Lanthanide(III) Complexes of Novel Mixed Carboxylic-Phosphorus Acid Derivatives of Diethylenetriamine: A Step towards More Efficient MRI Contrast Agents

Jan Kotek, [a, b] Petra Lebdusková, [a, b] Petr Hermann, [b] Luce Vander Elst, [c] Robert N. Muller, [c] Carlos F. G. C. Geraldes, [d] Thomas Maschmeyer, [a] Ivan Lukes, [b] and Joop A. Peters*[a]

Abstract: Three novel phosphorus-containing analogues of H₅DTPA (DTPA = diethylenetriaminepentaacetate) were synthesised (H₆L₁, H₅L₂, H₅L₃). These compounds have a -CH₂-P(O)(OH)-R function (R = OH, Ph, CH₂NBn₂) attached to the central nitrogen atom of the diethylenetriamine backbone. An NMR study reveals that these ligands bind to lanthanide(III) ions in an octadentate fashion through the three nitrogen atoms, a P=O oxygen atom and four carboxylate oxygen atoms. The complexed ligand occurs in several enantiomeric forms due to the chirality of the central nitrogen atom and the phosphorus atom upon coordination. All lanthanide complexes studied have one coordinated water molecule. The residence times ($t_{298}$) of the coordinated water molecules in the gadolinium(III) complexes of H₆L₁ and H₅L₂ are 88 and 92 ns, respectively, which are close to the optimum. This is particularly important upon covalent and noncovalent attachment of these Gd³⁺ chelates to polymers. The relaxivity of the complexes studied is further enhanced by the presence of at least two water molecules in the second coordination sphere of the Gd³⁺ ion, which are probably bound to the phosphate/phosphinate moiety by hydrogen bonds. The complex [Gd(L³)(H₂O)]²⁻ shows strong binding ability to HSA, and the adduct has a relaxivity comparable to MS-325 (40 s⁻¹mm⁻¹ at 40 MHz, 37°C) even though it has a less favourable $t_{34}$ value (685 ns). Transmetallation experiments with Zn²⁺ indicate that the complexes have a kinetic stability that is comparable to—or better than—those of [Gd(dtpa)(H₂O)]²⁻ and [Gd(dpabma)(H₂O)]⁻.

Keywords: chelates - imaging agents - lanthanides - NMR spectroscopy - phosphate complexes - phosphonate complexes

Introduction

Metal chelates of the polyaminocarboxylates DTPA⁻⁻ (DTPA⁻⁻ = diethylenetriamine-N,N',N'',N''',N''''-pentaacetate), DOTA⁴⁺ (DOTA⁴⁺ = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate) and derivatives thereof have found widespread use in medical diagnosis (e.g. Magnetic Resonance Imaging, MRI; Positron Emission Tomography, PET; or Single-Photon Emission Computed Tomography, SPECT) and in radiotherapy.¹⁻³ These complexes have high thermodynamic and kinetic stabilities, essential features for in vivo applications, since the metal aqua ions as well as their ligands are toxic, whereas the complexes are not. The applicability of radioactive complexes also requires that complexation should be rapid enough to allow radiolabelling by a simple procedure just prior to the diagnostic procedure or the treatment. Complexes of DTPA⁻⁻ meet this requirement, while the formation of complexes of DOTA⁴⁺ is usually very slow.
MRI contrast agents are mostly Gd\(^{3+}\) complexes, as this paramagnetic ion has a relatively long electronic relaxation time, which leads to high nuclear relaxation efficiency. This is usually expressed as the relaxivity, \(r_1\), which is the enhancement of the water proton relaxation rate in s\(^{-1}\)mm\(^{-1}\). Other important parameters governing the relaxivity are the rotational correlation time (\(\tau_{\text{R}}\)), the number of Gd\(^{3+}\)-bound water molecules (\(q\)), their residence time (\(\tau_{\text{RD}}\)) and the electron spin relaxation times (\(T_{\text{IR}}\)), \(i=1,2\). Theory predicts optimal efficiency for high-molecular-weight gadolinium(III) chelates if the residence time, \(\tau_{\text{RD}}\), is in the range of 20–50 ns.\(^4\) All current commercial Gd\(^{3+}\)-based contrast agents have low molecular weights and are hydrophilic. Consequently, these compounds are distributed rather unspecifically over the extracellular fluids. More efficient contrast agents are being developed that may be directed to targets of interest, thereby achieving higher local concentrations at lower dosages.\(^5\) These agents usually are conjugates of one or more Gd\(^{3+}\) chelates and a targeting vector. The criterion regarding the water exchange rate is particularly critical to achieve optimal efficiency for this new class of compounds. The current commercial Gd\(^{3+}\) chelates show water exchange rates that are an order of magnitude lower than the optimal value.\(^1,2,4,6\) Recently, it was shown that phosphorus-containing analogues of the commercially used [Gd(dota)(H\(_2\)O)]\(^{3+}\) complex have faster water exchange than the parent system.\(^7\) Similar results were observed on pyridine-containing macrocyclic ligands with phosphonic acid pendant arms.\(^8,9\) Moreover, these compounds show rapid complex formation, which makes them suitable for radiodiagnostic and radiotherapeutic applications. The phosphorus-containing arm can be functionalised (e.g. with an ester moiety or some alkyl or aryl group) to afford bifunctional ligands that can be easily linked to a biologically active compound that determines the biodistribution of the final complex. The interaction of a paramagnetic Gd\(^{3+}\) complex with a macromolecule results in an increase in relaxivity due to the elongation of \(\tau_{\text{R}}\).

We have extended these studies to phosphorus-containing analogues of open-chain DTPA\(^{5-}\) complexes. In this paper, we describe the synthesis and physicochemical characterisation of lanthanide complexes of three novel DTPA\(^{5-}\) derivatives with a phosphorus acid pendant arm on the central nitrogen atom of the diethylenetriamine backbone, H\(_5\)L\(_1\), H\(_5\)L\(_2\) and H\(_5\)L\(_3\) (see Scheme 1). Ligand H\(_5\)L\(_1\) is the parent structure, while H\(_5\)L\(_2\) is a ligand that, after appropriate substitution of the phenyl group, can be linked covalently to a polymer. Ligand H\(_5\)L\(_3\) has a dibenzylamino moiety attached to the phosphorus function, a structural motif that has some similarity with that occurring in MS-325.\(^{10}\) The Gd\(^{3+}\) complex of the latter ligand is known to be a very efficient blood-pool contrast agent due to its ability to bind noncovalently to human serum albumin (HSA).

**Results and Discussion**

**Synthesis of the ligands:** Attempts to build up the ligands from benzylamine (2) by treatment with tosylaziridine (1), followed by deprotection of the tosyl groups and alkylation with ethyl bromoacetate to give a H\(_5\)DTPA analogue with a N-benzyl-protected central amino group of the skeleton (5) were not successful due to the formation of a very stable lactam (6) after debenzylation (Scheme 2). This lactam was found to be extremely stable towards hydrolysis; it could be hydrolysed only under very harsh conditions (i.e., 20% NaOH, 90°C, overnight), affording the tetraetacetic derivative (7). Therefore, it was decided first to attach the phosphorus-containing moiety to the diethylenetriamine backbone. This was achieved by a Mannich-type reaction between N\(_2\)N'-bis(phthaloyl)diethylenetriamine (8), paraformaldehyde and the appropriate phosphorus derivative, followed by deprotection of phthaloyl moieties with hydrazine. Then, alkylation of intermediate (10) with ethyl bromoacetate and hydrolysis of the ester groups afforded the desired compounds H\(_5\)L\(_1\), H\(_5\)L\(_2\) and H\(_5\)L\(_3\) in overall isolated yields of 50–80% (Scheme 3).

**Determination of the ligand-protonation constants by using \(^1\)H and \(^31\)P NMR chemical-shift titrations:** Since the thermodynamic stability of the Ln\(^{3+}\) complexes of aminocarboxylates is related to the summed protonation constants of the free ligand,\(^{11-13}\) insight into the structural effects on these constants is desirable. Therefore, the protonation constants of all the ligands were determined by using the pH dependence of \(^1\)H and \(^31\)P NMR chemical shifts. The chemical shift curves (see Figure 1) display sharp changes at several ranges of pH values; they may be ascribed to the shift dependence on the changes of the protonation state of the ligand concerned.

Since the protonation equilibria are fast on the NMR timescale, the chemical shift of each signal can be given as a weighted average of the shifts of the various protonated species (see [Eq. (1)])\(^{14}\)

\[
\delta_{\text{obs}} = \sum X_i \cdot \delta_i
\]

Here \(\delta_{\text{obs}}\) is the observed chemical shift of a given signal, \(X_i\) is the molar fraction of species \(i\) and \(\delta_i\) is its chemical shift. The observed \(^1\)H and \(^31\)P chemical shifts were fitted simultaneously according to Equation (1) by using the dissociation constants (pK\(_a\)) and the values of \(\delta_i\) as adjustable pa.
parameters. The fits of experimental data points are shown in Figure 1, and the resulting $p_{K_{a}}$ and $d_{i}$ values are compiled in Table 1 and the Supporting Information (Tables S1–S3), respectively. For comparison, $p_{K_{a}}$ values for H5DTPA reported in the literature[15] are included in Table 1.

