# Modest effectiveness of carbostyril 124 as a sensitising chromophore in europium and terbium amide complexes based on 1,4,7,10-tetraazacyclododecane

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**A tetraazamacrocyclic ligand-containing four carboxamide coordinating arms has been prepared, which incorporates a 7-amino-4-methyl-2(1*H*)-quinolinone group (carbostyril 124). The europium and terbium complexes of this ligand have been isolated and sensitisation of the characteristic metal luminescence, following excitation of the organic chromophore, is observed. The results are contrasted with recent work using the same chromophore as sensitiser. Measurement of the luminescence quantum yields shows that sensitisation of terbium is more efficient than for europium although both the intensity and lifetime of the terbium emission are significantly reduced through a thermally activated back energy transfer process of deactivation which is operative at room temperature.**

## Introduction

Luminescent lanthanide complexes are of growing interest because the long lifetime of emission allows time-resolved detection procedures to be employed under ambient conditions.[1,2] This unusual property makes such compounds particularly attractive for use as luminescent labels or probes in biological systems as it provides a means of discriminating against short-lived background fluorescence. Potential applications include their use in time-resolved fluoroimmunoassay and in biodistribution studies.[3]

Owing to the low molar absorptivities of the lanthanide ions, a suitable ligand must not only be able to form a lanthanide complex of high stability with respect to metal decomplexation in aqueous media, but also should incorporate a sensitising group or 'antenna', whose function is to absorb strongly at a suitable wavelength (preferably in excess of about 330 nm) and to transfer this excitation energy to the metal which may then emit its characteristic luminescence.[2] In this way, the effective absorbance of the lanthanide ion is greatly increased. In assessing the suitability of such complexes for practical applications, one of the most important quantities which needs to be determined is the overall quantum yield of emission by the metal following excitation of the sensitiser, denoted here by $\varphi_{net}$. Clearly, this net quantum yield will depend in part on the emissive quantum yield of the metal ion, $\varphi_{em}$; that is, on the probability that the lanthanide ion re-emits light after it has acquired an appropriate amount of energy. However, since the emission is induced indirectly through excitation into the sensitising group, the overall quantum yield will also depend both on the efficiency of formation ($\varphi_A$) of the state from which energy transfer occurs (in most cases, this is thought to be the $T_1$ state of the antenna) and on the efficiency of the energy transfer process itself ($\eta_{et}$). This is indicated in eqn. (1),

$$\varphi_{net} = \varphi_A \eta_{et} \varphi_{em} \quad (1)$$

or, since

$$\varphi_{em} = k^o \tau_{obs} \quad (2)$$

where $\tau_{obs}$ is the observed luminescence lifetime and $k^o$ is the radiative rate constant in the absence of other deactivating processes, then eqn. (3) results.

$$\varphi_{net} = \varphi_A \eta_{et} k^o \tau_{obs} \quad (3)$$

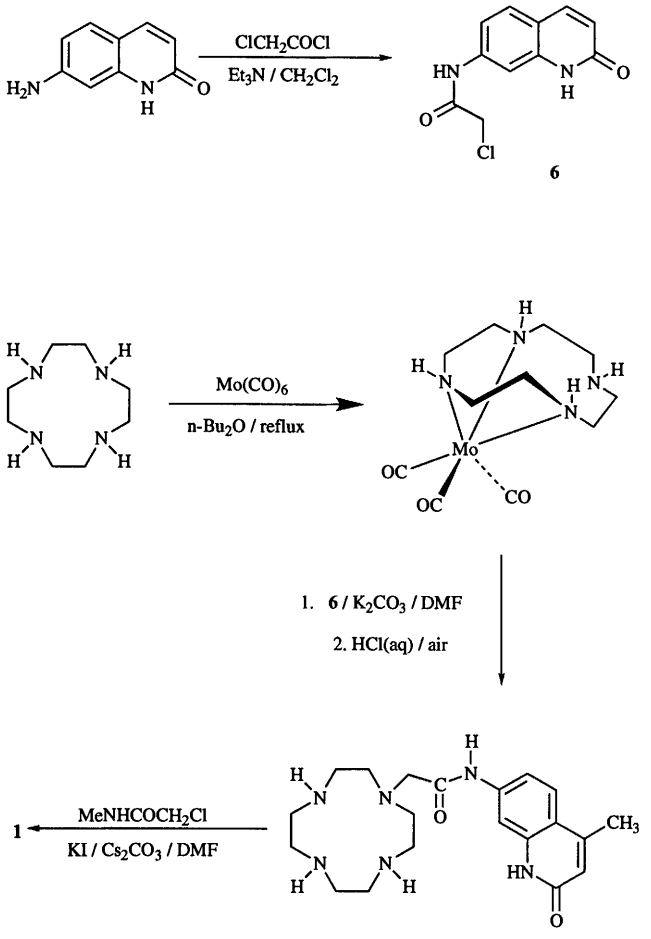
The number of different types of sensitiser which have been investigated in conjunction with highly stable, water soluble complexes of europium and terbium is quite limited and most have been based on the tris-bipyridyl ligands first defined by Lehn and co-workers.[4] A recent report described the use of a $Eu^{3+}$ complex of a DTPA-based ligand modified with a 7-amino-4-methyl-2(1*H*)-quinolinone group as sensitiser.[5] This chromophore has the trivial name carbostyril 124 and is denoted cs124 in subsequent discussions. Two subsequent reports have appeared; one on the use of the analogous terbium complex[6] and a second describing the use of the same chromophore linked, as an amide, through one of the carboxylates of TTHA, TETA or DOTA.†[7]

