Supplementary Information for

$[Gd(CyPic3A)(H_2O)_2)]^-$: A Stable, Bis(aquated) and High-Relaxivity Gd(III) Complex

Eric M. Gale, Nathaniel Kenton and Peter Caravan*

Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, 149 Thirteenth Street, Charlestown, MA 02129, USA

E-mail: caravan@nmr.mgh.harvard.edu

Table of Contents

Experimental Methods

General. All chemicals and solvents were purchased commercially and used without further purification. NMR spectra were recorded on either a 500 MHz or 400 MHz Varian spectrometer equipped with a 5 mm broadband probe. Chemical shifts are reported in δ (ppm). For ^{1}H NMR spectra, the peak from residual protio solvent was used as an internal reference.[1] For ^{13}C NMR spectra, the solvent peaks were used as an internal reference, except when acquired in D_2O, in which case dioxane was used as the internal reference.[1] Liquid chromatography-electrospray mass spectrometry (LC-MS) was performed using an Agilent 1100 Series apparatus with an LC/MSD trap and Daly conversion diode detector with UV detection at 220, 254 and 280 nm. Characterization of CyPic3A, $[Gd(CyPic3A)(H_2O)_2]^-$, $[Eu(CyPic3A)(H_2O)_2]^-$ and ligand competition experiments were performed using a Kromasil C18 reversed-phase column (250 mm x 4.6 mm) by the following method: the mobile phase was a mixture of 10 mM aqueous ammonium formate (eluent A) and a solution of acetonitrile/ 10% 10 mM aqueous ammonium formate (eluent B). Starting from 5% B, the fraction of B increased to 95% over 12 minutes. The column was washed with 95% B for 2 min and than ramped to 5% B. The system was re-equilibrated at 5% B. Reversed-phase semi-preparative purification was performed on the Rainin Dynamax HPLC system with UV detection at 254 nm using a Kromasil C4 (250 x 21.8 cm) column. The method used for purification is as follows: the mobile phase was a mixture of water (eluent A) and acetonitrile (eluent B), each containing 0.1% TFA. Starting from 5% B, the fraction of B increased to 25% over 23 minutes. The column was washed with 95% B for 2 minutes and then ramped to 5% B. The system was re-equilibrated at 5% B. Gadolinium concentrations were determined using an Agilent 7500a ICP-MS system. All samples were diluted with 0.1% Triton X-100 in 5% nitric acid containing 20 ppb of Lu (as internal standard). The ratio of Gd (157.25)/Lu (174.97) was used to quantify the Gd concentration. A linear calibration curve ranging from 0.1 ppb to 200 ppb was generated daily for the quantification. pH was measured using a ThermoOrion pH meter connected to a VWR Symphony glass electrode. Ultrafiltration was performed using Ultrafree-MC Microcentrifuge filters with a 5,000 Da cut-off PLCC cellulosic membrane. Incubation of samples was performed using a New Brunswick Scientific Inova4000 Incubator Shaker.

Relaxometry. Relaxivity measurements were performed on a Bruker mq60 or Bruker mq20 Minispec at 1.41 T and 0.47 T, respectively, and 37 °C. Longitudinal (T_1) relaxation was acquired via an inversion recovery experiment on 10 inversions of duration ranging between 0.05 x T_1 and 5 x T_1. Relaxivity (r_1) was determined from the slope of a plot of $1/T_1$ vs [Gd] for at least 4 concentrations of Gd(III). The transverse (T_2) relaxation times of ^{17}O were acquired at 500 MHz using a CPMG pulse sequence at temperatures ranging from 278 to 368 K. Reduced relaxation rates ($1/T_{2r}$) were calculated by dividing the $[Gd(CyPic3A)(H_2O)_2]^-$ imparted increase in $1/T_2$ relative to neat H_2O at pH 3 by the mole fraction of coordinated water molecules. This data was plotted

against reciprocal temperature (1000/T (K^{-1})) and fit to a four-parameter model as described previously.[2] The Gd-O hyperfine coupling constant, $A/\hbar$, was assumed to be -3.79 x 10^6 rad/s.[3] Samples were prepared in neat H_2O adjusted and enriched with a small amount of $H_2{}^{17}O$.

Luminescence. Luminescence lifetime measurements were recorded on a Hitachi f-4500 fluorescence spectrophotometer on samples containing ~50 mM Eu(III). Samples in D_2O were lyophilized and reconstituted three times to ensure minimization of residual protio solvent. Excitation was achieved at 396 nm and emission was recorded at 616 nm. A total of 80 replicates were acquired for each sample and the results averaged. Temporal resolution was set to 0.04 ms (0-20 ms) and the PMT voltage was set to 400 V. The luminescent lifetimes were ascertained though monoexponential fits of the data.

Ligand challenge. Mixtures of precisely defined concentrations of $[Gd(CyPic3A)(H_2O)_2]^-$ and DTPA or MS-325-L were prepared in pH 4 citrate buffer (25 mM) and pH 7.4 Tris buffer (25 mM). Equilibration of each solution was monitored by LC-MS. Relative distributions of $[Gd(CyPic3A)(H_2O)_2]^-$ and CyPic3A were determined by integrating the corresponding absorbance traces at 280 nm. MS-325 and MS-325-L were analyzed analogously at 220 nm. K_{comp} values determined between $[Gd(DTPA)(H_2O)]^{2-}$ and various ligands were determined from conditional formation constants (K_{cond})[4] values taken from the literature.[5-10] Direct comparisons were only made between K_{cond} values determined under identical conditions.

Kinetic inertness. Modified conditions of Muller and co-workers were used for this experiment.[11,12] Solutions containing 2.5 mM $[Gd(CyPic3A)(H_2O)_2]^-$ and 2.5 mM $Zn(OTf)_2$ were combined in pH 7.0 phosphate buffer (50 mM) and placed in an incubator shaker set to 310 K. Progress of the transchelation was monitored via the decrease in $1/T_1$ with time. Prior to measurement, aliquots were placed in small glass inserts and centrifuged to bring all insoluble material to the bottom so as not to interfere with the measurement.

