KEYNOTE

# Luminescent sensors for pH, $pO_2$, halide and hydroxide ions using phenanthridine as a photosensitiser in macrocyclic europium and terbium complexes

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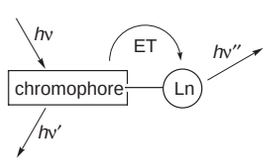
**The metal-based luminescence of the Eu and Tb complexes of the octadentate ligands $L^1$ and $L^{2a/2b}$ is a function of pH in aqueous solution: in $[EuL^1]$, the red-emission is switched on following *N*-protonation of the sensitising phenanthridine group ($pK_a = 4.2$, $pK_{S_1} = 4.4$) with a luminescence enhancement of over 500 for $\lambda_{exc} \geqslant 370$ nm. With the Tb complex, the green luminescence switches off following protonation ($pK_{T_1} = 5.7$) because back-energy transfer occurs rapidly to the low-lying triplet state only in the protonated complex ($E_T = 21\,300$ cm$^{-1}$) and not in the unprotonated ($E_T = 22\,000$ cm$^{-1}$) form. In the corresponding *N*-methylated complexes, the metal-based emission is quenched by halide ions (*e.g.* $K_{sv}^{-1} = 40$ mmol dm$^{-3}$ for $Cl^-$ in $[MeEuL^1]^+$) and, for the terbium complexes only, by molecular oxygen ($K_{sv}^{-1} = 45$ Torr for $[MeTbL^1]^+$).**

Single-component and chemically robust luminescent chemosensors are required for the analysis of bioactive ions or molecules in solution.[1a] In addition, such responsive luminescent probes may find application in monitoring or assaying the ionic composition of aqueous samples of interest to environmental or industrial process analysis. Several successful fluorescent chemosensors have been devised for determining, *in vitro*, the concentration of species such as $H^+$,[2] $Ca^{2+}$,[3] $Zn^{2+}$[4] and chloride.[5] There is a need for methods of measuring chloride in the physiological range (*ca.* 100 mM extracellular and 5 to 75 mM intracellular) due to its important rôle in controlling endosomal acidification.[1b] Chloride is an essential anion in maintaining a proper water distribution and in regulating both the normal anion/cation balance and the osmotic pressure. In particular, chloride ion channels are involved in the facilitated exchange of $Cl^-$ for $HCO_3^-$ in erythrocytes; malfunction of this system has been implicated in patients suffering from cystic fibrosis.[6] Whilst there have been many reports of 'synthetic receptors' for the chloride anion, few—if any—exhibit good selectivity in aqueous media, although some show promise for application when immobilised in a suitable membrane.[7–10]

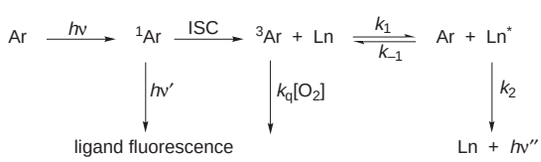
There is also strong current interest in determining $pO_2$ in aqueous media or at an interface. Whilst electroanalytical devices such as the Clark electrode have been used successfully for many years, there is currently much interest in optical sensors. Most of these employ an aromatic chromophore wherein luminescence from the excited state is quenched by triplet (ground-state) oxygen. Many have involved ruthenium diimine complexes with excited state lifetimes in the microsecond range and second-order rate constants for quenching have been determined which are typically of the order of $2 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$.[11,12] Other *longer-lived* luminescent oxygen-sensitive probes of higher sensitivity have been based on the phosphorescent porphyrins of platinum and palladium; for example, their octaethyl porphyrins exhibit natural lifetimes of 0.09 and 0.99 ms respectively, in a polystyrene matrix in the absence of oxygen.[12,13]

Recently the long-lived (ms) luminescence of lanthanide complexes has been exploited in certain bio-assays.[14,15] Time-gating permits easy distinction from the shorter-lived (sub-μs) background present in most biological systems, and also obviates problems associated with Rayleigh scattering and autofluorescence. As a result of the low extinction coefficients associated with Laporte-forbidden lanthanide f–f transitions, direct excitation is only practicable with lasers and it is then more convenient to incorporate a sensitising chromophore into the complex structure. This 'built-in' chromophore preferably should absorb incident light at wavelengths of ⩾340 nm thereby avoiding the need for quartz optics and eliminating the unwanted excitation of biomolecules in the sample. Many different aromatic chromophores can be used for this purpose, under ambient conditions, provided that they possess a triplet energy that is close to, but at least 1700 cm$^{-1}$ *above*, that of the emissive lanthanide state. Under these conditions, efficient intramolecular energy transfer should occur from the excited chromophore to the proximate lanthanide (Scheme 1).[16] When the energy gap,




**Scheme 1**

$\Delta E$, between the aryl triplet and the emissive lanthanide state ($^5D_4$ and $^5D_0$ for Tb and Eu respectively) is less than 1500 cm$^{-1}$, then the rate of thermally activated back-energy transfer increases (proportional to $e^{-\Delta E/kT}$; $kT \sim 208$ cm$^{-1}$ at 298 K). Depending upon the relative rates of intramolecular energy transfer and of metal emission ($k_1$, $k_{-1}$ and $k_2$ in Scheme 2), the

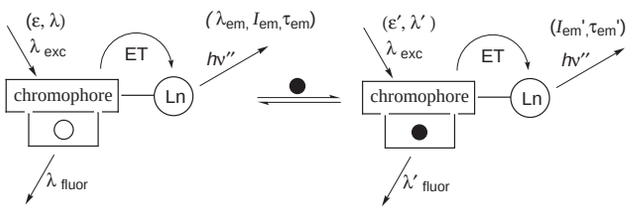



**Scheme 2**

metal-based luminescence may become sensitive to the presence of dissolved oxygen.[1,16,17,18] That is, $k_1$ may be comparable to $k_q[O_2]$. Of course this offers a means of assessing oxygen concentration, as the intensity of the metal (or ligand) based luminescence is then a function of $pO_2$.[19]

Some reports have appeared recently in which protonation of a basic site suppresses photoinduced electron transfer (PET) from a spaced chromophore to a proximate luminescent metal

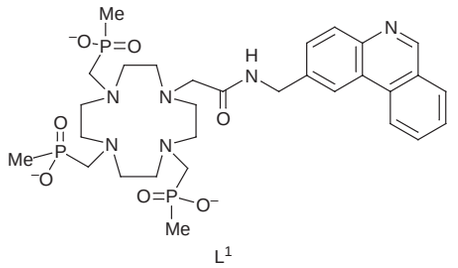
centre. For example a $Ru(bpy)_3^{2+}$ reporter has been conjugated to a calixarene and phenoxide protonation is accompanied by an enhancement of the metal-based emission.[20] A pentadentate terpyridyl ligand bearing aminomethyl substituents forms a relatively weak 1:1 complex with Tb or Eu in water, and protonation of two basic nitrogen sites in the ligand suppresses electron transfer from the singlet excited state of the aryl moiety to the $Ln^{3+}$ centre, leading to luminescence enhancements of the $Ln^{3+}$ emission intensity[21] of up to a factor of 16 (in the presence of a 100-fold excess of $Ln^{3+}$ ions). The 'PET sensor' principle is not the only means of allowing changes in pH (or other ionic concentrations) to be signalled and quantified. For example, when ion binding modulates the absorption characteristics of the chromophore ($\lambda_{abs}$, $\varepsilon_{abs}$; Scheme 3), then this



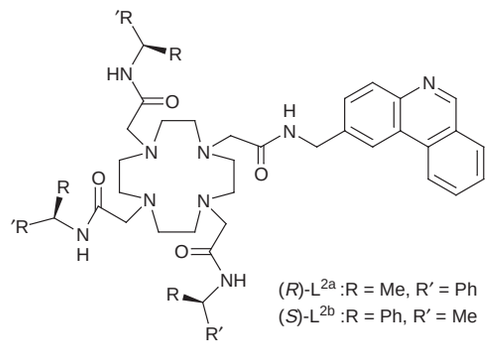

**Scheme 3** Ion binding may modulate the absorption characteristics of the chromophore or the rate of energy transfer (ET) and hence perturb the lanthanide-based emission intensity ($I_{em}$) or emission lifetime ($\tau_{em}$)

too may be signalled by changes in the lanthanide emission intensity.

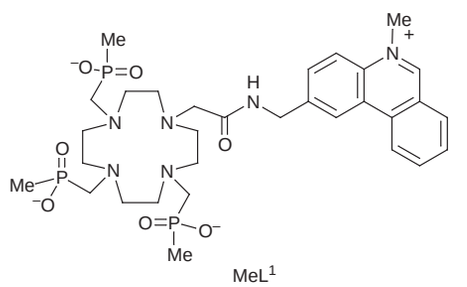
Recent work has established that octadentate monoamidetriphosphinate ligands based on 1,4,7,10-tetraazacyclododecane (cyclen), form kinetically robust 1:1 complexes in aqueous solution with lanthanide ions such as $Y^{3+}$, $Eu^{3+}$, $Gd^{3+}$ and $Tb^{3+}$.[22,23] One predominant stereoisomer exists in solution with less than one water molecule, on average, close to the lanthanide centre. In addition, chiral tetraamide derivatives of cyclen also form kinetically stable mono-hydrated 1:1 $Ln^{3+}$ complexes, wherein the remote chirality α to the amide group directs the helicity of the pendant arms and determines the macrocyclic ring configuration.[24,25] Both types of ligand may be modified easily to allow the introduction of a chromophore, absorbing light at wavelengths of ⩾340 nm. A phenanthridine group is suitable for this purpose: it is easily functionalised and the basicity of the heteroatom may be controlled by substitution at C-2 and C-6. The ease of *N*-alkylation allows the formation of phenanthridinium salts, whose fluorescence is known to be quenched selectively by halide anions.[1,5] In the *N*-protonated or *N*-alkylated form, the phenanthridine group may serve as an effective sensitising chromophore for europium emission in particular, because the ligand-to-metal charge transfer process that constrains the overall luminescence efficiency of most europium complexes in solution, is less likely to occur. With this in mind, lanthanide complexes of the macrocyclic ligands $L^1$–$L^4$ were prepared and their photochemical properties studied in detail as a function of pH, $pO_2$ and halide ion concentration.



