Preparation of some new intercalating europium(III) sensitizers

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The synthesis of phenanthridinium salts linked to a chelating phenanthroline-2,9-dicarboxylic acid group, as in 1 and 2, is described. These derivatives behave as useful probes for the identification of DNA single strands in a new homogeneous assay.

We have recently described the use of the intercalating sensitizers **1** and **2** in a new assay for DNA.[1] The principle of this assay is outlined in Fig. 1. In this the lanthanide ion is tethered to a probe DNA molecule. After hybridisation to a target, the intercalator/sensitizer can fit into the double-strand duplex and 'hunt' along the base pairs until the metal chelator can reach and bind to the lanthanide ion. The cooperative effect is the switching on of the lanthanide luminescence by the sensitizer once a hit (target–probe match) is made.

The sensitizing probes are critical to the success of this assay. In designing these, certain properties are required:

(i) The probe must be water soluble, albeit only sparingly, as the working concentrations are in the range 10^{-6}–10^{-11} mol dm^{-3}.

(ii) The probes must be stable in aqueous solution in the pH range 7–9, the normal range for using DNA.

(iii) The linker joining the intercalating unit from the sensitizer must be at an optimum length; too long and the advantages of cooperativity (Fig. 1) are lost; too short and the chelating group (sensitizer) will not be able to reach the lanthanide ion.

(iv) The interchelating group and the sensitizer must not adversely interact with one another to prevent the overall signalling system from working.

In this paper we describe the design and synthesis of the sensitizer/intercalators **1** and **2**.[1]

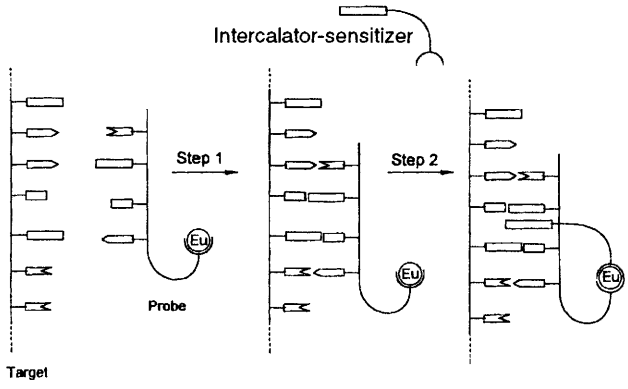


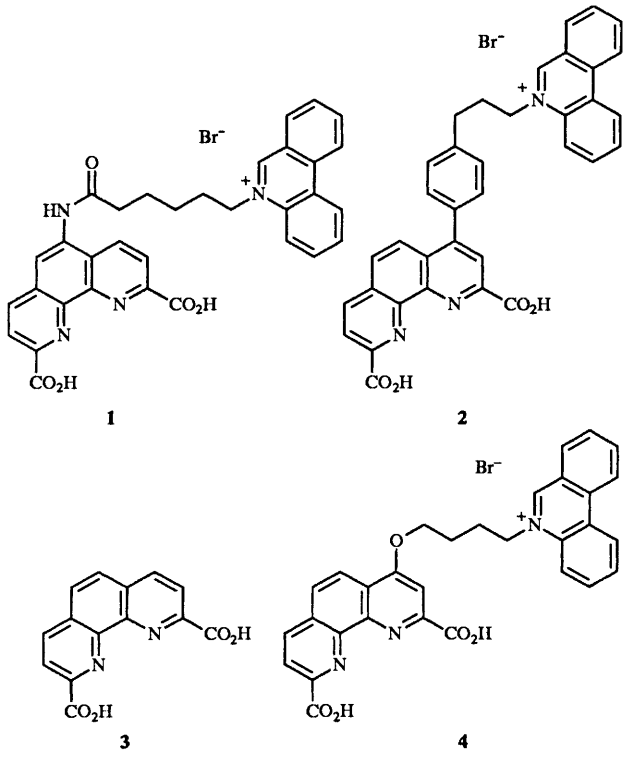
Fig. 1 Step 1: labelled probe seeks and hybridizes to target DNA strand.

Step 2: intercalator-sensitizer hunts along duplex until it reaches and binds to the metal ion. Only the final, cooperative complex gives rise to a signal upon irradiation.

Results and discussion

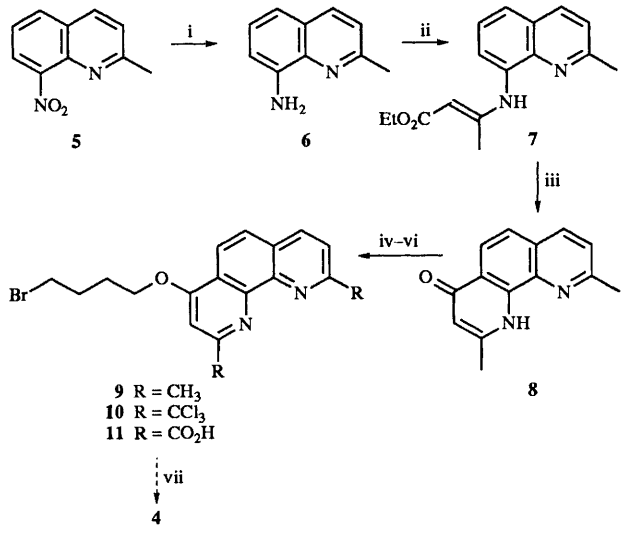
The choice of sensitizer was based on the known agent, 1,10-phenanthroline-2,9-dicarboxylic acid **3**.[2] This is one of the simplest sensitizing species and has the advantage that it is conformationally rigid.[3] A consequence of this is that the rate at which it can chelate to a lanthanide ion such as Eu^{3+} is relatively fast, *i.e.* in a matter of seconds, whereas more flexible chelators, such as ethylenediaminetetraacetic acid (EDTA) take hours to reach equilibrium.

The sensitizer has to be linked by a short chain to the intercalator. The length of the linking groups, in both the intercalator/sensitizer and the DNA probe linking the lanthanide chelator was chosen after consideration of molecular models and some literature precedent. Thus Hélène and co-workers[4] have reported that a length of at least 6–12 atoms is required when an intercalator is attached to a DNA probe in order to allow enough freedom for the handle to fold back and reach into the duplex DNA formed when the reagent is hybridised to a target. For the intercalator/sensitizer unit, the handle should be just long enough to allow intercalation to occur, without interference from the metal chelating unit, as well as to avoid interference from the DNA phosphate groups during binding of the lanthanide ion.



Two initial approaches for the introduction of the linking group were made. The first was to bind the linker *via* a hydroxy

group, as in the target **4**. The route to this is outlined in Scheme 1. The commercially available 2-methyl-8-nitroquinoline **5** was reduced by catalytic hydrogen transfer to give the amine **6**. Conrad–Limpach formation of the 1,10-phenanthroline **8** was achieved by initial condensation with ethyl acetoacetate, to give the enamine **7** followed by thermal cyclisation at 255 °C in boiling diphenyl ether.[5] The product **8**, obtained in overall yield of 72% from the nitroquinoline, existed mainly as the keto-tautomer indicated, the NH group being strongly hydrogen bonded to the adjacent ring nitrogen atom. However *O*-alkylation could readily be effected, for example using 1,4-dibromobutane and caesium carbonate as base in acetonitrile, to give the ether **9**.

