Experimental assessment of the efficacy of sensitised emission in water from a europium ion, following intramolecular excitation by a phenanthridinyl group

Ian M. Clarkson, Andrew Beeby, James I. Bruce, Linda J. Govenlock, Mark P. Lowe, Céline E. Mathieu, David Parker* and Kanthi Senanayake

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

Received (in Cambridge, UK) 16th February 2000, Accepted 7th April 2000

The overall quantum yields for phenanthridinium sensitised emission from a europium ion have been measured in H_2O and D_2O for a series of five structurally related, octadentate ligands in which the distance from the phenanthridinium chromophore to the Eu ion varies from 2.5 to *ca.* 8.2 Å. Overall quantum yields (pD $\leqslant$ 2) range from 0.25 to 0.012 suggesting that the experimental distance for 50% efficiency of intramolecular energy transfer lies close to 5.5 Å for this system.

An important aspect in the development of practicable luminescent probes is the overall efficiency of the photochemical process, revealed by measurements of the emission quantum yield, φ_{em}. Recently there have been several reports of emissive lanthanide complexes in which the metal-based emission results from intramolecular energy transfer from an antenna chromophore which is located near the lanthanide ion. Substituted aryl or heterocyclic groups have usually been used for this purpose including 2,2′-bipyridyls and 1,10-phenanthrolines,[1] coumarins,[2] substituted phenyl groups,[3] aryl ketones[4] and benzimidazoles.[5] In each of these chromophores, the lowest energy excited triplet state lies above the emissive Eu $^5D_0$ level at 17 200 cm^{-1}. Sensitising chromophores which possess a small singlet-triplet energy gap, thereby allowing excitation at wavelengths above 350 nm (*ca.* 28 500 cm^{-1}) are much less common.[4,6] Moreover, such antennae need to be engineered into a ligand system that forms a stable lanthanide complex in competitive aqueous media, if practicable systems are to be devised. Long-wavelength excitation (*i.e.* $\geqslant$350 nm) is an important issue in this respect, as it obviates the need for quartz optics and allows many samples containing biomolecules to be addressed directly. Considerable promise has been exhibited by lanthanide complexes of octadentate ligands which incorporate a phenanthridinium chromophore.[6,7] The neutral chromophore possesses a singlet excited state around 28 600 cm^{-1} which lowers by *ca.* 2000 cm^{-1} on N-protonation (or alkylation). The chromophore also absorbs well at 365 nm by introduction of a conjugating electron-withdrawing group in the 6-position, *i.e.* α to the ring nitrogen. The triplet excited state lies around 22 000 cm^{-1}, lowering to *ca.* 21 300 cm^{-1} on N-protonation or alkylation.[6] The energy gap to the $^5D_0$ Eu level is then *ca.* 4000 cm^{-1} which is small enough to allow efficient energy transfer but not so small as to permit thermally activated back energy transfer at room temperature.

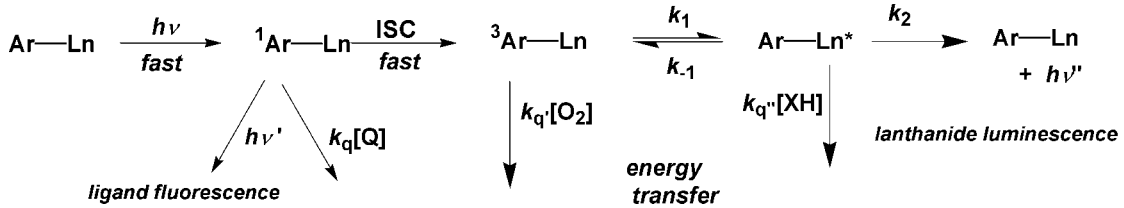
For a process in which an aryl triplet excited state serves as an intermediate in the sensitised emission (Scheme 1) then the measured quantum yield, φ_{em}, is related to four key parameters by eqn. (1):

$$\varphi_{em} = [\varphi_T \eta_{ET}](k_0 \tau_{obs}) \quad (1)$$

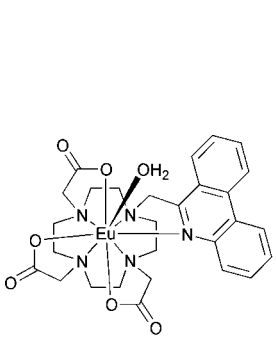
where φ_T is the quantum yield for triplet formation, η_{ET} is the efficiency of the energy transfer process, k_0 is the natural radiative rate constant for the $^5D_0$ state of the europium(III) complex and τ_{obs} is the observed lifetime for europium decay.

In this work, we have sought to assess the efficacy of the sensitised emission process, by examining a series of structurally related monohydrated europium(III) complexes which possess a phenanthridinyl group. These range from $[EuL^1]$ and $[EuL^2]^{3+}$ in which the phenanthridinyl nitrogen is directly bound to europium, to $[EuL^3]$, $[EuL^4]^{3+}$ and $[EuL^5]$ where the aromatic moiety is more distant but is *relatively* rigidly oriented with respect to the lanthanide centre. Thus, in $[EuL^3]$ and $[EuL^4]^{3+}$, the phenanthridinyl amide group is coordinated to the europium centre and such complexes have been shown to adopt rigid square antiprismatic complexes in aqueous solution.[3,7] With $[EuL^5]$, a valine spacer has been introduced in the expectation that the short chain will adopt the preferred extended *trans*-amide conformation.

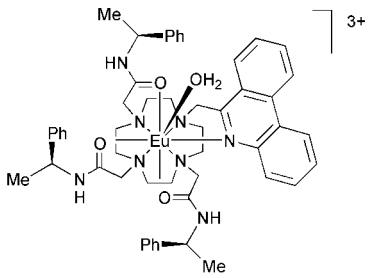
Notwithstanding the residual conformational mobility inherent in the complexes of L^3, L^4 and L^5, reasonable estimates of the distance between the europium ion and the nearest phenanthridinyl ring atom may be made. For $[EuL^1]$ and $[EuL^2]^{3+}$, this distance will be the Eu–N bond length, which, by analogy with the X-ray data for $[GdL^6]$ (Gd–N 2.55 Å), will be very close to 2.55 Å.[8] For $[EuL^3]$ and $[EuL^4]^{3+}$, the best model is the europium complex of the chiral tetra-amide $[EuL^7]^{3+}$,[3] and the nearest distance between the aryl carbons and Eu from the reported X-ray



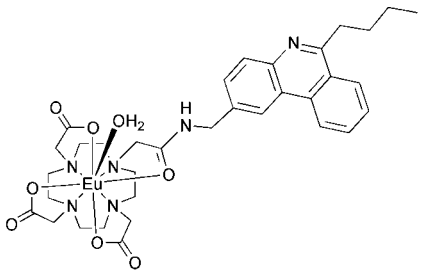
Scheme 1



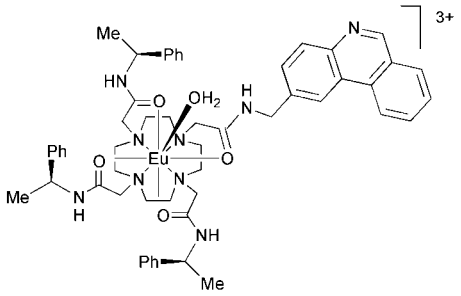
[EuL^1]



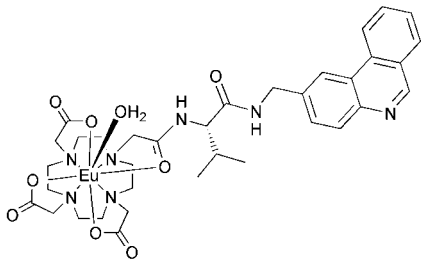
[EuL^2]$^{3+}$



[EuL^3]



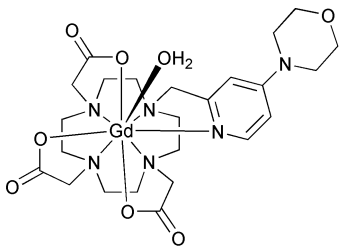
[EuL^4]$^{3+}$



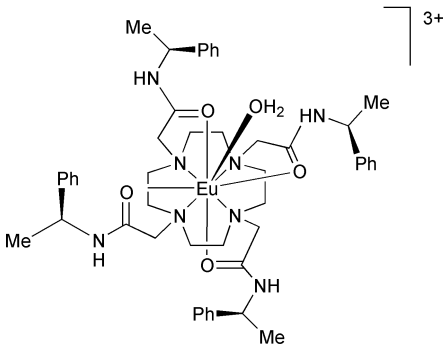
[EuL^5]

structures is 5.2 ± 0.1 Å (CCDC data[3]). Examination of the system by simple molecular modeling packages (Biosym, CaChe) suggest that the phenanthridine group *cannot* get closer than 5.0 Å from the Eu centre, in any conformer. Finally, for [EuL^5], the insertion of a valine moiety (in the preferred extended *anti* conformation) is expected to increase the separation by *ca.* 3 Å, albeit with a greater degree of uncertainty, associated with the freedom of rotation about the phenanthridine 2-substituent.

With this series of complexes to hand, we set out to measure the values of φ_{em} and τ_{obs} for each in H_2O and D_2O, in the presence/absence of acid. Protonation of the phenanthridine nitrogen prevents the europium ion quenching the intermediate singlet excited state by electron transfer,[6] while measurements in D_2O allow the quenching effect of exchangeable OH and NH oscillators to be minimised.[9] Thus this comparative study set out to highlight the relative importance of each of these factors; the assessment of the φ_{em} values in acidic D_2O sought to allow the relative efficiency of the energy transfer step to be highlighted.



