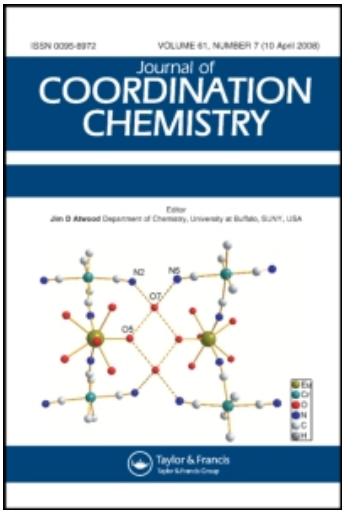

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Synthesis, structure, and DNA-binding studies of a new tetracopper(II) complex bridged by dissymmetrical *N*-benzoato-*N'*-(2-aminoethyl)oxamide

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Synthesis, structure, and DNA-binding studies of a new tetracopper(II) complex bridged by dissymmetrical *N*-benzoato-*N*′-(2-aminoethyl)oxamide

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A new oxamido-bridged tetracopper(II) complex, $[Cu_4(oxbe)_2(bpy)_2](ClO_4)_2 \cdot 2H_2O$, where H_3oxbe and bpy stand for *N*-benzoato-*N*′-(2-aminoethyl)oxamide and 2,2′-bipyridine, has been synthesized and characterized by elemental analyses, molar conductivity, infrared and electronic spectra, and single-crystal X-ray diffraction. It crystallizes in triclinic system, space group *P-1*, with crystallographic data: $a = 7.829(5)$ Å, $b = 12.680(5)$ Å, $c = 13.420(5)$ Å, $\alpha = 104.665(5)^\circ$, $\beta = 95.275(5)^\circ$, $\gamma = 106.931(5)^\circ$, and $Z = 1$. The circular tetranuclear copper(II) cation $[Cu_4(oxbe)_2(bpy)_2]^{2+}$ with an embedded center of inversion is assembled by a pair of *cis*-oxbe-bridged dinuclear copper(II) units through coordination between carboxyl and copper(II). One copper(II) is located in a slightly distorted square-planar environment, while the other is in a distorted square-pyramidal geometry. In the crystal structure, abundant hydrogen bonds and aromatic stackings link the tetranuclear copper(II) units into an overall 3-D framework. Interactions of the tetranuclear copper(II) complex with herring sperm DNA (HS-DNA) are investigated by using UV absorption and fluorescence spectra, electrochemical techniques, and viscometry. The results suggest that the tetranuclear copper(II) complex interacts with DNA by intercalation with an intrinsic binding constant of 1.47×10^5 mol^{-1} L.

Keywords: Crystal structure; Tetranuclear copper(II) complex; μ-Oxamidato-bridge; DNA interaction

1. Introduction

Interactions of transition-metal complexes with DNA are of interest to gain insight into the interaction model between DNA and small molecules, and obtain information about drug design and tools of molecular biology [1–7].

Transition-metal complexes can interact with DNA through electrostatic interaction, groove binding, and intercalation. Binding to DNA through an intercalation mode with

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planar ligands intercalating into the adjacent base pairs of DNA [8, 9] correlates to the planarity of the ligand, coordination geometry of the metal ion, and donor type of the ligand [10, 11]. The metal ion type and its valence play important roles in the extent of binding to DNA [12]. Understanding these observations is important to explore potential chemotherapeutical agents. Many copper(II) complexes possess biologically accessible redox potentials and demonstrate high nucleobase affinity of potential chemotherapeutical interest [13–23]. Compared to the number of studies dealing with mono- and other polynuclear complexes [24–33], relatively few studies on tetranuclear complexes have been reported [34–37]. *N*,*N*′-disubstituted oxamides are versatile bridging ligands as bi-, tri-, or tetradentate are capable of forming polynuclear complexes [10]. Using mononuclear Cu(II) complexes of some *N*,*N*′-bis(coordinating group substituted)oxamides, many oxamido-bridged polynuclear complexes have been prepared and studied magnetically [10, 38–44]. Only nine cyclic tetranuclear complexes with oxamide bridges have been synthesized and structurally characterized by single-crystal X-ray diffraction [45–50], and none has been studied for the interaction with DNA. The enhancement of DNA cleavage activity for tetranuclear complexes [34–37] stimulates us to design and synthesize new tetranuclear complexes to evaluate DNA-binding and cleavage properties.

To investigate the DNA-binding properties of oxamido-bridged tetranuclear copper(II) complexes in solution, in this article, a new tetracopper(II) complex bridged by dissymmetrical *N*-benzoato-*N*′-(2-aminoethyl)oxamide (oxbe) and end-capped with 2,2′-bipyridine (bpy), $[Cu_4(oxbe)_2(bpy)_2](ClO_4)_2 \cdot 2H_2O$, has been synthesized and structurally characterized by single-crystal X-ray diffraction. Its DNA-binding properties were also explored by using electronic absorption spectroscopy, fluorescence spectroscopy, voltammetry, and viscosity measurements. These results should be valuable in understanding the interaction with DNA as well as laying a foundation for the rational design of powerful agents for probing and targeting nucleic acids.

2. Experimental

The ligand *N*-benzoato-*N*′-(2-aminoethyl)oxamide (H_3oxbe) and its mononuclear copper(II) complex $Na[Cu(oxbe)] \cdot 1.5H_2O$ were synthesized according to the literature method [51]. All chemicals used in the synthesis were of reagent grade. Doubly-distilled water was used to prepare buffers. Ethidium bromide (EB) and herring sperm DNA (HS-DNA) were purchased from Sigma Corp. and used as received.