The previous work with carbostyril as a sensitiser did not report the overall emissive quantum yields for the complexes, following excitation of the cs124 chromophore, so it is difficult to be certain about the effectiveness of the cs124 group as a sensitiser for europium and terbium.

## Results and discussion

In connection with our ongoing work on luminescent lanthanide complexes,[8-12] it was of interest to determine the overall quantum yields attainable through the use of cs124 as a sensitiser. This, after all, is the factor which determines the suitability of a given system for the applications in mind and not just the quantum yield of emission alone. The tetraamide ligand **1** was therefore prepared using the procedure shown in Scheme 1. The use of the molybdenum tricarbonyl complex of 1,4,7,10-tetraazacyclododecane, $12N_4$, provides a convenient route for the selective functionalisation of just one of the four ring nitrogen atoms.[13] Thus, reaction of 1,4,7,10-tetraazacyclododecane with $Mo(CO)_6$ in dibutyl ether followed by alkylation of the resulting molybdenum complex with *N*(4-methyl-2-oxo-1,2-dihydro-7-quinolyl)-2-chloroethanamide in dimethylformamide yielded the monosubstituted $12N_4$ intermediate. Subsequent reaction with excess 2-chloro-*N*-methylethanamide in DMF in the presence of caesium carbonate and potassium iodide gave the desired compound **1** which was purified by reverse-phase HPLC. The terbium and europium complexes were prepared by reaction of the ligand with the appropriate

† TTHA = triethylenetetraamine hexaacetic acid, TETA = tetraazacyclotetradecanetetraacetic acid and DOTA = 1,4,7,10-tetraazacyclododecanetetraacetic acid.




**Scheme 1**

metal triflate salt in solution in acetonitrile at 50 °C. Complexation occurred very rapidly under these conditions and the complexes were isolated by precipitation from diethyl ether in a similar manner to that described previously for the related tetraamide ligands **2** and **3**.[8] It is important to note that this ligand involves a tertiary amide link between the lanthanide binding moiety and the sensitiser, in contrast to prior work where a secondary amide bond forms the link.

The characteristic metal-based luminescence was observed for both complexes in aqueous solution following excitation of the cs124 chromophore at 320 nm. The metal luminescence excitation spectra closely resembled the UV absorption profiles indicating the occurrence of an energy transfer process from the sensitiser to the metal ion. The excitation spectrum in aqueous solution at 295 K (Fig. 1, for the terbium complex) is similar to that reported in related work.[7] An excitation spectrum was also recorded in ethanol at 150 K and it too is shown in Fig. 1. Compared with the room temperature spectrum in aqueous solution, a small bathochromic shift is noted and there appear to be some additional bands. These may well reflect a partial resolution of the vibrational fine structure on reducing the temperature.

For both europium and terbium, the detailed appearance of the metal luminescence spectra were very similar to those of the corresponding complexes of the ligands **2** and **3**, reported previously.[8] For example, the terbium spectra displayed no discernible splitting of the $J = 6$ and $J = 5$ bands, in complete contrast to the extensive splitting observed under the same conditions in the spectra of related complexes containing phosphinate as opposed to amide coordinating arms, *e.g.* [Tb·**5**]. Again, an enhanced intensity of the $J = 6$ band was noted in the tetraamide complex compared with the phosphinate complexes. Similarly, the $Eu^{3+}$ spectra (Fig. 2) resemble the complexes of DOTA, **2** and **3** much more closely

