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Influence of the Copper Coordination Geometry on the DNA Cleavage Activity of Clip-Phen Complexes Studied by DFT

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Six different Cu(Clip-Phen)$^{2+/+}$ complexes, with or without a coordinating chloride ligand, have been investigated by DFT calculations to evaluate the influence of the length and functional substituents of the bridge linking the two phenanthroline units on the DNA cleavage activity. The changes of the structural and energetic profiles imposed by the bridge of these complexes have been analyzed by comparison with the well studied nuclease active agent Cu(phen)$_2^{2+/+}$. The present studies show that the bridge length of these complexes is critical for the consequent geometry, and both the strain and ligand binding energies. Upon reduction (needed for the DNA cleavage activity), the geometry of Cu(phen)$_2$ changes drastically. The behavior of the complexes with a 4- or 5-carbon bridge resembles the behavior of Cu(phen)$_2^{2+/+}$. However, the geometries of the complexes with two- or three-carbon bridges are markedly different from the one of unbridged Cu(phen)$_2^+$, as a result of constraints enforced by the short bridge. The results suggest that the cleaving activities of these bis-phenanthroline complexes are influenced by the different ligand environment geometries imposed by the bridge, and not by the change of the redox properties. It appears that the influence of the different bridges on the redox properties of the complexes is minor.

Introduction

Redox-active chemical nucleases that are able to irreversibly damage DNA have received a lot of interest because of their potential application as biological tools or as drugs.[1,2] The best studied complex systems are Fe-bleomycin,[3,4] Fe-edta[5,6] and Cu(phen)$_2$.[7,8] These complexes are able to oxidize the deoxyribose unit of DNA in the presence of dioxygen or dihydrogen peroxide. The mechanism of action of Cu(phen)$_2^{2+}$ allegedly involves: (i) its reduction in solution to Cu(phen)$_2^+$[9] (ii) the reversible binding to DNA[10] (iii) the reaction with dihydrogen peroxide to generate the unknown reactive species (iv) the abstraction of the protons H-1′, H-4′ and H-5′ from the deoxyribose unit in the minor groove of DNA, leading ultimately to DNA scission.[11] Unfortunately, the low binding constant of the second phenanthroline ligand limits its applicability,[12] because copper complexes with only one coordinated phenanthroline are less efficient DNA cleaving agents.[13–16] To prevail over this problem, the two phenanthroline ligands have been coupled, via their C2 or C3 atom, using a serinol bridge. As a result, the nuclease activity is found to increase by a factor of 2 or 60, for 2-Clip-Phen and 3-Clip-Phen, respectively.[17,18] A second advantage of this strategy is the possible, straightforward functionalization of the serinol bridge, allowing for example, an improvement of the sequence selectivity.[19–21] The mechanism of action of these Cu(Clip-Phen) complexes is similar to that of the Cu(phen)$_2$ complex.[20] Pitié et al. have also investigated the influence of the length and nature of the bridge of 2- or 3-Clip-Phen derivatives on the corresponding cleavage activity.[22] The length of the bridge has been varied from two to five methylene groups (Figure 1, complexes **2**, **3**, **6** and **7**). For the three-carbon bridge, the central carbon atom has been functionalized with either an amine or an acetamide group (Figure 1, complexes **4** and **5**). Interestingly, clear differences have been found in the cleaving activity of these complexes, and the following order was observed: **4** >> **5** > **3** > **2** = **6** >> **7** > **1**. It has been concluded that the complexes bearing an amine or an acetamide group are more active, because of an increased affinity for DNA.[22,23] The optimal length of the bridge linking the phenanthrolines has been estimated to three carbon atoms. Complexes **2**, **3**, **6** and **7** probably interact with DNA in a comparable manner, namely by partial intercalation of the phenanthroline unit, and no clear differences are observed in their respective Cu^{II}/Cu^{I} redox potentials, although complex **7** shows a quasi-irreversible Cu^{II}/Cu^{I} reduction cycle.

