Physicochemical characterization of the dimeric lanthanide complexes \([\text{en}\{\text{Ln(DO3A)}(\text{H}_2\text{O})\}_2]\) and \([\text{pi}\{\text{Ln(DTTA)}(\text{H}_2\text{O})\}_2]\)^{2-}: a variable-temperature \(^{17}\text{O}\) NMR study

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The Gd(III) complexes of the two dimeric ligands \([\text{en(DO3A)}]_2\) \(\left[\text{N,N’-bis[1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-N,N’-ethylenediamine}\right]\) and \([\text{pi(DTTA)}]^{8-}\) \([\text{bisdiethylene-triaminepentaacetic acid (trans-1,2-cyclohexanediamine)}]\) were synthesized and characterized. The \(^{17}\text{O}\) NMR chemical shift of \(\text{H}_2\text{O}\) induced by \([\text{en(DO3A)}]_2\) and \([\text{pi(DTTA)}]^{2-}\) at pH 6.80 proved the presence of 2.1 and 2.2 inner-sphere water molecules, respectively. Water proton spin–lattice relaxation rates for \([\text{en(Gd(DO3A))(H}_2\text{O})]_2\) and \([\text{pi(Gd(DTTA))(H}_2\text{O})]^{2-}\) at 37.0 ± 0.1 °C and 20 MHz are 3.60 ± 0.05 and 5.25 ± 0.05 ms⁻¹ s⁻¹ per Gd, respectively. The EPR transverse electronic relaxation rate and \(^{17}\text{O}\) NMR transverse relaxation time for the exchange lifetime of the coordinated \(\text{H}_2\text{O}\) molecule and the \(^{2}\text{H}\) NMR longitudinal relaxation rate of the deuterated diamagnetic lanthanum complex for the rotational correlation time were thoroughly investigated, and the results were compared with those reported previously for other lanthanide(III) complexes. The exchange lifetimes for \([\text{en(Gd(DO3A))(H}_2\text{O})]_2\) (769 ± 10 ns) and \([\text{pi(Gd(DTTA))(H}_2\text{O})]^{2-}\) (910 ± 10 ns) are significantly higher than those of \([\text{Gd(DOTA)(H}_2\text{O})]^{−}\) (243 ns) and \([\text{Gd(DTPA)(H}_2\text{O})]^{−}\) (303 ns) complexes. The rotational correlation times for \([\text{en(Gd(DO3A))(H}_2\text{O})]_2\) (150 ± 11 ps) and \([\text{pi(Gd(DTTA))(H}_2\text{O})]^{2-}\) (130 ± 12 ps) are slightly greater than those of \([\text{Gd(DOTA)(H}_2\text{O})]^{−}\) (77 ps) and \([\text{Gd(DTPA)(H}_2\text{O})]^{−}\) (58 ps) complexes. The marked increase in relaxivity \((r_T)\) of \([\text{en(Gd(DO3A))(H}_2\text{O})]_2\) and \([\text{pi(Gd(DTTA))(H}_2\text{O})]^{2-}\) result mainly from their longer rotational correlation time and higher molecular weight. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: NMR; EPR; \(^{1}\text{H}\) NMR; \(^{2}\text{H}\) NMR; \(^{17}\text{O}\) NMR; Gd(III) complexes; paramagnetic complexes; proton relaxation

INTRODUCTION

Several lanthanide [e.g. Gd(III)] and transition metal [e.g. Mn(II) and Fe(III)] complexes of polyaminocarboxylates are either commercially available or in clinical trials for use as magnetic resonance imaging (MRI) contrast agents. The MR signal of body fluids can be altered by the presence of paramagnetic water relaxation agents to result in enhanced image contrast. The general design criteria for safe and efficacious MRI contrast agents have been reviewed by a number of investigators.¹ ² However, they remain areas for further research and development of MRI contrast agents, particularly those with enhanced relaxivity and improved tissue targeting functions. The main barrier to this endeavor is the low sensitivity of MRI contrast agents when they are coupled with the low flux of most biochemical processes. This problem can be addressed by developing contrast agents that possess higher spin–lattice relaxivity \((r_L)\). The dimeric MRI contrast agents possess two gadolinium(III) ions and will increase relaxivity.

