Relaxometric and luminescence behaviour of triaquahexaazamacrocyclic complexes, the gadolinium complex displaying a high relaxivity with a pronounced pH dependence

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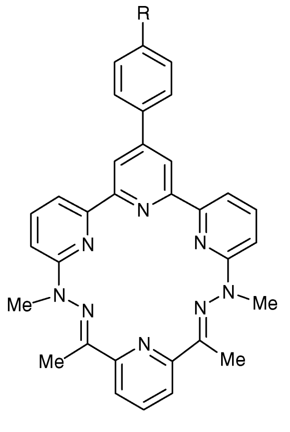
The relaxivity of $[GdL^1]^{3+}$ in aqueous solution has been studied as a function of temperature and magnetic field strength. The complex possesses a high relaxivity at pH 6 (12.8 $mmol^{-1}$ s^{-1}) that diminishes as successive water molecules are deprotonated, leading to a complex species whose relaxivity is typical of a purely outer-sphere complex. Measurements of the luminescence lifetime of a very similar complex, $[YbL^2]^{3+}$, in H_2O and D_2O were used to confirm that the complex possesses three bound waters at pH 5.5, but above pH 11 there are no bound OH groups in the putative di-oxo-bridged dimer.

Macrocyclic lanthanide complexes are finding increasing applications in medicine, both as diagnostic agents and as therapeutic agents. In the diagnostics area, one of the earliest and certainly the most widespread example of their use is as NMR contrast agents, of which $[Gd–DOTA]^-$ and $[Gd–DTPA]^{2-}$ are well-known.[1] For *in vitro* use only, Eu and Tb cryptates have recently been developed as biological markers.[2] In a therapeutic setting, La– and Lu–texaphyrin complexes are presently under investigation for photodynamic therapy,[3] and in the field of antisense technology, macrocyclic Eu and Dy complexes have emerged with a rôle as phosphoryl transfer catalysts.[4] All of these applications share a common requirement; the compounds need to be sufficiently stable to allow use under physiological conditions, *i.e.* they should possess a high kinetic stability with respect to the loss of the metal cation from the ligand. For a given ligand, stability can be maximised by coordinatively saturating the metal centre with suitable hard donor atoms, thereby minimising its susceptibility to nucleophilic attack. However, for two of the afore-mentioned applications—as contrast and antisense agents—the cations ideally require coordination sites for ancillary ligands. In the former case, this simply allows exchange of water molecules at the paramagnetic centre and in the latter allows the binding of RNA phosphodiester oxygens during transesterification.[5] Lanthanide complexes with a high kinetic stability and 'free' coordination sites at the metal centre may thus prove useful for both of these applications. Complexes with a high positive charge at the centre are particularly attractive as acid-promoted dissociation pathways will be disfavoured by electrostatic repulsion while anion coordination may be favoured. An example of multiple applications from a single family of complexes is provided by the lanthanide texaphyrins, wherein different lanthanide metals in a common ligand system may give rise to contrast agents, photodynamic therapy agents and antisense reagents.[6] The availability of a variety of structurally homologous complexes of the type L^1, originally conceived as phosphodiester transesterification catalysts, prompted the screening of a representative example, $[GdL^1]Cl_3$, as a magnetic resonance imaging (MRI) contrast agent.

