Fe(III) Templated Gd(III) Self-Assemblies – a New Route Toward Macromolecular MRI Contrast Agents[1]

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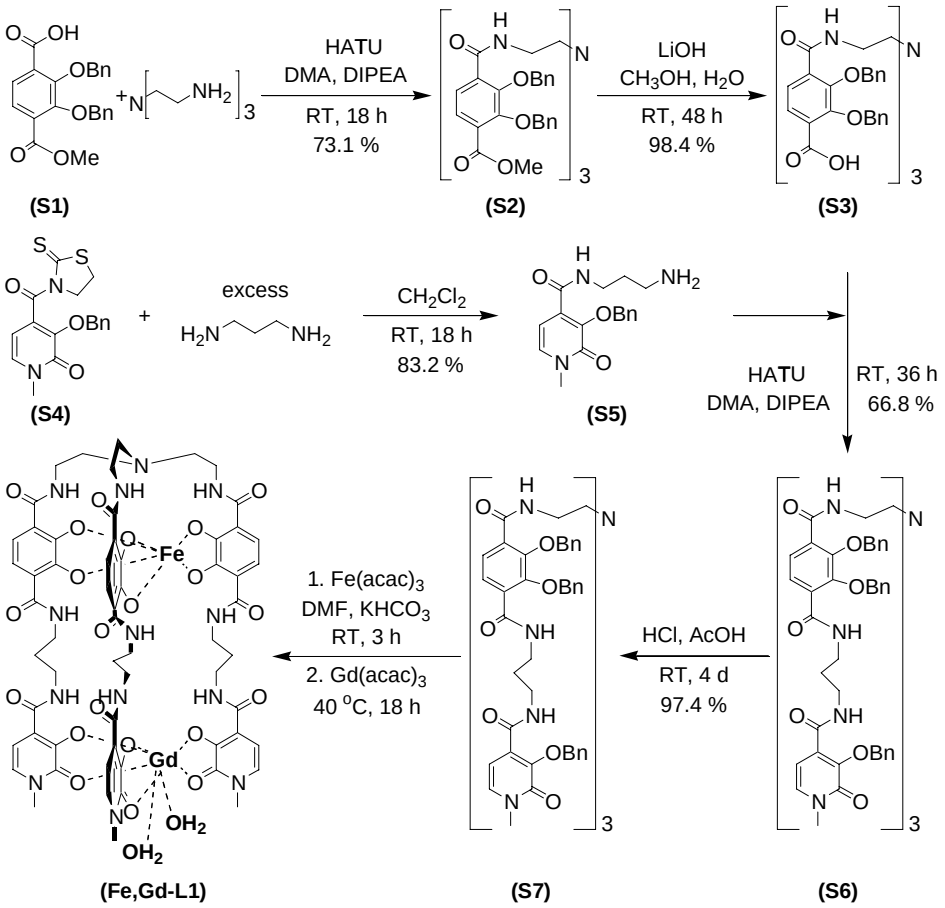
SUPPORTING INFORMATIONS

Experimental procedures and characterization data for the synthesis of the complexes Fe,Gd-L_A, Fe,Gd-L_B and Fe,2Gd-L_C

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 S1. Synthesis of **Fe,Gd-L$_A$.**

TREN-tris(TAM-Bn$_2$-CO$_2$Me) (S2). *O*-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 1.14 g, 3.01 mmol) and TAM-Bn$_2$-CO$_2$Me **(S1)**[1] (1.28 g, 2.73 mmol) were dissolved in anhydrous dimethylacetamide (10 mL) and diisopropylethylamine (10 mL). The mixture was stirred at room temperature for 20 min until complete dissolution of the HATU. A solution of tris-2-aminoethyl amine (121 mg, 0.827 mmol) in dimethylacetamide (5 mL) and diisopropylethylamine (5 mL) was then added to the reaction mixture, which was stirred at room temperature for 18 h. The solvents were then removed under reduced pressure and the crude product was partitioned between methylene chloride (100 mL) and 1 M HCl$_{aq}$ (100 mL). The organic phase was washed with 1 M HCl$_{aq}$ (2 × 100 mL) and brine (1 × 100 mL). The organic phase was dried with anhydrous magnesium sulfate and the solvent was removed under

[1] Pierre, V. C. Ph.D. dissertation, University of California, Berkeley, 2005.

reduced pressure. The crude product was purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 90 % CH_2Cl_2 / 10 % CH_3OH. Removal of the solvents under reduced pressure yielded **(S2)** (0.767 g, 73.1 %) as a colorless solid that was dried under high vacuum at room temperature for 18h. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 2.37 (t, 6H, N(H)-CH_2-C*H*$_2$-N, *J* = 6.0), 3.23 (t, 6H, N(H)-C*H*$_2$-CH_2-N, *J* = 6.0), 3.88 (s, 9H, C(O)-O-C*H*$_3$), 5.09 (b, 12H, Ar-C*H*$_2$-O), 7.29-7.45 (m, 30H, Ar-*H*), 7.56 (d, 3H, TAM Ar-*H*, *J* = 8.0), 7.67 (d, 3H, TAM Ar-*H*, *J* = 8.0), 7.83 (t, 3H, C(O)-N(*H*)-CH_2, *J* = 7.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 38.5, 52.3, 65.0, 76.5, 125.6, 125.9, 126.8, 127.3, 128.2, 128.3, 128.4, 128.5, 128.7, 135.8, 136.4, 151.3, 151.7, 165.6; MALDI-MS m/z = 1269.4 (M^+), (Calcd. 1269.5); Anal. Found (Calcd.). C 74.29 (74.58), H 5.96 (5.65), N 3.28 (3.74).

