

A benzene-core trinuclear Gd^{III} complex: towards the optimization of relaxivity for MRI contrast agent applications at high magnetic field

João Bruno Livramento,^a Lothar Helm,^a Conlin O'neil,^b Angélique Sour,^a André E. Merbach,^a and Éva Tóth^{a,c*}

^a Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, ISIC, BCH, CH-1015 Lausanne, Switzerland. Fax: 00 41 21 693 9875 Tel: 00 41 21 693 98 71
lothar.helm@epfl.ch

^b Laboratoire de médecine régénérative et de pharmacobiologie, École Polytechnique Fédérale de Lausanne, EPFL-AAB; CH-1015 Lausanne, Switzerland, CH-1015 Lausanne, Switzerland.

^c Centre de Biophysique Moléculaire, CNRS, rue Charles Sadron, 45071 Orléans, France. Fax: 00 33 2 3863 1517; Tel: 00 33 2 38 25 76 25; E-mail: eva.jakabtoth@cnrs-orleans.fr

Equations used for the analysis of ¹⁷O NMR and NMRD data.

Tables S1-3: Proton relaxivities (Table S1) and variable temperature reduced transverse and longitudinal ¹⁷O relaxation rates and chemical shifts of [Gd₃L(H₂O)₆]³⁻ (Tables S2-3).

Details of HPLC separation to obtain compound 6.

FigS1. HPLC separation (R = DTTA). Top: detection method ESI-MS; bottom: detection method UV-Vis spectroscopy.

Equations used for the analysis of ^{17}O NMR and NMRD data.

^{17}O NMR spectroscopy

From the measured ^{17}O NMR relaxation rates and angular frequencies of the paramagnetic solutions, $1/T_1$, $1/T_2$ and ω , and of the acidified water reference, $1/T_{1A}$, $1/T_{2A}$ and ω_A , one can calculate the reduced relaxation rates and chemical shift, $1/T_{1r}$, $1/T_{2r}$ and $\Delta\omega_r$ (Eq. 1-3), where $1/T_{1m}$, $1/T_{2m}$ are the relaxation rates of the bound water and $\Delta\omega_m$ is the chemical shift difference between bound and bulk water, τ_m is the mean residence time or the inverse of the water exchange rate k_{ex} and P_m is the mole fraction of the bound water.^[1,2]

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

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

$$\Delta\omega_r = \frac{1}{P_m} (\omega - \omega_A) = \frac{\Delta\omega_m}{(1 + \tau_m T_{2m}^{-1})^2 + \tau_m^2 \Delta\omega_m^2} + \Delta\omega_{os} \quad (3)$$

Previous studies have shown that outer sphere contributions to the ^{17}O relaxation rates are negligible on poly(amino carboxylate) complexes of Gd^{3+} .^[3] Eqs. 1 and 2 can be further simplified:

$$\frac{1}{T_{1r}} = \frac{1}{T_{1m} + \tau_m} \quad (4)$$

$$\frac{1}{T_{2r}} = \frac{1}{T_{2m} + \tau_m} \quad (5)$$

The exchange rate is supposed to assume the Eyring equation. In Eq. 6 ΔS^\ddagger and ΔH^\ddagger are the entropy and enthalpy of activation for the water exchange process, and k_{ex}^{298} is the exchange rate at 298.15 K.

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_B T}{h} \exp \left\{ \frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT} \right\} = \frac{k_{ex}^{298} T}{298.15} \exp \left\{ \frac{\Delta H^\ddagger}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right\} \quad (6)$$

In the transverse relaxation the scalar contribution, $1/T_{2sc}$, is the most important (Eq. 7). $1/\tau_{s1}$ is the sum of the exchange rate constant and the electron spin relaxation rates $1/T_{1e}$ or $1/T_{2e}$.

$$\frac{1}{T_{2m}} \cong \frac{1}{T_{2sc}} = \frac{S(S+1)}{3} \left(\frac{A}{\hbar} \right)^2 \left(\tau_{s1} + \frac{\tau_{s2}}{1 + \omega_S^2 \tau_{s2}^2} \right) \quad (7)$$

$$\frac{1}{\tau_{si}} = \frac{1}{\tau_m} + \frac{1}{T_{ie}} \quad \text{with } i=1,2$$