2.1. *Synthesis of* $[Cu_4(oxbe)_2(bpy)_2](ClO_4)_2 \cdot 2H_2O$

To a stirred methanol solution (5 mL) containing $Cu(ClO_4)_2 \cdot 6H_2O$ (0.0371 g, 0.1 mmol), a methanol solution (10 mL) of $Na[Cu(oxbe)] \cdot 1.5H_2O$ (0.0362 g, 0.1 mmol) was added dropwise at room temperature. After stirring it for 30 min, a methanol solution (5 mL) of bpy (0.0156 g, 0.1 mmol) was added dropwise. The mixture was stirred at 333 K for 5 h, the resulting green solution was filtered, and blue cube crystals suitable for X-ray analysis were obtained by slow evaporation at room temperature for 14 days.

Yield: 0.0375 g (61%). Anal. Calcd for $Cu_4C_{42}H_{40}N_{10}O_{18}Cl_2$ (%): C, 38.87; H, 3.11; N, 10.79. Found (%): C, 38.78; H, 3.22; N, 10.49. ES-MS, m/z (%): 1060 (100) $[M]^+$.

2.2. *Physical measurements*

Carbon, hydrogen, and nitrogen analyses were performed with a Perkin Elmer elemental analyzer Model 240. The mass spectra (ES-MS) was measured with a Waters Q-TOF GLOBLE mass spectrometer. Molar conductance was measured with a Shanghai DDS-11A conductometer. The infrared (IR) spectrum was recorded using KBr pellets in a Nicolet model Impact 470 FT-IR spectrophotometer from 4000 to 400 cm^{-1}. The UV-Vis spectrum was recorded in a 1 cm path-length quartz cell on a Cary 300 spectrophotometer. Fluorescence was tested on an Fp-750w fluorometer. A CHI 832 electrochemical analyzer (Shanghai CHI Instrument, Shanghai, China) in connection with a glassy carbon working electrode (GCE), a saturated calomel reference electrode (SCE), and a platinum wire counter electrode was used for electrochemical measurements. The GCE surface was freshly polished to a mirror prior to each experiment with 0.05 μm α-Al_2O_3 paste and then cleaned in water for 5 min. Viscosity measurements were carried out using an Ubbelodhe viscometer immersed in a thermostatic water bath maintained at 289($\pm$0.1) K.

2.3. *Crystal structure determination*

The single crystal used for data collection of the tetracopper(II) complex was selected and mounted on a Bruker APEX area-detector diffractometer with graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å) at 296 K. The crystal structures were solved by direct methods followed by Fourier syntheses. Structure refinements were performed by full-matrix least-squares procedures using SHELXL-97 on F^2 [52]. The water molecules (O9 and O10) were treated with a constrained site occupancy of 0.5 and the hydrogens were found in a difference Fourier map and were treated as riding, with a fixed U_{iso}(H) of 0.08 $Å^2$. The remaining hydrogens were placed in calculated positions, with C–H = 0.97 Å (methylene) or 0.93 Å (aromatic C) and N–H = 0.90 Å, and refined as riding, with $U_{iso}(H) = 1.2U_{eq}$ (carrier atoms). Crystal data and structural refinement parameters for the complex are summarized in table 1. The selected bond distances and angles are listed in tables 2 and 3.

2.4. *DNA-binding studies*

All experiments involving HS-DNA were performed in (hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution (pH 7.31). The solution of DNA in Tris–HCl buffer gave the ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of *ca* 1.9, indicating that the DNA was sufficiently free of protein [53]. The concentration of the prepared DNA stock solution was determined according to its absorbance at 260 nm using $\varepsilon 260 = 6600$ L mol^{-1} cm^{-1} [54]. The stock solution of DNA was stored at 277 K and used after no more than 4 days. Concentrated stock solution of the tetranuclear copper(II) complex was prepared by dissolving the complex in dimethyl sulfoxide (DMSO) and diluted with Tris–HCl buffer to obtain the required concentrations for all

Table 1. Crystallographic data and details of the structure determination for the complex.

Empirical formula	$Cu_4C_{42}H_{40}N_{10}O_{18}Cl_2$
Formula weight	1297.94
Wavelength (Å), Mo-Kα	0.71073
Crystal system	Triclinic
Space group	*P-1*
Unit cell dimensions (Å, °)	
a	7.829(5)
b	12.680(5)
c	13.420(5)
α	104.665(5)
β	95.275(5)
γ	106.931(5)
Volume (Å^3), *Z*	1213.1(10), 1
Calculated density (g cm^{-3})	1.777
Absorption coefficient (Mo-Kα) (mm^{-1})	1.926
F(0 0 0)	656
Crystal size (mm^3)	$0.08 \times 0.16 \times 0.20$
Radiation (Å) (Mo-Kα)	0.71073
θ range for data collection (°)	2.8–25.2
Total unique data	7162, 4036 [R(int) = 0.051]
Observed data [$I > 2\sigma(I)$]	1923
R indices	$R_1 = 0.1439$, $wR_2 = 0.4622$
S	1.51
Maximum average shift/error	0.000/0.000

Table 2. Selected bond distances (Å) and angles (°) for the complex.

