DALTON
PERSPECTIVE

Getting excited about lanthanide complexation chemistry

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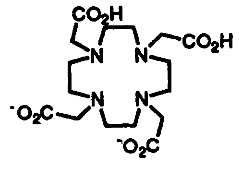
Lanthanide complexation chemistry in solution is an active research theme because of the importance of gadolinium complexes as contrast agents in magnetic resonance imaging, and of luminescent europium and terbium complexes as probes in biochemistry. The physicochemical principles and theory underpinning this work are being refined to account for recent developments such as the reappraisal of complex hydration state.

Over the past ten years there has been a renewal of interest in the complexation chemistry of the lanthanides and the rare-earth elements. Whereas a quarter of a century ago activity was focused on the use of complexes of Eu, Pr and Yb as shift reagents for NMR spectroscopy, now the interest centres on the *in vivo* application of related paramagnetic gadolinium complexes as commercially important contrast agents in magnetic resonance imaging.[1] In luminescence research attention has shifted from the development of solid-state phosphors (in television screens) and lasers [*e.g.* yttrium aluminium garnet (YAG) lasers] to solution-state methods exemplified by the development of time-resolved luminescent assays.[2] Our own interest in the complexation chemistry of these elements was triggered by the need to devise complexes of the β-emitting radioisotope, ^{90}Y, that were stable with respect to dissociation *in vivo*.[3] Yttrium-90 is a therapeutic radioisotope that has sufficient energy (2.25 MeV, *ca.* 3.6×10^{-13} J) to cause cell death following double-strand DNA cleavage.[4] By targeting the cytotoxic isotope selectively to tumour cells, effective radiotherapy results provided that the isotope is not prematurely released from the targeting vector. What was required for this work therefore was a ligand that bound ^{90}Y rapidly in aqueous solution (^{90}Y, $t_{\frac{1}{2}}$ = 64 h) to form a complex that resisted acid- or cation-mediated decomplexation *in vivo*. Octadentate macrocyclic ligands based on cyclen (1,4,7,10-tetraazacyclododecane) were suitable for this purpose and representative examples include L^1 (called dota) and its phosphinate analogues L^2.[4,5] Functionalised derivatives of L^1 and L^2 were prepared that allowed conjugation of the ligand to a suitable targeting vector, such as an engineered human antibody fragment (Fab),[3] as occurs in the trivalent conjugate L^3.[6]

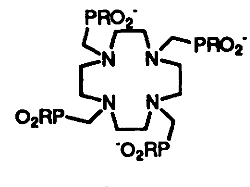
The complexation chemistry of yttrium closely resembles that of the late-middle lanthanides and this work highlighted the structural features that are required for the formation of a complex that is kinetically stable in acidic aqueous media.

Structure and Stability of Lanthanide Complexes

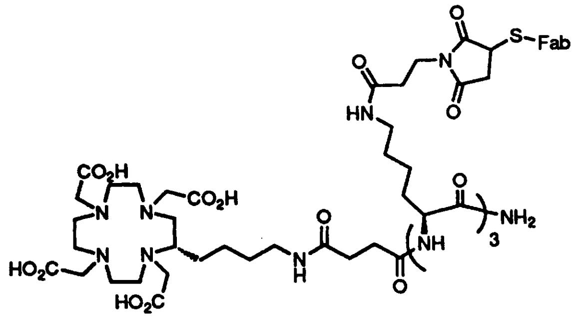
The most common co-ordination numbers of lanthanide ions are eight and nine. In order to form a stable complex in aqueous solution the ligand must possess donor atoms that satisfy the demands of a hard, polarising lanthanide ion. Thus, among neutral donors, more polarisable amine nitrogens are preferred to ether oxygens, and donors possessing large ground-state dipole moments, such as carboxamides and sulfoxides, interact better in ion–dipole and/or ion-induced dipole bonds than less polar substituents such as alcohols. Of course, hard anionic donors bind well to the triply charged centre, so that carboxylates, phosphonates and phosphinates are attractive groups. Thus compounds such as L^1 (dota),[7] L^4 (dtpa) and L^2 [8] form well defined 1 : 1 complexes with all of the lanthanide ions which possess both high formation constants and, more



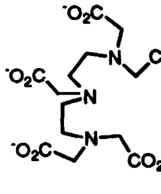
L^1 (dota)



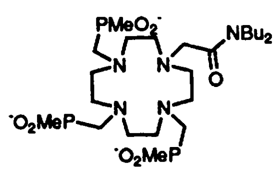
L^{2a} R = Me
L^{2b} R = CH$_2$Ph



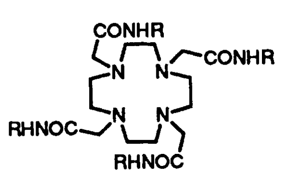
L^3



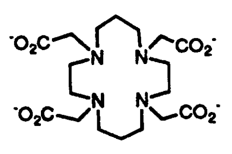
L^4 (dtpa)



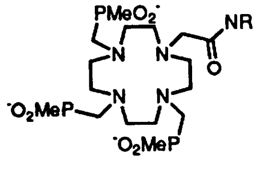
L^5



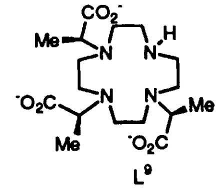
L^{6a} R = H
L^{6b} R = Me



L^7 (teta)



L^8



L^9

importantly, are kinetically inert with respect to proton- or cation-mediated dissociation pathways (Table 1).[4,5] Of course, the stability with respect to acid-catalysed dissociation is a sensitive function of the overall charge on the complex: the charge neutral monoamide complex of L^5 dissociates eight times more slowly than the monoanionic analogue.[5] The nature of the anionic donor is also important, and the more acidic phosphinates, L^2, form complexes that tend to resist protonation and hence exhibit excellent kinetic stability in acidic media.[5]

The importance of electrostatic repulsion is highlighted in the kinetic stability of cationic lanthanide complexes. The gadolinium complex of L^{6a} apparently possesses a 1 : 1 formation constant (log β_{ML}) of only 10.6,[10] yet the corresponding europium complex is remarkably stable with respect to dissociation [$t_{\frac{1}{2}}$ (60 °C, pH 2) = 50 d].[11]

Effect of Charge

A large family of compounds has now been defined that is based upon the macrocyclic skeleton 1,4,7,10-tetraazacyclododecane (cyclen). This compound, appended with three or four additional donors, figures prominently in the definition of important lanthanide complexes. The cyclen ring is predisposed to bind co-operatively to the face of a square antiprism, and crystal structures of tetraamides, tetracarboxylates (*e.g.* 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate or dota, L^1) and tetraphosphinates based on cyclen (*e.g.* L^2) clearly show that the macrocyclic ring adopts the same 'quadrangular' conformation in the free form and in the lanthanide complex (*e.g.* Fig. 1).[8] Depending upon the charge on the donor group, cationic, charge-neutral or anionic complexes may result (Scheme 1). Of the anionic complexes, the complexes of dota (L^1) are the most important. Following the pioneering work of Desreux,[7] the anionic gadolinium complex of dota[12] is now used as an extracellular contrast agent for general use in magnetic resonance imaging (MRI). The related gadolinium phosphinate complexes, L^2, do not possess a metal-bound water molecule[8] and their lipophilicity may be controlled through variation of the substituent at phosphorus.[9,13] The tetrabenzylphosphinate, L^{2b}, for example, forms anionic complexes $[LnL^{2b}]^-$ that bind with some avidity to proteins (*e.g.* albumin, $K_d = 3 \times 10^{-4}$ dm^3 mol^{-1}) and this lipophilic anion is recognised *in vivo* by the 'organic anion transporter' found in liver cells which usually handles species such as bilirubin.[8,9] This non-covalent binding interaction perturbs the biodistribution of the complex and leads to predominant biliary, rather than renal, clearance of the complex.

Table 1 Parameters defining the kinetic and thermodynamic stability of selected gadolinium complexes[a]

Complex	log β_{ML} (298 K, $I = 0.1$ mol dm^{-3})	$10^3\ k_{obs}{}^b$/s^{-1} (310 K), pH 1	pK_a of complex[c] (298 K, $I = 0.1$ mol dm^{-3})
$[Gd(dota)]^-$	25.8	3.2×10^{-3}	2.8
$[GdL^{2a}]^-$	19.8	10.4×10^{-3}	1.3
$[GdL^5]^-$	—	1.3×10^{-3}	< 1.1
$[Gd(dtpa)]^{2-}$ [d]	22.5	4.6	(2.4)[e]

[a] Data from refs. 4, 5, 9 and 1. [b] Measured rate of dissociation of the complex, leading to loss of the gadolinium ion. [c] Protonation constant of the given complex. [d] The corresponding diamide complexes dissociate more rapidly. [e] This value seems rather low.

The ligands possessing an L_5X_3 donor set have been the subject of much interest because injection of their complexes does not lead to significant pain as may occur when charged complexes are administered, *e.g.* $[GdL^4]^{2-}$.[14,15] This is an important factor in the clinical use of such complexes. For example, a typical adult may receive 3–4 g of $[GdL^1]^-$ ('Dotarem') or $[GdL^4]^{2-}$ (Magnevist), and the high osmolality of the complexes may lead to discomfort. The neutral complex of L^{10} marketed as 'Prohance' (Scheme 1) does not suffer from such a disadvantage.[14] Finally, cationic complexes are being studied as species that may promote RNA cleavage by phosphate ester transesterification.[11] Effort has focused on octadentate tetraamides, and the larger La^{3+} complexes, adopting higher co-ordination numbers (nine, ten), have been shown to promote RNA cleavage whereas corresponding complexes of the smaller Eu^{3+} ion (eight, nine) are inactive. Evidently complexes of Eu and Yb with triamide derivatives of cyclen having two available binding sites may be worthy of further investigation. Cationic lanthanide complexes of octadentate ligands bind phosphate anions in aqueous solution[16] and the lanthanide complexes of L^{6b} have recently been shown to act as extracellular ^{31}P NMR shift reagents for inorganic phosphate in serum, allowing the measurement of intra- *versus* extra-cellular phosphate concentration.[17]

Stereoisomerism in Macrocyclic Lanthanide Complexes

The majority of stable lanthanide complexes are formed with octadentate ligands and the most common polyhedra for a co-ordination number of eight are the square antiprism and the