The ^{17}O longitudinal relaxation rates in Gd(III) solutions are the sum of the contributions of the dipole-dipole and quadrupolar (in the approximation developed by Halle) mechanisms as expressed by Eq. 10-12 for non-extreme narrowing conditions, where γ_S is the electron and γ_I is the nuclear gyromagnetic ratio ($\gamma_S = 1.76 \times 10^{11} \text{ rad s}^{-1} \text{ T}^{-1}$, $\gamma_I = -3.626 \times 10^7 \text{ rad s}^{-1} \text{ T}^{-1}$), r_{GdO} is the effective distance between the electron spin of Gd(III) and the ^{17}O nucleus (point dipole approximation^[4]), I is the nuclear spin (5/2 for ^{17}O), χ is the quadrupolar coupling constant and η is an asymmetry parameter :

$$\frac{1}{T_{1m}} = \frac{1}{T_{1dd}} + \frac{1}{T_{1q}} \quad (8)$$

with:

$$\frac{1}{T_{1dd}} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{GdO}^6} S(S+1) \times [3J(\omega_I; \tau_{d1}) + 7J(\omega_S; \tau_{d2})] \quad (9)$$

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

In Eq. 3, the chemical shift of the bound water molecule, $\Delta\omega_m$, depends on the hyperfine interaction between the Gd(III) electron spin and the ^{17}O nucleus and is directly proportional to the scalar coupling constant, $\frac{A}{\hbar}$, as expressed in Eq. 11.^[5]

$$\Delta\omega_m = \frac{g_L \mu_B S(S+1) B}{3k_B T} \frac{A}{\hbar} \quad (11)$$

The isotopic Landé g factor is equal to 2.0 for the Gd(III), B represents the static magnetic field, and k_B is the Boltzmann constant.

The outer sphere term of the chemical shift was found proportional to $\Delta\omega_m$, through an empirical constant C_{os} .^[6]

$$\Delta\omega_{os} = C_{os} \Delta\omega_m \quad (12)$$

For slowly rotating species with the possibility of internal motion, the spectral density functions are described by the Lipari-Szabo approach.^[7,8] In this model we distinguish two statistically independent motions; a rapid local motion with a correlation time τ_l and a slower global motion with a correlation time τ_g . Supposing the global molecular reorientation is isotropic, the relevant spectral density functions are expressed as in Eq. 13-16, where the general order parameter S^2 describes the degree of spatial restriction of the local motion. If

the local motion is isotropic, $S^2 = 0$; if the rotational dynamics is only governed by the global motion, $S^2 = 1$.

$$J(\omega_I; \tau_{d1}) = \left(\frac{S^2 \tau_{d1g}}{1 + \omega_I^2 \tau_{d1g}^2} + \frac{(1 - S^2) \tau_{d1}}{1 + \omega_I^2 \tau_{d1}^2} \right) \quad (13)$$

$$J(\omega_S; \tau_{d2}) = \left(\frac{S^2 \tau_{d2g}}{1 + \omega_S^2 \tau_{d2g}^2} + \frac{(1 - S^2) \tau_{d2}}{1 + \omega_S^2 \tau_{d2}^2} \right) \quad (14)$$

$$\frac{1}{\tau_{dig}} = \frac{1}{\tau_m} + \frac{1}{\tau_g} + \frac{1}{T_{ie}} \quad i = 1, 2 \quad (15)$$

$$\frac{1}{\tau} = \frac{1}{\tau_g} + \frac{1}{\tau_l} \quad (16)$$

$$J_n(\omega_l) = \left(\frac{S^2 \tau_g}{1 + n^2 \omega_l^2 \tau_g^2} + \frac{(1 - S^2) \tau}{1 + n^2 \omega_l^2 \tau^2} \right) \quad n = 1, 2 \quad (17)$$

