

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

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The Gd(III) complexes of the two dimeric ligands $[\text{en}(\text{DO3A})_2]$ [*N,N'*-bis[1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-*N,N'*-ethylenediamine] and $[\text{pi}(\text{DTTA})_2]^{8-}$ [bisdiethylenetriaminepentaacetic acid (*trans*-1,2-cyclohexanediamine)] were synthesized and characterized. The ^{17}O NMR chemical shift of H_2O induced by $[\text{en}\{\text{Dy}(\text{DO3A})\}_2]$ and $[\text{pi}\{\text{Dy}(\text{DTTA})\}_2]^{2-}$ 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 $[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$ and $[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$ at $37.0 \pm 0.1^\circ\text{C}$ and 20 MHz are 3.60 ± 0.05 and $5.25 \pm 0.05 \text{ mM}^{-1} \text{ s}^{-1}$ per Gd, respectively. The EPR transverse electronic relaxation rate and ^{17}O NMR transverse relaxation time for the exchange lifetime of the coordinated H_2O molecule and the ^2H 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 $[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$ ($769 \pm 10 \text{ ns}$) and $[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$ ($910 \pm 10 \text{ ns}$) are significantly higher than those of $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ (243 ns) and $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$ (303 ns) complexes. The rotational correlation times for $[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$ ($150 \pm 11 \text{ ps}$) and $[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$ ($130 \pm 12 \text{ ps}$) are slightly greater than those of $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ (77 ps) and $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$ (58 ps) complexes. The marked increase in relaxivity (r_1) of $[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$ and $[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$ result mainly from their longer rotational correlation time and higher molecular weight. Copyright © 2004 John Wiley & Sons, Ltd.

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

INTRODUCTION

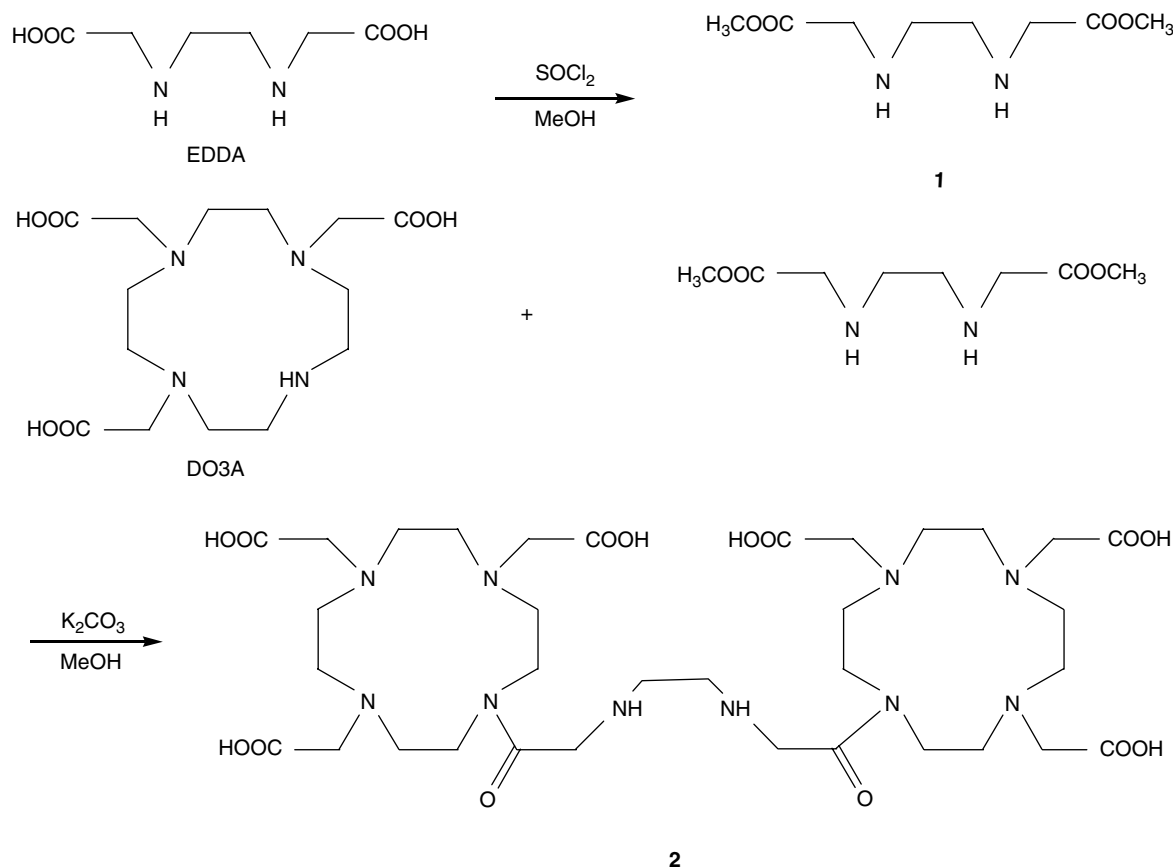
Several lanthanide [e.g. Gd(III)] and transition metal [e.g. Mn(II) and Fe(III)] complexes of polyaminocarboxylates are either commercially available or in clinical trials for use as magnetic resonance imaging (MRI) contrast agents. The MR signal of body fluids can be altered by the presence of paramagnetic 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_1). The dimeric MRI contrast agents possess two gadolinium(III) ions and will increase relaxivity.