Synthesis of CyPic3A. The synthesis comprised the following steps:

N-BOC-N'-((6-methyl picol-2-yl)methyl)-*trans*-1,2-diaminocyclohexane (3). A batch of 349 mg (1.63 mmol) N-BOC-*trans*-1,2-diaminocyclohexane (**1**)[14] was added to 269 mg (1.63 mmol) of methyl 6-formylpyridine-2-carboxylate (**2**)[15] stirring in 10 mL MeOH. Small aliquots of this reaction were removed and concentrated to dryness for NMR analysis to confirm full Schiff base formation. After 2h, the reaction was cooled to 0° C and 66 mg (1.75 mmoL) $NaBH_4$ was added in 1 mL MeOH forming a deep red solution within minutes. After 2h of stirring at 0° C, the reaction was quenched with satd. $NaHCO_{3(aq)}$ and MeOH was removed via rotary evaporation. The volume of the resultant solution was doubled via addition of CH_2Cl_2 and the reaction was brought to pH 7 using 1M $HCl_{(aq)}$. The organic layer was separated the aqueous phase washed 3x with CH_2Cl_2. The CH_2Cl_2 was pooled, dried over Na_2SO_4 and concentrated to 502 mg (1.38 mmol, 85 %) of **3** as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.97 (d, 1H), 7.75 (t,1H), 7.54 (d, br, 1H), 5.18 (d, br, 1H, N*H*), 4.08 (d, 1H), 3.99-3.95 (m, 4H), 3.32 (m, br, 1H), 2.37 (m, 1H), 2.09-2.01 (m, 2H), 1.66-1.62 (m, 2H), 1.41 (s, 9H), 1.29-1.05 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 165.95, 161.82, 156.21, 147.24, 137.33, 125.70, 123.35, 79.07, 64.67, 60.42, 54.75, 51.28, 33.18, 31.97, 28.49, 24.97, 24.61. ESI^+ ($M + H^+$) *m/z* = 364.3; calcd: 364.2.

N-((6-methyl picol-2-yl)methyl)-*trans*-1,2-diaminocyclohexane (4). A batch of 611 mg (1.68 mmol) **3** was dissolved in 3 mL each CH_2Cl_2: TFA. After 45 min the reaction mixture was concentrated to a pink oil which was taken up in 30 mL CH_2Cl_2 and stirred over a large excess of K_2CO_3. After 3h, the resultant light yellow solution was filtered and concentrated to 266 mg (1.00 mmol, 60 %) of **4** as a yellow oil. It should be noted that the filtrate often contained very little product when the reaction was performed on a larger scale; when this occurred, the slurry containing product and K_2CO_3 could be carried directly through to the next step (assuming 100% product) and afforded excellent yields. ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 8.00 (d, 1H), 7.81 (t, 1H), 7.67 (d, 1H), 4.17 (d, 1H), 4.40-3.96 (m, 4H), 2.45 (m, 1H), 2.13 (m, 2H), 1.92-1.67 (m, 7H, NH_2 and N*H* found as broad resonance), 1.30-1.07 (m, 4H). ^{13}C NMR ($CDCl_3$, 125.7 MHz), δ (ppm): 166.08, 161.88, 147.42, 137.47, 125.80, 123.55, 63.95, 55.53, 52.52, 36.14, 31.74, 35.39, 25.33. ESI^+ ($M + H^+$): *m/z* = 264.2; calcd: 264.2.

N-((6-methyl picol-2-yl)methyl)-N,N',N'-tri(*tert*-butylacetate)-*trans*-1,2-diaminocyclohexane (5). To 266 mg (1.13 mmol) of **4**, 535 mg (3.22 mmol) of potassium iodide and 922 mg (7.13 mmol) diisopropylethylamine stirring in 3 mL DMF was added 732 mg (3.75 mmol) *tert*-butyl bromoacetate at RT. After 16 h, the resultant yellow, heterogeneous solution was diluted with 50 mL CH_2Cl_2, washed with satd. $K_2CO_{3(aq)}$, copious water and brine. The organic portion was than dried with Na_2SO_4 and concentrated to a yellow oil. After chromatography on silica gel using 9:1 to 3:1 hexane: EtOAc, 2.600 g (4.29 mmol, 75 %) of **5** was isolated as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 8.05 (d, 1H), 7.92 (t,

1H), 7.76 (t, 1H), 4.12 (d, 1H), 3.94 (s, 3H), 3.80 (d, 1H), 3.45-3.20 (m, 6H), 2.62 (m, br, 1H), 2.53 (t, br, 1H). 2.07-1.99 (m, 2H), 1.66 (s, br, 2H), 1.36 (s, 27H), 1.09-1.00 (m, 4H). ^{13}C NMR ($CDCl_3$, 125.7 MHz), δ (ppm): 171.56, 171.49, 166.05, 161.57, 146.76, 137.37, 127.89, 123.57, 80.76, 80.54, 63.18, 61.70, 58.13, 55.75, 52.83, 28.07 (C(*C*H_3) peaks are coincidental), 26.78, 25.81, 25.66, 18.38. ESI$^+$ (M + H$^+$): *m/z* = 606.4; calcd: 606.4.

N-((6-methyl picol-2-yl)methyl)-N,N',N'-triacetate-*trans*-1,2-diaminocyclohexane (CyPic3·2TFA). To a batch of 173 mg (0.29 mmol) **5** in 4 mL 1:1 THF: H2O was added 28 mg (1.17 mmol) lithium hydroxide. After 4h stirring at RT, the reaction was concentrated to dryness and the resultant residue re-dissolved in 8 mL TFA and stirred at RT. After 16 h, the reaction mixture was concentrated to dryness and purified by preparative HPLC using the method described above (general methods). After pooling of the fractions containing product followed by lyophilization, 84 mg CyPic3A•2TFA was isolated as a white powder. The NMR spectra of the isolated ligand affords broad and ill-resolved peaks when dissolved in D_2O untreated. ^{1}H NMR (D_2O, 500 MHz), δ (ppm): 8.53 (t, 1H), 8.31 (d, 1H), 8.14 (s, br, 1H), 4.60-3.03 (m, br, 10H), 2.28 (d, 1H), 2.13 (s, br, 1H), 1.85 (s, br, 2H), 1.51-1.26 (m, br, 4H). ^{1}H NMR (D_2O, pH $\geq$ 10, 500 MHz), δ (ppm): 7.87 (t, 1H), 7.79 (d, 1H), 7.63 (d, 1H), 3.71 (q, 2H), 3.40 (d, 1H), 3.32 (d, 1H), 2.99 (d, 1H), 2.73 (d, 1H), 2.58-2.49 (m, 2H), 2.39 (t, 1H), 2.15-2.07 (m, 2H), 1.90 (d, 1H), 1.71 (t, 2H), 1.18-1.09 (m, 3H), 0.90 (q, 1H). ^{13}C NMR (D_2O, pH $\geq$ 10, 125.7 MHz), δ (ppm): The ^{13}C resonances were stronger and more visible at alkaline pH: 181.76, 181.44, 181.19, 174.03, 157.62, 153.67, 139.04, 127.42, 122.78, 61.44, 59.00, 58.02, 57.30, 54.23, 52.29, 25.63, 25.50, 24.72, 24.59. ESI$^+$ (M + H$^+$): *m/z* = 424.2; calcd: 424.2