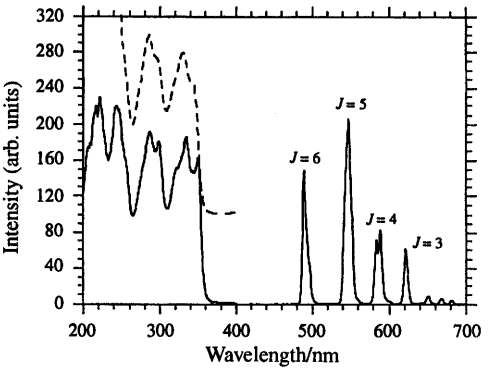
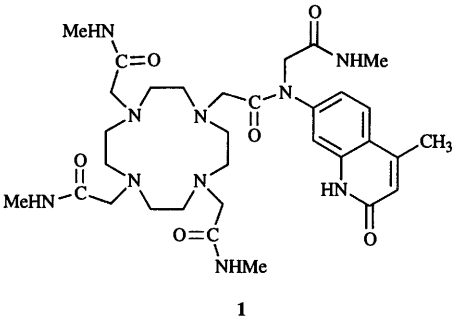
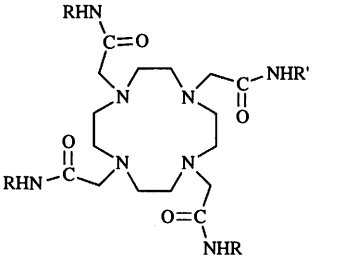
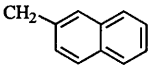


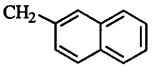

**Fig. 1** Left: metal luminescence excitation spectra ($\lambda_{em}$ = 545 nm) of $[Tb\cdot\mathbf{1}]^{3+}$ in water at 298 K (– – –) and in ethanol at 150 K (—). Right: corrected emission spectrum ($\lambda_{ex}$ = 330 nm) of $[Tb\cdot\mathbf{1}]^{3+}$ in solution in ethanol (20 μM) at 150 K. The bands arise from $^5D_4 \rightarrow {}^7F_J$ transitions; the $J$ values are shown on the spectrum. Excitation and emission monochromator slit widths set to 2.5 nm in each case.



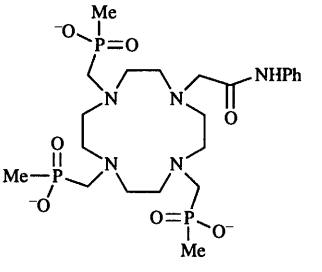
**1**



**2** R = Me R' = 

**3** R = R' = 

**4** R = R' = H



**5**

than the phosphinate complexes, such as [Eu·**5**], with a higher $J = 1/J = 2$ intensity ratio and a $J = 2$ band which is dominated by the shorter wavelength component.[12] These observations may reflect the expected distinction in the structures of the amide and phosphinate complexes, the former probably possessing square antiprismatic structures[14] whilst

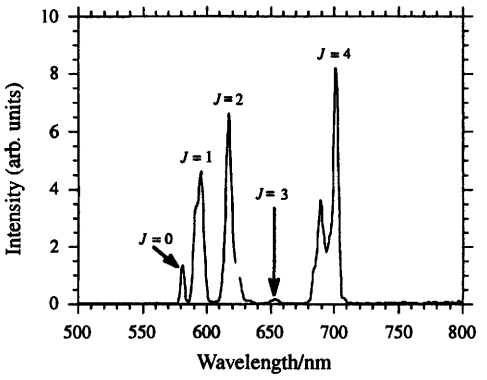



**Fig. 2** Metal luminescence emission spectrum of $[Eu\cdot\mathbf{1}]^{3+}$ in $D_2O$ (20 μM, 293 K) following excitation at 345 nm. Excitation and emission monochromator slit widths of 5 nm were employed. The bands arise from $^5D_0 \rightarrow {}^7F_J$ transitions; the $J$ values are shown on the spectrum.

the latter are thought to adopt an inverted square antiprismatic geometry.[15]

Lifetimes, fluorescence quantum yields of the cs124 group and metal luminescence quantum yields have been determined for the two complexes in solution in $H_2O$ and $D_2O$ and the results are shown in Table 1. The difference in excited state lifetimes between $H_2O$ and $D_2O$ is expected, owing to the greater efficiency of energy transfer to the vibrations of O–H bonds of coordinated water molecules compared with O–D bonds.[16] Use of the conventional Horrocks analysis [eqn. (4)]

$$q = A_{Ln}(k_{H_2O} - k_{D_2O}) \qquad A_{Tb} = 4.2\ A_{Eu} = 1.05 \quad (4)$$

allows the hydration state ($q$) of the metal to be estimated,[9,17,18] where $k = 1/\tau$ = rate constant for the depopulation of the excited state.

