

Highly relaxing Gadolinium based MRI contrast Agent responsive to Mg^{2+} sensing

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Supplementary information (7 pages)

Table S1. Full results of the potentiometric data with experimental details.

Table S2. Longitudinal relaxivity values (r_1) at pH=7.4, 25°C, in different conditions and experimental details of the relaxivity measurements.

Figure S1. Relaxivity of [GdL] at different concentrations of added MgCl_2 , pH=7.4, 25°C

Table S3. Atomic coordinates obtained for $[\text{GdH}_2\text{L}]^{3-}$ B3LYP/6-31G(d), aqueous solution (IEFPCM), 0 imaginary frequencies and full experimental details for the DFT modeling.

Table S1. Successive protonation constants ($\log K_{LH_n}$) of the free ligand **L**,¹ formal stability constants ($\log K_{ML}$), successive protonation constants ($\log K_{MLH_n}$) and pM^e values of the lanthanides(III) complexes (Ln(III) = La(III), Nd(III), Eu(III), Tb(III), Er(III) and Lu(III)) with **L**.^a

Constant	H ⁺	M = Ln(III)						
		La	Nd	Eu	Gd	Tb	Er	Lu
$\log K_{LH}$	11.21(2)							
$\log K_{LH2}$	10.29(2)							
$\log K_{LH3}$	8.04(4)							
$\log K_{LH4}$	6.49(6)							
$\log K_{LH5}$	5.53(8)							
$\log K_{LH6}$	4.19(9)							
$\log K_{ML}$		25.5(3)	27.1(2)	25.7(2)	^d	29.7(4)	29.7(7)	29.3(3)
$\log K_{MLH}$		9.4(3)	8.8(2)	9.4(2)	^d	7.6(5)	7.8(7)	7.4(4)
$\log K_{MLH2}$		7.3(4)	6.6(3)	7.5(3)	^d	^c	^c	^c
$\log K_{M(OH)}^b$		-8.83	-8.20	-7.78	-	-7.66	-7.54	-7.29
					7.85			
$pM(\mathbf{L})$		21.2	22.1	21.5	^d	23.6	23.7	23.1
$pM(\mathbf{L}')^2$		^d	^d	^d	17.5	^d	^d	^d
$pM(\text{DOTA})^3$		17.8	17.8	18.4	19.6	19.6	19.6	20.3
$pM(\text{DOTP})^4$		21.5	21.2	21.5	22.5	22.6	24.3	24.3

^a Solvent: water; $I = 0.1 \text{ M}$ (NaClO_4); $T = 25.0(2) \text{ }^\circ\text{C}$. Errors = 3σ with σ = standard deviation. $K_{LH_n} = [\text{LH}_n]/[\text{LH}_{n-1}][\text{H}]$, $K_{ML} = [\text{ML}]/([\text{L}][\text{M}]$ and $K_{MLH_m} = [\text{MLH}_m]/[\text{MLH}_{m-1}][\text{H}]$. Charges have been omitted for the sake of clarity. ^b The hydrolysis constants used for the processing of the potentiometric data on Ln(III) complexes with **L** have been taken from ref. 5. ^c non determined because of precipitation of the Ln(III) complexes below pH 6-6.5. ^d not determined. ^e $pM = -\log [\text{Ln}^{3+}]_{\text{free}}$ with $[\text{L}]_{\text{tot}} = 10^{-5} \text{ M}$ and $[\text{Ln(III)}]_{\text{tot}} = 10^{-6} \text{ M}$ at pH 7.4.⁶

The thermodynamics of the Ln(III) complexes (Ln = La, Nd, Eu, Tb, Er and Lu) with ligand **L** was elucidated using potentiometric titrations as well as metal exchange competition experiments (with Cu(II) at pH 2.0) monitored by UV-visible absorption spectrophotometry. The formal stability K_{ML} constants were determined with, however, poor accuracy due to the formation of a precipitate below pH 6-6.5 regardless of the nature of the lanthanide(III) cation considered. This phenomenon is a common feature of several linear amino-

