Enhancement of luminescence of europium(III) ions in water by use of synergistic chelation. Part 2. 1:1:1 Complexes

John Coates, Peter G. Sammes * and Richard M. West

Molecular Probes Unit, Department of Chemistry, Brunel University, Uxbridge, Middlesex, UK UB8 3PH

The design and choice of shielding ligands that enhance the sensitized luminescence of europium(III) ions in aqueous solution is reported. The cooperation of the shielding and sensitizing ligands allows for the development of novel homogeneous assay systems.

In the preceding paper[1] we described conditions for the formation of 1:1 and 2:1 sensitizer–europium(III) complexes in water and some of their properties. These studies showed that provided the coordination sites around the ion are not over-occupied, a second ligand can also approach and coordinate. In this paper we describe work on the formation of 1:1:1 complexes involving dissimilar ligands, only one of which is a sensitizer, but with the other as a non-sensitizing 'shield'.

The aim was to develop a luminescence trigger, suitable for use in certain biochemical assays, in which the sensitizer (L^2) approaches the shielded cation ($L^1 \cdot Eu^{3+}$) to form a 1:1:1 complex that exhibits efficient luminescence, Scheme 1.[2]

$$L^1 + Eu^{3+} \overset{K^1_{ass}}{\rightleftharpoons} [L^1 \cdot Eu^{3+}] + L^2 \overset{K^2_{ass}}{\rightleftharpoons} [L^1 \cdot Eu^{3+} \cdot L^2]$$

Non-luminescent Luminescent

Scheme 1

In aqueous solution, free europium ions do not luminesce efficiently[3] and in order to observe emission, both a sensitizer and a method for excluding water from the coordination shell is required. Thus, in Scheme 1, a ligand (L^1) is required that acts as a shield, the main purpose of which is to displace coordinated water from the solvation shell around the metal ion. Ideally, such a shielding ligand must be very tightly bound to the ion without saturating the available coordination sites. The tight binding is required to help fix the location of the lanthanide ion, since, under assay conditions, it is essential that this complex is kinetically stable. Furthermore, the resulting shielded species should not be luminescent since such a species would then be permanently active (switched on) and not amenable to the development of new homogeneous assays. Coupled with the shielding ligand should be a second species (L^2) that can approach and coordinate to the shielded ion and act as a sensitizer.

The binding constant of this second ligand for the shielded ion ($L^1 \cdot Eu^{3+}$) must be reasonable, but should not be too high ($\gtrsim 10^{10}$ dm^3 mol^{-1}) so as to prevent its formation in free solution under the assay conditions; if this condition is not met, high background signals would be observed. The assay conditions must be designed to help bring the components together at the target, thus aiding formation of the 1:1:1 species.

Work in our previous paper[1] concentrated on the selection of the sensitizing species, the most useful of which belonged to the 1,10-phenanthroline class of reagents.[4] This paper deals with the design and selection of the shield.

Our approach was to select a shield that did not occupy all the possible binding sites (normally about nine) found for the Eu^{3+} ion. Furthermore, it was initially considered that approach by a sensitizer, *e.g.* 1,10-phenanthroline-2,9-dicarboxylic acid **1** (PDCA), which at neutral pHs would be expected to exist as the monoanion, would be discouraged if the shielded species itself were negatively charged. As a consequence of these constraints we initially prepared the aza-crown derivative **5**,[5] since it was argued that the shielded ion, *cf.* **6**, would bear a net positive charge capable of electrostatically attracting the sensitizer PDCA. The aza-crown **5** ('[2.2.1]'), a known chelator of europium,[5] $K^1_{ass} > 10^{11}$ dm^3 mol^{-1}, fitted our requirements and although it can donate up to a maximum of seven chelating groups, models showed that sufficient space was left about the ion to allow PDCA to approach.