The hydration state of the terbium complex is about 1, indicating one bound water molecule, an observation which is consistent with the behaviour of the terbium complexes of **2** and **3**. In contrast, the europium complex displays a significantly higher $q$ value of 1.28, a value which is higher than might be expected given that one water molecule only is bound to europium in the related more hydrophilic complex $[Eu\cdot\mathbf{4}]^{3+}$. This behaviour is consistent with an N–H/N–D exchange process of the type described previously for the europium complexes of the amide ligands **2** and **3**. There, it was shown that there exists an additional deactivation mechanism for the excited europium $^5D_0$ state through coupling to amide N–H vibrational levels.[8] Such a deactivation mechanism will be much less efficient for amide N–D vibrations. Dissolution of the complexes in $D_2O$ results in rapid N–H/N–D exchange, probably mediated by the relative ease of reduction of $Eu^{3+}$ to $Eu^{2+}$. Thus, the measured hydration state for the europium complex is higher than that which would arise solely from the substitution of a bound $H_2O$ molecule by $D_2O$.

The overall metal luminescence quantum yields, $\varphi_{net}$, following excitation at 320 nm were determined using quinine sulfate as the standard for the terbium complex and $[Ru(bipy)_3]^{2+}(Cl^-)_2$ as a standard for the europium complex. Sensitisation of terbium is clearly the more efficient (Table 1) with quantum yields an order of magnitude higher than those observed for europium ($\varphi_{net}$ = 0.15 and 0.014, respectively in $D_2O$). The lower quantum yield of the europium complex could be due, at least in part, to a lower efficiency of the energy transfer process, $\eta_{et}$. However, a second reason also becomes apparent when the quantum yields of fluorescence of the cs124 moiety itself are considered. It is clear from Table 1 that the fluorescence of the chromophore is significantly reduced in the europium complex compared with the terbium analogue. Bearing in mind the similarity of the spin-orbit coupling constants of the two elements, this is unlikely to reflect a greater

**Table 1** Metal luminescence lifetimes ($\tau$) and quantum yields ($\varphi$) for the europium and terbium complexes of ligand **1**

| | $\tau$ (298 K)/ms | | $\varphi$ (298 K)/ms | | | |
|---|---|---|---|---|---|---|
| Complex | $D_2O$ | $H_2O$ | $D_2O$ | $H_2O$ | $q^a$ | $\varphi_{fl}{}^b$ |
| $[Eu\cdot\mathbf{1}]^{3+}$ | 1.47 | 0.51 | 0.014 | 0.006 | 1.28[c] | 0.018 |
| $[Tb\cdot\mathbf{1}]^{3+}$ | 1.12 | 0.88 | 0.15 | 0.13 | 1.02 | 0.044 |
| $[Tb\cdot\mathbf{1}]^{3+}$ (degassed) | 2.09 | 1.38 | 0.28 | 0.25 | 1.04 | 0.043 |

[a] $q$ is the hydration state obtained using the Horrocks equation. [b] $\varphi_{fl}$ is the fluorescence quantum yield of the carbostyril 124 group itself. A value of 0.075 was obtained for the ligand **1** in water. [c] Each of the three amide N–H oscillators effectively contributes *ca.* 0.08 to the apparent $q$ value measured: see refs. 8 and 9 for a thorough discussion.

enhancement of the rate of intersystem crossing to the triplet state in the europium complex. A more likely explanation is that there is present in the europium complex an additional deactivation pathway for the excited singlet state of the chromophore, involving electron transfer from the chromophore to the metal, with transient formation of $Eu^{2+}$ and a radical cation. Such a process is favoured by the relative ease of reduction of $Eu^{3+}$ to $Eu^{2+}$, but is disfavoured in the terbium complex owing to the relative inaccessibility of $Tb^{2+}$. There is considerable precedent for such an explanation on the basis of both inter- and intra-molecular studies using aromatic hydrocarbons as sensitisers.[19] Clearly, such a competing process will necessarily have the effect of reducing the proportion of the absorbed energy which is transferred to the metal, with the result that the net metal luminescence quantum yield will be reduced.

It may be noted in passing that the quantum yield of fluorescence for the cs124 moiety is lower in the terbium complex than in the free ligand (Table 1). This could be interpreted in terms of energy transfer from the excited singlet state of the chromophore to the bound metal. On the other hand, since almost all previous photophysical studies involving a variety of sensitising groups have shown that energy transfer occurs primarily from the triplet state,[4,20,21] a rather more likely explanation is that the lanthanide ion, with its high spin-orbit coupling constant, is able to enhance the rate of intersystem crossing, thereby diminishing the fluorescence quantum yield.

A further feature of the terbium complex is also apparent from Table 1, namely that a substantial increase in both the quantum yield of metal emission and of the luminescence lifetime is observed upon degassing of the solution. It is well known that oxygen has little direct effect on lanthanide luminescence. Hence, this phenomenon most likely reflects a back energy transfer process from the metal to the triplet state of the chromophore, which is then subject to deactivation by dissolved molecular oxygen. Such effects are well documented for certain other sensitising chromophores.[4,20] The energy gap between the triplet level of the cs124 and the emissive $^5D_4$ level of terbium is not known but, by analogy with structurally related compounds, is likely to be less than 30 kJ $mol^{-1}$. Under such circumstances, significant back energy transfer at room temperature would not be unreasonable.