TREN-tris(TAM-Bn$_2$-CO$_2$H) (S3). LiOH (147 mg, 6.14 mmol) was added to a solution of TREN-tris(TAM-Bn$_2$-CO$_2$Me) **(S2)** (0.754g, 0.594 mmol) in methanol (30 mL) and Millipore water (5 mL). The solution was stirred at room temperature for 48 h. The solvents were then removed under reduced pressure and the crude product re-dissolved in Millipore water (100 mL). The solution was cooled down to 0 °C and slowly acidified to pH 1 with 6 M HCl_{aq}. The resulting white precipitate was filtered, rinsed with 1 M HCl_{aq} (2 × 15 mL) and dried under high vacuum at 40 °C for 48 h. (0.770 g, 98.4 %). ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) = 3.40 (t, 6H, N(H)-CH_2-C*H*$_2$-N, *J* = 6.0), 3.85 (t, 6H, N(H)-C*H*$_2$-CH_2-N, *J* = 6.0), 5.15 (s, 6H, Ar-C*H*$_2$-O), 5.19 (s, 6H, Ar-C*H*$_2$-O), 7.28-7.51 (m, 30H, Ar-*H*), 7.59 (s, 6H, TAM Ar-*H*), 8.63 (t, 3H, N(*H*)-C(O), *J* = 6.0); MALDI-MS m/z = 1225.8 (M^+), (Calcd. 1226.5); Anal. Found (Calcd.) for **(S3)**· 5H_2O C 65.48 (65.64), H 6.02 (5.81), N 4.18 (4.25).

HOPO-Bn-C3-NH$_2$ (S5). A solution of HOPO-Bn-thiaz **(S4)**[2] (2.04 g, 5.66 mmol) in methylene chloride (500 mL) was slowly added over 48 h to a solution of 1,3-diaminopropane (8.30 g, 113 mmol) in methylene chloride (100 mL). The reaction mixture was stirred at room temperature for 5 h. The solvents were then removed under reduced pressure and the crude product purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 78 % CH_2Cl_2 / 16 % CH_3OH / 6 % NEt_3.

[2] HOPO-Bn-thiaz (**S4**) was synthesized as described previously: Xu, J.; Franklin, S. J.; Whisenhunt, D. W.; Raymond, K. N. *J. Am. Chem. Soc.* **1995,** *117*, 7245-7246.

HOPO-Bn-C3-NH_2 **(S5)** (1.48 g, 83.2 %) was obtained as a colorless oil that was dried under high vacuum at room temperature for 18 h. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 1.52 (t, 2H, NH-CH_2-C*H*$_2$-CH_2-NH_2, *J* = 6.4), 2.65 (t, 2H, NH-CH_2-CH_2-C*H*$_2$-NH_2, *J* = 6.4), 3.31 (dt, 2H, NH-C*H*$_2$-CH_2-CH_2-NH_2, *J* = *J'* = 6.4), 3.59 (s, 3H, Ar-N-C*H*$_3$), 3.85 (bs, 2H, CH_2-N*H*$_2$), 5.38 (s, 2H, Ar-C*H*$_2$-O), 6.73 (d, 1H, HOPO Ar-*H*, *J* = 7.2), 7.12 (d, 1H, Ar-*H*, *J* = 7.2), 7.27-7.43 (m, 5H, Ar-*H*), 8.10 (t, 1H, C(O)-N*H*-CH_2, *J* = 6.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 30.8, 36.8, 37.7, 38.5, 104.8, 128.3, 128.8. 128.9, 129.1, 130.3, 132.1, 136.2, 146.5, 159.6, 163.8; MALDI-MS m/z = 315.8 (M^+), (Calcd. 315.2); Anal. Found (Calcd.). C 64.31 (64.74), H 7.04 (6.71), N 12.71 (13.32).

TREN-tris(TAM-Bn$_2$-C3-HOPO-Bn) (S6). *O*-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 1.91 g, 5.02 mmol) and TREN-tris(TAM-Bn_2-CO_2H) **(S3)** (0.620 g, 0.471 mmol) were dissolved in a mixture of anhydrous methylene chloride (10 mL), anhydrous dimethylacetamide (5 mL) and triethylamine (5 mL). The mixture was stirred at room temperature for 20 min until complete dissolution of the HATU. A solution of HOPO-Bn-C3-NH_2 **(S5)** (0.490 g, 1.55 mmol) in methylene chloride (5 mL) and triethylamine (5 mL) was then added to the reaction mixture, which was stirred at room temperature for 36 h. The solvents were then removed under reduced pressure and the crude product was partitioned between methylene chloride (100 mL) and 1 M HCl_{aq} (100 mL). The organic phase was washed with 1 M HCl_{aq} (4 × 100 mL) and brine (1 × 100 mL). The organic phase was dried with anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 90 % CH_2Cl_2 / 10 % CH_3OH. Removal of the solvents under reduced pressure yielded **(S6)** (0.665 g, 66.8 %) as a beige solid that was dried under high vacuum at room temperature for 18h. ^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 1.45 (t, 6H, N(H)-CH_2-C*H*$_2$-CH_2-N(H), *J* = 6.4), 2.46 (t, 6H, N(H)-CH_2-C*H*$_2$-N, *J* = 6.0), 3.19 (dt, 12H, N(H)-C*H*$_2$-CH_2-C*H*$_2$-N(H), *J* = *J'* = 6.4), 3.27 (dt, 6H, N(H)-C*H*$_2$-CH_2-N, *J* = *J'* = 6.0), 3.50 (s, 9H, Ar-N-C*H*$_3$), 5.10 (s, 6H, TAM O-C*H*$_2$-Ar), 5.12 (s, 6H, TAM O-C*H*$_2$-Ar), 5.37 (s, 6H, HOPO O-C*H*$_2$-Ar), 6.61 (d, 3H, HOPO Ar-*H*, *J* = 7.2), 7.12 (d, 3H, HOPO Ar-*H*, *J* = 7.2), 7.27-7.46 (m, 45H, Ar-*H*), 7.55 (d, 3H, TAM Ar-*H*, *J* = 8.0), 7.62 (d, 3H, TAM Ar-*H*, *J* = 8.0), 7.78 (t, 3H, CH_2-N(*H*)-C(O), *J* = 7.0), 7.87 (t, 3H, CH_2-N(*H*)-C(O),

J = 7.0), 7.98 (t, 3H, CH_2-N(*H*)-C(O), *J* = 6.0); ^{13}C NMR (100 MHz, CD_2Cl_2) δ (ppm) = 29.7, 37.4, 37.8, 38.1, 54.4, 74.8, 77.4, 104.6, 126.1, 128.6, 128.8, 129.0, 129.1, 129.2, 129.3, 129.5, 131.3, 131.6, 131.7, 133.1, 136.6, 137.0, 146.5, 150.9, 159.9, 163.8, 164.9; MALDI-MS m/z = 2118.8 (M^+), (Calcd. 2118.9); Anal. Found (Calcd.). C 69.48 (69.71), H 6.23 (5.85), N 8.09 (8.59).

