DNA Cleavage by Copper Complexes of 2- and 3-Clip-Phen Derivatives

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The DNA cleavage activity of copper complexes of phenanthroline has been increased by the use of "2-Clip-Phen" and "3-Clip-Phen" ligands containing two phenanthroline entities linked by a serinol bridge. In order to optimize DNA cleavage activity, two series of new Clip-Phen ligands (L) with two phenanthroline residues linked through their C-2 or C-3 carbon atoms by different bridges have been synthesized. The physicochemical properties and the DNA cleavage activities of the corresponding (Clip-Phen)copper complexes were investigated in the presence of a reductant and air. X-ray analyses of single crystals showed that the cupric complexes of Clip-Phen were able adopt dimeric double-helical geometries with L_2Cu_2 stoichiometries in the solid state. These complexes adopt different geometries and stoichiometries in solution, with monomeric LCu species predominant. The compounds with a bridge connected at C-2 are less active as DNA cleavage agents than complexes with the bridge at the C-3 positions. Copper complexes with a bridge containing three methylene units appear to give the best results in DNA cleavage experiments. This activity is increased in the case of 3-Clip-Phen·$CuCl_2$ by the presence of the primary amine function of the serinol bridge. This amine function is probably coordinated to the copper atom in the case of 2-Clip-Phen·$CuCl_2$, decreasing the reactivity toward DNA.

Introduction

1,10-Phenanthroline (Phen) is a well-known motif for the preparation of a large range of strongly chelating ligands for various metal ions. The complexing capability of this easily accessible ligand has been used to develop biomimetic models of metalloenzymes, to design analytical reagents, cleavers, or conformational probes for nucleic acids, or to prepare supramolecules for molecular recognition and self-assembling systems.[1−13]

Redox-active copper complexes of phenanthroline are efficient artificial nucleases.[13−15] This versatile ligand is able to chelate copper ions to give mono- or bis(Phen)-copper complexes in oxidation states +I or +II. These interact efficiently with double-stranded DNA from the minor groove, probably by partial intercalation.[20,21] In the presence of hydrogen peroxide the $(Phen)_2Cu^{I}$ complex mediates single-strand cleavage of double-stranded DNA by oxidative attacks at C-1′ and C-4′ of 2-deoxyribose units.[13,16−18] Hydrogen peroxide can be generated in close proximity to DNA by $(Phen)_2Cu^{II}$ itself in the presence of a reductant and molecular oxygen.

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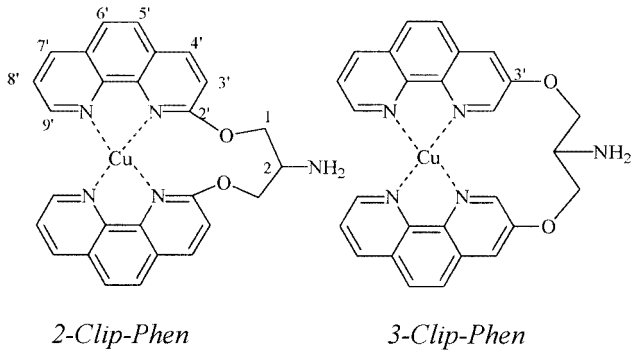
$(Phen)_2Cu^{I}$ is significantly more active than $(Phen)Cu^{I}$, but the association constant for the second phenanthroline ligand is only $10^{5.5}$ M^{-1}, too low for biological use at submicromolar concentrations of these complexes.[22] For this reason, we have recently prepared 2- and 3-Clip-Phen derivatives (Scheme 1) containing two Phen ligands linked through their 2′- or 3′-positions by a serinol bridge in order to favor the coordination of two Phen units around the same copper ion.[23,24] The oxidative nuclease activity of the copper complexes of these new ligands was found to be higher than with Phen itself; by a factor of 2 for 2-Clip-Phen, but by a factor of 60 for 3-Clip-Phen. The nuclease activity of 3-Clip-Phen·$CuCl_2$ is due to C-1′, C-4′, and C-5′ deoxyribose oxidations resulting from the cleavage interaction within the DNA minor groove.[25] 3-Clip-Phen·$CuCl_2$ itself appeared to be poorly sequence-selective, but the exogenous amine of its serinol bridge allowed the preparation of a conjugate with an analogue of distamycin (a minor groove binder that interacts selectively with five successive A·T base pairs) that appeared to be a sequence-selective DNA cleaver.[25,26] Conjugates of 2-Clip-Phen with the natural polyamine spermine (a minor groove binder)[27] or intercalators such as acridine derivatives[28] also exhibit enhanced nuclease activity.

Copper complexes of Clip-Phen have high DNA cleavage activity. This activity is preserved (and sometimes enhanced) when conjugated to a variety of vectors, which also retain their previous biological characteristics. This remarkable combination of properties opens wide perspectives for

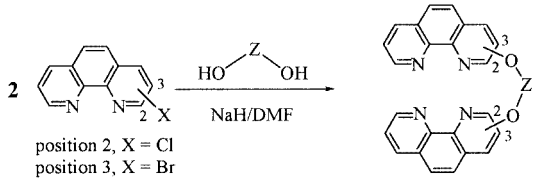


Scheme 1. Structures of 2-Clip-Phen and 3-Clip-Phen; numbering corresponds to NMR assignments; the type of coordination with Cu attempted from the design of the ligands is shown

the use of Clip-phen derivatives. The compounds can be used as biological tools to study DNA structures, as new DNA footprinting agents or as potential antitumoral or antiviral agent mimics of iron bleomycin, a well-known antitumoral drug able to cleave the DNA of cancer cells.[29,30] In this context, Clip-Phen's properties of cell penetration and specificity for DNA might be modulated by the preparation of conjugates incorporating many different vectors.

The serinol bridge included in 2- and 3-Clip-Phen was initially chosen because of a number of different parameters: (i) its length was well adapted to favor "crab-pincer" copper complexes with two Phen entities of the same ligand chelating the same copper atom, (ii) its flexibility allowed the conformational changes that occur during Cu^{I}/Cu^{II} redox cycles, (iii) its amine function facilitated the synthesis of conjugates without modification of the Phen chelating entities, (iv) its C_2 symmetry axis avoided the formation of stereoisomers, and (v) its commercial accessibility. Both 2- and 3-Clip-Phen do, however, contain the same junction bridge even though the steric constraints in their copper complexes are not the same. On the other hand, a comparison of their physicochemical properties needs to be done.

Our interest in improving the efficiency of the Clip-Phen·Cu scission reagents prompted us to synthesize new Clip-Phen ligands with different short bridges between the two Phen entities in order to optimize the linker parameters (Figure 1). The length of the linker between C-2 or C-3 of the two Phen moieties was varied from two to five methylene units (ethyl-, propyl-, butyl-, or pentyl-Clip-Phen derivatives). Larger variations in the linker length were tested in the case of the 3-Clip-Phen series, on the assumption that that these ligands were sterically more constrained than the 2-Clip-Phen ones, in order to produce "crab-pincer" copper complexes. The influence of the primary amine of Clip-Phen on the nuclease activity was also studied, as was that of amide residues such as those included in conjugates with different vectors, by comparison with acetyl-Clip-Phen derivatives. In this work the geometry and the redox activity of these new copper complexes of phenanthroline have been analyzed under conditions similar to those used for DNA cleavage experiments. Determination of their respective DNA cleavage efficiency was performed. This study allows the parameters increasing the DNA cleavage activity of copper complexes of Clip-Phen to be clearly defined and provides information concerning the different types of complexes that can be generated in solution with the same ligand.



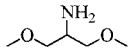
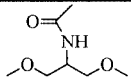
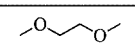
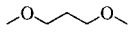
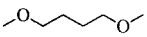
−O−Z−O−	Bridge on C2 of Phen	Bridge on C3 of Phen
	**1** 2-Clip-Phen	**5** 3-Clip-Phen
	**2** 2-acetyl-Clip-Phen	**6** 3-acetyl-Clip-Phen
	**3** 2-ethyl-Clip-Phen	**7** 3-ethyl-Clip-Phen
	**4** 2-propyl-Clip-Phen	**8** 3-propyl-Clip-Phen
	-	**9** 3-butyl-Clip-Phen
	-	**10** 3-pentyl-Clip-Phen

Figure 1. Structure of Clip-Phen derivatives **1**−**10** and general reaction pathways used for the preparation of Clip-Phen ligands

Results and Discussion

Preparation of the Ligands

The compounds **1**, **3**−**5**, and **7**−**10** were prepared by condensation of 1 equiv. of diol and 2 equiv. of halogenated phenanthroline (2-chloro or 3-bromo derivatives). Products substituted on C-2 of phenanthroline were obtained in good yields at 4 °C (70−80%). Products substituted at C-3, however, appeared to be more difficult to be prepared and were only obtained in poor yields (20−30%). As previously observed for the synthesis of 3-Clip-Phen (**5**), the low reactivity of 3-bromophenanthroline towards nucleophilic substitution necessitated a reaction temperature of 55 °C, and a proportion of the 3-BrPhen was lost due to a reduction process, giving back phenanthroline itself.[24] Ligands **2** and **6** were easily obtained by acetylation of 2- and 3-Clip-Phen, respectively (yield ca. 90%).

Characterization of the Copper Complexes

Ligands **1**−**10** were metallated with 1 equiv. of $CuCl_2$ in a DMF/methanol mixture and then precipitated with diethyl ether to produce polycrystalline green materials. We chose to use a copper chloride salt, since other DNA cleavage experiments have been carried out in the presence of NaCl in order to mimic physiological conditions. Analytical data are consistent with the formation of L/Cu^{II} 1:1 complexes,

but the coordination chemistry of the Clip-Phen family of ligands (L) with copper ions appeared to be more complicated than expected.

Crystal Structures

Copper complexes of Clip-Phen derivatives were unfortunately difficult to crystallize, and several attempts with 1 equiv. of different copper salts and various solvents were performed in order to obtain monocrystals of the copper complexes of **1**−**10** suitable for X-ray analysis. After several unsuccessful attempts, it was found to be possible to obtain suitable monocrystals of **1**·$Cu(BF_4)_2$, **3**·$CuCl_2$, **7**·$CuCl_2$, and also **1**·$ZnCl_2$ (Figures 2, 3, 4, and 5, respectively).