Table 1 Enhancement of luminescence with ternary complexes using the shielding ligand [2.2.1], **5**

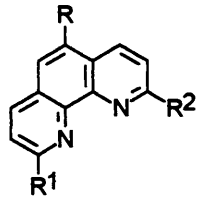
Sensitizing ligand	λ_{ex}/nm	λ_{em}/nm	I_{max} for 1:1:1 complex[a]	pH_{max}
1	289	613.5	257	8.0
2	288	615	46	7.6
3	285	615	95	9.7

[a] **[5]**, 1×10^{-5} mol dm^{-3}; [Eu^{3+}], 1×10^{-5} mol dm^{-3}; [sensitizer] 1×10^{-6} mol dm^{-3}.

No luminescence was observed from solutions of the 1:1 complex of [2.2.1] to Eu^{3+} when irradiated in the region of 280–300 nm. However, on addition of a solution of PDCA, **1**, a rapid onset of luminescence occurred to give a strong signal characteristic of excited Eu^{3+} similar in intensity to that observed from the 2:1 PDCA:Eu^{3+} complex[1] (Table 1).

The emission behaviour of the 1:1:1 complex from **1** and **5** was sensitive to pH changes[2] and strong emission was observed only in a relatively narrow pH 'window' (from 5 to 9). This was assumed to reflect changes in the protonation state of both the sensitizer and shield. At higher acidities, protonation of the carboxyl groups would occur and these are much weaker ligands for lanthanide ions than the charged carboxylate groups. At higher pH values, competition from hydroxide ions occurs and slow formation of insoluble hydroxy–oxides of the metal takes place, often resulting in precipitation.

Changing the sensitizer to the monocarboxylic acid **2**, which can also act as a sensitizer, gave a similar enhancement by using the [2.2.1] shield, although this was weaker than that produced by the dicarboxylic acid **1**. The diol **3**, however, gave a slightly stronger signal than the acid **2** (see Table 1). The two hydroxy groups in the diol **3** help to orientate the sensitizer about the europium ion in a more organized manner than that apparent with the monocarboxylic acid derivative **2**. The approximate binding constants of the latter two ligands for the shielded europium ion were lower (*ca.* 10^4–10^5) compared with that of the dicarboxylic acid **1** (*ca.* 10^6–10^7). The results were again very pH dependent,[2] the region of