TREN-tris(TAM-C3-HOPO) (S7). The protected ligand 2C3-TAM-Bn_2-$(HOPO\text{-}Bn)_2$ **(S6)** (650 mg, 0.307 mmol) was dissolved in a mixture of glacial acetic acid (20 mL), Millipore water (5 mL) and concentrated $HCl_{(aq)}$ (4 mL). 10 % Pd / C (216 mg) was added to the solution and the resulting suspension was stirred under $H_{2(g)}$ (80 atm) at room temperature for 3 days. The Pd / C was then filtered, the solvents removed under reduced pressure and the residual solvents were co-evaporated with methanol (3 × 30 mL). The crude oil was redissolved in methanol (30 mL), filtered, and the filtrate precipitated in diethyl ether (500 mL). The resulting suspension was filtered, and the residue was rinsed with diethyl ether (3 × 15 mL). The resulting white solid was redissolved in glacial acetic acid (7 mL) and 12 M $HCl_{(aq)}$ (20 mL) and stirred at room temperature for 4 days. The solvents were removed under reduced pressure and the resulting oil was co-evaporated with methanol (3 × 30 mL). The crude oil was redissolved in methanol (30 mL), filtered, and the filtrate precipitated in diethyl ether (500 mL). The resulting suspension was filtered, and the residue was rinsed with diethyl ether (3 × 15 mL). The ligand was obtained as a beige powder (449 mg, 97.4 %). ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm) = 1.80 (t, 6H, N(H)-CH_2-C*H*$_2$-CH_2-N(H), *J* = 6.8), 3.18 (b, 6H, N(H)-CH_2-C*H*$_2$-N), 3.46 (s, 9H, Ar-N-C*H*$_3$), 3.54 (m, 12H, N(H)-C*H*$_2$-CH_2-C*H*$_2$-N(H)), 3.78 (b, 6H, N(H)-C*H*$_2$-CH_2-N), 6.53 (d, 3H, HOPO Ar-*H*, *J* = 7.2), 7.19 (d, 3H, HOPO Ar-*H*, *J* = 7.2), 7.36 (s, 6H, TAM Ar-*H*), 8.58 (t, 3H, CH_2-N(*H*)-C(O), *J* = 5.6), 9.00 (bt, 3H, CH_2-N(*H*)-C(O), *J* = 5.6), 9.16 (bt, 3H, CH_2-N(*H*)-C(O), *J* = 5.6); MALDI-MS m/z = 1308.9 (M^-), (Calcd. 1307.5); Anal. Found for **(S7)**· 1.3 H_2O· 4.1 CH_3OH· 1 HCl (Calcd.). C 52.32 (51.31), H 5.84 (5.99), N 12.05 (12.14).

Fe,Gd-L_A. A solution of $Fe(acac)_3$ (11.8 mg, 33.4 μmol) in methanol (3 mL) was added to a solution of the ligand TREN-tris(TAM-C3-HOPO) **(S7)** (50.1 mg, 33.4 μmol) in dimethylformamide (3 mL). The pH was adjusted to ≈ 9 with $KHCO_{3\ (aq)}$ and the dark red solution was stirred under $N_{2(g)}$ for 3 h. A solution of $Gd(acac)_3$ (17.0 mg, 33.5 μmol)

in methanol (3 mL) was then added to the reaction mixture which was stirred at 40 °C for 18 h. Removal of the solvents under reduced pressure yielded **Fe,Gd-L_A** as a deep red powder that was dried under high vacuum at room temperature for 18 h. ES-MS = 503.9 (M^{3-}), (Calcd. 504.1) The isotopic distribution corresponds to the theoretical one. A detailed mass spectra analysis is shown in Figure S1.

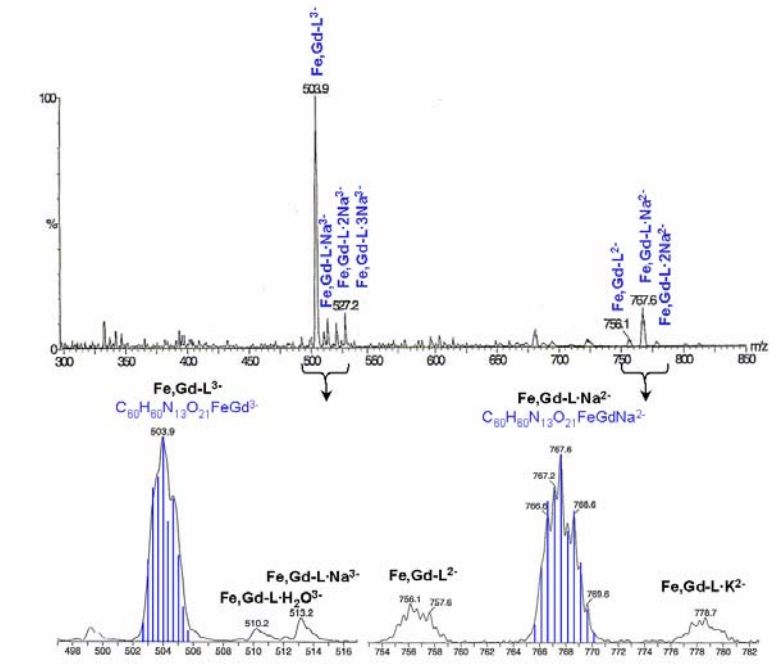
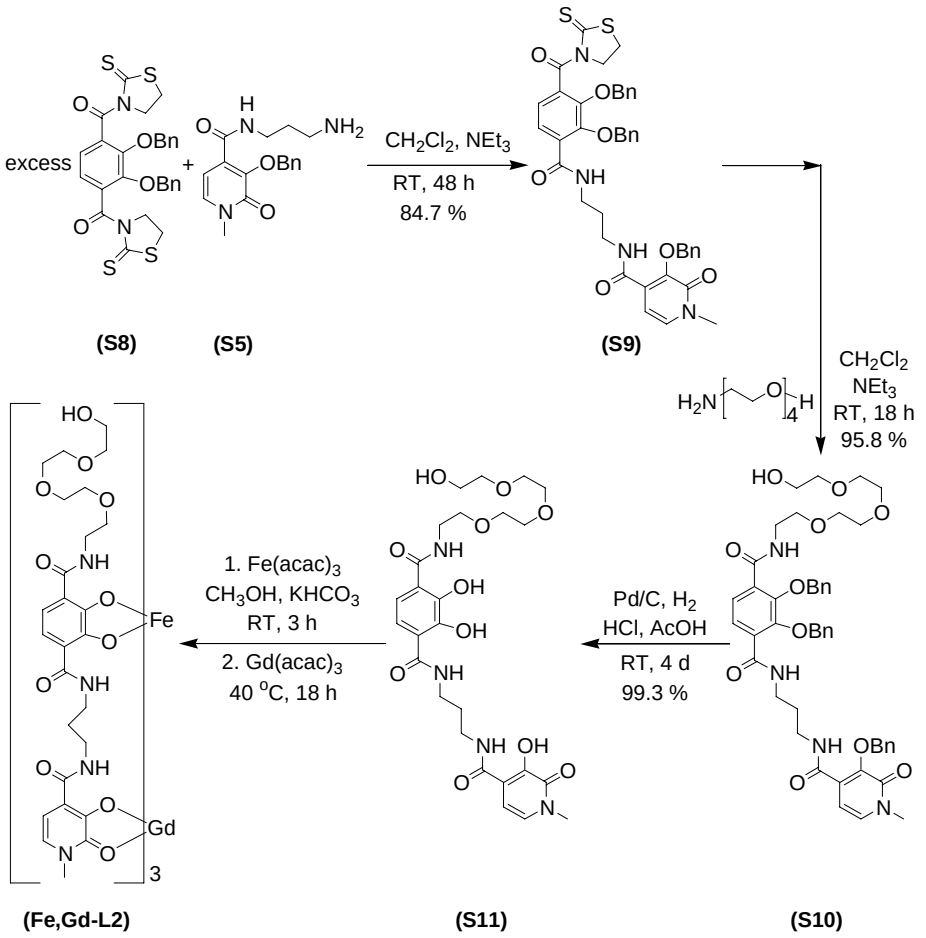


