Extent of hydration of octadentate lanthanide complexes incorporating phosphinate donors: solution relaxometry and luminescence studies

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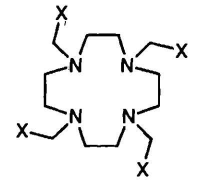
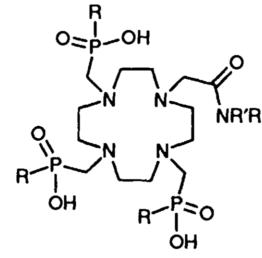
The behaviour of luminescent (Eu, Tb) and highly paramagnetic (Gd) complexes of 1,4,7,10-tetraazacyclododecane containing one carboxamide and three phosphinate substituents has been studied in solution. Analyses of variable-temperature nuclear magnetic relaxation dispersion profiles indicate that there is no water molecule directly co-ordinated to gadolinium(III) ions. It has been found that the observed relaxation enhancement of solvent protons is determined, in addition to the contribution from water molecules diffusing in close proximity to the paramagnetic complex, by a relatively distant water molecule in the 'second co-ordination sphere'. This is possible because the amide carbonyl oxygen can participate in hydrogen bonding (as a hydrogen-bond acceptor) to a local water molecule, which brings the water molecule close to the metal ion. The luminescence spectra of the complexes of Eu and Tb in water and D_2O are also consistent with such a hydration scheme and there is a good correlation between the non-integral q value (number of inner-sphere water molecules) determined by this method and the distance between the metal ion and the water proton estimated by relaxometric methods. It is proposed that the hydration states $q = 0$ and 1 may be considered to represent boundary conditions and a given complex in solution may possess intermediate values.

The lanthanide complexes of octadentate macrocycles have been studied intensively recently because of the clinical importance of paramagnetic gadolinium complexes as contrast agents in magnetic resonance imaging (MRI)[1,2] and the utility of luminescent europium and terbium complexes as probes in biochemical analyses.[3,4] An important feature of the behaviour of these complexes in solution is the hydration state, q, which is a factor determining the relaxivity† of gadolinium complexes[5] and the lifetime of the excited state of europium and terbium complexes.[6] The 'inner-sphere' contribution to ionic relaxivity with gadolinium complexes is directly proportional to the number of metal-bound water molecules. Thus $[Gd(dota)]^-$ (H_4dota = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and $[Gd(dtpa)]^{2-}$ (H_5dtpa = diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid) with one water molecule bound to the metal exhibit relaxivities of 3.7 and 3.8 $dm^3\ mmol^{-1}\ s^{-1}$ (298 K, 20 MHz) respectively, while the gadolinium complexes of the tetraphosphinic acids H_4L^2 and H_4L^1 show reduced relaxivities of 2.44 and 1.85 $dm^3\ mmol^{-1}\ s^{-1}$, consistent with there being few or no water molecules close to the paramagnetic ion.[7] Measurements of the excited-state lifetime of europium (and terbium) complexes in water and D_2O allow an independent measurement of the extent of solvation of the excited state,[5] and reasonably good correlations have been demonstrated between relaxometric and luminescence data for complexes with $q \geqslant 1$.[8]

We have been examining the lanthanide complexation chemistry of tri- and tetra-basic alkylphosphinate complexes based on the the 1,4,7,10-tetraazacyclododecane ([12]aneN_4) macrocycle.[7,9,10] A series of neutral and anionic complexes has been prepared (Ln = Eu, Gd, Tb or Dy) with monoamide triphosphinic acids, *e.g.* H_3L^3–H_3L^8, or tetraphosphinic acid donors, *e.g.* H_4L^1 and H_4L^2, of varying lipophilicity. The anionic lipophilic gadolinium complexes are promising biliary-imaging agents which also show some selective tumour-tissue retention while the neutral amide gadolinium complexes possess lower osmolality and somewhat higher intrinsic relaxivities *in vitro*.



H_4L^1 X = $P(CH_2Ph)O_2H$
H_4L^2 X = $PMeO_2H$
H_4dota X = CO_2H



H_3L^3 R = Me, R′ = H, R″ = Me
H_3L^4 R = Me, R′ = H, R″ = Ph
H_3L^5 R = R′ = R″ = Me
H_3L^6 R = Me, R′ = R″ = CH_2Ph
H_3L^7 R = Me, R′ = R″ = Bu^n
H_3L^8 R = Bu, R′ = H, R″ = Me

We now report more detailed studies of the relaxometric properties of representative neutral gadolinium complexes, and investigations into the luminescence properties of certain neutral europium and terbium complexes. This has allowed us to assess critically the extent of hydration of these complexes, each of which has been shown to exist as one predominant stereoisomer in solution.[7,11]

Results and Discussion

Luminescence behaviour of europium and terbium complexes

The emission spectra for the complexes of europium and terbium with macrocycles H_3L^3–H_3L^7 have been recorded (293 K, water) following excitation at 250 nm. Representative spectra (Fig. 1) are shown for $[EuL^5]$ and $[EuL^6]$, showing the assignments in terms of the expected ${}^5D_0 \longrightarrow {}^7F_n$ transitions. A single weak ${}^5D_0 \longrightarrow {}^7F_0$ ($\Delta J = 0$) transition (disallowed in D_{4h}, D_{4d} or D_4 symmetry) was observed at 579 nm for all of

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† Relaxivity is the increment of the water proton-relaxation rate per unit concentration of the paramagnetic contrast agent.