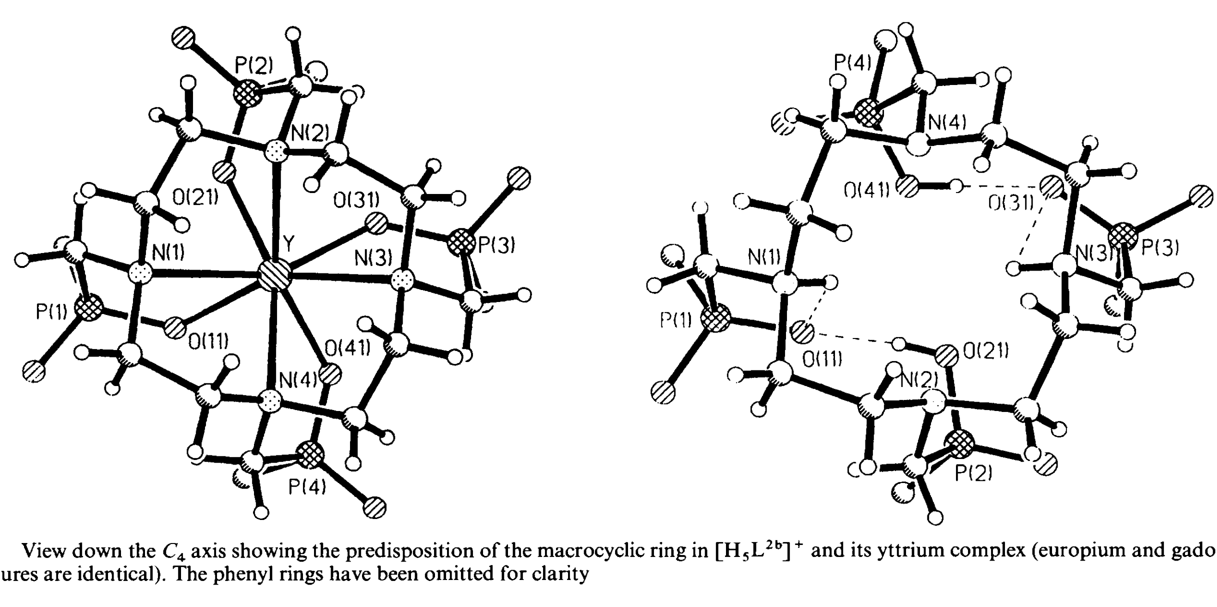
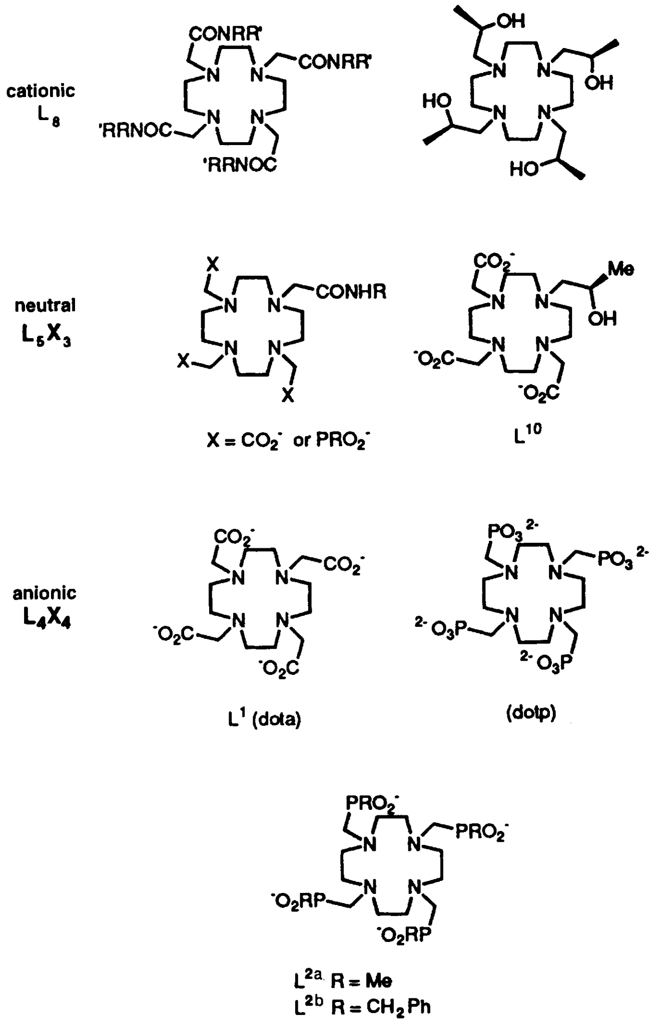


Fig. 1 View down the C_4 axis showing the predisposition of the macrocyclic ring in $[H_5L^{2b}]^+$ and its yttrium complex (europium and gadolinium structures are identical). The phenyl rings have been omitted for clarity



Scheme 1 Structure of representative compounds based on cyclen that form stable lanthanide complexes

dodecahedron.[18] The latter geometry is known with the terbium complex of 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetate or teta (L^7), albeit in a rather distorted co-ordination polyhedron.[19] The former is more commonly found, and occurs[8,20] in the isostructural complexes of Y, Gd and Eu with L^{2b} where the angle of twist around the four-fold axis is only 29° which is well short of that found in an idealised antiprism (45°). Such a geometry may be termed an 'inverted square antiprism' to distinguish it from the more regular square-antiprismatic geometry found with lanthanide complexes of L^1 (dota), for which the twist angle is 40° and in which a single water molecule caps the O_4 face.[7,12,21] In aqueous solution, NMR studies on [Ln(dota)] complexes have pinpointed the existence of both of these stereoisomers[10,22] ('inverted' and regular square antiprism) which are in relatively fast exchange on the NMR time-scale. Interconversion may occur through a concerted rotation of the acetate arms while maintaining the ring in a rigid [3333] conformation (Scheme 2).[22,23] Each of these complexes is chiral, and substitution at C on the macrocyclic ring or at the carboxymethylene group renders the corresponding complexes diastereoisomeric. The analogous tetraphosphinate complexes $[LnL^2]^-$ exist as one predominant diastereoisomer in solution ($\geq 90\%$), that possesses an *RRRR* (or *SSSS*) configuration at the four stereogenic phosphorus centres.[8] When one of the phosphinate groups is replaced by a carboxamide substituent, *e.g.* L^8, then 32 isomers of the lanthanide complex may exist. One chiral diastereoisomer predominates in solution again, presumably with an *RRR* or *SSS* configuration at phosphorus. In the shifted ^{31}P NMR spectra of lanthanide complexes of L^8 (*e.g.* R = R = Me or CH_2Ph) three resonances are observed for the diastereotopic phosphorus nuclei in the major diastereoisomer. For the ytterbium complex the ^{31}P NMR shift may be strictly related to geometrical coordinates of the P nuclei. The paramagnetic shifts are 'pseudo-contact' in origin and therefore may be closely linked to the polar coordinates of the P nuclei. Taking advantage of the magnetic field dependence of the Curie-spin relaxation mechanism, ^{31}P relaxation rate measurements as a function of magnetic field allow P–Yb distances (r) to be computed [equation (1)] that are in excellent agreement

$$R_{1p} = D_1\tau_S + \frac{\chi_1 f(\tau_R) B_0^2}{r^6} \qquad (1)$$

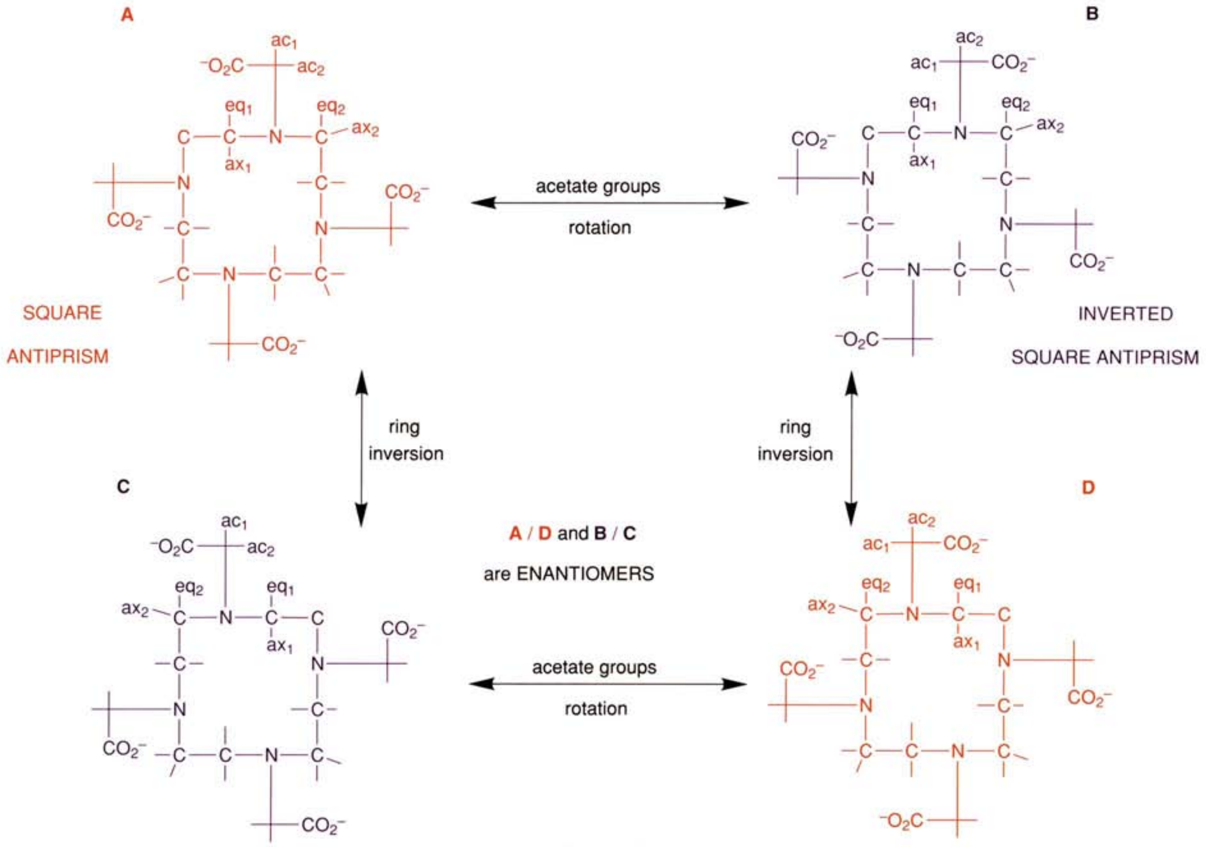
with those obtained by crystallographic measurements.[23] Here R_{1p} represents the paramagnetic contribution to the observed relaxation rate, τ_S is the electronic relaxation time for the Yb^{3+} ion ($\approx 10^{-13}$ s), B_0 is the applied field strength, D_1 and χ_1 are proportionality constants and τ_r is the reorientational correlation time which may be estimated to be 100 ps, by analogy with those of the corresponding gadolinium complexes.[24]

Lanthanide Luminescence in Solution

Under favourable conditions, many of the lanthanide(III) ions are able to exhibit long-lived luminescence following excitation into higher electronic states. This phenomenon is often observed in the solid state, where there is often little competition from other non-radiative deactivation processes. The property has been exploited in phosphors for television screens and in the ubiquitous YAG lasers, to name but two examples.[25,26] The observation of intense, long-lived emission from lanthanide compounds in solution is less common owing to a number of additional, highly competitive non-radiative deactivation pathways which are likely to arise under such conditions, especially in water.[27,28] Before going on to describe the challenges faced in preparing lanthanide complexes which are highly emissive in aqueous solution, it is useful to begin by outlining why one might wish to do this in the first place.

Solution assays

The use of lanthanide complexes as luminescent labels for analyses in biological media has attracted a great deal of attention, in particular as an alternative to radioimmunoassay.[2,29,30] This technique is widely used in the detection and quantification of important biological molecules. Typical systems consist of two components: a targeting group which may be a protein or antibody fragment raised by immunological methods to recognise selectively and bind strongly to the target molecule of interest; and a reporter group which incorporates a radioisotope to allow detection at extremely low levels. It has long been recognised that luminescence can potentially provide an equally sensitive detection method where the obvious drawbacks and problems associated with the handling and disposal of radiolabelled substances do not arise. A number of systems are indeed available which make use of fluorescent reporter groups.[29] The concept is by no means limited to immunological assays, however. Systems based on simpler, reversible targeting species for detecting smaller molecules may be envisaged, for example the use of small peptides,[31] nucleic acid strands[32] or cyclodextrins.[33] In such cases the use of a luminescent label may provide further advantages over radioisotopes as the binding of the analyte may cause a direct effect on the luminescence, for example a triggering or quenching of the emission or a shift in the emission or excitation wavelength maxima. In addition to detection systems of this



Scheme 2

sort, such luminescent compounds may also be used as 'tags' to monitor the fate of molecules or ions within cells.

In any application intended for use in biological media, however, the use of fluorescent labels faces a disadvantage, namely the problem of background fluorescence leading to decreased sensitivity. Any biological sample, including serum, is likely to contain a large number of fluorescent compounds [the aromatic amino acids tyrosine and tryptophan, for example, along with substances such as reduced nicotinamide adenine dinucleotide (NADH) or bilirubin] and will therefore exhibit substantial and variable levels of background fluorescence. This interferes with the signal from the fluorescent label and cannot be corrected for properly. Scattered light and Raman bands are further potential problems, especially where the Stokes shift is small.

