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

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Studies on the formation of complexes of europium(III) ions in water with various sensitizers are reported. The sensitizers utilized are derivatives of 1,10-phenanthroline-2,9-dicarboxylic acid and 2,2′:6′,2″-terpyridine-6,6″-dicarboxylic acid. Both 1:1 and 2:1 complexes form, the latter being particularly efficient luminescence enhancers.

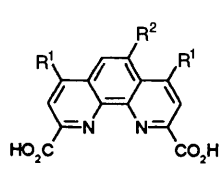
Introduction

Lanthanide luminescence, particularly that involving terbium and europium ions, is being increasingly employed in a range of biological assays and diagnostic kits.[1] Studies with europium(III) ions have been used for several reasons: (*a*) luminescence is relatively long-lived, allowing use of time-resolved measurements that eliminate interference problems from background and auto-fluorescence, as well as light scattering; (*b*) emission occurs at a much longer wavelength than excitation (a large Stokes' shift) and therefore no self-quenching occurs; (*c*) characteristic sharp emission spectra are obtained that are relatively unaffected by solvents, oxygen, *etc.*; (*d*) since the luminescence arises from the ions, they are robust and do not show 'ageing' commonly associated with organic fluorophores, enabling storage over long periods of time.

For luminescence to occur, two main conditions have to be met.[2] Europium(III) ions exhibit very weak absorption coefficients, since the excitation is formally forbidden. Use of energy donors, for example triplet sensitizers, overcomes this problem.[3] Since the triplet–triplet energy transfer process is close range (collision-encountered), chelating sensitizers are required. The second condition is more difficult to meet. Whereas in non-protic solvents excited state Eu^{3+} ions can exhibit relatively efficient luminescence in collapsing to the ground state, in protic solvents such as water, quenching of the excited state occurs by a dark reaction involving vibronic coupling processes to the O–H bonds.[4]

Two approaches have been adopted in order to avoid aqueous quenching of the excited state. In the first, methods for removing the water prior to estimating luminescence are used. In the DELFIA system[5] the europium ion, tightly chelated to ligands such as EDTA and DTPA, is used as a tag on a biological system in a similar manner to that used with radioactive tags. After separation the reagent–target complex is isolated and the europium then released from the complex by sequestration with a sensitizer such as an aromatic β-diketone in the presence of surfactants and metal coordinators, such as trioctylphosphine oxide ('enhancers'), to form hydrophobic micelles in which the luminescence of the europium is maximized. In the CyberFluor system,[6] a derivative of the known sensitizer, 1,10-phenanthroline-2,9-dicarboxylic acid **1** (PDCA), such as the bathophenanthroline compound **2**, is conjugated to a biological reagent and the reagent–target complex separated before adding an excess of Eu^{3+}, removing the excess of the lanthanide ions and measuring the luminescence, generally after the removal of water by drying. Neither of these methods lend themselves to homogeneous assays.

In an alternative approach several groups have developed ligands more able to saturate the coordination sites (about nine) around the ions. Lehn and co-workers[7] have developed the cryptand **5** whilst Toner and co-workers[8] have recommended use of the terpyridyl derivative **6**. Both these approaches use the lanthanide as a permanently switched on tag.

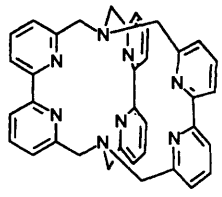


1 $R^1 = R^2 = H$

2 $R^1 = C_6H_4SO_2Cl$, $R^2 = H$

3 $R^1 = H$, $R^2 = NO_2$

4 $R^1 = C_6H_5$, $R^2 = H$



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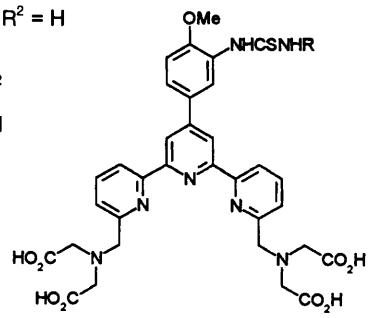


6

We have adopted an alternative, synergistic approach, which involves the use of two cooperating ligands.[9] One of these ligands acts as a shield whilst the other acts as a sensitizer. This system has certain advantages over current systems in that it can act as a molecular switch (Fig. 1). The system requires a shielding ligand that binds tightly and essentially irreversibly with the europium ions at biological pH (6–8), but that is not coordinatively saturated so that it can also accommodate the chelating groups from the sensitizing ligand. The binding constant of the ligand for the shielded Eu^{3+} species need not necessarily be very large since one requires interaction of this only at the biological target and not in free solution.

As a prelude to the design of a shielding component[10] we required an understanding of the interaction of the Eu^{3+} ion with sensitizing ligands on their own; this paper reports on these studies.

Results and discussion

Selection of the sensitizer

Although many possible sensitizers of Eu^{3+} luminescence are known,[11] the compounds **1**, **3**, **4** and **7**–**17** have been selected for study. The parent 1,10-phenanthroline-2,9-dicarboxylic acid systems **1**[12] and **4**[13] are known sensitizers; compounds **3** and **7**–

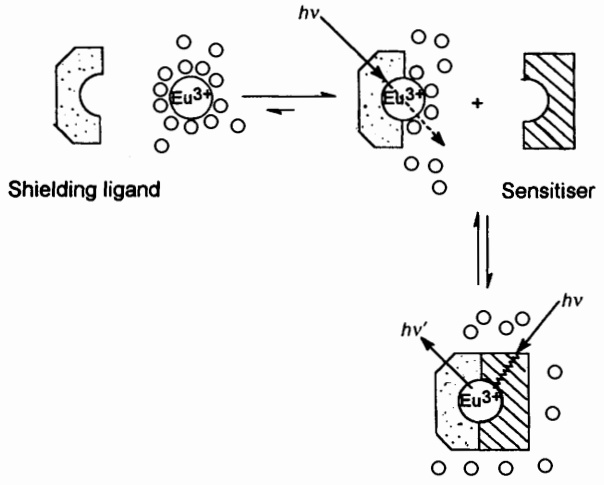
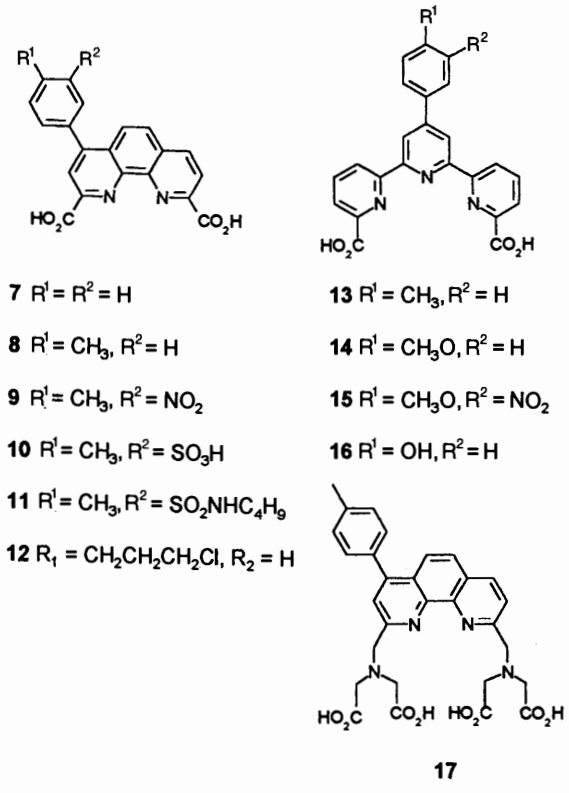