This has been confirmed for \(\text{H}_6\text{BO(DO3A)}_2^{3−}\) in which the Gd(III) complex incorporates several desirable features. As the ligand is a DOTA \([\text{DOTA} = 1,4,7,10\text{-tetraaza-1,4,7,10-tetrakis(carboxymethyl)cyclododecane}]\) derivative, we can expect high kinetic inertness and thermodynamic stability, and as a neutral complex it is preferable from the point of view of application (a less painful injection because of lower osmolality). The fact that two Gd\(^3+\) ions are bound to one molecule allows for smaller injection volumes for the same total amount in mmol Gd kg\(^{-1}\) body weight. Finally,
the increased molecular weight and volume may result in a longer rotational correlation time, and thus in a higher proton relaxivity.

To extend these observations, [en(DO3A)2]6− and [pi(DTTA)2]8−, the derivatives of DO3A (1,4,7,10-tetraaza-cyclododecane-1,4,7-triaacetic acid) and DTPA [1,1,4,7,7-pentakis(carboxymethyl)-1,4,7-triazahetapepane] were synthesized. The number of inner-sphere water molecules was determined from the 17O NMR chemical shift of the water as a function of Dy(III) concentration. The water proton spin–lattice relaxivity $r_1$ of the [enGd(DO3A)H2O]2+ and [piGd(DTTA)H2O]2+ complexes at various temperatures and pH values are described. The EPR and 17O NMR transverse relaxation rate data were analyzed together in a simultaneous multiple-parameter least-squares fitting procedure to determine the water residence lifetime. 2HN M R spectroscopy was used to determine the rotational correlation time.

EXPERIMENTAL

Materials
The acid forms of the free ligand, DO3A, were synthesized and characterized in accordance with the literature.4

Ethylene diaminediacetic acid dimethyl ester (1).
To a solution of EDDA (ethylene diaminediacetic acid, 28.41 mmol) in MeOH (150 ml) warmed to 40–50 °C was added SOCl2 (8.24 ml, 113.64 mmol) dropwise slowly. After 20 h, solvent was removed from the fraction containing the product by rotary evaporation. The residue was dried under vacuum and a white powder was obtained (4.76 g, 80.21%). 1HN M R (D2O), $\delta$ (ppm): 3.48 (NCH2CH2N), 3.75 (NCH2COOCH3), 4.03 (NCH2COOCH3).

ent(DO3A)2 (2) (Scheme 1).
DO3A (1.27 g, 3.66 mmol) and anhydrous methanol (120 ml) were mixed in a single-necked flask and the pH of the solution was adjusted to 11.59 with NH4OH. A water-bath was used to keep the temperature at 40–50 °C. Compound 1 (0.49 g, 2.4 mmol) was dissolved in anhydrous methanol (240 ml) and slowly added to a single-necked flask which contained DO3A and K2CO3 (10 g). After 48 h, the reaction solution was concentrated under reduced pressure to a pale yellow oil. The residue was dissolved in 50 ml of distilled water and made alkaline to pH 11.0 with ammonia solution. The solution was then applied to an AG1-X8 anion-exchange resin column (200–400 mesh, HCO3− form, 60 ml of resin and 3.0 cm column diameter). After passing through an anion-exchange resin column, the product eluted in the 0.035 M formic acid fraction. Solvent was removed from the fraction containing the product by rotary evaporation and co-evaporated five times with 200 ml of water to remove the formic acid. The residue was dried in vacuum and a pale yellow hygroscopic powder was obtained (1.374 g, 88%) 13CN M R (50 MHz, D2O) (ppm): 179.2, 178.0, 173.0, 166.7, 58.6, 57.4, 54.2, 53.1, 51.8, 50.8, 47.1, 46.8, 45.0, 42.6. Anal.
Variable-temperature $^{17}$O NMR of dimeric lanthanide complexes

Calculated for C$_{35}$H$_{64}$N$_{10}$O$_{15}$: C, 48.60; H, 7.46; N, 16.19. Found: C, 48.55; H, 7.40; N, 16.10%.

Diethylenetriamine-$N'$-acetic acid-$N,N'$-dianhydride (3). Pyridine (50 ml) and acetonitrile (50 ml) warmed to 50°C were mixed with a solution of DTPA (50 mmol, 19.7 g) in acetic anhydride (318 mmol, 32.4 g). After 24 h, solvent was removed from the fraction and the residue was washed with acetic anhydride and diethyl ether. The solid was then dried under vacuum and a white powder was obtained (16.6 g, 92%). $^1$H NMR (200 MHz, DMSO-d$_6$), $\delta$ (ppm): 3.71 (s, 8H, terminal NC$_2$H$_2$CO$_2$), 3.30 (s, 2H, central NC$_2$H$_2$CO$_2$), 2.72 (t, 4H, NCH$_2$CH$_2$N), 2.59 (t, 4H, NCH$_2$CH$_2$N).

