Synthesis, Variable Temperature and Pressure $^{17}$O NMR Study of Bis(alkylamide) Derivatives of [[Gd-DTPA](H$_2$O)$_2$]$^{2-}$ – An Assessment of the Substitution Effect on Water Exchange Kinetics


Keywords: Water exchange / MRI contrast agents / DTPA-bis(amide) derivates / Substituent effects / $^{17}$O

In this work, primary, secondary and tertiary bis(amide) derivatives of DTPA have been synthesized and the residence time of the water molecule coordinated to their gadolinium complexes has been measured by variable temperature $^{17}$O NMR spectroscopy. The influence of substituent characteristics (molecular mass, hydrophobicity, volume) has been examined. For the primary and secondary bis(amide) complexes, increasing the length of the lateral chains decreases the water residence time. It varies from 1171±38 ns for [Gd(DTPA-BA)(H$_2$O)] to 673±32 ns for [Gd(DTPA-BIpepta)(H$_2$O)]. The activation volumes of 5 bis(amide) complexes ([Gd(DTPA-BA)(H$_2$O)], [Gd(DTPA-BEA)(H$_2$O)], [Gd(DTPA-BbBA)(H$_2$O)], [Gd(DTPA-BAA)(H$_2$O)], and [Gd(DTPA-BHA)(H$_2$O)]) have been measured by a variable pressure $^{17}$O NMR spectroscopic study. No change in the activation volume is observed when the substituent size is increased. The inner sphere is therefore not disturbed by the substitution on the amide groups. The slight decrease in water residence time can be attributed to the hydrophobicity increase of the complex which, in turn, leads to a hydrophobic environment favorable to water molecule exchange. The water residence time of tertiary bis(amide) complexes is similar to the fastest secondary bis(amide) complex. For the tertiary bis(amide) complexes, a length increase by the substituent does not change the residence time. According to these results, we suggest that a disruption of the hydration shell of the complexes by amide substituents occurs that can influence coordinated water exchange by a factor of two, at most. (© Wiley-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002)

Introduction

Most of the contrast agents used in Magnetic Resonance Imaging (MRI) are paramagnetic complexes of gadolinium. Their efficiency, expressed by their relaxivity, $r_1$ (water proton relaxation rate enhancement induced by 1 millimole per liter of paramagnetic complex), depends on water exchange kinetics, electronic relaxation, and molecular tumbling, as well as on the number of water molecules in the first coordination sphere of the gadolinium cation ($q$). The influence of these parameters has been extensively described and the ways in which to modify some of them, for instance the rotational correlation time of the complex ($\tau_r$) and the number $q$, are well known.\textsuperscript{[1-10]} By comparison, the strategies towards changing the electronic relaxation times ($\tau_{so}$, $i = 1, 2$) and the residence time of the water-coordinated molecule ($\tau_M$) are poorly understood.

$\tau_M$ is not a limiting factor for the relaxivity of commercially available $T_1$ low molecular weight agents, like Magnesvit$^\text{®}$ (Schering, Berlin, Germany), Omniscan$^\text{®}$ (Amer- sham Health, Amersham, United Kingdom), Dotarem$^\text{®}$ (Guerbet, Aulnay, France) or Prohance$^\text{®}$ (Bracco, Milan, Italy), at physiological temperatures and magnetic fields normally used in MRI, but this parameter may play an important role when covalent or noncovalent coupling to a macromolecule is involved.\textsuperscript{[11-23]} Indeed, in order to improve the relaxivity, high molecular weight contrast agents have been developed. To reach optimal relaxivity, the residence time must, however, fulfill the condition: $T_{1M} > \tau_M > \tau_{so}$, where $T_{1M}$ is the longitudinal relaxation time of nuclei in the first coordination sphere. The adjustment of $\tau_M$ is therefore of paramount importance.

Up to now, it has been reported that the water exchange rate of monoaquagadolinium chelates decreases by several orders of magnitude as compared to the aqueous ion.\textsuperscript{[4]} Micksi et al. showed that the water exchange rate of [Gd(DTPA)(H$_2$O)$_2$]$^{2-}$ is about 200 times slower than that for aqueous gadolinium [Gd(H$_2$O)$_3$]$^{3+}$.\textsuperscript{[13]} The presence of amide groups further decreases the exchange rate of Ln$^{3+}$, DTPA derivatives.\textsuperscript{[6-9]} The determination of water exchange rates for a set of lanthanide complexes of DTPA-

\[ \text{[686]} \]
BMA (from Nd<sup>3+</sup> to Ho<sup>3+</sup>) has shown that the exchange rate increases when the ionic radius of the lanthanide decreases.<sup>104</sup> These two latter observations show the importance of steric hindrance in the coordination cage on the water exchange rate. Furthermore, the charge has also been invoked as a factor affecting residence times which are particularly long, as for [Gd(DTPA)(H<sub>2</sub>O)]<sup>3+</sup> (τ<sub>M</sub> = 17 μs at 278 K),<sup>111</sup> or short, as for [Gd(PCTP-13)(H<sub>2</sub>O)]<sup>3+</sup> (τ<sub>M</sub> = 8 ns at 278 K).<sup>112</sup> Further substitution of the DTPA backbone on the ethylene bridge decreases the residence time by about 30% as compared to [Gd(DTPA)(H<sub>2</sub>O)]<sup>2-</sup> at 310 K as is the case for the Gd<sup>3+</sup> chelates of EOB-DTPA and MS-325.<sup>113,114</sup>

From the literature, no simple relationship can be clearly established between the structure of Gd complexes and water exchange kinetics. To unravel some relationship, we have studied a series of equally charged but diversely amide-substituted chelates of Gd-DTPA bis(amide) derivatives (Table 1). Proton and oxygen-17 relaxometry at variable temperatures and pressures have been carried out to analyze the influence of the lengthening of the amide substituent on the water residence time.

