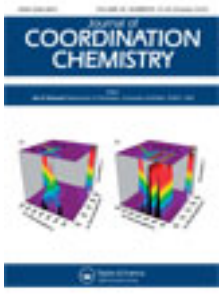

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Synthesis, structure, anticancer activities, and DNA-binding properties of a 1-D polymeric copper(II) complex alternately bridged by oxamide and terephthalate

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Synthesis, structure, anticancer activities, and DNA-binding properties of a 1-D polymeric copper(II) complex alternately bridged by oxamide and terephthalate

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A 1-D polymeric copper(II) complex alternately bridged by *N*,*N*′-bis(N-hydroxyethylamino-propyl)oxamide ($heap^{2-}$) and terephthalate (tpa^{2-}), $[Cu_2(heap)(tpa)]_n$, has been synthesized and characterized by single-crystal X-ray diffraction. The crystal structure reveals that the asymmetric unit of the copper(II) polymer is half a dinuclear copper(II) complex, $[Cu_2(heap)(tpa)]$, in which Cu(II) is located in a square-pyramidal coordination environment. Separations of Cu(II) through $heap^{2-}$ and tpa^{2-} bridges are 5.2459(6) and 11.1375(6) Å, respectively. The complex chains, accompanied with glide planes parallel to the *a*0*c* plane, can be classified to two groups according to their extending direction. Hydrogen bonds occur between a complex chain and any adjacent ones in the other orientation. Consequently, a 3-D supramolecular network is completed. The polymeric copper(II) complex exhibits potent anticancer activities against human hepatocellular carcinoma cell SMMC-7721 and human lung adenocarcinoma cell A549 tested by sulforhodamine B assays. The interactions of the polymeric copper(II) complex with herring sperm DNA (*HS*-DNA) are investigated by using electronic absorption titration, fluorescence titration, electrochemical titration, and viscometry measurements. The results suggest that the polymeric copper(II) complex interacts with *HS*-DNA *via* intercalation with intrinsic binding constant of $1.8 \times 10^6 (\text{mol L}^{-1})^{-1}$.

Keywords: μ-Oxamido-bridge; μ-Terephthalate-bridge; 1-D polymeric copper(II) complex; Crystal structure; Anticancer activity; DNA-binding property

1. Introduction

Synthesis and interaction of transition-metal complexes with DNA is a fundamental requirement to elucidate the mechanism involved in the site specific recognition of DNA, to determine the principles governing the recognition, for understanding models

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for protein–nucleic acid interactions, for application of probes of DNA structure, and to synthesize new types of pharmaceutical molecules [1–3].

Modes of DNA non-covalent interaction with metal complexes include electrostatic, groove binding, and intercalation. The effectiveness mainly depends on the mode and affinity of binding between the complexes and DNA [4]. Many transition metal complexes have been synthesized and interactions with DNA were studied [5, 6]. Significant developments have occurred in the chemistry of polymer–metal complexes. In particular, studies of the interaction of these polymer–metal complexes with DNA have been of great interest [7, 8]. However, examples of such polymer–metal complexes are still few, and comparatively little attention has been given to systems in which the transition metal ions are propagated both by oxamido and phenyldicarboxylate bridges, although oxamido [9] and phenyldicarboxylate [10–12] have been shown to be good for constructing polymer–metal complexes. It is thus of considerable interest to synthesize and study the DNA-binding properties of polymer–metal complexes with bridging oxamido and phenyldicarboxylate groups to gain insight into the DNA-binding properties and antitumor activities of these kinds of compounds.

In continuation of our interest in polymer–metal complexes [13], here is detailed a new 1-D polymeric copper(II) complex formulated as $[Cu_2(heap)(tpa)]_n$. The polymer has been synthesized and structurally characterized by using both *N*,*N*′-bis(N-hydroxyethylaminopropyl)oxamide ($heap^{2-}$) and terephthalate (tpa^{2-}) as polyatomic bridging ligands. The anticancer activities and DNA-binding properties of the polymeric copper(II) complex were studied. This is the first report about the DNA-binding and anticancer activities of a 1-D polymeric copper(II) complex alternately bridged by phenyldicarboxylate and *N*,*N*′-bis(substituted)oxamide with hydroxyl group coordination.

2. Experimental

2.1. *Materials and chemicals*

All chemicals were of analytical grade. *N*,*N*′-bis(N-hydroxyethylaminopropyl)oxamide (H_2heap) was synthesized according to the reported method [14]. Ethidium bromide (EB) and herring sperm DNA (*HS*-DNA) were purchased from Sigma Corp. and used as received.

2.2. *Physical measurements*

The C, H, and N microanalyses were performed on a Perkin-Elmer 240 elemental analyzer. Molar conductance was measured with a Shanghai DDS-11A conductometer. Infrared spectra were recorded on a Nicolet-470 spectrophotometer from 4000 to 400 cm^{-1} as KBr pellets. 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. Cyclic voltammetric experiments were carried out using a CHI 832B electrochemical analyzer in connection with a glassy carbon working electrode, saturated calomel reference, and a platinum wire auxiliary electrode.

Viscosity measurement was carried out using an Ubbelodhe viscometer immersed in a water bath maintained at 289(±0.1) K.

2.3. *Synthesis of [Cu$_2$(heap)(tpa)]$_n$*

To a methanol solution (5 mL) containing H_2heap (0.0145 g, 0.05 mmol) and piperidine (0.0085 g, 0.1 mmol) was added an aqueous solution (5 mL) of copper(II) chloride dihydrate (0.0170 g, 0.1 mmol) with continuous stirring. The mixture was stirred quickly for 30 min, and then an aqueous solution (5 mL) of terephthalic acid (0.0168 g, 0.1 mmol) and piperidine (0.0170 g, 0.2 mmol) was added dropwise into the mixture. The reaction solution was continuously stirred at 60°C for 4 h. The resulting blue precipitates were collected, washed with methanol, and dried in a desiccator. Yield: 85%. Blue crystals suitable for X-ray analysis were obtained by slow diffusion of two individual aqueous solutions in an H-tube (one contained H_2heap, piperidine, and copper(II) chloride dehydrate, and the other contained terephthalic acid neutralized by piperidine). Anal. Calcd for $CuC_{10}H_{14}N_2O_4$ (%): C, 41.45; H, 4.87; N, 9.67. Found (%): C, 41.40; H, 4.82; N, 9.70.

