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Dramatic increase of the DNA cleavage activity of Cu(Clip-phen) by fixing the bridging linker on the C^3 position of the phenanthroline units

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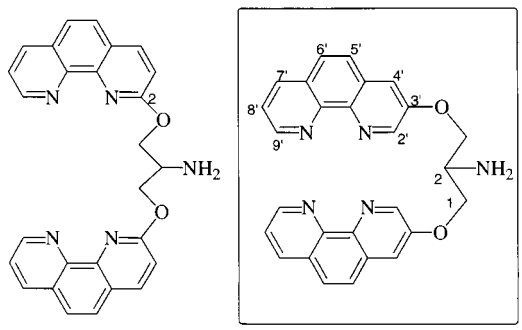
The synthesis of a new ligand with two phenanthrolines bridged on their C^3 carbon by a serinol is reported; Cu(3-Clip-phen) cleaves DNA more efficiently than the parent Cu(2-Clip-phen) bridged on the C^2 carbon.

The redox activity of cuprous complexes of 1,10-phenanthroline (phen) is well known as artificial nuclease. They are able to realise single-strand cleavages of DNA in the presence of H_2O_2 by oxidative attack on deoxyribose units from the minor groove of DNA.[1,2] $Cu^I(phen)_2$ is significantly more reactive[3] than $Cu^I(phen)$ but the association constant for the second phenanthroline ligand is only $10^{5.5}$ dm^3 mol^{-1} which is too low for a biological use at submicromolar concentrations of these complexes.[4] For this reason, we have recently prepared Clip-phen (renamed 2-Clip-phen in the present study, Scheme 1) with two phenanthroline entities linked *via* their C^2 carbon by a short flexible arm in order to favor the 2 : 1 phen–Cu stoichiometry.[5] In the presence of a reductant and air, we observed an increase of DNA cleavage activity on ΦX174 by a factor two for Cu(2-Clip-phen) compared to $Cu(phen)_2$. In addition, the serinol bridge between the two phenanthrolines of 2-Clip-phen allowed further functionalisation on its primary amino group with different possible vectors (polyamines, intercalators, oligonucleotides) in order to increase or to modulate the binding domain of these DNA cleavers. So, the attachment of the natural polyamine spermine (a minor groove binder) to 2-Clip-phen afforded a new conjugate with enhanced nuclease efficiency.[6]

The bridging serinol was fixed on the C^2 carbon of phen units in 2-Clip-phen, near the chelating nitrogen atoms, in order to decrease the length between the two phen entities. But, having in mind that this substitution on C^2 carbon has been observed to disactivate the DNA cleavage activity of Cu–phen complexes,[1,7] we decided to prepare a new bis-chelating ligand, 3-Clip-phen, with the serinol bridge between the C^3 carbons of phen units. The 3-position is known to have fewer effects on redox activity[4] and may also allow the use of a small bridge like serinol between the two phen units in order to favor a mononuclear Clip-phen complex $[Cu^{II}(Clip\text{-}phen)]^{2+}$. However the C^3 monosubstitution of the phen ligand, which limits steric constraints for DNA interactions and for conformational changes during the reduction of Cu^{II} to Cu^I, has been little studied although a conjugate of acridine on the C^3 carbon of phen has been described to cleave DNA.[8]



Scheme 1 Structures of 2-Clip-phen and 3-Clip-phen. Numbering corresponds to NMR assignments.