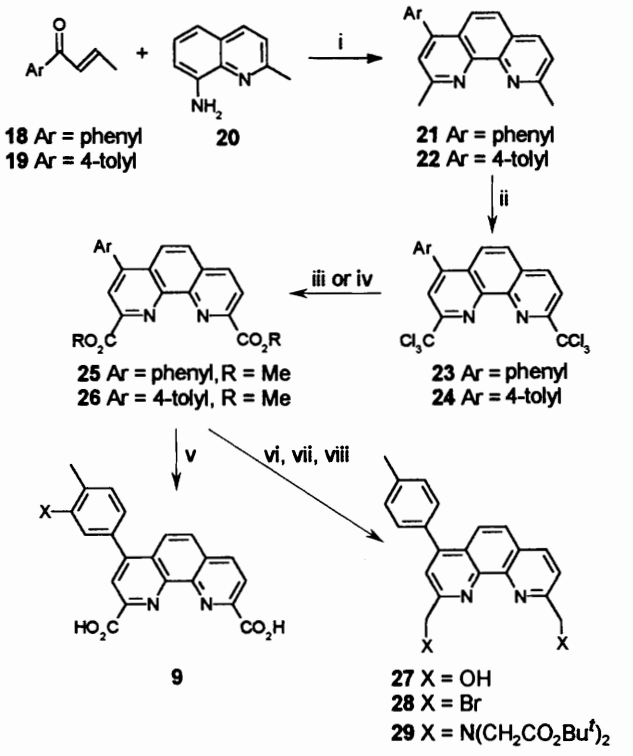
Fig. 1 The shielding ligand acts to displace most of the water (O) from the solvation sphere of the Eu^{3+} ions. The shielded complex is not luminescent. Addition of the sensitizer displaces most of the residual water to form a luminescent complex.



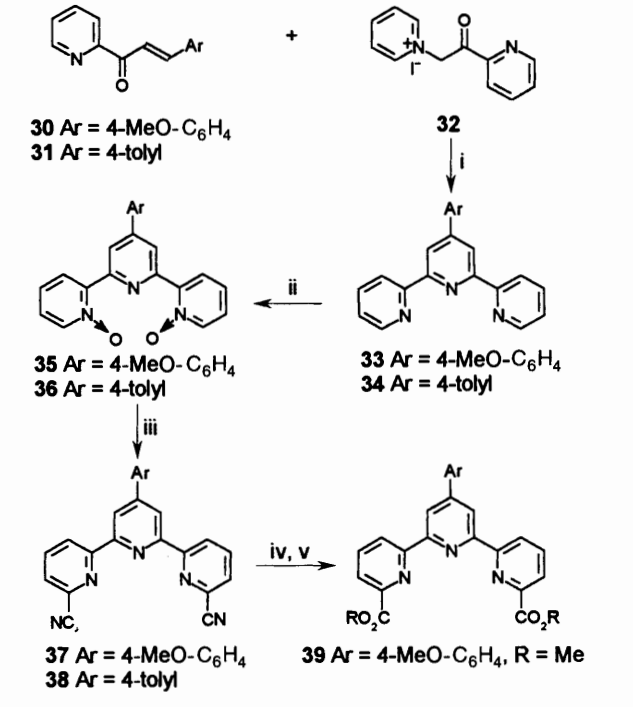
12 are new. The planar structure of these compounds makes them not very sterically demanding,[14] thus allowing one unit to approach a Eu^{3+} ion without coordinatively saturating it, so leaving enough free space for a second molecule to approach and bind to the same ion. Furthermore, since the two carboxylate groups point in a similar direction, entropic effects should help make the binding to the lanthanide ion kinetically fast. Some pioneering studies on the parent compound **1** have been reported previously.[12] Recently terpyridyl derivatives have been reported as Eu^{3+} sensitizers[5,15] and compounds **13–16** were studied for comparison.

The synthesis of the sensitizers was accomplished using standard methods, where required, using the outlined Schemes 1 and 2 (see Experimental section for details).

The sensitizers all possessed some fluorescence properties, as listed in Table 1; in general the excitation maximum ($\lambda_{max.em}$) corresponded closely to the main absorption maximum (λ_{max}) and the intensity is pH dependent;[10] although steady



Scheme 1 *Reagents:* i, Heat/KH_2AsO_3; ii, *N*-chlorosuccinimide/H^+; iii, H_2SO_4 then ROH; iv, H_3O^+; v, X^+, H_2SO_4; vi, $NaBH_4$; vii, HBr; viii, $HN(CH_2CO_2Bu^t)_2$



Scheme 2 *Reagents:* i, Heat, AcOH; ii, *m*CPBA; iii, Me_3SiCN, PhCOCl; iv, H^+, ROH; v, H_3O^+

fluorescence occurred in the range pH 5–11, the fluorescence fell away outside this range without any major changes in the observed pattern; Fig. 2 depicts the pattern observed for 4-phenyl-1,10-phenanthroline-2,9-dicarboxylic acid **7** at pH 11. The fluorescence spectra were absent in the complexes prepared with Eu^{3+} ions.

1:1 Complexes

Addition of an aqueous solution of the phenanthroline sensitizers to a slight molar excess of europium(III) ions, adjusted to pH 6.2 in buffer, gave rise to the appearance of

Table 1 UV absorption and fluorescence emission properties of the sensitizers. Concentrations 1×10^{-5} mol dm^{-3}, pH 11.0 in aqueous NaCl (0.1 mol dm^{-3})

	UV absorption		Fluorescence spectra		
Sensitizer	λ_{max}/nm	A (1 cm cell)	Excitation λ_{max}/nm	Emission λ_{max}/nm	Emission I_{max}
1	283.6	0.259	283.0	346.0	13.5
3	279.1	0.197	294.0	428.5	3.61
4	292.4	0.365	290.0	390.0	137.4
	323.4	0.153	320.6	390.0	85.3
7	287.9	0.301	286.0	379.5	54.9
	317.5	0.107	319.5	379.5	30.4
8	289.6	0.287	287.0	384.0	133.9
	320.5	0.112	322.5	384.0	78.1
9	287.9	0.371	289.0	323.0	0.05
	318.9	0.155	289.0	387.9	0.29
			339.1	387.9	0.18
10	288.0	0.340	287.0	384.0	80.9
	320.6	0.140	325.0	384.0	47.6
11	287.4	0.318	286.5	382.0	45.7
	320.0	0.125	319.5	382.0	25.6
12	289.3	0.292	288.5	396.0	126.2
	320.1	0.114	322.0	396.0	71.7
13	290.2	0.338	287.0	347.5	177.0
			319.0	362.0	101.8
14	290.1	0.324	286.0	449.5	66.8
			321.0	449.5	58.9

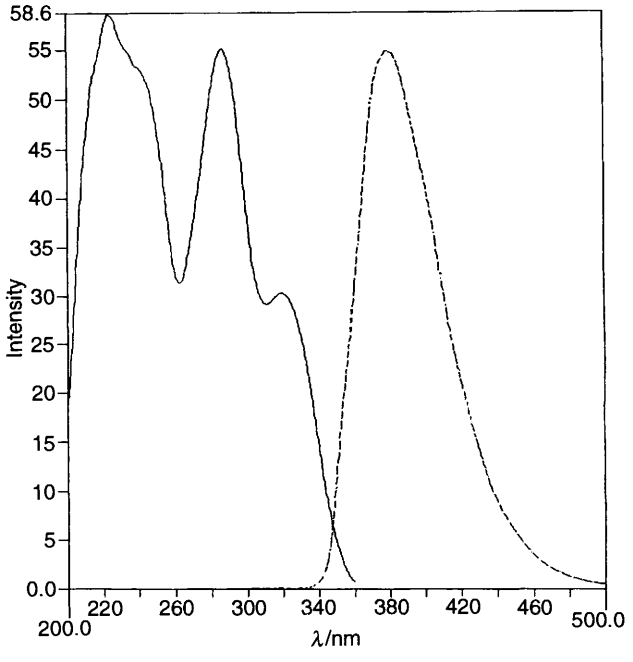


Fig. 2 Excitation and emission spectra for **7**, pH 11; — excitation (380 nm), - - - emission (286)

characteristic lanthanide emission bands when irradiated in the region 285–296 nm (Table 2).

