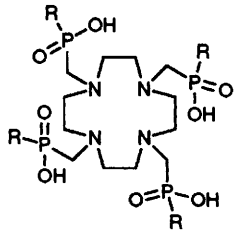
Luminescence Behaviour of Stable Europium and Terbium Complexes of Tetraaza Phosphinates: Efficient Through-space Energy Transfer from Phenyl to Terbium

Marcella Murru, David Parker,* Gareth Williams and Andrew Beeby

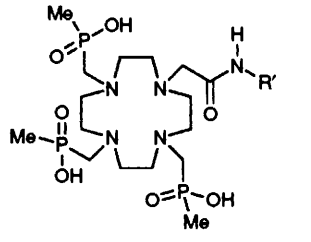
Department of Chemistry, University of Durham, South Road, Durham, UK DH1 3LE

Anionic and neutral complexes of europium and terbium with tetraaza phosphinate ligands are formed rapidly and are kinetically stable *in vivo*; the tetrakis(benzylphosphinate) terbium complex luminesces strongly *via* excitation into the proximate benzyl group involving efficient intramolecular energy transfer.

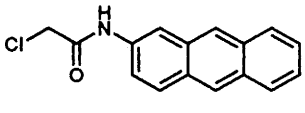
Luminescent lanthanoid complexes are being studied intensively[1] and are used in two commercially available immunoassay kits in clinical biochemistry.[2] The large Stokes' shift (*ca.* 300 nm), long excited state lifetime (typically of the order of 1 ms) and narrow visible emission spectra (*e.g.* for Eu: 616 and 592 nm) exhibited by europium(III) and terbium(III) complexes render them attractive as luminescent labels. A complexing agent for these ions should preferably possess the following properties: it should rapidly form kinetically stable complexes of Eu^{3+} and Tb^{3+} in aqueous solution; it should have reasonable water solubility and be readily synthesised; it should form highly luminescent complexes wherein the ligand possesses a large absorbance at the excitation wavelength (an 'antenna') and where energy transfer to the bound metal ion is reasonably efficient; it should shield the metal ion from water molecules (with europium a directly bound H_2O molecule causes quenching of the emissive $^5D_0$ state owing to coupling of the excited state with the third vibrational overtone of the O–H stretch of the coordinated H_2O)[3] in order to lengthen the excited state lifetime. Finally, the complexing agent should have a structure that may be easily modified to allow conjugation to a targeting vehicle such as an antibody or fragment thereof.[4] Many of these properties are similar in nature to those needed for gadolinium complexes used as contrast agents in magnetic resonance imaging (MRI)[5a] and in antibody conjugates of complexes of the β-emitter ^{90}Y which is being used in radioimmunotherapy. In these two cases, we have recently reported the synthesis, complexation behaviour and application of functionalised tetraaza phosphinate ligands.[4,5b,5c] Ligands **1**, **2** and **3** for example bind $^{90}Y^{3+}$ rapidly (95% radiolabelling yield, 5 μmol dm^{-3}, 15 min, 37 °C, pH 6) and form complexes that are kinetically stable *in vivo* (≥99.9% excretion of $^{90}Y^{3+}$ and $^{153}Gd^{3+}$ complexes in mice after 164 h).



1; R = Me
2; R = CH_2Ph



3; R′ = Me
4; R′ =



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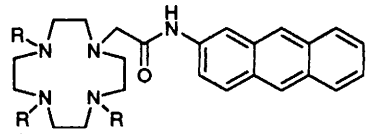

6; R = H
7; R = CH_2PMeO_2Et

In an attempt to enhance the absorption characteristics of the complex an anthryl group was introduced, as in **4**, that may conjugate to the bound europium or terbium ion *via* amide carbonyl ligation. The ligand was prepared by reaction of the molybdenum tricarbonyl complex of 1,4,7,10-tetraazacyclododecane with the chloroacetamide **5** [dimethylformamide (DMF), 60 °C, 30 min] to give after deprotection (aq. HCl, 20 °C, 15 h, air) the monosubstituted derivative **6** (81%). Condensation with $MeP(OEt)_2$ and paraformaldehyde [tetrahydrofuran (THF), 60 °C, 4 Å sieves, 15 h] yielded the triester **7** (51%) as a mixture of diastereoisomers.[4] Ester hydrolysis (aq. NaOH, 20 °C, 12 h, MeOH) yielded the triphosphinic acid, **4**.† The $^{153}Gd^{3+}$ (γ, $t_{\frac{1}{2}}$ 242 days) complex of **4** is formed readily (pH 6.5, 95%, 30 min) and its biodistribution profile in mice as a function of time (*e.g.* at 24 h < 0.1% of activity in skeleton, <0.2% in liver) was typical of a complex that is cleared predominantly through the renal system intact.[6]