2.4. *Determination of X-ray crystal structure*

Crystal structure analysis was carried out on a Bruker *APEX* area-detector diffractometer with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The crystal structure was solved by direct methods followed by Fourier syntheses. Structure refinement was performed by full-matrix least-squares on F^2 using *SHELXL-97* [15]. The hydroxyl hydrogen was found in an electron-density difference map and then treated as riding, with $U_{iso}(H) = 1.2U_{eq}(O)$. All other hydrogen atoms were placed in calculated positions, with C–H = 0.97 (methylene), 0.93 (aromatic), and N–H = 0.91 (amine), and refined in riding mode, with $U_{iso}(H) = 1.2U_{eq}$ (carrier atoms). Crystal data and refinement conditions are summarized in table 1.

2.5. *DNA-binding experiments*

All the experiments involving *HS*-DNA were performed in *tris*-(hydroxymethyl)aminomethane-HCl (*tris*-HCl) buffer solution (pH = 7.29). *Tris*-HCl buffer was prepared using deionized and sonicated triply distilled water. Solutions of *HS*-DNA in *tris*-HCl gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.8–1.9, indicating that the DNA was sufficiently free from protein [16]. The concentration of *HS*-DNA was determined by UV absorbance at 260 nm. The extinction coefficient, ε_{260}, was taken as 6600 L mol^{-1} cm^{-1} [17]. Stock solution of *HS*-DNA was stored at 277 K and used after no more than 4 days. Concentrated stock solution of the copper(II) complex was prepared by dissolving the copper(II) complex in *tris*-HCl buffer to required concentrations for all the experiments. Absorption spectral titration was performed by keeping the concentration of the copper(II) complex constant while varying *HS*-DNA concentration. Equal solution of *HS*-DNA was added to the copper(II) polymer 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

Table 1. Crystallographic data for the copper(II) complex.

Empirical formula	$CuC_{10}H_{14}N_2O_4$
Formula weight	289.77
Crystal system	Orthorhombic
Space group	*Pbcn*
Unit cell dimensions (Å, °)	
a	13.3017(2)
b	13.9226(4)
c	12.1243(3)
α	90.00
β	90.00
γ	90.00
Volume (Å^3), *Z*	2245.35(9), 8
Calculated density (g cm^{-1})	1.714
Calculated density (Mo-Kα) (mm^{-1})	1.95
Scan-mode	φ and ω scan
F(000)	1192
Crystal size (mm^3)	$0.23 \times 0.15 \times 0.05$
θ range	2.1–27.6
Total, unique data, *R*(int)	8257, 2619, 0.034
Observed data [$I > 2\sigma(I)$]	1902
R, wR_2	0.033, 0.091
S	1.04
Max., min. shift/error	0.000, 0.000

(L mmol L^{-1}) was added to 1 mL of *HS*-DNA solution (at saturated binding levels) [18], stored in dark for 2 h. Then solution of the copper(II) polymer was titrated into the DNA/EB mixture and diluted in *tris*-buffer to 5 mL, producing solutions with varied mole ratio of copper(II) polymer 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. The electrochemical titration experiments were performed by keeping the concentration of the copper(II) polymer constant while varying *HS*-DNA concentration using solvent of *tris*-HCl buffer. In viscosity measurement, *HS*-DNA samples approximately 200 base pairs in length were prepared by sonication in order to minimize complexities arising from DNA flexibility [19]. Flow times were measured with a digital stopwatch and each sample was measured three times with an average flow time calculated. Relative viscosities for *HS*-DNA in the presence and absence of copper(II) polymer 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 [20], where η is the viscosity of *HS*-DNA in the presence of the copper(II) polymer, and η_0 is the viscosity of DNA alone.

2.6. In vitro *antitumor activity evaluated by sulforhodamine B assays*

In vitro antitumor activities of the copper(II) polymer and *cis*-platin were evaluated against two cancer cell lines, SMMC-7721 and A549, by using the sulforhodamine B (SRB) assays. All cells were cultured in RPMI 1640 supplemented with 10%(v/v) fetal bovine serum, 1%(w/v) penicillin (104 U mL^{-1}) and 10 mg mL^{-1} streptomycin. Cell lines were maintained at 310 K in a 5%(v/v) CO_2 atmosphere with 95%(v/v) humidity.

