Preparation of the New Bis(phenanthroline) Ligand "Clip-Phen" and Evaluation of the Nuclease Activity of the Corresponding Copper Complex

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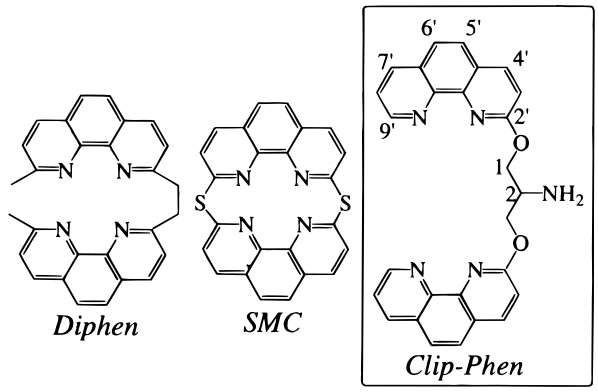
The best nuclease activities of 1,10-phenanthroline−copper complexes have been observed for a chelate/metal stoichiometry of 2/1. To favor this stoichiometry, the "Clip-Phen" ligand was synthesized with two 1,10-phenanthroline units linked via their C2-carbons by a short flexible arm. An exogenous amine function was present to allow future vectorization of this new chelating ligand. X-ray analysis of monocrystals of Clip-Phen confirmed its structure. An EPR study of (Clip-Phen)$CuCl_2$ showed that the cupric ion was pentacoordinated with a water molecule as the possible fifth ligand. The Cu(I/II) redox couple was found to be near 85 mV vs SCE, close to the redox couple of $(Phen)_2CuCl_2$. A comparison of the nuclease activity of copper complexes of Clip-Phen and 1,10-phenanthroline indicated that (Clip-Phen)Cu was the more active.

Introduction

1,10-Phenanthroline (Phen) is a well-known motif to prepare a large range of strong chelating ligands for various metal ions. Its complexing capability has been used to develop biomimetic models of metalloenzymes, to design analytical reagents, and to prepare supramolecules for molecular recognition and self-assembling systems.[1] Redox-active copper complexes of 1,10-phenanthroline derivatives, which are able to catalyze the single-strand cleavage of nucleic acids in the presence of dioxygen and a reductant, have been used as footprinting agents and conformational probes, or as more specific DNA cleavers when attached to intercalators, proteins, nucleic acids, or minor groove binders.[2−4] In fact, the design of artificial nucleases is a growing research area because major advancements in this field might have possible important applications as tools in molecular biology and as potential chemotherapeutic agents.[4]

However, the best nuclease activity has been observed for $(Phen)_2Cu^{II}$ complexes[2,3] and some problems result from this necessary 2/1 ratio for the chelate/metal stoichiometry:[5] (i) the association constant of $(Phen)Cu^{I}$ for the second 1,10-phenanthroline[6] is only $10^{5.5}$ M^{-1} and, to enhance the formation of $(Phen)_2Cu^{I}$ in the concentration range used in DNA cleavage experiments, a significant excess (often 10 equiv) of 1,10-phenanthroline is generally added. However, free phenanthroline is a weak competitive inhibitor of the binding of $(Phen)_2Cu^{I}$ to nucleic acids;[7] (ii) the reductants, used in large excess in DNA cleavage experiments for the activation of copper(II), e.g. thiols, can compete with 1,10-phenanthroline for copper coordination on $(Phen)Cu^{I}$, decreasing then the quantity of $(Phen)_2Cu^{I}$ available as cleaver; (iii) $(Phen)Cu^{I}$ has a residual cleavage activity, but its sequence preference is different from that of $(Phen)_2Cu^{I}$ and this phenomenon can complicate the analyses of the DNA cleavage pattern; (iv) the large majority of conjugates used as molecular biology tools included only one modified 1,10-phenanthroline ligand, which could significantly decrease their nuclease activity.[3,8]

Chart 1. Structures of Diphen, SMC, and Clip-Phen[a]



[a] Numbering corresponds to NMR assignments.

