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# Synthesis, characterization and DNA interaction of copper (II) complexes with Schiff base ligands derived from 2-pyridinecarboxaldehyde and polyamines

Xin-Bin Yang [a,*], Yu Huang [b], Jin-Sheng Zhang [c], Shu-Kai Yuan [a], Ren-Quan Zeng [a]

[a] Department of Basic Science, Rongchang Campus, Southwest University, Chongqing 402460, PR China
[b] Pharmacy College, Ningxia Medical University, Yinchuan 750004, PR China
[c] School of Chemistry and Material Science, Guizhou Normal University, Guiyang 550001, PR China

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ABSTRACT

A series of copper (II) complexes **a**–**d** with Schiff bases ligands derived from the condensation reactions between 2-pyridinecarboxaldehyde and different polyamines (ethylenediamine, diethylenetriamine, triethylenetetramine and tetraethylenepentamine) were synthesized and characterized by elemental analysis, FT-IR spectroscopy, HRMS, molar conductance and molecular modeling studies. The interactions of the copper complexes **a**–**d** with DNA were investigated by the UV spectra, viscosity measurements and gel electrophoresis under physiological conditions. The experimental results indicated that four complexes could bind to DNA via an intercalative mode and showed a different DNA cleavage activity with the sequence: **d**>**c**>**a**>**b**.



Investigations on the interaction between transition metal complexes and DNA has attracted many interests due to their importance in cancer therapy and molecular biology [1–9]. Among them, Schiff base metal complex is a kind of attractive reagent due to their special activities in pharmacology and physiology [10–16]. During the last decade, transition metal complexes of Schiff base derived from 2-pyridinecarboxaldehyde and different amines have received considerable attention on the part of synthetic and biological activities of DNA binding and cleavage with high sequence and structure selectivity [17–20]. However, to the best of our knowledge, less attention was paid on the interaction of DNA and Schiff base metal complexes derived from 2-pyridinecarboxaldehyde and polyamines such as ethylenediamine, diethylenetriamine, triethylenetetramine and tetraethylenepentamine. In this communication we describe the synthesis, DNA binding and cleavage abilities of a series of copper (II) complexes with Schiff base ligands derived from 2-pyridinecarboxaldehyde and different polyamines (Scheme 1).

The copper (II) complexes **a**–**d** were prepared by a typical procedure. Polyamine (0.12 g, 2 mmol) was added to 2-pyridinecarboxaldehyde (0.43 g, 4 mmol) and refluxed in methanol for 4 h. Copper (II) chloride dehydrate (0.34 g, 2 mmol) dissolved in methanol was added to this solution and refluxing was continued for another 1 h. Then the mixture was concentrated under reduced pressure. The resulting green solid was filtered off, washed with diethyl ether and dried in vacuo. We got the likely composition of complexes: $[CuL_1]Cl_2{\cdot}2H_2O$ (**a**), $[CuL_2]Cl_2{\cdot}2H_2O$ (**b**), $[CuL_3]Cl_2{\cdot}H_2O$ (**c**) and $[CuL_4]Cl_2{\cdot}H_2O$ (**d**) [21–24] through elemental analyses, HRMS, IR spectra and molar conductivity measurements. Since no single crystals suitable for X-ray determination could be isolated, structural information for these complexes were obtained from the B3LYP/6-311G** optimization calculations as shown in Fig. 1. The lengths of coordination bonds are labeled in the figure.

The mode of the four complexes bound to DNA was investigated by absorption spectra. Hyperchromic effect and hypochromic effect are the spectra features of DNA concerning its double-helix structure. This spectra change process reflects the changes of DNA in its conformation and structures after the complex binds to DNA [25,26]. Hypochromism results from the contraction of DNA in the helix as well as from the conformation on DNA, while hyperchromism results from the damage of the DNA double-helix structure. The absorption spectra of DNA in the absence and the presence of copper (II) complexes **a**–**d** were given in Fig. 2 respectively. The addition of copper (II) complexes to DNA caused appreciable increase in the absorption intensity, which was a typical hyperchromic effect. These results suggested that the binding of copper (II) complexes with DNA was an intercalative mode.