Results and Discussion

Primary and Secondary Bis(amide) DTPA Gd<sup>3+</sup>-Chelates

The proton relaxivity of primary and secondary chelates measured at 20 MHz and 310 K is given in Table 2. The data for the variable temperature study is presented in Figure 1. The proton longitudinal relaxivity reflects the molecular mass increase of the complexes as expected by the Solomon–Bloembergen theory.<sup>115,116</sup> The relaxivity is the sum of inner- and outer-sphere contributions. The outer-sphere relaxation is not influenced by the residence time of water and increases when the temperature decreases due to the reduction of the water molecule translational diffusion. On the contrary, the inner-sphere contribution depends on the water residence time as expressed in Equations (1) and (2).

When the water residence time is short as compared to T<sub>1,Mr</sub> (T<sub>1r</sub> << T<sub>1,Mr</sub>), the inner-sphere contribution increases and the total relaxivity is enhanced when the temperature decreases, as for [Gd(DTPA)(H<sub>2</sub>O)]<sup>2-</sup>.<sup>117</sup> If the water exchange is slow, the inner-sphere contribution decreases when the temperature is reduced, as is the case for [Gd(DTPA-BMA)(H<sub>2</sub>O)]. As a result, the total relaxivity may remain approximately constant or decrease at low temperatures. The proton relaxivity of all the bis(amide) complexes studied in this work is clearly limited at low temperatures and reflects a long water residence time (Figure 1).

For the determination of the residence time from <sup>17</sup>O NMR spectroscopic measurements, the experimental <sup>17</sup>O NMR spectroscopic data were fitted assuming q = 1 (the temperature dependence of the logarithm of the <sup>17</sup>O reduced transverse relaxation rates is shown in Figure 2 for four of the studied complexes). This assumption was supported by the values of the hyperfine-coupling constant (A/ h) and relaxivities that are similar to those measured for DTPA complexes with one coordinated water molecule. Table 2 shows the parameters obtained by the fitting of the experimental points. Since the <sup>17</sup>O transverse relaxation rates have been measured at one magnetic field (7.05 T in this study), the electronic parameters obtained are relatively

Table 1. Structures of the ligands

<table>
<thead>
<tr>
<th>Bis(amide) derivative</th>
<th>Ligand abbreviation</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(amide)</td>
<td>DTPA-SA</td>
<td>R&lt;sub&gt;1&lt;/sub&gt; = R&lt;sub&gt;2&lt;/sub&gt; = H</td>
<td></td>
</tr>
<tr>
<td>Bis(ethylamide)</td>
<td>DTPA-BMA</td>
<td>R&lt;sub&gt;1&lt;/sub&gt; = CH&lt;sub&gt;3&lt;/sub&gt;, R&lt;sub&gt;2&lt;/sub&gt; = H</td>
<td></td>
</tr>
<tr>
<td>Bis(tert-butylamide)</td>
<td>DTPA-BeA</td>
<td>R&lt;sub&gt;1&lt;/sub&gt; = CH&lt;sub&gt;3&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;, R&lt;sub&gt;2&lt;/sub&gt; = H</td>
<td></td>
</tr>
<tr>
<td>Bis(hexylamide)</td>
<td>DTPA-BePA</td>
<td>R&lt;sub&gt;1&lt;/sub&gt; = CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;, R&lt;sub&gt;2&lt;/sub&gt; = H</td>
<td></td>
</tr>
<tr>
<td>Bis(hexylamide)</td>
<td>DTPA-BnPA</td>
<td>R&lt;sub&gt;1&lt;/sub&gt; = CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;, R&lt;sub&gt;2&lt;/sub&gt; = H</td>
<td></td>
</tr>
<tr>
<td>(R,S)-Bis(2-hydroxypropylamide)</td>
<td>DTPA-(R,S)BnPA</td>
<td>R&lt;sub&gt;1&lt;/sub&gt; = CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;, R&lt;sub&gt;2&lt;/sub&gt; = H</td>
<td></td>
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</table>