¹ (a) S. Abada, A. Lecointre, M. Elhabiri, L. J. Charbonnière, *Dalton Trans.* **2010**, 39, 9055-9062. (b) S. Abada, A. Lecointre, I. Déchamps-Olivier, C. Platas-Iglesias, C. Christine, M. Elhabiri, L. Charbonnière, *Radiochim. Acta*, **2011**, 99, 663-678.
² L. Pellegatti, J. Zhang, B. Drahos, S. Villette, F. Suzenet, G. Guillaumet, S. petoud, É. Tóth, *Chem. Commun.* **2008**, 6591-6593.
³ W. P. Cacheris, S. K. Nickle, A. D. Sherry, *Inorg. Chem.* **1987**, 26, 958-960.
⁴ A. D. Sherry, J. Ren, J. Huskens, E. Brücher, É. Tóth, C. F. C. G. Geraldés, M. M. C. A. Castro, W. P. Cacheris, *Inorg. Chem.* **1996**, 35, 4604-4612.
⁵ G. D. Klungness, R. H. Byrne, *Polyhedron* **2000**, 19, 99-107.
⁶ W. R. Harris, C. J. Carrano, K. N. Raymond, *J. Am. Chem. Soc.* **1979**, 101, 2213.

polymethanephosphonates⁷ and indicates that some protonated Ln(III) complexes are poorly soluble in water at concentrations about 2×10^{-3} M. As a consequence, the processing of the potentiometric data in this constrained pH range ($6.5 \leq \text{pH} \leq 11$) led to the determination of only the higher protonation constants (K_{MLH} and K_{MLH_2} , Table S1). The progressive and monotonous increase in the formal K_{ML} stability constants as well as in the conditional K_{ML}^* stability constants measured at pH 2 ($\log K_{\text{ML}}^* = 3.8(1)$ (La); 4.0(1) (Nd); 4.1(5) (Eu); 4.4(1) (Tb) and 4.42(7) (Er)) along the Ln(III) cation series is characteristic of chelates that are rather flexible and show increasing stability with charge density on the cation. Interestingly, the protonation constants decrease as the stabilities of the Ln(III) complexes increase indicating that the protons first attack the secondary amine units sites which are most likely involved in N-Ln(III) coordination bonds. Even though very scarce thermodynamic data were reported for the acetate analog of **L** (Table S1), the substitution of the acetate binding units by phosphonates seemingly leads to more stable lanthanide(III) species as a result of the higher overall basicity of the phosphonate-based chelator (for instance, $\log \beta_{\text{LH}_4} = 36.03$ for **L** and $\log \beta_{\text{L}'\text{H}_4} = 22.66$ for **L'**). This feature was already reported for many binding systems as illustrated in Table S1 for DOTA and DOTP. On the other hand, ligand **L** forms very stable Ln(III) complexes (as illustrated by the high pLn values, see Table S1) with respect to those formed with DOTA or DOTP.

Relaxivity measurements

The samples were prepared in situ by mixing the appropriate amounts of ligand and $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%; Aldrich) in mQ water followed by adjustment of the pH with NaOH solution in water (1M and 0.1M). Gd(III) stock solutions were prepared by dissolving the appropriate amounts of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ in water. The exact Gd(III) ion concentration was determined by colorimetric titration in acetate buffer (pH=4.5) using standardized $\text{H}_2\text{Na}_2\text{edta}$ solutions (Aldrich) and xylenol orange as the indicator. The absence of free gadolinium was checked by the xylenol orange test.

Similar conditions of concentration and pH have been used for the relaxivity studies of all the complexes ($C^\circ = 2$ to 5mM). A stock solutions (~2 M) of MgCl_2 was prepared by dissolving a precisely weighed mass of the salt in H_2O and neutralizing it to pH~7.4 with KOH or HCl solutions. The relaxivities of the complexes in the presence of Mg(II) were measured in

⁷ K. Sawada, M. Kuribayashi, T. Suzuki, H. Miyamoto, *J. Sol. Chem.* **1991**, *20*, 829-839.

solutions prepared by mixing appropriate volumes of the corresponding Gd(III) complex and the anion stock solutions. The relaxivity at 200 MHz in presence of anion was compared to that in pure H₂O at the same pH.

For the measurement in the serum, solutions have been prepared by mixing appropriate volumes of a stock solution of the corresponding complex, at pH~7.4 in water in fetal bovine serum (ligand~0.2 mM)

The 1/T₁ NMRD profiles were obtained at 298 K from 0.01 to 35 MHz using a Spinmaster FFC (Fast Field Cycling) NMR Relaxometer (Stelar, Italy).