Clearly, such a process will require thermal activation as the triplet energy of the chromophore will certainly exceed that of the terbium $^5D_4$ level. A variable temperature study of the lifetimes and intensities of the metal emission was therefore undertaken in order to probe the effect in more detail. The results are shown in Fig. 3, for an aerated solution of $[Tb\cdot\mathbf{1}]^{3+}$ in ethanol. It is immediately apparent that temperature has a very significant effect: the lifetime at 335 K is almost four times its value at 225 K and the intensities are greatly affected. These observations are entirely consistent with the proposed thermally activated back energy transfer as a deactivation

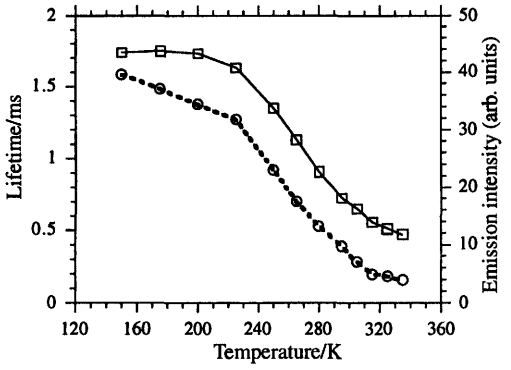



**Fig. 3** Effect of temperature on the metal luminescence intensity (○) and lifetime (□) for $[Tb\cdot\mathbf{1}]^{3+}$ in ethanol (aerated), following excitation at 320 nm

pathway for the excited terbium $^5D_4$ state. In fact, the magnitude of the effect of temperature on the luminescence lifetimes is quite similar to that which has been observed for the terbium complex of a tris(2,2′-bipyridine) cryptand, wherein thermally activated back energy transfer occurs from the terbium emissive state to the triplet level of the bipyridine.[21] In that case, the pertinent energy gap was estimated to be about 14 kJ mol$^{-1}$. Thus, it seems reasonable to suppose that a similar energy gap exists in the present instance. With respect to this hypothesis, it is noteworthy that the effect of both temperature and oxygen was even more dramatic for $[Tb\cdot\mathbf{2}]^{3+}$, where the luminescence was barely detectable under aerated conditions at room temperature.[22] This might be expected to reflect a smaller energy gap between the terbium $^5D_4$ state and the triplet naphthyl: indeed the energy difference in this case was estimated by means of a flash photolysis study to be of the order of 4.5 kJ mol$^{-1}$.

The previous work on the cs124 systems cited above[5–7] reported no temperature effect on excited-state lifetimes although only two temperatures were examined, 278 and 295 K. The effect over this range would be quite small and might have been dismissed as being within the experimental error of the measurements. It is possible, of course, that no back energy transfer occurs in these carbostyril containing polyaminopolycarboxylates which are linked to the antenna by a secondary amide link. The observation of longer luminescence lifetimes, compared with that for $[Tb\cdot\mathbf{1}]^{3+}$, is consistent with such a possibility. It is rather surprising that such a significant temperature effect should occur for **1**, with a tertiary amide linkage, but not for the closely related complexes involving polyaminocarboxylate ligands and a secondary amide linkage. If valid, then this comparison serves at least as a useful caveat to those engaged in designing suitable energy-matched chromophores as antennae for lanthanide complexes.

## Conclusions

In summary, the carbostyril 124 moiety in a tetraamide macrocyclic ligand is a reasonably effective sensitiser for a proximate bound terbium ion although limited in efficiency by thermally activated back energy transfer from the excited metal ion to the carbostyril triplet. This effect is significant at room temperature. With europium, a competitive charge transfer deexcitation pathway limits the efficiency of sensitisation.

## Experimental

### General

All reactions were carried out under an atmosphere of dry argon. Solvents were dried from an appropriate drying agent where required and water was purified by the Milli Q system. Preparative column chromatography was carried out using neutral alumina (Merck Aluminium Oxide 90, activity II–III, 70–230 mesh) pre-treated with ethyl acetate. Dichloromethane for chromatography was Analar® grade; other eluants were distilled prior to use. Analysis by HPLC was performed by means of a Varian 9010/9065 Polychrom system using a Technicol Hypersil 5ODS reverse-phase column and a flow rate of 1.4 cm$^3$ min$^{-1}$.

IR spectra were recorded with a Perkin-Elmer 1600 FT spectrometer with GRAMS Analyst operating software. $^1H$ and $^{13}C$-$\{^1H\}$ NMR spectra were obtained with a Brüker AC250 operating at 250.13 and 62.90 MHz respectively, and referenced internally to residual protio-solvent resonances. Chemical shifts are quoted in ppm and coupling constants in Hz. Mass spectra (EI and CI) were recorded with a VG7070E spectrometer whilst electrospray ionisation mass spectra were recorded using a VG Platform spectrometer.