Synthesis of new ligands with two covalently bridged phenanthrolines might provide a solution to these problems. Few examples of stable copper complexes have been reported with these types of ligands, e.g. Diphen [1,2-bis(9-methyl-1,10-phenanthrolin-2-yl)ethane]; however, this ligand is derived from the inactive neocuproin.[9,10] The rigid SMC ligand, with two phenanthrolines doubly bridged by two sulfur atoms, has a very poor cleavage activity (see Chart 1 for structures of Diphen and

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SMC).[11] In addition, an oligonucleotide conjugate containing two 1,10-phenantholines linked by a very long 15-atom tether, in fact too long to have both Phen entities on the same copper ion, failed to increase efficiently the nuclease activity.[12]

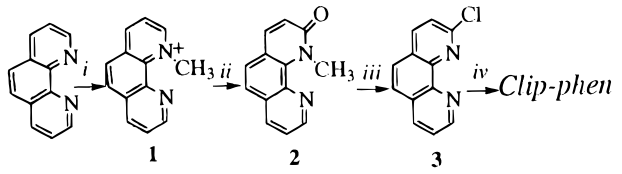
This paper describes the synthesis of the new ligand "Clip-Phen" (see Chart 1 for structure) with two 1,10-phenanthroline units linked via their C2-carbons by a short flexible arm adapted for having both Phen moieties on the same copper ion. Furthermore, this ligand has an exogenous primary amine to facilitate the attachment of different possible vectors in order to increase or to modulate the binding domain of these DNA cleavers. The X-ray structure of the Clip-Phen ligand was determined and confirmed the structure and the ability of Clip-Phen to behave as a strong chelating agent. Physicochemical studies provided data on its metalation with 1 equiv of copper(II) and on its electrochemical properties. The nuclease activity of (Clip-Phen)Cu^{II} was evaluated and compared to that obtained with $(Phen)_2Cu^{II}$.

Results and Discussion

Preparation of the Clip-Phen Ligand. The Clip-Phen ligand was designed in order to obtain a good crab-pincer for metal ions and to have an anchor for the fixation of a tether to link this potential DNA cleaver to different vectors to enhance and target its nuclease activity. The two 1,10-phenanthrolines were linked by taking into consideration the following criteria: (i) the bridge must be fixed on the C2-carbons of the 1,10-phenanthrolines, near the nitrogen atoms chelating copper, to be short; (ii) the length of this bridge must be sufficient to allow the flexibility necessary for conformational changes during the reduction of the corresponding Cu^{II} to Cu^{I}; (iii) this bridge must be functionalized to facilitate the attachment of different vectors without modification of the phenanthroline skeleton, and such functionalization of the bridge must be possible without inhibiting the ability of both phenanthroline groups to chelate copper or to interact with nucleic acids; (iv) the C9-carbon of Phen must be without functionalization to avoid a neocuproine-like ligand which has an affinity for nucleic acids but does not form a redox-active 2/1 complex (two ligands for one copper) due to steric interactions between the ortho methyl groups (which inhibit redox cycling through the square planar cupric complex); consequently Phen must be monofunctionalized on its C2-carbon; (v) and finally the new ligand must be as simple as possible and easy to prepare in large quantities if needed; therefore a C_2 symmetry is desired.

Clip-Phen was prepared in 72% yield in one step by condensation of 1 equiv of serinol (which is symmetric, is relatively short, and has an amine function) and 2 equiv of 2-chloro-1,10-phenanthroline,[13] which was easily prepared in large quantities from 1,10-phenanthroline in good yield (70% overall yield after the three steps) by the method of Halcrow[14] optimized by Lewis.[15] Scheme 1 summarizes this synthesis. Clip-Phen was easily purified by precipitation from the reaction mixture. The amine function allows further functionalization with DNA or RNA binders. All the intermediates of the Clip-Phen synthesis were characterized by proton NMR and mass spectrometry. Elementary analyses were correct.

Scheme 1. Preparation of Clip-Phen from Phenanthroline[a]



[a] Reagents: (i) $(CH_3)_2SO_4$ in C_6H_6; (ii) $K_3[Fe(CN)_6]$, NaOH (yield = 91% for (i) + (ii)); (iii) PCl_5 in $POCl_3$ (81%); (iv) serinol, NaH in DMF (72%).