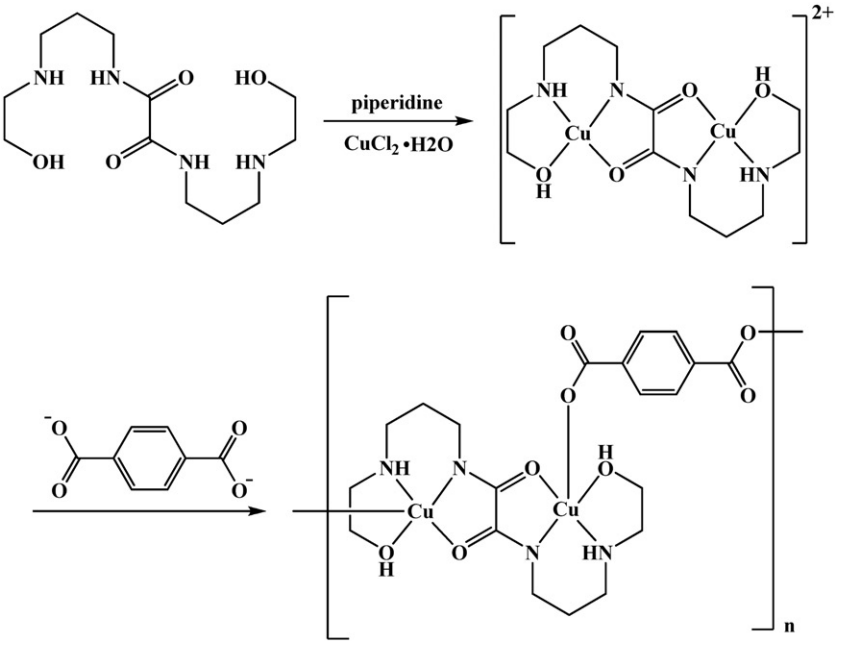
Cultures were passaged weekly using trypsin-EDTA to detach the cells from their culture flasks. The copper(II) polymer was dissolved in DMSO and diluted to the required concentration with culture medium when used. The content of DMSO in the final concentrations did not exceed 0.1%. At this concentration, DMSO was found to be non-toxic to the cells tested. Rapidly growing cells were harvested, counted, and incubated at the appropriate concentration in 96-well microplates for 24 h. The copper(II) polymer dissolved in culture medium was then applied to the culture wells to achieve final concentrations ranging from 10^{-3} to 10^{2} μg mL^{-1}. Control wells were prepared by addition of culture medium without cells. The plates were incubated at 310 K in a 5% CO_2 atmosphere for 48 h. Upon completion of the incubation, the cells were fixed with ice-cold 10% trichloroacetic acid (100 mL) for 1 h at 277 K, washed five times in distilled water and allowed to dry in air and stained with 0.4% SRB in 1% acetic acid (100 mL) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mmol L^{-1} unbuffered *tris*-base (100 mL) and the optical density of each well was measured at 540 nm on a microplate spectrophotometer. The IC_{50} values were calculated from curves constructed by plotting cell survival (%) *versus* the polymeric copper(II) complex concentration (μg mL^{-1}).

3. Results and discussion

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

Synthesis of polymeric metal–organic frameworks (MOFs) has been a challenging field for coordination chemists. An effective method to construct MOFs is the self-assembly of inorganic second building units (SBUs) and organic linkers [21]. *N*,*N*′-bis(substituent)oxamides are versatile bridging ligands, of which the *trans* conformation is more stable than the *cis* one in the lack of steric hindrance between substituents on the amine nitrogen and allow formation of *trans*-oxamidato-bridged binuclear SBUs [22]. Hence, this family of ligands has played an important role in constructing polymeric MOFs. Apart from inorganic SBUs, organic linker molecules with versatile coordination modes are indispensable to construct polymeric MOFs. Benzene dicarboxylate dianions with versatile bonding modes and peculiar structures involving carboxylates, which are non-coplanar with the benzene ring, could be good candidates for the preparation of polymeric MOFs [10, 13]. Many new metal–polymers with interesting structures based on the bridging *N*,*N*′-bis(substituent)oxamides have been synthesized and their magnetic properties studied extensively [23, 24]. However, structures of polymeric MOFs involving coordinating hydroxyl groups in the *N*,*N*′-bis(substituent)oxamide bridging ligand appear to be rare.

In this study, we chose *N*,*N*′-bis(N-hydroxyethylaminopropyl)oxamide ($heap^{2-}$) to synthesize the SBU with the *trans*-heap-bridged bicopper(II) building block $[Cu_2(heap)]^{2+}$. The terephthalato (tpa^{2-}) ion is used as the organic linker attaching these bicopper(II) building blocks to form a 1-D copper(II) coordination polymer. Elemental analyses indicate that the reaction of $heap^{2-}$ with $CuCl_2 \cdot 2H_2O$ and tpa^{2-} in 1 : 2 : 1 mole ratio yielded the 1-D polymeric copper(II) complex, $[Cu_2(heap)(tpa)]_n$, as expected. The synthetic pathway for complexation is represented by scheme 1.



Scheme 1. The synthetic pathway for the polymeric copper(II) complex.

The polymeric copper(II) complex is insoluble in non-polar solvents and common polar solvents, moderately soluble in water, methanol, and acetonitrile, and very soluble in DMF and DMSO to give stable solutions at room temperature, implying the polymeric nature of the copper(II) complex [25]. In the solid state, the polymeric copper(II) complex is fairly stable in air allowing physical measurements. The molar conductance value of the polymeric copper(II) complex ($\Lambda = 15\,\Omega^{-1}\,cm^2\,mol^{-1}$ in DMF) falls in the expected range for non-electrolytes [26], suggesting that all tpa^{2-} ligands in the copper(II) polymer are involved with coordination. The copper(II) complex exists as a whole polymer entity in solution. These observations coincide with the following spectral characterization.

3.2. *IR spectra*

The most salient feature in the IR spectra of the polymeric copper(II) complex is the existence of a strong band at $1619\,cm^{-1}$, attributed to ν(N–C=O) stretching vibration band of bridging $heap^{2-}$ [27]. The polymeric copper(II) complex exhibits characteristic stretching vibrations of ν_{as}(COO) ($1574\,cm^{-1}$) and ν_s(COO) ($1319\,cm^{-1}$) for coordinated carboxylate [28] of the bridging ligand tpa^{2-}. The Δ value, which represents the separation of ν_{as}(COO) and ν_s(COO), reflects the coordination modes of carboxylate [12, 28]. The polymeric copper(II) complex shows Δ of $255\,cm^{-1}$, indicating monodentate coordination in tpa^{2-} [28, 29]. The unidentate coordination of carboxylates in tpa^{2-} is supported by the crystal structure of $[Cu_2L_2(2,2'\text{-bpy})_2(H_2O)_2]$ [12], and the determination of the crystal structure for the present polymeric copper(II) complex (*vide infra*).