¹H-NMRD

The measured longitudinal proton relaxation rate, R_l^{obs} is the sum of a paramagnetic and a diamagnetic contribution as expressed in Eq. 18, where r_l is the proton relaxivity:

$$R_l^{obs} = R_l^d + R_l^p = R_l^d + r_l [\text{Gd}^{3+}] \quad (18)$$

The relaxivity can be divided into an inner and an outer sphere term as follows:

$$r_l = r_{lis} + r_{los} \quad (19)$$

The inner sphere term is given in Eq. 20, where q is the number of inner sphere water molecules^[9].

$$r_{lis} = \frac{1}{1000} \times \frac{q}{55.55} \times \frac{1}{T_{lm}^H + \tau_m} \quad (20)$$

The longitudinal relaxation rate of inner sphere protons, $1/T_{lm}^H$ is expressed by Eq. 21, where r_{GdH} is the effective distance between the electron spin and the ¹H nucleus, ω_l is the proton resonance frequency and ω_S is the Larmor frequency of the Gd(III) electron spin.

$$\frac{1}{T_{lm}^H} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{GdH}^6} S(S+1) \times [3J(\omega_l; \tau_{d1}) + 7J(\omega_S; \tau_{d2})] \quad (21)$$

$$\frac{1}{\tau_{di}} = \frac{1}{\tau_m} + \frac{1}{\tau} + \frac{1}{T_{ie}} \quad (22)$$

The spectral density functions are given by Eq. 13-16.

In Solomon-Bloembergen-Morgan theory the longitudinal and transverse electronic relaxation rates, $1/T_{1e}$ and $1/T_{2e}$ are expressed by Eq. 23-24, where τ_V is the correlation time for the modulation of the transient zero-field-splitting interaction, E_V the corresponding activation energy and Δ^2 is the mean square of the transient zero-field-splitting energy. In general an Arrhenius behaviour is assumed for temperature dependence of τ_V (Eq. 25).

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25} \Delta^2 \tau_V \{4S(S+1) - 3\} \left(\frac{1}{1 + \omega_S^2 \tau_V^2} + \frac{4}{1 + 4\omega_S^2 \tau_V^2} \right) \quad (23)$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2 \tau_V \left(\frac{5.26}{1 + 0.372\omega_S^2 \tau_V^2} + \frac{7.18}{1 + 1.24\omega_S \tau_V} \right) \quad (24)$$

$$\tau_V = \tau_V^{298} \exp \left\{ \frac{E_V}{R} \left(\frac{1}{T} - \frac{1}{298.15} \right) \right\} \quad (25)$$

The outer-sphere contribution can be described by Eq. 26 where N_A is the Avogadro constant, and J_{os} is the spectral density function ^[10,11].

$$r_{1os} = \frac{32N_A \pi}{405} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\hbar^2 \gamma_S^2 \gamma_I^2}{a_{GdH} D_{GdH}} S(S+1) [3J_{os}(\omega_I, T_{1e}) + 7J_{os}(\omega_S, T_{2e})] \quad (26)$$

$$J_{os}(\omega, T_{je}) = \text{Re} \left[\frac{1 + \frac{1}{4} \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2}}{1 + \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2} + \frac{4}{9} \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right) + \frac{1}{9} \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{3/2}} \right] \quad (27)$$

$j = 1, 2$

The correlation time for mutual translational diffusion between Gd(III) and outer sphere water protons, τ_{GdH} , is given by $\tau_{GdH} = a_{GdH}^2 / D_{GdH}$. The diffusion coefficient for the mutual diffusion constant between a Gd(III) complex and outer sphere water protons, D_{GdH} , is assumed to obey an Arrhenius law with an activation energy E_{GdH} , as given in Eq. 28. D_{GdH}^{298} is the diffusion coefficient at 298.15K.

$$D_{GdH} = D_{GdH}^{298} \exp \left\{ \frac{E_{GdH}}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right\} \quad (28)$$

References for Equations.