In all cases, the onset of luminescence was fast (within a few seconds, the time of mixing and measuring). In the phenanthroline cases, **1**, **3**, **4** and **7**–**12**, a species with an emission lifetime in the range 0.18–0.22 ms was formed, *e.g.* for compound **7**, see Fig. 3, curve (*a*). The lifetime of emission, around 0.2 ms, indicates a large number of remaining chelating (hydration) sites around the cation. Under these conditions the response is pH dependent. At very low pH we presume protonation of the carboxyl groups of PDCA occurs to form species that are less able to compete against water (*i.e.* formation of a protonated species with a lower association constant for the ion). At higher pH values europium starts to form hydroxide and oxide complexes and, eventually, at pH

Table 2 Emission maxima from 1:1 sensitizer–Eu^{3+} complexes; [Eu^{3+}] 2×10^{-5} mol dm^{-3}; [sensitizer] 5.0×10^{-6} mol dm^{-3}; BTP buffer, pH 6.2

Sensitizer	Excitation λ_{max}/nm	Emission λ_{max}/nm	Emission I_{max}	Lifetime τ/ms
1	286.5	613.5	6.8	0.21
3	284.8	614.0	3.69	0.18
4	296.0	613.5	11.2	0.21
7	292.5	613.5	8.6	0.22
8	293.3	613.5	8.6	0.20
9	291.0	613.5	8.2	0.20
10	292.5	613.5	9.8	0.20
11	292.5	613.5	8.6	0.21
12	293.5	613.5	9.5	0.21
13	290.0	615.5	13.4	0.25
14	291.0	615.5	10.4	0.25
17	282.5	616.5	24.5	0.58

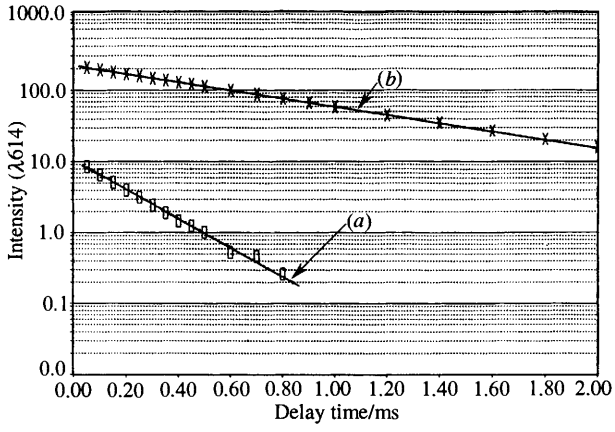


Fig. 3 Emission lifetimes for (*a*) the 1:1 complex and (*b*) the 2:1 complex between **7** and Eu^{3+}; conditions as described in Tables 2 and 3, respectively

values > 11, these lead to the formation of oxide precipitates. Solutions of the 1:1 complexes were only stable for long periods (days) at pH values < 7.

The terpyridyl complexes, **13** and **14**, both showed excitation, λ_{max} at around 290 nm, but with a slightly longer emission lifetime (0.25 ms) compared with that for the 1:1 phenanthroline diacid complexes. This probably reflects the presence of the extra chelating group (five coordinating sites) in the former.

The nature of the emission curve for the 1:1 complexes is known to be sensitive to the presence of different counteranions, such as phosphate and carbonate groups that can approach the vacant sites in the 1:1 complex. In order to avoid such complications we used non-chelating buffers and for this work 0.01 mol dm^{-3} 1,3-bis[tris(hydroxymethyl)-methylamino]propane (BTP) at pH 6.2; this gave reproducible results in the range 0.01–0.2 mol dm^{-3}.

The intensity of emission from these 1:1 complexes is relatively weak and only a slight variation with the nature of the substituents was observed (see Table 2). The weakest was the nitro-derivative **5**, whilst the nitro-substituted terpyridyl **15** did not give a signal, although in contrast, the nitro-tolylphenanthroline, **9**, was less affected. Presumably, for the latter, the pendant phenyl ring is twisted considerably out of the plane of the phenanthroline system so that the electron-attracting effect of the nitro-group is less predominant. Some of the terpyridyl phenol **16** was produced during our synthetic studies and this also failed to exhibit any signal in the presence of the lanthanide.

2:1 Complexes

With an excess of PDCA a 2:1 complex forms,[14] lifetime 0.75

Table 3 Emission maxima and lifetimes from 2:1 sensitizer–Eu^{3+} complexes; [Eu^{3+}] 5×10^{-6} mol dm^{-3}; [sensitizer] 2.0×10^{-5} mol dm^{-3}; BTP buffer, pH 6.2

Sensitizer	Excitation λ_{max}/nm	Emission λ_{max}/nm	Emission I_{max}	Lifetime τ/ms
1	292.5	613.5	187.3	0.79
3	290.0	614.5	33.5	0.45
4	304.5	613.0	571.2	1.01
7	295.6	613.5	243.5	0.76
8	299.0	613.5	228.0	0.75
9	297.0	613.5	133.4	0.70
10	299.5	613.5	205.9	0.73
11	304.0	613.0	235.4	0.99
12	303.0	613.0	325.6	1.06
13	296.7	612.5	139.7	1.64
14	297.5	612.5	71.1	1.59

Table 4 Qualitative results of extractions of 2:1 complexes. Complexes formed as described in Table 3. Aqueous solutions were treated with equal volumes of dichloromethane and the solutions examined under a UV light

Sensitizer	Observation
1	No luminescence in organic phase
7	Some luminescence in organic phase
12	Most luminescence in organic phase
4	All luminescence in organic phase

ms, consistent with the participation of eight binding sites from the two ligands and one molecule of water. The 2:1 complex also forms from the 1:1 complex at higher pH (>7) by a disproportionation process (see discussion above), europium being 'removed' by formation of oxide precipitates. The 2:1 complexes are stable over a much wider pH range (*ca.* 5–11) than the 1:1 species.

Similar studies on the other diacids [Table 3; see Fig. 3(*b*) for results with compound **7**] consistently exhibited longer lifetimes, ranging between 0.45 ms for the nitro-derivative **5**, to over 1.6 ms for the disubstituted terpyridyl complexes **13** and **14**. Complexes **13** and **14** are effectively saturated by the ligand and for these little interference from solvent water is evident.

Of interest were the longer lifetimes of 1.06 and 1.01 ms, respectively, observed for the derivatives **8** and **12**. Both of these ligands were only sparingly soluble in water and tended to form aggregates in the pH range 6–9. It is considered that the observed longer lifetimes in part reflect the formation of micelles, as observed in the DELFIA enhancer solutions; these micelles help to hinder the vibrational deactivation process by the solvent water. A similar effect may also explain the slightly longer lifetimes observed for the 1:1 complexes with these ligands.