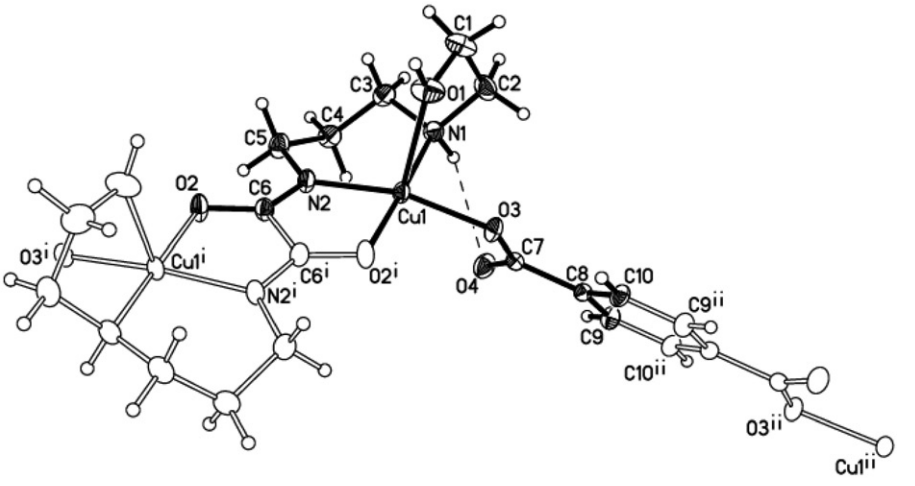


Figure 1. The molecular structure of $[Cu_2(heap)(tpa)]$ showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level. Dashed line indicates hydrogen bonds (symmetry codes: $^{i}1-x, y, 3/2-z$; $^{ii}1-x, 1-y, 1-z$).

3.3. *Electronic spectra*

To obtain further structural information, electronic spectra of the polymeric copper(II) complex were recorded in the UV-Vis region using DMSO as solvent. For the polymeric copper(II) complex, two absorptions with varied intensities are observed. The intense band at 230 nm was assigned to intra-ligand (π–π^*) transition associated with tpa^{2-} and a broad band at 613 nm matches well with d–d transition of five-coordinate copper(II) in the square-pyramidal geometry [30].

3.4. *Description of the structure*

The complex is a 1-D copper(II) polymer alternately bridged by tpa^{2-} and *trans*-$heap^{2-}$. The Cu···Cu separations through the two bridges are 5.2459(6) and 11.1375(6) Å, respectively. As shown in figure 1, copper(II) (Cu1) has an N2O3 square-pyramidal coordination geometry with $\tau = 0.22$ [31]. The basal plane is defined by three atoms from $heap^{2-}$ (N1, N2 and $O2^{i}$; symmetry code: ^{i} $-x, y, 3/2-z$) and a carboxyl oxygen (O3) of tpa^{2-} ion with displacements of the defined atoms from 0.1183(10) Å (N1) to 0.1373(11) Å ($O2^{i}$). Cu1 deviates 0.1012(11) Å from the plane. The apical position is occupied by a hydroxyl oxygen (O1) of $heap^{2-}$ with Cu1–O1 length of 2.352(2) Å (table 2). The Cu1–N1 bond is shorter than Cu1–N2, consistent with stronger donor abilities of nitrogen in sp^2 hybridization than in sp^3 [32].

The *trans*-$heap^{2-}$ coordinates quadridentate to Cu1. One "arm" [3-(2-hydroxyethylamino)propyl group] of the oxamido-bridge adopts a facial distribution of three coordination atoms and forms two puckered chelate rings. The five-membered ring has a twisted conformation with the puckering parameters [33] of Q (0.496(3) Å) and φ (308.3(3)°); the six-membered ring is between the envelope and the screwboat conformations, the corresponding puckering parameters are $Q = 0.602(3)$ Å, $\theta = 118.80(19)°$, and $\varphi = 343.0(3)°$. The facial disposition is different from the

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

Cu1–O1	2.352(2)	Cu1–N2	1.968(2)
Cu1–O2^{i}	1.9806(18)	O2–C6	1.271(3)
Cu1–O3	1.9838(17)	N2–C6	1.288(3)
Cu1–N1	2.018(2)		
O1–Cu1–O2^{i}	102.91(8)	O2^{i}–Cu1–N1	177.85(8)
O1–Cu1–O3	92.22(8)	O2^{i}–Cu1–N2	83.47(8)
O1–Cu1–N1	75.69(8)	O3–Cu1–N1	92.36(8)
O1–Cu1–N2	102.50(9)	O3–Cu1–N2	164.72(9)
O2^{i}–Cu1–O3	89.32(8)	N1–Cu1–N2	95.21(9)

Symmetry code: $^{i}1-x,\ y,\ 3/2-z$.

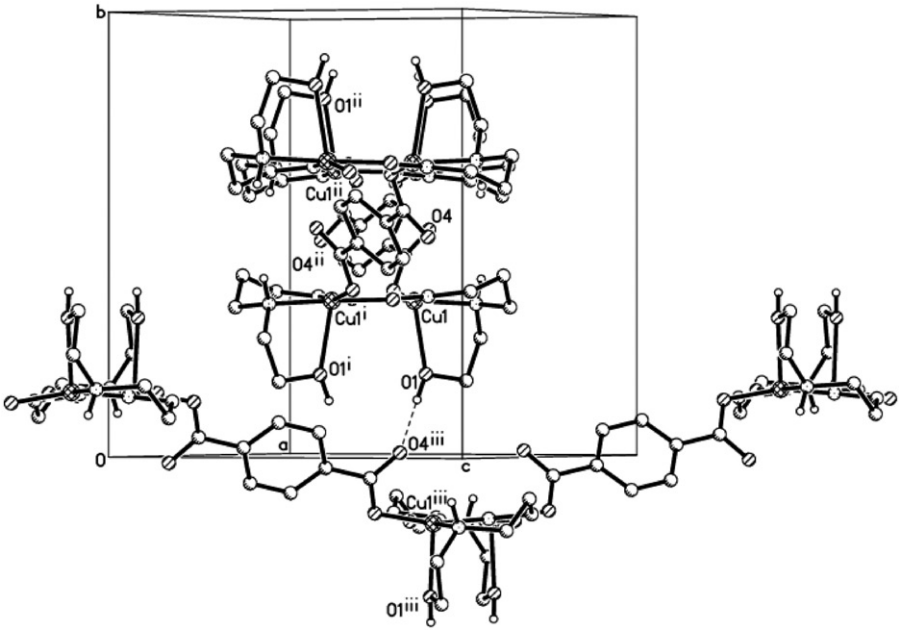


Figure 2. Two complex chains extending along the direction of [2 0 1] (upper) and [2 0 1] (below) linked by a strong O–H···O hydrogen bond. Both chains have glide planes parallel to the *a*0*c* plane through the middle of the *b*-axis and the origin, respectively (symmetry codes: $^{i}1-x,\ y,\ 3/2-z$; $^{ii}1-x,\ 1-y,\ 1-z$; $^{iii}1-x,\ -1/2+y,\ z$).