C1–O1	1.40(2)	Cu2–O2^i	2.370(14)
C1–O2	1.182(17)	Cu2–O3	1.967(10)
Cu1–O1	1.862(11)	Cu2–O4	1.933(10)
Cu1–N1	1.982(11)	Cu2–N4	1.960(13)
Cu1–N2	1.899(12)	Cu2–N5	1.942(14)
Cu1–N3	2.028(13)		
O1–Cu1–N1	95.4(5)	O1–Cu1–N2	175.0(7)
O1–Cu1–N3	96.7(5)	N1–Cu1–N2	85.8(5)
N1–Cu1–N3	168.0(5)	N2–Cu1–N3	82.2(5)
O2^i–Cu2–O3	89.3(5)	O2^i–Cu2–O4	100.7(5)
O2^i–Cu2–N4	92.3(6)	O2^i–Cu2–N5	95.8(6)
O3–Cu2–O4	85.1(4)	O3–Cu2–N4	97.5(5)
O3–Cu2–N5	174.9(7)	O4–Cu2–N4	166.8(7)
O4–Cu2–N5	94.2(5)	N4–Cu2–N5	82.1(6)
Cu1–O1–C1	127.8(8)	Cu2^i–O2–C1	110.8(10)
Cu1–O1–C1–O2	−161.5(13)	Cu2^i–O2–C1–O1	104.1(15)

Symmetry codes: $^i -x+2, -y, -z+1$.

the experiments. Absorption spectral titration was performed by keeping the concentration of the tetracopper(II) complex constant while varying the HS-DNA concentration. Equal amounts of the solution of HS-DNA was added to the copper(II) complex solution and reference solution to eliminate the absorbance of HS-DNA itself. In the EB fluorescence displacement experiment, 5 µL of the EB Tris–HCl solution (1 mmol L^{-1}) was added to 1 mL of HS-DNA solution (at saturated binding levels) [55], stored in dark for 2 h, and then the solution of the copper(II) complex was titrated into

Table 3. Hydrogen-bonding geometries for the complex (Å, °).

D–H···A	D–H	H···A	D···A	D–H···A
O9–H9B···O2	0.93	2.20	3.10(2)	161.0
O9–H9A···O10	0.88	1.87	2.73(3)	168.7
O10–H10C···O1^{ii}	0.90	1.87	2.74(2)	160.6
N3–H3B···O6^{iii}	0.90	2.44	3.23(3)	147.0
N3–H3A···O8	0.90	2.23	3.05(3)	150.3
C10–H10A···O5^{iv}	0.97	2.57	3.48(2)	156.2
C13–H13···O8^{i}	0.93	2.58	3.21(3)	125.6
C14–H14···O10^{v}	0.93	2.37	3.29(3)	168.1
C15–H15···O7^{vi}	0.93	2.55	3.42(3)	156.4
C19–H19···O2^{vii}	0.93	2.59	3.449(18)	154.0
C20–H20···O9^{vii}	0.93	2.58	3.37(3)	142.3

Symmetry codes: $^{i}-x+2, -y, -z+1$; $^{ii}-x+2, -y, -z$; $^{iii}x-1, y, z$; $^{iv}-x+3, -y+1, -z+1$; $^{v}-x+1, -y, -z+1$; $^{vi}x-1, y, z+1$; $^{vii}x, y+1, z+1$.

the DNA/EB mixture and diluted in Tris–HCl buffer to 5 mL, producing solutions with a varied mole ratio of the copper(II) complex to HS-DNA. Before measurements, the mixture was shaken and incubated at room temperature for 30 min. Fluorescence spectra of EB bound to HS-DNA were obtained at an emission wavelength of 584 nm in the Fluorometer. Electrochemical titration experiments were performed by keeping the concentration of the complex constant while varying the HS-DNA concentration using 50 mmol NaCl/5 mmol Tris–HCl buffer (pH 7.11) as solvent [6]. All voltammetric experiments were performed in a single compartment cell. The supporting electrolyte was the 50 mmol NaCl/5 mmol Tris–HCl buffer solution at pH 7.11 [6]. Solutions were deoxygenated by purging with nitrogen for 15 min prior to measurements; during measurements a stream of N_2 gas was passed over the solution. In viscosity measurements, HS-DNA samples, approximately 200 base pairs in length, were prepared by sonication in order to minimize the complexities arising from DNA flexibility [56]. Flow times were measured with a digital stopwatch; each sample was measured three times, and an average flow time was calculated. Relative viscosities for HS-DNA in the presence and absence of the complex were calculated from the relation $\eta = (t - t_0)/t_0$, where t is the observed flow time of DNA-containing solution and t_0 is that of Tris–HCl buffer alone. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio [57], where η is the viscosity of DNA in the presence of copper(II) complex and η_0 is the viscosity of DNA alone.

3. Results and discussion

3.1. *Synthetic route and general properties of the tetranuclear copper(II) complex*