The $^1$H NMR chemical shifts, $d_{i}$, calculated for each proton $H_{i}$ at the various degrees of protonation of the ligands (L1)6/C0, (L2)5/C0 and (L3)5/C0 (see Tables S1–S3) were then used to evaluate the protonation fractions $f_{i}$ ($i = 1–5$) at the nitrogen and oxygen basic sites of the ligands (Scheme 1c) for their successive protonated forms, with the empirical procedure of Sudmeier and Reilley.[14] This assumes that the chemical shifts of methylene protons in aminocarboxylates can be estimated by considering the effects of protonation of various basic sites to be additive and characteristic for the position of the given methylene group with respect to the protonation site, as expressed in Equation (2).

$$
\Delta d_{i} = \sum C_{N}f_{N} + \sum C_{O}f_{O} + \sum C_{P}f_{P}
$$

The protonation shifts of the methylene groups of the diethylenetriamine backbone reflect the protonation fractions of each of the terminal N(1) ($f_{1}$) and the central N(2) ($f_{2}$) nitrogen atoms of the ligands through the shielding constants $C_{N}$ and $C_{N}'$ for the protonation of those amino groups, when they are at an $\alpha$ or $\beta$ position, respectively, relative to those methylene groups (values of $C_{N} = 0.75$ ppm and $C_{N}' = 0.35$ ppm have been listed).[14,15] The protonation fractions of the oxygen atoms at the terminal carboxylates O(3) ($f_{3}$) and at the central phosphonate group

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$p_{K_{a1}}$</th>
<th>$p_{K_{a2}}$</th>
<th>$p_{K_{a3}}$</th>
<th>$p_{K_{a4}}$</th>
<th>$p_{K_{a5}}$</th>
<th>$\Sigma p_{K_{a}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H6L1</td>
<td>10.747(5)</td>
<td>7.88(2)</td>
<td>6.92(7)</td>
<td>2.7(2)</td>
<td>2.17(6)</td>
<td>30.4</td>
</tr>
<tr>
<td>H5L2</td>
<td>9.60(5)</td>
<td>9.10(4)</td>
<td>2.63(19)</td>
<td>2.15(12)</td>
<td>0.72(14)</td>
<td>24.2</td>
</tr>
<tr>
<td>H5L3</td>
<td>10.1(1)</td>
<td>9.4(2)</td>
<td>7.13(2)</td>
<td>1.32(5)</td>
<td>28.0 (20.8)</td>
<td></td>
</tr>
<tr>
<td>H5DTPA[15]</td>
<td>10.2</td>
<td>8.6</td>
<td>4.2</td>
<td>2.9</td>
<td>1.8</td>
<td>27.7</td>
</tr>
</tbody>
</table>

[a] Values in parenthesis exclude the $p_{K_{a}}$ value associated with protonation of side-chain nitrogen atom.

Scheme 2. Unsuccessful approach to ligand synthesis—formation of lactam (6), reagents: i) HBr; ii) BrCH2COOEt, NaOH; iii) H2, Pd/C; iv) NaOH.

Scheme 3. Synthesis of H6L1 (R = OH), H5L1 (R = Ph) and H5L3 (R = CH2N(CH2Ph)2); reagents: i) HP(O)(R)(OEt), CH2O; ii)N2H4; iii) BrCH2COOEt; iv) HCl or NaOH.
O(4) \( f_4 \) were also calculated by using shielding constants \( C_0 = 0.20 \) ppm for \( \alpha \)-carboxylate protonation\(^{[15]} \) and \( C_P = 0.20 \) ppm for \( \alpha \)-phosphonate/phosphinate protonation\(^{[16,17]} \).

The results are given in Table 2 together with data for DTPA\(^{\text{4-}} \) reported previously\(^{[15]} \). It can be seen that the first two protons bind exclusively to the backbone nitrogens in the three cases. The first protonation of the phosphonate ligand \((HL_1)^{\text{5-}} / C_0\) takes place mainly on the central nitrogen atom \( f_2 \), whereas in HDTPA\(^{\text{4-}} \) and \((HL_3)^{\text{4-}} / C_0\), the preference for the central nitrogen is somewhat less, so that the central nitrogen atom is protonated to about the same extent as the sum of the two terminal ones. This is in agreement with the basicity of the nitrogen atom in aminomethylphosphonates, which is generally higher than that in aminomethylcarboxylates\(^{[13]} \). However, aminomethylphosphonates are less basic than the corresponding aminomethylcarboxylates; this explains why the central nitrogen atom \( f_2 \) of the phenylphosphinic derivative \((L_2)^{\text{5-}} \) is only poorly protonated in the monoprotonated species \((HL_2)^{\text{4-}} \). In all cases, the protons of the \((H_2L)^{\text{3+}} \) species are located mainly on the outer nitrogen atoms \( f_1 \), this can be rationalised by the electrostatic repulsion between the two incoming protons.

For \( n > 2 \), the protonation also involves the basic atoms located at the pendant arms of the ligands. For the ligand \((L_2)^{\text{6-}} \), the \( f_1 \) values show that the third protonation step mainly occurs at the phosphonate moiety; this is in agreement with its value of \( pK_{a3} \) (6.92) being close to the \( pK_a \) values commonly observed for phosphonates\(^{[18]} \).
Table 2. Percent protonation fractions of the different basic sites of the ligands (L^2) \textsuperscript{2+}, (L^2) \textsuperscript{3+}, (L^3) \textsuperscript{3+}, and DTPA \textsuperscript{-} in the protonated forms H\textsubscript{3}L\textsuperscript{i} \textsuperscript{+} at increasing values of n (for DTPA \textsuperscript{-}, f\textsubscript{1} and f\textsubscript{2} correspond, respectively, to the terminal and central carboxylates. The errors in f\textsubscript{i} values are ±10%).

<table>
<thead>
<tr>
<th>H\textsubscript{3}L\textsuperscript{i}+</th>
<th>f\textsubscript{1}</th>
<th>f\textsubscript{2}</th>
<th>f\textsubscript{3}</th>
<th>f\textsubscript{4}</th>
<th>f\textsubscript{5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H\textsubscript{3}L\textsuperscript{+}) \textsuperscript{2+}</td>
<td>n=1</td>
<td>13</td>
<td>74</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n=2</td>
<td>92</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n=3</td>
<td>100</td>
<td>17</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
<td>100</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n=5</td>
<td>100</td>
<td>76</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>(H\textsubscript{3}L\textsuperscript{+}) \textsuperscript{3+}</td>
<td>n=1</td>
<td>46</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n=2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n=3</td>
<td>100</td>
<td>8</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
<td>100</td>
<td>48</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>(H\textsubscript{3}L\textsuperscript{+}) DTPA \textsuperscript{2+} [19]</td>
<td>n=1</td>
<td>23</td>
<td>54</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n=2</td>
<td>94</td>
<td>12</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>n=3</td>
<td>98</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
<td>100</td>
<td>18</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

The hydration numbers of the lanthanide complexes of H\textsubscript{3}L\textsuperscript{1}, H\textsubscript{3}L\textsuperscript{2} and H\textsubscript{3}L\textsuperscript{3} as determined from the lanthanide-induced water \textsuperscript{17}O NMR shifts: The \textsuperscript{17}O NMR chemical shifts for water oxygen in 0.2 M solutions of complexes of all ligands with 14 different Ln\textsuperscript{3+} ions were measured at 40.7 MHz, 70°C and pH 5–6. Under these conditions, the exchange between the Ln\textsuperscript{3+}-bound and bulk-water protons was rapid on the NMR timescale. Therefore, the observed chemical shifts with respect to free water (δ\textsubscript{obs}, see Table S4 in the Supporting Information) are related to those of Ln\textsuperscript{3+}-bound water (δ\textsubscript{w}) by Equation (3), in which q is the number of water molecules in the first coordination sphere of Ln\textsuperscript{3+} and ρ\textsubscript{w} is the Ln\textsuperscript{3+}/water molar ratio in the sample. The bound shifts comprise diamagnetic (δ\textsubscript{d}), contact (δ\textsubscript{c}) and pseudocontact contributions (δ\textsubscript{p}) [see Eq. (4)][20]

δ\textsubscript{obs} = q · ρ\textsubscript{w} · δ\textsubscript{d} (3)

δ\textsubscript{d} = δ\textsubscript{c} + δ\textsubscript{q} + δ\textsubscript{p} (4)

The contact contribution is the result of a through-bond transmission of unpaired-electron-spin density from the central ion to the ligand nucleus, and the pseudocontact contribution results from a dipolar interaction between the magnetic moment of the central ion and the nucleus in question. Both paramagnetic contributions, δ\textsubscript{c} and δ\textsubscript{p}, can be expressed as the product of lanthanide-dependent but ligand-independent constants (S\textsubscript{i}) and C\textsubscript{D}, respectively and terms characteristic for the nucleus under study (F and G, respectively) as given in Equation (5).

\[ \Delta = \delta_{\text{obs}}/\rho_{w} = q(S_{i} \cdot F + C_{D} \cdot G + \delta_{d}) \] (5)

Values for (S\textsubscript{i}) and C\textsubscript{D} are tabulated in the literature.[21–25] For isosstructural complexes, the ligand-dependent parameters F and G for the water \textsuperscript{17}O nucleus are the same for all paramagnetic Ln\textsuperscript{3+} ions, and thus Equation (5) can be linearised in two different ways [Eqs. (6) and (7), in which Δ = Δ−qδ\textsubscript{d}][20] In other words, if the observed data afford linear plots according to Equations (6) and (7), it may be concluded that the complexes concerned are isosstructural.

\[ \frac{\Delta - q \cdot \delta_{d}}{C_{D}} = \frac{\Delta}{C_{D}} \cdot \frac{(S_{i})}{(S_{i})} \cdot q \cdot F + q \cdot G \] (6)

\[ \frac{\Delta - q \cdot \delta_{d}}{(S_{i})} = q \cdot F + C_{D} \cdot (S_{i}) \cdot q \cdot G \] (7)

It has previously been shown that the value of parameter F is in the narrow range of 70±11 for one coordinated oxygen-donor atom, independent of the nature of that atom and of the other ligands present in the Ln\textsuperscript{3+} complex.[20] Thus, the slopes of the plots of the experimental data according to Equation (6) are proportional to the number of complexes of the first two ligands will be comparable with those of the corresponding H\textsubscript{3}DTPA complexes, whereas the protonation of the side-chain nitrogen of the third ligand is expected to lead to a somewhat reduced stability of the corresponding complexes.