Lanthanide probes

The use of luminescent lanthanide complexes potentially provides an elegant solution to this problem. The background fluorescence of biological materials is almost invariably short-lived compared to the long luminescence lifetimes which may be observed for Eu^{III} and Tb^{III} (the latter are of the order of milliseconds, compared to typical fluorescence lifetimes in the nanosecond to microsecond region). This allows a time-resolved detection procedure to be employed, whereby a delay is set between the excitation pulse and the measurement of the lanthanide luminescence, during which time the background fluorescence and scattered light decay to negligible levels. Provided that the luminescence decay is highly reproducible, the measured luminescence over the integration time will be directly proportional to the concentration of the analyte. In principle, any phosphorescent molecule with a sufficiently long phosphorescence lifetime could be used in this way. However, in most cases, the observation of long-lived phosphorescence requires the use of deoxygenated solutions and, in many cases, low temperatures, in order to reduce competitive deactivation processes. The attraction of the lanthanides lies in the fact that long-lived luminescence may be observed under ambient conditions. Two other advantages of lanthanide luminescence may be cited. First their emission spectra show bands which are very narrow and remarkably insensitive to environmental changes: the wavelengths barely shift more than ± 2 nm on changing conditions such as temperature or indeed the co-ordination site. Secondly, the use of sensitised emission gives rise to a large Stokes shift (often > 200 nm) such that there is unlikely to be any overlap of the emission bands with strong absorbance bands: concentration-dependent self-absorption problems therefore do not arise.

Despite these attractions, there are **three important problems** which need to be dealt with if lanthanide luminescence is to be exploited in such applications. One obstacle is that the bands observed in lanthanide(III) absorbance spectra are normally very weak, with molar absorption coefficients usually < 1 dm^3 mol^{-1} cm^{-1}.[34] This stems from the fact that the transitions of interest involve states of the same f^n configuration. Electric dipole transitions between such states are parity (Laporte) forbidden. Whilst the interaction with the ligand field does relax this selection rule to some extent, the weak nature of the interaction means that the oscillator strength remains low and the absorbance bands are weak. The outcome is that the excited states are not readily populated by conventional light sources. Obviously, this poses a disadvantage for the use of lanthanides as luminescent probes as it places an unsatisfactory limit on detection levels. Fortunately, sensitised emission provides a possible solution to this problem.[35] The concept here is that the ligand should incorporate a chromophore (sometimes referred to as an antenna[36]) which absorbs strongly at a suitable wavelength and transfers its excitation energy to the metal which, in accepting this energy, becomes excited to the emissive state. If the antenna has a high absorption coefficient and the energy transfer process is efficient, then the 'effective' molar absorption coefficient of the metal is vastly increased and

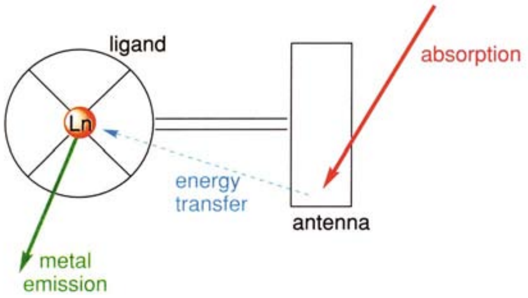


Fig. 2 The principle of sensitised emission: the antenna effect. Energy transfer is favoured by a short distance between the antenna chromophore and the metal ion

intense luminescence may result following excitation by conventional light sources (Fig. 2).

A second problem relates to the deactivation of the metal emissive states by vibrational energy transfer often primarily involving the solvent water molecules as alluded to earlier. This effect arises through an energy transfer process from the metal excited state to the O–H stretching vibrations of co-ordinated or closely diffusing water molecules.[28] The underlying theory is discussed in more detail later; here it should be noted that it serves to reduce both the lifetime and intensity of the emission and is indeed so profound for most of the aqueous lanthanide ions as to reduce their lifetimes from the theoretical values in the millisecond region to less than 2.5 μs.[34] Only for Gd^{3+}, Eu^{3+} and Tb^{3+} the lifetimes remain in excess of 0.1 ms in aqueous solution. Most research has focused on the latter two since the emission of Gd^{3+} occurs at an inconvenient wavelength in the UV (312 nm) whereas the other two ions emit in the visible. Even for these ions, however, the deactivating effect of water molecules leads to a reduction of an order of magnitude in the intensity of emission. Thus, in the design of luminescent lanthanide complexes, a second function of the ligand becomes apparent, namely to shield the metal as efficiently as possible from the solvent water molecules.

The final problem to be addressed concerns the stability of the complex with respect to metal ion dissociation. Since the applications in mind are concerned with the detection of molecules at low concentration, it is clearly essential that metal dissociation is negligible at high dilution, otherwise quantitative measurements will not be possible. In other words, suitable complexes should display very low dissociation constants (high association constants) in water over an appropriate pH range and also offer high kinetic stability. The most luminescent complexes of the lanthanide ions are those formed with certain β-diketone ligands.[37] However, the majority of these neither have sufficient water solubility nor stability for use in aqueous solution.

In summary, the challenge in this area lies in the preparation of lanthanide complexes which are simultaneously strongly luminescent and stable under aqueous conditions. Scheme 3 shows some representative examples of the types of compound which have been investigated in the pursuit of this goal.

Luminescent lanthanide complexes

The preparation of the tris(bipyridine) cryptand **1** in the mid-1980s[38] initiated a good deal of work on the use of 2,2′-bipyridine as an antenna chromophore.[39–41] The europium and terbium complexes of this cryptand displayed bipyridine-sensitised metal emission although the intensities were limited by competing photophysical processes as explained in subsequent sections below.[42] This ligand is only modestly effective at shielding the metal from the solvent water molecules with about 2.5 H_2O molecules estimated to be co-ordinated. The branched macrocyclic compound **2** is superior in this respect.[41]

A number of research groups have examined the luminescence properties of lanthanide complexes of calixarenes.[39] The calix[4]arenes have received the most attention. For example, europium and terbium ions have been encapsulated in the tetraacetamide **3**; the complexes are relatively stable and are water soluble.[43] Energy transfer from the phenyl groups to the metal ions is observed. Some related calix[4]arenes have been prepared in which two or four of the amide groups are replaced by bipyridine[44] groups. However, whilst some favourable observations were made concerning the luminescence properties of these complexes, it should be noted that all of the reported measurements were carried out in MeCN. The luminescence efficiency in water would be expected to be substantially lower, owing to incomplete shielding from the solvent water molecules with their O–H deactivating effect. Similarly, the work of Sato and Shinkai[45a] on analogous aryl ketone-appended calixarenes shows that, whilst good results are obtained in MeCN, the luminescence intensities fall dramatically in MeOH and would be expected to be lower still in water. Analogous problems would be expected to arise in the case of some recent work on phenanthrene-substituted calixarene complexes,[45b] in which it is likely that the calixarene aryl ether groups serve as the antenna for the bound terbium ion, rather than the phenanthryl group itself.

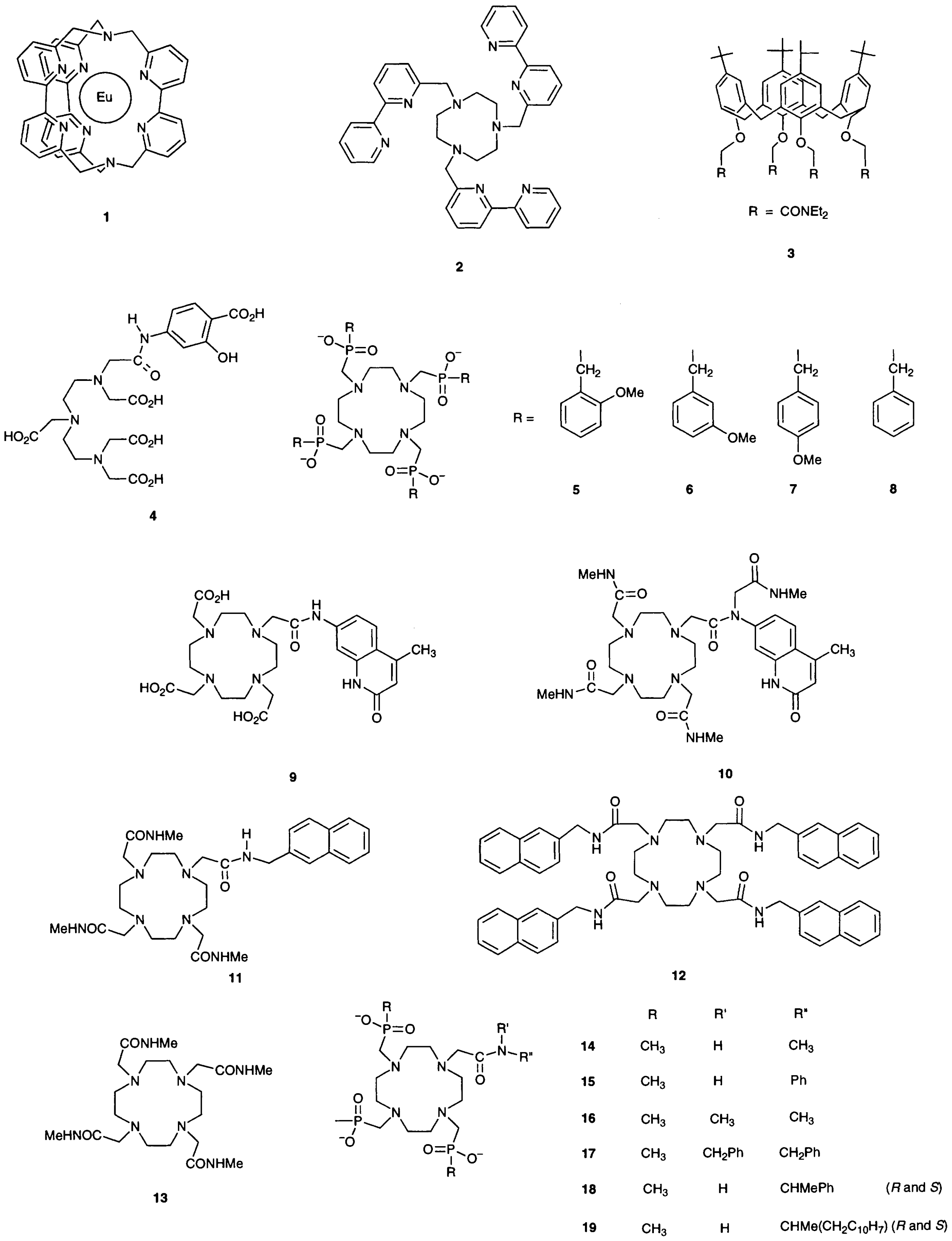
Polyaminopolycarboxylates and related ligands have also been studied. The acyclic compound L^4 (dtpa) forms complexes of high thermodynamic stability with lanthanide ions[46] (log K_{ML} *ca.* 22). Attempts have been made to append it with a strong chromophore in the hope that this would act as an antenna and give rise to strongly luminescent, stable lanthanide complexes. The reaction of the anhydride of dtpa with *p*-aminosalicylic acid gave a suitable compound **4** which formed a luminescent terbium complex.[47] It was proposed in the original report that the OH and CO_2^- groups of the aryl ring may bind to the metal, enhancing both the complex stability and energy transfer efficiency. However, it is clear from the structure that if the amide carbonyl binds to the metal then there is no way in which these groups will also be able to bind. In fact, this compound was not purified or characterised and may therefore have consisted of a mixture of dtpa and dtpa-*p*-aminosalicylic acid adducts. Nevertheless, the reagent has been linked to proteins by reaction with the NH_2 group of a suitable amino acid and a number of subsequent studies with proteins, hormones and oligonucleotides have revealed favourable detection limits.[48] Complexes based on polyazamacrocyclic ligands have also been examined, usually incorporating an antenna chromophore. Examples involve compounds **5–19** which possess additional carboxylate, carboxamide or phosphinate co-ordinating arms (appropriate references are provided in subsequent sections).