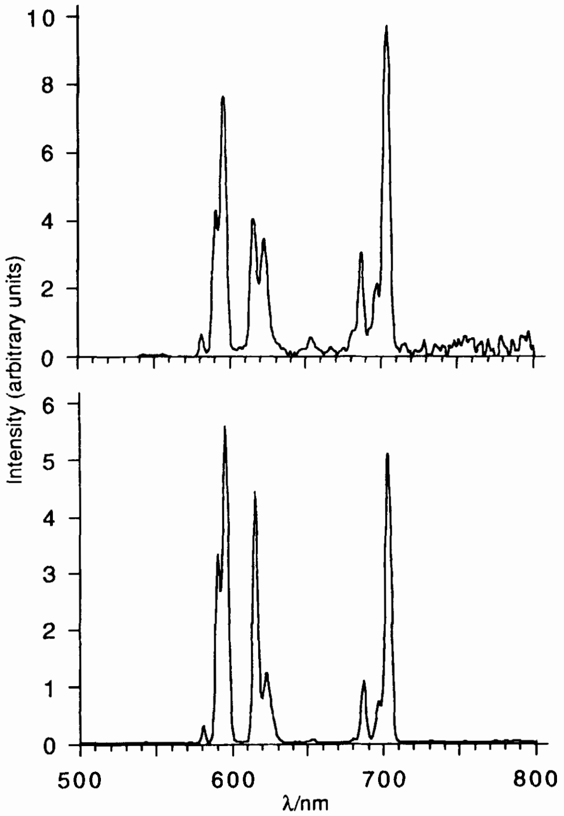

Fig. 1 Corrected emission spectrum for [EuL^5] (upper) and [EuL^6] (pH 5.5, water, 293 K) with an excitation wavelength of 250 nm showing $^5D_0 \longrightarrow {}^7F_n$ transitions

these complexes, consistent with there being one major isomer present in solution.[6,12,*] The $\Delta J = 1$ transition is magnetic-dipole allowed and has an intensity that is relatively unaffected by the co-ordination environment at europium. Strictly, three components are expected for the $^5D_0 \longrightarrow {}^7F_1$ transition in non-axial ligand fields, only two components in axially symmetric complexes.[13,14] Only two components were observed under the given experimental conditions, which were similar in relative intensity to those observed with [Eu(dota)]$^-$ and the C_4-symmetric phosphinate complexes, [EuL^1]$^-$ and [EuL^2]$^-$.[7] The $\Delta J = 2$ transition is electric-dipole allowed and is hypersensitive, so that the emission intensity of the bands observed is a very sensitive probe of ligand polarizability.[15,16] Again, two main bands were observed for each of the complexes {and also for [EuL^1]$^-$, [EuL^2]$^-$ and [Eu(dota)]$^-$}, but their relative intensity varied significantly and their absolute intensity, compared to the $\Delta J = 1$ transition, also changed markedly with each complex (Table 1). Upon consideration of these data, there appear to be two limiting cases represented by [Eu(dota)]$^-$ and [EuL^1]$^-$. In the former case the $\Delta J = 2$ transition is weaker than that of $\Delta J = 1$, and the band at lower wavelength (602 nm) is much the more intense. With [EuL^1]$^-$, the reverse is true. It is tempting to correlate this behaviour with the solution structure of the complexes: [Eu(dota)]$^-$ adopts a square-antiprismatic structure[17,18] while [EuL^1]$^-$ possesses an 'inverted square-antiprismatic' geometry which is obtained from the square antiprism either through a concerted rotation of the NCH$_2$X donor arms or by inverting the configuration of the macrocyclic ring by keeping the NCH$_2$X groups fixed in their position.[11] The neutral europium complexes incorporating amide donors show intermediate behaviour between these limiting cases. For example [EuL^7] behaves very similarly to [Eu(dota)]$^-$ (Table 1). It is also evident that the nature of the substituent at N has a significant effect on the characteristics of the $\Delta J = 2$ transition: [EuL^6] behaves quite differently from [EuL^5] [N(CH$_2$Ph)$_2$ *versus* NMe$_2$]. This effect was also noted with the corresponding ytterbium complexes of these same macrocycles when ^{31}P NMR studies indicated marked differences in their Yb–P distances induced by the proximate amide substituent.[11]

Table 1 Characteristics of the hypersensitive ($\Delta J = 2$) transition in europium complexes (298 K, H$_2$O)[a]

Complex	Relative band intensity (high/low wavelength) 602:616 nm	Relative overall intensity ($\Delta J = 1/\Delta J = 2$)
[Eu(dota)]$^-$	5:1	1.7
[EuL^6]	2.6:1	1.44
[EuL^5]	1.1:1	1.25
[EuL^4]	1.3:1	1.15
[EuL^3]	1.25:1[b]	0.90
[EuL^2]	0.5:1	0.71
[EuL^1]	0.3:1	0.71

[a] Intensities are from the corrected emission spectra. [b] Poorly resolved bands.

