Highly relaxing gadolinium based MRI contrast agents responsive to Mg^{2+} sensing[i]

Sabah Abada[a], Alexandre Lecointre[a], Mourad Elhabiri[b], David Esteban-Gómez[c], Carlos Platas-Iglesias[c], Gaylord Tallec[d], Marinella Mazzanti[d] and Loïc J. Charbonnière[a*]

[a] Laboratoire d'Ingénierie Moléculaire Appliquée à l'Analyse, IPHC, UMR 7178 CNRS/UdS, ECPM 25 rue Becquerel, 67087 Strasbourg Cedex 2, France

[b] Laboratoire de Chimie Bioorganique et Médicinale, UMR 7509 CNRS-UdS, ECPM, Université de Strasbourg, 25 rue Becquerel, 67200 Strasbourg, France

[c] Departamento de Química Fundamental, Universidade da Coruña, Campus da Zapateira-Rúa da Fraga 10, 15008 A Coruña, Spain

[d] Laboratoire de Reconnaissance Ionique et Chimie de Coordination, INAC, CEA Genoble, UMR-E 3 CEA-UJF, 38054 Grenoble Cedex 9, France

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Abstract

A Gd complex based on a polyphosphonated pyridyl ligand shows a very high stability in aqueous solution (log K_{EuL} = 25.7), a high relaxivity (8.5 mM^{-1} s^{-1} at 25 °C and 20 MHz) and a marked and selective relaxivity enhancement (37%) in the presence of Mg^{2+}, opening interesting perspectives for the design of cation responsive contrast agents.

Keywords: MRI contrast agents; DFT calculations; gadolinium complexes; relaxivity; magnesium sensing

Stable Gd^{3+} complexes with polyaminocarboxylate ligands are commonly used as contrast agents in Magnetic Resonance Imaging (MRI).[1] Contrast agents are paramagnetic molecules that enhance the image contrast by shortening the longitudinal and/or transversal relaxation times of protons of water molecules in the vicinity of the chelate.

Considering the efficiency of the commercially available gadolinium-based contrast agents, current efforts in this field of research are directed toward the fundamental understanding of the parameters governing the relaxivity in existing complexes or their derivatives,[1] the preparation of nanoscopic scaffolds such as micelles[2] or dendrimers,[3] with enhanced relaxivities due to an increase of the rotational correlation time, or toward the design of smart probes responsive to the presence of enzymes,[4] proteins[5] or cations.[6] Among these, smart probes for the detection of biologically relevant cations such as Cu^{2+}, Zn^{2+}, Ca^{2+} or Mg^{2+} are of particular interest for monitoring and imaging of the cellular flux of these cations.[7]

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[*] l.charbonn@unistra.fr

The essential prerequisites for efficient Gd-based relaxation agents are the highest possible relaxivity and high thermodynamic and kinetic stabilities that can be reached by the use of phosphonated functions.[8] We recently synthesized ligand **LH**$_8$ (Scheme 1),[9] based on a pyridyl moiety functionalized by two amino(methyl)bis(methylphosphonic) acid, which proved to be very efficient for the complexation of Cu^{2+},[9,10] thus indicating further potentialities for the coordination to lanthanide cations too.[11]
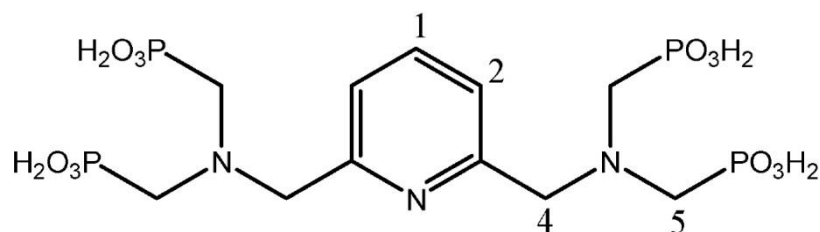
Scheme 1