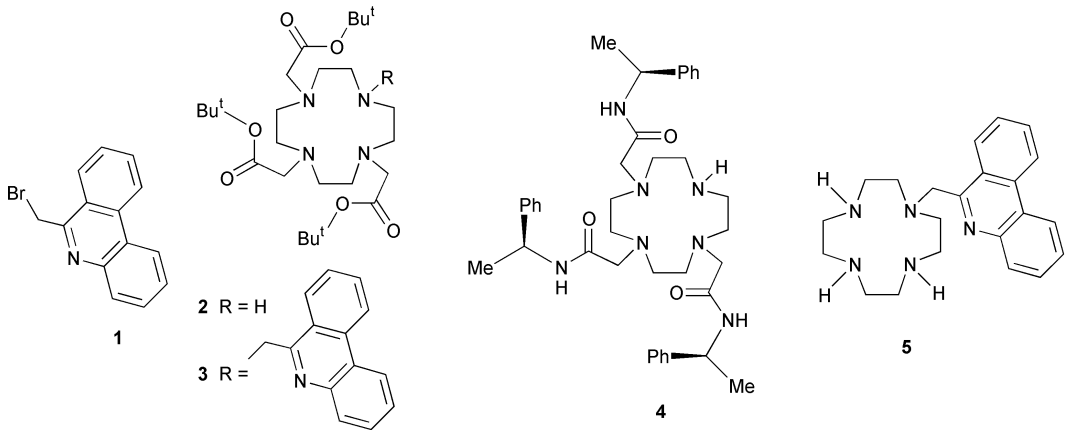
[GdL^6]



(SSSS)-Δ-[EuL^7]$^{3+}$

Synthesis and characterisation of Eu complexes

The europium complex [EuL^4]$^{3+}$ has previously been reported[6] and for the synthesis of the other complexes only minor modifications were made to established methods. In the synthesis of ligand H_3L^1, benzylic bromination of 6-methylphenanthridine yielded the bromomethyl derivative **1** which was sufficiently sterically hindered to allow purification by chromatography on silica. Direct alkylation of the tri-ester **2** in DMF afforded the tetra-amine **3** and deprotection with CF_3CO_2H followed by complexation of europium [$Eu(OAc)_3$–MeOH] yielded the neutral complex [EuL^1], purified by chromatography on reverse phase silica. For the related cationic complex, [EuL^2]$^{3+}$, various attempts to achieve direct alkylation of the more sterically hindered triamide (*SSS*)-**4** failed to yield the desired product in significant amounts. Instead, alkylation of an excess of the parent macrocycle, 1,4,7,10-tetraazacyclododecane (cyclen) with **1** (MeCN, Cs_2CO_3) gave the mono-substituted derivatives **5** and reaction with 3 equivalents of (*S*)-*N*-chloroethanoyl-2-phenylethylamine yielded the triamide, L^2. Complexation with $Eu(CF_3SO_3)_3$ in dry MeCN allowed the desired cationic complex to be prepared. The synthesis of [EuL^3] was carried out in a similar manner to that of [EuL^1] and required the preparation of the α-chloroamide, **9**. This was prepared by selective butylation of 2-bromophenanthridine at the 6-position, following reaction with butylmagnesium chloride in Et_2O. Cyanation of the 6-butyl derivative, **6**, (CuCN, DMF) followed by reduction with $BH_3 \cdot THF$ yielded the primary amine **8**, from which the desired α-halomide **9** was prepared



by EDC coupling with chloroacetic acid. Alkylation of **2** by **9** (MeCN, K_2CO_3) gave the triester **10** in 62% yield, and acidic deprotection (80% TFA–CH_2Cl_2) followed by Eu(III) complexation allowed the neutral complex [EuL^3] to be isolated. Finally, for the synthesis of H_3L^5, coupling of the primary amine **8** with BOC valine (EDC, HOBT, Et_3N, CH_2Cl_2) gave the mono-amide **11** and removal of the BOC group (96% TFA–H_2O) and coupling with chloroacetic acid afforded the diamide, **13**, from which the desired Eu(III) complex was prepared *via* the intermediate triester, **14**, using the methods described for [EuL^1].

Absorption and emission spectra

Absorption spectra for each of the Eu(III) complexes were recorded in acidic (pH $\leqslant$ 2) and neutral media (pH 7.5), (Table 1 and Fig. 1). The complexes [EuL^1] and [EuL^2]$^{3+}$ exhibited no pH dependence in their absorption spectra over the pH range 2–10. Spectra for both of these complexes were similar, with a strong absorption band at 355–360 nm, shifted by *ca.* 10 nm to longer wavelength from that observed for similar unbound neutral phenanthridines, *e.g.* **14**. In the case of [EuL^3], protonation caused a more marked shift to longer wavelength and significant hyperchromism (Fig. 1), with an isosbestic point at 306 nm. Monitoring the change in absorbance at 365 nm as a function of pH allowed the ground-state pK_a to be determined as 5.4 (295 K, $I = 0.1$ M NMe_4NO_3). The pK_a of the singlet excited state was also estimated by monitoring the change in fluorescence emission ($\lambda_{exc} = 365$ nm, $\lambda_{em} = 405$ nm) from the phenanthridyl group with pH: a value of 5.6 ($\pm$0.1) was determined. A similar apparent pK_a—reflecting the protonation constant of the first excited singlet state—was determined by observing the pH dependence of the europium emission (Fig. 2), monitoring the intensity of any of the $\Delta J = 0$, 1 or 2 bands at 580, 592 or 616 nm), following excitation at 365 nm.

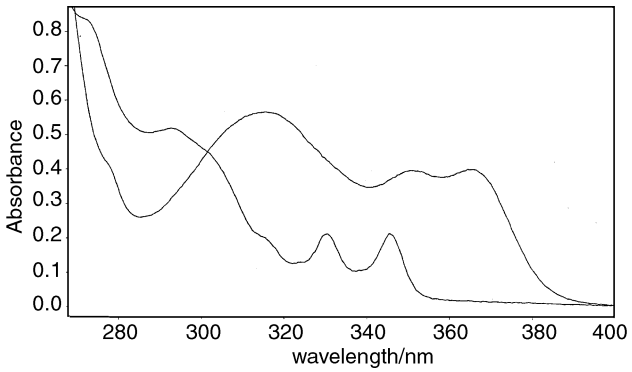
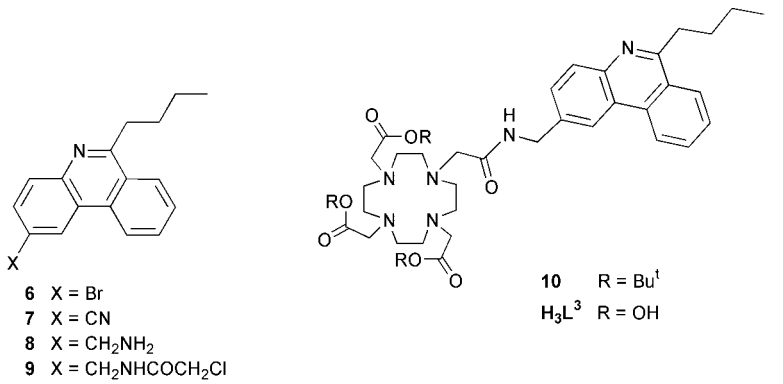


Fig. 1 Absorption spectra for [EuL^3] at pH 7.5 (lower profile) and for [$EuHL^3$]$^+$ at pH 2.0, showing the shift to longer wavelength accompanying N-protonation.



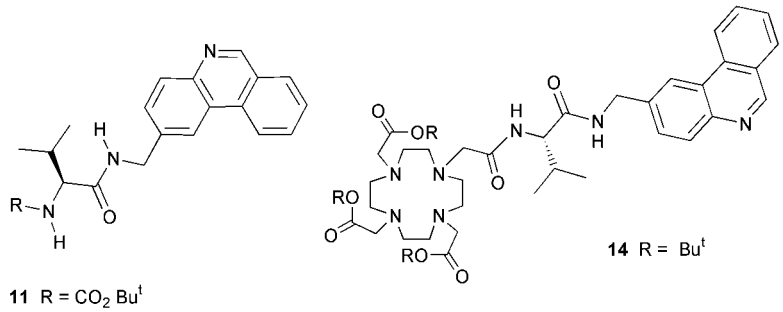


Table 1 Selected physical properties of the europium complexes of ligands L^1–L^5 (295 K)

Complex	$\lambda_{abs}(H_2O)^a$/nm (ε/M^{-1} cm^{-1})	$pK_a{}^b$ (I = 0.1 M NMe_4ClO_4)	^{1}H NMR chemical shift of H_{ax} (D_2O)
$[EuL^1]$	355(4×10^3) 335(4.5×10^3) 306(9.4×10^3) 280(1.4×10^4) 250(5×10^4)	Not determined	37.5 28.8 (2H) 20.5
$[EuL^2]^{3+}$ [c]	359(4×10^3) 345(5×10^3) 306(8×10^3)	Not determined	39.2[d] 35.6 25.2 22.3
$[EuL^3]$	345(2.5×10^3)[366(5×10^3)] 330(2.5×10^3)[352(5×10^3)] 293(6×10^3)[317(7×10^3)]	5.60 (5.4)	33.8 32.1 (2H) 31.6
$[EuL^4]^{3+}$	348(2.4×10^3)[370(5×10^3)] 330(2.3×10^3)[320(8.9×10^3)] 295(7.2×10^3)[250(5×10^4)]	3.40[e] (3.3)[e]	29.8[d] 29.1 (2H) 28.4
$[EuL^5]$	347(2.2×10^3)[368(4.5×10^3)] 330(2.2×10^3)[322(8×10^3)] 295(6.7×10^3)	4.45 (4.3)	38.4[d] 35.8 35.1 33.8

[a] Values in square brackets are for the protonated complexes. [b] Values refer to the pK_a of the Eu complex, monitoring Eu emission intensity (λ_{em} = 616 nm, λ_{exc} = 365 nm); values in parenthesis refer to pK_a values of the ground state, observing changes in absorbance at 365 nm. Uncertainty in values = ± 0.1. [c] Measured in H_2O–MeOH (4 : 1, v/v). [d] Measured in CD_3OD. [e] Data from ref. 6.

For the valine-spaced complex, $[EuL^5]$, protonation constants of 4.3 (λ_{abs} = 365 nm) for the ground state and 4.45 for the excited state (λ_{exc} = 365, λ_{em} = 405 (phen) or 616 (Eu)) were determined. These values are lower than those found with $[EuL^3]$ but very similar to literature values reported for related substituted phenanthridines.[6,10] The slightly higher pK_a of the 6-butylphenanthridinyl moiety in $[EuL^3]$ simply relates to the weak electron releasing effect of the 6-alkyl substituent.