Quantum yields and excited-state lifetimes for the europium and terbium complexes in water and D$_2$O are given in Table 2. Energy transfer to the O–H vibrational manifold of the co-ordinated water molecules affords an effective non-radiative de-excitation pathway for all of the complexes studied which is absent in D$_2$O.[20] Using Horrocks' method,[6] this allowed the determination of the hydration state, q, of each complex which was found to be less than unity in all cases. Emission was much weaker from the europium complexes (compared to the terbium analogues) owing to a competitive charge-transfer-transition mechanism of energy capture by the Eu^{3+} ion. For all of the terbium monoamide complexes there was a good match between absorption and excitation spectra and a fairly structureless band at 250 nm was apparent. The nature and position of this band was independent of the amide nitrogen substituent, suggesting that it may be a ligand (carbonyl oxygen) to metal charge-transfer band. The apparently lower quantum yields obtained with the NHPh amide complexes, [EuL^4] and [TbL^4], may at least in part simply relate to competitive absorption of light quanta at 250 nm by the aryl chromophore. The lifetimes in H$_2$O (Table 2) for the secondary amide complexes are all slightly shorter than for the related tertiary amide complexes. This is evidence for the quenching of the metal excited-state by coupling to the amide N–H vibrational manifold (ν_{NH} *ca.* 3400 cm^{-1}). This effect has been clearly defined in related tetraamide complexes based on [12]aneN$_4$,[21] and for each N–H oscillator the rate constant for energy transfer has been estimated to be 0.085 ms^{-1}, thereby effectively raising the q value by 0.09, for europium complexes.

Nuclear magnetic relaxation dispersion profiles of gadolinium complexes

The relaxation rate of the water protons R_1^{obs} in solutions containing paramagnetic gadolinium(III) complexes is usually analysed in terms of the sum of three contributions [eqn. (1)], where R_1^{d}, the 'diamagnetic' component, is the solvent

$$R_1^{\mathrm{obs}} = R_{1\mathrm{p}}^{\mathrm{is}} + R_{1\mathrm{p}}^{\mathrm{os}} + R_1^{\mathrm{d}} \qquad (1)$$

relaxation rate in the absence of the paramagnetic solute and $R_{1\mathrm{p}}^{\mathrm{is}}$ and $R_{1\mathrm{p}}^{\mathrm{os}}$ represent the paramagnetic contributions arising from the dipolar coupling between the electron magnetic moment of Gd$^{\mathrm{III}}$ with the nuclear spins of the water molecules in the inner and outer co-ordination spheres of the complex,

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[*] Direct laser excitation of the europium complexes of L^3–L^6 (λ_{exc} = 488 nm) gave rise to spectra of higher resolution, and again only one band was discerned at 579 nm.

Table 2 Luminescence data for europium and terbium complexes (298 K)

Complex	$\tau_{298\,K}$/ms		Φ(298 K)		
	D_2O	H_2O	D_2O	H_2O	q[a]
[Eu(dota)]$^{-}$ [b]	2.44	0.64	1.1×10^{-3}	—	1.1
[Tb(dota)]$^{-}$	2.54	1.51	—	—	1.1
[EuL^2]$^{-}$ [c]	1.85	1.25	1.0×10^{-3}	5.9×10^{-4}	0.27
[TbL^2]$^{-}$ [c]	3.71	2.96	9×10^{-3}	1.0×10^{-2}	0.27
[EuL^1]$^{-}$ [c]	2.07	1.59	1.5×10^{-3}	0.9×10^{-3}	0.15
[TbL^1]$^{-}$ [c]	4.44	4.13	0.49	0.44	0.07
[EuL^3]	1.85	0.76	1.3×10^{-3}	0.5×10^{-3}	0.81
[TbL^3]	4.30	3.20	0.28	0.16	0.34
[EuL^4]	2.03	0.7	0.35×10^{-3}	0.1×10^{-3}	0.98
[TbL^4]	4.13	3.09	4.4×10^{-3}	4.7×10^{-3}	0.34
[EuL^5]	1.97	0.84	1.4×10^{-3}	0.74×10^{-3}	0.72
[TbL^5]	4.30	3.53	0.12	0.10	0.21
[EuL^6]	1.93	0.80	1.4×10^{-3}	0.6×10^{-3}	0.77
[TbL^6]	4.03	3.32	0.11	0.10	0.22

[a] For [EuL^3] and [EuL^4], the effect of the quenching of the excited state by the amide N–H oscillator contributes 0.09 to the q value given.[21] [b] Data from refs. 6 and 13. [c] From refs. 7 and 19.

respectively;[1] R^{os}_{1p} is then related to the modulation of the electron–nucleus interaction through translational diffusion of the water molecules in the proximity of the metal complex and it is quantitatively described by the equation originally postulated by Freed[22] in a study of the relaxation of organic solvent molecules in the presence of a stable free radical. The relevant physicochemical parameters involved are the distance of closest approach (a) between the metal complex and the outer-sphere water protons, the electronic relaxation time (τ_s) of the metal ion and the relative diffusion constants D for the solvent and solute.