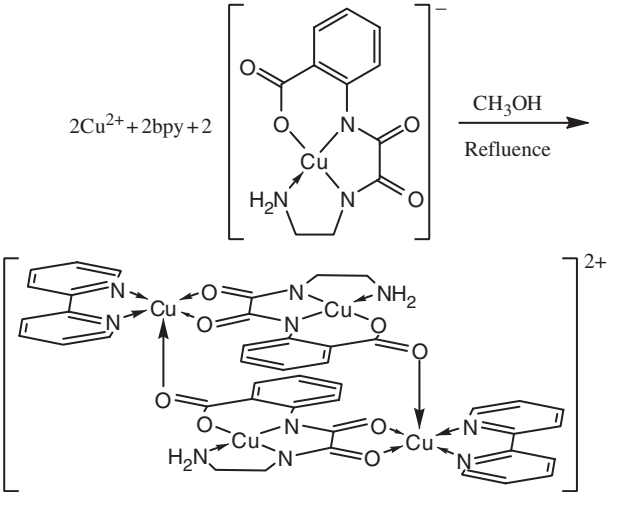
Two synthetic strategies are generally available for the preparation of polynuclear complexes. The first is to use a binucleating ligand that offers either the coordination geometry or the ligand field strength suitable for metal ions. The second is to use a "complex ligand" that contains a potential donor group capable of coordinating to another metal ion. In this study, our aim was to obtain tetranuclear copper(II) complex,

and therefore, the latter synthetic method was adopted. For this purpose, the mononuclear fragment *N*-benzoato-*N*′-(2-aminoethyl)oxamidocopper(II), $Na[Cu(oxbe)] \cdot 1.5H_2O$, was chosen as "complex ligand" because it can coordinate to metal ions through not only carbonyl oxygens and nitrogens of oxamido but also oxygens of carboxylate [50]. Simultaneously, 2,2′-bipyridine (bpy) was used as the terminal ligand. Indeed, elemental analyses indicate that the reaction of $Na[Cu(oxbe)] \cdot 1.5H_2O$ with $Cu(ClO_4)_2 \cdot 6H_2O$ and bpy in 1 : 1 : 1 mole ratio yielded the tetranuclear complex $[Cu_4(oxbe)_2(bpy)_2](ClO_4)_2 \cdot 2H_2O$, as expected. The synthetic pathway for the complexation may be represented by scheme 1.

The tetranuclear copper(II) complex is insoluble in non-polar solvents and common polar solvents, and moderately soluble in dimethylformamide (DMF) and DMSO, implying the polymeric nature of the complex [58]. In the solid state the complex is fairly stable in air allowing physical measurements. The molar conductance values of the tetranuclear copper(II) complex ($\Lambda = 138$ and $112\,\Omega^{-1}\,cm^{-2}\,mol^{-1}$ in DMF and DMSO, respectively) fall in the expected range for 1 : 2 electrolytes [59], suggesting that the two perchlorate anions in the complex are situated outside the coordination sphere and balance the charge of the tetranuclear complex. These observations coincide with the following spectral characterization.

3.2. *IR and electronic spectra*

IR spectra provide information regarding coordination in the tetranuclear copper(II) complex and were analyzed in comparison with that of the mononuclear fragment $Na[Cu(oxbe)] \cdot 1.5H_2O$. The antisymmetric stretching vibration of the carboxylate ($\nu_{as(COO)}$), together with the carbonyl stretching vibration of oxamidate ($\nu_{C=O}$) at $1644\,cm^{-1}$ for the mononuclear complex $Na[Cu(oxbe)] \cdot 1.5H_2O$ is considerably shifted to a higher frequency ($1674\,cm^{-1}$) in the tetranuclear copper(II) complex, suggesting that carbonyl oxygens of oxamido and oxygens of carboxylate in the "complex ligand",



Scheme 1. The synthetic pathway for the complexation.

Na[Cu(oxbe)] · 1.5H_2O, coordinate with copper(II) to form the tetranuclear complex [50]. This shift has often been used as a diagnostic indicator for oxamido-bridged structures [10]. Because deprotonated amide is coordinated to the metal ion, amide I band shifts to lower wave number. However, in the case of an oxamide dianion coordinated to two metal ions as a bridging ligand, the amide I band reverts to a position near its original one (in the protonated species) [10]. Although the amide I is due to a composite N–C=O vibration, it can essentially be seen as $\nu_{C=O}$. It is likely that the bond order of C=O (carbonyl) in the tetranuclear complex is higher than that in the corresponding mononuclear complex Na[Cu(oxbe)] · 1.5H_2O. Bands associated with $\nu_{C=N}$ and $\nu_{C=C}$ from the aromatic ring of bpy shift to 1497, 1471, and 1447 cm^{-1}, indicating that nitrogens are coordinated to copper(II) [60]. A broad and intense band centered at 1092 cm^{-1}, and a strong sharp band at 624 cm^{-1}, typical for a non-coordinated perchlorate [61], are also observed.

Electronic spectra of the tetranuclear complex were measured from 200 to 800 nm in DMSO. Three absorption bands with varied intensities are observed. The intense band at 235 nm of the tetranuclear complex is attributed to bpy (π–π*) transitions, while the less intense band at 300 nm is typical of charge transfer between bpy and metal (LMCT, ligand-to-metal charge transfer). The broad band at 602 nm corresponds to the d–d transitions of copper(II) [62]. These spectroscopic data of the tetranuclear complex are consistent with the crystal structure.