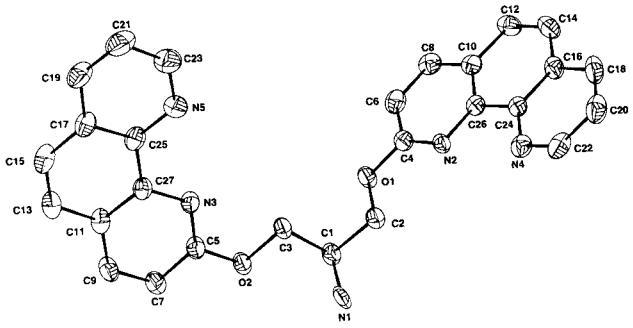


Figure 1. ORTEP drawing of Clip-Phen.

Structure Determination of Clip-Phen. X-ray data were collected on suitable monocrystals of Clip-Phen obtained by slow recrystallization of this ligand from methanol. Figure 1 shows the ORTEP drawing of Clip-Phen. The two phenanthroline units are perpendicular, and the amino group is statistically distributed on two sites with a 0.6/0.4 occupation ratio. Crystallizations of copper complexes of Clip-Phen are in progress.

Characterization of (Clip-Phen)Cu^{II}. The Clip-Phen ligand was metalated by using 1 equiv of $CuCl_2$. The corresponding polycrystalline green material was studied by electron spin resonance. The g values of (Clip-Phen)$CuCl_2$ were: $g_\perp = 2.14$ and $g_\parallel = 2.09$, but the solid was presumably not sufficiently magnetically diluted to yield ESR spectra indicative of the solid-state stereochemistry. On the other hand, an ESR spectrum was also obtained for 2.5 mM (Clip-Phen)$CuCl_2$ in a 80/20 mixture of CH_2Cl_2/DMF (v/v) glass at 100 K. The spectrum exhibited two identifiable magnetic g values along with the corresponding copper ($I = {}^3/_2$) hyperfine splitting constants with the following values: $g_\perp = 2.20$, $A_\perp = 70$ G; $g_\parallel = 2.055$, $A_\parallel = 110$ G. These g and A values were consistent with reported cases of trigonal bipyramidal copper(II) complexes and are comparable to values observed in the case of the $[(Diphen)Cu^{II}]^{2+}$ complex.[10] So, although no superhyperfine structures have been observed, it seems reasonable to propose that (Clip-Phen)$Cu^{II}Cl_2$ has a structure analogous to $[(Diphen)Cu^{II}]^{2+}$, with each phenanthroline unit of the Clip-Phen ligand providing an equatorial and an axial nitrogen atom to the copper(II) ion (the fifth ligand might be a water molecule as observed for (Diphen)Cu^{II}).

The Cu(II/I) redox potential of (Clip-Phen)$CuCl_2$ has been compared to the $(Phen)_2CuCl_2$ potential in aqueous solution since nucleic acid cleavage experiments are performed in water. Two different supporting electrolytes were used: (i) KCl, since cleavage experiments have been carried out in the presence of NaCl; (ii) KNO_3, because NO_3^- (like SO_4^- but unlike Cl^-) does not form strong complexes with cuprous ions.[6] Results are summarized in Table 1.

(Clip-Phen)$CuCl_2$ was more easily reduced than $(Phen)_2CuCl_2$ and had similar $E_{1/2}$ values, 85 and 45 mV, with both supporting

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Table 1. Comparison of Cyclic Voltammetry Data for (Clip-Phen)$CuCl_2$ and $(Phen)_2CuCl_2$, 0.4 mM in Water[a]

	$E_{1/2}$ (ΔE_p), mV	$E_{1/2}$ (ΔE_p), mV	no. of e^- exchanged[b]
(Clip-Phen)$CuCl_2$	85 (229)	47 (289)	1
$(Phen)_2CuCl_2$	−56 (68)	−42 (86)[c]	1
supporting electrolyte	KCl	KNO_3	KCl

[a] When unspecified, cyclic voltammetry was carried out at 0.1 V/s with 0.1 M supporting electrolyte under argon. $\Delta E_p = E_{p_{ox}} - E_{p_{red}}$. SCE was used as reference. [b] Carried out by coulometry. [c] Measured at 1 and 3 V/s because the oxidation of cuprous species was unobservable at 0.1 V/s.