Figure S1. Calculated (blue) and experimental electrospray (black) mass spectra of Fe,Gd- **L_A**



Scheme S2. Synthesis of **Fe,Gd-3L2.**

HOPO-Bn-C3-TAM-Bn$_2$-thiaz (S9). A solution of HOPO-Bn-C3-NH$_2$ **(S5)** (0.320 g, 1.02 mmol) in methylene chloride (100 mL) and triethylamine (20 mL) was added dropwise over 1 h to a solution of TAM-Bn$_2$-thiaz$_2$ **(S8)**[3] (2.96 g, 5.10 mmol) in methylene chloride (20 mL). After 48 h of stirring, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 95 % CH_2Cl_2 / 5 % CH_3OH. Removal of the solvent under reduced pressure yielded the activated **(S9)** as a yellow foam (0.671 g, 84.7 %). ^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 1.38 (p, 2H, NH-CH_2-C*H*$_2$-CH_2-NH, *J* = 6.8), 3.03 (t, 2H, N-CH_2-C*H*$_2$-S, *J* = 7.6), 3.14 (p, 4H, NH-C*H*$_2$-CH_2-C*H*$_2$-NH, *J* = 6.0), 3.52 (s, 3H, ArN-C*H*$_3$), 4.40 (t, 2H, N-C*H*$_2$-CH_2-S, *J* = 7.2), 5.11 (s, 2H, Ar-C*H*$_2$-O), 5.13 (s, 2H, Ar-C*H*$_2$-O), 5.38 (s, 2H, Ar-C*H*$_2$-O), 6.64 (d, 1H, HOPO Ar-*H*, *J* = 7.2), 7.11 (d,

[3] Doble, D. M. J.; Botta, M.; Wang, J.; Aime, S.; Barge, A.; Raymond, K. N. *J. Am. Chem. Soc.* **2001,** *123*, 10758-10759.

1H, HOPO Ar-*H*, *J* = 7.2), 7.16 (d, 1H, TAM Ar-*H*, *J* = 8), 7.26-7.43 (m, 15H, Ar-*H*), 7.76 (t, 1H, N*H*-C(O), *J* = 5.6), 7.79 (d, 1H, TAM Ar-*H*, *J* = 8), 7.96 (t, 1H, N*H*-C(O), *J* = 5.6); ^{13}C NMR (100 MHz, CD_2Cl_2) δ (ppm) = 29.4, 29.8, 37.3, 37.4, 38.0, 54.4, 59.4, 74.9, 76.6, 77.3, 104.7, 124.5, 126.9, 127.6, 128.6, 128.7, 128.9, 129.0, 129.1, 129.2, 129.2, 129.3, 129.5, 129.6, 131.0, 131.2, 132.8, 134.1, 136.5, 137.1, 137.6, 146.7, 150.0, 150.7, 160.0, 163.8, 164.7, 167.2, 202.3; MALDI-MS m/z = 776.8 (M^+), (Calcd. 776.2); Anal. Found (Calcd.). C 64.78 (64.93), H 5.42 (5.19), N 6.98 (7.21), S 8.14 (8.25).