### Luminescence measurements

UV absorbance spectra were recorded using an ATI Unicam UV-2 spectrometer. Fluorescence spectra were obtained with a Perkin-Elmer LS50B spectrofluorimeter equipped with a Hamamatsu R928 photomultiplier tube. Metal luminescence emission and excitation spectra were recorded using the same instrument operating in time-resolved mode, with a delay time of 0.1 ms and a gate time of 10 ms. Excitation and emission monochromator band passes of 2.5 nm were used and the emission spectra were corrected for the wavelength dependence of the photomultiplier tube. The metal luminescence excitation spectra were acquired by monitoring emission at 619 nm for $Eu^{III}$ and 545 nm for $Tb^{III}$.

Quoted lifetimes ($\tau$) are the average of at least five separate measurements, each of which was obtained by monitoring the emission intensity at 619 nm (for $Eu^{III}$) or 545 nm (for $Tb^{III}$) after 20 different delay times spanning a range of at least two lifetimes ($\lambda_{ex}$ = 320 nm). Slit widths of 15 nm were used and the gate time was 0.1 ms. The phosphorescence decay curves were fitted with good residuals to an equation of the form $I(t) = I(0)\exp(-t/\tau)$ using a curve-fitting program [where $I(t)$ is the intensity at time $t$ and $I(0)$ the initial intensity]. Lifetimes were found to be independent of concentration over the range examined.

Luminescence quantum yields were obtained by the method described by Haas and Stein[23] using as standards $[Ru(bipy)_3]^{2+}$ ($\varphi$ = 0.028 in water[24]) for the $Eu^{3+}$ complexes and quinine sulfate ($\varphi$ = 0.546 in 0.5 M $H_2SO_4$[25]) for the $Tb^{3+}$ complexes. The observed phosphorescence ($P$) was related to the total phosphorescence emission ($P_T$) through eqn. (5)[26]

$$\frac{P_T}{P} = \frac{1 - \exp(-20/\tau)}{\exp(-t_d/\tau) - \exp[-(t_d + t_g)/\tau]} \qquad (5)$$

where $\tau$ is the phosphorescence lifetime, $t_d$ the delay time and $t_g$ the gate time (values in ms and for a cycle time of 20 ms). Fluorescence quantum yields of the cs124 group were also measured using quinine sulfate as the standard.

Low temperature spectra were acquired using a variable temperature liquid nitrogen cryostat, Oxford Instruments model DN1704, controlled by an Oxford Instruments ITC 4 temperature controller. The LS50B was adapted to hold the cryostat in such a way that the sample was positioned in a reproducible position at the intersection of the excitation and emission beam paths of the instrument. In recording spectra at temperatures exceeding 298 K, a thermostatted water bath was used, which rapidly circulated warm water through a channel bored into the sample holder of the spectrometer. Ethanol was chosen as the solvent for variable temperature studies as its freezing point is sufficiently low for a large range of temperatures to be available in fluid solution (the range 150–330 K was used).

## Synthesis

**Chloro-*N*-[(4-methyl-2-oxo-1,2-dihydro-7-quinolyl)ethanamide.** 7-Amino-4-methyl-2(1*H*)-quinolone (carbostyril 124, 200 mg, 1.15 mmol) was taken into dry dichloromethane (2 $cm^3$) and triethylamine (0.19 $cm^3$, 1.38 mmol) was added. Chloroacetyl chloride (0.11 $cm^3$, 1.38 mmol) was added dropwise to the stirred suspension under argon with cooling to −10 °C. The mixture was allowed to warm to room temperature and stirred for a further 2 h. The resulting white solid was separated by centrifuge and washed with HCl(aq) (0.1 M, 2 × 4 $cm^3$) followed by water (4 × 4 $cm^3$) and finally methanol (4 × 4 $cm^3$). The solid was dried under vacuum to give the required product (210 mg, 73%), mp >250 °C. $\delta_H$([$^2H_6$]DMSO) 2.38 (3 H, s, $CH_3$), 4.29 (2 H, s, $ClCH_2$), 6.28 (1 H, s, C=CH–CO), 7.34 (1 H, dd, *J* 2.0 and 8.7, Ar–H position-6), 7.66 (1 H, d, *J* 8.7, Ar–H position-5), 7.74 (1 H, d, *J* 2.0, Ar–H position-8), 10.57 (1 H, s, $ClCH_2CONH$) and 11.61 (1 H, s, C=CH–CO*NH*). The compound was not sufficiently soluble to obtain $^{13}C$ NMR spectra. *m/z* (CI) 253 (33, $M^+ + 1$ for $^{37}Cl$), 251 (100, $M^+ + 1$ for $^{35}Cl$), 217 (33, [$M^+ - Cl + H$] + 1); *m/z* (EI) 252 (26, $M^+$ for $^{37}Cl$), 250 (78, $M^+$ for $^{35}Cl$) and 174 (100, $M^+ - ClCHCO$); $\nu$(KBr)/$cm^{-1}$ 3171m (N–H str), 3025m (Ar–H str), 2972m and 2919m (aliphatic C–H str), 1675s (amide I band for open-chain amide), 1647s (amide I band for ring amide), 1613s (C=C conjugated to C=O), 1585m (amide II band for open-chain amide), 1547s, 1459m, 1403s, 1269m, 885m and 856m (Found: C, 53.52; H 4.66; N, 10.40. $C_{12}H_{11}ClN_2O_2{\cdot}H_2O$ requires C, 53.63; H, 4.84; N, 10.43%).