Alternatively the effect may be more local, the more hydrophobic ligands helping to repel the last molecule of coordinated water making the complexes more hydrophobic.

Some evidence for this increased hydrophobicity of the 2:1 complexes could be qualitatively examined by partitioning the aqueous solutions with dichloromethane, see Table 4. Whereas the luminescence from the PDCA complex remained completely in the aqueous layer, luminescence from the complexes of the diphenyl- and chloropropylphenyl-substituted derivatives, **4** and **12**, was observed mainly from the organic phase.

The intensity of the emissions from the 2:1 complexes are again sensitive to the nature of the ring substituents. The nitro-derivative **5** was the weakest emitter, possibly indicating some intramolecular electron transfer process that is depleting the excited state. Very strong emission was observed from the bathophenanthroline derivative **4** (I_{max} 571 units) and the 4-(chloropropyl)phenyl-derivative **12** (I_{max} 326 units) again suggesting some aggregation effects. Rather surprising, given the long lifetimes observed, were the relatively weak emissions from the terpyridyl derivatives **13** (I_{max} 140 units) and **14** (I_{max} 71 units) despite the fact that for these complexes, coordination around the europium ion is saturated by the ligands (a maximum 10 coordination sites for the 2:1 complexes).

In order to estimate the upper limit for the number of coordinating sites permitted that allow 2:1 complexes to be formed one further ligand was briefly examined, the tetraacid **17**, which possesses a maximum of eight coordination sites. Although this formed a stable 1:1 complex (see Table 2), the observed spectrum was unchanged by adding an excess of ligand, indicating formation of a stable 1:1 species only. Thus the maximum number of coordination sites that can be accommodated by the first chelating molecule that allows ingress of a second sensitizer molecule, *e.g.* the phenanthroline dicarboxylic acids, is less than eight. Saturation of further ligand sites with smaller chelating agents is, of course, well known and even the europium complex of the cryptate **3**, for example, allows entry of one or more fluoride ions to complete the saturation of its coordination sphere.[16]

Emission patterns

The emission spectra show bands for the transitions from the $^5D_0$ level to the $^7F_0$ to $^7F_4$ levels. The strongest of these is the $^5D_0$ to $^7F_2$ transition, centred at 615 nm. The band at 580 nm corresponds to the $^5D_0$ to $^7F_0$ band and the ratio of these 'hypersensitive' transitions gives important information on the nature of coordination about the ion.[17] Typical curves for compound **7** are illustrated in Fig. 4. For the 1:1 complex [Fig. 4(*a*)] the pattern of emission clearly shows the 580 nm peak, whereas in the 2:1 complex [Fig. 4(*b*)] this has virtually disappeared. The symmetry expected for the 1:1 complex is low and consistent with C_2 symmetry, whilst the pattern exhibited by the 2:1 complex is consistent with a D_2 symmetry, as exhibited by a number of crystalline complexes.[10,11,18]

Binding constants

For PDCA, **1**, a 1:1 complex is formed having an association constant, K^1_{ass}, in the order of 4×10^8 dm^3 mol^{-1},[10] whilst the second binding constant, K^2_{ass}, for the 2:1 complex is reported to be about 2×10^6 dm^3 mol^{-1}. We have estimated approximate binding constants for the other phenanthroline complexes, **4**–**12**[19] and similar K^1_{ass} values were observed for each of these. Titrations of the 1:1 complexes with more ligand allowed an estimation of the second binding constant, K^2_{ass}, and these were in the range 10^5–10^6 dm^3 mol^{-1}; Fig. 5(*a*) illustrates a typical titration curve obtained for PDCA, **1**, and Fig. 5(*b*) the corresponding curve using the ratio of emissions at 614 and 580 nm as ordinate. Both curves indicate a cut-off for emissions consistent with the formation of a 2:1 complex.

To conclude, the above studies show that, in aqueous solutions, the europium(III) ion shows complex chelating properties, but that, under suitably controlled conditions discrete 2:1 complexes with ligands can be prepared provided the chelating group involved does not coordinatively saturate the ion, as shown for the ligand **17**, which has eight coordinating sites.

Experimental

Distilled, deionised water was used throughout this work. All volumetric flasks and quartz cells were carefully pre-cleaned with Caro's acid (1:1 v/v 30% H_2O_2 and 98% sulfuric acid) at room temperature. They were then rinsed with distilled water, 3 mol dm^{-3} HCl, distilled water and finally HPLC grade methanol, before air drying prior to use. Europium(III) chloride stock solutions were prepared from 99.99% europium(III)

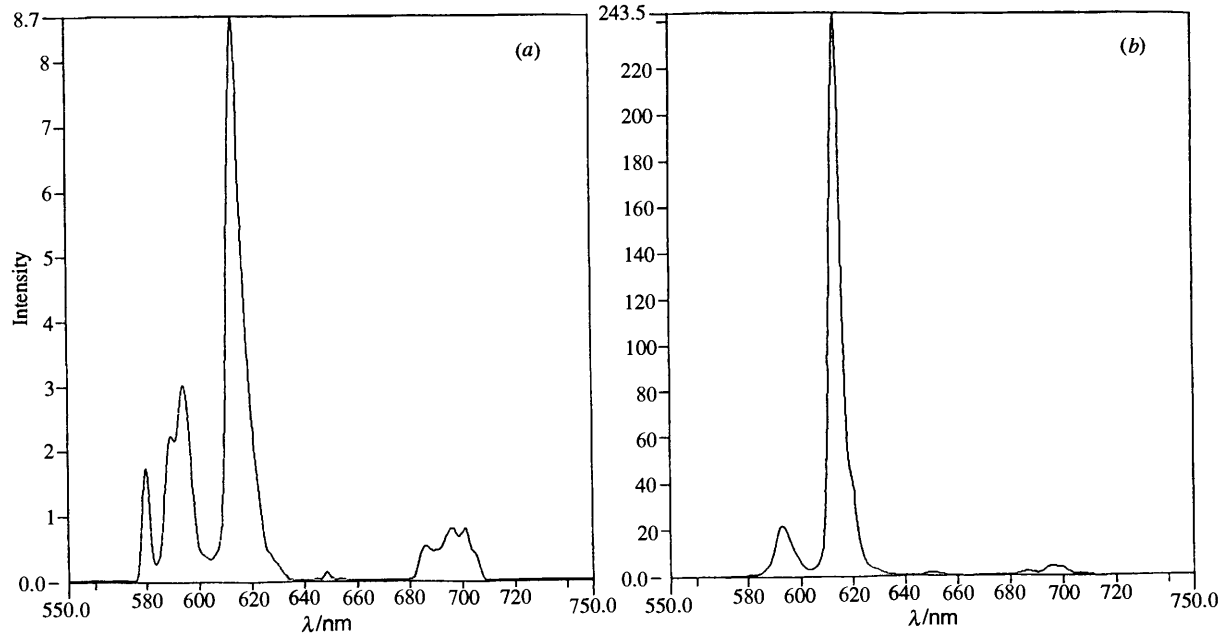


Fig. 4 (*a*) Emission spectra of 1 : 1 complex of **7** with Eu^{3+} (λ_{ex} 292 nm); (*b*) emission spectra of 2 : 1 complex of **7** and Eu^{3+} (λ_{ex} 296 nm); conditions as described in Tables 2 and 3, respectively