Excitation of $[EuL^3]$ and $[EuL^5]$ at their isosbestic wavelengths gave rise to emission from the phenanthridine excited singlet state the relative intensity of which varied with pH. For $[EuL^3]$, emission spectra recorded under acidic and neutral conditions (Fig. 3), showed the expected shift to longer wavelength in the protonated form, with a 2.5 fold enhancement in the total fluorescence intensity compared to the neutral form. For $[EuL^5]$, where the separation of the Eu ion and phenanthridine group is about 3 Å greater, the relative total intensity change was a factor of 1.2. The Eu(III) ion is well known to quench the fluorescence of proximate aryl excited states[11] by an electron (or charge) transfer mechanism, and this process is switched off by protonation, as the phenanthridinium ion is much less readily oxidised than a neutral phenanthridine.[6,11] The electron transfer quenching mechanism is likely to show a variation of rate with the distance between the donor and acceptor that follows the classical exp($-\beta r/2$) relationship.[12] If this is so, then for Δr = 3 Å and a two-fold change in the rate of charge transfer, the β value is likely to be near to 0.8 $Å^{-1}$, close to the established lower limit for electron transfer processes, studied in proteins.[12]

A representative europium emission spectrum is shown in Fig. 4 for (*SSS*)-$[EuL^2]^{3+}$, together with its circularly polarised emission spectrum. Comparison of these spectra with those reported for analogous chiral complexes, *e.g.* (*SSSS*)-$[EuL^7]^{3+}$,[3] reveals a similar spectral form and g_{em} values of +0.13 (586 nm), −0.09 (591 nm) (ΔJ = 1 transitions), +0.12 (648 nm, ΔJ = 3) and −0.07 (685 nm, ΔJ = 4) were also similar in magnitude and sign suggesting that (*SSS*)-$[EuL^2]^{3+}$ also adopts the Δ-configuration with a right-handed helicity in the layout of the ring nitrogen substituents.

For each of the Eu complexes examined (Table 1), similar paramagnetically shifted ^{1}H NMR spectra were recorded in D_2O or CD_3OD; for example the most shifted 'pseudo-axial' ring protons in the 12-N_4 macrocycle resonated at 293 K as four distinct broadened singlets in the range 39–20 ppm, con-

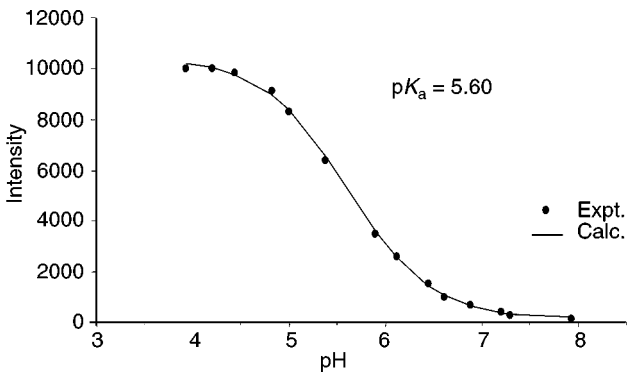


Fig. 2 Variation of europium emission intensity (λ_{em} = 616 nm) as a function of pH for $[EuL^3]$ (295 K, I = 0.1 M NMe_4ClO_4), showing the fit (curve) to the experimental data.

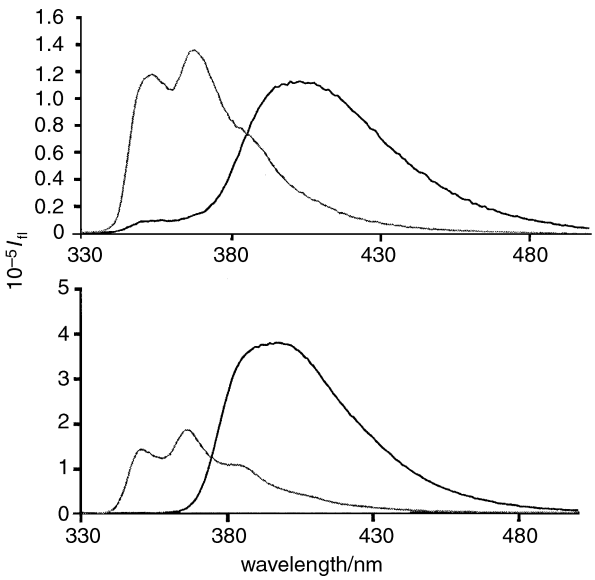


Fig. 3 Fluorescence emission spectra for $[EuL^3]$ (upper) and $[EuL^5]$ (lower) at pH 7.5 and 2 (295 K, λ_{exc} = 306 nm), following excitation at the isosbestic wavelength.

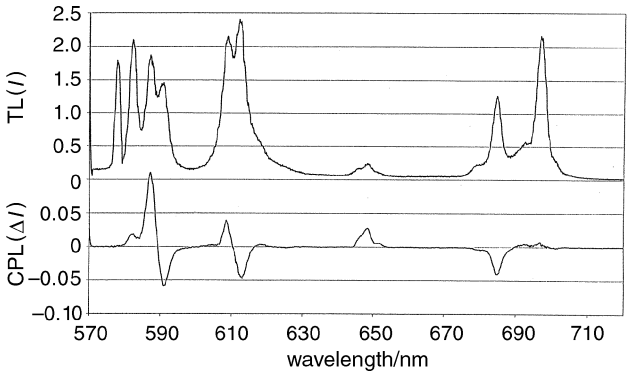


Fig. 4 Total (TL) and circularly polarised (CPL) luminescence spectrum for Δ-(*SSS*)-$[EuL^2]^{3+}$ (295 K, MeOH, $\lambda_{exc} = 355$ nm).

sistent with a common coordination environment and geometry for the complex in solution.

Measurement of quantum yields and radiative rate constants

The radiative rate constants for decay of the metal-based luminescence for each of the europium complexes were measured in H_2O and D_2O (Table 2). The values obtained are consistent with a hydration state of one, for each of the complexes studied.[9] This information, taken together with their similar solution NMR and Eu emission spectral behaviour, and by comparison with the properties of related complexes[3,6,7,9] suggests that each complex adopts a preferred mono-capped square-antiprismatic structure in solution. The overall quantum yield for Eu luminescence (φ_{em}) was also measured in H_2O and D_2O under neutral (pH 7.5 ± 0.5) and strongly acidic (⩽pH 2) conditions in aerated and degassed solution (Table 2). The standards used in this work were Rhodamine 101 and Cresyl Violet; both are known to exhibit similar emission spectra to Eu(III), and also possess a similar absorbance, in the UV region, to the complexes studied here (*e.g.* at 270 nm for Rhodamine 101 and 300 nm in the case of Cresyl Violet).[13,14] The values quoted in Table 2 were consistent with respect to either standard used, and represent the mean value of four independent measurements, with an estimated experimental error of ±10%.

The values of φ_{em} measured in D_2O were consistently three to four times larger than in H_2O, reflecting the deactivating effect of vibrational quenching by OH oscillators which is also revealed by the higher rate constants for luminescence decay in H_2O. For $[EuL^3]$, $[EuL^4]^{3+}$ and $[EuL^5]$, protonation of the phenanthridine nitrogen inhibits the quenching of the intermediate singlet excited state by charge transfer to Eu^{3+}, leading to higher φ_{em} values in acidic media. This effect is most marked with $[EuL^4]^{3+}$, which may reflect the greater ease of reduction of the Eu^{3+} centre in the tripositive complexes, compared to the neutral complex $[EuL^3]$. The effect is least marked for $[EuL^5]$ where, as discussed above in terms of the variation of fluorescence emission intensity from the phenanthridine group (Fig. 3), the aryl antenna is 3 Å more distant from the Eu ion, attenuating this quenching effect.

Relatively high quantum yields were measured for $[EuL^1]$ (0.19 in D_2O) and $[EuL^2]^{3+}$ (0.13 in D_2O), where the sensitising chromophore is closest to the lanthanide centre (*ca.* 2.55 Å). Obviously these complexes are not readily protonated and there may still be some residual quenching of the intermediate phenanthridinyl S_1 state. The measured quantum yields were the same in aerated and degassed media, consistent with a process in which the rate of energy transfer is fast with respect to bimolecular quenching by molecular oxygen. Only for the valine spaced complex was any difference in measured quantum yield observed on degassing the sample consistent with a slower energy transfer step: in H_2O, the quantum yield increased by 90% in degassed solution at pH 9 but was only enhanced by 14% at pH 2.

A striking feature of the behaviour of the complexes $[EuL^3]$ and $[EuL^4]^{3+}$ is that the quantum yields in acidic D_2O† are also quite high and once again did not change on degassing the sample. Referring back to eqn. (1), then the product ($\varphi_T\eta_{ET}$) may be estimated,

$$\varphi_{em} = (\varphi_T \eta_{ET})(k_0 \tau_{obs}) \quad (1)$$

provided that a reliable value for k_0 can be chosen. The term k_0 is the natural radiative rate constant for the Eu ion in the given coordination environment. The natural radiative lifetime for the Eu aqua ion has been *calculated* to be 9.67 ms, equivalent to a k_0 value of 0.103 ms^{-1}.[15] However *all* measured lifetimes, in the literature, for Eu(III) complexes with multidentate ligands in D_2O are considerably lower than this. The highest value reported in D_2O with an octadentate ligand is for polydeuteriated DOTA† (1,4,7,10-tetraazacyclododecane tetraacetate) for which $k_{D_2O} = 0.24$ ms^{-1}, in which the level of

† Acidification involved use of CF_3CO_2H; prior work[6] established that Cl^- quenched the alkylated or protonated phenanthridinium excited singlet state with $K_{SV} = 50$ mM^{-1} and 26 mM^{-1}, for formally neutral or tripositive Eu complexes, respectively, monitoring the Eu luminescence intensity change.