meridional in reported heap^{2-} complexes (CSD ref code [34]: BUTTOA [35], KICCIJ [36], and MUFVAL [37]). These complexes also have square-pyramidal copper(II). The hydroxyl oxygen atoms lie in the basal plane with longer Cu–O distances than Cu–O (oxamide) bonds only by 0.047, 0.03 and 0.018 Å, respectively. However, in the polymeric copper(II) complex, due to facial disposition, the hydroxyl oxygen (O1) is at the apical position of the coordination square-pyramidal. While apical bonds in Cu(II) systems are invariable elongated, the Cu1–O1 (hydroxyl) bond is longer, 0.371(3) Å, than Cu1–O2^{i} (oxamide) in the basal plane.

Although all the complex chains are exactly the same through symmetry, they do not extend along the same direction, but extend along two directions. As shown in figure 2, one extends along the [2 0 1] direction such as the chain containing the original

Table 3. Hydrogen-bond geometry (Å, °) for the copper(II) complex.

D–H···A	D–H	H···A	D···A	D–H···A
N1–H1···O4	0.91	2.35	2.981(3)	126
O1–H1C···O4^{iii}	0.89	1.77	2.658(3)	175

Symmetry code: $^{iii}1-x, -1/2+y, z$.

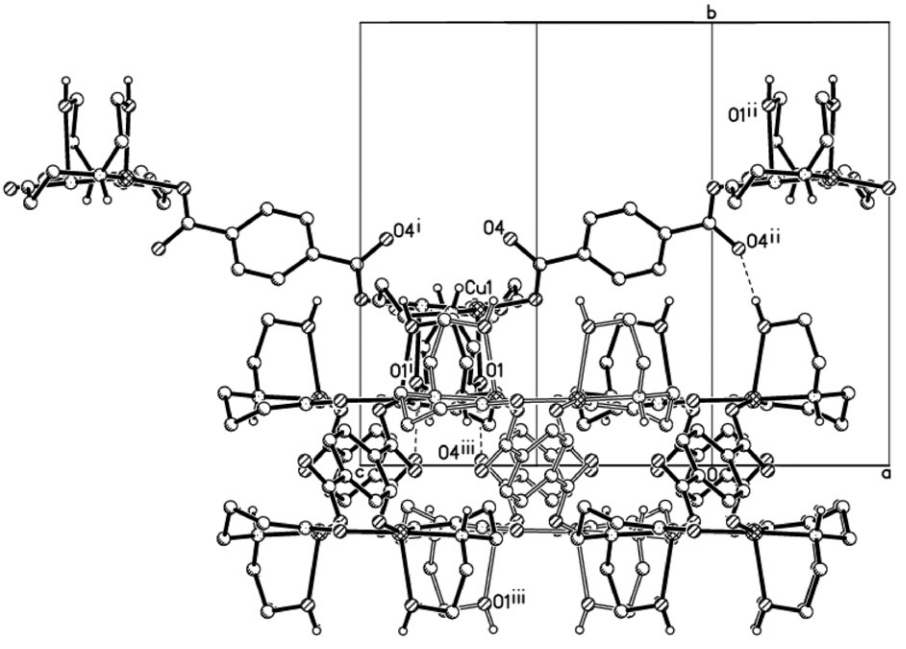


Figure 3. The hydrogen bonds between a complex chain and ones in a neighboring layer (symmetry codes: $^{i}1-x, y, 3/2-z$; $^{ii}1-x, 1-y, 1-z$; $^{iii}1-x, -1/2+y, z$).

asymmetric unit, which is accompanied with a glide plane through the middle of the *b*-axis and parallel to the *a*0*c* plane while the other is toward the [2 0 1] direction and the glide plane is just the *a*0*c* plane. Consequently, these copper(II) complexes form an alternating layer structure parallel to the *a*0*c* plane. Each layer consists of the complex chains with the same orientation, but chains belonging to neighboring layers are in different directions. Although there are no intermolecular interactions among these complex chains in the same layer (e.g. hydrogen bonds, π–π stacking), a strong hydrogen bond involving the hydroxyl (O1) of a heap^{2-} and the uncoordinated carboxyl oxygen (O4^{iii}; symmetry code: ^{iii} $1/2-x, -1/2+y, z$) of a tpa^{2-} anion exists between the neighboring layers (figure 2, table 3). Through the hydrogen bonds, as shown in figure 3, a complex chain can link chains in both adjacent layers. As a result, a 3-D supramolecular structure is formed.

If we compare the structure of the present polymeric copper(II) complex $[Cu_2(heap)(tpa)]_n$ with that of a previously reported analogous polymeric copper(II) complex $[Cu_2(dmapox)(tpa)(H_2O)_2]_n$ [13], we find that the two polymeric copper(II) complexes have similar *N*,*N*′-bis(substituent)oxamide skeletal structures (heap^{2-} and

dmapox^{2-}) and the same phenyldicarboxylate (tpa^{2-}). The main difference between $[Cu_2(heap)(tpa)]_n$ and $[Cu_2(dmapox)(tpa)(H_2O)_2]_n$ is that the substituents on the amine nitrogen of the oxamido-bridge ligands are 3-(2-hydroxylethylamino)propyl groups in the former case (heap^{2-}), and 3-(dimethylamino)propyl groups in the latter (dmapox^{2-}), which result in three differences between the two polymeric copper(II) complexes. The first difference is that although the copper(II) coordination environments in the two polymeric copper(II) complexes are both square-pyramidal, the apical position is occupied by a hydroxyl oxygen (O1) of heap^{2-}, while in the latter case, a water occupied the apical position. The second difference is the orthorhombic crystal system in $[Cu_2(heap)(tpa)]_n$, and triclinic one in $[Cu_2(dmapox)(tpa)(H_2O)_2]_n$. The third difference of the space group for $[Cu_2(heap)(tpa)]_n$ is *Pbcn*, accompanied with the glide planes, and that for $[Cu_2(dmapox)(tpa)(H_2O)_2]_n$ is $P\bar{1}$. These differences of structures for the two polymeric copper(II) complexes also have effects on the DNA-binding properties.