This has been confirmed for $\text{H}_6\text{BO}(\text{DO3A})_2$,³ in which the Gd(III) complex incorporates several desirable features. As the ligand is a DOTA [DOTA = 1,4,7,10-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,

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Scheme 1. Synthesis of $en(DO3A)_2$.

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-tetraazacyclododecane-1,4,7-triacetic acid) and DTPA [1,1,4,7,7-pentakis(carboxymethyl)-1,4,7-triazaheptane] were synthesized. The number of inner-sphere water molecules was determined from the ^{17}O NMR chemical shift of the water as a function of Dy(III) concentration. The water proton spin–lattice relaxivity r_1 of the $[en\{Gd(DO3A)(H_2O)\}_2]$ and $[pi\{Gd(DTTA)(H_2O)\}_2]^{2-}$ complexes at various temperatures and pH values are described. The EPR and ^{17}O NMR transverse relaxation rate data were analyzed together in a simultaneous multiple-parameter least-squares fitting procedure to determine the water residence lifetime. 2H NMR 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

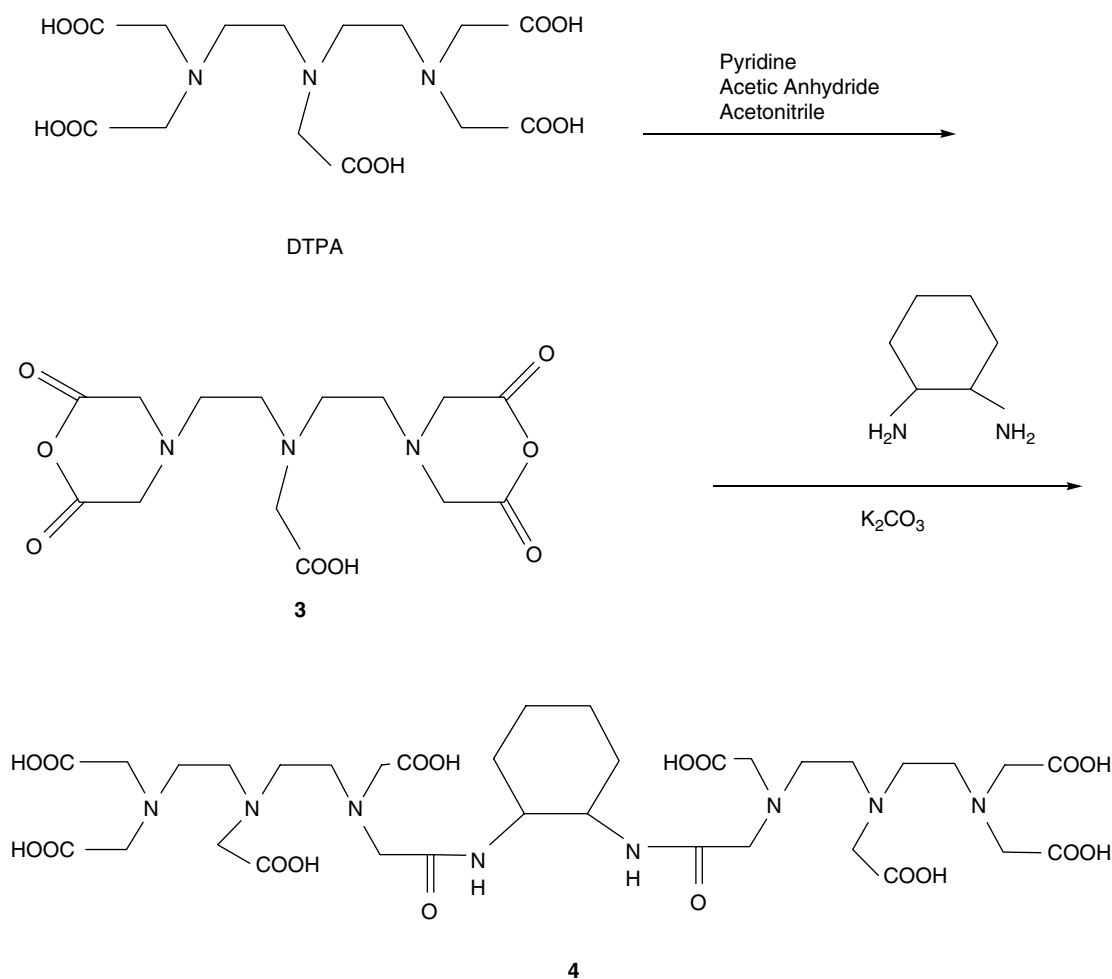
Ethylenediaminediacetic acid dimethyl ester (1).