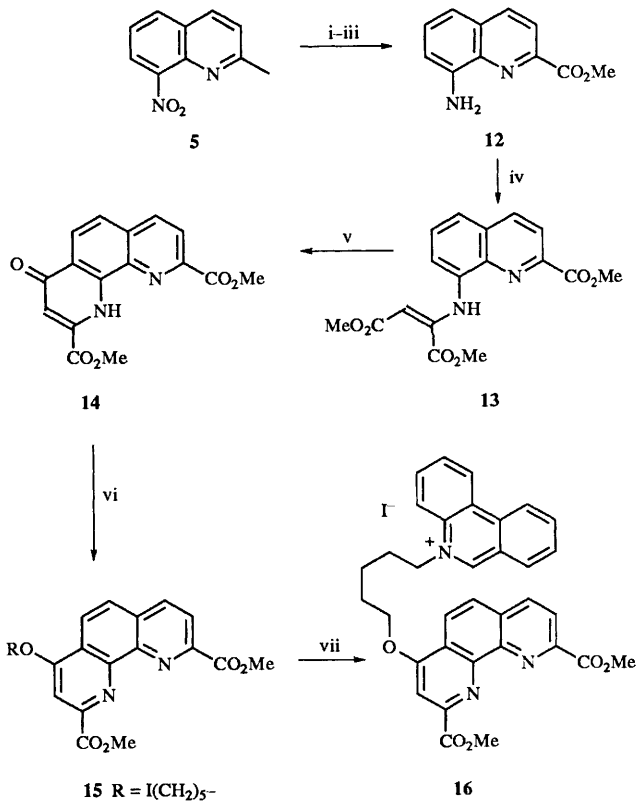


Scheme 1 *Reagents and conditions:* i, 10% Pd/C, N_2H_4·H_2O, CH_3OH; ii, ethyl acetoacetate, H^+; iii, heat, 255 °C; iv, $Br(CH_2)_4Br$, Cs_2CO_3, acetonitrile; v, NCS, $CHCl_3$–CCl_4; vi, AcOH, NaOAc; vii, phenanthridine, 125 °C

Oxidation of the methyl groups to carboxylic acids was attempted using the Newcombe method.[6] Thus, chlorination with *N*-chlorosuccinimide (NCS) in carbon tetrachloride, using 3-chloroperbenzoic acid as a catalyst, afforded the hexachloride **10**. Initially this was assumed to be a free radical process. However, observations on the ease of exchange of the methyl protons with deuterium under acid catalysis suggested that an ionic chlorination might also be possible, involving the intermediate enamines. Chlorinations, using a trace of either 3-chlorobenzoic acid or benzoic acid in place of the peracid, proved to be just as effective and resulted in cleaner reaction products. No chlorination occurred in the absence of either the free radical initiator or acid catalyst.

Attempts to hydrolyse the trichloromethyl groups with sulfuric acid failed, because of concomitant cleavage of the ether function. Use of buffered acetic acid overcame this problem and the diacid **11** could be obtained in low yield (27%). Attempts to quaternise the bromobutyl group with phenanthridine, in order to form the phenanthridinium salt—a known intercalating species[7]—were also disappointing and the product obtained was an intractable mixture which could not be purified.

As an alternative means to the target dicarboxylic acid **4** the route outlined in Scheme 2 was followed. It was envisaged that quaternisation of the diester intermediate **15** would lead to the more soluble salt **16**, which could then be hydrolysed to give the target. The 8-aminoquinoline-2-carboxylate ester **12** was prepared from the nitroquinoline **5** using standard methods (see Experimental section). Condensation of **12** with dimethyl acetylenedicarboxylate afforded the enamine **13** as a bright yellow solid. This cyclised when heated in diphenyl ether, to



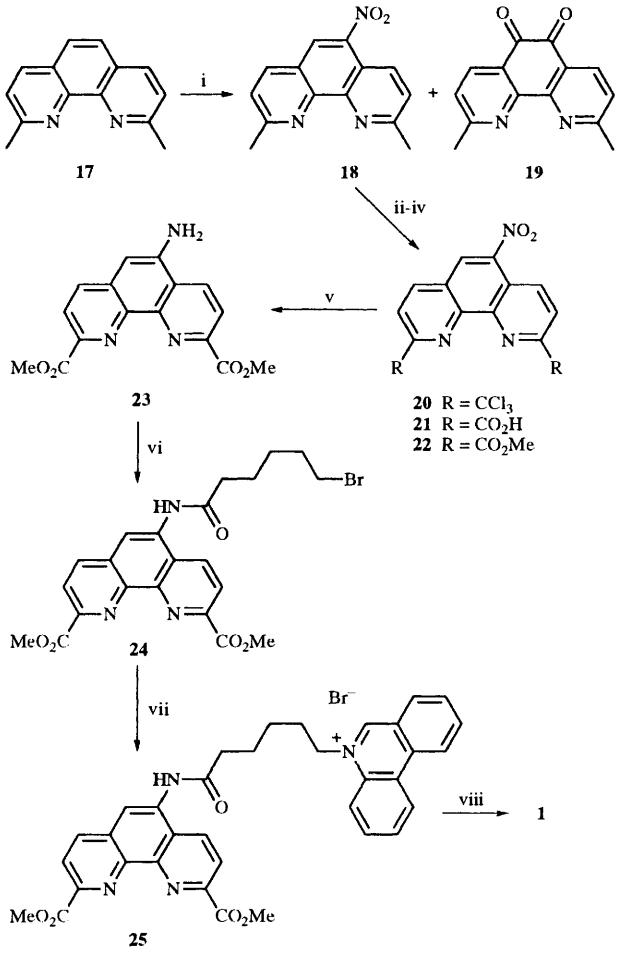
Scheme 2 *Reagents and conditions:* i, NCS, CCl_4; ii, H_2SO_4, MeOH; iii, 10% Pd/C, cyclohexene, MeOH; iv, dimethyl butynedioate; v, heat, 225 °C; vi, $I(CH_2)_5I$, Ag_2CO_3, benzene; vii, phenanthridine, 125 °C

produce the phenanthridone **14** in overall 60% yield from the ester **12**.

O-Alkylation of **14** with 1,5-diiodopentane, using anhydrous silver carbonate as base in benzene,[8] gave the ether **15**; in this case use of caesium carbonate in acetonitrile failed, instead forming a complex mixture of products. Surprisingly, attempted purification of the ether proved difficult, extensive decomposition occurring on silica and alumina columns. Similarly, attempted quaternisation of the alkyl group with phenanthridine only afforded a low yield of the required quaternary ester **16** and the ester function of this could only be hydrolysed with difficulty. In both steps, loss of the alkyl ether group seemed to be occurring.