3.5. *DNA-binding studies*

3.5.1. Electronic absorption titration. Electronic absorption spectroscopy is an effective method to examine the binding modes of the metal complexes with DNA. In general, hypochromism and red-shift are associated with binding of complexes to DNA, due to intercalation involving a strong stacking interaction between the aromatic chromophore of the complexes and the base pairs of DNA [38]. Absorption spectra of the polymeric copper(II) complex in the absence and presence of *HS*-DNA are given in figure 4. When titrated by *HS*-DNA, the polymeric copper(II) complex has significant

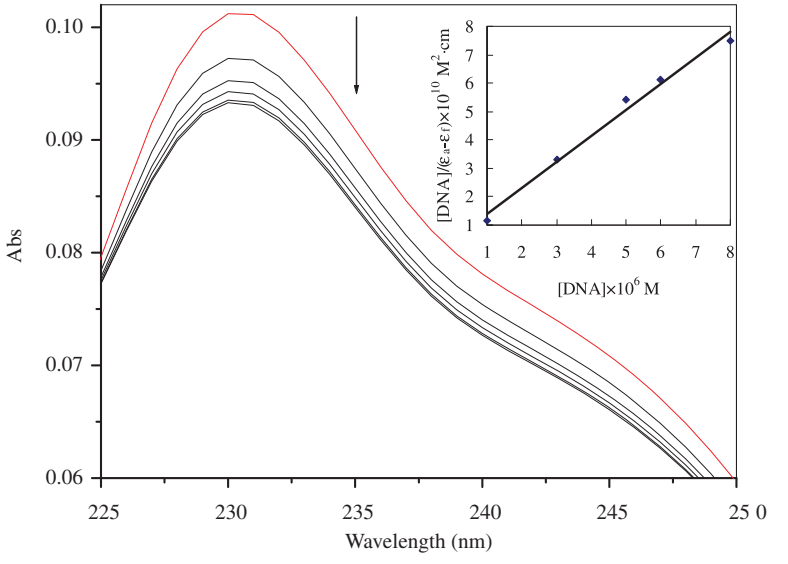


Figure 4. Absorption spectra of the complex upon titration of *HS*-DNA. The arrow indicates the change upon increasing DNA concentration. Inset: plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *vs.* [DNA] for the absorption titration of *HS*-DNA with the copper(II) complex.

hypochromism centered at 230 nm, accompanied by slight red-shifts of 2 nm in the absorbance maxima. These spectral characteristics reveal that the polymeric copper(II) complex interacts with *HS*-DNA through intercalation that involves π–π stacking between the polymeric copper(II) complex and the base pairs of DNA.

To evaluate quantitatively the affinity of polymeric copper(II) complex with *HS*-DNA, the intrinsic binding constant K_b was determined by monitoring the changes in absorbance at 230 nm using the following equation [39]:

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

where [DNA] is the concentration of *HS*-DNA and ε_a, ε_f, and ε_b correspond to the extinction coefficient, respectively, for each addition of the DNA to the copper(II) complex, for the free copper(II) complex, and for the copper(II) complex in the fully bound form. A plot of $[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA], gives K_b, the intrinsic binding constant as the ratio of slope to intercept. From the $[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA] plots (inset in figure 4), the binding constant for the polymeric copper(II) complex was estimated to be $1.8 \times 10^6 (\mathrm{mol\,L^{-1}})^{-1}$ ($R = 0.9967$ for five points).

The K_b value of the present polymeric copper(II) complex has the same level as that for classical intercalators (e.g. EB-DNA, $\sim 10^6 (\mathrm{mol\,L^{-1}})^{-1}$) [40] and is very much higher than that for dicopper(II) complexes previously reported by our group, using *N*,*N*′-bis(substituted)oxamides as bridging ligands and polypyridines as terminal ligands, such as $[Cu_2(heap)(H_2O)_2](pic)_2 \cdot 2H_2O$ (K_b, $2.67 \times 10^4 (\mathrm{mol\,L^{-1}})^{-1}$) [35], $[Cu_2(pdmaeox)(bpy)(H_2O)](pic) \cdot H_2O$ (K_b, $3.39 \times 10^4 (\mathrm{mol\,L^{-1}})^{-1}$) [41], and $[Cu_2(oxpep)(phen)]ClO_4$ (K_b, $4.5 \times 10^5 (\mathrm{mol\,L^{-1}})^{-1}$) [42]. This shows that the present polymer–copper(II) complex binds very strongly with *HS*-DNA compared to other μ-oxamido-bridged dicopper(II) complexes known in the literature [35, 41, 42].

A comparison of the two polymeric copper(II) complexes, $[Cu_2(heap)(tpa)]_n$ and $[Cu_2(H_2O)_2(dmapox)(tpa)]_n$ [13], shows that they share the same metal ion and analogous skeletal structures. The main difference is the N-substituents on the oxamido-bridge ligands. The reason the binding constant of $[Cu_2(heap)(tpa)]_n$ to *HS*-DNA is much higher than $[Cu_2(dmapox)(tpa)(H_2O)_2]_n$ ($1.45 \times 10^4 (\mathrm{mol\,L^{-1}})^{-1}$) may be that the electron-pushing substituents ($-CH_3$) on bridging $heap^{2-}$ in $[Cu_2(heap)(tpa)]_n$ increase the electric charge density of the bridging ligand, and decrease the binding affinity between $[Cu_2(H_2O)_2(dmapox)(tpa)]_n$ and *HS*-DNA.