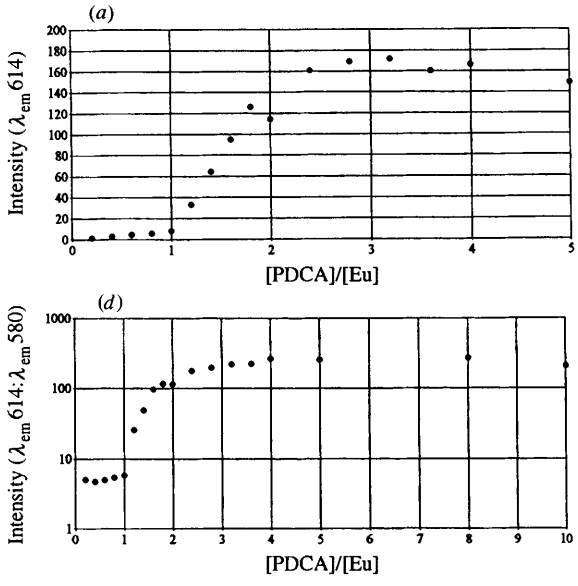


Fig. 5 Titration of $[Eu^{3+}]$ against PDCA, **1**; $[Eu^{3+}] = 5 \times 10^{-6}$ mol dm^{-3}, pH 6.2. (*a*) Plot against λ_{em} 614 nm, intensity in arbitrary units; (*b*) plot against the ratio λ_{em} 614 nm : λ_{em} 580 nm.

chloride hexahydrate, *ex* Sigma Aldrich. A stock solution of 1.0×10^{-3} mol dm^{-3} was prepared, acidified to pH < 3 with conc. HCl to prevent hydroxide–oxide formation.[20] Ethylènediaminetetraacetic acid, disodium salt, 99 + %, was obtained from Sigma Aldrich, as was the dianhydride; the latter was dried over P_2O_5 overnight at 50 °C before use. Other compounds were prepared by the methods described below.

Fluorescence and UV measurements were obtained on the ligands at 1.0×10^{-5} mol dm^{-3} in 0.1 mol dm^{-3} NaCl solutions adjusted to pH 11 with aqueous NaOH on their own in water. Luminescent measurements were performed on a Perkin-Elmer LS50B spectrofluorimeter fitted with a red-sensitive photomultiplier, using a 1 cm optically flat cuvette at room temperature (20–23 °C) without external temperature control. Europium luminescent measurements were unaffected by small ambient temperature changes. For europium luminescence measurements standard phosphorescent instrument settings were used with a 0.05 ms delay time, excitation slit width of 10 nm and emission slit width of 2.5 nm, using a 350 nm emission filter. Both emission and excitation spectra were recorded. The band shape experiments (Fig. 4) were carried out with the emission slit at its smallest setting (2.5 nm). Output was to an IBM PC interfaced *via* the Perkin-Elmer Fluorescence Data Manager (FLDM) package.

Solutions were buffered with 0.01 mol dm^{-3} 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP) at pH 6.2, pH adjustments being made with 0.1 mol dm^{-3} hydrochloric acid or sodium hydroxide before final dilution of samples to volume. Readings were generally recorded, in duplicate, after allowing solutions to stand for several hours in order to reach equilibrium. Representative samples of solutions were heated to 95 °C and then cooled to ambient temperature in order to check equilibrium had been reached.

^1H NMR spectra were recorded on a JEOL FX200 instrument, unless otherwise stated, in deuteriochloroform solution using tetramethylsilane as an internal reference; chemical shifts are in ppm and coupling constants are measured in hertz. Microanalyses were carried out by MEDAC Ltd., Brunel University.

1,10-Phenanthroline-2,9-dicarboxylic acid 1

This was prepared using the method of Newkome *et al.*,[21] mp 238 °C decomp. (lit.,[22] 238 °C decomp.) (Calc. for $C_{14}H_8N_2O_4 \cdot H_2O$: C, 58.55; H, 3.4; N, 9.8. Found: C, 58.55; H, 3.5; N, 9.8%).

5-Nitro-1,10-phenanthroline-2,9-dicarboxylic acid monohydrate[23] 3

Mp 218–220 °C (lit.,[23] 218–220 °C) (Calc. for $C_{14}H_7N_3O_6 \cdot H_2O$: C, 50.8; H, 2.7; N, 12.70. Found: C, 50.8; H, 2.8; N, 12.4%).

4,7-Diphenyl-1,10-phenanthroline-2,9-dicarboxylic acid 4

A mixture of bathocuproine (3.6 g, 10 mmol) and *N*-chlorosuccinimide (9.35 g, 7 mmol) in chloroform (25 cm^3) and carbon tetrachloride (150 cm^3) and a catalytic quantity of 3-chloroperbenzoic acid (10 mg) were heated to reflux for 16 h before cooling and filtering. The organic phase was washed with 10% w/v aqueous sodium carbonate solution and 0.1 mol dm^{-3} sodium thiosulfate solution before drying and evaporating to dryness. The residual solid was recrystallized from chloroform–ethanol to afford 4,7-diphenyl-2,9-bis(trichloromethyl)-1,10-phenanthroline (4.31 g, 76%) as white crystals, mp 278–279 °C.

A mixture of the hexachloride (0.57 g, 1 mmol) in formic acid (10 cm^3) and water (1 cm^3) was heated at reflux for 24 h. The yellow solution was poured into water and the precipitate collected (0.45 g, 100%). The product was recrystallized from aqueous formic acid to afford the title diacid (0.39 g, 89%); mp 206 °C decomp., ν_{max}/cm^{-1} 3500–3300, 1730, 1300, 790 and 720; δ_H 8.28 (2 H, s), 8.01 (2 H, s), 7.63 (10 H, br s) and 3–5 (br, exch.) (Calc. for $C_{26}H_{16}N_2O_4 \cdot H_2O$: C, 71.2; H, 4.1; N, 6.4. Found: C, 70.4; H, 4.1; N, 6.25%).

4-Phenyl-1,10-phenanthroline-2,9-dicarboxylic acid 7

The quinoline **20** (0.79 g, 5 mmol) was dissolved in conc. HCl (40 cm^3) and the ketone **18** (2.19 g, 15 mmol) and potassium dihydrogen arsenate (1.00 g, 5.5 mmol) added. The mixture was stirred at 80 °C for 7 h before cooling and basifying to *ca.* pH 10 with 40% w/v aq. NaOH. The mixture was extracted with chloroform (3 × 50 cm^3), the organic extracts dried, treated with decolourizing charcoal and filtered. The filtrate was evaporated under reduced pressure and the residue treated with chloroform (5 cm^3) and ether (80 cm^3) before leaving at 0 °C for four days. The crystalline precipitate was collected and dried to give 4-phenyl-2,9-dimethyl-1,10-phenanthroline **21** (0.9 g, 63%). A sample was recrystallized from benzene to give pale yellow crystals, mp 203–205 °C, δ_H 2.97 (3 H, s), 2.98 (3 H, s), 7.43 (1 H, s), 7.49 (1 H, d, *J* 8.3), 7.52 (5 H, br s), 7.61 (1 H, d, *J* 9), 7.80 (1 H, d, *J* 9) and 8.09 (1 H, d, *J* 8.3) (Found: C, 84.6; H, 5.7; N, 9.6. $C_{20}H_{16}N_2$ requires C, 84.5; H, 5.7; N, 9.85%).