Photophysical Properties which need to be investigated

In assessing the suitability of a given lanthanide complex for use as a luminescent probe, a number of photophysical properties need to be investigated and, if possible, optimised. First, it must be possible to excite the complex at a suitable wavelength. Biological samples absorb strongly below about 330 nm; excitation wavelengths below this value are therefore undesirable as only a small proportion of the incident light will be absorbed by the complex, with a resulting loss of sensitivity. Detection limits will be at their lowest if the absorbance of the antenna is strong; hence a high absorption coefficient at the excitation wavelength is required.

Secondly, if a single light-emitting species is present and there is no quenching of the emissive state by other excited state molecules, then monoexponential decay of the luminescence is expected. That is, the intensity at time t after an excitation pulse will be given by equation (2) where I_0 is the intensity at time t =

$$I_t = I_0 \exp(-k_{obs}t) \qquad (2)$$



	R	R'	R"	
14	CH_3	H	CH_3	
15	CH_3	H	Ph	
16	CH_3	CH_3	CH_3	
17	CH_3	CH_2Ph	CH_2Ph	
18	CH_3	H	CHMePh	(*R* and *S*)
19	CH_3	H	$CHMe(CH_2C_{10}H_7)$ (*R* and *S*)	

Scheme 3

0. The decay curve is characterised by k_{obs}, the observed rate constant for deactivation of the emissive state, which is given by the sum of the radiative rate constant (k^0) and the first-order rate constants for competing non-radiative processes (k^{nr}), equation (3). It is usual to quote the lifetime, τ, of the emission, which is simply the reciprocal of k_{obs}.

$$k_{obs} = k^0 + \Sigma k_i^{nr} \qquad (3)$$

A long luminescence lifetime is clearly a requirement for time-resolved assays. Lifetime measurements also provide an indication of the efficiency of the light-emitting process (*cf.* quantum yields below): a long lifetime (approaching the calculated natural luminescence lifetime, $\tau^0 = 1/k^0$) indicates that non-radiative processes are not competing efficiently with luminescence.

Finally, the quantum yield of emission is perhaps the most useful piece of information, being the ratio of the number of photons emitted to the number of photons absorbed and hence a direct measure of the efficiency of the emission process. The emissive quantum yield φ_{em} of an excited electronic state is the ratio of the radiative rate constant k^0 to the sum of all rate constants for processes that deactivate the emitting state [equation (4)] and as such is the fraction of molecules that are

$$\varphi_{em} = \frac{k^0}{k^0 + \Sigma k_i^{nr}} \tag{4}$$

deactivated by emission of light. Here, Σk_i^{nr} is the sum of the rate constants for unimolecular or pseudo-unimolecular non-radiative deactivation processes. The quantity $(k^0 + \Sigma k_i^{nr})^{-1}$ is the observed luminescence lifetime τ_{obs} as discussed above and hence we obtain expression (5) which indicates that there is a

$$\varphi_{em} = k^0\tau_{obs} \tag{5}$$

direct dependence of the quantum yield on the luminescence lifetime. Of course this reflects the fact that a high quantum yield is more likely to be obtained when the non-radiative pathways of deactivation are not able to compete efficiently with luminescence. For ligand-sensitised lanthanide luminescence, the overall emissive quantum yield will also be dependent both on the efficiency of formation of the state from which energy transfer occurs (in most cases this is thought to be the T_1 state of the antenna) *and* on the efficiency of the energy transfer process itself, η_{et}. Thus equation (6) is applicable where φ_D is the

$$\varphi_{em} = \varphi_D\eta_{et}k^0\tau_{obs} \tag{6}$$

quantum yield of formation of the antenna state from which the energy transfer occurs, following excitation into ground state absorption bands. The value of k^0 is not expected to vary substantially from one complex to another. Hence, in designing efficient luminescent lanthanide probes, it is necessary to optimise the quantities φ_D and η_{et} (determined by the choice of antenna), and τ_{obs}, governed by the co-ordination environment of the metal in the complex.

Factors influencing the efficiency of energy transfer

The occurrence of antenna-to-metal energy transfer (as opposed to direct metal excitation) may be probed by recording a luminescence excitation spectrum. In such an experiment the emission intensity at a given wavelength, at which the metal is known to emit strongly (*e.g.* 545 nm for terbium, 619 nm for europium), is monitored as a function of the excitation wavelength. The resulting excitation spectrum shows the profiles of the band or bands responsible for metal emission. A close resemblance to the UV/VIS absorption spectrum of the antenna chromophore is indicative of sensitised emission and energy transfer. An example is provided by $[TbL]^-$ (aq) (L = **5** in Scheme 3) for which the metal luminescence excitation spectrum is shown in Fig. 3 with the UV absorption spectrum overlaid. The absorbance band at 270 nm is that of the methoxybenzyl chromophore and the close match with the excitation spectrum is firm evidence for energy transfer to the metal.[49]

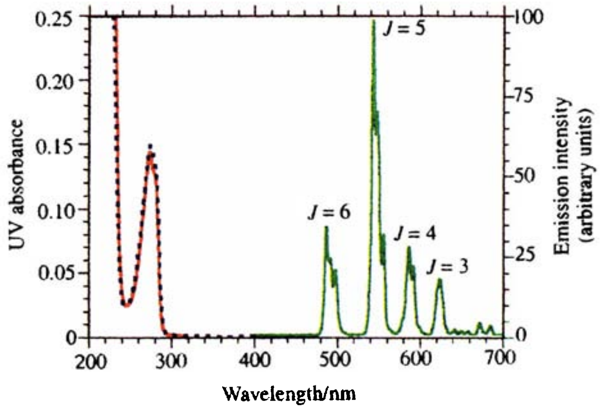


Fig. 3 Blue: metal luminescence excitation spectrum of $[TbL]^-$ in aqueous solution (6.5×10^{-5} mol dm^{-3}, 295 K); emission monitored at 545 nm. The red line overlaid is the UV absorption spectrum of this solution. Green: corrected metal luminescence emission spectrum of the same solution, 1 ms after excitation at 276 nm. The bands arise from $^5D_4 \longrightarrow {}^7F_J$ transitions; the *J* values of the bands are labelled

There are two well known theories concerned with the mechanism of energy transfer in general, namely Förster theory which treats the process as a purely through-space effect occurring by a dipole–dipole mechanism, and the theory of Dexter which invokes electron exchange and requires significant overlap of the electron clouds of the donor and acceptor.[50] Common to both theories, however, is a dependence on the extent of overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor. In other words, energy transfer should be favoured by a matching of the pertinent energy levels of the donor and acceptor, with the former preferably being slightly the higher in energy in order to ensure that the forward process is exothermic.

Whilst this prediction has been vindicated to a large extent, a problem arises if the energy gap is too small. Under such circumstances, thermally activated back energy transfer can occur from the excited electronic state of the metal to the triplet state of the antenna, which may subsequently be deactivated through the usual pathways. Such a process will clearly lead to a reduction in both the intensity and lifetime of the emission and the effect will be more significant at higher temperatures. Indeed such a mechanism was first put forward in the 1960s in order to account for the temperature-dependent quantum yields and lifetimes observed for some terbium complexes of β-diketone ligands.[37,51] The advent of laser flash photolysis and the technique of transient absorption spectroscopy has allowed such processes to be investigated in more detail. The terbium complex of the cryptand **1** provides an example which has been studied in considerable detail.[42] Here, the energy gap between the triplet state of the bipyridine and the emissive $^5D_4$ level of the metal ion was estimated to be 14 kJ mol^{-1}. Luminescence lifetimes were of the order of six times longer at 77 K compared to those at 300 K and the intensities were affected similarly. It was also noted that degassing of the solutions at room temperature resulted in a substantial increase in intensity and lifetime, despite the fact that it is widely observed that oxygen has little direct effect on lanthanide luminescence. This was interpreted in terms of the efficient deactivation of aryl triplet states by triplet oxygen: the bipyridine triplet will be deactivated in this manner and the observation of such a profound effect of dissolved molecular oxygen is thus strong evidence in support of an equilibrium between the aryl donor and metal acceptor levels at room temperature.

Factors limiting sensitised terbium luminescence

In the case of terbium, where the emissive state is quite high in energy (Fig. 4), this problem puts a relatively stringent lower limit on the energy of the antenna triplet. Clearly, the effect is reduced as the energy gap increases and may not be distinguished if an insufficient range of temperatures is

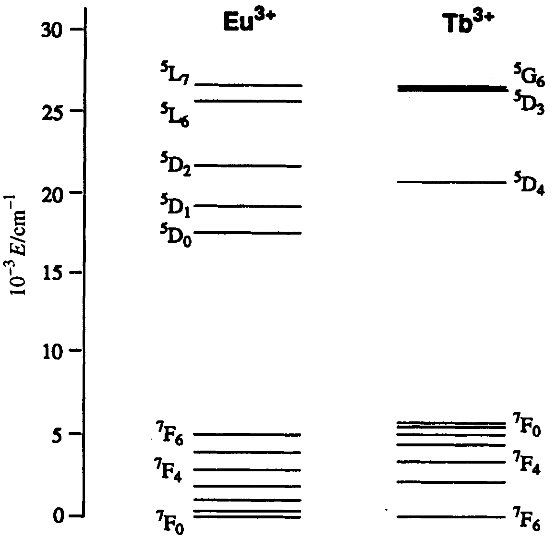


Fig. 4 The lower energy levels of Eu^{3+} and Tb^{3+}

examined. For example, there is apparently no significant back energy transfer to the quinolinone triplet in [TbL] [52] (H_3L = **9** in Scheme 3) (and in some related compounds containing the same chromophore) although, in the structurally related complex [TbL]$^{3+}$ (L = **10** in Scheme 3), albeit involving a tertiary amide rather than a secondary amide link, a significant back energy transfer process is operative at room temperature.[53] Clearly then small structural changes can have a profound effect on the efficiency of energy transfer, as was noted in some calixarene lanthanide complexes.[45b] At the other extreme, where the energy gap is very small, the effect of oxygen and temperature is enormous. Thus, for [TbL]$^{3+}$ (L = **11** in Scheme 3), for example, where the naphthyl triplet was estimated to lie only 4–5 kJ mol^{-1} above the $^5D_4$ state of the metal, an aerated aqueous solution at room temperature displayed negligible metal emission following naphthyl excitation, whilst a degassed solution under the same conditions exhibited an excellent quantum yield of 0.1.[54] Other effects which diminish the deactivation pathways available to the antenna triplet states should also lead to an enhancement in metal luminescence. For example, addition of β-cyclodextrin to an aqueous solution of [TbL]$^{3+}$ (L = **11**) of appropriate concentration leads to a significant increase in both τ and φ, reflecting the fact that the triplet naphthyl formed as a result of the back energy transfer is subject to a reduced degree of non-radiative deactivation (*e.g.* triplet–triplet annihilation) on formation of an adduct with the cyclodextrin[54] (*cf.* the enhanced *phosphorescence* which is commonly observed for aryl groups included inside cyclodextrins[55]).

Whilst there is a tendency to focus on the matching of the antenna triplet level with the emissive state of the metal ($^5D_4$ for Tb or $^5D_0$ for Eu), it is also important to consider the possible transient involvement of higher metal electronic states. For example, the benzyl and methoxybenzyl groups have been found to be particularly efficient sensitisers for terbium (*e.g.* complexes involving ligands **5**–**8**, quantum yields φ_{em} for which are about 0.5) despite the fact that the energy gap between the aryl triplet and the metal $^5D_4$ emissive level is very large (about 100 kJ mol^{-1}).[49,56] The high efficiencies observed in these cases probably reflect the fact that the higher $^5D_3$ level (313 kJ mol^{-1}) is well matched with the triplet levels of the aryl groups (literature values for toluene and methoxybenzene, possible model compounds, are 346 and 338 kJ mol^{-1} respectively).[57] It is likely that energy transfer initially occurs to this higher metal state which, once populated, undergoes rapid internal conversion and vibrational relaxation to the emissive $^5D_4$ state.