3.5.2. Fluorescence titration. The EB fluorescence displacement experiment has been widely used to investigate the interaction of metal complexes with DNA. In order to further investigate the interaction between the polymer–copper(II) complex and *HS*-DNA, EB fluorescence displacement experiments were employed. 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 because of its intercalation into the 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 the DNA available for EB will be decreased, and hence the fluorescence intensity of EB will be quenched [43]. In our experiment, as illustrated in figure 5, the fluorescence intensities of EB bound to *HS*-DNA at 584 nm show remarkable decreasing trends with increasing concentration of the polymeric copper(II) complex, indicating that some EB molecules were released into solution after

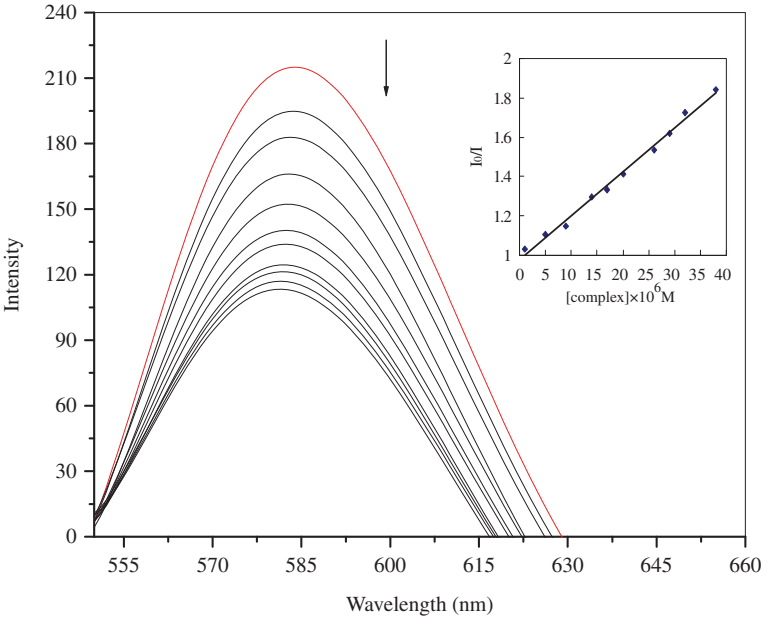


Figure 5. Emission spectra of the *HS*-DNA-EB system upon titration of the complex. The arrow shows the change upon increasing complex concentration. Inset: plot of I_0/I *vs.* [complex] for the titration of the copper(II) complex to *HS*-DNA-EB.

the exchange with the polymeric copper(II) complex, and resulted in the fluorescence quenching of EB. This observation may be interpreted as intercalation of the polymeric copper(II) complex between base pairs of *HS*-DNA or the stacking of the polymeric copper(II) complex on the outside of *HS*-DNA. Such a quenched fluorescence behavior of EB bound to *HS*-DNA caused by the interaction between the polymeric copper(II) complex and the DNA is also found in the literature for terephthalate-bridged tetranuclear copper(II) complex [44]. The quenching of EB bound to DNA by the polymeric copper(II) complex is in agreement with the linear Stern–Volmer equation [45]:

$$I_0/I = 1 + K_{sv}[Q], \tag{2}$$

where I_0 and I represent the fluorescence intensities in the absence and presence of quencher, respectively, K_{sv} is a linear Stern–Volmer quenching constant, and Q is the concentration of quencher. In the quenching plot (inset in figure 5) of I_0/I *versus* [complex], K_{sv} is given by the slope. The K_{sv} value for the polymeric copper(II) complex is 2.28×10^4 ($R = 0.9963$ for 10 points).

3.5.3. Electrochemical titration. The application of cyclic voltammetry to study interaction between metal complexes and DNA provides a useful complement to the previously used spectral studies [46]. In this study, it has been employed to understand the nature of DNA-binding of the polymeric copper(II) complex, and the result is shown in figure 6. In the absence of *HS*-DNA (curve a), the polymeric copper(II) complex displays a couple of well-defined redox waves corresponding to the

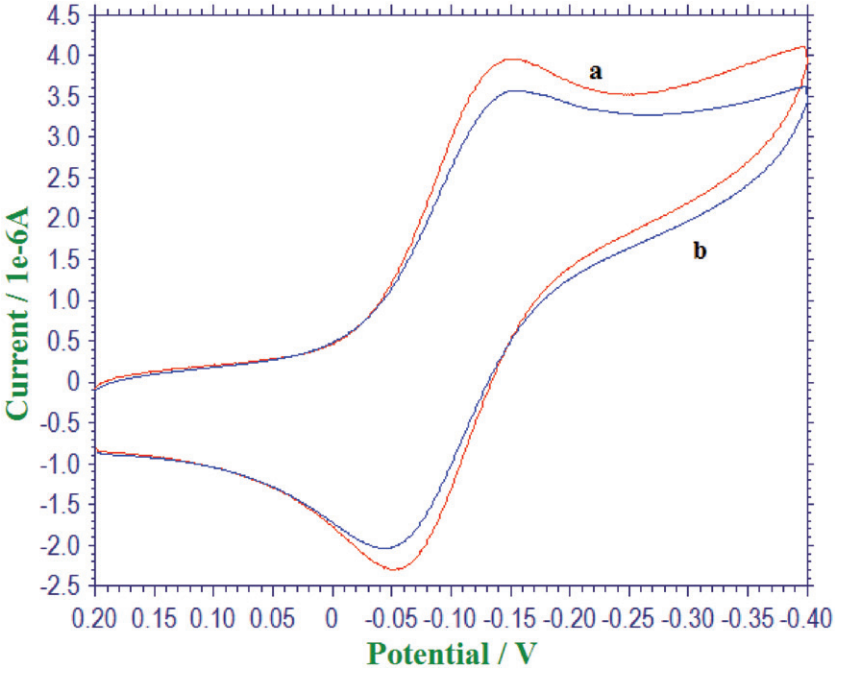


Figure 6. Cyclic voltammograms of the copper(II) complex in the absence (a) and presence (b) of *HS*-DNA.