[Gd(CyPic3A)(H_2O)$_2$]$^-$. To a batch of 54.5 mg (0.0837 mmol) CyPic3A·2TFA in H_2O was added 31.1 mg (0.0837 mmol) of $GdCl_3·6H_2O$ and the solution adjusted to pH 6.5. Full chelation was affirmed by the testing of small aliquots of this solution in Arsenazo III (0.01 mM in 0.15 M pH 7 ammonium acetate buffer). ESI$^+$ (MW + 2H$^+$): *m/*z = 579.0; calcd: 579.1. Less than 1% free ligand was observed by LC-MS.

[Eu(CyPic3A)(H_2O)$_2$]$^-$. To a batch of 54.8 mg (0.0841 mmol) CyPic3A·2TFA in H_2O was added 24.3 mg (0.0663 mmol) of $EuCl_3·6H_2O$ was added and the solution adjusted to pH 7.2. Analysis by LC-MS revealed full chelation with a slight excess of ligand species present. This was done to ensure against free Eu(III) during the luminescence measurments. ESI$^+$ (MW + 2H$^+$): *m/z* = 574.0; calcd: 574.1

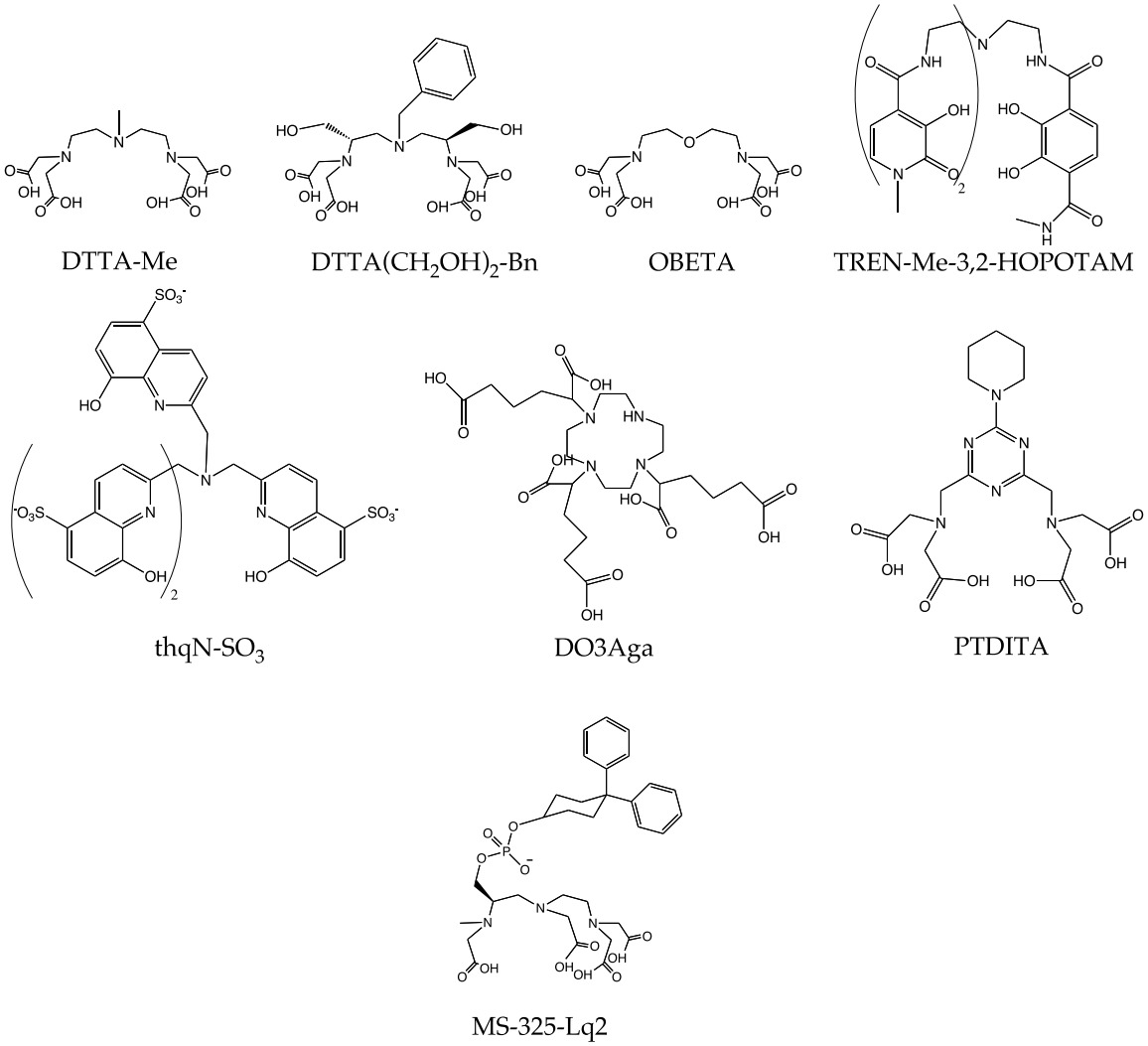

Figure S1. Additional ligands (DTTA-Me,[16] DTTA(CH_2OH)$_2$-Bn,[17] OBETA,[18] thqN-SO_3,[19] DO3Aga,[20] PTDITA[21] and MS-325-Lq2[22]) that support Gd(III) complexes of q = 2.

