Synthesis of Two Acridine Conjugates of the Bis(phenanthroline) Ligand "Clip-Phen" and Evaluation of the Nuclease Activity of the Corresponding Copper Complexes

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The synthesis of three novel derivatives [*N*-acetyl-Clip-Phen (**1**), Clip-Phen-hexylaminoacridine (**4**) and Clip-Phen-hexylchloromethoxyaminoacridine (**5**)] of the bis(phenanthroline) ligand "**Clip-Phen**", are described. Complexation of these ligands with copper(II) afforded the 1:1 complexes as hexafluorophosphate salts. The relaxation of ϕX 174 DNA was used as a DNA cleavage assay, with the following results. $[Cu(\mathbf{1})]^{2+}$ was found to show a diminished activity relative to the parent complex $[Cu(\mathbf{Clip\text{-}Phen})]^{2+}$. However, the acridine-containing complexes $[Cu(\mathbf{4})H]^{3+}$ and $[Cu(\mathbf{5})H]^{3+}$ exhibited significantly enhanced cleavage efficiencies, which has been attributed to increased affinity of the complexes for DNA. UV/Vis-spectral data of the complexes in the presence of calf-thymus DNA was consistent with an intercalative mode of binding.

Introduction

The artificial nuclease capability of redox-active copper complexes of the chelating ligand 1,10-phenanthroline (Phen) is well established.[1][2] Single-strand cleavage of nucleic acids is observed in the presence of dioxygen and a reductant, by oxidative attack on deoxyribose units from the DNA minor groove.[1] The reactive species $(Phen)_2Cu^{I}$ is significantly more active than the $(Phen)Cu^{I}$ species,[3] although the association constant for the second Phen ligand[4] is only $10^{5.5}$ M^{-1}. Competitive binding of copper can thus be a problem in cleavage experiments, and may hamper the use of (Phen)copper compexes in vivo. With these concepts in mind, we recently reported the synthesis of the novel bis(phenanthroline) ligand "Clip-Phen", whereby two Phen ligands are linked at the 2′ position by a serinol bridge.[5] The artificial nuclease activity of this new ligand was found to be higher than Phen itself (by a factor of 2) and, in addition, the serinol bridge contains an exogenous amine group, which allows further functionalisation.

Attachment of the natural polyamine spermine to Clip-Phen also afforded a new conjugate with even greater nuclease efficiency.[6] This was attributed to the high affinity of the spermine moiety for nucleic acids, through electrostatic interactions with the anionic phosphate backbone.[7] Cleavage reactions on restriction fragment DNA showed that there was very little sequence specificity of this Clip-Phen-spermine bioconjugate.

Attachment of an intercalating agent to Clip-Phen was the motivation for the current work. DNA binding by intercalation of polyaromatic species between adjacent base pairs was first proposed by Lerman[8] in 1961. Efficient intercalators must be planar and often contain three fused six-membered rings. The presence of a positive charge usually aids in the binding process.[9] Although intercalation has been studied by various groups, the overall binding process is complex, partly due to ambiguities in the actual binding mode (intercalation, minor-groove binding, or electrostatic interactions) and difficulties in measuring association constants.[10] Certain intercalators, such as daunomycin, adriamycin, and *m*-AMSA exhibit anti-tumour activity, in a process which is believed to involve the disruption of topoisomerase activity in vivo.[11] Association constants for intercalation are broadly comparable, normally between 10^5 and 10^6 M^{-1}, and there is a general lack of correlation between affinity and pharmacological efficacy. Overall, there is little sequence specificity observed in the interactions of intercalators with DNA.[9]

We wished to quantify the nuclease efficiency of an intercalator−Clip-Phen hybrid, such that the reactive Clip-Phen fragment would be anchored to the DNA. We chose to attach acridine derivatives for several reasons. Firstly, acridines are one of the most studied of the intercalating groups. Secondly, acridines are known to rapidly enter cells and accumulate in the nucleus,[12] which is an important consideration with regard to in vivo activity. For example, a netropsin−acridine hybrid has been reported to accumulate in the cell nucleus, using the acridine moiety as the cell-penetration vector,[13] whereas netropsin itself exhibits slow kinetics of cell penetration and poor uptake by the nucleus.[14] Finally, conjugation to acridines was considered to be the most straightforward and least expensive synthetic route available. Synthetic strategies to oligonucleotide-intercalator hybrids[15] and minor-groove binder-intercalator hybrids[16] have been reviewed recently in the literature.

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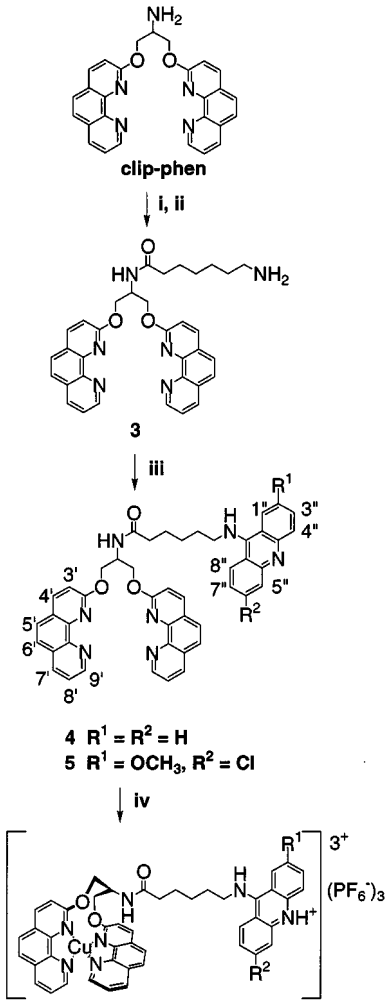
Results and Discussion

Ligand Synthesis

Clip-Phen was readily prepared according to the previously reported procedure.[5] The primary amine of Clip-Phen presents a convenient site for further functionalisation. However, previous reports[17] have suggested that the reactive fragments in intercalator hybrids show greater efficacy if separated by a polymethylene linker. We chose, therefore, to protect the amine function of 6-aminohexanoic acid (with the BOC group) allowing facile attachment of the carboxylate to Clip-Phen by amide bond formation. Deprotection of Clip-Phen-hexyl-BOC (**2**) proceeded smoothly to afford Clip-Phen-hexylamine (**3**) in 80% yield (see Scheme 1).



