed by the Microanalytical Laboratory and Mass Spectrometry Laboratory, respectively, at the College of Chemistry at the University of California at Berkeley. Microanalytical analysis of all complexes including the analysis of metal content were performed by Desert Analytics, Tucson, Arizona. Matrix Assisted Laser Desorption Ionization mass spectra (MALDI-MS) were recorded on an Applied Biosystems Voyager System 6322.



Scheme S 1. Synthesis of **Gd-L1** and **Gd-L3**.

TAM-Bn_2-CO_2Me (S2). TAM-Bn_4 **(S1)** (11.5 g, 20.5 mmol) and LiOH (0.492 g, 20.5 mmol) were dissolved in methanol (800 mL) and distilled water (100 mL). The solution was stirred at room temperature for 15 h. The solvents were then evaporated *in vacuo* and the crude product was purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 86 % CH_2Cl_2 / 14 % CH_3OH. The mono ester protected TAM was obtained as a white powder (7.02 g, 87.0 %). ^{1}H NMR (CDCl3) δ = 3.91 (s, 3H, O-C*H*$_3$), 5.16 (s, 2H, Ar-O-C*H*$_2$-Ar), 5.30 (s, 2H, Ar-O-C*H*$_2$-Ar), 7.33-7.49 (m, 10H, Ar-*H*), 7.65 (d, 1H, TAM Ar-*H*, *J* = 8), 7.91 (d, 1H, TAM Ar-*H*, *J* = 8); ^{13}C NMR (CDCl3) δ = 52.6, 76.4, 76.8, 126.5, 126.6, 127.3, 128.6, 128.7, 128.9, 129.4, 129.5, 131.6, 134.2, 136.0, 151.6, 151.9, 165.2; LR FAB-MS m/z = 393 (MH+), (Calcd. 393); Anal. Found (Calcd.) for **(S2)** C 70.22 (70.40), H 5.28 (5.14).

TREN-bis(HOPO-Bn)-TAM-Bn_2-CO_2Me (S4). *O*-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 2.20 g, 5.79 mmol) and TAM-Bn_2-CO_2Me[1] **(S2)** (2.11 g, 5.38 mmol) were dissolved in anhydrous dimethylacetamide (50 mL) and diisopropylethylamine (20 mL). The solution was stirred at room temperature for 30 min. A solution of TREN-bis(HOPO-Bn)[2] **(S3)** (3.64 g, 5.79 mmol) dissolved in anhydrous dimethylacetamide (20 mL) and diisopropylethylamine (20 mL) was then added to the TAM solution. The reaction mixture was stirred at room temperature for 18 h. The solvents were then removed under reduced pressure and the crude product was purified by flash chromatography eluting with a gradient of 100 % CH_2Cl_2 to 93 % CH_2Cl_2 / 7 % CH_3OH. The protected ligand **(S4)** was obtained as a white foam (4.14 g, 84.0 %) that was further dried under high vacuum at room temperature for 18 h. ^{1}H NMR ($CDCl_3$) δ = 2.26 (b, 6H, N-C*H*$_2$-CH_2-NH-C(O)-HOPO and N-C*H*$_2$-CH_2-NH-C(O)-TAM), 3.28 (b, 6H, N-CH_2-C*H*$_2$-NH-C(O)-HOPO and N-CH_2-C*H*$_2$-NH-C(O)-TAM), 3.56 (s, 6H, ArN-C*H*$_3$), 3.83 (s, 3H, C(O)-O-C*H*$_3$), 5.06 (s, 2H, O-C*H*$_2$-ArTAM), 5.08 (s, 2H, O-C*H*$_2$-ArTAM), 5.27 (s, 4H, O-C*H*$_2$-ArHOPO), 6.58 (d, 2H, HOPO Ar-*H*, *J* = 8), 7.06 (d, 2H, HOPO Ar-*H*, *J* = 8), 7.24 – 7.43 (m, 20H, Ar-*H*), 7.52 (d, 1H, TAM Ar-*H*, *J* = 8), 7.69 (b, 2H, TAM Ar-*H* and CH_2-N(*H*)-TAM, *J* = 5), 7.81 (t, 2H, CH_2-N(*H*)-HOPO, *J* = 5); ^{13}C NMR ($CDCl_3$) δ = 37.1, 37.4, 37.9, 43.1, 51.6, 52.2, 53.5, 55.2, 67.0, 74.1, 104.1, 125.1, 125.6, 126.4, 127.9, 128.2, 128.3,128.4, 128.6, 130.4, 132.2, 135.2, 135.7, 136.0, 136.3, 145.8, 150.9, 151.6, 159.1, 163.1, 164.4, 164.8; LR FAB-MS m/z = 851 (MH^+), (Calcd. 851); HR-FAB for $C_{45}H_{50}N_6O_{11}$ = 851.3630 (MH^+) (Calcd. 851.3616); Anal. Found (Calcd.). C 63.18 (63.52), H 6.30 (5.92), N 9.77 (9.88).

TREN-bis(HOPO-Bn)-TAM-Bn_2-CO_2H (S5). TREN-bis(HOPO-Bn)-TAM-Bn_2-CO_2Bn **(S4)** (3.01 g 2.78 mmol) was dissolved in methanol (50 mL) and 1 M $KOH_{(aq)}$ (20 mL). The solution was stirred at room temperature for 24 h. The solvents were then removed under reduced pressure and the crude product was partitioned between methylene chloride (150 mL) and 1M $HCl_{(aq)}$ (75 mL). The organic phase was further washed with 1M $HCl_{(aq)}$ (1 × 75 mL) and brine (1 × 75 mL) then dried with anhydrous magnesium sulfate. Removal of the solvent under reduced pressure yielded the

[1] TAM-Bn_2-CO_2Me was synthesized as previously reported, see Doble, D. M. J.; Botta, M.; Wang, J.; Aime, S.; Barge, A.; Raymond, K. N. *J. Am. Chem. Soc.* **2001,** *123*, 10758-10759.

[2] TREN-bis(HOPO-Bn) was synthesized as previously reported, see Cohen, S. M.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2000,** *39*, 4339-4346.

free acid **(S5)** as an off-white foam (2.40 g, 87.2 %). ^{1}H NMR ($CDCl_3$) δ = 2.33 (b, 6H, N-C*H*$_2$-CH$_2$-NH-C(O)-HOPO and N-C*H*$_2$-CH$_2$-NH-C(O)-TAM), 3.12 (b, 6H, N-CH$_2$-C*H*$_2$-NH-C(O)-HOPO and N-CH$_2$-C*H*$_2$-NH-C(O)-TAM), 3.57 (s, 6H, ArN-C*H*$_3$), 5.05 (s, 2H, O-C*H*$_2$-ArTAM), 5.12 (s, 2H, O-C*H*$_2$-ArTAM), 5.23 (s, 4H, O-C*H*$_2$-ArHOPO), 6.55 (d, 2H, HOPO Ar-*H*, *J* = 8), 7.18 (d, 2H, HOPO Ar-*H*, *J* = 8), 7.23 – 7.33 (m, 20H, Ar-*H*), 7.45 (d, 1H, TAM Ar-*H*, *J* = 8), 7.44 (m, 2H, CH$_2$-N(*H*)-HOPO), 7.56 (b, 1H, TAM Ar-*H*), 7.93 (b, 1H, CH$_2$-N(*H*)-TAM); ^{13}C NMR ($CDCl_3$) δ = 37.0, 37.3, 37.7, 43.0, 51.4, 52.0, 53.4, 55.1, 74.0, 104.1, 125.0, 125.4, 126.3, 127.8, 128.0, 128.2,128.3, 128.5, 130.3, 132.1, 135.0, 135.5, 135.8, 136.1, 145.5, 150.7, 151.4, 159.0, 163.0, 164.3, 164.7; LR FAB-MS m/z = 988 (MH^+), (Calcd. 988); Anal. Found (Calcd.). C 67.66 (68.00), H 6.02 (5.71), N 9.77 (9.88).