The $Cu(BF_4)_2$ complex of 2-Clip-Phen (**1**) crystallized on slow diffusion of CH_2Cl_2 into a solution of the complex, dissolved in a DMF/CH_3OH mixture, as a double-helical structure of general formula bis[(**1**)Cu][BF_4]$_2$ (Figure 2). Table 1 presents selected average bond lengths and angles of this complex. Each copper center shows the same coordination geometry and can be described as a distorted square-planar-pyramidal arrangement around the metal ion with the aliphatic amine and two phenanthroline residues (one from each Clip-Phen derivative ligand of the double helix). The equatorial positions are occupied by the aliphatic amine and three nitrogen atoms of the phenanthroline residues, with similar bond lengths of 2.02−2.05 Å. The axial bond length at 2.18 Å is longer. The phenanthroline nitrogen atom in the α-position to the bridge is equatorial. However, the angles in the crystal structure deviate from ideal values for a square-planar pyramid, although EPR results are in accordance with this geometry (see Exp. Sect. for these EPR values). Accordingly, the geometry could also be described as a distorted trigonal bipyramid with each nitrogen atom of a phenanthroline residue occupying one axial and one equatorial position.

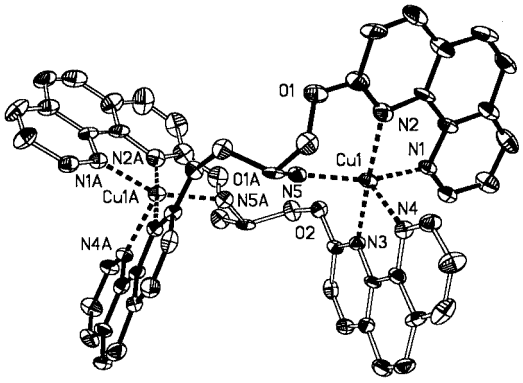


Figure 2. Crystal structure of bis[(**1**)Cu][BF_4]$_2$; hydrogen atoms, non-coordinated counter-ions, and solvent molecules are omitted for clarity

The double-helical motif of copper(II) complexes of Clip-Phen derivatives is also observed in the structures of **3**·$CuCl_2$ and **7**·$CuCl_2$, but with some major differences. The $CuCl_2$ complex of 2-ethyl-Clip-Phen (**3**) crystallizes in aqueous methanol as a double-helical structure of general formula [(**3**)CuCl]$_2$Cl$_2$ (Figure 3). Table 2 presents selected average bond lengths and angles of this complex. The coordination geometries are similar for each metal center and can be described as a distorted trigonal-bipyramidal arrangement with one chloride ion and two phenanthroline residues (one from each 2-ethyl-Clip-Phen ligand of the double-helix) around the copper atom. Each phenanthroline group occupies one axial and one equatorial position. The nitrogen atom in the α-position to the bridge is in an axial position for one phenanthroline residue and equatorial for the other. The third equatorial position is occupied by the chloride ion, with close Cu−Cl distances of 2.27 and 2.32 Å, respectively, for each copper center. Each coordinated chloride ion points in opposite direction of the double helix. The bond lengths between the copper atom and the phenanthroline nitrogen atoms are approximately 2.0 Å. The N−Cu−N angle formed by the nitrogen atoms of one phenanthroline residue and the central atom is smaller than the ideal value of 90° for a trigonal-bipyramid geometry, but the observed values of approximately 80° are typical for copper complexes of phenanthroline.[31] In addition, the axial Cu−N distances are always slightly compressed, consistently with other known trigonal-bipyramidal bis(phenanthroline)Cu^{II} crystal structures.[32]

Table 1. Selected bond lengths [Å] and angles [°] for bis[(**1**)Cu][BF_4]$_2$

Cu−N(1)	2.014(9)
Cu−N(2)	2.051(10)
Cu−N(3)	2.051(8)
Cu−N(4)	2.180(8)
Cu−N(5)	2.005(9)
N(5)−Cu−N(1)	153.6(4)
N(5)−Cu−N(2)	93.0(4)
N(1)−Cu−N(2)	81.7(4)
N(5)−Cu−N(3)	90.2(3)
N(1)−Cu−N(3)	93.7(4)
N(2)−Cu−N(3)	175.0(4)
N(5)−Cu−N(4)	111.6(3)
N(1)−Cu−N(4)	94.7(3)
N(2)−Cu−N(4)	103.7(4)
N(3)−Cu−N(4)	78.7(3)

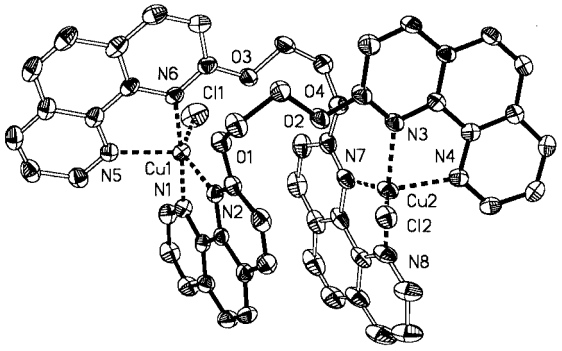


Figure 3. Crystal structure of [(**3**)CuCl]$_2$Cl$_2$; hydrogen atoms, non-coordinated counter-ions, and solvent molecules are omitted for clarity

Table 2. Selected bond lengths [Å] and angles [°] for [(**3**)$CuCl]_2Cl_2$

Cu(1)−N(1)	1.985(5)	Cu(2)−N(3)	1.981(4)
Cu(1)−N(6)	1.987(4)	Cu(2)−N(8)	1.984(5)
Cu(1)−N(5)	2.140(5)	Cu(2)−N(4)	2.139(5)
Cu(1)−N(2)	2.141(5)	Cu(2)−N(7)	2.169(5)
Cu(1)−Cl(1)	2.275(2)	Cu(2)−Cl(2)	2.3229(15)
N(1)−Cu(1)−N(6)	175.17(18)	N(3)−Cu(2)−N(8)	174.13(19)
N(1)−Cu(1)−N(5)	94.70(18)	N(3)−Cu(2)−N(4)	81.00(17)
N(6)−Cu(1)−N(5)	81.03(18)	N(8)−Cu(2)−N(4)	94.27(19)
N(1)−Cu(1)−N(2)	80.44(18)	N(3)−Cu(2)−N(7)	97.31(19)
N(6)−Cu(1)−N(2)	98.98(18)	N(8)−Cu(2)−N(7)	80.5(2)
N(5)−Cu(1)−N(2)	111.57(16)	N(4)−Cu(2)−N(7)	104.74(17)
N(1)−Cu(1)−Cl(1)	92.20(14)	N(3)−Cu(2)−Cl(2)	92.59(14)
N(6)−Cu(1)−Cl(1)	91.16(14)	N(8)−Cu(2)−Cl(2)	92.38(15)
N(5)−Cu(1)−Cl(1)	106.20(13)	N(4)−Cu(2)−Cl(2)	111.42(13)
N(2)−Cu(1)−Cl(1)	141.94(13)	N(7)−Cu(2)−Cl(2)	143.58(12)

Ligands **3** and **7** are isomers differing only in the position of the bridge − at C-2 or C-3 of the phenanthroline group in the cases of **3** and **7**, respectively. Figure 4 provides a view of the $CuCl_2$ complex of 3-ethyl-Clip-Phen (**7**). Because of the poor quality of the very small and weakly diffracting crystals, we were not able to refine the structure satisfactorily. Nevertheless, our model is good enough for discussion of the main features of this complex. Complex **7**·$CuCl_2$ crystallizes in a DMF/methanol (2:1) mixture in the form of a double-helical structure of general formula [(**7**)$CuCl]_2Cl_2$. In the case of [(**7**)$CuCl]_2Cl_2$ − as in that of [(**3**)$CuCl]_2Cl_2$ − the coordination geometries of each copper center are similar and are best described as a distorted trigonal-bipyramidal arrangement with one chloride ion and two phenanthroline residues (one from each ethyl-Clip-Phen ligand of the double-helix) around the copper atom. The main difference is the Cu···Cu distances, 11.00 Å in [(**7**)$CuCl]_2Cl_2$ and 6.48 Å in [(**3**)$CuCl]_2Cl_2$.

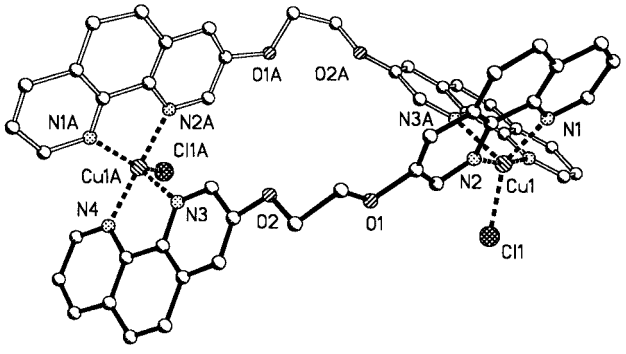


Figure 4. Complex of [(**7**)$CuCl]_2Cl_2$ in the crystalline solid state; hydrogen atoms, non-coordinated counter-ions, and solvent molecules are omitted for clarity

The double-helix units in all L_2Cu_2 copper complexes are chiral, but the three structures are in centrosymmetric space groups, which means that there is a racemic mixture of left- and right-turning helices present in the crystal.