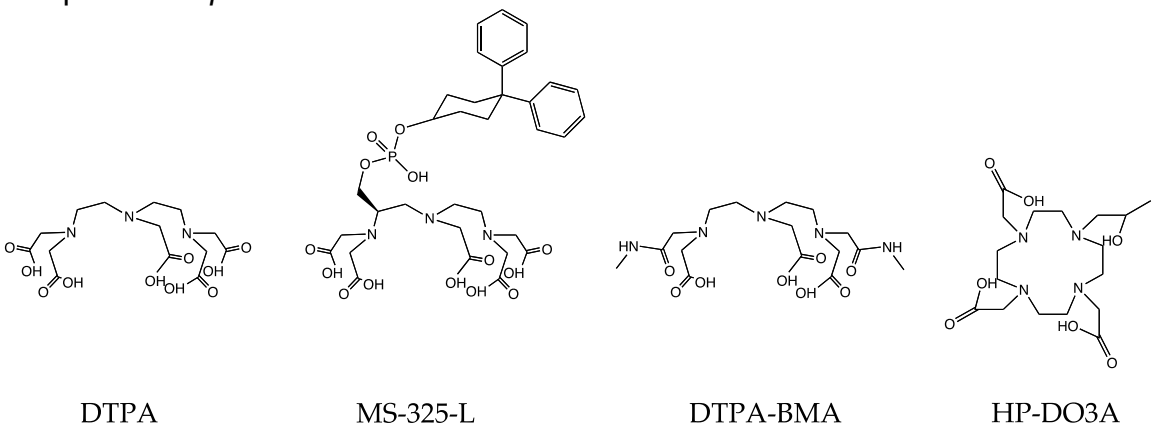

Figure S4. Gd(III) chelators that CyPic3A is compared against in this manuscript. DTPA, MS-325-L DTPA-BMA and HP-DO3A are the ligand components of the

clinically utilized Magnevist®, Ablavar® (MS-325), Omniscan® and ProHance®; each of which forms a ternary Gd(III) complex of q=1.

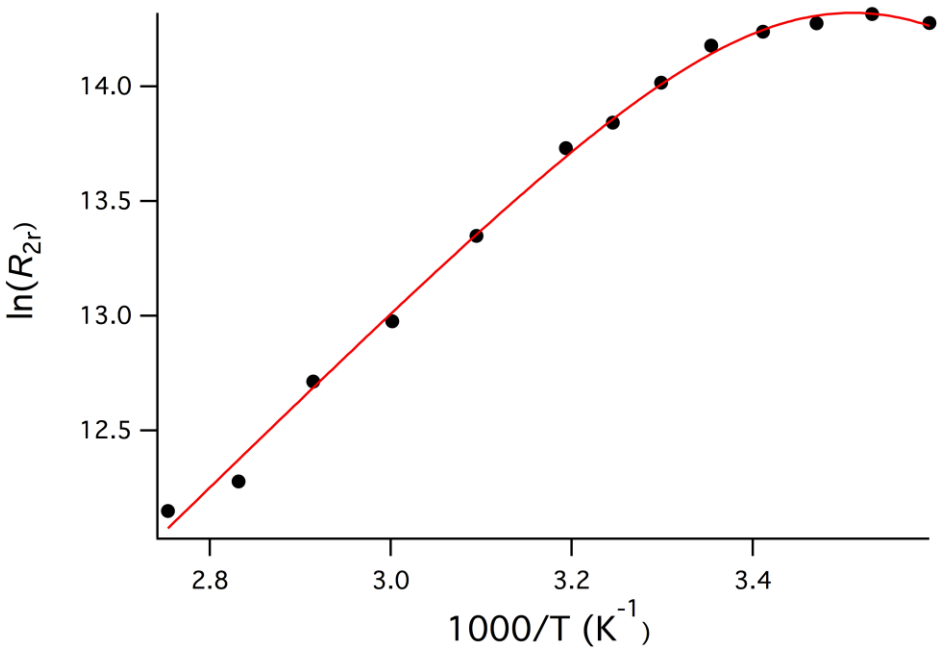

Figure S3. Temperature dependence on the reduced transverse relation rates of $[Gd(CyPic)(H_2O)_2]^-$. Solid red line represents the fit to the data.

	k_{ex}^{298} (x10^6 s^{-1})	τ_m^{310} (ns)	$\Delta H^{\neq}$ (kJ/mol)	T_{1e}^{298} (ns) (11.7T)	ΔE_{T1e} (kJ/mol)
[Gd(CyPic3A)(H_2O)$_2$]$^-$	44	14±1	29.1±1.5	104	35.1±13.3
[Gd(DTPA)(H_2O)]$^{2-}$ [23]	3.3±0.2	130	51.6±1.4	481[c]	--
MS-325 (2*R*, 4*S*) [2]	5.9±2.8	79±37	56±8	37[d]	-12.7±2.3
MS-325 (2*R*, 4*R*) [2]	3.2±1.8	140±74	59±11	37[d]	-12.7±2.3
[Gd(DTPA-BMA)(H_2O)] [23]	0.45±0.01	220	47.6±1.1	539[c]	--
[Gd(HPDO3A)(H_2O)] [24,a]	1.6	320[b]	53±8	72[c]	--
[Gd(TREN-Me-3,2-HOPOTAM)(H_2O)$_2$]$^-$ [25]	91±30	9.5[b]	7±0.8	--	--
[Gd(DO3A)(H_2O)$_2$] [20]	6.3	76[b]	44	7[e]	--
[Gd(DO3Aga)(H_2O)$_2$] [20]	33.3	--	--	--	--
[Gd(PCTA)(H_2O)$_2$] [26]	14.3	33[b]	45	13[e]	--
[Gd(AAZTA)(H_2O)$_2$]$^-$ [27]	11.1	--	--	1,280[c]	--
[Gd(DTTA(CH_2OH)$_2$-Bn)(H_2O)$_2$]$^-$ [17]	77±10	100[b]	12.9±3.5	2,670[c]	--
[Gd(PTDITA)(H_2O)$_2$]$^-$ [21]	3.3±2	160[b]	37.7±1.1	779[c]	--
[Gd(MS-325-Lq2)(H_2O)$_2$)]$^{2-}$ [22]	0.44±0.21	1160(60)	40±9.5	43[d]	-15±28

Table S1. Parameters obtained from fitting the transverse relaxation rate data of water in the presence of [Gd(CyPic)(H_2O)$_2$]$^-$ and parameters previously reported for select Gd(III) complexes. [a]Values reported for major isomer (square anti-prismatic). [b] τ_m^{310} values calculated from reported k_{ex}^{298} and $\Delta H^{\neq}$.[2] [c] T_{1e}^{298} values calculated from reported magnitude of transient zero-field splitting (Δ^2), the correlation time (τ_v) and activation energy (ΔE_v) for this perturbation.[2] [d]Recorded at 7T. [e]Recorded at 2.1 T.