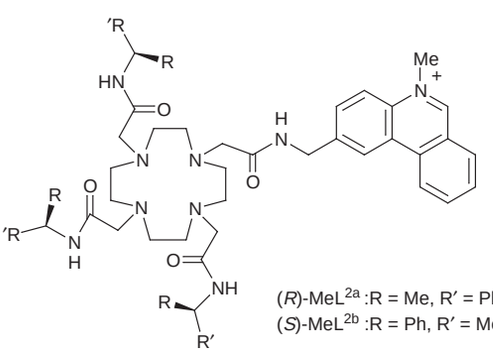
$L^1$



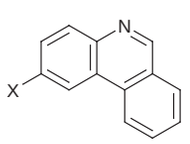
(*R*)-$L^{2a}$ :R = Me, R′ = Ph
(*S*)-$L^{2b}$ :R = Ph, R′ = Me



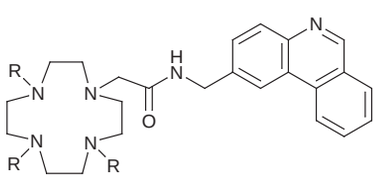
$MeL^1$



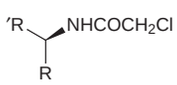
(*R*)-$MeL^{2a}$ :R = Me, R′ = Ph
(*S*)-$MeL^{2b}$ :R = Ph, R′ = Me



**1** X = Br
**2** X = CN
**3** X = $CH_2NH_2$
**4** X = $CH_2NHCOCH_2Cl$



**5** R = H
**6** R = $CH_2PMeO_2Et$



**7a** R′ = Me, R = Ph
**7b** R′ = Ph, R = Me

## Results and discussion

### Synthesis of ligands and lanthanide complexes

The cyanation of 2-bromophenanthridine was carried out in hot DMF using cuprous cyanide and subsequent nitrile reduction with $BH_3$·THF afforded 2-aminomethylphenanthridine in 79% overall yield. Coupling of this primary amine **3** with chloroacetic acid occurred efficiently in the presence of EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] and *N*-hydroxybenzotriazole, to afford the corresponding α-chloroamide **4**. Monoalkylation of 1,4,7,10-tetraazacyclododecane (cyclen) with **4** was achieved *via* the intermediacy of the molybdenumtricarbonyl complex of cyclen, using well-established methodology.[22] The mono-substituted cyclen **5** was treated with paraformaldehyde and $MeP(OEt)_2$, in the presence of 4 Å molecular sieves in THF to yield the triphosphinate ester as a mixture of diastereoisomers. Base hydrolysis at room temperature gave the octadentate ligand $L^1$. The tetraamide (*R*)-$L^{2a}$ was formed by reaction of **5** with 3 equivalents of **7a** in warm MeCN, in the presence of caesium carbonate and KI. The enantiomer (*S*)-$L^{2b}$ was formed in parallel using (*S*)-**7b**. Europium and terbium complexes of $L^1$ were prepared by reaction

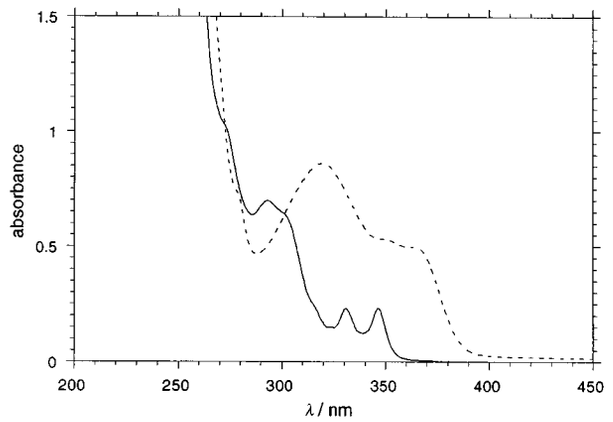



**Fig. 1** Absorption spectra for $[EuL^1]$ (solid line pH 6.8) and $[HEuL^1]^+$ (dashed line, pH 1.5)

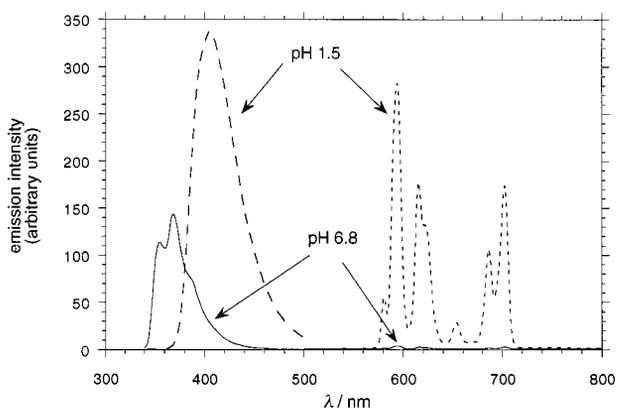



**Fig. 2** Effect of pH on phenanthridinium fluorescence ($\lambda_{exc}$ 320, $\lambda_{em}$ 405 nm) and on europium luminescence ($\lambda_{exc}$ 370, $\lambda_{em}$ 594 nm, delay 0.1 ms) in $[EuL^1]$ at pH 1.5 and 6.8 ($I = 0.1$ M $NMe_4ClO_4$)

of the ligand with an equimolar quantity of the lanthanide nitrate salt in water, and were purified by chromatography on a short column of neutral alumina (eluent: $CH_2Cl_2$–MeOH). Analysis of $[EuL^1]$ and $[TbL^1]$ by $^{31}P$ NMR (pD 5.5, 293 K) revealed that in each case there was one (≥90%) predominant stereoisomer in solution $[EuL^1]$: $\delta_p$ 101.1, 88.9, 70.3; $[TbL^1]$: $\delta_p$ 648, 621, 484), as has been noted previously in related monoamidetriphosphinate complexes.[22,23] Complexes of $L^2$ were prepared in anhydrous acetonitrile using the appropriate trifluoromethanesulfonate salt, $Ln(CF_3SO_3)_3$ and were purified by precipitation onto anhydrous diethyl ether. In the lanthanide complexes of $L^1$ and $L^2$, the metal serves as a protecting group for the bound ring and pendant arm heteroatoms. Selective *N*-alkylation of the phenanthridine group in $L^1$ and $L^2$ occurred under mild conditions (excess MeI, MeCN, 35/40 °C) to give $L^3$ and $L^4$, respectively, and the reaction was conveniently monitored by observing the formation of a shifted band at *ca.* 375 nm in the absorption spectrum of the product.

### pH Dependence of complex absorption and emission

In the neutral complexes $[EuL^1]$ and $[TbL^1]$, the absorption spectrum reveals distinct bands in water (pH 6.8) at 346, 330, 315 (sh), 304 and 295 nm (Fig. 1). At pH 1.5, the absorption spectrum changes considerably, and only two distinctive bands are observed at 370 and 330 nm, each of which is at least twice as intense as the corresponding band in the unprotonated form.† The *N*-protonated complex may therefore be selectively excited at wavelengths of greater than, say 375 nm. The same pH dependence was observed with $[LnL^2]^{3+}$ complexes (Ln = Eu, Tb).

† These absorption maxima, and their shifts upon protonation, are similar to those of phenanthridine itself in a polar solvent such as methanol.[26]

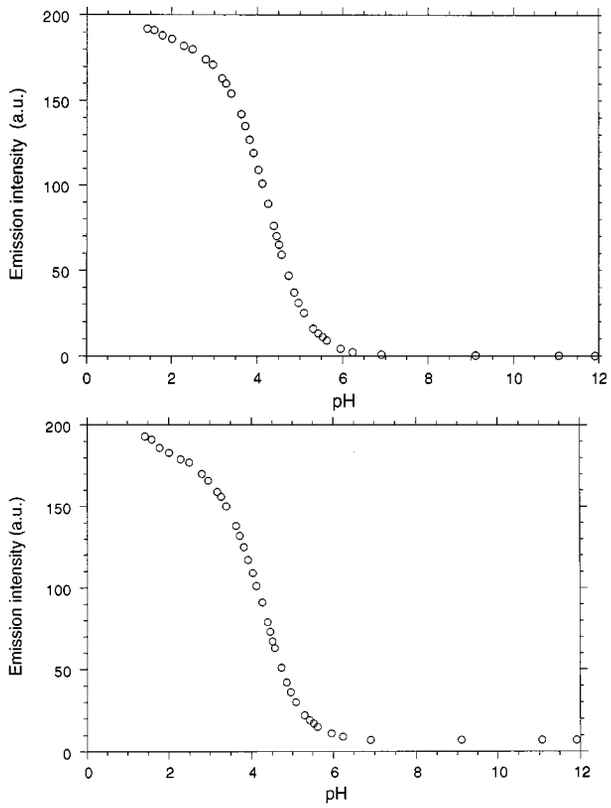



**Fig. 3** *upper*: Variation of Eu luminescence emission intensity ($\lambda_{exc}$ 370 nm, $I = 0.1$ M $NMe_4ClO_4$, $\lambda_{em}$ 594 nm) as a function of pH; *lower*: variation of fluorescence emission intensity ($\lambda_{exc}$ 370; $\lambda_{em}$ 405 nm) in $[EuL^1]$ as a function of pH

Excitation of $[EuL^1]$ at 370 nm gave rise to fluorescence from the phenanthridine group and sensitised emission from the Eu centre that was a sensitive function of pH (Fig. 2).[27] Excitation at pH 6.8 gave a *very* weak emission from the Eu centre, indeed using an excitation wavelength of ≥378 nm, this delayed luminescence could not be observed. Upon addition of trifluoroacetic acid the intensity of the metal-based emission increased dramatically, and a luminescence enhancement factor of ≥500 was estimated ($\lambda_{exc} = 370$ nm) for the *switch* to the protonated species. Changes in the ligand-based fluorescence also occurred following protonation (Fig. 2) and formation of a relatively intense fluorescence emission band was observed at 405 nm following excitation at 320 nm. A titration was carried out, monitoring both the 405 nm fluorescence band and the 594 nm ($\Delta J = 1$) europium emission and, from the resultant data (Fig. 3), the p$K_a$ of the excited singlet state was estimated to be 4.4 (±0.1) ($I = 0.1$ M $NMe_4ClO_4$, 293 K). The *same* value was derived by analysis of the ligand fluorescence and the metal-based phosphorescence, and this compares to ground-state p$K_a$ values of 4.47 and 4.76 reported for phenanthridine and 3-methylphenanthridine.[28] Monitoring the change in absorption with pH for $[EuL^1]$, gave a p$K_a$ of 4.2 (±0.1), in accord with the literature value and similar to the singlet excited state p$K_a$. It is important to note that excitation of $[EuL^1]$ at 380 nm gave rise to no measurable luminescence at pH ≥ 6, so that a genuine pH switch has been devised, wherein protonation switches on the red emission from the Eu metal centre.