Table 2 Quantum yields and mean rate constants (k/ms^{-1}) or decay of the europium $^5D_0$ excited state for the Eu complexes of ligands 1–5 in aerated solution (295 K)

Complex	φ_{H_2O}	φ_{D_2O}	$(\varphi_T\eta_{ET})^a$ (in D_2O)	k_{H_2O}	k_{D_2O}	q^b	Estimated separation Eu–phen/Å
$[EuL^1]^c$	0.063	0.19	0.43	1.57	0.45	1.04	2.55
$[EuL^2]^{3+c}$	0.039	0.13	0.31	1.52	0.44	0.80	2.55
$[EuL^3]$	0.014	0.055	0.11	1.48	0.41	0.92	5.2
$[EuL^3H]^+$	0.062	0.25	0.50	1.49	0.40	0.92	
$[EuL^4]^{3+d}$	0.004	0.012	0.025	1.72	0.42	0.90	5.2
$[EuL^4H]^{4+d}$	0.021	0.12	0.25	1.71	0.41	0.91	
$[EuL^5]^e$	0.0038	0.012	0.037	1.63	0.57	0.89	8.2
$[EuL^5H]^{+e}$	0.0045	0.014	0.043	1.62	0.56	0.89	

[a] This product is given by $\varphi_T\eta_{ET} = \varphi_{em}/k_0\tau_{obs}$ where k_0 is the estimated radiative rate constant for $[Eu(d^{24}\text{-DOTA})]^-$ [0.2 ms^{-1}] and τ_{obs} is the observed lifetime in D_2O. [b] This hydration number is given by $q^{Eu} = 1.2\ [(k_{H_2O} - k_{D_2O}) - 0.25\text{–}0.075x]$ where x is the number of proximate amide NH oscillators, which when equidistant are assumed to quench in an equivalent manner. [c] No change was observed in emission intensity and absorption spectra over the pH/D range 2–8. [d] Data from ref. 6. [e] In degassed media, the measured quantum yield for $[EuL^5]$ in H_2O increased by 90% and for $[EuL^5H]^+$ by 14%; in all other cases, values for aerated and degassed samples were within ±10%.

deuteriation was estimated to be in the range 75–85%.[9] A more reasonable value therefore for k_0 is 0.2 ms^{-1}, although such a value should be regarded as no more than an 'informed' estimate. Using this value, then the product of ($\varphi_T \eta_{ET}$) may be assessed for each of the europium complexes studied (Table 1). The highest values are found for $[EuL^3H]^+$ (0.50) and for $[EuL^1]$ (0.43) and lend support to the idea that k_0 must be >0.1 otherwise this product ($\varphi_T \eta_{ET}$) is equal to unity in the former case! The term φ_T (quantum yield of triplet formation) has *not* been measured in this work and will undoubtedly vary from one complex to another in the series examined. There is no literature value for φ_T for phenanthridine itself; for phenanthrene the value of φ_T is 0.73.[16] Notwithstanding our ignorance of this value, an estimate can be made for the distance at which energy transfer is 50% efficient, independent of any theoretical interpretation.

A priori, here are two mechanisms that seek to describe triplet–singlet energy transfer from a donor to an acceptor. In Dexter transport, the exciton hops between molecules directly in a short-range process that is dependent on the nature and degree of orbital overlap between the two components. The symmetry of the two components is preserved in this case so that triplet–singlet energy transfer is formally not possible by the Dexter mechanism. However, a change in spin symmetry is possible if the donor exciton breaks up and reforms on the acceptor by incoherent electron exchange. In general, such a process is relatively unlikely as it requires dissociation of the donor exciton.[17] The alternative theory was developed by Förster wherein molecular transition dipoles couple and exchange energy.[18] In this situation, the efficiency of energy transfer, η_{ET} is given by eqn. (2), where k_{ET} is the rate of energy transfer, k_r and k_{nr} are the rates of radiative and non-radiative decay of the donor r is the donor–acceptor separation and r_0 is the distance for 50% efficient transfer. In Förster theory, k_{ET} and k_r are proportional to the oscillator strength of the transition. The efficiency of energy transfer is approximately independent of oscillator strength if $k_r \ll k_{nr}$, *i.e.* if the donor is efficiently phosphorescent, thereby allowing triplet to singlet energy transfer to be described by this mechanism. Indeed such transfers were predicted by Förster[19] and were confirmed first by Ermolaev and Sveshnikova.[20] Very recently, the approach has been experimentally validated in a system with a phosphorescent metal complex donor and an acceptor dye.[21] Taking a value of $r_0 = 5.5$ Å, then $\eta_{ET} = 58\%$ if $r = 5.2$ Å. Despite the evident uncertainty in this estimate as a consequence of the number of assumptions made in the calculation, it is clear that for $[EuL^3H]^+$ η_{ET} must be $>50\%$ (Table 2), so that the distance for 50% efficient energy transfer is certainly >5 Å and is likely to be close to 5.5 Å.

$$\eta_{ET} = k_{ET}/(k_{ET} + k_r + k_{nr}) = \frac{1}{[1 + (r/r_0)^6]} \quad (2)$$

Conclusion

This study reveals that it is possible to get quite high overall quantum yields for sensitised europium luminescence even using a phenanthridinium chromophore that is located >5 Å from the metal ion. It highlights again the intrinsic limitations in operating with monohydrated complexes and those in which the intermediate antenna singlet excited state can be competitively quenched, *e.g.* by Eu^{3+} itself or by halide anions. Such problems are obviated of course when the complexes are not hydrated, *e.g.* with nonadentate ligands or using sterically encumbered octadentate ligands such as those incorporating phosphinate donors.[22] In addition, in the absence of back energy transfer, the advantage of using the corresponding Tb^{3+} complexes are again apparent, as vibronic and charge transfer quenching mechanisms are intrinsically less important.[1,11,23]

Experimental

Solvents were dried from an appropriate drying agent when required and water was purified by the "$Purite_{STILL}$ plus" system. Thin layer chromatography was carried out using neutral aluminium oxide plates (Merck Art 5550) or silica plates (Merck Art 5554), both of these being fluorescent on irradiation at 254 nm. Preparative column chromatography was carried out using silica (Merck silica gel 60, 230–400 mesh). IR spectra were recorded with a Perkin-Elmer 1600 FT-IR spectrophotometer operating with GRAMS Analyst software using a 'Golden Gate' accessory.

Mass spectra (CI) were recorded using a VG 7070E spectrometer using ammonia as the impingent gas. Electrospray mass spectra were recorded using a VG II Platform spectrometer (Fisons Instruments) with methanol as the carried solvent. Accurate masses were measured by the EPSRC Mass Spectroscopy Service at the University of Wales at Swansea.

Proton NMR spectra were recorded on a Varian VXR 400 (65.26 MHz), Varian VXR 200 (199.99 MHz), Varian Gemini 200 (199.99 MHz), Varian Mercury 200 (199.99 MHz) or Varian Unity 300 (299.91 MHz), carbon NMR spectra using the Varian Mercury 200 (50.29 MHz), Varian Unity 300 (75.41 MHz), Varian VXR 400 (100.58 MHz) or a Bruker AMX 500 spectrometer (125.77 MHz). Spectra were referenced to solvent residual proton resonances. All chemical shifts (δ) are reported in ppm and coupling constants are reported in Hz.

Melting points were measured using a Reichart–Köfler block and are uncorrected. Optical rotations were measured on a Bellingham and Stanley Ltd. P20 polarimeter.

Ultraviolet absorbance spectra were recorded on a Unicam UV2 spectrometer operating with Unicam Vision software, with absorption coefficients reported in parentheses, units of M^{-1} cm^{-1}. Luminescence spectra and luminescent lifetimes were recorded either using a Perkin Elmer LS 50B or an Instruments SA Fluorolog 3-11 equipped with a Spex 1934D3 phosphorimeter. Corrected spectra were obtained taking into account the wavelength-dependent response of the instrument, particular attention being paid to the response characteristics of the Hamamatsu R928 PM tube response in the range 650–750 nm.

Non-zero baseline effects were allowed for by running a blank sample and subtracting this from the obtained spectra. Second order diffraction effects were obviated by using a cut-off filter to remove the scattered light before it enters the emission monochromator, *e.g.* by use of a 380 nm cut-off filter when acquiring Eu emission spectra.

Europium lifetime measurements

Excited state lifetime measurements for europium and terbium were made on either the Perkin Elmer LS 50B (using *Phlemming* data acquisition written by Dr. A. Beeby, University of Durham) or the Instruments SA Fluorolog (using DataMax for Windows v2.1). Lifetimes were measured by excitation of the sample by a short pulse of light (355 nm for terbium, 397 nm for europium) followed by monitoring the integrated intensity of light (545 nm for terbium, 594 nm or 619 nm for europium) emitted during a fixed gate time, t_g, a delay time, t_d, later. At least 20 delay times were used covering three or more lifetimes. Typically gate times of between 100 and 250 μs were used, and the excitation and emission slits were set to 5–15 nm bandpass. The obtained decay curves were fitted to a simple mono-exponential decay curve using either Grafit 3.0 (Erithacus software) or Microsoft Excel.

Quantum yield determinations

Measurements were made relative to two known standards. For europium complexes these were Rhodamine 101 in ethanol ($\varphi = 1$) and Cresyl Violet in methanol ($\varphi = 0.54$). These standards were chosen as they emit in a similar spectral window to europium and possess a similar absorbance to the aromatic antenna at the excitation wavelength, in the studied complexes. For each of the standards and the unknown, five solutions with absorbances between 0.02 and 0.1 were used. For each of these solutions the absorbance at the excitation wavelength and the total integrated emission intensity was determined. Errors in quantum yield determinations can arise due to the inner filter effect or errors in the amount of absorbed light. The first of these can be very important when using references such as Rhodamine 101 and Cresyl Violet as these are both strongly absorbing in the emission region. This effect was minimised by only using samples with absorbances below 0.2. Errors in the amount of light absorbed by each sample were minimised by choosing the excitation wavelength to be on a relatively flat area of the absorption curve (*i.e. ca.* 270 nm) and by using a small bandpass for excitation.

Preparations

The preparation of $[EuL^4(H_2O)](CF_3SO_3)_3$ was reported in ref. 6.

