Modulating water-exchange rates of lanthanide(III)-containing polyaminopolycarboxylate-type complexes using polyethylene glycol

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General Experimental Procedures

Commercially available chemicals were of reagent-grade purity and were used without further purification unless otherwise noted. Water was purified using a PURELAB Ultra Mk2 (ELGA) water purification system. Flash chromatography was carried out with silica gel 60, 230−400 mesh (EMD chemicals).[1] Thin-layer chromatography was performed on ASTM TLC plates with a silica gel 60 F_{254} coating (250 μm layer thickness). Visualization of TLC was carried out with a UV lamp followed by staining with potassium permanganate (2 g $KMnO_4$, 20 g K_2CO_3, 5 mL 5% w/v aqueous NaOH, and 300 mL H_2O). Spectra/Por Biotech cellulose ester dialysis membranes of 100−500 and 500−1000 Da molecular weight cut off (MWCO) were used for dialysis. Freeze drying was carried out using a LABCONCO FreeZone 2.5 freeze dryer. Resin reactions were performed in Poly-Prep chromatography columns on a Barnstead/Thermolyne LABQUAKE rotator. Centrifugation was carried out using a Fisher Scientific Centrific centrifuge 225. High-performance liquid chromatography (HPLC) was carried out on a Shimadzu HPLC system equipped with fluorescence (λ_{ex} = 273, λ_{em} = 622 and λ_{ex} = 396, λ_{em} = 593 nm, for Gd^{III} and Eu^{III} complexes, respectively), photodiode array (traces at 210 nm included in the SI), and refractive index detectors and a C4 column (RESTEK Ultra C4, 5.0 μm × 250 mm). Aqueous size-exclusion chromatography (SEC) was performed on the same HPLC system using three aquagel-OH columns in series (VARIAN PLaquagel-OH-mixed, 8 μm × 300 mm). A binary gradient method (pump A: H_2O, pump B: CH_3CN; 95−5% B over 20 min; flow rate: 1 mL/min) was used with the C4 column, and an isocratic method (100% H_2O; flow rate: 1 mL/min) was used with the aquagel-OH columns.

^1H NMR spectra were acquired using a Varian Unity 400 (400 MHz) or a Varian-500S (500 MHz) spectrometer, and ^{13}C NMR spectra were acquired using a Varian Unity 400 (101

MHz) or a Varian-500S (125 MHz) spectrometer. Chemical shifts (ppm) for ^{1}H NMR spectra are reported relative to residual $CHCl_3$ in $CDCl_3$ (7.27 ppm) or CH_3OH in CD_3OD (3.30 ppm). Multiplicities are reported as "s" = singlet, "m" = multiplet, and "brs" = broad singlet. The elements responsible for particular shifts are noted with italicized font. Chemical shifts for ^{13}C NMR spectra are reported relative to $CDCl_3$ (77.23 ppm) in $CDCl_3$ or CD_3CN (118.26 ppm) as an internal standard in D_2O. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained on an electrospray time-of-flight high-resolution Waters Micromass LCT Premier XE mass spectrometer. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry was performed on a Waters SYNAPT G2 mass spectrometer (Manchester, UK) equipped with a commercial MALDI source and a Nd:YAG laser (355 nm, 200 Hz) or a Bruker Ultraflex MALDI-TOF mass spectrometer. α-Cyano-4-hydroxycinnamic acid (5 mg in 50:50 CH_3CN/H_2O with 0.1% formic acid) was used as the matrix. Prior to plating, samples (~1 μg/mL in water with 0.1% formic acid or ~1 mg/mL in water) and matrix were mixed in 1:1 or 1:100 v/v ratios. *N*-(*tert*-Butoxycarbonyl)-*N'*-aminoacetylchloride, **4**, was synthesized according to a published procedure.[2]

Inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements were performed on a HORIBA Jobin Yvon *ULTIMA* spectrometer or by Columbia Analytical Services Inc., Tucson, Arizona, USA. Samples measured with the *ULTIMA* spectrometer were diluted with nitric acid (2% v/v, aqueous), and standards were prepared by serial dilution of Gd, Eu, and Y standards (High-Purity Standards).

Water proton relaxation rate data were obtained using a Bruker mq 60 NMR Analyzer (1.4 T) at 37 °C for Gd^{III}-containing conjugates **1a–d** in phosphate-buffered saline (pH 7.4). The relaxivities of Gd^{III}-containing conjugates were obtained from the slopes of the linear plots of

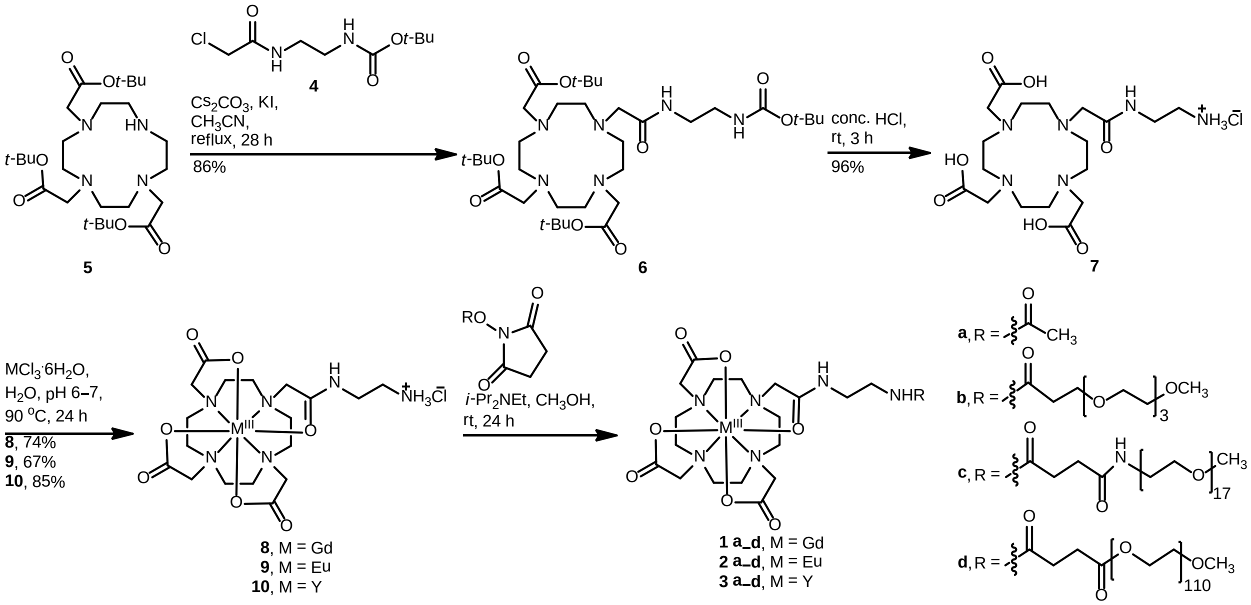
$1/T_1$ versus Gd^{III} concentration. Measurements were repeated three times with independently prepared solutions for each Gd^{III}-containing conjugate. The Gd^{III} concentration was verified by ICP–OES.

Variable-temperature ^{17}O NMR measurements of Gd^{III}-containing conjugates **1a** (6 mM), **1b** (6 mM), **1c** (4 mM), and **1d** (17 mM) and their diamagnetic Y^{III} analogues (**3a–d**) in H_2O were carried out on a Varian-500S spectrometer. Enrichment in ^{17}O (1%) was achieved using ^{17}O-enriched water (10% $H_2{}^{17}O$, Cambridge Isotope Laboratories, Inc.). Line widths at half height were measured at 20 (or 25), 30, 40, 50, 60, and 70 °C. $A/\hbar$ and ΔE were fixed to -3.8×10^{-6} rad/s and 2.5×10^{-11} J/mol, respectively, for Gd^{III}-containing conjugates, **1a–d**. The water-coordination number, q, was set to the value obtained from luminescence-decay measurements for Eu^{III}-containing conjugates **2a–d**. The least-squares fits of the ^{17}O NMR relaxation data were calculated using origin software (8.0951 B951) following a previously published procedure[3] to obtain the water-exchange rates of Gd^{III}-containing conjugates, **1a–d**. Gd^{III} and Y^{III} concentrations were verified by ICP–OES.

Luminescence-decay measurements of Eu^{III}-containing conjugates **2a–d**, in H_2O and D_2O were acquired using a HORIBA Jobin Yvon Fluoromax-4 spectrofluorometer in decay by delay scan mode using the phosphorescence lifetime setting. Excitation and emission wavelengths of 393 and 596 nm were used, respectively, while the other parameters were kept constant: excitation and emission slit widths (5 nm), flash count (100), initial delay (0.01 ms), maximum delay (2 ms for solutions in H_2O and 8 ms for solutions in D_2O), and delay increment (0.01 ms). The number of coordinated water molecules, q, was determined using the method developed by Horrocks and coworkers.[4]

Electron paramagnetic resonance (EPR) measurements of **1a–d** in water were performed on a Bruker EMX X-band spectrometer. From the EPR spectra, the electronic Landé *g* factors, g_{L}, peak-to-peak line widths, ΔH_{pp}, and transverse electronic relaxation rates, $1/T_{2e}$, were obtained according to a previously reported method.[5]

Synthetic Procedures



1,4,7-Tris(*tert*-butyloxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (5): This procedure is based on a previous report with modifications as noted in the following text.[6] To a mixture of cyclen (1.00 g, 5.80 mmol, 1 equiv) and $NaHCO_3$ (1.61 g, 19.2 mmol, 3.3 equiv) in anhydrous CH_3CN (15 mL) at 0 °C under Ar was added a solution of *tert*-butyl bromoactetate (2.90 mL, 19.4 mmol, 3.3 equiv) in anhydrous CH_3CN (20 mL) over a period of 18 h (instead of 30 min as described in the previous report). The reaction mixture was allowed to warm to ambient temperature during the addition of *tert*-butyl bromoactetate (instead of after the addition as previously reported). The reaction mixture was stirred under Ar for 48 h after the addition of *tert*-butyl bromoactetate. The reaction mixture was filtered through celite, and the filtrate was reduced to dryness under reduced pressure to yield a pale yellow solid that was purified using silica gel column chromatography (9:1 $CHCl_3/CH_3OH$) (not described in the previous report). Fractions with R_f values (9:1 $CHCl_3/CH_3OH$) between 0.39 and 0.63 were combined, and the solvent was removed under reduced pressure. The resulting solid was recrystallized from hot toluene, dissolved in saturated $NaHCO_3$ (50 mL), extracted with $CHCl_3$ (3 × 50 mL), and dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure to obtain 1.46 g (49%) of **5** as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.34−1.52 (m, C*H*$_3$, 27H), 2.70−2.91 (m, C*H*$_2$C*H*$_2$, 12H), 3.00 (brs, C*H*$_2$C*H*$_2$, 4H), 3.30 (s, C*H*$_2$C=O, 2H), 3.35 (s, C*H*$_2$C=O, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 28.20 (*C*H$_3$), 28.24 (*C*H$_3$), 47.8 (*C*H$_2$*C*H$_2$), 50.4 (*C*H$_2$C=O), 51.3 (*C*H$_2$*C*H$_2$), 51.7 (*C*H$_2$ *C*H$_2$), 58.1 (*C*H$_2$C=O), 81.5 (*C*(CH$_3$)$_3$), 81.6 (*C*(CH$_3$)$_3$), 170.4, 171.1; R_f = 0.47 (9:1 CH_3Cl/CH_3OH); HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $C_{26}H_{51}N_4O_6$, 515.3809; found 515.3817.

