# Syntheses, Crystal Structure, and Nuclease Activity of Oxalato-Bridged Dicopper(II) Complexes with Planar N-Donor Heterocyclic Bases

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Oxalato-bridged dicopper(II) complexes [{CuL($H_2O$)}$_2$(μ-ox)]-($ClO_4$)$_2$ (**1**–**4**), where L is a bidentate chelating heterocyclic base such as 2,2′-bipyridine (bpy, **1**), 1,10-phenanthroline (phen, **2**), dipyridoquinoxaline (dpq, **3**) and dipyridophenazine (dppz, **4**), were prepared by treatment of copper(II) perchlorate hexahydrate with sodium oxalate and L in aqueous ethanol. Complex **3** has been structurally characterised. The coordination geometry of the copper centres is essentially square-pyramidal. The {CuL($H_2O$)} units are bridged by an oxalate moiety to form the discrete dimeric core. Magnetic studies in the 20–300 K temperature range show the presence of an antiferromagnetically coupled dicopper(II) unit, giving $-2J$ values in the 346–384 $cm^{-1}$ range. The redox-active complexes retain the dimeric core in solution, and two quasireversible $Cu^{II}/Cu^{I}$ couples are observed in the potential range from 0.2 to –0.4 V vs. SCE in a DMF/tris–HCl/0.1 M KCl buffer system (1:1, v/v, pH = 7.2) with a glassy carbon working electrode. The binding and cleavage of DNA by the complexes have been studied. The intercalating ability of the planar bases, determined from the decrease of the fluorescence intensity of ethidium bromide bound calf thymus DNA on addition of the complex, gave the order: **3** > **4** > **2** >> **1**. The nuclease activity of the complexes with supercoiled pUC19 DNA in DMF/tris–HCl/NaCl buffer (pH = 7.2) in the presence of ascorbic acid follows the order: **3** > **2** >> **4** ≈ **1**. The dpq complex exhibits the most efficient DNA binding and cleavage activity. The dppz complex shows good binding ability but poor nuclease activity.


## Introduction

Coordination complexes that oxidatively cleave DNA under physiological conditions are of current interest in the development of artificial nucleases.[1−3] In view of the important role played by copper ions in enzymatic catalysis, efforts to develop the chemistry of redox-active copper complexes as chemical nucleases are underway.[1−6] It has been suggested that metallointercalators such as the bis(1,10-phen) complex of copper(I), on activation by hydrogen peroxide in the presence of a reducing agent, cleave DNA through the formation of a hydrogen-abstracting active oxygen species.[6] It has also been postulated that the active oxo species react with the H1′ or H4′ deoxyribose protons of the nucleotide in the vicinity of the binding site at the minor groove to initiate a series of reactions resulting in the cleavage of DNA.[5a,7] The cleavage efficiency is generally dependent on the DNA binding ability and the metal-based redox potential of the complex.

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Although the true binding mode of the bis(phen) complex to DNA is currently unknown, it has been indicated by several research groups that the nuclease activity of the complex is related to the partial intercalation or binding of one phen ligand to the minor DNA groove, with the other phen ligand making favourable contacts within the groove.[8−11] Rill and co-workers have observed that the phen complex displays efficient nuclease activity at a phenanthroline/copper ratio of greater than 2:1, regardless of the reducing agent used in the cleavage reaction.[8] This current work stems from our interest in covalently linking two mono(phen) and related copper units through an oxalate moiety to form a dinuclear complex with an N-donor base/copper ratio of 1:1 and studying the DNA binding and nuclease activity of the complexes. The objective of the use of the oxalate as a linker is to provide a planar skeleton in the {LCu−ox−CuL} moiety. In addition, the bio-relevant oxalate ligand is an oxidized product of ascorbic acid (vitamin C), used extensively in copper-mediated enzymatic reactions.[12] An essentially planar $N_4O_4Cu_2$ unit with planar N-donor bases should be expected to show good binding ability to the DNA minor groove. Previous studies on oxalato-bridged dicopper(II) complexes have shown that an estimate of the planarity of the core can readily be obtained from the singlet-triplet energy gap, which varies over a wide range depending upon the nature of the terminal

Table 1. Spectral and magnetic data for the complexes $[\{CuL(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2$ (**1**−**4**)

| Complex | IR[a]/cm$^{-1}$ oxalate | $ClO_4^-$ | d-d band[b] $\lambda_{max}$/nm | $\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ | Magnetic data[c] $-2J$/cm$^{-1}$ | $g$ ($\rho$) | $\mu_{eff}/\mu_B$ |
|---|---|---|---|---|---|---|---|
| **1** | 1653 | 1091 | 650 | 140 | 358 | 2.18 (0.01) | 1.3 |
| **2** | 1651 | 1098 | 665 | 120 | 370 | 2.19 (0.029) | 1.5 |
| **3** | 1655 | 1085 | 667 | 114 | 384 | 2.19 (0.015) | 1.4 |
| **4** | 1662 | 1077 | 710 | [d] | 346 | 2.19 (0.049) | 1.4 |