<sup>16</sup> The abbreviation BAA [Bisamydilamide] for the penta derivative has been selected to avoid confusion with the propyl derivative (BnPA)
Table 2. Parameters obtained from the fitting of experimental $^{17}$O NMR spectroscopic data at variable temperature and proton longitudinal relaxivity of the studied bis(amide) complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>$r_1$ [s$^{-1}$ mm$^{-1}$] at 20 MHz</th>
<th>$\tau_{1/2}$ [ns]</th>
<th>$\Delta H^*$ [kJ mol$^{-1}$]</th>
<th>$\Delta S^*$ [J mol$^{-1}$ K$^{-1}$]</th>
<th>$\lambda/\eta$ [s$^{-1}$]</th>
<th>$B$ [10$^{16}$ s$^{-2}$]</th>
<th>$\tau_v$ [ps] (298 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Gd(DTPA)$_2$(H$_2$O)$_2$]$^{a}$</td>
<td>3.8</td>
<td>143±25</td>
<td>51.5±0.3</td>
<td>52.1±0.6</td>
<td>-3.4±0.1</td>
<td>2.60±0.06</td>
<td>12.3±0.3</td>
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<tr>
<td>[Gd(DTPA-BAA)$_2$(H$_2$O)$_2$]</td>
<td>3.6</td>
<td>1171±38</td>
<td>43.3±0.1</td>
<td>82.0±0.1</td>
<td>-2.8±0.1</td>
<td>2.45±0.07</td>
<td>36.5±1.2</td>
</tr>
<tr>
<td>[Gd(DTPA-BMA)$_2$(H$_2$O)$_2$]$^{b}$</td>
<td>3.8</td>
<td>967±36</td>
<td>48.0±0.1</td>
<td>24.9±0.2</td>
<td>-3.2±0.1</td>
<td>2.04±0.06</td>
<td>21.2±0.6</td>
</tr>
<tr>
<td>[Gd(DTPA-BEA)$_2$(H$_2$O)$_2$]</td>
<td>3.7</td>
<td>927±31</td>
<td>46.8±0.1</td>
<td>21.2±0.1</td>
<td>-2.8±0.1</td>
<td>2.73±0.10</td>
<td>39.2±1.4</td>
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<tr>
<td>[Gd(DTPA-BenPA)$_2$(H$_2$O)$_2$]</td>
<td>4.0</td>
<td>845±35</td>
<td>47.2±0.1</td>
<td>23.4±0.2</td>
<td>-2.8±0.1</td>
<td>2.09±0.18</td>
<td>24.8±2.1</td>
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<tr>
<td>[Gd(DTPA-BnBA)$_2$(H$_2$O)$_2$]</td>
<td>4.4</td>
<td>713±31</td>
<td>48.5±0.1</td>
<td>29.2±0.2</td>
<td>-3.4±0.2</td>
<td>2.03±0.21</td>
<td>17.1±1.9</td>
</tr>
<tr>
<td>[Gd(DTPA-BAA)$_2$(H$_2$O)$_2$]</td>
<td>4.3</td>
<td>575±29</td>
<td>48.4±0.1</td>
<td>20.4±0.3</td>
<td>-3.6±0.1</td>
<td>1.96±0.17</td>
<td>14.8±1.3</td>
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<tr>
<td>[Gd(DTPA-BHA)$_2$(H$_2$O)$_2$]</td>
<td>4.5</td>
<td>681±23</td>
<td>45.7±0.1</td>
<td>20.5±0.2</td>
<td>-2.8±1.6</td>
<td>1.37±0.05</td>
<td>25.2±0.8</td>
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<tr>
<td>[Gd(DTPA-BHepA)$_2$(H$_2$O)$_2$]</td>
<td>4.5</td>
<td>673±32</td>
<td>45.7±0.1</td>
<td>20.5±0.2</td>
<td>-2.8±0.3</td>
<td>2.27±0.14</td>
<td>17.9±1.1</td>
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<tr>
<td>[Gd(DTPA-BBMA)$_2$(H$_2$O)$_2$]</td>
<td>3.8</td>
<td>624±44</td>
<td>48.9±0.1</td>
<td>31.5±0.4</td>
<td>-3.5±0.1</td>
<td>3.46±0.31</td>
<td>25.9±2.4</td>
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<tr>
<td>[Gd(DTPA-BBVE)$_2$(H$_2$O)$_2$]</td>
<td>3.9</td>
<td>654±28</td>
<td>48.3±0.1</td>
<td>29.2±0.2</td>
<td>-3.3±0.4</td>
<td>2.00±0.16</td>
<td>18.5±1.5</td>
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<tr>
<td>[Gd(DTPA-BBPA)$_2$(H$_2$O)$_2$]</td>
<td>4.5</td>
<td>672±38</td>
<td>42.3±0.1</td>
<td>9.4±0.2</td>
<td>-3.0±0.3</td>
<td>1.67±0.23</td>
<td>22.2±3.8</td>
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<tr>
<td>[Gd(DTPA-BBPA)$_2$(H$_2$O)$_2$]</td>
<td>4.1</td>
<td>654±29</td>
<td>52.6±0.1</td>
<td>42.9±0.1</td>
<td>-4.5±1.6</td>
<td>3.34±0.31</td>
<td>15.2±1.3</td>
</tr>
<tr>
<td>[Gd(DTPA-BS)$_2$(H$_2$O)$_2$]</td>
<td>4.2</td>
<td>659±27</td>
<td>44.2±0.1</td>
<td>15.8±0.2</td>
<td>-3.0±0.1</td>
<td>2.56±0.08</td>
<td>35.9±1.2</td>
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<tr>
<td>[Gd(DTPA-BS)$_2$(H$_2$O)$_2$]$^{c}$</td>
<td>4.5</td>
<td>1128±95</td>
<td>43.2±0.1</td>
<td>8.1±0.3</td>
<td>-2.8±1.1</td>
<td>1.58±0.15</td>
<td>26.7±3.2</td>
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</table>

$^{a}$ See ref. [13] $^{b}$ The reduced $^{17}$O transverse relaxation rates measured at variable temperature have been refitted with the equations used in this study.

