Relaxivity and Water Exchange Studies of a Cationic Macrocyclic Gadolinium(III) Complex

Daniele M. Corsi,[a] Luce Vander Elst,[b] Robert N. Muller,[b] Herman van Bekkum,[a] and Joop A. Peters*[a]

Abstract: We conducted relaxometric and water exchange studies of the cationic [Gd((*S,S,S,S*)-THP)(H_2O)]$^{3+}$ complex (THP = 1,4,7,10-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane). While the NMRD profiles obtained are typical for DOTA-like complexes (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate), variable-temperature ^{17}O NMR investigations revealed a relatively high water exchange rate ($k_{ex}^{298} = 1.89 \times 10^7$ s^{-1}). These results differ from those reported for other cationic tetraamide macrocyclic Gd^{III} complexes, which exhibit characteristically low exchange rates. Since the low exchange rates are attributed partially to the geometry of the *M* isomer (square antiprismatic) in the tetraamide derivatives, the atypical water exchange rate observed in [Gd((*S,S,S,S*)-THP-(H_2O)]$^{3+}$ may result from a twisted square antiprismatic structure in this complex and from the relatively high steric strain at the water coordination site as a result of the presence of methyl groups at the α-position with respect to the Gd^{III}-bound O atoms of THP.

Keywords: contrast agents · gadolinium · lanthanides · macrocyclic ligands · magnetic resonance imaging · NMR spectroscopy

Introduction

Since Gd^{III}-based contrast agents for MRI were first used, considerable research has focused on understanding the inherent features of the chelates which govern their effectiveness.[1–4] The parameters which influence relaxation enhancement depend upon the solution structure and/or dynamics of the Gd^{III} complexes. For instance, the rotational correlation time (τ_R) is influenced by the molecular tumbling and local motions of the complex, while the electronic relaxation time of the paramagnetic Gd^{III} ion (T_{1e}) is related to flexibility and/or symmetry in the complex. The lifetime of a bound water molecule (τ_m) is believed to be determined by several factors including the geometry and steric crowding at the water binding site as well as the overall charge of the complex. Owing to the number of factors which influence water exchange dynamics and to the significance of this parameter in affecting the relaxivity of contrast agents, there has been a great deal of interest surrounding the determinants of water exchange in Gd^{III} complexes.

The macrocyclic contrast agents [Gd(DOTA)]$^-$ and [Gd(HP-DO3A)] (HP-DO3A = 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate), derived from 1,4,7,10-tetraazacyclododecane (cyclen) are of particular interest because of their high thermodynamic and kinetic stability. A common feature of cyclen-based macrocyclic Ln^{III} complexes is the formation of various isomers, which may display dynamic behavior on the NMR time scale. The intrinsic structural aspects of these complexes give rise to two independent motions, which provide sources of chirality: a) rotation of the cyclen ethylene moieties about the C–C bond relative to the Ln^{III}-N-N plane producing the $\lambda\lambda\lambda\lambda$ versus $\delta\delta\delta\delta$ conformation of the ethylene rings and b) clockwise or counterclockwise flipping of the pendant groups yielding the Δ versus Λ orientation of the pendant arms.[5] In principle, these motions may occur separately or in concert to generate four stereoisomers which exist as two enantiomeric pairs (Figure 1). The $\Delta(\lambda\lambda\lambda\lambda)/\Lambda(\delta\delta\delta\delta)$ diastereomers (usually called *M* isomers) are characterized by a square antiprismatic (SAP) geometry with a twist angle of 40° between the two square planes formed by the four N atoms of the cyclen backbone and the four O atoms of the carboxylate arms, whereas the $\Delta(\delta\delta\delta\delta)/\Lambda(\lambda\lambda\lambda\lambda)$ diastereomers (*m* isomers) have a twisted SAP (TSAP) geometry with a twist angle of approximately 30°.

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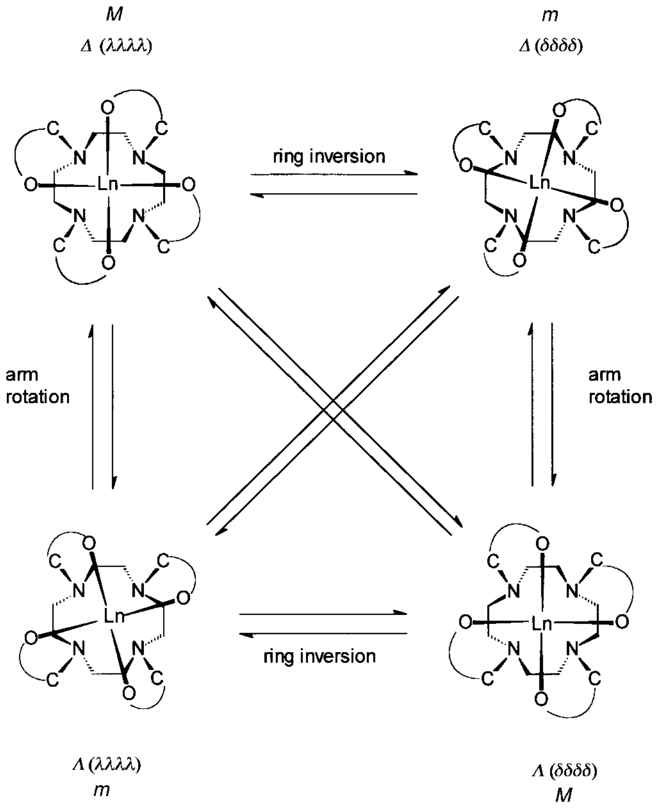
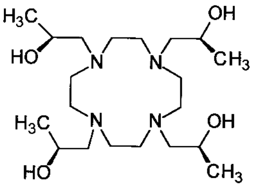


Figure 1. Interconversion of stereoisomers in lanthanide complexes of cyclen-based macrocylic ligands.



