Characterization of lanthanide-mediated DNA cleavage by intercalator-linked hydroxamic acids: comparison with transition systems

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Phenanthridine-linked hydroxamic acids with alkyl chain of variable length (n = 3, 4, 5, 6 and 7) have been newly synthesized. Their DNA cleavage activities have been investigated by Col E1 plasmid relaxation assay in the presence of transition(II) (ferrous and vanadyl) or lanthanide(III) (lutetium) metal ions. Linker length is extremely determinant for lutetium-mediated DNA cleavage. The increase in cleavage activity of the lutetium system with increasing pH suggests the involvement of metal-bound hydroxide as catalytically active species. Middle (samarium and europium) and late (thulium, ytterbium and lutetium) trivalent lanthanide systems are effective for DNA cleavage. For maximal DNA cleavage activity, the lutetium/hydroxamic acid ratio has been found to be 2 whereas that of the transition system has been found to be 1. We propose a hydrolytic mechanism in which two lanthanide ions are acting in concert for effective DNA cleavage.

Development of artificial metallonucleases that can bind DNA and induce cleavage is of great concern. To date, transition metal complexes capable of cleaving DNA have been extensively studied[1] and conjugation of them with small molecular DNA binding agents such as intercalator[2] and groove binder[3] have been successfully achieved. These artificial metallonucleases usually employ oxidative chemistry to decompose the deoxyribose moiety.

Hydroxamic acid is a bidentate oxygen ligand possessing affinity for a variety of metal ions.[4] Desferrioxamine, a well-known siderophore comprising trihydroxamate ligands, forms a stable octahedral coordination complex with transition metal ions.[5] Joshi and Ganesh have demonstrated that the copper(II), cobalt(III) and nickel(II) complexes of desferrioxamine can induce oxidative DNA cleavage.[6] Our attention was focused on the monohydroxamic acid–lanthanide complex because certain lanthanide complexes which have overall positive charge catalyse the hydrolysis of phosphate ester[7] and RNA[8] under mild conditions. In addition, Komiyama *et al.* have demonstrated that lanthanide complexes are also active for the DNA hydrolysis.[9] These findings prompted us to evaluate the DNA-cleaving properties of monohydroxamic acid–metal complexes.

Our preliminary study has demonstrated that intercalator-linked monohydroxamic acid can induce effective plasmid DNA cleavage at relatively low concentrations in the presence of transition (ferrous, ferric and vanadyl) or lanthanide(III) (lutetium, thulium and europium) metal ions.†[10] In an inhibition study, the lanthanide and transition systems were presumed to proceed by a hydrolytic and oxidative mechanism, respectively. In order to aid an understanding of the hydrolytic chemistry it was thought useful to compare the characteristic features of DNA cleavage for the lanthanide and transition systems. In the present study, we have prepared hydroxamic acid homologues having alkyl chains of variable length for which the cleavage activities have been investigated in both the transition and lanthanide systems. We describe the effect of linker length, pH of the reaction, various lanthanide ions and the stoichiometry of hydroxamic acid and metal ion on metal-mediated DNA cleavage by phenanthridine-linked hydroxamic acid in order to compare the lanthanide system with the transition system.

† Quite recently, the hydrolytic cleavage of plasmid DNA by lanthanide cations in the presence of polyamines or a cryptand has also been reported: J. Rammo, R. Hettich, A. Roigk and H. J. Schneider, *Chem. Commun.*, 1996, 105.

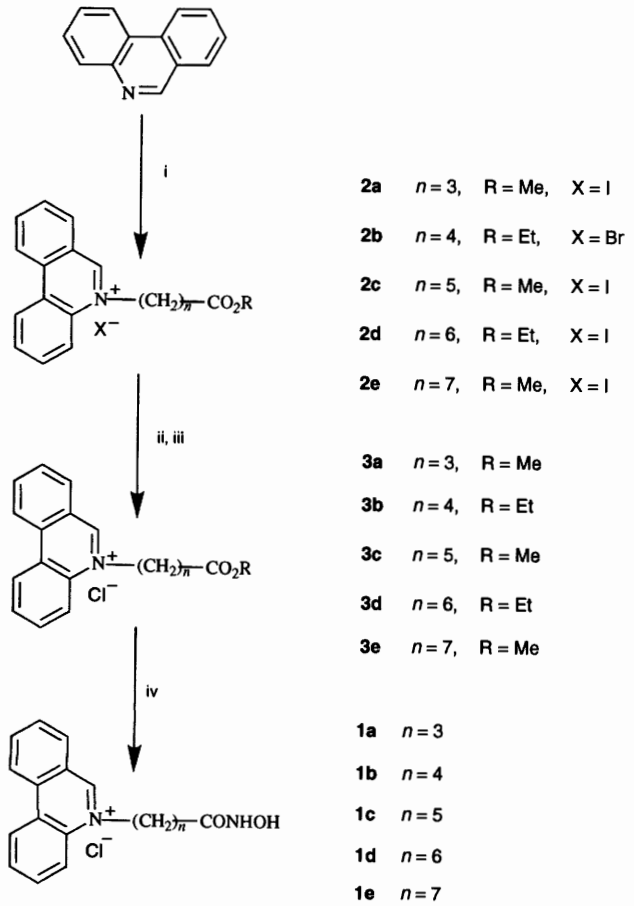
Results

Synthesis of hydroxamic acids

In the molecular design and construction of an intercalator-linked hydroxamic acid, we chose the phenanthridine ring as an intercalation moiety, the intercalation properties of such compounds having been well established[11] and the intact ring system being easily functionalized with side chains at the N-10 position.[12] The syntheses of phenanthridine-linked hydroxamic acids (**1a–e**) are outlined in Scheme 1. Initially the nitrogen of phenanthridine was alkylated by the corresponding iodo- or bromo-substituted aliphatic esters to give the expected quaternized products (**2a–e**) after recrystallization. Metathesis of the iodide or bromide and subsequent generation of the chloro compounds (**3a–e**) was accomplished by a modification of Lippard's method.[13] The silver halides were quantitatively recovered in the reactions and the products were purified by recrystallization. The hydroxamic acids (**1a–e**) were successfully obtained by a modification of Hauser and Renfrow's method.[14] Exposure to atmospheric oxygen was minimized throughout the reaction to avoid oxidation of free hydroxylamine and the concurrent formation of undesirable product. Final purification of hydroxamic acids was performed by a short-column of XAD-2 followed by recrystallization from MeOH–EtOH. All hydroxamic acids obtained showed a positive Fe^{III} reaction which is characteristic of hydroxamic acid and gave satisfactory spectroscopic data.