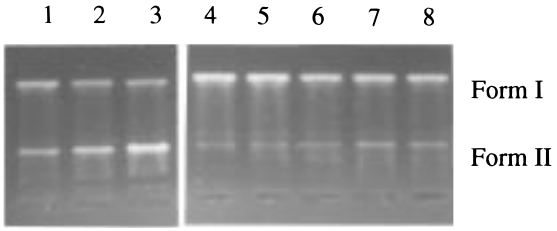

Figure 2. Comparison of ΦX 174 cleavage efficiency between Phen and Clip-Phen in the presence of 1 μM $CuCl_2$ and 5 mM MPA. Lane 1: 1 μM Phen. Lane 2: 2 μM Phen. Lane 3: 1 μM Clip-Phen. Lane 4: control DNA. Lane 5: control DNA plus 5 mM MPA. Lane 6: 1 μM $CuCl_2$, and 5 mM MPA without ligand. Lanes 7 and 8: 2 μM Phen and 1 μM Clip-Phen, respectively, with 5mM MPA but without $CuCl_2$.

electrolytes, KCl and KNO_3, respectively. These values are only 88 and 140 mV higher than that observed for $(Phen)_2CuCl_2$ under the same experimental conditions. These differences are less important than the $E_{1/2}$ differences (>400 mV) observed between $(Phen)_2Cu$ and the inactive (as DNA cleaver) neocuproine copper complexes.[6,16] So the presence of the bridge of Clip-Phen seems to have a limited influence on the redox activity of its phenanthroline units complexed to copper. However the Cu^{II}/Cu^{I} reduction of the Clip-Phen complex was only quasi-reversible since the ΔE_p values were 229 and 286 mV with KCl and KNO_3, respectively. This monoelectronic metal-centered process was confirmed by coulometry with KCl supporting electrolyte and by the observation of a large decrease in the EPR signal ($g = 2.102$ at 100 K) of (Clip-Phen)$Cu^{II}Cl_2$ after being reduced under the same conditions. There was no appearance of a new signal indicating the formation of a radical on the ligand (the same experiment was also carried out with $(Phen)_2CuCl_2$, and a similar result was obtained).

DNA Cleavage Activity. The DNA cleavage activity of the (Clip-Phen)Cu complex was compared to that of $(Phen)_nCu$. The concentration of copper ions was always 1 μM, and DNA cleavage was monitored by relaxation of supercoiled circular ΦX 174 DNA (form I) into nicked circular (form II) and linear (form III). Initiation of the redox activity of the copper complex was carried out by addition of 5 mM mercaptopropionic acid (MPA) in the presence of air. Figure 2 summarizes the results obtained with $CuCl_2$ as the copper source.

The comparison of 1/1 Clip-Phen/Cu (lane 3) with Phen−Cu complexes was carried out under two different conditions: Phen/Cu stoichiometries of 1/1 (lane 1) and 2/1 (lane 2), the latter case being formally equivalent to the stoichiometry of the phenanthroline units of Clip-Phen for copper, but it must noted that, with Phen, copper ions are only partially present in solution as the $(Phen)_2Cu^{I}$ complex (the more active form) since the affinity constants of copper for Phen have been calculated as being $10^{10.3}$ and $10^{5.5}$ M^{-1} for the first and the second 1,10-phenanthroline fixations, respectively.[6] Since the presence of a second equivalent of 1,10-phenanthroline increased the amount of single-strand breaks expressed as μ values (Table 2) (μ = 0.55 versus 0.88 in lanes 1 and 2, respectively), Clip-Phen−copper was clearly more active ($\mu = 1.4$ in lane 3). These results indicated that the binding of two phenanthroline units to copper, as with Clip-Phen, had a beneficial effect on the cleavage activity of the copper complexes and that this chelating effect compensated possible steric constraints resulting from the bridge between the C2-carbons of both phenanthroline units. These results were confirmed when $CuSO_4$ was used as the copper precursor (Table 2). In this case, the cleavage efficiency was also as follows: Clip-Phen/Cu > 2 Phen/Cu > Phen/Cu.