[a] KBr phase. [b] Aqueous DMF (1:1, v/v). [c] $\mu_{eff}$ per copper at 298 K; $\rho$: paramagnetic impurity. [d] Not measured due to solubility problem.

ligands.[13−17] Here we report the synthesis, structure and nuclease activity of the dicopper(II) complexes $[\{CuL(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2$ (**1**−**4**) with planar heterocyclic bases as terminal ligands [L: 2,2′-bipyridine (bpy), **1**; 1,10-phenanthroline (phen), **2**; dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (dpq), **3**; and dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz), **4**]. The significant result of this study is the observation of varied DNA binding and nuclease activity of the structurally similar complexes **1**−**4**. A preliminary report on the crystal structure of complex **1** has already appeared.[18]

## Results and Discussion

### Synthesis and Characterization

Complexes **1**−**4**, prepared by treatment of $Cu(ClO_4)_2\cdot 6H_2O$ with the bidentate N-donor heterocyclic base (L) and sodium oxalate in aqueous ethanol, belong to the general class of oxalato-bridged dicopper(II) complexes of formulation $[LCu(\mu\text{-}C_2O_4)CuL]X_n$, where X is a counteranion or solvent molecule.[13] The physicochemical data for **1**−**4** are given in Table 1. The complexes are moderately soluble in methanol and MeCN, soluble in DMF or DMSO. While the bpy and phen complexes are moderately water-soluble, the dpq and dppz complexes show poor solubility in water. They display characteristic infrared bands in the 1651−1662 and 1077−1098 cm$^{-1}$ ranges for the oxalate and perchlorate, respectively. The complexes show a d-d band in the 650−710 nm range in aqueous DMF solution.

### Crystal Structure and Magnetic Properties

Complex **3**, crystallised as $[Cu_2(ox)(dpq)_2(H_2O)_2](ClO_4)_2\cdot 2H_2O$, has been structurally characterised by single-crystal X-ray diffraction. A perspective view of the complex is shown in Figure 1. The crystallographic data and the data collection parameters are summarized in Table 5 and the selected bond lengths and angles are given in Table 2. The structure consists of a centrosymmetric complex in which $Cu(dpq)(H_2O)$ units are bridged by oxalate to give a copper−copper distance of 5.138 Å. Each copper centre is essentially in a 4 + 1 coordination environment, with the N(1), N(2), O(1), and O(2) atoms forming a basal plane and the Ow(1) atom of the bound water occupying the axial position. The perchlorate anion, involved in the hydrogen-bonding interactions with the water molecules, also shows a very weak axial contact to the copper centre through oxygen atom O(11) [Cu(1)···O(11), 2.696 Å]. The planar dpq ligand exhibits a bidentate chelate mode of bonding and non-involvement of the quinoxaline moiety in any metal−ligand interaction. The deviation of the copper atom from the basal plane is 0.062 Å, which is the shortest among known copper−oxalate species.[14] The angle formed between the basal plane containing the copper atom and the oxalate plane is 2.55°. This angle gives a measure of the coplanarity, an important factor for derivation of the orbital topologies.

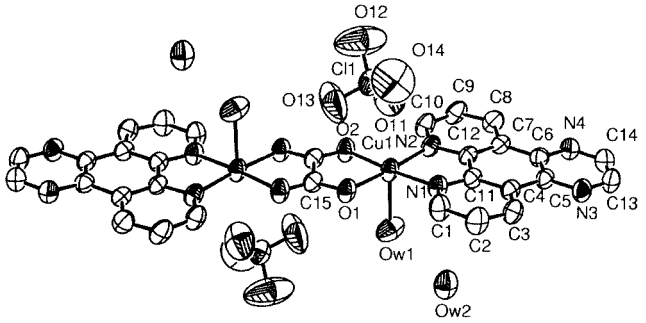



Figure 1. An ORTEP view of $[\{(dpq)Cu(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2\cdot 2H_2O$ (**3**·2H$_2$O) showing the atom labelling scheme and thermal ellipsoids at a 50% probability level