The synthetic strategy used to prepare 3-Clip-phen was based on the synthesis of the 2-Clip-phen parent compound. Two equivalents of halogenated phenanthroline (600 mg, 2.3 mmol of 3-bromophenanthroline prepared according to ref. 9) and one equivalent of serinol (109 mg, 1.18 mmol) were stirred for 24 h in dry DMF (14 mL) in presence of 9 equivalents of NaH (423 mg, 10.5 mmol of a 60% dispersion in mineral oil). At 0 °C, 2-Clip-phen was obtained in good yield, but 3-Br-phen gave only 4% of 3-Clip-phen under the same conditions. The low reactivity of 3-Br-phen for nucleophilic aromatic substitution required warming of the reaction mixture.[10] Unfortunately the quantitative reduction of 3-Br-phen by NaH at 80 °C forced us to choose intermediate heating conditions at 50 °C. Under these conditions 3-Clip-phen was only obtained in 27% yield after purification [addition of ethanol and water in the reaction mixture in order to destroy the excess of NaH, then extraction with chloroform, precipitation with hexane to remove unreacted 3-Br-phen and phen and a neutral alumina column ($CHCl_3$ with 0–5% of methanol)].†

3-Clip-phen was metallated with one equivalent of $CuCl_2$ and its DNA cleavage activity was compared to that of $[Cu(2\text{-}Clip\text{-}phen)]Cl_2$. Relaxation of supercoiled ΦX174 DNA (form I) into relaxed circular (form II) and linear (form III) conformations was used to quantify the relative cleavage efficiency of these copper complexes. The nuclease activity of these Cu(II) complexes (1 μM) was initiated by addition of 5 mM mercaptopropionic acid (MPA) in the presence of air. Fig. 1 summarises the results obtained.‡

As expected, no degradation of DNA was observed in the absence of reductant. The comparison of lanes 4 and 5 shows that Cu(3-Clip-phen) complex exhibited a significantly higher activity than Cu(2-Clip-phen) since all form I disappeared, to give forms II and III (50% of the starting material) and a smear (corresponding to multifragmented DNA), whereas form I was

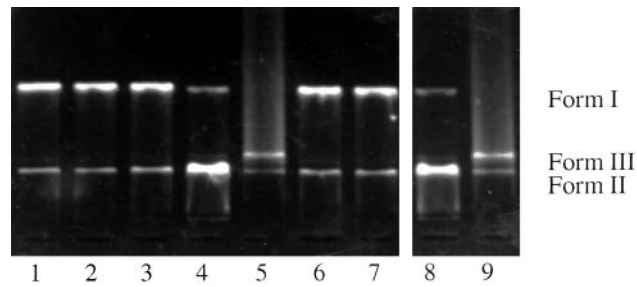


Fig. 1 Comparison of ΦX174 cleavage efficiency between 2-Clip-phen and 3-Clip-phen in the presence of $CuCl_2$ and 5 mM MPA. Lane 1: control DNA. Lane 2: 1 μM 2-Clip-phen and $CuCl_2$ without MPA. Lane 3: 1 μM 3-Clip-phen and $CuCl_2$ without MPA. Lane 4: 1 μM 2-Clip-phen and $CuCl_2$. Lane 5: 1 μM 3-Clip-phen and $CuCl_2$. Lane 6: control DNA with 5 mM MPA. Lane 7: control DNA with 1 μM $CuCl_2$ and 5 mM MPA. Lane 8: 1 μM 2-Clip-phen and 2 μM $CuCl_2$. Lane 9: 1 μM 3-Clip-phen and 2 μM $CuCl_2$.

still present for the same concentration of Cu(2-Clip-phen) under identical conditions. Assuming a non-specific single-strand cleavage of the DNA double-helix, as previously observed for Cu(2-Clip-phen),[6] the number of single-strand breaks per ΦX174 DNA molecule was calculated according to ref. 11. Cu(3-Clip-phen) gave 32 ± 4 single strand breaks per ΦX174 DNA compared with 1.4 ± 0.2 for Cu(2-Clip-phen). The comparison of lanes 5 and 9 shows also that Cu(3-Clip-phen) gave the same number of single strand breaks when metallated by 1 equivalent of copper salt [1 Cu for 2 phen entities, corresponding to a Cu(3-Clip-phen) complex] or 2 equivalents of copper salt [1 Cu per phen entity, corresponding to a putative Cu_2(3-Clip-phen) compound] as expected for a ligand able to chelate the same copper ion with its two phen subunits.