$[\mu$(DTTA)$_2$]$_2$ (4) (Scheme 2).

Compound 3 (2.3 g, 6.88 mmol), K$_2$CO$_3$ (10 g) and DMSO (100 ml) were mixed in a single-necked flask and trans-1,2-cyclohexanediamine (0.33 ml, 2.75 mmol) was slowly added. After 12 h, the solution was fractioned and filtered by ultrafiltration using ultrafiltration membranes YM3 (diameter 25 mm, MW cut-off = 3000) and YM1 (diameter 25 mm, MW cut-off = 1000). Solvent was removed by rotary evaporation. The residue was dried under vacuum and a pale-yellow oil was obtained (1.37 g, 43%). $^{13}$C NMR (100 MHz, D$_2$O), $\delta$ (ppm): 172.91, 170.80, 166.81, 166.72, 56.64, 56.22, 54.40, 52.93, 52.44, 52.34, 52.03, 50.67, 50.38, 49.89, 31.28, 24.04. Anal. Calculated for C$_{34}$H$_{56}$N$_{8}$O$_{18}$: C, 47.22; H, 6.53; N, 12.96. Found: C, 47.16; H, 6.50; N, 12.64%.

General

GdCl$_3 \cdot 6$H$_2$O (99.9%), DyCl$_3 \cdot 6$H$_2$O (99.9%) and LaCl$_3 \cdot 7$H$_2$O (99.9%) were obtained from Aldrich and used without further purification. The concentrations of Gd$^{3+}$, Dy$^{3+}$ and La$^{3+}$ were determined by chelatometric titration with EDTA using xylene orange as indicator. All other reagents used for the synthesis of the ligand were purchased from commercial sources unless noted otherwise. $^1$H and $^{13}$C NMR spectra and elemental analyses were used to confirm the composition of the products. $^{17}$O-enriched water (20.1%) was purchased from Isotec.

Deuteration

The lanthanum complexes were synthesized by reaction of La$_2$O$_3$ with ligands in water and precipitated by addition of acetone. Deuteration of lanthanum complexes at the $\alpha$-position with respect to carboxylate groups was performed using the procedure described by Wheeler and Legg. Deuteration was confirmed by $^1$H NMR spectroscopy.

Complexation

The Dy(III) and Gd(III) complexes were prepared by mixing solutions of hydrated LnCl$_3$ (10 mM) and ligand (10 mM) in a
2:1 ratio. The pH was maintained at 7.50 with 1.0 M NaOH. Complex formation was instantaneous at room temperature. The solution was then evaporated under reduced pressure and the residue dried overnight at 60 °C.

**Proton T$_1$ measurements**

The samples were prepared by dissolving a measured amount of the Gd(III) chelates in water at pH 6.80 using the buffer solution (0.10 M) PIPES (pipperazine-$N,N'$-bis-2-ethanesulfonic acid)–NaOH. The buffer solution was used to maintain a constant ionic strength (i.e. 0.10 M). The 0.10 M buffer was sufficient to maintain the solution pH at 6.80. The buffered Gd(III) chelate solutions were all allowed to equilibrate for at least 2 h. The pH of these solutions was determined immediately before relaxation time ($T_1$) measurements.

Relaxation times of aqueous solutions of gadolinium(III) complexes with [en(DO3A)$_2$]$_2^{2-}$ and [pip(DTTA)$_2$]$_2^{2-}$ were measured to determine the relaxivity $r_1$. All measurements were made at 20 MHz as a function of temperature on a Bruker Minispec NMS-120 NMR spectrometer. The samples were contained in 5 mm glass tubes. The spectrometer was tuned and calibrated before each measurement. The values of $T_1$ were measured from eight data points generated by an inversion–recovery pulse sequence. The slope of plots of $1/T_1$ versus the concentration of Gd(III) complex gives $r_1$ in mM$^{-1}$ s$^{-1}$.

**EPR measurements**

EPR spectra were recorded at the X-band (0.34 T) using a Bruker ER 200D-SRC spectrometer operated in the continuous-wave mode. The samples were contained in the 1 mm glass tubes. The cavity temperature was stabilized using electronic temperature control of the gas flowing through the cavity. Temperature was verified by substituting a thermometer for the sample tube. Measurements were made from 273 to 363 K. The peak-to-peak linewidth was measured from the recorded spectra using the instrument’s software.