Factors limiting sensitised europium luminescence

Back energy transfer is less likely to be a problem for europium as its emissive state ($^5D_0$) is substantially lower in energy than that of terbium ($^5D_4$) (Fig. 4). Sensitisers having lower triplet energies may therefore be employed. However, in the case of europium, another problem crops up regularly. Owing to the relative ease of reduction of Eu^{3+} (f^6) to Eu^{2+} (f^7), competitive non-radiative deactivation of the excited states of the antenna, especially the singlet state, may occur through photoinduced electron transfer from the chromophore to the metal, with transient formation of Eu^{2+} and a radical cation. A recent example is provided by [EuL]$^{3+}$ (L = **11** in Scheme 3) where the naphthyl-sensitised metal emission was found to be very weak (φ *ca.* 10^{-4} in aqueous solution).[58] Evidence that this is due to photoinduced electron transfer deactivating the naphthyl excited singlet state is provided by the *fluorescence* spectrum of the naphthyl moiety: comparison with equiabsorbent solutions of the complexes of Gd^{3+} and Tb^{3+} showed that the fluorescence intensity in the case of Eu^{3+} was only 10% that measured for the complexes of the other two metals. Photoinduced electron transfer is disfavoured in these cases owing to the relative inaccessibility of Tb^{II} and Gd^{II}. A similar effect occurs for [EuL]$^{3+}$ (L = **10**), albeit less pronounced.[53]

Photoinduced electron transfer or charge transfer

The significance of competitive photoinduced electron transfer (PET) is expected to depend on the free energy change ΔG_{ET} for the electron transfer process. This quantity may be estimated from the difference in the energy of the excited singlet state E_S of the chromophore and that required to bring about the electron transfer process, as calculated from the appropriate reduction and oxidation potentials, equation (7). The final term reflects

$$\Delta G_{ET}/\text{J mol}^{-1} = F[E_{\frac{1}{2}}^{ox}(\text{aryl}) - E_{\frac{1}{2}}^{red}(\text{Eu}^{III}\text{-Eu}^{II})] - E_S - \Delta G_C \quad (7)$$

the attractive potential energy between the radical ion pair formed, which favours the PET process, and has a value of about 5 kJ mol^{-1} for an aryl-to-metal distance of 7 Å.[57a] Using the relevant values for 2-methylnaphthalene as a model for the naphthyl group in compound **11** ($E_{\frac{1}{2}}^{ox}$ = 1.45 V, E_S = 374 kJ mol^{-1})[57b] and the standard electrode potential for Eu^{3+}–Eu^{2+} (aq) of −0.35 V, we can *crudely* estimate ΔG_{ET} for PET from the S_1 state of 2-methylnaphthalene to Eu^{3+} to be $F[1.45 - (-0.35)] - 374 \times 10^3 - 5 \times 10^3 = -205$ kJ mol^{-1}. In view of this highly exothermic free energy change, it is not surprising that electron transfer is of such significance in this system. An intermolecular study has shown that k_Q for electron-transfer quenching of naphthalene by Eu^{3+} has a value of 7.2×10^9 dm^3 mol^{-1} s^{-1} in MeCN.[59]

Clearly the value of $E_{\frac{1}{2}}^{ox}$(aryl) is critical in determining the extent to which PET competes. Sensitising chromophores which are not readily oxidised should be preferable. An elegant study is provided by Abusaleh and Meares,[60] who examined complexes of ethylenedinitrilotetraacetate (edta) appended with aromatic sidechains. The cyanobenzyl group was found to be 15 times more efficient at sensitising emission of Eu^{3+} than the methoxybenzyl analogue, an observation which is probably related directly to the difference in oxidation potentials ($E_{\frac{1}{2}}^{ox}$ = 3.3 V for 4-cyanotoluene, 1.8 V for 4-methoxytoluene in MeCN).

In general terms, the rate of any photoinduced electron transfer process is strongly dependent on the distance between the donor and acceptor. If the distance is small then the rate may be comparable with that of the absorption of a photon, in which limit the process is better described as ligand-to-metal charge transfer (l.m.c.t.) and gives rise to additional bands in the UV spectrum. Again, however, their effect is to reduce the proportion of the absorbed light energy which finds its way to the triplet and is subsequently transferred to the metal. A well documented example of this effect is provided by the europium(III) complexes of cryptands such as **1** and cryptand [221] (4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane) where l.m.c.t. involves the amine lone pairs. In the latter case the l.m.c.t. bands are clearly visible in the 270–300 nm region.[39]

Deactivation Pathways determined by the Co-ordination Environment

Effect of water

The deactivating effect of water molecules was first noted by Kropp and Windsor[28] in the 1960s who pioneered the investigations into the effect. They showed that the luminescence of Eu^{3+} and Tb^{3+} was more intense in D_2O than in water. Moreover, the ratio of the emission intensity of a given state of Ln^{3+} in D_2O to that of the same state in water bore, at least to some extent, an inverse relation to the energy gap between the emissive state and the next lower level. The luminescence lifetimes were affected similarly. They concluded that the quenching of luminescence in aqueous solutions occurred *via* energy transfer into O–H stretching vibrations and that the rate was proportional to the number of O–H oscillators associated with the lanthanide ion. In general terms, the probability of an A–B oscillator becoming excited from the ground vibrational level, v'', to a higher level, v', decreases rapidly as v' increases, owing to the poorer Franck–Condon overlap of the two wavefunctions. Thus, energy transfer into O–D vibrations is much less efficient than for O–H as the former has a smaller vibrational stretching frequency: bridging of a given energy gap ΔE will require excitation to a higher vibrational level, v', for D_2O than for water. This gives rise to the observed enhancement of the luminescence intensity in D_2O. This interpretation also accounts for the observed variation in the magnitude of the effect for the different lanthanide ions: energy transfer into O–H (or O–D) vibrations becomes less probable as the energy gap ΔE between the emissive and next lower level increases. For example, the effect is more pronounced for Eu^{3+} (ΔE = 12 300 cm^{-1}) than for Tb^{3+} (14 700 cm^{-1}). Subsequent work showed that the deactivating effect of co-ordinated water molecules could be partitioned from the other modes of depopulation of the emissive state, equation (8) where ${k_{obs}}^{H_2O}$ is the observed

$$ {k_{obs}}^{H_2O} = k^0 + \Sigma {k_i}^{nr} + k_{OH} \qquad (8) $$

luminescence decay constant in water, k_{OH} is the rate constant for non-radiative energy transfer to the O–H oscillators of co-ordinated water molecules and the other terms have the meanings given earlier. In D_2O the k_{OH} term may be neglected and so equation (8) reduces to (9). Hence we obtain expression

$$ {k_{obs}}^{D_2O} = k^0 + \Sigma {k_i}^{nr} \qquad (9) $$

(10). The work of Heller and of Haas and Stein[61] showed that

$$ \Delta k_{obs} = {k_{obs}}^{H_2O} - {k_{obs}}^{D_2O} = k_{OH} \qquad (10) $$

OH oscillators act independently in the de-excitation process and so k_{OH} is directly proportional to the number of water molecules, q, associated with the metal ion, equations (11) and

$$ \Delta k_{obs} = k_{OH} = A'q \qquad (11) $$

(12) where τ is the luminescence lifetime (reciprocal of k) and A'

$$ q = A_{Ln}(\tau_{H_2O}^{-1} - \tau_{D_2O}^{-1}) \qquad (12) $$

and A_{Ln} are proportionality constants specific to a given lanthanide ion, Ln.

Empirical proportionality constants (for europium and terbium complexes) have been determined by Horrocks and Sudnick[62] from a study of several crystalline solids and solutions where the number of co-ordinated water molecules was known in the solid state from X-ray crystallography. Plots of $\Delta\tau^{-1}$ *versus* the known number of co-ordinated water molecules, q, were linear, with high correlation coefficients, in agreement with the hypothesis. The proportionality constants obtained were $A_{Eu} = 1.05$ and $A_{Tb} = 4.2$ for τ in milliseconds in equation (12). Thus, lifetime measurements in H_2O and D_2O may be used to provide an estimate of the hydration state of lanthanide complexes.

Metal-bound and unbound water molecules: are intermediate cases possible?

The luminescence lifetime of $[TbL^{2b}]^-$ in water was found to be 4.13 ms.[56] This is an exceptionally long lifetime, indeed it appears to be the longest reported to date for a terbium complex in aqueous solution. It indicates that competitive, non-radiative deactivation pathways for the metal emissive state are minimal in this compound. In particular, it implies that the ligand is shielding the metal very efficiently from solvent water molecules. In D_2O the lifetime was only slightly higher, 4.44 ms, and application of equation (12) provides a value of 0.07 for the hydration state, q. Other observations, both in the solid state (crystal structures of the related yttrium, gadolinium and europium complexes[8]) and in solution [the shifts in the ^{17}O NMR resonance of water induced by the dysprosium(III) complex and the nuclear magnetic relaxation dispersion (NMRD) profile of the Gd^{3+} complex, see below] confirm that there are no metal-bound water molecules and that the complex approaches the limit of purely 'outer-sphere' behaviour. This is an unprecedented observation for lanthanide complexes based on the [12]aneN_4 (1,4,7,10-tetraazacyclododecane) skeleton. That the value of q as determined by the luminescence results is non-zero implies that deactivation of the excited state may occur over longer distances than those associated with 'bound' water molecules. With respect to this possibility, the related phosphinate complexes of compounds **14**–**19** provide an interesting series.[23,63] The q values have been calculated in each case from the lifetime measurements in water and D_2O. All of these neutral amide complexes display non-integral q values between 0 and 1, which are significantly higher than those obtained for the tetraphosphinate complexes (limiting outer-sphere behaviour) but lower than the values for $[Eu(dota)]^-$ and $[Tb(dota)]^-$ which, as is well known, have only one metal-bound water molecule.[7]

At first sight one possible explanation would be that the compounds exist in solution as a mixture of isomers, some of which have $q = 0$ and others having $q \geqslant 1$. The observed q values would then be averages, determined by the proportions of these isomers in solution. However, such an explanation can be dismissed on the grounds that solution NMR studies have shown that there is only one major isomer in solution.[23] Moreover, only one $\Delta J = 0$ transition is observed in the high resolution emission spectra of the europium complexes, which is also very strong evidence for the presence of a single predominant isomer (see below).

A second possibility is simply to dismiss the non-integral q values as arising from the error inherent in the method of analysis: Horrocks quotes an error of ±0.5 in q. This is an unsatisfactory explanation especially bearing in mind the high reproducibility of the lifetime measurements. It should be noted that the Horrocks A_{Ln} values are purely empirical parameters being derived from a study of a limited number of complexes and a correlation with the number of metal-bound water molecules as obtained from crystal structure analyses. The plots were derived from complexes where q took integral values from

0 to 9 in the solid state but there is of course no guarantee that the behaviour in solution will be the same as that in the solid state.

Indeed, irrespective of such empirical calculations and the possibility of an intrinsic error therein, one is faced with a group of complexes which display Δk values (*i.e.* $k_{H_2O} - k_{D_2O}$ as defined earlier) which are intermediate between those observed for the complexes of compounds **5**–**8** ('pure' outer sphere) and those of dota (L^1) (one metal-bound water). This *must* reflect a degree of deactivation by water O–H oscillators that is rather less than in a model 'one-bound water' complex (*e.g.* those of dota) but is more significant than in 'purely' outer-sphere complexes such as those of **8**. One obvious possibility is that the equilibrium position of the nearest water molecule is further away from the metal than in dota complexes. One would certainly expect the O–H deactivating effect to be strongly distance dependent. In assessing this possibility the information provided by an analysis of the NMRD profiles of related gadolinium(III) complexes has proved to be useful (see below).