Effect of linker length on DNA cleavage activity by hydroxamic acid homologues

It has been shown previously that lanthanide-mediated DNA cleavage by **1c** might involve metal-ligated hydroxide, currently believed to be the active species in hydrolytic chemistry,[9] whereas the transition mechanism would take place with a reactive oxygen species such as a hydroxyl radical. For the cleavage to occur, a reactive oxygen species, because of its



Scheme 1 *Reagents:* i, $X(CH_2)_nCO_2R$; ii, $AgNO_3$–DMF; iii, aqueous NaCl; iv, $HONH_2$·HCl–KOH–MeOH

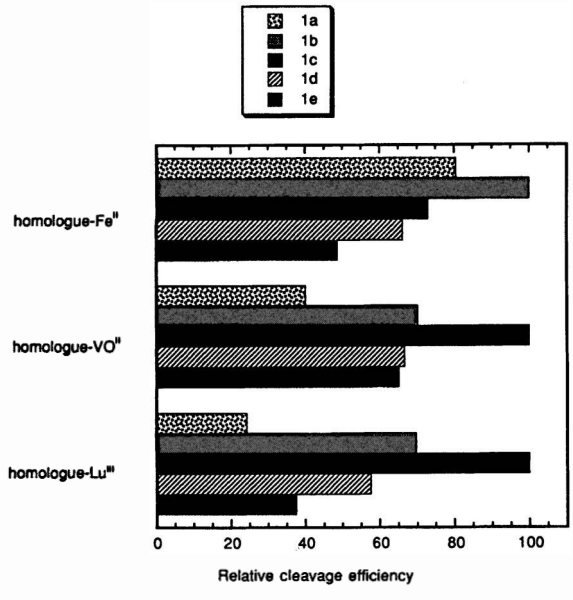


Fig. 1 Effect of linker length on metal-mediated DNA cleavage by hydroxamic acid homologues. Col E1 supercoiled DNA was incubated with homologues in the presence of metal ions in 40 mmol dm^{-3} TRIS·HCl buffer (pH 8.0) at 37 °C for 1.0 h (ferrous and vanadyl) or 2.0 h (lutetium). Concentrations of each homologue and metal ions were as follows: 5 μmol dm^{-3} $FeSO_4$ + 5 μmol dm^{-3} homologue; 10 μmol dm^{-3} $VOSO_4$ + 10 μmol dm^{-3} homologue and 30 μmol dm^{-3} $LuCl_3$ + 30 μmol dm^{-3} homologue.

diffusible nature,[15] need not be produced at the deoxyribose moiety; in contrast, a metal-ligated hydroxide needs to be produced in the immediate vicinity of the phosphorus atom of DNA. A correlation of linker length with DNA cleavage activity may reflect the positional requirements of the DNA-cleaving species. Fig. 1 shows the relative cleavage activity of metal-mediated DNA cleavage by hydroxamic acid homologues with alkyl chains of various lengths. The activity was examined by following the conversion of supercoiled Col E1 DNA (Form i) to open circular (Form ii), the relative value being determined as a ratio of Form ii DNA production (%) by each homologue to that by **1b** (ferrous system) and **1c** (vanadyl and lutetium systems). In the ferrous system, the activity gradually decreased with increasing linker length except for **1b**. The vanadyl system showed that **1c** was the most active, although the activity was scarcely influenced by the linker length for other homologues except for **1a**. The lutetium system, on the other hand, showed a quite remarkable length dependence with maximum activity shown by **1c**. Assuming that the binding affinity for DNA of each compound is almost the same, this result indicates that the linker length is an important factor which severely controls the activity of the lanthanide system. We chose to use **1c** for comparison of the lanthanide and transition systems in the following experiment because of its high activity in both systems.

Effect of pH on DNA cleavage activity by 1c

The pH dependence of the metal-mediated DNA cleavage by **1c** was studied over the pH range 7.0–9.0 in 40 mmol dm^{-3} TRIS–HCl buffer. The pH profile of each cleavage system is shown in Fig. 2. In the ferrous system, a decrease of cleavage activity was observed above pH 8.0. The pH profile of the vanadyl system showed a bell-shaped curve, in which the activity was almost independent of pH between pH 7.5 and 8.5. In contrast to the transition system, the activity of the lutetium system increased with increasing pH and levelled off at pH 8.5. A similar trend was observed for cerium and thulium systems. As predicted from the pK_a value for deprotonation of butyrohydroxamic acid (pK_a = 7.48),[16] hydroxamic acid interacts strongly with metal ions at alkaline pH. Thus, the activities of both the transition and lanthanide systems seemed to increase in the present pH region. However, only the lanthanide system displayed a marked increase of activity. Taking into account that lanthanide ions tend to precipitate from solution at alkaline pH, hydroxamic acid may prevent such precipitation and thus retain the activity of the species to provide lanthanide-bound hydroxide as the catalytically active species.

Effect of various lanthanide ions on DNA cleavage activity by 1c

Along the series of trivalent lanthanide members from lanthanum to lutetium, a smooth decrease in the pK_a of lanthanide-bound hydroxide is generally observed,[17] so that the relative activity of each lanthanide system is expected to increase systematically with an increase in atomic number. Our previous study has indicated that europium, thulium and lutetium systems are effective for DNA cleavage and their cleavage activities are in the following order: Eu^{III}·**1c** > Tm^{III}·**1c** > Lu^{III}·**1c**. We also investigated the activities of other members of the lanthanide series and the results are shown in Fig. 3. The middle and late lanthanide systems exhibited high activities compared to those of early members. This tendency is roughly consistent with above mentioned expectation. The europium system showed the highest activity of the eight lanthanide ions studied. The activity of tetravalent cerium, which is said to be by far the most active for the hydrolysis of DNA,[18] was found to be the least active in our system (data not shown). Hydroxamic acid is known to be oxidized by aqueous ceric ammonium nitrate[19] and decomposition of the Ce^{IV}·**1c** complex seems to have occurred because of concurrent redox reactions.