**1-[(4-Methyl-2-oxo-1,2-dihydro-7-quinolyl)carbamoylmethyl]-1,4,7,10-tetraazacyclododecane.** A mixture of 1,4,7,10-tetraazacyclododecane (138 mg, 0.8 mmol) and molybdenum hexacarbonyl (210 mg, 0.8 mmol) in dibutyl ether (10 $cm^3$) was heated to reflux under argon for 2 h. A bright yellow precipitate of the 1,4,7,10-tetraazacyclododecane–molybdenum tricarbonyl complex was formed, which was filtered under argon and dried under vacuum. The yellow complex was suspended in dry, degassed dimethylformamide (5 $cm^3$) in the presence of anhydrous potassium carbonate (166 mg, 1.2 mmol) and potassium bromide (95 mg, 0.8 mmol). The chloroacetylated carbostyril (200 mg, 0.8 mmol) was added and the mixture stirred at 70 °C for 6 h under an atmosphere of argon, after which the solvent was removed under reduced pressure with mild heating. The resulting brown residue was taken into HCl (aq) (1 M, 10 $cm^3$) and the mixture stirred at room temperature and open to the air for 18 h. The pH of the solution was raised to 14 with KOH pellets with cooling and the dark green molybdenum residues filtered off giving a clear pale yellow solution. Extraction into dichloromethane (6 × 15 $cm^3$) followed by removal of solvent under reduced pressure gave a pale yellow oil (170 mg, 55%). $\delta_H$($CDCl_3$) 2.44 (1 H, s, $CH_3$), 2.74 (16 H, br, $CH_2$ ring), 2.88 (3 H, br, amine N–H in ring), 3.35 (2 H, s, $NCH_2CO$), 6.35 (1 H, s, C=CH–CO), 7.55 (1 H, d, *J* 8.8, Ar–H position-5), 7.60 (1 H, d not sufficiently resolved to obtain *J* value, Ar–H position-8), 7.91 (1 H, dd, larger *J* 8.8, Ar–H position-6) and 10.56 (1 H, s, $ClCH_2CONH$); $\delta_C$\{$^1H$\}($CDCl_3$) 19.6 ($CH_3$), 46.2, 46.7, 47.6 and 47.7 ($CH_2$ ring), 60.3 ($NCH_2CO$), 106.0 ($CH_3$-C=*C*H), 117.4 ($CH_3C$=CH), 115.0, 119.3 and 125.7 (tertiary aromatic carbons), 139.7, 141.2 and 149.2 (aromatic quaternary carbons), 165.0 ($CH_3C$=CH*C*=O) and 171.5 ($NCH_2CO$); *m/z* (ES +) 387 (100, $M^+ + 1$) and 409 ($M + Na^+$); $\nu$(thin film)/$cm^{-1}$ 2940m and 2843m (HC–H str), 1674s (amide I band for open-chain amide), 1654s (amide I band for ring amide), 1607m (C=C conjugated to C=O), 1541s, 1459m, 1405m, 1356m, 1293m and 819w.