In order to understand this high DNA cleavage activity of Cu(3-Clip-phen), work on interactions of the complex with DNA are in progress. New conjugates of 3-Clip-phen with DNA binders are also in preparation in order to increase the nuclease activity of this promising new family of DNA cleavers.

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Notes and references

† 3-Clip-phen has been characterised by ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.14 (dd, 2H, *J* 4.3, 1.7 Hz, H9′), 8.96 (d, 2H, *J* 2.9 Hz, H2′), 8.20 (dd, 2H, *J* 8.1 and 1.7 Hz, H8′), 7.78 and 7.72 (AX, 2H, *J* 8.9 Hz, H5′, H6′), 7.60 (d, 2H, *J* 2.9 Hz, H4′), 7.56 (dd, 2H, *J* 8.1, 4.3 Hz, H7′), 4.36 (m, 4H, H1), 3.82 (q, 1H, *J* 5.4 Hz, H2); MS (CDI, NH_3): *m*/*z* (%) = 448 (M + H, 100), 268 (14.0), 252 (22.7), 197 (75.9); UV–VIS (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}): 240 (59 500), 272 (44 100), 294 (23 900, sh), 314 (8200, sh), 328 (5700), 344 (3800). Anal. Calc. for $C_{27}H_{21}N_5O_2 \cdot 4\ H_2O$: C, 62.42; H, 5.63; N, 13.48. Found: C, 62.38; H, 5.09; N, 13.66%.

‡ Complexes were prepared as 1 mM solutions in DMF–water (2 : 3) then diluted to 4 μM with water prior to the addition of 5–10 μL of a solution of supercoiled ΦX174 DNA (7 nM, 40 μM in bp) in 80 mM sodium phosphate buffer (pH 7.2), 100 mM NaCl and 20 mM $MgCl_2$. After 30 min at room temperature, DNA cleavage was initiated by addition of 5 μL of a 20 mM aquous solution of mercaptopropionic acid and incubated at 37 °C for 1 h prior to being loaded on a 0.8% agarose gel containing 1 μg mL^{-1} of ethidium bromide. Bands were located by UV light, photographed and quantified by microdensity. The correction coefficient 1.47 was used for the decrease in stainability of form I DNA *versus* forms II and III.[12]

1 D. S. Sigman, A. Mazumder and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2295.
2 G. Pratviel, J. Bernadou and B. Meunier, *Adv. Inorg. Chem.*, 1998, **45**, 251.
3 J. M. Veal, K. Merchant and R. L. Rill, *Nucleic Acids Res.*, 1991, **19**, 3383.
4 B. R. James and R. J. P. Williams, *J. Chem. Soc.*, 1961, 2007.
5 M. Pitié and B. Meunier, *Inorg. Chem.*, 1998, **37**, 3486.
6 M. Pitié and B. Meunier, *Bioconjugate Chem.*, 1998, **9**, 604.
7 J. Gallagher, C. B. Chen, C. Q. Pan, D. M. Perrin, Y. Cho and D. S. Sigman, *Bioconjugate Chem.*, 1996, **7**, 413; T. B. Thederan, M. D. Kuwabara, T. A. Larsen and D. S. Sigman, *J. Am. Chem. Soc.*, 1989, **111**, 4941.
8 F. C. K. Chiu, R. T. C. Brownlee, K. Mitchell and D. R. Phillips, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2721.
9 D. Tzalis, Y. Tor, S. Failla and J. S. Siegel, *Tetrahedron Lett.*, 1995, **36**, 3489.
10 D. Tzalis and Y. Tor, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2666 and references therein.
11 R. P. Hertzberg and P. B. Dervan, *Biochemistry*, 1984, **23**, 3934.
12 J. Bernadou, G. Pratviel, F. Bennis, M. Girardet and B. Meunier, *Biochemistry*, 1989, **28**, 7268.

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