TREN-bis(HOPO-Bn)-TAM-Bn$_2$-Asp(OBn)-OBn (S6). *O*-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 0.286 g, 0.753 mmol) and TREN-bis(HOPO-Bn)-TAM-Bn$_2$-CO$_2$H **(S5)** (0.698 g, 0.706 mmol) were dissolved in anhydrous methylene chloride (20 mL) and diisopropylethylamine (8 mL). The solution was stirred at room temperature for 30 min. A solution of H_2N-Asp(OBn)OBn · Ts (0.534 g, 1.10 mmol) dissolved in anhydrous dimethylacetamide (5 mL) and diisopropylethylamine (2 mL) was then added to the TAM solution. The reaction mixture was stirred at room temperature for 18 h. The solvents were then evaporated under reduced and the crude product was purified by flash chromatography eluting with a gradient of 100 % CH_2Cl_2 to 90 % CH_2Cl_2 / 10 % CH_3OH. The protected ligand **(S6)** was obtained as a white foam (661 mg, 79.8 %) that was further dried under high vacuum at room temperature for 18 h. ^{1}H NMR ($CDCl_3$) δ = 2.39 (bm, 6H, N-C*H*$_2$-CH$_2$-NH-HOPO and N-C*H*$_2$-CH$_2$-NH-TAM), 2.82-298 (m, 2H, C(H)-C*H*$_2$-C(O)-O), 3.12 (bm, 6H, N-CH$_2$-C*H*$_2$-NH-HOPO and N-CH$_2$-C*H*$_2$-NH-TAM), 3.51 (s, 6H, ArN-C*H*$_3$), 4.08 (bm, 12H, C(O)-O-C*H*$_2$-Ar and O-C*H*$_2$-Ar, *J* = 11), 6.44 (d, 2H, Ar-*H*, *J* = 7), 7.03 (d, 2H, Ar-*H*, *J* = 7), 7.19 - 7.34 (m, 30H, Ar-*H*), 7.50 (d, 1H, Ar-*H*, *J* = 8), 7.66 (d, 1H, Ar-*H*, *J* = 8), 7.74 (t, 1H, C(O)-N(*H*)-CH$_2$, *J* = 7), 7.88 (t, 2H, C(O)-N(*H*)-CH$_2$, *J* = 5), 8.73 (d, 1H, C(O)-N(*H*)-CH-, *J* = 7); ^{13}C NMR ($CDCl_3$) δ = 14.12, 27.13, 30.40, 37.19, 37.35, 37.61, 51.90, 52.19, 52.37, 60.53, 61.49, 74.70, 104.56, 125.55, 126.24, 128.49, 128.52, 128.61, 128.64, 128.72, 128.89, 130.49, 132.20, 132.26, 135.85, 135.96, 136.30, 146.25, 150.12, 150,68, 159.41, 163.27, 164.26, 164.72, 171.62, 172.28; LR FAB-MS m/z = 1174.6 (MH^+), (Calcd. 1174.5); HR FAB-MS for $C_{65}H_{71}N_7O_{14}$ m/z = 1174.5152, (Calcd. 1174.5137); Anal. Calcd (Found). C 68.83 (69.20), H 6.03 (5.73), N 7.35 (7.63).

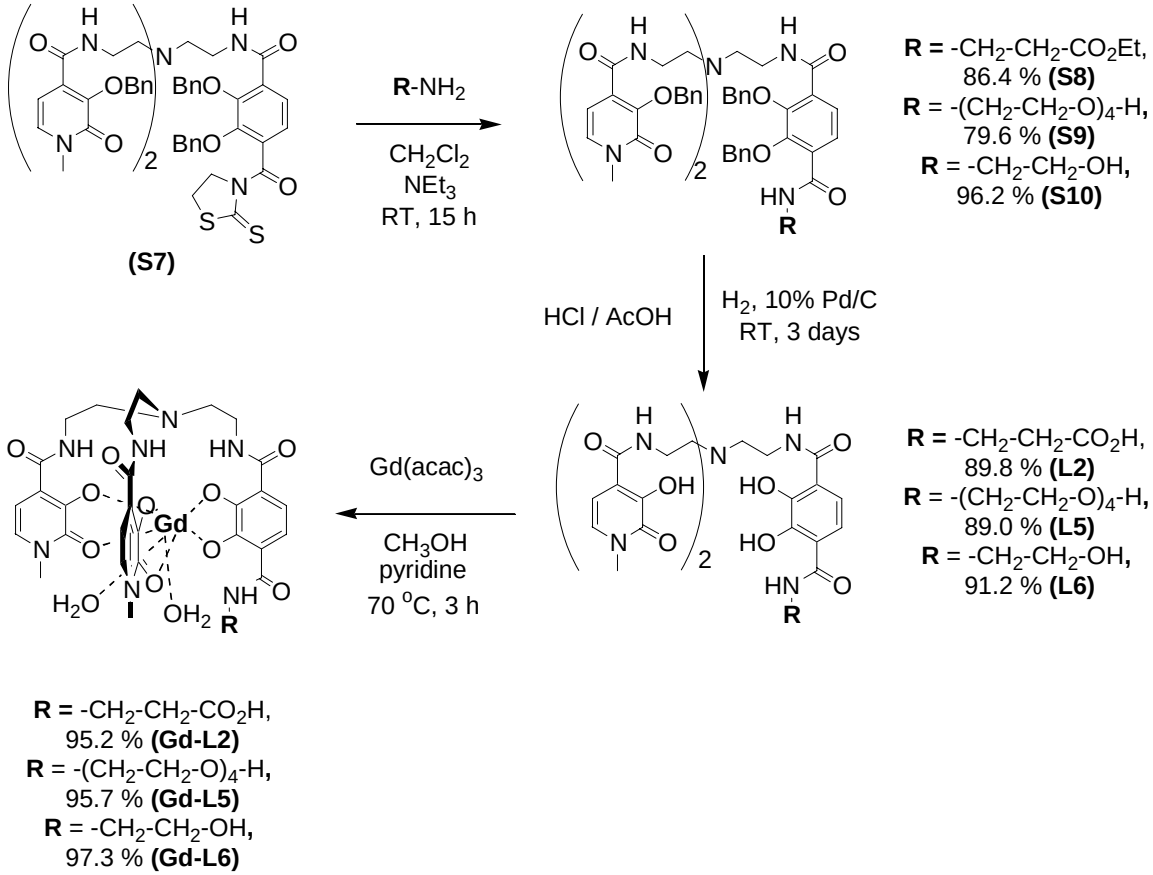
L1. TREN-bis(HOPO-Bn)-TAM-Bn$_2$-Asp(OBn)-OBn **(S6)** (710 mg, 553 μmol) was dissolved in glacial acetic acid (15 mL). Millipore water (5 mL), concentrated HCl (4 mL) and 10 % wet Pd/C (125 mg) were then added to the solution. The reaction mixture was stirred under H_2 (1500 PSI) at room temperature for 3 days. The Pd/C was then filtered, washed with acetic acid, and the filtrate was evaporated to dryness. The resulting white solid was dissolved in methanol (50 mL) and the solvent removed under reduced pressure. This last step was repeated until no more acetic acid could be detected. The product was then redissolved in methanol (30 mL) and added dropwise to diethyl ether (500 mL) under vigorous agitation. The white suspension was stirred at room temperature for 18 h. The resulting precipitate was filtered, rinsed with diethyl ether, and dried under