**1,4,7-Tris(methylcarbamoylmethyl)-10-[*N*-(4-methyl-2-oxo-1,2-dihydro-7-quinolyl)-*N*-(methylcarbamoylmethyl)carbamoylmethyl]-1,4,7,10-tetraazacyclododecane 1.** The monoalkylated macrocycle (150 mg, 0.39 mmol) was dissolved in dry dimethylformamide (2 $cm^3$) and anhydrous caesium carbonate (190 mg, 0.58 mmol) and potassium iodide (65 mg, 0.39 mmol) were added. Chloro-*N*-methylethanamide (210 mg, 1.95 mmol) was added and the mixture stirred under an atmosphere of argon at 75 °C for 48 h. The solvent was removed under reduced pressure and the residue taken into dichloromethane, filtered and the dichloromethane removed from the resulting clear solution leaving a yellow oil. The product was purified by alumina column chromatography (gradient elution from dichloromethane to 3% methanol–dichloromethane; $R_f$ = 0.6, 10% methanol–dichloromethane) giving a yellow oil. The product obtained required further purification which was achieved by means of preparative reverse-phase HPLC, using a Rainin Dynamax 60 Å column, gradient elution from 10% A:90% B at *t* = 0 to 90% A:10% B at *t* = 20 min, flow rate = 10 $cm^3$ $min^{-1}$, $\lambda_{obs}$ = 330 nm, retention time = 10.4 min (A = $CH_3CN$ containing 0.1% trifluoroacetic acid; B = $H_2O$ containing 0.1% trifluoroacetic acid). Removal of solvent by freeze-drying gave the required compound as a pale yellow solid (45 mg, 13%). $\delta_H$($D_2O$) 2.13 (3 H, s, CH=CC*H*$_3$), 2.23 (3 H, s, NHC*H*$_3$), 2.34 (3 H, s, NHC*H*$_3$), 2.38 (6 H, s, NHC*H*$_3$), 3.6–2.7 (24 H, v br, $CH_2$ ring + $NCH_2CO$), 6.20 (1 H, s, $CH_3C$=CH), 6.95 (1 H, br s, Ar–H position-8), 7.26 (1 H, br d, *J* 8.5, Ar–H position-6) and 7.45 (1 H, d, *J* 8.5, Ar–H position-5); $\delta_C$\{$^1H$\}($D_2O$) 18.1 (CH=C*C*H$_3$), 25.6 and 25.7 (NHC$H_3$), 48.9 and 50.5 ($CH_2$ ring), 54.8, 55.0 and 55.4 ($NCH_2CO$), 104.6 ($CH_3C$=*C*H), 115.6, 117.3 and 126.6 (tertiary aromatic C), 118.4 ($CH_3$*C*=CH), 138.6, 140.0 and 150.4 (quaternary aromatic C), 163.6 ($CH_3C$=CH*C*O) and 169.6 ($NCH_2CO$); *m/z* (ES +) 672 (34, $M^+ + 1$) and 356 (100, $M + Ca^{2+}$); $\nu$(KBr)/ $cm^{-1}$ 3303m (N–H str), 3104 m (Ar–H str), 2973m and 2919m (aliphatic C–H str), 1684s, 1677s, 1655s and 1648s (amide I bands), 1585m (amide II band), 1429m, 1397m, 1205s, 1133m, 801m and 722m.

**[Eu·1]$^{3+}$.** The ligand **1** (its diprotonated trifluoroacetate salt, 15 mg, 0.0167 mmol) was dissolved in anhydrous acetonitrile (1 $cm^3$) to which was added a solution of europium triflate (10 mg, 0.0167 mmol) in acetonitrile (2 $cm^3$). The mixture was warmed to 50 °C for 2 h after which the solvent was removed under reduced pressure. The residue was taken up into the minimum volume of acetonitrile and added dropwise to a large volume of anhydrous diethyl ether (5 $cm^3$) in a centrifuge tube, with vigorous shaking between additions (Whirlimix). The complex precipitated as a fine microcrystalline solid (10 mg, 47%). (The complex proved to be too hygroscopic to obtain a reliable microanalysis; sample homogeneity was confirmed by analytical HPLC using the conditions described in the preparation of the ligand **1**, retention time 7.4 min); *m/z* (ES +) 1120 {6, $[M^{3+} + (CF_3SO_3^-)_2]^+$}, 971 {2, $[M^{3+} + (CF_3SO_3^-) + e^-]^+$}, 822 {3, $[M^{3+} + 2e^-]^+$}, 486 {78, $[M^{3+} + (CF_3SO_3^-)]^{2+}$} and 410 {45, $[M^{3+} + e^-]^{2+}$}; $\nu$(KBr)/$cm^{-1}$ 3151w (Ar–H str), 2985w, 2927w (aliphatic C–H str), 1639s (amide I band), 1545m (amide II), 1418m, 1279s, 1251s, 1171s, 1031s and 640s.

**[Tb·1]$^{3+}$.** The terbium complex was prepared in a similar manner to that described above for the europium complex, from the ligand **1** (15 mg, 0.0167 mmol) and terbium triflate (10 mg, 0.0167 mmol). (Yield = 11 mg, 52%); *m/z* (ES +) 1128 {3, $[M^{3+} + (CF_3SO_3^-)_2]^+$}, 829 {12, $[M^{3+} + 2e^-]^+$}, 489 {48, $M^{3+} + (CF_3SO_3^-)]^{2+}$} and 414 {100, $[M^{3+} + e^-]^{2+}$}. IR spectroscopic data are identical to that of the europium complex.

Terbium triflate and europium triflate were prepared as described previously.[8]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