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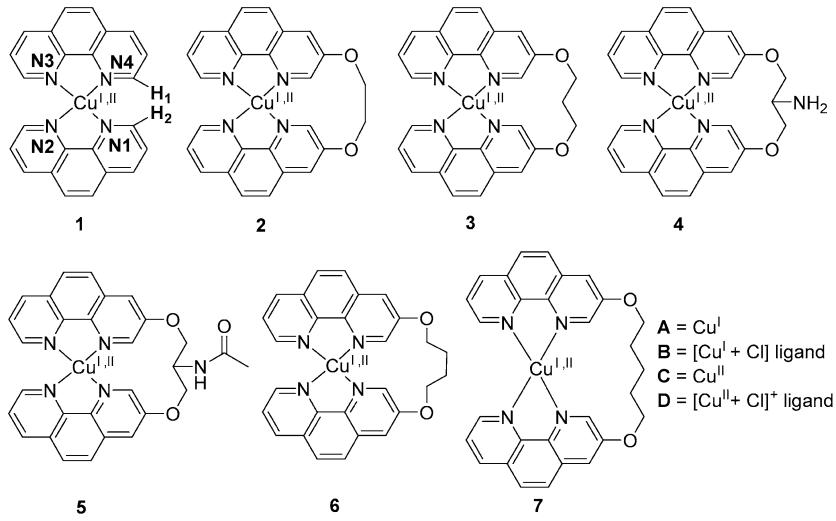

Figure 1. The optimized structures of complexes **1** $Cu(phen)_2$, **2** Cu(3-ethyl-Clip-Phen), **3** Cu(3-propyl-Clip-Phen), **4** Cu(3-Clip-Phen), **5** Cu(3-acetyl-Clip-Phen), **6** Cu(3-butyl-Clip-Phen) and **7** Cu(3-pentyl-Clip-Phen) were calculated for the two oxidation states and with or without chloride as a fifth ligand, namely Cu^{I} (**A**), [Cu^{I}–Cl] (**B**), Cu^{II} (**C**) and [Cu^{II}–Cl]$^{+}$ (**D**). The nitrogen atoms close to the bridges are numbered N1 and N4.

Despite the excellent nuclease properties of these copper-phenanthroline complexes, only few modeling and quantum chemical studies have been performed to investigate their mechanism of action.[23–30] In the present study, Cu^{I} and Cu^{II} complexes (Figure 1) in the presence of a coordinating chloride anion are investigated by theoretical calculations. Their geometry and electronic properties have been calculated in the gas phase by density functional theory (DFT). A thorough comparison of these complexes has been made in order to reveal: (i) the influence of the length of the bridge and of the substituents on the copper coordination geometry, and (ii) the variations of the redox potentials. These studies are aimed at better understanding the influence of the ligand conformation at the copper center on the cleavage activity of phenanthroline-based complexes. Such investigations are expected to be highly beneficial for the rational design and preparation of new copper-based nuclease active agents.

Computational Details

All Density Functional Theory (DFT) calculations were performed using ADF.[31–33] The complexes were subjected to full geometry optimization with Slater type (STO) basis sets of triple ζ with two polarization functions (TZ2P), and a frozen core approximation at the BP86[34,35] level of theory. The copper ion was calculated with a basis set of quadruple ζ with four polarization functions (QZ4P). The optimizations were performed using the zeroth-order regular approximation (ZORA)[36–38] for relativistic effects. Single-point energies (SPE) were calculated with the same conditions. Open-shell Cu^{II} complexes were treated with a spin-unrestricted formalism. In order to relate the structural changes in copper complexes occurring upon the redox process with their cleavage efficiency, the reorganization energy for complexes **1**–**7** was estimated. According to the Marcus theory, the rate of electron transfer is given by Equation (1).

$$k_{ET} = \frac{2\pi}{h}\frac{H_{DA}^2}{\sqrt{4\pi\lambda RT}}\exp\left(\frac{-\left(\Delta G^0+\lambda\right)^2}{4\lambda RT}\right) \quad (1)$$

H_{DA} is the electronic coupling element, which is a function of the overlap between the wave functions of the two states, ΔG^0 is the free energy change of the redox reaction, and λ is the reorganization energy, i.e., the energy associated with relaxing the geometry of the system after electron transfer. In particular, for metal complexes, the inner-sphere reorganization energy (λ_i) is associated with the structural changes between the reduced and oxidized forms. λ_i is the sum of two contributions, λ_{red} and λ_{ox}, which are calculated as follows: λ_{ox} is the difference between the energy of Cu^{II} at its optimal geometry and its energy at the optimal geometry of Cu^{I}. Likewise, λ_{red} is the difference between the energy of Cu^{I} at its optimal geometry and its energy at the optimal geometry of the Cu^{II} complex.[39–43] Inner-sphere reorganization energies of coordination compounds typically range from 1 to 50 kcal/mol (4–200 kJ/mol).[39,42]