6-Bromomethylphenanthridine 1. 6-Methylphenanthridine (1.4 g, 7.18 mmol) was dissolved in a mixture of CCl_4 (10 mL) and $CHCl_3$ (50 mL). *N*-Bromosuccinimide (1.5 g, 8.42 mmol, 1.1 eq.) and a catalytic amount of benzoyl peroxide (3 mg) were added and the resultant orange solution stirred under reflux for 5 h. The solution was cooled, filtered and the solvent removed under vacuum. The residue was purified by chromatography over silica [CH_2Cl_2–hexane (3 : 1), R_f 0.3] to afford the title compound (1.6 g, 82%) as a pale yellow solid, mp 107–108 °C. $\delta_H(CDCl_3$, 200 MHz): 4.98 (2H, s, CH_2Br), 7.67–7.80 (3H, m, ArH), 7.89 (1H, t, $J = 7.0$, ArH), 8.04 (1H, d, $J = 8.0$, ArH), 8.22 (1H, d, $J = 8.0$, ArH), 8.42 (1H, d, $J = 8.0$, ArH), 8.52 (1H, d, $J = 8.0$, ArH). $\delta_C(CDCl_3$, 200 MHz): 32.3 (CH_2), 122.2, 122.8, 124.2 (4 °C), 124.6 (4 °C), 126.5, 127.6, 127.8, 129.1, 130.3, 131.1, 133.6 (4 °C), 142.8 (4 °C), 156.2 (4 °C). *m/z* (ESMS+): 272/274 (65%, M + H), 295/297 (30%, M + Na^+). Found C, 61.6; H, 3.96; N, 5.01. $C_{14}H_{10}BrN$ requires: C, 61.6; H, 3.70; N, 5.15%.

1-(6′-Phenanthridylmethyl)-4-7,10-tris(*tert*-butoxycarboxymethyl)-1,4,7,10-tetraazacyclododecane 3. A suspension of 6-bromomethylphenanthridine **1** (85 mg, 0.313 mmol), 1,4,7 - tris(*tert*-butoxycarboxymethyl) - 1,4,7,10 - tetraazacyclododecane **2**, (123 mg, 0.239 mmol) and anhydrous potassium carbonate (37 mg, 0.268 mmol) in dry acetonitrile (5 mL) was heated at reflux for 18 h under argon. The solvents were removed under reduced pressure and the residue suspended in dichloromethane (20 mL) and filtered. Removal of solvents under reduced pressure gave a crude yellow–brown solid. Column chromatography on silica [gradient elution, 0.5, 2 then 4% MeOH–CH_2Cl_2, $R_f = 0.4$ (10% MeOH–CH_2Cl_2)] yielded a pale yellow solid (90 mg, 0.13 mmol, 54%). $\delta_H(CDCl_3)$: 1.10 (br s, 18H, 2-OBu^t), 1.52 (s, 9H, OBu^t), 1.8–3.4 (br, 24H), 7.42 (t, 1H, H-8, $J = 7.8$), 7.57 (t, 1H, H-9, $J = 8.0$), 7.69 (t, 1H, H-3, $J = 7.9$), 7.85 (t, 1H, H-2, $J = 8.1$), 8.03 (d, 1H, H-7, $J = 7.2$), 8.24 (d, 1H, H-4, $J = 8.1$), 8.52 (d, 1H, H-10, $J = 7.8$), 8.64 (d, 1H, H-1, $J = 8.4$). *m/z* (ESMS+): Found: 706.4541 (M + H)$^+$; $C_{40}H_{60}N_5O_6$ requires: 706.4544. $\delta_C(CDCl_3)$: 28.0, 28.2, 51–53 (br), 53.7, 56.1, 57.6, 82.1, 82.4, 106.8, 117.8, 123.9, 124.9, 125.2, 126.9, 128.0, 128.7, 129.7, 131.2, 132.8, 143.4, 158.0, 173.1 (C=O), 173.4 (C=O).

1-(6′-Phenanthridylmethyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane H_3L^1. A solution of 1-(6′-phenanthridylmethyl) - 4,7,10 - tris(*tert*-butoxycarboxymethyl) - 1,4,7,10-tetraazacyclododecane (90 mg, mmol) in trifluoroacetic acid (99%, 2 mL) was stirred for 18 h then the solvent was removed under reduced pressure. The resulting oil was triturated with dichloromethane (5 × 5 mL) to give a pale yellow solid (68 mg, 0.13 mmol, 99%), mp 124–126 °C. Found: C, 64.5; H, 8.91; N, 9.21; $C_{40}H_{59}N_5O_6 \cdot 2H_2O$ requires: C, 64.8; H, 8.56; N, 9.44%. $\delta_H(CD_3OD)$: 2.9–3.9 (m, 20H), 4.25 (s, 2H), 5.31 (s, 2H), 7.6–7.8 (m, 3H), 7.91 (dd, 1H, $J = 7.8$), 8.12 (d, 1H, $J = 8.4$), 8.25 (d, 1H, $J = 7.8$), 8.63 (d, 1H, $J = 7.8$), 8.75 (d, 1H, $J = 8.1$); $\delta_C(CD_3OD)$: 49.1, 51.7, 52.3, 52.7, 55.7, 57.6, 122.2, 122.8, 123.4, 124.5, 124.7, 128.0, 128.2, 129.5, 130.0, 132,1, 133.5, 142.0, 150.5, 168.3 (C=O), 172.6 (C=O); ν/cm^{-1}: 3400 (br, OH) 3041 (br, aryl CH) 2918(m, CH) 1669(s, C=O), 1427(m), 1348(m). λ_{max}(MeOH)/nm: 248 (18 100), 274 (2700), 304 (2100), 330 (1100), 348 (1200).

[$EuL^1(H_2O)$]. A suspension of ligand H_3L^1 (68 mg, 0.127 mmol) and europium triacetate (64 mg, 0.191 mmol) in methanol (2 ml) was stirred at 60 °C for 18 h under argon. Solvents were removed under reduced pressure and the residue purified by chromatography on reverse phase silica [EtOH, $R_f = 0.2$(20% H_2O–EtOH)] to yield an off-white solid, (51 mg, 0.074 mmol, 59%). $\delta_H(D_2O$–10% CD_3OD, 200 MHz, 293 K) a complex broadened spectrum comprising one main isomer for which a partial assignment is: 37.5 (1H), 28.8 (2H), 20.5 (br, 1H) (ring H_{ax}). *m/z* (ES+): 708 (M + Na^+, ^{151}Eu), 710 (M + Na^+, ^{153}Eu), 724 (M + K^+, ^{151}Eu), 726 (M + K^+, ^{153}Eu). Found: C, 44.0; H, 5.59; N, 8.90 $C_{28}H_{32}N_5O_6Eu \cdot 4H_2O$ requires: C, 44.3; H, 5.31; N, 9.23%.

1-(6′-Phenanthridylmethyl)-1,4,7,10-tetraazacyclododecane 5. 6-Bromomethylphenanthridine **1** (220 mg, 0.809 mmol), cyclen (443 mg, 2.57 mmol, 3.2 equiv.) and caesium carbonate (805 mg, 2.47 mmol) were stirred in acetonitrile (3 mL) overnight under argon at room temperature. The solvent was removed under reduced pressure and the residue taken up into $CHCl_3$ (20 mL) and washed with water (3 × 30 mL), dried (Na_2SO_4), filtered and solvent removed under vacuum to give a pale yellow oil (230 mg, 78% crude yield) which was used without purification. $\delta_H(CDCl_3$, 200 MHz): 2.37–2.76 (16H, m, ring CH_2), 4.24 (2H, s, CH_2), 7.53–7.64 (3H, m, ArH), 7.71 (1H, t, $J = 8.2$Hz, ArH), 8.05 (1H, d, $J = 8.2$Hz, ArH), 8.42–8.47 (2H, m, ArH), 8.51 (1H, d, $J = 8.2$Hz, ArH). *m/z* (ESMS+): 364 (100%, M + H). Found 364.2504; $C_{22}H_{30}N_5$ requires 364.2502.

1-(6′-Phenanthridylmethyl)-4,7,10-tris-[(*S*)-1-(1-phenyl)-ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane L^2. 1-(6′-Phenanthridylmethyl)-1,4,7,10-tetraazacyclododecane **5** (200 mg, 0.554 mmol), (*S*)-*N*-chloroethanoyl-2-phenylethylamine (340 mg, 1.72 mmol, 3.1 equiv.) and caesium carbonate (550 mg, 1.68 mmol) were stirred overnight in a mixture of MeCN (2 mL) and CH_2Cl_2 (2 mL) at 60 °C under argon. The solvent was removed under vacuum and the residue taken up into CH_2Cl_2 (20 mL), washed with water (70 mL), dried (Na_2SO_4), filtered and evaporated to dryness to afford the title compound (340, mg, 73%) as an orange oily residue. $\delta_H(CDCl_3$, 200 MHz): 1.22 (3H, d, $J = 6.8$Hz, CH_3), 1.33–1.43 (6H, m, CH_3), 2.40–2.83 (20H, m, ring NCH_2, pendant arm NCH_2), 3.88–3.91 (4H, m, pendent arm NCH_2), 4.91–5.10 (3H, m, CH), 7.04–7.28 (18H, m, phenyl H + amide NH), 7.47–7.78 (4H, m, phen H), 7.89 (1H, t, $J = 8.0$ Hz, phen H), 8.26 (1H, t, $J = 8.0$ Hz, phen H), 8.37–8.57 (2H, m, phen H). *m/z* (ESMS+): 444 (100%, M^{2+}, M + Ca^{2+}), 847 (35%, M + H), 869 (85%, M + Na^+). Found 847.5011 (M + H); $C_{52}H_{62}N_8O_3$ requires 847.5023.