1,4,7-Tris(*tert*-butyloxycarbonylmethyl)-10-(*N*-(2-*tert*-butoxycarbonylaminoethyl) acetamide-1,4,7,10-tetraazacyclododecane (6): To a mixture of **5** (0.394 g, 0.765 mmol, 1 equiv), Cs_2CO_3 (0.584 g, 1.79 mmol, 2.3 equiv), and KI (0.278 g, 1.67 mmol, 2.2 equiv) in anhydrous CH_3CN (16 mL) was added a solution of **4** (0.219 g, 0.925 mmol, 1.2 equiv) in anhydrous CH_3CN (16 mL) under Ar. The reaction mixture was heated at reflux under Ar for 28 h. The reaction mixture was cooled to ambient temperature and filtered through celite, and the solvent was removed under reduced pressure. The resulting residue was dissolved in $CHCl_3$ (40 mL) and washed sequentially with H_2O (40 mL) and saturated aqueous KCl (3 × 40 mL). The organic layer was dried over anhydrous K_2CO_3 and concentrated under reduced pressure to obtain 0.470 g (86%) of **6** as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.32−1.52 (m, C*H*$_3$, 36H), 2.51 (brs, C*H*$_2$C*H*$_2$, 4H), 2.68 (brs, C*H*$_2$C*H*$_2$, 4H), 2.89 (brs, C*H*$_2$C=O, 6H), 3.07 (s, C*H*$_2$C=O, 2H), 3.16−3.58 (m, C*H*$_2$C*H*$_2$, 12H), 5.99 (brs, N*H*, 1H), 8.79 (brs, N*H*, 1H); ^{13}C NMR (101 MHz, CDCl$_3$, δ): 27.9 (*C*H$_3$), 28.0 (*C*H$_3$), 28.2 (*C*H$_3$), 28.6 (*C*H$_3$), 39.6 (*C*H$_2$*C*H$_2$), 41.1 (*C*H$_2$*C*H$_2$), 51.7 (*C*H$_2$*C*H$_2$), 52.3 (*C*H$_2$C=O), 53.7 (*C*H$_2$C=O), 55.1 (*C*H$_2$*C*H$_2$), 56.2 (*C*H$_2$*C*H$_2$), 57.1 (*C*H$_2$*C*H$_2$), 57.8 (*C*H$_2$C=O), 79.1 (*C*(CH$_3$)$_3$), 80.9 (*C*(CH$_3$)$_3$), 81.0 (*C*(CH$_3$)$_3$), 81.9 (*C*(CH$_3$)$_3$), 155.9, 156.5, 170.6, 171.8, 172.7; HRESIMS (*m*/*z*): [M + H]$^+$ calcd for $C_{35}H_{67}N_6O_9$, 715.4970; found 715.4976.

1,4,7-Tris(carbonylmethyl)-10-(aminoethyl-*N′*)acetyl)-1,4,7,10-tetraazacyclododecane (7): To *tert*-butylester **6** (0.248 g, 0.347 mmol) was added concentrated HCl (25 mL), and the resulting mixture was stirred at ambient temperature for 3 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was dissolved in H$_2$O and freeze dried to obtain 0.161 g (96%) of **7** as a yellow–brown solid. ^{1}H NMR (400 MHz, CD$_3$OD, δ at 55 °C): 3.02−3.24 (m, C*H*$_2$C*H*$_2$, 10H), 3.36−3.58 (m, C*H*$_2$C*H*$_2$, and C*H*$_2$C=O, 12H), 3.70 (s, C*H*$_2$C=O, 2H), 3.94 (s, C*H*$_2$C=O, 4H); ^{13}C NMR (125 MHz, D$_2$O, δ at 65 °C): 35.4 (*C*H$_2$*C*H$_2$), 38.3 (*C*H$_2$*C*H$_2$), 48.2 (*C*H$_2$*C*H$_2$), 48.3 (*C*H$_2$*C*H$_2$), 48.9 (*C*H$_2$*C*H$_2$), 49.0 (*C*H$_2$*C*H$_2$), 52.1 (*C*H$_2$C=O), 52.3 (*C*H$_2$C=O), 52.9 (*C*H$_2$C=O), 169. 0, 169.3, 170.0; HRESIMS (*m*/*z*): [M + H]$^+$ calcd for $C_{18}H_{35}N_6O_7$, 447.2567; found 447.2562.

General procedure for the synthesis of Ln^{III} (8 and 9) and Y^{III} complexes (10):

Ligand **7** (0.100 g, 0.207 mmol, 1 equiv) was dissolved in H$_2$O (20 mL) and the pH of the solution was adjusted to between 6 and 7 using 1 M NH$_4$OH. To the resulting solution was added MCl$_3$·6H$_2$O (0.311 mmol, 1.5 equiv), and the pH of the solution was adjusted to between 6 and 7 using 1 M NH$_4$OH. The reaction mixture was heated at 90 °C for 24 h and then cooled to ambient temperature. The pH of the solution was increased to 11 by adding 1 M NH$_4$OH followed by centrifugation, and the supernatant was filtered through a 0.2 µm hydrophilic syringe filter (Millipore, IC MILLEX-LG). The filtrate was dialyzed in a 500 Da molecular weight cut off (MWCO) dialysis membrane against H_2O (4 L). The dialysis reservoir was changed at 2–4, 6–8, and 10–14 h. After the last change, dialysis was continued for 7 h. Contents within the dialysis membrane were freeze dried to obtain 0.0948 g (74%) of **8**, 0.0851 g (67%) of **9**, or 0.0981 g (85%) of **10** as off-white solids.

Gd^{III} complex (8): HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $GdC_{18}H_{32}N_6O_7$, 599.1559; found 599.1579. SEC chromatogram is on page S24.

Eu^{III} complex (9): HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $EuC_{18}H_{32}N_6O_7$, 595.1531; found 595.1523. SEC chromatogram is on page S24.

Y^{III} complex (10): HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $YC_{18}H_{32}N_6O_7$, 533.1391; found 533.1396. SEC chromatogram is on page S24.

General procedure for the synthesis of Ln^{III} (1a–c and 2a–c) and Y^{III} conjugates (3a–c):

To a flask containing N-acetoxysuccinimide, **a**, or a succinimidyl ester derivative of PEG, **b** or **c**, (5 equiv), was added dropwise a mixture of Ln^{III} complex **8** or **9**, or Y^{III} complex **10**, (1 equiv), and diisopropylethylamine (5 equiv) in anhydrous CH_3OH (5 mL). The resulting mixture was allowed to stir at ambient temperature under Ar for 24 h. The resulting reaction mixture was added to aminomethylated polystyrene HL (100–200 mesh) resin (5 equiv, pre-swollen in ethanol for 0.5–1 h), and the resulting mixture was allowed to rotate for 15 to 18 h. The liquid portion of the reaction mixture was separated from the resin via filtration, and the resin was washed with ethanol (3 × 7 mL). The washings were combined with the liquid portion of the reaction mixture, and the solvents were removed under reduced pressure to obtain an oil that was dissolved in H_2O (10 mL) and washed with hexanes (4 × 10 mL). The H_2O layer was dialyzed in either a 500 (**1a**, **2a**, **3a**, **1b**, **2b**, and **3b**) or 1000 Da (**1c**, **2c**, and **3c**) MWCO dialysis membrane against H_2O (4 L). The dialysis reservoir was changed at 2–4, 6–8, and 10–14 h. After the last change, dialysis was continued for 7 h. Contents within the dialysis membrane were freeze dried and the resulting solids were washed with CH_3CN (3 × 5 mL) to yield Ln^{III} conjugates **1a–c** and **2a–c** and Y^{III} conjugates **3a–c** as white solids. The purity of conjugates **1a–c**, **2a–c**, and **3a–c** was verified by HPLC, and the chromatograms are on pages S25–S27.

Conjugate 1a: 18.6 mg (46%), HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $GdC_{20}H_{34}N_6O_8$, 641.1664; found 641.1640.

Conjugate 1b: 29.1 mg (43%), HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $GdC_{28}H_{50}N_6O_{12}$, 817.2713; found 817.2708.

Conjugate 1c: 18.8 mg (51%), MALDI-MS: median peak $[M + H]^+$ calcd for $GdC_{57}H_{107}N_7O_{26}$, 1463.65; found 1463.19

Conjugate 2a: 18.9 mg (47%), HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $EuC_{20}H_{34}N_6O_8$, 637.1637; found 637.1634.

Conjugate 2b: 22.5 mg (34%), HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $EuC_{28}H_{50}N_6O_{12}$, 813.2685; found 813.2682.

Conjugate 2c: 12.87 mg (49%), MALDI-MS: median peak $[M + H]^+$ calcd for $EuC_{57}H_{107}N_7O_{26}$, 1458.65; found 1458.19

Conjugate 3a: 16.0 mg (48%), HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $YC_{20}H_{34}N_6O_8$, 575.1497; found 575.1498.

Conjugate 3b: 27.2 mg (39%), HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $YC_{28}H_{50}N_6O_{12}$, 751.2545; found 751.2543.

Conjugate 3c: 16.1 mg (49%), MALDI- MS: median peak $[M + H]^+$ calcd for $YC_{57}H_{107}N_7O_{26}$, 1394.63; found 1394.19

General procedure for the synthesis of Ln^{III}- (1d and 2d) and Y^{III}-PEG conjugates (3d): To a flask containing succinimidylester derivative of PEG **d** (1 equiv), was added dropwise a mixture of Ln^{III} complex **8** or **9**, or Y^{III} complex **10**, (3 equiv), and diisopropylethylamine (3 equiv) in anhydrous CH_3OH (5 mL). The resulting mixture was allowed to stir at ambient temperature under Ar for 24 h. The resulting reaction mixture was concentrated under reduced pressure to obtain a white solid that was dissolved in H_2O (10 mL) and washed with hexanes (4 × 10 mL). The H_2O layer was dialyzed in a 1000 Da MWCO dialysis membranes against H_2O (4 L). The dialysis reservoir was changed at 2–4, 6–8, and 10–14 h. After the last change, dialysis was continued for 7 h. Contents within the dialysis membrane were freeze dried and the resulting solids were washed with CH_3CN (3 × 5 mL) to yield Ln^{III} conjugates **1d** and **2d**, and Y^{III} conjugate **3d**, as white solids. The purity of **1d**, **2d**, and **3d** was verified by aqueous SEC, and the chromatograms are on page S28.

Conjugate 1d: 42.6 mg (45%), MALDI- MS: median peak $[M + H]^+$ calcd for $GdC_{228}H_{448}N_6O_{112}$, 5222.87; found 5222.03

Conjugate 2d: 44.6 mg (48%), MALDI- MS: median peak $[M + H]^+$ calcd for $EuC_{228}H_{448}N_6O_{112}$, 5217. 87; found 5217.01

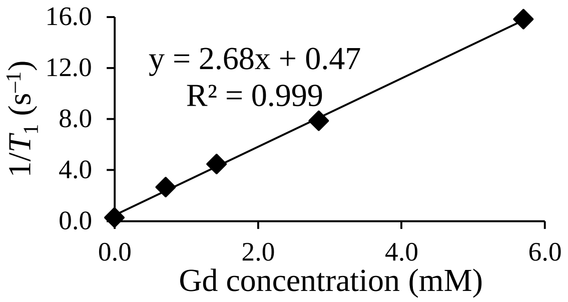
Conjugate 3d: 41.9 mg (44%), MALDI- MS: median peak $[M + Na]^+$ calcd for $YC_{228}H_{447}N_6O_{112}Na$, 5175.84; found 5175.88

Water-Proton Relaxation Rate Data

1a

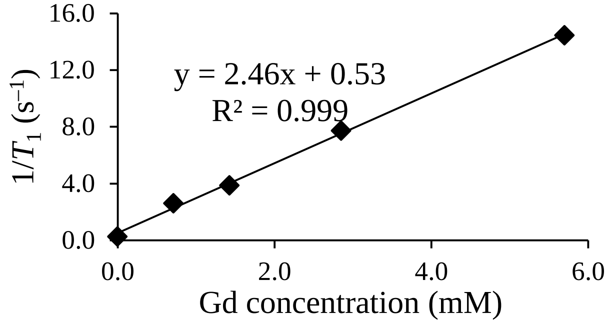
Trial 1

Concn (mM)	1/T_1 (s^{-1})	T_1(s)
5.70	15.8	0.0633
2.85	7.84	0.128
1.43	4.44	0.225
0.713	2.64	0.379
0.000	0.259	3.87



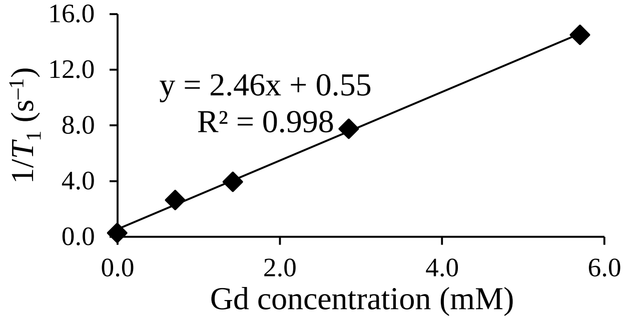
Trial 2

Concn (mM)	1/T_1 (s^{-1})	T_1(s)
5.70	14.5	0.0692
2.85	7.73	0.129
1.43	3.88	0.258
0.713	2.62	0.382
0.000	0.262	3.81