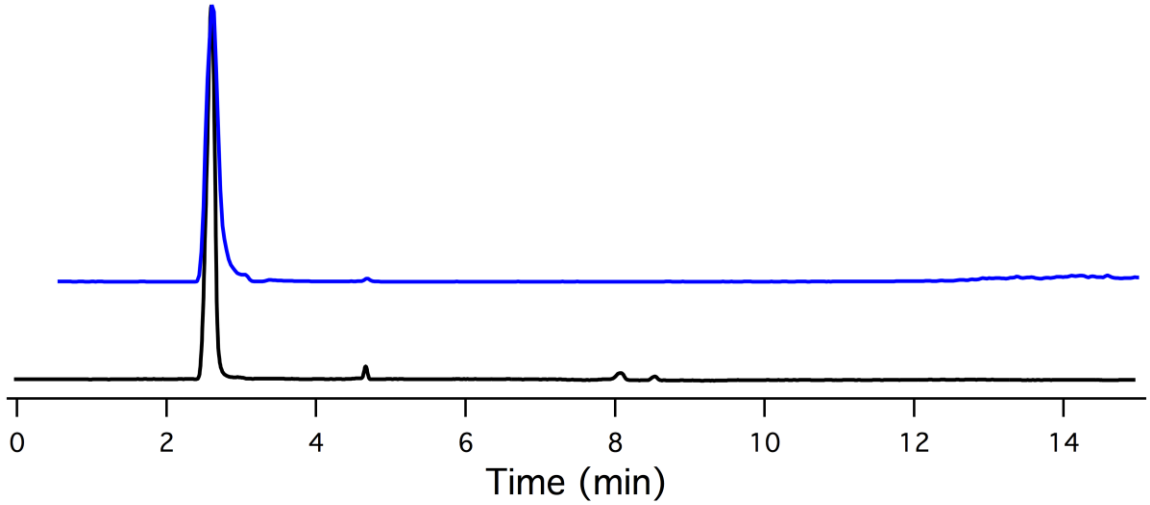

Figure S4. LC of CyPic3A at 280 nm detection (black) and MS chromatogram of extracted *m/z* = 424.2 [MW + H^+] (blue).

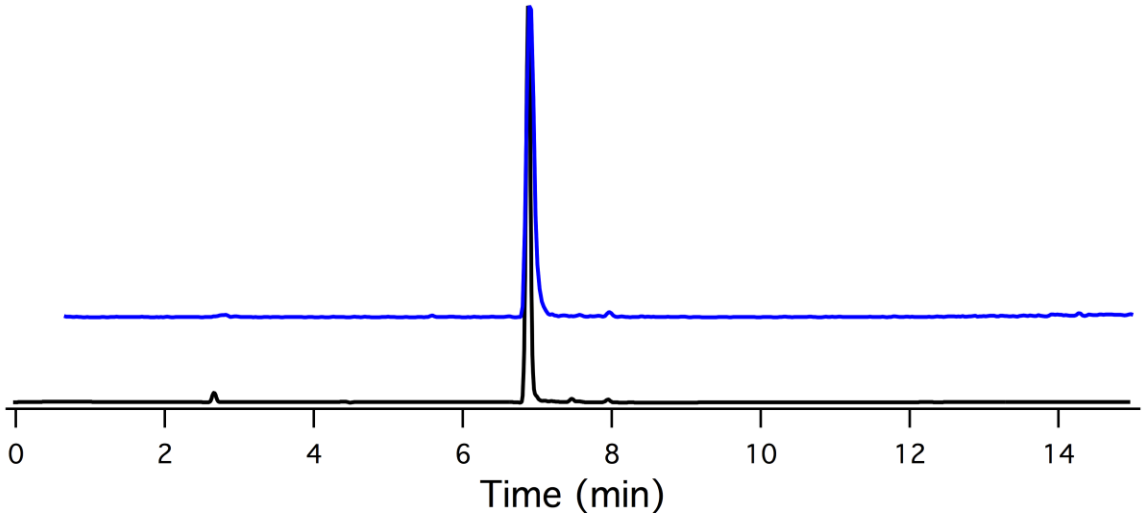

Figure S5. LC of $[Gd(CyPic3A)(H_2O)_2]^-$ at 280 nm detection (black) and MS chromatogram of extracted *m/z* = 579.0 [MW + $2H^+$] (blue).

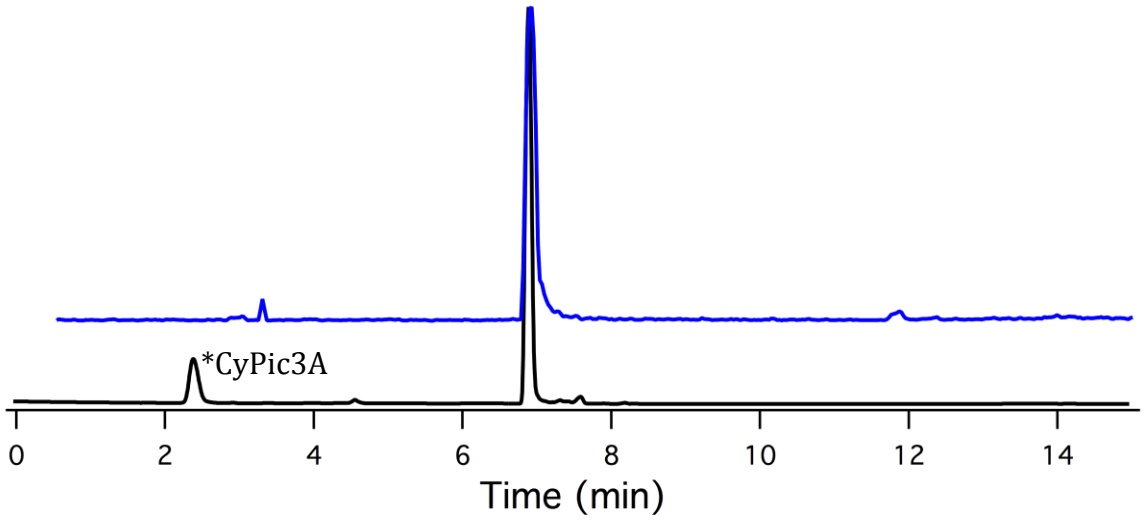

Figure S6. LC of $[Eu(CyPic3A)(H_2O)_2]^-$ at 280 nm detection (black) and MS chromatogram of extracted *m/z* = 574.0 [MW + $2H^+$] (blue). The species eluting at 2.48 min (*) is CyPic3A, added in excess to ensure full chelation of Eu(III) during the luminescence lifetime measurements.