The proton is mainly equally distributed over the four carboxylate oxygens, while the fifth shows a preference for the central nitrogen. In the cases of the phosphinate derivative ligands, (L^2) \textsuperscript{2+} and (L^3) \textsuperscript{3+}, the central phosphinate oxygen is never protonated (f\textsubscript{1} = 0) under the conditions applied (2 < pH < 13), but the value of pK\textsubscript{a} of the second ligand (7.13) corresponds to protonation of the dibenzylamino moiety, as shown by the value f\textsubscript{1} = 100% (Table 2) and the shifts of the protons e and f next to the N(5) atom of its side chain (Figure 1f). Thus, for the (L^3) \textsuperscript{3+} ligand, the third proton is about equally distributed over the four carboxylate oxygens, while the fourth binds preferentially to the central nitrogen. However, in the case of (L^2) \textsuperscript{2+}, the fourth proton is almost equally distributed over the four carboxylate oxygens and the central nitrogen atom.

This protonation scheme is qualitatively confirmed by the \textsuperscript{31}P NMR titration curves (Figure 1a,c,e). Protonation of N(2) dramatically affects the charge density of the phosphorus atom of the phosphonate group, and to a lesser extent that of the phosphinate group. This can be ascribed to formation of a strong intramolecular hydrogen bond between N(2)H\textsuperscript{+} and the phosphonate O\textsuperscript{-}, forming stable five-membered rings and resulting in a shift of the \textsuperscript{31}P resonance to low frequency.[17,19] This can be seen in Figure 1, where low-frequency \textsuperscript{31}P shifts are observed when N(2) and/or N(5) are protonated. De-protonation of N(2) or protonation of the phosphonate/phosphinate group leads to high-frequency \textsuperscript{31}P shifts, as both processes lead to the disappearance of the internal NH\textsuperscript{+}O\textsuperscript{-} bonds.[17]

The sum of the pK\textsubscript{a} values of the atoms of the ligand that are involved in binding to the Ln\textsuperscript{3+} ion of H\textsubscript{3}L\textsuperscript{1}, H\textsubscript{3}L\textsuperscript{2} and H\textsubscript{3}L\textsuperscript{3} (for the last one, thus excluding the pK\textsubscript{a} value associated with the protonation of the side-chain nitrogen atom, Table 1) is a good indication of the thermodynamic stability of the lanthanide complexes. Their values indicate that the bonds.

\[ D = \frac{\Delta - q \cdot \delta_{d}}{C_{D}} = \frac{\Delta}{C_{D}} \cdot \frac{(S_{i})}{(S_{i})} \cdot q \cdot F + q \cdot G \] (6)

\[ \frac{\Delta - q \cdot \delta_{d}}{(S_{i})} = q \cdot F + C_{D} \cdot (S_{i}) \cdot q \cdot G \] (7)
water molecules coordinated in the inner sphere of the Ln\(^{3+}\) ion.

The observed chemical shifts for the diamagnetic La\(^{3+}\) and Lu\(^{3+}\) complexes of the ligands under study were taken as \(q\Delta_\phi\). Plots of the values of \(\Delta\) for the Ln\(^{3+}\) complexes of the ligands studied according to Equation (6) were perfectly linear (see Figure 2a,c,e). The slopes of the lines and thus the values of \(q\) for the Ln\(^{3+}\) complexes of the lanthanide-induced \(^{17}\text{O}\) NMR shifts observed in D\(_2\)O solutions of the Ln\(^{3+}\) and Lu\(^{3+}\) ions, whereas the plots according to Equation (7) are linear. Such a break, observed exclusively in the former plots, can be ascribed to some small gradual changes of complex geometry\(^{[27]}\) and/or to a change of crystall-field parameters along the lanthanide series.\(^{[28]}\)

The plots according to Equation (7) (see Figure 3b,d,f) show a break between lighter (\(\text{Ln} = \text{Ce} \rightarrow \text{Eu}\)) and heavier (\(\text{Ln} = \text{Tb} \rightarrow \text{Yb}\)) Ln\(^{3+}\) ions, whereas the plots according to Equation (6) are linear. Such a break, observed exclusively in the former plots, can be ascribed to some small gradual changes of complex geometry\(^{[27]}\) and/or to a change of crystall-field parameters along the lanthanide series.\(^{[28]}\)

The pH dependence of the \(^{17}\text{O}\) NMR shifts was studied in some detail for the Dy\(^{3+}\) complex of H\(_6\)L\(_1\). A plot of the \(\Delta / C_0\) values for this system as a function of the pH (see Supporting Information Figure S1) shows that, at pH close to 0, the value of \(\Delta (\approx 21000 \text{ ppm})\) is almost the same as that observed for the free Dy\(^{3+}\)-aqua ion \((\approx 21685 \text{ ppm})\), which has eight water molecules coordinated to the Dy\(^{3+}\) ion. Upon increase of the pH to 2.5, the value of \(\Delta\) changes to about \(-3600 \text{ ppm};\) this reflects the substitution of 7 coordinated water molecules by the organic ligand, H\(_5\)L\(_1\). Between pH 2.5 and 9.5, \(\Delta\) is invariant, and no precipitation of hydroxides is observed; once again, this demonstrates the high stability of the complex formed.

Structure of the lanthanide complexes of H\(_6\)L\(_1\) and H\(_5\)L\(_3\) in solution as determined from \(^{13}\text{C}\) and \(^{31}\text{P}\) relaxation enhancements: The coordination number of Ln\(^{3+}\) ions in complexes of polyaminocarboxylates is, in general, nine. The \(^{17}\text{O}\) NMR measurements described above show that one water molecule is bound in the first coordination sphere of the Ln\(^{3+}\) ion in the complexes of H\(_6\)L\(_1\), H\(_5\)L\(_2\) and H\(_5\)L\(_3\). Therefore, it is most likely that these ligands are bound in an octadentate fashion, with binding occurring through the three nitrogen atoms of the backbone, four carboxylate oxygen atoms and one phosphonate/phosphinate oxygen atom. This is a binding mode similar to that of H\(_5\)DTPA itself. The NMR spectra of the Ln\(^{3+}\) complexes all displayed multiple resonances for the various types of nuclei. For example, the \(^{13}\text{C}\) NMR spectrum of the diamagnetic complexes \([\text{Y(L}^1\text{)(H}_2\text{O})]^\text{3-}\) and \([\text{Y(L}^2\text{)(H}_2\text{O})]^\text{3-}\) at 25°C showed two resonances in the carboxylate region (intensities 1:1) and some broad signals for the aliphatic \(^{13}\text{C}\) nuclei. This indicates that several isomers of these complexes exist in solution and are in exchange with each other.

To support the coordination mode proposed, we evaluated the Nd\(^{3+}\)-C and Nd\(^{3+}\)-P distances from the \(^{13}\text{C}\) and \(^{31}\text{P}\) paramagnetic lanthanide-induced longitudinal-relaxation-rate enhancements. The Nd\(^{3+}\) ion was selected for this purpose, since it has the longest electron-spin-relaxation times among the light Ln\(^{3+}\) ions (\(\text{Ln} = \text{Ce} \rightarrow \text{Eu}\)). The measurements were performed at 80°C, at which temperature the spectra displayed relatively sharp lines \((\Delta\nu_{1/2} < 10 \text{ Hz})\). In order to correct for diamagnetic contributions, the relaxation rates of the corresponding La\(^{3+}\) complexes were subtracted from the measured values for the Nd\(^{3+}\) complexes. Under the conditions employed, the \(^{13}\text{C}\) NMR spectra of the Nd\(^{3+}\) complexes of H\(_6\)L\(_1\) and H\(_5\)L\(_2\) showed two carboxyl resonances, two resonances of carboxymethyl methylenes, two signals of the diethylenetriamine backbone and one doublet for the central methylene carbon, whereas the \(^{31}\text{P}\) NMR spectra showed a single resonance. Since the outer-sphere contribution to longitudinal relaxation rates \((1/T_{1,\text{os}})\) becomes significant only for remote
nuclei, this was neglected. From the electron-spin relaxation for Nd\(^{3+}\) \((T_1\approx 10^{-10}\text{ s})\),\cite{29} it can be estimated that the contact contribution to the paramagnetic relaxation is negligible. Therefore, two contributions are of importance: the dipolar relaxation and the Curie relaxation. These are represented by a combination of a simplified Solomon–Bloembergen equation with one for Curie relaxation, giving Equation (8).\cite{20} 

\[
\frac{1}{T_1} = \left[ \frac{4}{3} \left( \frac{\mu_0}{4\pi} \right)^2 \cdot \bar{r}^2 \cdot \gamma_i^2 \cdot \beta^2 \cdot T_{\text{Curie}} \right] + \left( \frac{6}{5} \left( \frac{\mu_0}{4\pi} \right)^2 \cdot \gamma_i^2 \cdot H_i^2 \cdot \mu_4^2 \cdot \beta^4 \right) \cdot \frac{1}{T_{\text{Curie}}} 
\]

(8)

Here \(\mu_0/4\pi\) is the permeability of a vacuum, \(\mu\) is the effective magnetic moment of Nd\(^{3+}\), \(\gamma_i\) is the gyromagnetic ratio of the nucleus under study \((^{13}\text{C})\), \(\beta\) is the Bohr magneton, \(T_{\text{Curie}}\) is the Curie spin relaxation time for Nd\(^{3+}\). \(H_0\) is the strength of the magnetic field, \(T_0\) is the Boltzmann constant, \(T\) is the temperature, \(\tau_{\text{rot}}\) is the rotational correlation time for the complex species and \(r\) is the distance of Nd\(^{3+}\) to the nuclei in the complex. This equation can be used to calculate the Nd\(^{3+}\)–C and Nd\(^{3+}\)–P distances \(r\). For these calculations, values of \(T_0\) at 80 °C of the corresponding Gd\(^{3+}\) complexes \((23.1\text{ ps})\) for \([\text{Nd}(L_1)(\text{H}_2\text{O})]\) and \(33.2\text{ ps}\) were employed. These values were evaluated by variable-concentration \(^1\text{H}\) NMR data performed on the deuterated La\(^{3+}\) complexes by using the activation energy for \(\tau_{\text{rot}}\) as obtained from the fitting of the \(^{13}\text{C}\) and nuclear magnetic relaxation dispersion (NMRD) data (see below).