high vacuum at room temperature for 18 h. The deprotected ligand **(L1)** (491 mg, 92.7 %) was obtained as white powder. ^{1}H NMR (DMSO-d_6) δ = 2.84 (t, 2H, CH-C*H*$_2$-C(O)-OH, *J* = 6), 3.38 – 3.43 (bm, 6H, N(H)-CH$_2$-C*H*$_2$), 3.45 (s, 6H, ArN-C*H*$_3$), 3.56 – 3.83 (b, 10H, N(H)-C*H*$_2$-CH$_2$), 4.78 (dd, 1H, NH-C*H*-CH$_2$, *J* = 5), 6.42 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.02 (bdd, 1H, TAM-Ar-*H*), 7.08 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.31 (bdd, 1H, TAM-Ar-*H*), 8.70 (b, 2H, N(*H*)-C(O)-HOPO), 9.08 (b, 1H, N(*H*)-C(O)-TAM), 9.20 (b, 1H, N(*H*)-C(O)-TAM); ^{13}C NMR (DMSO-d_6) δ = 36.7, 37.3, 43.1, 43.2, 46.5, 52.78, 55.4, 55.5, 102.8, 125.9, 130.4, 132.0, 132.7, 136.0, 136.7, 146.4, 152.6, 158.2, 162.7, 167.0; LR FAB-MS m/z = 744 (MH^+), (Calcd. 744); HRFAB-MS for $C_{32}H_{38}N_7O_{14}$ = 744.2467 (MH^+) (Calcd. 744.2476); Anal. Found (Calcd.) for **(L1)**· 6H_2O· 2CH_3OH· 1HCl C 42.70 (42.68), H 6.07 (5.74), N 10.23 (9.95).

Gd-L1. A solution of Gd(acac)$_3$ (39.9 mg, 78.5 μmol) in degassed methanol (1.5 mL) was added to a solution of **L1** (74.9 mg, 78.2 μmol) in degassed methanol (15 mL). The solution was purged with N_2 and pyridine (2 drops) was added. The solution was refluxed for 6 h under N_2 during which time a beige precipitate appeared. The suspension was cooled to room temperature and precipitated in diethyl ether (250 mL). The ether suspension was stirred at room temperature for 1 h. The precipitate was then filtered, rinsed with diethyl ether (2 × 15 mL) and dried under high vacuum for 15 h at room temperature. The Gd complex **(Gd-L1)** was obtained as a beige powder (82.0 mg, 96.5 %). ESI-MS m/z = 448.1 (Calcd. 448.5). The isotropic distribution corresponded to that calculated for the doubly charged complex. MALDI-MS m/z = 896.4 (M^- Calcd. 897.1). The isotropic distribution corresponded to that calculated for a mono-charged complex. Anal. Found (Calcd.) for **Gd-L1**·10H_2O C 35.11 (35.47), H 5.03 (5.00), N 9.38 (9.05), Gd 15.05 (14.51).

L3. TREN-bis(HOPO-Bn)-TAM-Bn$_2$-CO_2Bn **(S4)** (838 mg, 776 μmol) was dissolved in glacial acetic acid (10 mL). Millipore water (3 mL), concentrated HCl (3 mL) and 10 % wet Pd/C (275 mg) were then added to the solution. The reaction mixture was stirred under H_2 (850 PSI) at room temperature for 4 days. The Pd/C was then filtered away and washed with acetic acid. The filtrate was evaporated to dryness. The resulting white solid was dissolved in methanol (50 mL) and the solvent removed under reduced pressure. This last step was repeated until no more acetic acid could be detected. The product was then redissolved in methanol (10 mL) and added dropwise to diethyl ether (500 mL) under vigorous agitation. The white suspension was stirred at room temperature for 1.5 h. The resulting precipitate was filtered, rinsed with diethyl ether, and dried under high vacuum at 60 °C for 18 h. The deprotected ligand **(L3)** (489 mg, 80.6 %) was obtained as a white powder. ^{1}H NMR (DMSO-d_6) δ = 3.41 (bm, 6H, N(H)-CH$_2$-C*H*$_2$), 3.45 (s, 6H, ArN-C*H*$_3$), 3.68 (b, 6H, N(H)-C*H*$_2$-CH$_2$), 6.45 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.13 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.20 (d, 1H, TAM-Ar-*H*, *J* = 9), 7.31 (d, 1H, TAM-Ar-*H*, *J* = 9), 8.69 (b, 2H, N(*H*)-C(O)-HOPO), 9.13 (b, 1H, N(*H*)-C(O)-TAM); ^{13}C NMR (DMSO-d_6) δ = 37.2, 43.2, 43.3, 52.67, 55.5, 125.9, 130.5, 132.1, 132.2, 136.1, 136.9, 146.8, 152.9, 158.0, 163.9, 166.0; LR FAB-MS m/z = 629 (MH^+), (Calcd. 629); HRFAB-MS for $C_{28}H_{33}N_6O_{11}$ = 629.2197 (MH^+) (Calcd. 621.2207); Anal. Found (Calcd.) for **(L3)**· 4H_2O· 1.4CH_3OH· 1HCl C 45.23 (45.24), H 5.63 (6.01), N 10.51 (10.75).

Gd-L3. A solution of $Gd(acac)_3$ (65.1 mg, 128 μmol) in degassed methanol (1.5 mL) was added to a solution of **L3** (100 mg, 128 μmol) in degassed methanol (12 mL). The solution was purged with N_2 and pyridine (8 drops) was added. The solution was refluxed for 3 h under N_2 during which time a beige precipitate appeared. The solution was cooled to room temperature and precipitated in diethyl ether (250 mL). The ether suspension was stirred at room temperature for 3 h. The precipitate was then filtered, rinsed with diethyl ether (2 × 15 mL) and dried under high vacuum for 15 h at room temperature. The Gd complex **(Gd-L3)** was obtained as a beige powder (140 mg, 94.6 %). FAB-MS m/z = 782.1 (Calcd. 782.1). The isotropic distribution corresponded to that calculated.Anal. Found (Calcd.) for **(Gd-L3)**·$3H_2O$·$2CH_3OH$·1.6Py C 39.59 (36.64), H 4.42 (4.33), N 9.20 (9.26), Gd 13.61 (13.63).