To a solution of the phenanthroline **21** (0.5 g, 1.75 mmol) in chloroform (5 cm^3) and carbon tetrachloride (30 cm^3) was added *N*-chlorosuccinimide (1.54 g, 11.5 mmol) and a few mg of 3-chloroperoxybenzoic acid before heating the mixture to reflux for 12 h. The mixture was cooled, filtered and the organic phase was washed with 5% w/v sodium carbonate solution (3 × 30 cm^3), 0.1 mol dm^{-3} sodium thiosulfate solution (50 cm^3) then brine (50 cm^3). The organic extract was dried, filtered and evaporated to give the crude hexachloride **23** as a pale yellow solid (0.94 g, 100%). The product was purified by column chromatography through silica gel, using 2:3 dichloromethane–light petroleum as eluent, followed by recrystallization from chloroform–ethanol to give the hexachloride **23** as an off-white solid (0.75 g, 87%), mp 218–220 °C, δ_H 7.6 (5 H, s), 7.88 (1 H, d, *J* 9.3), 8.05 (1 H, d, *J* 9.3), 8.25 (1 H, s), 8.32 (1 H, d, *J* 8.8) and 8.43 (1 H, d, *J* 8.8) (Found: C, 49.0; H, 2.1; N, 5.55; Cl, 43.3. $C_{20}H_{10}N_2Cl_6$ requires C, 48.9; H, 2.05; N, 5.7; Cl, 43.3%).

Hydrolysis of the hexachloride **23** was achieved in aqueous formic acid, as described for the preparation of compound **4**. The solid diacid **7** (81%) showed mp 215 °C decomp., ν_{max}/cm^{-1} 3420, 3060, 1720, 1710, 1690, 1620, 1275 and 1220; $\delta_H([^2H_6]DMSO)$ 8.72 (1 H, d, *J* 8.3), 8.42 (1 H, d, *J* 8.3), 8.25 (1 H, s), 8.17 (1 H, d, *J* 9), 8.00 (1 H, d, *J* 9), 7.65 (5 H, s) and 3–4 (br s, D_2O exch.) (Found: C: 64.1; H, 3.95; N, 7.4. $C_{23}H_{12}N_2O_4 \cdot H_2O$ requires C, 66.3; H, 3.9; N, 7.7%).

4-(4-Methylphenyl)-1,10-phenanthroline-2,9-dicarboxylic acid 8

The aminoquinoline **20** (0.79 g, 5 mmol) was reacted with the ketone **19** (2.40 g, 15 mmol) in the manner described above, to afford 4-(4-methylphenyl)-2,9-dimethyl-1,10-phenanthroline **22** (1.2 g, 81%), mp 140–142 °C; δ_H 8.10 (1 H, d, *J* 8.3), 7.83 (1 H, d, *J* 9.3), 7.62 (1 H, d, *J* 9.3), 7.49 (1 H, d, *J* 8.3), 7.45–7.32 (5 H, m), 2.96 (6 H, s) and 2.48 (3 H, s) (Found: C, 84.1; H, 6.1; N, 9.3. $C_{21}H_8N_2$ requires C, 84.5; H, 6.1; N, 9.4%).

Hexachlorination of the 2,9-dimethylphenanthroline **22** was achieved using *N*-chlorosuccinimide in the manner described above. The hexachloride **24** (73%) showed mp 192–193 °C (chloroform–ethanol), δ_H 8.43 (1 H, d, *J* 8.3), 8.33 (1 H, d, *J* 8.3), 8.24 (1 H, s), 8.09 (1 H, d, *J* 9.3), 7.98 (1 H, d, *J* 9.3), 7.50–7.38 (4 H, m) and 2.51 (3 H, s) (Found: C, 50.1; H, 2.5; N, 5.5. $C_{21}H_{12}N_2Cl_6$ requires C, 49.9; H, 2.4; N, 5.55%).

The hexachloride **24** (0.5 g, 10 mmol) was mixed with formic acid (5 cm^3) and heated to reflux for 24 h, water (0.5 cm^3) was added and refluxing continued a further 16 h. The solution was quenched into water and the precipitate collected and recrystallized from formic acid–water to give diacid **8** (0.25 g, 67%) as an off-white solid, mp 193 °C (decomp.); ν_{max}/cm^{-1} 3600–3200, 1730 and 1280; $\delta_H([^2H_6]DMSO)$ 8.71 (1 H, d, *J* 8.3), 8.45 (1 H, d, *J* 8.3), 8.26 (1 H, s), 8.13 (1 H, d, *J* 9.3), 8.02 (1 H, d, *J* 9.3), 7.6–7.4 (4 H, m) and 2.46 (3 H, s) (Found: C, 66.6; H, 4.2; N, 7.4. $C_{21}H_{14}N_2O_4 \cdot H_2O$ requires C, 67.0; H, 4.3; N, 7.4%).

4-(4-Methyl-3-nitro)phenyl-1,10-phenanthroline-2,9-dicarboxylic acid 9

The dicarboxylic acid **8** (2.26 g, 6 mmol) was added portionwise to sulfuric acid (98%, 30 cm^3) cooled in an ice-bath to < 10 °C. After the solid had dissolved, nitric acid (70%, 0.675 mg, 7.5 mmol) was added dropwise to the solution, with stirring, to give a bright yellow solution. The mixture was stirred at 0 °C for 5 min and then allowed to warm to room temperature over 20 min. The solution was quenched in ice–water to produce a gelatinous precipitate. The aqueous suspension was warmed to 80 °C for a few minutes to help produce a more granular solid, which was then collected, washed well with water and dried under reduced pressure to afford the title product (2.51 g, 99%) as a pale yellow solid, mp 208–210 °C decomp.; ν_{max}/cm^{-1} 3600–3200, 1730, 1530, 1360 and 1270; $\delta_H([^2H_6]DMSO)$ 8.71 (1 H, d, *J* 8.3), 8.41 (1 H, d, *J* 8.3), 8.31 (1 H, s), 8.23 (1 H, d, *J* 2.0), 8.16 (1 H, d, *J* 9.3), 7.99–7.89 (2 H, m), 7.75 (1 H, d, *J* 8.3), 7–3 (4 H, br, exch.) and 2.66 (3 H, s) (Found: C, 59.8; H, 3.5; N, 9.9. $C_{21}H_{13}N_3O_6 \cdot H_2O$ requires C, 59.9; H, 3.6; N, 10.0%).

4-(4-Methyl-3-sulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid 10

The hexachloride **24** (1.0 g) was added to cold conc. sulfuric acid (98%, 3 cm^3) and heated to 90 °C for 5 h. The dark brown solution was cooled and quenched in ice–water. The solid precipitate was collected by filtration and then dissolved in hot 2 mol dm^{-3} sulfuric acid (*ca.* 150 cm^3) before reducing the volume 50 cm^3 before cooling. A yellow solid formed, which was collected and washed with small portions of 1 mol dm^{-3} hydrochloric acid, followed by acetone, before drying under reduced pressure over anhydrous calcium chloride, to give the sulfonated acid **10** (0.6 g, 55%), mp > 300 °C decomp., $\delta_H([^2H_6]DMSO)$ 8.81 (1 H, d, *J* 8.3), 8.45 (1 H, d, *J* 8.3), 8.27 (1 H, s), 8.26 (1 H, d, *J* 9.3), 8.07 (1 H, d, *J* 9.3), 8.00 (1 H, d, *J* 1.6), 7.55 (1 H, dd, *J* 7.8, 1.6), 7.45 (1 H, d, *J* 7.8) and 2.08 (3 H, s).