Table 2. Estimation of the FX 174 Cleavage Efficiency after 1 h at 37 °C in the Presence of 5 mM MPA[a]

	no copper	$CuCl_2$ (1 μM)	$CuSO_4$ (1 μM)
no ligand	0.07	0.08	0.09
Phen (1 μM)		0.55	0.25
Phen (2 μM)	0.12	0.88	0.67
Clip-Phen (1 μM)	0.09	1.4	1.05

[a] Single-strand breaks are expressed as μ values representing −ln(fraction of form I).

Experimental Section

General Methods. Proton and carbon-13 NMR spectra were recorded on Bruker 250 and 400 MHz instruments, respectively; $\delta^1H-\delta^1H$ GE-COSY and $\delta^1H-\delta^{13}C$ GE-HMQC (^1J and ^3J) correlations allowed NMR assignments. UV−vis spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer using cuvettes of 1 cm path length. EPR spectra were recorded on a Bruker ESP 300 in X-band, with an ER035 M gaussmeter and an EIP 548 hyperfrequencymeter. Chemical syntheses were monitored by thin-layer chromatography on Macherey-Nagel Alugram Sil G/UV 254, eluted by toluene/methanol, 7/3 (v/v), with saturation of the tank by ammonia, and spots were visualized with UV light (violet spots at 254 nm and blue fluorescence at 365 nm for Clip-Phen). DMF was dried over calcium sulfate. Other commercial reagents were used without purification.

2-Chloro-1,10-phenanthroline. This compound was prepared according to refs 14 and 15. Benzene was used as solvent in the methylation step (see below), and all our attempts to exchange it for safer solvents (toluene or dichloromethane) resulted in a dramatically decreased reaction yield. Briefly, 1,10-phenanthroline (10 g, 50.4 mmol) was methylated with dimethyl sulfate (6.4 mL, 67.8 mmol) in 100 mL of benzene to give 1-methylphenanthrolidiniumyl sulfate (**1**, Scheme 1). After removal of the solvent, **1** was oxidized with potassium ferricyanide (39.3 g, 119.3 mmol) in aqueous sodium hydroxyde to give 1-methyl-2-phenanthrolone, **2** (9.64 g, 45.9 mmol, yield = 91% for these two steps). **2** was then heated in 85 mL of phosphorus oxychloride with phosphorus pentachloride (12.14 g, 58 mmol) to give 2-chloro-1,10-phenanthroline, **3** (7.93 g, 37 mmol, yield = 81%).

2-Amino-1,3-bis(1′,10′-phenanthrolin-2′-yloxy)propane (Clip-Phen). To a solution of sodium hydride (1.49 g, 37.1 mmol) in 21.6 mL of dry DMF on an ice bath were added 2-chloro-1,10-phenanthroline (1.08 g, 5.0 mmol) and 2-amino-1,3-propanediol (230 mg, 2.5 mmol). After the mixture was stirred for 24 h and allowed to warm to room temperature, the crude product was dissolved in 65 mL of DMF and 22 mL of ethanol before being precipitated with 216 mL of water. After filtration and precipitation from 120 mL of hot methanol, the product was dissolved in chloroform and precipitated with hexane to give Clip-Phen as a pale yellow powder (817 mg, yield = 72%). ^1H NMR (CD_2Cl_2, 250 MHz): $\delta = 3.81$ (quintet, 1H, $J = 5.5$ Hz, H2), 4.87 (d, 4H, $J = 5.5$ Hz, H1), 7.19 (d, 2H, $J = 8.6$ Hz, H3′), 7.61 (dd, 2H, $J = 7.9$, 4.3 Hz, H8′), 7.69 (d, 2H, $J = 8.6$ Hz, H6′), 7.78 (d, 2H, $J =$