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Morrow and co-workers investigated the ^{1}H and ^{13}C NMR spectra of Ln^{III} complexes formed with a single stereoisomer of 1,4,7,10-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane having an *S* configuration at all α-carbons ((*S*,*S*,*S*,*S*)-THP, **1**).[6] Like other cyclen-based macrocycles (e.g. DOTA), the isomeric forms defined by the Δ/Λ and $\lambda\lambda\lambda\lambda/\delta\delta\delta\delta$ configurations may be envisaged for lanthanide(III) THP complexes. In (*S*,*S*,*S*,*S*)-THP complexes, the additional methyl group on the α-carbons adds four chiral centers, thus giving rise to two possible diastereomeric pairs rather than enantiomeric pairs (Figure 1). However, ^{1}H and ^{13}C NMR spectra of La^{III} and Lu^{III} complexes of (*S*,*S*,*S*,*S*)-(−)-THP do not display the fluxional behavior seen in [Ln(DOTA)]$^-$ complexes over the temperature range from 18 to 100 °C, indicating the presence of only one diastereomer in solution.[6] In the crystal structure of the racemic [Eu(THP)]$^{3+}$ complex, the unit cell was shown to consist of two discrete [Eu(THP)(H$_2$O)]$^{3+}$ ions that were diastereomers of each other (both with the $\lambda\lambda\lambda\lambda$ configuration of the ethylene rings), differing in the configuration at the chiral carbon (i.e. *R*,*R*,*R*,*S* versus *S*,*S*,*S*,*R*) and consequently the handedness of the helix. The THP ligand coordinated the Eu^{III} ion through four N atoms of the macrocycle and four hydroxy O atoms of the pendant arms; the ninth coordination site was filled by a bound water molecule. Interestingly, the coordination geometry is best described as monocapped twisted SAP with an average twist angle of ca. 20°, which is consistent with an m-isomeric structure (Λ ($\lambda\lambda\lambda\lambda$)).

Assuming that the solid-state structure is retained in solution, an investigation of the water exchange dynamics in [Gd(THP)(H$_2$O)]$^{3+}$ provides an interesting comparison with other related cationic Gd^{III} complexes which exhibit moderate to high *M*/*m* isomer ratios in solution. This report highlights the variable-temperature ^{17}O NMR and NMRD measurements carried out in order to gain a better understanding of the water exchange and relaxation processes involved in the THP system.

Results and Discussion

Synthesis of [Gd((*S*)-THP)(H$_2$O)]$^{3+}$: A single stereoisomer of THP having an *S* configuration at all α-carbons ((*S*,*S*,*S*,*S*)-THP (**1**), hereafter denoted simply as THP) was isolated in high yield from the free-base form of cyclen and (*S*)-propylene oxide.[6] The presence of a single stereoisomer was confirmed by ^{1}H and ^{13}C NMR spectroscopy of the ligand. The complexation of THP in aqueous solution is unfavorable because of the formation of insoluble lanthanide hydroxide precipitates. However, under anhydrous conditions a complex formed between THP and gadolinium(III) triflate [Gd(CF$_3$SO$_3$)$_3$].[6] The resulting [Gd(THP)]$^{3+}$ complex was stable upon dissolution in water at neutral pH and room temperature.

Variable-temperature ^{17}O NMR measurements: Determination of the ^{17}O NMR longitudinal and transverse relaxation rates and chemical shifts as a function of temperature may furnish information on the rotational correlation times, electronic relaxation and water exchange rates of Ln^{III} complexes.[7] The temperature dependence (280 to 378 K) of the ^{17}O relaxation rates and chemical shifts for [Gd(THP)(H$_2$O)]$^{3+}$ is shown in Figure 2. The [Gd(THP)(H$_2$O)]$^{3+}$ complex was found to dissociate at high temperature, a fact evident in the data points recorded above 360 K in the ^{17}O chemical shift plot.

From the measured ^{17}O NMR relaxation rates ($1/T_1$ and $1/T_2$) and angular frequencies (ω) of the Gd^{III} solution and of the acidified water reference ($1/T_{1A}$, $1/T_{2A}$, and ω_A) it is possible to calculate the reduced relaxation rates and chemical shifts ($1/T_{1r}$, $1/T_{2r}$ and ω_r) using Equations (1)–(3).[8–11]

$$\frac{1}{T_{1r}}=\frac{1}{P_m}\left[\frac{1}{T_1}-\frac{1}{T_{1A}}\right]=\frac{1}{T_{1m}+\tau_m}+\frac{1}{T_{1os}} \tag{1}$$

$$\frac{1}{T_{2r}}=\frac{1}{P_m}\left[\frac{1}{T_2}-\frac{1}{T_{2A}}\right]=\frac{1}{\tau_m}\frac{T_{2m}^{-2}+\tau_m^{-1}T_{2m}^{-1}+\Delta\omega_m^2}{(\tau_m^{-1}+T_{2m}^{-1})^2+\Delta\omega_m^2}+\frac{1}{T_{2os}} \tag{2}$$

$$\Delta\omega_r=\frac{1}{P_m}(\omega-\omega_A)=\frac{\Delta\omega_m}{(1+\tau_m T_{2m}^{-1})^2+\tau_m^2\Delta\omega_m^2)}+\Delta\omega_{os} \tag{3}$$