In order to unravel the complexation behaviour of **LH**$_8$ with lanthanide cations, spectrophotometric titration experiments (absorption and fluorescence) were first carried out by adding Eu^{3+} or Tb^{3+} salts into a solution of the ligand in buffered aqueous solutions (Fig. 1), keeping in mind that these cations are prone to lead to luminescent complexes thanks to the antenna effect. Monitoring the emission intensities at fixed wavelengths revealed a marked inflection point at a 1 : 1 M : L ratio, followed by the formation of polynuclear species.
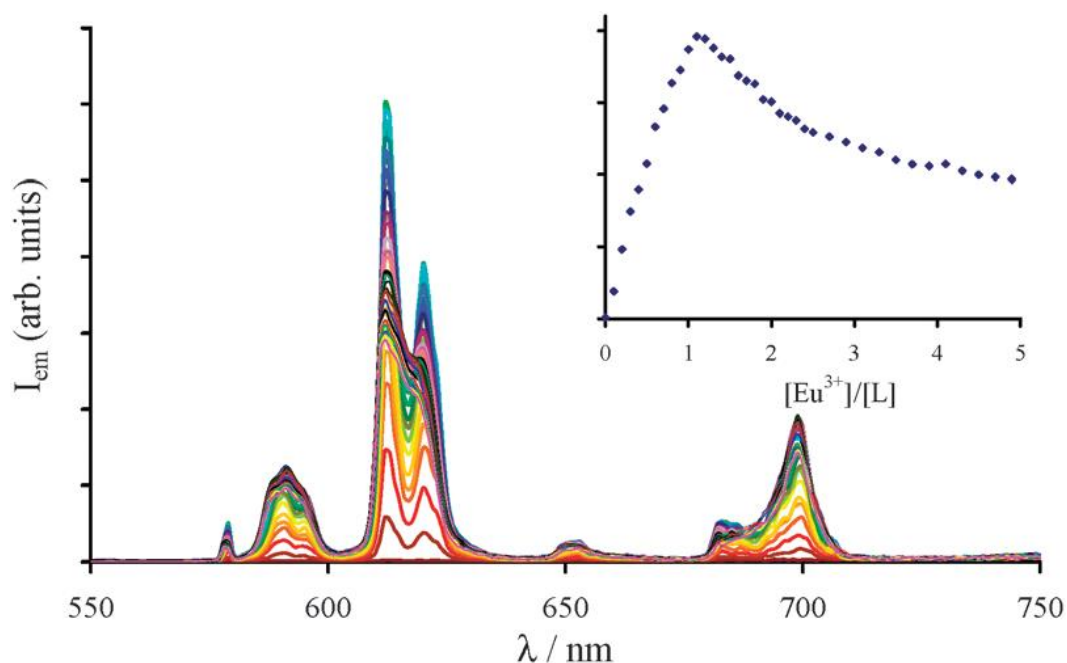
Fig. 1. Evolution of the emission intensity during the addition of EuCl$_3$·6H$_2$O (8.2×10^{-4} M) to an aqueous solution of **LH**$_8$ (8.5×10^{-5} M, in 0.01 M Tris/HCl, pH = 7.0). Inset: Evolution of the emitted intensity at 620 nm as a function of the [Eu]/[**L**] ratio.

Potentiometric titrations were then performed on solutions containing equimolar amounts of the ligand and trivalent lanthanide salts (Ln = La, Nd, Tb, Eu, Nd and Lu). Despite the precipitation of the complexes at acidic pH for the heaviest lanthanides, it was possible to evaluate the thermodynamic stability constants (Table 1), using the previously reported protonation constants of the ligand (pK_a = 11.21, 10.29, 8.04, 6.49, 5.53 and 4.19).[9] Table 1 also provides the pLn values (pLn = $-\log[Ln^{3+}]_{free}$ with $[L]_{tot}$ = 10^{-5} M and $[Ln]_{tot}$ = 10^{-6} M at pH 7.4)[12] for **L**, DOTA[13] and DOTP,[14] the phosphonated analogue of DOTA (full details of the potentiometric experiments are available in the ESI[i]).

Table 1. Stability constants (log K_{ML}), successive protonation constants (log K_{MLH_m}) and pLn values of lanthanide complexes of **L**[a,b]

	La	Nd	Eu	Tb	Er	Lu
log K_{ML}	25.5(4)	27.1(3)	25.7(3)	29.7(6)	29.7(1.0)	29.3(4)
log K_{MLH}	9.4(4)	8.8(3)	9.4(3)	7.6(7)	7.8(1.0)	7.4(6)
log K_{MLH_2}	7.3(6)	6.6(4)	7.5(4)	c	c	c
log $K_{M(OH)}$[b]	−8.83	−8.20	−7.78	−7.66	−7.54	−7.29
pLn$_\mathbf{L}$	21.2	22.1	21.5	23.6	23.7	23.1
pLn$_{DOTA}$	17.8	17.8	18.4	19.6	19.6	20.3
pLn$_{DOTP}$	21.5	21.2	21.5	22.6	24.3	24.3

[a] Solvent: water; I = 0.1 M (NaClO$_4$); T = 25.0(2) °C. Errors = 4σ with σ = standard deviation. K_{ML} = [ML]/[L][M] and K_{MLH_m} = [MLH$_m$]/[MLH$_{m-1}$][H]. Charges have been omitted for the sake of clarity. [b] The hydrolysis constants used for the processing of the potentiometric data on Ln complexes with **L** have been taken from ref. 16. [c] Not determined because of precipitation of the Ln complexes below pH 6–6.5.