In the inner-sphere contribution, R^{is}_{1p}, the dipolar coupling involves the metal ion and the water molecule(s) held in a fixed position near the paramagnetic centre (either co-ordinated, as is normally the case, or not) for a time longer than that necessary for solute and solvent to diffuse apart. The paramagnetic effect is then propagated to the bulk of the solvent by chemical exchange. Given that the relaxation time, T_{1M}, of the protons of inner-sphere water molecules is of the order of 10^{-6}–10^{-5} s for small gadolinium(III) chelates, the relaxivity enhancement is a maximum[23] when the exchange lifetime τ_M is much shorter than T_{1M} (fast-exchange condition), as established in equation (2) where q is the number of inner-

$$R^{is}_{1p} = \frac{[C]q}{55.6(T_{1M} + \tau_M)} \qquad (2)$$

sphere water molecules and [C] the molar concentration of the paramagnetic complex.

According to the Solomon–Bloembergen–Morgan theory,[24] T_{1M} is given by equations (3)–(5) where γ_H is the gyromagnetic

$$\frac{1}{T_{1M}} = \frac{2}{15}\frac{\gamma_H{}^2 g^2 \beta^2 S(S+1)}{r^6} \times \left(\frac{7\tau_C}{1 + \omega_S{}^2\tau_C{}^2} + \frac{3\tau_C}{1 + \omega_H{}^2\tau_c{}^2}\right) \qquad (3)$$

$$\frac{1}{\tau_C} = \frac{1}{\tau_R} + \frac{1}{\tau_S} + \frac{1}{\tau_M} \qquad (4)$$

$$\frac{1}{\tau_S} = \frac{1}{5\tau_{SO}}\left(\frac{1}{1 + \omega_S{}^2\tau_V{}^2} + \frac{4}{1 + 4\omega_S{}^2\tau_V{}^2}\right) \qquad (5)$$

ratio of proton nuclei, g the electronic Landé factor, β the Bohr magneton, S the electronic spin quantum number, r the distance of the inner-sphere water protons from the paramagnetic metal ion, τ_R the molecular reorientational correlation time and ω_S and ω_H are the Larmor frequencies for the electron and proton respectively. The electronic relaxation time τ_S is itself frequency dependent through equation (5) and is determined by its value at zero magnetic field τ_{SO} and by a field-independent correlation time τ_V related to the modulation of the ligand field by collision with solvent molecules.

The structural and dynamic parameters involved in the paramagnetic relaxation theory are best evaluated by fitting the calculated and experimental magnetic field dependence of the water proton-relaxation rates, the so-called nuclear magnetic relaxation dispersion (NMRD) profiles. However, since different sets of best-fitting parameters may well reproduce the experimental data, the solution has to be based on a judicious, chemically sound choice, or an independent evaluation of the value of some of the parameters. Fortunately, in recent years a large data set has been accumulated which allows the source of error and the uncertainty of the results to be limited by providing a reasonable choice of the range of possible values for the various parameters. A further improvement may be obtained by simultaneously fitting the data recorded at different temperatures and using as constraints the known temperature dependence of several parameters.

This approach to the study of the magnetic field-dependent solvent-relaxation enhancement promoted by paramagnetic complexes often affords very good results.[25] It provides a satisfactory picture of the structural and dynamic properties of Gd^{III}-co-ordinated solvent molecules, as well as of the water molecules diffusing near the surface of the complex, but it leaves some ambiguity in the characterization of water molecules in the second co-ordination sphere, as thoroughly discussed by Lauffer.[2] In fact, the effects of solvated water molecules are well accounted for by the translational diffusion model (outer sphere) when their exchange lifetime with the bulk is of the same order of magnitude as the time necessary for the complex and the molecules of the bulk to diffuse apart. On the other hand, when their residence lifetime τ_M on the complex becomes longer than the translational diffusion time their effects are indistinguishable from those of metal-co-ordinated water molecules and therefore can be described by equations (3)–(5).

In Fig. 2 we report, as a representative example, the $1/T_1$ NMRD profile of the complex [GdL^7] as recorded at 298 K in the frequency range 0.03–400 MHz. The profile has the typical shape for small gadolinium(III) chelates with a single co-ordinated water molecule, characterized by a dispersion centred at $\approx$5 MHz corresponding to the condition $\omega_S\tau_C = 1$ and thus to a τ_C value of $\approx$50 ps. However, the magnitude of the profile