Trial 3

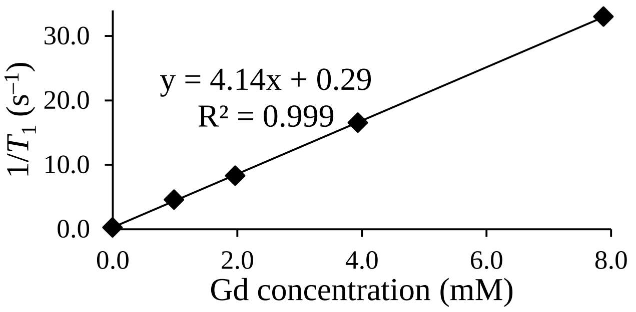
Concn (mM)	1/T_1 (s^{-1})	T_1(s)
5.70	14.5	0.0690
2.85	7.75	0.129
1.43	3.94	0.254
0.713	2.63	0.381
0.000	0.262	3.81



1b

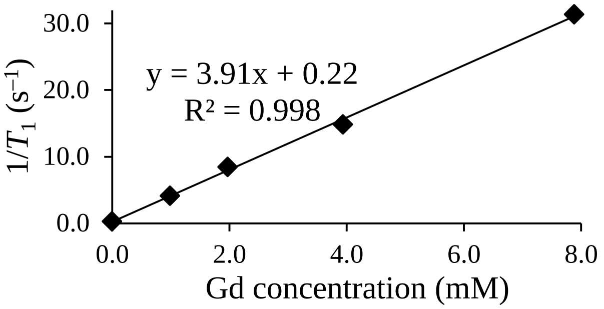
Trial 1

Concn (mM)	1/T_1 (s^{-1})	T_1(s)
7.88	33.0	0.0303
3.94	16.5	0.0606
1.97	8.29	0.121
0.990	4.60	0.217
0.000	0.259	3.87



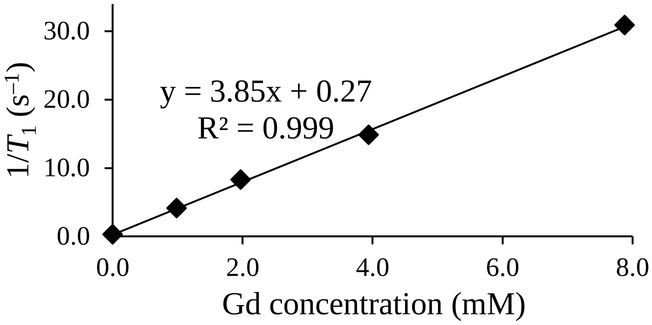
Trial 2

Concn (mM)	1/T_1 (s^{-1})	T_1(s)
7.88	31.4	0.0319
3.94	14.8	0.0675
1.97	8.40	0.119
0.990	4.10	0.244
0.000	0.262	3.81



Trial 3

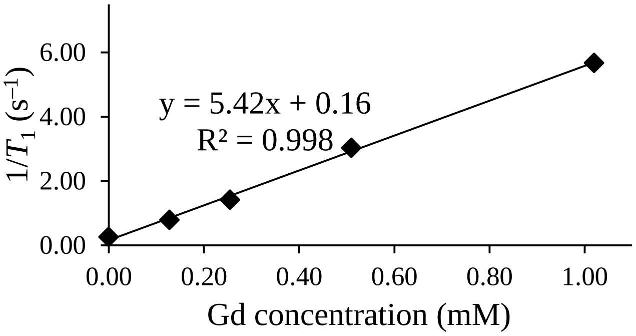
Concn (mM)	1/T_1 (s^{-1})	T_1(s)
7.88	30.9	0.0324
3.94	14.8	0.0675
1.97	8.26	0.121
0.990	4.11	0.2436
0.000	0.262	3.81



1c

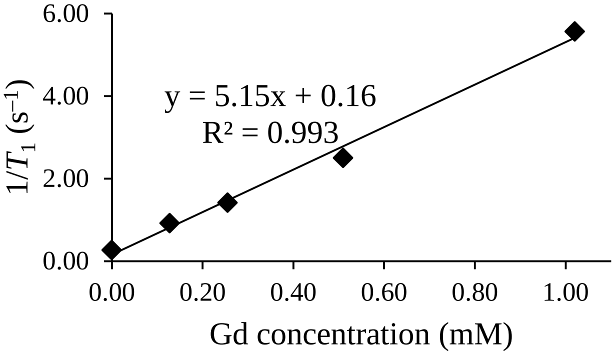
Trial 1

Concn (mM)	1/T_1 (s^{-1})	T_1(s)
1.02	5.68	0.176
0.510	3.03	0.330
0.255	1.42	0.704
0.128	0.790	1.27
0.000	0.260	3.85



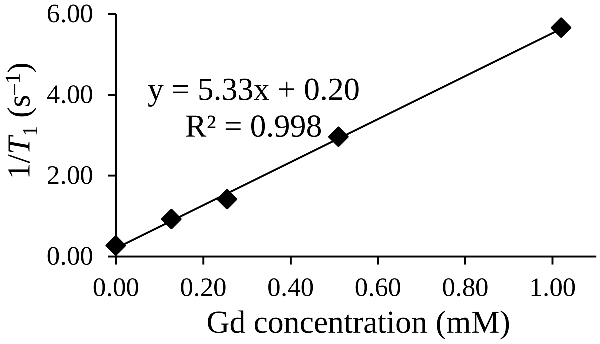
Trial 2

Concn (mM)	1/T_1 (s^{-1})	T_1(s)
1.02	5.68	0.176
0.510	3.03	0.330
0.255	1.42	0.704
0.128	0.790	1.27
0.000	0.262	3.81



Trial 3

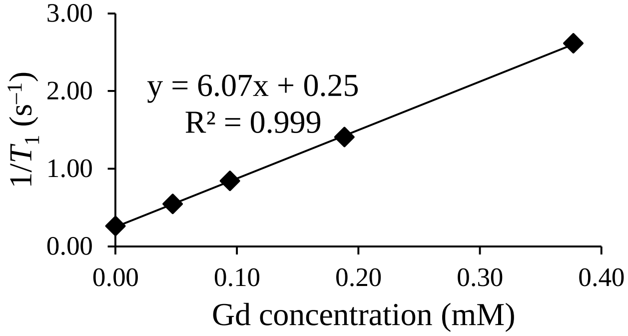
Concn (mM)	$1/T_1$ (s^{-1})	T_1(s)
1.02	5.68	0.176
0.510	3.03	0.330
0.255	1.42	0.704
0.128	0.790	1.27
0.000	0.262	3.81



1d

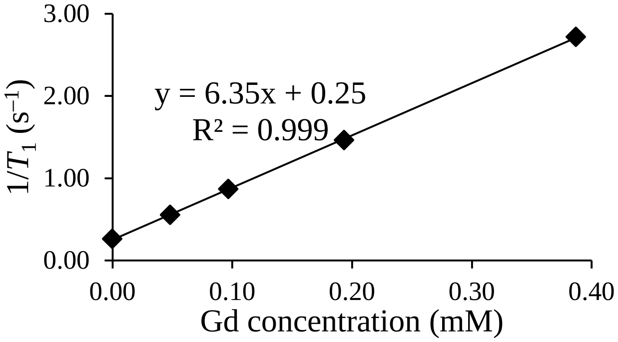
Trial 1

Concn (mM)	$1/T_1$ (s^{-1})	T_1(s)
0.387	2.61	0.383
0.194	1.41	0.712
0.097	0.842	1.19
0.048	0.547	1.83
0.000	0.260	3.85



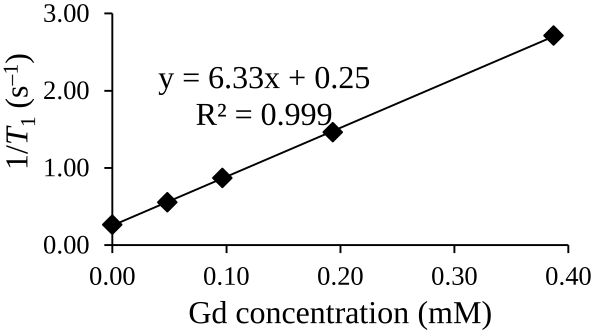
Trial 2

Concn (mM)	$1/T_1$ (s^{-1})	T_1(s)
0.387	2.72	0.368
0.194	1.46	0.685
0.097	0.867	1.15
0.048	0.555	1.80
0.000	0.262	3.81



Trial 3

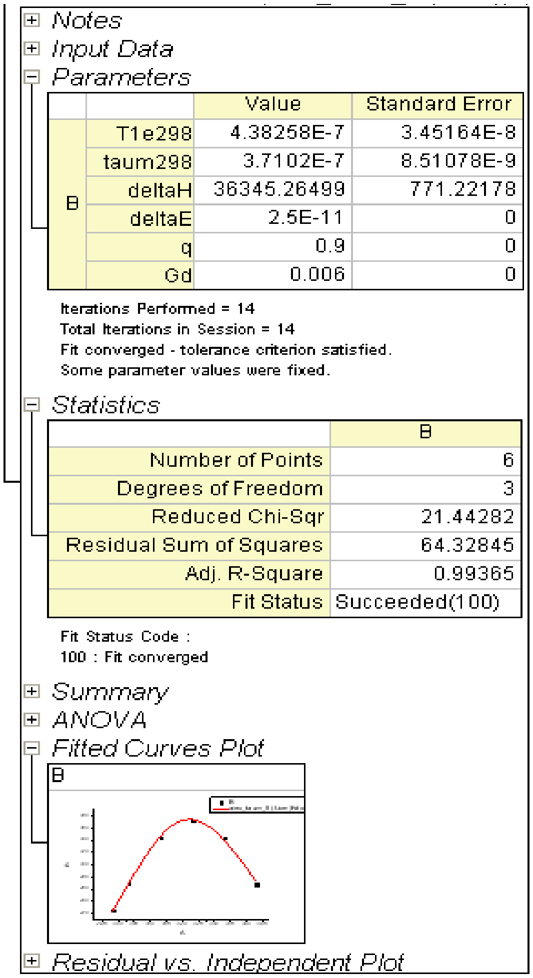
Concn (mM)	$1/T_1$ (s^{-1})	T_1(s)
0.387	2.71	0.369
0.194	1.46	0.684
0.097	0.870	1.15
0.048	0.554	1.80
0.000	0.262	3.81



Variable-Temperature ^{17}O NMR Data

1a

Temperature (°C)	Linewidth at half height (Hz)	
	1a	**3a**
70	114.08	28.87
60	141.92	32.27
50	155.88	37.10
40	151.51	42.31
30	136.03	50.52
25	130.05	58.68



		Value	Standard Error
B	T1e298	4.38258E-7	3.45164E-8
	taum298	3.7102E-7	8.51078E-9
	deltaH	36345.26499	771.22178
	deltaE	2.5E-11	0
	q	0.9	0
	Gd	0.006	0

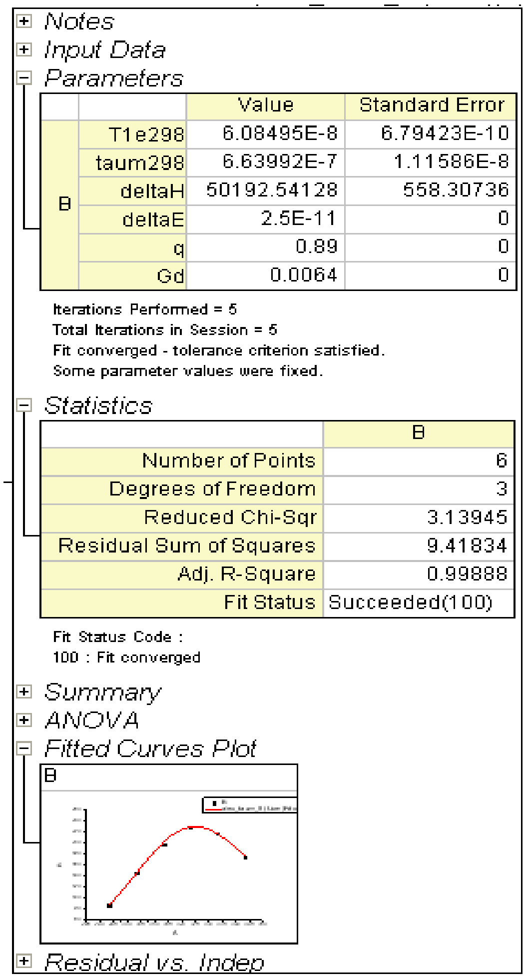
Iterations Performed = 14
Total Iterations in Session = 14
Fit converged - tolerance criterion satisfied.
Some parameter values were fixed.