As a consequence of these stability problems, **4** was abandoned as a suitable target and attention turned to the probe **1**. The route to this is outlined in Scheme 3. Nitration of 2,9-dimethylphenanthroline (neocuproine) **17** afforded the 5-nitro derivative **18**, formed along with considerable amounts of the diketone **19**. Hexachlorination of **18** gave a clean conversion into the hexachloride **20**, which, unlike most of this family of chelating agents, could be readily purified. Hydrolysis of the trichloromethyl groups to the corresponding carboxylic acids, as in **21**, was effected with conc. sulfuric acid and quenching with water; alternatively, use of a methanol quench gave the dimethyl ester **22**. Reduction of the nitro group was tried under several conditions, of which transfer hydrogenation using palladium-on-charcoal in methanol and cyclohexene was most effective, giving the amine **23** as a bright yellow solid. The linker unit was 6-bromohexanoyl chloride and this was coupled to the amine in either dry chloroform or acetonitrile, using diisopropylethylamine as base. The amide **24** was isolated in over 80% yield.

Heating the bromohexanoyl derivative with phenanthridine in a variety of solvents, such as nitrobenzene and xylene, failed to afford the desired quaternary salt **25** and either recovered starting material or the degradation products being obtained. Ultimately we used phenanthridine as a melt at 115–120 °C, to

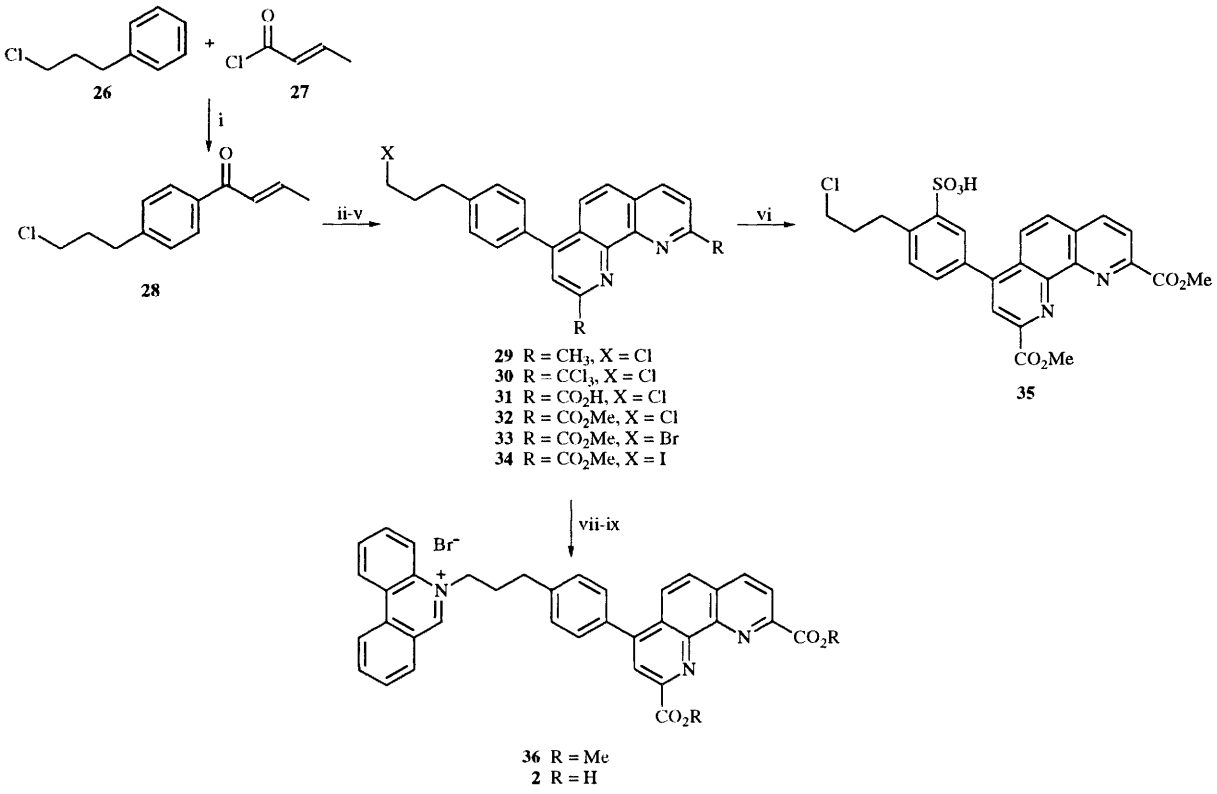


Scheme 3 *Reagents and conditions:* i, HNO_3–H_2SO_4; ii, NCS, CCl_4; iii, H_2SO_4; iv, MeOH, H^+; v, 10% Pd/C, cyclohexene–MeOH; vi, $Br(CH_2)_5COCl$, Pr^i_2NEt, acetonitrile; vii, heat, phenanthridine; viii, H_2O–HBr, pH 4

which the bromide was added in small portions with stirring. After addition and further heating for about 1 h, the melt was cooled and the hard brown solid triturated with large quantities of ether. The desired salt **25** was obtained as a pale brown powder. As well as being insoluble in all but a few polar solvents, such as methanol, this proved difficult to crystallise, so a reprecipitation from acetone or methanol with ether was utilised. Formation of the quaternary salt was indicated by the appearance in its ^1H NMR spectrum of a downfield proton, at δ_H 11.0 ppm, indicative of the proton at C-6 of the phenanthridinium ring, as well as appearance of a strong molecular ion and by formation of a methylene signal adjacent to the quaternary nitrogen atom at *ca.* 5.2 ppm.

The final step in formation of the required probe **1** was hydrolysis of the ester groups in **25**. Base-catalysed hydrolysis was ruled out as the phenanthridinium system is sensitive to nucleophilic attack at position 6,[9] so acid conditions, to which they are stable, were examined. Use of low pH systems gave rise to a mixture of products, primarily due to cleavage of the amide group. However, hydrolysis with a trace of hydrobromic acid (to ensure a common anion was maintained) at pH 4 gave mainly the required diacid, accompanied by small quantities of the monomethyl ester and hydrolysis products from cleavage of the amide group.

The other target, **2**, was selected since this has no amide group that can be broken during deprotection of the required carboxylic acid groups. The starting materials for this synthesis (Scheme 4) were the 8-aminoquinoline **6** and the chloropropylphenyl ketone **28**, which was prepared as the major isomer by Friedel–Crafts acylation of 3-chloropropylbenzene **26** with crotonyl chloride **27**. The condensation of **6** and **28** gave a dihydroaromatic system, which had to be oxidised to produce the required aromatic phenanthroline **29**. Initial attempts with ferric chloride as oxidant failed, mainly because of the formation of insoluble iron complexes with the product phenanthroline which proved difficult to filter and separate. Use of arsenic acid as oxidant [10] overcame these problems and, after purification, gave the phenanthroline in 57% yield.