At pH 1.5 and 6.8 the *lifetime* of the Eu emission in $[EuL^1]$ was 0.71 ms, and in $D_2O$ the corresponding values were 1.95 and 1.92 ms. The overall absolute quantum yield for metal-based emission was measured at pH 6.8 and 1.5, and values of 0.011 and 0.030 were found in $H_2O$ (0.034 and 0.10 in $D_2O$) which were independent of excitation wavelength in the range

**Table 1** Luminescence data[a] for europium[b] and terbium complexes of $L^1$, $L^2$, $L^3$ and $L^4$ (293 K, pH 6.5–7 for unprotonated and 1.5 for protonated complexes)

| Parameter | $[TbL^1]$ | $[TbL^1H]^+$ | $[TbL^{2a}]^{3+}$ | $[HTbL^{2a}]^{4+}$ | $[TbL^3]^+$ | $[TbL^{4a}]^{4+}$ |
|---|---|---|---|---|---|---|
| $\tau_{H_2O}$ (degassed) | 1.82 | 0.83 | 1.56 | 0.94 | 1.45 | 1.17 |
| $\tau_{H_2O}$ (aerated) | 0.98 | 0.1 | 0.85 | 0.09 | 0.1 | <0.1 |
| $\varphi$ (degassed) | 0.12 | $4.6 \times 10^{-2}$ | 0.15 | $6.9 \times 10^{-2}$ | $0.9 \times 10^{-2}$ | $1.7 \times 10^{-2}$ |
| $\varphi$ (aerated) | $2.5 \times 10^{-2}$ | $9.1 \times 10^{-4}$ | $5.1 \times 10^{-2}$ | $0.73 \times 10^{-2}$ | *c* | *c* |
| Parameter | $[EuL^1]$ | $[EuL^1H]^+$ | $[EuL^{2a}]^{3+}$ | $[HEuL^{2a}]^{4+}$ | $[EuL^3]^+$ | $[EuL^{4a}]^{4+}$ |
| $\tau_{D_2O}$ | 1.92 | 1.95 | 2.38 | 2.44 | 1.94 | 1.61 |
| $\tau_{H_2O}$ | 0.71 | 0.72 | 0.58 | 0.58 | 0.73 | 0.55 |
| $\varphi_{H_2O}$ (aerated) | $1.1 \times 10^{-2}$ *d* | $3 \times 10^{-2}$ | $0.4 \times 10^{-2}$ *e* | $2.2 \times 10^{-2}$ *f* | $1.4 \times 10^{-2}$ | $1.1 \times 10^{-2}$ |

[a] Lifetimes are given in milliseconds and quantum yields are absolute values which are independent of the excitation wavelength in the range 300 to 375 nm. The counterion in the protonated complexes is trifluoroacetate and, for the complexes of $L^3$ and $L^4$, iodide is present. [b] Europium lifetimes were independent of the presence of $O_2$ within experimental error (±10%). The effect of oxygen on the photophysical properties of the europium complexes is discussed in ref. 29. [c] The short lifetimes of the terbium complexes in aerated solution do not allow reliable quantum yields to be determined using the pulsed spectrometer employed here. [d] $3.4 \times 10^{-2}$ in $D_2O$. [e] $1.6 \times 10^{-2}$ in $D_2O$. [f] $12 \times 10^{-2}$ in $D_2O$.

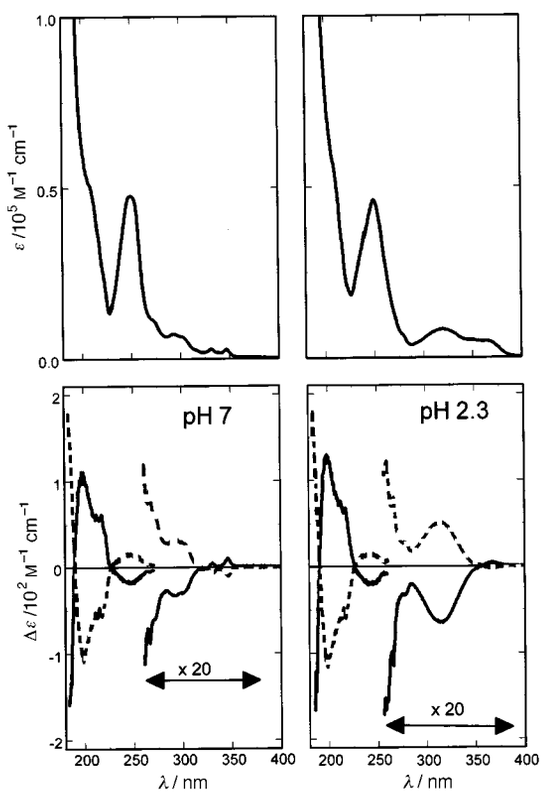



**Fig. 4** Circular dichroism spectra for (*R*)-$[EuL^{2a}]^{3+}$ (– – –) and (*S*)-$[EuL^{2b}]^{3+}(CF_3SO_3)_3$ (——) at pH 7 and 2.3, showing the effect of protonation at higher wavelength

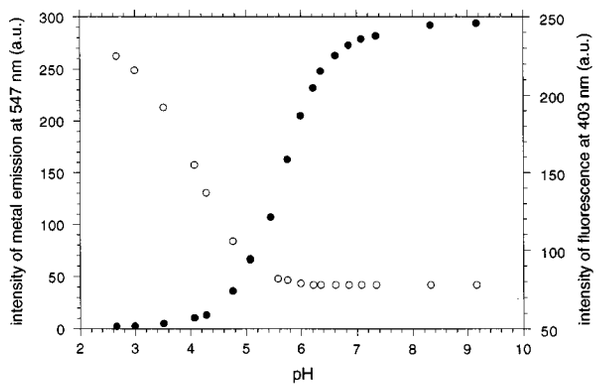



**Fig. 5** Effect of pH on phenanthridinium fluorescence (open circles, $\lambda_{exc}$ 304, $\lambda_{em}$ 403 nm) and on terbium luminescence (filled circles, $\lambda_{exc}$ 304, $\lambda_{em}$ 547 nm) in $[TbL^1]$ as a function of pH, highlighting the p$K_a$ of the singlet (4.2) and triplet (5.75) excited states respectively

300 to 370 nm. Excitation of $[EuL^1]$ at the isosbestic wavelength[27] (304 nm, Fig. 1), at pH 1.5 and 6.8 gave rise to a luminescence enhancement factor of 3, consistent with the ratio of the quantum yields for $[EuL^1H]^+$ and $[EuL^1]$. Such behaviour is consistent with a process (Schemes 2 and 3, above), wherein absorption of light by the phenanthridyl chromophore is accompanied by some ligand-based fluorescence which competes with inter-system crossing in the deactivation of the excited singlet state. Energy transfer from the phenanthridyl triplet is fast and the energy levels of the protonated and unprotonated forms are likely to be considerably higher than the emissive Eu $^5D_0$ level (17 277 $cm^{-1}$), given the observed insensitivity of the Eu emission lifetime to pH (and $pO_2$). Subsequent luminescence ($k_2$ in Scheme 2) is slow with respect to energy transfer. With this complex, the luminescence enhancement of 3, observed for a $\lambda_{exc}$ of 304 nm, relates to the suppression of photoinduced electron transfer, associated with protonation. Similar values have been found (per basic N site) in related sensors which operate simply on the 'PET' principle.[1,20,21] The much larger enhancements observed in this work, when using an excitation wavelength of ⩾370 nm, relate almost entirely to the pH dependence (in $\lambda_{abs}$ and $\varepsilon_{abs}$) of the sensitising 'antenna' chromophore, and the Eu luminescence reports this faithfully. The pH dependence of the photophysical behaviour of $[EuL^{2a}]^{3+}$ mirrored that of $[EuL^1]$ (Table 1). Charge repulsion disfavours *N*-protonation and a p$K_a'$ value of 3.4 (±0.1) was measured by monitoring either the ligand fluorescence intensity changes ($\lambda_{em}$ 405 nm) or the Eu emission ($\lambda_{em}$ 594 nm). With the chiral complexes $[EuL^{2a}]^{3+}$ and $[EuL^{2b}]^{3+}$, pH-dependent circular dichroism spectra were recorded (Fig. 4). The observed circular dichroism was almost identical in the range 190–275 nm, but marked differences were observed in the range 280–380 nm, consistent with the pH-dependence observed in the related absorption spectra. A strong CD band was observed at 320 nm in the protonated form, which was absent in the free-base form: evidently observation of the dichroism at 304 nm (the isosbestic wavelength) may be used to signal and measure the extent of protonation.

### Terbium complexes: observation of the triplet p$K_a$ and definition of rates of energy transfer and quenching

Excitation of $[TbL^1]$ at its isosbestic wavelength gave rise to pH-dependent ligand-based fluorescence and metal-based luminescence from the terbium $^5D_4$ excited state. The fluorescence from the phenanthridyl chromophore at 403 nm increased in intensity by a factor of about 3 following protonation of the aryl ring nitrogen (Fig. 5). Such behaviour—matching almost exactly the situation found for $[EuL^1]$ for excitation at the isosbestic wavelength—is consistent with the luminescence

enhancements associated with the suppression of photoinduced electron transfer. The p$K'_a$ of the excited singlet was 4.2 (±0.1), very similar to the value of 4.4 (±0.1) found for [EuL$^1$]. The metal-based luminescence behaved quite differently (Fig. 5): the neutral complex was quite strongly emissive ($\varphi_{H_2O} = 0.025$; $\tau_{H_2O} = 0.98$ ms) but the luminescence was switched off following protonation ($\varphi_{H_2O} = 9 \times 10^{-4}$; $\tau_{H_2O} = 0.1$ ms) (Table 1). A luminescence enhancement factor of ~125 was observed ($\lambda_{exc}$ 304 nm; 293 K), and an apparent p$K_a$ of 5.7 (±0.1) was estimated from the pH dependence of the terbium $\Delta J = +1$ emission at 547 nm. The intensity of the metal-based emission was observed to be quite sensitive to the presence of dissolved oxygen. De-aeration of the sample led to an increase in the overall quantum yield and $\varphi_{Tb} = 0.12$ when the complex was not protonated and p$O_2$ was zero. The measured lifetime under these conditions was 1.82 ms. In the protonated complex under de-aerated conditions, values of $\varphi_{H_2O} = 0.046$ and $\tau_{H_2O} = 0.83$ ms were measured.