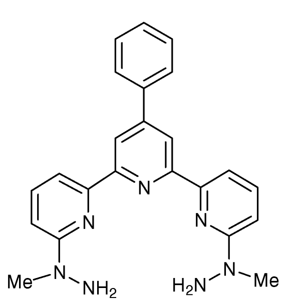
Results and Discussion

Synthesis and preliminary evaluation of stability

The synthesis of the complex $[GdL^1]Cl_3$ was performed *via* a template-directed condensation of the reported terpyridine ligand **1**[7] with 2,6-diacetylpyridine in the presence of $GdCl_3$. The orange complex was purified by precipitation from methanol with Et_2O. Some simple TLC studies (SiO_2; $MeOH–H_2O$–aq. KOAc; R_f for $[GdL^1]Cl_3$ is 0.29) were undertaken to examine the stability of the complex in aqueous media at room temperature. Incubation at pH 4.5, 6 and 7 revealed a very small degree of decomposition after 6 days, with a faster running spot discerned in each case, corresponding to that of the precursor **1**. When challenged separately with an 0.5 mol dm^{-3} solution of Na_4EDTA (pH 8) and NaH_2PO_4 (pH 7.5), an aqueous solution of $[GdL^1]^{3+}$ gave no detectable decomposition over 72 h, after formation of a small amount of precipitate. On the other hand, in 15% aqueous ammonia solution, or at pH 4, decomposition was observed with a half-life of *ca.* 2.5 days at ambient tem-



L^1 R = H
L^2 R = NH_2



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perature. Despite this sensitivity to acid- or base-promoted dissociation, further experiments were undertaken to probe the relaxation behaviour of the gadolinium complex.

Relaxation properties of $[\mathrm{GdL}^1]^{3+}$

A great deal of structural and dynamic information on paramagnetic metal chelates in solution can be obtained by the relaxometric technique. This reports on the properties of the solute by measuring the nuclear magnetic relaxation behaviour of the solvent nuclei.[8] Though largely utilised for studying diamagnetic proteins in aqueous solution, relaxometry represents one of the best approaches in the case of solutions containing paramagnetic compounds. Such solutions often cannot be directly investigated by standard high-resolution NMR procedures. The water proton relaxation times (T_1 and T_2) are markedly decreased in a solution of a paramagnetic metal complex, as a result of the strong electron-nucleus interactions occurring at the water–solute boundary.[9] The complexes of $\mathrm{Gd^{III}}$ (and $\mathrm{Mn^{II}}$) are particularly effective in this respect. The metal ion is endowed with a high magnetic moment and a long electronic relaxation time (τ_{s1} and τ_{s2}), two basic requirements for optimal enhancement of the solvent nuclear relaxation rates ($1/T_{1,2}$). Indeed, low molecular weight $\mathrm{Gd^{III}}$ chelates are widely used for altering the contrast in MRI.[1,10]

For such small $\mathrm{Gd^{III}}$ complexes, the magnetic interaction with the solvent is represented by the electron-nucleus dipolar coupling between the metal ion and the water molecules. This interaction involves both directly coordinated water molecules, with a lifetime τ_{M} (*inner-sphere* interaction), and those diffusing near the complex (*outer-sphere* interaction). The measured increase of longitudinal proton relaxation rate, compared to pure water and normalised to millimolar $\mathrm{Gd^{3+}}$, is called relaxivity and contains contributions from both inner-sphere and outer-sphere relaxivities:

$$R^{\mathrm{obs}}_{1\mathrm{p}} = R^{\mathrm{is}}_{1\mathrm{p}} + R^{\mathrm{os}}_{1\mathrm{p}} \tag{1}$$

The inner-sphere relaxation rate is described in terms of the following set of equations:[9]

$$R^{\mathrm{is}}_{1\mathrm{p}} = \frac{Mq}{55.6}\,\frac{1}{T^{\mathrm{H}}_{1\mathrm{M}} + \tau_{\mathrm{M}}} \tag{2}$$

$$\frac{1}{T_{1\mathrm{M}}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{h^2\gamma_{\mathrm{s}}^2\gamma_{\mathrm{I}}^2}{r^6_{\mathrm{GdH}}}\,S(S+1) \times \left[\frac{3\tau_{\mathrm{c1}}}{1+\omega_{\mathrm{I}}^2\tau_{\mathrm{c1}}^2} + \frac{7\tau_{\mathrm{c2}}}{1+\omega_{\mathrm{s}}^2\tau_{\mathrm{c2}}^2}\right] \tag{3}$$