Scheme 4 *Reagents and conditions:* i, $AlCl_3$, $CHCl_3$; ii, **6**, KH_2AsO_4, HCl, AcOH; iii, NCS, CCl_4; iv, H_2SO_4 then H_2O; v, MeOH, H^+; vi, H_2SO_4, MeOH; vii, LiBr; viii, heat, phenanthridine; ix, H_2O, HCO_2H, HBr

Table 1 Luminescence emission (λ_{em} 615 nm) for 1:1:1 complexes of compounds **1**–**3** with Eu^{3+} and EDTA[a]

Probe	Control[c]	1	2	3
Intensity[b]	0.00	70.2	204.6	115.8

[a] Tris buffer with 0.1 mol dm^{-3} NaCl at pH 7.8; [EDTA:Eu] at 2×10^{-5} mol dm^{-3}; compounds at 2×10^{-6} mol dm^{-3}. [b] Measured on a Perkin-Elmer LS50B spectrofluorimeter; intensity in arbitrary units. [c] No sensitizer present.

Further manipulation used the hexachlorination–hydrolysis sequence described above, to give the diacid **31** *via* the chloride **30**.

The acid **31** was only sparingly soluble in organic solvents and attempts to quaternise this directly with phenanthridine failed, because of degradation and decarboxylation. The carboxylate groups could be esterified with methanol and the ester **32** proved to be more readily manipulated. Direct formation of the diester from the hexachloride by quenching the concentrated sulfuric acid mixture with methanol rather than water, was not possible, the major product being not the diester **32** but, instead, the sulfonated product **35**.

Attempts to quaternise the chloropropyl derivative **32** failed and mainly unchanged material was isolated from melts with phenanthridine; use of higher temperatures only produced elimination products. The chloride group was therefore displaced with either iodide or bromide before quaternisation to give the corresponding halides **33** and **34**. Of these derivatives the bromide proved most useful, affording the phenanthridinium salt **36** in good yield (> 50%); use of the iodide led to concomitant demethylation of the ester groups to give inseparable mixtures. De-esterification of the diester **36** was effected by boiling the salt in dilute formic-hydrobromic acid for several h which gave the required target molecule **2** as a pale yellow powder in reasonable yield. Again, MS and highfield NMR studies confirmed the presence of the phenanthridinium species.

The probes **1** and **2** were both sparingly soluble in water but solutions could be obtained (at 10^{-5} mol dm^{-3}) by first dissolving the compounds in dimethyl sulfoxide followed by washing into a large volume of buffer at pH 7.5. The use of these probes in DNA assays will be reported elsewhere but initial studies, using 1:1:1 mixtures of EDTA:Eu^{3+}:probe, with the parent 1,10-phenanthroline-2,9-dicarboxylic acid **3** as a control, are reported in Table 1. These show that, under comparable conditions, the probe **1** gives lower signal strengths than the reference **3**, whilst the phenyl-substituted system **2**, gives stronger responses. Furthermore, the latter probe gives strong signals at excitation wavelengths ≥320 nm, whereas both the reference **3** and probe **1** only produce weak signals at excitation wavelengths > 300 nm.

Experimental

Mps were determined on a hot-stage apparatus and are uncorrected. All chromatographic purifications were carried out using Sorbasil silica gel and redistilled solvents. Thin layer chromatography (TLC) was performed on Whatman 2.5 × 7.5 cm glass-backed plates with a 0.25mm layer of silica gel 60 F_{254}. Solvents were redistilled and, where necessary, dried before use.[11] Solutions were generally dried over anhydrous Na_2SO_4 before removal of the solvent on a rotary evaporator and removal of volatile residues at an oil pump.

^1H NMR spectra were obtained on either a Varian CFT20 (80 MHz) or JEOL FX200 instrument and, unless otherwise stated, for solutions in deuteriochloroform, using tetramethylsilane as an internal reference. *J* Values are given in Hz. Mass spectra were recorded on an AEI MS902 spectrometer; the high resolution and FAB spectra were obtained on the EPSRC facilities, Department of Chemistry, University College, Swansea. Infrared spectra were obtained on a Perkin-Elmer 1420 ratio recording spectrophotometer as KBr discs. The luminescence spectra, as detailed in Table 1, were obtained on a Perkin-Elmer LS50B luminescence spectrometer. Microanalyses were carried out by MEDAC Ltd, Brunel University.

1,10-Phenanthroline-2,9-dicarboxylic acid 3

2,9-Dimethyl-1,10-phenanthroline (neocuproine) (5.0 g, 0.024 mol) in carbon tetrachloride (200 cm^3) containing *N*-chlorosuccinimide (NCS) (19.2 g, 0.144 mol) was heated at reflux in the presence of 3-chloroperbenzoic acid (20 mg) for 12 h. After evaporation of the mixture, the residue was dissolved in chloroform and the solution washed with an excess of 10% w/v aq. $NaHCO_3$, dried, filtered and concentrated under reduced pressure to afford a crystallising oil. The product was chromatographed (silica gel; 3:2 dichloromethane–light petroleum) and crystallised from methanol–dichloromethane to give 2,9-bis(trichloromethyl)-1,10-phenanthroline as a white crystalline solid (8.57 g, 86%), mp 212–214 °C (lit,[12] 212–214 °C).

A solution of the hexachloride (2.0 g, 4.82 mmol) in 98% sulfuric acid (10 cm^3) was heated at 80–90 °C under nitrogen for 6 h. The resulting brown solution was allowed to cool to room temperature and the viscous solution was slowly poured over crushed ice. The crude diacid was precipitated as an off-white solid, which was filtered off, washed well with cold water and then recrystallised from aq. THF to give the title diacid **3** as a white solid (1.18 g, 91%), mp 238 °C (lit.,[13] 238 °C) (Found: C, 58.55; H, 3.5, N, 9.8. $C_{14}H_8N_2O_4 \cdot H_2O$ requires C, 58.55; H, 3.4; N, 9.8%).

Reduction of 2-methyl-8-nitroquinoline

To a solution of the nitroquinoline **8** (10 g, 0.053 mol) in methanol (200 cm^3) was added hydrazine hydrate (15.9 g, 0.138 mol) and 10% Pd/C (200 mg). The mixture was heated at reflux for 1 h, after which a further portion of Pd/C (200 mg) was added and the mixture reheated to reflux; this procedure was repeated once more, to give a total reflux time of 3 h. The mixture was filtered hot through Celite, the filtrate concentrated to a small volume and 3 mol dm^{-3} hydrochloric acid (100 cm^3) added. The resulting orange precipitate was basified with 3 mol dm^{-3} aq. NaOH whilst cooling, to afford a light-brown solid. This was filtered off, washed with water, dried and recrystallised from light petroleum to give 8-amino-2-methylquinoline **6** as pale yellow crystals (8.0 g, 95%), mp 56–57 °C (lit.,[14] 56 °C); δ_H 2.67 (3 H, s), 4.0–5.55 (2 H, br s, exch. D_2O) and 6.7–8.0 (5 H, m).