	B
Number of Points	6
Degrees of Freedom	3
Reduced Chi-Sqr	21.44282
Residual Sum of Squares	64.32845
Adj. R-Square	0.99365
Fit Status	Succeeded(100)

Fit Status Code :
100 : Fit converged

1b

Temperature (°C)	Linewidth at half height (Hz)	
	1b	**3b**
70	85.90	30.30
60	102.03	32.84
50	110.20	37.08
40	105.90	42.82
30	97.05	50.93
20	91.20	63.56



Parameters

		Value	Standard Error
B	T1e298	6.08495E-8	6.79423E-10
	taum298	6.63992E-7	1.11586E-8
	deltaH	50192.54128	558.30736
	deltaE	2.5E-11	0
	q	0.89	0
	Gd	0.0064	0

Iterations Performed = 5
Total Iterations in Session = 5
Fit converged - tolerance criterion satisfied.
Some parameter values were fixed.

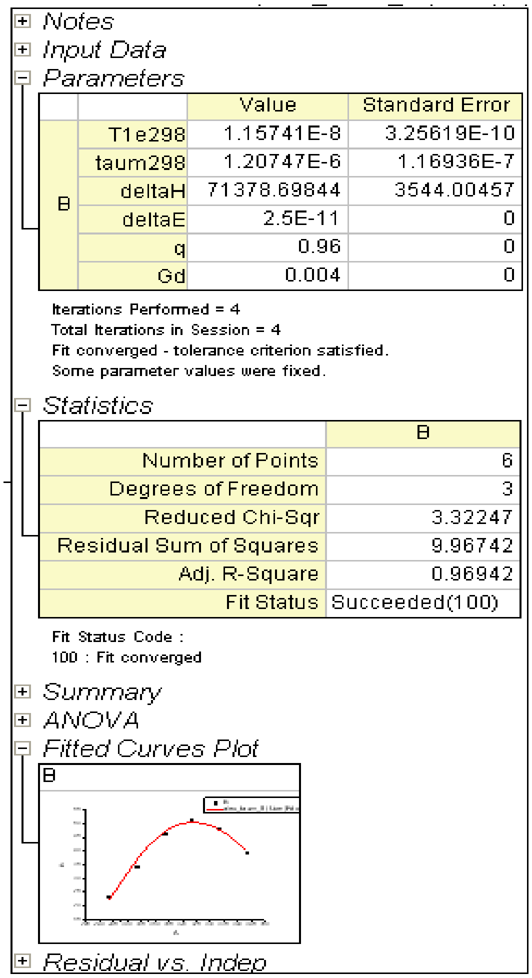
Statistics

	B
Number of Points	6
Degrees of Freedom	3
Reduced Chi-Sqr	3.13945
Residual Sum of Squares	9.41834
Adj. R-Square	0.99888
Fit Status	Succeeded(100)

Fit Status Code :
100 : Fit converged

1c

Temperature (°C)	Linewidth at half height (Hz)	
	1c	**3c**
70	42.16	29.56
60	48.22	32.88
50	53.56	37.10
40	57.82	43.01
30	62.26	51.34
20	71.26	63.73



		Value	Standard Error
B	T1e298	1.15741E-8	3.25619E-10
	taum298	1.20747E-6	1.16936E-7
	deltaH	71378.69844	3544.00457
	deltaE	2.5E-11	0
	q	0.96	0
	Gd	0.004	0

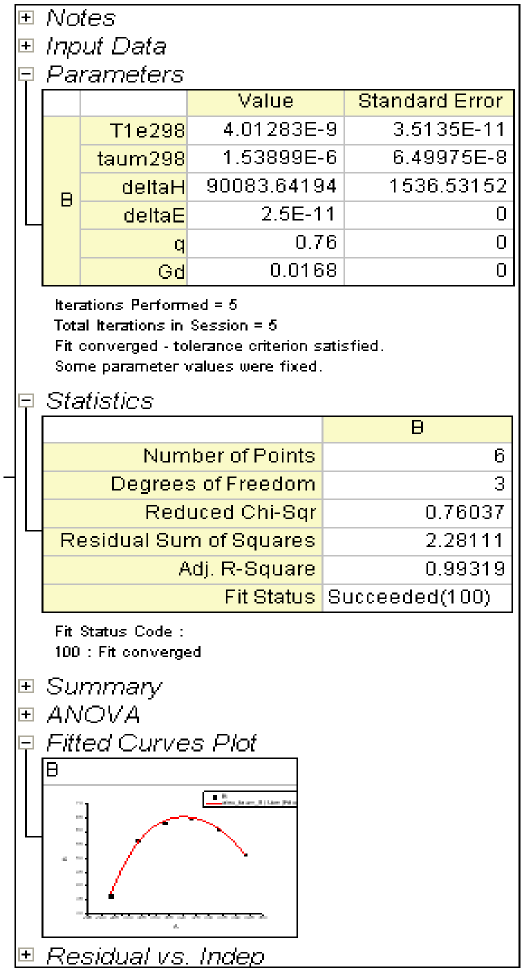
Iterations Performed = 4
Total Iterations in Session = 4
Fit converged - tolerance criterion satisfied.
Some parameter values were fixed.

	B
Number of Points	6
Degrees of Freedom	3
Reduced Chi-Sqr	3.32247
Residual Sum of Squares	9.96742
Adj. R-Square	0.96942
Fit Status	Succeeded(100)

Fit Status Code :
100 : Fit converged

1d

Temperature (°C)	Linewidth at half height (Hz)	
	1d	**3d**
70	46.59	30.11
60	53.57	34.15
50	59.60	38.95
40	65.80	45.61
30	72.63	54.55
20	79.79	68.18



Notes

Input Data

Parameters

		Value	Standard Error
B	T1e298	4.01283E-9	3.51135E-11
	taum298	1.53899E-6	6.49975E-8
	deltaH	90083.64194	1536.53152
	deltaE	2.5E-11	0
	q	0.76	0
	Gd	0.0168	0

Iterations Performed = 5
Total Iterations in Session = 5
Fit converged - tolerance criterion satisfied.
Some parameter values were fixed.

Statistics

	B
Number of Points	6
Degrees of Freedom	3
Reduced Chi-Sqr	0.76037
Residual Sum of Squares	2.28111
Adj. R-Square	0.99319
Fit Status	Succeeded(100)

Fit Status Code :
100 : Fit converged

Summary

ANOVA

Fitted Curves Plot

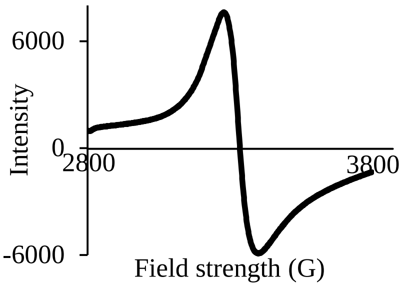
Residual vs. Indep

Luminescence-Decay Rate Data

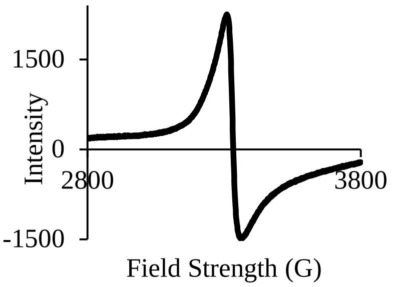
Complex	Luminescence-decay rates (s^{-1})					
	Trial 1		Trial 2		Trial 3	
	in H_2O	in D_2O	in H_2O	in D_2O	in H_2O	in D_2O
2a	1.48	0.419	1.46	0.428	1.48	0.428
2b	1.46	0.427	1.48	0.434	1.47	0.432
2c	1.52	0.404	1.52	0.404	1.48	0.419
2d	1.33	0.422	1.32	0.422	1.39	0.421

Electron Paramagnetic Resonance Spectra

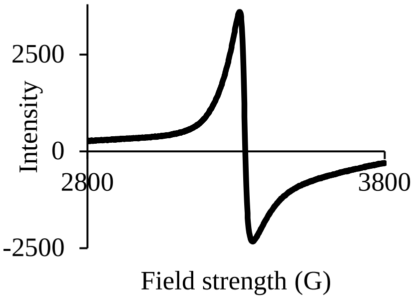
1a



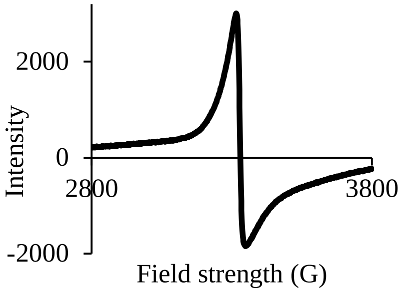
1b



1c



1d



Complex	ΔH_{pp} (G)
1a	118
1b	53.8
1c	43.0
1d	33.2

Estimation of Rotational Correlation Time (τ_R)

To obtain an estimation of τ_R, we started with equation 1 that relates observed relaxivity, r_1^{obs}, to inner- and outer-sphere relaxivities, r_1^{IS} and r_1^{OS}, respectively, at 1.4 T (60 MHz).[7]

$$r_1^{obs} = r_1^{IS} + r_1^{OS} \qquad \text{equation 1}$$

Assuming that complexes **1a–d** behave similarly to small molecular contrast agents, r_1^{obs} is composed of approximately equal contributions from r_1^{IS} and r_1^{OS}.[7] Therefore, r_1^{IS} can be expressed as half of r_1^{obs} as shown in equation 2.

$$0.5r_1^{obs} = r_1^{IS} \qquad \text{equation 2}$$

r_1^{IS} is related to the number of coordinated water molecules (q), longitudinal relaxation time of the coordinated water proton (T_{1m}), and residence lifetime of the coordinated water molecule in the inner-sphere (τ_m, reciprocal of water-exchange rate, k_{ex}) as expressed in equation 3.[7]

$$r_1^{IS} = \frac{q}{55{,}500}\left[\frac{1}{T_{1m} + \tau_m}\right] \qquad \text{equation 3}$$

Equations 2 and 3 can be combined to obtain equation 4, which enables the calculation of T_{1m} by substituting r_1^{obs}, τ_m, and q with values obtained experimentally for complexes **1a–d** (r_1^{obs} and τ_m) and **2a–d** (q).

$$T_{1m} = \frac{q}{27{,}750r_1^{obs}} - \tau_m \qquad \text{equation 4}$$

T_{1m} is composed of a dipole–dipole contribution (DD) and a scalar (SC) contribution to longitudinal proton relaxation as shown in equation 5.[7]

$$\frac{1}{T_{1m}} = \frac{1}{T_1^{DD}} + \frac{1}{T_1^{SC}} \qquad \text{equation 5}$$

The scalar contribution to overall longitudinal proton relaxation is negligible at field strengths greater than 10 MHz.[7] Therefore, at field strengths above 10 MHz, the longitudinal proton relaxation becomes equal to the *DD* contribution that can be expressed as shown in equation 6.[7]

$$\frac{1}{T_{1m}} = \frac{1}{T_1^{DD}} = \frac{2}{15}\left(\frac{\gamma_I^2 g^2 \mu_B^2}{r_{GdH}^6}\right) S(S+1)\left(\frac{\mu_0}{4\pi}\right)^2 \left(7\frac{\tau_{C2}}{1+\omega_S^2\tau_{C2}^2} + 3\frac{\tau_{C1}}{1+\omega_I^2\tau_{C1}^2}\right) \qquad \text{equation 6}$$

γ_I, nuclear gyromagnetic ratio = 2.67×10^8 s^{-1} T^{-1}

g, electron g-factor obtained for complexes **1a–d** from EPR spectra

μ_B , Bohr magneton = 9.274×10^{-24} J T^{-1}

$\mu_{0,}$ vacuum permeability = $4\pi \times 10^{-7}$ T mA^{-1}

r_{GdH}, electron spin-proton distance = 3.1×10^{-10} m (from reference 7 for DOTA-based Gd$^{\mathrm{III}}$-containing complexes)

S, electron spin for Gd = 3.5

$\omega_S = \gamma_S B$

$\omega_I = \gamma_I B$

γ_S, electron gyromagnetic ratio = 1.76×10^{11} s^{-1} T^{-1}

ω_S and ω_I are the electron and nuclear Larmor frequencies, respectively, at magnetic field strength B (1.4 T).

$$\frac{1}{\tau_{C_2}} = \frac{1}{\tau_R} + \frac{1}{\tau_m} + \frac{1}{T_{2e}} \qquad \text{equation 7}$$

$$\frac{1}{\tau_{C_1}} = \frac{1}{\tau_R} + \frac{1}{\tau_m} + \frac{1}{T_{1e}} \qquad \text{equation 8}$$

T_{1e} and T_{2e} are the longitudinal and transverse electronic relaxation times, respectively. T_{1e} (obtained from the fitting of ^{17}O NMR data) and T_{2e} (obtained from EPR spectra) for complexes **1a–d** were used in equations 7 and 8 that were combined with equation 6 to solve for τ_R for complexes **1a–d**.