$$\frac{1}{\tau_{\mathrm{c}i}} = \frac{1}{\tau_{\mathrm{M}}} + \frac{1}{\tau_{\mathrm{R}}} + \frac{1}{\tau_{\mathrm{S}i}} \qquad i = 1, 2 \tag{4}$$

$$\left(\frac{1}{\tau_{\mathrm{S1}}}\right) = \frac{1}{5\tau_{\mathrm{S0}}}\left(\frac{1}{1+\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2} + \frac{4}{1+4\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2}\right) \tag{5}$$

$$\left(\frac{1}{\tau_{\mathrm{S2}}}\right) = \frac{1}{10\tau_{\mathrm{S0}}}\left[3 + \frac{5}{1+\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2} + \frac{2}{1+4\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2}\right] \tag{6}$$

In eqn. (2)–(6), M is the molar concentration of the paramagnetic complex, q is the number of water molecules in the inner coordination sphere of the metal ion, τ_{M} is their mean residence lifetime, $T_{1\mathrm{M}}$ is their longitudinal relaxation time; S is the electron spin quantum number, γ_{I} is the proton nuclear magnetogyric ratio, r_{GdH} is the distance between the metal ion and the protons of the coordinated water molecules, ω_{I} and ω_{S} are the proton and electron Larmor frequencies, respectively, and τ_{R} is the molecular reorientational correlation time. Finally, τ_{S1} and τ_{S2} are the longitudinal and transverse electron spin relaxation times, which are frequency dependent, according to eqn. (5) and (6), and are characterised by the correlation time associated with the modulation of the zero-field splitting interaction (τ_{v}) and their value at zero magnetic field (τ_{S0}).

The outer-sphere contribution to proton relaxivity can be described by eqn. (7):

$$R^{\mathrm{os}}_{1\mathrm{p}} = \frac{6400 N_{\mathrm{A}}\pi}{81}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{h^2\gamma_{\mathrm{s}}^2\gamma_{\mathrm{I}}^2}{a_{\mathrm{GdH}}D_{\mathrm{GdH}}} \times S(S+1)[3J_{\mathrm{os}}(\omega_{\mathrm{I}}, \tau_{\mathrm{S1}}) + 7J_{\mathrm{os}}(\omega_{\mathrm{S}}, \tau_{\mathrm{S2}})] \tag{7}$$

where N_{A} is the Avogadro constant, D_{GdH} is the relative diffusion coefficient for solute and solvent, a_{GdH} is the shortest distance of approach of an outer-sphere water molecule to the paramagnetic centre and J_{os} is the spectral density function.[11]

For small $\mathrm{Gd^{III}}$ polyaminopolycarboxylate complexes with $q = 1$, the two mechanisms make a comparable contribution to the overall relaxivity at any value of the magnetic field strength.

pH Dependence

The proton relaxivity of $[\mathrm{GdL}^1]^{3+}$, measured at 20 MHz and 25 °C, assumes a constant value of 12.8 $\mathrm{mM^{-1}\ s^{-1}}$ in the pH range 1.5 to 6.0 (Fig. 1). This value is quite high, about three times greater than that found for the nine-coordinate mono-aqua complexes such as $[\mathrm{Gd(DOTA)}]^-$ and $[\mathrm{Gd(DTPA)}]^{2-}$.[8,12–14] At this magnetic field strength, the relaxivity of low molecular weight $\mathrm{Gd^{III}}$ chelates is controlled primarily by q, τ_{R} and the $1/r^6$ terms in eqn. (2), (3) and (4) (τ_{R} is about two orders of magnitude shorter than τ_{M} and τ_{S}). Given that the variation of τ_{R} and r is rather small among low molecular weight complexes, the measured relaxivity enhancement suggests a higher hydration for $[\mathrm{GdL_1}]^{3+}$, presumably with q of 2 or 3. Under the same experimental conditions, the aqua ion $[\mathrm{Gd(H_2O)_8}]^{3+}$ has a relaxivity of 13.0 $\mathrm{mM^{-1}\ s^{-1}}$, as a result of a smaller τ_{R} and a higher q value.[15] However, rapid dissociation of the complex in this pH range can be excluded since $R_{1\mathrm{p}}$ does not change upon addition of a ten-fold excess of $\mathrm{H_4EDTA}$ or $\mathrm{H_5DTPA}$. Indeed, in the pH range studied, the kinetic stability of the complex is evidently quite high as its integrity is maintained even in the presence of DTPA, one of the strongest lanthanide(III) chelators ($\log K_{\mathrm{f}} = 22.5$).[16]