3.3. *Crystal structure*

The molecular structure of the tetranuclear copper(II) complex is illustrated in figure 1, and selected bond distances and angles are given in table 2. The asymmetric unit can be

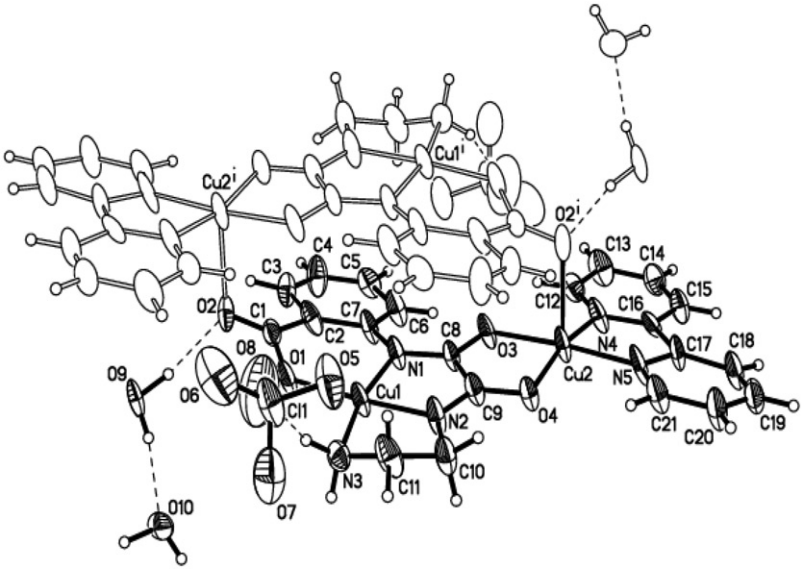


Figure 1. An ORTEP of the complex with thermal ellipsoids at 30% probability. Hydrogens are shown as small spheres of arbitrary radii. For clarity, the atoms of the asymmetric residue unit and its counterpart are represented in different styles. Hydrogen bonds are shown as dotted lines [Symmetry code: $^{i}-x+2, -y, -z+1$].

considered as a *cis*-oxamido-bridged dinuclear copper(II) cation linked with two halves of water and a perchlorate anion by hydrogen bonds, namely $[Cu_2(oxbe)(bpy)](ClO_4)\cdot H_2O$]. The waters of O9 and O10 are disordered on a special position (1 0 0) of a higher inversion symmetry than the molecules can take [the distance of $O9\cdots O10^{-x+2,-y,-z}$ is only 1.51(3) Å], thus the occupancies are constrained to 0.5. A pair of units assembles to form a circular tetranuclear system through carboxyl bridges with an embedded inversion center. The $Cu\cdots Cu$ separations through the oxamido and the carboxyl bridges are 5.186(3) and 5.225(4) Å, respectively. The *cis*-oxamide coordinates to Cu1 and Cu2 in the usual chelating mode with bite angles of 85.8(5)° and 85.1(4)°, respectively. The carboxyl bridges copper(II) ions in a skew–skew fashion, with the corresponding torsion angles being −161.5(13)° of Cu1–O1–C1–C2 and 104.1(15)° of $Cu2^{i}$–O2–C1–O1 [symmetry code (i) $-x+2, -y, -z+1$].

Cu1 has distorted square-planar geometry, formed by N1, N2, N3, and O1 from $oxbe^{3-}$. The maximum displacement from the coordination plane is 0.051(9) Å (N2). Cu1 is 0.034(8) Å out of the plane. The behavior of $oxbe^{3-}$ results in the formation of two five- and one six-membered rings around Cu1. The five-membered Cu1–N2–C10–C11–N3 ring has a twist conformation with puckering parameters [63] of $Q = 0.342(19)$ Å and $\varphi = 299(3)°$, while the other five-membered Cu1–N1–C8–C9–N2 ring is almost planar. The puckering parameters of the six-membered ring Cu1–O1–C1–C2–C7–N1 are $Q = 0.246(16)$ Å, $\theta = 78(4)°$ and $\varphi = 106(4)°$. The Cu1–N3 bond [2.028(13) Å] is longer than Cu1–N1 [1.982(11) Å] and Cu1–N2 bond [1.899(12) Å], consistent with the stronger donor ability of the deprotonated amido nitrogen compared with the amino nitrogen [64]. The deprotonated amide nitrogens make the structure more stabile by forming a conjugated π bond between the oxamidate skeleton and the phenyl ring.

The environment around Cu2 can best be described as a distorted square-pyramidal geometry with a τ value of 0.13 [65]. Cu2 coordinates to the *exo*-oxygens of *cis*-$oxbe^{3-}$ (O3 and O4). The other two atoms of the basal plane (N4, N5) are both from bpy, while the apical position is occupied by a carboxyl oxygen ($O2^{i}$) with Cu2–$O2^{i}$ bond length of 2.370(14) Å. Deviations of N4, N5, O3, and O4 from the basal plane are in the range of 0.063(7)–0.067(8) Å, and Cu2 displaces 0.152(8) Å out of the plane.

Hydrogen bonds dominate the crystal structure. The complex cations and perchlorate anions are connected by classical hydrogen bonds listed in table 3 into a 1-D chain parallel to *a*-axis. These chains are linked by water molecules into a 2-D hydrogen bonding network extending along (0 1 0) plane (figure 2). The layers are further assembled by non-classical hydrogen bonds to a 3-D supramolecular hydrogen bonding system. The system is consolidated by two kinds of π–π stacking (figure 3). One is between the pyridine ring containing N4 and the benzene ring of the neighboring complex associated by the symmetry operation $-x+1, -y, -z+1$ (^{v}). The nearest separation is 3.46(2) Å ($N4^{v}$). The other is observed between pyridine rings containing N5 related to $-x+2, -y+1, -z+2$ (viii), the smallest separation is 3.41(3) Å for $C20^{viii}$.

Electron paramagnetic resonance (EPR) and magnetic susceptibility data are very important for investigating the geometry around Cu centers in polynuclear Cu(II) complexes [66, 67]. However, the structure of the complex has been solved by single-crystal X-ray diffraction. Therefore, the focus of this article was to investigate DNA-binding properties of the tetranuclear copper(II) complex in solution (*vide infra*).