**$^{17}$O NMR**

The hydration numbers of [en{Dy(DO3A)$_2$}]$_2^{2-}$ and [pip{Dy(DTTA)$_2$}]$_2^{2-}$ were determined using the method described by Alpoim et al. The $^{17}$O NMR spectra were recorded on a Varian Gemini-400 spectrometer at 25°C. Induced $^{17}$O shift (d.i.s: dysprosium(III) induced $^{17}$O NMR water chemical shift) measurements were made using D$_2$O as an external standard. Dy(III) chelate solutions were prepared by combining solutions of Dy(III) and ligand in a 2:1 ratio, and a stoichiometric amount of standardized NaOH was added so that the complex was fully formed. Six solutions of various dysprosium(III) concentrations were prepared by serial dilution of the stock solution.

Measurement of the $^{17}$O transverse relaxation rate was carried out with a Varian Gemini-300 (7.05 T, 40.65 MHz) spectrometer, equipped with a 5 mm probe, by using an external D$_2$O lock. Experimental settings were spectral width 10 000 Hz, pulse width 7 µs, acquisition time 10 ms and no sample spinning. A Varian VT-J103 temperature control unit was used to stabilize the temperature. The value of the transverse relaxation rate was obtained by evaluating the linewidth at half-height ($\Delta f/2$) of the water $^{17}$O signal ($R_2 = \pi \Delta f/2$). Solutions containing 2.6% of the $^{17}$O isotope were used.

$^2$H NMR

The rotational correction time values of [en{La(DO3A)$_2$}]$_2^{2-}$ and [pip{La(DTTA)$_2$}]$_2^{2-}$ were determined by $^2$H NMR spectroscopy. The samples were prepared by dissolving the La$^{3+}$ complexes in D$_2$O at pH 6.80. The measurement was carried out in a 10 mm o.d. tube on a Varian Gemini-400 (9.4 T) spectrometer equipped with a broadband probe and measured by a substitution technique as described elsewhere.

**RESULTS AND DISCUSSION**

**Dy(III)-induced water $^{17}$O NMR shifts**

Figure 1 shows the Dy(III)-induced water $^{17}$O NMR shifts versus Dy(III) chelate concentration in D$_2$O at 25.0 ± 0.1 °C.

![Figure 1](imageurl)

Figure 1. Dy(III)-induced water $^{17}$O NMR shift versus Dy(III) chelate concentration in D$_2$O at 25.0 ± 0.1 °C.

The slope of plots of $\Delta f$ versus the concentration of Gd(III) complex gives $r_2$ for $^{17}$O. Therefore, $r_2$ is linear to $C_1$, the concentration of Dy(III) complexes. The slopes obtained for [en{Dy(DO3A)$_2$}]$_2^{2-}$ and [pip{Dy(DTTA)$_2$}]$_2^{2-}$ are $-10.2$ ppm mM$^{-1}$ ($r^2 = 0.9764$) and $-107.8$ ppm mM$^{-1}$ ($r^2 = 0.9762$). On the other hand, the slope for DyCl$_3$ is $-382.8$ ppm mM$^{-1}$ ($r^2 = 0.999$), and eight hydration numbers have been proposed for the dysprosium(III) ion.$^7$ The slopes obtained for [en{Dy(DO3A)$_2$}]$_2^{2-}$ and [pip{Dy(DTTA)$_2$}]$_2^{2-}$ are $-10.2$ ppm mM$^{-1}$ ($r^2 = 0.9764$) and $-107.8$ ppm mM$^{-1}$ ($r^2 = 0.9762$). On the other hand, the slope for DyCl$_3$ is $-382.8$ ppm mM$^{-1}$ ($r^2 = 0.999$), and eight hydration numbers have been proposed for the dysprosium(III) ion.$^7$ Therefore, [en{Dy(DO3A)$_2$}]$_2^{2-}$ and [pip{Dy(DTTA)$_2$}]$_2^{2-}$ complexes contain 2.1 and 2.2 inner-sphere water molecules, respectively, at pH 6.80. The actual number of inner sphere water molecules coordinated to the metal center for [en{Dy(DO3A)$_2$}]$_2^{2-}$ and [pip{Dy(DTTA)$_2$}]$_2^{2-}$ is one per Dy(III) ion. This result is similar to those for [pip{Gd(DO3A)(H$_2$O)$_2$}]$_2^{2-}$ and [bisoxa{Gd(DO3A)(H$_2$O)$_2$}]$_2^{2-}$.
Relaxometric studies of the gadolinium(III) complexes