At pH > 6.0 the relaxivity decreases markedly and passes from 12.8 to about 2 $\mathrm{mM^{-1}\ s^{-1}}$, without traces of decomposition, even in concentrated solutions. This decrease of $R_{1\mathrm{p}}$ at high pH has been previously observed for $\mathrm{Gd^{III}}$ complexes with $q \geqslant 2$ and was attributed to either the formation of OH-bridged polymeric species, the formation of ternary complexes with dissolved carbonate ions[17] or the partial replacement of coordinated water molecules by $\mathrm{OH^-}$ ions.[18] The formation of polymers is unlikely since the relaxivity behaviour with pH is perfectly reversible, whereas the formation of ternary complexes with carbonate has been shown to be significant only at pH > 8. Moreover, the same pH dependence is measured for a solution containing a ten-fold excess of DTPA (*i.e.*, the same as in Fig. 1). A possible explanation for the relaxivity decrease is a reduction of q due to coordination of $\mathrm{OH^-}$ anions. The limiting value of the relaxivity at basic pH is close to the typical value expected for a purely outer-sphere complex.[18,19]

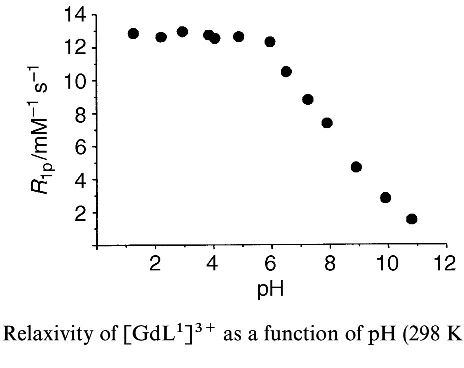


Fig. 1 Relaxivity of $[\mathrm{GdL}^1]^{3+}$ as a function of pH (298 K, 20 MHz)

It is plausible that at pH > 11 we only have in solution a neutral complex [GdL$_1$(OH)$_3$], which is characterised by a very long lifetime of the hydroxyl ligands, resulting in a negligible inner-sphere contribution to the relaxivity [$\tau_M \gg T_{1M}$ in eqn. (2)]. However, there is no precedent for this behaviour and fast prototropic exchange might still be expected on/off the bound OH groups, as was found recently for a cationic tetraamide complex.[20] A pH-metric titration of [GdL^1]$^{3+}$ was undertaken ($I = 0.1$ NMe$_4$NO$_3$, 298 K) and in the pH range 6.0 to 11, 3.3 equiv. of base were consumed. Over this experimental timescale, some base-consuming complex decomposition had been noted in the preliminary stability studies, so this value is likely to be an overestimate of the number of protons lost from the intact complex. The reduced relaxivity could involve reversible dimer formation, involving loss of two protons per metal ion, associated with a decrease in the coordination number at Gd to eight.