As anticipated, the replacement of carboxylate functions by phosphonate ones led to a very large increase of the thermodynamic stability constants in the lanthanide series. The log K_{ML} values obtained for the Eu and Tb complexes (Table 1) are several orders of magnitude higher than that reported for the Gd complex of **L′**, the analogue of **L** containing carboxylate functions instead of phosphonate ones (log $K_{GdL'}$ = 18.6, pGd = 17.5).[15] Despite the non-macrocyclic structure of the ligand, the pM values reported in Table 1 clearly show that the complexes of **L** are more stable than the DOTA analogues, and almost as stable as those of DOTP,[14] suggesting sufficient stability of the complexes for their use in biological media.

As previously observed for Ln complexes with ligands containing phosphonate groups,[17] the potentiometric data revealed the formation of mono- and di-protonated species in solution (Table 1). Both the mono- and di-protonated forms of the complexes are expected to exist at physiological pH. In the case of Eu, the relative abundance of non-protonated, mono-protonated and di-protonated forms are 0.4, 44.1 and 55.5% at pH = 7.4 ($[L]$ = $[Eu]$ = 10^{-3} M).

Considering the interest of stable hydrated Gd complexes as contrast agents for magnetic resonance imaging, the NMRD profile of the Gd complex was measured in water at pH = 7.4 and 25 °C (Fig. 2). The relaxivity of the Gd complex at 20 MHz appeared to be very high (r_1 = 8.5 mM^{-1} s^{-1} at 25 °C) when compared to conventional monohydrated contrast agents such as [Gd(DOTA)]$^-$ (r_1 = 4.2 mM^{-1} s^{-1})[18] or [Gd(DTPA)]$^{2-}$ (r_1 = 4.3 mM^{-1} s^{-1}).[18] The NMRD profile (Fig. 2) indicated a behaviour typical of small complexes.[1] The relaxivity determined at 25 °C and 20 MHz is very similar to those reported for Gd complexes with ligands containing phosphonate groups and one inner-sphere water molecule,[19] which are endowed with relatively

high relaxivities as a consequence of an important second-sphere contribution. Luminescence lifetimes of the europium complex were also measured in water (0.57 ms) and D_2O (2.21 ms) to determine the hydration number by Horrocks' method,[20a] using refined coefficients obtained by Beeby *et al.*,[20b] revealing a *q* value of 1.0 ± 0.1.
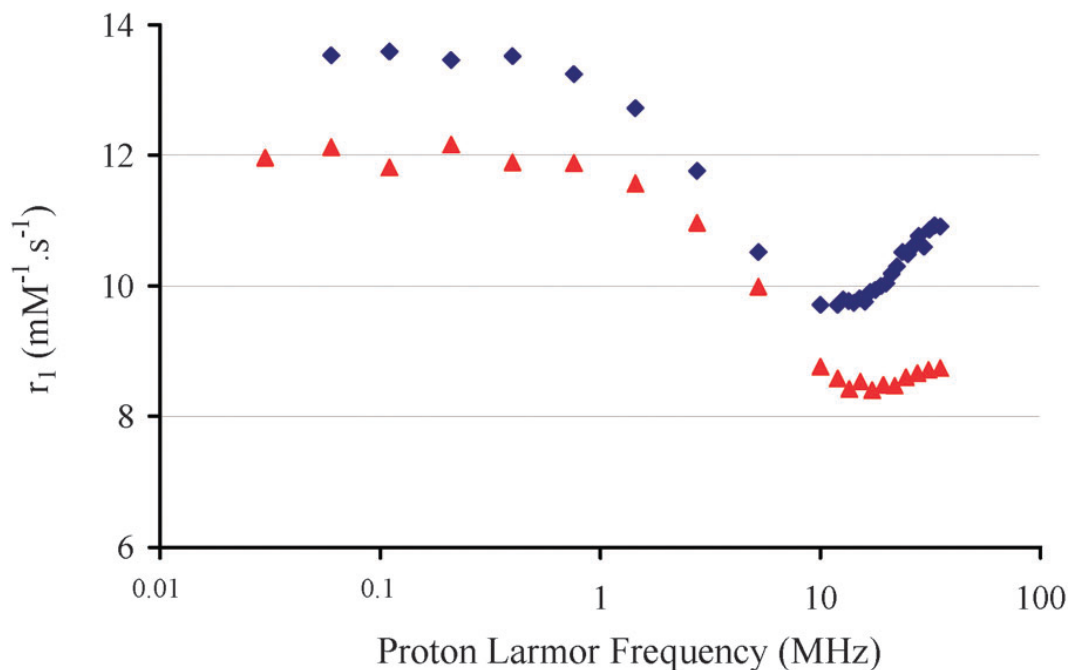