Scheme S 2. Synthesis of **Gd-L2**, **Gd-L5** and **Gd-L6**.

TREN-bis(HOPO-Bn)-TAM-βAla(Et)-Bn_2 (S8). β-Alanine ethyl ester (44.4 mg, 3.79 mmol) was added to a solution of TREN-bis(HOPO-Bn)-TAM-Bn_2-thiaz[3] **(S7)** (827 mg, 0.759 mmol) in methylene chloride (50 mL) and triethylamine (10 mL). The reaction mixture was stirred at room temperature for 18 h. The solvents were then removed under reduced pressure and the crude oil purified by flash chromatography over

[3] TREN-bis(HOPO-Bn)-TAM-Bn_2-thiaz[3] **(S7)** was synthesized as previously reported. See Pierre, V. C.; Botta, M.; Aime, S.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 5344-5345.

silica eluting with a gradient of 100 % CH_2Cl_2 to 86 % CH_2Cl_2 / 14 % CH_3OH. The product **(S8)** was obtained as a white foam (712 mg, 86.4 %) that was further dried under high vacuum for 15 h at room temperature. ^{1}H NMR ($CDCl_3$) δ = 1.20 (t, 3H, O-CH_2-C*H*$_3$, *J* = 7), 2.24 (t, 4H, N-(-C*H*$_2$-CH_2-NH-HOPO)$_2$, *J* = 6), 2.31 (t, 2H, N-C*H*$_2$-CH_2-NH-TAM, *J* = 6), 2.47 (t, 2H, CH_2-C*H*$_2$-C(O)-O, *J* = 6), 3.08 (q, 4H, N-(-CH_2-C*H*$_2$-NH-HOPO)$_2$, *J* = 6), 3.14 (q, 2H, N-CH_2-C*H*$_2$-NH-TAM, *J* = 6), 3.55 (s, 6H, ArN-C*H*$_3$), 3.57 (m, 2H, C*H*$_2$-CH_2-C(O)-O), 4.06 (q, 2H, O-C*H*$_2$-CH_3, *J* = 7), 5.06 (s, 2H, Ar-C*H*$_2$), 5.13 (s, 2H, Ar-C*H*$_2$), 5.27 (s, 4H, Ar-C*H*$_2$), 6.59 (d, 2H, Ar-*H*, *J* = 7), 7.06 (d, 2H, Ar-*H*, *J* = 7), 7.05-7.36 (m, 20H, Ar-*H*), 7.55 (bt, 1H, N(*H*)-C(O)-TAM), 7.64 (d, 1H, Ar-*H*, *J* = 8), 7.74 (d, 1H, Ar-*H*, *J* = 8), 7.81 (bt, 2H, N(*H*)-C(O)-HOPO), 8.10 (bt, 1H, N(*H*)-C(O)-TAM); ^{13}C NMR ($CDCl_3$) δ = 1.0, 14.1, 33.8, 35.2, 37.2, 37.3, 37.6, 51.2, 52.3, 60.6, 71.7, 74.7, 77.3, 104.6, 125.7, 126.1, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 130.3, 130.5, 131.6, 132.2, 135.9, 136.0, 136.3, 146.3, 150.2, 150.4, 159.4, 163.3, 164.4, 164.7, 172.0; LR FAB-MS m/z = 1088 (MH^+), (Calcd. 1088); HR FAB-MS for $C_{61}H_{65}N_7O_{12}$ m/z = 1088.4774, (Calcd. 1088.4769); Anal. Found (Calcd.) C 66.57 (66.84), H 6.05 (5.80), N 8.90 (9.25).

TREN-bis(HOPO-Bn)-TAM-dPEG4-Bn$_2$ (S9). Amino-dPEG$_4$ (506 mg, 2.62 mmol) was added to a solution of TREN-bis(HOPO-Bn)-TAM-thiaz-Bn$_2$ (**S7**) (2.75 g, 2.52 mmol) in methylene chloride (20 mL) and diisopropylethylamine (5 mL). The reaction mixture was stirred at room temperature for 18 h. The solvents were then removed under reduced pressure and the crude oil purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 86 % CH_2Cl_2 / 14 % CH_3OH. The product (**S9**) was obtained as a white foam (2.94 g, 79.6 %) that was further dried under high vacuum for 15 h at room temperature. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.23 (t, 4H, N-C*H*$_2$-CH_2-NH-C(O)-HOPO, *J* = 7), 2.29 (t, 2H, N-C*H*$_2$-CH_2-NH-C(O)-TAM, *J* = 7), 3.06 (bm, 4H, N-CH_2-C*H*$_2$-NH-C(O)-HOPO), 3.12 (bm, 2H, N-CH_2-C*H*$_2$-NH-C(O)-TAM), 3.53 (s, 6H, Ar-N-C*H*$_3$), 3.39-3.61 (m, 16H, C*H*$_2$-O of dPEG$_4$), 5.04 (s, 2H, TAM-O-C*H*$_2$-Ar), 5.11 (s, 2H, TAM-O-C*H*$_2$-Ar), 5.26 (s, 4H, HOPO-O-C*H*$_2$-Ar), 6.56 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.04 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.25 – 7.36 (m, 20H, Ar-*H*), 7.58 (t, 1H, N(*H*)-C(O)-TAM, *J* = 5), 7.63 (d, 1H, TAM-Ar-*H*, *J* = 7), 7.67 (d, 1H, TAM-Ar-*H*, *J* = 7), 7.79 (t, 1H, N(*H*)-C(O)-HOPO, *J* = 5), 8.10 (bt, 1H, N(*H*)-C(O)-TAM); ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 11.9, 17.3, 18.6, 37.1, 37.6, 39.6, 51.9, 52.3, 53.5, 61.4, 69.5, 70.0, 70.1,70.3, 70.5, 72.4, 74.6, 76.5, 76.9, 104.6, 125.7, 125.8, 128.4, 128.5, 128.6, 128.7, 128.9, 130.5, 131.1, 131.2, 132.2, 136.1, 136.3, 146.2, 150.2, 150.3, 159.4, 163.3, 164.8; LR FAB-MS m/z = 1164.4 (MH^+), (Calcd. 1164.5); HR FAB-MS for $C_{64}H_{74}N_7O_{14}$ m/z = 1164.5263 (Calcd. 1164.4293); Anal. Found (Calcd.) C 66.28 (66.02), H 6.46 (6.32), N 8.35 (8.42).