The number of metal-bound water molecules in the lanthanoid complex was determined by an ^{17}O NMR method, wherein the ^{17}O shift of bulk water is monitored as a function of the added dysprosium complex.[7] The dysprosium(III) complex is preferred (over Eu or Tb) because of the dominance of the contact shift over the pseudo-contact contribution. Using the Dy^{3+} complexes of dota (H_4dota = tetrazacyclododecane-1,4,7,10-tetraacetic acid) and dtpa (H_5dtpa = diethylenetriaminepentaacetic acid) as controls (both possessing one bound H_2O), the ^{17}O data are consistent with there being no water coordinated to **1** and **2** and one water bound to the charge neutral complex of **3**. Such information is in agreement with relaxivity measurements made on the gadolinium complexes of **1** and **2** {[Gd–**1**] (298 K, 1.5 T) r_1 = 2.09, r_2 = 2.34 dm^3 $mmol^{-1}$ s^{-1}: [Gd–**2**] r_1 = 1.9, r_2 = 2.1 dm^3 $mmol^{-1}$ s^{-1} (*cf.* r_1 = 3.50, r_2 = 4.27 dm^3 $mmol^{-1}$ s^{-1} for [Gd(dota)])}[5a] and with a preliminary crystal structure analysis of the yttrium(III) complex of **2**, that has revealed that there is no metal-bound water molecule.[8]

The absorption and luminescence properties of the Eu^{III} and Tb^{III} complexes of **1**, **2**, **3** and **4** have been investigated (298 K, H_2O and D_2O solutions) (Fig. 1 and Table 1). The relative lack of variation (H_2O *vs.* D_2O) of the luminescence lifetimes of the Eu and Tb complexes with **1** and **2** is consistent with there being no metal-bound water as suggested by the ^{17}O NMR and Gd-relaxivity measurements. Usually in Eu^{3+} and Tb^3 complexes, as a result of the exceedingly low molar absorption coefficients of the metal-centred bands, strong luminescence may only be observed by excitation in ligand-centred or charge-transfer bands, followed by energy transfer to the luminescent metal-centred levels. Indeed with $[Eu\text{–}\mathbf{1}]^-$ and $[Tb\text{–}\mathbf{1}]^-$ only weak luminescence characteristic of the bound ion was observed {[Eu–**1**] max 616 nm ($^5D_0 \rightarrow {}^7F_2$); [Tb–**1**] max 545 nm ($^5D_4 \rightarrow {}^7F_5$)} following excitation into charge-transfer bands at 250 nm. Much stronger luminescence

† Ligands and complexes gave FAB mass spectral, ^1H and ^{31}P NMR, and microanalytical data in accord with the proposed structures.

Table 1 Luminescence data[a]

Complex	τ (298 K)/ms		Φ (298 K)		
	D_2O	H_2O	D_2O	H_2O	q[d]
[Eu(dota)]$^-$[b]	2.44	0.64	1.1×10^{-3}	—	1.2
[Tb(dota)]$^-$[c]	2.03	1.4	—	—	0.9
[Eu-**1**]$^-$	1.85	1.25	1.0×10^{-3}	5.9×10^{-4}	0.27
[Tb-**1**]$^-$	3.71	2.96	9×10^{-3}	1.0×10^{-2}	0.27
[Eu-**2**]$^-$	2.07	1.59	1.5×10^{-3}	0.9×10^{-3}	0.15
[Tb-**2**]$^-$	4.44	4.13	0.49	0.44	0.07
[Eu-**3**]	1.85	0.76	1.3×10^{-3}	0.5×10^{-3}	0.8
[Tb-**3**]	4.30	3.20	0.28	0.16	0.34
[Eu-**4**][e]	0.17, 1.21	0.07, 0.60	3.2×10^{-3}	0.75×10^{-3}	0.9
[Tb-**4**][e]	0.26, 0.80	0.17, 0.63	4.1×10^{-3}	0.56×10^{-3}	1.4

[a] Data were obtained using a Perkin-Elmer LS50B instrument. Quantum yields were measured[9] using $[Ru(bpy)_3]^{2+}$ (for Eu) or quinine sulfate (Tb) as standards (bpy = 2,2′-bipyridine). Uncertainties in lifetimes are ≤10% and quantum yields ≤25%. Excitation at 250 nm, except for **4** at 303 mm. Quantum yields were independent of concentration, gate time and gate delay. [b] The free europium aqua ion is reported to have a lifetime of 0.1 ms.[10] [c] For the related chiral tetrapropionate derivative.[13] [d] q is the number of metal-bound water molecules calculated according to Horrocks,[3] who quotes an error of (±0.5). [e] Fluorescence quantum yields (anthryl) were: [Eu-**4**]$^-$ 0.005 (H_2O and D_2O); [Tb-**4**]$^-$ 0.015.