The longitudinal relaxivity $r_1$ values of $[\text{en}]\text{Gd(DO3A)(H}_2\text{O)}]_2$ and $[\pi\text{Gd(DTTA)(H}_2\text{O)}]_2^{2-}$ are 3.60 mm$^{-1}$s$^{-1}$ per Gd and 5.25 mm$^{-1}$s$^{-1}$ per Gd at pH 6.80, 37.0 ± 0.1 °C and 20 MHz, respectively. The $r_1$ value of $[\text{en}]\text{Gd(DO3A)(H}_2\text{O)}]_2$ is significantly higher than those of $[\text{Gd(DOTA)}]^{-}$ (3.38 mm$^{-1}$s$^{-1}$ per Gd, 37.0 °C)$^{11}$ and $[\text{BO}](\text{Gd(DO3A)(H}_2\text{O)}]_2$ (4.61 mm$^{-1}$s$^{-1}$ per Gd, 37.0 ± 0.1 °C)$^3$ but lower than those of $[\pi\text{Gd(DO3A)(H}_2\text{O)}]_2$ (5.8 mm$^{-1}$s$^{-1}$ per Gd, 40.0 °C)$^{12}$ and $[\text{bisoxa}](\text{Gd(DO3A)(H}_2\text{O)}]_2$ (4.9 mm$^{-1}$s$^{-1}$ per Gd, 40.0 °C).$^{12}$ Also, the longitudinal relaxivity $r_1$ value of $[\pi\text{Gd(DTTA)(H}_2\text{O)}]_2^{2-}$ is significantly higher than that of the monomer $[\text{Gd(DTPA)}]^{2-}$.

The origin of paramagnetic relaxation enhancement is generally divided into two components, inner-sphere and outer-sphere:$^{13}$

$$\frac{1}{T_1}_{ip} = \frac{1}{T_1}_{inner-sphere} + \frac{1}{T_1}_{outer-sphere} \quad i = 1, 2 \quad (1)$$

Inner-sphere relaxation refers to relaxation enhancement of a solvent molecule directly coordinated to the paramagnetic ion, and outer-sphere relaxation refers to relaxation enhancement of solvent molecules in the second coordination sphere and beyond (i.e. bulk solvent). The inner-sphere relaxation contribution is obtained with the equation$^{14}$

$$r_{ip} = C\eta/\{55.6(T_{1M} + \tau_M)\} \quad (2)$$

where $C$ is the molar concentration of the gadolinium(III) complex, $\eta$ is the number of water molecules bound to metal ion, $T_{1M}$ is the longitudinal relaxation time of the bound water protons and $\tau_M$ is the residence lifetime of the bound water. Because of the inverse temperature dependence of $T_{1M}$ and $\tau_M$, two cases can be considered: (1) fast water-exchange ($T_{1M} \gg \tau_M$), $r_{ip}$ increases as temperature decreases; (2) slow water-exchange ($T_{1M} \ll \tau_M$), $r_{ip}$ decreases as temperature decreases. Figure 2 displays a monoeponential decrease of observed relaxivity with increasing temperature in the range 278–343 K. This is characteristic of fast chemical exchange behavior, occurring when the $\tau_M$ of the coordinated water molecule is much shorter than $T_{1M}$ of the bound water proton. In fact, Eqn (3)$^{15}$ can express $T_{1M}$:

$$T_{1M} = \frac{2}{15} r_{ip}^2 S(S + 1)\beta^2 \left( \frac{3\tau_{C1}}{1 + \omega_1^2\tau_{C1}^2} + \frac{7\tau_{C2}}{1 + \omega_2^2\tau_{C2}^2} \right)$$

(3)

$$\frac{1}{\tau_{C1}} = \frac{1}{\tau_r} + \frac{1}{\tau_m} + \frac{1}{\tau_k} \quad (4)$$

where $S$ is the electron spin quantum number (7/2 for Gd$^{3+}$), $\gamma_1$ is the proton nuclear magnetogyric ratio, $\beta$ is the Bohr magneton, $g$ is the Landé factor for the free electron, $\tau_r$ is the distance between the metal ion and the bound water protons, $\omega_1$ and $\omega_2$ are the respective proton and electron Larmor frequencies and $\tau_{C1}$ ($i = 1, 2$) is the correlation time of the modulation of the dipolar-electron-proton coupling. The overall correlation time $\tau_{C2}$ receives contributions from $\tau_{C2}$, $\tau_{C2}$ and $\tau_r$ (the electronic relaxation time of the metal ion) [Eqn (4)]. To understand how $\tau_M$ and $\tau_{C2}$ influence

**Figure 2.** Temperature dependence of the relaxivity for $[\text{en}]\text{Gd(DO3A)(H}_2\text{O)}]_2$ and $[\pi\text{Gd(DTTA)(H}_2\text{O)}]_2^{2-}$ at pH 6.80 and 20 MHz.