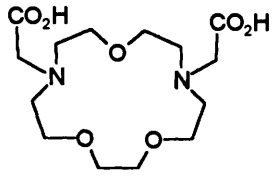


1, R = H, $R^1 = R^2 = CO_2H$

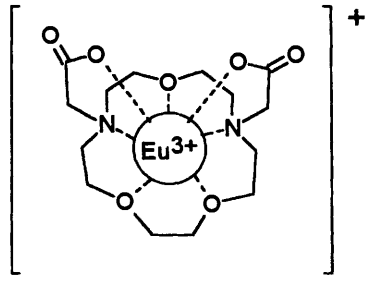
2, $R = R^1 = H$, $R^2 = CO_2H$

3, R = H, $R^1 = R^2 = CH_2OH$

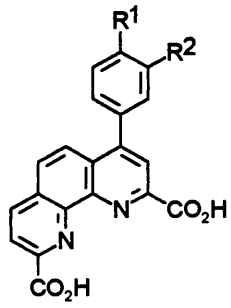
4, $R = NO_2$, $R^1 = R^2 = CO_2H$



5



6



10, $R^1 = R^2 = H$

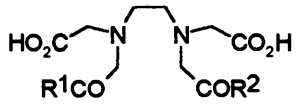
11, $R^1 = CH_3$, $R^2 = H$

12, $R^1 = CH_3$, $R^2 = NO_2$

13, $R^1 = CH_3$, $R^2 = SO_3H$

14, $R^1 = CH_3$, $R^2 = SO_2NHBu$

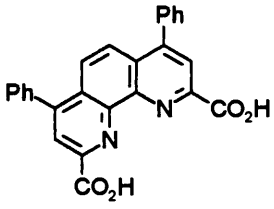
15, $R^1 = Cl(CH_2)_3$, $R^2 = H$



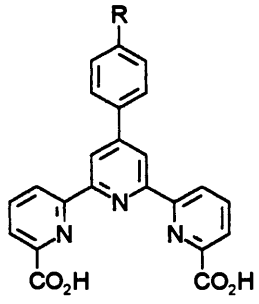
7, $R^1 = R^2 = OH$

8, $R^1 = OH$, $R^2 = NHBu$

9, $R^1 = R^2 = NHBu$



16



17, $R = CH_3O$

18, $R = CH_3$

maximum emission intensities (I_{max}) being narrow and, as a consequence of these pH effects, other shielding ligands were sought.

Our attention turned to EDTA and its derivatives; in the pH range 6–8 the dianion is a major contributor to the ionized forms present.[6] Of these, interesting results were obtained with EDTA **7**, and its mono- **8** and bis(butylamide) **9** derivatives. The behaviour of these latter ligands, as 1:1 complexes with europium(III) cations, were studied with the sensitizers **1**, **4** and **10**–**18** (Table 2), the intensity of the emission centred at 614 nm being measured. The bathocuproine derivative **16** was only measured with Eu^{3+}–EDTA (**7**) because of solubility problems associated with this sensitizer.[1]

The following conclusions were drawn from these studies. The onset of luminescence, by the addition of the solution of the phenanthroline sensitizers to the preformed shielded Eu^{3+} ion, was in all cases relatively fast (within mixing times, in the order of seconds).

In contrast, changes in the emission intensity upon addition of the shielding ligand, EDTA **7** and its derivatives, **8** and **9**, to 1:1 solutions of Eu^{3+}:sensitizer were remarkably slow, reflecting a kinetically sluggish process for full coordination of the EDTA species with the lanthanide ion. Such solutions only reached equilibrium values (maximum emission values) after leaving to stand at room temperature for several hours. Although it is known that the coordination of Eu^{3+} ions with EDTA in water is complex, forming more than one species,[7] the rate of formation of the complexes has not been thoroughly examined and this cooperative method, using PDCA as a monitor, opens up a different approach to study this process.

The parent phenanthroline, PDCA, **1** gave stable 1:1:1 complexes with all three of the shielding ligands. Of interest was the observation that both the butylamide derivatives gave slightly stronger emission intensities than for the parent EDTA, whilst only marginal changes in the lifetime of the emission signals were observed (Table 2). This trend was also followed with the other sensitizers. Presumably the hydrophobic nature of the butylamide group acts to slow the molecular motion of the sensitizer about the lanthanide ion, so marginally increasing the efficiency of the energy transfer step. The

Table 2 Ternary complexes with EDTA 7, monoamide **8** and bisamide **9**[a]

Sensitizing ligand	EDTA, 7			Monoamide, **8**			Bisamide, **9**		
	λ_{ex}/nm	I_{max}	Lifetime τ/ms	λ_{ex}/nm	I_{max}	Lifetime τ/ms	λ_{ex}/nm	I_{max}	Lifetime τ/ms
1	289	41.3	0.78	289	76.4	0.78	289	61.1	0.81
4	282	0.27	*b*	281	1.14	*b*	280	0.87	*b*
10	294	74.1	0.76	295.5	115.6	0.77	295	93.7	0.78
11	296.5	69.6	0.75	295.5	103.4	0.77	297	63.9	0.76
12	295.5	54.2	0.72	295.5	78.6	0.72	295.5	52.9	0.72
13	295.5	59.2	0.75	294	71.7	0.77	292	59.7	0.87
14	295	58.8	0.74	296	83.3	0.73	295	75.1	0.75
15	296.5	60.1	0.80	296	103.2	0.77	297	96.0	0.77
16	299	55.1	0.71	—	—	—	—	—	—
17	296.5	(8.3)[c]	*b*	292.5	(8.4)[c]	*b*	290.5	(8.4)[c]	*b*
18	295.5	(18.1)[c]	*b*	293	(11.6)[c]	*b*	294	(16.7)[c]	*b*