Fig. 2. NMRD profile of the Gd complex (pH = 7.4, 25 °C) alone (▲) and in the presence of 30 equivalents of $MgCl_2$ (◆).

Considering the fruitless efforts for crystallizing the complexes, we turned our attention toward density functional theory (DFT) calculations at the B3LYP level (see ESI[i] for full details),[8] taking into account solvent effects (water) by using a polarizable continuum model (PCM). Fig. 3 represents the minimum energy conformation obtained for the $[Gd(H_2\mathbf{L})(H_2O)]^{3-}$ system, which is expected to be the major form of the complex in solution at physiological pH. According to our calculations the ligand provides an asymmetrical coordination of the metal ion due to the presence of an inner-sphere water molecule. The conformation adopted by the ligand in the complex implies the occurrence of two helicities: one associated with the layout of the methylenephosphonate arms (absolute configuration Δ or Λ), and the other to the four five-membered chelate rings formed by the binding of the N–CH₂–P–O units (each of them showing absolute configuration δ or λ).[21] The minimum energy conformation calculated for $[Gd(H_2\mathbf{L})(H_2O)]^{3-}$ corresponds to the Δ(δδδδ) [or Λ(δδδδ)] form. Two of the methylenephosphonate groups are placed above and below the mean plane of the pyridyl fragment, while the remaining two methylenephosphonate groups lie close to that plane. The distance between Gd and the inner-sphere water molecule (2.609 Å) is *ca.* 0.1 Å longer than those observed for different Gd polyaminocarboxylates,[1b] probably because the PCM solvation model does not take into account specific interactions between the inner-sphere water molecules and the second hydration sphere of the complex.

The conformation adopted by the ligand in the complex according to our DFT calculations is in agreement with the NMR spectra recorded for the diamagnetic Lu complex (Fig. 3). The ^{1}H NMR spectrum recorded at 278 K shows an AB spin system for protons H4 at 4.21 and 3.61 ppm (^2J = 15.0 Hz) and 4 distinct signals for the methylene protons adjacent to the phosphonate functions. This points to an overall C_2 symmetry of