HOPO-Bn-C3-TAM-Bn$_2$-dPEG$_4$ (S10). A solution of HOPO-Bn-C3-TAM-Bn$_2$-thiaz **(S9)** (0.650 g, 0.837 mmol) and amino-dPEG$_4$ (0.200 g, 1.03 mmol) in methylene chloride (20 mL) and triethylamine (5 mL) was stirred at room temperature for 18 h. The solvents were then removed under reduced pressure and the product was purified by flash chromatography over silica eluting with a gradient of 100 % CH_2Cl_2 to 95 % / 5 % CH_2Cl_2. Removal of the solvents under reduced pressure yielded the protected ligand as a colorless glass (0.679 g, 95.8 %). ^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 1.38 (p, 2H, NH-CH_2-C*H*$_2$-CH_2-NH, *J* = 6.4), 3.15 (2p, 4H, NH-C*H*$_2$-CH_2-C*H*$_2$-NH, *J* = *J'* = 6.4), 3.52 (s, 6H, Ar-N-C*H*$_3$), 3.46 (t, 2H, O-CH_2-C*H*$_2$-OH, *J* = 5.2), 3.53-3.56 (m, 14H, C*H*$_2$-C*H*$_2$-O), 5.13 (s, 2H, Ar-C*H*$_2$-O), 5.16 (s, 2H, Ar-C*H*$_2$-O), 5.36 (s, 2H, Ar-C*H*$_2$-O), 6.63 (d, 1H, HOPO Ar-*H*, *J* = 7.2), 7.10 (d, 1H, HOPO Ar-*H*, *J* = 7.2), 7.27-7.44 (m, 15H, Ar-*H*), 7.71 (d, 1H, TAM Ar-*H*, *J* = 8.8), 7.77 (d, 1H, TAM Ar-*H*, *J* = 8.4), 7.94 (t, 1H, N*H*-C(O), *J* = 6), 8.04 (t, 1H, N*H*-C(O), *J* = 6); ^{13}C NMR (100 MHz, CD_2Cl_2) δ (ppm) = 29.7, 37.4, 38.0, 40.2, 54.4, 62.0, 70.1, 70.6, 70.7, 70.9, 71.0, 72.9, 74.9, 77.4, 77.6, 104.7, 126.3, 126.5, 129.1, 129.2, 129.2, 129.3, 129.4, 129.6, 131.1, 131.3, 132.2, 132.8, 136.6, 136.7, 137.1, 146.7, 150.8, 151.1, 160.0, 163.9, 164.9, 165.0, MALDI-MS m/z = 851.2 (MH^+), (Calcd. 851.4); Anal. Found (Calcd.). C 66.03 (66.34), H 6.68 (6.40), N 6.32 (6.58).

HOPO-C3-TAM-dPEG$_4$ (S11). The protected ligand HOPO-Bn-C3-TAM-Bn$_2$ **(S10)** (650 mg, 0.763 mmol) was dissolved in a mixture of glacial acetic acid (20 mL), Millipore water (4 mL) and concentrated $HCl_{(aq)}$ (3 mL). 10 % Pd / C (227 mg) was added to the solution and the resulting suspension was stirred under an atmosphere of $H_{2(g)}$ at room temperature for 4 days. 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 with diethyl ether (500 mL). The ether was decanted, and the residual oil was dissolved in methanol (20 mL) and lyophilized under high vacuum at room temperature for 24 h. The deprotected ligand **(S11)** was obtained as a beige solid (450 mg, 99.3 %). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) = 1.94 (q, 2H, CH_2-CH_2-CH_2, *J* = 6.4), 3.51-3.56 (m, 6H, CH_2-CH_2-CH_2 and CH_2-CH_2-OH), 3.58 (s, 3H, Ar N-CH_3), 3.61-3.68 (m, 14H, CH_2-O-CH_2), 6.64 (d, 1H, HOPO Ar-*H*, *J* = 7.2), 7.08 (d, 1H, HOPO Ar-*H*, *J* = 7.2), 7.27 (d, 1H, TAM Ar-*H*, *J* = 8.4), 7.31 (d, 1H, TAM Ar-*H*, *J* = 8.4); MALDI-MS m/z = 581.9 (M^+), (Calcd. 581.2); Anal. Found for **(S11)**· 0.4 H_2O· 0.2 CH_3OH (Calcd.). C 52.93 (52.91), H 6.03 (6.38), N 9.18 (9.43).

Fe,Gd-3L$_B$. A solution of $Fe(acac)_3$ (5.95 mg, 16.9 μmol) in methanol (3 mL) was added to a solution of the ligand HOPO-C3-TAM-dPEG$_4$ **(S11)** (30.3 g, 51.0 mmol) in methanol (3 mL). The pH was adjusted to ≈ 9 with $KHCO_{3\ (aq)}$ and the dark red solution was stirred under $N_{2(g)}$ for 3 h. A solution of $Gd(acac)_3$ (8.68 mg, 17.0 μmol) in methanol (3 mL) was then added to the reaction mixture which was stirred at 40 °C for 18 h. Removal of the solvents under reduced pressure yielded **Fe,Gd-3L$_B$** as a deep red powder that was dried under high vacuum at room temperature for 18 h. ES-MS = 973.3 (M^{2-}), (Calcd. 972.8). The isotopic distribution corresponds to the theoretical one. A detailed mass spectra analysis is shown in Figure S2.

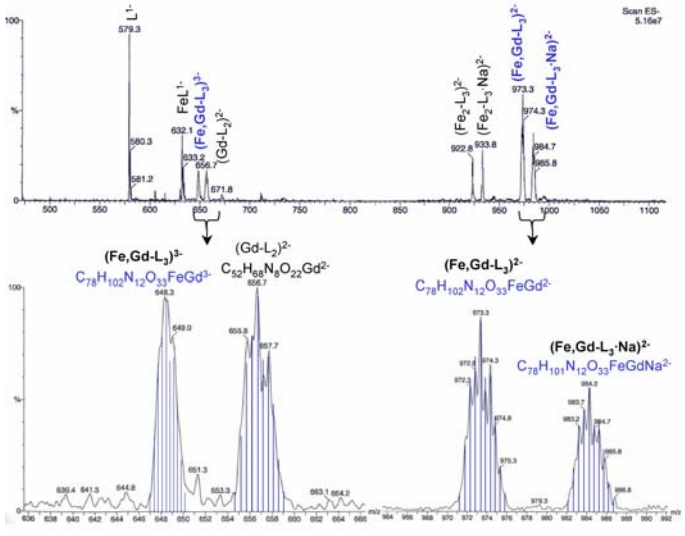
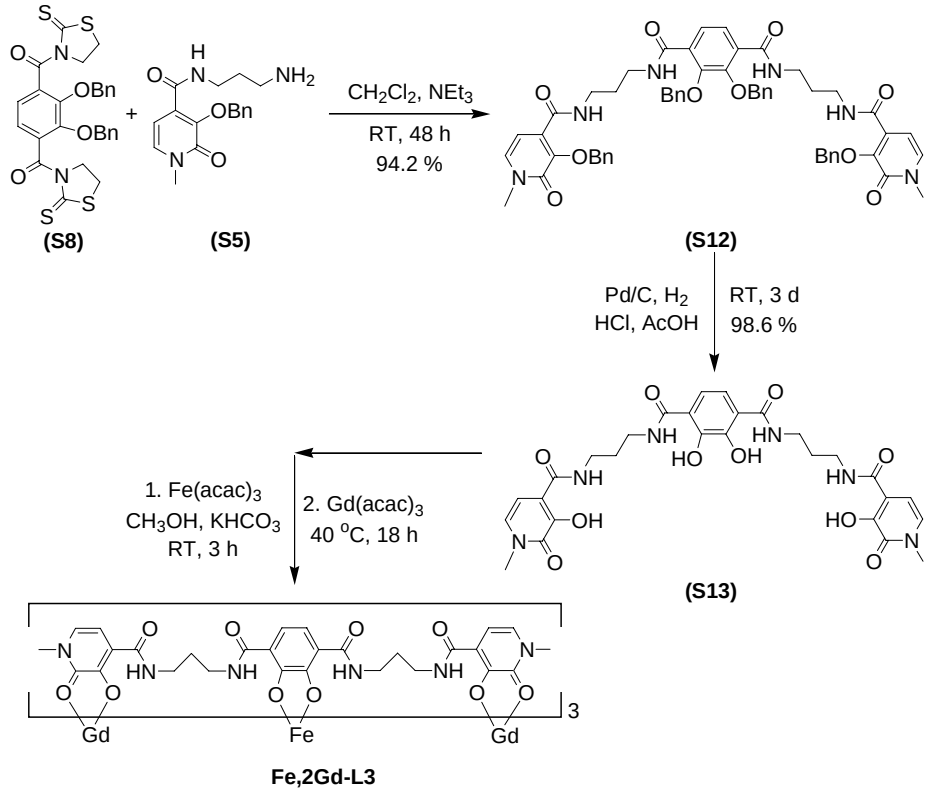