Table 3 lists the experimental values of longitudinal relaxation rates observed for Nd\(^{3+}\) and La\(^{3+}\) complexes, together with the Nd\(^{3+}\)–C and Nd\(^{3+}\)–P distances calculated from them by Equation (8). For comparison, results obtained for complexes of H\(_5\)DTPA and its bis(amides) derivatives reported previously\cite{26,30,31} were used in the Table. The similarity of these values confirms the proposed octadentate binding mode (similar to structures of well-known complexes of H\(_5\)DTPA) of the new ligands in their lanthanide complexes.**

Table 3. Observed longitudinal relaxation rates in La\(^{3+}\) and Nd\(^{3+}\) complexes of ligands H\(_5\)L\(_1\) and H\(_6\)L\(_1\) and calculated nonbonding distances \(r_{\text{(Nd-P)}}\) and \(r_{\text{(Nd-C)}}\)\n
<table>
<thead>
<tr>
<th>atom</th>
<th>La–H(_5)L(_1)</th>
<th>Nd–H(_5)L(_1)</th>
<th>La–H(_6)L(_1)</th>
<th>Nd–H(_6)L(_1)</th>
<th>H(_5)L(_1)</th>
<th>H(_6)L(_1)</th>
<th>distances from Nd(^{3+}) [Å]</th>
<th>H(_5)DTPA(^{[1]})</th>
<th>H(_6)DTPA-bis(amides)(^{[2]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P)</td>
<td>0.356</td>
<td>10.06</td>
<td>0.291</td>
<td>9.73</td>
<td>3.49</td>
<td>3.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>0.17(^{[5]})</td>
<td>6.02–6.25(^{[5]})</td>
<td>0.10(^{[5]})</td>
<td>6.29–8.93(^{[5]})</td>
<td>3.22–3.24(^{[5]})</td>
<td>3.03–3.22(^{[5]})</td>
<td>3.15–3.20</td>
<td>3.14–3.30</td>
<td></td>
</tr>
<tr>
<td>N–CH(_2)–CO</td>
<td>2.56(^{[5]})</td>
<td>7.04–7.46(^{[5]})</td>
<td>2.63(^{[5]})</td>
<td>7.52–7.69(^{[5]})</td>
<td>3.33–3.35(^{[5]})</td>
<td>3.33–3.35(^{[5]})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N–CH(_2)–P</td>
<td>3.00(^{[5]})</td>
<td>5.39(^{[5]})</td>
<td>1.72</td>
<td>8.71</td>
<td>3.76(^{[5]})</td>
<td>3.15</td>
<td>3.17(^{[5]})</td>
<td>3.14–3.30</td>
<td></td>
</tr>
<tr>
<td>CH(_2)–N–CH(_2)–P</td>
<td>3.11</td>
<td>6.99</td>
<td>3.10</td>
<td>7.52</td>
<td>3.47</td>
<td>3.41</td>
<td>3.48(^{[5]})</td>
<td>3.04–3.48(^{[5]})</td>
<td></td>
</tr>
<tr>
<td>CH(_2)–N–CH(_2)–CO</td>
<td>2.82</td>
<td>7.09</td>
<td>3.11</td>
<td>8.40</td>
<td>3.41</td>
<td>3.30</td>
<td>3.21</td>
<td>3.04–3.48</td>
<td></td>
</tr>
<tr>
<td>P–C (arom)</td>
<td>–</td>
<td>–</td>
<td>0.11</td>
<td>1.96</td>
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<td>3.94</td>
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<td>–</td>
</tr>
<tr>
<td>C (arom-o)</td>
<td>–</td>
<td>–</td>
<td>0.72</td>
<td>1.29</td>
<td>–</td>
<td>4.79</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C (arom-m)</td>
<td>–</td>
<td>–</td>
<td>0.70</td>
<td>0.89</td>
<td>–</td>
<td>5.75</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C (arom-p)</td>
<td>–</td>
<td>–</td>
<td>1.28</td>
<td>1.41</td>
<td>–</td>
<td>6.13</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

[a] Taken from ref. [26]. [b] Taken from ref. [31]. [c] Two different signals of equal intensity are found in \(^{13}\text{C}\) NMR spectra. [d] Not determined. [e] The signal has low intensity and overlaps others; this makes it unsuitable for the determination of relaxation rates. [f] Value corresponding to the signal of an acetate pendant moiety bound to the central nitrogen atom. [g] Value corresponding to the signal of a backbone carbon atom bound to the central nitrogen atom.
Evaluation of rotational correlation times by $^3$H NMR: The rotational correlation time, $\tau_R$, is one of the parameters governing the relaxivity of a Gd$^{3+}$ complex. Usually, a relatively large discrepancy exists between the $\tau_R$ values evaluated from the $^1$H and $^{31}$P NMR data. Therefore, we decided to determine the rotational correlation times independently using the deuteron longitudinal relaxation rates of the deuterated ligands [D$_8$]H$_6$L$_1$, [D$_8$]H$_5$L$_2$ and [D$_8$]H$_5$L$_3$ in their diamagnetic La$^{3+}$ complexes.[34] In such a diamagnetic system, the deuteron relaxation depends only on quadrupolar interactions and is given by Equation (9):

$$R_i = \frac{1}{\tau_i} = \frac{3}{8} \left( \frac{\epsilon^2 q Q}{h} \right)^2 r_R$$

The quadrupolar coupling constant ($\epsilon^2 q Q/h$) has a value of 170 x 2π kHz for an sp$^3$-hybridised C=H bond. It has been demonstrated that $\tau_R$ values obtained in this way agree well with those obtained from $^3$H NMRD measurements.[34] The $1/\tau_i$ values and, therefore, also the $\tau_R$ values for $^2$H in samples of the La$^{3+}$ complexes of the deuterated ligands were found to be dependent on the concentration of the complex for concentrations varying between 4 and 200 mm (Figure 3). Extrapolation of the curves in Figure 3 to the concentration used in the NMRD measurements (1 mm, see below) gave estimated values of 86, 110 and 121 ps for $\tau_R^{298}$ of the Gd$^{3+}$ complexes of [D$_8$]H$_6$L$_1$, [D$_8$]H$_5$L$_2$ and [D$_8$]H$_5$L$_3$, respectively. The trend of these $\tau_R^{298}$ values agrees with the expected increase of the rotational correlation time upon increase of the molecular volume.

![Figure 3. Rotational correlation times at 298 K ($\tau_R^{298}$) obtained from solutions of [La([D$_8$]L$_2$)(H$_2$O)]$^{2+}$ (•••), [La([D$_8$]L)($\text{H}_2$O)]$^{3-}$ (○) and [La([D$_8$]L$_3$)(H$_2$O)]$^{2-}$ (◦) in H$_2$O at different concentrations.](image)
and a water proton was fixed at 3.1 Å, whereas the distance of closest approach of a water molecule to Gd\(^{3+}\), was fixed at 3.5 Å. The value of \(E_V\), the activation energy of the correlation time \(t_v\), was fixed at 1 kJmol\(^{-1}\). Attempts to unfix this parameter led to negative values of the activation energy. The hyperfine coupling constants \(A_i\) were fixed at the values calculated from the \(^{17}\)O NMR studies described above and by using Equation (12), in which \(\gamma\) is the Bohr magneton, \(k\) is the Boltzmann constant and \(\gamma_{I}\) is the \(^{17}\)O magnetogyric ratio. Furthermore, the quadrupolar coupling constant of the bound water, \(c(1 + \eta^2)^{1/2}\), was taken equal to that determined recently for the complex [Gd(dota)(H\(_2\)O)]\(^{2-}\) of 5.2 MHz.[37]

\[
F = \frac{\beta}{3kT\gamma_1} \frac{A}{h} \cdot 10^6
\]  

(12)

Fitting of the data with a single rotational correlation time resulted in bad fits, whereas separate fitting of the \(^{17}\)O and NMRD data resulted in good fits, but with different \(r_e\) values. This may be ascribed to the large difference in concentration at which the \(^{17}\)O (200 mM) and the NMRD measurements (1 mM) were carried out (see above).\[^{34}\] Furthermore, the \(^{17}\)O and \(^1\)H relaxation rates are modulated by rotation of the Gd–O and the Gd–H vectors, respectively. It may be expected that these rotations have different correlation times.\[^{37}\] Therefore, two rotational correlation times were taken into consideration, \(r_{eR}\) and \(r_{eH}\). The parameter \(r_{eH}\) was fixed at the values obtained from the \(^2\)H NMR measurements (see above).