Table S2 . Longitudinal relaxivity values (r₁) at pH=7.4, 25°C, in different conditions.

	r ₁ (mM ⁻¹ .s ⁻¹)			
	35MHz	200MHz	500MHz	nbr d'eq
AL090	9,25	8,78	7,17	
AL090_carbonates		7,39	6,36	200
AL090_serum	15,75	7,16	3,92	
AL090_Ca ²⁺		8,87		3
		8,48		30
		8,48		200
AL090_Mg ²⁺		8,59		0
		9,01		1
		9,09		3
		9,53		5
		10,05		10
		10,23		20
		10,89		25
	10,91	11,56		30
		11,91		50
		11,94		100
		12,03		200

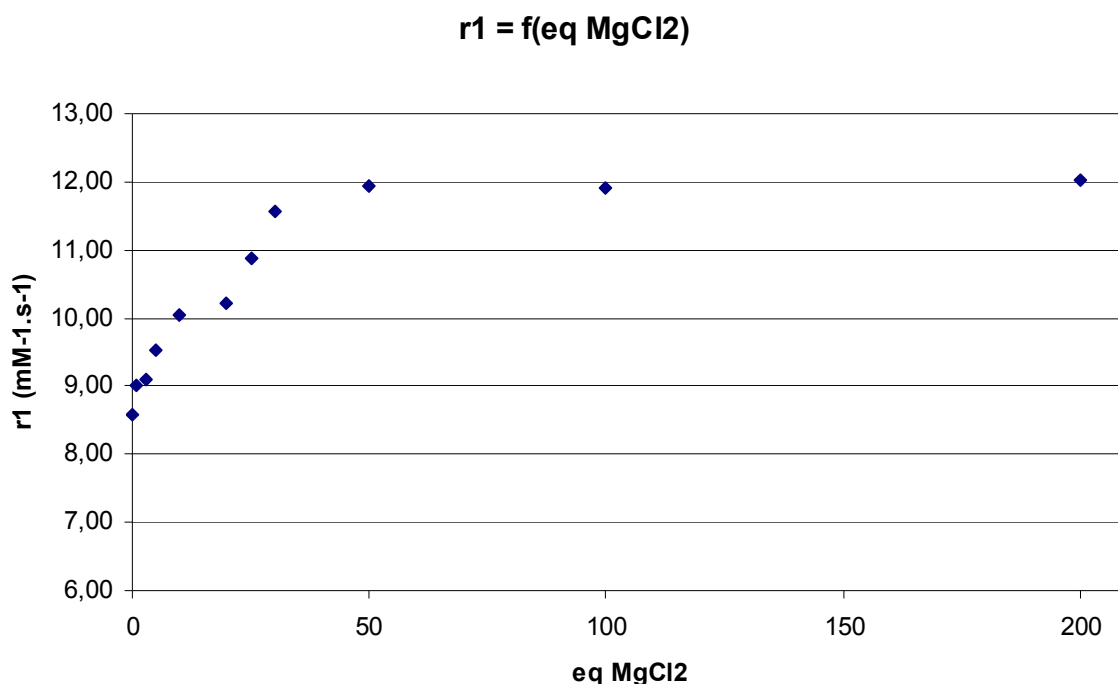


Figure S1 Relaxivity of [GdL] at different concentrations of added MgCl₂, pH=7.4, 25°C

Computational details. All calculations were performed employing hybrid DFT with the B3LYP exchange-correlation functional,^{8,9} and the Gaussian 09 package (Revision C.01).¹⁰ Full geometry optimizations of the [GdH₂L]³⁻ system were performed in aqueous solution by using the effective core potential (ECP) of Dolg et al. and the related [5s4p3d]-GTO valence basis set for the lanthanides,¹¹ and the 6-31G(d) basis set for C, H, N, O and P atoms. No symmetry constraints have been imposed during the optimizations. The default values for the integration grid (“fine”) and the SCF energy convergence criteria (10⁻⁸) were used. The

⁸ A. D. Becke, *J. Chem. Phys.* 1993, **98**, 5648-5652.

⁹ C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* 1988, **37**, 785-789.