Strain energies were calculated as the difference between the energy of $Cu(phen)_2^{2+/+}$ at its optimal geometry and that of a $Cu(phen)_2^{2+/+}$ fragment at the optimal geometry of the corresponding clipped complexes. The strain energy is herein defined as the energy that would be required to deform the $Cu(phen)_2^{2+/+}$ complex to the geometries adopted by the ligands in the bridged complexes.

Ligand binding energies were computed as the difference in energy between the optimal geometry of the complex, and the sum of the optimized ligand and the $Cu^{II/I}$ or (optimized) $[CuCl]^{+/0}$ complex.

Results and Discussion

Calculated Structures of Complexes 1–7

The accuracy of the DFT calculations (BP86) was confirmed by comparison of the unbridged complexes **1A**, **1C**, and **1D**, with experimental structures (with different anions)[44–58] and with earlier reported theoretical results (see supplementary material).[23–25,28,30,59] Furthermore, the basis set and the relativistic effects were tested with complex **4A** (unpublished results). Only minor differences in the total energy are observed between the basis sets TZP, TZ2P and QZ4P (only for copper). The relativistic effects, considered by the ZORA relativistic approximation, are very important. Indeed, calculations using the same basis set, but taking into account the relativistic effects or not, resulted in deviations of about 20 kJ mol^{-1}.

Pitié et al. reported several crystal structures of copper complexes with Cu(Clip-Phen). In all cases, a helical geometry with two copper ions and two ligands (L_2/Cu_2 stoichiometry) was found. Mass spectrometry analyses demonstrated that these complexes mainly exist in solution in a 1:1 ligand-to-copper stoichiometry.[22] The minimized 1:1 L/Cu structures at the BP86/TZ2P/QZ4P level (copper only) for complexes **1AC**, **3AC**, **6AC** and **7AC** with a Cu^{I} (**A**) or a Cu^{II} (**C**) ion are shown in Figure 2. The structures of complexes **1**–**7** with a chloride anion are shown in Figure S1, together with the structures of compounds **2**, **4** and **5** which are related to complex **3**. The chloride anion has been selected as the fifth ligand, because $CuCl_2$ is the copper salt typically used in DNA cleavage studies.[22] Moreover, during the oxidation of DNA, a fifth ligand capable of abstracting a proton from the deoxyribose unit is likely to bind to the copper moiety. Therefore, the structural features of such five-coordinate complexes are of special interest to rationalize the nuclease activity of copper phenanthroline compounds.

In accordance with previous reports, $Cu(phen)_2^+$ (Figure 2, **1A**) has a tetrahedral geometry, which slightly deviates from the D_{2d} symmetry.[23–25,28,30,44–59] The dihedral angle (N1–N2–N3–N4) between the two phenanthrolines amounts to 80° (Table 1). The coordination geometry of $Cu(phen)_2$ drastically changes upon oxidation of the copper ion. However, it has been experimentally shown that bridged Cu^{II} complexes similar to those studied in the present computational investigation are able to retain their four-coordinate environment in solution.[60] The complex $Cu(phen)_2^{2+}$ (Figure 2, **1C**) is significantly more planar than the corresponding $Cu(phen)_2^+$ complex. This structural feature results in a larger N1–Cu–N3 angle (Table 1, 124° for complex **1A** and 144° for complex **1C**) and a smaller N1–N2–N3–N4 torsion angle (Table 1, 80° for complex **1A** and 40° for complex **1C**). The Cu–N distances of **1C** are identical, namely 2.00 Å. The $Cu(phen)_2^+$ and $Cu(phen)_2^{2+}$ complexes holding a chlorido ligand, i.e. **1B** and **1D** (Figure S1 and Table 1), exhibit a trigonal bipyramidal geometry. However, the geometry of compound **1B** is distorted. The equatorial plane is formed by a chlorido ligand and two nitrogen atoms of two different phenanthrolines. The axial positions are occupied by the other two nitrogen atoms of the phenanthroline units. The axial Cu–N distances are typically longer. The Cu–Cl distances of all complexes (**1B**,**D-7B**,**D**) are approximately 2.24 Å, which is in the range of experimental values.[44,47,51,61,62] Complex **1D** displays a Cu–N3 distance of 2.19 Å, which is 0.07 Å longer than the average experimental values. The axial Cu–N lengths of the copper^{I} complex **1B** are very long, namely 2.25 Å for Cu–N1, and 2.42 Å for Cu–N3.