2,9-Dimethyl-1,10-phenanthrolin-4-ol 8[5]

This compound was prepared according to the method of Case.[5] From the aminoquinoline **6**, the intermediate enamine **7** was obtained as a light brown solid (6.7 g, 99%) and this was used without further purification in the cyclisation. The crude phenanthroline **8** was recrystallised from water to give the title product **8** as its hydrate (4.3 g, 77%), mp 237–238 °C (lit.,[5] 237–238 °C) (Found: C, 67.9; H, 5.7; N, 11.3. $C_{14}H_{12}N_2O \cdot 1.25H_2O$ requires C, 68.1; H, 5.9; N, 11.4%).

4-(4-Bromobutoxy)-2,9-dimethyl-1,10-phenanthroline 9

A mixture of the phenanthroline **8** (0.25 g, 1.1 mmol), caesium carbonate (1.1 g) and 1,4-dibromobutane (0.72 g, 3.35 mmol) in acetonitrile (50 cm^3) was heated at reflux under N_2 for 5 h, and

then cooled and filtered. The filtrate was concentrated under reduced pressure to give a brown solid which was dissolved in chloroform and the solution washed with water and dried. After concentration of the mixture, the residue was chromatographed over silica gel (1 : 1 benzene–acetone) to afford the *title ether* **9** (0.28 g, 70%) as a white solid, mp 100–102 °C (decomp.); δ_H 1.73 (2 H, exch. D_2O), 2.15 (4 H, m), 2.87 (3 H, s), 2.91 (3 H, s), 3.54 (2 H, t, *J* 6), 4.24 (2 H, t, *J* 6), 6.82 (1 H, s), 7.50 (1 H, d, *J* 9), 7.52 (1 H, d, *J* 9.1), 8.05 (1 H, d, *J* 9) and 8.07 (1 H, d, *J* 9.1) (Found: C, 57.5; H, 5.55, N, 7.25. $C_{18}H_{19}BrN_2O{\cdot}H_2O$ requires C, 57.30; H, 5.6; N, 7.4%).

4-(4-Bromobutoxy)-2,9-bis(trichloromethyl)-1,10-phenanthroline 10

To the ether **9** (0.4 g, 1.1 mmol) in carbon tetrachloride (20 cm^3) with *N*-chlorosuccinimide (0.98 g, 7.33 mmol) was added 3-chloroperbenzoic acid (15 mg) as an initiator. The mixture was heated at reflux for 5 h, cooled to room temperature and filtered. The filtrate was evaporated to a small volume and the residue chromatographed through silica gel (6 : 4 benzene–light petroleum) to give the *title chloride* (0.41 g, 65%) as a white solid, mp 171–172 °C; δ_H 2.19 (4 H, m), 3.52 (2 H, m), 4.37 (2 H, m), 7.57 (1 H, s), 7.85 (1 H, d, *J* 7) and 8.25–8.40 (3 H, m) (Found: C, 38.4; H, 2.5; N, 4.9. $C_{18}H_{13}BrCl_6N_2$ requires C, 38.2; H, 2.3; N, 4.95%).

The reaction was repeated, using either 3-chlorobenzoic acid or benzoic acid as initiator, to afford the hexachloride **10** in higher yields (75%).

4-(4-Bromobutoxy)-1,10-phenanthroline-2,9-dicarboxylic acid 11

To a 4 : 1 mixture of acetic acid and water (50 cm^3) was added the hexachloride **10** (0.40 g, 0.7 mmol) and the mixture was heated to reflux. At 30 min intervals, anhydrous sodium acetate (0.35 g, 4.24 mmol) was added in three equal portions and hydrolysis was continued for a further 2 h. The mixture was evaporated under reduced pressure and the residue recrystallised from aq. THF to give the *dicarboxylic acid* **11** as a yellow powder (80 mg, 27%), mp > 250 °C (decomp.); ν_{max}/cm^{-1} 3600–2900 (CO_2H), 1720 (C=O), 1290–1220 (C–O) and 1130 (C–O–C); δ_H[$(CD_3)_2SO$] 2.28 (4 H, m), 3.49 (2 H, m), 4.38 (2 H, m), 8.21–8.36 (3 H, m) and 8.6–8.8 (2 H, d, *J* 8) (Found: C, 49.3; H, 3.7; N, 6.9. $C_{18}H_{15}BrN_2O_5{\cdot}H_2O$ requires C, 49.4; H, 3.9; N, 6.9%).

Methyl 8-aminoquinoline-2-carboxylate[14] 12

This compound was prepared according to the method of Roth and Erlenmeyer,[14] in four steps starting from 2-methyl-8-nitroquinoline to give the aminoquinoline **12** in 30% overall yield, mp 98–99 °C (lit.,[15] 97–99 °C); *m/z* 202 (M^+; 4.9%), 171 (51) and 143 (100).

Dimethyl 4-hydroxy-1,10-phenanthroline-2,9-dicarboxylate 14

To a solution of the aminoquinoline **12** (1.4 g, 6.9 mmol) in dry methanol (50 cm^3) was added dimethyl butynedioate (1.03 g, 7.3 mmol) and the solution heated at reflux under N_2 for 12 h. Evaporation of the mixture under reduced pressure afforded, as a yellow solid, the enamine **13** (2.4 g, 100%). This was used without further purification.

The enamine (2.0 g, 5.8 mmol) was added portionwise to molten diphenyl ether (150 g) at 250 °C. After addition was complete the mixture was heated at reflux for a further 2 h, allowed to cool and then triturated with light petroleum. The fine, light brown precipitate that formed was filtered off (1.08 g, 60%). A portion was recrystallised from hot water to give the *phenanthroline* **14** as off-white needles, mp 220 °C; ν_{max}/cm^{-1} 3350 (NH), 1760 (C=O), 1690 (C=O) and 1170 (C–O–C); δ_H 1.82 (H_2O), 4.08 (6 H, s, MeO), 7.18 (1 H, s), 7.66 (1 H, d, *J* 9), 8.34 (2 H, s) and 8.41 (1 H, d, *J* 9) (Found: C, 60.6; H, 3.8; N, 8.5. $C_{16}H_{12}N_2O_5{\cdot}0.25H_2O$ requires C, 60.76, H, 4.0; N, 8.8%).