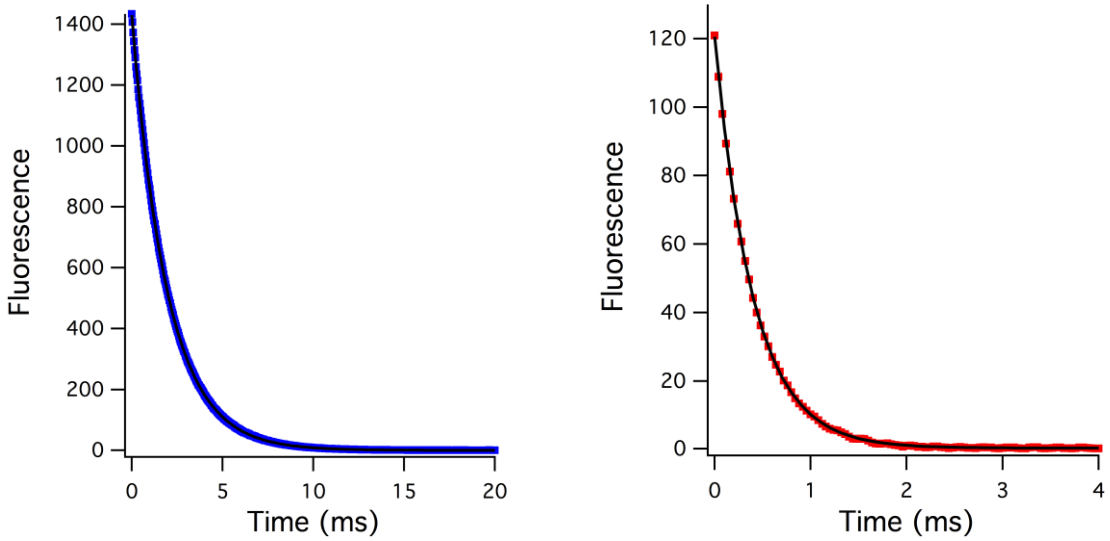

Figure S7. Time-dependence on luminescence intensity of $[Eu(CyPic)(H_2O)_2]^-$ in D_2O (left) and H_2O (right); monoexponential fits are in black. Note the different scale for the faster decaying sample in H_2O.

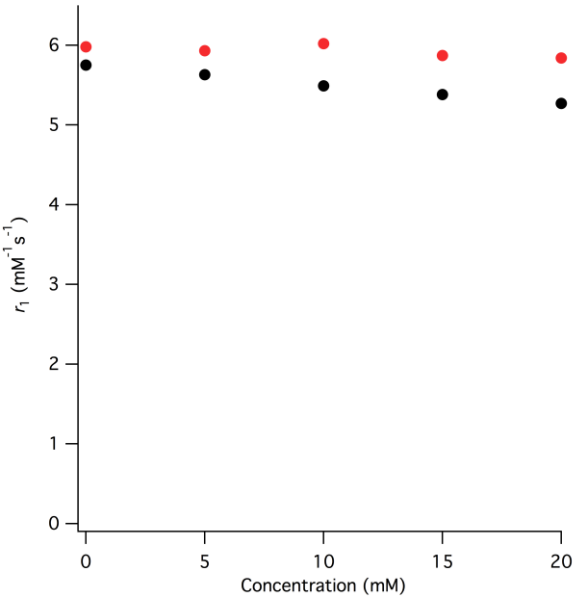

Figure S8. Relative change in r_1 of 1 mM $[Gd(CyPic3A)(H_2O)_2]^-$ in pH 7.4 HEPES buffer (50 mM) as a function of carbonate (red) and *L*-lactate (black) concentration.

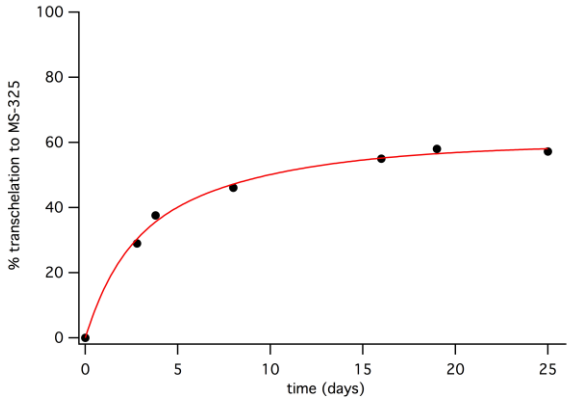

Figure S9. Time profile of $[Gd(CyPic3A)(H_2O)_2]^-$ (0.53 mM at t = 0) conversion to MS-325 during challenge with 1 mol-equiv. MS-325-L in 25 mM pH 7.4 Tris buffer.