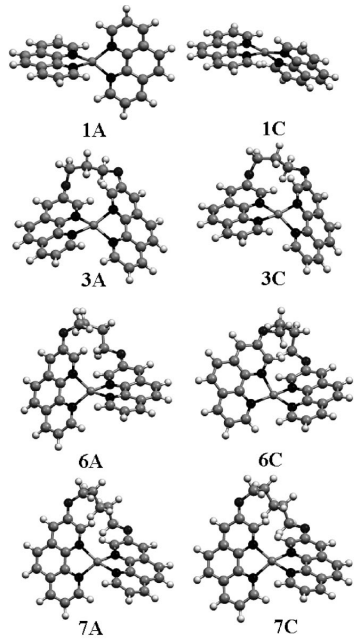

Figure 2. DFT-optimized geometries of complexes **1AC**, **3AC**, **6AC** and **7AC**. The letters **A** and **C** symbolize the structures holding a Cu^{I} or a Cu^{II} moiety, respectively.

In the Clip-Phen complexes, the same characteristic is observed for the Cu–N distances of the complexes **2B**–**7B**. Some of these [Cu^{I}LCl] complexes even show Cu–N distances up to 2.6 Å, which suggests a low stability. The structures of the Cu^{I} complexes **2A**–**7A** significantly differ from the $Cu(phen)_2^+$ one. Due to the presence of the bridge, complexes **2A**–**7A** can not adopt a tetrahedral geometry, as is evidenced by the N1–Cu–N3 angle and the N1–N2–N3–N4 dihedral angle. The N1–Cu–N3 angle for $Cu(phen)_2^+$ is 124°, whereas this angle varies from 159° to 166° for complexes **2A**–**5A**. Complexes **6A** and **7A** possess a bridge of respectively, four and five methylene groups; therefore, the N1–Cu–N3 angle amounts to respectively 131° and 130°, which are values much closer to the one observed for $Cu(phen)_2^+$. Similarly, complexes **2A**–**7A** exhibit torsion angles of respectively 31°, 38°, 38°, 41°, 51° and 54°, far below the value of 80° displayed by complex **1A**.

For complex **2A**, the small dihedral angle of 31° results in very close contacts between the protons H1 and H2 (H1···H2 2.20 Å), and steric repulsion can be expected. The Cu–N1 (2.28 Å) and Cu–N3 (2.15 Å) distances are also longer, compared to those of complexes with a longer bridge. This H1···H2 distance becomes even smaller upon

Table 1. Selected bond lengths and angles for complexes **1**–**7**.