Here, $1/T_{1m}$ and $1/T_{2m}$ represent the relaxation rates for bound water molecules, τ_m is the residence time of water molecules in the inner coordination sphere, $\Delta\omega_m$ is the

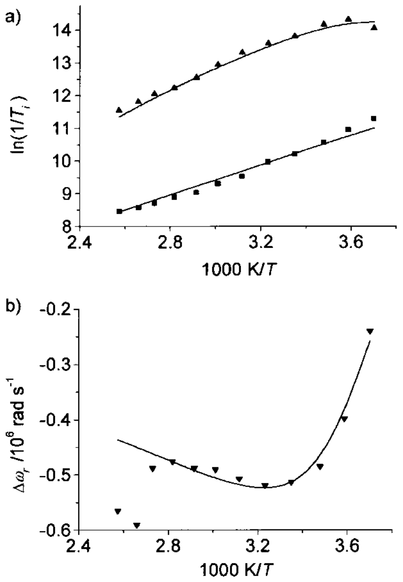

Figure 2. Temperature dependence of the reduced ^{17}O (a) transverse (▲) and longitudinal (■) relaxation rates, expressed as $\ln(1/T_{ir})$, and (b) chemical shifts (▼), $\Delta\omega_r$.

chemical shift difference between the bound water molecules and bulk water (in the absence of a paramagnetic interaction), and P_m is the mole fraction of bound water. The overall outer-sphere contributions to the reduced relaxation rates and chemical shifts are given by $1/T_{1os}$, $1/T_{2os}$, and $\Delta\omega_{os}$.

Previous investigations have shown that the outer-sphere contributions to the relaxation rates listed in Equations (1) and (2) may be omitted.[7, 12] Furthermore, if the contribution of $\Delta\omega_m$ in Equation (2) is assumed to be negligible, then Equations (1) and (2) reduce to Equations (4) and (5).

$$\frac{1}{T_{1r}} = \frac{1}{T_{1m} + \tau_m} \quad (4)$$

$$\frac{1}{T_{2r}} = \frac{1}{T_{2m} + \tau_m} \quad (5)$$

A changeover between the fast- and slow-exchange limits is also apparent from the jump in the curve of $\Delta\omega_r$ at 273–285 K (Figure 2b). At elevated temperatures, the inner-sphere contribution to $\Delta\omega_r$ is determined by the chemical shift of the Gd^{III}-bound water molecules, which is governed by the hyperfine interaction between the Gd^{III} electron spin and the ^{17}O nucleus [Eq. (6)].

$$\Delta\omega_m = \frac{g_L \mu_B S(S+1) B}{3 k_B T} \frac{A}{\hbar} \quad (6)$$

Here, g_L is the isotropic Landé g factor ($g_L = 2.0$ for Gd^{III}), S is the electron spin for Gd^{III} ($S = 7/2$), B is the magnetic field, k_B is the Boltzmann constant and $A/\hbar$ is the hyperfine or scalar coupling constant. The outer-sphere contribution to $\Delta\omega_r$ may be assumed to have a temperature dependence similar to $\Delta\omega_m$, which is given by Equation (7), where C_{os} is an empirical constant.

$$\Delta\omega_{os} = C_{os}\Delta\omega_m \quad (7)$$

The ^{17}O longitudinal relaxation rates for bound water molecules in Gd^{III} solutions are dominated by dipole–dipole and quadrupolar interactions[7, 12] and are given by Equation (8).

$$\frac{1}{T_{1m}} = \left[\frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{GdO}^6} S(S+1)\right] \times \left[6\tau_{d1} + 14\left(\frac{\tau_{d2}}{1+\omega_S^2\tau_{d2}^2}\right)\right] + \frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}\chi^2(1+\eta^2/3)\tau_R \quad (8)$$

Here, $\mu_0/4\pi$ is the magnetic permeability in a vacuum, $\hbar$ is the Dirac constant, γ_I is the nuclear gyromagnetic ratio ($\gamma_I = -3.626 \times 10^7\,\mathrm{rad\,s^{-1}T^{-1}}$ for ^{17}O), $\gamma_S = g_L\mu_B/\hbar$ is the electron gyromagnetic ratio ($\gamma_S = 1.76 \times 10^{11}\,\mathrm{rad\,s^{-1}}T^{-1}$ for $g_L = 2.0$), r_{GdO} is the distance between the electron charge and the ^{17}O nucleus, $\tau_{di}^{-1} = \tau_m^{-1} + T_{ie}^{-1} + \tau_R^{-1}$ ($i = 1,2$), T_{ie} is the electronic relaxation time, τ_R is the rotational correlation time for the Gd^{III}–O vector, I is the nuclear spin ($I = 5/2$ for ^{17}O), χ is the quadrupolar coupling constant and η is the asymmetry parameter for the electric field gradient. We estimate a value of $r_{GdO} = 2.5$ Å based on the crystal structures of $[Ln(DOTA)(H_2O)]^-$ (Ln = Gd, Eu).[13, 14] It may be assumed that the rotational correlation time, τ_R, follows a simple exponential temperature dependence, shown in Equation (9), where τ_R^{298} is the correlation time at 298.15 K and E_R is the activation energy.

$$\tau_R = \tau_R^{298}\exp\left[\frac{E_R}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right] \quad (9)$$

The ^{17}O transverse relaxation rates of bound water molecules in Gd^{III} chelates are governed by an electron–nucleus scalar mechanism and may be expressed in terms of Equation (10), where $\tau_{is}^{-1} = \tau_m^{-1} + T_{ie}^{-1}$.[7, 12, 15]

$$\frac{1}{T_{2m}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left[\tau_{1s} + \frac{\tau_{2s}}{1+\omega_S^2\tau_{2s}^2}\right] \quad (10)$$