Figure 1. Temperature dependence of the proton relaxivity of linear bisamide complexes of [Gd(DTPA)]$_2$(H$_2$O)$_2$ and of the parent compounds at 20 MHz

Figure 2. Temperature dependence of the reduced transverse $^{17}$O relaxation rate for four linear bis(amide) complexes at 7.05 T; the straight lines represent the fitted curves

since it could reduce the energy necessary to reach the transition state assumed to have identical energy whatever the complex.

The $^{17}$O transverse relaxation rate of the paramagnetic solutions, $1/T_2$, and of the acidified water reference, $1/T_2^{wa}$, were also measured at high pressure. This allows the calculation of the reduced transverse relaxation rate as described above in the variable temperature section. For the fitting of the high pressure data, $T_{SM}^2$ was then fixed to the value calculated at 400 MHz with the parameters obtained at 300 MHz in the variable temperature study using Equations (7)–(10). The pressure dependence of $1/T_2$ at 298.15 K is presented in Figure 4 for five of the studied complexes. The decrease of $1/T_2$ as pressure increases, indicates a slowing down of the water exchange process, which has been checked to be reversible for all the studied Gd$^{III}$ chelates. The fitted parameters are given in Table 3. The replacement of two carboxylic groups by two amide groups induces a nearly twofold decrease in the activation volume, as seen by
Figure 3. Dependence of the water resonance time for linear bisamide complexes as a function of the group mass and of the hydrophobicity of the ligand (log D).

Figure 4. Pressure dependence of the reduced transverse \(^1\)H NMR relaxation rate at 298.15 K and 9.4 T for [Gd(DTPA-BA\(\cdot\)H\(_2\)O)\(\cdot\)H\(_2\)O] (black diamonds), [Gd(DTPA-BMA\(\cdot\)H\(_2\)O)] (white triangles), [Gd(DTPA-BMA\(\cdot\)H\(_2\)O)] (black triangles), [Gd(DTPA-BA\(\cdot\)H\(_2\)O)] (black stars) and [Gd(DTPA-BMA\(\cdot\)H\(_2\)O)] (white circles) as well as the straight lines to represent the fitted curves.

Table 3. Parameters obtained from the fit of the high pressure \(^1\)H NMR spectroscopic data; the high pressure study has been performed at 9.4 T and at \(T = 298.15\) K if not stated otherwise; the values in parentheses are the water exchange rate constants obtained from the fit of variable temperature \(^1\)H NMR spectroscopic data at 298.15 K; \(T_{298}\) was fixed for the fit.

<table>
<thead>
<tr>
<th>Gd complexes</th>
<th>(k_{\text{ex}} V/10^9) s(^{-1})</th>
<th>(\Delta T/\text{cm}^2/\text{mol}^{-1})</th>
<th>(T_{298}/10^{17}) s</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Gd(DTPA(\cdot)H(_2)O(\cdot)H(_2)O)](^{2-})</td>
<td>1.37</td>
<td>+12.5±0.2</td>
<td>—</td>
</tr>
<tr>
<td>[Gd(DTPA-BA(\cdot)H(_2)O)]</td>
<td>0.44±0.01 (0.42)</td>
<td>+6.3±0.4</td>
<td>1.30</td>
</tr>
<tr>
<td>[Gd(DTPA-BMA(\cdot)H(_2)O)](^{2+})</td>
<td>0.30±0.02 (0.04)</td>
<td>+7.5±0.2</td>
<td>1.38</td>
</tr>
<tr>
<td>[Gd(DTPA-BMA(\cdot)H(_2)O)]</td>
<td>0.52±0.01 (0.49)</td>
<td>+7.3±0.2</td>
<td>1.54</td>
</tr>
<tr>
<td>[Gd(DTPA-BA(\cdot)H(_2)O)]</td>
<td>0.55±0.01 (0.64)</td>
<td>+7.0±0.2</td>
<td>1.54</td>
</tr>
<tr>
<td>[Gd(DTPA-BA(\cdot)H(_2)O)]</td>
<td>0.85±0.01 (0.73)</td>
<td>+7.1±0.2</td>
<td>1.54</td>
</tr>
<tr>
<td>[Gd(DTPA-BMA(\cdot)H(_2)O)]</td>
<td>0.62±0.01 (0.71)</td>
<td>+6.5±0.2</td>
<td>1.14</td>
</tr>
</tbody>
</table>

\(T = 298.2\) K, \(\Delta T = 0.6\) K.
activation volume is not affected by the nature of the substituent.

In order to characterize the hydrophobicity of our bis(amide) complexes carrying aliphatic linear chains, the log D coefficient of the corresponding ligands was used. At a fixed pH, this log D coefficient reflects the hydrophobicity for the different ligands in solution and takes into account the contribution of ionizable groups. For the calculation of log D, the pH was set to 12 since the ligand structure is similar to the one in the complex (the carboxylate groups and nitrogen atoms are not protonated). The calculated hydrophobicity of bis(amide) ligands is reported in Figure 3. The residence time decreases with increasing hydrophobicity and remains quite similar for larger groups. This plateau could be explained due to the fact that only the first carbon atoms of the chain disturb the hydration shell that contributes to the exchange process.

The influence of the substituent volume on the water residence time was also investigated. The volumes of 4 substituents (R1 = hydrogen, methyl, isopropyl, tert-butyl) were calculated using the van der Waals radii of atoms. The volumes of linear chains were not calculated because their flexibility does not allow a correct description of their volumes. The evolution of the residence times shown in Figure 5 is similar to the one observed in Figure 3. The measured values for [Gd(DTPA-BnBA)(H₂O)] and [Gd(DTPA-BtBA)(H₂O)] (Table 2) confirm a fact previously observed in this study: the only hindrance arising from the first carbon atoms of the substituent leads to a shortening of the residence time.