Table 2. Selected bond lengths [Å] and bond angles [°] for the complex $[\{Cu(dpq)(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2\cdot 2H_2O$ (**3**.2H$_2$O)

| | | | |
|---|---|---|---|
| Cu(1)···Cu(1)′ | 5.138(1) | O(1)−Cu(1)−O(2) | 84.87(12) |
| Cu(1)−O(1) | 1.979(3) | O(1)−Cu(1)−Ow(1) | 88.54(16) |
| Cu(1)−O(2) | 1.970(3) | O(2)−Cu(1)−Ow(1) | 93.77(14) |
| Cu(1)−Ow(1) | 2.256(4) | O(2)−Cu(1)−N(1) | 175.83(15) |
| Cu(1)−N(1) | 1.992(4) | O(1)−Cu(1)−N(1) | 98.00(13) |
| Cu(1)−N(2) | 1.977(4) | O(2)−Cu(1)−N(2) | 94.04(13) |
| O(1)−C(15) | 1.253(5) | O(1)−Cu(1)−N(2) | 175.66(15) |
| O(2)−C(15)′ | 1.232(5) | N(2)−Cu(1)−Ow(1) | 95.73(16) |
| C(15)−C(15)′ | 1.555(8) | N(2)−Cu(1)−N(1) | 82.88(14) |
| N(3)−C(5) | 1.347(5) | N(1)−Cu(1)−Ow(1) | 89.35(15) |
| N(3)−C(13) | 1.324(7) | O(1)−C(15)−C(15)′ | 115.7(5) |
| N(4)−C(14) | 1.320(6) | O(2)′−C(15)−O(1) | 126.7(4) |
| N(4)−C(6) | 1.353(6) | O(2)′−C(15)−C(15)′ | 117.3(5) |
| C(13)−C(14) | 1.386(8) | C(14)−N(4)−C(6) | 116.2(4) |
| C(5)−C(6) | 1.401(6) | C(13)−N(3)−C(5) | 115.7(4) |

The complexes **1**−**4** are antiferromagnetically (AF) coupled dimeric species. Magnetic susceptibility measurements from 20 to 300 K with polycrystalline samples of the complexes gave a singlet-triplet separation ($-2J$) in the 346−384 cm$^{-1}$ range from theoretical fitting of the $\chi_M T$ vs. $T$ plots (Figure 2; Table 1). It has been theoretically predicted that, for a coplanar orbital topology with the $d_{x^2-y^2}$-type orbital coplanar with the oxalate molecule [i.e., both singly occupied molecular orbitals (SOMOs) being in the

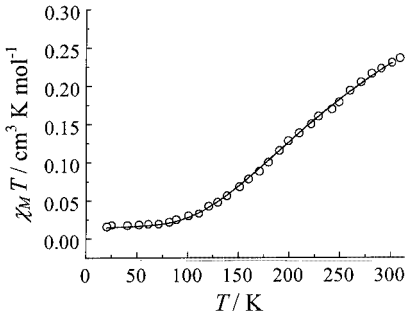



Figure 2. Plot of $\chi_M T$ vs. $T$ for a polycrystalline sample of [{(phen)Cu(H$_2$O)}$_2$(μ-ox)](ClO$_4$)$_2$ (**2**); the solid line shows a theoretical fit to the experimental data

plane of the oxalate bridge], the $-2J$ (S-T gap) value should fall in the 300−400 cm$^{-1}$ range, with the singlet being the ground state.[13] The observed $-2J$ values for the complexes **1**−**4** suggest a coplanar orbital topology in which the copper(II) $d_{x^2-y^2}$ orbitals, being in the plane of the oxalate molecule, result in the strong overlap of both SOMOs and the oxalate-based molecular orbitals.

### Electrochemical and Catalytic Properties

In a DMF−50 mM tris−HCl/0.1 M KCl (1:1, v/v) buffer (pH = 7.2) and with a glassy carbon working electrode, complexes **1**−**3** show two cyclic voltammetric responses attributable to the $Cu_2^{II}/Cu^{II}Cu^{I}$ and $Cu^{II}Cu^{I}/Cu_2^{I}$ couples in the 0.2 to −0.4 V (vs. SCE) potential range (Figure 3; Table 3). The observation of these redox couples, with similar current heights, suggests a dimeric nature of the complexes in solution. The ratio of unity of the cathodic to anodic peak currents ($i_{pc}/i_{pa}$) at different scan rates and the $\Delta E_p$ values in the 100−250 mV range at 50 mV·s$^{-1}$ indicate the quasireversible nature of the electron-transfer processes. Because of poor solubility, the redox chemistry of the dppz complex was studied by differential pulse voltammetry (DPV). The electrochemical data suggest the stability order for the copper(I) species as: **4** (dppz) > **3** (dpq) > **2** (phen) > **1** (bpy). The observed differences in the $E_{1/2}$ values could be due to the effect of the quinoxaline and phenazine moieties in dpq and dppz ligands, stabilizing the copper(I) state. When the potential is scanned up to −1.7 V, the complexes show an irreversible reduction peak in the range of −1.0 to −1.5 V and two anodic counterparts, with the one near −0.1 V showing high anodic current indicating absorption of the reduced species on the electrode surface.