TREN-bis(HOPO-Bn)-TAM-EA-Bn$_2$ (S10). TREN-bis(HOPO-Bn) (1.84 g, 2.94 mmol), and TAM-thiaz-EA-Bn$_2$ (1.00 g, 1.91 mmol), were stirred in freshly distilled methylene chloride (100 mL) at room temperature for 24 h. The solvent was then evaporated *in vacuo* and the raw product purified by flash chromatography over silica eluting with 95% CH_2Cl_2/ 5% CH_3OH to 90% CH_2Cl_2/ 10% CH_3OH (1.89 g, 96.2 %). mp 62 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.56 (s, 1H, O*H*), 2.26 (bt, 4H, C*H*$_2$-N), 2.34 (t, 2H, C*H*$_2$-N, *J* = 7), 3.09 (m, 4H, C*H*$_2$-N), 3.19 (m, 2H, C*H*$_2$-N), 3.45 (m, 2H, CH_2 -

C*H*$_2$-OH), 3.55 (s, 3H, N-C*H*$_3$), 3.67 (m, C*H*$_2$- CH$_2$-OH), 5.06 (s, 2H, C*H*$_2$Ar), 5.14 (s, 2H, C*H*$_2$-Ar), 5.26 (s, 4H, C*H*$_2$-Ar), 5.60 (d, 2H, HOPO Ar*H*, *J* = 6.2), 7.070 (d, 2H, HOPO Ar*H*. *J* = 6.2), 7.27-7.37 (m, 20H, Ar-*H*), 7.57 (s, br, 1H, N*H*-CO), 7.59 (d, 1H, TAM Ar*H*, *J* = 7), 7.73 (d, 1H, TAM Ar*H*, *J* =7), 7.77 (s, br, 2H, N*H*-CO), 8.22 (s, br, 1H, N*H*-CO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 37.67, 43.2, 43.3, 46.5, 52.67, 55.5, 64.4, 73.98, 76.70, 103.00, 125.89, 128.23, 128.28, 128.51, 128.61, 128.66, 129.06, 130.51, 132.08, 132.24, 136.05, 136.89, 146.82, 152.96, 158.95, 163.89, 166.03; LR FAB-MS m/z 1032.5 (MH^+), (Calcd. 1033.1); Anal. Found (Calcd.) for $C_{30}H_{37}N_7O_{11}$.HCl.5$H_2$0: C 45.47 (45.14), H 6.08 (6.06), N 12.16 (12.28).

L2. All glassware were dried in the oven overnight. BBr_3 (2.10 mL, 22.2 mmol) was added slowly at - 48 °C and under N_2 to a solution of TREN-bis(HOPO-Bn)-TAM-β-Ala(Et) **(S8)** (650 mg, 0.597 mmol) in freshly distilled methylene chloride (60 mL). The reaction mixture was stirred under N_2 at - 48 °C for 20 min and at room temperature for 24 h. Solvents were then removed under reduced pressure and the crude product was dissolved in methanol (50 mL) and refluxed for 24 h. The solvent was removed under reduced pressure the product redissolved in methanol (10 mL) and distilled water (10 mL). 12 M $HCl_{(aq)}$ (1 mL) was added to the solution which was stirred at room temperature for 18 h. Solvents were removed under reduced pressure, and the resulting beige solide redisolved in methanol (10 mL). The product was then precipitated in diethyl ether (500 mL) and filtered. The deprotected ligand **(L2)** was obtained as a white powder (375 mg, 89.8 %) that was further dried under high vacuum for 15 h. ^{1}H NMR (DMSO-d_6) δ = 2.60 (t, 2H, CH$_2$-C*H*$_2$-C(O)-OH, *J* = 7), 3.51 (bm, 6H, N(H)-CH$_2$-C*H*$_2$), 3.57 (s, 6H, ArN-C*H*$_3$), 3.67 (b, 10H, N(H)-C*H*$_2$-CH$_2$ + C*H*$_2$-CH$_2$-C(O)-OH), 6.39 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.09 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.23 (d, 1H, TAM-Ar-*H*, *J* = 10), 7.28 (d, 1H, TAM-Ar-*H*, *J* = 10), 8.60 (b, 2H, N(*H*)-C(O)-HOPO), 8.95 (b, 1H, N(*H*)-C(O)-TAM), 9.49 (b, 1H, N(*H*)-C(O)-TAM); ^{13}C NMR (DMSO-d_6) δ = 37.2, 38.4, 43.2, 43.3, 46.5, 52.67, 55.5, 64.4, 73.9, 76.7, 103.0, 125.9, 130.5, 132.1, 132.2, 136.1, 136.9, 146.8, 152.9, 158.0, 163.9, 166.0; LR FAB-MS m/z = 699 (MH^+), (Calcd. 699); Anal. Found (Calcd.) for **(L2)**· 11H_2O C 41.58 (41.47), H 6.24 (6.62), N 11.06 (10.91).

L5. TREN-bis(HOPO-Bn)-TAM-dPEG4-Bn$_2$ (**S9**) (2.29 g, 1.96 mmol) was dissolved in glacial acetic acid (100 mL). Concentrated 12 M $HCl_{(aq)}$ (3 mL), millipore water (4 mL), and 10 % wet Pd/C (455 mg) were then added to the solution. The reaction mixture was stirred under H_2 (1200 PSI) at room temperature for 72 h. The Pd/C was then filtered off and washed with acetic acid. The filtrate was evaporated to dryness. The resulting white solid was dissolved in methanol (50 mL) and the solvent removed under reduced pressure. This last step was repeated until no more acetic acid could be detected. The product was then redissolved in methanol (5 mL) and added dropwise to diethyl ether (500 mL) under vigorous agitation. The resulting precipitate was filtered, rinsed with diethyl ether, and dried under high vacuum at 50 °C for 18 hours. The deprotected ligand (**L5**) was obtained as white powder (1.40 g, 89.0 %). ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 3.36 – 3.72 (m, 28H, C*H*$_2$-O of dPEG$_4$ + ArN-C*H3* + N-C*H*$_2$-CH$_2$-N(H)), 3.72 (b, 6H, N-CH$_2$-C*H*$_2$-N(H)), 6.44 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.13 (d, 2H, HOPO-Ar-*H*, *J* = 7), 7.32 (d, 1H, TAM-Ar-*H*, *J* = 10), 7.34 (d, 1H, TAM-Ar-*H*, *J* = 10), 8.67 (b, 2H, N(*H*)-C(O)-HOPO), 8.97 (b, 1H, N(*H*)-C(O)-TAM), 9.10 (b, 1H, N(*H*)-C(O)-TAM); ^{13}C