Figure S2. Calculated (blue) and experimental electrospray (black) mass spectra of Fe,Gd-3**L$_B$**.



Scheme S3. Synthesis of **Fe,2Gd-3L$_C$.**

2C3-TAM-Bn$_2$-(HOPO-Bn)$_2$ (S12). A solution of HOPO-Bn-C3-NH$_2$ **(S5)** (0.350 g, 1.11 mmol) in methylene chloride (20 mL) and triethylamine (5 mL) was added to a solution of TAM-Bn$_2$-thiaz$_2$ **(S8)** (0.292 g, 0.502 mmol) in methylene chloride (5 mL). The solution was stirred at room temperature for 48 h. The solvents were 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 90 % CH_2Cl_2 / 10 % CH_3OH. Removal of the solvents under reduced pressure yielded the protected ligand **(S12)** (0.460 g, 94.2 %) as a white foam that was dried under high vacuum at room temperature for 18 h. ^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 1.40 (p, 2H, NH-CH_2-C*H*$_2$-CH_2-NH, *J* = 6.8), 3.16 (m, 4H, NH-C*H*$_2$-CH_2-C*H*$_2$-NH), 3.51 (s, 6H, ArN-C*H*$_3$), 5.15 (s, 4H, Ar-C*H*$_2$-O), 5.36 (s, 4H, Ar-C*H*$_2$-O), 6.63 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 7.10 (s, 2H, HOPO Ar-*H*, *J* = 7.2), 7.30-7.45 (m, 20H, Ar-*H*), 7.76 (s, 2H, TAM Ar-*H*), 7,77 (t, 2H, N(*H*)-C(O), *J* = 6.0), 7.95 (t, 2H, N(*H*)-C(O), *J* = 5.6); ^{13}C NMR (100 MHz, CD_2Cl_2) δ (ppm) = 29.7, 37.4, 38.0, 54.4, 74.9, 77.6, 104.7, 126.5, 129.1, 129.2, 129.3, 129.4, 129.6, 131.2, 131.5, 132.8, 136.5, 137.1, 146.7, 150.9, 160.0, 163.9, 164.8; MALDI-MS

m/z = 972.7 (M^+), (Calcd. 972.4); Anal. Found (Calcd.). C 69.40 (69.12), H 6.18 (5.80), N 8.36 (8.64).

2C3-TAM-(HOPO)$_2$ (S13). The protected ligand 2C3-TAM-Bn$_2$-(HOPO-Bn)$_2$ **(S12)** (420 mg, 0.432 mmol) was dissolved in a mixture of glacial acetic acid (30 mL), Millipore water (5 mL) and concentrated $HCl_{(aq)}$ (4 mL). 10 % Pd / C (192 mg) was added to the solution and the resulting suspension was stirred under an atmosphere of $H_{2(g)}$ at room temperature for 3 days. 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 oil was redissolved in methanol (30 mL), filtered, and the filtrate 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 40 °C for 15 h. The deprotected ligand **(S13)** was obtained as a white powder (280 mg, 98.6 %).^{1}H NMR (400 MHz, CD_3OD) δ (ppm) = 1.93 (p, 4H, NH-CH_2-C*H*$_2$-CH_2-NH, *J* = 6.8), 3.51 (t, 8H, NH-C*H*$_2$-CH_2-C*H*$_2$-NH, *J* = 6.4), 3.57 (s, 6H, ArN-C*H*$_3$), 6.64 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 7.10 (d, 2H, HOPO Ar-*H*, *J* = 7.2), 7.24 (s, 2H, TAM Ar-*H*), 7.33 (t, 1H, N*H*-C(O), *J* = 7), 7.45 (t, 1H, N*H*-C(O), *J* = 7); MALDI-MS m/z = 612.3 (M^+), (Calcd. 612.2); Anal. Found for **(S13)**· 1.4 CH_3OH (Calcd.). C 53.88 (53.70), H 5.47 (5.77), N 12.39 (12.77).

Fe$_2$,Gd$_2$-3L$_C$. A solution of $Fe(acac)_3$ (5.31 mg, 15.0 μmol) in methanol (3 mL) was added to a solution of the ligand 2C3-TAM-HOPO$_2$ **(S13)** (30.1 g, 45.7 μmol) in methanol (3 mL). The pH was adjusted to ≈ 9 with $KHCO_{3\ (aq)}$ and the dark red solution was stirred under N_2 for 3 h. A solution of $Gd(acac)_3$ (15.5 mg, 30.5 mmol) in methanol (3 mL) was then added to the reaction mixture which was stirred at 40 °C for 18 h. Removal of the solvents under reduced pressure yielded **Fe$_2$,Gd$_2$-3L$_C$** as a deep red powder that was dried under high vacuum at room temperature for 18 h ES-MS = 1117.2 ($M+K^{2-}$), (Calcd. 1117.1). The isotopic distribution corresponds to the theoretical one. A detailed mass spectra analysis is shown in S3.