Temperature dependence

The relaxation rate of [GdL^1]$^{3+}$ has been measured as a function of temperature in the range of 273 to 335 K at 20 MHz and pH = 5.4. R_{1p} increases exponentially with decreasing temperature (Fig. 2) and this behaviour is consistent with the occurrence of the fast-exchange condition for the coordinated water molecules [$\tau_M \ll T_{1M}$ in eqn. (2)] over the entire range of values. Indeed, at this magnetic field strength, the temperature dependence of the relaxivity coincides with the temperature dependence of τ_R (and D), which is assumed to follow a simple exponential law. By using the published values of the relaxation parameters of [Gd(DTPA)]$^{2-}$ and by adopting $q = 3$, a value for T_{1M} of 1 μs at 298 K was estimated.[10] This means that the mean residence lifetime of the inner-sphere waters at pH 5.4 has an upper limit of about 10 ns and therefore it does not influence the relaxivity at all.

NMRD profiles

The qualitative predictions discussed above can be quantified more precisely through a magnetic field dependence study. The set of relaxivity data measured as a function of magnetic field strength, over a large range of values, represents what is termed a nuclear magnetic relaxation dispersion (NMRD) profile. The experimental profile, measured with specialised instrumentation (a relaxometer) developed over the years by Koenig and Brown,[21] is fitted to eqn. (2)–(7) for inner- and outer-sphere contributions and reliable values of the relaxation parameters may be obtained.

The NMRD profiles of [GdL^1]$^{3+}$ at 4, 25 and 39 °C and pH = 5 are reported in Fig. 3. The three profiles have a similar shape, with a minimum near 10 MHz at 4 °C that shifts towards higher frequencies with the temperature, and a pronounced 'hump' around 100 MHz. At high fields the profiles are not influenced by τ_M and τ_S and therefore the only parameters that enter into the fit are q, r, τ_R, a and D. From the available X-ray data the Gd—O distance of the coordinated water falls in a narrow range of values centred around 2.5 Å, which corresponds to a Gd—H distance of around 2.9 Å. By comparison with previous studies and with the relaxivities of Gd^{III} complexes of analogous size (and therefore analogous outer-sphere contribution), a hydration number $q = 3$ may be estimated. For a and τ_R (which depend on the molecular dimension of the complex) values of about 4 Å and 100 ps (at 25 °C), respectively, may be assumed.[22] At low fields the electronic relaxation time, but not τ_M, plays an important contribution to the inner- and outer-sphere relaxivities and this simplifies the analysis. By changing the temperature only τ_{SO}, τ_v, τ_R and D vary and their temperature dependence is assumed to follow an exponential law:

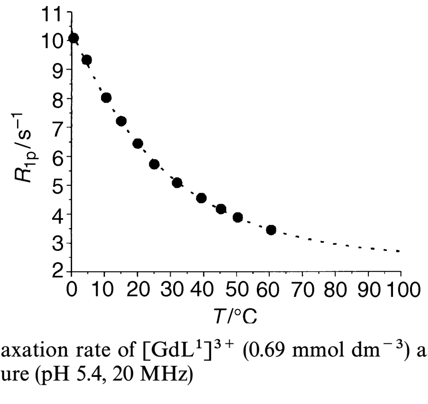


Fig. 2 Relaxation rate of [GdL^1]$^{3+}$ (0.69 mmol dm^{-3}) as a function of temperature (pH 5.4, 20 MHz)

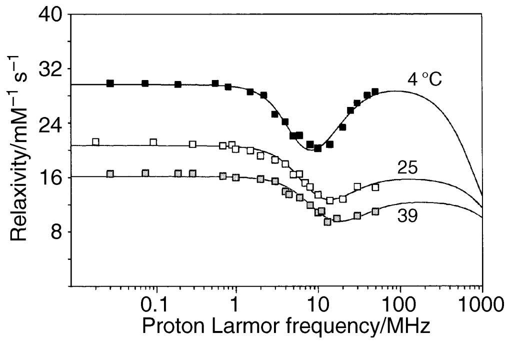


Fig. 3 NMRD profiles for [GdL^1]$^{3+}$ at 4, 25 and 39 °C (pH 5)

$$\tau = \tau^{298} \exp\left\{\frac{E}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right\} \tag{8}$$

where τ^{298} is the value at 298.15 K and E is the activation energy.[14]