Scheme 1. Synthesis of ligands **4** and **5**, and their corresponding Cu^{II}/PF_6^- complexes; conditions: (i) 6-BOC-amino-1-hexanoic acid, EtOCOCl; (ii) 25% CF_3COOH in CH_2Cl_2; (iii) appropriate 9-phenoxyacridine, CH_3CN, 10 equiv. CH_3COOH; (iv) $Cu(OAc)_2 \cdot 2\,H_2O$, MeOH, then NH_4PF_6

Primary amines are known to react with 9-chloroacridine derivatives in hot phenol (by in situ 9-phenoxyacridine formation) to give the 9-amino acridines in high yield.[18] We encountered several problems with this reaction, only being able to isolate products in very low yield after chromatographic separation. ^{1}H-NMR data from side-products obtained suggested that this was due to decomposition of the Clip-Phen fragment in hot phenol, possibly due to nucleophilic attack of phenol at the C-2′ position of the phenanthroline. We chose, therefore, to isolate the 9-phenoxyacridine derivatives prior to reaction with **3**. Reaction of 9-phenoxyacridine with **3** in refluxing acetonitrile afforded the Clip-Phen-hexylaminoacridine (**4**) in 30−40% yield after chromatography. However, the corresponding reaction between 6-chloro-9-phenoxy-2-methoxyacridine and **3** proceeded in very poor yield. Addition of 5−10 equivalents of acid (usually acetic acid) was found to greatly improve the yield by increasing the electrophilicity of the C-9′′ carbon atom.[19] Subsequent addition of acid to the reaction between 9-phenoxyacridine and **3** also led to a significant enhancement in yield (to 74%). Products **4** and **5** (see Scheme 1) were bright yellow, fluorescent solids, which made chromatographic purification simple, and gave satisfactory ^{1}H-NMR and mass spectra and analytical data.

Since the Clip-Phen fragments of both **4** and **5** contain an amide function in place of the primary amine, we also wished to prepare an analogous Clip-Phen amide derivative (without an intercalating group) to allow direct comparison of reactivities. This was readily achieved by reaction of acetic anhydride with Clip-Phen in CH_2Cl_2, to afford *N*-acetyl Clip-Phen (**1**) (see Figure 1) in 94% yield. The DNA cleavage reactivity of (phenathroline)Cu^{II} derivatives necessitates reduction to Cu^{I}, and thus formation of a tetrahedral intermediate. By converting the amine of Clip-Phen to the amide in **1**, we would thus avoid any differences in the coordination sphere or any steric constraints upon reduction.

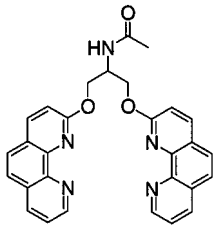


Figure 1. *N*-Acetyl Clip-Phen (**1**)

Complex Formation with Cu^{II} Salts

The coordination chemistry of the Clip-Phen family of ligands (L) with Cu^{II} is not straightforward.[20] The 5-atom bridge between the two phenanthrolines leads to little thermodynamic stability of the mononuclear species $[Cu(L)]^{2+}$, with respect to higher nuclearity species $[Cu_n(L)_n]^{2n+}$, in a process that may be dependent upon concentration and solvent. Complexes with a Cu/L ratio of 2:1 have also been observed when the Cu^{II} concentration is higher than that of the ligand. In general, we found that isolation of solid products of overall stoichiometry 1:1 (L/Cu) was problematic when $CuCl_2 \cdot 2\,H_2O$ or $Cu(SO_4) \cdot 4\,H_2O$ were used as the starting copper salts (data not

shown). We found that a reproducible procedure for complex formation with **1**, **4**, and **5** was to treat one equivalent of $Cu(OAc)_2 \cdot H_2O$ with one equivalent of ligand in warm methanol for two hours. Addition of an excess of NH_4PF_6 gave a precipitate which was redissolved by addition of acetone. Upon concentration of this solution, the products precipitated and could be isolated by centrifugation. Analytical data and mass spectra were consistent with the formation of L/Cu^{II} 1:1 complexes. The pK_a of the acridine fragment (> 10) is sufficiently high to allow proton transfer from the ammonium ion of NH_4PF_6 ($pK_a = 9.3$), such that the intercalator is present as an acridinium hexafluorophophate in the complexes of both **4** and **5** (see Scheme 1). This did not present a problem, however, since the intercalators are also protonated under biological conditions, and under the conditions used for the DNA experiments described (phosphate buffer, pH = 7.2). The UV/Vis spectra of the complexes exhibited the expected ligand absorptions between 200 and 450 nm, in addition to a broad Cu^{II} d-d band at circa 810 nm ($\varepsilon \approx 150\ M^{-1}\ cm^{-1}$).

The 1:1 copper complex of Clip-Phen was prepared by using the previously reported synthesis of [Cu(Clip-Phen]Cl_2.[5] After equimolar quantities of Clip-Phen and anhydrous $CuCl_2$ were stirred in DMF for 4 h, the chloride salt was precipitated with ether. This was redissolved in MeOH and an excess of NH_4PF_6 was added to afford a green precipitate, which was isolated by centrifugation. The analytical data was consistent with the overall stoichiometry [Cu(Clip-Phen)]$(PF_6)_2$. The absence of a third hexafluorophosphate anion in this complex indicates that the exogenous primary amine of Clip-Phen is not protonated.

DNA Cleavage Activity

Relaxation of supercoiled circular Φx 174 DNA (form I) into relaxed circular (form II) and linear (form III) conformations was the assay used to assess the relative cleavage efficiency of the four complexes. The redox activity of the Cu^{II} centres was initiated by the addition of 5 mM mercaptopropionic acid (MPA) in the presence of air.