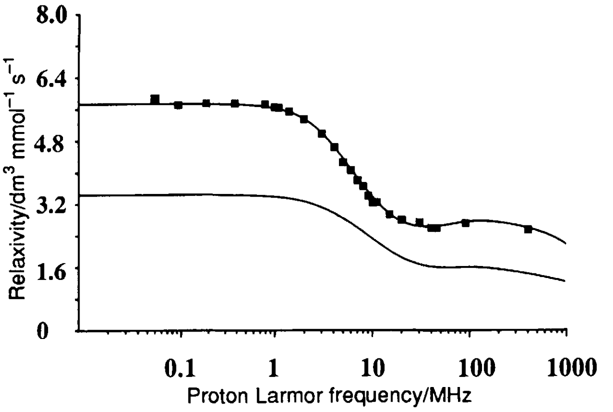


Fig. 2 The $1/T_1$ NMRD profile of a 1 mmol dm^{-3} aqueous solution of $[GdL^7]$ at 298K and pH 7. The curve through the data points has been calculated with the parameters of Table 1, obtained through a best-fitting procedure to equations for inner- and outer-sphere paramagnetic relaxation. The lower curve represents the outer-sphere component

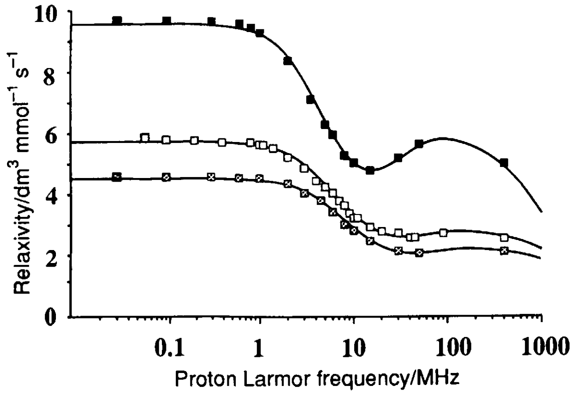
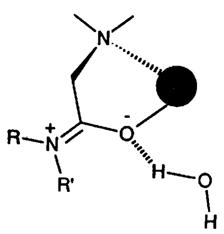


Fig. 3 The $1/T_1$ NMRD profiles of $[GdL^7]$ at 278 (■) and 308 K (⊠) (pH 7), compared with that at 298 K (□). The solid lines fitting the experimental data are calculated from the parameters of Table 1. In the fitting procedure of the data taken at 278 and 308 K, *q*, *r* and *a* were kept fixed at their 298 K values

is significantly lower than those known for analogous 'inner-sphere' complexes where, for example, the relaxivity passes from 8–12 in the flat region at low fields, to *ca.* 4–6 dm^3 mmol^{-1} s^{-1} at 20 MHz.[26–28] Here, the relaxivity of $[GdL^7]$ falls between the values 5.9 (0.03) and 2.8 dm^3 mmol^{-1} s^{-1} (20 MHz). On the other hand, the hypothesis of the absence of metal-co-ordinated water molecules is excluded on the basis of (*i*) the low value of the inflection point and (*ii*) the comparison with the profile, taken at the same temperature, for the analogous tetra(methylphosphinate) complex. In fact, 'outer-sphere' complexes such as $[Cd(ttha)]^{3-}$ (H_6ttha = triethylenetetramine-*N,N,N′,N″,N‴,N‴*-hexaacetic acid),[29] $[GdL^1]^-$ and $[GdL^2]^-$ (ref. 7) present the inflection point at ≈ 10 MHz, corresponding to a diffusional correlation time of ≈ 20 ps and lower relaxivity values over the entire magnetic field range. So, assuming $q = 0$ for $[GdL^7]$ would imply for this complex either a significantly shorter molecular radius or a dramatic change of the diffusional dynamics of its aqueous solution. Thus, for $[GdL^7]$ we have to admit the existence of both inner- and outer-sphere contributions to the observed paramagnetic enhancement of the solvent longitudinal relaxation rates. The outer-sphere component is expected to be similar to that of $[GdL^2]^-$ and is presumably a little lower because of a longer value of *a* due to the larger size of the complex. The reorientational correlation time τ_R is strictly related to the molecular dimension and therefore we expect it to be of the order of 80–100 ps, as found for the analogous tetraphosphonate complex.[28] The temperature dependence of the relaxivity, measured at 20 MHz in the range 274–323 K, indicates the fast-exchange condition ($\tau_M \ll T_{1M}$) and therefore a τ_M value of the order of nanoseconds. Interestingly, the profile of Fig. 2 shows a little hump of relaxivity around 100 MHz which reproduces, on a much more reduced scale and at a different position, the characteristic relaxivity peak of slowly tumbling systems (metalloproteins,[1] covalent or non-covalent conjugates with macromolecules, adducts with micelles[30] and cyclodextrins[31]) for which the reorientational correlation time τ_R becomes so long as to make visible the field dependence of τ_S according to equation (5). Here, this indicates a rather shorter value of the electronic relaxation time and a dependence of the relaxivity upon the rotational dynamics of the complex. This substantiates the hypothesis of the presence of an 'inner-sphere' contribution. These aspects are even more evident in the profile recorded at low temperature (Fig. 3) because of the further increase of τ_R. Given all the above observations we are left with the need to explain the unusually low relaxivity values: if attention is focused in the high-field region and the reasonable assumption is made of an outer-sphere contribution of ≈ 2.4 dm^3 mmol^{-1} s^{-1}, as found for $[GdL^2]^-$, then it turns out that the inner-sphere relaxivity is about 0.5 dm^3 mmol^{-1} s^{-1}. Given that R^{is}_{1p} in the high-field region is proportional to the term $q\tau_R/r^6$ and also given the hypothesis of $q = 1$ and a reorientational correlation time of the order of 90 ps, then a Gd–H distance for the co-ordinated water molecule of *ca.* 3.6 Å results. This value is noticeably longer than the corresponding ones (3.0–3.2 Å) found for gadolinium(III) complexes with both linear and macrocyclic polyaminopolycarboxylic ligands. Thus, the conclusion is that the increase in relaxivity observed on passing from purely outer-sphere complexes, such as $[GdL^1]^-$ and $[GdL^2]^-$ to $[GdL^7]$ has to be ascribed to the presence of a tightly bound water molecule in the second co-ordination sphere of the metal ion. The occurrence of this type of solvent interaction must then be related to the substitution of a phosphinate group by a carboxamide. In this respect one could envisage the formation of a hydrogen bond between the carboxamide oxygen atom and a water molecule (Scheme 1) which results in both a suffcently long lifetime for the interaction and a metal–proton distance short enough to affect the solvent relaxation. Strictly similar data have been obtained and thus analogous observations have been made for the related complexes of Gd^{III} with macrocycles H_3L^3, H_3L^4, H_3L^6 and H_3L^8.