To a solution of EDDA (ethylenediaminediacetic acid, 28.41 mmol) in MeOH (150 ml) warmed to 40–50 °C was added $SOCl_2$ (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%). 1H NMR (D_2O), δ (ppm): 3.48 (NCH_2CH_2N), 3.75 (NCH_2COOCH_3), 4.03 (NCH_2COOCH_3).

$en(DO3A)_2$ (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 NH_4OH . 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 K_2CO_3 (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, HCO_2^- 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%) ^{13}C NMR (50 MHz, D_2O) (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.



Scheme 2. Synthesis of $\pi(\text{DTTA})_2$.

Calculated for $\text{C}_{35}\text{H}_{64}\text{N}_{10}\text{O}_{15}$: 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%). ^1H NMR (200 MHz, $\text{DMSO}-d_6$), δ (ppm): 3.71 (s, 8H, terminal NCH_2CO_2), 3.30 (s, 2H, central NCH_2CO_2), 2.72 (t, 4H, $\text{NCH}_2\text{CH}_2\text{N}$), 2.59 (t, 4H, $\text{NCH}_2\text{CH}_2\text{N}$).

[$\pi(\text{DTTA})_2$] (4) (Scheme 2).

Compound 3 (2.3 g, 6.88 mmol), K_2CO_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 fractionated 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_2O), δ (ppm): 172.91, 170.80, 166.81, 166.72, 56.64, 56.22, 54.40, 52.93, 52.44, 52.34, 53.03, 50.67, 50.38, 49.89, 31.28,

24.04. Anal. Calculated for $\text{C}_{34}\text{H}_{56}\text{N}_8\text{O}_{18}$: C, 47.22; H, 6.53; N, 12.96. Found: C, 47.16; H, 6.50; N, 12.64%.

General

$\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (99.9%), $\text{DyCl}_3 \cdot 6\text{H}_2\text{O}$ (99.9%) and $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ (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 xylenol orange as indicator. All other reagents used for the synthesis of the ligand were purchased from commercial sources unless noted otherwise. ^1H 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_2O_3 with ligands in water and precipitated by addition of acetone. Deuteration of lanthanum complexes at the α -position with respect to carboxylate groups was performed using the procedure described by Wheeler and Legg.⁵ Deuteration was confirmed by ^1H 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 (PIPES = piperazine-*N,N'*-bis-2-ethanesulfonic acid)–NaOH. The buffer solution was used to maintain a constant ionic strength (i.e. 0.10M). 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 $[\text{en}(\text{DO3A})_2]^{6-}$ and $[\text{pi}(\text{DTTA})_2]^{8-}$ 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 $\text{mM}^{-1} \text{s}^{-1}$.

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 $[\text{en}\{\text{Dy}(\text{DO3A})\}_2]$ and $[\text{pi}\{\text{Dy}(\text{DTTA})\}_2]^{2-}$ were determined using the method described by Alpoim *et al.*⁶ 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_2O 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_2O 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

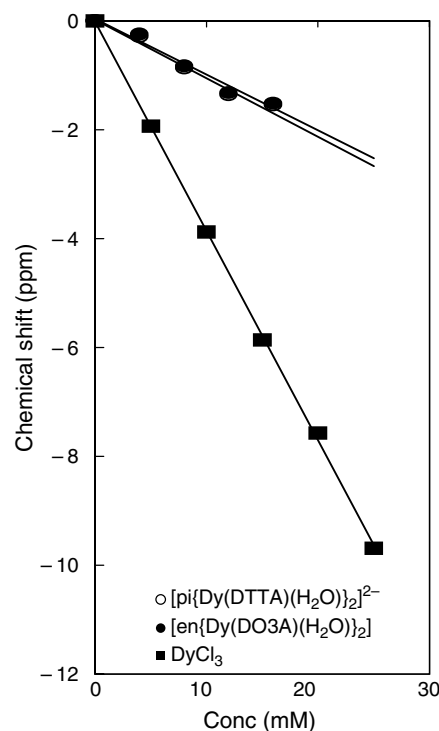


Figure 1. Dy(III)-induced water ^{17}O NMR shift versus Dy(III) chelate concentration in D_2O at 25.0 ± 0.1 °C.