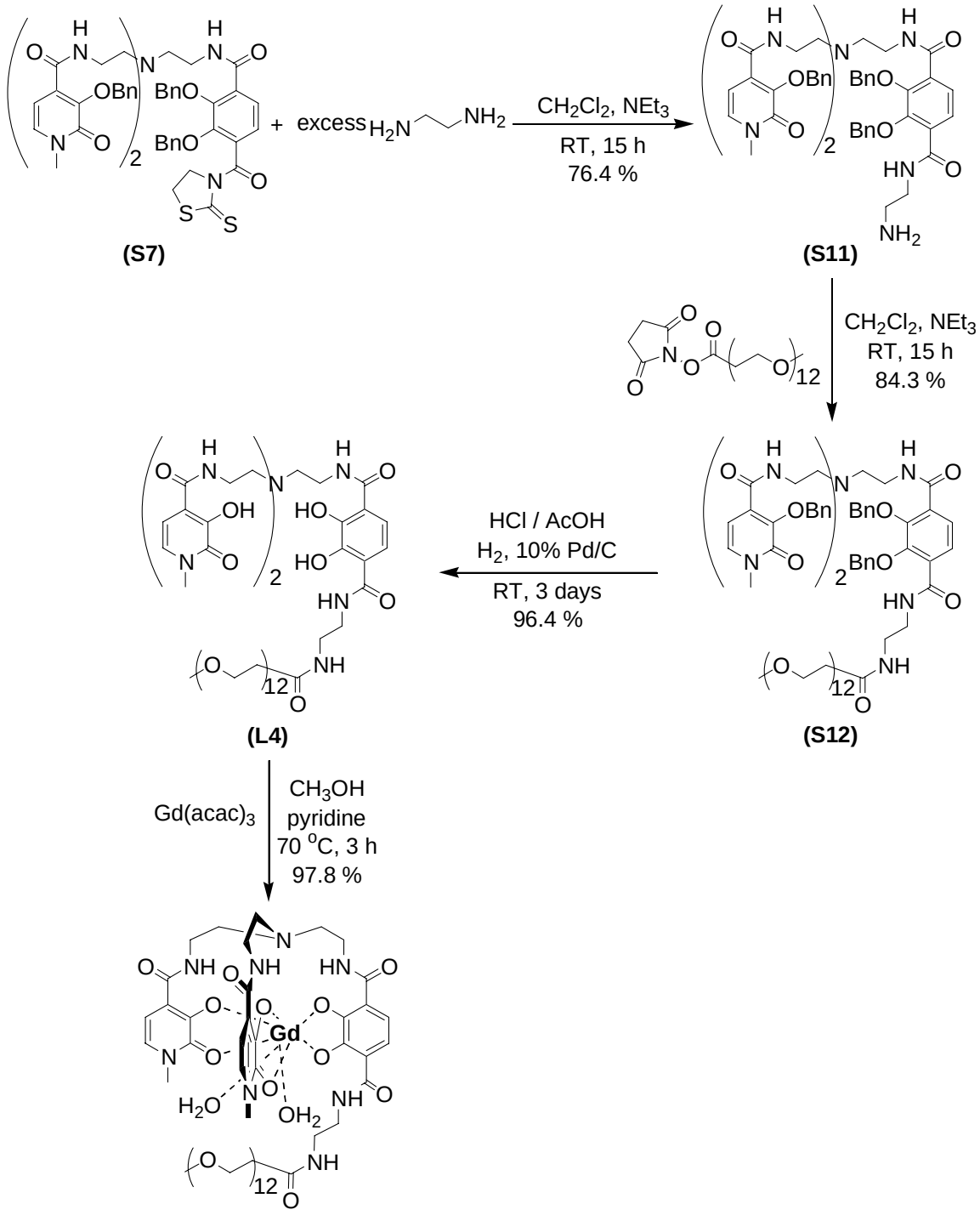
NMR (DMSO-d_6, 100 MHz) δ = 11.9, 17.3, 18.6, 37.1, 37.6, 39.6, 51.9, 52.3, 53.5, 61.4, 69.5, 70.0, 73.9, 76.7, 103.0, 125.9, 130.5, 132.1, 132.2, 136.1, 136.9, 146.8, 152.0, 158.0, 163.9, 166.0; LR FAB-MS m/z = 805.1 (MH^+), (Calcd. 804.3); Anal. Found (Calcd.) for (2-17)· $7H_2O$ C 46.72 (46.50), H 6.90 (6.83), N 10.63 (10.54).

L6. TREN-bis(HOPO-Bn)-TAM-EA-Bn_2 (**S10**) (0.897 g, 0.868 mmol), and 10% Pd supported on C (189 mg) were stirred in glacial acetic acid (80 mL) and Millipore water (10 mL) under H_2 for 72 h. The Pd catalyst was then filtered off and washed with distilled water. The filtrate was evaporated to a thick oil *in vacuo* and stirred in methanol / HCl at room temperature for 15 h. The solution was evaporated to dryness, dissolved in water and dried again. The resulting white precipitate was dissolved in methanol and evaporated to dryness. The resulting off-white powder (0.532 g, 91.2 %) was dried overnight under high vacuum. mp 77 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 6.45 (d, 2H, Ar*H*, *J* = 6.2), 7.14 (d, 2H, Ar*H*, *J* = 6.2), 7.36 (d, 1H, Ar*H*, *J* = 7), 7.40 (d, 1H, Ar*H*, *J* = 7), 8.72 (s, 2H, N*H*CO), 8.95 (s, 1H, N*H*-CO) 9.16 (s, 1H, N*H*CO), the CH_2-N protons were covered by the water peak and were thus analyzed in MeOH-d_4, 400 MHz: 3.53 (m, 6H, N-CH_3), 3.63 (m, 4H, CH_2-CH_2-N), 3.68 (m, 4H, CH_2-CH_2-N), 3.70 (m, 4H, CH_2-CH_2-N), 3.88 (m, 4H, CH_2-CH_2-N); ^{13}C NMR (DMSO-d_6, 100 MHz) δ = 37.67, 43.2, 43.3, 46.5, 52.67, 55.5, 64.4, 73.98, 76.70, 103.00, 125.89, 130.51, 132.08, 132.24, 136.05, 136.89, 146.82, 152.96, 158.95, 163.89, 166.03; IR 3384 (s, O-H), 3263 (s, Ar-H), 1656 (s, C=O), 1604 (m, NHCO); LR FAB-MS m/z 672 (MH^+), (Calcd 671.6); Anal. Found (Calcd.) C 67.05 (67.49), H 5.32 (5.96), N 9.16 (9.50).

Gd-L2. A solution of $Gd(acac)_3$ (42.8 mg, 84.1 μmol) in degassed methanol (1 mL) was added to a solution of **L2** (75.1 mg, 84.0 μmol) in degassed methanol (10 mL). The solution was purged with N_2 and pyridine (2 drops) was added. The solution was refluxed for 3 h under N_2 during which time a white precipitate appeared. The suspension was cooled to 0°C and precipitated in diethyl ether (200 mL). The resulting precipitate was filtered, rinsed with diethyl ether (50 mL) and dried under high vacuum for 15 h at room temperature. The Gd complex **(Gd-L2)** was obtained as a beige powder (72.6 mg, 95.2 %). ES-MS m/z = 853.1 (Calcd. 853.3). The isotopic distribution corresponded to the calculated one. Anal. Found (Calcd.) for **(Gd-L2)**· $3H_2O$ C 38.50 (38.75), H 4.66 (4.72), N 10.38 (10.20), Gd 16. 43 (16.36).

Gd-L5. A solution of $Gd(acac)_3$ (40.6 mg, 79.8 μmol) in degassed methanol (1 mL) was added to a solution of **L5** (75.0 mg, 79.9 μmol) in degassed methanol (10 mL). The solution was purged with N_2 and pyridine (2 drops) was added. The solution was refluxed for 3 h under N_2 during which time a white precipitate appeared. The suspension was cooled to 0 °C and precipitated in diethyl ether (200 mL). The resulting precipitate was filtered, rinsed with diethyl ether (50 mL) and dried under high vacuum for 15 h at room temperature. The Gd complex (**Gd-L5**) was obtained as a beige powder (85.5 mg, 95.7 %). ES-MS m/z = 957.2 (Calcd. 957.3). The isotopic distribution corresponded to the calculated one. Anal. Found (Calcd.) for (2-6)·$9H_2O$ C 38.82 (38.63), H 5.50 (5.67), N 8.93 (8.76), Gd 14.23 (14.05).