A comparison of the values of the fitted parameters of the complexes under study with those of [Gd(dtpa)(H\(_2\)O)]\(^{2-}\) (see Table 4) reveals significant differences in the parameters related to the electronic relaxation (the square of the zero-field-splitting tensor, \(D^2\), and the corresponding correlation time, \(r_e\), and, particularly, in the diffusion coefficient, \(D_{GdH}\), (Eq. (13)).

\[
D_{GdH} = D_{complex} + D_{water}
\]  

(13)

Since \(D_{water}\) at 298 K is \(2.23 \times 10^{-9}\) m\(^2\) s\(^{-1}\), it should be expected that \(D_{GdH}^{208}\) is larger than this value. The relatively low
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Table 4. Parameters for Gd³⁺ complexes as obtained from the simultaneous fitting of ¹⁷O NMR and ¹H NMRD data by using a model including second-sphere water molecules and a model without second-sphere water molecules (see text), compared with literature values for [Gd(dtpa)(H₂O)]²⁻ complex.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[Gd(L¹)(H₂O)]³⁻ no 2nd sphere</th>
<th>[Gd(L¹)(H₂O)]³⁻ 2nd sphere</th>
<th>[Gd(L²)(H₂O)]³⁻ no 2nd sphere</th>
<th>[Gd(L²)(H₂O)]³⁻ 2nd sphere</th>
<th>[Gd(dtpa)(H₂O)]²⁻ no 2nd sphere</th>
<th>[Gd(dtpa)(H₂O)]²⁻ 2nd sphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>τ₂⁰ [ns]</td>
<td>62 ± 20</td>
<td>88 ± 26</td>
<td>74 ± 25</td>
<td>92 ± 29</td>
<td>543 ± 120</td>
<td>685 ± 297</td>
</tr>
<tr>
<td>ΔH² [kJ mol⁻¹]</td>
<td>38 ± 10</td>
<td>41 ± 8</td>
<td>36 ± 9</td>
<td>37 ± 8</td>
<td>29 ± 10</td>
<td>37 ± 9</td>
</tr>
<tr>
<td>E₂ [kJ mol⁻¹]</td>
<td>14.2</td>
<td>21.3 ± 3</td>
<td>15.3 ± 3</td>
<td>19 ± 5</td>
<td>20 ± 3</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>BₘgH₂O [°/s]</td>
<td>2.3 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>3.5 ± 0.4</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>τ₁² [ps]</td>
<td>30 ± 3</td>
<td>22 ± 3</td>
<td>34 ± 3</td>
<td>26 ± 3</td>
<td>31 ± 3</td>
<td>25²[b]</td>
</tr>
<tr>
<td>Δτ [¹⁰⁻⁶ s⁻¹]</td>
<td>0.29 ± 0.03</td>
<td>0.45 ± 0.09</td>
<td>0.22 ± 0.02</td>
<td>0.32 ± 0.06</td>
<td>0.26 ± 0.03</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>qₐ [¹⁰⁻⁶]</td>
<td>5.2 ± 2</td>
<td>6.1 ± 2</td>
<td>8.1 ± 2</td>
<td>8 ± 3</td>
<td>12 ± 2</td>
<td>11 ± 2²[b]</td>
</tr>
<tr>
<td>Aₘg [¹⁰⁻¹ rad s⁻¹]</td>
<td>−3.26[b]</td>
<td>−3.28[b]</td>
<td>−4.2</td>
<td>−3.61[b]</td>
<td>−3.69[b]</td>
<td>−3.69[b]</td>
</tr>
<tr>
<td>DₘgH₂O [¹⁰⁻⁶ m² s⁻¹]</td>
<td>14.3 ± 0.7</td>
<td>22.75[b]</td>
<td>13.2 ± 0.5</td>
<td>22.75[b]</td>
<td>12.4 ± 0.6</td>
<td>22.75[b]²[e]</td>
</tr>
<tr>
<td>E₀[¹⁰⁻³] [MHz]</td>
<td>28.7 ± 3</td>
<td>−39.6 ± 3</td>
<td>−</td>
<td>25 ± 3</td>
<td>−</td>
<td>19.4²[b]</td>
</tr>
<tr>
<td>q₈</td>
<td>0</td>
<td>2.2 ± 0.4</td>
<td>0</td>
<td>2.0 ± 0.3</td>
<td>0</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>r⁺[Å]</td>
<td>−</td>
<td>35 ± 8</td>
<td>−</td>
<td>50 ± 9</td>
<td>−</td>
<td>60 ± 10²[b]</td>
</tr>
<tr>
<td>ΔH₂ [kJ mol⁻¹]</td>
<td>−36 ± 11</td>
<td>−48 ± 12</td>
<td>−</td>
<td>35 ± 10</td>
<td>−</td>
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</tr>
<tr>
<td>ln[(1/Tₑ⁺) exp⁰]</td>
<td>23.65</td>
<td>23.47</td>
<td>23.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln[(1/Tₑ⁺) calcul]</td>
<td>22.53</td>
<td>22.24</td>
<td>22.35</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[a] Taken from ref. [36]. [b] Parameters were fixed during the fitting. [c] Calculated with Equation (14). [d] Determined from EPR line widths; [e] Calculated by using fitted parameters.

values obtained for DₘgH₂O indicate that the outer-sphere contribution to the total relaxivity is overestimated in the calculations. Most likely, this is due to an unaccounted contribution of water molecules in the second coordination sphere of Gd³⁺, which may be bound to the ligand through hydrogen bonds to, for example, the negatively charged phosphinate/phosphonate group.[39] To account for such a contribution of second-sphere water molecules, we included a series of equations that are similar to those for the inner-sphere contribution (see Supporting Information). It should be noted, however, that it is very difficult to evaluate the second-sphere parameters because strong correlations exist among some of them. Moreover, the second-sphere water protons probably do not occupy a unique location but may exchange among various sites. We fixed the distance between the Gd³⁺ ion and the protons of the second-sphere water molecules, r₂⁰ [Å], at 3.5 Å in the fitting procedure. Furthermore, the values of Dₘgwater at various temperatures were calculated with the semiemipirical relationship proposed by Hindman [Eq. (14)].[38] The size of the Gd³⁺ complexes of H₂L¹, H₂L² and H₂L³ is much larger than that of water and, consequently, the self-diffusion constants of these complexes will be much smaller than that of water. In the present fittings Dcomplex was, therefore, neglected.

$$-\ln D_{water}^T = \ln \left[ 3.1185 \times 10^{-4} e^{1.6258 \times 10^9/T} + 1.54792 \times 10^2 e^{1.6203 \times 10^9/T} \right]$$ (14)

A good fit was obtained by assuming about two second-sphere water molecules (q₂≈2). The resulting optimised parameters are included in Table 4, and the results are also shown as curves in Figure 4g-i. Now, the optimised parameters obtained all compare well with those previously report-
ed for [Gd(dtpa)(H₂O)]²⁻.[38] The residence times of these second-sphere water molecules (τ₂⁰ = 35–60 ps) are of the same magnitude as those obtained for other systems.[39–41] Although the accuracy of the second-sphere parameters obtained may be low due to the many assumptions made, it is clear that at least two second-sphere water molecules, with a residence time that is sufficiently long to be detected, have to be included in the model to adequately explain the NMRD profile. Aime et al.[45] have reported that two second-sphere water molecules are present in [Gd(2tp2a)- (H₂O)]⁻¹, a complex of a pyridine containing macrocycle bearing one methylenephosphonic and two acetate arms. Apparently, phosphonate/phosphinate groups are capable of forming hydrogen bonds to two water molecules. Previously, it has been shown that second-sphere water molecules contribute to the relaxivity of several other phosphonate-bearing ligands including [Gd(dotp)]⁻¹⁻(HDOTP = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraakis(methylphosphonic acid)).[42] While the results of simultaneous fits of the present data gave a r₂⁰/rₚH ratio of 2.7±3.0, the concentration dependence of rₚH as determined from ¹H NMR (see above) gave an estimate of this ratio of about 1.4 for equal concentrations. The latter value (1.4) is in agreement with that reported by Dunand et al. for the [Gd(dota)(H₂O)]⁻¹⁻ complex.[37] The differences between r₂⁰/H and rₚH may be ascribed to differences in the rotation rates of the Gd⁺⁻H and Gd⁺⁻O vectors.

The residence time of the Gd⁺⁻bound water molecule, rₚM, is an important parameter with regard to the efficiency of an MRI contrast agent. The theoretical curve of the relaxivity as a function of rₚM has a sharp maximum between 20 and 50 ns.[1,2,4] The values of τ₂⁰ for the [Gd(L¹)(H₂O)]⁻¹⁻ and [Gd(L²)(H₂O)]⁻²⁻ systems (88 and 92 ns, respectively)
are close to the optimum value. These $\tau_m$ values are lower than those of the current commercial contrast agents. For example, $[\text{Gd(dtpa)}(\text{H}_2\text{O})]^2^+\text{ and }[\text{Gd(dota)}(\text{H}_2\text{O})]^2^+$ have $\tau_m$ values of 303 and 243 ns, respectively. Consequently, it may be expected that very efficient MRI contrast agents can be obtained from $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ by increasing its $\tau_m$ value through covalent or noncovalent binding to macromolecules. Unfortunately, the $\tau_m$ value of the $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ complex is considerably higher (685 ns) and, thus, it may be expected that for this complex $\tau_m$ is limiting the relaxivity upon binding to a high-molecular-weight compound.