Spectroscopic data are necessary, but not sufficient to support a binding mode. Hydrodynamic methods such as viscosity measurements which are sensitive to length increase of DNA are regarded as the most effective means of studying the binding mode of complexes to DNA in the absence of crystallographic structural data and NMR [27]. For further clarification of the binding mode, viscosity measurements were carried out. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to an increase in separation of base pairs at intercalation site and hence an increase in overall DNA length. However, a partial and/or nonclassical intercalation of complex may bend (or kink) the DNA helix, resulting in the decrease of its effective length and, concomitantly, its viscosity [28,29]. The effect of the copper (II)

* Corresponding author.
*E-mail address:* yangxbqq@126.com (X.-B. Yang).

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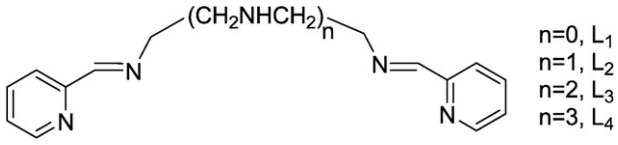


**Scheme 1.** Schiff base ligands derived from 2-pyridinecarboxaldehyde and different polyamines.

complexes on the viscosity of CT-DNA solution is given in Fig. 3. The plots show that the relative viscosity of DNA increased with the addition of the copper (II) complexes, which are ascribed to classical intercalative binding mode. From the relative viscosity values, it is clear that complex **d** shows much higher binding affinity than the complex **a, b** and **c**.

Besides the above methods, interactions between the copper (II) complexes and DNA were also investigated by the cleavage assay of plasmid DNA (pUC 19). The cleavage of the plasmid DNA was analyzed by monitoring the conversion of supercoiled circular DNA (Form I) to nicked DNA (Form II). The amounts of strand scission were assessed by agarose gel electrophoresis.

First we compared the cleavage abilities of the copper (II) complexes **a–d** at a concentration of 1.25 mM and an incubation time of 12 h. Fig. 4 showed the results. Lane 1 in the figure showed the control DNA without any additives. It is obvious that copper (II) complex **d** (lane 5) catalyzed the cleavage of plasmid DNA (pUC 19) much more efficiently than copper (II) complex **a** (lane 2), **b** (lane 3) and **c** (lane 4) under physiological conditions. Electrophoresis and densitometry indicated that the copper (II) complexes **a–d** resulted in 35%, 4%, 54% and 82% of nicked DNA respectively. Therefore, our subsequent efforts focus on the reactivity of the copper (II) complex **d**.

The cleavage of DNA by different concentrations of the copper (II) complex **d** was studied for 24 h. The intensity of the nicked DNA (Form II) band increased apparently with the increase of the complex concentration as shown in Fig. 5. Increasing the concentration of complex **d** in the order of 0.25, 0.75, 1.25 and 1.75 mM resulted in 24%, 55%, 76% and 97% nicked DNA, respectively.

In summary, a series of the copper (II) complexes **a–d** with Schiff base ligands derived from 2-pyridinecarboxaldehyde and polyamines were synthesized and characterized. The interactions of complexes **a–d** with DNA were studied by UV spectra, viscosity and gel electrophoresis under physiological conditions. The results indicate that the Schiff base copper (II) complexes **a–d** are capable of binding DNA by an intercalative mode and cleaving DNA without the use of any exogenous agents. Moreover, complex **d** shows the considerably high binding and cleavage abilities. The results revealed that the structure difference on the polyamine might lead to obvious