- [1] T. J. Swift, R. E. Connick, *J. Chem. Phys.* **1962**, *37*, 307.
- [2] J. R. Zimmermann, W. E. Brittain, *J. Phys. Chem.* **1957**, *61*, 1328.
- [3] K. Micskei, L. Helm, E. Brücher, A. E. Merbach, *Inorg. Chem.* **1993**, *32*, 3844.
- [5] [O. V. Yazyev, L. Helm, V. G. Malkin O. L. Malkina, *J. Phys. Chem. A* **109** (2005) 10997.
- [5] H. G. Brittain, J. F. Desreux, *Inorg. Chem.* **1984**, *23*, 4459.
- [6] G. Gonzalez, H. D. Powell, V. Tissières, A. E. Merbach, *J. Phys. Chem.* **1994**, *98*, 53.
- [7] G. Lipari, S. Szabo, *J. Am. Chem. Soc.* **1982**, *104*, 4546.
- [8] G. Lipari, S. Szabo, *J. Am. Chem. Soc.* **1982**, *104*, 4559.
- [9] Z. Luz, S. Meiboom, *J. Chem. Phys.* **1964**, *40*, 2686.
- [10] J. H. Freed, *J. Chem. Phys.* **1978**, *68*, 4034.
- [11] S.H. Koenig, R. D. Brown III, *Prog. Nucl. Magn Reson. Spectrosc.* **1991**, *22*, 487.

Table S1 – Proton relaxivities, r_1 ($\text{mM}^{-1}\text{s}^{-1}$) of $[\text{Gd}_3\text{L}(\text{H}_2\text{O})_6]^{3-}$ ($c_{\text{Gd}} = 2.00 \text{ mM}$).

v/MHz	5 °C	25 °C	37 °C
0.01	32.50	26.24	22.40
0.01	32.68	26.20	22.44
0.02	32.69	26.28	22.64
0.03	32.53	26.20	22.69
0.04	32.91	26.32	22.74
0.05	32.65	26.30	22.72
0.07	32.66	26.30	22.56
0.10	32.44	26.16	22.82
0.15	32.43	26.14	22.73
0.20	32.72	26.20	22.62
0.29	32.13	25.65	22.58
0.40	31.64	25.72	22.49
0.56	30.94	25.64	22.31
0.78	30.28	24.90	21.88
1.09	29.48	24.20	21.38
1.53	27.89	23.31	20.44
2.14	26.89	21.99	19.50
2.99	25.55	20.84	18.18
4.18	23.92	19.45	16.78
5.85	23.15	17.64	15.33
8.18	24.44	18.06	15.24
11.44	26.47	18.72	15.37
16.00	29.17	19.33	15.32

20.00	30.35	20.10	15.66
30.00	31.89	21.22	15.93
40.00	32.98	21.56	16.19
60.00	32.54	21.63	16.04
100.00	28.88	20.49	15.81
200.00	19.54	17.02	14.05
400.00	10.05	10.69	10.16

Table S2 - Variable temperature reduced transverse and longitudinal ^{17}O relaxation rates and chemical shifts of a $[\text{Gd}_3\text{L}(\text{H}_2\text{O})_6]^{3-}$ solution at 9.4 T. ($C_{\text{Gd}} = 30.54$ mmol/kg; $P_m = 1.10 \times 10^{-3}$)

T / K	1000/T	T_{1A}/s	T_1/s	$\ln(1/T_{1r})$	T_{2A}/s	T_2/s	$\ln(1/T_{2r})$	ν_A/Hz	ν/Hz	$\Delta\omega_r$ / 10^6 rad s^{-1}
277.05	3.61	3.74E-03	2.50E-03	11.70	3.81E-03	6.15E-04	14.03	3361.45	3294.67	-0.38
283.85	3.52	4.66E-03	3.08E-03	11.51	4.76E-03	6.16E-04	14.07	3314.62	3266.50	-0.27
291.65	3.43	5.99E-03	3.91E-03	11.30	6.09E-03	6.64E-04	14.01	3310.29	3200.40	-0.63
303.25	3.30	8.48E-03	5.57E-03	10.93	8.49E-03	8.21E-04	13.82	3236.29	3084.09	-0.87
315.05	3.17	1.07E-02	7.15E-03	10.65	1.07E-02	1.05E-03	13.57	3210.59	3087.45	-0.70
326.65	3.06	1.36E-02	9.06E-03	10.42	1.35E-02	1.50E-03	13.20	3196.15	3056.76	-0.80
336.65	2.97	1.65E-02	1.12E-02	10.18	1.63E-02	2.12E-03	12.83	3192.22	3082.90	-0.62
345.05	2.90	1.86E-02	1.29E-02	9.97	1.84E-02	2.78E-03	12.54	3117.32	3017.12	-0.57
357.05	2.80	2.24E-02	1.59E-02	9.72	2.22E-02	3.94E-03	12.15	3091.27	2995.93	-0.54
371.05	2.70	2.60E-02	1.97E-02	9.34	2.58E-02	6.03E-03	11.66	3087.54	2981.82	-0.60