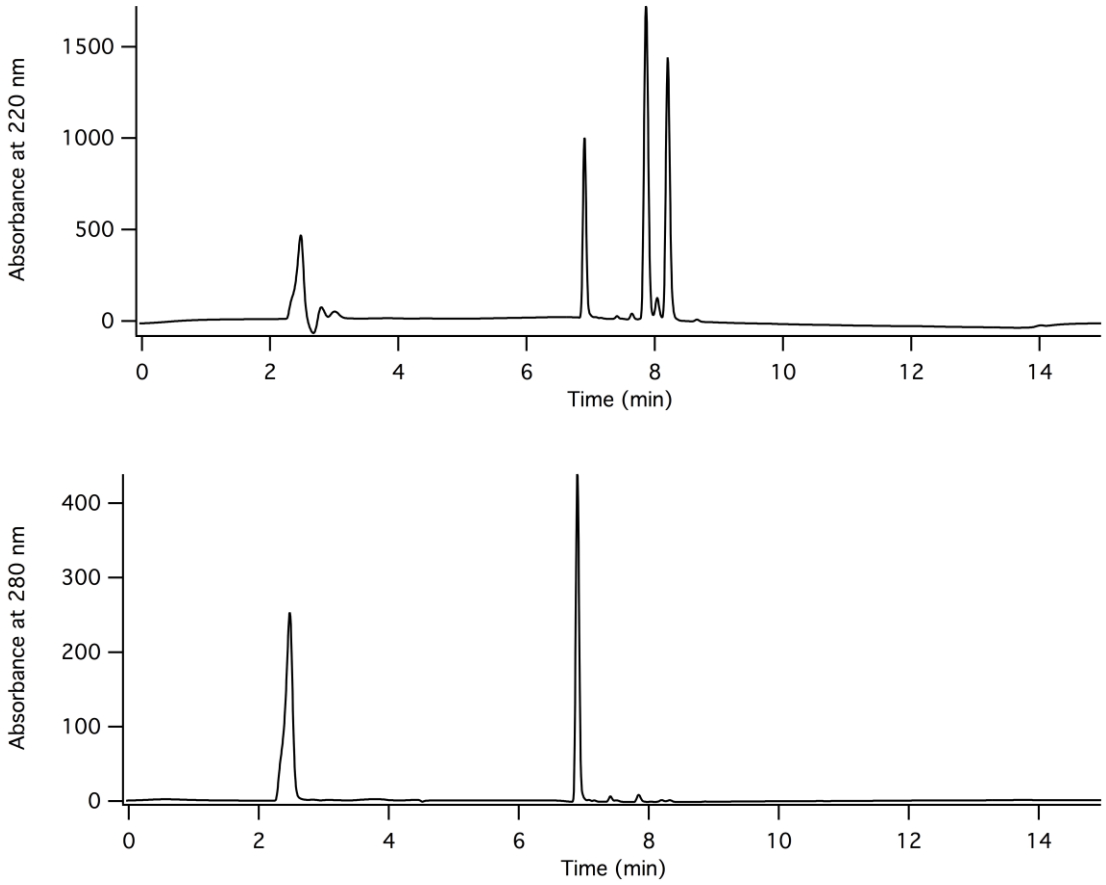

Figure S10. LC traces of $[Gd(CyPic3A)(H_2O)_2]^-$ vs. 1 mol-equiv. MS-325-L. Top: LC at 220 nm, where MS-325-L (7.88 min) and MS-325 (8.21 min) are most easily differentiated. Bottom: LC at 280 nm, where CyPic3A (2.48 min) and $[Gd(CyPic3A)]^-$ (6.90 min) are most easily differentiated. These particular traces were taken at 25 days.

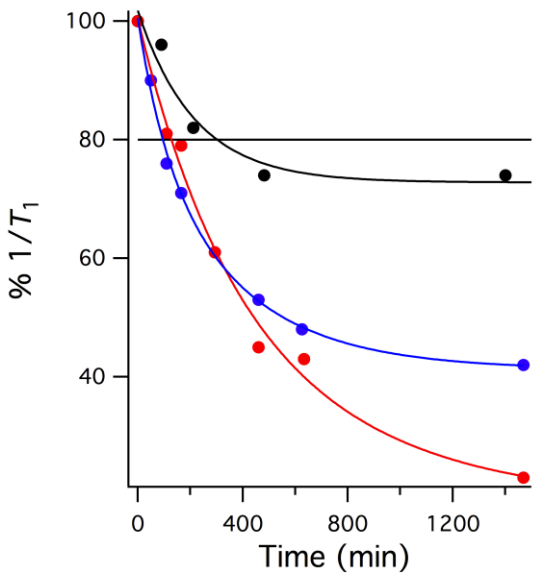

Figure S11. Time profile of transmetallation of $[Gd(CyPic3A)(H_2O)_2]^-$ (blue), $Gd(DTPA\text{-}BMA)(H_2O)$ (red) and $[Gd(DTPA)(H_2O)]^{2-}$ (black) with 1 equiv. Zn(II). The reaction is monitored by following $1/T_1$ with time, as the liberated Gd(III) precipitates as $Gd_2(PO_4)_3$ and does not contribute to T_1. (2.5 mM Gd(III)-complex, 2.5 mM $Zn(OTf)_2$, pH 7 phosphate buffer (50 mM), 310 K).

	MW	q	r_1 ($mM^{-1}s^{-1}$) 0.47 T, 310 K	r_1 ($mM^{-1}s^{-1}$) 1.41 T, 310 K	r_1 ($mM^{-1}s^{-1}$) 0.47 T, 298 K	r_1 ($mM^{-1}s^{-1}$) 1.41 T, 298 K
[Gd(CyPic3A)(H_2O)$_2$]$^-$	577.1	2	6.9	5.7	8.3	7.9
[Gd(DTPA)(H_2O)]$^{2-}$	564.0	1[28]	3.4[29]	3.3	4.3[28]	--
MS-325	907.1	1[30]	5.8[29]	5.2 (1.5 T)[29]	--	--
[Gd(HP-DO3A)(H_2O)]	577.1	1[28]	3.1[29]	2.9 (1.5 T)[29]	--	--
[Gd(DTPA-BMA)(H_2O)]	592.1	1[28]	3.5[29]	3.3 (1.5 T)[29]	--	--
[Gd(AAZTA)(H_2O)$_2$]$^-$	537.1	2[27]	--	--	7.1[27]	--
[Gd(HOPO)(H_2O)$_2$]	790.2	2[31]	10.5[28]	--	--	--
[Gd(DO3A)(H_2O)$_2$]	536.1	2[29]	4.8[28] (312K)	--	--	--
[Gd(DO3Aga)(H_2O)$_2$]	793.9	2[20]	--	--	12.3[20]	--
[Gd(PCTA)(H_2O)$_2$]	569.6	2.4[32]	--	--	6.9[32]	--
[Gd(PTDITA)(H_2O)$_2$]-	643.7	2[21]	--	--	10.2[21]	--
[Gd(DTTA-Me)(H_2O)$_2$]$^-$	538.6	2[16]	--	--	6.2[16]	--
[Gd(DTTA-CH_2OH)$_2$(H_2O)$_2$]$^-$	674.7	2[17]	--	--	8.7[17]	--
[Gd(OBETA)(H_2O)$_2$]$^-$	525.4	2[18]	--	--	7.0[18]	--
[Gd(thqN-SO_3)(H_2O)$_2$]	916.0	2[19]	--	--	5.7[19]	--
[Gd(MS325Lq2)(H_2O)$_2$)]$^{2-}$	881.9	2[22]	7.8[22] (308K)	--	--	--

Table S2.Comparison of r_1 values of [Gd(CyPic3A)(H_2O)$_2$]$^-$ to select FDA approved Gd(III) complexes and previously studied Gd(III) complexes of q = *2.*