Since copper(II) complexes of Clip-Phen derivatives are difficult to crystallize, we attempted to obtain more information on their geometry through the preparation of the corresponding zinc(II) complexes. We chose Zn^{II} since its coordination behavior and ionic radius are very close to those of Cu^{II} and because it − like Cu^{II} − forms penta- and hexadentate complexes, which could be isostructural to the Cu^{II} ones. Major differences, but also some interesting similarities, could be observed on comparison of the copper and zinc complexes of the same 2-Clip-Phen ligand **1**. Complex **1**·$ZnCl_2$ crystallizes in DMSO as a monomeric structure of general formula (**1**)$ZnCl_2$ (Figure 5). Table 3 presents selected average bond lengths and angles of this complex. The $P\bar{1}$ spatial group is centrosymmetric, in accordance with a racemic mixture in which the two enantiomers exits in the crystal in 1:1 proportions. As would be expected from the ionic radii of the divalent metal ions, the average of the Zn^{II}−N bonds is longer than those involving Cu^{II}. The coordination geometry is again best described as a distorted trigonal-bipyramidal arrangement around the metal atom, and the phenanthroline residue again occupies one axial and one equatorial position. Only one Phen residue of the ligand is coordinated; however; the other positions around the zinc(II) ion are occupied by two chloride ions in equatorial positions and the primary amine of the bridge in an axial one. The differences in geometry between **1**·$Cu(BF_4)_2$ and **1**·$ZnCl_2$ could easily be explained by the exchange of Cu^{II} by Zn^{II} and also the presence of chloride ions, which are better ligands than tetrafluoroborate, but in any case the coordination of the amine of the bridge of 2-Clip-Phen is strong and competes with the coordination of the second Phen residue of the ligand.

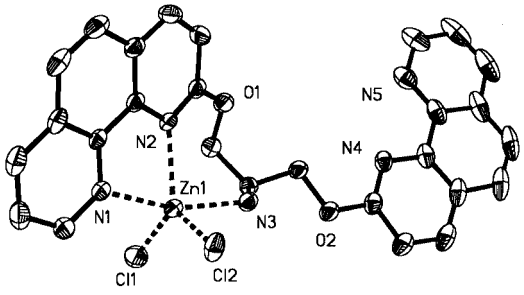


Figure 5. Crystal structure of (**1**)$ZnCl_2$; hydrogen atoms and solvent molecules are omitted for clarity

Mass Analysis

It is remarkable that, although we could legitimately assume that copper(II) complexes of Clip-Phen adopted a double-helical geometry with a copper/ligand stoichiometry of 2:2, these species were particularly difficult to observe by mass spectrometry (Figure 6). Electrospray mass spectra of the different complexes generally reflected a 1:1 copper/ligand stoichiometry. Analyses were conducted in a CH_3OH/H_2O 1:1 mixture, chosen because our DNA cleavages were performed in aqueous solution. High concentrations of $CuCl_2$ complexes (10^{-4} and 10^{-5} M) and different orifice voltages (50, 5, but also 0 V) were tested with the goal of promoting the observation of L_2Cu_2 complexes (see ref.[9]

Table 3. Selected bond lengths [Å] and angles [°] for (**1**)$ZnCl_2$

Zn(1)−N(3)	2.1294(16)
Zn(1)−N(1)	2.1431(16)
Zn(1)−N(2)	2.2645(16)
Zn(1)−Cl(1)	2.2929(6)
Zn(1)−Cl(2)	2.3452(6)
N(3)−Zn(1)−N(1)	161.42(7)
N(3)−Zn(1)−N(2)	88.34(6)
N(1)−Zn(1)−N(2)	75.73(6)
N(3)−Zn(1)−Cl(1)	98.82(5)
N(2)−Zn(1)−Cl(1)	121.81(4)
N(3)−Zn(1)−Cl(2)	88.48(5)
N(1)−Zn(1)−Cl(2)	89.90(5)
N(2)−Zn(1)−Cl(2)	115.77(4)
Cl(1)−Zn(1)−Cl(2)	122.05(2)

and references therein for a discussion about these analytical conditions generally best used in order to promote the detection of double-helical structures by electrospray mass spectrometry). The 1:1 stoichiometry was confirmed by comparison with theoretical isotopic patterns, which could be analyzed unambiguously thanks to the presence of $^{63/65}Cu$ and $^{35/37}Cl$ isotopes, the *m*/*z* values being determined from the spacing between two isotope peaks.

The LCu^+ species was always observed, in agreement with the expected reduction of Cu^{II} in a mass spectrometer.[33] The monocationic species $LCu^{II}Cl$, however, was also clearly observed; LCu^{2+} species only being observed at lower activation voltages. The L_2Cu_2 species with or without chloride ion(s) or different charge states, though, were only observed − and only as minor products − for concentrated solutions with a low orifice voltage. It is reasonable to speculate that the dimeric species are poorly stable in solution and dissociate during analysis, or that they are absent or only present in small quantities in solution and are, in this last case, in equilibrium with monomeric LCu species in solution. No direct evidence for this last hypothesis was obtainable, but it seems reasonable since equilibria between monomeric and dimeric copper complexes of bis(phenanthroline) ligands have been observed previously, during the synthesis of a trefoil knot for example.[34] The geometries of monomeric species are probably similar to those of other previously described pincer-crab bis(phenanthroline)-copper complexes.[32]

It should be noted that different attempts were made to perform ^1H NMR analysis of cuprous complexes of **1**, **2**, **4**, or **5** (obtained by reduction of deaerated solutions of the cupric complexes in DMSO/H_2O mixtures by addition of ascorbate) or zinc complexes of the same ligands. The ^1H NMR spectra showed broader signals at room temperature, sharpening when the temperature increased, consistent with mixtures of species in rapid exchange on the NMR timescale (results not shown). Only the spectra of **1**·$ZnCl_2$ seem to be resolved at room temperature, but these also showed complex signals for the bridge protons, and irradiation experiments also confirmed the presence of different species in solution in this case (see the Exp. Sect. for these NMR spectroscopic data).

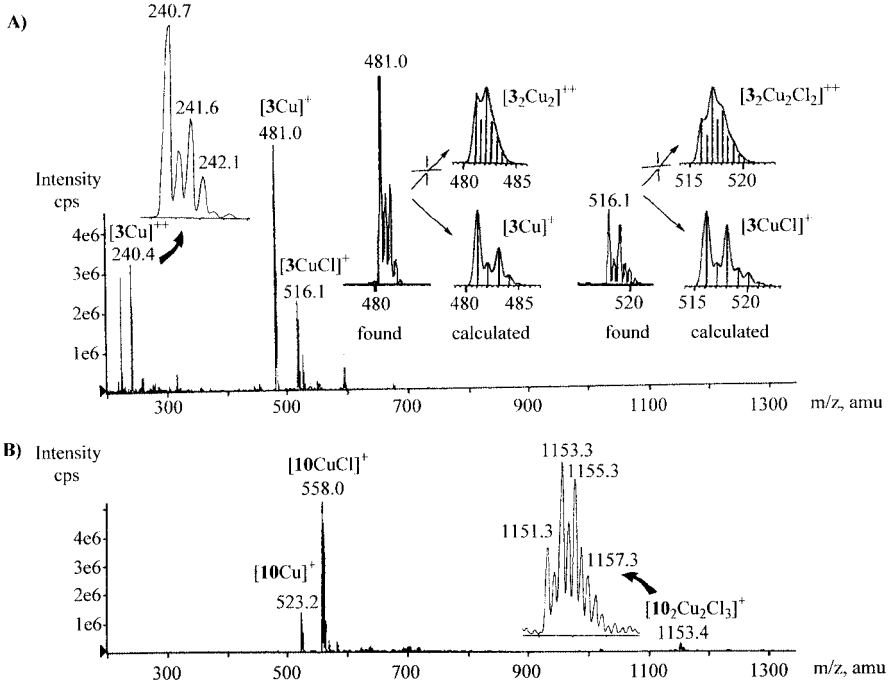


Figure 6. Typical examples of electrospray mass spectra analyses of copper(II) complexes of Clip-Phen derivatives in CH_3OH/H_2O (1:1): A) mass spectrum analysis of 2-ethyl-Clip-Phen·$CuCl_2$ (**3**·$CuCl_2$) (10^{-4} M, orifice voltage = 5 V); B) observation of traces of L_2Cu_2 species in the case of 3-pentyl-Clip-Phen·$CuCl_2$ (**10**·$CuCl_2$) (10^{-4} M, orifice voltage = 0 V)

It cannot be discounted that the copper complexes of Clip-Phen derivatives may adopt different geometries or stoichiometries in solution and that the relative proportions of these species could vary with the concentrations of the complexes (with the monomeric species being favored in dilute solutions, such as those used for DNA cleavage experiments).

As observed in the single-crystal analyses, L_2Cu_2 cupric complexes can form left- and right-turning double-helices. Cuprous and cupric LCu monomeric complexes, however, could form Λ and Δ enantiomers. Separations of these enantiomers have not been performed, but from model studies the two enantiomers probably have different DNA-binding modes. However, such studies are well behind the scope of this paper (for recent studies on DNA interaction with enantiomeric metal complexes, and metal helicates in particular, see refs.[35,36] and references therein).

EPR Data

Copper(II) complexes of the Clip-Phen derivatives were analyzed by electron spin resonance (Table 4). None of the complexes was magnetically sufficiently diluted to yield EPR spectra indicative of the solid-state geometry. X-EPR spectra of these green polycrystalline samples are best described as isotropical signals centered at $g = 2.12-2.14$ (except for **1**·$CuCl_2$ and **10**·$CuCl_2$, for which two g values were observed, reflecting axial symmetry). Superhyperfine coupling to nitrogen was not apparent but hyperfine coupling constants were obtained in DMF/CH_2Cl_2/CH_3OH (2:4:1) and H_2O/$C_2H_6O_2$ (1:2) glasses. Two g values were generally observed, in accordance with an axial symmetry of the g tensor, but different families of complexes could be observed.

The spectrum of the cupric complex of 2-ethyl-Clip-Phen (**3**·$CuCl_2$) was not well resolved in the two types of glasses tested, but the spectra of the cupric complexes of all the other compounds substituted at C-2 of the phenanthroline residues and in which the linker contained three carbon atoms (ligands **1**, **2**, and **4**) were well resolved. They had similar geometries in DMF/CH_2Cl_2/CH_3OH glass, since they exhibited similar EPR spectra consistent with a single configuration for a randomly oriented sample. The g and A values are in agreement with reported cases of trigonal-bipyramidal copper(II) complexes.[32] They exhibit two identifiable magnetic g values, together with the corresponding copper ($I = 3/2$) hyperfine splitting constants. The following values were observed: $g_{\|} = 2.20-2.23$, $A_{\|} = 100-110$ G, $g_{\perp} = 2.01$, $A_{\perp} = 78-90$ G. The parallel or perpendicular attribution of g values did not appear clearly in the spectra but was confirmed by integration of the corresponding signal area, the $g_{\|}$ area being, as expected, roughly twice that of the $g_{\perp}$ one. Crystallographic data obtained previously confirm that the complexes could adopt this trigonal-bipyramidal geometry. The fifth ligand might be a Cl^- or a water molecule, but the primary amine function can also play this role in the particular case of 2-Clip-Phen (**1**).