$r_1$ of $[\text{en}]\text{Gd(DO3A)(H}_2\text{O)}]_2$ and $[\pi\text{Gd(DTTA)(H}_2\text{O)}]_2^{2-}$, $^{17}$O and $^2$H NMR spectra were used to determine the values of $\tau_M$ and $\tau_r$.

**Water-exchange lifetime studies of Gd(III) complexes**

The measured peak-to-peak line widths, $\Delta H_{pp}$, of the derivative spectrum can be related to the overall transverse electronic relaxation rate, $1/T_{2e}$ via Eqn (5), where $g_L$ is the isotropic Landé $g$ factor ($g_L = 2.0$ for Gd$^{3+}$):$^{16}$

$$\frac{1}{T_{2e}} = \frac{g_L\mu_B\sqrt{3}}{h} \Delta H_{pp} \quad (5)$$

The temperature dependence of transverse electronic relaxation rates at the X-band (0.34 T) at pH 6.80 for 50 mM solution of $[\text{en}]\text{Gd(DO3A)(H}_2\text{O)}]_2$ and $[\pi\text{Gd(DTTA)(H}_2\text{O)}]_2^{2-}$ are shown in Figs 3 and 4. The data were fitted simultaneously with the following $^{17}$O NMR results. Analysis of the temperature dependence of the transverse relaxation rate for the $^{17}$O water nuclei is the most accurate method for evaluating the exchange lifetime of the water molecules directly coordinated to the metal in a paramagnetic Gd$^{3+}$ chelate. According to the Swift and Connick theory,$^{16}$ the paramagnetic contribution ($R_{2p}^2$) to the observed transverse relaxation rate is given by

$$R_{2p}^2 = \frac{C\eta/\{55.6(T_{1M}^{-1} + 2\omega_2^2\tau_{C2}^{-1}) + \Delta \omega_2^2\}}{[2\tau_{C2} + (T_{1M}^{-1})]} \quad (6)$$
where \( R_{2M}^{O} \) represents the \(^{17}\text{O} \) transverse relaxation rate of the coordinated water molecule and \( \Delta \delta_{M}^{O} \) the chemical shift difference between the coordinated and bulk water \(^{17}\text{O} \) NMR resonances.

\[ R_{2M}^{O} = \frac{1}{3} \left( \frac{A}{h} \right)^2 S(S+1) \left( \frac{\tau_{e1} + \frac{\tau_{e2}}{1 + \omega_{0}^{2} \tau_{e2}}}{} \right) \]  

and

\[ \tau_{e1}^{-1} = \tau_{M}^{-1} + \tau_{e1}^{-1} \]  

where \( S \) is the electronic spin quantum number \([7/2 \text{ for Gd(III)}] \), \( A/h \) is the \(^{17}\text{O} \) scalar coupling constant and \( \tau_{e} \) \((i = 1, 2) \) represents the correlation time of the processes modulating the scalar interaction. This modulation may occur through both the longitudinal and the transverse average electronic relaxation times \( (T_{1e} \text{ and } T_{2e}) \) and the mean residence lifetime \( (\tau_{M}^{O}) \) of the water molecule at the paramagnetic site.

The temperature dependence of \( R_{2M}^{O} \) is determined by the temperature effect on \( \tau_{M}^{O} \), \( \tau_{e} \) (the correlation time for modulation of the zero field splitting interaction) and \( \Delta \delta_{M}^{O} \) according to

\[ (\tau_{M}^{O})^{-1} = \frac{(\tau_{e}^{-1})^{288.15T}}{298.15} \exp \left( \frac{\Delta H_{j}}{R} \left( \frac{1}{298.15} - \frac{1}{T} \right) \right) \]  

\[ \Delta \delta_{M}^{O} = \frac{g_{j} \mu_{B} S(S+1) B}{k_{B} T} A \]  

where the subscript \( j \) refers to the different correlation times, \( \Delta H_{j} \) is the activation enthalpy for the corresponding dynamic process, \( B \) is the applied magnetic field strength and \( k_{B} \) is the Boltzmann constant.