	K_{comp} MS-325 vs. L, pH 4	K_{comp} MS-325 vs. L pH 7.4	K_{comp}, $[Gd(DTPA)(H_2O)]^{2-}$ vs. L, pH 4	K_{comp}, $[Gd(DTPA)(H_2O)]^{2-}$ vs. L, pH 7.4	Time to 80% r_1 (min)
CyPic3A	0.031	0.56	0.18	.172	95
DTPA	0.58[5]	0.35[5]	1	1	383
MS-325-L	1	1	1.74[5]	2.88[5]	3800[11]
HP-DO3A	0.16[5,6]	0.07[5,6]	0.22[6]	0.19[6]	inert[11]
DTPA-BMA	--	--	0.37[7]	.00027[7]	124
AAZTA	--	--	0.091[9]	.001[9]	--
HOPO	--	--	0.0079[8,9]	0.63[8,9]	--
DO3A	0.00046[5,6]	0.00017[5,6]	0.00048[5,6]	0.00050[5,6]	inert[11]
PCTA	--	--	0.064[10]	0.019[10]	--
PTDITA	--	--	93.3[21]	0.19[21]	--
DTTA-Me	--	--	.00041[16]	.00019[16]	44[16]
OBETA	--	--	.0017[18]	.00085[18]	--
MS-325-Lq2	.0002[5,22]	.0028[5,22]	.0007[5,22]	.015[5,22]	--

Table S3. Comparison of K_{comp} of $[Gd(DTPA)(H_2O)]^{2-}$ and MS-325 vs. L obtained through ligand challenge (for CyPic3A) or calculation (all other L) from thermodynamic data at pH 4 and 7.4, and time to 80% $1/T_1$ from Zn(II) challenge experiment.

References.

1. G.R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg. *Organometallics*, 2010, **29**, 2176.
2. P. Caravan, G. Parigi, J. M. Chasse, N. J. Cloutier, J. J. Ellison, R. B. Lauffer, C. Luchinat, S. A. McDermid, M. Spiller and T. J. McMurry. *Inorg. Chem.* 2007, **46**, 6632.
3. P. Caravan, A. V. Astashkin and A. M. Raitsimring, *Inorg. Chem*. 2003, **42**, 3972.
4. D. C. Harris, in *Quantitiative Chemical Analysis*, 2nd edn., W.H. Freeman and Company, New York, 2003.
5. P. Caravan, C. Comuzzi, W. Crooks, T. J. McMurry, G. R. Choppin and S. R. Woulfe. *Inorg. Chem*. 2001, **40**, 2170.
6. K. Kumar, C. A. Chang, L. C. Francesconi, D. D. Dischino, M. F. Malley, J. Z. Gougoutas and M. F. Tweedle. *Inorg. Chem*. 1994, **33**, 3567.
7. L. Moriggi, C. Cannizzo, C. Prestinari, F. Berrière and L. Helm. *Inorg. Chem*. 2008, **47**, 8357.
8. D. M. J. Doble, M. Melchior, B. O'Sullivan, C. Siering, J. Xu, V. C. Pierre and K. N. Raymond. *Inorg. Chem*. 2003, **42**, 4930.
9. Z. Baranyai, F. Uggeri, G. B. Giovenzana, A. Bényei, E. Brücher and S. Aime. *Chem. Eur. J*. 2009, **15**, 1696.
10. G. Tircsó, Kovács and A. D. Sherry. *Inorg.Chem*. 2006, **45**, 9269.
11. S. Laurent, L. Vander Elst, F. Copoix and R. N. Muller. *Invest. Radiol*. 2001, **36**,115.
12. S. Laurent, L. Vander Elst, C. Henoumon and R. N. Muller. *Contrast Media Mol. Imag*. 2010, **5**, 305.
13. M. Polasek and P. Caravan. *Inorg. Chem*. 2013, **52**, 4084.
14. P. D. Garimella, A. Datta, D. W. Romanini, K. N. Raymond and M. B. Francis. *J. Am. Chem. Soc*. 2011, **133**, 14704.
15. C. Platas-Iglesias, M. Mato-Iglesias, K. Djanashvili, R. N. Muller, L. Vander Elst, J. A. Peters, A. de Blas and T. Rodríguez-Blas. *Chem. Eur. J*. 2004, **10**, 3579.
16. L. Morriggi, C. Cannizzo, C. Prestinari, F. Berrière and L. Helm. *Inorg. Chem*. 2008, **47**, 8357.
17. S. Silvério, S. Torres, A. F. Martins, J. A. Martins, J. P. André, L. Helm, M. I. M. Prata, A. C. Santos and C. F. G. C. Geraldes. *Dalton Trans.* 2009, 4656.
18. Z. Baranyai, M. Botta, M. Fekete, G. B. Giovenzana, R. Negri, L. Tei and C. Platas-Iglesias. *Chem. Eur. J.* 2012, **18**, 7680.
19. G. Tallec, D. Imbert, P. H. Fries and M. Mazzanti. *Dalton Trans*. 2010, **39**, 9490.
20. D. Messeri, M. P. Lowe, D. Parker and M. Botta. *Chem. Commun.* 2001, 2742.

21. Z. Baranyai, L. Tei, G. B. Giovenzana, F. K. Kálmán and M. Botta. *Inorg. Chem.* 2012, *51*, 2597.
22. P. Caravan, J. C. Amedio Jr., S. U. Dunham, M. T. Greenfield, N. J. Cloutier, S. A. McDermid, M. Spiller, S. G. Zech, R. J. Looby, A. M. Raitsimring, T. J. McMurry and R. B. Lauffer. *Chem. Eur. J*. 2005, **11**, 5866.
23. K. Micskei, L. Helm, E. Brücher and A. Merbach. *Inorg. Chem*. 1993, **32**, 3844.
24. D. D. Castelli, M. C. Caligara, M. Botta, E. Terreno and S. Aime. *Inorg. Chem.* 2013, **52**, 7130.
25. S. M. Cohen, J. Xu, E. Radkov, K. N. Raymond, M. Botta, A. Barge and S. Aime. *Inorg. Chem*. 2000, **39**, 5747.
26. S. Aime, M. Botta, S. G. Crich, G. Giovenzana, R. Pagliarin, M. Sisti and E. Terreno. *Magn. Res. Chem.* 1998, **36**, S200.
27. S. Aime, L. Calabi, C. Cavallotti, E. Gianollo, G. B. Giovenzana, P. Losi, A. Malocchi, G. Palmisano and M. Sisti. *Inorg. Chem.* 2004, **43**, 7588.
28. P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer. *Chem. Rev.* 1999, **99**, 2293.
29. M. Rohrer, H. Bauer, J. Mintorovitch, M. Requardt and H.-J. Weinmann. *Invest. Radiol*. 2005, **40**, 715.
30. P. Caravan. *Acc. Chem. Res*. 2009, **42**, 851.
31. A. Datta and K. N. Raymond, *Acc. Chem. Res*. 2009, **42**, 938.
32. W. D. Kim, G. E. Kiefer, F. Maton, K. McMillan, R. N. Muller and A. D. Sherry. *Inorg. Chem*. 1995, **34**, 2233