Structures	Distance [Å]		Angle [°]		Dihedral angle [°]		Distance [Å]	
	Cu–N1	Cu–N2	Cu–N3	Cu–N4	Cu–Cl	N1–Cu–N3	N1–N2–N3–N4	H1–H2
1A	2.00	2.00	2.00	2.00		124	80	4.56
2A	2.28	1.98	2.15	1.99		166	31	2.20
3A	2.08	2.04	2.05	2.06		164	38	2.56
4A	2.07	2.04	2.05	2.05		165	38	2.59
5A	2.04	2.03	2.02	2.05		159	41	2.61
6A	2.00	2.04	1.99	2.11		131	51	3.15
7A	2.01	2.03	2.01	2.05		130	54	3.07
1B	2.25	2.10	2.42	2.04	2.23	167	37	2.75
2B	2.58	2.06	2.28	2.03	2.23	163	23	2.14
3B	2.50	1.99	2.05	2.63	2.23	146	30	2.61
4B	2.32	2.11	2.13	2.25	2.24	160	29	2.63
5B	2.48	2.05	2.32	2.03	2.23	168	28	2.68
6B	2.02	2.25	2.02	2.65	2.23	166	37	2.81
7B	2.02	2.45	2.03	2.33	2.24	172	40	2.91
1C	2.00	2.00	2.00	2.00		144	40	2.52
2C	2.05	2.03	2.04	2.01		173	23	1.98
3C	2.01	2.05	2.02	2.04		172	26	2.20
4C	2.00	2.05	2.00	2.05		170	28	2.33
5C	2.00	2.04	2.01	2.04		167	28	2.33
6C	1.98	2.01	2.00	2.01		141	40	2.77
7C	2.00	2.00	2.00	2.01		143	40	2.57
1D	2.00	2.11	2.02	2.19	2.25	178	47	2.90
2D	2.08	2.27	2.05	2.09	2.22	165	32	2.16
3D	2.02	2.20	2.02	2.17	2.24	167	35	2.51
4D	2.01	2.15	2.02	2.21	2.24	167	36	2.61
5D	2.02	2.23	2.01	2.14	2.24	169	36	2.53
6D	2.1	2.03	2.19	2.05	2.23	171	46	3.06
7D	2.21	2.03	2.09	2.02	2.24	175	48	3.05

oxidation (complex **2C**), with a value reaching 1.98 Å. The Cu^{II} complexes with a 3-carbon bridge (complexes **3C**–**5C**) also show short H1···H2 contact distances, ranging from 2.20 to 2.33 Å. Due to a longer bridge, complexes **1C**, **6C** and **7C** have H1···H2 separation distances of 2.52, 2.77 and 2.57 Å, respectively.

Interestingly, the geometry of the complexes with two or three methylene-bridges (complexes **2A**–**5A**) does not change drastically upon oxidation (complexes **2C**–**5C**) of the copper(I) center. The dihedral angle (N1–N2–N3–N4) decreases on average by 10°, and the N1–Cu–N3 angle experiences an increase of about 8°. 2- and 3-carbon bridges thus prevent large geometrical changes upon oxidation or reduction of the corresponding complexes, in contrast to the significant structural variations observed with longer bridges or in the absence of bridges. The complexes **1C**, **6C** and **7C** present a dihedral angle (N1–N2–N3–N4) between the aromatic rings of 40°, and an N1–Cu–N3 angle of approximately 143°. The Cu–N distances of all the complexes are close to 2.00 Å.

Similar findings are noted with the $[Cu^{II}Cl]^+$ structures of complexes **2D**–**5D**. Due to their short bridging unit, the complexes **2D**–**5D** have distorted trigonal bipyramidal geometries. The torsion angle N1–N2–N3–N4 ranges from 32° to 36° and the N1–Cu–N3 angle varies from 165° to 169° for complexes **2D** and **5D**, respectively. $[Cu^{II}(phen)_2Cl]^+$ (**1D**) has a trigonal bipyramidal geometry, with a torsion angle N1–N2–N3–N4 of 47°, and a N1–Cu–N3 angle of 178°. Complexes **6D** and **7D** share their structural arrangement with complex **1D**. The N1–N2–N3–N4 torsion angles are 46° and 48°, and the N1–Cu–N3 angles are 171° and 175° for complexes **6D** and **7D**, respectively. The axial positions are occupied by two nitrogen atoms from two different phenanthroline ligands. The axial Cu–N distances in compounds **1D**, **3D**–**7D** are close to 2.20 Å, while complex **2D** has a bond length of 2.27 Å. These values strongly differ from the calculated ones for the $[Cu^{I}Cl]$ structures (Cu–N bond lengths up to 2.6 Å).

It should be noted that the amine and acetamide groups of compounds **4** and **5** do not interact with the central copper ion (the stereo-isomers in which these groups are folded to the inner sides of the bridges are sterically unfavorable and even then these functional groups do not interact with the central copper ion). The experimentally observed effects of functional groups in the bridge probably are caused by their interaction with the DNA strand.