Such behaviour is consistent with the establishment of a photo-equilibrium involving the phenanthridyl triplet excited state, wherein thermally-activated back-energy transfer occurs from the Tb $^5D_4$ state at 20 500 cm$^{-1}$ to the phenanthridyl triplet. Similar p$O_2$ dependent behaviour was observed with [TbL$^2$]$^{3+}$ and in order to clarify the issue further, the phenanthridine triplet energies were measured at 77 K in a MeOH–EtOH glass for the corresponding Gd complexes, in [GdL$^1$] and [GdL$^{2a}$]$^{3+}$. The measured values were 22 000 cm$^{-1}$ for the unprotonated complexes and 21 300 cm$^{-1}$ for the protonated complexes. These values are 1500 and 800 cm$^{-1}$ respectively higher than the energy of the $Tb^{3+}$ emissive $^5D_4$ state, so that the rate of thermally activated back-energy transfer (proportional to $e^{-\Delta E/RT}$) is much faster for the protonated complex (Fig. 6). A kinetic scheme accounting for this behaviour may be devised [eqns. (1) to (4)].

$$[\text{phen}]_{S_0} \xrightarrow[h\nu]{\text{fast}} [\text{phen}]^*_{S_1} \xrightarrow[k_1]{\text{ISC}} [\text{phen}]^*_{T_1} \quad (1)$$

$$[\text{phen}]^*_{T_1} + O_2 \xrightarrow{k_q} [\text{phen}]_{S_0} \quad (2)$$

$$[\text{phen}]^*_{T_1} + \text{Tb} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [\text{phen}]_{S_0} + \text{Tb}^* \quad (3)$$

$$(^5D_4)\text{Tb}^* \xrightarrow{k_3} \text{Tb} + h\nu \quad (4)$$

As part of a fuller photophysical study examining six different lanthanide complexes of L$^{2a}$, the rates of decay of the phenanthridyl triplet in degassed and aerated conditions have been measured by observing the time-dependence of the depletion of the $T_2 \leftarrow T_1$ transition at 600 nm by flash photolysis. In addition the rate of formation and decay ($k_2$ and $k_3$) of the lanthanide emission was monitored. Full details are being reported elsewhere[29] but for [HTbL$^{2a}$]$^{4+}$ in $H_2O$ at 293 K, the rate of decay of the phenanthridyl triplet ($k_q[O_2] + k_2 + k'_{nr}$) was $1.1 \times 10^5$ s$^{-1}$ in degassed solution and $2.5 \times 10^5$ s$^{-1}$ ‡ in aerated solution, ($[O_2] = 0.29 \times 10^{-3}$ mol dm$^{-3}$ in water at atmospheric pressure and 20 °C).

However, in degassed solution a double-exponential decay was observed and a second, much slower rate process was measured with a rate constant of 1000 s$^{-1}$, consistent with repopulation of the triplet state by back-energy transfer, ($k_{-2}$), from the metal to the phenanthridine. The value of $k_q$ was therefore of the order of $0.5 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ and similar second-order quenching rate constants have been measured in studies of aryl triplet quenching by molecular oxygen.[18] Observation of the 'grow-in' of the lanthanide emission at 545 nm gave values for $k_2$ of $2 \times 10^5$ s$^{-1}$ in degassed solution and $3.3 \times 10^5$ s$^{-1}$ in the aerated sample. The decay of the terbium emission was 1140 s$^{-1}$ in degassed solution, and this value rose to 9800 s$^{-1}$ in the aerated sample when back-energy transfer to the triplet occurred and oxygen quenching was competitive.

‡ Errors on luminescence rate constants are ±10% and ±15% for values derived from transient triplet state observations; $k'_{nr}$ relates to other radiationless decay pathways.

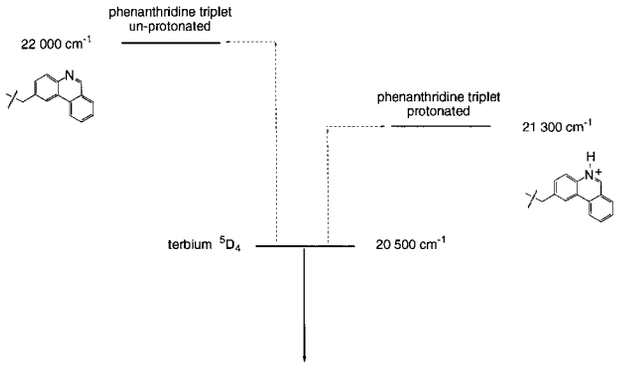
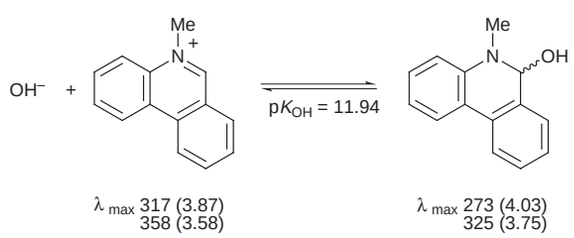



**Fig. 6** Schematic representation of the energy levels of the emissive $^5D_4$ Tb ion and the phenanthridine triplet states

Taken together, these data explain the pH and p$O_2$ dependence observed in [TbL$^1$] and [TbL$^{2a}$]$^{3+}$. In the protonated complex only, back-energy transfer from the metal $^5D_4$ level to the phenanthridyl triplet state occurs at a significant rate, and the metal-based emission reports the p$K_a$ of the excited triplet state of the phenanthridine (5.75 [±0.1]). This value is close to the p$K_a$ value of 5.7 reported for triplet phenanthridine itself.[30] As a further consequence of a significant rate of back-energy transfer, the rate of decay of the terbium emission (*i.e.* its lifetime) is now also a sensitive function of the concentration of dissolved oxygen; *i.e.* $k_q[O_2]$ is of similar magnitude to $k_2$ [eqns. (2) and (3)]. Thus both pH and p$O_2$ determine the lifetime of the terbium emission in a predictable manner. Given that the pH dependence in the range 2 to 9, can be eliminated by *N*-methylation and that the p$K_a$ of the phenanthridyl group can be 'tuned' by substitution into the heterocyclic ring, then a family of pH and p$O_2$ sensors can be envisaged.

**Hydroxide and halide sensitivity of [MeLnL$^1$]$^+$ and [MeLnL$^{2a}$]$^{4+}$**

It has been established that *N*-methylphenanthridinium ions are attacked by the hydroxide ion in a reversible reaction,[31] involving formation of a pseudo-base (Scheme 4). Attack by




**Scheme 4**

hydroxide occurs at C-6, α to the ring nitrogen, and leads to the disappearance of the longest wavelength absorption band. Addition of increasing amounts of aqueous potassium hydroxide to [MeEuL$^1$]$^+$ ($I = 0.1$ M $NMe_4ClO_4$, 293 K) was monitored by examining the metal-based luminescence at 594 nm following excitation at 360 nm. The intensity of emission fell (Fig. 7), and was zero above pH 13.5: the behaviour was fully reversible over at least five cycles. An effective dissociation constant of 16 mM (±3) was estimated from this information, equivalent to p$K'_{OH} = 12.2$ (±0.15) in good agreement with similar values reported in the literature for related ground-state ions.[31] Reversible carbon–oxygen bond formation was also marked by changes in the *N*–Me phenanthridinium fluorescence spectrum,

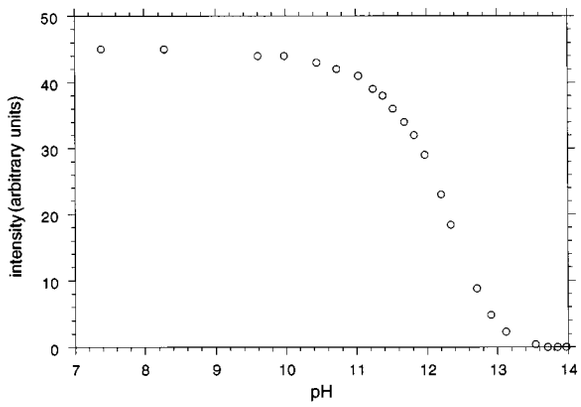



**Fig. 7** Variation of luminescence emission from $[MeEuL^1]^+$ as a function of pH ($\lambda_{exc}$ 380, $\lambda_{em}$ 594 nm, gate time 0.1 ms, $I = 0.1$ M $NMe_4ClO_4$). No change in emission intensity was observed in the pH range 2 to 9.

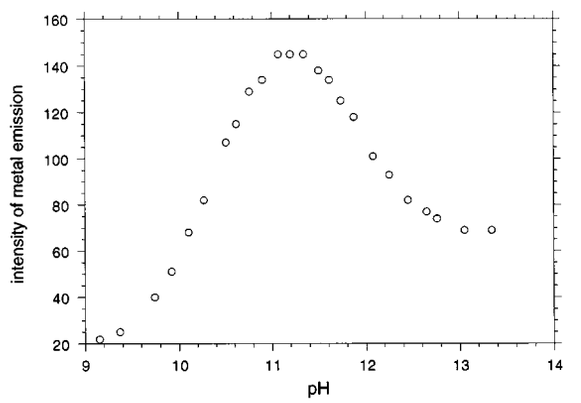



**Fig. 8** Variation of luminescence emission from $[MeTbL^{2a}]^{4+}$ as a function of pH ($\lambda_{exc}$ 380, $\lambda_{em}$ 545 nm, delay 0.1 ms, $I = 0.1$ M $NMe_4ClO_4$)

*e.g.* the disappearance of the lowest energy emission band at 405 nm as a function of hydroxide ion concentration. Thus europium emission was independent of pH in the range 2 to 9 but in basic solution, the red luminescence from the europium ion was switched off, and a luminescence diminution factor of ⩾200 was measured in association with this process. Using $[MeEuL^{2a}]^{4+}$, parallel observations were made and a $pK_{OH}$ value of 10.6 (±0.15) was obtained, consistent with the assistance to hydroxide attack from the Coulombic attraction of the tetrapositively charged complex. More complex behaviour was observed with $[MeTbL^{2a}]^{4+}$ (Fig. 8). Addition of hydroxide switched on the green terbium emission reaching a maximum at a pH of 11.2 ($\lambda_{exc} = 340$ nm), before reducing to a limiting value beyond pH 13. The initial enhancement may be readily rationalised as in the 'pseudo-base' adduct (Scheme 4), the triplet energy of the chromophore will undoubtedly be significantly higher than that of the *N*-methylphenanthridinium moiety (at 21 300 $cm^{-1}$), so that in the former case, competitive back-energy transfer does not occur. The factor of 2 reduction in intensity between pH 11.2 and 13 is less readily rationalised, but was found to be reversible over several acid/base cycles.