An alternative explanation can be proposed. The bis(alkylamide) DTPA derivatives present four diastereoisomers in solution. It has been shown for the complex [Eur(DOTAM)(H₂O)]⁺ that the diastereoisomers have different exchange rates. Recently, the study of interconversion mechanisms between the diastereoisomers proved that the global value of the residence time was mainly fixed by the minor stereoisomer. The exchange rates measured for the bis(alkylamide) derivatives are therefore averages weighted by the molar fractions of the stereoisomers. Like for tetraakis(amide) DOTA derivatives, the alkyl chains could influence the equilibrium between the stereoisomers. According to Figure 5, the lengthening of lateral chains could favor the faster stereoisomer, but this must still be demonstrated.

**Study of Tertiary Bis(amide) DTPA Complexes**

For the tertiary bis(amide), the evolution of proton relaxivity at varying temperatures also demonstrates a limitation by the residence time (Figure 6). The values of the residence times are similar to those measured for the larger linear bis(amide) complexes [Gd(DTPA-BnBA)(H₂O)], [Gd(DTPA-BA)(H₂O)], [Gd(DTPA-BtBA)(H₂O)], and [Gd(DTPA-BtHeptA)(H₂O)] (Table 2). The reduction of the

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**Table 4. Distances obtained from crystallographic data**

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<tbody>
<tr>
<td>Gd–N</td>
<td>2.64</td>
<td>2.33–2.42</td>
<td>2.39</td>
<td>Na₃[Gd(DTPA(H₂O))][³⁻]</td>
</tr>
<tr>
<td>2.61–2.74</td>
<td>2.44</td>
<td>2.38</td>
<td>2.42</td>
<td>[Gd(DTPA-BMAA)(H₂O)][²⁻]</td>
</tr>
<tr>
<td>2.67</td>
<td>2.49</td>
<td>2.38</td>
<td>2.44</td>
<td>[Gd(DTPA-BHAA)(H₂O)][²⁻]</td>
</tr>
<tr>
<td>2.70</td>
<td>2.42</td>
<td>2.36</td>
<td>2.42</td>
<td>[Gd(DTPA-BEAA)(H₂O)][²⁻]</td>
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</tbody>
</table>

[¹] From ref. [²] [³] [⁴] From ref. [⁵] BBA = Bis(benzylamide).

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**Figure 5. Volume substituent dependence of the residence time for bis(amide) complexes**

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**Figure 6. Temperature dependence of the proton relaxivity for tertiary bis(amide) complexes of [Gd(DTPA(H₂O))²⁻]**

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NMR Study of Bisalkylamido) Derivatives of [[Gd-DTPA-6]H(O)]

The residence time of [[Gd-DTPA-6]BMA]([H(O)]) compared to [[Gd-DTPA-BMA][H(O)]], as well as the similarity of the residence time of the tertiary bis(amides), once again confirm the importance of steric hindrance at the level of the nitrogen atom from the amide group.

Conclusions

For the first time an extensive series of Gd-DTPA bisamido) derivatives has been studied to assess the influence of the amide substituents on the water exchange rate. 

1H NMR spectroscopic studies carried out at variable temperatures and high pressures reveal that the substituents, which are close to the first coordination sphere, influence the residence time of bisamide) DTPA derivatives without modifying the activation volume. However, this influence is relatively weak since the water exchange rate is doubled, at the most, for [[Gd-DTPA-6]BMA][H(O)] as compared to [[Gd-DTPA-BHepA][H(O)]] and does not reach the exchange rate of [[Gd-DTPA-BMA][H(O)]].

To explain the decrease of $\tau_m$ along the series, both the hydrophobicity and the hindrance of the complexes have been addressed, since these complexes are all neutral. The relative influence of these factors depends on the complexes studied. However, another hypothesis is that the lengthening of alky groups favors the stereoisomers with the faster exchange rates and therefore influences the global exchange rate.

Experimental Section

Chemicals: DTPA-BMA was provided by Nycomed (Amersham Health, Amersham, United Kingdom). All chemicals and GdCl₃·6H₂O were purchased from Aldrich (Bornem, Belgium).

Ligand Synthesis: The bisamido) derivatives of DTPA were synthesized by adding excess of amine to DTPA-bisamido) (2,2,2). Amine (16.8 mmol) was added to a stirred suspension of DTPA-bisamido) (2 g, 5.6 mmol) in anhydrous DMF (50 mL) and heated at 325 K. After 12 h, the yellow solution was concentrated under reduced pressure. The residue was dissolved in water (50 mL) and the pH of the aqueous phase was adjusted to 7 with saturated NaHCO₃ solution. The aqueous phase was extracted with diethyl ether (5 x 30 mL). The organic phase was discarded and the pH of the aqueous solution was adjusted to 2-3 with 3 M HCl and the solvents evaporated once again. The residue was dissolved in a minimum of methanol and filtered. Solvent A (Table 5) was slowly added whilst stirring. The white precipitate was filtered, suspended in solvent B (Table 5) and heated until dissolution. The warm solution was filtered and cooled to 273 K. Cool solvent C (Table 5) was then slowly added whilst stirring to precipitate the ligand. The ligand was filtered, washed with diethyl ether and dried under vacuum. The yields are reported in Table 5. For the synthesis of DTPA-BHepA, when the pH of the aqueous phase was adjusted to 7 with saturated NaHCO₃ solution, the aqueous phase was continuously extracted with diethyl ether for 48 h (to avoid emulsion formation). The purity and structure of the compounds were identified by 1H, 13C NMR spectroscopy with a Bruker AMX-300 (Bruker, Karlsruhe, Germany) and by ESIMS mass spectrometry (VG Analytical Autospec-6F, Manchester, UK). For this technique, samples were dissolved in water and deposited on a glycerol matrix.