Complex	$T_{1m} \times 10^{-6}$ (s)	$\tau_R \times 10^{-12}$ (s)
1a	13	46
1b	2.9	79
1c	5.3	110
1d	7.4	220

Estimation of Relaxivity, r_1^{obs}, Based on τ_R

Estimated relaxivity values for complex **1a** were obtained using τ_R values from complexes **1a–d**, and τ_m and q values from complex **1a**: $\tau_m = 3.7 \times 10^{-7}$ s and $q = 0.9$. τ_{C1} and τ_{C2} were calculated by substituting τ_m, T_{1e}, and T_{2e} obtained for **1a** and τ_R from **1a–d** into equations 7 and 8 (page S20). The calculated τ_{C1} and τ_{C2} values together with other constants were used to calculate values for T_{1m} using equation 6 (page S20), and the calculated T_{1m} values and fixed τ_m and q values (from **1a**) were substituted in equation 3 (page S19) to obtain r_1^{IS} values that were used in equation 2 (page S19) to obtain the estimated relaxivity values for **1a** based on changes in τ_R associated with complexes **1a–d**.

Complex	Observed r_1 (mM^{-1} s^{-1})	Complex	Estimated r_1 (mM^{-1} s^{-1})
1a	2.5	**1a**	2.5
1b	4.0	**1a** with τ_R from **1b**	4.2
1c	5.3	**1a** with τ_R from **1c**	5.7
1d	6.3	**1a** with τ_R from **1d**	11

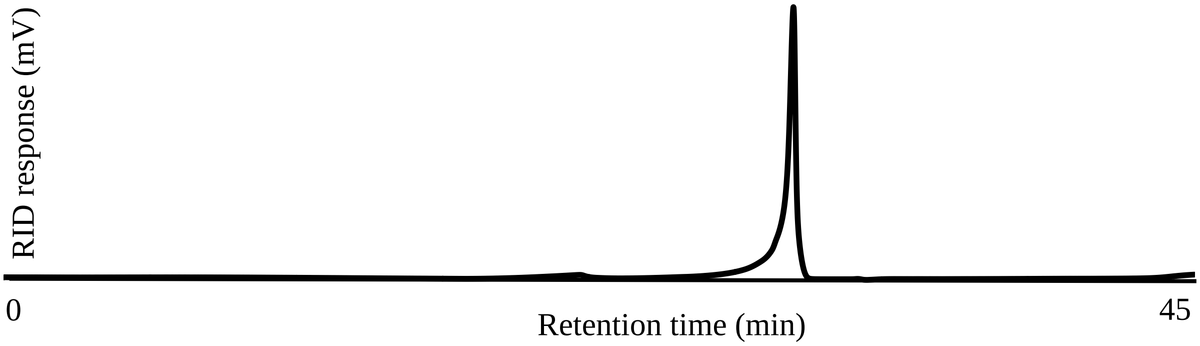
References

1. W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
2. A.-M. Fanning, S. E. Plush and T. Gunnlaugsson, *Chem. Commun.*, 2006, 3791.
3. J. Garcia, J. Neelavalli, E. M. Haacke and M. J. Allen, *Chem. Commun*., 2011, **47**, 12858.
4. R. M. Supkowski and W. D. Horrocks, Jr., *Inorg. Chim. Acta*, 2002, **340**, 44.
5. D. H. Powell, O. M. N. Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer and A. E. Merbach, *J. Am. Chem. Soc.*, 1996, **118**, 9333.
6. A. Dadabhoy, S. Faulkner and P. G. Sammes, *J. Chem. Soc., Perkin Trans. 2*, 2002, 348.
7. P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev*., 1999, **99**, 2293.

High Performance Liquid Chromatograms

SEC Chromatograms for Complexes 8–10

8



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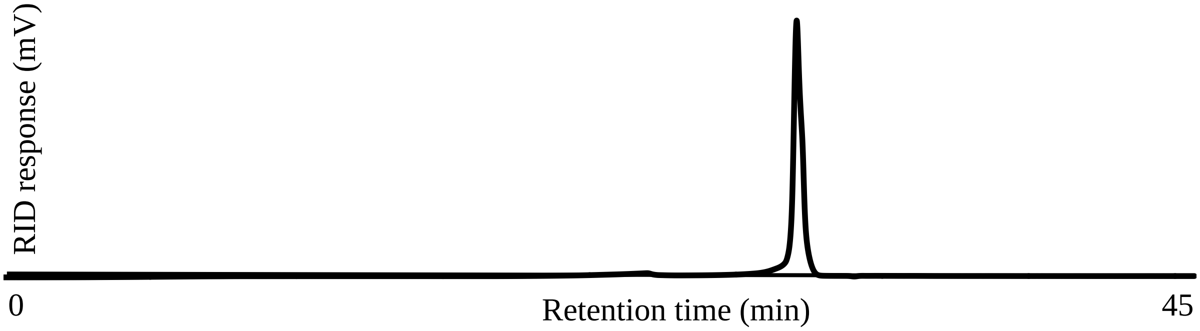

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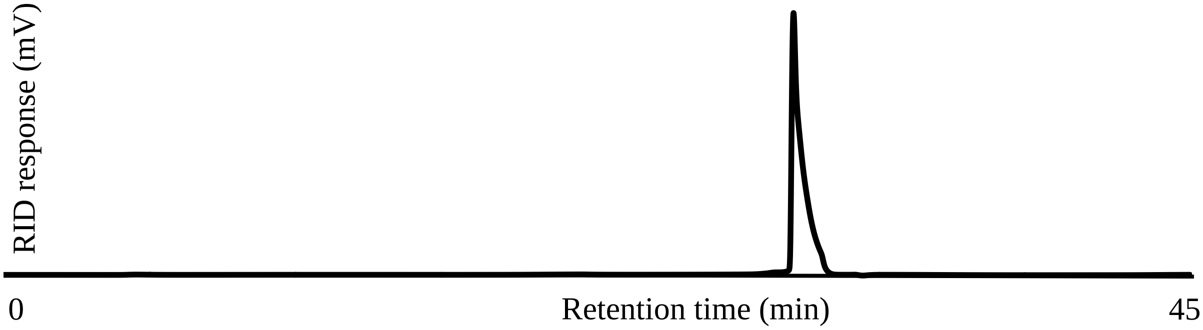
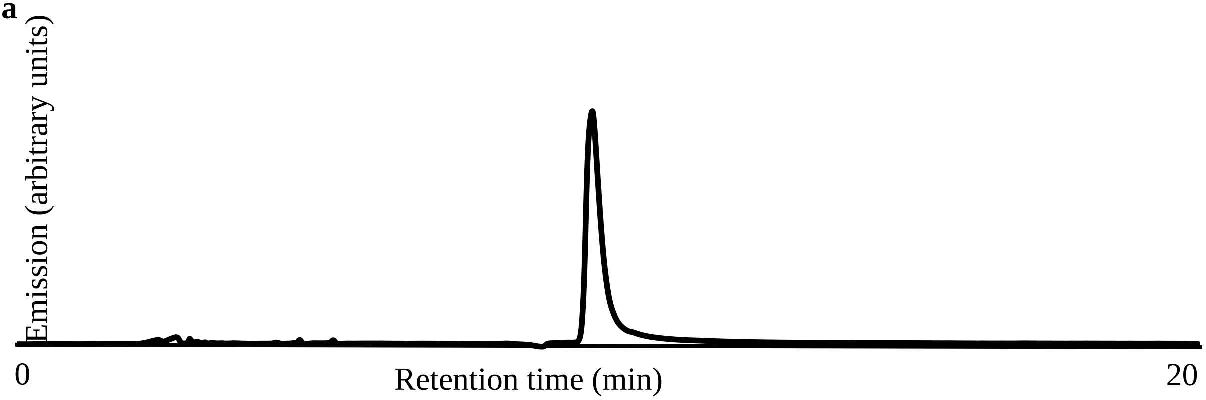
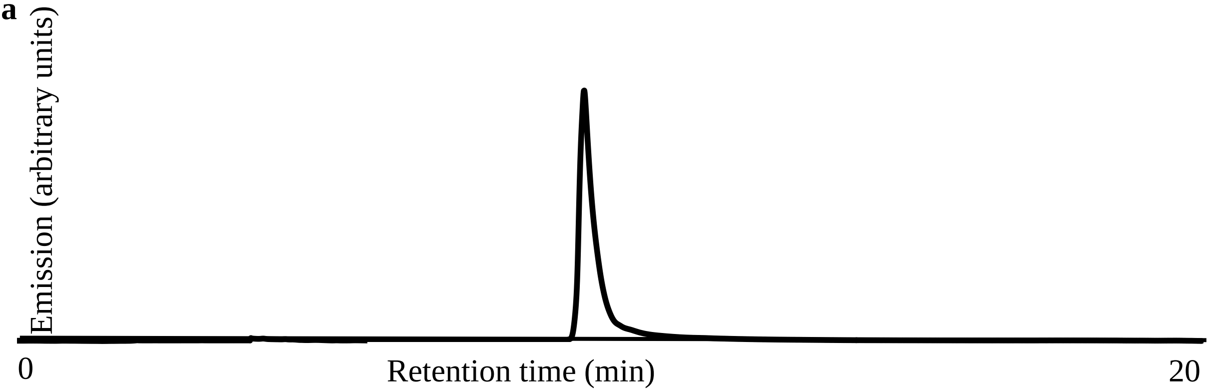