Possible role of other high energy X–H vibrations

The efficiency of O–H bonds at deactivating the lanthanide emissive states stems from their high vibrational stretching frequencies (*ca.* 3400 cm^{-1}). It may be noted that the N–H bonds of amides and amines have rather similar stretching frequencies, in excess of 3000 cm^{-1}, whilst C–H bonds are also relatively high (2900–3100 cm^{-1}). The possibility of metal deactivation through energy transfer to such bonds is not usually considered. If present, it could of course be represented explicitly through separation of the pertinent rate constants from the term Σk_i^{nr} in equation (8) as in equation (13). Recent

$$k_{obs}^{H_2O} = k^0 + \Sigma k_i^{nr} + k_{OH} + k_{NH} + k_{CH} \qquad (13)$$

work on the europium(III) complexes of [12]aneN_4-based ligands incorporating secondary amide co-ordinating arms, –CONHR, has shown that deactivation through energy transfer into amide N–H bonds can indeed occur.[57,64] Following dissolution of the europium complexes of compounds **11**–**13** in D_2O, rapid N–H/N–D exchange occurs and this leads to a lengthening of the luminescence lifetime by an extent which is substantially greater than that predicted to arise solely from the substitution of the single metal-bound H_2O molecule by D_2O. This is reflected in the q values which are 1.3–1.4 in each case, rather than the expected value of 1. Measurements in MeCN before and after deuteriation have confirmed this explanation and the effect of a single N–H bond was estimated to be 75 s^{-1} (*cf.* 450 s^{-1} for an inner-sphere O–H oscillator). It is interesting that, whilst the deuteriation at N occurred effectively instantaneously for the europium complexes, no such effect was observed for the analogous terbium complexes, or for the yttrium analogues where exchange took several hours as monitored by ^1H NMR spectroscopy. This may be ascribed to the ease of reduction of the europium complex (Eu^{III} to Eu^{II}) in such a way that the europium acts as a charge sink in the H/D exchange mechanism, promoting amide enolisation (Scheme 4). A related deactivating effect of an amine N–H bond has been noted previously with the seven-co-ordinated europium complex of a ligand based on [12]aneN_4, wherein the single ring secondary amine underwent NH/ND exchange and led to a lengthening of the excited state lifetime.[65]

An analogous observation has also been made concerning C–H bonds following an examination of $[Eu(dota)]^-$ in D_2O.[64] After heating such a solution to 60 °C for 18 h at pD 11, 90% deuterium incorporation into the acetate groups was obtained; *i.e.* an average of 7.2 of the eight methylene C–H protons had undergone exchange. Deuteriation does not occur at ambient temperature and pH. The luminescence decay constant was found to be reduced by 180 s^{-1} following deuteriation. This amounts to an average effect of about 25 s^{-1} per C–H oscillator. As expected in view of the lower vibrational stretching frequency, the effect is smaller than that caused by proximate O–H or N–H oscillators. The quenching effect will be a sensitive function of the distance and the orientation of the oscillator with respect to the metal. Thus in $[Eu(dota)]^-$, where the $CH_2CO_2^-$ hydrogens are only 3.5 Å from the europium ion, the effect is well defined but in $[EuL^{2b}]^-$ exchange of the more distant benzylic hydrogens (4.1 Å from the Eu) led to no significant effect.

Scheme 4 Possible role of the europium ion in promoting N–H/N–D exchange

Characteristics of the Lanthanide Emission Spectra

A representative emission spectrum for a terbium complex is shown in Fig. 3, that of $[TbL]^-$ (L = **5** in Scheme 3).[49] Four intense bands are observed, arising from transitions from the emissive $^5D_4$ level to the $^7F_6$, $^7F_5$, $^7F_4$ and $^7F_3$ levels of the ground manifold. Two of the important features mentioned earlier, namely the sharpness of the bands and the large Stokes shift, are clearly apparent. Some fine structure is visible in the first three bands. This arises from the removal of the degeneracy of the $2J + 1$ states per J level which occurs on bringing a lanthanide ion from the gas phase (or a hypothetical spherical environment) into the complex where the symmetry is lower. The extent to which the degeneracy is removed depends on the symmetry of the metal's environment: complete loss of degeneracy, giving $2J + 1$ non-degenerate bands, only occurs when the symmetry is low. Owing to the large J values associated with the pertinent terbium energy levels, terbium emission bands normally consist of a large number of transitions which can rarely be fully resolved. Thus, it is not usually possible to infer much about the local symmetry of the metal ion from an analysis of the fine structure.

Europium proves to be a more useful ion for this purpose, owing to the lower J values associated with the observed transitions. In particular, the $^5D_0 \longrightarrow {}^7F_0$ transition (579 nm) is unique for a given chemical environment, since both the initial and final states are non-degenerate.[66] The number of components observed for this transition is then related to the number of chemically distinct environments of the Eu^{3+} ion and the transition has been used both in excitation and emission as a probe of sample homogeneity. For example, the 0–0 bands for $[EuL^{2b}]^-$ and $[EuL^8]$ (R = R′ = Me) at high resolution are shown in Fig. 5. It is clear that there is only one band in each case, consistent with the presence of a single predominant isomer (enantiomers are not resolved through this technique).

Some trends in the intensities of the europium bands have also become apparent.[63] The emission spectra of $[EuL^{2b}]^-$ and $[EuL]^{3+}$ (L = **12** in Scheme 3) are shown in Fig. 6. The $\Delta J = 1$ band is magnetic dipole allowed and is little affected by changes in the co-ordination environment, whilst the $\Delta J = 2$ band is an electric dipole transition and is sensitive to the environment. Careful integration of the band intensities shows that the $\Delta J = 2/\Delta J = 1$ intensity ratio is substantially higher for the former. Moreover, the intensities of the two components within the $\Delta J = 2$ band are reversed relative to one another in the two complexes. Such changes may be related to the different co-ordination geometries of the two complexes; the former possesses an inverted square antiprismatic structure[8] whilst the

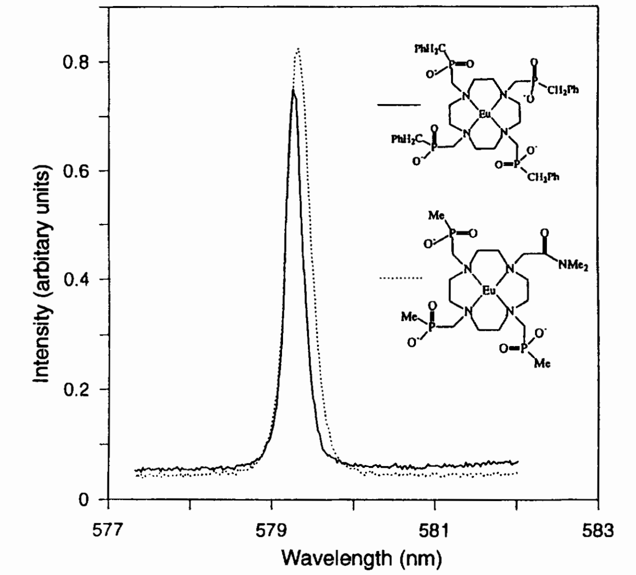


Fig. 5 High resolution emission spectra of the 0–0 transition of $[EuL^{2b}]^-$ (solid line) and $[EuL^8]$ (dotted line) following excitation of the metal at 553.43 nm (water, 298 K). The bands have been normalised to similar intensities for comparison

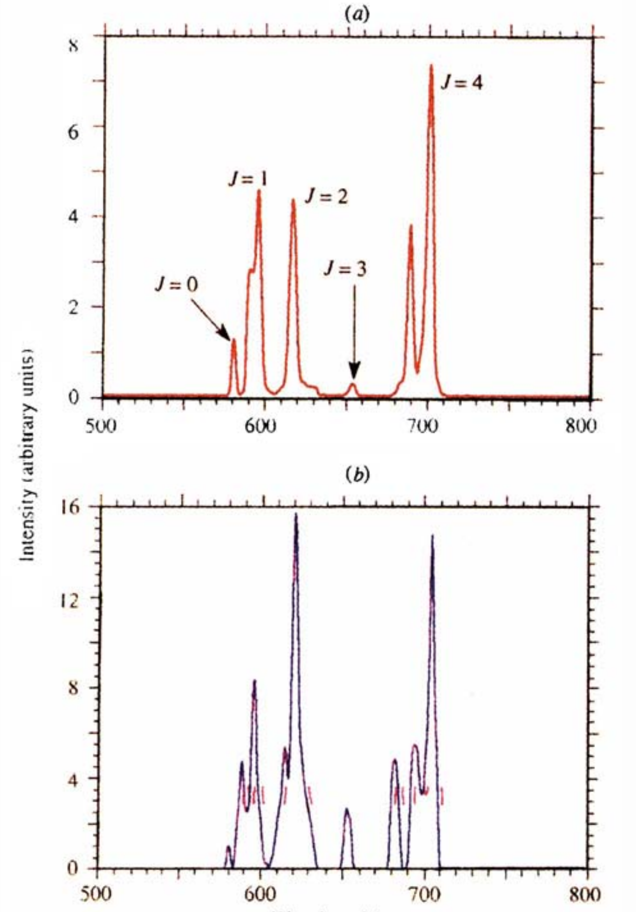


Fig. 6 Corrected metal emission spectra of (*b*) $[EuL^{2b}]^-$ (blue) and (*a*) $[EuL]^{3+}$ (L = **12** in Scheme 3) (red) in D_2O (293 K). Excitation and emission slit widths of 10 and 2.5 nm respectively were used

tetraamide complex possesses a square antiprismatic geometry, very similar to that adopted by $[Eu(dota)]^-$.[7]

Circularly Polarised Luminescence (CPL)

Enantiomeric complexes give rise to identical emission spectra. One technique that does allow discrimination between enantiomers, however, is that of CPL.[67] This phenomenon arises from the differential emission of right and left circularly polarised light by a chiral complex and is particularly well suited to the lanthanides owing to the high intensities of emission which may be achieved under ambient conditions. No commercial instrumentation is available for the measurement of CPL; most of the instrumental arrangements which have been described in the literature are essentially identical to a commercial fluorescence spectrometer but with the addition of a circular analyser between the sample and the emission monochromator.