The water-exchange rates for \([\text{en(Gd(DO3A)(H}_{2}\text{O)})]_{2} \) and \([\text{pip(Gd(DT TA)(H}_{2}\text{O)})]_{2}^{2-} \) were obtained by measuring the \(^{17}\text{O} \) NMR transverse relaxation rate \( (R_{2p}^{O}) \) as a function of temperature. The data and its best simulation according to Eqns (5)–(10) are shown in Figs 5 and 6. As there are a large number of parameters to be determined in the quantitative analysis of the \(^{17}\text{O} \) NMR transverse relaxation rate \( (R_{2p}^{O}) \) versus \( T \) profiles, it is convenient to fix some of them. On this basis, in addition to the values of \( q \) and \( A/h(-3.8 \times 10^{9}\text{ rad s}^{-1}) \), the value of \( \Delta H_{M} \) is fixed at 30 kJ mol\(^{-1}\).\(^{10} \) The parameters which provide the best fit of the data for \([\text{en(Gd(DO3A)(H}_{2}\text{O)})]_{2} \) and \([\text{pip(Gd(DT TA)(H}_{2}\text{O)})]_{2}^{2-} \) are listed in Table 1. By varying the temperatures over a wide range, \( R_{2p}^{O} \) is dominated by \( 1/\tau_{M} \) in the slow kinetic region at low temperatures and is dominated by \( 1/\tau_{e} \) in the fast kinetic region at high temperature.

As shown in Table 1, the water-exchange lifetime \( \tau_{M}^{288} \) of \([\text{en(Gd(DO3A)(H}_{2}\text{O)})]_{2} \) \((769 \pm 10 \text{ ns}) \) is similar to those of \([\text{pip(Gd(DO3A)(H}_{2}\text{O)})]_{2}^{2-} \) \((666 \text{ ns})^{10} \) and \([\text{bisoxa(Gd(DO3A)(H}_{2}\text{O)})]_{2} \) \((714 \text{ ns})^{12} \) but higher than that of \([\text{Gd(DOTA)(H}_{2}\text{O})]^{2-} \) \((243 \text{ ns})^{18} \). The higher water-exchange lifetimes for \([\text{en(Gd(DO3A)(H}_{2}\text{O)})]_{2} \), \([\text{pip(Gd(DO3A)(H}_{2}\text{O)})]_{2}^{2-} \) and \([\text{bisoxa(Gd(DO3A)(H}_{2}\text{O)})]_{2} \) is perhaps due to the decreased number of carboxylate moieties which bind to the Gd(III) ion, so that the ligand is pulled less tightly around the metal center and is therefore less crowded around the water binding site.\(^{10} \) The water-exchange lifetime \( \tau_{M}^{288} \) of \([\text{pip(Gd(DT TA)(H}_{2}\text{O)})]_{2}^{2-} \) \((910 \pm 10 \text{ ns}) \) is significantly higher.
where the quadrupolar coupling constant $\Delta$ on the hybridization state of the C-atom carrying the $^2H$ atom, its value being $\sim 170$ kHz in the case of an sp$^3$ C-atom. The measurement was performed on diamagnetic lanthanum(III) complexes deuterated in the $\alpha$-position to the carboxylate groups. The values of $\Delta$ for [Gd(DOTA)]$_2$ than that of [Gd(DTPA)(H$_2$O)]$^{2-}$ (303 ns). As described in the literature, a $\tau_M$ of 1000 ns creates a situation wherein the exchange rate of water is a significant limiting factor determining relaxivity ($r_1$) when $r_1$ increases due to the $\tau_M$ increase in these multidentate chelates, making a major contribution even for dimers. Therefore, the difference in $r_1$ is not only one between rigid and non-rigid linkages, but is also due to $\tau_M$ in the higher molecular weight molecule.

**Rotational correlation time studies of La(III) complexes**

In diamagnetic molecules, the relaxation rate of the $^2H$ nucleus is predominantly determined by the quadrupolar mechanism, which is strictly intramolecular and modulated by the sole rotation of the molecule. For fast-tumbling systems, the relaxation rate is thus directly related to the rotational correlation time ($\tau_M$):