HPLC Chromatograms for complexes 1a–c, 2a–c, and 3a–c

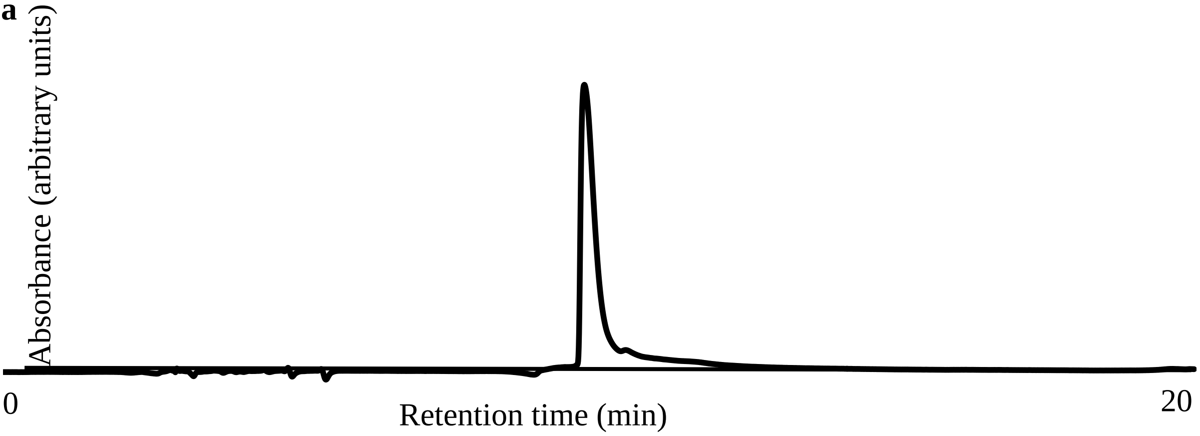
1a



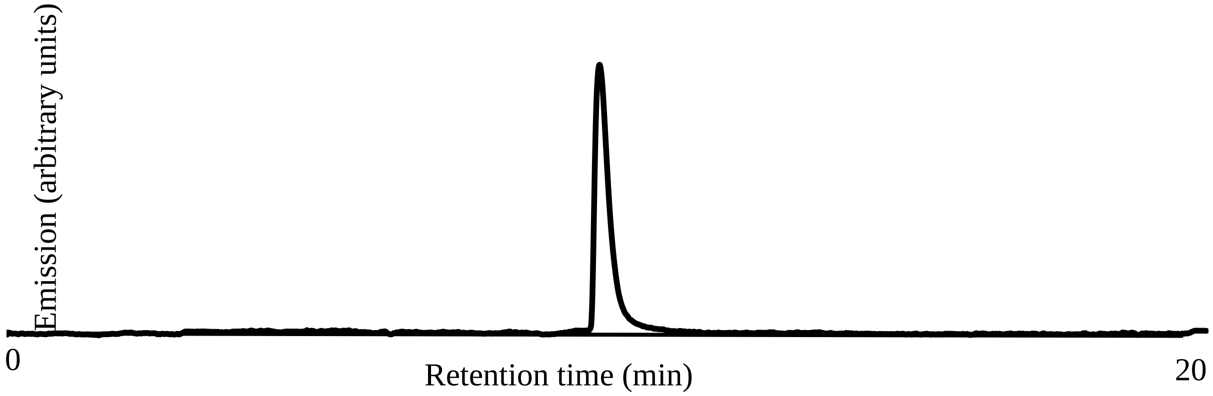
2a



3a



1b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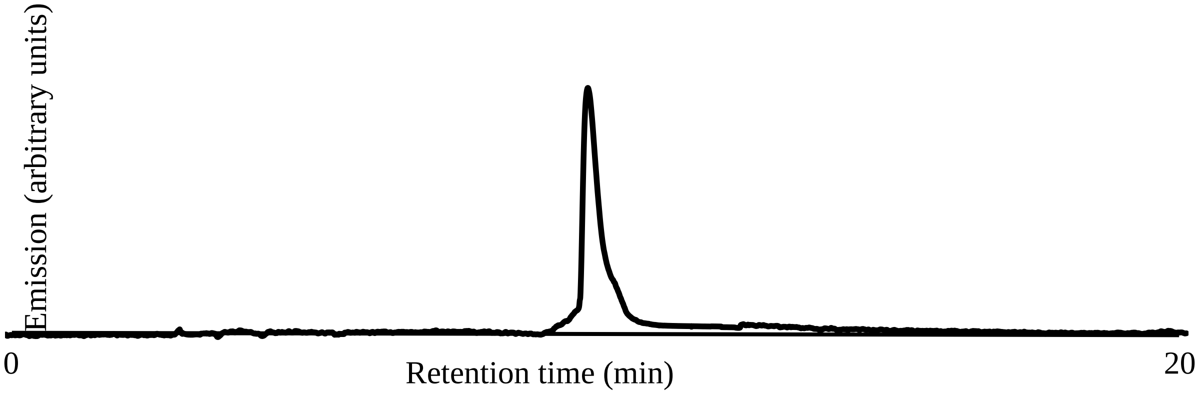
Retention time (min)
20

2b


Emission (arbitrary units)
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Retention time (min)
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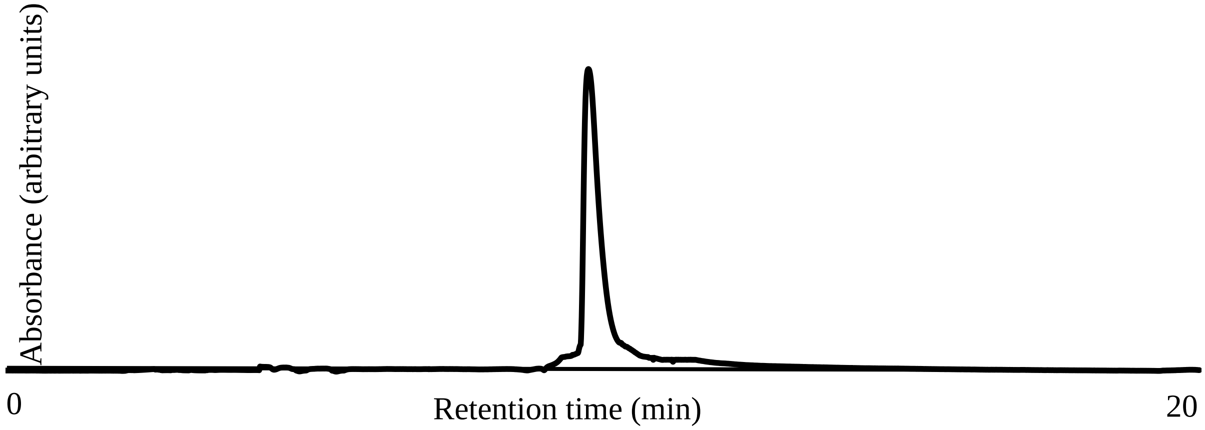
3b


Absorbance (arbitrary units)
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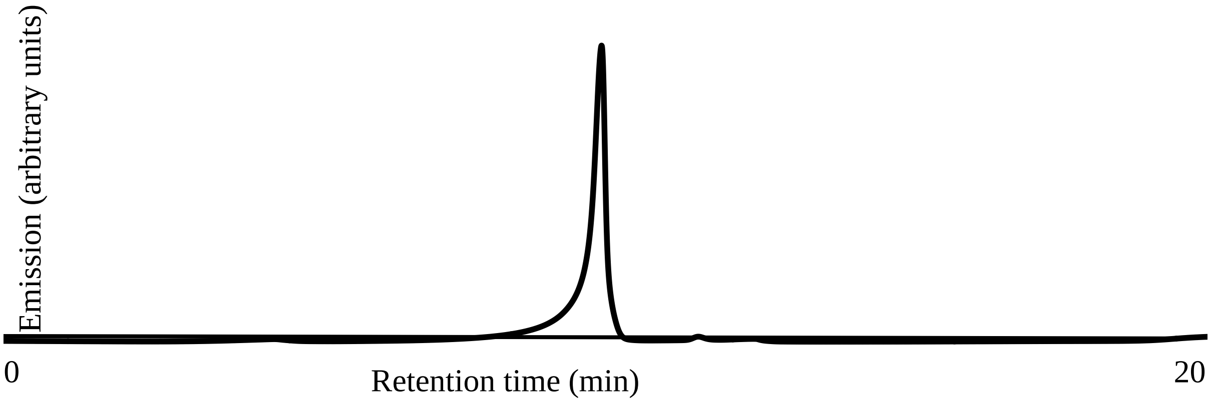
Retention time (min)
20

1c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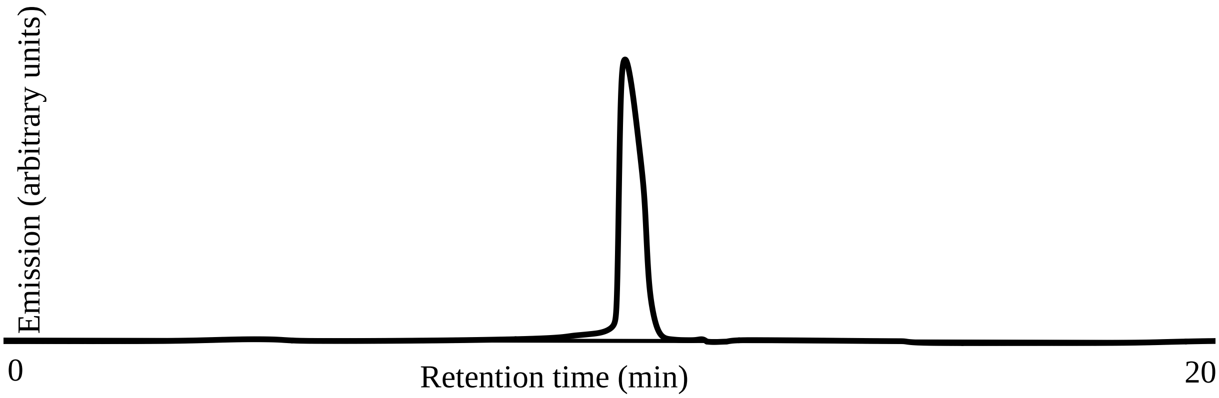
2c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Retention time (min)
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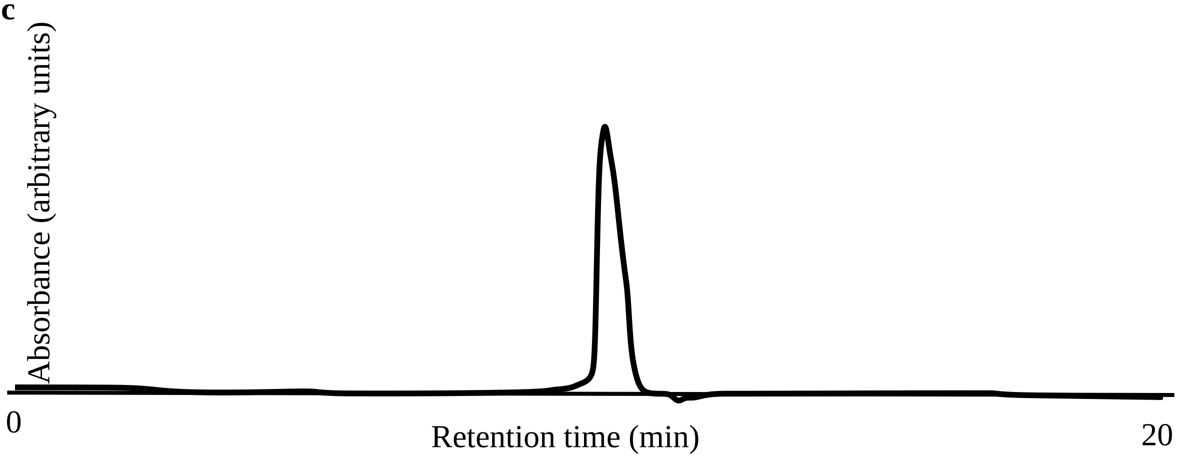
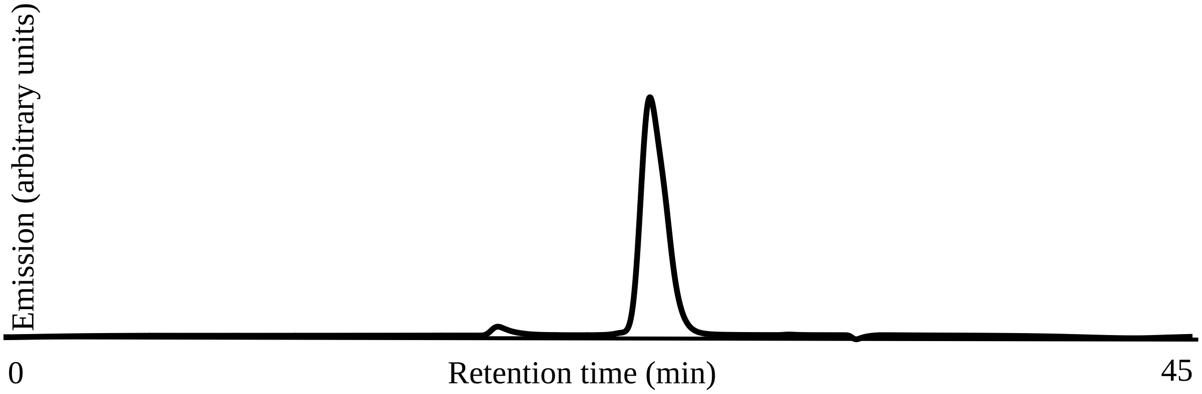
3c