The quantities measured in the CPL experiment are $\Delta I = I_L - I_R$ and $I = I_L + I_R$ where I_L and I_R are proportional to the number of left and right circularly polarised photons emitted, respectively. A CPL spectrum is a plot of ΔI against wavelength; its features may be used to obtain information on the chirality of the excited state of the molecule and may provide a 'fingerprint' which is unique for a given structural geometry. By analogy with circular dichroism, it is usual to report a luminescence (or emission) dissymmetry ratio, $g_{lum}{}^{\lambda}$, equation (14). Observation

$$g_{lum}{}^{\lambda} = \Delta I/0.5I \tag{14}$$

of CPL normally requires the sample to consist of a single enantiomer or, failing that, an excess of one enantiomer over the other. A racemic mixture is no use as the effects for the two enantiomers will be equal and opposite and therefore cancel each other out (*cf.* the lack of optical rotation for a racemic mixture). The use of circularly polarised excitation of the sample provides a way round this limitation as it allows one enantiomer of a racemate to be preferentially excited over the other, to an extent which is determined by the values of $g_{abs}{}^{\lambda}$ at the excitation wavelength [$g_{abs}{}^{\lambda}$ is the absorbance dissymmetry factor of circular dichroism, given by $(A_R - A_L)/\frac{1}{2}(A_L + A_R)$ where A_L and A_R are the absorbances of left and right circularly polarised light respectively]. Circularly polarised emission may then be detected in the same way as if there were an excess of this enantiomer in the mixture. The measured luminescence dissymmetry factor g_{obs} is then given by equation (15).

$$g_{obs} = \tfrac{1}{2} g_{abs}{}^{\lambda ex} g_{lum}{}^{\lambda em} \tag{15}$$

The transitions which display the largest values of g_{abs} and g_{lum} are those which obey magnetic dipole selection rules, that is $\Delta J = \pm(2n + 1)$. The CPL properties of the complexes of Eu^{3+} and Tb^{3+} with L^{2b} have been examined by Jim Riehl and co-workers at Michigan Technological University.[67b] The complexes consist of a 1 : 1 mixture of enantiomers (*RRRR* and *SSSS* in the inverted square antiprismatic geometry). Separation of the two enantiomers has not been achieved to date and so the use of CP excitation is essential if any CPL is to be detected. For $[TbL^{2b}]^-$ the ${}^7F_6 \longrightarrow {}^5D_4$ transition was excited by means of an argon ion laser. Here, $\Delta J = -2$ but, despite the low enantioselectivity (low $g_{abs}{}^{\lambda}$) expected for this transition, some surprisingly strong CPL was observed for the ${}^5D_4 \longrightarrow {}^7F_5$, ${}^7F_4$ and ${}^7F_3$ emission bands. Maximum g_{obs} values were about 0.03, 0.015 and 0.014 respectively and the spectra for the third of these are shown in Fig. 7. Clearly, these results indicate that the *emission* dissymmetry factors must be very high for this compound. This is conclusive evidence for the chirality of the terbium environment and shows that there is no interconversion of enantiomers during the excited state lifetime of the terbium. The spectra provide a useful 'fingerprint' of the specific co-ordination environment.

For $[EuL^{2b}]^-$ a new approach was adopted, namely excitation of the ${}^7F_2 \longrightarrow {}^5D_1$ transition at 553 nm using a tunable dye laser. Although the ${}^7F_2$ state is only about 1% populated at room temperature, the advantage of this approach lies in the fact that the transition satisfies the $\Delta J = \pm(2n + 1)$ selection rule, which imparts a high degree of circular dichroism

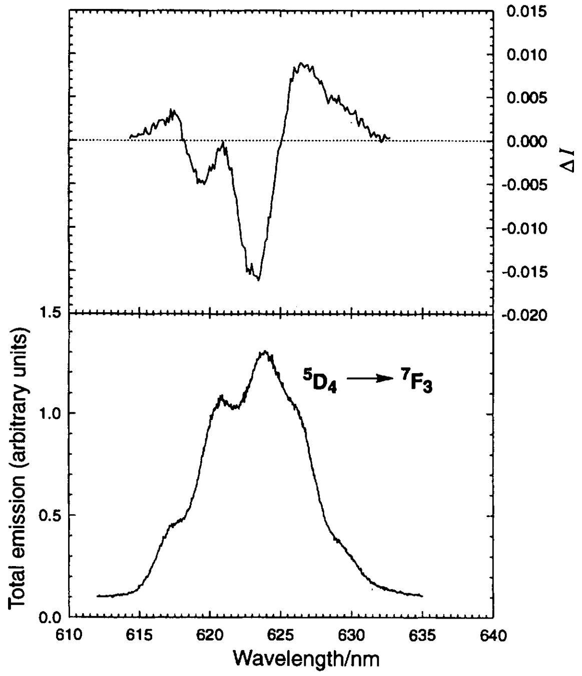


Fig. 7 Circularly polarised and total luminescence emission spectra of the $^5D_4 \longrightarrow {}^7F_3$ transition of $[TbL^{2b}]^-$ in aqueous solution on excitation at 488 nm

to the transition; *i.e.* a high g_{abs}^{λ}, reflected in large discriminations between the two possible circular polarisations. Circularly polarised emission was again detected, albeit quite weak (maximum g_{obs} is -0.016 for $^5D_0 \longrightarrow {}^7F_1$). Again, this indicates that the environment of the europium is chiral. Moreover, in the absence of a circularly polarising quarter-wave plate in the excitation beam, no CPL was observed, consistent with the presence of a racemic mixture of the two enantiomers.

Gadolinium Complexes in Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a diagnostic imaging technique that relies upon the detection of the spatially localised NMR signals of water protons.[68,69] Spatial information is obtained by making the water resonance frequency position dependent and this occurs when a gradient magnetic field is imposed on the sample. The water signal intensity is dependent upon a number of factors particularly the values of the T_1 and T_2 water proton relaxation times. The relaxation time of water protons decreases substantially when the water oxygen is close to a highly paramagnetic complex such as those of Gd^{3+} (f^7) or Mn^{2+} (d^5). The ability of the paramagnetic complex to shorten the water proton relaxation rate is defined as relaxivity, *i.e.* the increment of the water proton relaxation rate per unit concentration of the paramagnetic agent. Paramagnetic 'contrast' agents are used routinely in clinical practice to enhance the signal intensity obtained in an MRI image. Attention is focused on the Gd^{3+} ion which couples a large magnetic moment ($S = \frac{7}{2}$) with a long electron–spin relaxation time (*ca.* $10^{-9.5}$ s, *cf.* 10^{-13} s for Eu^{3+}, Yb^{3+} and Dy^{3+}), two properties which allow efficient nuclear spin relaxation.[70] The aqua Gd^{3+} ion is toxic and poorly tolerated by the body so the contrast agent must be administered as a complex that is well tolerated and is kinetically stable with respect to gadolinium dissociation for the period that the complex is in the body.

Parameters determining the Effectiveness of a Paramagnetic Contrast Agent

In general, water proton relaxation rates are altered by the presence of a contrast agent by two distinct mechanisms, outer and inner sphere. The inner sphere contribution to the relaxivity (R_p^{is}) originates from dipolar interactions between the paramagnetic ion and the proton nuclei of directly co-ordinated water molecules (q is the number of metal bound water molecules). These dipolar interactions are modulated by the molecular reorientation (τ_R), electron-spin relaxation (τ_S) and chemical exchange (τ_M) times. This latter phenomenon is usually sufficiently fast for gadolinium complexes to provide the means of transferring the interaction to the bulk water.

The outer sphere mechanism, representing the sole contribution to relaxivity (R_{1p}^{os}) for complexes without a bound water molecule ($q = 0$), involves the electron–nuclear magnetic dipolar coupling which occurs when solvent molecules diffuse close to the metal complex. In this case the interaction is modulated by the relative translational diffusion of solute and solvent, D, and by the electronic relaxation time, τ_S. The net paramagnetic contribution to the measured water proton relaxation rate (R_{1p}^{meas}) in a solution containing the metal complex is given by the sum of these two terms; equation (16). The inner sphere contribution, as described by the

$$R_{1p}^{meas} = R_{1p}^{is} + R_{1p}^{os} \qquad (16)$$

Solomon–Bloembergen–Morgan theory, is given,[70,71] by equations (17)–(19) where c is the molar concentration of the

$$R_{1p}^{is} = \frac{cq}{55.6} \frac{1}{T_{1M} + \tau_M} \qquad (17)$$

$$T_{1M} = \frac{K}{r^6} f(\tau_C, \omega_I, \omega_S) \qquad (18)$$

$$\frac{1}{\tau_C} = \frac{1}{\tau_R} + \frac{1}{\tau_S} + \frac{1}{\tau_M} \qquad (19)$$

paramagnetic complex, q is the number of bound water molecules, T_{1M} their longitudinal proton relaxation time and τ_M their mean residence lifetime in the co-ordination sites. The relaxation time of the bound water molecules [equation (18)] depends on the inverse of the sixth power of the distance r between the metal ion and the water protons, on the Larmor frequencies for the proton (ω_I) and electron (ω_S) and on the correlation time τ_C for the modulation of the dipolar interaction which is itself dependent inversely on τ_R, τ_M and τ_S [equation (19)].

The outer sphere term R_{1p}^{os} may constitute a significant contribution to the observed relaxation rate for low molecular weight gadolinium(III) complexes[72] and it is normally described through Freed's equation[73] (20) which was originally suggested

$$R_{1p}^{os} = (32\pi/405)\gamma_H^2 g^2 \mu_B^2 S(S+1) \times (N_A/1000)(C/aD) f(\tau_S, \tau_D, \omega_T, \omega_S) \qquad (20)$$

for the interaction between stable free radicals and organic solvent molecules, where N_A is the Avogadro number, a is the distance of closest approach between the paramagnetic centre and the water molecules and $\tau_D = a^2/D$. The longitudinal water proton relaxation rate is dependent on the magnetic field not only directly through equations (18) and (20), but also because the parameter τ_S which enters the same equations is itself field dependent, as given by the Morgan equation (21), where τ_{SO} is

$$\tau_S^{-1} = \frac{1}{5\tau_{SO}} \left(\frac{1}{1 + \omega_S^2 \tau_V^2} + \frac{4}{1 + 4\omega_S \tau_V^2} \right) \qquad (21)$$

the value of the electronic relaxation time at zero field and τ_V is the correlation time characterising the time dependence of the interaction responsible for the relaxation process.

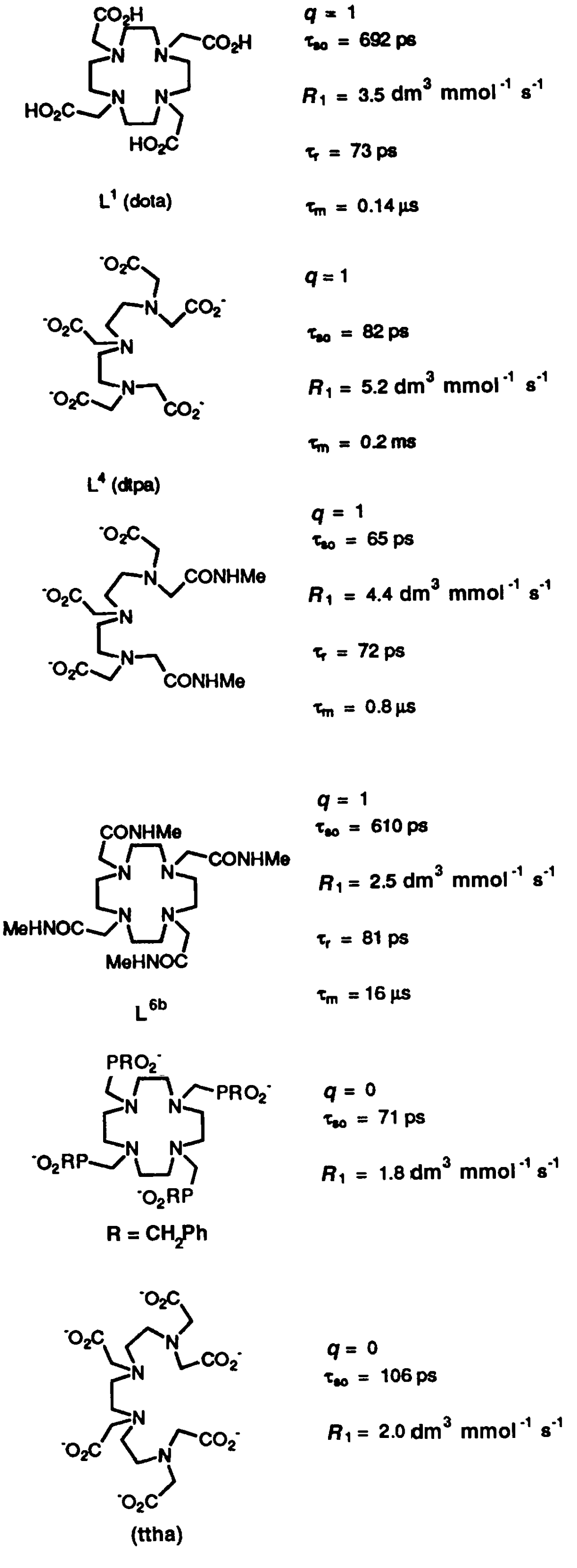
The large structural and dynamic information content of solvent relaxation rate determinations when gadolinium(III) complexes are present in solution can only be exploited through a magnetic field dependent study. Experimentally this can be done with the field-cycling relaxometer developed by Koenig and Brown,[74] that allows the measurement of longitudinal water proton relaxation rates in a magnetic field range corresponding to proton Larmor frequencies of 0.01–50 MHz. A NMRD profile is obtained, which can be analysed with the above equations. A substantial number of such profiles have been recorded over the last few years for gadolinium(III) complexes of multidentate polyaminopolycarboxylate ligands and some regular and general trends have been defined.[75] The relaxivity in the high magnetic field region ($\approx$10–50 MHz) is mainly controlled by the value of τ_R, directly proportional to the molecular volume, and this has about a 50% contribution from the outer sphere mechanism. At low magnetic fields the relaxivity receives a sizeable contribution from τ_S, whose value has been shown to depend upon the symmetry around the metal ion and the chemical nature of the co-ordinating group.[76]