Reactions were carried out by using either the isolated hexafluorophosphate salts described, or by premetallating the ligands with $CuCl_2$ in DMF/H_2O (40:60, 1 mM in ligand, 1 mM in $CuCl_2$) for 2 h prior to dilution and addition to DNA. The DNA/complex mixtures were allowed to equilibrate for 30 min at room temperature prior to addition of MPA. Several reactions were also carried out by pre-equilibration of the ligand (1 μM, 15 min) with DNA, followed by $CuCl_2$ (1 μM, 15 min), or by pre-equilibration of $CuCl_2$ (1 μM, 15 min) with DNA, followed by ligand (1 μM, 15 min). The choice of counterion (chloride or hexafluorophosphate) or order of addition was found to have a negligible effect upon the degree of reactivity (within experimental error). Since stock solutions of the hexafluorophosphate salts appeared to decompose over time (becoming an orange-brown colour), we chose to freshly prepare our complexes by metallation with $CuCl_2$ prior to reaction.

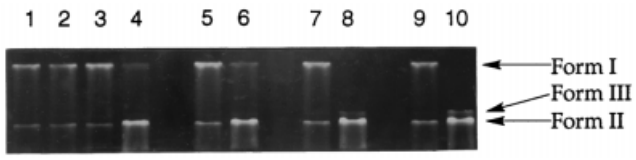


Figure 2. Comparison of Φx 174 cleavage efficiency between Clip-Phen, **1**, **4**, and **5** (1 μM) in the presence of $CuCl_2$ (1 μM) and MPA (5 mM); lane 1: control, no complex, no MPA; lane 2: control, no complex, with MPA; lanes 3, 5, 7, and 9: Clip-Phen, **1**, **4**, and **5**, respectively, without addition of Cu; lanes 4, 6, 8, and 10: Clip-Phen, **1**, **4** and **5**, respectively, in the presence of Cu

Figure 2 shows a representative gel illustrating the relative reactivities of the 4 complexes at 1 μM concentration. The reactivity of Clip-Phen-copper (1.4 ± 0.1 strand breaks per plasmid) is consistent with the previously reported data,[5] showing a cleavage activity which is approximately twice that of phenanthroline itself. However, *N*-acetyl Clip−Phen−copper shows a diminished reactivity (0.9 ± 0.1 strand breaks per plasmid), either due to the lack of a primary amine to coordinate or due to the steric effects of the bulkier amide group. The lack of protonation of the primary amine in the [Cu(Clip-Phen)]$(PF_6)_2$ complex is consistent with coordination to the Cu^{II} centre, although it is unlikely to be coordinated upon reduction to Cu^{I}. The copper complexes of the acridine-containing ligands **4** and **5** both exhibit cleavage activities of circa 4 ± 1, which is significantly higher than the two non-acridine-containing ligands. A direct comparison between **1** and **4** suggests an approximate fourfold increase in reactivity. Since the estimation of cleavage efficiency is less accurate after the disappearance of form 1, the variation in reactivity with concentration was also measured for **1** and **4**, this data being presented in Figure 3. Enhanced reactivity is thus observed at all concentrations.

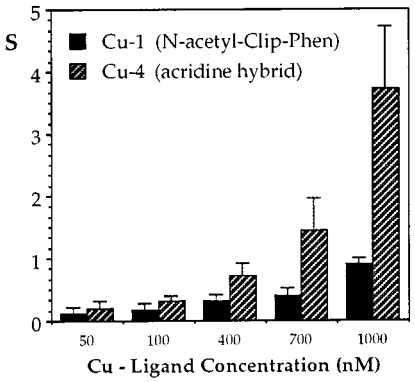


Figure 3. Concentration dependence of Φx 174 cleavage efficiency for **1** (solid) and **4** (hatched) in the presence of equimolar $CuCl_2$

Spectrophotometric Studies of DNA Binding

The binding of the complexes and ligands described herein to DNA is expected to be complex, since both moieties (the acridine fragment and the Cu−Clip-Phen frag-

ment) each have a separate affinity for DNA. Intercalative binding of acridine derivatives is well established, and gives rise to small (but significant) bathochromic and hypochromic shifts in the visible spectrum.[21] This technique was therefore used as a guide to whether intercalation was taking place with derivatives **4** and **5**. The alkylamino derivatives 9-(3-methoxyprop-1-ylamino)acridine (**6**) and 6-chloro-2-methoxy-9-(3-methoxyprop-1-ylamino)acridine (**7**) (see Figure 4) were also prepared and studied, since these analogues should have the same chromophores as **4** and **5**, without containing the clip-phen fragment.

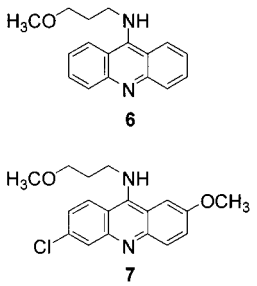


Figure 4. Alkylaminoacidire derivatives **6** and **7**

The spectrophotometric studies were carried out under identical buffer and salt conditions to the DNA cleavage experiments. Spectral shifts were recorded for DNA base-pair ratios of 0, 1, 4, 12, and 20 equivalents. We did not attempt to interpret this data in terms of binding constants, since the Cu−Clip-Phen fragment may also be binding in the minor groove.

Compounds **4**, **4** + $CuCl_2$, and **6** all gave bathochromic shifts of around 4 nm and hypochromic shifts of around 30% upon the addition of calf-thymus DNA (see Table 1), which were in good agreement with data previously reported in the literature.[21] We conclude from these results that the binding process of the free ligand (**4**), its copper complex (**4** + $CuCl_2$) and a related alkylaminoacridine (**6**) all involve intercalative binding of the acridine fragment. The spectral shifts of **4** + $CuCl_2$ and **6** upon titration with calf-thymus DNA are shown in Figure 5.

The data obtained from the 9-amino-6-chloro-2-methoxyacridine derivatives was more complex, as can be evidenced from the data in Table 1. Compounds **5**, **5** + $CuCl_2$, and **7** all show similar λ_b and ε_b in the presence of excess DNA, suggesting that the acridine fragment is in a similar environment for all three upon DNA binding. However, in the absence of DNA, whilst the free acridine compound **7** exhibits the expected behaviour, **5** and its copper complex show anomalous behaviour. This is probably due to stacking interactions of the acridine fragment with the phenanthroline rings in the absence of DNA, particularly with free **5**, which shows a 12-nm red-shift, relative to its Clip-Phen-free analogue **7**.