Comparison of the Calculated Energies of Complexes 1–7

The ligand binding energies and the strain energies, as defined in the computational details section, are reported in Table 2. In general, the $Cu(Clip\text{-}Phen)^{2+}$ complexes have the strongest ligand binding energies and the $[Cu(Clip\text{-}Phen)Cl]^0$ complexes the weakest, following the net charges of the complexes. This observation fits with the elongated Cu–N bond lengths observed in the previous section. Furthermore, the complexes **2**–**6** (Figure 1), characterized by short bridges, have weaker complex ligand binding energies than complex **1**. Complex **2** exhibits the weakest ligand

Table 2. Ligand binding energies and strain energies (on the ligand coordination) of complexes **1**–**7**.

Complex	Ligand binding energy [kJ mol^{-1}]	Strain energy [kJ mol^{-1}]	Complex	Ligand binding energy [kJ mol^{-1}]	Strain energy [kJ mol^{-1}]
1A	–759	0	**1C**	–1892	0
2A	–656	110	**2C**	–1818	95
3A	–692	76	**3C**	–1867	58
4A	–662	76	**4C**	–1838	56
5A	–727	69	**5C**	–1920	53
6A	–711	49	**6C**	–1892	35
7A	–730	26	**7C**	–1922	11
1B	–232	0	**1D**	–707	0
2B	–194	48	**2D**	–631	74
3B	–204	38	**3D**	–673	43
4B	–180	36	**4D**	–645	42
5B	–215	35	**5D**	–696	45
6B	–214	21	**6D**	–686	24
7B	–214	5	**7D**	–709	3

binding energy with one exception: the ligand binding energy is stronger for **2B** than for **4B**. A reasonable assumption to explain this weaker interaction obtained for **4B** is the steric hindrance of the chloride atom by the oxygen from the acetamide group of the bridge.

In order to investigate the geometrical constraints imposed by the bridge of the Clip-Phen-based complexes, the strain energy (see computational details) was also calculated (Table 2 and Figure 3). The Cu^{I} structures **2A**–**7A** show the highest strain energies, which can be explained by the significant difference between the geometries of these complexes and the tetrahedral environment of $Cu(phen)_2^+$ (**1A**). Upon binding of the chloride, the geometry of **1B** changes drastically to a trigonal bipyramidal environment, comparable to the environment of complexes **2B**–**7B**. As a result, the strain energies of the $[Cu^{I}Cl]$ complexes **2B**–**7B** are much lower. The small variations in strain energy between the Cu^{II} and $[Cu^{II}Cl]^+$ structures are ascribed to the minor structural differences between **1C** and **1D**. As expected, complex **2** holding the shortest bridge has the highest strain energy, which can be justified by steric repulsions between the protons H1 and H2 of the phenanthrolines. Strain energies are lower for the complexes with a three-methylene bridge, i.e. complexes **3**–**5** (Figure 1). No significant differences are observed between these three complexes, indicating that the functionalization of the bridge has no influence on the magnitude of the strain energy. A further increase of the bridge length leads to computed structures closely related to the one of complex **1**. Thus, complexes **6** and **7** show markedly lower strain energies than complexes **2**–**5**. In particular, complex **7** displays a very low strain energy, which clearly indicates that the long bridge does not induce significant geometrical constraints. The coordination characteristics of **7** are therefore very close to those of $Cu(phen)_2^{2+/+}$ complexes.

The effect of the bridge on the redox properties was estimated for these complexes, by calculation of the inner-sphere reorganization energy (see computational details). The redox properties of the complexes are important because the compounds have to go through a full redox cycle during their cleavage activity. Table 3 displays the λ_{red}, λ_{ox} and λ_i values of complexes **1**–**7** in vacuo. $Cu(phen)_2$ has a λ_{red} of 19 kJ mol^{-1} and a λ_{ox} of 25 kJ mol^{-1}. The sum of these components, i.e. λ_i, is 44 kJ mol^{-1}. Complexes **2**–**4**, **6** and **7** have comparable inner-sphere reorganization energies; only minor differences are noticed. Complexes **3** and **4** have a slightly lower inner-sphere reorganization energy, which can be expected, since the structural differences between the reduced and oxidized structures are less significant than for complex **1**. Complex **2** has a higher λ_i compared to compound **1**, possibly due to the distorted Cu^+ structure. Only the λ_i value of complex **5** is markedly higher than those of the other complexes. In general, the complexes bearing a chlorido ligand (**1BD**-**7BD**) have higher λ_i values compared to the compounds without chlorido ligand (**1AC**-**7AC**). The highly distorted $[Cu^{I}Cl]$ structures of all the complexes are reflected by the large differences observed for the λ_{ox} values for the chloride-containing complexes. Consequently, it is not possible to draw any general conclusions from the differences in the λ_i values of the complexes with a chlorido ligand, other than that distortion by additional ligands can affect the redox properties rather drastically. Cyclic voltammetry experiments performed by Pitié