unit was used to stabilize the temperature. The value of the transverse relaxation rate was obtained by evaluating the linewidth at half-height ($\Delta\nu_{1/2}$) of the water ^{17}O signal ($R_2 = \pi\Delta\nu_{1/2}$). Solutions containing 2.6% of the ^{17}O isotope were used.

^2H NMR

The rotational correlation time values of $[\text{en}\{\text{La}(\text{DO3A})\}_2]$ and $[\text{pi}\{\text{La}(\text{DTTA})\}_2]^{2-}$ were determined by ^2H NMR spectroscopy. The samples were prepared by dissolving the La^{3+} complexes in D_2O 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.⁵

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 for solutions of DyCl_3 , $[\text{en}\{\text{Dy}(\text{DO3A})\}_2]$ and $[\text{pi}\{\text{Dy}(\text{DTTA})\}_2]^{2-}$ in D_2O at 25 °C. The slopes obtained for $[\text{en}\{\text{Dy}(\text{DO3A})\}_2]$ and $[\text{pi}\{\text{Dy}(\text{DTTA})\}_2]^{2-}$ at pH 6.80 are $-102.6 \text{ ppm mM}^{-1}$ ($r^2 = 0.9764$) and $-107.8 \text{ ppm mM}^{-1}$ ($r^2 = 0.9762$). On the other hand, the slope for DyCl_3 is $-382.8 \text{ ppm mM}^{-1}$ ($r^2 = 0.999$), and eight hydration numbers have been proposed for the dysprosium(III) ion.^{7–9} Therefore, $[\text{en}\{\text{Dy}(\text{DO3A})\}_2]$ and $[\text{pi}\{\text{Dy}(\text{DTTA})\}_2]^{2-}$ 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 $[\text{en}\{\text{Dy}(\text{DO3A})\}_2]$ and $[\text{pi}\{\text{Dy}(\text{DTTA})\}_2]^{2-}$ is one per Dy(III) ion. This result is similar to those for $[\text{pip}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$ and $[\text{bisoxa}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$.¹⁰

Relaxometric studies of the gadolinium(III) complexes

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

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

$$(1/T_i)_p = (1/T_i)_{\text{inner-sphere}} + (1/T_i)_{\text{outer-sphere}} \quad i = 1, 2 \quad (1)$$

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 equation¹⁴

$$r_{1p}^{\text{is}} = Cq/[55.6(T_{1M} + \tau_M)] \quad (2)$$

where C is the molar concentration of the gadolinium(III) complex, q is the number of water molecules bound to metal ion, T_{1M} is the longitudinal relaxation time of the bound water protons and τ_M^{298} is the residence lifetime of the bound water. Because of the inverse temperature dependence of T_{1M} and τ_M^{298} , two cases can be considered: (1) fast water-exchange ($T_{1M} \gg \tau_M$), r_{1p}^{is} increases as temperature decreases; (2) slow water-exchange ($T_{1M} \ll \tau_M$), r_{1p}^{is} decreases as temperature decreases. Figure 2 displays a monoexponential decrease of observed relaxivity with increasing temperature in the range 278–343 K. This is characteristic of fast chemical exchange behavior, occurring when the τ_M^{298} 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} :

$$\frac{1}{T_{1M}} = \frac{2}{15} \frac{\gamma_H^2 g^2 S(S+1) \beta^2}{r_H^6} \left(\frac{3\tau_{C1}}{1 + \omega_H^2 \tau_{C1}^2} + \frac{7\tau_{C2}}{1 + \omega_S^2 \tau_{C2}^2} \right) \quad (3)$$

$$\frac{1}{\tau_{Ci}} = \frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{\tau_{Si}} \quad (4)$$

where S is the electron spin quantum number ($7/2$ for Gd^{3+}), γ_H is the proton nuclear magnetogyric ratio, β is the Bohr magneton, g is the Landé factor for the free electron, r_H is the distance between the metal ion and the bound water protons, ω_H and ω_S are the respective proton and electron Larmor frequencies and τ_{Ci} ($i = 1, 2$) is the correlation time of the modulation of the dipolar electron-proton coupling. The overall correlation time τ_{Ci} receives contributions from τ_M^{298} , τ_R^{298} and τ_S (the electronic relaxation time of the metal ion) [Eqn (4)]. To understand how τ_M^{298} and τ_R^{298} influence