It is remarkable that these cupric complexes can adopt different geometries and that their geometries change in more polar media, as observed in H_2O/$C_2H_6O_2$ glasses. The resolution of the EPR spectra was poor, probably due to the presence of different conformations, but these spectra indicated that copper complexes of **1**, **2**, and **4** would be able to adopt geometries similar to those of cupric complexes of 3-Clip-Phen in water solution (the solvent used for DNA cleavage experiments).

The cupric complexes of 3-Clip-Phen derivatives **5**−**10** probably have poorer capabilities to change their geometries than the 2-Clip-Phen ones, since the EPR spectra for all these complexes are nearly superimposable in the two media tested. The spectra of **5**−**10**·$CuCl_2$ are consistent with a single configuration for a randomly oriented sample. They were in agreement with classical five- or six-coordinated copper(II) in a square-pyramidal or octahedral geometry with four equatorial ligands in a deformed square-planar geometry.[36] Two identifiable magnetic g values were observed, along with the corresponding copper ($I = 3/2$) hyp-

Taable 4. EPR data of **1**−**10**·$CuCl_2$ complexes

Complex	Solid	DMF/CH_2Cl_2/CH_3OH glass					H_2O/$C_2H_6O_2$ glass		
		$g_{\|}$	$A_{\|}$ (G)	$g_{\perp}$	$A_{\perp}$ (G)	$g_{\|}$	$A_{\|}$ (G)	$g_{\perp}$	$A_{\perp}$ (G)
1·$CuCl_2$	$g_{\|} = 2.14$ $g_{\perp} = 2.09$	2.20	110	2.005	78	2.26	bad resolved	2.07	bad resolved
2·$CuCl_2$	$g_{iso} = 2.14$	2.22	102	2.01	90	2.29	160	2.08	105
3·$CuCl_2$	$g_{iso} = 2.12$	unresolved complex signal					unresolved complex signal		
4·$CuCl_2$	$g_{iso} = 2.12$	2.23	101	2.01	83	2.29	135	2.08	30
5·$CuCl_2$	$g_{iso} = 2.12$	2.26	155	2.07	60	2.28	150	2.07	–
6·$CuCl_2$	$g_{iso} = 2.12$	2.25	155	2.06	–	2.28	155	2.07	–
7·$CuCl_2$	$g_{iso} = 2.12$	2.27	153	2.06	–	2.28	150	2.07	–
8·$CuCl_2$	$g_{iso} = 2.12$	2.26	153	2.06	–	2.28	154	2.08	–
9·$CuCl_2$	$g_{iso} = 2.12$	2.26	166	2.06	–	2.28	150	2.06	–
10·$CuCl_2$	$g_{\|} = 2.12$ $g_{\perp} = 2.06$	2.27	159	2.06	–	2.28	158	2.07	–
$CuCl_2$	$g_1 = 2.24$ $g_2 = 2.18$ $g_3 = 2.04$	2.40	117	2.08	14	2.42	121	2.08	–

erfine splitting constants with the following values: $g_{\|}$ = 2.28, $A_{\|}$ = 150−158 G, $g_{\perp}$ = 2.07; $A_{\perp}$(Cu) was unobservable.

These results indicated that the EPR values of the complexes, and consequently their geometries, depend on the solvent and on the position of the bridge, at C-2 or C-3 of the phenanthroline entity. The length of the bridge, however, or the incorporation of substituents in this linker, in particular in the case of 3-Clip-Phen derivatives, had a limited influence on EPR spectra and probably also on the geometries of the cupric complexes.

Electrochemical Properties

The copper(II/I) redox potentials of the different copper complexes were compared in aqueous solution with KCl as supporting electrolyte, since nucleic acid cleavage experiments are performed in water in the presence of NaCl. The cyclic voltammograms consisted of only one oxidation and one reduction wave in all cases except for that of the copper complex of 3-pentyl-Clip-Phen (**10**·$CuCl_2$), for which the oxidation wave was not observable. As in the case of the EPR analyses, copper complexes of 2- and 3-Clip-Phen derivatives showed different behavior although the results were similar for all 2-Clip-Phen and all 3-Clip-Phen derivatives, respectively. Results are summarized in Table 5. $(Phen)_2CuCl_2$ was also used for comparison. $\Delta Ep = Ep_{ox} - Ep_{red}$ (= separation of its Cu^{II}/Cu^{I} couple) approached the 56/*n* mV separation expected for a reversible one-electron process. This one-electron metal-centered process has been confirmed for this complex previously, but also for 2-Clip-Phen·$CuCl_2$ by coulometry and EPR.[23]

Table 5. Comparison of cyclic voltammetry data for **1**−**10**·$CuCl_2$ complexes (1 mM in water); cyclic voltammetry measurements were carried out at 0.1 V/s with 0.1 M KCl as supporting electrolyte under argon and at room temperature; $\Delta Ep = Ep_{ox} - Ep_{red}$; SCE was used as a reference

	Ep_c [mV/ECS]	Ep_a [mV/ECS]	Ip_c/Ip_a	$E_{1/2}$ [mV/ECS]	ΔEp [mV]
1·$CuCl_2$	−25	+237	0.60	+106	262
2·$CuCl_2$	+34	+297	1.89	+165	263
3·$CuCl_2$	+39	+219	0.84	+129	180
4·$CuCl_2$	+38	+303	0.93	+170	265
5·$CuCl_2$	−76	+157	1.12	+40	233
6·$CuCl_2$	−72	+92	1.50	+10	163
7·$CuCl_2$	−278	+134	6.80	−15	412
8·$CuCl_2$	−110	+93	18.4	+8	203
9·$CuCl_2$	−99	+74	2.36	−12	172
10·$CuCl_2$	−91	–	–	–	–
$(Phen)_2CuCl_2$	−68	+1	0.67	−33	69

The ΔEp separation for copper complexes of 2-Clip-Phen derivatives (**1**−**4**) ranges from 180 to 263 mV and is best described as quasi-reversible. In all cases, the reduction potentials of the copper complexes of 2-Clip-Phen derivatives were shifted to more positive values relative to the Cu^{II}/Cu^{I} couple of $(Phen)_2CuCl_2$, reflecting their more facile reduction and indicating that 2-Clip-Phen ligands stabilized the copper(I) state for their complexes. This phenomenon is well known in the cases of phenanthroline derivatives substituted on their C-2 and C-9 carbon atoms, in the α-position to the chelating nitrogen atom. They are known to stabilize the tetrahedral copper(I) complexes relative to copper(II) complexes, mostly through steric effects that inhibit the pentahedral or octahedral geometries favorable for copper(II).[3,4,7,22,38] In the case of 2-Clip-Phen derivatives only substituted at the C-2 position, however, this phenomenon was less important.

Further bulk electrolysis was performed with 2-Clip-Phen·$CuCl_2$ and **3**·$CuCl_2$, which form dimers in the crystals. This gave an *n* value of 1 for both. The observation of only one wave of oxidation and reduction and the observed *n* value were in accordance with the presence of monomeric species under the conditions used for electrolyses. The absence of two distinct redox states in cyclic voltammograms should not, however, be interpreted as definitive proof of monomeric species in solution, since double-helical dimeric structures can also exhibit a single redox state when both metal centers are strictly equivalent (as previously observed for some other copper dimetallic complexes[8,32,39,40]). Cyclic voltammetry was performed on the electrochemically reduced product, and again only one oxidation and one reduction wave were observed, with small differences in comparison with the cyclic voltammetry data of the oxidized form attributed to geometrical exchanges occurring between the +II and +I oxidation states. This reduced complex was then reoxidized electrochemically, and the cyclic voltammetry of the reoxidized form remained similar to that of the initial copper(II) complex, confirming the quasi-reversibility of the system (results not shown). Finally, it could be noted that the ΔEp increased when the scan rate increased in the case of 2-Clip-Phen·$CuCl_2$, this last result also being in accordance with a slow quasi-reversible system.

Surprisingly, the copper complexes of the 3-Clip-Phen derivatives (**5**−**10**) were only poorly electroactive. More strongly negative reduction potentials were observed than for $(Phen)_2CuCl_2$, which could reflect the greater stabilization of the copper(II) ion in these complexes with 3-Clip-Phen derivative ligands. However, the Cu^{II}/Cu^{I} reduction is quasi-irreversible for cupric complexes of **5**−**9** and seems to be irreversible for **10**·$CuCl_2$. The cupric complex of 3-pentyl-Clip-Phen (**10**) is in fact the most difficult of this series to reduce, but also the most irreversible (and is also the worst DNA cleaver in the 3-Clip-Phen series).

DNA Cleavage Activity

The DNA cleavage activities of the different (Clip-Phen)-copper complexes were compared. Relaxation of supercoiled circular ΦX174 DNA (form I) into relaxed (form II) and linear (form III) forms was used to quantify the relative cleavage efficiencies of the different complexes. Redox activity of the Cu^{II} complexes was initiated by the addition of 5 mM mercaptopropionic acid (MPA) in the presence of air. Control experiments clearly showed the necessity of the presence of copper complexes and a reductant for noticeable DNA cleavage (Table 7). Residual DNA cleavage was

observed in the case of 3-Clip-Phen (**5**) and for its cupric complex (the more active compound) without MPA. This was probably due to buffer impurities.

As previously observed for copper complexes of 2- and 3-Clip-Phen (**1** and **5**), DNA cleavage experiments performed in the presence of different concentrations of complexes showed that all compounds produced single-strand cleavages in DNA, since they transformed form I into form II. Form III resulted from multiple cleavage of form II and appeared only when full cleavage of form I was practically achieved. A smear corresponding to multifragmented DNA was also observed for higher DNA cleavage activities (results not shown).