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8.7 Hz, H5′), 8.15 (d, 2H, J = 8.6 Hz, H4′), 8.27 (dd, 2H, J = 7.9, 1.8 Hz, H7′), 9.11 (dd, 2H, J = 4.3, 1.8 Hz, H9′). ^{13}C NMR (CD_2Cl_2, 100.62 MHz): δ = 49.80 (C2), 67.61 (C1), 113.11 (C3′), 121.93 (C8′), 123.12 (C6′), 125.59 (C5′), 135.42 (C7′), 138.40 (C4′), 148.96 (C9′). MS (CDI, NH_3), m/z (%): 448 (M + 1, 100), 252 (16), 225 (7), 197 (34), 152 (4), 102 (34). UV−vis (CH_3OH): 226 nm (ϵ = 78 400 mol^{-1} cm^{-1}), 274 (52 200) 332 (2700), 346 (1700). Anal. Calcd for $C_{27}H_{21}N_5O_2 \cdot {}^1/_3CHCl_3 \cdot {}^1/_2H_2O$: C, 66.15; H, 4.50; N, 14.11. Found: C, 66.05; H, 4.62; N, 14.12.

X-ray Analysis of Clip-Phen. X-ray diffraction analysis on Clip-Phen were carried out on a STOE IPDS (imaging plate diffraction system) equipped with an Oxford cryosystems cooler device. The crystal-to-detector distance was 80 mm; 125 exposures were obtained with $0 < \varphi < 250°$ and with the crystal oscillated through 2.0° in φ. Coverage of the unique set was over 94% complete to at least 2θ = 48.4°. Crystal decay was monitored by measuring 200 reflections per image. The final unit cell was obtained by the least-squares refinement of 5000 reflections using Mo Kα radiation (λ = 0.71073 Å). Only statistical fluctuations were observed in the intensity monitoring over the course of the data collection. No absorption corrections were applied to the data.

The structure was determined from a triclinic crystal of dimensions: 0.4 × 0.15 × 0.05 mm^3 (space group $P\bar{1}$), with unit cell a = 6.8882(1) Å, b = 12.707(2) Å, c = 14.663(2) Å, α = 91.77(2)°, β = 92.88(2)°, γ = 99.48(2)°, and V = 1259.6(2) Å^3. The unit cell contained two molecules; ρ_{calcd} = 1.35 g cm^{-3}, μ = 0.9 cm^{-1}, and F_{000} = 2931. A total 9988 reflections were measured (3732 independent) with $R_{average}$ = 0.04.

The structure was solved by direct methods (SIR92)[17] and refined by least-squares procedures on F_o. H atoms were located on a difference Fourier map, but they were introduced into the calculations in idealized positions (d(C−H) = 0.96 Å) and their atomic coordinates were calculated after each cycle of refinement. They were given isotropic thermal parameters 20% higher than those of the carbons to which they were attached. All non-hydrogen atoms were refined anisotropically. A disorder was observed on an N(1) atom; in fact, an electronic density statistically distributed on two sites was noted with a ratio of occ = 0.6/0.4 for this atom. Least-squares refinements were carried out by minimizing the function $\sum w(||F_o| - |F_c||)^2$, where F_o and F_c are the observed and calculated structure factors. A weighting scheme was used.[18] The model reached convergence with $R = \sum(||F_o| - |F_c||)/\sum|F_o|$ and $R_w = [\sum w(||F_o| - |F_c||)^2/\sum w(|F_o|)^2]^{1/2}$. The final R and R_w values were 0.054 and 0.065, respectively, for 2193 reflections ($I > 3\sigma(I)$) and 365 variables. The calculations were carried out with the aid of the CRYSTALS package programs[19] running on PC. The drawing of the molecule was realized with CAMERON[20] with thermal ellipsoids at the 30% probability level. The atomic scaterring factors were taken from ref 21. Further details of the crystal structure investigation are available on request from the Director of the Cambridge Crystallographic Data Centre, 12 Union Road, GB-Cambridge CB21EZ, U.K., on quoting the full journal citation.