The in vivo activity of compounds **4** and **5** is currently being investigated and will be reported in due course.

Table 1. UV/Vis data in the region 370−470 nm for **4**, **5**, **4** + $CuCl_2$, **5** + $CuCl_2$, **6**, and **7** (10 μM) in phosphate buffer (pH = 7.2): ε_f and λ_f are the values in the absence of calf-thymus DNA; ε_b and λ_b are the values in the presence of 20 base-pair equivalents of calf-thymus DNA

	λ_f (ε_f)	λ_b (ε_b)
4	396 (7000) 415 (8600) 437 (8100)	398 (4400) 419 (5700) 440 (5400)
4 + $CuCl_2$	394 (4200) 414 (6800) 437 (5500)	397 (2700) 417 (4100) 440 (3700)
6	395 (4500) 410 (8100) 432 (6900)	398 (2900) 416 (5800) 436 (5000)
5	433 (7800) 455 (7700)	428 (9700) 450 (9500)
5 + $CuCl_2$	426 (2900) 444 (2700)	427 (8100) 451 (7700)
7	420 (13300) 443 (12700)	429 (6800) 451 (6900)

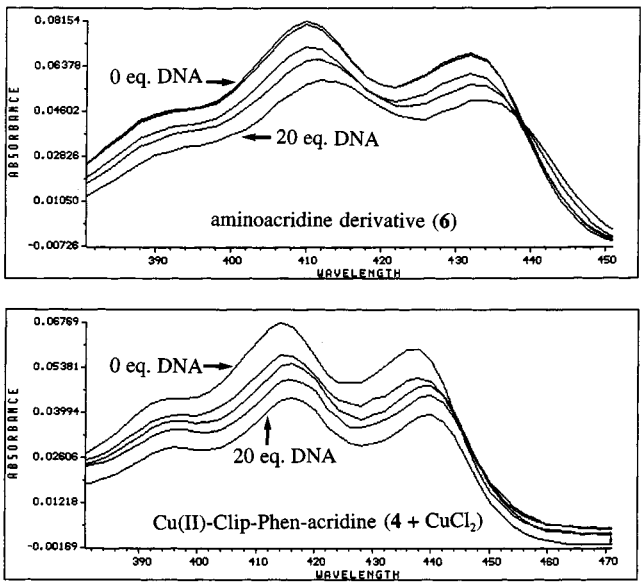


Figure 5. UV/Vis titration of **6** (top) and **4** + $CuCl_2$ (bottom) (all 10 μM) against calf-thymus DNA (0, 1, 4, 12, and 20 base-pair equivalents) in phosphate buffer (pH = 7.2) in the presence of NaCl (50 mM) and $MgCl_2$ (10 mM)

Conclusions

We have described the synthesis of two novel conjugate ligands (**4** and **5**) and their copper(II) complexes, which contain a reactive DNA cleavage unit attached to an intercalating DNA recognition unit. The presence of the intercalator has been shown to significantly enhance the DNA cleavage activity, in spite of the diminished activity which is found upon blockage of the exogenous primary amine of

the parent Clip-Phen from which they are derived. Thus, these species are of potential interest for in vivo applications.

Experimental Section

Warning: Acridines and ethidium bromide are mutagenic and should always be handled with appropriate precautions.

General: Reactions were monitored by thin-layer chromatography using Macherey-Nagel Alugram Sil G/UV 254 plates and spots were visualized with UV light. − Proton NMR spectra were recorded with a Bruker 250 MHz instrument and referenced to deuterated solvent. Aromatic proton signals due to phenanthroline are denoted by a single prime (′), those due to acridine are denoted by double prime (′′). − UV/Vis spectra were recorded with a Hewlett-Packard 8452A diode-array spectrophotometer in cuvettes of 1 cm path length. − Electrospray mass spectra were recorded with a Perkin-Elmer SCIEX API 100 instrument. − Ligand metallation and DNA reactions were carried out in Nanopure deionized water. DMF was dried with calcium sulfate. Other commercial reagents were used without purification.

Syntheses

2-Amino-1,3-bis(1′,10′-phenanthrolin-2′-yloxy)propane (Clip-Phen) was prepared by reaction of 2-chloro-1,10-phenathroline with serinol according to the method described previously.[5]

6-(Butoxycarbonylamino)-1-hexanoic Acid: 6-Aminohexanoic acid (1 g, 7.6 mmol), MgO (296 mg, 7.6 mmol), and NaOH (305 mg, 7.6 mmol) were added to 40 mL of dioxane/H_2O (6:1). Di-*tert*-butyl dicarbonate (1.83 g, 8.39 mmol) was then added in portions over 20 min, the solution was stirred for 18 h at room temperature, filtered, and concentrated in vacuo. The residue was dissolved in H_2O (20 mL), washed with diethyl ether (20 mL), acidified to pH = 3 with acetic acid (10%), and extracted with ethyl acetate (2 × 50 mL). The organic was then dried ($MgSO_4$) and concentrated to afford the product as a colourless oil (1.56 g, 89%). − ^{1}H NMR (CD_2Cl_2): δ = 1.41 [s, 9 H, C(CH_3)$_3$], 1.3−1.5 (m, 4 H, CH_2CH_2), 1.59 (m, 2 H, CH_2), 2.34 (t, 2 H, *J* = 7.5 Hz, CH_2COOH), 3.06 [m, 2 H, CH_2NHC(O)O], 4.67 (br. s, 1 H, CH$_2$N*H*). − DCI MS; *m*/*z* (%): 232 (63) [MH$^+$], 249 (56) [MNH$_4^+$]. − $C_{11}H_{21}NO_4$ (231.3): calcd. (+ 0.5 H_2O) C 54.9, H 9.15, N 5.82; found C 55.5, H 8.91, N 5.84.