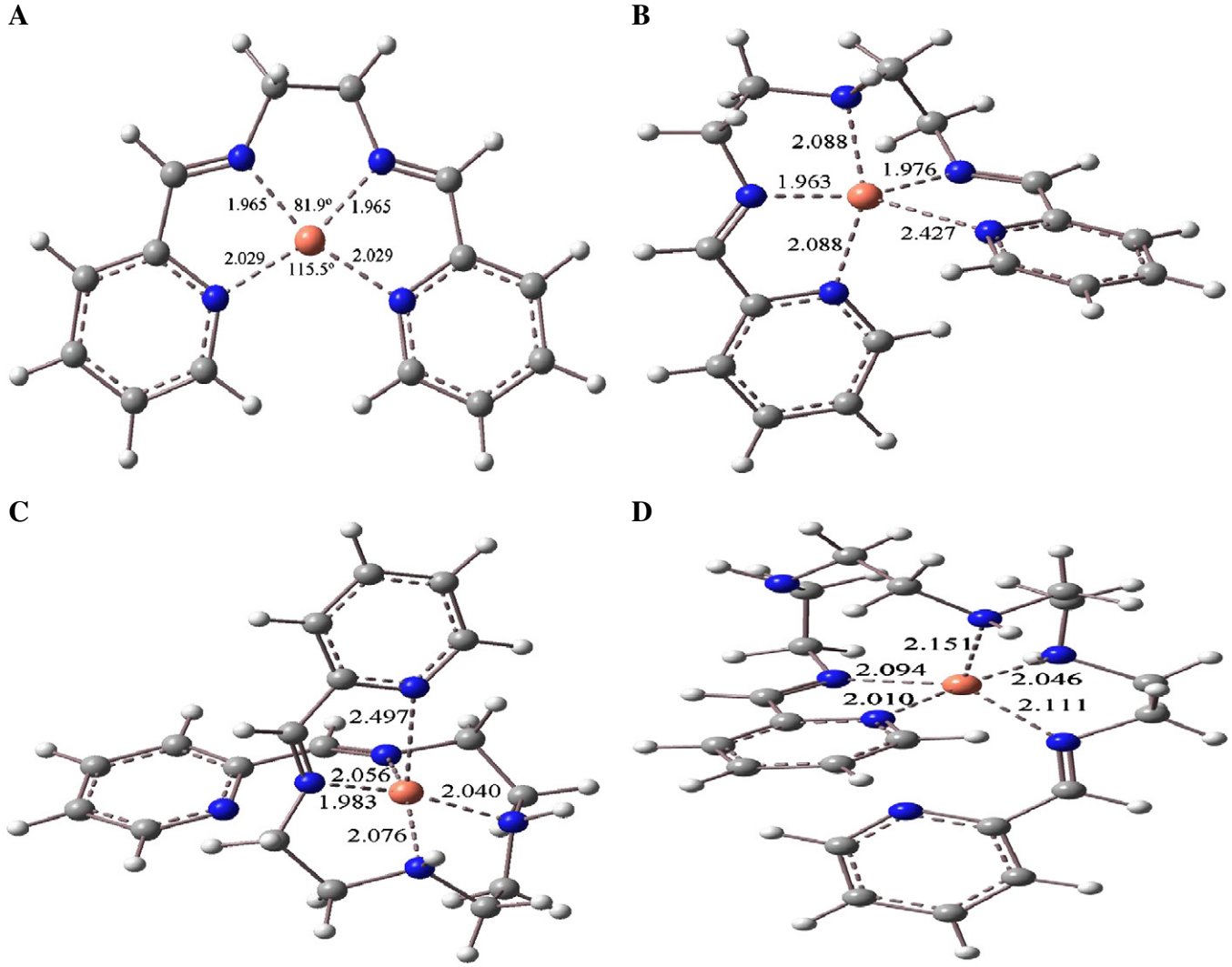


**Fig. 1.** The optimized structures of the complexes **a–d** with lengths of coordination bonds. The red ball stands for copper atom, the blue balls for nitrogens, the grey balls for carbons and the white balls for hydrogens.