All complexes were tested under the same experimental conditions (1 μM concentration for a reaction time of 1 h at 37 °C), in order to provide direct comparison of their DNA cleavage efficiencies. To facilitate this analysis, attempts to quantify the number *S* of single-strand cleavages per ΦX174 molecule were carried out according to refs.[41,42] This assumes random cleavage of the DNA target, as previously observed for **1**·$CuCl_2$ and **5**·$CuCl_2$.[25,27] Table 6 indicates that the relative DNA cleavage efficiencies of the different copper complexes were in the following order: **5**·$CuCl_2$ >> **6**·$CuCl_2$ > **8**·$CuCl_2$ > **7**·$CuCl_2$ ≈ **9**·$CuCl_2$ > **4**·$CuCl_2$ > **10**·$CuCl_2$ > **1**·$CuCl_2$ > **2**·$CuCl_2$ > **3**·$CuCl_2$. Comparison of the cleavage patterns of copper complexes of **1** versus **5**, **2** versus **6**, **3** versus **7**, and **4** versus **8** (*S* = 1.2 and 35, 0.8 and 21, 0.7 and 10, 6 and 15, respectively) clearly indicated that, for the same bridge, copper complexes of 3-Clip-Phen derivatives were always more active than their isomers with a junction at C-2 of the phenanthroline.

Differences between 2- and 3-Clip-Phen series also clearly appeared during electrochemical analyses of the different copper complexes (Table 5). All these data suggest that the lower DNA cleavage efficiency of copper complexes of 2-Clip-Phen derivatives might be correlated with their higher stabilization of cuprous species. It can be seen, however, that no correlation between the redox activity and the DNA cleavage efficiency within each series was observed. Other parameters such as steric or electrostatic constraints in the vicinity of the DNA target probably modulate the cleaving activities of the complexes.

The reactivities of the complexes are largely governed by the position of the bridge, at C-2 or C-3 of each phenanthroline residue. However, the length and the nature of this bridge also appeared to be significant for the DNA cleavage activity of these copper complexes. Hence, **4**·$CuCl_2$ of the 2-Clip-Phen series, for example, is a better DNA cleaver than **10**·$CuCl_2$ of the 3-Clip-Phen series.

Effect of the Length of the Bridge on the DNA Cleavage Efficiency

Comparison of cleavage patterns obtained with copper complexes of **3** and **4** on one hand and **7**−**10** on the other allowed the optimal length of the bridge for high DNA cleavage patterns to be determined, since these compounds differed only in the number of methylene units in the bridge.

Table 6 shows that for copper complexes of ethyl (**3**) and propyl (**4**) derivatives of 2-Clip-Phen, a small change due to the addition of one methylene unit to the bridge has a dramatic effect on DNA cleavage efficiency. For 1 μM of **3**·$CuCl_2$, 50% of DNA form I was present after 1 h of reac-

Table 6. Comparison of ΦX174 DNA cleavage efficiency of **1**−**10**·$CuCl_2$ complexes (1 μM in the presence of 5 mM MPA and air for 1 h at 37 °C); *S* corresponds to the average number of single-strand breaks per DNA molecule, assuming a random cleavage of the DNA target by the activated copper complexes

	% Form I	% Form II	% Form III	% Forms (I + II +III)[a]	S
DNA control	85±5	15±5	0	100	0.16±0.05
MPA control	85±5	15±5	0	100	0.16±0.05
$CuCl_2$/MPA	80±5	20±5	0	100	0.22±0.05
1·$CuCl_2$/MPA	30±5	70±5	0	100	1.2±0.2
2·$CuCl_2$/MPA	45±5	55±5	0	100	0.80±0.1
3·$CuCl_2$/MPA	50±5	50±5	0	100	0.70±0.1
4·$CuCl_2$/MPA	1±1	95±5	5±2	100	6±2
5·$CuCl_2$/MPA	0	35±10	65±10	50±10	35±5
6·$CuCl_2$/MPA	0	50±5	50±5	95±5	21±1
7·$CuCl_2$/MPA	0	85±5	15±5	100	10±2
8·$CuCl_2$/MPA	0	70±5	30±5	95±5	15±1
9·$CuCl_2$/MPA	5±2	80±5	15±5	100	10±2
10·$CuCl_2$/MPA	55±5	45±5	2±2	100	2±1
$(Phen)_2CuCl_2$/MPA	40±5	60±5	0	100	0.92±0.1
$(Phen)CuCl_2$/MPA	60±5	40±5	0	100	0.51±0.05
1·$CuCl_2$	80±5	20±5	0	100	0.22±0.05
4·$CuCl_2$	80±5	20±5	0	100	0.22±0.05
5·$CuCl_2$	70±5	30±5	0	100	0.36±0.05
8·$CuCl_2$	70±5	30±5	0	100	0.36±0.05
1/MPA	85±5	15±5	0	100	0.16±0.05
5/MPA	60±5	40±5	0	100	0.51±0.05
1	85±5	15±5	0	100	0.16±0.05
5	70±5	30±5	0	100	0.36±0.05

[a] For values less of 100%, the other part of DNA appeared as a smear on the gel.

tion. With $S = 0.7$, **3**·$CuCl_2$ has a rather poor DNA cleavage activity, just below that of $(Phen)_2$·$CuCl_2$ itself ($S = 0.9$). However, **4**·$CuCl_2$ appeared to be an efficient DNA cleaver under the same experimental conditions, converting nearly all supercoiled DNA not only into form II (95%), but also into form III (5%). With an S value estimated as 6, **4**·$CuCl_2$ appeared to be roughly ten times more active than **3**·$CuCl_2$. It is reasonable to propose that the poor DNA cleavage activity of **3**·$CuCl_2$, with the smallest bridge between the two phenanthroline residues, is due to steric constraints around the copper ion.

For copper complexes of ethyl (**7**), propyl (**8**), butyl (**9**), and pentyl (**10**) derivatives of 3-Clip-Phen ($S = 10$, 15, 10, and 2, respectively), variation by one methylene unit in the bridge had more limited effects. However, better DNA cleavage activity was obtained for **8**·$CuCl_2$, with three methylene units in the bridge, while, for a longer bridge such as in **10**·$CuCl_2$ (with five methylene units), the DNA cleavage efficiency significantly decreased. It is remarkable that 3-Clip-Phen (**5**) has the same bridge length as **8**. Thus, though serinol was chosen for reasons previously described, it also appears to have an optimal length to favor a high level of DNA cleavage activity.

Effect of the Nature of the Bridge on the DNA Cleavage Efficiency

Table 6 indicates that the nuclease activity of the copper complexes is also modulated by the chemical composition of the bridge. Three types of bridges of the previously observed optimized length were studied: unsubstituted ones [copper complexes of 2- and 3-propyl-Clip-Phen (**4** and **8**)], bridges including an amine residue such as in Clip-Phen [copper complexes of 2- and 3-Clip-Phen (**1** and **5**)], and bridges with an acetamido residue mimicking the amide junction used for the vectorization of Clip-Phen [copper complexes of 2- and 3-acetyl-Clip-Phen (**2** and **6**)].

Increases in DNA cleavage activity had previously been observed for copper complexes of conjugates of 2- or 3-Clip-Phen with DNA-binding molecules.[26−28] Acetylation of the amine residue of 2- and 3-Clip-Phen, however, resulted in a decrease in the DNA cleavage efficiency of their copper complexes by roughly 50%. This phenomenon was observed on comparison of the DNA cleavage activities of **1**·$CuCl_2$ and **5**·$CuCl_2$ ($S = 1.2$ and 35, respectively) with those of the corresponding acetylated derivatives **2**·$CuCl_2$ and **6**·$CuCl_2$ ($S = 0.8$ and 21, respectively). This difference was probably due not only to the steric effects of the bulkier amido group on the interaction with the DNA target, but also on the geometry changes during the Cu^I/Cu^{II} redox cycle. While this last proposal is not supported by the electrochemical data (Table 5), the phenomenon is probably enhanced in the case of a complex confined in the vicinity − or, more probably, in the floor − of the DNA minor groove, as suggested by DNA cleavage mechanism studies.[25] However, these phenomena were overcompensated by the increase of DNA affinity in conjugates with DNA binders, resulting in higher nuclease activities.

It is remarkable, though, that the amine on the bridge produced different effects on DNA cleavage for copper complexes of 2-Clip-Phen and 3-Clip-Phen derivatives. This phenomenon was clear on comparison of the DNA cleavage activities of **1**·$CuCl_2$ and **5**·$CuCl_2$ ($S = 1.2$ and 35, respectively) with those of their derivatives without the amine function **4**·$CuCl_2$ and **8**·$CuCl_2$ ($S = 6$ and 15, respectively). These data suggested that the presence of the amine decreased the DNA cleavage activity in the case of 2-Clip-Phen derivatives but increased it in the case of 3-Clip-Phen ones. Since the X-ray structures of the monocrystals of copper and zinc complexes of 2-Clip-Phen indicate that this amine could coordinate the metal ion, it is reasonable to propose that this type of coordination could also occur under DNA cleavage conditions. DNA cleavage results indicated that this coordination has an unfavorable effect on the DNA cleavage activity. However, geometrical constraints did not allow this type of coordination in the case of 3-Clip-Phen. It is reasonable to propose that, in the case of the copper complex of 3-Clip-Phen (**5**), the free amine residue could be protonated under the conditions used for DNA cleavage experiments, generating a cationic species with a higher affinity for the polyanionic DNA target, consequently increasing the DNA cleavage activity.

On the other hand, these last results indicate that the benefit of bridging on the C-2 carbon atom of Phen had previously been greatly underestimated, since data were obtained with the copper complex of 2-Clip-Phen (**1**), with the free primary amine function able to perturb the coordination sphere of the copper ion.

Conclusion

Different bis(phenanthroline) ligands were prepared in order not only to study their copper complexes but also to optimize the DNA cleavage activity of Clip-Phen derivatives. Physicochemical analysis showed that these copper complexes appear to be particularly versatile. As shown by structural analysis, the cupric complexes can adopt double-helical L_2Cu_2 geometries, but this type of geometry seems to be labile. The mass spectra results showed that the presence of monomeric structure L_1Cu_1 in solution could not be discounted. NMR studies showed that the complexes adopt different geometries, existing in equilibrium in solution. EPR analyses indicated that the geometries of the cupric complexes of 2-Clip-Phen derivatives could change with the composition of the solvent employed for their dilution. From electrochemical studies it also appears that all the ligands related to 2-Clip-Phen stabilize Cu^I more than their 3-Clip-Phen counterparts. Copper complexes of this latter ligand family appear to be surprisingly poorly redox-active.