The best fitting parameters are reported in Table 1 and confirm the qualitative predictions made before. The data are consistent with the presence of three, fast exchanging, water molecules in the inner-coordination sphere of the Gd^{III} ion at a distance r_{GdH} of 2.91 Å. The electronic relaxation time is short and is similar to the value calculated for the Gd^{III} complexes with linear and flexible polyaminopolycarboxylic ligands. It is much lower than those found for the highly rigid and symmetric macrocyclic DOTA-like complexes. The concomitant occurrence of a short τ_{SO} and a relatively long τ_R is responsible for the appearance of the relaxivity peak at high field, which becomes more pronounced and shifts towards low fields on lowering the temperature. The results suggest that the complex adopts an unsymmetrical solution structure, which is considerably distorted from planarity and may be rather fluxional. This is consistent with the reported X-ray structure for [EuL^2]$^{3+}$, in which there are two bound water molecules and a coordinated chloride anion. The six metal-

Table 1 Best fitting parameters for the NMRD profiles of [GdL^1]$^{3+}$ in Fig. 3[a]

	Temperature/°C		
	4	25	39
Parameter			
τ_{SO}/ps	72.7	59.1	46
τ_v/ps	20.9	13.4	9.1
τ_R/ps	192.4	105	87
q	3	3	3
r/Å	2.91	2.91	2.91
a/Å	3.6	3.6	3.6
10^5D/cm^2 s^{-1}	1.29	2.40	3.15

[a] The value of τ_M has been fixed at 10 ns throughout the fitting procedure. However, the results are insensitive to a variation of one order of magnitude of this value. Errors for the shown values are consistent with those reported in the literature for similar such analyses.[12]

coordinated N atoms form a distorted boat with the bound anion on the convex side and the coordinated water molecules on the concave side.[5]

Luminescence behaviour

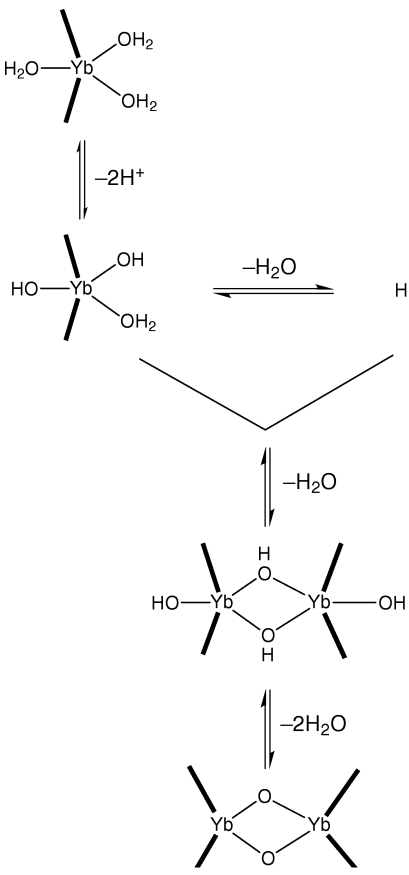
Preliminary experiments with the analogous $[TbL^2]^{3+}$ and $[EuL^2]^{3+}$ complexes revealed that with an excitation wavelength of between 300 and 370 nm in H_2O or D_2O (with and without degassing), no measurable metal-based luminescence could be detected. Even in the presence of 2 equiv. of trifluoroacetic acid and with excitation by a laser at 355 nm, no metal-based emission was observed. For $[EuL^2]^{3+}$ this lack of luminescence may be a direct consequence of efficient competitive photoinduced electron transfer. It is more probable that in both cases the ligand-based triplet is too low in energy to facilitate energy transfer (ET) to the emissive Eu and Tb states or else there is a very inefficient population of the aryl triplet.[23]