Stoichiometry of hydroxamic acid–metal interaction

In order to discover the stoichiometry of the hydroxamic acid–

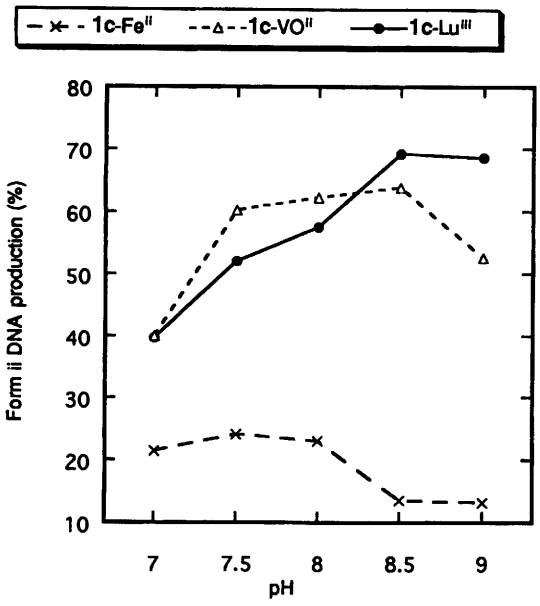


Fig. 2 Effect of pH on metal-mediated DNA cleavage by **1c**. Col E1 supercoiled DNA was incubated with **1c** in the presence of metal ions in 40 mmol dm^{-3} TRIS·HCl buffer at various pH values. Concentrations of **1c** and metal ions were the same as in Fig. 1.

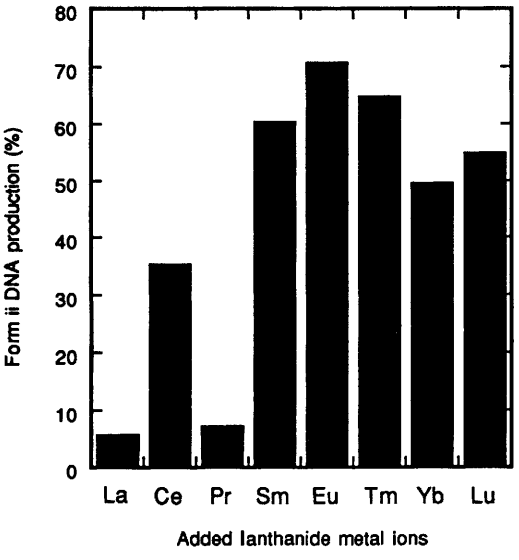


Fig. 3 Trivalent lanthanides-mediated DNA cleavage activity by **1c**. Col E1 supercoiled DNA was incubated with **1c** in the presence of lanthanide ions in 40 mmol dm^{-3} TRIS·HCl buffer (pH 8.0). Concentrations of **1c** and lanthanide ions were 30 μmol dm^{-3} each.

metal interaction responsible for DNA cleavage, the dependence of cleavage activity on increasing concentration of **1c** was studied, the metal concentration being kept constant; the results from these studies are shown in Fig. 4. Although the activity of the vanadyl system continued to increase slightly above a molar ratio of 1.0, the activity of both ferrous and vanadyl systems was almost at a maximum at this ratio. This result suggests that 1:1 complex is the active species in the transition system. Interestingly, the activity of the lutetium system increased sharply at a lower ratio and reached a maximum at a molar ratio of 0.5. Further addition of **1c** resulted in suppression of the cleavage, which implies that a bimetal system is the active species. The activity of this bimetal cleavage system may be significantly modified by the stoichiometry of DNA–hydroxamic acid–lanthanide ternary interaction.

Discussion

The activity of metal-mediated DNA cleavage by phenanthridine-linked hydroxamic acids was significantly influenced by the linker length (Fig. 1). Quite remarkable length dependence was observed for lanthanide-mediated cleavage compared to that of the transition system. Lanthanide cations behave as typical hard acids and interact preferentially with hard bases such as oxygen rather than with softer bases such as nitrogen.[20] Hence, the lanthanide–hydroxamic acid complex should interact preferably with the hard oxygen sites of the phosphate diester backbone of DNA. Fixed placement of the complex close to the phosphorus atom of DNA is necessary because hydrolytic cleavage employs lanthanide-ligated hydroxide whereas oxidative cleavage usually employs diffusible reactive species. Model-building studies indicate that **1a** is not long enough to ensure proximity of the complex to the phosphorus atom of DNA either at the intercalation site or one base pair away, assuming an intercalation geometry with the linker moiety of the compound protruding into the minor groove; **1e**, however, has a high degree of freedom to orientate to the phosphorus atom of DNA. Of the five homologues, **1c** provides the optimum proximity effect for the complex. These considerations are compatible with the observed increase, maximum and decrease in the activity pattern in length dependence of the lutetium system.

On the basis of the results of pH dependence and stoichiometric experiment, we propose one possible mechanism for DNA cleavage by a hydroxamic acid–lanthanide complex (see Fig. 5). The phosphorus oxygen sites of DNA and hydroxamic acid are thought to compete for lanthanide ion binding, although the thermodynamics of lanthanide binding for them has yet to be examined. One lanthanide ion coordinated with hydroxamic acid could act as a general base to provide ligated hydroxide nucleophile, whose local concentration is increased by intercalative binding of the phenanthridine ring. A second lanthanide ion could then act as a Lewis acid by binding to the phosphorus oxygen and thus make the phosphorus atom more susceptible to nucleophilic attack by hydroxide. Such cooperation of two lanthanide ions as a general acid and base catalyst could bring about effective DNA cleavage. Recently, remarkable hydrolytic activity for phosphate esters has been achieved by use of bimetallic cooperation systems, which involve a mechanism where Lewis acid activation and intramolecular nucleophilic attack of hydroxide are acting in concert.[21] These reports also support the concept that DNA cleavage by **1c** proceeds through the cooperation of two lanthanide ions.