DNA cleavage experiments allowed optimization of the bridge between the two phenanthroline residues of Clip-Phen to provide higher nuclease activity. The compounds with a bridge linked at C-3 were more active DNA cleavers than complexes with the bridge in the C-2 position. A tether length corresponding to three methylene units gave better

DNA cleavage activities, but incorporation of voluminous substituents within the linker could decrease the DNA cleavage efficiency. Surprisingly, the presence of an amine group in the bridge had different effects on DNA cleavage activity for the copper complexes of 2-Clip-Phen and of 3-Clip-Phen, and allowed the proposal that, in the case of 2-Clip-Phen·$CuCl_2$, this amine group could be coordinated to the copper ion under the DNA cleavage conditions.

Experimental Section

General Remarks: 2-Chloro-1,10-phenanthroline,[43] 3-bromo-1,10-phenanthroline,[44] 2-Clip-Phen (**1**),[23] 3-Clip-Phen (**5**),[24] 2-acetyl-Clip-Phen (**2**),[28] 3-propyl-Clip-Phen (**8**),[45] and 2-Clip-Phen·$CuCl_2$ (**1**·$CuCl_2$)[23] were synthesized by literature procedures. Proton NMR spectra were recorded with a Bruker 250 MHz instrument. The ESI-MS spectrometer was a Perkin−Elmer SCIEX API 365 used in positive mode; samples were introduced into the electrospray source with a Havard Apparatus syringe pump. UV/Vis spectra were recorded with a Hewlett Packard 8452A diode array spectrophotometer. EPR spectra were recorded with a Bruker ESP 300 in X Band, with an ER035 M gaussmeter (NMR probe) and an EIP 548 hyperfrequencemeter. Syntheses were monitored by thin layer silica chromatography (Macherey−Nagel Alugram Sil G/UV 254) eluted by CH_2Cl_2/methanol (9:1, v/v) to which 1% of concentrated aqueous ammonia (30%) had been added, and spots were viewed under UV light (violet spots at 254 nm). DMF was dried with 4 Å molecular sieves. Other commercially available reagents and solvents were purchased from standard chemical suppliers and used without further purification.

General Method for the Synthesis of 2-alkyl-Clip-Phen 3 and 4: 2-Chloro-1,10-phenanthroline (200 mg, 0.93 mmol) and the diol (ca. 0.46 mmol of ethylene glycol or 1,3-propanediol) were added to a suspension of sodium hydride (275 mg of a 60% suspension in mineral oil, 6.85 mmol) in 4 mL of dry DMF, cooled by an ice bath. After the mixture had been stirred for 24 h and allowed to warm to room temperature, the crude product was dissolved in 4 mL of ethanol before being precipitated with 40 mL of water. After filtration, the product was precipitated from 15 mL of hot methanol to give 2-alkyl-Clip-Phen.

2,2′-Ethane-1,2-dioxybis(1,10-phenanthroline) (2-ethyl-Clip-Phen, 3): Yield = 146 mg (75%) as white needles. ^{1}H NMR ($CDCl_3$): δ = 5.25 ppm (s, 4 H, 1-H), 7.21 (d, *J* = 8.7 Hz, 2 H, 3′-H), 7. 58 (dd, *J* = 8.1, 4.2 Hz, 2 H, 8′-H), 7.66 and 7.75 (AB, *J* = 8.7 Hz, 4 H, 5′-H and 6′-H), 8.12 (d, *J* = 8.7 Hz, 2 H, 4′-H), 8.23 (dd, *J* = 8.1, 1.8 Hz, 2 H, 7′-H), 9.18 (dd, *J* = 4.2, 1.8 Hz, 2 H, 9′-H). MS (CDI, NH_3): *m*/*z* (%) = 419 (100) [M + 1], 223 (67), 197 (65). UV/Vis (CH_3OH): λ (ε) = 228 nm (73600 $mol^{-1}·cm^{-1}$), 276 (52300), 292 (16400, sh), 316 (4000, sh), 332 (2300), 350 (1500). $C_{26}H_{18}N_4O_2$ (418): calcd. (+ 0.25 H_2O) C 73.86, H 4.41, N 13.25; found C 73.53, H 4.17, N 13.11.

2,2′-Propane-1,3-dioxybis(1,10-phenanthroline) (2-propyl-Clip-Phen, 4): Yield = 157 mg (78%) as a white powder. ^{1}H NMR (CD_2Cl_2): δ = 2.52 ppm (quint, *J* = 6.2 Hz, 2 H, 2-H), 4.93 (t, *J* = 6.2 Hz, 4 H, 1-H), 7.15 (d, *J* = 8.6 Hz, 2 H, 3′-H), 7. 59 (dd, *J* = 8.1, 4.2 Hz, 2 H, 8′-H), 7.68 and 7.76 (AB, *J* = 8.7 Hz, 4 H, 5′-H and 6′-H), 8.13 (d, *J* = 8.6 Hz, 2 H, 4′-H), 8.25 (dd, *J* = 8.1, 1.8 Hz, 2 H, 7′-H), 9.11 (dd, *J* = 4.2, 1.8 Hz, 2 H, 9′-H). MS (CDI, NH_3): *m*/*z* (%) = 433 (100) [M + 1], 235 (5), 197 (6). UV/Vis (CH_3OH): λ (ε) = 228 nm (67700 mol^{-1} cm^{-1}), 276 (48700), 292 (16400, sh), 314 (4400, sh), 332 (2300), 348 (1400). $C_{27}H_{20}N_4O_2$ (432): calcd. (+ 0.75 H_2O) C 72.74, H 4.85, N 12.56; found C 72.83, H 4.57, N 12.40.

General Method for the Synthesis of 3-Alkyl-Clip-Phen 7, 9, and 10: The appropriate diol (ca. 0.77 mmol of ethylene glycol, 1,4-butanediol, or 1,5-pentanediol) was added to a suspension of sodium hydride (156 mg of a 60% suspension in mineral oil, 3.9 mmol) in 7 mL of dry DMF. The mixture was stirred for 1 h at 55 °C, and then cooled to 4 °C with an ice bath. 3-Bromo-1,10-phenanthroline (400 mg, 1.54 mmol) was added, and the mixture was warmed at 55 °C overnight. After return to room temperature, 2 mL of ethanol was added, then 20 mL of water. The precipitate was filtered, dissolved in dichloromethane, and washed with a 5% solution of ammonia. The organic layer was concentrated and the product was crystallized from 3 mL of methanol before being dissolved in dichloromethane and precipitated with hexane to give pure 3-alkyl-Clip-Phen as white powder. *Warning:* The number of NaH equivalents should be optimized according to the intrinsic quality of reagents and solvents (ultrapure quality is preferred).

3,3′-Ethane-1,2-dioxybis(1,10-phenanthroline) (3-ethyl-Clip-Phen, 7): Yield = 74 mg (23%). ^{1}H NMR (CD_2Cl_2): δ = 4.67 ppm (s, 4 H, 1-H), 7.59 (dd, *J* = 4.4, 8.1 Hz, 2 H, 8′-H), 7.69 (d, *J* = 3.0 Hz, 2 H, 4′-H), 7.83 and 7.78 (AB, *J* = 8.9 Hz, 4 H, 5′-H and 6′-H), 8.25 (dd, *J* = 1.8, 8.1 Hz, 2 H, 7′-H), 8.93 (d, *J* = 3.0 Hz, 2 H, 2′-H), 9.09 (dd, *J* = 1.8, 4.4 Hz, 2 H, 9′-H). MS (CDI, NH_3): *m*/*z* (%) = 419 (100) [M + 1]. UV/Vis (CH_3OH): λ (ε) = 240 nm (69500 $mol^{-1}·cm^{-1}$),·272 (51700), 294 (27400, sh), 304 (17100, sh), 314 (8400, sh), 328 (5500), 342 (3600). $C_{26}H_{18}N_4O_2$ (418): calcd. (+ 0.5 CH_2Cl_2) C 69.06, H 4.15, N 12.16; found C 69.08, H 4.05, N 12.34.

3,3′-Butane-1,4-dioxybis(1,10-phenanthroline) (3-butyl-Clip-Phen, 9): Yield = 80 mg (23%). ^{1}H NMR (CD_2Cl_2): δ = 2.20 ppm (m, 4 H, 2-H), 4.30 (t, *J* = 5.4 Hz, 4 H, 1-H), 7.56 (dd, *J* = 4.2, 8.1 Hz, 2 H, 8′-H), 7.58 (d, *J* = 3.0 Hz, 2 H, 4′-H), 7.79 and 7.74 (AB, *J* = 8.9 Hz, 4 H, 5′-H and 6′-H), 8.23 (dd, *J* = 1.8, 8.1 Hz, 2 H, 7′-H), 8.87 (d, *J* = 3.0 Hz, 2 H, 2′-H), 9.08 (dd, *J* = 1.8, 4.2 Hz, 2 H, 9′-H). MS (CDI, NH_3): *m*/*z* (%) = 447 (100) [M + 1]. UV/Vis (CH_3OH): λ (ε) = 242 nm (72600 $mol^{-1}·cm^{-1}$), 272 (48200), 294 (24300, sh), 306 (14500, sh), 314 (7000, sh), 328 (5000), 344 (3300). $C_{28}H_{22}N_4O_2$ (446): calcd. (+ 0.1 CH_2Cl_2) C 74.18, H 4.92, N 12.31; found C 73.84, H 4.33, N 12.18.