Ytterbium complexes possess a much lower emissive state (at 10 200 cm^{-1}) and so sensitisation is possible from aryl triplets that are much lower in energy.[24] Accordingly, the analogous ytterbium complex of L^2 was prepared and its luminescence properties studied in water and methanol. The triplet energy of $[YbL^2]^{4+}$ was measured by examining the triplet phosphorescence emission (0–0 transition) in a EtOH–MeOH glass at 77 K and was found to be 220 kJ mol^{-1}, *i.e.*, below the level of the emissive $^5D_0$ and $^5D_4$ states of Eu and Tb. The lifetime of the ytterbium luminescence ($\lambda_{em} \approx 980$ nm) was monitored in protiated and deuteriated solvents (Table 2) allowing the quenching effect of bound (and proximate) OH oscillators to be assessed using eqn. (9):

$$q = A(k_{H_2O} - k_{D_2O}) - B \qquad (9)$$

where $A = 1.0$ μs,[24] k_{H_2O/D_2O} are the quenching rate constants ($k = 1/\tau$) and B is a term representing the quenching effect of closely diffusing water molecules ($0.1 < B < 0.3$).[24] The value of q determined in this way was 2.8 (± 0.2), consistent with the presence of three bound, quenching water molecules. Given that ytterbium is one of the smallest lanthanides and therefore usually possesses a coordination number that is lower than (or the same as) that of Eu/Gd/Tb, it is very likely that the $[GdL^1]^{3+}$ complex also possesses three bound water molecules in acidic aqueous solution, *i.e.*, below pH 6.

The luminescent lifetime of the choride salt was measured at pH 12 in H_2O and D_2O and the emissive lifetime was much longer. In H_2O, the value was 2.79 μs and in D_2O, at the same base concentration, the value was 5.14 μs. Using eqn. (9), and taking B to be 0.2, then $q = 0$ (± 0.2). Clearly there are no longer any quenching OH oscillators bound to the Yb ion. A tentative scheme that accounts for this behaviour has been formulated (Scheme 1). Nucleophilic attack by a metal-bound OH group on an eight-coordinate aqua complex generates, after elimination of water, a bis(μ-oxo) dimer. In the dimer there are no bound OH groups and each Yb centre may be eight-coordinate. This tentative postulate is in accord with the neutralisation of at least two protons per Yb centre (see Scheme 1) deduced from the potentiometric titration on the analogous Gd complex. The formation of such dimeric species has ample precedent in the hydrolysis of a variety of aqua ions,[25] although in this case, further oligomerisation must be inhibited by the bulky hexadentate ligand.



Scheme 1

Table 2 Radiative rate constants for $[YbL^{2+}]^{4+}$ (293 K). Errors on rate measurements are ±10%

Solvent	$k/\mu s^{-1}$
H_2O (pH 5.5)	3.09
H_2O (pH 12)	0.36
D_2O (pD 5.1)	0.11
D_2O (pD 11.6)	0.19
CH_3OH	0.60
CD_3OD	0.967

In summary the gadolinium complex described possesses a high relaxivity that is pH dependent in the range 6 to 10. The pH dependence offers the possibility of examining, by MRI methods, pH gradients *in vivo*. The decrease of the relaxivity may be ascribed to formation of a dimeric complex that possesses no bound water molecules or hydroxyl groups. Support for this assertion is provided by the luminescence studies on the analogous Yb complex at pH 5.5 and 12. Indeed, this is the first time that Yb lifetime measurements have been applied to define the solution hydration state of a complex.

Experimental

Luminescence

Lifetime measurements were carried out using the signal observed with a nitrogen-coded germanium photodiode/amplifier (North Coast EO-817-P) operating in high-sensitivity mode. Decays were analysed by iterative reconvolution and nonlinear least-squares analysis of the instrument response profile with a single exponential function.[24] Good fits were obtained in all cases, as judged by the randomness of the residuals and a satisfactory (<1.05) reduced χ^2 value. An excitation wavelength of 355 nm was used for $[YbL^2]^{4+}$, using the third harmonic of a Nd-YAG laser.