the complex in solution with different signals for the in plane (ip) and out-of-plane (op) methylenephosphonate groups. This was further confirmed by the presence of two peaks with the same intensity in the ^{31}P-NMR spectrum at 18.9 and 21.6 ppm.
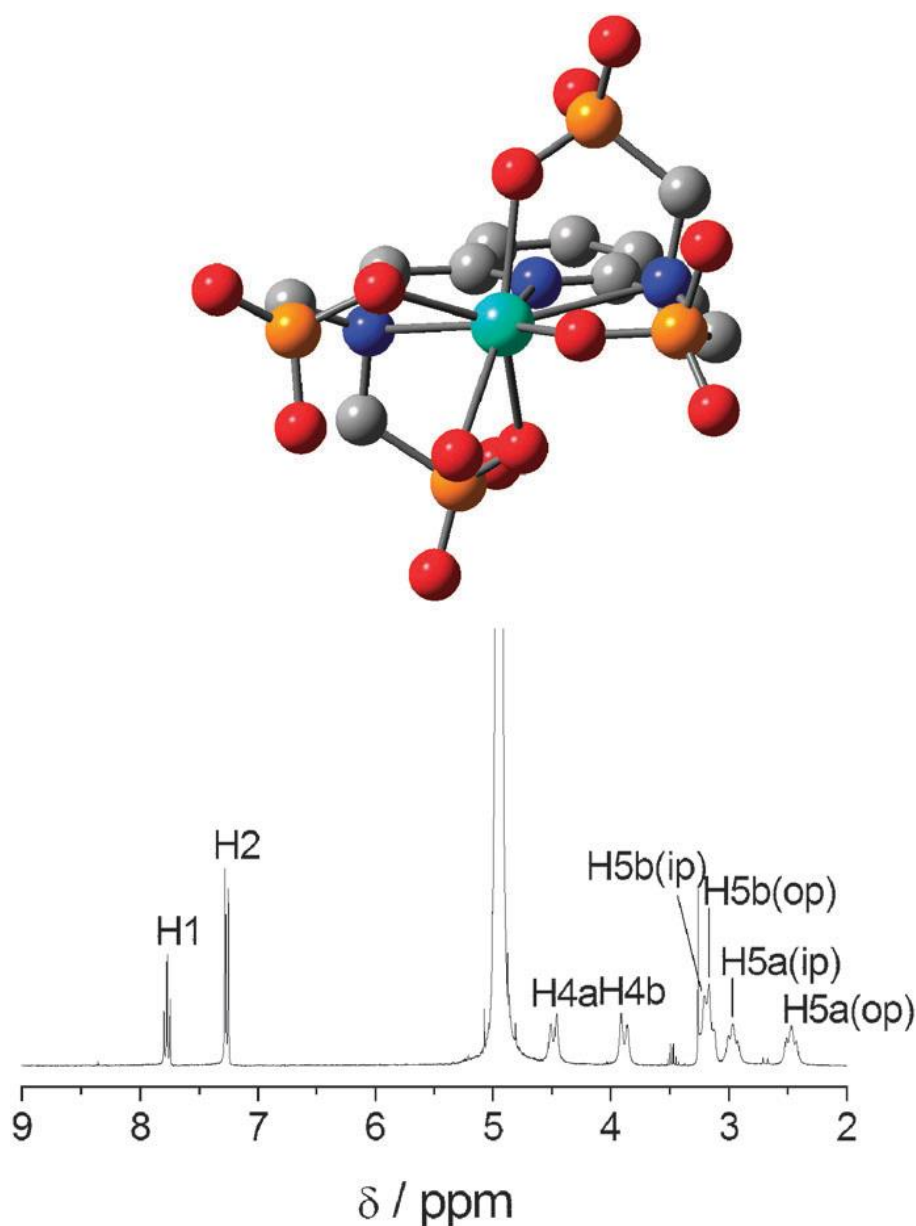

Fig. 3. (top) Minimum energy conformation obtained from DFT calculations (B3LYP) in aqueous solution for the $[Gd(H_2\mathbf{L})(H_2O)]^{3-}$ system ($d_{Gd-Npyr} = 2.714$ Å, $d_{Gd-Nalk} = 2.771$ and 2.824 Å and $d_{Gd-O} = 2.29–2.42$ Å). (bottom) ^{1}H NMR spectrum of the Lu complex of **L** recorded in D$_2$O solution at 278 K (pD = 10.5); see Scheme 1 for labeling. Ip and op refer to in-plane and out-of-plane methylenephosphonate groups, respectively.

Considering the negative charge of the Gd complex at pH close to neutrality, its possible interactions with cations were investigated by monitoring the changes of the relaxivity in the presence of Ca^{2+} and Mg^{2+}. In the presence of up to 200 equivalents of CaCl$_2$, almost no impact was observed on the relaxivity of the complex which varied from 8.78 to 8.48 mM^{-1} s^{-1}(pH = 7.4, 25 °C, 200 MHz) without and with Ca^{2+}. In contrast, addition of MgCl$_2$ resulted in a 37% enhancement of the relaxivity (see Fig. S1, ESI[i]), rising up to 12.0 mM^{-1} s^{-1} in the same conditions.

The absence of free gadolinium was checked in all samples by the xylenol orange test, ruling out the possibility of gadolinium release in the presence of magnesium. The NMRD profile of the Gd complex in the presence of 30 equivalents of $MgCl_2$ (Fig. 2) confirmed the large enhancement of the relaxivity at all frequencies, but also revealed a larger enhancement at high frequencies, generally associated to the increase of the rotational tumbling time of the complex.[1] This behaviour is likely related to the formation of polynuclear clusters as often observed with phosphonate based lanthanide complexes.[22]

In conclusion, the interaction of the non-macrocyclic ligand **L** with lanthanide complexes led to the formation of very stable [Ln**L**] complexes, which displayed a high relaxivity for the Gd complex. In addition, [Gd**L**] showed a marked and selective relaxivity enhancement in the presence of Mg^{2+} and no changes in the presence of Ca^{2+}. Such simple polyphosphonated scaffolds may provide a large interest in the design of new Gd based complexes for magnetic resonance imaging and our current efforts are devoted toward the deep understanding of their properties and the preparation of bifunctional chelatorsfor labelling of biomaterial with this kind of ligand.

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