For Gd^{III} complexes, the electronic relaxation rates may be interpreted in terms of the zero-field splitting (ZFS) interaction,[16] which results from transient distortions of the complex and a spin rotation (SR) mechanism [Eq. (11)].[17–19]

$$\frac{1}{T_{ie}} = \left(\frac{1}{T_{ie}}\right)^{ZFS} + \left(\frac{1}{T_{ie}}\right)^{SR} \quad (11)$$

The ZFS contribution to the longitudinal electronic relaxation rates may be described by Equation (12).[20] It has been shown that the transverse relaxation rates of Gd^{III} complexes can be well described by the semiempirical Equation (13).[16] Here, Δ^2 represents the mean square ZFS energy and τ_v is the correlation time describing the modulation of the electronic spin-state splitting. The correlation time, τ_v, is assumed to display Arrhenius behavior, represented in Equation (14), where τ_v^{298} is the value for τ_v at 298.15 K and E_v is the activation energy.

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25}\Delta^2\tau_v[4S(S+1)-3]\left(\frac{1}{1+\omega_S^2\tau_v^2} + \frac{4}{1+4\omega_S^2\tau_v^2}\right) \quad (12)$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2\tau_v\left(\frac{5.26}{1+0.372\omega_S^2\tau_v^2} + \frac{7.18}{1+1.24\omega_S\tau_v}\right) \quad (13)$$

$$\tau_v = \tau_v^{298}\exp\left[\frac{E_v}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right] \quad (14)$$

The spin rotation (SR) contribution is a magnetic-field-independent mechanism that may be approximated with Equation (15), where $\delta g_L^2 = \Sigma_i \delta g_{Li}^2$, which refers to the deviations from the free-electron value of g_L.[17]

$$\left(\frac{1}{T_{1e}}\right)^{\mathrm{SR}} = \frac{\delta g_L^2}{9\tau_{\mathrm{R}}} \tag{15}$$

It should be noted that the transverse electronic relaxation rates usually have a negligible influence on the ^{1}H and ^{17}O relaxation rates. In addition, the intermolecular dipole–dipole contribution to the longitudinal electronic relaxation rates was found to be insignificant.

Finally, the temperature dependence of τ_m (or the exchange rate, $k_{\mathrm{ex}} = 1/\tau_m$) is assumed to obey the Eyring Equation [Eq. (16)], where $\Delta H^{\neq}$ corresponds to the enthalpy of activation for the exchange process and k_{ex}^{298} is the exchange rate at 298.15 K.

$$\frac{1}{\tau_m} = k_{\mathrm{ex}} = \frac{k_{\mathrm{ex}}^{298} T}{298.15} \exp\left[\frac{\Delta H^{\neq}}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \tag{16}$$

NMRD studies, water proton relaxation: The efficacy of $[\mathrm{Gd(THP)(H_2O)}]^{3+}$ was evaluated with the use of water ^{1}H longitudinal relaxation time measurements at 25 and 37 °C with magnetic field strengths varying between 2.5×10^{-4} and 1.2 T. The NMRD profiles are shown in Figure 3.

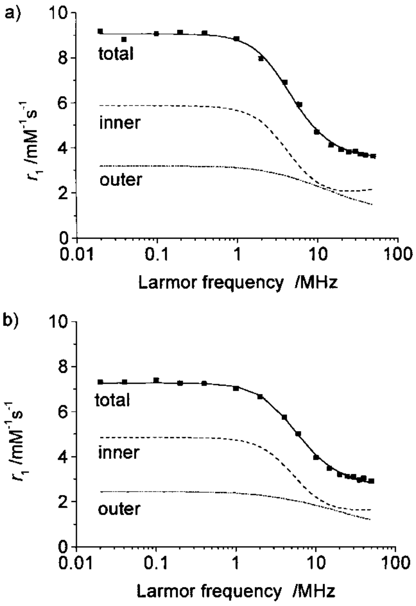

Figure 3. NMRD profiles of the $[\mathrm{Gd(THP)(H_2O)}]^{3+}$ at (a) 25 °C and (b) 37 °C expressed as relaxivity ($\mathrm{mM^{-1}\,s^{-1}}$) versus ^{1}H Larmor frequency (MHz).

In NMRD studies, the longitudinal proton relaxation rate enhancements are generally expressed in terms of the relaxivity, r_1 ($\mathrm{s^{-1}\,mM^{-1}}$). The observed paramagnetic relaxivity results from a sum of contributions originating from the inner- (r_1^{is}) and outer-sphere mechanisms (r_1^{os}) [Eq. (17)].

$$r_1 = r_1^{\mathrm{is}} + r_1^{\mathrm{os}} \tag{17}$$

Inner-sphere relaxation enhancement results from water molecules bound in the first coordination sphere of the paramagnetic metal and exchanging with the bulk solvent. By convention, all other long-range interactions of unbound water located within the vicinity of the paramagnetic center are incorporated into the outer-sphere contribution. Recent studies have provided evidence of a well-defined second coordination sphere in certain systems, which may contribute to the total relaxivity.[21] This contribution is related to the hydrogen-bonding interactions between suitable functional groups on the chelating ligand and exchanging water molecules located at an "intermediate" distance from the metal ion.