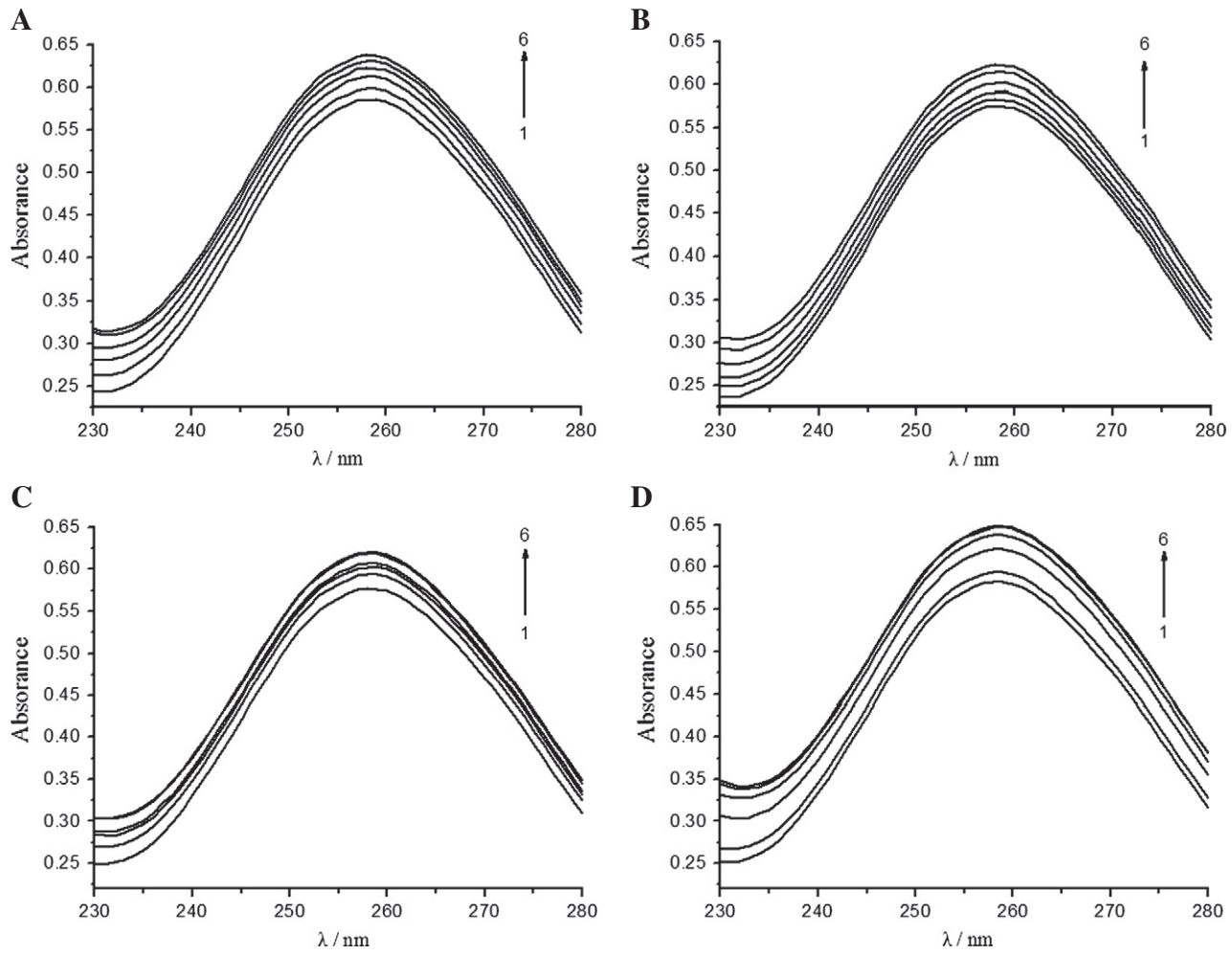


**Fig. 2.** Absorption spectra of CT-DNA in the absence and presence of copper complex **a** (i), **b** (ii), **c** (iii) and **d** (iv). [DNA] = $0.9\times10^{-4}$ mol/L, $[CuL]^{2+}$ = 0, 0.5, 1.0, 1.5, 2.0, and 2.5 μM respectively. The arrow shows the intensity changes on increasing the copper complex concentration.

difference of DNA binding and cleavage abilities of the complexes. The –NH– of polyamine might play a key role in the DNA recognition process. More in-depth studies about binding and cleavage mechanisms will be continued in our laboratory.

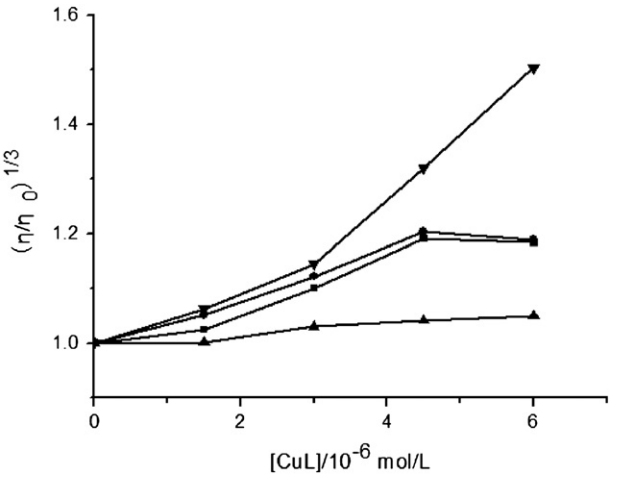


**Fig. 3.** Effects of increasing amount of copper complexes **a** (■), **b** (●), **c** (▲) and **d** (▼) on the viscosity of CT-DNA, [DNA] = $1.0\times10^{-4}$ mol/L.