4-[4-Methyl-3-(*N*-butylaminosulfo)phenyl]-1,10-phenanthroline-2,9-dicarboxylic acid 11

The dicarboxylic acid **8** (0.75 g, 2 mmol) was added, portionwise with stirring, to chlorosulfonic acid (97%, 3 cm^3) maintained at 0 °C in an ice-bath. After addition was complete the red solution was heated to 60 °C for 1 h, cooled and then quenched in ice–water to give a pale yellow precipitate. This was collected at 0 °C and the damp solid freeze-dried under reduced pressure to produce the *chlorosulfonyl derivative*, mp 320 °C decomp. (Found: C, 52.5; H, 3.1; N, 5.9; S, 7.0. $C_{21}H_{13}N_2ClO_6S \cdot H_2O$ requires C, 53.1; H, 3.2; N, 5.9; S, 7.0%).

The sulfonyl chloride (0.54 g, 1.2 mmol) was added to a solution of butylamine (0.73 g, 10 mmol) in 0.5 mol dm^{-3} aqueous NaOH (10 cm^3) at 0 °C with stirring. The solid slowly dissolved to afford a yellow solution, when it was allowed to warm to ambient temperature for 2 h before finally heating briefly to 60 °C. The solution was cooled to 0 °C in an ice-bath before acidifying with 3 mol dm^{-3} HCl. The white precipitate that formed was collected, washed with a little water and then dried (0.51 g, 84%). A small sample was recrystallized from water–tetrahydrofuran to give the title amide as a white solid, mp 255 °C decomp.; ν_{max}/cm^{-1} 3500–3200, 1740, 1340 and 1170;

δ_H(trifluoroacetic acid) 9.00–7.89 (8 H, m), 3.18 (2 H, t, *J* 6.6), 2.94 (3 H, s), 1.60 (2 H, m), 1.40 (2 H, m) and 0.92 (3 H, t, *J* 7.3) (Found: C, 58.55; H, 4.6; N, 7.7. $C_{25}H_{23}N_3O_6S{\cdot}H_2O$ requires C, 58.7; H, 4.9; N, 8.2%. Found: MH^+, 494.1360. $C_{25}H_{24}N_3O_6S$ requires 494.1385).

4-[4-(3-Chloropropyl)phenyl]-1,10-phenanthroline-2,9-dicarboxylic acid 12

This was prepared as described previously,[23] mp 190 °C decomp.

2,9-Bis-[*N*,*N*-bis(carboxymethyl)aminomethyl]-4-(4-methylphenyl)-1,10-phenanthroline 17

The acid **8** (1.1 g, 3 mmol) was heated in refluxing thionyl chloride (10 cm^3) for 1 h under nitrogen before cooling and removing the solvent. To the solid residue was added methanol (25 cm^3) and the mixture heated to reflux for 30 min. Removal of the solvent gave essentially pure methyl ester **26** (1.15 g, 100%), which was used without further purification. To the ester was added ethanol (20 cm^3) and the solute dissolved by warming before adding a suspension of sodium borohydride (0.68 g, 18 mmol) in ethanol (20 cm^3) and heating the mixture to reflux for 3 h. A small portion of acetone was then added and the bulk of the solvent removed by evaporation. The residual solid was partitioned between water and chloroform and the organic layer collected, dried, filtered and evaporated to produce the crude diol **27** as a pale yellow foam (1.05 g, 100%). The crude diol was dissolved in 49% HBr (12 cm^3) to give a pale yellow solution which was heated to reflux for 3 h, cooled and carefully neutralized with solid sodium hydrogen carbonate before extracting with chloroform (3 × 30 cm^3). The organic extracts were combined, dried, filtered and evaporated to produce a light brown solid. The solid was dissolved in chloroform before chromatographing through silica gel, using chloroform as eluent, to afford 2,9-bis(bromomethyl)-4-(4-methylphenyl)-1,10-phenanthroline dibromide **28** (0.45 g, 32%), mp > 120 °C with gradual decomp.; ν_{max}/cm^{-1} 1610, 1575, 1540 and 1490; δ_H 8.21 (1 H, d, *J* 8.4), 7.9 (1 H, d, *J* 9.2), 7.86 (1 H, d, *J* 8.5), 7.77 (1 H, s), 7.67 (1 H, d, *J* 9.2), 7.2 (4 H, s) and 2.47 (3 H, s) (Found: C, 54.4; H, 3.6; N, 6.0. $C_{21}H_{16}N_2Br_2{\cdot}0.5H_2O$ requires C, 54.2; H, 3.7; N, 6.0%).

The dibromide (140 mg, 0.3 mmol) and di-*tert*-butyl bis(iminoacetate) (0.147 mg, 0.6 mmol) were dissolved in dry acetonitrile (8 cm^3), solid sodium carbonate (220 mg) added and the mixture heated at reflux for 16 h under nitrogen. The mixture was cooled and filtered and the filtrate evaporated to afford a yellow oil, which was diluted with chloroform (5 cm^3) followed by ether (25 cm^3). On leaving at 0 °C for several days a colourless crystalline precipitate formed which was collected and dried to afford 2,9-bis-[*N*,*N*-bis-(*tert*-butoxycarbonylmethyl)aminomethyl]-4-(4-methylphenyl)-1,10-phenanthroline **29** (200 mg, 85%), mp 110 °C decomp., ν_{max}/cm^{-1} 1730; δ_H 8.32 (1 H, d, *J* 8.2), 7.93 (1 H, d, *J* 9.2), 7.77 (1 H, d, *J* 9.2), 7.70 (1 H, d, *J* 8.2), 7.50 (1 H, s), 7.44–7.37 (4 H, m), 4.39 (2 H, s), 4.37 (2 H, s), 3.62 (4 H, s), 2.49 (3 H, s) and 1.39 (36 H, s) (Found: C, 60.0; H, 6.8; N, 6.2. $C_{45}H_{60}N_4O_8{\cdot}NaBr{\cdot}H_2O$ requires C, 59.9; H, 7.3; N, 6.2%).

The tetra-*tert*-butyl ester **29** (100 mg) was dissolved in trifluoroacetic acid (2 cm^3) and the solution left at 25 °C for 2 h before removing the solvent under reduced pressure to give a pale yellow solid. Trituration with ether gave the product tetra-acid **17** as a cream-coloured solid (60 mg, 85%); ν_{max}/cm^{-1} 3400, 3000, 2550(br), 1740–1600, 1400, 1380 and 1190; δ_H([$^2H_6$]-DMSO) 8.74–7.46 (9 H, m), 4.46 (2 H, s), 4.44 (2 H, s), 3.69 (4 H, s), 3.67 (4 H, s) and 2.47 (3 H, s).

***N*-(2-Pyridylcarbonylmethyl)pyridinium iodide 32**

This was prepared according to the literature method[24] to give the title salt, mp 200 °C decomp. (lit., 198–199 °C, decomp.).

1-(2-Pyridyl)-3-(4-methoxyphenyl)prop-2-en-1-one 30

This was prepared by the literature method,[25] mp 85–86 °C (lit., 84–85 °C).

4′-(4-Methoxyphenyl)-2,2′:6′,2″-terpyridine 33

Prepared according to the method of Spahni and Calzaferri;[26] mp 163–164 °C (lit., 153–154 °C).