Scheme 1

The best-fitting results of the NMRD profiles taken at three temperatures for the five complexes are reported in Table 3. In each case the fitting was undertaken with a *q* value of 1, and the *D* values were estimated from the analysis of $[Gd(ttha)]^{3-}$ and $[Gd(teta)]^-$ (H_4teta = 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid),[32] the best values for *a* and *r* obtained at 298 K were employed in the analysis of the data at the other two temperatures. The values of τ_M are those affected by the largest uncertainty because of the occurrence of the fast-exchange condition and its limited contribution to τ_C. A τ_M value of 0.7 ns at 298 K seems, however, quite reasonable since

Table 3 Relaxation parameters obtained from the fitting of the experimental NMRD profiles by the relaxation theory for gadolinium(III) complexes

	[GdL^3]*			[GdL^4]			[GdL^6]		
T/K	278	298	308	278	298	308	278	298	308
τ_{SO}/ps	90.3	79	75.7	110	114	95.6	94	109	71
τ_V/ps	25.7	14.2	11.0	21.6	8.1	4.35	18.8	11.1	5.5
τ_M/ns	1.3	0.8	0.59	1.9	0.7	0.4	1.3	0.8	0.7
τ_R/ps	152	98.7	78.7	135	96.9	75	189	95	80.7
r/Å	3.42	3.42	3.42	3.42	3.42	3.42	3.56	3.56	3.56
a/Å	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
10^{-5} D/cm^2 s^{-1}	1.0	2.4	3.0	1.0	2.4	3.0	1.0	2.4	3.0

	[GdL^7]			[GdL^8]		
T/K	278	298	308	278	298	308
τ_{SO}/ps	116	108	85	127	88.5	89.4
τ_V/ps	10.4	5.5	4.2	19.2	8.6	5.6
τ_M/ns	1.4	0.8	0.6	1.6	0.8	0.6
τ_R/ps	180	96	74	160	102	82
r/Å	3.53	3.53	3.53	3.47	3.47	3.47
a/Å	4.0	4.0	4.0	4.1	4.1	4.1
10^{-5} D/cm^2 s^{-1}	1.0	2.4	3.0	1.0	2.4	3.0

* For the related $-CONH(CH_2)_4NH_3^+$ derivative (instead of CONHMe).

it is sizeably shorter than the residence lifetime of metal-co-ordinated water molecules and sensibly longer than the diffusional correlation time. Furthermore, a very similar value has been reported by Koenig *et al.*[33] for the case of water molecules hydrogen bonded to fluoromethaemoglobin. Finally, for τ_R, τ_M and τ_V an Arrhenius-type temperature dependence has been assumed throughout the fitting procedure.

It is worth commenting that although the occurrence of water molecules tightly bound in the second co-ordination sphere of a small gadolinium(III) chelate is certainly a common phenomenon, this represents the first case where their contribution to the overall relaxation enhancement is finely determined and analysed.† These findings may be very useful in the design of novel, safer and more effective contrast agents for magnetic resonance imaging.

Conclusion

Correlation of NMRD and luminescence data: complex hydration state

The fitting of the observed NMRD profiles of the gadolinium complexes of H_4L^1, H_4L^2, H_4dota and several of the monoamide complexes allows an estimation of the mean distance, r, between the ion and the nearest water proton. This information may be compared with that obtained by luminescence measurements where the average hydration state, q, of the europium (or terbium) complexes is evaluated, Table 4. In each of the chosen cases solution NMR studies have indicated unequivocally that there is predominantly one major isomer in solution, so that non-integral q values ($0 > q > 1$) may not be attributed to the presence of various solution species with different hydration states. This idea was supported by the observation of only one $\Delta J = 0$ transition for the analogous europium complexes in their phosphorescence emission spectra. The correlation between the NMRD-derived r value and the q value measured by luminescence spectroscopy is quite good, Fig. 4 ($R^2 = 0.93$).