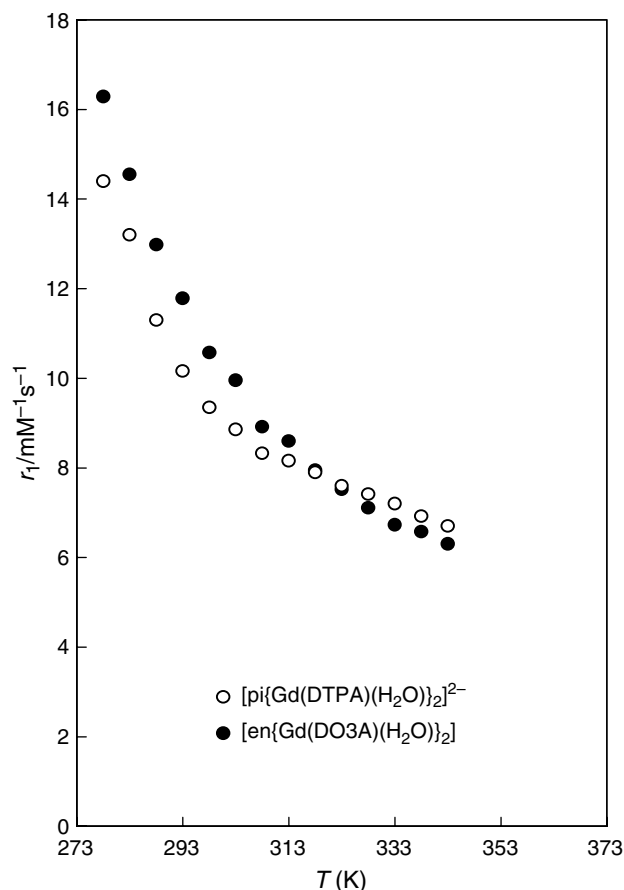


Figure 2. Temperature dependence of the relaxivity for $[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$ and $[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$ at pH 6.80 and 20 MHz.

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

Water-exchange lifetime studies of Gd(III) complexes

The measured peak-to-peak line widths, ΔH_{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

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

The temperature dependence of transverse electronic relaxation rates at the X-band (0.34 T) at pH 6.80 for 50 mM solution of $[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$ and $[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$ 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.¹⁷ According to the Swift and Connick theory,¹⁴ the paramagnetic contribution (R_{2p}^{O}) to the observed transverse relaxation rate is given by

$$R_{2p}^{\text{O}} = \frac{Cq}{55.6} (\tau_M^{\text{O}})^{-1} \frac{R_{2M}^{\text{O}2} + (\tau_M^{\text{O}})^{-1} R_{2M}^{\text{O}} + \Delta\omega_M^{\text{O}2}}{[R_{2M}^{\text{O}} + (\tau_M^{\text{O}})^{-1}]^2 + \Delta\omega_M^{\text{O}2}} \quad (6)$$

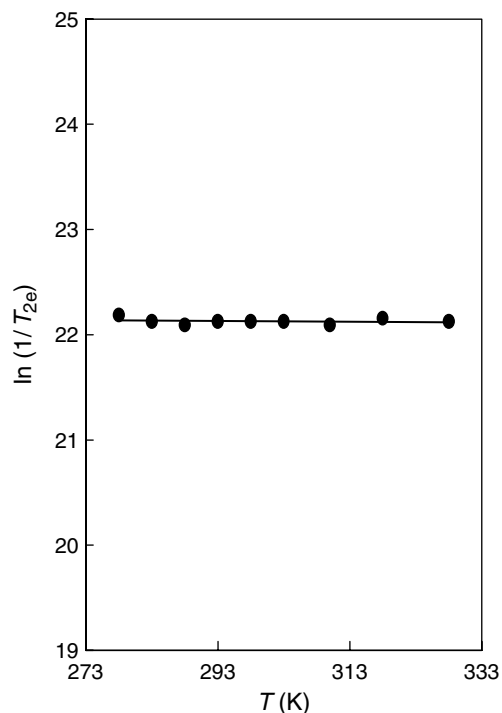


Figure 3. Temperature dependence of transverse electronic relaxation rates at the X-band (0.34 T) and pH 6.80 for a 50 mM solution of [en{Gd(DO3A)(H₂O)₂}].