4′-(4-Methoxyphenyl)-2,2′:6′,2″-terpyridine *N*,*N*″-dioxide 35

To a solution of terpyridyl **34** (4.48 g, 13 mmol) in dichloromethane (80 cm^3) was added a solution of 85% 3-chloroperbenzoic acid (8.1 g) in dichloromethane (100 cm^3) and the solution stirred at room temperature for 16 h before washing with 10% w/v aqueous sodium carbonate solution, the organic phase dried and evaporated to dryness and the residue triturated with acetone to afford the title compound as a white crystalline product (4.31 g, 88%) and was recrystallized from methanol; mp 195–196 °C; δ_H 8.38, (2 H, dt, *J* 0.6, 6.4), 8.33 (2 H, dd, *J* 2.2, 8.0), 7.80 (2 H, m), 7.49 (2 H, tt, *J* 0.6, 7.5), 7.31 (2 H, dt, *J* 2.2, 7.1), 7.00 (2 H, m) and 3.87 (3 H, s) (Found: C, 71.1; H, 4.6; N, 11.2. $C_{22}H_{17}N_3O_3$ requires C, 71.15; H, 4.6; N, 11.3%).

4′-(4-Methoxyphenyl)-2,2′:6′,2″-terpyridine-6,6″-dinitrile 37

To a solution of the di-*N*-oxide **36** (1.72 g, 4.6 mmol) in dichloromethane (35 cm^3) was added trimethylsilylcyanide (3 cm^3, 22.5 mmol). After 5 min benzoyl chloride (2.9 cm^3, 25 mmol) was slowly added (over 10 min). A slight exotherm set in and a crystalline precipitate formed. The mixture was stirred for an extra 3 h, left to stand at 5 °C overnight and the precipitate collected to afford, after recrystallisation from dioxan, the title dinitrile (1.47 g, 82%), mp 187–189 °C; δ_H 8.90 (2 H, dd, *J* 0.97, 8.3), 8.64 (2 H, s), 8.26 (2 H, t, *J* 7.8), 8.10 (2 H, dd, *J* 1.0, 7.8), 7.92, 7.15 (4 H, aromatic H) and 3.87 (3 H, s) (Found: 74.0; H, 3.8:, N, 18.0. $C_{24}H_{15}N_5O$ requires C, 74.0; H, 3.9; N, 18.0%).

4′-(4-Methoxyphenyl)-2,2′:6′,2″-terpyridine-6,6″-dicarboxylic acid 14

The dinitrile **37** (0.30 g, 0.8 mmol) was heated in a mixture of conc. HCl (40 cm^3) and acetic acid (20 cm^3) at reflux for 1 h. On cooling, a yellow crystalline precipitate formed that was collected and recrystallized from aq. dioxan to give the title diacid[8] (0.21 g, 64%), mp 242 °C decomp.; δ_H([$^2H_6$]DMSO) 8.78 (4 H, m), 8.18 (4 H, m), 7.94, 7.20 (4 H, aromatic H) and 3.68 (3 H, s); *m*/*z* (FAB) 428 (M + H, 100%), 450 (M + Na).

1-(2-Pyridyl)-3-(4-methylphenyl)prop-2-en-1-one 31

This was prepared by the literature method.[25] The isolated chalcone formed in 74% yield; mp 85–87 °C (lit., 83 °C).

4′-(4-Methylphenyl)-2,2′:6′,2″-terpyridine 34

This was prepared by the literature method.[26] The product was formed in 45% yield; mp 168–170 °C (lit., 158–159 °C).

4′-(4-Methylphenyl)-2,2′:6′,2″-terpyridine *N*,*N*″-dioxide 36

The terpyridine **34** (1.62 g, 5 mmol) was reacted with 3-chloroperbenzoic acid in the manner described above and, after isolation afforded the title *N*,*N*-dioxide (1.55 g, 89%), mp 249–250 °C; δ_H 9.21 (2 H, s), 8.35 (2 H, m), 8.22 (2 H, m), 7.75 (2 H, m), 7.43–7.27 (6 H, m) and 2.41 (3 H, s) (Found: C, 74.1; H, 4.75; N, 11.8. $C_{23}H_{17}N_3O_2$ requires C, 74.35; H, 4.8; N, 11.8%).

4′-(4-Methylphenyl)-2,2′:6′,2″-terpyridine 6,6″-dinitrile 38

Obtained from compound **36**, using trimethylsilylcyanide–benzoyl chloride in the same manner as described for the preparation of compound **37**. The dinitrile showed mp 268–270 °C; δ_H([$^2H_6$]DMSO) 8.95 (2 H, d, *J* 8), 8.65 (2 H, s), 8.29 (2 H, t, *J* 8), 7.87–7.42 (4 H, m) and 2.42 (3 H, s) (Found: C, 77.1; H, 4.1; N, 18.7. $C_{24}H_{15}N_5$ requires C, 77.2; H, 4.05; N, 18.8%).

4′-(4-Methylphenyl)-2,2′:6′,2″-terpyridine-6,6″-dicarboxylic acid 13

A solution of the dinitrile (0.37 g, 1 mmol) in acetic acid (20 cm^3) and conc. HCl (40 cm^3) was heated to reflux for 16 h, cooled and the solid diacid product was collected after recrystallization from aq. dioxan the title diacid (0.32 g, 78%), mp 227–228 °C; $\delta_H([^2H_6]DMSO)$ 8.89 (2 H, m and 2 H, s), 8.22 (2 H, t, *J* 7.6), 8.18 (2 H, dd, *J* 1.2, 7.6), 7.44–7.87 (4 H, m) and 2.43 (3 H, s) (Found: C, 68.6; H, 4.5; N, 9.4. $C_{24}H_{17}N_3O_4 \cdot 1/2H_2O$ requires C, 68.6; H, 4.2; N, 10.0%).

4′-(4-Hydroxyphenyl)-2,2′:6′,2″-terpyridine-6,6″-dicarboxylic acid 16

The ether **14** (200 mg, 0.5 mmol) was heated in glacial acetic acid (5 cm^3) and 49% HBr (25 cm^3) at reflux for 18 h. The solution was cooled and the yellow solid that separated was collected and recrystallized from dioxan to give the title phenol diacid as a yellow solid (0.18 g, 85%), mp 269 °C decomp.; $\delta_H([^2H_6]DMSO)$ 13.3 (2 H, br s), 9.98 (1 H, br s), 8.90 (2 H, dd, *J* 1.3, 7.5), 8.86 (2 H, s), 8.26 (2 H, t, *J* 7.6), 8.18 (2 H, dd, *J* 1.3, 7.6) and 7.03–7.86 (4 H, m); *m/z* 413 (M^+, 5%), 369 ($M^+ - CO_2$, 50) and 325 ($M^+ - 2CO_2$, 100).

4′-(4-Methoxy-3-nitrophenyl)-2,2′:6′,2″-terpyridine-6,6″-dicarboxylic acid 15

This was prepared either by a total synthesis from the corresponding nitrated chalcone or from direct nitration of the methoxydiacid **14**; for the latter route extensive demethylation accompanied nitration. The product nitro-compound was recrystallized from aq. dioxan; mp 263 °C decomp.; $\delta_H([^2H_6]DMSO)$ 8.89 (4 H, m), 8.45 (1 H, d, *J* 2.2), 8.27 (1 H, dd, *J* 2.2, 8.8), 8.19 (4 H, m), 7.61 (1 H, d, *J* 8.8) and 4.04 (3 H, s).

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