### Halide quenching of luminescence in europium complexes

*N*-Alkyl-quinolinium and -phenanthridinium ions possess a fluorescence which is quenched selectively by addition of halide ions.[5] Indeed this sensitivity has been harnessed in the development of *in vitro* assays for the determination of chloride ion in biological samples, as the effect typically is manifest over the range 10 to 100 mM. This range coincides with the reputed intracellular $Cl^-$ concentration range and is close to the average extracellular figure (*ca.* 100–110 mmol $dm^{-3}$). Quenching is believed to occur by charge transfer from the halide to the singlet excited state of the electron-poor cation, so that the quenching effect follows the sequence $I^- > Br^- > Cl^-$, consistent with the ease of oxidation of the halide ion.

**Table 2** Stern–Volmer quenching constants[a,b] ($K_{sv}^{-1}$, mmol $dm^{-3}$)[c] for the effect of halide ions on $[MeEuL^1]^+$ and $[MeEuL^{2a}]^{4+}$

| | $[MeEuL^1]^+$ | | $[MeEuL^{2a}]^{4+}$ | |
|---|---|---|---|---|
| Anion[d] | Phenanthridine fluorescence | Metal[e] emission | Phenanthridine[f] fluorescence | Metal[f] emission |
| $Cl^-$ | 40 | 50 | 6.0 | 26 |
| $Br^-$ | 8.0 | 16 | 2.5 | 12 |
| $I^-$ | 6.4 | 4.9 | 1.9 | 1.2 |

[a] Values given have a 15% error. [b] Concentration of the Eu complex used (as its triflate salt) was $5 \times 10^{-5}$ mol $dm^{-3}$ ($I = 0.1$ M $NMe_4ClO_4$, 293 K). [c] $I_0/I = \tau_0/\tau = 1 + K_{sv}[X^-]$, where $I_0$ and $\tau_0$ are the intensity and lifetime in the absence of halide. [d] Addition of added sodium phosphate (5 mmol $dm^{-3}$), citrate (1 mmol $dm^{-3}$) or hydrogen carbonate (30 mmol $dm^{-3}$) led to no change in the measured $K_{sv}$ values, within the stated error. [e] No significant change in the europium emission lifetime was observed during the course of these measurements ($\tau_{H_2O} = 0.73$ ms, pH 6.8; $\varphi = 0.014$ for iodide salt, 0.035 for $Cl^-$ salt). [f] With $[MeTbL^{2a}]^{4+}$ at a concentration of $6.6 \times 10^{-5}$ mol $dm^{-3}$, $K_{sv}$ values for halide quenching were 4.5 mmol $dm^{-3}$ for $Cl^-$, 1.9 mmol $dm^{-3}$ for $Br^-$ and 1.5 mmol $dm^{-3}$ for $I^-$. These values were derived from fluorescence intensity measurements: metal-based emission did reduce as a function of $[X^-]$, but was much less sensitive.

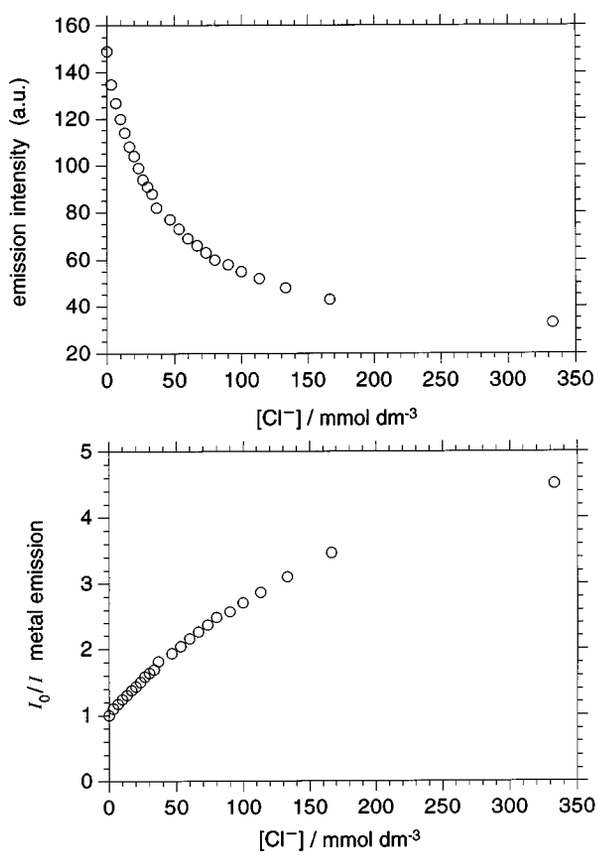



**Fig. 9** Dependence of metal-based luminescence from $[MeEuL^1]^+$ as a function of chloride ion concentration ($\lambda_{em}$ 594 nm, $I = 0.1$ M $NMe_4ClO_4$). The lower figure shows the Stern–Volmer plot giving $K_{sv}^{-1} = 50$ mmol $dm^{-3}$. No significant change occurred with added $HCO_3^-$, $HPO_4^{2-}$, citrate or lactate (⩽30 mmol $dm^{-3}$).

Addition of aqueous potassium chloride solution to $[MeEuL^1]^+$ was monitored by observing the changes in the ligand fluorescence at 405 nm and in the metal emission at 594 nm. The reduction in the intensity of the luminescence in each case was similar (Fig. 9), and some negative curvature was noted in the Stern–Volmer plot at higher chloride ion concentrations. Over the chloride ion concentration range 0–0.3 mol

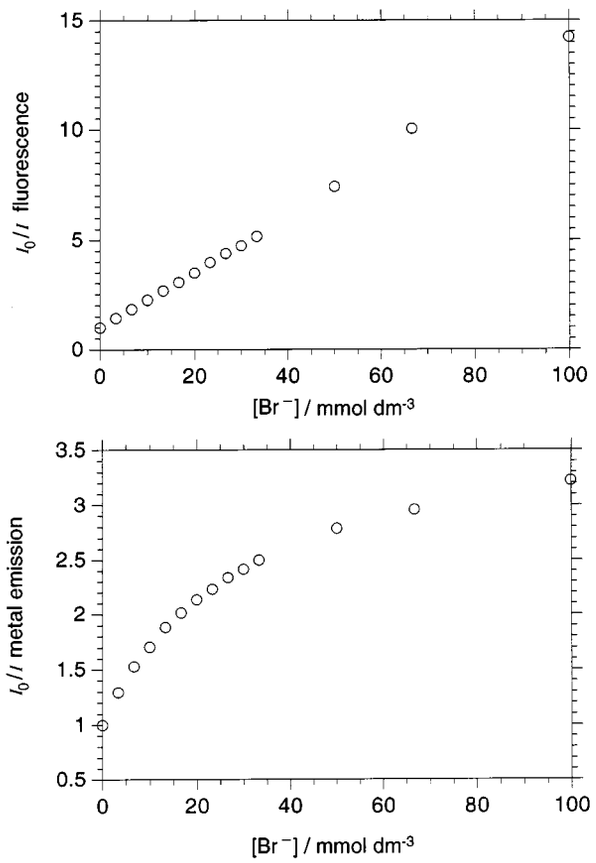



**Fig. 10** Dependence of phenanthridine fluorescence ($\lambda_{exc}$ 320, $\lambda_{em}$ 405 nm) and europium emission $[MeEuL^1]^+$ as a function of bromide ion concentration ($K_{sv}^{-1} = 8$ mmol dm$^{-3}$ from analysis of the upper and 16 mmol dm$^{-3}$ from the lower 'curve')

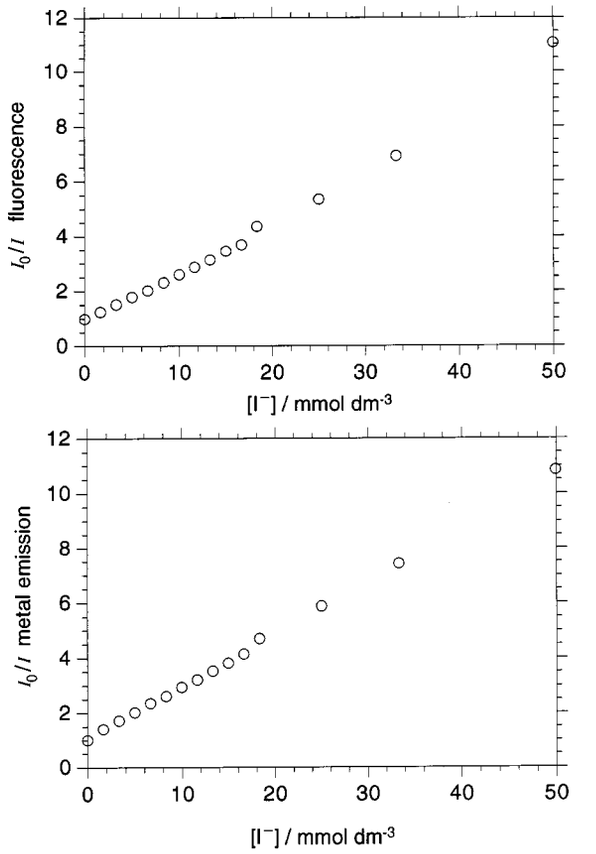



**Fig. 11** Stern–Volmer analyses of the dependence of phenanthridine fluorescence ($\lambda_{em}$ 405 nm) and europium luminescence ($\lambda_{em}$ 594 nm) in $[MeEuL^1]^+$ as a function of iodide ion concentration ($I = 0.1$ M $NMe_4ClO_4$)

dm$^{-3}$, there was a reduction in both the fluorescence band at 405 nm and the Eu emission band at 594 nm of a factor of 5. The Stern–Volmer quenching constant of 40/50 mmol dm$^{-3}$ (Table 2) falls in the expected range for such a process.[5] Addition of up to 30 mmol dm$^{-3}$ of either sodium citrate, lactate, hydrogen carbonate or hydrogen phosphate caused no effect to the ligand or metal–based luminescence intensity, either in the presence of chloride or in its absence.

Addition of bromide was monitored in a similar manner and in this case, the reduction in intensity occurred at lower added concentrations (Fig. 10). Monitoring the changes in ligand fluorescence intensity, a linear Stern–Volmer plot was derived, whereas the variation of the metal-based luminescence intensity with [Br$^-$], gave rise to a pronounced curvature. In this case, the rate of quenching of the phenanthridinium singlet excited state may now be competitive with the rate of inter-system crossing that leads to population of the triplet, which in turn is the state from which energy transfer to the europium ion occurs. Finally with iodide, linear Stern–Volmer plots giving more-or-less the same $K_{sv}$ value were obtained (Fig. 11). Iodide is a heavy atom and the validity of the description of the excited states as singlets or triplets becomes questionable in its presence.