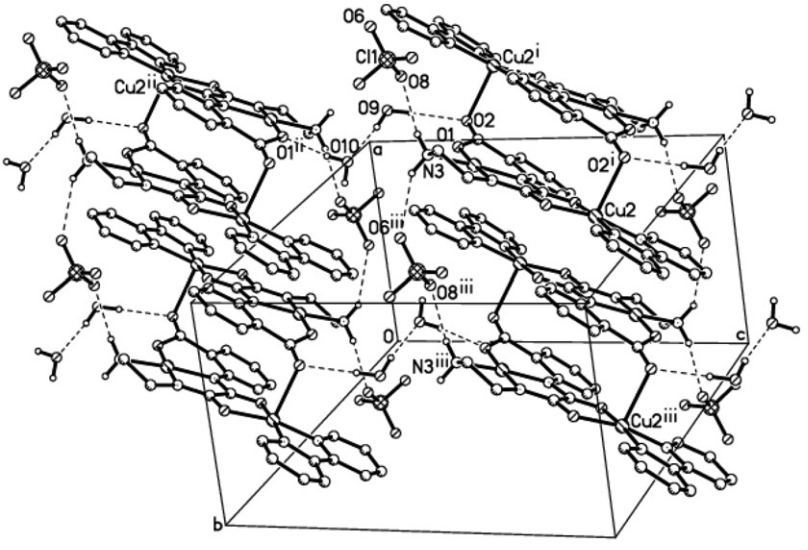


Figure 2. The 2-D classical hydrogen-bonding structure parallel to *a*0*c* plane. Hydrogen bonds are shown as dotted lines [Symmetry codes: [ii] $-x+2, -y, -z$; [iii] $x-1, y, z$].

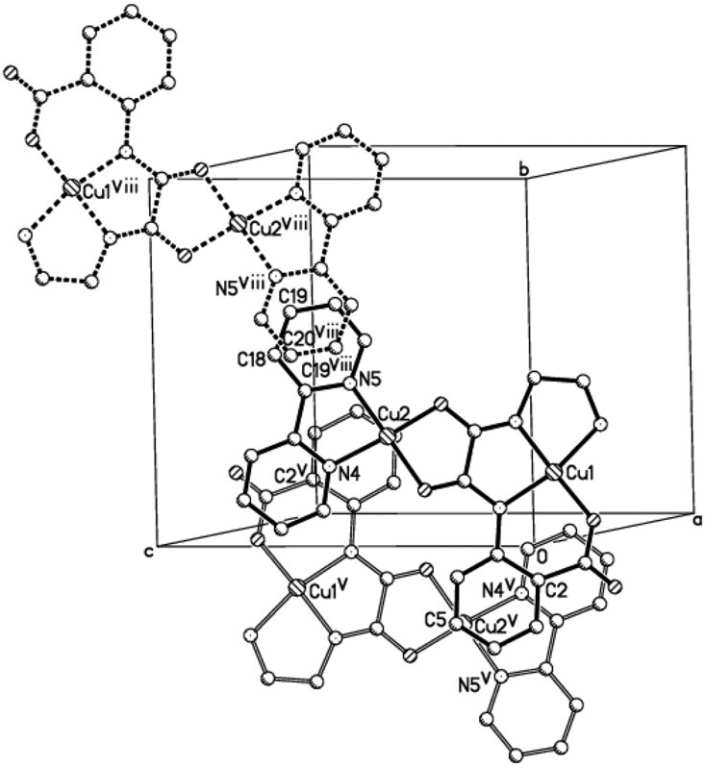


Figure 3. Schematic representation of the π–π stacking interactions in the crystal. The two fragments joined by the bold dotted lines and the open lines are on the front and back of the paper, respectively. Only halves of the tetranuclear complexes are drawn and hydrogens have been omitted for clarity [Symmetry code: [v] $-x+1, -y, -z+1$; [viii] $-x+2, -y+1, -z+2$].

3.4. *DNA-binding studies*

3.4.1. Electronic absorption titration. Electronic absorption spectroscopy is effective to examine the binding modes and the binding extent of metal complexes with DNA. In general, hypochromism and red shift are associated with the binding of the complexes to the DNA helix, due to the intercalative mode involving a strong stacking interaction between the aromatic chromophore of the complexes and the base pairs of DNA [68]. The absorption spectra of the tetranuclear copper(II) complex in the absence and presence of HS-DNA are given in figure 4. As shown in figure 4, when titrated by HS-DNA, the tetranuclear complex shows significant hypochromism accompanied by slight red shifts in the absorbance maxima. The observed hypochromicity and the bathochromic shifts were 10.4% with a red shift of 2 nm at a ratio of [DNA]/[complex] = 11. Obviously, the spectral characteristics suggest that the tetranuclear copper(II) complex interacts with HS-DNA most likely through the intercalation mode, which can be rationalized by the following reasons. When the tetranuclear complex intercalates the base pairs of HS-DNA, the π^* orbital of the intercalated ligand in the complex can couple with the π orbital of the base pairs of HS-DNA, thus decreasing the π–π^* transition energy and resulting in bathochromism. Furthermore, the coupling π-orbital is partially filled by electrons, thus decreasing the transition probabilities and concomitantly resulting in hypochromism.