It has been shown that the water exchange in $\text{Gd}^{3+}$-polyaminocarboxylates with one $\text{Gd}^{3+}$-bound water generally takes place through a dissociative mechanism. Then, steric strain at the water site may increase the energy of the initial state and, therefore, decrease the activation energy. The decrease in $\tau_m$ upon replacement of the central $-\text{CH}_2\text{COO}^-$ moiety in $[\text{Gd(dtpa)}(\text{H}_2\text{O})]^2^+$ by a $-\text{CH}_2\text{PO}_3^-$ to form $[\text{Gd(L)}^2(\text{H}_2\text{O})]^3^+$ may be rationalised by an increase in steric strain around the $\text{Gd}^{3+}$-bound water molecule due to the relatively large size of the $-\text{PO}_3^-$ function compared with the $-\text{COO}^-$ function. An inspection of molecular models shows that the phenyl group in $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ is in the proximity of the $\text{Gd}^{3+}$-bound water. Most likely, $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ has a preferred conformation with the phenyl groups at large distances from the water site. The nitrogen atom of the dibenzylamino moiety is protonated phenyl groups at large distances from the water site. The ni-


Figure 5. Relaxivity at 20 MHz as a function of temperature for $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ (○), $[\text{Gd(L)}^2(\text{H}_2\text{O})]^3^+$ (□) and $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ (●). Experimental data were not fitted—the lines are only guides for the eye.

Figure 6. Proton longitudinal paramagnetic relaxation rates in solutions containing 4% HSA and increasing amounts of $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ (●) measured at 20 MHz and 310 K. The full line corresponds to the fitting of the data, and the dashed line represents $R^p_1$ in an aqueous solution in the absence of albumin.

**Evaluation of the electron-spin relaxation times $T_{2e}$ of the $\text{Gd}^{3+}$ complexes from EPR measurements:** The X-band (0.34 T) EPR spectra of the $\text{Gd}^{3+}$ complexes in aqueous solution at 298 K give approximately Lorentzian lines of $g \approx 2.0$. The transverse electronic relaxation rates ($1/T_{2e}$) were calculated from the experimental peak-to-peak line widths, $\Delta H_{pp}$, by using Equation (15), in which the symbols have their usual meaning.\[1/T_{2e} = \left(g_\mu_B\tau\sqrt{3h}\right)\Delta H_{pp}\] (15)

The experimental values of $\Delta H_{pp}$ obtained at 298 K were $0.122 \pm 0.05 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$, $0.103 \pm 0.05 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$ and $0.182 \pm 0.04 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$. The corresponding values of $\ln(1/T_{2e})_{\text{calc}}$ are compared in Table 4 with the $\ln(1/T_{2e})_{\text{calc}}$ values calculated using Equation (S9) (see Supporting Information), from the values of the parameters $A^*$ and $\tau_m$ obtained from the simultaneous fitting of the $^{17}\text{O}$ NMR and $^1\text{H}$ NMRD data. Although the relative experimental and calculated $1/T_{2e}$ values follow very similar trends in the three $\text{Gd}^{3+}$ complexes, the experimental values are systematically larger by a factor of about three. This discrepancy has been noted before\[36,44,45\] and corrected by introducing both static and dynamic zero-field-splitting effects in the electronic relaxation mechanisms of $\text{Gd}^{3+}$\[46\].

**Interaction of $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ with human serum albumin:** To study the interaction between $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ and HSA, a solution of the complex was added stepwise to a $4\%$ solution of HSA in water. A nonlinear increase of the paramagnetic relaxation rate of a solution containing $0.81 \text{ mM}$ of $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ to $0.05 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$ and $0.103 \pm 0.05 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$ and $0.182 \pm 0.04 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$.

\[1/T_{2e} = \left(g_\mu_B\tau\sqrt{3h}\right)\Delta H_{pp}\] (15)

The experimental values of $\Delta H_{pp}$ obtained at 298 K were $0.122 \pm 0.05 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$, $0.103 \pm 0.05 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$ and $0.182 \pm 0.04 \text{ mT } \left([\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+\right)$. The corresponding values of $\ln(1/T_{2e})_{\text{calc}}$ are compared in Table 4 with the $\ln(1/T_{2e})_{\text{calc}}$ values calculated using Equation (S9) (see Supporting Information), from the values of the parameters $A^*$ and $\tau_m$ obtained from the simultaneous fitting of the $^{17}\text{O}$ NMR and $^1\text{H}$ NMRD data. Although the relative experimental and calculated $1/T_{2e}$ values follow very similar trends in the three $\text{Gd}^{3+}$ complexes, the experimental values are systematically larger by a factor of about three. This discrepancy has been noted before\[36,44,45\] and corrected by introducing both static and dynamic zero-field-splitting effects in the electronic relaxation mechanisms of $\text{Gd}^{3+}$\[46\].

Interaction of $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ with human serum albumin:

To study the interaction between $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ and HSA, a solution of the complex was added stepwise to a $4\%$ solution of HSA in water. A nonlinear increase of the water-proton paramagnetic longitudinal relaxation rate was observed (see Figure 6) when plotted as a function of the concentration of the $\text{Gd}^{3+}$ complex. The paramagnetic relaxation rate of a solution containing $0.81 \text{ mM}$ of $[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ in $4\%$ HSA is $3.9\text{ times higher than that of }0.81 \text{ mM of }[\text{Gd(L)}^2(\text{H}_2\text{O})]^2^+$ in pure water; this indicates a strong interaction between the complex studied and HSA. This interaction is characterised by a stability constant ($K_{\text{SS}}$) of an adduct of HSA with the $\text{Gd}^{3+}$ complex $[\text{Eq.}...$
The proton relaxivity data obtained in HSA, \( R_1^{\text{obs}} \), were fitted to Equation \( (17) \); here \( p^0 \) is the protein concentration, \( s^0 \) is the concentration of the paramagnetic complex, \( n \) is the number of independent interaction sites and \( r_i^0 \) and \( r_i \) are the relaxivities of the \([Gd(L^\text{i})(H_2O)]^{2-}\) complex when noncovalently bound to HSA and free, respectively. In this fitting procedure, the association constant, \( K_{AS} \) and \( r_i^0 \) were used as adjustable parameters.

\[
n GdL + \text{HSA} \rightarrow (GdL)_n - \text{HSA} \quad K_{AS} = \frac{[GdL)_n - \text{HSA}]}{[GdL]^n [\text{HSA}]}
\]

\[
R_1^{\text{obs}} = 1000 \times \left[ (r_i^0 - s^0) + \frac{1}{2}(r_i - r_i^0) \left( (n \cdot p^0) + s^0 \right) \right] + K_{AS}^{-1} \sqrt{((n \cdot p^0) + s^0 + K_{AS}^{-1} - 4N \cdot s^0 \cdot p^0)}
\]

A good fit between experimental and calculated values was obtained by using a model for one binding site \( (n = 1) \) with an association constant \( K_{AS} = 4500 \pm 175 \text{m}^{-1} \). The relaxivity of noncovalently bound complex \( (r_i) \) was calculated to be \( 43 \pm 0.4 \text{ s}^{-1} \text{mm}^{-1} \), while the value of the relaxivity of free complex \( (r_i^0) \) is \( 5.9 \pm 0.3 \text{ s}^{-1} \text{mm}^{-1} \) (see above). Thus, in a solution containing \( 0.81 \text{ m}\text{m} \) of \([Gd(L^\text{i})(H_2O)]^{2-}\) and 4% (0.6 mHSA), 48.4% of Gd\(^{3+}\) complex interacts with the protein.

Longitudinal relaxation rates of the solution containing 4% HSA and \([Gd(L^\text{i})(H_2O)]^{2-}\) \((0.81 \text{ m}\text{m})\) were measured at 310 K over the range of magnetic fields \( 4 \times 10^4 \text{–} 7.05 \text{T} \). The corresponding NMRD profile (Figure 7a) shows the expected hump, characteristic for interactions with macromolecules, appearing in the high-frequency part (\( \approx 20 \text{ MHz} \)) of the recorded profile; this represents the combined contributions of the bound and free Gd\(^{3+}\) complex in the solution.

The theoretical \( ^1\text{H} \) NMRD profile of the \([Gd(L^\text{i})(H_2O)]^{2-}\)–HSA adduct was then calculated from the known NMRD profile of free \([Gd(L^\text{i})(H_2O)]^{2-}\) and the concentrations of free and bound complex obtained from the estimated stability constant of the adduct (see above) (Figure 7b). A lower number of second-sphere water molecules always resulted in calculated relaxivities too low for the low-field part (<10 MHz) of the NMRD curve. The results of the fitting possibly reflect the presence of mobile HSA protons that are dipolarly relaxed by the proximity of the Gd\(^{3+}\) ion. Alternatively, reduced mobility of solvent molecules in the second coordination sphere of Gd\(^{3+}\) upon noncovalent binding of \([Gd(L^\text{i})(H_2O)]^{2-}\) to HSA can explain this result.