[EuL^2(H$_2$O)](CF$_3$SO$_3$)$_3$. The Eu(III) complex was prepared by stirring ligand L^2 (42 mg, 0.05 mmol) with 1 equivalent of Eu(CF$_3$SO$_3$)$_3$ in dry MeCN (1 mL) at 80 °C overnight. The solvent was removed under vacuum and the complex precipitated by slowly adding an acetonitrile solution (0.5 ml) of the complex to dry THF (10 ml). The resultant residue was taken up into a mixture of methanol (1 mL) and distilled water (10 mL) and removal of solvents by lyophilisation gave the complex as a pale yellow glassy solid. *m/z* (ESMS+): 499 (85%, $[M + H]^{2+}$), 574 (100%, $[M + CF_3SO_3]^{2+}$), 1297 (5%, $[M + (CF_3SO_3)_2]^{+}$). Found: C, 44.85; H, 4.28; N, 7.82. $C_{55}H_{62}N_8EuF_9O_{12}S_3 \cdot 2H_2O$ requires C, 44.5; H, 4.48; N, 7.55%. δ_H(CD$_3$OD: >90% of one stereoisomer) 39.2, 35.6, 25.2, 22.3 (s, 1H each, ring H_{ax}); 15.8 (br s, 1H, phen H), 13.1 (1H), 11.2 (3H), 10.0 (1H), 9.0–0.0 (m, 30H aryl H, CH + CH$_3$), −1.2 (2H), −2.0 (2H), −2.7 (1H), −3.2 (1H), −7.8 (2H), −11.4 (2H), −12.5 (1H), −13.0 (1H), −14.9 (1H), −16.4 (1H), −19.2 (1H), −20.4 (1H), −20.9 (1H, NCHCO).

2-Bromo-6-butylphenanthridine 6. 2-Bromophenanthridine (0.98, 3.8 mmol) was dissolved in dry diethyl ether (20 ml) under argon. The suspension was cooled down to −10 °C, a solution of butylmagnesium chloride in dry diethyl ether (2.2 ml, 4 mmol) was added dropwise and the mixture stirred for 2 h at −10 °C. The solution was allowed to warm to room temperature overnight. The orange solution was quenched with hydrochloric acid (5 ml, 0.1 M solution), extracted with diethyl ether (3 × 20 ml) and with dichloromethane (4 × 20 ml). The combined organic layers were washed with brine (2 × 20 ml), and dried over potassium carbonate. Solvent was removed under reduced pressure, the residue dissolved in dichloromethane (50 ml) and manganese dioxide (1 g) added. The suspension was stirred overnight at room temperature, the solid filtered off and the residue was purified by chromatography on silica gel (R_f = 0.3, dichloromethane) to give a pale yellow solid (0.93 g, 78%), mp 118–119 °C δ_H(CDCl$_3$): 8.66 (d, 1H, H-1, *J* = 2), 8.56 (d, 1H, H-4, *J* = 8), 8.26 (d, 1H, H-7, *J* = 8), 7.80 (d, 1H, H-10, *J* = 8.6), 7.85–7.73 (m, 3H, H-3, H-9, H-8), 3.35 (t, 2H, C*H*$_2$Ar, *J* = 8), 1.90 (m, 2H, C*H*$_2$CH$_2$Ar), 1.55 (tq, 2H, C*H*$_2$CH$_3$), 1.01 (t, 3H, CH$_3$). *m/z* (ES$^+$): Found: 314.0545, $C_{17}H_{16}BrN$ requires: 314.0546 $(M + H^+)$.

2-Cyano-6-butylphenanthridine 7. 2-Bromo-6-butylphenanthridine (0.9 g, 2.8 mmol) was dissolved in anhydrous dimethylformamide (20 ml). The solution was heated at 100 °C for 1 h and copper(I) cyanide (0.55 g, 5.7 mmol) added. The reduced solution was stirred at 140 °C under argon for 2 days. Solvent was removed under reduced pressure and the residue was taken up in concentrated hydrochloric acid (30 ml, 6 M) to give a yellow solution. The pH of the solution was increased to 12 by addition of potassium hydroxide pellets and the product was extracted using dichloromethane (4 × 20 ml). The combined organic layers were washed with aqueous potassium carbonate solution (0.1 M, 2 × 20 ml), dried over potassium carbonate and the residue was recrystallised twice from ethanol to give a pale yellow solid (0.57 g, 77%), mp 110–111 °C. δ_H(CDCl$_3$): 8.83 (d, 1H, *J* = 1, H-1), 8.56 (d, 1H, *J* = 5.4, H-4), 8.28 (d, 1H, *J* = 5.6, H-7), 8.15 (d, 1H, *J* = 6, H-10), 7.90–7.78 (m, 3H, H-3, H-9, H-8), 3.37 (t, 2H, *J* = 5.2, C*H*$_2$Ar), 1.90 (m, 2H, CH$_2$C), 1.55 (tq, 2H, CH$_2$C), 1.01 (t, 3H, CH$_3$). δ_C(CDCl$_3$): 164.8 (C-6), 144.3 (*C*CN), 130.5, 130.2, 129.6, 129.0, 127.5, 126.6, 125.5, 124.4, 122.6, 121.3, 118.1 (Ar-C), 108.4 (CN), 35.1 (*C*H$_2$Ar), 30.3 (*C*H$_2$C), 22.0 (*C*H$_2$C), 12.9 (CH$_3$). *m/z* (ES$^+$): 260.9 $(M + H)^+$. ν_{max}/cm^{-1} (thin film): 2940 (CH), 2215 (CN). Found: C, 81.79; H, 6.08; N, 10.48. $C_{18}H_{16}N_2$(+0.25 H$_2$O) requires C, 80.7; H, 6.25; N, 10.60%.

2-Aminomethyl-6-butylphenanthridine 8. 2-Cyano-6-butylphenanthridine (0.1 g, 0.38 mmol) was dissolved in borane–tetrahydrofuran solution (1 M, 15 ml) under argon and the mixture heated at 70 °C for 4 days. The reaction was monitored by analysis of a quenched sample by IR spectroscopy. The solution was cooled to room temperature and methanol (20 ml) was added carefully, dropwise. The solvent was removed and the residue was taken up in methanol (20 ml); this procedure was repeated three times and finally the residue was dissolved in hydrochloric acid (1 M, 20 ml) and heated at 80 °C for 1 h. The temperature was maintained at 60 °C overnight. The acid solution was washed using diethyl ether (30 ml), the pH was raised to 13 and the product extracted using dichloromethane (5 × 20 ml). The combined extracts were washed with brine (2 × 20 ml), dried over potassium carbonate and the solvent removed under pressure to yield a brown solid, 91 mg (90%) which was used directly without further purification. δ_H(CDCl$_3$): 8.53 (d, 1H, H-4, *J* = 8), 8.36 (s, 1H, H-1), 8.13 (d, 1H, H-7, *J* = 8.2), 7.89 (d, 1H, H-10, *J* = 6), 7.70 (t, 1H, H-3), 7.90–7.78 (m, 2H, H-9, H-8), 4.0 (br, s, 2H, NH$_2$), 3.37 (s, 2H, CH$_2$N), 3.23 (t, 2H, *J* = 5.2, CH$_2$C), 1.80 (m, 2H, CH$_2$C), 1.45 (tq, 2H, *J* = 3.2, CH$_2$C), 0.91 (t, 3H, CH$_3$). δ_C(CDCl$_3$): 162.2 (C-6), 142.9, 132.9, 130.3, 129.7, 128.2, 127.3, 126.4, 125.3, 123.7, 122.6, 120.0 (Ar-C), 50.5 (*C*H$_2$Ar), 36.2 (*C*H$_2$Ar), 31.9 (*C*H$_2$C), 23.3 (*C*H$_2$C), 14.3 (CH$_3$). *m/z* (ES$^+$): 264.9 $(M + H)^+$.

***N*-Chloroacetyl-2-aminomethyl-6-butylphenanthridine 9.** 2-Aminomethyl-6-butylphenanthridine (0.32 g, 1.24 mmol) was dissolved in tetrahydrofuran (30 ml) under argon. Chloroacetic acid (0.068 ml, 1.26 mmol) and 1-hydroxybenzotriazole (HOBT) (0.116 g, 1.26 mmol) were added and the solution was cooled at 0 °C. EDC [1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] (0.245 g, 1.29 mmol) was then added and the solution stirred at 0 °C for 1 h and at room temperature for a further 3 h. The solvent was removed and the residue washed with cold tetrahydrofuran (20 ml). The product was extracted using dichloromethane (3 × 25 ml), washed using a saturated solution of sodium carbonate (2 × 20 ml) and finally dried over potassium carbonate. Solvent was removed to yield a white solid, 0.33 g (78%), mp > 250 °C. δ_H(CDCl$_3$): 8.63 (d, 1H, *J* = 8, H-4), 8.39 (s, 1H, H-1), 8.20 (d, 1H, *J* = 8.2, H-7), 8.03 (d, 1H, *J* = 6, H-10), 7.80 (t, 2H, H-3), 7.65–7.50 (m, 2H, H-9, H-8), 6.95 (br s, 1H, NH), 4.67 (d, 2H, *J* = 6, C*H*$_2$NH), 4.10 (s, 2H, CH$_2$Cl), 3.30 (t, 2H *J* = 5.2, C*H*$_2$Ar), 1.83 (m, 2H, CH$_2$C), 1.45 (m, 2H, CH$_2$C), 0.93 (t, 3H, CH$_3$). δ_C (CDCl$_3$): 165.9 (C6), 162.6, 143.1, 135.1, 132.4, 130.2, 130.0, 128.1, 127.4, 126.3, 125.2, 123.5, 122.3, 121.1 (Ar-C); 44.0 (CH$_2$Cl), 42.6 (CH$_2$N), 36.1 (*C*H$_2$Ar), 31.6 (*C*H$_2$C), 23.0 (*C*H$_2$C), 14.0 (CH$_3$). *m/z* (ES$^+$) (MeOH): 340.9, 342.9 $(M + H)^+$. Found: 341.1421; $C_{20}H_{22}ClN_2O$ requires 341.1421 $(M + H)^+$. Found: C, 70.11; H, 6.61; N, 8.05. $C_{20}H_{21}ClN_2O$ requires C, 70.47; H, 6.21; N, 8.21%. ν_{max}/cm^{-1} (thin film): 3103, 1637, 1539, 1226, 1051.