In order to quantitatively investigate the binding strength of the tetranuclear complex with HS-DNA, the intrinsic binding constant K_b was obtained by monitoring the changes in absorbance at 300 nm for the complex with increasing the concentration of HS-DNA using the following equation [68]:

$$[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

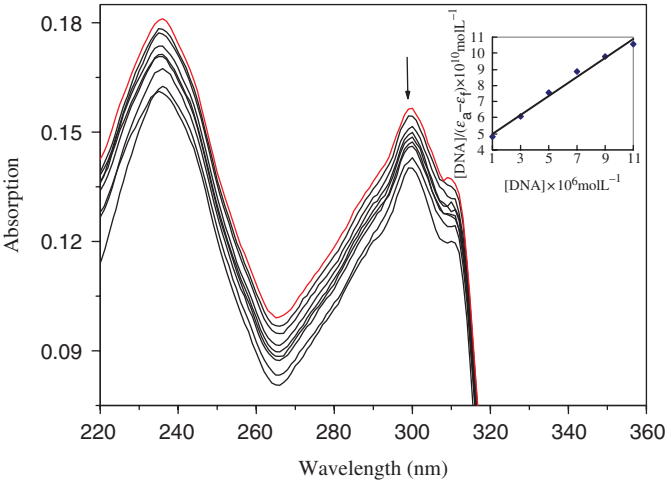


Figure 4. Absorption spectra of the complex upon titration of HS-DNA. Arrow indicates the change upon increasing the DNA concentration. Inset: Plot of $[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f)$ *vs.* [DNA] for the absorption titration of HS-DNA with the complex.

where ε_a, ε_f, and ε_b correspond to the extinction coefficient, respectively, for each addition of HS-DNA to the tetranuclear copper(II) complex, for the free tetranuclear copper(II) complex, and for the tetranuclear copper(II) complex in the fully bound form. From the plot (insets in figure 4) of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA], the binding constant K_b is given by the ratio of the slope to the intercept. The K_b of the complex is $1.47 \times 10^5\,mol^{-1}L$ ($r = 0.9936$ for six points). The K_b value of the complex is lower than that observed for a classical intercalator such as EB-DNA, $\sim 10^6\,mol^{-1}$ [69], but higher than some well-established intercalation agents [33, 70, 71].

3.4.2. Fluorescence titration. To better understand the interaction mode between the tetranuclear copper(II) complex and HS-DNA, the EB fluorescence displacement experiments were also used. The intrinsic fluorescence intensity of DNA is very low, and that of EB in Tris–HCl buffer is also not high due to quenching by solvent. However, on addition of DNA, the fluorescence intensity of EB will be enhanced due to its intercalative binding to DNA. Thus, EB can be used to probe the interaction of complexes with DNA. If the complexes can intercalate into DNA, the binding sites of DNA available for EB will be decreased, and hence the fluorescence intensity of EB will be quenched [72]. In our experiment, as illustrated in figure 5, for the tetranuclear complex the fluorescence intensities of EB bound to HS-DNA at 584 nm showed a remarkable decrease with the increasing concentration of the tetranuclear copper(II) complex, indicating that some EB molecules were released into the solution after an exchange with the tetranuclear copper(II) complex, which resulted in the fluorescence quenching of EB. The present observation is often the characteristic of intercalation and coincides with other intercalators [73].

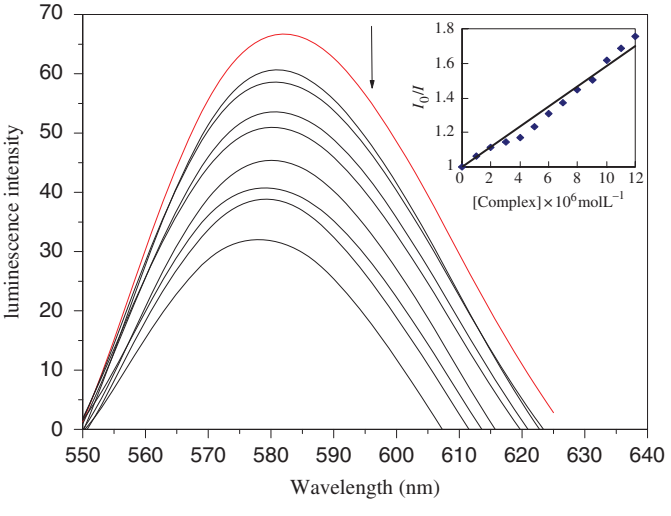


Figure 5. Emission spectra of the HS-DNA–EB system upon titration of the complex. Arrow shows the change upon increasing the complex concentration. Inset: Plot of I_0/I *vs.* [complex] for the titration of the complex to HS-DNA–EB.

In order to quantitatively understand the magnitude of the binding strength of the tetranuclear copper(II) complex with HS-DNA, the linear Stern–Volmer equation is employed [74]:

$$I_0/I = 1 + K_{sv}[Q] \quad (2)$$

where I_0 and I represent the fluorescence intensities in the absence and presence of the quencher, respectively, Q is the concentration of the quencher, and K_{sv} is a linear Stern–Volmer quenching constant. As shown in figure 5, in the quenching plot of I_0/I *versus* [complex], K_{sv} of the complex is given by the slope. The K_{sv} value for the tetranuclear copper(II) complex is 5.83×10^4 ($r = 0.9871$ for 13 points), which is in agreement with that derived by electronic absorption spectra measurements.