NMR Spectroscopic Data

DTPA-B0PA (4): C₆H₅H₂O₅N₃O₅; 475.54 amu. 1H NMR (D₂O, 298 K, pH = 6); δ = 0.55 (t, 6 H, 2 × CH₂), 1.2 (sext, 4 H, 2 × CH₂), 2.5 (t, 4 H, 2 × CH₂), 2.9 (t, 4 H, 2 × CH₂), 3.0 (6 H, 2 × CH₂), 3.1 (m, 8 H, 4 × CH₂), 3.55 (s, 2 H, 1 × CH₂) ppm. 13C NMR (D₂O, 298 K, pH = 6, reference pyridine); δ = 10.0, 21.1, 40.1, 49.6, 51.9, 53.6, 58.1, 58.2, 169.5, 172.2, 177.8 ppm.

DTPA-BA (6): C₆H₅H₂O₅N₃O₅; 531.64 amu. 1H NMR (D₂O, 298 K, pH = 13); δ = 0.75 (t, 6 H, 2 × CH₂), 1.1-1.3 (m, 8 H, 4 × CH₂), 1.4 (quint, 4 H, 2 × CH₂), 2.3-2.6 (m, 8 H, 4 × CH₂), 2.9-3.2 (m, 14 H, 2 × CH₂ + 5 × CH₃) ppm. 13C NMR (D₂O at 298 K, pH = 13, reference MeOH); δ = 14.0, 22.4, 28.9, 32.6, 39.3, 52.1, 52.6, 58.6, 59.1, 59.4, 173.4, 179.2, 179.6 ppm.

DTPA-BHepA (8): C₆H₅H₂O₅N₃O₅; 587.75 amu. 1H NMR (D₂O, 298 K, pH = 11); δ = 0.7 (t, 6 H, 2 × CH₂), 1.1-1.3 (m, 16 H, 8 × CH₂), 1.3-1.5 (quint, 4 H, 2 × CH₂), 1.8 (m, 4 H, 2 × CH₂), 2.3-2.6 (m, 6 H, 3 × CH₂), 2.9-3.3 (m, 12 H, 6 × CH₂) ppm. 13C NMR (D₂O, 298 K, pH = 11, reference pyridine); δ = 13.5, 22.2, 23.1, 23.2, 26.6, 28.7, 28.8, 31.5, 38.8, 52.1, 52.6, 57.9, 58.3, 58.9, 172.6, 178.5, 181.2 ppm.

Table 5. Solvents used and yields for the syntheses of bisamido) derivatives

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Solvent A</th>
<th>Solvent B</th>
<th>Solvent C</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTPA-B0PA</td>
<td>acetone</td>
<td>2-propanol</td>
<td>diethyl ether</td>
<td>69</td>
</tr>
<tr>
<td>DTPA-BA</td>
<td>acetone</td>
<td>ethanol</td>
<td>diethyl ether</td>
<td>51</td>
</tr>
<tr>
<td>DTPA-BHepA</td>
<td>diethyl ether</td>
<td>ethanol</td>
<td>acetone</td>
<td>55</td>
</tr>
<tr>
<td>DTPA-BHepA</td>
<td>diethyl ether</td>
<td>ethanol/ethyl acetate (2:2)</td>
<td>diethyl ether</td>
<td>57</td>
</tr>
<tr>
<td>DTPA-BHepA</td>
<td>diethyl ether</td>
<td>ethanol/ethyl acetate (5:5)</td>
<td>diethyl ether</td>
<td>49</td>
</tr>
<tr>
<td>DTPA-BHepA</td>
<td>diethyl ether</td>
<td>2-propanol</td>
<td>diethyl ether</td>
<td>47</td>
</tr>
<tr>
<td>DTPA-BHepA</td>
<td>diethyl ether</td>
<td>2-propanol/ethanol (3:1)</td>
<td>acetone</td>
<td>47</td>
</tr>
</tbody>
</table>

FULL PAPER

CHL 3.9 (s, 4 H, 2 × CH2), 4.0 (s, 4 H, 2 × CH2) ppm. 13C NMR (δ-D0, 288 K, pH = 6, reference pyridine): δ = 17.9, 38.7, 43.8, 51.0, 53.3, 53.5, 162.7, 168.1, 169.2 ppm. 1H NMR δ-D0, 288 K, pH = 6: 6.9 (δ-D0, 2 × CH2), 2.8 (t, 4 H, 2 × CH2), 2.9–3.0 (m, 8 H, 4 × CH2), 3.0–3.2 (m, 8 H, 4 × CH2), 3.55 (s, 2 H, 1 × CH2), 3.65 (2 H, sex, 2 × CH) ppm. 13C NMR δ-D0, 288 K, pH = 6, reference pyridine: δ = 18.9, 45.3, 49.7, 52.3, 53.8, 58.2, 63.2, 65.4, 169.7, 172.8, 178.0 ppm.