Cu(II)/Cu(I) couple. The cathodic peak potential (E_{pc}) and the anodic peak potential (E_{pa}) are −155 mV and −52 mV. The value of the formal potential $E_{1/2}$, taken as the average of E_{pc} and E_{pa}, is −104 mV. In the presence of *HS*-DNA, the voltammetric peak currents decreased, indicating that there exist interactions between the polymeric copper(II) complex and *HS*-DNA [47]. The drop of the voltammetric current in the presence of *HS*-DNA may be attributed to slow diffusion of the polymeric copper(II) complex bound to *HS*-DNA. The cyclic voltammograms of the polymeric copper(II) complex exhibited slight shifts in the anodic and cathodic peak potentials followed by decrease in peak current, indicating the interaction existing between polymeric copper(II) complex and *HS*-DNA. The $E_{1/2}$ value for the polymeric copper(II) complex in the presence of *HS*-DNA (curve b) is −98 mV and exhibits positive shift of 6 mV. The positive shifts in $E_{1/2}$ of the polymeric copper(II) complex in the presence of *HS*-DNA indicate that the polymeric copper(II) complex can interact with DNA by intercalation [48]. The shift in the value of the formal potential ($\Delta E^{o\prime}$) can be used to estimate the ratio of equilibrium binding constants (K_R/K_O) according to the model of interaction described by Bard and Carter using the equation [49]:

$$\Delta E^{o\prime} = E_b^o - E_f^{o\prime} = 0.059\log\left[K_{Cu(I)}/K_{Cu(II)}\right], \quad (3)$$

where $K_{Cu(I)}$ and $K_{Cu(II)}$ are the binding constants of Cu(I) and Cu(II) to DNA, respectively. The ratio of constants for the binding of the Cu(I) and Cu(II) ions to *HS*-DNA was estimated to be 1.26, suggesting stronger binding affinity in the Cu(I) state compared to the Cu(II) state for the polymeric copper(II) complex. Thus, the

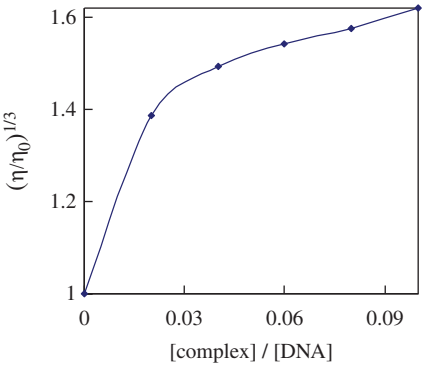


Figure 7. Effect of the increasing amount of the complex on the relative viscosity of *HS*-DNA at 289(±0.1) K, [*HS*-DNA] = 0.1 mmol L^{-1}.

electrochemical results are in agreement with the spectral studies, which reinforce the conclusion that the polymeric copper(II) complex binds to DNA by intercalation.

3.5.4. Viscosity measurement. To clarify the interaction mode of the polymeric copper(II) complex and *H*S-DNA, viscosity measurements were carried out. 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. Therefore, viscosity measurement is regarded as the least ambiguous and most critical means studying the binding mode of complexes with DNA in solution and provides strong arguments for intercalative binding [50, 51]. For polymeric copper(II), as shown in figure 7, the relative viscosity of *HS*-DNA increased obviously with increasing concentration of the polymeric copper(II) complex. The viscosity increase can be ascribed to the length increase of the *HS*-DNA double helix due to intercalation of the polymeric copper(II) complex between base pairs of DNA [52, 53]. Thus, the results of viscosity studies validate those obtained from electronic absorption titration, fluorescence titration, and electrochemical titration. Furthermore, the above studies of the DNA-binding properties on the polymeric copper(II) complex prompt us to explore cytotoxic activities.

3.6. In vitro *antitumor activity studies*

In vitro anticancer activities of the polymeric copper(II) complex and *cis*-platin against two cancer cell lines human hepatocellular carcinoma cell line SMMC-7721 and human lung adenocarcinoma cell line A549 were conducted. The polymeric copper(II) complex has significant cytotoxicities against the two cancer cell lines. Although the measured cytotoxic activity is less than that of *cis*-platin ($1.6 \pm 0.1\,\mu g\,mL^{-1}$ for SMMC-7721 and $2.3 \pm 0.2\,\mu g\,mL^{-1}$ for A549), inhibition of cell proliferation produced by the copper(II) complex on the same batch of cell lines and under identical experimental conditions is still rather active (IC_{50} values of 27 ± 2 and $32 \pm 2\,\mu g\,mL^{-1}$, respectively).

4. Conclusion

This article has investigated anticancer activities and interaction of a polymeric metal complex with DNA. $[Cu_2(heap)(tpa)]_n$ was synthesized using *N*,*N*′-bis(N-hydroxyethylaminopropyl)oxamide ($heap^{2-}$) and terephthalate (tpa^{2-}) as bridging ligands and its structure was characterized by single-crystal X-ray diffraction. The cytotoxicities of the polymeric copper(II) complex were tested *in vitro* by SRB assays against human hepatocellular carcinoma cell SMMC-7721 and human lung adenocarcinoma cell A549, suggesting that the copper(II) complex exhibits cytotoxic effects against the two cell lines. The DNA-binding results obtained by using absorption, emission spectral and electrochemical techniques, and viscometry suggest that the polymeric copper(II) complex interacts with *HS*-DNA by intercalation. These results have confirmed that in this system the affinity of the polymeric metal complexes toward *HS*-DNA may be modified and tuned by variation of the N-substituents on the oxamido-bridging ligands, and this strategy should be valuable in understanding the binding properties of polymeric metal complexes with DNA as well as laying a foundation for the rational design of powerful agents for probing and targeting nucleic acids.

Supplementary material

Crystallographic data for the structural analysis of the polymeric copper(II) complex have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 842 175. Some information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1233-336-033; E-mail: deposit@ccdc.cam.ac.uk).

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