Conclusions

The distinguishing features of lanthanide-mediated DNA cleavage by intercalator-linked hydroxamic acids are summarized as follows: (i) hydroxamic acid with five methylene units shows maximal cleavage activity, which dramatically decreases for compounds of shorter and longer linker length; (ii) in TRIS–HCl buffer (pH 7.0–9.0), the activity increases with increasing pH; (iii) the europium system is the most active of eight trivalent lanthanide ions; (iv) two lanthanide ions participate in efficient DNA cleavage. A suitable molecular design for a hydroxamic acid–lanthanide system would aid the development of an artificial hydrolytic metallonuclease which could, ultimately, access the enormous catalytic activity of enzymes.

Experimental

General information

All solvents and reagents were of reagent grade quality and were used without further purification. Polystyrene resin DIAION SP 207 was purchased from Mitsubishi Chemical Co.

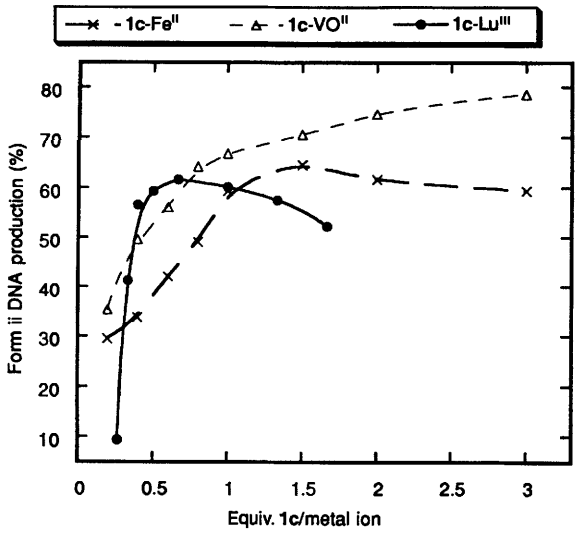


Fig. 4 Stoichiometry of the hydroxamic acid–metal ion interaction. Col E1 supercoiled DNA was incubated with increasing concentration of **1c** in the presence of metal ions in 40 mmol dm^{-3} TRIS·HCl buffer (pH 8.0). The concentration of metal ions were fixed at 10 μmol dm^{-3} (ferrous and vanadyl) or 30 μmol dm^{-3} (lutetium). The form ii DNA production is plotted *vs.* the equivalents of **1c** added per equivalent of metal ions.

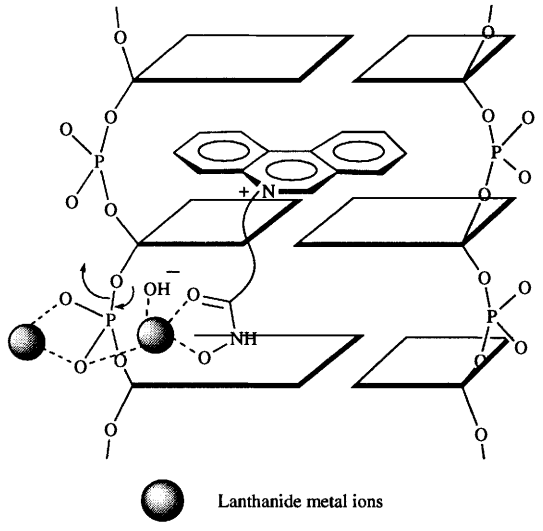


Fig. 5 Proposed mechanism for the hydrolytic DNA cleavage by hydroxamic acid–lanthanide complex

Melting points were determined on a Yanaco micro melting-point apparatus and are uncorrected. TLC was carried out with pre-coated silica gel plates (Merck 60 F_{254}) using butanol–AcOH–H_2O (4:1:2) as eluent, with visualization at 254 nm. IR spectra were obtained as KBr discs on a Shimadzu IR-470 machine and UV spectra were taken on a Shimadzu UV-2100 instrument. The ^{1}H NMR spectra were recorded on a JEOL JNM-EX 400 (400 MHz) spectrometer; *J* values are recorded in Hz. $SiMe_4$ in $CDCl_3$ solution and the solvent peak in CD_3OD solution were used as internal reference for measurement. The low-resolution EI mass spectra were obtained using a Hitachi GC M-80 spectrometer. The low-resolution FAB mass spectra and the accurate mass measurements were provided by a JEOL JMS-SX102A machine. Elemental analyses were carried out at Daiichi Pharmaceutical Co. (Tokyo) using a Perkin-Elmer 2400 II instrument. Evaporation of solvents were performed under reduced pressure by a rotary evaporator.

Reagent grade inorganic salts, namely, $FeSO_4$, $VOSO_4$, $LaCl_3$, $CeCl_3$, $Ce(NH_4)_2(NO_3)_6$, $EuCl_3$ and $YbCl_3$ were obtained from Wako Pure Chemical Co., and $PrCl_3$, $SmCl_3$, $TmCl_3$, $LuCl_3$ were from Aldrich. These metal salts were used without further purification. The Col E1 plasmid DNA was purchased from Nippon Gene Co. and ethanol-precipitated to remove EDTA contained in the solution prior to the reaction.

General procedure for the phenanthridinium bromide or iodide derivatives 2a, 2b, 2c, 2d and 2e

A mixture of phenanthridine and the appropriate N5 quaternizing agents (1.2 mol equiv.) in xylene was stirred at 110 °C overnight. After evaporation of the solvent, the oily residue was recrystallized twice from CH_2Cl_2–AcOEt.

***N*-[3-(Methoxycarbonyl)propyl]phenanthridinium iodide 2a.** Hygroscopic yellow prisms (79.7%), R_F 0.37; mp 153–155 °C (Found: C, 51.33; H, 4.22; N, 2.99. $C_{18}H_{18}NO_2I·0.7H_2O$ requires C, 51.49; H, 4.65; N, 3.33%); $\nu_{max}(CHCl_3)$/cm^{-1} 1720s and 1625m; $\delta(CDCl_3)$ 2.52 (2 H, m), 2.85 (2 H, t, *J* 6.0), 3.73 (3 H, s), 5.53 (2 H, t, *J* 8.2), 8.00 (1 H, dd, *J* 7.4 and 7.4), 8.07 (1 H, dd, *J* 8.4 and 8.4), 8.13 (1 H, dd, *J* 7.2 and 7.2), 8.29 (1 H, dd, *J* 7.6 and 7.6), 8.77 (1 H, d, *J* 8.8), 8.80 (1 H, d, *J* 8.4), 8.90 (2 H, dd, *J* 7.0 and 7.0) and 11.74 (1 H, s); *m/z* 280 (M^+ − I, 14%), 220 (5.4), 194 (33) and 179 (100).