1-{Methylcarbonyl[2-aminomethyl(6-butylphenanthridinyl)]}-4,7,10-tris-(*tert*-butoxycarboxymethyl)-1,4,7,10-tetraazacyclododecane 10. 1,4,7-Tris(*tert*-butoxycarboxymethyl)-1,4,7,10-tetraazacyclododecane (0.15 g, 0.29 mmol) and *N*-chloroacetyl-2-aminomethyl-6-butylphenanthridine (0.1 g, 0.2 mmol) were dissolved in dry acetonitrile (20 ml). Potassium carbonate (0.02 g, 0.15 mmol) was added and the mixture was heated at 70 °C for 4 h. Solvent was removed under reduced pressure and the residue taken in dichloromethane, washed with water (2 × 20 ml) and dried over potassium carbonate. The product was purified on alumina chromatography column (R_f = 0.2, 5% EtOH-CH$_2$Cl$_2$), to give a colourless solid (0.1 g, 62%). δ_H(CDCl$_3$): 10.2 (1H, t), 8.70 (d, 1H, *J* = 8.4, H-4), 8.57 (s, 1H, H-1), 8.18 (d, 1H, *J* = 8.2, H-7), 7.93 (d, 1H, *J* = 8.4, H-10), 7.80–7.58 (m, 3H, H-3, H-9, H-8), 4.64 (d, 2H, *J* = 5.7, C*H*$_2$NH), 3.31 (t, 2H, *J* = 8.1, C*H*$_2$Ar), 2.60–2.2 (br m, 16H, CH$_2$ ring), 1.80 (m, 2H,

CH_2C), 1.50 (m, 2H, CH_2C), 1.29 (27H, m, Bu^t), 0.97 (t, 3H, CH_3). $\delta_C(CDCl_3)$: 172.4 (C=O), 161.8 (C-6), 142.8, 138.7, 133.3, 130.4, 129.3, 129.1, 127.2, 126.2, 125.2, 123.6, 123.3, 121.3 (Ar-C); 82.0, 81.9 [$C(CH_3)_3$], 58.2 (CH_2NH), 56.3–55.8 (8C, ring CH_2N), 43.3 (C-2′), 36.4 (CH_2Ar), 31.5 (CH_2C), 28.3, 28.1, 28.0 [$C(CH_3)_3$], 23.3 (CH_2C), 14.2 (CH_3). *m/z* (ES+) (MeOH): 842 $(M + Na)^+$.

1-{Methylcarbonyl-[2-aminomethyl(6-butylphenanthridinyl)]}-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane H_3L^3. The *tert*-butyl ester (0.1 g, 0.12 mmol) was dissolved in a solution of 80% trifluoroacetic acid in dichloromethane (10 ml). The mixture was stirred at room temperature for 3 h, solvents were removed under reduced pressure and the residue taken up into water (1 ml). Solvent was removed by lyophilisation to yield colourless solid (0.073 g, 95%), which was used without further purification. $\delta_H(D_2O$, pD 5): 8.40 (d, 1H, $J = 8.4$, H-4), 8.16 (m, 2H, H-1, H-7), 7.90 (d, 1H, H-10), 7.75–7.55 (m, 3H, H-3, H-9, H-8), 4.39 (br d, 2H, CH_2NH), 3.85 (br s, 1H, NH), 3.2–2.8 (br m, 16H, CH_2 ring), 1.45 (m, 2H, CH_2C), 1.15 (2H, m, CH_2C), 0.64 (m, 3H, CH_3). *m/z* (ES+): 647 $(M + H)^+$.

[$EuL^3(H_2O)$]. Europium acetate (15 mg, 45 μmol) and ligand H_3L^3 (25 mg, 38 μmol) were dissolved in water (3 ml). The pH was adjusted to 5.5 and the solution heated at 80 °C for 2 h. The product was purified on a short alumina chromatography column (eluent 10% MeOH-CD_2Cl_2), to give a colourless solid (27 mg, 90%). δ_H (D_2O, pD = 5.5): 33.8 (1H), 32.1 (2H), 31.6 (1H) (ring H_{ax}); 8.1–7.0 (m, 7H, phen H), 4.0–1.0 (m, 11H, CH_2N + butyl chain), 0.1 (br s, 2H, $H_{eq'}$), −2.4 (1H, $H_{eq'}$), −3.1 (2H, $H_{eq'}$), −4.8 (1H, H_{eq}), −6.0 (1H, H_{eq}), −7.2 (2H, $H_{ax'}$, H_{eq}), −7.6 (1H, $H_{ax'}$), −11.5 (2H, $H_{ax'}$); −11.9 (1H, CHCO), −12.4 (1H, CHCO), −14.8 (2H, CHCO), −15.6 (1H, CH′CO), −16.0 (1H, CHCO), −17.0 (1H, CH′CO), −17.5 (1H, CHCO). *m/z* (ES+) (MeOH): 801 $(M + H)^+$, 839 $(M + K)^+$: Found: 800.2270; $C_{34}H_{44}N_6O_7Eu$ requires 800.2272 $(M + H)^+$.

***tert*-Butyl {1-[(2′-phenanthridylmethyl)carbamoyl]-2-methylpropyl}carbamate 11.** A solution of BOC-valine (1.540 g, 7.10 mmol), 2-aminomethyl-6-butylphenanthridine **8**, (1.48 g, 7.1 mmol), hydroxybenzotriazole (0.96 g, 7.1 mmol), EDC (1.35 g, 7.1 mmol) and triethylamine (1.0 mL, 7.2 mmol) in dichloromethane (50 mL) was stirred at 20 °C for 18 h. The solution was then washed with water (25 mL), citric acid solution (10% in water, 25 mL), and sodium hydrogen carbonate solution (5% in water, 25 mL). The combined organic layers were dried over anhydrous potassium carbonate and solvents removed under reduced pressure to yield a colourless residue. Column chromatography on silica [gradient elution, CH_2Cl_2, then 4% MeOH–CH_2Cl_2, $R_f = 0.25$, (5% MeOH–CH_2Cl_2)] yielded an off-white semicrystalline solid (2.2 g, 5.40 mmol, 76%), mp 118–120 °C Found: C, 70.7; H, 7.12; N, 10.1. $C_{24}H_{28}N_3O_3$ requires: C, 70.9; H, 6.94; N, 10.3%. $\delta_H(CDCl_3)$: 0.94 [t, 6H, $J = 7$, $(CH_3)_2CH$], 1.35 [s, 9H, $(CH_3)_3C$], 2.13 [hept, 1H, $J = 7$, $(CH_3)_2CH$], 4.08 (br t, $J = 8.0$, 1H), 4.47 + 4.61 (dd + dd, 2H, $J = 5.6$, 15.2, CH_2), 5.57 (d, 1H, $J = 8.0$), 7.40 (d, 1H, $J = 8.1$), 7.53 (dd + br t, 2H, $J = 7.1$, NHCO + H-8), 7.64 (dt, 1H, $J = 7.7$), 7.80 (d, 1H, $J = 7.8$), 7.88 (d, 1H, $J = 8.4$), 8.13 (s, 1H, H-1), 8.28 (d, 1H, $J = 8.1$), 9.02 (s, 1H, H-6); $\delta_C(CDCl_3)$: 18.2, 19.5, 28.4, 31.0, 43.4 (CH_2phen), 60.5 (NH*C*HCO), 79.8 ($OCMe_3$), 120.7, 121.8, 123.8, 126.2, 127.4, 128.0, 128.5, 130.1, 130.8, 132.1, 137.1, 143.5, 153.2, 156.2 (NHCO), 172.3 (NHCO) *m/z* (ES+): 408 (100%, $M + H^+$), 430 (80%, $M + Na^+$), 815 (40%, $2M + H^+$), 837 (60%, $2M + Na^+$); ν/cm^{-1}: 3294 (br, NHCO), 2962 (m, CH), 1671 (m, CO) 1646 (s, NHCO) 1520(s); λ_{max}(MeOH)/nm: 210 (29 000), 221 (23 000), 249 (42 000), 273 (sh) (9300), 294 (6700), 331 (2400); 347 (2300); $[\alpha]_D = 14$ (*c* 1.08, MeOH).

2-Ammonio-*N*-(2′-phenanthridylmethyl)-3-methylbutyramide trifluoroethanoate 12. A solution of the *tert*-butyl ester (870 mg, 2.14 mmol) in trifluoroacetic acid (2 mL) was stirred at room temperature for 18 h; solvent was removed under reduced pressure and the resulting oil was triturated with dichloromethane (5 × 5 mL) to give a yellow solid (0.9 g, 2.14 mmol, 98%), mp 99–101 °C. $\delta_H(CD_3OD)$: 1.11 [d + d, 6H, $J = 6.6$, $(CH_3)_2CH$], 2.33 [hept, 1H, $J = 6.6$, $(CH_3)_2CH$], 3.95 (d, 1H, $J = 5.7$, ^+H_3NCH), 4.66 + 4.50 (d + d, 2H, $J = 15.9$, CH_2Ar), 7.70 (d, 1H, $J = 8.7$), 7.74–7.86 (m, 2H), 7.99 (dd, 1H, $J = 7.5$), 8.24 (d, 1H, $J = 8.1$), 8.29 (s, 1H), 8.34 (d, 1H, $J = 8.4$), 9.36 (s, 1H). *m/z* (ES+): 308 (100%, M^+), 615 (5%, $2M^+$); $[\alpha]_D = +15.9$ (*c* 2.57, MeOH).