***N*-Acetyl Clip-Phen (1):** Clip-Phen (447 mg, 1 mmol) was dissolved in CH_2Cl_2, to which was added Et_3N (1 mL) and acetic anhydride (1 mL). Upon standing for 2 h at room temperature, the solvent was removed in vacuo, the residue redissolved in CH_2Cl_2 (10 mL), washed with aqueous NaOH (10 mL, 1 M), and dried ($MgSO_4$). The solution was then concentrated to ca. 2 mL, and hexane was added to precipitate the product as a white solid (460 mg, 94%), which was filtered off and dried in vacuo. − ^{1}H NMR (CD_2Cl_2): δ = 2.03 (s, 3 H, CH_3), 4.84 (m, 3 H, serinol bridge), 5.04 (m, 2 H, serinol bridge), 7.15 (d, 2 H, *J* = 8.5 Hz, 3′-H), 7.59 (dd, 2 H, *J* = 8, 4.25 Hz, 8′-H), 7.70 (d, 2 H, *J* = 8.75 Hz, 6′-H), 7.77 (d, 2 H, *J* = 8.75 Hz, 5′-H), 8.14 (d, 2 H, *J* = 8.5, 4′-H), 8.27 (dd, 2 H, *J* = 1.5, 8 Hz, 7′-H), 9.07 [br. s, 1 H, CH_3C(O)N*H*], 9.07 (dd, 2 H, *J* = 1.5, 4.25 Hz, 9′-H). − DCI MS; *m*/*z* (%): 490 (100) [MH$^+$]. − $C_{29}H_{23}N_5O_3$ (489): calcd. (+ 0.33 $CHCl_3$, 0.5 H_2O) C 65.4, H 4.43, N 13.1; found C 65.4, 4.49, N 13.4.

Clip-Phen-hexyl-BOC (2): 6-Butoxycarbonylamino-1-hexanoic acid (1.3 g, 5.62 mmol) was dissolved in CH_2Cl_2 (10 mL) at 0°C, to which was then added Et_3N (567 mg, 5.62 mmol) and EtOC(O)Cl (609 mg, 5.62 mmol). After stirring for 30 min, the solvent was removed in vacuo, the residue dissolved in CH_2Cl_2, Clip-Phen (1 g, 2.23 mmol) added, and the solution stirred at room temperature for 3 h. The solvent was removed in vacuo, the residue redissolved in CH_2Cl_2 (50 mL), and the solution was washed with aqueous $KHCO_3$ (5%, 3 × 50 mL), H_2O (3 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo to afford the product as an oily solid (1.22 g, 83%). − ^{1}H NMR (CD_2Cl_2): δ = 1.0−1.2 (m, 4 H, CH_2CH_2), 1.38 [s, 9 H, C(CH_3)$_3$] 1.41 (m, 2 H, CH_2), 2.24 [t, 2 H, *J* = 7.5 Hz, CH_2C(O)NH], 2.88 [m, 2 H, CH_2NHC(O)O], 4.55 [br. s, 1 H, OC(O)N*H*], 4.84 (m, 3 H, serinol bridge), 5.06 (m, 2 H, serinol bridge), 7.14 (d, 2 H, *J* = 8.5 Hz, 3′-H), 7.62 (dd, 2 H, *J* = 8, 4.25 Hz, 8′-H), 7.71 (d, 2 H, *J* = 8.75 Hz, 6′-H) 7.77 (d, 2 H, *J* = 8.75 Hz, 5′-H), 8.14 (d, 2 H, *J* = 8.5 Hz, 4′-H), 8.27 (dd, 2 H, *J* = 1.5, 8 Hz, 7′-H), 8.69 [d, 1 H, *J* = 5.8 Hz, CH$_2$C(O)N*H*], 9.10 (dd, 2 H, *J* = 1.5, 4.25 Hz, 9′-H). − DCI MS; *m*/*z* (%): 661 (72) [MH$^+$]. − $C_{38}H_{40}N_6O_5$ (660).

Clip-Phen-hexylamine (3): 2 (1 g, 1.52 mmol) was dissolved in 25% (v/v) of CF_3COOH/CH_2Cl_2 (40 mL) and the solution stirred for 1 h at room temperature. The solvent was then removed in vacuo, the residue dissolved in H_2O, and the pH adjusted to 12 (1 M NaOH). Extraction of this solution with CH_2Cl_2, followed by drying ($MgSO_4$) and concentration in vacuo afforded the product as a pale yellow solid (823 mg, 97%). − ^{1}H NMR (CD_2Cl_2): δ = 1.0−1.2 (m, 4 H,CH_2CH_2), 1.41 (m, 2 H, CH_2), 2.05 (br. s, 2 H, CH$_2$NH_2), 2.21 [t, 2 H, *J* = 7.8 Hz, CH_2C(O)NH], 2.44 (t, 2 H, *J* = 6.5 Hz, CH_2NH$_2$), 4.85 (m, 3 H, serinol bridge), 5.06 (m, 2 H, serinol bridge), 7.14 (d, 2 H, *J* = 8.5 Hz, 3′-H), 7.58 (dd, 2 H, *J* = 8, 4.25 Hz, 8′-H), 7.69 (d, 2 H, *J* = 8.75 Hz, 6′-H), 7.76 (d, 2 H, *J* = 8.75 Hz, 5′-H), 8.13 (d, 2 H, *J* = 8.5 Hz, 4′-H), 8.26 (dd, 2 H, *J* = 1.5, 8 Hz, 7′-H), 8.69 [d, 1 H, *J* = 5.8 Hz, CH$_2$C(O)N*H*], 9.06 [dd, 2 H, *J* = 1.5, 4.25 Hz, 9′-H]. − DCI MS; *m*/*z* (%): 561 (100) [MH$^+$].

9-Chloroacridine: Acridone (2 g, 10.3 mmol) and $POCl_3$ (20 mL) were placed in a round-bottomed flask and heated at reflux for 1 h. The unused $POCl_3$ was then removed by distillation under vacuum, the residue cooled to 50°C, diluted with $CHCl_3$ (20 mL), and poured into aqueous ammonia (30%, 10 mL) and ice (25 g). After the organic layer was separated and the aqueous further extracted with $CHCl_3$ (3 × 50 mL), the organic layers were combined, dried ($MgSO_4$), and concentrated to 10 mL, and then hexane was added. Filtration afforded the product as a yellow solid (1.99 g, 91%). − ^{1}H NMR (CD_2Cl_2): δ = 7.67 (dd, 2 H, *J* = 9.9 Hz, 2′′-H or 3′′-H), 7.82 (dd, 2 H, *J* = 9.9 Hz, 2′′-H or 3′′-H), 8.20 (d, 2 H, *J* = 9 Hz, 1′′-H or 4′′-H), 8.44 (d, 2 H, *J* = 9 Hz, 1′′-H or 4′′-H). − DCI MS; *m*/*z* (%): 214 (100) [MH$^+$]. − $C_{13}H_8ClN$ (213): calcd. C 73.0, H 3.77, N 6.56; found C 72.7, H 3.35, N 6.41.