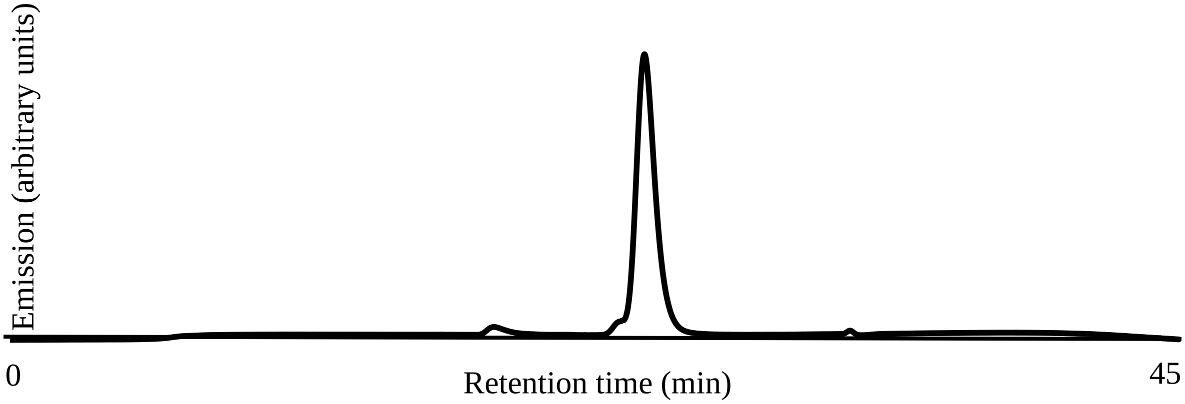

Absorbance (arbitrary units)
0
Retention time (min)
20

SEC for complexes 1d, 2d, and 3d

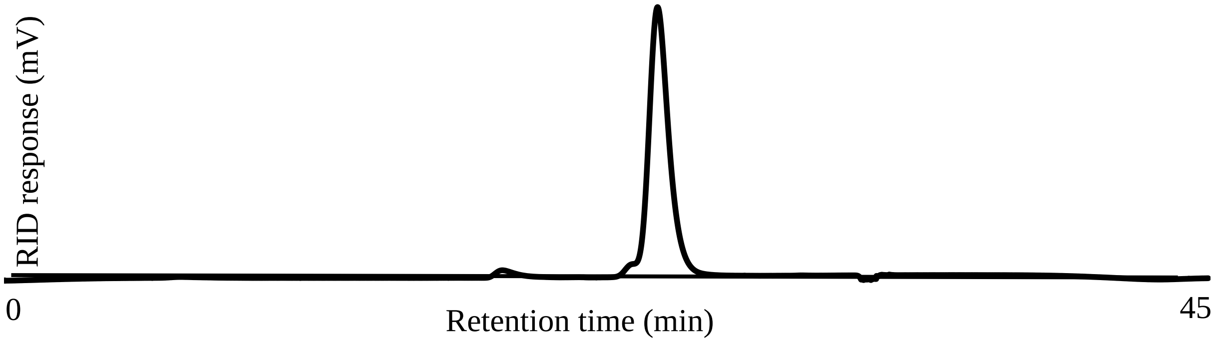
1d

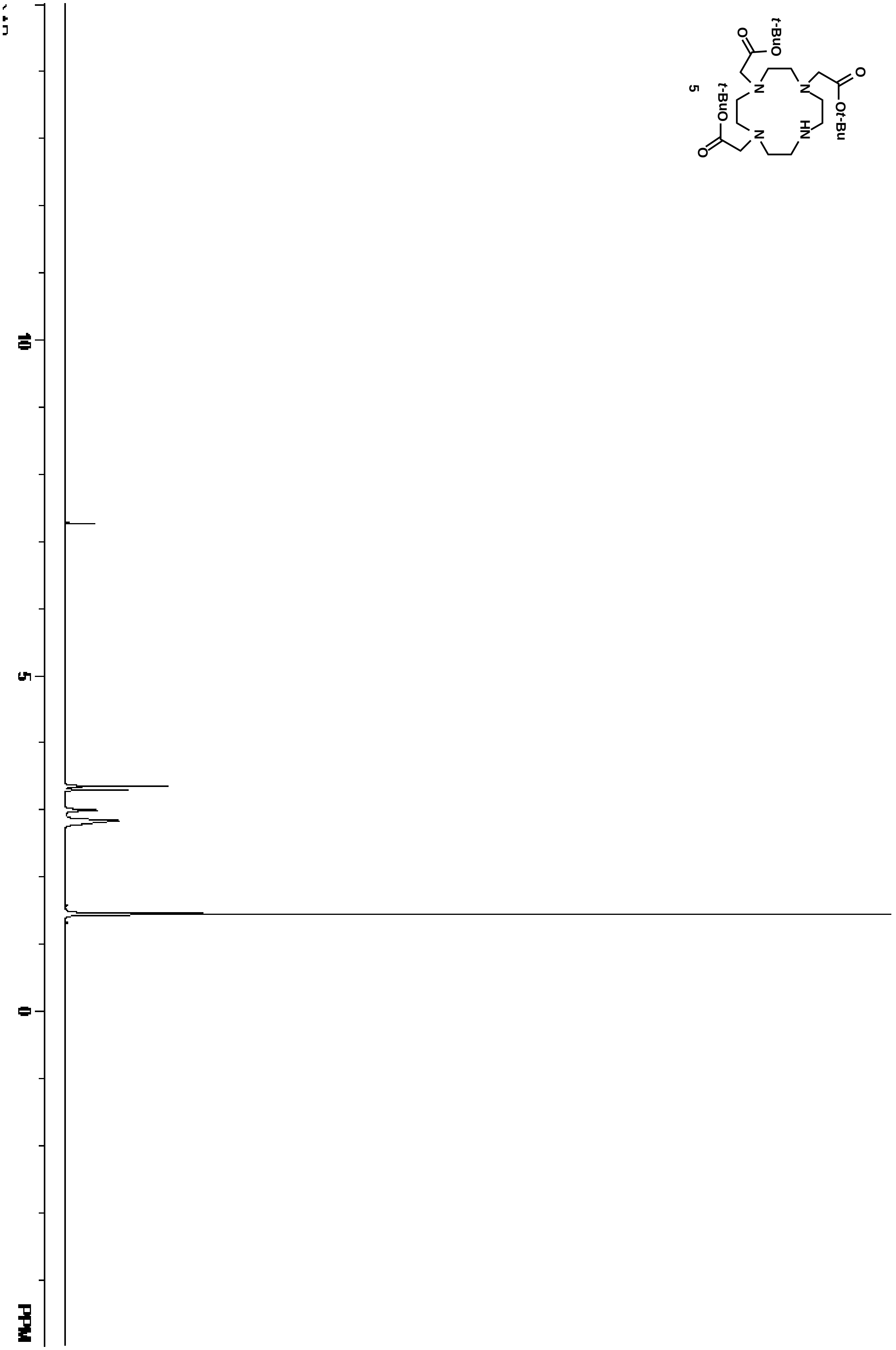


2d



3d





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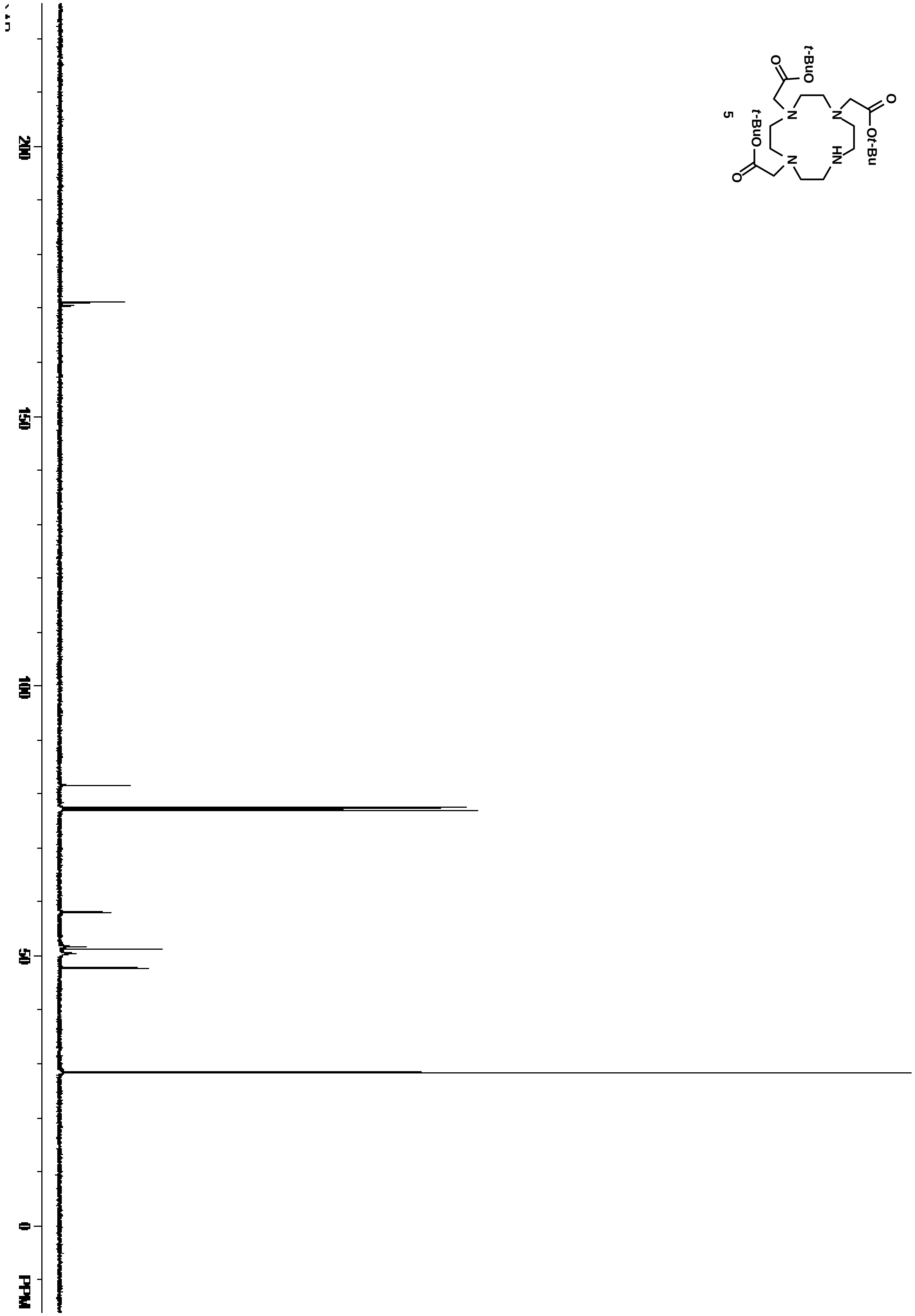
PPM
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100
150
200
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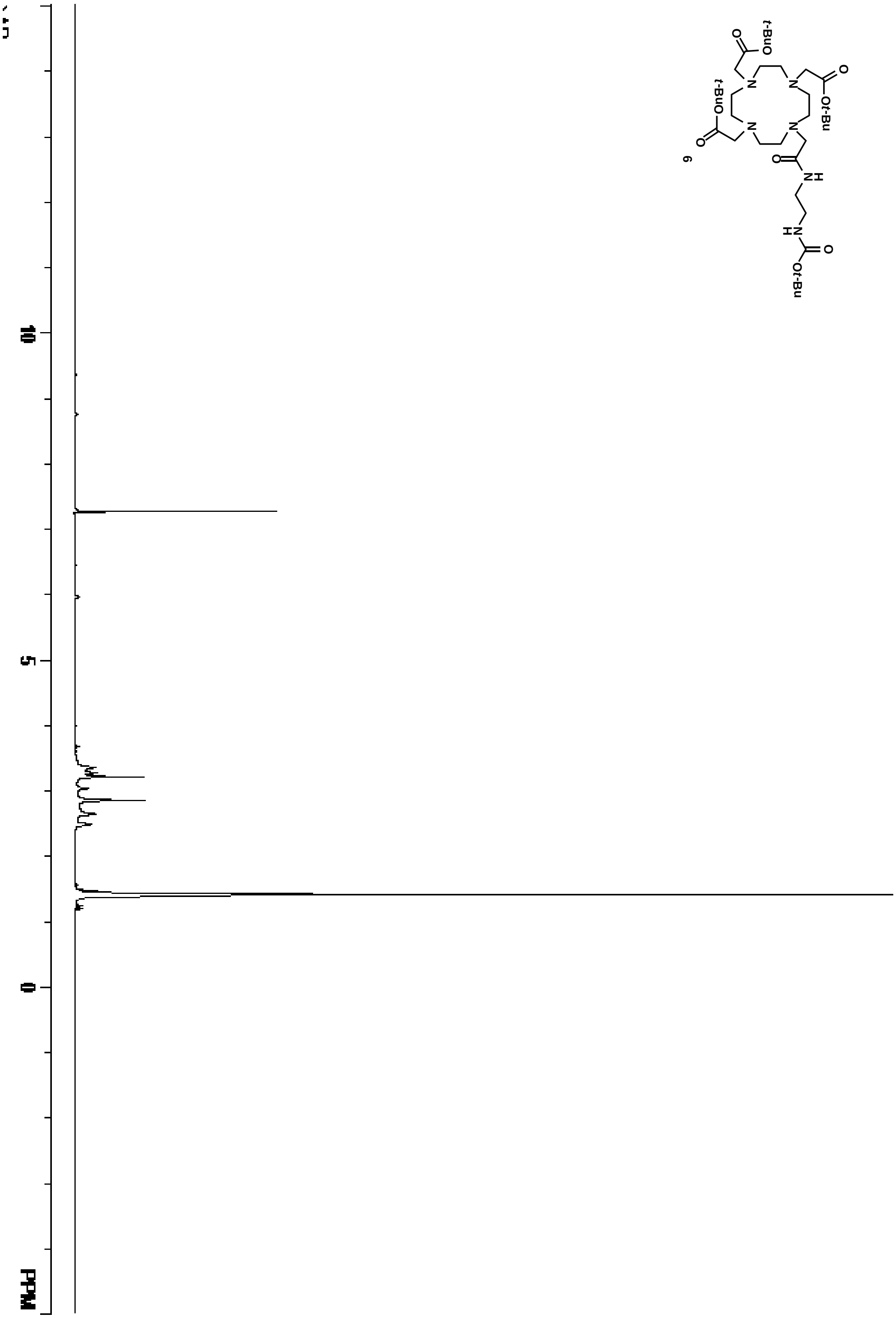
t-BuO
Ot-Bu
N
N
N
HN
O
O
O
5