***N*-[4-(Ethoxycarbonyl)butyl]phenanthridinium bromide 2b.** Hygroscopic pale yellow prisms (59.7%), R_F 0.41; mp 104–106.5 °C (Found: C, 56.90; H, 6.28; N, 3.24. $C_{20}H_{22}NO_2$-Br·$2H_2O$ requires C, 56.60; H, 6.13; N, 3.30%); ν_{max}(CH-Cl_3)/cm^{-1} 1720s and 1625m; $\delta(CDCl_3)$ 1.20 (3 H, t, *J* 6.8), 1.95 (2 H, m), 2.26 (2 H, m), 2.45 (2 H, t, *J* 7.0), 4.07 (2 H, q, *J* 6.9), 5.51 (2 H, t, *J* 7.6), 7.99 (1 H, dd, *J* 7.6 and 7.6), 8.04 (1 H, dd, *J* 7.6 and 7.6), 8.10 (1 H, dd, *J* 7.4 and 7.4), 8.28 (1 H, dd, *J* 8.0 and 8.0), 8.39 (1 H, d, *J* 8.8), 8.79 (1 H, d, *J* 8.8), 8.89 (1 H, d, *J* 8.4), 8.96 (1 H, d, *J* 8.0) and 11.83 (1 H, s); *m/z* 308 (M^+ − Br, 0.8%), 279 (1.1), 220 (1.4) and 179 (100).

***N*-[5-(Methoxycarbonyl)pentyl]phenanthridinium iodide 2c.** Yellow prisms (57.7%), R_F 0.38; mp 136–137.5 °C (Found: C, 55.03; H, 5.08; N, 3.14. $C_{20}H_{22}NO_2I$ requires C, 55.17; H, 5.06; N, 3.22%); ν_{max}(KBr)/cm^{-1} 3030vw, 2920vw, 2850vw, 1720s, 1625m and 1530w; $\delta(CDCl_3)$ 1.67 (2 H, m), 1.73 (2 H, m), 2.22 (2 H, m), 2.34 (2 H, t, *J* 7.2), 3.63 (3 H, s), 5.42 (2 H, t, *J* 7.8), 8.00 (1 H, dd, *J* 7.8 and 7.8), 8.05 (1 H, dd, *J* 7.6 and 7.6), 8.09 (1 H, dd, *J* 6.8 and 6.8), 8.29 (1 H, dd, *J* 7.8 and 7.8), 8.37 (1 H, d, *J* 8.4), 8.80 (1 H, d, *J* 8.8), 8.89 (1 H, d, *J* 8.0), 8.96 (1 H, d, *J* 8.0) and 11.60 (1 H, s); *m/z* 308 (M^+ − I, 12%), 220 (7.2), 194 (19) and 179 (100).

***N*-[6-(Ethoxycarbonyl)hexyl]phenanthridinium iodide 2d.** Bright yellow prisms (49.6%), R_F 0.44; mp 129–130 °C (Found: C, 56.96; H, 5.83; N, 2.92. $C_{22}H_{26}NO_2I$ requires C, 57.01; H, 5.61; N, 3.02%); ν_{max}(KBr)/cm^{-1} 3570w, 3450w, 2950w, 1715s, 1620m and 1530w; $\delta(CDCl_3)$ 1.23 (3 H, t, *J* 7.0), 1.44 (2 H, m), 1.59–1.65 (4 H, m), 2.20 (2 H, m), 2.29 (2 H, t, *J* 7.4), 4.09 (2 H, q, *J* 7.2), 5.41 (2 H, t, *J* 7.6), 7.99 (1 H, dd, *J* 7.6 and 7.6), 8.07 (1 H, dd, *J* 8.4 and 8.4), 8.10 (1 H, dd, *J* 6.8 and 6.8), 8.29 (1 H, dd, *J* 7.2 and 7.2), 8.38 (1 H, d, *J* 8.4), 8.80 (1 H, d, *J* 8.8), 8.90 (1 H, d, *J* 7.6), 8.96 (1 H, d, *J* 8.0) and 11.58 (1 H, s); *m/z* 336 (M^+ − I, 1.4%) and 179 (100).

***N*-[7-(Methoxycarbonyl)heptyl]phenanthridinium iodide 2e.** Bright yellow prisms (61.1%), R_F 0.44; mp 176–178 °C (Found: C, 57.05; H, 5.64; N, 2.84. $C_{22}H_{26}NO_2I$ requires C, 57.02; H, 5.62; N, 3.02%); ν_{max}(KBr)/cm^{-1} 2920w, 2850w, 1720s, 1620m and 1530w; $\delta(CDCl_3)$ 1.31 (2 H, m), 1.39 (2 H, m), 1.55–1.60 (4 H, m), 2.18 (2 H, m), 2.27 (2 H, t, *J* 7.4), 3.63 (3 H, s), 5.40 (2 H, t, *J* 7.8), 7.99 (1 H, dd, *J* 7.8 and 7.8), 8.05 (1 H, dd, *J* 8.0 and 8.0), 8.11 (1 H, dd, *J* 7.0 and 7.0), 8.29 (1 H, dd, *J* 7.8 and 7.8), 8.37 (1 H, d, *J* 8.8), 8.81 (1 H, d, *J* 8.0), 8.91 (1 H, d, *J* 8.0), 8.95 (1 H, d, *J* 8.0) and 11.56 (1 H, s); *m/z* 336 (M^+ − I, 22%), 220 (7.7), 194 (40) and 179 (100).

General procedure for the phenanthridinium chloride derivatives 3a, 3b, 3c, 3d and 3e

To a solution of the appropriate phenanthridinium bromide or iodide derivative in DMF was added dropwise a solution of silver nitrate (1.1 mol equiv.) in DMF. The reaction mixture

was stirred at room temperature in the dark overnight. After a heavy precipitate of silver halide had been filtered off, the filtrate was concentrated and the residue was taken up in $CHCl_3$. The organic solution was washed with two portions of brine, dried (Na_2SO_4) and evaporated. The oily residue was recrystallized twice from $CHCl_3$–AcOEt. An analytically pure sample was prepared by supporting the product on a DIAION SP207 column and washing with the 5% aqueous NaCl, deionized water and eluting with 50% aqueous MeOH followed by recrystallization from $CHCl_3$–AcOEt.