Halide quenching by the tetra-cationic complex $[MeEuL^{2a}]^{4+}$ was studied in parallel (Table 2), and in all cases the enhanced electrostatic attraction gave rise to lower $K_{sv}$ values. Again some deviations from linearity were observed in the Eu emission Stern–Volmer quenching plots with bromide and also—but to a lesser extent—with chloride. The enhanced sensitivity of this cationic complex extends the working range of these useful halide sensors.

The situation with the corresponding terbium complexes is less favourable: metal-based emission is rather weak and oxygen sensitive. The ligand-based fluorescence was quenched in the same manner as in the Eu complexes (Table 2, footnote *f*) and the metal-based emission was only slightly reduced in intensity with added halide ions. Thus, for addition of 30 mmol dm$^{-3}$ halide to $[MeTbL^{2a}]^{4+}$, the intensity of the terbium emission at 545 nm was 14, 18 and 50% of the initial intensity value for Cl$^-$, Br$^-$ and I$^-$ respectively and the reduction was even less with $[MeTbL^1]^+$.

### Oxygen sensors based on $[MeTbL^1]^+$ and $[MeTbL^{2a}]^{4+}$

The observed sensitivity to molecular oxygen of the metal-based emission of the terbium complexes suggested that the phenomenon should be studied in a little more detail. Sensors for $pO_2$ that depend upon the quenching of an aromatic triplet excited state are well-known [11,12] and include polyazaruthenium and platinum and palladium porphyrin complexes. There have been no reports of lanthanide-based sensors presumably because the line-like emission of lanthanide ions following *direct* excitation is independent of $pO_2$. On the other hand, the phenomenon of back-energy transfer to an aryl triplet is commonplace and *indirect* excitation of lanthanide complexes often is characterised by a dependence of the metal-based emission intensity on $pO_2$.[17,18]

The emission intensity of $[MeTbL^1]^+$ and $[MeTbL^{2a}]^{4+}$ was measured as a function of $pO_2$ (Fig. 12). Linear Stern–Volmer plots were obtained with $K_{sv}^{-1} = 45$ and 58 Torr respectively. The terbium emission lifetime in $[MeTbL^1]^+$ was 0.83 and 0.1 ms in the absence and presence of oxygen respectively, and the corresponding values for $[MeTbL^{2a}]^{4+}$ were 0.94 and 0.09 ms respectively. Thus terbium emission lifetime measurements may also be used to characterise the $pO_2$ dependence, and these are independent of pH in the range 2 to 9 and are relatively insensitive to the presence of anions, particularly in the case of $[MeTbL^1]^+$.

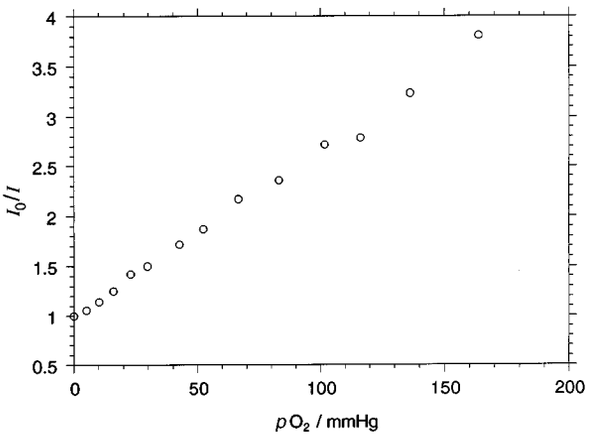



**Fig. 12** Stern–Volmer plot for the dependence of the terbium emission intensity ($\lambda_{exc}$ 370, $\lambda_{em}$ 545 nm), in $[MeTbL^1]^+$ ($I = 0.1$ M $NMe_4ClO_4$), upon the concentration of dissolved molecular oxygen, giving $K_{sv}^{-1} = 58$ mmHg

## Summary

The physico-chemical properties of the lanthanide complexes of $L^1$ and $L^{2a}$ offer several attractive features in the development of practicable analytical processes. The neutral europium and terbium complexes of $L^1$ in particular exhibit *genuine* pH-dependent switching with luminescence enhancements of over 500, covering the pH range 3.5 to 6.5. This range may be readily extended to cover the physiological pH range, by substitution α to the aryl nitrogen atom allowing variation of the p$K_a$ of the aryl singlet and triplet excited states. In the corresponding terbium complexes, lifetime measurements—obviating the problems with emission intensity quantification and standardisation—may be calibrated to measure pH. Quaternisation of the phenanthridyl nitrogen gives a complex salt whose ligand- and metal-based luminescence is sensitive to halide ion concentration. In particular, the mono-cationic europium complex of $L^1$ is sensitive to chloride ions in the physiological range while the tetra-cationic complex of $L^{2a}$ is more sensitive: in each case the delayed europium emission intensity may be measured (at several frequencies, associated with its $\Delta J = 0, \pm1, 2, 3$ and 4 transitions) to afford the anion concentration. Interference from other bioactive oxyanions is minimal.

The *N*-methylated terbium complexes of $L^1$ and $L^{2a}$ are sensitive to $pO_2$ in aqueous media in the pH range 2 to 9 and again lifetime measurements may be used as an alternative means of measuring the delayed terbium luminescence intensity. Finally, the properties of the *N*-alkylated lanthanide complexes of $L^{2a}$ and $L^{2b}$ are being studied primarily with a view to exploring their behaviour as luminescent probes of DNA structure. These rigid, enantiopure complexes have been shown recently[32] to bind strongly to *B*-DNA and further reports of the stereoselectivity of this interaction and its relationship to DNA structure will form the basis of subsequent publications.

## Experimental

### Synthesis

Details of the experimental techniques employed have been described previously, together with the spectroscopic instrumentation used for the characterisation of the products.[22,18] *J* Values are given in Hz.

**2-Bromophenanthridine 1.** This compound was prepared according to a literature procedure.[33] A suspension of phenanthridine (10 g, 56 mmol) and *N*-bromosuccinimide (10 g, 56 mmol) in carbon tetrachloride (120 $cm^3$) was heated at reflux for 48 h, in the presence of a trace amount of benzoyl peroxide. The red solution which formed was filtered whilst hot and the remaining sticky solid residue washed with more carbon tetrachloride. The solvent was reduced to one third of the initial volume and allowed to crystallise. The crystals were separated and further purified by repetitive recrystallisations from hot ethanol or by chromatography on silica (eluent $CH_2Cl_2$, $R_f = 0.25$, 10% EtOH–$CH_2Cl_2$). Mp 155–158 °C (lit.,[33] 160–162.5 °C).

**2-Cyanophenanthridine 2.** 2-Bromophenanthridine (1.5 g, 6 mmol) and copper(I) cyanide were taken into dry, degassed dimethylformamide (50 $cm^3$) and the suspension heated at 170 °C to give a clear orange solution. Heating was continued for 48 h, after which the solvent was removed under high vacuum and the residue treated with hydrochloric acid (6 M, 50 $cm^3$) to give a clear solution. The product was extracted using dichloromethane (5 × 50 $cm^3$). More product was isolated by increasing the pH of the aqueous phase to 13 and further extraction with dichloromethane (5 × 50 $cm^3$). The crude product was purified by recrystallisation from hot ethanol. Yield 0.8 g, 68%, mp 200–205 °C (dec). $\delta_H(CDCl_3)$ 7.72 (1H, dd, *J* 7.0, 7.0, H-9), 7.93 (1H, dd, *J* 7.5, 7.5, H-8), 7.96 (H, d, *J* 7.0, H-10), 8.12 (1H, d, *J* 7.5, H-7), 8.26 (1H, d, *J* 8.0, H-3), 8.58 (1H, d, *J* 7.5, H-4), 8.92 (1H, s, H-1), 9.39 (1H, s, H-6); $\delta_C\{^1H\}$ ($CDCl_3$) 121.8 (1C, s), 127.9 (1C, s), 128.8 (2C, s), 129.2 (2C, s), 130.2 (2C, s), 131.3 (1C, s), 132.0 (1C, s), 156.4 (1C, s, ring C=N), 183 (1C, s, CN); $\nu_{max}/cm^{-1}$ 2224 (CN) (Found: C, 82.35; H, 3.98; N, 13.81. $C_{14}H_8N_2$ requires C, 82.33; H, 3.94; N, 13.71%); *m*/*z* ($ES^+$) 204 ($M^+$).

**2-(Aminomethyl)phenanthridine 3.** 2-Cyanophenanthridine (1.0 g, 5 mmol) was taken into a solution of borane–THF (1 M) under argon and heated at reflux temperature for 40 h to give a clear solution. The progress of the reaction was monitored by observing the disappearance of the nitrile band in the infra-red (2224 $cm^{-1}$). Excess borane was quenched by the careful addition of methanol and the solvent removed under reduced pressure. This procedure was repeated three times to give a waxy solid. The residue was taken into hydrochloric acid (1 M, 50 $cm^3$) and heated at 100 °C for 15 h. The acid solution was washed with diethyl ether (3 × 50 $cm^3$) and the pH then raised to 13. The product was extracted using dichloromethane (3 × 50 $cm^3$) and dried with potassium carbonate to give a pale yellow solid. Yield 0.88 g (87%). $\delta_H(CDCl_3)$ 1.67 (2H, br s, $NH_2$), 4.10 (2H, s, $CH_2NH_2$), 7.60 (1H, dd, *J* 7.5, 7.5, H-8), 7.64 (2H, d dd, *J* 7.5, 7.3, 7.3), 7.98 (1H, d, *J* 7.5, H-7), 8.21 (1H, d, *J* 7.5, H-2), 8.45 (1H, s, H-1), 8.56 (1H, d, *J* 7.5, H-3); $\delta_C\{^1H\}$ ($CDCl_3$) 46.41 (1C, s, $CH_2NH_2$), 119.8 (1C, s, Ar-CH), 121.7 (1C, s, Ar-CH), 123.8 (1C, s, Ar-C), 126.2 (1C, s, Ar-C), 127.2 (1C, s, Ar-CH), 127.9 (1C, s, Ar-CH), 128.5 (1C, s, Ar-CH), 130.0 (1C, s, Ar-CH), 130.6 (1C, s, Ar-CH), 132.1 (1C, s, Ar-C), 141.7 (1C, s, Ar-C), 143.3 (1C, s, Ar-C), 152.7 (1C, s, C=N) (Found: C, 80.92; H, 5.91; N, 13.55. $C_{14}H_{12}N_2$ requires C, 80.74; H, 5.80; N, 13.45%). *m*/*z* ($ES^+$) 208.9 ($M^+$), 249 (M + $K^+$).