Relaxometry

Variable temperature proton solvent longitudinal relaxation times were measured at 20 MHz on a Spinmaster spectrometer [Stelar, Mede (PV), Italy] by means of the inversion-recovery technique (16 experiments, 4 scans). The reproducibility in T_1 measurements was within less than 1%. The temperature was controlled by a Jeol airflow heater equipped with a copper thermocouple: the temperature in the probehead was measured with a Fluke 52 k/j digital thermometer with an uncertainty of 0.5 K.

The water proton NMRD profiles were measured on a Koenig–Brown field-cycling relaxometer over a continuum of magnetic fields from 2.4×10^{-4} to 1.2 T, corresponding to 0.01 to 50 MHz proton Larmor frequency. The temperature inside the probe was controlled by circulation of perfluoroalkanes. Data at higher field strength were obtained from Jeol EX-90 and EX-400 spectrometers.

Synthesis and characterisation

UV spectroscopy was carried out with a Shimadzu UV-160A spectrometer. Electrospray mass spectra were recorded in positive ion mode with a VG-Platform II using 10 μmol dm^{-3} solutions of complex. Matrix-assisted laser desorption ionisation time-of-flight mass spectra were recorded on a Linear Scientific LDI 1700 machine in the negative mode: samples were dissolved in a matrix solution containing ammonium citrate and 2,4,6-trihydroxyacetophenone (THA).

$[GdL^1]Cl_3 \cdot 5H_2O$. To a suspension of the terpyridine ligand **1**[7] (5.0 g, 12.6 mmol) in methanol (500 cm^3) was added gadolinium chloride (4.67 g, 12.6 mmol) and the mixture was heated slowly under reflux to give a clear yellow solution, which changed into a thicker yellow suspension. A solution of 2,6-diacetylpyridine (2.59 g, 15.1 mmol) in methanol (50 cm^3) was added, together with a few drops of concentrated hydrochloric acid. The mixture was boiled under reflux, giving a clear orange solution. After 4 days, the solution was cooled and the solvent evaporated to give an orange solid. The orange residue was dissolved in the minimum volume of cold methanol and diethyl ether was added to precipitate an orange solid (9.9 g, 98%). The complex could be recrystallised from aqueous isopropanol. R_f 0.29 [SiO_2; MeOH–H_2O–sat. aq. KOAc (1 : 1 : 1)]. ν_{max} (ε, H_2O) 364 (17100), 249 nm (38500 dm^3 mol^{-1} cm^{-1}). *m/z* (MALDI-TOF-MS) 1017 $(C_{32}H_{28}N_8Gd \cdot THA)^{2-}$ requires 1016; (ES-MS) 751.20 $(C_{32}H_{31}N_8GdClO_2)^+$ requires 751.25, 356.2 and 358.0 $[L^1GdCl - H]^+$ with good agreement between observed and calculated isotope patterns. Anal. found: C, 44.6; H, 4.40; N, 12.4; Cl, 11.7%; $C_{32}H_{28}N_8GdCl_3 \cdot 5H_2O$ requires C, 43.8; H, 4.40; N, 12.6; Cl, 12.1%.

$[YbL^2]Cl_3 \cdot HCl \cdot 6H_2O$. This was prepared, as described above, using L^2 [5] and $YbCl_3$. ν_{max} (ε, H_2O) 363 (17000), 250 nm (38700 dm^3 mol^{-1} cm^{-1}). *m/z* (MALDI-TOF-MS) 783 $(C_{32}H_{29}N_9YbCl_2)^-$ requires 783; (ES-MS) 782.8. Anal. found: C, 39.8; H, 4.3; N, 13.1; Cl, 14.7%; $C_{32}H_{29}N_9YbCl_3 \cdot HCl \cdot 6H_2O$ requires C, 39.8; H, 4.3; N, 13.1 Cl, 14.7%.

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