The inner-sphere contribution to the observed relaxivity is given by Equation (18), where q is the number of inner-sphere water molecules in the Gd$^{\mathrm{III}}$ chelate and T_{1m} is the longitudinal relaxation time of bound water. The ^{1}H longitudinal relaxation rate of inner-sphere water molecules is dominated by dipolar interactions and may be expressed by the Solomon–Bloembergen Equation[22, 23] [Eq. (19)], where γ_I is the nuclear gyromagnetic ratio ($\gamma_I = 2.675 \times 10^8\,\mathrm{rad\,s^{-1}\,T^{-1}}$ for protons), ω_I and ω_s are the proton and electron Larmor frequencies, respectively, and r_{GdH} is the effective distance between the gadolinium electron spin and the water protons.

$$r_1^{\mathrm{is}} = \left(\frac{q}{1000 \times 55.5}\right) \frac{1}{T_{1m} + \tau_m} \tag{18}$$

$$\frac{1}{T_{1m}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2 \gamma_s^2 \gamma_I^2}{r_{\mathrm{GdH}}^6} S(S+1) \left[\frac{3\tau_{d1}}{1 + \omega_I^2 \tau_{d1}^2} + \frac{7\tau_{d2}}{1 + \omega_s^2 \tau_{d2}^2}\right] \tag{19}$$

The outer-sphere contribution to the observed relaxivity is described by Equation (20)[24] where N_{A} is Avogadro's number, a_{GdH} is the distance of closest approach of an outer-sphere water molecule to the Gd$^{\mathrm{III}}$ ion, and τ_{GdH} is the correlation time, which corresponds to $a_{\mathrm{GdH}}^2/D_{\mathrm{GdH}}$.

$$r_1^{\mathrm{os}} = \frac{32\pi}{405}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_I^2 \gamma_s^2 \hbar^2 S(S+1) \frac{N_{\mathrm{A}}}{a_{\mathrm{GdH}} D_{\mathrm{GdH}}} [3J_{\mathrm{os}}(\omega_I, T_{1e}) + 7J_{\mathrm{os}}(\omega_s, T_{2e})] \tag{20}$$

The electronic relaxation dependence is expressed by the spectral density functions, $J_{\mathrm{os}}(\omega, T_{je})$ ($j = 1,2$) in Equation (21).

$$\begin{aligned} J_{\mathrm{os}}(\omega, T_{je}) = R_e\{&\{1 + \tfrac{1}{4}[i\omega\tau_{\mathrm{GdH}} + (\tau_{\mathrm{GdH}}/T_{je})]^{1/2}\}/ \\ &\{1 + [i\omega\tau_{\mathrm{GdH}} + (\tau_{\mathrm{GdH}}/T_{je})]^{1/2} + \tfrac{4}{9}[i\omega\tau_{\mathrm{GdH}} + (\tau_{\mathrm{GdH}}/T_{je})] \\ &+ \tfrac{1}{9}[i\omega\tau_{\mathrm{GdH}} + (\tau_{\mathrm{GdH}}/T_{je})]^{3/2}\}\} \end{aligned} \tag{21}$$

The diffusion coefficient, D_{GdH}, is assumed to have an exponential temperature dependence [Eq. (22)], where D_{GdH}^{298} is the diffusion coefficient at 298.15 K and E_{DGdH} is the activation energy.

$$D_{\mathrm{GdH}} = D_{\mathrm{GdH}}^{298} \exp\left[\frac{E_{\mathrm{DGdH}}}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \tag{22}$$

Fitting of the ^{17}O NMR and NMRD data: The variable-temperature ^{17}O and NMRD data were fit simultaneously using Equations (1)–(22) in order to impose constraints on common parameters. Following previous studies, additional constraints have been introduced by fixing the gadolinium electron spin distances from the ^{17}O nucleus (r_{GdO}) and the water protons (r_{GdH}) at 2.5 Å and 3.1 Å, respectively (for $q = 1$).[17, 25] The distance of closest approach of a water molecule to Gd$^{\mathrm{III}}$, a_{GdH}, was fixed at 3.5 Å. In addition, the C_{os} and δg_{L}^2 parameters were fixed at 0, since variation resulted in small negative values from the fittings.

Weighting factors were introduced in order to compensate for the variation in magnitude of the data: ^{17}O relaxation rates, ^{17}O chemical shifts, and ^{1}H relaxivities were weighted with factors of 10, 0.1, and 1, respectively. The parameters obtained from the fittings are listed in Table 1 with the curve fits shown in Figures 2 and 3. For comparison, previously published data for [Gd(DOTA)(H$_2$O)]$^-$ have been included in Table 1.[17]

Table 1. Parameters obtained from the analysis of ^{17}O NMR and NMRD data.

Parameter	[Gd(THP)]$^{3+}$	[Gd(DOTA)]$^{-}$[a]
$\Delta H^{\ddagger}$ [kJ mol^{-1}]	33.8 ± 5.7	49.8 ± 1.5
k_{ex}^{298} [s^{-1}]	18.9 (±4) × 10^6	4.1 (±0.2) × 10^6
τ_R^{298} [ps]	73 ± 3	77 ± 4
E_{R} [kJ mol^{-1}]	19.1 ± 0.2	16.1 ± 7.4
τ_{v}^{298} [ps]	4.8 ± 0.5	11 ± 1
E_{v} [kJ mol^{-1}]	15 ± 0.6	1.0[b]
$A/\hbar$ [10^6 rad s^{-1}]	– 3.4 ± 0.1	– 3.7 ± 0.2
Δ^2 (10^{19} s^{-2})	6.5 ± 1.1	1.6 ± 0.1
C_{os}	0[b]	0.21 ± 0.04
$\delta g_L^2/10^{-2}$	0[b]	1.9 ± 0.3
D_{GdH}^{298} [10^{-10} m^2 s^{-1}]	40 ± 2	22 ± 1
E_{DGdH} [kJ mol^{-1}]	20.9 ± 3.4	20.2 ± 1.1
$\chi(1 + \eta^2/3)^{1/2}$ [MHz]	8.92 ± 2.24	7.58[b]

[a] The data listed for [Gd(DOTA)]$^-$ have been reported previously by Powell et al.[17] and are provided here for comparisons. [b] These parameters were fixed in the fitting procedures.