9-Phenoxyacridine: 9-Chloroacridine (680 mg, 3.2 mmol) was added to a solution of NaOH (200 mg, 5 mmol) in phenol (3 g, 32 mmol), the mixture was stirred at 100°C for 2 h, then poured into NaOH solution (2 M, 50 mL). The solution was then extracted with $CHCl_3$ (3 × 50 mL) and the solution dried ($MgSO_4$) and concentrated in vacuo. Recrystallisation of the residue from hot MeOH/H_2O (50:50) afforded the product as pale yellow needles (770 mg, 89%). − ^{1}H NMR (CD_2Cl_2): δ = 6.87 (m, 2 H, phenoxy 2-H), 7.05 (m, 1 H, phenoxy 4-H), 7.27 (m, 2 H, phenoxy 3-H) 7.47 (dd, 2 H, *J* = 9.9 Hz, 2′′-H or 3′′-H), 7.78 (dd, 2 H, *J* = 9, 9 Hz, 2′′-H or 3′′-H), 8.11 (d, 2 H, *J* = 9 Hz, 1′′-H or 4′′-H), 8.25 (d, 2 H, *J* = 9 Hz, 1′′-H or 4′′-H). − DCI MS; *m*/*z* (%): 272 (100) [MH$^+$]. $C_{19}H_{13}NO$ (271).

6-Chloro-2-methoxy-9-phenoxyacridine: 6,9-Dichloro-2-methoxyacridine (2 g, 7 mmol) was added to a solution of NaOH (400 mg, 10 mmol) in phenol (10 g, 106 mmol), the mixture was stirred at

100°C for 2 h and then poured into NaOH solution (2 M, 50 mL). After this solution had been extracted with $CHCl_3$ (3 × 50 mL), the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Recrystallisation of the residue from hot MeOH afforded the product as yellow needles (2.02 g, 84%). − ^{1}H NMR (CD_2Cl_2): δ = 3.79 (s, 3 H, OC*H*$_3$), 6.86 (d, 2 H, *J* = 12 Hz, phenoxy 2-H), 7.06 (t, 1 H, *J* = 7 Hz, phenoxy 4-H), 7.16 (d, 1 H, *J* = 2.75 Hz, 1′′-H or 5′′-H), 7.26 (m, 2 H, phenoxy 3-H), 7.37 (dd, 1 H, *J* = 9.25, 2 Hz, 3′′-H or 7′′-H), 7.45 (dd, 1 H, *J* = 2.75, 9.5 Hz, 3′′-H or 7′′-H), 7.97 (d, 1 H, *J* = 9.25 Hz, 4′′-H or 8′′-H), 8.08 (d, 1 H, *J* = 9.5 Hz, 4′′-H or 8′′-H), 8.17 (d, 1 H, *J* = 2 Hz, 1′′-H or 5′′-H). − DCI MS; *m*/*z* (%): 336 (100) [MH$^+$].

Clip-Phen-hexylaminoacridine (4): Compound **3** (200 mg, 0.36 mmol), 9-phenoxyacridine (195 mg, 0.72 mmol), and acetic acid (216 mg , 3.6 mmol) were added to CH_3CN (15 mL) and the solution was heated at reflux for 18 h. The solvent was then removed in vacuo, the residue was dissolved in H_2O and the pH adjusted to 12 (1 M NaOH). The solution was extracted with $CHCl_3$ (2 × 50 mL), the combined organic layers dried ($MgSO_4$), concentrated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N, 92:7:1) on silica gel to afford the product (R_f = 0.3) as a bright yellow solid (194 mg, 74%). − UV/Vis (phosphate buffer, pH = 7.2): λ_{max} (ε M^{-1}cm^{-1}) = 226 (87500), 268 (90600), 396 (7000), 415 (8600) and 437 (8100). − ^{1}H NMR (CD_2Cl_2): δ = 1.23 (m, 2 H, NHCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$), 1.51 (m, 4 H, NHCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$), 2.31 (t, 2 H, *J* = 7.25 Hz, NHCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$), 3.57 (t, 2 H, *J* = 7 Hz, NHC*H*$_2$-CH$_2$CH$_2$CH$_2$CH$_2$), 4.84 (m, 3 H, serinol bridge), 4.99 (m, 3 H, serinol bridge), 7.08 (d, 2 H, *J* = 8.5 Hz, 3′-H), 7.25 (m, 2 H, 2′′-H or 3′′-H), 7.51 (dd, 2 H, *J* = 8, 4.25 Hz, 8′-H), 7.58 (d, 2 H, *J* = 8.75 Hz, 6′-H), 7.59 (m, 2 H, 2′′-H or 3′′-H), 7.65 (d, 2 H, *J* = 8.75 Hz, 5′-H), 7.96 (d, 2 H, *J* = 8.75 Hz, 1′′-H or 4′′-H), 8.04 (d, 2 H, *J* = 8.5 Hz, 4′-H), 8.06 (d, 2 H, *J* = 8.75 Hz, 1′′-H or 4′′-H), 8.16 (dd, 2 H, *J* = 1.5, 8 Hz, 7′-H), 8.92 (br. d, 1 H, *J* = 5.75 Hz, N*H*CO), 9.02 (dd, 2 H, *J* = 1.5, 4.25 Hz, 9′-H). − DCI MS; *m*/*z* (%): 738 (18) [MH$^+$], 542 (38) [MH$^+$ − phenanthrolyloxy], 197 (100) [hydroxyphenathrolineH$^+$]. − $C_{46}H_{39}N_7O_3$ (737): calcd. (+ 2 H_2O) C 71.4, H 5.56, N 12.6; found C 71.6, H 5.39, N 12.5.