Preparation of the Cu(II) Complex of Clip-Phen, (Clip-Phen)-$CuCl_2$. To a solution of Clip-Phen (10 mg, 22.37 μmol) in dry DMF (2.24 mL) was added $CuCl_2$ (3 mg, 22.37 μmol). After 4 h of stirring at room temperature, the product was precipitated with diethyl ether to afford (Clip-Phen)$CuCl_2$ as a pale green powder (11.39 mg, 92%). MS (ES, positive mode), m/z: = 547.0 (monocation, M − Cl), 509.0 (cuprous complex), 313.0, 252.0. UV−vis (CH_3OH): 226 nm (ϵ = 67 700), 276 (50 000), 332 (3400, sh), 346 (2000, sh), 704 (140). Anal. Calcd for $C_{27}H_{21}N_5O_2CuCl_2 \cdot {}^1/_2H_2O$: C, 54.88; H, 3.75; N, 11.85. Found: C, 54.72; H, 3.88; N, 11.94. EPR data: from solid state, $g_\perp$ = 2.14, $g_\parallel$ = 2.09; from a DMF/CH_2Cl_2 glass at 110 K, $g_\perp$ = 2.20 ($A_\perp$ = 70 G), $g_\parallel$ = 2.055 ($A_\parallel$ = 110 G).

Electrochemistry of (Clip-Phen)$CuCl_2$. Electrochemical measurements were carried on a homemade potentiostat using the interrupt method to minimize the uncompensated resistance (*IR*) drop.[22] Electrochemical experiments were performed at room temperature in an airtight three-electrode cell connected to a vacuum argon/N_2 line. The reference electrode consisted of a saturated calomel electrode (SCE) separated from the solutions by a bridge compartment. The counter electrode was a spiral of ca. 1 cm^2 apparent surface area, made of Pt wire 8 cm long and 0.5 mm in diameter. The working electrode was Pt (1 mm) for cyclic voltammetry. For electrolysis experiments, a Pt gauze was used. The supporting electrolyte was KCl or KNO_3 (Prolabo RP, Normapur), and both salts were used as received. All solutions measured were 0.4 mM in copper complex and 0.1 M in supporting electrolyte. All experiments were conducted under an argon atmosphere. Stock solutions (2 mM) of (Clip-Phen)$CuCl_2$ and (Phen)$_2CuCl_2$ were prepared in water (Clip-Phen became soluble in water at 2 mM concentration after complexation with $CuCl_2$).

DNA Cleavage Experiments. To 10 μL of ΦX 174 (7 nM, 40 μM in bp) in 80 mM sodium phosphate buffer (pH 7.2), 100 mM NaCl, and 20 mM $MgCl_2$ was added 5 mL of desired complex (4 μM). Metalations were carried out using a 1 mM $CuCl_2$ (or $CuSO_4$) concentration with 1 or 2 equiv of ligand in a DMF/water mixture (1/1, v/v) for 4 h at room temperature before dilution of the complex with water to 4 μM. After 30 min at room temperature, cleavage was initiated by addition of an aqueous solution of mercaptopropionic acid (5 μL, 20 mM), and samples were incubated 1 h at 37 °C under aerobic conditions. A 7.5 μL portion 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 samples were immediately loaded onto agarose gel (0.8%) containing 1 μg/mL ethidium bromide. Electrophoreses were run at constant current (25 mA for 15 h) in TBE buffer. Bands were located by UV light (254 nm), photographed, and quantified by microdensity. The correction coefficient of 1.47 was used for decreased stainability of form I DNA vs forms II and III. 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]

Abbreviations. Phen = 1,10-phenanthroline (oP has also been used as an abbreviation in many articles devoted to DNA cleavage mediated by bis(phenanthroline)copper; MPA = 3-mercaptopropionic acid; TBE = Tris−borate−EDTA buffer.

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Supporting Information Available: Tables giving crystal data and details of the structure determination of Clip-Phen, all atom coordinates, bond lengths, bond angles, and anisotropic thermal parameters (9 pages). Ordering information is given on any current masthead page.

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