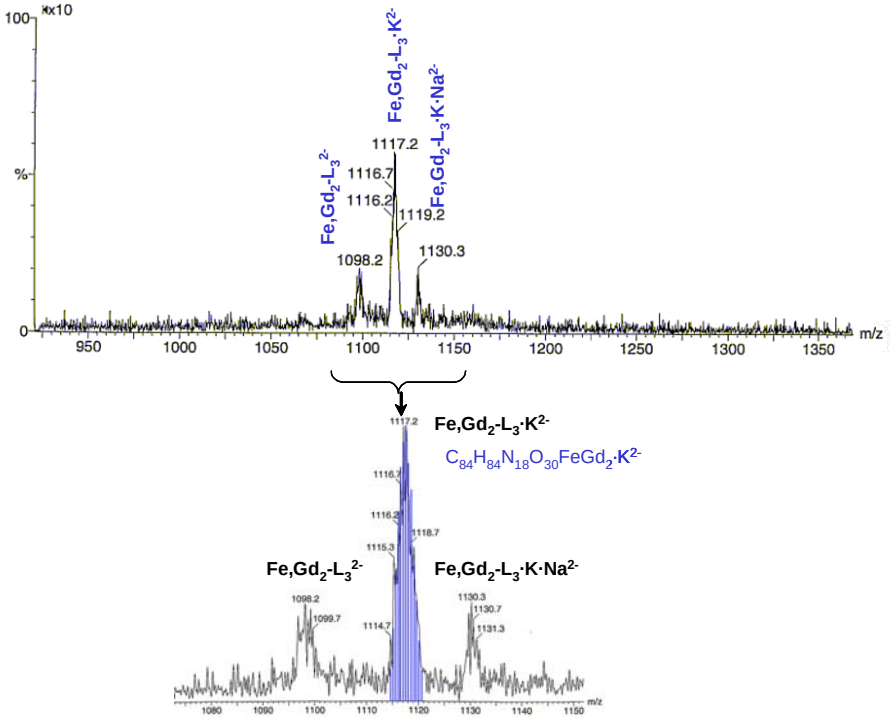


Figure S2. Calculated (blue) and experimental electrospray (black) mass spectra of Fe,2Gd-3$\mathbf{L_C}$.

pH-dependence of the selectivity of Fe(III) for TAM over HOPO ligands.

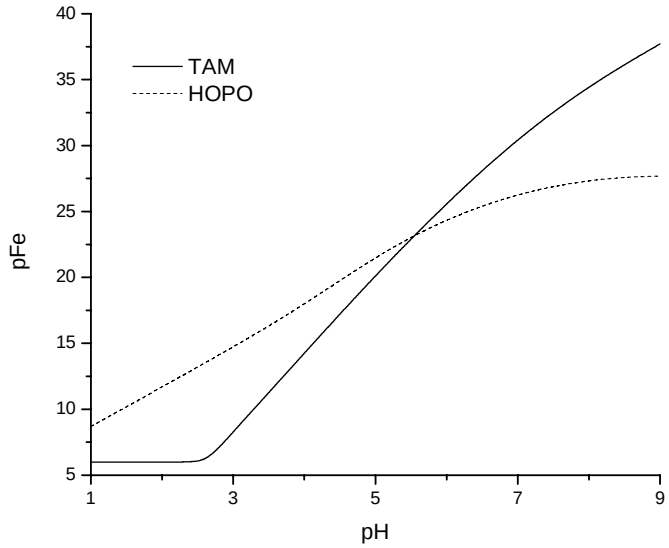


Figure S1. Selectivity of Fe(III) for 2,3-dihydroxyterephthalamide (TAM) over 3-hydroxy-2-pyridinone (HOPO) as a function of pH. Calculated from references [7,8].

Inner- and outer-sphere contributions to the NMRD profiles of the assemblies. MM3 geometry minimization of the assemblies

Fe,Gd-L_A
25°C – pH = 7.4

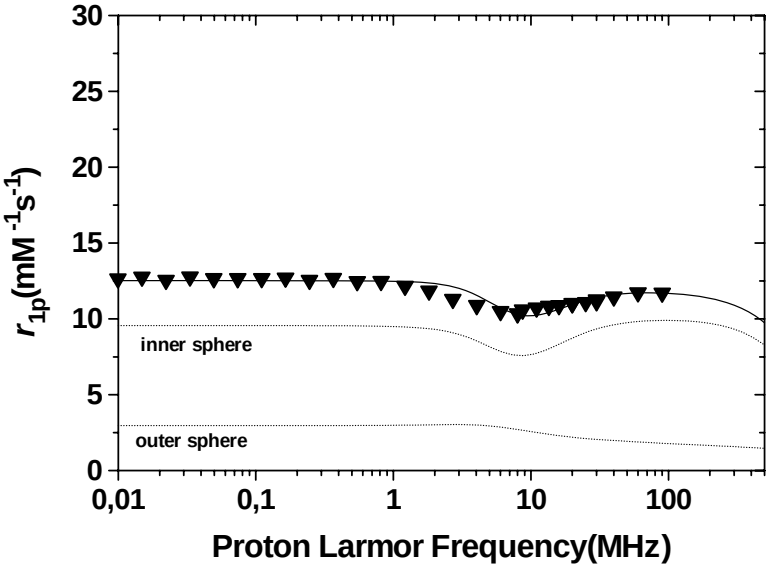


Figure S4. Inner- and outer-sphere contributions to the $1/T_1$ NMRD profile at 298 K and pH 7.4 of Fe,Gd- $\mathbf{L_A}$.

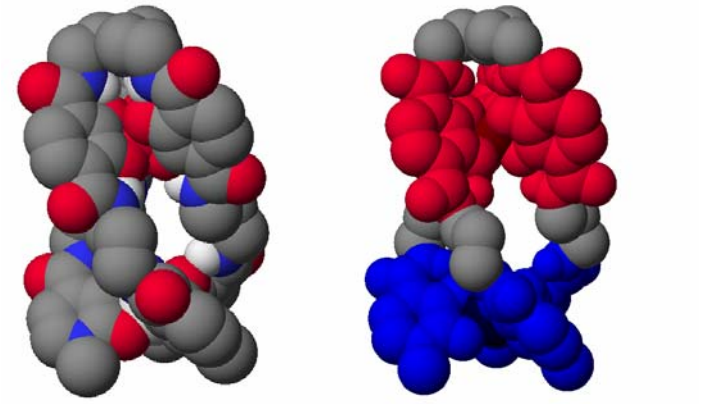


Figure S5. MM3 geometry minimization of Fe,Gd- $\mathbf{L_A}$.[4] The Fe(III) complexation is shown in red, that of Gd(III) is shown in blue.