Inspection of the data listed in Table 1 reveals some similarities in the relaxivity parameters for [Gd(THP)(H$_2$O)]$^{3+}$ and [Gd(DOTA)(H$_2$O)]$^-$. The scalar coupling constant ($A/\hbar$), which is principally determined from the ^{17}O chemical shifts, gauges the Gd$^{\mathrm{III}}$ electron spin density present at the ^{17}O nucleus. The number obtained from the fittings is similar to values reported for [Gd(DOTA)(H$_2$O)]$^-$ and other Gd$^{\mathrm{III}}$ polyamino carboxylate complexes having one inner-sphere water molecule and therefore confirms that $q = 1$.[12, 17, 26, 27] Likewise, the value obtained for the rotational correlation time (τ_R^{298}) is in reasonable agreement with [Gd(DOTA)(H$_2$O)]$^-$, which is of a similar molecular size.

The parameters describing electronic relaxation of the Gd$^{\mathrm{III}}$ ion are expressed in terms of the zero-field splitting interaction (τ_{v}^{298}, E_{v}, and Δ^2). Values determined for these parameters from the fittings are notably different from those for [Gd(DOTA)(H$_2$O)]$^-$ but are in the range usually observed for Gd$^{\mathrm{III}}$ chelates of polyaminocarboxylates.[1]

Another variation in our findings is the substantially higher value obtained for the diffusion coefficient (D_{GdH}^{298}). Since [Gd(THP)(H$_2$O)]$^{3+}$ and [Gd(DOTA)(H$_2$O)]$^-$ are of similar bulk, such a large variation in values for D_{GdH}^{298} is unexpected. It should be noted, however, that the value of a_{GdH}, the distance of closest approach between a diffusing water molecule and the Gd$^{\mathrm{III}}$ ion of [Gd(THP)(H$_2$O)]$^{3+}$, was fixed at 3.5 Å during the fitting procedure. Therefore, the large value for D_{GdH}^{298} might be the result of a compensation for too low a value fixed for a_{GdH}.

The most striking result from the data fittings is the value obtained for the water exchange rate of [Gd(THP)(H$_2$O)]$^{3+}$ at 298 K. The value of k_{ex}^{298} was determined as 18.9×10^6 s^{-1}, corresponding to a residence time (τ_m^{298}) of 53 ns, a value which is atypical for a cationic Gd$^{\mathrm{III}}$ compound. Earlier studies suggested that optimization of the water exchange rate was possible only by enlisting complexes with increasing negative charge.[4] Table 2 provides a comparison of water exchange lifetimes for Ln$^{\mathrm{III}}$ complexes with various charges.

The water exchange mechanism for nine-coordinate Gd$^{\mathrm{III}}$ complexes with a hydration number of 1 proceeds through a dissociative pathway whereby the transition state is characterized as an eight-coordinate non-hydrated species.[12] Water exchange studies for a series of isostructural Ln$^{\mathrm{III}}$ complexes revealed that an increase in water exchange rate accompanied a decrease in the Ln$^{\mathrm{III}}$ ionic radius.[28] These findings were explained in terms of stabilization of the eight-coordinate transition state and/or destabilization of the nine-coordinate ground state following an increase of steric crowding at the water binding site. Therefore, the exchange rate of a bound water molecule in a nine-coordinate Ln$^{\mathrm{III}}$ complex is dependent upon the nature of the coordination geometry at the metal center.

The subtle influence of geometry at the Gd$^{\mathrm{III}}$ center is demonstrated nicely by water exchange studies of the cationic [Eu(DOTAM)(H$_2$O)]$^{3+}$ complex (DOTAM = 1,4,7,10-tetrakis(carbamoyl)-1,4,7,10-tetraazacyclododecane), which exists as two isomeric forms (m and M) in solution.[29, 30] From the variable-temperature ^{1}H NMR spectra, the exchange rate was

Table 2. Comparisons of physical and structural parameters for various charged and neutral Ln$^{\mathrm{III}}$ macrocyclic complexes.

Complex	r_1[a] [mM^{-1} s^{-1}]	k_{ex}^{298} [s^{-1}]	Dominant isomer	Ln–OH$_2$ [Å]	Ref.
[Gd(DOTA)]$^-$	4.7	4.1 × 10^6	SAP	2.458 (Gd)	[13,17]
[Gd(DOTMA)]$^-$	3.8	14.7 × 10^6	TSAP	–	[4,35]
[Gd(HP-DO3A)]	4.2	2.9 × 10^6	–	2.50 (Gd)	[34,41]
[Gd(THP)]$^{3+}$	3.9	18.9 × 10^6	TSAP	2.507 (Eu)	[6], present study
[Gd(DOTTA)]$^{3+}$	3.0	1.3 × 10^5	TSAP	–	[31]
[Gd(DTMA)]$^{3+}$	2.5	5.9 × 10^4	SAP	2.461 (Gd)	[31]
[Gd(DOTAM)]$^{3+}$	2.5	5.3 × 10^4	SAP	2.442 (Eu)	[31]
M-[Eu(DOTAM)]$^{3+}$	–	8.3 × 10^3	(SAP)	–	[30]
m-[Eu(DOTAM)]$^{3+}$	–	3.3 × 10^5	(TSAP)	–	[30]