Water *vs.* Proton Exchange

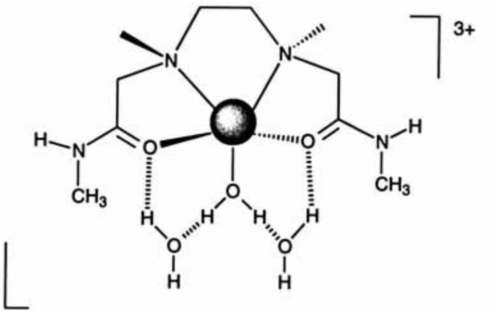
The exchange lifetime normally neither contributes to τ_C nor influences the NMRD profile since it has an estimated value of the order of tens of nanoseconds and is then shorter than T_{1M} [equation (17): fast exchange condition]. Recently, Merbach and co-workers[17,77] have reported that the mean residence lifetime τ_M, in $[Gd(dota)]^-$ and $[Gd(dtpa)]^{2-}$ is longer than initially surmised (*ca.* 0.2 μs at 298 K) but this does not limit their effectiveness as contrast agents, as T_{1M} the longitudinal relaxation time of water protons in the first co-ordination sphere [equation (17)], is still the factor limiting the proton relaxation rate. For the bis(methylamide) derivative of dtpa (Scheme 5) the water lifetime is four times longer and this is particularly evident in the temperature dependence of relaxivity where a flatter profile is observed.[78] A clear-cut example of the exchange lifetime limiting the measured relaxivity at ambient temperature is provided by the tetraamide complex, $[GdL^{6b}]^{3+}$. In this case, the mean residence lifetime is of the order of 20 μs (R_1 = 2.5 dm^3 mmol^{-1} s^{-1}, 298 K, 20 MHz) and the slowness of water exchange reduces the measured relaxivity, for this $q = 1$ complex.[64,79] The observed relaxivity is essentially due to the contribution from closely diffusing water molecules (*i.e.* outer sphere waters and those hydrogen bonding to the amide carbonyl group) (Scheme 6). The pK_a of the bound water molecule in $[GdL^{6a}]^{3+}$ is 7.90, and measurements of ^{17}O transverse relaxation times as a function of temperature prove that the Gd-bound water molecule is exchanging at the same rate at the different pH values. In the basic (pH > 8) regime, the water proton relaxation rate increases significantly {at pH 12 the relaxivity is very similar to that of [Gd(dota)]}. This behaviour is reversible and in the absence of fast water molecule exchange must involve base catalysed prototropic exchange. In this unique case then the contribution to the overall relaxivity arising from prototropic exchange has been assessed.[79]

Maximising Relaxivity

In most cases $T_{1M} \gg \tau_M$ and the relaxivity of simple gadolinium complexes at high field is a function of q and τ_R. The value of q may be measured independently for the isostructural complexes of Eu and Tb using luminescence methods.[28,62,64] When $q = 0$, as occurs in the gadolinium complexes of ttha (triethylenetetraminehexaacetate) and of L^{2b} (a cyclen-based tetrabenzylphosphinate), the relaxivity is due to the 'outer sphere' contribution only, which is of the order of 1.7 to 2 dm^3 mmol^{-1} s^{-1}. There has been much interest in devising gadolinium complexes with higher q values ($q > 1$) that will possess higher relaxivities as a result. A practical limitation is that they still must be kinetically inert *in vivo*. The compound L^9 is a step in this direction but, despite being heptadentate, q is only 1.4,[80] as measured by luminescence. When a low molecular weight gadolinium complex binds to a macromolecule the increase in τ_R leads to an increase in τ_C [equation (19)]. The rotational correlation time τ_R (typically 80–100 ps for a complex of M 500–1000) increases with increasing molecular volume leading to increased relaxivity, provided that the motion of the paramagnetic centre is coupled to that of the macromolecule to which it is linked.



Scheme 5 Relaxation parameters for representative gadolinium contrast agents of the compounds shown (298 K, 20 MHz)



Scheme 6 Possible hydration scheme around $[LnL^{6b}]^{3+}$ ($pK_a = 8.5$, 298 K). Certain counter anions may also form hydrogen bonds to the metal-bound water molecule

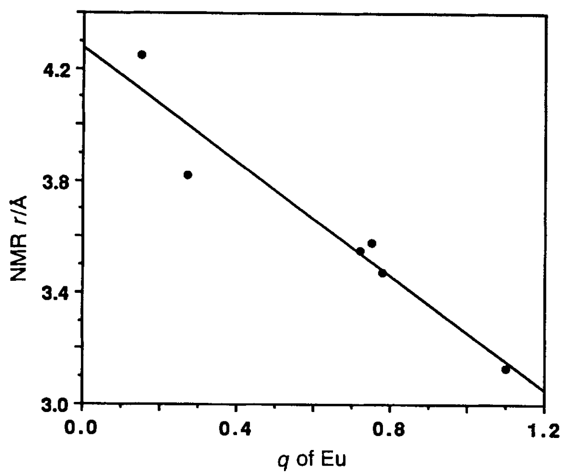


Fig. 8 Correlation of NMRD-derived distances, r, and q values measured by luminescence on complexes of Gd and Eu with L^1, L^2, L^5 and L^8

Table 2 Europium–water bond lengths in nine-co-ordinate complexes

Complex *	Average Eu–O/Å	Ref.	Number of co-ordinated water molecules, q
$[Eu(dota)]^-$	2.48	19	1
$[Eu(bopta)]^{2-}$	2.49	—	1
$[Eu(12\text{-crown-}4)]^{3+}$	2.43	82	5
$[Eu\{HO(CH_2CH_2O)_5H\}]^{3+}$	2.43	83	3
$[EuCl(18\text{-crown-}6)]^{2+}$	2.43	83	2
$[EuCl\{HO(CH_2CH_2O)_4H\}]^{2+}$	2.43	84	3
$[Eu(agly)_3]$	2.43	85	3
$[EuL^{6a}]^{3+}$	2.44	10	1
$[Eu(R^1[12]aneN_4)]^{3+}$	2.43	16	1
$[Eu(R^2[12]aneN_4)]^{3+}$	2.50	86	1

* bopta is a benzyloxymethyl derivative of dtpa; 12-crown-4 = 1,4,7,10-tetraoxacyclododecane; 18-crown-6 = 1,4,7,10,13,16-hexaoxacyclooctadecane; agly = *N*-acetylglycinate; $R^1 = CH_2C(O)NHCHMePh$; $R^2 = CH_2CH(OH)Me$.

This effect (proton relaxation enhancement) may therefore increase with molecular volume up to the point where one of the other correlation times may dominate (*e.g.* τ_S or τ_M). The relaxivity of hydrophilic complexes such as $[Gd(dtpa)]^{2-}$ or $[Gd(dota)]^-$ is the same in blood, water and soft tissue and little or no binding of the complexes to membranes or proteins occurs. With $[GdL^{2b}]^-$, an outer sphere agent, $q = 0$, non-covalent binding to serum albumin occurs ($K_d = 2.8 \times 10^{-4}$ $dm^3\ mol^{-1}$)[8] and the relaxivity increases by a factor of four. Larger relaxivity gains are achieved for $q = 1$ complexes when the gadolinium complex is covalently linked to a macromolecule.[81] In the future, systems which combine a long τ_{SO} value, a short τ_M and involve a stable paramagnetic gadolinium complex whose motion is coupled to that of a biocompatible macromolecule will provide a second generation of contrast agents. They may find use in blood-pool imaging, for example. At the same time, gadolinium complexes which will target specific tissues or organs or respond to particular biochemical stimuli *in vivo* are being sought. Targeted agents are required that bind non-covalently to a receptor site with sufficient avidity to allow good localisation but not too strongly otherwise the rate of clearance will be adversely affected and undesirable toxic effects are more likely to result. A challenge indeed for a chemist!

The Degree of Hydration of Lanthanide Complexes

An additional parameter which may be obtained from the fitting of observed NMRD profiles of gadolinium complexes is the distance between the paramagnetic centre and the water proton [equations (18) and (20)]. This information may then be compared with that obtained by luminescence methods where the mean hydration state q is evaluated. The fact that not only bound-OH oscillators but also closely diffusing OH oscillators contribute to the quenching of the europium excited state (in a distance dependent manner) strongly suggested that non-integral 'q' values are quite reasonable[64] (see above). For a series of structurally closely related complexes (*e.g.* of L^1, L^2, L^5 and L^8) in which one major isomer is present in solution and for which partial hydration was suggested ($0 < q < 1.3$), the correlation of the NMRD-derived distance r with the hydration state q, determined by europium luminescence is good (Fig. 8). This supports the notion that lanthanide complexes (or indeed any metal complex) in water may possess partial hydration states and, in particular for octadentate lanthanide complexes, values of q of zero and one simply represent boundary conditions and a given complex in solution may possess intermediate values.[63]

Conclusion

The determination of complex hydration state by solution luminescence methods is dependent on the nature of the coupling between the europium $^5D_0$ state and nearby OH oscillators. Such vibronic quenching of the emissive state is a function of the distance between the europium ion and the OH oscillator. This distance parameter may be compared to that occurring between a paramagnetic gadolinium centre and the nearest proton of a water molecule, determined by careful fitting of the NMRD profiles of related gadolinium complexes.

For europium complexes which have been established to possess bound water molecules, where a bound water is defined to be one with an Eu–O distance of < 2.55 Å, there is little variation in Eu–O distances (Table 2, crystallographic data). Therefore the quenching effect of these bound water molecules may be expected to be very similar from one complex to another. Closely diffusing water molecules also quench the europium excited state. The measured difference in radiative rate constants in water and D_2O is proportional to the sum of the effect of bound *and* closely diffusing OH oscillators, in addition to any effect from proximate NH and exchangeable CH oscillators. Thus in the complex of europium with the tetrabenzylphosphinate, L^{2b}, where the nearest water is > 5.5 Å away from the Eu in the crystal lattice, the measured Δk value is 0.15 ms^{-1} (equivalent to an apparent $q = 0.16$). For the more hydrophilic complex with PMe substituents, $\Delta k = 0.26\ ms^{-1}$ ($q = 0.27$). In the complex of Eu with dota, L^1, $\Delta k = 1.06$; this value is clearly made up of terms due to bound (approximately 0.85) and unbound water molecules (*ca.* 0.21).[64]

In certain complexes intermediate hydration states may be exhibited particularly when the ligand contains a local hydrogen-bond acceptor group (such as an amide carbonyl oxygen). These complexes could be considered to possess a long Eu–O interaction. The apparent q value measured by luminescence methods (or r value estimated from an analysis of the NMRD profiles of gadolinium complexes) is a function of both the hydrophobicity of the local environment, determined by the proximate substituents, and of the charge density at the metal centre. Thus the mean distance of closest approach of a water molecule to a ligand bound lanthanide ion may vary between say 2.4 to 2.5 Å ($q = 1$) and of the order of 3.8 Å ($q = 0$) according to the absence or presence of local hydrogen-bonding groups.[63]

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