[a] $[Eu^{3+}]$, 1.0×10^{-5} mol dm^{-3}; [shield], 1.1×10^{-5} mol dm^{-3}; [sensitizer], 5.0×10^{-6} mol dm^{-3}. [b] Decay plot revealed more than one luminescent species present. [c] Approximate equilibrium reading after 24 h.

lifetimes of all the phenanthroline dicarboxylate sensitizers with the shielded lanthanide suggest approximately one bound water of solvation around the ion and this was confirmed by the appropriate measurements using deuterium oxide in place of water.[8]

The nitro-PDCA **4** gave surprisingly poor results, its ternary complexes being practically non-luminescent and attempts to measure the lifetime of these complexes showed that a mixture of species was present. Low luminescent efficiencies were previously observed during the studies on the 1:1 and 2:1 complexes with this acid,[1] and it is believed that this is primarily due to a poor energy transfer step between this sensitizer and the lanthanide ion.

The emission spectra of the product complexes derived from the phenanthroline diacids were all of the same appearance as for those for the 1:2 EuL_2 complexes, with nearly all of the emitted light arising from the $^5D_0 \rightarrow {}^7F_2$ transition around 613–615 nm; only a very weak peak was observed at around 580 nm. The intensity of the emission signals was much stronger than those observed for the 1:1 sensitizer:Eu^{3+} complexes, but not quite as strong as those observed with the 2:1 sensitizer:Eu^{3+} system. This observation was as expected since, for the 1:1:1 complexes, only one of the ligands can act as an absorber of light and then sensitizer, the shielding ligand playing a passive role.

The luminescence decay plots were all exponential, except for the nitro-PDCA **4** mentioned above. The lifetime values for the PDCA derivatives were all in the range 0.70–0.87 ms, similar to many of the EuL_2 complexes reported earlier.[1]

The two terpyridine diacid sensitizers, **17** and **18**, gave relatively poor results. Luminescence did not develop rapidly upon mixing, as was observed with the phenanthroline ligands. Instead, luminescence appears gradually at room temperature, taking up to a day to give stable readings. The final intensity values were low and decay plots indicated the presence of at least two luminescent species, with lifetimes similar to the corresponding EuL and EuL_2 chelates. It was concluded that these terpyridine diacids were not suitable for use in the ternary assay systems.

Use of a larger shielding ligand was investigated, by using diethylenetriaminepentaacetic acid (DTPA) in place of EDTA. The former can donate up to eight coordinating ligands thus practically saturating the coordination sphere around the europium ion. Under these conditions it was doubted that a second separate ligand would be able to compete to allow sensitization of luminescence. In the event, no luminescence was observed whenever any of the sensitizer ligands were added to a solution of Eu^{3+}–DTPA.

In order to characterize further the binding interaction between the parent PDCA **1** and the europium–EDTA chelates,

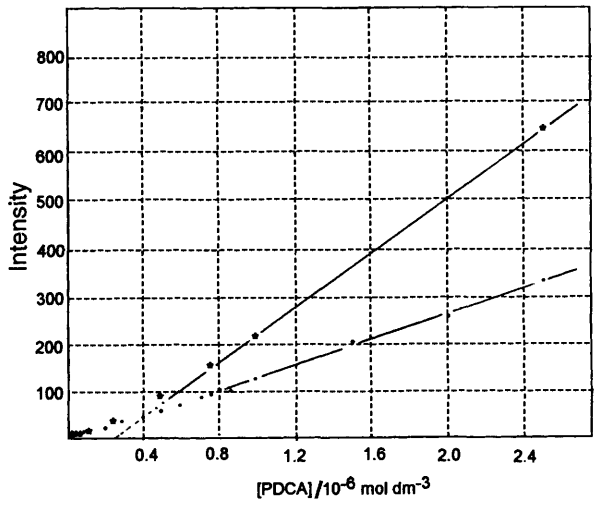


Fig. 1 Plot of intensity of emission (I_{max}, *ca.* 614 nm) *vs.* [PDCA]; · with EDTA, 7; * with monobutylamide **8** (see Tables 3 and 4 for data)