Preparation of the Gadolinium Complexes: The gadolinium complexes were prepared by mixing a slight excess (5%) of ligand with gadolinium chloride. The pH of the solution was adjusted to 6–7. The absence of free gadolinium ions was checked with xylene orange indicator. In order to eliminate the excess of ligand, the bistetramidel complexes were filtered through a Sep-Pak Vac 6cc cartridge, OH- form from Waters (Brussels, Belgium) and eluted with demineralized water. The mass was confirmed by ElectroSpray spectrometry (Micromass, Manchester, UK) and the purity of the chelates was checked by HPLC (Waters, Milford, USA), C18 column, inverse phase, pH = 6.5, triethylammonium buffer 0.05 M, flow 1 ml/min, diode array and fluorescence detectors. The concentration of the Gd complex solutions was measured by proton relaxometry after decomposition of GdO 4 and by Induced Coupled Plasma methods (Jobin-Yvon 38+, Jobin-Yvon ISA, Longjumeau, France).

Variable Temperature and High Pressure 17O NMR Spectroscopy: Variable temperature 17O NMR spectroscopic measurements were recorded with a Bruker AMX-300 spectrometer (7.05T, 40.65 MHz). The temperature was controlled by a BVT-2000 unit. All 17O NMR spectroscopic measurements at natural abundance were performed in 2-mL samples contained in 10-mm (o.d.) tubes. The concentration of the solutions was lower than 30 mM. 17O diamagnetic transverse relaxation times of water were measured using a Carr-Purcell-Meiboom-Gill sequence (90° and 180° pulse lengths were 25 and 50 μs, respectively). All 17O NMR spectra were proton-decoupled. 17O transverse relaxation times of water in solutions containing Gd complexes were calculated from the spectral linewidth. High pressure 17O NMR spectroscopic measurements were performed up to 200 MPa at 298.15 K and with a Bruker ARX 400 spectrometer (9.4T, 54.2 MHz) equipped with a homebuilt probe head. The Gd 3+ complex solutions were prepared in 3%, using in all cases 10% 17O-enriched water (Yeda R&D Co., Rehovot, Israel). Their Gd 3+ concentration was checked as previously described. The pH of the solutions, measured with a combined glass electrode, was between 4.0 and 6.1. The reference used was 2% 17O-enriched bidistilled water acidified with perchloric acid. The temperature was externally controlled by a circulating flow from an external temperature bath and measured using a built-in Pt resistor. The transverse relaxation times were obtained by the CPMG spin-echo technique.

Proton Relaxometry: The relaxivities were measured at 0.47T with a Bruker Minispec PC-120 thermostatted by a tetrahydroethylene flow at temperatures between 278.15 and 318.15 K. To avoid prototropic exchange, measurements were carried out at pH = 6–7.

Data Analysis: Fitting of variable temperature 17O NMR spectroscopic data was performed with Minuit software (CERN library). Least-squares fits of the 17O high pressure data were performed by Scientist for Windows. The log D coefficients were calculated using log D Suite developed by Advanced Chemistry Development (Toronto, Canada). The volumes of substituents were calculated with the software Molecular Modeling Pro (ChemSW Software, Fairfield, USA). All the equations that are used in the treatment of 17O NMR spectroscopic data and relaxivity measurements are given here.

Relaxivity Measurement: The measured proton relaxivities contain both inner- and outer-sphere contributions [Equation (1)].

\[
\frac{1}{T_1^p} = \frac{P_q}{T_{w} + T_M} + \frac{P_q}{T_{M} + T_{M}^{-1} + \Delta \omega M^2} \cdot \frac{T_{M}^{-1}}{T_{M}^{-1} + T_{M}^{-1}} + \Delta \omega M^2 
\]

The inner-sphere term is given by Equation (2), where \( P \) is the molar fraction of paramagnetic centers and \( q \) is the number of coordinated water molecules.

\[
\frac{1}{T_1^p} = \frac{P_q}{T_{w} + T_M} \quad (2)
\]

Variable Temperature 17O NMR Spectroscopy: The residence time of inner-sphere water molecules can be estimated by the analysis of the temperature dependence of the 17O transverse relaxation rate. The observed transverse relaxation rate is the sum of a paramagnetic (1/T2P) and a diamagnetic (1/T2D) contribution [Equation (3)].

\[
\frac{1}{T_1^p} = \frac{1}{T_{w}} + \frac{1}{T_{M}} \quad (3)
\]

The paramagnetic term itself arises from outer-sphere (1/T2P) and inner-sphere (1/T2D) mechanisms [Equation (4)].

\[
\frac{1}{T_1^p} = \frac{1}{T_{w}} + \frac{1}{T_{M}} \quad (4)
\]

The inner-sphere contribution is given by the classical Equation (5), where \( \Delta \omega M \) is the chemical shift difference between bound and bulk water and \( T_{2M} \) is the transverse relaxation time of the bound water.

\[
\frac{1}{T_1^p} = \frac{P_q}{T_{M}} \cdot T_{M}^{-2} + T_{M}^{-1} \text{ Re} \bigg[ \frac{T_{M}^{-1} + \Delta \omega M^2}{T_{M}^{-1} + T_{M}^{-1}} \bigg] + \Delta \omega M^2 
\]