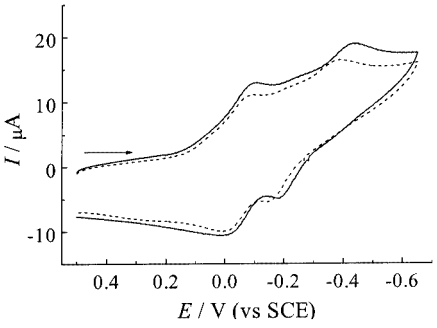


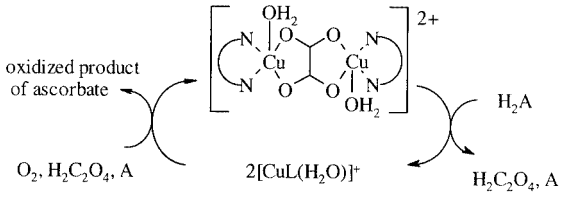

Figure 3. Cyclic voltammograms showing the $Cu_2^{II}/Cu^{II}Cu^{I}$ and $Cu^{II}Cu^{I}/Cu_2^{I}$ couples for [{LCu(H$_2$O)}$_2$(μ-ox)](ClO$_4$)$_2$ in DMF−50 mM tris−HCl/0.1 M KCl buffer (1:1, v/v, pH = 7.2) at 50 mV s$^{-1}$ [L = bpy, −; phen, ---]

Table 3. Electrochemical data for the complexes [{CuL(H$_2$O)}$_2$(μ-ox)](ClO$_4$)$_2$ (**1**−**4**) in DMF−50 mM tris−HCl/0.1 M KCl buffer (1:1, v/v, pH = 7.2) at 50 mV s$^{-1}$

| Complex[a] | $E_{1/2}$/V ($\Delta E_p$/mV) $Cu_2^{II}/Cu^{II}Cu^{I}$ | $Cu^{II}Cu^{I}/Cu_2^{I}$ | $E_{pc}$/V ($E_{pa}$/V)[b] |
|---|---|---|---|
| **1** | −0.05 (100) | −0.31 (250) | −1.45 (0.25, −0.17) |
| **2** | −0.04 (100) | −0.27 (240) | −1.30 (0.30, −0.10) |
| **3** | 0.02 (200) | −0.22 (250) | −1.15 (0.36, −0.11) |
| **4** | 0.07 | −0.20 | |

[a] CV data for **1**−**3**; DPV data for **4**. [b] Irreversible process.

The complexes undergo facile reduction with ascorbic acid (H$_2$A) in a DMF/50 mM tris−HCl buffer medium (1:1, v/v, pH = 7.2) to form an unstable brown copper(I) species, which converts into the oxidized species on exposure to dioxygen (Scheme 1). The catalytic cycle is effective with mol ratios of H$_2$A and the complex of ca. 100 for **1** and **2** and ca. 60 for **3** and **4**. The product from such a reaction in the presence of excess ascorbic acid has been analysed as polymeric copper(II) oxalate by the powder X-ray diffraction method.[19] The oxalic acid is formed by hydrolytic ring-rupture and oxidation of dehydroascorbate through the formation of L-threonic and 2,3-diketo-L-gulonic acids.[12] Additionally, the reduced species of **3** and **4** are susceptible to dimer formation through the extended aromatic rings of the quinoxaline and phenazine moieties.[8] Attempts to isolate the cuprous complex of **1** under anaerobic condition over a period of 7 d were unsuccessful. The brown single crystals formed in low yield were analysed from the unit cell dimensions as the bis(bpy) complex of copper(I).[20] The reduced species isolated by rapid precipitation of the solid from the reaction mixture was tentatively analysed as [Cu(bpy)(H$_2$O)(ClO$_4$)]. The solid mass did not show any IR band for the oxalate ion. We believe that the dicopper cores in **1**−**4** undergo cleavage due to a concomitant two-electron and two-proton transfer process by ascorbic acid [H$_2$A → A + 2 H$^+$ + 2 e$^-$]. The reduced monomeric species regenerates the dimeric core on exposure to dioxygen in the presence of oxalic acid in solution.