a variety of titration experiments were performed. Solutions were prepared in which the concentration of the europium chelate was kept constant at 1.0×10^{-5} mol dm^{-3} in a 0.01 mol dm^{-3} tris–HCl buffer at pH 8.0 and the PDCA **1** concentrations ranged from 1.0×10^{-8} to 2.5×10^{-6} mol dm^{-3}, *i.e.* in a region where the 1:1:1 complexes formed. After leaving the solutions to stand for a while their luminescence spectra were recorded and the results for the EDTA and EDTA monobutylamide are given in Tables 3 and 4 and plotted on Fig. 1.

These experiments showed that a wide range of linearity of signal strength *vs.* concentration could be achieved. However, at very low PDCA concentrations, a second luminescent component with a lifetime in the order of 0.22 ms was present, characteristic of the formation of a small quantity of an excited state species with several water molecules in the solvation shell. This was assigned as the free 1:1 Eu^{3+}·EDTA species. At low concentrations of PDCA **1**, relatively little of the ternary complex would be expected to form. Excitation of the ternary complex, followed by the relatively rapid intramolecular energy transfer to the europium ion could be followed by dissociation of the sensitizer to leave the excited 1:1 species. Some evidence that this is the case was obtained by removal of this short-lived species by addition of an excess of the EDTA shield (50% plus). In the presence of the excess of the shield any released excited 1:1 species interacts with the extra EDTA ligand, in direct competition with water, thus giving an excited state species of longer lifetime.

Table 3 Titration of PCDA **1** against Eu^{3+}·EDTA complex[a]

$[\mathbf{1}]/10^{-6}$ mol dm^{-3}	I_{max}	$[\mathbf{1}]/10^{-6}$ mol dm^{-3}	I_{max}
0.010	0.54	0.500	58.8
0.025	1.26	0.600	72.1
0.050	2.46	0.700	89.2
0.075	4.11	0.750	99.4
0.090	4.76	0.800	104.7
0.100	5.30	0.900	100.7
0.200	15.9	1.000	131.3
0.250	23.3	1.500	204.6
0.300	28.5	2.000	264.0
0.400	41.2	2.500	321.6

[a] $[Eu^{3+}]$, 1.0×10^{-5} mol dm^{-3}; [EDTA], 1.05×10^{-5} mol dm^{-3}, 0.01 mol dm^{-3} tris–HCl buffer at pH 8.0. Linearity in instrument response assumed.

Table 4 Titration of PDCA **1** against Eu^{3+}·monoamide **8** complex[a]

$[\mathbf{1}]/10^{-6}$ mol dm^{-3}	I_{max}	$[\mathbf{1}]/10^{-6}$ mol dm^{-3}	I_{max}
0.010	0.45	0.250	31.5
0.025	1.07	0.500	83.7
0.050	2.63	0.750	148.1
0.075	4.68	1.000	212.0
0.100	7.73	2.500	643.0

[a] $[Eu^{3+}]$ 1.0×10^{-5} mol dm^{-3}; [monobutylamide **8**] 1.05×10^{-5} mol dm^{-3}, 0.01 mol dm^{-3} tris–HCl buffer at pH 8.0. Linearity in instrument response assumed.