Transmetallation: An important parameter determining the toxicity of Gd\(^{3+}\)-based contrast agents is the kinetic stability of the complexes. Transmetallation by endogenous metal ions may afford free Gd\(^{3+}\) ion, which is highly toxic. To get an impression of the kinetic stability of the phosphorus-containing complexes studied, we performed some transmetallation studies with Zn\(^{2+}\) according to a previously described protocol.\(^{[49]}\)

Samples containing the Gd\(^{3+}\) complexes of H\(_6\)L\(_1\), H\(_5\)L\(_2\), H\(_4\)L\(_3\) and a phosphate buffer containing ZnCl\(_2\) were monitored by measuring the \( ^1\text{H} \) relaxivity at 20 MHz. Upon transmetallation with Zn\(^{2+}\), the free Gd\(^{3+}\) formed immediately precipitated as the phosphate salt and, therefore, did not contribute to the total relaxivity any more. The resulting decrease in the proton-relaxation rate observed is a good estimate of the extent of transmetallation and, therefore, also for the kinetic stability of the Gd\(^{3+}\) complex. The results of the transmetallation experiments are displayed in Figure 8, while Table 5 shows the percentage of Gd\(^{3+}\) complexes left in the solution after 3 d. Thus, the kinetic stability decreases in the following order: \([Gd(dtpa)(H_2O)]^{2-}\) \(\approx\) \([Gd(L^\text{i})(H_2O)]^{2-}\) \(\geq\) \([Gd(L^\text{ii})(H_2O)]^{2-}\) \(\geq\) \([Gd(dtpa-bma)(H_2O)]^{2-}\) \(\geq\) \([Gd(L^\text{iii})(H_2O)]^{2-}\). Therefore, all complexes studied are less stable towards Zn\(^{2+}\) transmetallation than \([Gd(dtpa)(H_2O)]^{2-}\), but \([Gd(L^\text{i})(H_2O)]^{2-}\) and \([Gd(L^\text{ii})(H_2O)]^{2-}\) are slightly more kinetically stable than \([Gd(dtpa-bma)(H_2O)]^{2-}\).
Figure 8. Evolution of the relative water proton paramagnetic longitudinal relaxation rate $R_1(t)/R_1(0)$ vs. time for [Gd(dtpa)(H$_2$O)$_2$]$^{3-}$ (○), [Gd(L)$_3$(H$_2$O)$_2$]$^{3-}$ (□) and [Gd(L)$_3$(H$_2$O)$_2$]$^{2-}$ (△). The lines are only guides for the eye. The solution initially contained Gd$^{3+}$ complex (2.5 mM), ZnCl$_2$ (2.5 mM) and phosphate (H$_2$PO$_4^-$, HPO$_4^{2-}$, PO$_4^{3-}$, 67 mM).

Table 5. Percentage of remaining Gd$^{3+}$ complexes after 3 d of transmetallation with Zn$^{2+}$.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>$R_1(0)/R_1(3d)/R_1(0)$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Gd(L)$_3$(H$_2$O)$_2$]$^{3-}$</td>
<td>13</td>
</tr>
<tr>
<td>[Gd(L)$_3$(H$_2$O)$_2$]$^{2-}$</td>
<td>1.9</td>
</tr>
<tr>
<td>[Gd(L)$_3$(H$_2$O)$_2$]$^{2-}$</td>
<td>11</td>
</tr>
<tr>
<td>[Gd(dtpa-bma)(H$_2$O)]$^-$</td>
<td>49</td>
</tr>
</tbody>
</table>

Conclusion

A useful synthetic approach for a new class of H$_2$DTPA-based ligands in which the central pendant arm has a –P(OH)O–R moiety is reported. Their Ln$^{3+}$ complexes show structural features analogous to the H$_5$DTPA complexes, including the presence of one water molecule coordinated in the first coordination sphere of the metal ion. The phosphonate (H$_4$L$_3$) and the phenylphosphinate derivatives (H$_5$L$_2$) have $R_1$ values that are close to optimal (88 and 92 ns, respectively). Therefore, these complexes are very suitable for attachment to polymers, which should result in compounds with very high relaxivity. The relaxivity will be limited by water exchange than in most conjugates of chelates of the H$_2$DTPA or H$_2$DOTA type. Furthermore, an additional increase in the relaxivity is obtained by virtue of the presence of two water molecules in the second coordination sphere and which are probably bound to the phosphonate/phosphinate moiety through hydrogen bonds. The [Gd(L)$_3$(H$_2$O)$_2$]$^{3-}$ complex has a less favourable water-exchange rate ($R_1^{H_2O} = 685$ ns), but it has a high affinity to HSA and a relaxivity that is comparable with that of the well-known blood pool contrast agent MS-325.

Experimental Section

Materials and methods: Commercially available benzylamine (2), diethylenetriamine, phthalanhydride, ethyl chloroformate, ethyl bromoacetate, diethylyphosphate and phenylphosphinic acid had synthetic purity and were used as received. The 10% Pd/C catalyst for the hydrogenation reaction was obtained from Acros. Bis(phenylaldehyde)diethylenetriamine (8), ditosyl-ethanolamine, N-tosylaziridine (1), and ethyl phenylphosphinate were prepared by published methods. Paraformaldehyde was obtained by filtration of aged aqueous formaldehyde solutions and was dried in a desiccator over concentrated sulfuric acid.

Preparation of N-benzyldiethylenetriamine (4): N-tosylaziridine (1) (320 g, 162 mmol) was dissolved in ethanol (150 mL). Benzylamine (2) (8.00 g, 75 mmol) was added, and the solution was heated under reflux for 3 days. Then, the mixture was cooled and concentrated aqueous ammonia (5 mL) was added to quench the excess of tosylaziridine. The mixture obtained was heated under reflux for 15 min. Solvents were removed, and the residual brown oil was dissolved in a mixture of concentrated HBr/AcOH (300 mL, 1:1, v/v). The solution was heated under reflux for 24 h. After cooling, the reaction mixture was evaporated to dryness leaving a brown oil, which solidified upon cooling. This solid was dissolved in NaOH (100 mL, 15%), and the solution obtained was extracted with chloroform (7 × 50 mL). The organic phases were combined and evaporated to dryness to leave the mixture of monotosylated intermediate and the required product as a yellow oil. These compounds were separated by chromatography on silica by using gradient elution with increasing concentration of concentrated ammonia in ethanol from a ratio of 1:25 (mixture A) to 1:5 (mixture B), detection by ninhydrine (purple spots).

Note: extension of the reaction period to 36–48 h reduces the yield of the final product due to decomposition.

Yield: 8.25 g (57%), $R_1$ (mixt. A) = 0.1, $R_1$ (mixt. B) = 0.3; $^{1}H$ NMR (CDCl$_3$): δ = 2.05 (br, 4H; NH$_2$), 2.52, 2.75 (2m, 2H; NHCH$_2$CH$_2$N), 3.38 (s, 2H; NH$_2$Ph), 7.31 (m, 5H; Ph); $^{13}C$ NMR (CDCl$_3$): δ = 34.56 (2C), 57.05 (2C), 127.67 (2C), 127.82 (1C), 129.01 (2C), 129.45 (2C), 130.20 (2C), 132.31 (1C), 133.83 (1C), 135.17 (1C), 139.85 (1C), 140.08 (1C), 145.97 (1C).

$N$-Tosyl-$N'$-benzyldiethylenetriamine (7): Yield: 4.45 g (18%); $R_1$ (mixt. A) = 0.8, $R_1$ (mixt. B) = 0.9; $^{1}H$ NMR (CDCl$_3$): δ = 2.40 (s, 3H; CH$_3$), 2.49 (m, 2H), 2.58 (m, 2H), 2.76 (m, 2H) and 2.95 (m, 2H; all NHCH$_2$CH$_2$N), 3.05 (br, 1H; NH$_2$), 3.52 (s, 2H; NH$_2$Ph), 7.20 (m, 2H; Ts), 7.27 (m, 5H; Ph), 7.73 (m, 1H; Ts); $^{13}C$ NMR (CDCl$_3$): δ = 22.12 (1C; CH$_2$), 39.73, 42.11, 53.14, 55.99 (4×1C; NHCH$_2$CH$_2$N), 59.83 (13C; NH$_2$Ph), 67.12 (2C), 127.82 (1C), 129.01 (2C), 129.45 (2C), 132.20 (12C), 137.82 (1C), 139.51 (1C), 143.58 (1C); ES-MS: positive $m/z$: 348.3 ($^{14}M+H^+$).

Preparation of $N$-benzyldiethylenetriamine–$N$-$N'$-$N''$-tetraacetic acid (5): $N$-benzyldiethylenetriamine (4) (1.00 g, 5.2 mmol) was dissolved in water (5 mL) and then 1 mL of a solution of NaOH (2.5 g, 62.5 mmol, dissolved in 10 mL of water) was added. The mixture was heated to 90°C. Ethyl bromoacetate (4.34 g, 26 mmol) and the remaining NaOH solution were added in 6 portions during 1.5 h. The solution was then heated under reflux for 24 h to hydrolyse the ester groups. After cooling, the mixture was poured onto a column containing a strong cation exchange resin (150 mL, Dowex 50) in the H$^+$ form. The column was washed with water and the product was eluted with diluted aqueous ammonia (1:3). The eluate was evaporated to dryness to leave the crude
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13C NMR of intermediate (precipitated as a nonstoichiometric hydrochloride (2.5±3 equiv HCl per a strong anion exchanger (Dowex1) in the acetate form. After washing with water, some yellow impurities were removed with 10% acetic acid. The precipitate was washed with acetone and air-dried. Yield: 10.15 g (76%); m.p. 133–135°C (dec); 1H NMR (D,O, pD 0.7): ̇δ = 3.01 (2H, NHCH2), 3.22 (4H, NHCH2CH3), 3.39 (4H, NHCH2CH2N), 3.96 (8H, NHCO); 13C NMR (Di-O, pD 0.7): ̇δ = 49.64 (d, DCP = 147 Hz, 1C, NHCH2), 50.89 (2C, PCH2NCH2), 51.86 (2C, PCH2NCH2CH3), 55.30 (4C, NHCO), 169.56 (4C, CO); 13P NMR (D,O, pD 0.7): ̇δ = 167.5; elemental analysis calculated (%) for [H2]+OH4C3(H2(OH)2N2P2O4, M = 483.79]: C 32.72, H 5.63, Cl 7.33, N 8.69; found: C 31.90, H 5.49, Cl 7.33, N 8.45; ESI-MS: positive m/z: 430.3 [M+H]+; negative m/z: 428.3 [M–H]−.