***N*-[3-(Methoxycarbonyl)propyl]phenanthridinium chloride 3a.** Pale yellow prisms (17.8%), R_F 0.34; mp 100–110 °C; λ_{max}(TRIS–acetate buffer, pH 8.0)/nm 248 (ε/dm^3 mol^{-1} cm^{-1} 39 200) and 320 (8330); ν_{max}(KBr)/cm^{-1} 3470m, 3350m, 1715s, 1620m and 1530w; δ($CDCl_3$) 2.51 (2 H, m), 2.86 (2 H, t, *J* 6.0), 3.72 (3 H, s), 5.60 (2 H, t, *J* 8.2), 7.99 (1 H, dd, *J* 7.6 and 7.6), 8.05 (1 H, dd, *J* 7.4 and 7.4), 8.13 (1 H, dd, *J* 7.4 and 7.4), 8.29 (1 H, dd, *J* 7.8 and 7.8), 8.77 (2 H, dd, *J* 9.4 and 9.4), 8.88 (1 H, d, *J* 8.0), 8.94 (1 H, d, *J* 8.0) and 12.03 (1 H, s); *m*/*z* 280 (M^+ − Cl, 37%), 266 (14), 194 (100) and 180 (79) (Found: M^+ − Cl, 280.1340. $C_{18}H_{18}NO_2$ requires *M* − Cl, 280.1334).

***N*-[4-(Ethoxycarbonyl)butyl]phenanthridinium chloride 3b.** Pale yellow prisms (17.4%), R_F 0.40; mp 141–144.5 °C; ν_{max}(KBr)/cm^{-1} 3430w, 2980vw, 1720s, 1620m and 1530w; δ($CDCl_3$) 1.20 (3 H, t, *J* 7.2), 1.94 (2 H, m), 2.26 (2 H, m), 2.45 (2 H, t, *J* 7.0), 4.06 (2 H, q, *J* 7.2), 5.52 (2 H, t, *J* 7.6), 7.98 (1 H, dd, *J* 7.6 and 7.6), 8.04 (1 H, dd, *J* 7.6 and 7.6), 8.10 (1 H, dd, *J* 7.6 and 7.6), 8.29 (1 H, dd, *J* 7.6 and 7.6), 8.38 (1 H, d, *J* 8.4), 8.79 (1 H, d, *J* 8.8), 8.90 (1 H, d, *J* 8.0), 8.96 (1 H, d, *J* 8.4) and 11.88 (1 H, s); *m*/*z* 308 (M^+ − Cl, 6.3%), 278 (1.8), 220 (7.2) and 179 (100) (Found: M^+ − Cl, 308.1654. $C_{20}H_{22}NO_2$ requires *M* − Cl, 308.1646).

***N*-[5-(Methoxycarbonyl)pentyl]phenanthridinium chloride 3c.** Pale yellow prisms (23.7%), R_F 0.40; mp 101.5–104 °C; λ_{max}(TRIS–acetate buffer, pH 8.0)/nm 248 (ε/dm^3 mol^{-1} cm^{-1} 38 300) and 319 (6670); ν_{max}(KBr)/cm^{-1} 3400w, 2950vw, 1715s, 1625m and 1530w; δ($CDCl_3$) 1.64 (2 H, m), 1.71 (2 H, m), 2.21 (2 H, m), 2.33 (2 H, t, *J* 7.0), 3.60 (3 H, s), 5.49 (2 H, t, *J* 7.4), 7.97 (1 H, dd, *J* 7.2 and 7.2), 8.06 (1 H, dd, *J* 7.6 and 7.6), 8.09 (1 H, dd, *J* 8.4 and 8.4), 8.30 (1 H, dd, *J* 7.8 and 7.8), 8.38 (1 H, d, *J* 8.8), 8.82 (1 H, d, *J* 8.8), 8.94 (2 H, dd, *J* 8.0 and 8.0) and 11.80 (1 H, s); *m*/*z* 308 (M^+ − Cl, 60%), 278 (9.6), 220 (53), 194 (100) and 179 (55) (Found: M^+ − Cl, 308.1657. $C_{20}H_{22}NO_2$ requires *M* − Cl, 308.1646).

***N*-[6-(Ethoxycarbonyl)hexyl]phenanthridinium chloride 3d.** Colourless prisms (22.9%), R_F 0.43; mp 110.5–113 °C (Found: C, 66.94; H, 7.04; N, 3.49. $C_{22}H_{26}NO_2Cl{\cdot}1.3H_2O$ requires C, 66.84; H, 7.29; N, 3.54%); ν_{max}(KBr)/cm^{-1} 3420m, 3380m, 2930w, 1715s, 1625s and 1530w; δ($CDCl_3$) 1.23 (3 H, t, *J* 7.2), 1.43 (2 H, m), 1.57–1.65 (4 H, m), 2.17 (2 H, m), 2.28 (2 H, t, *J* 7.2), 4.09 (2 H, q, *J* 7.2), 5.47 (2 H, t, *J* 7.6), 7.98 (1 H, dd, *J* 7.6 and 7.6), 8.04 (1 H, dd, *J* 7.2 and 7.2), 8.08 (1 H, dd, *J* 8.0 and 8.0), 8.28 (1 H, dd, *J* 7.8 and 7.8), 8.32 (1 H, d, *J* 8.4), 8.78 (1 H, d, *J* 8.8), 8.90 (1 H, d, *J* 8.0), 9.00 (1 H, d, *J* 7.6) and 11.94 (1 H, s); *m*/*z* 336 (M^+ − Cl, 5.2%), 292 (1.3), 220 (6.7) and 179 (100) (Found: M^+ − Cl, 336.1980. $C_{22}H_{26}NO_2$ requires *M* − Cl, 336.1958).