Scheme 1. Catalytic oxidation reaction of ascorbic acid (H$_2$A) by dioxygen, mediated by [{LCu(H$_2$O)}$_2$(μ-ox)](ClO$_4$)$_2$ (L = bpy, **1**; phen, **2**; dpq, **3**; dppz, **4**) in a DMF/tris−HCl buffer (1:1, v/v, pH = 7.2)

### DNA Binding and Cleavage Studies

The binding of the complexes **1**−**4** and $[Cu(phen)_2(H_2O)](ClO_4)_2$ to calf thymus DNA was studied by fluorescence spectroscopy. Measurements were made with ethidium bromide bound (EB-bound) DNA, and showed enhanced emission intensity relative to the free EB. Competitive binding of the copper complex to CT DNA results in the displacement of the bound EB. This causes a reduction in the emission intensity, due to fluorescence quenching of the free EB by the solvent molecules.[21−23] Figure 4 shows the decrease in the emission intensity of EB on addition of the complexes, including the bis(phen)copper(II) complex. Complexes **3** and **4**, showing higher slopes than the bis(phen) complex, indicate significant binding abilities of the dpq and dppz complexes (Figure 4). The bpy complex, with a near zero slope, does not show any binding affinity to CT DNA. The binding ability of the complexes to the CT DNA follows the order: **3** ≥ **4** > **2** ≥ $Cu(phen)_2^{2+}$ >> **1**. The

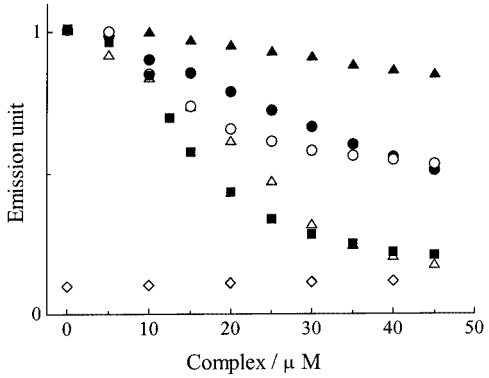



Figure 4. Effect of addition of $[\{LCu(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2$ (L = bpy, black triangle; phen, whithe circles; dpq, black squares; dppz, whithe triangles) and the bis(phen)copper(II) species (black circles) on the emission intensity of the CT DNA bound ethidium bromide (12.5μM) at different complex concentrations in 5 mM tris−HCl + 50 mM NaCl buffer (pH = 7.2) containing 3% DMF at 25 °C; the concentration of CT DNA was 125 μM; the emission intensity of EB (in the absence of DNA) at various concentrations of the $[\{(dpq)Cu(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2$ complex is also shown (whithe diamonds)

binding constant ($K_b$) for the bis(phen)copper(I) complex is known to be $5\times10^4$ $M^{-1}$ (base pairs).[9] The $K_b$ value for the copper(II) species is 2.7 times lower, as evidenced by differential pulse voltammetric (DPV) studies.[23] Although it was not possible for us to estimate the $K_b$ values for **1**−**4**, due to the absence of any intense band in the visible spectra of the complexes, the emission data suggest a significantly higher binding constant for **3** and **4** than for the bis(phen)-copper(II) species.

The nuclease activity of **1**−**4**, together with that of the bis(phen)copper complex, was studied with pUC19 DNA in a medium of DMF/tris−HCl buffer (1:1, v/v, pH = 7.2) in the presence of ascorbic acid. The cleavage efficiency of the complexes was determined by gel electrophoresis, from their ability to convert supercoiled (SC) to nicked circular

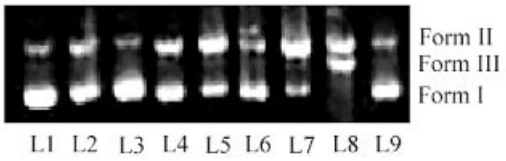



Figure 5. Cleavage of supercoiled pUC19 DNA (0.5 μg) by $[\{LCu(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2$ (**1**−**4**) in DMF in the presence of ascorbic acid ($H_2A$, 100 μM) in a 50 mM tris−HCl + 50 mM NaCl buffer at 37 °C; cleavage by the mono- and bis(phen) complexes of copper are also shown for comparison; Lane 1, DNA control; Lane 2, DNA + $H_2A$; Lane 3, DNA + complex **3**; Lane 4, DNA + $H_2A$ + mono(phen)copper(II) complex; Lane 5, DNA + $H_2A$ + bis(phen)copper(II) complex; Lane 6, DNA + $H_2A$ + **1**; Lane 7, DNA + $H_2A$ + **2**; Lane 8, DNA + $H_2A$ + **3**; Lane 9, DNA + $H_2A$ + **4**; complex concentration was 30 μM; forms I−III are supercoiled, nicked circular and linear, respectively