Table S3 - Variable temperature reduced transverse and longitudinal ^{17}O relaxation rates and chemical shifts of the $[\text{Gd}_3\text{L}(\text{H}_2\text{O})_6]^{3-}$ solution at 4.7 T. ($C_{\text{Gd}} = 30.54$ mmol/kg; $P_m = 1.10 \times 10^{-3}$)

T / K	1000/T	T_{1A}/s	T_1/s	$\ln(1/T_{1r})$	T_{2A}/s	T_2/s	$\ln(1/T_{2r})$	ν_A/Hz	ν/Hz	$\Delta\omega_r$ / 10^6 rad s^{-1}
277.45	3.60	3.91E-03	2.51E-03	11.77	3.93E-03	6.99E-04	13.88	-652.21	-694.26	-0.24
285.75	3.50	5.19E-03	3.28E-03	11.54	5.13E-03	7.34E-04	13.88	-661.94	-713.68	-0.30
298.55	3.35	7.23E-03	4.70E-03	11.12	7.22E-03	8.76E-04	13.72	-674.44	-748.54	-0.42
310.25	3.22	9.69E-03	6.27E-03	10.84	9.63E-03	1.19E-03	13.53	-692.19	-760.83	-0.39
317.85	3.15	1.14E-02	7.53E-03	10.61	1.12E-02	1.38E-03	13.27	-699.07	-763.67	-0.37
331.75	3.01	1.49E-02	1.00E-02	10.30	1.35E-02	2.09E-03	12.82	-718.12	-777.09	-0.34
343.95	2.91	1.81E-02	1.25E-02	10.02	1.77E-02	2.96E-03	12.45	-730.76	-790.18	-0.34
351.35	2.85	2.03E-02	1.45E-02	9.81	2.03E-02	3.73E-03	12.20	-741.56	-799.88	-0.33
370.05	2.70	2.67E-02	1.94E-02	9.47	2.61E-02	6.09E-03	11.65	-764.08	-816.72	-0.30

HPLC separation to obtain compound 6.

The analysis, separation and collection were performed using a Waters Ultrapurification system. The HPLC equipment was comprised of a sample manager for injection and collection (2767 Sample Manager,) a column manager for automated switching from analytical to preparative columns, a flow splitter from Dionex (Sunnyvale CA, USA) a makeup pump (515 Pump) to direct the flow from the splitter into the detectors, the main pump (2525 Pump,) a UV/visible detector (996 PDA,) and a single quadrupole mass spectrometer (ZQ) equipped with a dual ESI and APCI source. We used the Atlantis dC18 OBD 10 micron, 19x150 mm preparative column for the reverse phase separation. The conditions we used for the collection are as follows: Flow 20 mL/min, two injections of 300 μ l for optimization, 8 injections of 1500 μ l, then one injection of 1650 μ l. The gradient program is as follows: Mobile phase A 0.1% TFA, B acetonitrile. Initial composition 100% A, 0-2 min. 90% A, 2-14 80% A, 14-16 100% B, 16-18 100% A, end 20 min. 100% A. The MS detector settings were: Capillary voltage 3.0 keV, cone voltage 40 V, and extractor set to 5 V. The source temperature was set to 100 $^{\circ}$ C, desolvation to 200 $^{\circ}$ C, with a desolvation gas setting of 300 L/h. The instrument was set to scan a mass window of 50-1950 Da, scan time of 0.5 seconds and an inter scan delay of 0.1 seconds. Data was collected in centroid mode, using positive electrospray ionization (ESI⁺).

Fig. S1. The chromatogram obtained in the HPLC separation (R = DTTA). Top: detection method ESI-MS; bottom: detection method UV-Vis spectroscopy.