¹⁰ Gaussian 09, Revision A.1, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

¹¹ M. Dolg, H. Stoll, A. Savin, H. Preuss, *Theor. Chim. Acta* 1989, **75**, 173-194.

stationary points found on the potential energy surfaces as a result of the geometry optimizations have been tested to represent energy minima rather than saddle points via frequency analysis. Solvent effects were evaluated by using the polarizable continuum model (PCM), in which the solute cavity is built as an envelope of spheres centered on atoms or atomic groups with appropriate radii. In particular, we used the integral equation formalism (IEFPCM) variant as implemented in gaussian 09.¹²

Table S3. Atomic coordinates obtained for $[\text{GdH}_2\text{L}]^{3-}$ B3LYP/6-31G(d), aqueous solution (IEFPCM), 0 imaginary frequencies

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	64	0	0.009096	-0.712803	-0.015637
2	6	0	-1.132568	2.640901	0.634689
3	6	0	-1.211990	4.037110	0.661077
4	1	0	-2.055670	4.523791	1.139728
5	6	0	-2.181043	1.810824	1.347299
6	7	0	-2.427687	0.468264	0.786428
7	6	0	-3.057201	-0.409160	1.820538
8	1	0	-4.147605	-0.258718	1.849457
9	1	0	-2.669100	-0.114018	2.799726
10	6	0	-3.288626	0.485052	-0.422211
11	1	0	-3.700986	-0.523770	-0.509902
12	1	0	-4.131879	1.185002	-0.303046
13	1	0	-1.824802	1.671904	2.374586
14	1	0	-3.115587	2.390853	1.416846
15	15	0	-2.593110	-2.198815	1.621100
16	8	0	-1.014206	-2.071202	1.554376
17	8	0	-3.108891	-2.935428	2.844889
18	8	0	-3.123247	-2.688657	0.246467
19	15	0	-2.485263	0.756152	-2.065366
20	8	0	-3.430153	0.265478	-3.126597
21	8	0	-1.053255	0.189328	-1.993202
22	8	0	-2.347858	2.397161	-2.237689
23	1	0	3.836509	0.156263	-2.202143
24	6	0	2.865983	-0.230945	-1.848748
25	7	0	2.286676	0.657122	-0.800129
26	1	0	2.179592	-0.210886	-2.702434
27	15	0	3.021415	-2.017366	-1.308736
28	6	0	1.881768	1.949329	-1.377106
29	6	0	3.249468	0.823070	0.319680
30	8	0	1.606662	-2.239136	-0.603342
31	8	0	3.151569	-2.822873	-2.597400
32	8	0	4.169986	-2.123521	-0.301128
33	6	0	0.852262	2.712918	-0.568715
34	1	0	1.426281	1.740871	-2.352245
35	1	0	2.754136	2.595296	-1.565649

¹² J. Tomasi, B. Mennucci, R. Cammi, *Chem. Rev.* 2005, **105**, 2999-3093.

36	1	0	3.914699	-0.046605	0.296943
37	1	0	3.864991	1.725166	0.185557
38	15	0	2.534883	0.776388	2.016267
39	6	0	0.848445	4.109656	-0.567456
40	7	0	-0.113456	1.997941	0.040572
41	8	0	3.615956	0.427351	3.005639
42	8	0	1.271220	-0.099044	1.928992
43	8	0	1.985121	2.305602	2.341115
44	6	0	-0.199016	4.780505	0.062299
45	1	0	1.646536	4.655416	-1.060316
46	1	0	-0.230531	5.865960	0.074099
47	8	0	-1.037928	-2.716835	-1.317220
48	1	0	-1.849871	-2.850008	-0.719426
49	1	0	-0.332250	-3.309238	-1.006273
50	1	0	2.662010	2.773811	2.858142
51	1	0	-1.440954	2.678283	-2.037212

E(RB3LYP) = -2975.11310932 Hartree

Zero-point correction = 0.352282

Thermal correction to Energy = 0.385869

Thermal correction to Enthalpy = 0.386814

Thermal correction to Gibbs Free Energy = 0.289575

Sum of electronic and zero-point Energies = -2974.760827

Sum of electronic and thermal Energies = -2974.727240

Sum of electronic and thermal Enthalpies = -2974.726296

Sum of electronic and thermal Free Energies = -2974.823534