Dimethyl 4-(5-iodopentyloxy)-1,10-phenanthroline-2,9-dicarboxylate 15

To a stirred solution of the phenanthroline **14** (0.24 g, 0.64 mmol) in benzene (50 cm^3) was added silver carbonate (0.088 g, 0.32 mmol), followed by 1,5-diiodopentane (0.42 g, 1.28 mmol). The reaction mixture was protected from light and heated at reflux under N_2 for 36 h after which it was cooled to room temperature and filtered. The filtrate was evaporated to dryness and purification of the residue by column chromatography through silica gel (benzene) gave the *title ether* **15** as a light brown, amorphous powder (92 mg, 28%); ν_{max}/cm^{-1} 1720 (C=O), 1280 (C–O) and 1025 (O–CH_3); δ_H 1.91 (6 H, m), 2.06 (H_2O), 3.26 (2 H, t, *J* 6.8), 4.08 (3 H, s, MeO), 4.11 (3 H, s, MeO), 4.35 (2 H, m), 7.78 (1 H, s), 7.92 (1 H, d, *J* 9.0), 8.35 (1 H, d, *J* 9.0) and 8.36 (2 H, s); *m/z* 380 (M^+ − I, 3.9%), 322 (60.5), 263 (8) and 44 (100).

5-[5-(2′,9′-Bismethoxycarbonyl-1′,10′-phenanthrolin-4′-yloxy)-pentyl]phenanthridinium iodide 16

Phenanthridine (0.18 g, 0.98 mmol) was heated to 115 °C in a pear shaped flask under N_2 and the iodide **15** (50 mg) was added to the melt over 5 min. The mixture immediately turned dark brown and became very viscous. The mixture was heated for 1 h and then allowed to cool to room temperature after which it was triturated with benzene (10 cm^3) to give a light brown solid. The solid was filtered off and re-triturated with further batches of benzene. Finally the product was purified by dissolution in chloroform and then precipitation with a minimum volume of diethyl ether, to afford the quaternary salt **16** (12 mg, 15%), mp 203 °C (decomp.); δ_H 1.96 (6 H, m), 2.14 (H_2O), 4.08 (6 H, s, MeO), 4.34 (2 H, m), 5.23 (2 H, m, CH_2N^+), 7.3–9.12 (13 H, m) and 11.61 (1 H, s, 2-H, phenanthridinium) (Found: M^+, 560.2180. $C_{34}H_{30}N_3O_5$ requires *M*, 560.2185).

5-Nitro-2,9-bis(trichloromethyl)-1,10-phenanthroline 20

5-Nitro-2,9-dimethyl-1,10-phenanthroline[15] **18** was prepared by the standard method from neocuproine **17**. Considerable amounts of the dione **19** accompanied the nitro product. The nitro compound **18** (2.0 g, 7.9 mmol) was dissolved in carbon tetrachloride (150 cm^3) and dry chloroform (20 cm^3) after which *N*-chlorosuccinimide (6.7 g, 0.05 mol) and a portion of benzoic acid were added (10 mg). The mixture was heated to reflux for 24 h, cooled to room temperature and then filtered. The filtrate was washed with several portions of 10% (w/v) aq. Na_2CO_3, dried, filtered and evaporated. The crude product was chromatographed through silica gel ($CHCl_3$) to give the *title compound* **20** as a yellow, crystalline solid (2.9 g, 81%), mp 228–230 °C; ν_{max}/cm^{-1} 1510, 1330 and 820–740; δ_H 8.39 (1 H, d, *J* 8.4), 8.44 (1 H, d, *J* 9.1), 8.58 (1 H, d, *J* 8.4), 8.78 (1 H, s) and 9.19 (1 H, d, *J* 9.1) (Found: C, 36.4; H, 0.9; N, 8.9. $C_{14}H_5Cl_6N_3$ requires C, 36.6; H, 1.1; N, 9.1%).

5-Nitro-1,10-phenanthroline-2,9-dicarboxylic acid 21

The hexachloride **20** (2.5 g, 5.4 mmol) was added portionwise to 98% sulfuric acid (6 cm^3) at room temp. and the mixture was heated at 80–90 °C for 6 h under N_2. The dark-brown solution was cooled and the viscous product poured over crushed ice, to afford a yellow precipitate. The solid was filtered off, washed with cold water and recrystallised from hot aq. THF to give the *title diacid* **21** as a pale yellow solid (0.59 g, 85%), mp 218–

220 °C; ν_{max}/cm^{-1} 3500–2900 (CO_2H), 1735 (C=O), 1540, 1340 and 1150; δ_H[$(CD_3)_2SO$] 2.65 (H_2O), 8.44 (1 H, d, *J* 8.2), 8.48 (1 H, d, *J* 8.8), 8.90 (1 H, d, *J* 8.2), 9.03 (1 H, d, *J* 8.8) and 9.13 (1 H, s) (Found: C, 50.8; H, 2.7; N, 12.4. $C_{14}H_7N_3O_6 \cdot H_2O$ requires C, 50.8; H, 2.7; N, 12.7%).

Dimethyl 5-nitro-1,10-phenanthroline-2,9-dicarboxylate 22

The hexachloride **20** (2.5 g, 5.4 mmol) was added portionwise to 98% sulfuric acid (8 cm^3) and the mixture heated at 80–90 °C for 6 h. The mixture was cooled in ice before the *careful and slow* addition of methanol (15 cm^3). The mixture was heated at reflux for 2 h, cooled and then concentrated by the evaporation of the excess of methanol under reduced pressure. The residue was neutralised with aq. Na_2CO_3 to give a precipitate, which was collected, washed with water, dried and recrystallised from methanol–chloroform to give the *title ester* **22** as an off-white solid (1.59 g, 86%), mp 258–260 °C; ν_{max}/cm^{-1} 3500–3200 (OH), 1730 (C=O), 1540, 1340 and 1210; δ_H 3.12 (H_2O), 4.13 (6 H, s, MeO), 8.57 (2 H, s), 8.59 (1 H, d, *J* 8.8), 8.77 (1 H, s) and 9.15 (1 H, d, *J* 8.8) (Found: C, 55.4; H, 3.2; N, 11.9. $C_{16}H_{11}N_3O_6 \cdot 0.25H_2O$ requires C, 55.6; H, 3.2; N, 12.15%).

Dimethyl 5-amino-1,10-phenanthroline-2,9-dicarboxylate 23

The nitro ester **22** (0.5 g, 1.5 mmol) in methanol (50 cm^3) was heated to reflux to dissolve all the ester, after which the solution was cooled and cyclohexene (0.72 g, 8.8 mmol) and 10% Pd/C (0.1 g) were added. The mixture was reheated to reflux under N_2 for a further 2 h after which it was filtered hot through Celite and the filter cake was washed well with hot methanol. The combined filtrates were evaporated under reduced pressure to afford the *title amine* **23** as a yellow crystalline product (0.38 g, 83%). Purification by flash chromatography through silica gel (1:9 methanol–chloroform) gave a sample, mp 239–240 °C; ν_{max}/cm^{-1} 3320–3200 (NH_2), 1730 (C=O), 1510, 1300 and 1150; δ_H[$(CD_3)_2SO$] 3.91 (3 H, s, MeO), 3.97 (3 H, s, MeO), 4.88 (4 H, br s, exch. D_2O), 7.00 (1 H, s), 8.16 (2 H, s), 8.35 (1 H, d, *J* 8.8) and 8.94 (1 H, d, *J* 8.8) (Found: C, 58.1; H, 4.3; N, 12.6. $C_{16}H_{13}N_3O_4 \cdot H_2O$ requires C, 58.3; H, 4.5; N, 12.8%).