Clip-Phen-hexylchloromethoxyaminoacridine (5): Compound **3** (200 mg, 0.36 mmol), 6-chloro-2-methoxy-9-phenoxyacridine (241 mg, 0.72 mmol), and acetic acid (216 mg , 3.6 mmol) were added to CH_3CN (15 mL) and the solution was heated at reflux for 18 h. The solvent was then removed in vacuo, the residue was dissolved in H_2O and the pH was adjusted to 12 (1 M NaOH). The solution was extracted with $CHCl_3$ (2 × 50 mL), the combined organic layers dried ($MgSO_4$), concentrated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N, 92:7:1) on silica gel to afford the product (R_f = 0.35) as a bright yellow solid (189 mg, 66%). − UV/Vis (phosphate buffer, pH = 7.2): λ_{max} (ε M^{-1}cm^{-1}) = 228 (112000), 272 (101400), 433 (7800) and 455 (7700). − ^{1}H NMR (CD_2Cl_2): δ = 1.25 (m, 2 H, NHCH$_2$CH$_2$-C*H*$_2$CH$_2$CH$_2$), 1.51 (m, 4 H, NHCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$), 2.32 (t, 2 H, *J* = 7.25 Hz, NHCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$), 3.45 (t, 2 H, *J* = 7 Hz, NHC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 3.83 (s, 3 H, OC*H*$_3$), 4.84 (m, 3 H, serinol bridge), 4.98 (m, 3 H, serinol bridge), 7.05 (d, 2 H, *J* = 8.5 Hz, 3′-H), 7.12 (dd, 1 H, *J* = 2.25, 9.25 Hz, 3′′-H or 7′′-H), 7.20 (d, 1 H, *J* = 2.5 Hz, 1′′-H or 5′′-H), 7.30 (dd, 1 H, *J* = 2.5, 9.25 Hz, 3′′-H or 7′′-H), 7.49 (dd, 2 H, *J* = 8, 4.25 Hz, 8′-H), 7.54 (d, 2 H, *J* = 8.75 Hz, 6′-H), 7.61 (d, 2 H, *J* = 8.75 Hz, 5′-H), 7.86 (d, 1 H, *J* = 9.25 Hz, 4′′-H or 8′′-H), 7.91 (d, 1 H, *J* = 2 Hz, 1′′-H or 5′′-H), 7.96 (d, 1 H, *J* = 9.25 Hz, 4′′-H or 8′′-H), 8.01 (d, 2 H, *J* = 8.5 Hz, 4′-H), 8.13 (dd, 2 H, *J* = 1.5, 8 Hz, 7′-H), 8.99 (br. s, 1 H, N*H*CO), 9.00 (dd, 2 H, *J* = 1.5, 4.25 Hz, 9′-H). − DCI MS; *m*/*z* (%): 802 (1.5) [MH$^+$], 606 (100) [MH$^+$ − phenanthrolyloxy], 197 (89) [hydroxyphenathrolineH$^+$]. − $C_{47}H_{40}ClN_7O_3$ (801.5): calcd. (+ CH_2Cl_2) C 64.9, H 4.73, N 11.1; found C 65.1, H 4.50, N 11.2.

9-(3-Methoxyprop-1-ylamino)acridine (6): 3-Methoxypropylamine (50 mg, 0.56 mmol), 9-phenoxyacridine (140 mg, 0.52 mmol), and acetic acid (100 mg) were added to CH_3CN (5 mL) and the solution was heated at reflux for 1 h. The solvent was then removed in vacuo and the residue purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 90:9:1) on silica gel to afford the product as a bright yellow solid (104 mg, 76%). − ^{1}H NMR (CD_2Cl_2): δ = 2.15 (m, 2 H, C*H*$_2$), 3.41 (s, 3 H, C*H*$_3$), 3.62 (t, 2 H, *J* = 5.5 Hz, C*H*$_2$), 3.99 (t, 2 H, *J* = 7 Hz, C*H*$_2$), 7.09 (dd, 2 H, *J* = 9, 9 Hz, 2′′-H or 3′′-H), 7.41 (dd, 2 H, *J* = 9, 9 Hz, 2′′-H or 3′′-H), 7.72 (d, 2 H, *J* = 9 Hz, 1′′-H or 4′′-H), 8.09 (d, 2 H, *J* = 9 Hz, 1′′-H or 4′′-H). − DCI MS; *m*/*z* (%): 331 (100) [MH$^+$].

6-Chloro-2-methoxy-9-(3-methoxyprop-1-ylamino)acridine (7): 3-Methoxypropylamine (50 mg, 0.56 mmol), 6-chloro-2-methoxy-9-phenoxyacridine (100 mg, 0.30 mmol), and acetic acid (100 mg) were added to CH_3CN (5 mL) and the solution was heated at reflux for 1 h. The solvent was then removed in vacuo and the residue was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 90:9:1) on silica gel to afford the product as a bright yellow solid (80 mg, 81%). − ^{1}H NMR (CD_2Cl_2): δ = 1.97 (m, 2 H, C*H*$_2$), 3.45 (s, 3 H, C*H*$_3$), 3.67 (t, 2 H, *J* = 5.5 Hz, C*H*$_2$), 3.88 (t, 2 H, *J* = 6 Hz, C*H*$_2$), 3.93 (s, 3 H, OC*H*$_3$), 7.27 (dd, 1 H, *J* = 2, 9 Hz, 3′′-H or 7′′-H), 7.30 (d, 1 H, *J* = 2 Hz, 1′′-H or 5′′-H), 7.38 (dd, 1 H, *J* = 2, 9 Hz, 3′′-H or 7′′-H), 7.92 (d, 1 H, *J* = 9 Hz, 4′′-H or 8′′-H), 7.99 (d, 1 H, *J* = 2 Hz, 1′′-H or 5′′-H) and 8.12 (d, 1 H, *J* = 9 Hz, 4′′-H or 8′′-H). − DCI MS; *m*/*z* (%): 267 (100) [MH$^+$].