(NC) DNA (Table 4; Figure 5).[24] The dpq complex has the greatest ability to cleave DNA. The mechanistic aspects of the cleavage reactions are of importance. Oxalato-bridged dicopper(II) complexes containing terminal 2,2′-bipyridyl or 2,2′:6′,2"-terpyridyl ligands (L) are known to exist in equilibria with two mononuclear complexes of formulations $CuL^{2+}$ and CuL(ox) at pH values ranging between 2.0 and 6.0.[25,26] At pH values higher than 7.5, the mononuclear

Table 4. A comparison of the DNA cleavage efficiency of **1**−**4** along with the mono- and bis(phen) complexes of copper(II) in the presence of ascorbic acid

| Sl. no.[a] | Reaction condition[b] | Form (%) I | II | III |
|---|---|---|---|---|
| **1** | DNA control | 90 | 10 | |
| **2** | DNA + $H_2A$ | 83 | 17 | |
| **3** | DNA + $[Cu_2(dpq)_2(H_2O)_2(ox)](ClO_4)_2$ (**3**) | 90 | 10 | |
| **4** | DNA + $H_2A$ + $[Cu(phen)(H_2O)_2](ClO_4)_2$ | 70 | 30 | |
| **5** | DNA + $H_2A$ + $[Cu(phen)_2(H_2O)](ClO_4)_2$ | 40 | 60 | |
| **6** | DNA + $H_2A$ + $[Cu_2(bpy)_2(H_2O)_2(ox)](ClO_4)_2$ (**1**) | 80 | 20 | |
| **7** | DNA + $H_2A$ + $[Cu_2(phen)_2(H_2O)_2(ox)](ClO_4)_2$ (**2**) | 28 | 72 | |
| **8** | DNA + $H_2A$ + $[Cu_2(dpq)_2(H_2O)_2(ox)](ClO_4)_2$ (**3**) | 0 | 55 | 45 |
| **9** | DNA + $H_2A$ + $[Cu_2(dppz)_2(H_2O)_2(ox)](ClO_4)_2$ (**4**) | 78 | 22 | |

[a] The serial numbers correspond to the lanes shown in Figure 5. [b] Concentration: $H_2A$ 100 μM; copper complexes 30 μM. Reaction medium: DMF−50 mM tris−HCl/50 mM NaCl buffer (1:1, v/v, pH = 7.2). Forms I−III are supercoiled (SC) pUC19, nicked circular and linear DNA, respectively.

[Cu(L)(ox)] species predominates. Such mononuclear copper(II) complexes, with one terminal ligand, show poor cleavage activity when L is an intercalating ligand such as 1,10-phen. Although we cannot exclude the possibility of such an equilibrium involving the present complexes, the cleavage efficiency data indicate that the dimeric structure in **1**−**4** is generally retained in pH = 7.2 DMF/tris−HCl buffer.

There are several important findings in this study pertaining to the binding and cleavage of DNA. Complexes **1**−**4** are dicationic and have a Cu/heterocyclic base ratio of 1:1. The bpy complex has been found to be nuclease-inactive and a poor binder to DNA. This suggests that DNA binding in the form of intercalation could be an important factor for the cleavage activity. The luminescence experiments monitoring the emission of the DNA-bound ethidium bromide strongly support an intercalative binding mode for the copper(II) complexes. Complexes **2**−**4** are powerful binders to DNA. The high affinities of the dpq and dppz ligands for intercalative binding could be related to the presence of extended aromatic rings, making noncovalent interactions between the π-system of the ligand and the DNA base pairs more favourable. The ability of the dppz ligand to approach deep into the DNA helix would be expected to be greater than that of the dpq ligand. However, the emission data indicate equal CT DNA helix binding affinities for both complexes. The complexes differ drastically in their abilities to cleave DNA in the presence of a reducing agent such as ascorbic acid. The anomalous nuclease activity of **4** could be related either to the difference in groove selectivity[27−32] or to an enhancement of the stability of the cuprous complex in the presence of hydrophobic interactions.

## Conclusions

Four dicopper(II) complexes with $[Cu(\mu\text{-ox})Cu]^{2+}$ cores and planar N-donor heterocyclic bases as terminal ligands show interesting DNA-binding and -cleavage properties. The phen, dpq and dppz complexes were found to be powerful binders to CT DNA. While the phen and dpq complexes show good cleavage properties, the dppz complex is nuclease-inactive. The results are explained in terms of intercalative binding of the complexes to DNA. The cleavage activity of the dpq complex is significantly greater than that of the phen complexes. The results are of significance, as the dpq complex may find use as a new DNA-binding and -cleaving agent in nucleic acids chemistry.