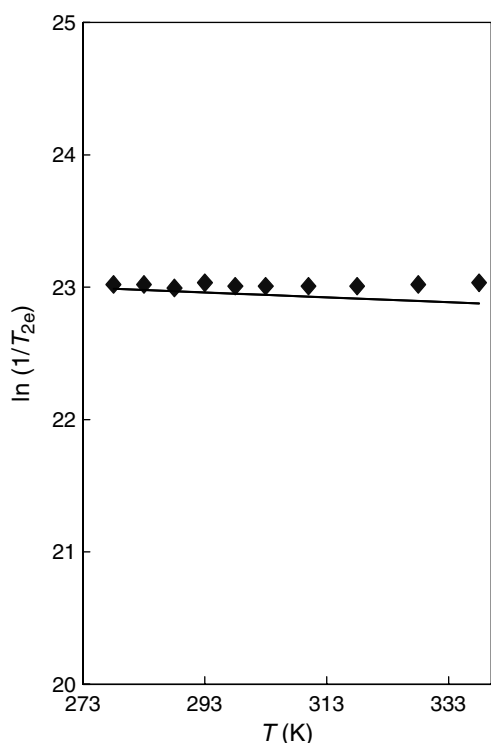


Figure 4. Temperature dependence of transverse electronic relaxation rates at the X-band (0.34 T) and pH 6.80 for a 50 mM solution of [pi{Gd(DTTA)(H₂O)₂}].

where R_{2M}^O represents the ^{17}O transverse relaxation rate of the coordinated water molecule and $\Delta\omega_M^O$ the chemical shift difference between the coordinated and bulk water ^{17}O NMR resonances.

R_{2M}^O is expressed by

$$R_{2M}^O = \frac{1}{3} \left(\frac{A}{\hbar} \right)^2 S(S+1) \left(\tau_{ei} + \frac{\tau_{e2}}{1 + \omega_s^2 \tau_{e2}^2} \right) \quad (7)$$

and

$$\tau_{ei}^{-1} = \tau_M^{O-1} + T_{ie}^{-1} \quad (8)$$

where S is the electronic spin quantum number [7/2 for Gd(III)], A/\hbar is the Gd- ^{17}O scalar coupling constant and τ_{ei} ($i = 1, 2$) 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} and T_{2e}) and the mean residence lifetime (τ_M^O) of the water molecule at the paramagnetic site.

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

$$(\tau_j)^{-1} = \frac{(\tau_j^{-1})^{298.15} T}{298.15} \exp \left[\frac{\Delta H_j}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right] \quad (9)$$

$$\Delta\omega_M^O = \frac{g_L \mu_B S(S+1) B A}{3 k_B T \hbar} \quad (10)$$

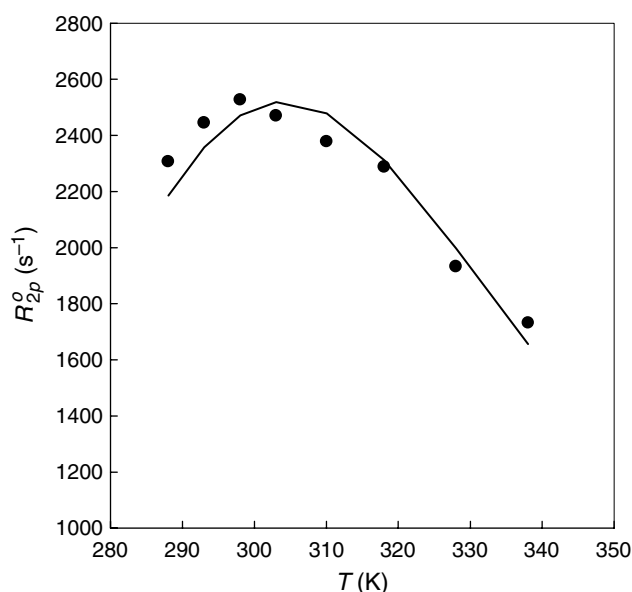
where the subscript j refers to the different correlation times, ΔH_j is the activation enthalpy for the corresponding dynamic process, B is the applied magnetic field strength and k_B is the Boltzmann constant.

The water-exchange rates for [en{Gd(DO3A)(H₂O)₂}] and [pi{Gd(DTTA)(H₂O)₂}]²⁻ were obtained by measuring the ^{17}O NMR transverse relaxation rate (R_{2p}^O) as a function of temperature. The data and its best simulation according to Eqns (5)–(10)^{16,18} 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}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/\hbar ($-3.8 \times 10^6 \text{ rad s}^{-1}$), the value of ΔH_M is fixed at 30 kJ mol^{-1} .¹⁰ The parameters which provide the best fit of the data for [en{Gd(DO3A)(H₂O)₂}] and [pi{Gd(DTTA)(H₂O)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 $1/\tau_{ei}$ in the fast kinetic region at high temperature.