***N*-(2′-Chloroethanoyl)-2-phenanthridylmethylamine 4.** Chloroacetic acid (0.23 g, 2.41 mmol) was added to a solution of 2-(aminomethyl)phenanthridine (0.5 g, 2.41 mmol) in anhydrous tetrahydrofuran (15 $cm^3$). Upon cooling to 0 °C, 1-hydroxybenzotriazole (0.33 g, 2.41 mmol) was added, followed by EDC (0.48 g, 2.5 mmol). The solution was allowed to warm to room temperature with stirring and then stirred for a further hour, to give a clear solution and a sticky lump of solid. The solution was decanted and the solid washed with cold tetrahydrofuran. The solvent was evaporated and the residue taken into dichloromethane (75 $cm^3$), washed with a saturated solution of sodium bicarbonate (2 × 50 $cm^3$) followed by water (2 × 50 $cm^3$) and dried over potassium carbonate. The solvent was evaporated to give a white solid and further purified (to remove a trace of starting amine) by recrystallisation from hot ethanol. Yield 0.6 g (88%), mp >250 °C. $\delta_H(CDCl_3)$ 4.18 (2H, s, $CH_2Cl$), 4.78 (2H, d, *J* 7.5, $CH_2NH$), 7.06 (1H, broad s, NH), 7.67 (1H, dd, *J* 8.0, 2.5, H-9), 7.74 (1H, dd, *J* 7.5, 7.5, H-8), 7.69 (1H, dd, *J* 7.5, 7.5, H-7), 8.07 (1H, d, *J* 7.5, H-3), 8.18 (1H, d,

*J* 8.0, H-6), 8.5 (1H, s, H-1), 8.60 (1H, d, *J* 8.0, H-4), 9.28 (1H, s, H-5); $\delta_C(CDCl_3)$ 41.7 (1C, s, Cl–$CH_2$), 43.03 (1C, s, $CH_2NH$), 120.4 (1C, s, Ar-CH), 120.8 (1C, s, Ar-CH), 123.1 (1C, s, Ar-C), 125.5 (1C, s, Ar-C), 126.8 (1C, s, Ar-CH), 127.3 (1C, s, Ar-CH), 127.8 (1C, s, Ar-CH), 129.8 (1C, s, Ar-CH), 130.11 (1C, s, Ar-CH), 131.2 (1C, s, Ar-C), 143.0 (1C, s, Ar-C), 152.8 (1C, s, C=N), 165.0 (1C, s, C=O) (Found: C, 67.52; H, 4.59; N, 9.81. $C_{16}H_{13}ClN_2O$ requires C, 67.49; H, 4.60; N, 9.84%); $\nu_{max}/cm^{-1}$ 1664 (CO); *m*/*z* ($ES^+$) 284.8 and 286.7 ($M^+$).

**1-(2′-Phenanthridylmethylcarbamoylmethyl)-1,4,7,10-tetraazacyclododecane 5.** A suspension of the molybdenum tricarbonyl complex of 1,4,7,10-tetraazacyclododecane (0.22 g, 0.61 mmol) was heated in dry, degassed dimethylformamide (30 $cm^3$), in the presence of compound **4** (0.18 g, 0.61 mmol) and potassium carbonate (0.13 g, 0.61 mmol), to 80 °C. Over a period of 2 h, the mixture darkened to give a brown solution. The solvent was removed under vacuum and the residue was taken into hydrochloric acid (1 M, 20 $cm^3$) and stirred for 16 h open to the air. The pH of the solution was raised to 14 using potassium hydroxide pellets. The desired product was extracted using dichloromethane (3 × 30 $cm^3$), and dried over potassium carbonate. The solvent was removed under vacuum to give a pale yellow oil. Yield 0.22 g (85%). $\delta_H(CDCl_3)$ 2.6–2.93 (16H, m, ring CH), 3.26 (2H, s, $NCH_2$), 4.72 (2H, d, *J* 5, $NHCH_2$), 7.68 (2H, br m, NH, $H_9$), 7.86 (1H, dd, *J* 7.5, H-7), 7.99 (1H, d, *J* 7.5, H-8), 8.02 (1H, d, *J* 7.5, H-3), 8.12 (1H, d, *J* 7.5, H-6), 8.53 (1H, s, H-1), 8.65 (1H, d, *J* 8.0, H-4), 9.24 (1H, s, H-5); $\delta_C\{^1H\}$ ($CDCl_3$) 43.15 (1C, s, $NHCH_2$), 45.5, 45.8, 46.6, 46.9, 47.7, 53.15 (8C, s, ring C), 58.9 (1C, s, $CH_2CO$), 121.2 (1C, s, Ar-CH), 121.7 (1C, s, Ar-CH), 123.6 (1C, s, Ar-C), 126.2 (1C, s, Ar-C), 127.5 (1C, s, Ar-CH), 128.0 (1C, s, Ar-CH), 128.5 (2C, s, Ar-CH), 130.0 (1C, s, Ar-CH), 130.9 (1C, s, Ar-CH), 132.0 (1C, s, Ar-C), 137.6 (1C, s, Ar-C), 143.4 (1C, s, Ar-C), 153.3 (1C, s, C=N), 171.6 (1C, s, C=O); *m*/*z* ($ES^+$) 421.6 ($M^+$). There was a significant amount of the unsubstituted macrocycle remaining (20%) but the crude product was successfully carried through without further purification.

**1-(2′-Phenanthridylmethylcarbamoylmethyl)-4,7,10-tris-[(ethoxymethylphosphinyl)methyl]-1,4,7,10-tetraazacyclododecane 6.** Compound **5** (0.23 g, 0.55 mmol) and paraformaldehyde (0.08 g, 2.67 mmol) were taken into anhydrous tetrahydrofuran and the reaction mixture heated to 60 °C. Methyldiethoxyphosphine (0.26 g, 1.92 mmol) was added and the solution was refluxed over 4 Å molecular sieves according to the general procedure described previously.[22] The product was purified by column chromatography on alumina (gradient elution from $CH_2Cl_2$ to 2% EtOH–$CH_2Cl_2$, $R_f$ = 0.5 by TLC using 10% EtOH–$CH_2Cl_2$) to give a pale yellow oil. Yield 0.2 g (42%). $\delta_H(CDCl_3)$ 1.19 (9H, m, $CH_2$ $CH_3$), 1.28 (9H, d, *J* 15, P–$CH_3$), 2.3–3.07 (22H, br m, N–$CH_2$, N–$CH_2$P), 3.18 (2H, s, $NCH_2CO$), 3.9 (6H, m, P–$OCH_2$), 4.72 (2H, br d, $CH_2$–Ar), 7.68 (2H, br, Ar-H), 7.84 (1H, dd, *J* 7.5, 7.5, Ar-H), 8.02 (1H, d, *J* 7.5, Ar-H), 8.09 (1H, d, *J* 7.5, Ar-H), 8.54 (1H, s, Ar-H-1), 8.61 (1H, d, *J* 8.0, Ar-H), 8.8 (1H, br, NH–$CH_2$), 9.23 (1H, s, H-5); $\delta_P(CDCl_3)$ 51.65, 51.61; *m*/*z* ($ES^+$) 781.7 ($M^+$).

**Ligand $L^1$.** A solution of potassium deuteroxide in $D_2O$ (15 $cm^3$) was added to the phosphinate ester (0.15 g, 0.16 mmol) and the mixture stirred at room temperature for 16 h to give a clear solution. The progress of the reaction was monitored by $^{31}P$ NMR. The solution was neutralised by addition of dilute aqueous hydrochloric acid and the solvent was removed under vacuum to give a hygroscopic glassy solid. Yield 0.1 g (87%). $\delta_H(D_2O)$ 1.35 (9H, br d, P–$CH_3$), 2.7–3.1 (22 H, br, $NCH_2$, $NCH_2P$), 3.36 (2H, br s, $NCH_2CO$), 4.72 (2H, br, $NHCH_2$), 7.6–8.3 (6H, br, Ar-H), 8.7 (1H, s, Ar-H), 9.1 (1H, br, Ar-H); $\delta_P(D_2O)$ 40.3; *m*/*z* ($ES^+$) 696 ($M^+$).

**[$EuL^1$].** Europium nitrate (60 mg, 0.14 mmol) was added to a solution of ligand $L^1$ (100 mg, 0.14 mmol) in water (10 $cm^3$) and the mixture heated at 70 °C for 16 h to give a clear solution. The progress of the reaction was monitored using $^{31}P$ NMR spectroscopy. The solvent was removed and the product was purified by chromatography on a short column of alumina (eluent 10% MeOH–$CH_2Cl_2$) to give a colourless solid. Yield 36 mg (30%). $\delta_H(D_2O)$ 28.26, 25.04, 14.97, 12.70 (ring $H_{ax}$), 10.04, 9.04, 8.45, 7.94, 7.75, 7.40, 7.01, 6.93 (Ar-H, couplings not resolved due to line broadening), 1.31 (ring $H_{eq}$), −0.76 (ring $H_{eq}$), −1.77 ($PCH_3$, $^2J$ 11.4), −3.23 (ring $H_{ax}$), −4.25 ($PCH_3$, $^2J$ 13.2), −5.42 ($NCH_2P$), −6.67 ($PCH_3$, $^2J$ 11.0), −7.27 ($NCH_2P$), −7.82 ($NCH_2P$), −7.99 (ring $H_{eq}$), −10.81, −11.02, −12.24, −12.97, −13.12, −15.47 (ring H and $NCH_2CO$). The partial assignment of the signals provided here is based on more complete assignments of related monoamide tris-phosphinate complexes, obtained using a combination of two-dimensional spectra and dipolar shift analysis.[34] The form of the spectra has been found to be very characteristic of lanthanide complexes with such ligands. $\delta_P(D_2O)$ 70.33, 88.9, 101.1; *m*/*z* ($ES^+$) 844 ($M^+$), 883 (M + $K^+$).

**[$TbL^1$].** The terbium complex was prepared similarly. $\delta_P(D_2O)$ 484.6, 621.1, 648.0; *m*/*z* ($ES^-$) 851 ($M^-$), 887 $(M^{2-} + K^+)^-$.

**[$MeEuL^1$]$^+I^-$.** The complex [$EuL^1$] (15 mg, 0.017 mmol) was taken into dry acetonitrile (1 $cm^3$) and methyl iodide (20 mg, 0.17 mmol) was added. The reaction vessel was sealed and the solution heated gently to 40 °C for 48 h to give a yellow precipitate. The solid was separated by filtration, washed with acetonitrile and dried under vacuum to give the methylated complex. Yield 13 mg (96%); $\delta_P(D_2O)$ 68.8, 87.9, 101.2; *m*/*z* ($ES^+$) 859 ($M^+$). [$TbL^3$]$^+I^-$ was prepared similarly, by methylation of [$TbL^1$]. $\delta_P(D_2O)$ 490.6, 622.2, 656.9; *m*/*z* ($ES^-$) 858 ($M^-$).