Dimethyl 5-(6-bromohexanoylamino)-1,10-phenanthroline-2,9-dicarboxylate 24

To a solution of the amine **23** (0.1 g, 0.32 mmol) and diisopropylethylamine (50 mg, 0.39 mmol) in acetonitrile (10 cm^3) was added, at room temperature, 6-bromohexanoyl chloride (60 mg, 0.32 mmol). The reaction mixture was stirred at room temperature under N_2 for 2 h after which it was quenched with water (10 cm^3) and extracted with chloroform (2 × 25 cm^3). The organic extracts were washed with saturated aq. $NaHCO_3$ (2 × 20 cm^3) and brine (2 × 20 cm^3), dried, filtered and evaporated under reduced pressure to afford the crude product. Chromatography, through silica gel (1:19, methanol–chloroform) gave the *title amide* **24** (100 mg, 66%), mp 129–131 °C; ν_{max}/cm^{-1} 3460, 1750 (C=O), 1560 and 1280; δ_H 1.6–2.0 (6 H, m), 2.66 (2 H, t, *J* 7.3), 3.13 (2 H, H_2O), 3.43 (2 H, t, *J* 6.7), 4.02 (3 H, s, MeO), 4.08 (3 H, s, MeO), 7.75 (1 H, d, *J* 8.5), 7.95 (1 H, s), 8.10 (1 H, d, *J* 8.5), 8.30 (1 H, d, *J* 8.5), 8.34 (1 H, d, *J* 8.5) and 9.49 (1 H, br s, exch. D_2O, NH) (Found: C, 51.9; H, 4.6; N, 8.2. $C_{22}H_{22}BrN_3O_5 \cdot H_2O$ requires C, 52.2; H, 4.8; N, 8.3%).

5-[6-(2′,9′-Bismethoxycarbonyl-1′,10′-phenanthrolin-5′-yl-amino)-6-oxohexyl]phenanthridinium bromide 25

The bromide **24** (0.24 g, 0.49 mmol) was added in portions over 10 min to a melt of phenanthridine (0.26 g, 1.48 mmol) in a pear shaped flask heated to 115 °C and under an atmosphere of N_2. The mixture was kept at this temperature for a further 2 h after which it was cooled and dissolved in chloroform (5 cm^3). The brown solution was added dropwise to ether (15 cm^3) to precipitate the product as a pale yellow solid. The solid was filtered off, redissolved in chloroform and reprecipitated as described above to give the *title product* **25** (0.25 g, 73%). A sample was recrystallised from boiling water, although recovery was poor (*ca.* 10%), mp 158 °C (decomp.); ν_{max}/cm^{-1} 3500 (OH, NH), 1740 (C=O) and 1630 (amide); δ_H(all peaks showed broadening) 1.63–2.55 (6 H, m), 2.90 (2 H, m), 3.31 (2 H, H_2O), 4.01 (6 H, s, MeO), 5.20 (2 H, m), 7.4–9.35 (13 H, m), 10.4 (1 H, br s, NH) and 11.42 (1 H, s, 2 phenanthridine-H); *m/z* (FAB) 587 (Found: C, 61.45; H, 4.7; N, 8.2. $C_{34}H_{31}BrN_4O_5 \cdot H_2O$ requires C, 61.3; H, 4.7; N, 8.2%).

5-[6-(2′,9′-Dicarboxy-1′,10′-phenanthrolin-5′-ylamino)-6-oxohexyl]phenanthridinium bromide 1

The quaternised ester **25** (100 mg, 0.15 mmol) was added to distilled water (10 cm^3) adjusted to pH 4 with hydrobromic acid. The mixture was heated to gentle reflux whereupon the solid gradually dissolved to form a bright yellow solution. After 24 h the solution was decanted from the trace of an oily residue and freeze-dried to yield the desired probe **1** as a bright orange solid. HPLC chromatography (ODS, reverse phase, acetonitrile–water) showed this was one major product (*ca.* 50% purity by peak height analysis), with a little of the mono-ester, starting diester and phenanthridine as contaminants. Variable purity material was obtained by repeating the hydrolysis (40–80% pure) and the purer samples were used in the DNA assay work.[1] The probe **1** could be purified by use of chromatography through an XAD-2 column using water–methanol mixtures as solvent[16] [Found: M^+ (FAB), 559.1973. $C_{33}H_{27}N_4O_5$ requires *M*, 559.1981]; δ_H[$(CD_3)_2SO$] 1.62–2.18 (6 H, m), 2.24 (2 H, m), 4.01 (2 H, m), 5.18 (2 H, m), 8.03–9.19 (13 H, m), 10.22 (1 H, br s, NH) and 10.45 (1 H, s).

Preparation of 1-[4-(3-chloropropyl)phenyl]prop-1-enyl ketone 28

To a cooled slurry of anhydrous aluminium chloride (8.0 g, 60 mmol) in chloroform (25 cm^3), *trans*-but-2-enoyl chloride (6.27 g, 60 mmol) was added slowly. When the solids had dissolved, (3-chloropropyl)benzene **26** (8.5 g, 55 mmol) was added dropwise over 10 min after which the mixture was warmed to 45 °C for 30 min, during which gas was evolved. The mixture was then cooled and poured over crushed ice. The organic layer was separated, washed with saturated aq. $NaHCO_3$, dried, filtered and evaporated. The gummy solid obtained was chromatographed through silica gel (2:3, dichloromethane–light petroleum) to give the *title ketone* **28** (6.5 g, 53%) as a pale yellow oil that solidified to a waxy solid with time; ν_{max}/cm^{-1} 1670 (C=O) and 1620 (C=C); δ_H 2.0 (3 H, dd, *J* 1.5 and 6.3), 2.03–2.13 (2 H, m), 2.85 (2 H, t, *J* 7.3), 3.53 (2 H, t, *J* 6.5), 6.90 (1 H, dq, *J* 1.5 and 15.1), 7.08 (1 H, dq, *J* 6.3 and 15.1), 7.32–7.27 (2 H, m) and 7.91–7.84 (2 H, m); *m/z* 224, 222 (M^+, 28 and 83%), 183 (49) and 181 (100) (Found: C, 69.9; H, 6.7. $C_{13}H_{15}ClO$ requires C, 70.1; H, 6.8%).