## Experimental Section

**General Methods:** All reagents and chemicals were purchased from commercial sources and used without further purification. Solvents were purified by standard procedures. The calf thymus (CT) DNA and supercoiled (SC) pUC19 DNA were purchased from Bangalore Genie (India). Agarose (molecular biology grade) and ethidium bromide were from Sigma (USA). Tris−HCl buffer solution was prepared with deionised, sonicated, triply distilled water. Dipyridoquinoxaline and dipyridophenazine ligands were prepared by literature methods.[24,33] The elemental analyses were done with a Perkin−Elmer 2400 CHN analyser. The infrared, electronic and fluorescence spectra were recorded with Bruker Equinox 55, Hitachi U-3000 and Perkin−Elmer LS 50B spectrophotometers, respectively. Variable-temperature magnetic susceptibility data in the 20−300 K range were obtained for polycrystalline samples with a George Associates Inc. Lewis-coil-force magnetometer system equipped with a closed-cycle cryostat (Air products) and a Cahn balance. $Hg[Co(NCS)_4]$ was used as a calibrant. Experimental susceptibility data were corrected for diamagnetic contributions[34] and temperature-independent paramagnetism ($N\alpha = 60\times10^{-6}$ $cm^3\cdot mol^{-1}$ per copper). The molar magnetic susceptibilities were fitted by the modified Bleaney−Bowers expression[35,36] based on the isotropic form of the Heisenberg−Dirac−van Vleck (HDvV) model with a spin Hamiltonian $H = -2JS_1S_2$ ($S_1 = S_2 = 1/2$ for $d^9-d^9$ configuration): $\chi_{Cu} = [Ng^2\beta^2/kT][3 + \exp(-2J/kT)]^{-1}(1 - \rho) + (Ng_{\parallel}^2\beta^2/4kT)\rho + N\alpha$, where ρ is the fraction of monomeric impurity and $-2J$ is the singlet-triplet energy separation, with the singlet being the ground state. The magnetic moments at various temperatures were calculated in $\mu_B$ units [$\mu_B \approx 9.274\cdot10^{-24}$ $J\cdot T^{-1}$]. Electrochemical measurements were made at 25 °C with an EG&G PAR Model 253 Versastat Potentiostat/Galvanostat with electrochemical analysis software 270 for voltammetric work with a three-electrode set-up comprising a glassy carbon working electrode, a platinum wire auxiliary electrode and a saturated calomel reference (SCE) electrode. The electrochemical data were uncorrected for junction potentials.

**Preparation of $[\{CuL(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2$ (1−4):** The complexes were prepared by a general procedure in which the heterocyclic base (L, 1.0 mmol; 156 mg bpy; 198 mg phen; 232 mg dpq; 282 mg dppz) was treated with $Cu(ClO_4)_2\cdot6H_2O$ (1.0 mmol; 371 mg) in aqueous ethanol (70 $cm^3$), followed by addition of an aqueous solution (5 mL) of sodium oxalate (0.5 mmol; 67 mg). The reaction mixture was stirred for 10 min. Complexes were isolated as pale blue solids after washing with ethanol and water followed by drying in vacuo over $P_4O_{10}$ (yield: 70%). ***Caution!*** Perchlorate salts are potentially explosive and should be handled in small quantities with care. Single crystals of **3**, suitable for X-ray studies, were obtained by slow concentration of an aqueous methanolic solution of the complex $C_{22}H_{16}Cl_2Cu_2N_4O_{12}$ (**1**) (726.38): calcd. C, 36.36, H 2.20, N 7.71; found C 36.51, H 2.48, N 7.62. $C_{26}H_{20}Cl_2Cu_2N_4O_{14}$ (**2**) (810.45): calcd. C, 38.52, H 2.47, N 6.91; found C 38.92, H 2.23, N 6.93. $C_{30}H_{20}Cl_2Cu_2N_8O_{14}$ (**3**) (914.55): calcd. C, 39.36, H 2.19, N 12.25; found C 38.97, H 2.34, N 11.96. $C_{38}H_{24}Cl_2Cu_2N_8O_{14}$ (**4**) (1014.64): calcd. C, 44.97, H 2.37, N 11.05%; found C 44.71, H 2.63, N 10.86.