10
5
0
PPM
6
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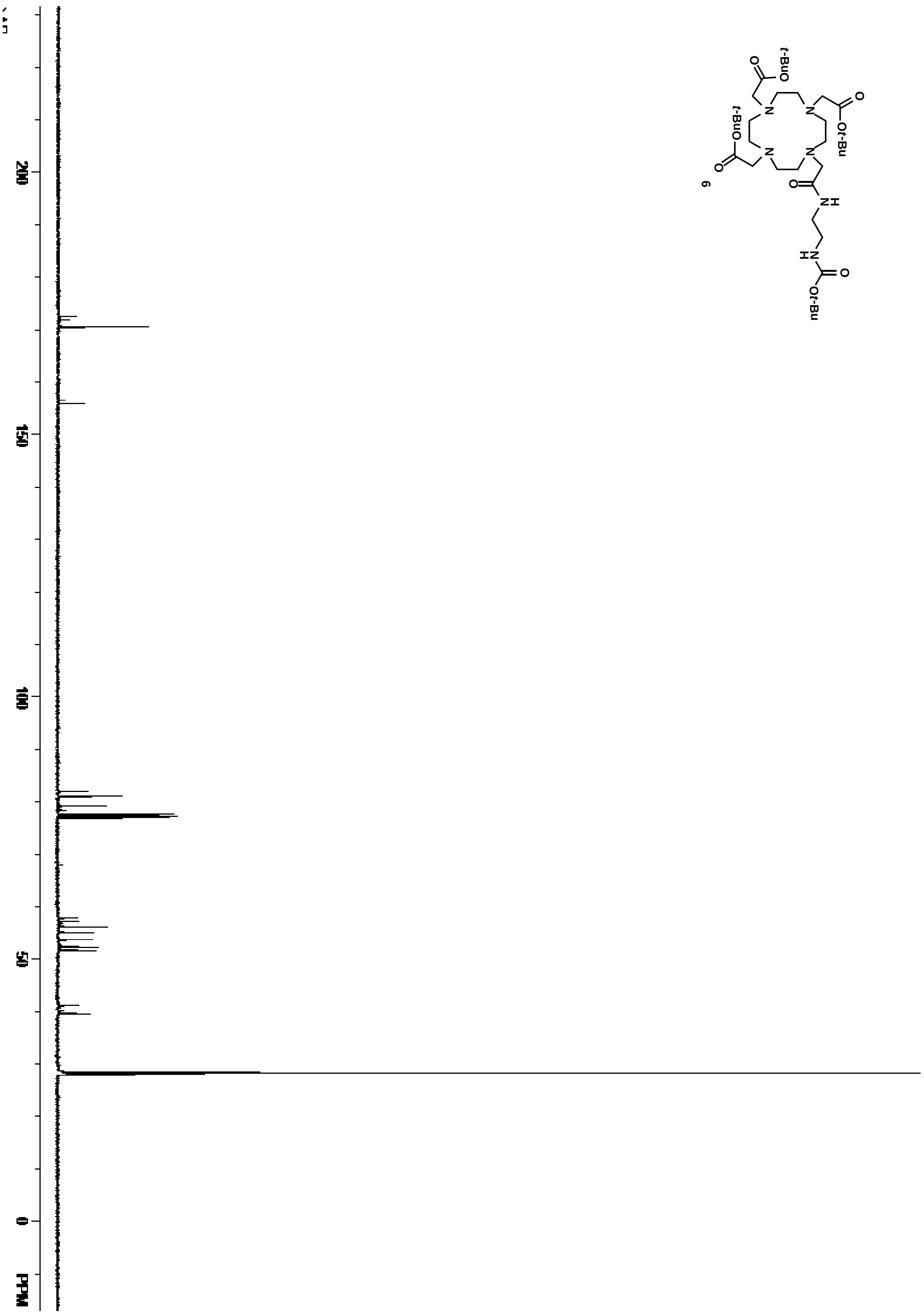
Ot-Bu
N
H
O



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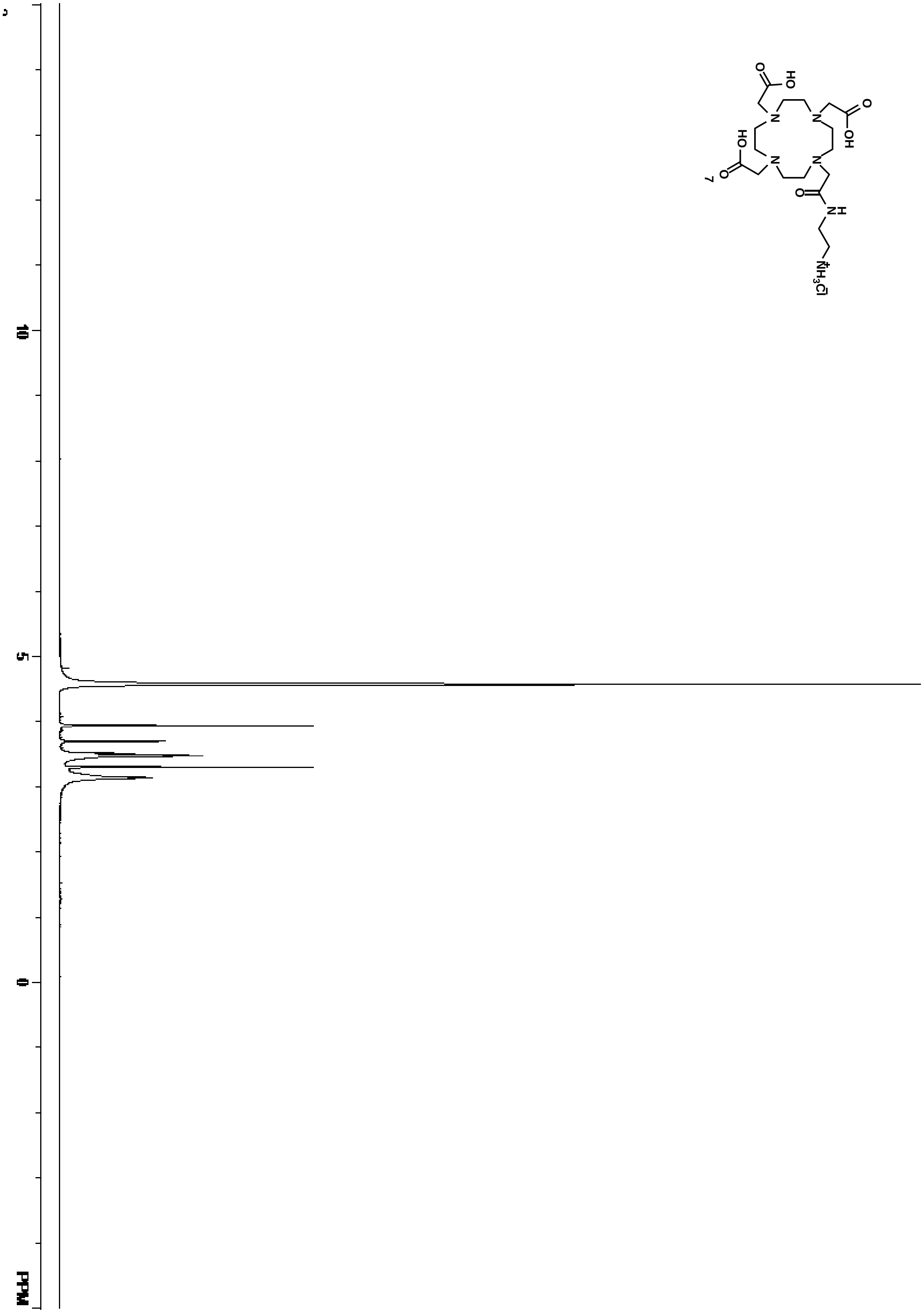

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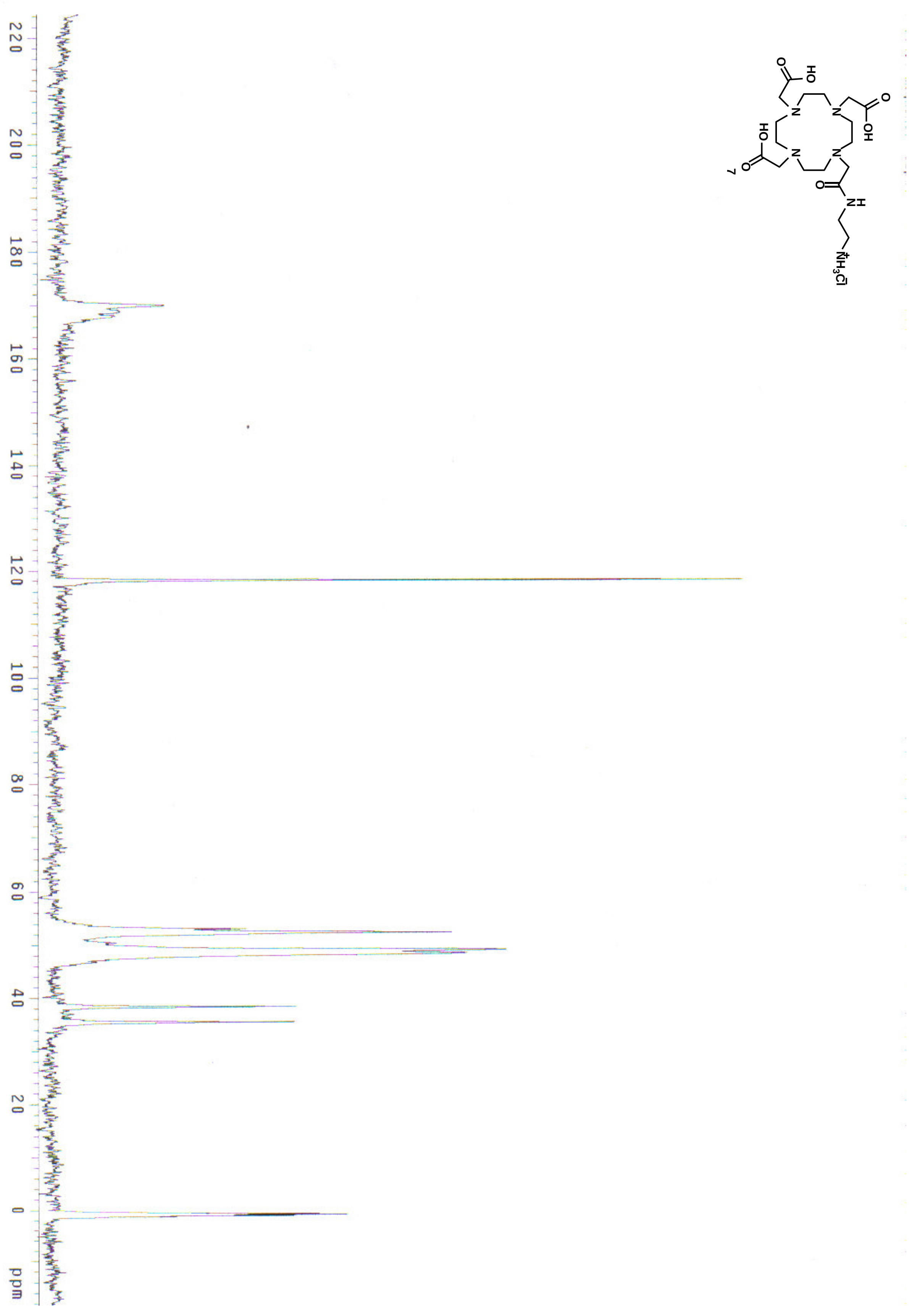
7
HO
O
OH
N
H
$\overset{+}{N}H_3\overset{-}{Cl}$
220
200
180
160
140
120
100
80
60
40
20
0
ppm