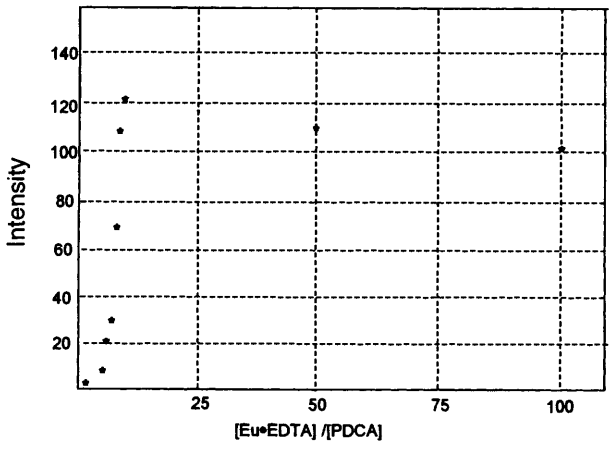


Fig. 2 Plot of titration of the EDTA **7** complex of Eu^{3+} *vs.* [PDCA]; ratio of $[Eu^{3+}]$:[**7**] = 1:1.1; [PDCA], 1.0×10^{-6} mol dm^{-3}; 0.01 mol dm^{-3} HEPES–NaOH buffer at pH 7.5

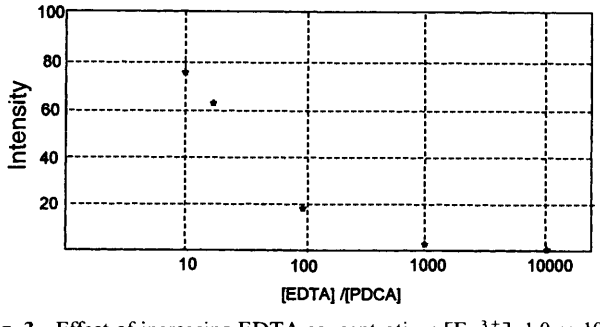


Fig. 3 Effect of increasing EDTA concentration; $[Eu^{3+}]$, 1.0×10^{-5} mol dm^{-3}; [PDCA], 1.0×10^{-6} mol dm^{-3}; 0.01 mol dm^{-3} HEPES–NaOH buffer at pH 7.5

Titration studies, where the PDCA concentration was kept constant at 1×10^{-6} mol dm^{-3} and the Eu^{3+}–EDTA concentrations varied upwards from this value, showed an increase in luminescence intensity until a peak was reached and thereafter, at very high ratios, a falling away (Fig. 2). The shape of these curves does not exactly fit the expected simple equilibrium

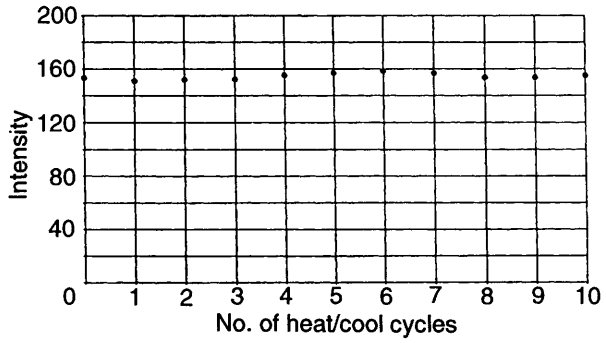


Fig. 4 Effect of heating to 95 °C for 1 min and then cooling to 25 °C over 10 min on the luminescence intensity of a solution of a 1:1:1 ternary complex: $[Eu^{3+}]$, 1.0×10^{-5} mol dm^{-3}; [monoamide **8**], 1.05×10^{-5} mol dm^{-3}; [**1**], 1.0×10^{-6} mol dm^{-3}; 0.01 mol dm^{-3} tris buffer, pH 7.5

situation between the shielded europium ion, Eu^{3+}·EDTA, and the 1:1:1 species. One complication is the dynamic nature of the EDTA–Eu^{3+} chelate structure.[7] For example, high resolution laser excitation studies have shown that this can exist in at least two forms in aqueous solutions.[9] Another factor is that the slight excess of EDTA present in the solution, at the higher concentrations, can compete with the sensitizer to form non-luminescent 2:1 complexes. Thus increasing the concentration of EDTA with respect to a constant concentration of PDCA and Eu^{3+} leads to a decrease in the emission signal (Fig. 3).