$$ R_1 = \frac{1}{T} = \frac{3}{8} \left( e^Q / h \right)^2 \tau_M $$

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where the quadrupolar coupling constant $(e^Q/h)$ depends on the hybridization state of the C-atom carrying the $^2H$ atom, its value being $\sim 170$ kHz in the case of an sp$^3$ C-atom. The measurement was performed on diamagnetic lanthanum(III) complexes deuterated in the $\alpha$-position to the carboxylate groups. The values of $\Delta$ for [Gd(DOTA)]$_2$ than that of [Gd(DTPA)(H$_2$O)]$^{2-}$ (303 ns). As described in the literature, a $\tau_M$ of 1000 ns creates a situation wherein the exchange rate of water is a significant limiting factor determining relaxivity ($r_1$) when $r_1$ increases due to the $\tau_M$ increase in these multidentate chelates, making a major contribution even for dimers. Therefore, the difference in $r_1$ is not only one between rigid and non-rigid linkages, but is also due to $\tau_M$ in the higher molecular weight molecule.

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**Table 1.** Kinetic and NMR parameters obtained from the simultaneous fit of $^{17}$O NMR and EPR data for Gd(III) complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\Delta^2$ (s$^{-2} \times 10^{20}$)</th>
<th>$\tau_M$ ($^2$H) (ps)</th>
<th>$\tau_M$ ($^2$H) (ms)</th>
<th>$\tau_M$ ($^2$H) (ps)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd$^{3+}$</td>
<td>1.19</td>
<td>7.3</td>
<td>1.2</td>
<td>41</td>
<td>15.3</td>
</tr>
<tr>
<td>[Gd(DOTA)(H$_2$O)]$^{-}$</td>
<td>0.16</td>
<td>11</td>
<td>243</td>
<td>77</td>
<td>49.8</td>
</tr>
<tr>
<td>[Gd(DTPA)(H$_2$O)]$^{2-}$</td>
<td>0.46</td>
<td>25</td>
<td>303</td>
<td>58</td>
<td>51.6</td>
</tr>
<tr>
<td>[pip(Gd(DO3A)(H$_2$O)]$_2^+$</td>
<td>0.17 ± 0.01</td>
<td>19 ± 2</td>
<td>666</td>
<td>171 ± 12</td>
<td>34.2 ± 1.8</td>
</tr>
<tr>
<td>[bisoxa(Gd(DO3A)(H$_2$O)]$_2^-$</td>
<td>0.21 ± 0.02</td>
<td>15 ± 1</td>
<td>714</td>
<td>106 ± 14</td>
<td>38.5 ± 1.8</td>
</tr>
<tr>
<td>[en(Gd(DO3A)(H$_2$O)]$_2^-$</td>
<td>0.49 ± 0.02</td>
<td>17 ± 1</td>
<td>769 ± 10</td>
<td>105 ± 11</td>
<td>35.2 ± 1.0</td>
</tr>
<tr>
<td>[pip(Gd(DTTA)(H$_2$O)]$_2^-$</td>
<td>0.90 ± 0.01</td>
<td>14 ± 2</td>
<td>910 ± 10</td>
<td>130 ± 12</td>
<td>45.0 ± 1.8</td>
</tr>
</tbody>
</table>

$^a$ Data from Ref. 11.

$^b$ Data from Ref. 15.
Gd(III) monomer and dimer complexes\(^\text{19}\) is shown in Fig. 7. The results show that there is a strong correlation between \(r_1\) and molecular weight, which means that the relaxivity value of the monomer generally increases with molecular weight. However, in order to maximize the relaxivity gain, the linking group and molecular weight must be taken into account for Gd(III) dimer complexes.

**CONCLUSION**

From analysis of the \(^{17}\)O NMR relaxometric properties, the larger water-exchange lifetime \(\tau_M\)\(^\text{28}\) for \([\text{en(Gd(DO3A)}(\text{H}_2\text{O}))_2]\) is perhaps due to a decrease in the number of carboxylate moieties, so that the ligand is pulled less tightly around the metal center and there is less crowding around the water binding site. We have demonstrated relaxivity \((r_1/\text{Gd mss}^{-1} \text{s}^{-1})\) enhancement through the incorporation of rigidifying elements in the linkers or increasing the molecular weight of chelates. The water proton spin–lattice relaxivity of \([\text{pi(DTTA)}(\text{H}_2\text{O}))_2]\)\(^2\) is higher than that for the monomer \([\text{Gd(DTPA)}]\)\(^2\) owing to its longer rotational correlation time and greater molecular weight. Furthermore, approaches aimed at enhancing relaxivity by modulating the water-exchange lifetime, \(\tau_M\)\(^\text{28}\), will be important for the future development of molecular MRI contrast agents used in imaging biochemical processes.

**Acknowledgement**

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**REFERENCES**