[a] At 298 K and 20 MHz.

found to be 40 times higher in the *m* isomer, which is characterized by the twisted square antiprismatic (TSAP) geometry (see Table 2). Since these two isomers contribute to the overall exchange rate, a similar trend may be observed in the k_{ex}^{298} values of the cationic Gd^{III} amide complexes, which have varying *m/M* isomer ratios (Table 2). In this series, the water exchange rates (k_{ex}^{298}) decrease from $1.3 \times 10^5\,s^{-1}$ for [Gd(DOTTA)(H$_2$O)]$^{3+}$ (predominately m isomer) (DOTTA = 1,4,7,10-tetrakis[(*N*,*N*-dimethylcarbamoyl)methyl]-1,4,7,10-tetraazacyclododecane) to $5.3 \times 10^4\,s^{-1}$ for [Gd(DOTAM)(H$_2$O)]$^{3+}$ (predominately *M* isomer).[31] Recently, a ^{17}O NMR study on tetra(carboxyethyl)DOTA showed once again that the water exchange lifetime at Gd^{III} correlates well with the proportion of the m isomer (TSAP geometry) observed in solution.[32]

This same behavior is observed for the [Gd(DOTMA)(H$_2$O)]$^-$ complex, which exists almost exclusively as the *m* isomer. Like DOTA, the DOTMA (1R,4R,7R,10R)-$\alpha,\alpha',\alpha'',\alpha'''$-tetramethyl-1,4,7,10-tetraazacyclododecane ligand has methyl substituents on the pendant arms adjacent to the N atoms of the cyclen ring. The k_{ex}^{298} value for [Gd(DOTMA)(H$_2$O)]$^-$ is $14.7 \times 10^6\,s^{-1}$,[4] which is quite similar to that found for [Gd(THP)(H$_2$O)]$^{3+}$ ($18.9 \times 10^6\,s^{-1}$). An increased steric demand at the metal center apparently favors the TSAP structure. Possibly, a relatively high local steric strain at the Gd^{III}-bound water facilitates its release in the dissociative mechanism. A relatively high steric strain in the TSAP structure might also be reflected in the Ln–OH$_2$ distances, which in the TSAP structures are generally about 0.05 Å greater than in the SAP structures (see Table 2).

NMR studies of [Ln(THP)(H$_2$O)]$^{3+}$ (Ln = La, Eu, Lu) complexes suggest the exclusive presence of only one of the m isomers (*Δ(δδδδ)* or *Λ(λλλλ)*) in solution; this indicates a high degree of strain at the metal center.[6] The crystal structure of [Eu(THP)(H$_2$O)](CF$_3$SO$_3$)$_3$ reveals an m-isomeric structure (*Λ(λλλλ)*) with a smaller binding cavity than the parent DOTA complex.[6] The twist angle defining the geometry at the metal center was found to be only 20°, compared with 40° or 30° for typical SAP or TSAP geometries. Inspection of molecular models shows that the methyl groups at the α-position with respect to the Ln^{III}-bound O atoms of THP give rise to a large steric strain around the water coordination site. Most other DOTA derivatives studied so far have substituents at the β-position with respect to the Ln^{III}-bound O atoms, which is further away from the bound water.

The first pK_a value of the OH groups of [Eu((*S*,*S*,*S*,*S*)-THP)] has been reported to be 7.7.[33] Therefore, the alcoholic groups in THP are not deprotonated to a substantial degree under the experimental conditions we used (pH 5 for ^{17}O NMR and pH 7 for NMRD measurements). In principle, then, the OH protons on the THP ligand could contribute to relaxation enhancement of solvent water. However, Aime et al. have shown for [Gd(HP-DO3A)] that this contribution is negligible at pH < 8.[21] We assume that the same holds in the present case. Unfortunately, a NMRD study at variable pH to substantiate this was not possible, owing to the instability of the complex under acidic or basic conditions.

The relaxivity profiles recorded at 25 °C and 37 °C are somewhat lower than those for [Gd(DOTA)(H$_2$O)]$^-$ at low fields, reflecting the difference in the electronic relaxation times or the zero-field splitting parameter (τ_v^{298}). The low-field region more closely resembles that of [Gd(HP-DO3A)(H$_2$O)].[34] At higher frequencies (20 MHz) and 298 K, the relaxivity of [Gd(THP)(H$_2$O)]$^{3+}$ is similar to that of [Gd(HP-DO3A)(H$_2$O)] and [Gd(DOTMA)(H$_2$O)]$^-$, with r_1 values of 3.9, 4.2, and 3.8 mM^{-1}s^{-1}, respectively (Table 2).[34, 35] The relaxivity of [Gd(THP)(H$_2$O)]$^{3+}$ is somewhat lower than that of [Gd(DOTA)(H$_2$O)]$^-$. A comparison of the calculated inner- and outer-sphere relaxivity curves for these compounds shows that this may be attributed to a reduced outer-sphere contribution.

Conclusion

Factors influencing the relaxivity and exchange dynamics in the cationic [Gd(THP)(H$_2$O)]$^{3+}$ complex were investigated by variable-temperature ^{17}O NMR and ^{1}H NMRD. Unlike other cationic complexes based on the macrocyclic cyclen chelate, we found increased longitudinal proton relaxivity at 20 MHz and 298 K and a much higher water exchange rate. To our knowledge, [Gd(THP)(H$_2$O)]$^{3+}$ possesses the highest water exchange rate yet observed for a nine-coordinate complex with $q = 1$.