The range of competing chelating species present under various conditions did not allow us to obtain an accurate measure of the binding constant for formation of the 1:1:1 complex between the sensitizer and the shielded europium species. From dilution studies, approximate binding constant values for PDCA were estimated to be in the range $1–10 \times 10^{-6}$ mol dm^{-3}. In these dilution studies, however, care had to be taken to avoid the kinetically low rate of dissociation of the sensitizer from the 1:1:1 species and diluted solutions had to be heated to 90 °C for several minutes in order to help the solutions reach new (thermodynamic) equilibrium values.

Once formed, the solutions of the 1:1:1 complexes were found to be stable to heating–cooling cycles between 25 and 90 °C, see Fig. 4.

Experimental

The preparation and sources of the sensitizers used in these studies were as described previously.[1] All solutions were made in freshly distilled and then deionized water. Before use all volumetric flasks and quartz cuvettes were cleaned in a 1:1 mixture of 30% aq. H_2O_2 and 98% sulfuric acid, before rinsing with distilled water, 3 mol dm^{-3} HCl, distilled water and finally HPLC grade methanol, followed by air drying.

Europium(III) chloride stock solution at 1.0×10^{-3} mol dm^{-3} was prepared, using 99.99% $EuCl_3 \cdot 6H_2O$ from Sigma Aldrich and adjusted to pH < 3 with conc. HCl to prevent hydroxide–oxide precipitation.[10] The EDTA and EDTA amide shielding chelates were prepared as stock solutions in deionized water at 1.0×10^{-3} mol dm^{-3} adjusted to pH 7.5–8.0 with NaOH; these were used to prepare the solutions of the shielded europium chelates by mixing with the appropriate proportion of the stock europium solution, diluting with 0.01 mol dm^{-3} 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP) generally adjusted to pH 8.5. These solutions contained europium chloride at 1.0×10^{-4} mol dm^{-3} and the EDTA derivative at 1.1×10^{-4} mol dm^{-3}; the slight excess of ligand ensured complete chelation of the europium ions. Solutions of the sensitizers were prepared as required. Other buffers used included HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid] and tris [tris(hydroxymethyl)aminomethane] obtained from Sigma Aldrich Ltd.

Fluorescence and luminescence spectra were recorded on a Perkin-Elmer LS-50B spectrofluorimeter with an excitation width of 10 nm and an emission slit width of 2.5 nm using 1 cm^3 quartz cells. The luminescence spectra were collected using the phosphorescence mode, with a delay time between excitation and collection of 0.05 ms and a collection time of 10.0 ms, using a 350 nm emission filter. Lifetime plots were processed on Aseasyas or Microsoft Works spreadsheet programs.

Ethylenediamine-*N,N,N',N'*-tetraacetic acid mono-butylamide, trisodium salt 8

To a slurry of ethylenediaminetetraacetic acid anhydride (2.0 g, 7.8 mmol) in dry tetrahydrofuran (40 cm^3) was added butylamine (0.57 g, 7.8 mmol) and the reaction mixture was stirred under nitrogen for 24 h. The solvent was removed under reduced pressure to give a white solid residue. This was dissolved in aqueous NaOH solution (15 cm^3, 0.94 g NaOH, 24 mmol) and washed with diethyl ether (2 × 15 cm^3) before removing the water from the solution by evaporation under reduced pressure and drying the residue under high vacuum. The residual, sticky solid was recrystallized from methanol to give the monobutylamide as a white, slightly hygroscopic solid (2.6 g, 76%), mp > 300 °C (Found: C, 40.4; H, 5.7; N, 10.05. Calc. for $C_{14}H_{22}N_3O_7 \cdot H_2O$: C, 40.5; H, 5.8; N, 10.1%).