3,3′-Pentane-1,5-dioxybis(1,10-phenanthroline) (3-pentyl-Clip-Phen, 10): Yield = 110 mg (31%). ^{1}H NMR (CD_2Cl_2): δ = 1.80 ppm (m, 2 H, 3-H), 1.99 (m, 4 H, 2-H), 4.20 (t, *J* = 6.3 Hz, 4 H, 1-H), 7.51−7.56 (m, 4 H, 4′-H and 8′-H), 7.79 and 7.74 (AB, *J* = 8.9 Hz, 4 H, 5′-H and 6′-H), 8.23 (dd, *J* = 1.8, 8.1 Hz, 2 H, 7′-H), 8.87 (d, *J* = 3.0 Hz, 2 H, 2′-H), 9.08 (dd, *J* = 1.8, 4.2 Hz, 2 H, 9′-H). MS (CDI, NH_3): *m*/*z* (%) = 461 (100) [M + 1]. UV/Vis (CH_3OH): λ (ε) = 240 nm (72400 $mol^{-1}·cm^{-1}$), 272 (51000), 294 (26600, sh), 304 (17000, sh), 314 (92000, sh), 328 (6000), 344 (4200). $C_{29}H_{24}N_4O_2$ (460): calcd. (+ 0.5 CH_2Cl_2 + 1.5 H_2O) C 66.85, H 5.32, N 10.57; found C 67.08, H 5.37, N 10.70.

2-Acetylamino-3,3′-propane-1,3-dioxybis(1,10-phenanthroline) (3-acetyl-Clip-Phen, 6): Acetic anhydride (50 μL, 50 mg, 0.49 mmol) was added to a solution of 3-Clip-Phen (50 mg, 0.11 mmol) in 2 mL of $CHCl_3$. The solution was stirred for 1 h at room temperature, 2 mL of water was then added, and the pH was adjusted to 9 with concentrated aqueous ammonia (30%). The organic layer was separated and washed twice with water, and the solvent was evaporated under vacuum to give **6** as a pale brown foam (50 mg, yield = 92%). ^{1}H NMR (CD_2Cl_2): δ = 2.16 ppm (s, 2 H, CH_3), 4.45 (m, 4 H, 1-H), 4.88 (m, 1 H, 2-H), 6.59 (d, *J* = 8.0 Hz, 1 H, NH), 7.55

(dd, J = 8.0, 4.4 Hz, 2 H, 8′-H), 7. 63 (d, J = 2.8 Hz, 2 H, 4′-H), 7.69 and 7.75 (AB, J = 8.7 Hz, 4 H, 5′-H and 6′-H), 8.19 (dd, J = 8.0, 1.7 Hz, 2 H, 7′-H), 8.90 (d, J = 2.8 Hz, 2 H, 2′-H), 9.11 (dd, J = 4.4, 1.7 Hz, 2 H, 9′-H). MS (CDI, NH_3): m/z (%) = 490 (81) [M + 1], 294 (100), 197 (60). UV/Vis (CH_3OH): λ (ε) = 240 nm (62700 $mol^{-1}\cdot cm^{-1}$), 272 (44500), 294 (22300, sh), 306 (12400, sh), 314 (6100, sh), 328 (4300), 342 (2700). $C_{29}H_{23}N_5O_3$ (489): calcd. (+ 0.4 $CHCl_3$) C 66.40, H 4.37, N 13.20; found C 66.19, H 4.51, N 13.22.

General Preparation of Copper Complexes (8·$CuCl_2$ exempted): $CuCl_2$ (1 equiv., previously dissolved at 58.7 mM concentration in DMF) was added to a solution of ligand (10 mg) in 1 mL of DMF/CH_3OH (1:1). After 1 h at room temperature, the complex was precipitated with 5 mL of diethyl ether overnight at −20 °C and then centrifuged for 10 min at 3000 rpm and washed twice with diethyl ether (2 × 6 mL) before being dried under vacuum.

Preparation of 8·$CuCl_2$: Solubility problems meant that the metallation of 3-propyl-Clip-Phen (**8**) was conducted under more dilute conditions than used for the General Preparation of the other copper complexes. A solution of $CuCl_2$ (23.1 μmol, 1 equiv.) in DMF was added over 2 h, as 5 aliquots of 100 μL, to a solution of 3-propyl-Clip-Phen (**8**, 10 mg, 23.1 μmol) in 23 mL of DMF (1 mM final concentration),. The mixture was stirred overnight and the volume was then reduced to 5 mL. The copper complex was precipitated with diethyl ether and centrifuged, the supernatant was removed, and the precipitate was washed twice with diethyl ether before being dried under vacuum.

2·$CuCl_2$: Treatment of **2** with $CuCl_2$ as described above on a 17.0 μmol scale afforded 11.4 mg (89%) of **2·$CuCl_2$** as a pale green powder. MS (ES, positive mode): m/z = 587 (monocation, M − Cl), 551 (cuprous complex). UV/Vis (H_2O): λ (ε) = 210 nm (59000 $mol^{-1}\cdot cm^{-1}$), 224 (66800), 276 (48400), 336 (2100, sh), 356 (1500, sh), 862 (160). $C_{29}H_{23}Cl_2CuN_5O_3$ (624): calcd. (+ 2.5 H_2O) C 52.07, H 4.21, N 10.47; found C 52.19, H 4.27, N 10.40.

3·$CuCl_2$: Treatment of **3** with $CuCl_2$ on a 23.1 μmol scale afforded 13.6 mg (98%) of **3·$CuCl_2$** as a pale green powder. MS (ES, positive mode): m/z = 516 (monocation, M − Cl), 481 (cuprous complex). UV/Vis (H_2O): λ (ε) = 208 nm (60500 $mol^{-1}\cdot cm^{-1}$), 218 (57300), 274 (38900), 340 (3300, sh), 356 (2600, sh), 825 (120). $C_{26}H_{18}Cl_2CuN_4O_2$ (553): calcd. (+ 2 H_2O) C 53.03, H 3.77, N 9.51; found C 53.11, H 4.20, N 9.48. Green crystals suitable for X-ray analysis were obtained by recrystallization of **3·$CuCl_2$** from hot methanol.

4·$CuCl_2$: Treatment of **4** with $CuCl_2$ on a 19.3 μmol scale afforded 12.2 mg (93%) of **4·$CuCl_2$** as a pale green powder. MS (ES, positive mode): m/z = 530 (monocation, M − Cl), 495 (cuprous complex). UV/Vis (H_2O): λ (ε) = 210 nm (61300 $mol^{-1}\cdot cm^{-1}$), 224 (69000), 274 (51300), 338 (2000, sh), 354 (1400, sh), 855 (160). $C_{27}H_{20}Cl_2CuN_4O_2$ (567): calcd. (+ 3.5 H_2O) C 51.48, H 4.32, N 8.89; found C 51.56, H 4.00, N 9.05.

5·$CuCl_2$: Treatment of **5** with $CuCl_2$ on a 20.3 μmol scale afforded 13.3 mg (93%) of **5·$CuCl_2$** as a green-brown powder. MS (ES, positive mode): m/z = 546 (monocation, M − Cl), 510 (cuprous complex). UV/Vis (H_2O): λ (ε) = 210 nm (51700 mol^{-1} cm^{-1}), 232 (56200), 280 (41100), 318 (10400, sh), 332 (7200, sh), 344 (3400, sh), 702 (70), 955 (40, sh). $C_{27}H_{21}Cl_2CuN_5O_2$ (582): calcd. (+ 4 H_2O) C 49.59, H 4.47, N 10.71; found C 49.64, H 4.10, N 10.85.

6·$CuCl_2$: Treatment of **6** with $CuCl_2$ on a 18.6 μmol scale afforded 11.4 mg (89%) of **6·$CuCl_2$** as a green-brown powder. MS (ES, positive mode): m/z = 587 (monocation, M − Cl), 552 (cuprous complex). UV/Vis (H_2O): λ (ε) = 208 nm (43000 mol^{-1} cm^{-1}), 230 (41900), 282 (31200), 316 (2100, sh), 330 (7400, sh), 344 (4000, sh), 693 (50), 900 (40, sh). $C_{29}H_{23}Cl_2CuN_5O_3$ (624): calcd. (+ 3.5 H_2O) C 50.70, H 4.40, N 10.19; found C 50.80, H 4.05, N 10.22.

7·$CuCl_2$: Treatment of **7** with $CuCl_2$ on a 23.8 μmol scale afforded 10.0 mg (76%) of **7·$CuCl_2$** as a green powder. MS (ES, positive mode): m/z = 516 (monocation, M − Cl), 481 (cuprous complex). UV/Vis (H_2O): λ (ε) = 208 nm (46900 mol^{-1} cm^{-1}), 232 (55700), 245 (50626, sh), 280 (45900), 320 (14700, sh), 332 (10300, sh), 346 (4300, sh), 700 (51). $C_{26}H_{18}Cl_2CuN_4O_2$ (553): calcd. (+ 0.1 H_2O) C 56.30, H 3.31, N 10.10; found C 56.39, H 3.50, N 9.98. Green crystals suitable for X-ray analysis were obtained from a DMF/methanol (2:1) solution of the complex after 3 d at −20 °C.

8·$CuCl_2$: Treatment of **8** with $CuCl_2$ on a 23.1 μmol scale afforded 10.3 mg (76%) of **8·$CuCl_2$** as a green powder. MS (ES, positive mode): m/z = 530 (monocation, M − Cl), 495 (cuprous complex). UV/Vis (H_2O): λ (ε) = 208 nm (49100 $mol^{-1}\cdot cm^{-1}$), 232 (54200), 245 (41900, sh), 280 (41100), 320 (14100, sh), 332 (11900, sh), 346 (4400, sh), 700 (59). $C_{27}H_{20}Cl_2CuN_4O_2$ (567): calcd. (+ 1.3 H_2O) C 54.93, H 3.86, N 9.49; found C 54.87, H 3.56, N 9.27.

9·$CuCl_2$: Treatment of **9** with $CuCl_2$ on a 22.3 μmol scale afforded 10.0 mg (74%) of **9·$CuCl_2$** as a green powder. MS (ES, positive mode): m/z = 544 (monocation, M − Cl), 509 (cuprous complex). UV/Vis (H_2O): λ (ε) = 208 nm (49000 $mol^{-1}\cdot cm^{-1}$), 232 (54600), 245 (43700, sh), 280 (40700), 320 (14100, sh), 332 (11800, sh), 346 (5100, sh), 720 (68). $C_{28}H_{22}Cl_2CuN_4O_2$ (581): calcd. (+ 1.5 H_2O) C 55.32, H 4.14, N 9.22; found C 55.09, H 4.02, N 9.48.