**(*R*)-*N*-(2-Chloroethanoyl)-2-phenylethylamine 7a.** Chloroacetyl chloride (0.78 $cm^3$, 9.9 mmol) in solution in dry diethyl ether (20 $cm^3$) was added dropwise to a stirred solution of (*R*)-1-phenylethylamine (1.1 $cm^3$, 8.3 mmol) and triethylamine (1.4 $cm^3$, 9.9 mmol) also in dry diethyl ether (30 $cm^3$) at −20 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Water (30 $cm^3$) was added to dissolve all the white precipitate ($Et_3NH^+Cl^-$) and the ether solution was separated, washed with aqueous hydrochloric acid (0.1 M, 50 $cm^3$) followed by water (3 × 30 $cm^3$) and dried over potassium carbonate. Removal of the solvent under reduced pressure gave a colourless solid, which was recrystallised from diethyl ether to yield clear needle-like crystals. Yield 1.20 g (75%). Mp 95–96 °C. $\delta_H(CDCl_3)$ 1.63 (3H, d, $^3J$ 7.1, $CH_3$), 4.14 (2H, s, $CH_2Cl$), 5.23 (1H, quintet, $^3J$ 7.3, PhCH), 6.92 (1H, br, CONH), 7.41–7.48 (5H, m, Ar-H); $\delta_C\{^1H\}$ ($CDCl_3$) 22.2 ($CH_3$), 43.2 ($CH_2Cl$), 49.8 (*C*HMe), 126.6 (*o*-Ar), 128.1 (*p*-Ar), 129.3 (*m*-Ar), 142.9 (*ipso*-Ar), 165.6 (C=O); $\nu_{max}/cm^{-1}$ (KBr) 3265 (N–H), 1652 (C=O) (Found: C, 60.6; H, 6.15; N, 6.90. $C_{10}H_{12}ClNO$ requires C, 60.8; H, 6.12; N, 7.08%). *m*/*z* (DCI) 198 ($M^+$). The enantiomeric compound **7b** was prepared similarly and gave identical spectroscopic data.

**Ligand $L^{2a}$.** Compound **7a** (167 mg, 0.84 mmol) was added to a solution of compound **5** (108 mg, 0.26 mmol) in dry, degassed dimethylformamide (5 $cm^3$), in the presence of caesium carbonate (293 mg, 0.90 mmol) and potassium iodide (141 mg, 0.85 mmol). The mixture was heated to 80 °C for 18 h, after which analysis by thin layer chromatography on alumina showed two closely running spots of $R_f$ 0.8 (eluent 10% MeOH–$CH_2Cl_2$). The solvent was removed under vacuum and the residue taken into $CH_2Cl_2$, washed twice with water and dried over anhydrous potassium carbonate. Removal of solvent under reduced pressure led to a pale brown glassy solid (225 mg) which was subsequently crystallised at room temperature from a small volume of acetonitrile (2 $cm^3$) and diethyl ether (1 $cm^3$). A further two recrystallisations led to a colourless solid (80 mg, 34%) of high purity by NMR. Mp 160–165 °C. $\delta_H(CDCl_3)$ 1.41 (9H, t, *J* 7.0, $CH_3$), 2.48 (16H, br, $CH_2$ ring), 2.75 (2H, s, $NCH_2CO$), 2.81 (2H, s, $NCH_2CO$), 2.82 (2H, s, $NCH_2CO$), 3.06 (2H, s, $NCH_2$-CO), 4.51 (1H, dd, phenth-CH*H*N, $^2J$ 14.6, $^3J$ 5.6), 4.68 (1H,

dd, phenth-C*H*HN, $^2J$ 14.6, $^3J$ 6.5), 5.06 (3H, q, $^3J$ 7.0), 6.68 (3H, d, CONH, $^3J$ 8.2), 6.85 (1H, d, CONH, $^3J$ 8.3), 7.24 (15H, m, phenyl H), 7.60 (1H, d, $^3J$ 8.5, H-10), 7.73 (1H, t, $^3J$ 7.7, H-8), 7.87 (1H, t, $^3J$ 7.7, H-9), 8.05 (1H, d, $^3J$ 7.9, H-7), 8.12 (1H, d, $^3J$ 8.3, H-3), 8.45 (1H, s, H-1), 8.58 (1H, d, $^3J$ 8.3, H-4), 9.25 (1H, s, H-6); $\delta_C\{^1H\}$ ($CDCl_3$) 22.1 ($CH_3$), 48.9 (Ph*C*Me), 51.9 (phenth-$CH_2$), 53.7, 53.9, 54.1 (ring C), 59.4, 59.7 ($NCH_2CO$), 122.2, 122.5, 126.8, 128.5, 129.3, 131.1, 131.9, 138.6, 143.6 (aryl C), 170.2 (C=O), 171.7 (C=O); $\nu_{max}$(solid)/$cm^{-1}$ 3285 (N–H), 1655 (C=O); *m*/*z* ($ES^+$) 926 [M + $Na^+$], 904 [M + $H^+$]; $[\alpha]_D^{20}$ = +75.48° (*c* 0.14, MeOH).

The enantiomeric compound $L^{2b}$ was prepared using the same procedure, starting from compound **7b**, and gave identical NMR spectra; $[\alpha]_D^{20}$ = −74.80 (*c* 0.025, MeOH).

**$[EuL^{2a}]^{3+}(CF_3SO_3^-)_3$.** Ligand $L^{2a}$ (30 mg, 33 μmol) and europium triflate (20 mg, 33 μmol) were dissolved in anhydrous acetonitrile (2 $cm^3$) and the solution heated under argon for 2 h. The volume of solution was reduced and added dropwise, with vigorous stirring, to diethyl ether (100 $cm^3$) contained in a centrifuge tube. The fine white precipitate was separated, dried and recrystallised from acetonitrile. Yield 30 mg (60%), mp 200–205 °C (dec); $\nu_{max}$(solid)/$cm^{-1}$ 3300 (br, N–H), 1620 (C=O); $\delta_H(CD_3OD)$ 29.8 (1H, s, $H_{ax}$), 29.1 (2H, s, $H_{ax}$), 28.4 (1H, s, $H_{ax}$), 10.6, 10.3, 9.6, 9.3, 9.1, 9.0 (phenanthridine signals, coupling not resolved due to lanthanide-induced line broadening), 6.7, 6.6, 6.5 (phenyl H), 1.24, 1.18, 0.94 (C*H*Me), −0.7, −1.2, 1.3, −1.7 (singlets, $H_{eq}$), −4.9, −5.3, −6.0 (2 overlapping signals), −6.2 (2 overlapping signals), −7.0, −7.1 (singlets $H_{eq}$ and $H_{ax}$), −12.4, −13.0, −13.3, −13.5 ($NCH_2CO$). The basis of assignment of NMR signals to axial and equatorial ring protons, through the magnitude of the lanthanide induced shifts, is described in more detail elsewhere.[34] *m*/*z* ($ES^+$) 527 $(M^{3+} + e^-)^{2+}$, 601 $(M^{3+} + CF_3SO_3^-)^{2+}$, 1352 $[M^{3+} + (CF_3SO_3^-)_2]^+$; $[\alpha]_D^{20}$ +110.98° (Found: C, 44.83; H, 4.45; N, 8.18. $C_{57}H_{65}EuF_9N_9O_{13}S_3\cdot H_2O$ requires C, 45.00; H, 4.44; N, 8.29%). The terbium complex was prepared in a similar manner, with an identical shift in the carbonyl stretching frequency relative to the free ligand as for the europium complex. *m*/*z* ($ES^+$) 606 $(M^{3+} + CF_3SO_3^-)^{2+}$, 1361 $[M^{3+} + (CF_3SO_3^-)_2]^+$. The severe line-broadening and shifting properties of the terbium inhibit the meaningful assignment of the NMR spectra. Similarly, for the gadolinium complex, $\nu_{max}$(solid)/$cm^{-1}$ 3284 (br, N–H), 1624 (C=O) and *m*/*z* ($ES^+$) 605 $(M^{3+} + CF_3SO_3^-)^{2+}$. The *N*-methylated complexes were prepared by reaction with methyl iodide in acetonitrile, as described for $[MeEuL^1]^+I^-$, and the pale yellow products were used without further purification.

### Absorbance and luminescence measurements

Absorbance and luminescence spectra were recorded using the instrumentation and procedures described previously.[18,35] Details on the measurements of lifetimes and quantum yields have also been reported in earlier accounts.[35] For the pH titrations, the pH was monitored using a Corning Semi-Micro Combination electrode operated through a Jenway 3020 pH meter, calibrated prior to use using standard buffer solutions of pH 4.0 and 10.0 at 20 °C. The volume of solution used in each case was 25 $cm^3$, containing $Me_4N^+ClO_4^-$ at a concentration of 0.1 M, to maintain an approximately constant ionic strength during the titration. The concentration of the complex was generally $5 \times 10^{-5}$ M, except where stated otherwise, corresponding to an absorbance of about 0.15 at 350 nm. Aliquots of 3 $cm^3$ were taken for absorbance and emission measurements in standard quartz cuvettes. Titrations were run from acid to alkaline pH and shown in each case to be fully reversible.

Titrations with halide ions were carried out using 3 $cm^3$ of an aqueous solution of the lanthanide complex, at a concentration of $5 \times 10^{-5}$ M, contained in a quartz cuvette. The halides used were their potassium salts, at a concentration of 0.1 or 1 M (as appropriate to the effective association constant for the system under investigation) and, typically, 10 μl increments of these solutions were added to the solution in the cuvette using a Gilson 20 μl pipette. These high concentrations of the stock solutions ensured a negligible effect of dilution on the fluorescence emission intensity.

Where necessary, solutions were degassed by means of three freeze–pump–thaw cycles in a degassing cell fitted with a Young's tap. A vacuum line equipped with a mercury diffusion pump was used for this purpose (base pressure < $10^{-3}$ mmHg). The concentration of oxygen in the sample could be varied subsequently by introducing a known pressure of air into the vacuum line and equilibration with the solution. The pressure was monitored by means of a Baratron (MKS Instruments Inc.), which produces a DC voltage proportional to the pressure in the range 0–1000 torr. The output was read using a digital voltmeter. Henry's law was assumed to apply.

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