Preparation of diethylentriamine-N,N′,N″,N‴-tetraacetic acid (H4L4): Bis(phthaloyl)diethylenetriamine (10.00 g, 27.5 mmol) was dissolved in dry ethanol (30 mL) and then the mixture was heated under reflux with a Dean–Stark trap. During the next 6 h, paraformaldehyde (2.48 g, 82.5 mmol, 3 equiv) was added in portions. The solvent in the trap was removed, and then the mixture was heated for another 14 h at 100°C. The mixture was cooled and filtered. Ethyl hydroxymethyl(phenyl)phosphinate formed as a by-product (δ = 40.1 ppm in 13P NMR spectra) and some starting ethyl phenylphosphinate were extracted with water (10–50 mL), the organic layer was dried over Na2SO4 and then evaporated to dryness, leaving a glassy solid, which was dissolved in small amount of water and crystallised by standing for several days. The white solid obtained was filtered, washed with acetone and air-dried. Yield: 10.15 g (76%); m.p. 133–135°C (dec); 1H NMR (D,O, pD 0.7): ̇δ = 3.01 (2H, NHCH2), 3.22 (4H, NHCH2CH3), 3.39 (4H, NHCH2CH2N), 3.96 (8H, NHCO); 13C NMR (Di-O, pD 0.7): ̇δ = 49.64 (d, DCP = 147 Hz, 1C, NHCH2), 50.89 (2C, PCH2NCH2), 51.86 (2C, PCH2NCH2CH3), 55.30 (4C, NHCO), 169.56 (4C, CO); 13P NMR (D,O, pD 0.7): ̇δ = 167.5; elemental analysis calculated (%) for [H2]+OH4C3(H2(OH)2N2P2O4, M = 483.79]: C 32.72, H 5.63, Cl 7.33, N 8.69; found: C 31.90, H 5.49, Cl 7.33, N 8.45; ESI-MS: positive m/z: 430.3 [M+H]+; negative m/z: 428.3 [M–H]−.
Preparation of diethylenetriamine-6.69, N 4.96.

Lanthanide Complexes of Diethylenetriamine 5899±5915

A mixture was heated to 60°C. Hypophosphorous acid (10.0 g of 50% aq. solution, 76.0 mmol) was added, and the reaction mixture was stirred at 60°C for 24 h. Then, chromatography on a strong cation exchanger (Dowex 1, 250 mL, acetate form, elution with water and 10% acetic acid) was used to purify the product. The residue was dissolved in chloroform (50 mL) and ethyl chloroformate (1 g) and pyridine (1.5 g) were added. After another 24 h, only one signal belonging to the desired ethyl ester was observed in the 1H NMR spectrum (35 ppm). The reaction mixture was washed with water (3×30 mL), dried (Na2SO4) and evaporated to dryness. The residue, a yellowish oil, was redissolved in toluene and evaporated in order to remove any excess of chloroformate. Yield: 3.30 g (11.0 mmol). The product showed a variable temperature 17O NMR study of Gd3+/C0 (dec.); 1H NMR (D2O, pH = 8.02): δ = 9.98 (dd, δGd/PH = 9.6, 8.4 Hz, 4H, CH2(NCH2)2) (5.90 g, 14.1 mmol) was stirred with BrCH2COOEt (11.8 g, 70.0 mmol) and K2CO3 (9.7 g, 70 mmol) in DMF (50 mL) at room temperature for 24 h. The reaction mixture was then extracted with toluene (100 mL) and the organic phase was extracted aqueous NaHCO3 (100 mL). After evaporation, a solution of NaOH (10 g) in water (50 mL) was added. Then, EtOH (50 mL) was added to obtain a homogeneous solution. This solution was stirred at room temperature for two days. The 31P NMR spectrum showed that the reaction was complete (one major peak at 35.9 ppm). The reaction mixture was evaporated until dryness. The residue was dissolved in the minimum amount of water and then purified on a column of a strong cation exchanger (Dowex 50, 200 mL, NH4+ form, with water (=1000 mL) as the eluent. After elution, a 31P NMR spectrum of the eluate was taken and showed the presence of H3L1. The fractions concerned were evaporated to obtain a yellow oil, which was crystallised at room temperature from an ethanol solution containing a small amount of acetone. The crystallisation started immediately. The product was filtered, washed with small amounts of ethanol and dried. Yield: 4.1 g, 84%.

Analysis of induced shifts in 17O NMR spectra of lanthanide(III) complexes with a complex concentration of about 0.2 M were prepared. The solid lanthanide(III) chlorides and solid ligands (±10% excess) were dissolved in a weighed amount of D2O in a small vial containing a stirring bar and a microscopic grain of methyl red indicator. The vials were capped with a septum, and then a solution of 10–15% sodium hydroxide in D2O was added dropwise from a syringe with stirring till a colour change of the indicator (pH 4–6). Then, the vial was weighed again. From the increase of the weight, the molar ratio of exchangeable oxygen atoms on the lanthanide ion can be calculated. Relaxation enhancements in 1H and 31P NMR spectra of lanthanide(III) complexes: For these experiments, the samples prepared for 1H NMR experiments described above were used. The longitudinal relaxation rates were measured at 30°C by using the inversion-recovery method.

Variable temperature 17O NMR study of Gd3+/C0 complexes: Solutions of Gd3+ complexes of H3L1 and H4L2 with a concentration of about 0.15 M were prepared by dissolution of exactly weighed solid GdCl3·6H2O and the solid ligands (H3L1 and H4L2) as the hydrochloride salts, ±10%
excess) in a weighed amount of deionised water. Calculated amounts of sodium hydroxide (9 and 8 equivalents for H$_2$L and H$_2$L$^-$ or H$_2$L$^{2-}$ respectively) in 0.1 M solution were added dropwise with stirring. After the mixture had stood for 30 min at room temperature, the pH was checked. All samples gave a negative Xylenol orange test for the presence of free Gd$^{3+}$. All NMR spectra were conducted without a frequency lock. To correct the $^{17}$O NMR shift for the contribution of the bulk magnetic susceptibility (BMS), the difference between chemical shifts of proton signals of acetone (or tert-butanol) in the paramagnetic sample and in pure water was used.$^{[31]}$ Longitudinal ($T_1$) and transversal ($T_2$) relaxation rates were obtained by the inversion-recovery method$^{[9]}$ and the Carr–Purcell–Meiboom–Gil pulse sequence.$^{[32]}$ Respectively. Experimental data were fitted with a computer program written by Dr. E. Töth and Dr. L. Helm (EPFL Lausanne, Switzerland) using the Micromath Scientist program, version 2.0 (Salt Lake City, UT, USA).

**Concentration dependence of $^1$H NMR longitudinal-relaxation time:** Deuterium-containing ligands were prepared by H-D exchange from H$_2$L$^-$ and H$_2$L$^{2-}$ in D$_2$O/K$_2$CO$_3$ (pD 10.5) by heating mixtures at 95°C for 5 d, analogously to the method reported in the literature.$^{[33]}$ The ligand and LaCl$_3$·7H$_2$O (equivalent amounts) were dissolved in deuterium-depleted water (1 mL). Then the pH was adjusted to a value close to neutral by addition of small portions of solid LiOH·H$_2$O. The transversal-relaxation rates were measured by using the inversion-recovery pulse sequence.

**EPR Measurements:** EPR spectra of the Ln$^{3+}$ complexes in aqueous solution were recorded on a Bruker ESP300E spectrometer operating at 9.43 GHz (0.34 T, X-band). 5 mM aqueous solutions of the complexes were measured at 298 K in a quartz flat cell. Typical parameters used were: sweep width 40 mT, microwave power 20 mW, modulation amplitude 0.32 mT and constant offset 0.02 s. The frequency was calibrated with diphenylpycrylhydrazyl (dpph) and the magnetic field with Mn$^{2+}$ in MgO.

**Measurements of NMRD profiles:** The samples were prepared by mixing the ligand under study with a slight excess of solid Gd(C$_6$I$_6$)$_2$ followed by dissolution in water. The pH values of the solutions were adjusted to about 7 with a NaOH solution. The solutions were then stirred overnight in the presence of Chlex 20 to remove the remaining free Gd$^{3+}$. The solids were removed in a centrifuge, and the remaining solutions were freeze-dried. The solid complexes were dissolved in an appropriate amount of water. The absence of free Gd$^{3+}$ was checked with an Arsenazo III indicator. The concentrations of Gd$^{3+}$ in 0.05 M Na$_2$SO$_4$ solutions were determined from proton-relaxivity measurements at 20 MHz and 37°C at complete purity. The purity of complex solutions was checked by ESI-MS; [Gd(L)$_3$]$^{3+}$: m/z: 651 [M+3Na$^+$]; 673 [M+4Na$^+$]; 695 [M+5Na$^+$]; 730 [M+4Na$^+$+K$^+$]; [Gd(L)$_2$]$^{2+}$: m/z: 711 [M+3Na$^+$]; 769 [M+2Na$^+$+K$^+$]; [Gd(L)]$^{+}$: m/z: 822 [M+2Na$^+$]; 844 [M+3Na$^+$]. The complexes [Gd(L)$_3$]$_2$(H$_2$O)$_2$]$^{2+}$, [Gd(L)$_2$]$_2$(H$_2$O)$_2$]$^{2+}$ and [Gd(L)]$_2$(H$_2$O)$_2$]$^{2+}$, were studied by $^1$H longitudinal-relaxation-time measurements. NMRD profiles were measured at 5, 25 and 37°C.

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**References**


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