Table 4 Correlation of Gd–H (H_2O distances), r, determined by NMRD with the hydration state, q, determined by luminescence measurements (298 K, H_2O) for octadentate lanthanide complexes

Complex	q Eu	Tb	Gd(NMRD) r/Å
[LnL^3]	0.81	0.34	3.42[a]
[LnL^5]	0.72	0.21	3.53[b]
[LnL^4]	0.98	0.34	3.42
[LnL^6]	0.77	0.22	3.56
[LnL^1]$^{-}$[c]	0.15	0.07	4.25
[LnL^2]$^{-}$[c]	0.27	0.27	3.82
[Ln(dota)]$^-$	1.1	0.9	3.13

[a] For the related $CONH(CH_2)_4NH_3^+$ derivative. [b] For the *N*,*N*-dibutylamide analogue. [c] From ref. 7; a crystal structure of the [YL^1]$^-$ complex revealed the nearest water molecule, in the crystal lattice, to be 5.62 Å from the metal ion.

These data therefore support the hypothesis that for lanthanide complexes of octadentate ligands the hydration states $q = 0$ and 1 simply represent boundary conditions and a given complex in solution may in effect possess *any* intermediate value. This value is a function of the charge density at the metal centre and the hydrophobicity of the local environment, which is determined by the nature of the substituents of the ligand at N or C. In other words, the mean distance of closest approach of a water molecule to the ligand-bound metal ion can vary between, say, 3.1 ($q = 1$) and > 4.5 Å (0), according to the absence or presence of local hydrogen-bonding groups. An alternative view is to consider that there exists a well defined 'second hydration sphere', promoted by the proximity of hydrogen-bond acceptor groups: of course there is little *real* distinction between these viewpoints.

Experimental

Compounds H_3L^3 and H_3L^5–H_3L^8 were prepared as previously reported,[11] as was H_3L^4.[7] The preparation and

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† Although unlikely, there is an alternative explanation for the relaxation enhancement displayed by the gadolinium(III) complexes considered in this work. This approach is based on the assumption that the inner-sphere contribution deals *only* with water molecules directly co-ordinated to the paramagnetic ion. This implies a Gd–H distance not longer than 3.20 Å. If the inner-sphere contribution is evaluated by keeping r in the range 3.10–3.20 Å and the reorientational correlation time has to be in the range 80–100 ps, then we have to assign to the hydration number q a fractional value in the range 0.4–0.6. Such a fractional number would be an indication that there is in solution a mixture of isomers, characterized by having $q = 0$ and 1. The principal argument against this interpretation is based on the observation of just one major isomer in the high-resolution NMR spectra along the lanthanide series (La^{III}, Eu^{III}, Tb^{III} and Yb^{III}) accounting for roughly 90% of the total intensity.[11]

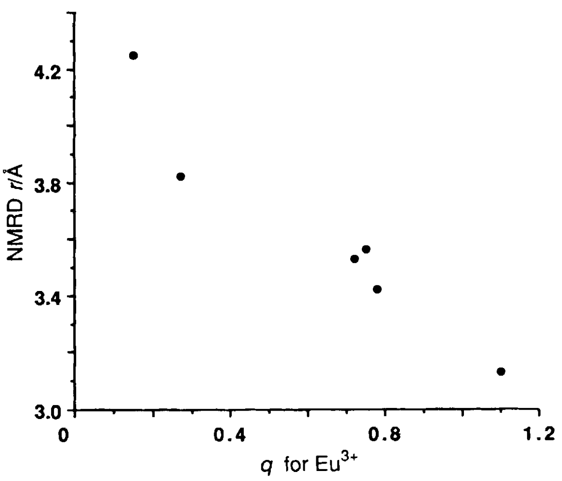

Fig. 4 Correlation between the *q* value measured by luminescence methods for the europium complexes of H_4L^1, H_4L^2, H_3L^3, H_3L^5 and H_3L^6 and the distance of closest approach of a water molecule for the corresponding gadolinium complexes, determined by NMRD methods

characterization of the europium complexes has been described.[11] The terbium complexes were prepared in an identical manner using terbium acetate or nitrate in aqueous solution, complexation proceeding at pH 6. The $^{31}P-\{^1H\}$ spectra were obtained with a Bruker AC250 spectrometer operating at 101.1 MHz, IR spectra with Perkin-Elmer 1600 FT spectrometer with GRAMS Analyst operating software and mass spectra (FAB, glycerol matrix) using a VG7070E spectrometer. It was possible to obtain ^1H NMR spectra of the terbium complexes (Bruker AC250 operating with a sweep width of 125 kHz or 500 ppm) but the severe paramagnetic line broadening and very large shifts induced by terbium (peaks have been observed between δ +300 and −250 and half-height widths are typically 3 ppm or more) has prevented any assignments from being made.

Proton solvent longitudinal relaxation times were measured at 20 MHz and variable temperature on a SpinMaster spectrometer [Stelar, Mede (PV), Italy] by means of the inversion-recovery technique (16 experiments, four scans). The reproducibility in T_1 measurements was ±0.4%. The temperature was controlled by a JEOL air-flow heater, equipped with a copper–constantan thermocouple; the actual temperature in the probehead was measured with a Fluke 52 k/j digital thermometer, with an uncertainty of 0.5 K.