As shown in Table 1, the water-exchange lifetime τ_M^{298} of [en{Gd(DO3A)(H₂O)₂}] ($769 \pm 10 \text{ ns}$) is similar to those of [pip{Gd(DO3A)(H₂O)₂}] (666 ns)¹⁰ and [bisoxa{Gd(DO3A)(H₂O)₂}] (714 ns)¹² but higher than that of [Gd(DOTA)(H₂O)₂]²⁻ (243 ns).¹⁸ The higher water-exchange lifetimes for [en{Gd(DO3A)(H₂O)₂}], [pip{Gd(DO3A)(H₂O)₂}] and [bisoxa{Gd(DO3A)(H₂O)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 τ_M^{298} of [pi{Gd(DTTA)(H₂O)₂}]²⁻ ($910 \pm 10 \text{ ns}$) is significantly higher

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

Complex	Δ^2 ($\text{s}^{-2} \times 10^{20}$)	τ_v^{298} (ps)	τ_M^{298} (ms)	τ_R^{298} (ps)	ΔH (kJ mol^{-1})
Gd^{3+a}	1.19	7.3	1.2	41	15.3
$[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^{-a}$	0.16	11	243	77	49.8
$[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-b}$	0.46	25	303	58	51.6
$[\text{pip}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]^b$	0.17 ± 0.01	19 ± 2	666	171 ± 12	34.2 ± 1.8
$[\text{bisoxa}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]^b$	0.21 ± 0.02	15 ± 1	714	106 ± 14	38.5 ± 1.8
$[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$	0.49 ± 0.02	17 ± 1	769 ± 10	105 ± 11	35.2 ± 1.0
$[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$	0.90 ± 0.01	14 ± 2	910 ± 10	130 ± 12	45.0 ± 1.8

^a Data from Ref. 11.^b Data from Ref. 15.**Figure 5.** Temperature dependence of the transverse water ^{17}O relaxation rate at 7.05 T and pH 6.80 for a 50 mM solution of $[\text{en}\{\text{Gd}(\text{DO3A})(\text{H}_2\text{O})\}_2]$.

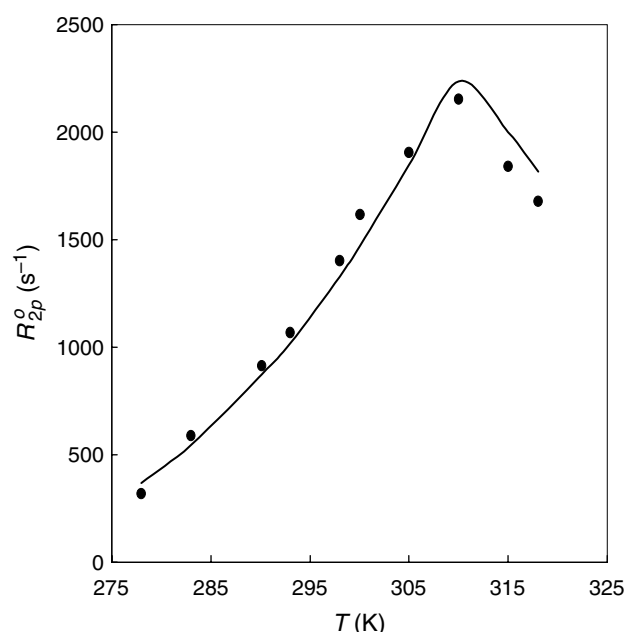
than that of $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$ (303 ns).¹⁰ As described in the literature,¹⁹ a τ_M^{298} of 1000 ns creates a situation wherein the exchange rate of water is a significant limiting factor determining relaxivity (r_1) when r_1 increases due to the τ_R^{298} increase in 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 τ_R^{298} in the higher molecular weight molecule.¹⁸

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,⁵ 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 (τ_R^{298}):

$$R_1 = \frac{1}{T_1} = \frac{3}{8} \left(\frac{e^2 q Q}{h} \right)^2 \tau_R \quad (11)$$

where the quadrupolar coupling constant ($e^2 q Q/h$) depends on the hybridization state of the C-atom carrying the ^2H

**Figure 6.** Temperature dependence of the transverse water ^{17}O relaxation rate at 7.05 T and pH 6.80 for a 50 mM solution of $[\text{pi}\{\text{Gd}(\text{DTTA})(\text{H}_2\text{O})\}_2]^{2-}$. The line represents the simultaneous least-squares fit to all data points as described in the text.