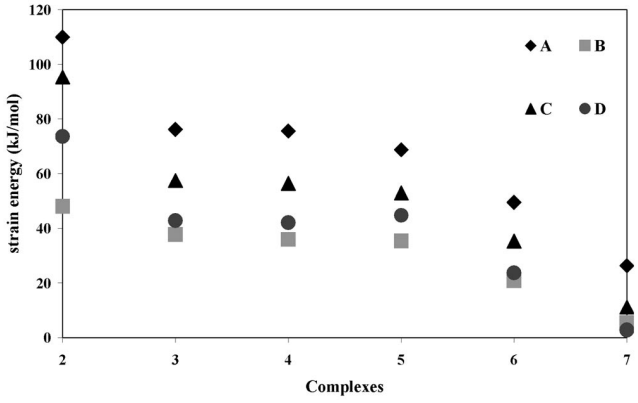

Figure 3. Strain energies of complexes **2**–**7**. The letters **A**, **B**, **C** and **D** symbolize the structures holding a Cu^{I}, $[Cu^{I}–Cl]$ Cu^{II} or $[Cu^{II}–Cl]^+$ moiety, respectively.

et al.[22] indicated that the redox properties of complexes **2**–**7** are analogous. The small differences noticed for the complexes without chlorido ligand reflect these experimental findings.

Table 3. Inner-sphere reorganization energies ($kJ\,mol^{-1}$) of complexes **1**–**7**.

Complex	λ_{red}	λ_{ox}	λ_i	Complex + Cl	λ_{red}	λ_{ox}	λ_i
1	19	25	44	**1** + Cl	26	50	76
2	22	23	46	**2** + Cl	37	40	77
3	23	18	41	**3** + Cl	19	60	79
4	21	17	38	**4** + Cl	22	34	55
5	25	30	55	**5** + Cl	60	78	138
6	19	27	46	**6** + Cl	35	48	83
7	25	24	48	**7** + Cl	25	63	88

Effect on the Nuclease Activity

The following nuclease activities were found for complexes **1**–**7**: **4** ($S = 35$) >> **5** ($S = 21$) > **3** ($S = 15$) > **2** ($S = 10$) = **6** ($S = 10$) >> **7** ($S = 2$) > **1** ($S = 0.9$), where S is a value to define the cleaving ability.[22] The limited activity of $Cu(phen)_2^{2+}$ (**1**) is presumably due to the loss of one phenanthroline ligand leading to the formation of $Cu(phen)^{2+}$, a much less active cleaving agent, at low complex concentrations, as a result of the low binding constant of the second phenanthroline ligand. However, the difference in cleaving activity between complexes **1** and **7** is minor, although compound **7** has a bridge that prevents the dissociation of the second phenanthroline. The computed coordination geometries of complexes **1** and **7** are comparable; only the geometry of complex **7A** is significantly different from the one of compound **1A**. Apparently, the geometry constraints generated by the short bridges are crucial for the nuclease activity.

Kinetic studies have demonstrated that the nuclease activity of $Cu(phen)_2^{2+}$ proceeds by an ordered mechanism: the freely diffusing $Cu(phen)_2^{2+}$ is first reduced to the Cu^I complex, i.e. $Cu(phen)_2^{+}$, which binds reversibly to DNA.[9] The relative planarity (dihedral angles of 30–50 degrees vs. 80 °) of the structures **2A**–**7A** (Figures 2 and S1) should favor the intercalation, and therefore increase the binding to DNA. It should be pointed out here that coordination compounds do not necessarily need to be fully planar to be able to enter the minor groove; for instance in the present series of complexes, if the torsion angle N1–N2–N3–N4 is small enough then the potential interaction with the minor groove is increased. A shortening of the bridge leads to a strong increase in the nuclease activity. However, complex **2** with a two-methylene bridge is less active than complex **3** which possesses a bridge of three carbon atoms. Apparently, a two-methylene bridge is too short, introducing too much strain, recognizable in elongated Cu–N distances and the very short H1–H2 distance (Table 1). Bridges *longer* than three carbon atoms (i.e. complexes **6** and **7**) do not apply enough constraint, and the geometries become less planar.