***N*-[7-(Methoxycarbonyl)heptyl]phenanthridinium chloride 3e.** Colourless prisms (21.4%), R_F 0.45; mp 136–139 °C (Found: C, 67.66; H, 7.14; N, 3.51. $C_{22}H_{26}NO_2Cl{\cdot}H_2O$ requires C, 67.77; H, 7.18; N, 3.59%); ν_{max}KBr/cm^{-1} 3400br, 2930w, 1730s, 1620m and 1530w; δ($CDCl_3$) 1.32 (2 H, m), 1.38 (2 H, m), 1.55–1.60 (4 H, m), 2.18 (2 H, m), 2.27 (2 H, t, *J* 7.4), 3.64 (3 H, s), 5.46 (2 H, t, *J* 7.4), 7.99 (1 H, dd, *J* 7.6 and 7.6), 8.04 (1 H, dd, *J* 8.8 and 8.8), 8.09 (1 H, dd, *J* 8.4 and 8.4), 8.29 (2 H, dd, *J* 9.4 and 9.4), 8.78 (1 H, d, *J* 8.4), 8.89 (1 H, d, *J* 8.4), 9.02 (1 H, d, *J* 8.0) and 11.97 (1 H, s); *m*/*z* 336 (M^+ − Cl, 64%), 306 (8.6), 220 (33), 194 (100) and 180 (44) (Found: M^+ − Cl, 336.1961. $C_{22}H_{26}NO_2$ requires *M* − Cl, 336.1958).

***N*-[3-(Hydroxylaminocarbonyl)propyl]phenanthridinium chloride 1a.** MeOH as the solvent was degassed with nitrogen gas to remove atmospheric oxygen prior to the reaction. Separate solutions of hydroxylamine hydrochloride (3.96 g, 57.0 mmol) in MeOH (27 ml), KOH (7.0 g, 125 mmol) in MeOH (40 ml) and **3a** (1.53 g, 4.84 mmol) in MeOH (10 ml) were prepared. The solution containing alkali was added *via* a syringe to the stirred hydroxylamine solution and the mixture was allowed to stand in ice–water for 3 min under a nitrogen atmosphere. To the alkaline mixture was added the solution of **3a** *via* a syringe, and the reaction mixture was stirred for 4.6 h at room temperature under a nitrogen atmosphere. The reaction was terminated by the addition of 2.0 mol dm^{-3} aqueous HCl (28.5 ml, 57 mmol) to the mixture which was then filtered to remove salt. The filtrate was evaporated and the residue was taken up in EtOH and filtered. This procedure was repeated once more after which the filtrate was evaporated. Two recrystallizations of the residue from MeOH–AcOEt gave brown crystals (0.56 g), the purification of which by a short-column of Serva XAD-2 (eluted with water) and recrystallization from MeOH–EtOH gave **1a** as light brown prisms (0.35 g, 22.6%), R_F 0.22; mp 203.5–205.5 °C (decomp.) (Found: C, 63.55; H, 5.47; N, 8.69. $C_{17}H_{17}N_2O_2Cl{\cdot}0.2H_2O$ requires C, 63.73; H, 5.47; N, 8.74%); λ_{max}(TRIS–acetate buffer, pH 8.0)/nm 248 (ε/dm^3 mol^{-1} cm^{-1} 41 700), 320 (8330) and 361 (3930); ν_{max}(KBr)/cm^{-1} 3400br, 3050m, 2850m, 1675s (CO), 1625m, 1530w and 1510w; δ(CD_3OD) 2.44–2.49 (4 H, m), 5.20 (2 H, t, *J* 7.4), 8.10 (2 H, q, *J* 7.3), 8.17 (1 H, dd, *J* 7.5 and 7.5) 8.38 (1 H, dd, *J* 7.4 and 7.4), 8.58 (1 H, d, *J* 7.6), 8.69 (1 H, d, *J* 8.4), 9.06 (1 H, d, *J* 8.4), 9.12 (1 H, d, *J* 7.6) and 10.20 (1 H, s); *m*/*z* (FAB-MS) 281 (M^+ − Cl, 100%), 220 (8.2), 193 (7.5) and 180 (24) (Found: M^+ − Cl, 281.1299. $C_{17}H_{17}N_2O_2$ requires *M* − Cl, 281.1286).

***N*-[4-(Hydroxylaminocarbonyl)butyl]phenanthridinium chloride 1b.** The ester function of **3b** (0.82 g) was converted into a hydroxamic acid by the method used for **1a**, except that the reaction was for 10 h and hydroxylamine and alkali were in 6- and 13-fold excess, respectively. After the reaction had been terminated, the mixture was concentrated to low volume and the residue was extracted with butanol–water. The extract was washed with three portions of water and the aqueous layer was back-extracted with two portions of butanol. The combined organic layers were evaporated, and the residue was taken up in MeOH–EtOH and filtered to remove insoluble material. After evaporation of the filtrate, the residue was recrystallized from MeOH–EtOH to give pale brown crystals (0.27 g). Purification of the crystals on a column of Serva XAD-2 (eluted with water) and recrystallization from MeOH–EtOH gave **1b** as light brown prisms (0.12 g, 15.6%), R_F 0.19; mp 201.5–203 °C (decomp.) (Found: C, 64.95; H, 5.79; N, 8.30. $C_{18}H_{19}N_2O_2Cl{\cdot}0.1H_2O$ requires C, 65.00; H, 5.81; N, 8.42%); ν_{max}(KBr)/cm^{-1} 3180m, 2880m, 1665s (CO), 1620m, 1530w and 1500w; δ(CD_3OD) 1.85 (2 H, m), 2.18–2.26 (4 H, m), 5.17 (2 H, t, *J* 7.6), 8.10 (2 H, q, *J* 7.1), 8.15 (1 H, dd, *J* 7.0 and 7.0), 8.39 (1 H, dd, *J* 8.6 and 8.6), 8.59 (2 H, dd, *J* 7.2 and 7.2), 9.06 (1 H, d, *J* 8.4), 9.13 (1 H, d, *J* 8.0) and 10.20 (1 H, s); *m*/*z* (FAB-MS) 295 (M^+ − Cl, 100%), 206 (7.5) and 180 (21) (Found: M^+ − Cl, 295.1440. $C_{18}H_{19}N_2O_2$ requires *M* − Cl, 295.1442).