It has been shown that for gadolinium complexes the outer-sphere contribution is negligible.\(^{144} \Delta \omega M \) is defined by Equation (6), where \( g_T \) is the Landé factor (equal to 2 for Gd\(^{3+} \)), \( g_B \) is the Bohr magneton, \( S \) is the electron spin (3.5 for Gd\(^{3+} \)), \( h \) is the Boltzmann constant, \( A_H \) is the hyperfine coupling constant, and \( T \) is the temperature.

\[
\Delta \omega M = \frac{g_T g_B S (S + 1) B_0 A_H}{3 h T} \quad (6)
\]

The transverse relaxation rate of 17O coordinated to the gadolinium ion is predominantly described by the scalar interaction [Equation (7)]. The dipolar and quadrupolar contributions can be neglected since their contribution to the relaxation is 5% at most.\(^{144} \) Therefore, \( T_{2M} \) is given by Equation (7), where \( \omega_0 \) is the resonance frequency of the electron.

\[
\frac{1}{T_2^M} = \frac{1}{3} \bigg[ \frac{\omega_0 \gamma g_T g_B S}{h} \bigg] \bigg[ \frac{1}{1 + \gamma g_B S (1/2 + 1/2)} \bigg] 
\]

The scalar correlation times \( \tau_s \) are defined by Equation (8).

\[
T_2^M = \frac{1}{\tau_s} + \frac{1}{\tau_w} + \frac{1}{\tau_p} \quad (8)
\]

The electronic relaxation times \( \tau_s \) are described by the contribution of the zero-field splitting interaction (no contribution of the spin rotation has been considered) and are expressed by Equation (9) and (10), where \( \tau_s \) is the correlation time related to the modulation of the zero-field splitting (ZFS) and \( B \) is a parameter related to the ZFS and is independent of the temperature.

\[
\frac{1}{\tau_p} = \frac{\gamma g_T \hbar}{1 + g_B S (1/2 + 1/2)} \quad (9)
\]

MR Study of Bist(alkylamido) Derivatives of [(Gd-DTPA)(H2O)]

\[
\frac{5}{2} + \frac{5}{2} \left( 1 + \frac{2}{5} \right) + \frac{2}{5} \left( 1 + \frac{2}{5} \right) \quad (10)
\]

At high magnetic fields, \( c_{Gd} \alpha \tau_m \gg 1 \) and Equation (7) becomes Equation (11).

\[
\frac{1}{\tau_m} = \frac{5}{3} \left( \frac{4}{3} \right) \tau_t \quad (11)
\]

A reduced relaxation rate given by Equation (12) was used in this study, where \( f \) is the ratio [paramagnetic complexes]/[water].

\[
\frac{1}{\tau} = \frac{1}{\tau_t} \quad (12)
\]

The reduced transverse relaxation depends thus on the electronic relaxation time \( \tau_t \), the hyperfine coupling constant \( A_{Sh} \), and the water residence time \( \tau_m \). The parameters \( A_{Sh} \) and \( B \) are independent of temperature. The temperature dependence of the water residence time and of the correlation time \( \tau_t \) can be described according to the theories of Eyring and Arrhenius (Equation (13) and (14)), where \( \Delta S^T \) and \( \Delta H^T \) are the entropy and enthalpy of activation, respectively, for the water exchange process and \( E_r \) is the activation energy related to \( \tau_t \).

\[
\frac{1}{\tau_w} = \frac{1}{\tau_m} + \frac{1}{\tau_t} \quad (13)
\]

\[
\tau_t = \frac{1}{\tau_m} \exp \left( \frac{\Delta S^T + \Delta H^T}{RT} \right) \quad (14)
\]

The temperature dependence of the \( ^{17}O \) reduced transverse relaxation rate, \( 1/T_2 \), can thus be used to determine the water residence time and the related activation parameters \( \Delta S^T \) and \( \Delta H^T \).

**Variable Pressure \( ^{17}O \) NMR Spectroscopy:** By neglecting \( \Delta H_T \) in Equation (5) and using Equation (12), the reduced transverse relaxation rate, \( 1/T_2 \), can be simplified by Equation (15), where \( 1/T_2 \) is expressed as in Equation (7).[21]

\[
\frac{1}{\tau} = \frac{1}{\tau_t} + \frac{1}{\tau_m} \quad (15)
\]

Previous studies have shown that the logarithm of the water exchange rate varied almost linearly with pressure, as long as the compressibility coefficient of activation is negligible, as expressed by Equation (16).[13] Where \( \Delta F \) is the activation volume independent of pressure and \( \delta_h \) is the water exchange rate at zero pressure and temperature \( T \); \( 1/T_2 \) is small, and therefore can be assumed to be independent of the pressure since a pressure dependence of \( 1/T_2 \) with \( \Delta F \) between +5 and -5 cm3 mol-1 does not affect the value of \( \Delta V^T \) within the experimental errors.

\[
\frac{1}{\tau_w} = \frac{1}{\tau_m} \exp \left( \frac{\Delta V^T}{RT} \right) \quad (16)
\]

**Acknowledgments**

We thank the Fonds pour la Recherche dans l’Industrie et l’Agriculture (FRIA) of Belgium. We are also grateful to the Swiss OFES as part of the EU COST D18 Action and the Swiss National Science Foundation. The authors thank Mrs. Patricia de Francisco for her help in preparing the manuscript.


Received February 20, 2002 [I02089]