Gd-L6. A solution of $Gd(acac)_3$ (38.1 mg, 74.8 μmol) in degassed methanol (1 mL) was added to a solution of **L6** (50.2 mg, 74.8 μmol) in degassed methanol (10 mL). The solution was purged with N_2 and pyridine (2 drops) was added. The solution was refluxed for 3 h under N_2 during which time a white precipitate appeared. The suspension was cooled to 0 °C and precipitated in diethyl ether (200 mL). The resulting precipitate was filtered, rinsed with diethyl ether (50 mL) and dried under high vacuum for 15 h at room temperature. The Gd complex (**Gd-L6**) was obtained as a beige powder (71.3 mg, 93.5 %). ES-MS m/z = 825.0 (Calcd. 825.1). The isotopic distribution corresponded to the calculated one. Anal. Found (Calcd.) for (**Gd-L6**)·$10H_2O$ C 35.27 (35.34), H 3.90 (4.40), N 9.53 (9.62), Gd 15.07 (15.42).



Scheme S 3. Synthesis of **Gd-L4**.

TREN-bis(HOPO-Bn)-TAM-Bn$_2$-NH$_2$ (S11). A solution of TREN-bis(HOPO-Bn)-TAM-Bn$_2$-thiaz **(S7)** (2.50 g, 2.29 mmol) in methylene chloride (400 mL) was slowly added over 6 h to a solution of 1,2-diaminoethane (1.82 g, 30.3 mmol) in methylene chloride (20 mL) and triethylamine (10 mL). The reaction mixture was further

stirred at room temperature for 1 h. The solvents were then removed under reduced pressure and the crude oil was further dried under high vacuum at room temperature for 15 h. The product was purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 86 % CH_2Cl_2 / 10 % CH_3OH / 4 % NEt_3. The ligand **(S11)** (1.80 g, 76.4 %) was obtained as a white foam that was further dried under high vacuum at room temperature for 1 h. ^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 1.92 (bs, 2H, CH_2-*NH*$_2$), *2*.30 (t, 4H, N-C*H*$_2$-CH_2-NH-HOPO, *J* = 6.8), 2.36 (t, 2H, N-C*H*$_2$-CH_2-NH-TAM, *J* = 6.4), 2.70 (t, 2H, NH-CH_2-C*H*$_2$-NH_2, *J* = 6), 3.12 (t, 4H, N-CH_2-C*H*$_2$-NH-HOPO, *J* = 6.8), 3.18 (t, 2H, N-CH_2-C*H*$_2$-NH-TAM, *J* = 6.4), 3.32 (t, 2H, C(O)-NH-C*H*$_2$-CH_2-NH_2, *J* = 6), 3.53 (s, 6H, ArN-C*H*$_3$), 5.09 (s, 2H, TAM O-C*H*$_2$-Ar), 5.15 (s, 2H, TAM O-C*H*$_2$-Ar), 5.26 (s, 4H, HOPO O-C*H*$_2$-Ar), 6.50 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 7.06 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 7.24-7.50 (m, 20H, Ar-*H*), 7.58 (d + t overlapping, 3H, TAM Ar-*H* + N(*H*)-C(O)-CH_2-CH_2-NH_2, *J* = 7.2, *J'* = 6), 7.76 (t, 2H, N(*H*)-C(O)-HOPO), 7.98 (t, 1H, N(*H*)-C(O)-TAM); ^{13}C NMR (100 MHz, CD_2Cl_2) δ (ppm) =10.6, 37.8, 37.9, 41.8, 43.3, 45.9, 46.5, 52.9, 53.0, 71.3, 74.9, 77.4, 77.5, 104.6, 125.9, 126.3, 127.4, 128.8, 128.9, 129.1, 129.2, 129.3, 129.5, 131.3, 131.4, 132.2, 132.9, 136.8, 136.9, 137.2, 146.6, 150.8, 151.0, 159.9, 163.8, 165.0, 165.1; MALDI-MS m/z = 1032.5 (MH^+), (Calcd. 1031.4); Anal. Found (Calcd.). C 67.73 (67.56), H 6.24 (6.06), N 10.48 (10.87).

TREN-bis(HOPO-Bn)-TAM-Bn$_2$-dPEG$_{12}$ (S12). A solution of the N-hydroxysuccinimide activated NHS-dPEG$_{12}$ (1.00 g, 1.46 mmol) in methylene chloride (5 mL) was added to a solution of TREN-bis(HOPO-Bn)-TAM-Bn$_2$-NH_2 **(S11)** (1.80 mg, 1.74 mmol) in methylene chloride (10 mL) and triethylamine (3 mL). The solution was stirred at room temperature for 18 h. The solvents were then removed under reduced pressure and the crude product was purified by flash chromatography over silica eluting with a gradient of 95 % CH_2Cl_2 / 5 % CH_3OH to 86 CH_2Cl_2 / 10 % CH_3OH / 4 % NEt_3. Removal of the solvents under reduced pressure yielded **(S12)** as a white foam that was further dried under high vacuum at room temperature for 18 h. (1.97g , 84.3 %), ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 2.29 (t, 4H, N-C*H*$_2$-CH_2-NH-C(O)-HOPO, *J* = 6.4), 2.35 (t, 2H, N-C*H*$_2$-CH_2-NH-C(O)-TAM, *J* = 6.4), 2.42 (t, 2H, NH-C(O)-C*H*$_2$-CH_2-O), *J* = 5.6), 3.13 (t, 4H, N-CH_2-C*H*$_2$-NH-C(O)-HOPO, *J* = 6.4), 3.20 (t, 2H, N-CH_2-C*H*$_2$-NH-C(O)-TAM, *J* = 6.4), 3.37 (t, 2H, TAM-C(O)-NH-CH2-C*H2*-NH-C(O), *J* = 5.6), 3.40 (s, 3H, O-CH_3), 3.44 (t, 2H, TAM-C(O)-NH-C*H2*-CH2-NH-C(O), *J* = 5.6), 3.55 (s, 6H, ArN-C*H*$_3$), 3.47-5.75 (m, 46H, O-C*H*$_2$-C*H*$_2$-O), 5.09 (s, 2H, TAM O-C*H*$_2$-Ar), 5.16 (s, 2H, TAM O-C*H*$_2$-Ar), 5.32 (s, 4H, HOPO O-C*H*$_2$-Ar), 6.64 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 6.96 (t, 1H, N*H*-C(O)-PEG, *J* = 6), 7.10 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 7.30-7.39 (m, 20H, Ar-*H*), 7.63 (t, 1H, N*H*-C(O)-TAM, *J* = 6), 7.68 (d, 1H, TAM Ar-*H*, *J* = 7.2), 7.71 (d, 1H, TAM Ar-*H*, *J* = 7.2), 7.82 (t, 2H, N*H*-C(O)-HOPO, *J* = 6), 8.20 (t, 1H, N*H*-C(O)-TAM, *J* = 6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 36.8, 37.1, 37.3, 37.6, 39.5, 39.6, 52.0, 52.2, 58.9, 67.0, 70.1, 70.2, 70.5, 71.9, 74.7, 77.1, 104.6, 125.7, 128.4, 128.6, 128.7, 128.8, 128.9, 130.6, 130.8, 131.3, 132.2, 135.9, 136.0, 136.3, 146.2, 150.3, 159.4, 163.3, 164.5, 165.4, 171.9; MALDI-MS m/z = 1601.2 (M^+), (Calcd. 1600.8); Anal. Found (Calcd.). C 62.76 (62.98), H 7.23 (7.05), N 6.67 (7.00).