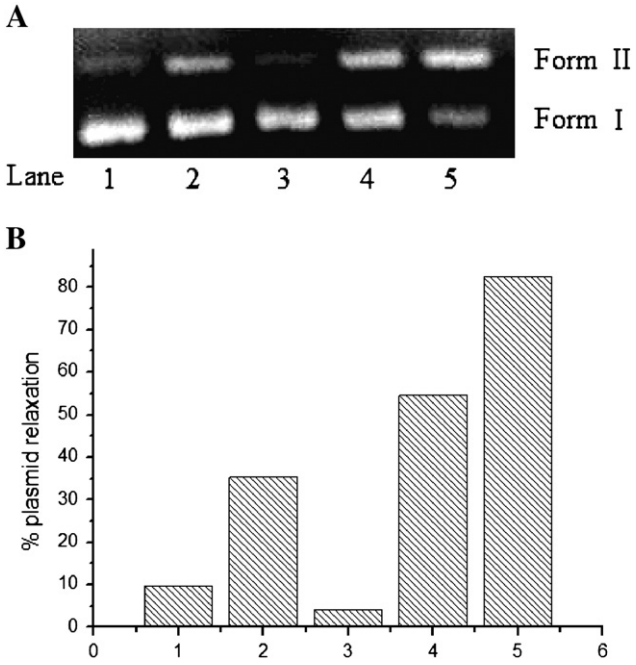


**Fig. 4.** Effect of different copper (II) complexes **a–d** (1.25 mM) on the cleavage reactions of pUC 19 DNA (12.5 μg/mL) in a Tris–HCl buffer (100 mM, pH 7.4) at 37 °C for 12 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control; lane 2, **a**; lane 3, **b**; lane 4, **c**; lane 5, **d**. (B) Quantitation of % plasmid relaxation (Form II%) relative to plasmid DNA per lane.

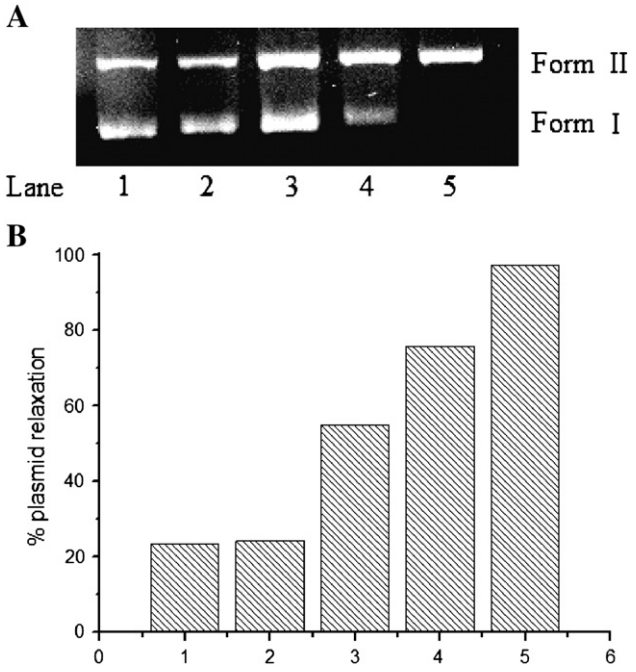


**Fig. 5.** Effect of concentration of complex **d** on the cleavage of pUC 19 DNA (12.5 μg/mL) in a Tris–HCl buffer (100 mM, pH 7.4) at 37 °C for 24 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control; lanes 2–5, [complex d] = 0.25, 0.75, 1.25 and 1.75 mM. (B) Quantitation of% plasmid relaxation (Form II%) relative to plasmid DNA per lane.