Interestingly, while the calculated geometries and the corresponding energies of complexes **3**–**5** are comparable, the cleavage activities are rather disparate, probably due to specific interactions of the amine or acetamide group with DNA. Nevertheless, it is clear that the 3-methylene bridges give the best cleavage activities, and from the calculations reported in the present study, it seems apparent that this is due to the planar geometry enforced by these short bridges.

Conclusions

A theoretical study has been performed with $Cu(phen)_2^{2+/+}$ and a series of $Cu(Clip\text{-}Phen)^{2+/+}$ complexes whose bridge length and functionalization have been varied. The calculations have been carried out in the presence and in the absence of an added chlorido ligand. This study confirms that upon oxidation of $Cu(phen)_2^{+}$, the structural environment of the metal center drastically changes from a tetrahedral geometry for the initial copper(I) species to a geometry in between tetrahedral and square planar for $Cu(phen)_2^{2+}$. The coordination of a chlorido ligand results in a trigonal bipyramidal environment for $[Cu(phen)_2Cl]^{+/0}$. In general, the $[Cu^ICl]$ structures of type **B** are highly distorted, reflected by some of their long Cu–N distances.

Complexes **6BCD** and **7BCD** exhibit similar structures, related to those of compounds **1BCD**. However, complexes **6A** and **7A** can not adopt a true tetrahedral geometry, as a result of spatial limitations induced by the bridge. The short bridges of complexes **2**–**5** force the compounds to adopt geometries, which are distinct from the one found in complex **1**. The Cu^{II} complexes **C** and **D** and the Cu^I complexes **A** and **B** show comparable geometries. The 2-carbon bridge of complex **2** forces the phenanthroline protons next to the nitrogen atom to be in close contact (within 2 Å), which obviously instigates steric hindrance. This decrease in stability is further proven by the corresponding ligand binding energy, which is the strongest obtained. An unambiguous relation between the number of methylene groups constituting the bridge and the strain energy is observed: the highest energies are observed with the shortest bridges, and vice versa.

For the redox properties of the complexes, no correlation is observed with their cleaving abilities. The redox properties of the complexes are only mildly influenced by the bridges. Also no significant influence of the bridge length is observed on the inner-sphere reorganization energies of complexes **2**–**7**. A higher energy is only noted for complex **5**. These results nicely correlate with earlier reported experimental findings.[22]

The cleaving abilities of the complexes with unfunctionalized bridges can be compared, because their interaction with DNA is mainly achieved by intercalation. The different cleaving abilities observed, i.e. **3** > **2** = **6** > **7**, can be explained as follows: (i) a shortening of the bridge gives rise to an increase of the planarity of the resulting Cu^I complexes, which is reflected by a subsequent higher affinity for DNA, and (ii) the structural changes occurring upon oxidation or reduction are less dramatic for the complexes possessing a short bridge. Accordingly, the kinetics of the

cleavage reaction are enhanced. The fact that complex **2** is less active than compound **3** may be explained by a lower stability. Indeed, the H1 and H2 proton are very close to each other, resulting in the highest strain and ligand binding energies and elongated Cu–N distances. Complexes **3**–**5** exhibit three-methylene bridges and complexes **4** and **5** have a substituent at the C2 position of their three-carbon bridge. The corresponding geometrical and energy profile disparities are minor; nonetheless, significant differences are observed in the cleaving activities. Most likely, the additional functional groups on the bridge improve the interaction with DNA, and thus the nuclease activity.

The extensive DFT study herein presented investigates the structural and electronic properties of a series of bridged copper phenanthroline complexes in order to ultimately promote the rational design and synthesis of novel phenanthroline-based chemical nucleases. Theoretical investigations of the interaction of some of these complexes with DNA are currently in progress.

Supporting Information: Comparison of the structure of **1** with experimental and theoretical results. See also the footnote on the first page of this article

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