L4. The protected ligand TREN-bis(HOPO-Bn)-TAM-Bn$_2$-dPEG$_{12}$ **(S12)** (1.91 g, 1.19 mmol) was dissolved in a mixture of glacial acetic acid (20 mL) and concentrated

$HCl_{(aq)}$ (20 mL). 10 % Pd / C (356 mg) was added to the solution and the resulting suspension was stirred under an atmosphere of $H_{2(g)}$ at room temperature for 24 h. The Pd / C was then filtered and the solvents removed under reduced pressure. The residual solvents were co-evaporated with methanol (3 × 30 mL) under reduced pressure. The crude foam was redissolved in methanol (10 mL) and precipitated in diethyl ether (500 mL). The resulting suspension was filtered, and the residue was rinsed with diethyl ether (3 × 15 mL) and dried under high vacuum at 60 °C for 15 h. The deprotected ligand **(L4)** was obtained as a beige solid (1.53 g, 96.4 %). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) = 2.29 (t, 4H, N-C*H*$_2$-CH$_2$-NH-C(O)-HOPO, *J* = 6.4), 2.35 (t, 2H, N-C*H*$_2$-CH$_2$-NH-C(O)-TAM, *J* = 6.4), 2.42 (t, 2H, NH-C(O)-C*H*$_2$-CH$_2$-O), *J* = 5.6), 3.13 (t, 4H, N-CH$_2$-C*H*$_2$-NH-C(O)-HOPO, *J* = 6.4), 3.20 (t, 2H, N-CH$_2$-C*H*$_2$-NH-C(O)-TAM, *J* = 6.4), 3.37 (t, 2H, TAM-C(O)-NH-CH2-C*H2*-NH-C(O), *J* = 5.6), 3.40 (s, 3H, O-CH$_3$), 3.44 (t, 2H, TAM-C(O)-NH-C*H2*-CH2-NH-C(O), *J* = 5.6), 3.55 (s, 6H, ArN-C*H*$_3$), 3.47-5.75 (m, 46H, O-C*H*$_2$-C*H*$_2$-O), 6.64 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 6.96 (t, 1H, N*H*-C(O)-PEG, *J* = 6), 7.10 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 7.63 (t, 1H, N*H*-C(O)-TAM, *J* = 6), 7.68 (d, 1H, TAM Ar-*H*, *J* = 7.2), 7.71 (d, 1H, TAM Ar-*H*, *J* = 7.2), 7.82 (t, 2H, N*H*-C(O)-HOPO, *J* = 6), 8.20 (t, 1H, N*H*-C(O)-TAM, *J* = 6); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) = 36.4, 36.7, 57.7, 66.8, 69.8, 69.9, 70.0, 70.1, 71.6, 173.2; MALDI-MS m/z = 1239.7 (M^+), (Calcd. 1240.6); Anal. Found (Calcd.). C 51.08 (51.09), H 7.43 (7.21), N 8.57 (8.42).

Gd-L4. A solution of Gd(acac)$_3$ (26.77 mg, 52.7 μmol) in degassed methanol (3 mL) was added to a solution of **L4** (70.5 mg, 52.9 μmol) in methanol (5 mL). Pyridine (5 drops) was added and the pale yellow solution was stirred at a light reflux under N_2 for 4 h. The solution was freeze-dried, re-dissolved in Millipore water (3 mL) and lyophilized. The complex was obtained as a beige powder (76.3 mg, 97.8 %). ES-MS m/z = 1394.6 (M^-), (Calcd. 1394.5). The isotopic distribution correspond to the calculated one; Anal. Found (Calcd.). for **(Gd-L4)**·3H_2O C 46.62 (46.43), H 6.41 (6.26), N 7.30 (7.74), Gd 10.93 (10.86).

General Method for Determination of pM values by Competition Batch Titration for ML Complexes

In this type of experiment, the protonation equilibria of the ligand can be neglected since this is removed under the constant pH conditions used in the experiment (pH = 7.4). In the following equilibria, 'L' is the ligand, 'C' is the competing ligand:

$M + L \leftrightarrow ML$
$M + C \leftrightarrow MC$
$\beta_{ML} = [ML]/[M][L]$
$\beta_{MC} = [MC]/[M][C]$

These formation constants can be considered conditional stability constants, as standard conditions are employed at pH 7.4. Hence, the difference in log β's is equivalent to the difference in pM values. The log/log plot used to determine ΔpM is derived as follows:

$$\log \beta_{ML} - \log \beta_{MC} = pM_{ML} - pM_{MC} = \log ([ML]/[M][L]) - \log ([MC]/[M][C])$$

$$= \log ([ML][C]/[L][MC])$$

$$= \log ([ML]/[MC]) + \log ([C]/[L])$$

The equation is then rearranged to give the following form which is used to generate the plots shown in Figures 2 and 4:

$$\log ([MC]/[ML]) = \log ([C]/[L]) - (pM_{ML} - pM_{MC})$$

$$\log ([MC]/[ML]) = \log ([C]/[L]) + \Delta pM$$

The plots directly give the difference in pM between each ligand and competitor (log ([C]/[L]) when log([MC]/[ML]) = 0, or when the concentration of competitor generates equal partition of metal between the ligand and competitor). In this case, since the pGd of DTPA is known to be 19.1, as calculated from protonation and complex formation constants found in Reference 21, the pGd's of each new ligand can be calculated (Table 1).

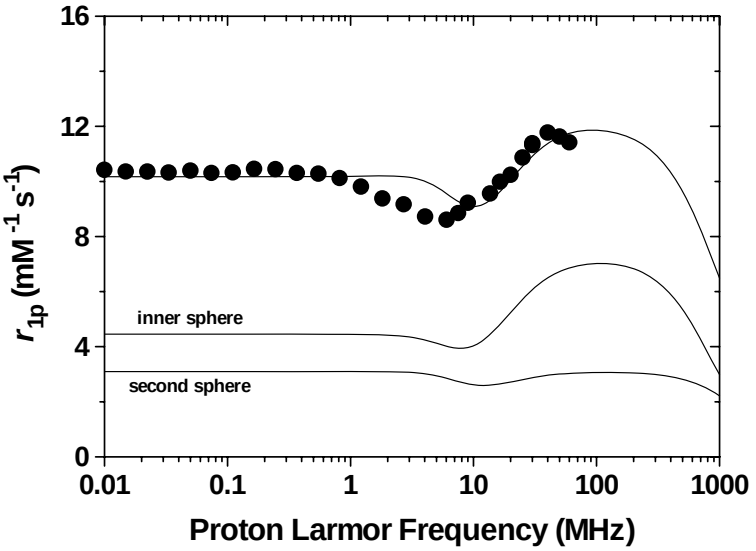


Figure S1. Inner sphere and second sphere contributions to the Nuclear Magnetic Resonance Dispersion profile of **Gd-L4**.