***N*-(2′-phenanthridylmethyl)-2-(carbamoylchloromethyl)-3-methylbutyramide 13.** A solution of chloroethanoic acid (39 mg, 0.41 mmol), 2-amino-*N*-(2′-phenanthridylmethyl)-3-methylbutyramide trifluoroethanoic acid salt (174 mg, 0.41 mmol), HOBT (55 mg, 0.41 mmol), EDC (79 mg, 0.41 mmol) and triethylamine (0.15 mL, 1.1 mmol) in dry tetrahydrofuran (50 mL) was stirred at 20 °C for 18 h. Solvents were removed under reduced pressure and the resulting residue was partitioned between dichloromethane (50 mL) and water (50 mL). The organic layer was washed with aqueous citric acid (10%, 50 mL), and aqueous sodium hydrogen carbonate (5%, 50 mL). The organic layer was dried over anhydrous potassium carbonate and then solvents removed under reduced pressure to yield a colourless residue. Column chromatography on silica [gradient elution, CH_2Cl_2, 2 then 10% MeOH–CH_2Cl_2, $R_f = 0.54$, (10% MeOH–CH_2Cl_2)] yielded an off-white crystalline solid (50 mg, 0.13 mmol, 32%), mp >220 °C. Found: C, 65.4; H, 5.97; N, 10.6. $C_{21}H_{22}ClN_3O_2$ requires: C, 65.7; H, 5.78; N, 10.9%. $\delta_H(CDCl_3$–CD_3OD, 10 : 1): 0.91 [d + d, 6H, $J = 6.8$, $(CH_3)_2CH$], 2.07 (hept, 1H, $J = 6.8$, $(CH_3)_2CH$], 4.00 (s, 2H, CH_2Cl), 4.18 (d, 1H, $J = 7.5$, NC*H*CO), 4.61 + 4.47 (d + d, 2H, $J = 15.0$, CH_2Ar), 7.57 (d, 1H, $J = 8.1$), 7.66 (dd, 1H, $J = 7.5$), 7.82 (dd, 1H, $J = 7.7$), 7.98 (d, 1H, $J = 8.1$), 8.02 (d, 1H, $J = 8.4$), 8.41 (s, 1H), 8.55 (d, 1H, $J = 8.1$), 9.13 (s, 1H). *m/z* (ES+): 384 (M + H), 406 $(M + Na^+)$.

1-{*N*-[1-((2′-phenanthridylmethyl)carbamoyl)-2-methylpropyl]carbamoylmethyl}-4,7,10-tris(*tert*-butoxycarboxymethyl)-1,4,7,10-tetraazadodecane 14. A suspension of *N*-(2′-phenanthridylmethyl)-2-(chloromethylamino)-3-methylbutyramide, (50 mg, 0.13 mmol), 1,4,7-tris(*tert*-butoxycarboxymethyl)-1,4,7-10-tetraazacyclododecane (67 mg, 0.13 mmol), caesium carbonate (42 mg, 0.13 mmol) and potassium iodide (22 mg, 0.13 mmol) in dry acetonitrile (5 mL) was heated at reflux for 18 h under an inert atmosphere of argon. Solvent was removed under reduced pressure and the resulting solids were suspended in dichloromethane (20 mL) and filtered. The filter cake was washed well with dichloromethane (3 × 20 mL). Removal of solvents under reduced pressure gave a crude yellow–brown solid. Column chromatography on silica gel [gradient elution: 0.5, 2 then 4% MeOH–CH_2Cl_2; $R_f = 0.65$ (10% MeOH–CH_2Cl_2)] yielded a pale yellow solid (101 mg, 0.12 mmol, 90%). *m/z* (ESMS+) Found: 862.5436 (M + H); $C_{47}H_{72}N_7O_8$ requires: 862.5443. $\delta_H(CDCl_3)$ 0.90 (d + d, 6H, CH_3). 1.42 (br, 27H, Bu^t), 1.6–3.9 (br m, 25H, CH_2N + CH), 4.30 (dd, 1H, CHN), 4.59 (m, 2H, CH_2phen), 4.82 (br, NHCO), 7.66 (t, 1H, $J = 7.5$), 7.8–7.9 (m, 2H), 7.97 (d, 1H, $J = 8$), 8.05 (d, 1H, $J = 8$), 8.45 (br, 1H, H-2), 8.86 (br, 1H), 9.18 (s, 1H, H-6).

1-{*N*-[1-((2′-Phenanthridylmethyl)carbamoyl)-2-methylpropyl]carbamoylmethyl}-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazadodecane H_3L^5. A solution of the tris(*tert*-butyl ester) **14** (101 mg, 0.12 mmol) in trifluoroacetic acid (2 mL) was stirred for 18 h. Solvent was removed under reduced pressure and the resulting oil was triturated with dichloro

methane (5 × 5 mL) to give an off-white solid (80 mg, 0.115 mmol, 98%) which was used directly without further purification. $\delta_H(F_3CO_2D)$: 1.08 (s + s, 6H, Me), 3.2–4.4 (br m, 16H, CH_2N), 3.67 (br m, 8H, CH_2CO), 4.91 + 5.12 (d + d, 2H, CH_2phen), 8.1–8.6 (m, 5H), 9.01 (s, 1H, H-2), 9.10 (d, 1H), 9.68 (s, 1H, H-6).

[EuL^5(H$_2$O)]. This was prepared in 56% yield as described for [EuL^1], and was purified by chromatography on neutral alumina (CH_2Cl_2, 2–4–10% MeOH gradient elution) to yield a colourless solid. *m/z* (ES+): 444 (90%, M + 2Na$^+$, ^{151}Eu), 445 (100%, M + 2Na$^+$, ^{153}Eu), 864 (10%, M + Na$^+$, ^{151}Eu), 866 (12%, M + Na$^+$, ^{153}Eu). Found: 842.2239 (M + H); $C_{35}H_{45}N_7O_8Eu$ requires: 842.2233. $\delta_H(CD_3OD)$ 38.4, 35.8, 35.1, 33.8 (4H, ring H_{ax}); 10–0.2 (br m, 18H), −0.9, −2.0, −2.8, −3.6 (4H, $H_{eq'}$); −4.8, −6.4, −6.5, −6.9 (4h, H_{eq}); −11.0, −12.8, −14.0, −14.5 (4H, $H_{ax'}$); −17.3, −17.7, −17.9, −18.5 (8H, CH_2CO).

We thank EPSRC, the Royal Society and BBSRC for support and Dr Bob Peacock (University of Glasgow) for the CPL spectrum.

References

1 (*a*) N. Sabbatini, M. Guardigli and J-M. Lehn, *Coord. Chem. Rev.*, 1993, **123**, 201; (*b*) G. Mathis, *Clin. Chem.*, 1995, **45**, 1391.
2 (*a*) P. R. Selvin, T. M. Rana and J. E. Hearst, *J. Am. Chem. Soc.*, 1994, **116**, 6029; (*b*) M. Li and P. R. Selvin, *Bioconjugate Chem.*, 1997, **8**, 127; (*c*) D. Parker and J. A. G. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1996, 1581.
3 (*a*) R. S. Dickins, J. A. K. Howard, C. L. Maupin, J. M. Moloney, D. Parker, J. P. Riehl, G. Siligardi and J. A. G. Williams, *Chem. Eur. J.*, 1999, **5**, 1095; (*b*) A. Casnati, C. Fischer, M. Guardigli, A. Isernia, I. Manet, N. Sabbatini and R. Ungaro, *J. Chem. Soc., Perkin Trans. 2*, 1996, 395.
4 M. H. V. Werts, M. A. Duin, J. W. Hofstraat and J. W. Verhoeven, *Chem. Commun.*, 1999, 799.
5 (*a*) S. Petoud, J.-C. G. Bunzli, F. Renaud, C. Piguet, K. J. Schenk and G. Hopfgartner, *Inorg. Chem.*, 1997, **36**, 5750; (*b*) M. Elhabiri, R. Scopelliti, J.-C. G. Bunzli and C. Piguet, *Chem. Commun.*, 1998, 2347.
6 D. Parker, P. K. Senanayake and J. A. G. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2129.
7 S. Aime, M. Botta, D. Parker and J. A. G. Williams, *J. Chem. Soc., Dalton Trans.*, 1995, 2259.
8 S. Aime, A. S. Batsanov, M. Botta, J. A. K. Howard, M. P. Lowe and D. Parker, *New J. Chem.*, 1999, **23**, 669.
9 A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans. 2*, 1999, 493.
10 B. R. T. Keene and P. Tissington, *J. Chem. Soc.*, 1965, 4426.
11 A. P. de Silva, H. Q. N. Gunaratne and T. E. Rice, *Angew. Chem. Int. Ed. Engl.*, 1996, 16, 2116; D. Parker, *Coord. Chem. Rev.* in press; A. Weller, *Pure Appl. Chem.*, 1968, **16**, 115.
12 (*a*) J. R. Winkler and H. B. Gray, *Chem. Rev.*, 1992, **92**, 369; (*b*) J. Jortner and M. Bixon, *J. Chem. Phys.*, 1988, **88**, 167; (*c*) G. L. Closs and J. R. Miller, *Science*, 1988, **240**, 440.
13 J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991.
14 *Handbook of Organic Photochemistry*, ed. J. C. Scaiano, CRC Press, Boca Raton, FL, 1989.
15 N. Sabbatini, M. Guardigli and I. Manet, in *Handbook on the Physics and Chemistry of Rare Earths*, ed. K. A. Gschneidner Jr. and L. R. Eyring, Elsevier, Amsterdam, 1996, vol. 23, ch. 154.
16 *Handbook of Photochemistry*, ed. S. L. Murov, I. Carmichael and G. L. Hug, 2nd edn., Dekker, New York, 1993.
17 M. Klessinger and J. Michl, *Excited States and Photochemistry of Organic Molecules*, VCH, New York, 1995.
18 T. Förster, *Ann. Phys. (Leipzig)*, 1948, **2**, 55.
19 T. Förster, *Discuss. Faraday Soc.*, 1959, **27**, 7.
20 V. L. Ermolaev and E. B. Sveshnikova, *Dokl. Akad. Nauk. SSSR*, 1963, **149**, 1295.
21 M. A. Baldo, M. E. Thompson and S. R. Forrest, *Nature*, 2000, **403**, 750.
22 (*a*) S. Aime, M. Botta, R. S. Dickins, C. L. Maupin, D. Parker, J. P. Riehl and J. A. G. Williams, *J. Chem. Soc., Dalton Trans.*, 1998, 881; (*b*) S. Aime, A. S. Batsanov, M. Botta, R. S. Dickins, S. Faulkner, C. E. Foster, A. Harrison, J. A. K. Howard, J. M. Moloney, T. J. Norman, D. Parker, L. Royle and J. A. G. Williams, *J. Chem. Soc., Dalton Trans.*, 1997, 3623.
23 D. Parker and J. A. G. Williams, *J. Chem. Soc., Dalton Trans.*, 1996, 3613.