[4] MM3 minimizations were performed on CAChe® WorkSystem Professional Version 6.1.10.

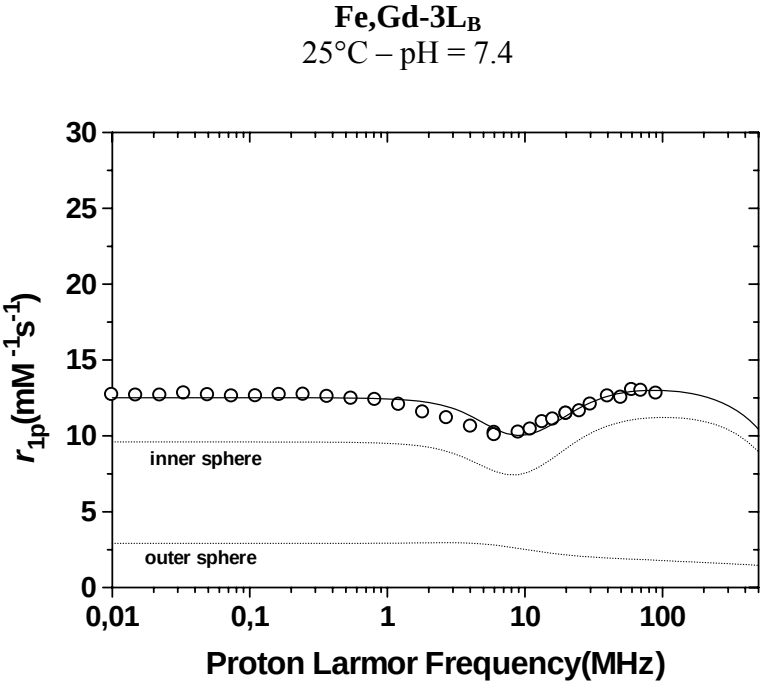


Figure S6. Inner- and outer-sphere contributions to the $1/T_1$ NMRD profile at 298 K and pH 7.4 of Fe,Gd-3 $\mathbf{L_B}$.

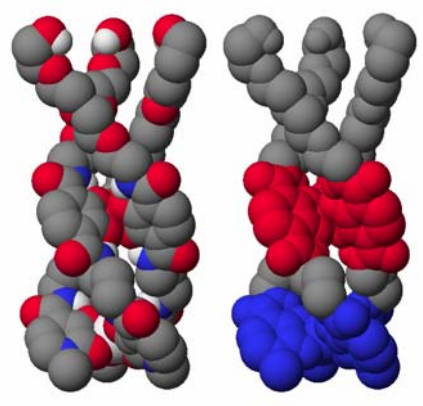


Figure S7. MM3 geometry minimization of Fe,Gd-3 $\mathbf{L_B}$. The Fe(III) complexation is shown in red, that of Gd(III) is shown in blue.

Fe,2Gd-3L$_C$

25°C – pH = 7.4

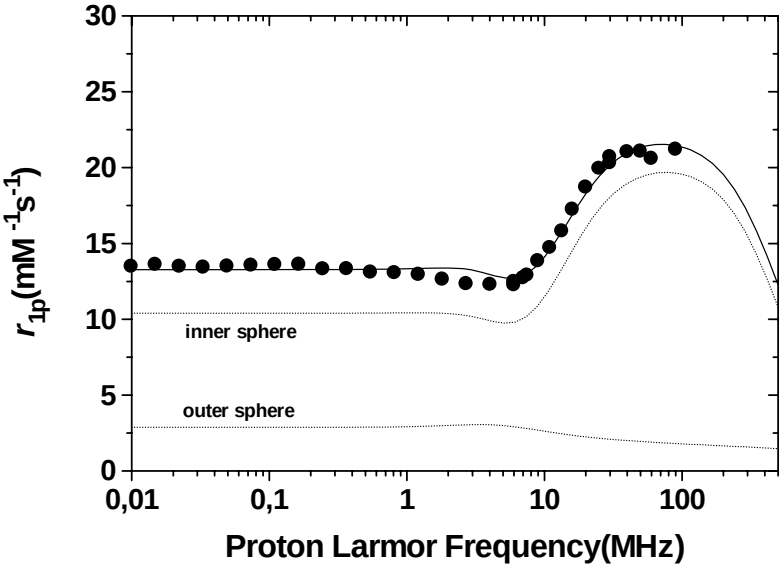


Figure S8. . Inner- and outer-sphere contributions to the $1/T_1$ NMRD profile at 298 K and pH 7.4 of Fe,2Gd-3 **L$_C$**.

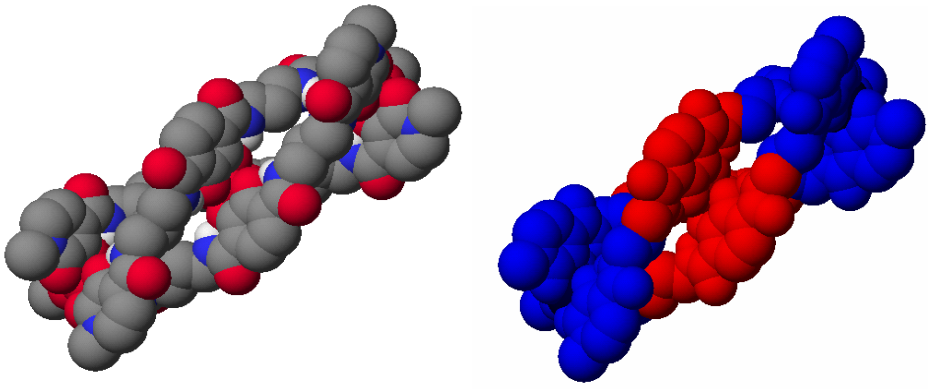


Figure S9. MM3 geometry minimization of Fe,Gd-3 **L$_C$**. The Fe(III) complexation is shown in red, that of Gd(III) is shown in blue.