Originally, it was believed that such high exchange rates could only be achieved through the use of negatively charged complexes. The high water exchange rate observed for [Gd(THP)(H$_2$O)]$^{3+}$ implicates a TSAP geometry (m isomer) which has been shown to facilitate water exchange in nine-coordinate Ln^{III} complexes. This study is further evidence of the importance of steric crowding at the bound water of Gd^{III}-based contrast agents in influencing exchange dynamics and ultimately achieving optimum relaxation efficiencies.

Experimental Section

Materials and methods: Gadolinium triflate [Gd(CF$_3$SO$_3$)$_3$] was purchased from Aldrich. (*S*)-(−)-Propylene oxide was obtained from Fluka. ^{17}O-enriched water (10% labeling) was purchased from Cortec (Paris, France). All other reagent-grade chemicals were purchased from commercial sources and used without further purification.

The precursor, cyclen (1,4,7,10-tetraazacyclododecane), was prepared following a method previously reported by Swinkels et al. which was slightly adapted by us.[36, 37]

(*S*,*S*,*S*,*S*)-(−)-THP was synthesized as described by Chin et al. using free-base cyclen and (*S*)-(−)-propylene oxide.[6] FAB MS *m*/*z*: 405 [*M*+H]$^+$; ^{1}H NMR (D$_2$O, 25 °C): $\delta = 1.07$ (d, $J = 6.3$ Hz, 12H; C*H*$_3$), 2.28–2.70 (16H; NC*H*$_2$C*H*$_2$N), 2.97 (d, $J = 9.6$ Hz, 8H; C*H*$_2$CH(OH)(CH$_3$)), 3.96 (m, 4H; C*H*(OH)(CH$_3$)); ^{13}C NMR (D$_2$O, 25 °C): $\delta = 21.0$ (*C*H$_3$), 51.6 (N*C*H$_2$*C*H$_2$N), 63.7 (*C*H$_2$CH(OH)(CH$_3$)), 65.7 (*C*H(OH)(CH$_3$)).

Preparation of [Gd(THP)]$^{3+}$: [Gd(THP)](CF$_3$SO$_3$)$_3$ was prepared as described previously (for Ln = La^{III}, Eu^{III}, and Lu^{III}) by refluxing equimolar amounts of [Gd(CF$_3$SO$_3$)$_3$] and THP in acetonitrile and trimethyl orthoformate for 1 h.[6] The procedure was modified slightly by carrying the reaction out in air and recrystallizing the crude material from a hot CH$_2$Cl$_2$ solution. A white crystalline product was isolated in 90% yield. Aqueous solutions of the sample were tested for uncomplexed lanthanide ions using xylenol orange as indicator.[38]

Physical methods: ^{1}H (300 MHz), ^{13}C (75.5 MHz), and ^{17}O (40.7 MHz) NMR spectra were recorded on a Varian INOVA-300 spectrometer using

5 mm sample tubes. Chemical shifts are reported as δ values. For measurements in D_2O, *tert*-butyl alcohol was used as an internal standard with the methyl signal calibrated at 1.2 ppm (^1H) or 31.2 ppm (^{13}C). D_2O (100%) was used as an external chemical shift reference for ^{17}O resonances. The pH of samples was measured at ambient temperature by means of a Corning 125 pH meter with a calibrated microcombination probe purchased from Aldrich. The pH values reported are direct meter readings (no correction for D-isotope effects was made). pH values were adjusted with the aid of dilute solutions of NaOH and HCl.

The $1/T_1$ nuclear magnetic resonance dispersion (NMRD) profiles were recorded at 25 and 37 °C with an IBM Research Relaxometer by the field cycling method over a range of magnetic fields from 2.5×10^{-4} to 1.2 T (corresponding to a proton Larmor frequency range of 0.01–50 MHz). The samples contained 1 mM of Gd^{III} as the THP complex at pH 7.

The variable-temperature ^{17}O measurements were made at a magnetic field of 7.05 T with a Varian INOVA-300 spectrometer equipped with a 5 mm probe. An aqueous solution of the Gd^{III} complex having a Gd^{III} concentration of 0.185 M was prepared for this study. The pH was adjusted to 5.1, where the stability at room temperature is optimal for a sample of this concentration. Instability of the complex at high temperatures prompted us to add a small amount of TTHA (5%) to bind any residual Gd^{III} ions which may be expelled from the THP under these conditions. Since Ln^{III} complexes of TTHA contain no inner-sphere water molecules, the measurements should be unaffected. In addition, an acidified water sample (pH 5.1) was used as a reference for these measurements. Solutions were prepared with ^{17}O-enriched water (5%) and the samples were sealed under an argon atmosphere. For each temperature, spectral parameters were collected for both the Gd^{III} complex and the acidified water sample. The measurements were conducted without a frequency lock and without sample spinning. The ^1H signal of *tert*-butyl alcohol (1% *w*/*w*, measured via the decoupler channel) was used as an internal reference. Longitudinal relaxation rates ($1/T_1$) were determined with the inversion–recovery method,[39] and the transverse relaxation rates ($1/T_2$) were obtained by the Carr–Purcell–Meiboom–Gill spin-echo technique.[40]

Calculations: Experimental variable-temperature ^{17}O NMR and NMRD data were fit with a computer program written by É. Tóth and L. Helm (University of Lausanne, Switzerland) using the Micromath Scientist program version 2.0 (Salt Lake City, UT, USA).

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