The $1/T_1$ NMRD profiles of water protons were measured over a continuum of magnetic fields from 0.000 24 to 1.2 T (corresponding to 0.01–50 MHz proton Larmor frequency) on the Koenig–Brown field-cycling relaxometer installed at the Department of Chemistry, University of Florence, Italy. The temperature inside the probe was controlled by circulation of perfluoroalkanes. Data at higher frequencies were also taken on JEOL EX-90 and EX-400 spectrometers. The gadolinium complexes were prepared (as described in refs. 10 and 11) in 1 mmol dm^{-3} concentration in aqueous solutions at pH ≈ 7.

Luminescence measurements

Lanthanide phosphorescence spectra were recorded using a Perkin-Elmer LS50B spectrofluorimeter operating in time-resolved mode and equipped with a Hamamatsu R928 photomultiplier tube. Typically, delay and gate times were set at 0.1 and 10 ms respectively and the most highly resolved spectra were obtained using excitation and emission slit widths of 5 and 2.5 nm respectively, following pulsed excitation at 250 nm. Emission spectra were corrected for the wavelength dependence of the photomultiplier tube. Phosphorescence excitation spectra were acquired by monitoring emission at 619 nm for Eu^{III} and 545 nm for Tb^{III}.

Quoted lifetimes (τ) are the average values from at least five separate measurements, each of which was made by monitoring the emission intensity at 619 (for Eu^{III}) or 545 nm (for Tb^{III}) after 20 different delay times spanning a range of at least two lifetimes (λ_{ex} = 250 nm). Slit widths of 15 nm were used and the gate time was 0.1 ms. The phosphorescence decay curves were fitted by an equation of the form $I(t) = I(0)\exp(-t/\tau)$ using a curve-fitting program. High correlation coefficients were observed in each case (typically 0.999 or higher). Lifetimes were found to be independent of concentration.

Luminescence quantum yields were obtained by the method described by Haas and Stein[34] using as standards $[Ru(bipy)_3]^{2+}$ (bipy = 2,2′-bipyridyl) (φ = 0.028 in water)[35] for the europium(III) complexes and quinine sulfate (φ = 0.546 in 0.5 mol dm^{-3} H_2SO_4)[36] for the terbium(III) complexes. The observed phosphorescence (P) was related to the total phosphorescence emission (P_T) through equation (6).[37] where τ

$$P_T/P = \frac{1-\exp(-20/\tau)}{\exp(-t_d/\tau)-\exp[-(t_d+t_g)/\tau]} \qquad (6)$$

is the phosphorescence lifetime, t_d the delay time and t_g the gate time (values in ms and for a cycle time of 20 ms).

Characterization data for terbium complexes

[TbL³]. $\delta[P-\{^1H\}](D_2O)$: 531, 599 and 603. *m/z* (FAB): 738 (100, $M^+ + 1$) (Found: C, 27.40; H, 6.50. $C_{17}H_{37}N_5O_7P_3Tb\cdot 4H_2O$ requires C, 27.40; H, 6.05%). IR (KBr): 3404s, 2974m, 2869m, 1639s, 1459m, 1412m, 1296m, 1230m, 1148s, 1070m, 1025s, 891m, 807s and 522s cm^{-1}.

[TbL⁴]. $\delta[P-\{^1H\}](D_2O)$: 468, 615 and 634. *m/z* (FAB): 676 (77, $M^+ + 1$) (Found: C, 33.10; H, 5.65. $C_{22}H_{39}N_5O_7P_3Tb\cdot 3H_2O$ requires C, 33.40; H, 5.70%). IR (KBr): 3390m, 2977s, 2871s, 1626s, 1596s, 1571s, 1455m, 1425m, 1294m, 1137s, 1070m, 1034s, 891m, 810m, 743m and 526m cm^{-1}.

[TbL⁵]. $\delta[P-\{^1H\}](D_2O)$: 569, 574 and 579. *m/z* (FAB): 691 (64, $M^+ + 1$) (Found: C, 27.30; H, 6.25; N, 8.55. $C_{18}H_{39}N_5O_7P_3Tb\cdot 6H_2O$ requires C, 27.10; H, 6.40; N, 8.75%). IR (KBr): 2974m, 2875m, 1625s, 1460m, 1295m, 1229m, 1144s, 1071m, 1030s, 976m, 892m, 743m and 542m cm^{-1}.

[TbL⁶]. $\delta[P-\{^1H\}](D_2O)$: 489, 695 and 748. *m/z* (FAB): 842 (100, $M^+ + 1$) (Found: C, 40.80; H, 6.25. $C_{30}H_{47}N_5O_7P_3Tb\cdot 2H_2O$ requires C, 41.05; H, 5.80%). IR (KBr): 2970m, 2869m, 1600s, 1455m, 1294m, 1228m, 1149s, 1071m, 1025s, 805m and 668m cm^{-1}.

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