atom, its value being ~ 170 kHz in the case of an sp^3 C-atom. The measurement was performed on diamagnetic lanthanum(III) complexes deuterated in the α -position to the carboxylate groups. The values of τ_R^{298} for La(III) complexes with $\text{en}(\text{DO3A})_2^{6-}$, $\text{pip}(\text{DO3A})_2^{6-}$,¹² $\text{bisoxa}(\text{DO3A})_2^{6-}$,¹¹ $[\text{pi}(\text{DTTA})_2]^{8-}$, DTPA¹⁰ and DOTA¹¹ at 310 K are given in Table 1. The τ_R^{298} values for Gd(III) dimers are significantly higher than those for Gd(III) monomers, $[\text{La}((^2\text{H}_{10})\text{DOTA})]^{-}$ (77 ps)¹¹ and $[\text{La}((^2\text{H}_{10})\text{DTPA})]^{2-}$ (58 ps).¹¹ Thus, the change in the molecular weight in these complexes alters τ_R significantly. On the other hand, the lower rotational correlation time (τ_R^{298}) values for $[\text{en}\{\text{La}((^2\text{H}_{10})(\text{DO3A}))\}_2]$ and $[\text{bisoxa}\{\text{La}((^2\text{H}_{10})(\text{DO3A}))\}_2]$ compared with that of $[\text{pip}\{\text{La}((^2\text{H}_{10})(\text{DO3A}))\}_2]$ ¹⁸ indicates that the more flexible linker between the two macrocyclic chelating moieties in $\text{en}(\text{DO3A})_2^{6-}$ and $\text{bisoxa}(\text{DO3A})_2^{6-}$ decreases the τ_R value and causes the lower relaxivity (r_1) of the Gd(III) complexes. The relationship between r_1 and the molecular weight of

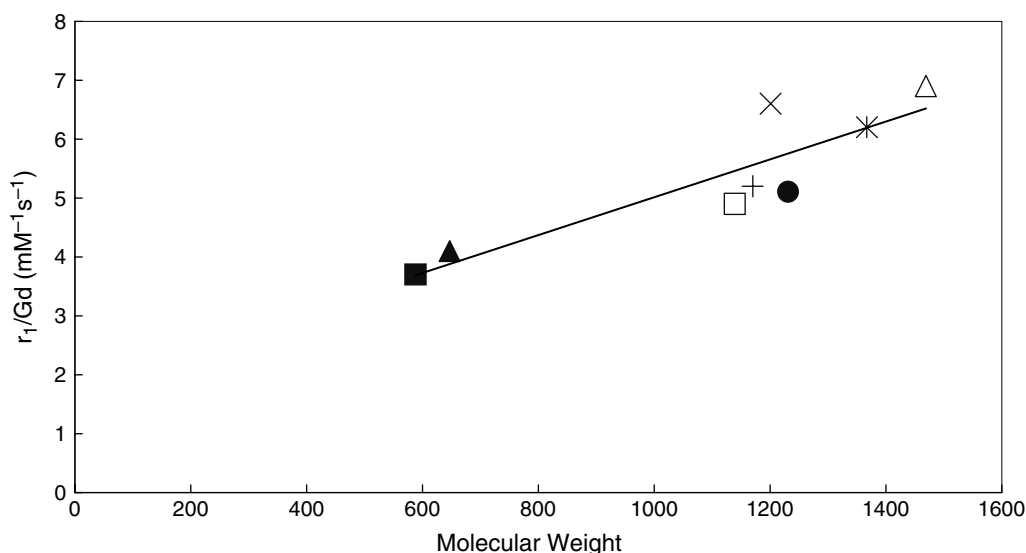


Figure 7. Correlation of molecular weight of gadolinium(III) complexes with HP-DO3A (■), PA-DO3A (▲), B22F (●), B22 (○), TU1 (×), TU2 (△), en(DO3A)₂⁶⁻ (□) and [pi(DTTA)₂]⁸⁻ (+) with relaxivity.

Gd(III) monomer and dimer complexes¹⁹ 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 ¹⁷O NMR relaxometric properties, the larger water-exchange lifetime τ_M^{298} for [en{Gd(DO3A)(H₂O)}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/Gd $\text{mM}^{-1}\text{s}^{-1}$) enhancement through the incorporation of rigidifying elements in the linkers or increasing the molecular weight of chelates. The water proton spin–lattice relaxivity of [pi{Gd(DTTA)(H₂O)}₂]²⁻ is higher than that for the monomer [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, τ_M^{298} , will be important for the future development of molecular MRI contrast agents used in imaging biochemical processes.

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