4-[4-(3-Chloropropyl)phenyl]-2,9-dimethyl-1,10-phenanthroline 29

8-Amino-2-methylquinoline **6** (0.40 g, 2.5 mmol), the ketone **28** (1.11 g, 5.0 mmol) and KH_2AsO_4 (0.50 g, 2.8 mmol) were dissolved in conc. HCl (10 cm^3) and acetic acid (10 cm^3) and the mixture was heated at 90 °C for 30 h under N_2. It was then cooled, basified with NaOH (10 mol dm^{-3}) and extracted with chloroform (3 × 30 cm^3). The organic extracts were washed with water, dried and evaporated to dryness to give a residue,

which was chromatographed through silica gel (dichloromethane then 2% v/v methanol) recrystallised from chloroform–ether to give the *title compound* **29** (0.52 g, 57%), mp 191 °C; ν_{max}/cm^{-1} 1620, 1590, 1190 and 880–850; δ_H 2.27 (2 H, m), 2.91 (2 H, t, *J* 7), 2.97 (6 H, s), 3.61 (2 H, t, *J* 6.3), 7.35–7.5 (4 H, m), 7.43 (1 H, s), 7.50 (1 H, d, *J* 7.8), 7.63 (1 H, d, *J* 9.3), 7.83 (1 H, d, *J* 9.3) and 8.11 (1 H, d, *J* 7.8) (Found: C, 76.4; H, 5.8; N, 7.8. $C_{23}H_{21}ClN_2$ requires C, 76.6; H, 5.9; N, 7.8%).

4-[4-(3-Chloropropyl)phenyl]-2,9-bis(trichloromethyl)-1,10-phenanthroline 30

The preceding compound was chlorinated with *N*-chlorosuccinimide in the manner described for **10**, to yield the *heptachloride* **30** (71%), mp 150–151 °C; ν_{max}/cm^{-1} 1620, 1590, 1550, 900 and 850–700; δ_H 2.27–2.13 (2 H, m), 2.94 (2 H, t, *J* 7), 3.63 (2 H, t, *J* 6.35), 7.42–7.54 (4 H, m), 7.89 (1 H, d, *J* 9.3), 8.08 (1 H, d, *J* 9.3), 8.24 (1 H, s), 8.33 (1 H, d, *J* 8.8) and 8.44 (1 H, d, *J* 8.8) (Found: C, 48.6; H, 2.6; N, 4.9. $C_{23}H_{15}Cl_7N_2$ requires C, 48.7; H, 2.7; N, 4.9%).

4-[4-(3-Chloropropyl)phenyl]-1,10-phenanthroline-2,9-dicarboxylic acid 31

The chloride **30** (1.13 g, 2 mmol) was dissolved in formic acid (7 cm^3) containing a few drops of water to give a yellow solution, which was heated at reflux for 24 h. The solution was cooled and poured into ice–water to produce an off-white precipitate, which was filtered off, washed with water and dried (0.88 g, 100%). A sample was recrystallised from aq. formic acid to give the *title diacid*, **31**, mp 190 °C (decomp.); ν_{max}/cm^{-1} 3400, 2600, 1730 (C=O), 1620, 1300 and 1160; δ_H 2.12–2.23 (2 H, m), 2.88 (2 H, t, *J* 7), 3.71 (2 H, t, *J* 6.5), 7.4–7.6 (4 H, m), 8.02 (1 H, d, *J* 9), 8.19 (1 H, d, *J* 9), 8.25 (1 H, s), 8.41 (1 H, d, *J* 8) and 8.74 (1 H, d, *J* 8) (Found: C, 62.7; H, 4.4; N, 6.3. $C_{23}H_{17}ClN_2O_4 \cdot H_2O$ requires C, 63.0; H, 4.4; N, 6.4%).

Formation of the esters 32–34

The acid **31** (0.31 g, 0.7 mmol) in methanol (50 cm^3) containing 2 drops of conc. H_2SO_4 was heated at reflux for 16 h. The solution was cooled, aq. $NaHCO_3$ added and the mixture partitioned between chloroform and water. The organic layer was separated, dried, filtered and evaporated to dryness to yield, after recrystallisation from methanol–chloroform, *dimethyl* 4-[4-(3-*chloropropyl*)*phenyl*]-1,10-*phenanthroline*-2,9-*dicarboxylate* **32** (250 mg, 76%), mp 191–193 °C; ν_{max}/cm^{-1} 3400, 1730, 1620, 1340, 1270 and 1150; δ_H 2.17 (2 H, m), 2.94 (2 H, t, *J* 7), 3.63 (2 H, t, *J* 6.4), 4.15 (6 H, s), 7.4–7.55 (4 H, m), 7.91 (1 H, d, *J* 9.3), 8.12 (1 H, d, *J* 9.3) and 8.4–8.55 (3 H, m) (Found: C, 66.9; H, 4.7; N, 6.2. $C_{25}H_{21}N_2O_4Cl$ requires C, 66.9; H, 4.7; N, 6.2%).

Exchange of the halogen was accomplished by heating the chloride **32** (0.5 mmol) in methanol (15 cm^3) at reflux with either sodium iodide (2.5 g) or sodium bromide (2.0 g). After 48 h substitution was complete for the iodide, whereas the treatment had to be repeated twice to ensure almost complete formation of the bromide (> 90%).

The iodide **34** showed a similar ^1H NMR spectrum to the chloride except that the triplet at 3.63 ppm was replaced by a triplet at δ_H 3.26 (2 H, *J* 7).

The bromide **33** showed a similar ^1H NMR spectrum to the chloride except for the replacement of the signal at 3.63 ppm by a triplet at δ_H 3.48 (2 H, *J* 7). Compounds **32**, **33** and **34** showed very similar IR spectra.

5-{3-[4-(2′,9′-Bismethoxycarbonyl-1′,10′-phenanthrolin-4′-yl)-phenyl]propyl}phenanthridinium bromide 36

The bromide **33** (100 mg, 0.2 mmol) and phenanthridine (110 mg, 0.6 mmol) were intimately mixed and then heated to 115–120 °C under N_2 for 3 h. The homogeneous melt thus formed was dissolved in chloroform (2 cm^3) and added dropwise to ether (25 cm^3) to give an off-white precipitate. The dissolution–precipitation step was repeated to give the *title salt* **36** (110 mg, 73%). HPLC analysis (ODS, reverse phase, acetonitrile–water) showed this was essentially a single compound (> 95% pure by peak heights).

5-{3-[4-(2′,9′-Dicarboxy-1′,10′-phenanthrolin-4′-yl)phenyl]-propyl}phenanthridinium bromide 2

The salt **36** (340 mg, 0.5 mmol) in water (7 cm^3) and formic acid (5 cm^3) containing 48% HBr (1 drop) was heated at reflux for 40 h after which the solution was evaporated until a crystalline solid formed. This was collected and dried to yield the required *probe* **2** (220 mg, 66%), mp 213 °C (decomp.); ν_{max}/cm^{-1} 3400, 3100–3000, 2340br, 1720, 1710, 1630, 1610, 1370 and 1160; *m/z* (FAB) 564 (100%, M^+), 520 (70), 476 (25) (Found: M^+, 564.1945. $C_{36}H_{26}N_3O_4$ requires *M*, 564.1945).

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