[(Clip-Phen)Cu^{II}](PF$_6$)$_2$: $CuCl_2$ (3 mg, 0.022 mmol) and Clip-Phen (10 mg, 0.022 mmol) were dissolved in DMF (2 mL) and the solution was stirred for 4 h. Diethyl ether was then added and the solution left at −20°C for 18 h. The green precipitate which had formed was isolated by centrifugation, washed with ether, and dried. This solid was then dissolved in MeOH (2 mL) and a solution of NH_4PF_6 (15.9 mg, 0.1 mmol) in MeOH/H_2O (2 mL, 50:50) was added to give immediately a green precipitate. This was isolated by centrifugation, washed with H_2O and dried in vacuo (11.8 mg, 78%). − UV/Vis (CH_3CN): λ_{max} (ε M^{-1}cm^{-1}) = 226 (71800), 272 (49800), 810 (132). − ES MS; *m*/*z* (%): 510 (26) [Cu(Clip-Phen)]$^+$. − $C_{27}H_{21}CuF_{12}N_5O_2P_2$ (800.5): calcd. (+ 2 H_2O) C 38.7, H 2.98, N 8.37; found C 38.7, H 2.61, N 8.21.

Complex Formation with Ligands 1, 4, and 5: Molar equivalents of ligand and $Cu(OAc)_2 \cdot 2\ H_2O$ were dissolved separately in a small volume (1−2 mL) of warm methanol, then the solutions were mixed and left to stand for 2 h. A fivefold excess of NH_4PF_6 was then added as an aqueous solution to afford a precipitate. Acetone was slowly added until the solid dissolved and the solution was left open to the air for 24−48 h. Evaporation of the acetone afforded the product as a precipitate, which was isolated by centrifugation, washed with water and dried in vacuo.

[(1)Cu^{II}](PF$_6$)$_2$: Reaction of **1** and $Cu(OAc)_2 \cdot H_2O$ on a 0.024-mmol scale afforded 14.9 mg (74%) of product as a pale green solid. − UV/Vis (CH_3CN): λ_{max} (ε M^{-1}cm^{-1}) = 226 (55900), 276 (44400), 818 (135). − ES MS; *m*/*z* (%): 550.9 (100) [Cu(**1**)]$^+$. − $C_{29}H_{23}CuF_{12}N_5O_3P_2$ (842.5): calcd. (+ 0.5 CH_3COCH_3) C 41.9, H 2.97, N 8.03; found C 42.2, H 3.00, N 8.31.

[(4)Cu^{II}](PF$_6$)$_2$ · HPF$_6$: Reaction of **4** and $Cu(OAc)_2 \cdot H_2O$ on a 0.025-mmol scale afforded 17.0 mg (55%) of product as a bright

yellow solid. − UV/Vis (phosphate buffer, pH = 7.2): λ_{max} (ε $M^{-1}cm^{-1}$) = 226 (97600), 274 (89900), 394 (4200), 414 (6800), 437 (5500) and 814 (180). − ES MS; *m/z* (%): 799.3 (73) $[Cu(\mathbf{4})]^+$. − $C_{46}H_{40}CuF_{18}N_7O_3P_3$ (1236.5): calcd. C 44.6, H 3.23, N 7.88; found C 45.0, H 3.22, N 7.92.

$[(5)Cu^{II}](PF_6)_2 \cdot HPF_6$: Reaction of **5** and $Cu(OAc)_2 \cdot H_2O$ on a 0.025-mmol scale afforded 20.2 mg (62%) of product as a bright yellow solid. − UV/Vis (phosphate buffer, pH = 7.2): λ_{max} (ε $M^{-1}cm^{-1}$) = 226 (122000), 274 (122000), 426 (2900), 444 (2700) and 818 (146). − ES MS; *m/z* (%): 865.3 (100) $[Cu(\mathbf{5})]^+$. − $C_{47}H_{41}ClCuF_{18}N_7O_4P_3$ (1301): calcd. (+ CH_3COCH_3) C 44.2, H 3.45, N 7.21; found C 44.2, H 3.30, N 7.46.

DNA Cleavage Experiments: Reactions were carried out in a total volume of 20 μL (10 μL DNA and buffer + 5 μL complex + 5 μL MPA) containing 250 ng of Φx174 viral DNA (7nM, 19 μM in base pairs) in 40 mM sodium phosphate (pH = 7.2), 50 mM NaCl and 10 mM $MgCl_2$ (final concentrations). Complexes were prepared as 1mM stock solutions in DMF, which were then diluted to 4 μM with water, prior to being added to DNA solutions for 30 min at room temperature. DNA cleavage was initiated by addition of mercaptopropionic acid (5 μL, 20mM, final concentration 5mM) and samples were incubated at 37°C for 1 h. 7.5 μL of a solution of 50% (v/v) glycerol/40 mM Tris. HCl buffer (pH = 8) and 0.05% bromophenol blue (w/v) was then added and the samples were immediately loaded on agarose gel (0.8%) containing 1 μg/mL of ethidium bromide. Electrophoresis was carried out at constant current (25 mA) for 15 h in TBE buffer. Bands were located by UV light, photographed, and quantified by microdensity, rates being averaged from at least 3 different sets of experimental data. The correction coefficient 1.47 was used for the decrease in stainability of form I DNA versus forms II and III.[22] The average number of single-strand scissions per DNA molecule, expressed as μ, was considered to be equal to −ln(fraction of form I) according to a Poisson distribution.[23]

UV/Vis-Spectrophotometric Titrations: Solutions were prepared with the appropriate acridine derivative (4 μL of a 2.5 mM solution in DMF), calf-thymus DNA (diluted from a 1 mg/mL solution), NaCl (final concentration 50 mM), $MgCl_2$ (final concentration 10 mM) and phosphate buffer (pH = 7.2, final concentration 40 mM) and diluted with H_2O to a total volume of 1 mL. After equilibration for 24 h, spectra were recorded against an analogous blank solution containing the same concentration of DNA/NaCl/$MgCl_2$ and phosphate buffer. The $CuCl_2$ complexes of **4** and **5** were preformed for 30 min (4 μL of 2.5 mM ligand solution and 1 μL of 10 mM $CuCl_2$ solution) prior to dilution with the appropriate salts and DNA.

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