**X-ray Crystal Structure Analysis:** All geometric and intensity data were collected with an automated Enraf−Nonius CAD4 diffractometer equipped with Mo-$K_\alpha$ radiation (λ = 0.71073 Å) with a crystal mounted on a fibre by epoxy cement (Table 5). Intensity data, collected by an ω-2θ scan technique, were corrected for Lorentz polarisation effects and for absorption.[37] The structure was solved by the combination of Patterson and Fourier techniques and refined by full-matrix least squares. During refinement, positional disorder of the oxygen atoms of the perchlorate anion were observed. The four oxygen peaks concerned were refined with restraints. The $ClO_4$ moiety refined well, but showed a highest peak of 1.01 e·Å$^{-3}$ near the O(11) atom ($x = 0.7932$; $y = 0.2161$; $z = 0.3594$). All the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located from the difference Fourier map and

Table 5. Crystallographic data for compound $[Cu_2(dpq)_2(H_2O)_2(ox)](ClO_4)_2 \cdot 2H_2O$

| | |
|---|---|
| Empirical formula | $C_{30}H_{24}N_8O_{16}Cl_2Cu_2$ |
| Formula mass | 950.55 |
| Crystal system | monoclinic |
| Space group | $P2_1/n$ (no. 1014) |
| $a$ [Å] | 8.738(2) |
| $b$ [Å] | 13.691(4) |
| $c$ [Å] | 15.181(4) |
| β [°] | 98.23(2) |
| $V$ [Å$^3$] | 1797.3(8) |
| $Z$ | 2 |
| $D_c$ [g cm$^{-3}$] | 1.756 |
| μ [mm$^{-1}$] | 1.419 |
| Crystal colour and habit | bluish green needle |
| Crystal size [mm] | 0.32 × 0.18 × 0.08 |
| $2\theta_{max}$ [°] | 50 |
| Index range | $0 \le h \le 10$, $0 \le k \le 16$, $-18 \le l \le 17$ |
| Reflns. collected | 3158 |
| Independent reflns [$I > 2\sigma(I)$] | 2536 |
| $R_{int}$ | 0.0118 |
| Parameters refined | 310 |
| $F(000)$ | 960 |
| $R$(obsd.data) | 0.0481 |
| $wR$(obsd.data)[a] | 0.1329 |
| $R$(all data) | 0.0611 |
| $wR$(all data) | 0.1411 |
| Goodness of fit | 1.078 |
| Largest diff. peak and hole [e Å$^{-3}$] | 1.011 and −0.562 |

[a] $w = 1/[\sigma^2(F_o^2) + (0.0690\ P)^2 + (3.0700\ P)]$ where $P = [\mathrm{Max}(F_o^2,0) + 2\ F_c^2]/3$.

were refined isotropically, except for those attached to the water oxygen atoms, which were treated as rigid groups. All calculations were performed with PC versions of the SHELX system of programs.[38] The perspective view of the complex was obtained by use of ORTEP.[39] CCDC-159021 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

**DNA-Binding and -Cleavage Experiments:** The concentration of the CT DNA was determined by recording the absorption intensity at 260 nm, with a known molar extinction coefficient value of 6600 dm$^3$ mol$^{-1}$ cm$^{-1}$.[40] The experiments were performed with 0.1 mM complex solution in the absence and in the presence of CT DNA, varying from 0 to 250 μM. In order to compare the relative binding of the complexes to DNA with that of the bis(phen) complex of copper(II), ethidium bromide bound CT DNA solution in tris−HCl/NaCl buffer (pH = 7.2) was treated with an increasing amount of the complex solution. The fluorescence intensities of the ethidium bromide in the bound form at 601 nm (510 nm excitation) were plotted against the complex concentration. The cleavage of DNA was monitored by agarose gel electrophoresis. Supercoiled pUC19 DNA (6 μL, ca. 500 ng) in tris−HCl buffer (50 mM) with 50 mM NaCl (pH = 7.2) was treated with the metal complex (30 μM) and ascorbic acid (100 μM), followed by dilution with the tris−HCl buffer to a total volume of 20 μL. The samples were incubated for 1 h at 37 °C, loading buffer [25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 μL)] was added and then loaded on 0.8% agarose gel containing 1.0 μg/mL ethidium bromide. Electrophoresis was carried out at 40 V for 3 h in TBE buffer. Bands were viewed under UV light and photographed. The cleavage efficiency was measured by determining the ability of the complex to convert the supercoiled DNA (SC) to nicked circular form (NC). After electrophoresis, the proportion of DNA in each fraction was estimated quantitatively from the intensities of the bands by use of the BIORAD Gel Documentation System. The fraction of the original supercoiled DNA, converted into the NC (nicked circular) at the end of the reaction, was calculated after correction for the low level of NC present in the original sample and the low affinity of ethidium bromide binding to SC compared to NC and linear forms of DNA.[24]

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