10·$CuCl_2$: Treatment of **10** with $CuCl_2$ on a 21.7 μmol scale afforded 10.3 mg (77%) of **10·$CuCl_2$** as a green powder. MS (ES, positive mode): m/z = 557 (monocation, M − Cl), 523 (cuprous complex). UV/Vis (H_2O): λ (ε) = 208 nm (48200 $mol^{-1}\cdot cm^{-1}$), 232 (50900), 245 (33700, sh), 280 (35900), 320 (12500, sh), 332 (11000, sh), 346 (5300, sh), 740 (71). $C_{29}H_{24}Cl_2CuN_4O_2$ (595): calcd. (+ 1.3 H_2O) C 56.33, H 4.34, N 9.06; found C 56.28, H 4.02, N 9.10.

Preparation of 1·$Cu(BF_4)_2$: $Cu(BF_4)_2\cdot 6\ H_2O$ (26.8 mg, 0.08 mmol) was added to 2-Clip-Phen (**1**, 50.6 mg, 0.11 mmol) dissolved in 600 μL of DMF/CH_3OH. The mixture was stirred for 1 h at room temperature, and green crystals of **1**·$Cu(BF_4)_2$ suitable for X-ray analysis were then obtained by slow diffusion of CH_2Cl_2 into the mixture. MS (ES, positive mode): m/z = 509 (monocharged monomer, **1**·Cu^+, major pick), 529 (monomer, **1**·$Cu^{++}F^-$), 1281 {monocharged dimer, $[(\mathbf{1})_2Cu_2][BF_4]_3$, minor pick}. $C_{27}H_{21}B_2CuF_8N_5O_2$ (685): calcd. (+ 1.5 H_2O) C 45.01, H 3.40, N 9.85; found C 44.91, H 3.38, N 9.60. EPR data: from solid state: $g_\perp$ = 2.09, $g_\parallel$ = 2.2; from a DMF/CH_2Cl_2/CH_3OH (2:4:1) glass (1 mM) at 100 K: $g_\perp$ = 2.09 ($A_\perp$ = 42 G, badly resolved), $g_\parallel$ = 2.24 ($A_\parallel$ = 67 G); from a H_2O/ethylene glycol (1:2) glass (1.1 mM) at 100 K: $g_\perp$ = 2.09, $g_\parallel$ = 2.27 ($A_\parallel$ = 75 G).

Preparation of 1·$ZnCl_2$: Compound **1** (10 mg, 22 μmol) was metallated with 1 equiv. of $ZnCl_2$ (3 mg, 22 μmol) in 0.5 mL of a CH_2Cl_2/CH_3OH (9:1) mixture. After 1 h at room temperature, the solvent was evaporated and the product was dissolved in 0.5 mL of [D_6]DMSO. White crystals suitable for X-ray analysis were obtained, directly in the NMR tube, after 2 d at room temperature. ^1H NMR ([D_6]DMSO): δ = 3.99 ppm (m, 1 H), 4.97 (dd, 2 H, 4.7 Hz and 12 Hz), [3.47 (d, J = 6.2 Hz), 3.99 (s) and 5.40 (br. d): 4 H in slow exchange on the NMR time scale as confirmed by selective irradiation of NMR signals], 7.29 (d, J = 8.7 Hz, 2 H, 3′-H), 7. 82 (dd, J = 7.9, 3.6 Hz, 2 H, 8′-H), 7.92 and 8.00 (AB, 4 H,

J = 8.8 Hz, 5′-H and 6′-H), 8.42 (d, J = 8.7 Hz, 2 H, 4′-H), 8.60 (br. d, 2 H, J = 7.9, 7′-H), 9.19 (br. d, 2 H, J = 3.6, 9′-H).

X-ray Crystallographic Study: Crystal data for all structures are presented in Table 7. Data for bis[(**1**)Cu][BF_4]$_2$ were collected at low temperature with a Stoe-IPDS diffractometer, and data for [(**3**)CuCl]$_2$Cl$_2$, [(**7**)CuCl]$_2$Cl$_2$, and (**1**)ZnCl$_2$ were collected at low temperatures with a Bruker-CCD with Mo-K_α (λ = 0.71073 Å). The structures were solved by direct methods by use of SIR92[46−47] {bis[(**1**)Cu][BF_4]$_2$} or SHELXS-97[48] {[(**3**)CuCl]$_2$Cl$_2$, [(**7**)CuCl]$_2$Cl$_2$, and (**1**)ZnCl$_2$} and refined with all data on F^2 by SHELXL-97.[49] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms of the molecules were geometrically idealized and refined using a riding model. In all cases non-coordinated solvent molecules were refined anisotropically with the help of ADP and distances restraints. The fact that the very small crystals of [(**7**)CuCl]$_2$Cl$_2$ diffracted weakly and the presence of highly disordered solvent molecules do not permit the structure to be discussed in detail. We have limited the discussion to the main features of the complex. [(**7**)CuCl]$_2$Cl$_2$ crystallizes in the monoclinic space group $C2/c$ with the cell parameters: a = 26.020(11), b = 10.363(5), c = 29.498(13) Å and β = 114.20(1)°. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre: CCDC-187474 {bis[(**1**)Cu][BF_4]$_2$}, -187475 {[(**3**)CuCl]$_2$Cl$_2$}, -187476 [(**1**)ZnCl$_2$]. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. [Fax: (internat.) + 44-1223/336-033, E-mail: deposit@ccdc.cam.ac.uk].

Electrochemistry: Electrochemical measurements were carried with a custom-made potentiostat, using the interrupt method to minimize the uncompensated resistance (IR) drop.[50] Electrochemical experiments were performed at room temperature in an air-tight 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. Cyclic voltammetry was conducted with a Pt (1 mm) working electrode in the potential range of −600 to +600 mV. The supporting electrolyte was KCl (Prolabo RP, Normapur) and was used as received. All solutions measured were 1 mM in complex and 0.1 M in supporting electrolyte. All experiments were conducted in water under argon. Stock solutions (2 mM) of ligand·$CuCl_2$ were prepared in water.

DNA Cleavage Experiments: The desired complex [4 μM, 5 μL, metallations carried out at 1 mM $CuCl_2$ concentration with 1 equiv. of ligand in DMF/water (1:1) for 4 h at room temperature before dilution of the complex with water to 4 μM] was added to ΦX174 (10 μL, 7 nM, 40 μM in bp) in sodium phosphate buffer (80 mM, pH = 7.2), NaCl (100 mM), and $MgCl_2$ (20 mM). After 30 min at room temperature, cleavage was initiated by addition of an aqueous solution of MPA (5 μL, 20 mM) and samples were incubated for 1 h at 37 °C. A solution of 7.5 μL of 50% (v/v) glycerol/40 mM Tris·Cl buffer (pH = 8)/0.05% bromophenol blue (w/v) was then added and samples were immediately loaded onto agarose gel (0.8%) containing 1 μg/mL of ethidium bromide. Electrophoresis was 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 1.47 was used for the decreased stainability of form I DNA vs. forms II and III.[41] When only forms I and II were present, the average number of single-strand scissions per DNA phage, S, was considered to be equal to

Table 7. Crystal structure data for bis-[(**1**)Cu][BF_4]$_2$, [(**3**)CuCl]$_2$Cl$_2$, and (**1**)ZnCl$_2$

	bis[(**1**)Cu][BF_4]$_2$	[(**3**)CuCl]$_2$Cl$_2$	(**1**)ZnCl$_2$
Empirical ormula	$[C_{54}H_{40}Cu_2N_{10}O_4][BF_4]_2$, C_3H_7NO, H_2O, 4 CH_4O, 0.8 CH_2Cl_2	$C_{26}H_{18}CuCl_2N_4O_2$· 4.75 H_2O	$C_{27}H_{21}Cl_2N_5O_2Zn$· 3 C_2H_6OS
Formula mass [g·mol^{-1}]	1647.8	638.46	818.14
Temperature [K]	180(2)	173(2)	193(2)
Crystal system	monoclinic	triclinic	triclinic
Space group	$P2/c$	$P\bar{1}$	$P\bar{1}$
a [Å]	11.141(5)	13.3616(10)	8.8789(10)
b [Å]	15.632(5)	14.2053(10)	14.7728(16)
c [Å]	21.496(5)	15.5438(11)	15.2291(16)
α [°]	90	80.81(10)	88.52(2)
β [°]	99.224(5)	87.56(10)	76.15(2)
γ [°]	90	68.48(10)	76.78(2)
Volume [Å^3]	3695(2)	2709.03(3)	1887.2(4)
Z	4	4	2
$D_{calcd.}$ [Mg/m^3]	1.481	1.565	1.440
Abs. coeff. [mm^{-1}]	0.736	1.056	1.004
Crystal size [mm]	0.25 × 0.30 × 0.42	0.05 × 0.1 × 0.6	0.05 × 0.1 × 0.1
F(000)	1666	1314	848
θ range [°]	1.45−24.2	1.33−24.71	1.38−28.28
Reflections collected/R(int)	16839/0.0499	15804/0.0275	19529/0.0832
Completeness to θ (%)	98.6	98.6	99.3
Absortion correction	numerical	semi-empirical	none
Max./min. transmission	0.80/0.41	1.00/0.78	−
Data/restraints/parameters	3306/326/592	9132/704/895	9313/114/489
Goodness-of-fit on F^2	1.486	1.022	1.038
R_1 [$I > 2\sigma(I)$]	0.1052	0.0491	0.0412
$wR2$ (all data)	0.3161	0.1487	0.1144
Largest diff. peak/hole [e·Å^{-3}]	1.058/−0.972	1.065/−0.749	1.096/−0.640

−ln(fraction of form I) according to a Poisson distribution. In case of the presence of the form III, the number of single-strand breaks per ΦX174 DNA molecule was calculated according to the equation: $I + II = \{1 - [S\cdot(2h + 1)/2L]\}^{S/2}$;[41,42] the (I + II) value is the percentage of form I and II, L is the number of DNA base pairs in ΦX174 (5386 bp), and h is the distance between nicks on opposite strands needed to produce a linear molecule. A value h = 16 bp previously proposed in ref.[42] was used since it allowed a good linearity of S as a function of the concentration of copper complexes of Clip-Phen derivatives.

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