Ethylenediamine-*N,N,N',N'*-tetraacetic acid, bisbutylamide disodium salt 9

To a slurry of ethylenediaminetetraacetic acid anhydride (2.0 g, 7.8 mmol) in dry tetrahydrofuran (40 cm^3) was added butylamine (1.14 g, 16 mmol) and the mixture stirred at room temperature under nitrogen for 24 h. The work-up was identical to that described above for the monobutylamide except that two equivalents of NaOH were employed. The obtained solid was recrystallized from methanol to give the title salt as a hygroscopic, colourless solid (2.4 g, 69%), mp 173–174 °C (Found: C, 46.4; H, 7.2; N, 11.85. $C_{18}H_{32}N_4Na_2O_6 \cdot H_2O$ requires C, 46.55; H, 7.4; N, 12.1%).

1,4,10-Trioxa-7,13-diazacyclopentadecane-*N,N'*-diacetic acid 5

This was prepared using the method of Gokel and co-workers.[11] The diacid was recrystallized from 80% v/v ethanol to give the diacid as white crystals, $\delta_H(D_2O)$ 3.57 (8 H, m, NCH_2), 3.81 (16 H, m, NCH_2CO_2, OCH_2); *m/z* 290 ($M^+ - CO_2$, 6%), 246 ($M^+ - 2CO_2$, 32), 44 (CO_2, 100) (Found: C, 50.0; H, 8.05; N, 8.1. Calc. for $C_{14}H_{26}N_2O_7$: C, 50.3; H, 8.05; N, 8.1%).

Acknowledgements

We thank the EPSRC for a research studentship (R. M. W.) and MEDAC Ltd. for financial support (J. C.). We thank the EPSRC Mass Spectrometry Service Centre, University College, Swansea, for FAB mass spectral information.

References

1 J. Coates, P. G. Sammes and R. M. West, *J. Chem. Soc., Perkin Trans. 2*, preceding paper.
2 P. G. Sammes, G. Yahioglu and G. D. Yearwood, *J. Chem. Soc., Chem. Commun.*, 1992, 1282.
3 G. Stein and E. Wurzberg, *J. Chem. Phys.*, 1975, **62**, 208; Y. Haas and G. Stein, *J. Phys. Chem.*, 1971, **75**, 3677.
4 P. G. Sammes and G. Yahioglu, *Chem. Soc. Rev.*, 1994, 327.
5 C. A. Chang, V. O. Ochaya and V. C. Sekhar, *J. Chem. Soc., Chem. Commun.*, 1985, 1724; C. A. Chang and V. O. Ochaya, *Inorg. Chem.*, 1986, **25**, 355.
6 G. Schwarzenbach, *Complexometric Titrations*, Chapman and Hall, London, 1957, p. 8; D. A. Skoog, D. M. West and F. J. Holler, *Fundamentals of Analytical Chemistry*, Saunders College Publishing, Orlanda, 6th edn., 1992, p. 289.
7 N. A. Kostromina and N. N. Tananaeva, *Russ. J. Inorg. Chem.*, 1971, **16**, 1256; G. Geler and C. K. Jørgensen, *Chem. Phys. Lett.*, 1971, **9**, 263.
8 W. DeW. Horrocks Jr. and D. R. Sudnick, *J. Am. Chem. Soc.*, 1972, **101**, 334.
9 C. C. Bryden and C. N. Reilley, *Anal. Chem.*, 1982, **54**, 610.
10 F. S. Richardson, *Chem. Rev.*, 1982, **82**, 541.
11 V. J. Gatto, K. A. Arnold, M. Viscariello, S. R. Miller, C. A. Morgan and G. W. Gokel, *J. Org. Chem.*, 1986, **51**, 5373.

Paper 6/01358I
Received 26*th February* 1996
Accepted 20*th March* 1996