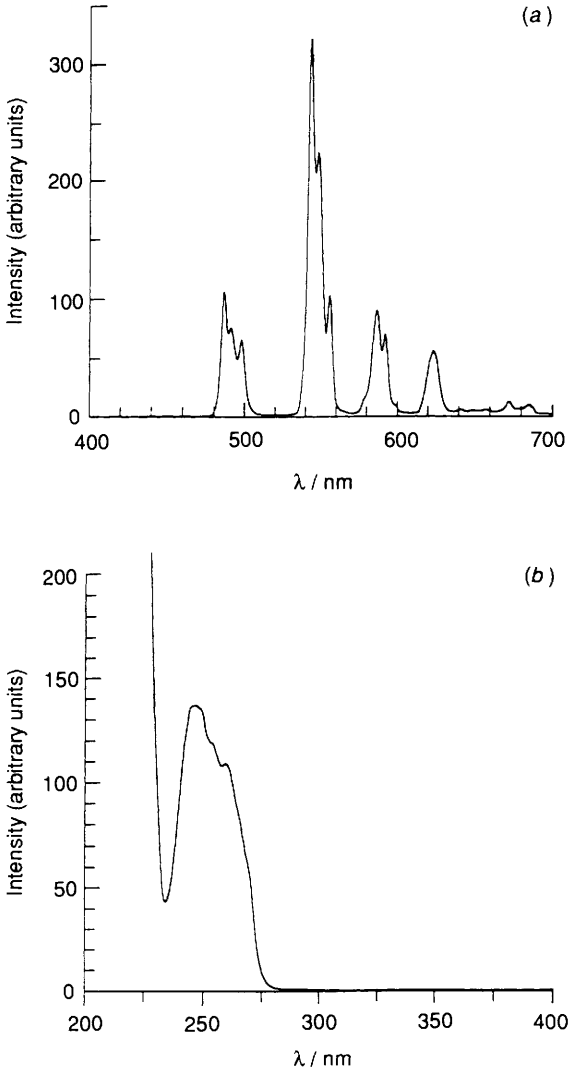

Fig. 1 (*a*) Corrected emission spectrum of [Tb-**2**], excitation wavelength 250 nm; (*b*) Corrected phosphorescence excitation spectrum of [Tb-**2**], emission wavelength 545 nm. For both spectra excitation and emission monochromator slits set to bandwidths of 2.5 nm, delay time 0.5 ms, gate 4.0 ms, conc. [Tb-**2**] = 2×10^{-6} mol dm^{-3} (H_2O, pH 5.5, 298 K).

was observed with [Tb-**2**]$^-$ (Φ = 0.44 in H_2O) and the corrected excitation spectra closely matched the corresponding absorption spectra over the UV region (Fig. 1). Evidently a through-space energy transfer[11] may be occurring *via* excitation of the π–π* state in each of the four equivalent phenyl rings.

Such processes may be described by a Förster-type dipole–dipole mechanism wherein the measured efficiency of energy transfer is related to the distance, r, separating the metal ion and the chromophore.[12] Given that $\Phi = \eta_{et} \times \eta_r$ (where η_r is the luminescence efficiency of the metal-centred level and η_{et} is the efficiency of energy transfer) and $\eta_{et} = [1 + (r/R_0)^6]^{-1}$,‡ then using a value of r = 5.48 Å (the distance from Y to the centre of the plane of each phenyl ring found in the X-ray structure of [Y-**2**]$^-$ H_3O^+:[8] *cf.* 4.74 Å to the phenyl edge) and making the assumption that there are four non-interacting donors per terbium acceptor and that η_r is unity, then the R_0 value is 3.88 Å. This value may be useful in assessing the relative distances separating Phe (phenylalanine) residues from Tb sites in modified proteins where sensitised Tb^{3+} emission is used quite commonly to measure distances between Tb ions and aromatic residues (*e.g.* Trp, Tyr, Phe). With Trp as a donor R_0 values varying between 2.81 and 3.40 Å have been calculated.[11,12]

The neutral terbium complex of the simple amide, [Tb-**3**], exhibits a relatively high quantum yield (0.28 in D_2O) and the excitation and absorption spectra match very well, with a band at 250 mm apparent. Emission is much weaker in [Eu-**3**] owing to a competitive charge transfer transition mechanism of energy capture by the Eu^{3+} ion. The presence of one metal-bound water molecule also shortens the luminescence lifetime.

A different situation arises with [Eu-**4**] and [Tb-**4**]. Excitation at 303 nm gives the characteristic metal-centred emissions and there is a competitive anthryl fluorescence (Table 1). Indeed phosphorescence excitation spectra (for both the Eu and Tb complex in H_2O and D_2O) reveal a broad band, at 308 nm. This is not the expected signature of the anthryl moiety and it is possible that it may be related to a formally disallowed excitation of the anthryl group (perturbed by the neighbouring heavy atom) from the S_0 to a triplet state

‡ r is the distance between the bound ion and the centre of the planar chromophore and R_0 is the distance for 50% efficient energy transfer.

followed by energy transfer to the metal from the anthryl T_1 state. Direct fluorescence from the anthryl group was also observed and its relative inefficiency suggests that there is an efficient quenching of the anthryl singlet state.

The functionalisation of ligands such as **2** and **3** to permit protein conjugation is straightforward[4] and should make possible the development of new, practicable, time-resolved fluoroimmunoassay methods.

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