3.4.3. Electrochemical titration. Application of cyclic voltammetry to the study of interaction between the metal complexes and DNA also provides a useful complement to the previously used spectral studies. As can be seen in figure 6, in the absence of HS-DNA (solid line), the tetranuclear copper(II) complex shows two quasi-reversible redox processes corresponding to Cu(I)/Cu and Cu(II)/Cu(I), respectively [21, 37, 75]. The separations of anodic and cathodic peaks (ΔE_p) are 0.804 and 0.083 V with formal potentials −0.422 and 0.141 V for Cu(I)/Cu and Cu(II)/Cu(I), respectively. In the presence of HS-DNA (dotted line) with $R = 4$ ($R = [DNA]/[complex]$) the voltammetric peak current decreased, indicating interaction between the tetranuclear complex and HS-DNA [76]. The drop of the voltammetric current in the presence of HS-DNA may be attributed to the slow diffusion of the tetranuclear complex bound to HS-DNA. The peak-to-peak separations became larger with $\Delta E_p = 0.814$ and 0.087 V for Cu(I)/Cu and Cu(II)/Cu(I), respectively, suggesting that the electron transfers of both the processes in the presence of HS-DNA become less reversible. The formal potentials of the two couples in binding are −0.393 and 0.149 V. The formal potentials ($E_b^{o'}$) of the tetranuclear copper(II) complex are both shifted positive, indicating that the complex could bind intercalatively to HS-DNA [77]. The shift in the formal potentials can be

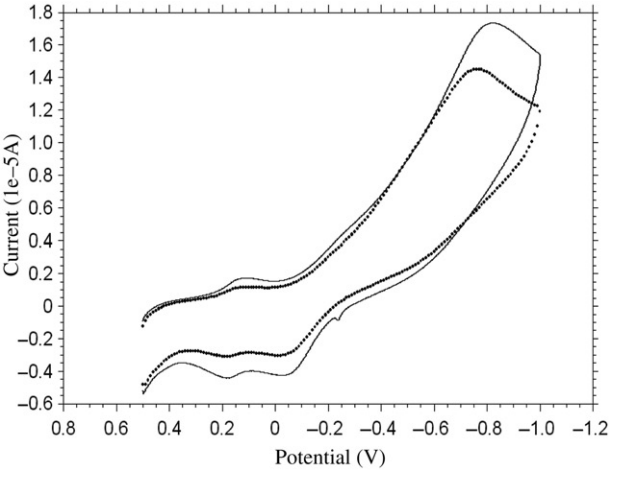
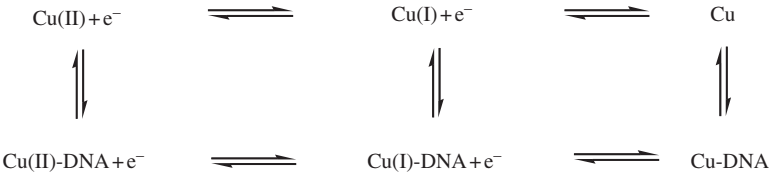


Figure 6. Cyclic voltammograms of the complex in the absence (solid line) and presence (dotted line) of HS-DNA.

used to estimate the ratio of binding constants for the reduced form and oxidized form to DNA using the equation as follows [77]:

$$E_b^{o'} - E_f^{o'} = 0.059 \log(K_{red}/K_{ox}) \quad (3)$$

where $E_f^{o'}$ is the formal potential of the Cu_{red}/Cu_{ox} couple in the free form and K_{red} and K_{ox} are the binding constants of Cu_{red} and Cu_{ox} forms to DNA, respectively. We calculate $K_{Cu(II)}/K_{Cu(I)}$ and $K_{Cu(I)}/K_{Cu}$ as 1.42 and 3.10, respectively, suggesting that the reduced form interacts more strongly than the oxidized one. The electron transfer system in which the oxidized and reduced forms were associated with a third species (DNA) in solution can be described as follows:



Thus, the electrochemical result is in agreement with the spectral results, which reinforce the proposal that the complex binds to the DNA in intercalation mode.

3.4.4. Viscosity measurements. Viscosity measurement, sensitive to changes in the length of DNA, is regarded as the least ambiguous and the most critical means for studying the binding mode of complexes with DNA in solution and provides stronger arguments for intercalative binding [78, 79]. In classical intercalation the DNA helix lengthens as base pairs are separated to accommodate the bound ligand leading to increased DNA viscosity, whereas a partial, non-classical ligand intercalation causes a bend in DNA helix reducing its effective length and thereby its viscosity. The effects of the tetranuclear complex on the viscosities of HS-DNA are shown in figure 7. On increasing the amount of the complex the relative viscosity of HS-DNA increases steadily, proving that the tetranuclear complex binds to HS-DNA by intercalation [27, 28]. Thus, the results obtained from viscosity studies validate those obtained from UV-Vis spectral titration, fluorescence spectra, and electrochemical titration.

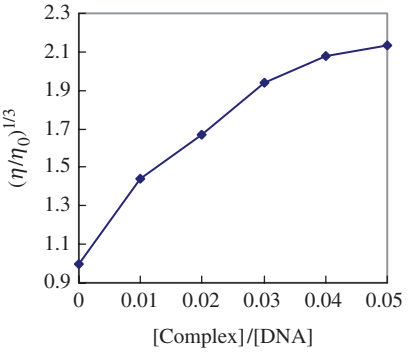


Figure 7. Effect of the increasing amount of the complex on the relative viscosity of HS-DNA at 289(±0.1) K, [DNA] = 0.1 mmol L^{-1}.

Further investigations are still required in order to get a deeper insight into the DNA-binding studies of the tetranuclear complexes and are in progress in our laboratory.

Supplementary material

Crystallographic data (excluding structure factors) for the structure reported in this work have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition number: CCDC 729358.

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