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[21] Preparation of complex **a**: ethylenediamine (0.12 g, 2 mmol) was added to 2-pyridinecarboxaldehyde (0.43 g, 4 mmol) and refluxed in methanol for 4 h. Copper (II) chloride dehydrate (0.34 g, 2 mmol) dissolved in methanol was added to this solution and refluxing was continued for another 1 h. Then the mixture was concentrated under reduced pressure. The resulting green solid was filtered off, washed with diethyl ether and dried in vacuo: Yield: 72%, IR (KBr, $cm^{-1}$): 3416; 2921, $\nu(CH_2)$; 1604, $\nu$(C N); 774, $\gamma$(C–H); 647, $\nu$(Cu–N). Anal. Calcd. for $[C_{14}H_{14}CuN_4]Cl_2\cdot 2H_2O$: C, 41.14; H, 4.44; N, 13.71%. Found: C, 41.64; H, 4.54; N, 13.77%. HRMS-ESI: m/z calcd for $C_{14}H_{13}CuN_4$ $[CuL_1 - H]^+$: 300.0514, found: 300.0544. Conductance: $CuCl_2$: 239 S $cm^2$ $mol^{-1}$, $[C_{14}H_{14}CuN_4]Cl_2\cdot 2H_2O$: 246 S $cm^2$ $mol^{-1}$.
[22] Preparation of complex **b**: this complex was prepared by a similar procedure to that of complex **a** by taking diethylenetriamine (0.21 g, 2 mmol), 2-pyridinecarboxaldehyde (0.43 g, 4 mmol) and copper (II) chloride dehydrate (0.34 g, 2 mmol). The resulting dark brown solid was obtained. Yield: 65%, IR (KBr, $cm^{-1}$): 3419; 2927, $\nu(CH_2)$; 1604, $\nu$(C N); 772, $\gamma$(C–H); 647, $\nu$(Cu–N). Anal. Calcd. For $[C_{16}H_{19}CuN_5]Cl_2\cdot 2H_2O$: C, 42.53; H, 5.13; N, 15.50%. Found: C, 42.21; H, 5.20; N, 15.74%. HRMS-ESI: m/z calcd for $C_{16}H_{19}ClCuN_5$ $[CuL_2 - H]^+$: 343.0936, found: 343.0974. Conductance: $CuCl_2$: 239 S $cm^2$ $mol^{-1}$, $[C_{16}H_{19}CuN_5]Cl_2\cdot 2H_2O$: 229 S $cm^2$ $mol^{-1}$.
[23] Preparation of complex **c**: this complex was prepared by a similar procedure to that of complex **a** by taking triethylenetetramine (0.30 g, 2 mmol), 2-pyridinecarboxaldehyde (0.43 g, 4 mmol) and copper (II) chloride dehydrate (0.34 g, 2 mmol). The resulting dark brown solid was obtained. Yield: 81%, IR (KBr), $cm^{-1}$: 3418; 2928, $\nu(CH_2)$; 1604, $\nu$(C N); 773, $\gamma$(C–H); 646, $\nu$(Cu–N). Anal. Calcd. For $[C_{18}H_{24}CuN_6]Cl_2\cdot H_2O$: C, 45.33; H, 5.50; N, 17.62%. Found: C, 45.19; H, 5.56; N, 16.68. HRMS-ESI: m/z calcd for $C_{18}H_{23}CuN_6$ $[CuL_3–H]^+$: 386.1358, found: 386.1362. Conductance: $CuCl_2$: 239 S $cm^2$ $mol^{-1}$, $[C_{18}H_{24}CuN_6]Cl_2\cdot H_2O$: 220 S $cm^2$ $mol^{-1}$.
[24] Preparation of complex **d**: This complex was prepared by a similar procedure to that of complex **a** by taking tetraethylenepentamine (0.38 g, 2 mmol), 2-pyridinecarboxaldehyde (0.43 g, 4 mmol) and copper (II) chloride dehydrate (0.34 g, 2 mmol). The resulting dark brown solid was obtained. Yield: 78%, IR (KBr, $cm^{-1}$): 3424; 2926, $\nu(CH_2)$; 1632, $\nu$(C N); 775, $\gamma$(C–H); 616, $\nu$(Cu–N). Anal. Calcd. For $[C_{20}H_{29}CuN_7]Cl_2\cdot H_2O$: C, 46.20; H, 6.01; N, 18.86%. Found: C, 47.06; H, 6.27; N, 18.74. HRMS-ESI: m/z calcd for $C_{20}H_{28}CuN_7$ $[CuL_4–H]^+$: 429.1780, found: 429.1851. Conductance: $CuCl_2$: 239 S $cm^2$ $mol^{-1}$, $[C_{20}H_{29}CuN_7]Cl_2\cdot H_2O$: 210 S $cm^2$ $mol^{-1}$.
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