***N*-[5-(Hydroxyaminocarbonyl)pentyl]phenanthridiniumchloride 1c.** Compound **1c** was prepared by the method used for **1a**, except that the reaction was for 6.9 h, and the hydroxylamine and alkali were in 5- and 11-fold excess, respectively. Compound **1c** was obtained as pale brown prisms (12.1%), R_F 0.19; mp 195–197 °C (decomp.) (Found: C, 65.36; H, 6.19; N, 7.87. $C_{19}H_{21}N_2O_2Cl{\cdot}0.2H_2O$ requires C, 65.49; H, 6.19; N, 8.03%); λ_{max}(TRIS–acetate buffer, pH 8.0)/nm 248 (ε/dm^3 mol^{-1} cm^{-1} 40 000), 319 (8000) and 360 (3670); ν_{max}(KBr)/cm^{-1} 3180br, 3000m, 2930m, 1670s (CO), 1620m, 1525w and 1505w; δ(CD_3OD) 1.57 (2 H, m), 1.75 (2 H, m), 2.14 (2 H, t, *J* 7.2), 2.20 (2 H, m), 5.14 (2 H, t, *J* 7.6), 8.11 (2 H, q, *J* 7.7), 8.16 (1 H, dd, *J* 8.4 and 8.4), 8.39 (1 H, dd, *J* 7.0 and 7.0), 8.58 (2 H, d, *J* 8.4), 9.08 (1 H, d, *J* 8.8), 9.14 (1 H, d, *J* 8.0) and 10.15 (1 H,

s); *m/z* (FAB-MS) 309 (M^+ − Cl, 100%), 206 (8.2), 180 (18) (Found: M^+ − Cl, 309.1592. $C_{19}H_{21}N_2O_2$ requires *M* − Cl, 309.1598).

***N*-[6-(Hydroxyaminocarbonyl)hexyl]phenanthridinium chloride 1d.** Compound **1d**, prepared by the method used for **1b**, was obtained as pale brown prisms (24.8%), R_F 0.21; mp 208.5–210 °C (decomp.) (Found: C, 66.10; H, 6.50; N, 7.61. $C_{20}H_{23}N_2O_2Cl{\cdot}0.2H_2O$ requires C, 66.27; H, 6.50; N, 7.72%); ν_{max}(KBr)/cm^{-1} 3180m, 3050m, 2930m, 2850m, 1655s (CO), 1620m, 1530w and 1505w; δ(CD_3OD) 1.43–1.49 (2 H, m), 1.54–1.68 (4 H, m), 2.11 (2 H, t, *J* 7.0), 2.14–2.22 (2 H, m), 5.14 (2 H, t, *J* 7.6), 8.11 (2 H, q, *J* 7.3), 8.17 (1 H, dd, *J* 7.8 and 7.8), 8.39 (1 H, dd, *J* 7.6 and 7.6), 8.58 (2 H, d, *J* 9.2), 9.07 (1 H d, *J* 8.0), 9.13 (1 H, d, *J* 8.4) and 10.19 (1 H, s); *m/z* (FAB-MS) 323 (M^+ − Cl, 100%), 248 (2.7), 206 (7.4), 193 (7.4) and 180 (20) (Found: M^+ − Cl, 323.1755. $C_{20}H_{23}N_2O_2$ requires *M* − Cl, 323.1754).

***N*-[7-(Hydroxyaminocarbonyl)heptyl]phenanthridiniumchloride 1e.** Compound **1e**, prepared by the method used for **1b**, was obtained as light brown prisms (34.1%), R_F 0.25; mp 211–212 °C (decomp.) (Found: C, 66.48; H, 6.66; N, 7.19. $C_{21}H_{25}N_2O_2Cl{\cdot}0.3H_2O$ requires C, 66.67; H, 6.82; N, 7.40%); ν_{max}(KBr)/cm^{-1} 3100br, 2920m, 2850m, 1665s (CO), 1625m and 1530w; δ(CD_3OD) 1.36–1.39 (2 H, m), 1.42–1.53 (2 H, m), 1.55–1.65 (4 H, m), 2.08 (2 H, t, *J* 7.4), 2.14–2.21 (2 H, m), 5.14 (2 H, t, *J* 7.8), 8.11 (2 H, q, *J* 7.5), 8.17 (1 H, dd, *J* 7.4 and 7.4), 8.39 (1 H, dd, *J* 7.8 and 7.8), 8.58 (2 H, dd, *J* 8.4 and 3.6), 9.07 (1 H, d, *J* 8.8), 9.13 (1 H, d, *J* 8.4) and 10.19 (1 H, s); *m/z* (FAB-MS) 337 (M^+ − Cl, 97%), 193 (6.1), 180 (11) and 154 (100) (Found: M^+ − Cl, 337.1928. $C_{21}H_{25}N_2O_2$ requires *M* − Cl, 337.1910).

Reaction of supercoiled Col E1 DNA with phenanthridine-linked hydroxamic acids and metal ions

Throughout the experiment work, metal solutions were freshly prepared. The reaction mixture (15 μl) contained supercoiled Col E1 DNA (0.3 μg), hydroxamic acid (dissolved in deionized water) and metal ion in 40 mmol dm^{-3} TRIS–HCl buffer. The reaction was started by addition of the metal ion to the desired concentration as the last component after which the mixture was kept at 37 °C in the dark.

Agarose gel electrophoresis and quantitation of DNA bands

The reaction was terminated by the addition of a solution (5 μl) of 50% glycerol–25 mmol dm^{-3} EDTA–0.025% Bromophenol Blue. The sample in a final volume of 20 μl was analysed by agarose gel electrophoresis [0.9% agarose containing 0.5 μg ml^{-1} ethidium bromide; running buffer, 40 mmol dm^{-3} TRIS·HCl–5 mmol dm^{-3} sodium acetate–1 mmol dm^{-3} EDTA (pH 8.0)]. The electrophoresis was carried out at 100V for 1.0 h. Bands of DNA were photographed under UV light and the relative amounts of form i and form ii of DNA were determined with a Shimadzu dual-wavelength flying spot scanner CS-9000. The area under form i DNA was multiplied by a factor of 1.07 to correct for reduced binding of ethidium bromide by form i DNA.[22] Form ii DNA production (%) described in the present paper are the averages of the results of at least three runs.

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