New polyaza macrobicyclic binucleating ligands and their binuclear copper(II) complexes: Electrochemical, catalytic and DNA cleavage studies

ARTICLE *in* POLYHEDRON · JANUARY 2009

Impact Factor: 2.01 · DOI: 10.1016/j.poly.2008.09.022

Polyhedron 28 (2009) 49–56

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

New polyaza macrobicyclic binucleating ligands and their binuclear copper(II) complexes: Electrochemical, catalytic and DNA cleavage studies

S. Anbu [a], M. Kandaswamy [a,*], Pon. Sathya Moorthy [b], M. Balasubramanian [b], M.N. Ponnuswamy [b]

[a] Department of Inorganic Chemistry, School of Chemical Sciences, University of Madras, Guindy Campus, Chennai 600 025, India
[b] Centre of Advanced Study in Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai 600 025, India

ARTICLE INFO

Article history:
Received 28 July 2008
Accepted 23 September 2008
Available online 6 November 2008

Keywords:
Tetraldehyde
Macrobicyclic ligands
Binuclear Cu(II) complexes
DNA cleavage

ABSTRACT

A new symmetrical polyaza macrocyclic binucleating ligand $\mathbf{L^1}$ and its permethylated derivative ligand $\mathbf{L^2}$ have been synthesized by using the precursor compound 2-[2-(2,6-diformyl-4-methylphenoxy)propoxy]-5-methylisophthalaldehyde and bis(aminoethyl) amine. The molecular structure of the precursor compound and the ligand $\mathbf{L^2}$ were determined by the single crystal XRD method. The ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ were treated with two equivalents of $Cu(ClO_4)_2 \cdot 6H_2O$ to afford the new binuclear complexes $[Cu_2L^1(\mu\text{-}OH)(ClO_4)_2](ClO_4)$ (**1**) and $[Cu_2L^2(\mu\text{-}OH)(ClO_4)_2](ClO_4)$ (**2**), respectively. Both the complexes were characterized by elemental and spectral methods. A cyclic voltammetric investigation of these Cu(II) complexes show a quasireversible followed by an irreversible one electron reduction processes around $E^1_{pc} = -0.59$ V, -0.55 V and $E^2_{pc} = -1.07$ V, -1.02 V. ESR spectra of the copper(II) complexes **1** and **2** show a broad signal at $g = 2.08$ and 2.10, and μ_{eff} values of 1.43 and 1.46 BM, respectively, which convey a spin–spin interaction between the two copper(II) ions. The initial rate (V_{in}) for the oxidation of 3,5-di-*tert*-butylcatechol to *o*-quinone by the binuclear Cu(II) complexes **1** and **2** are 1.15×10^{-4} and 1.66×10^{-4} Ms^{-1} respectively. Both the complexes remarkably promote the hydrolytic cleavage of supercoiled plasmid DNA under physiological conditions in the presence of H_2O_2 at pH 7.2 and 37 °C. The reaction profile for the complex **1** mediated reaction displayed approximately pseudo-first-order kinetic behavior, with $k_{obs} \sim 0.07$ min^{-1} and $R^2 = 0.984$.

1. Introduction

Polyaza macrocyclic Schiff bases have been studied as potential inorganic and organic cation receptors [1], electron transfer agents, homogeneous catalysts and DNA, RNA interacting agents [2]. Because of their unique and selective complexing properties [3], macrocyclic ligands are particularly suited for the synthesis of such complexes as they are more stable than their acyclic counterparts and the two metal ions are fixed in close proximity which has important implications for metal–metal interactions and binuclear metal reactivity [4]. They are smaller than enzymes, which allows them to attack macromolecular regions that are inaccessible to enzymes, and also their specificity and efficiency may be modulated. These properties have turned chemical nucleases into useful tools as adjuvants in PCR diagnostics [5], nucleic acid attacking and cleaving agents [6]. These polyaza copper(II) complexes are a beacon in modelling, and they serve as bioinorganic model compounds not only in enzymatic reactions, but also in catalytic synthetic oxidation reactions [7,9]. Hence, binuclear copper(II) complexes are of interest for availing magnetostructural correlations and as useful model systems for catalysis. Catechol oxidases are ubiquitous plant enzymes belonging to the oxidoreductase class that contain a dinuclear Cu center in their site, which catalyses the two-electron oxidation of a broad range of *o*-diphenols (catechol) to the corresponding quinones, coupled with the reduction of molecular oxygen to water [7]. Further, catechol oxidases exclusively catalyze the oxidation of catechols to quinones without tyrosine [10]. This reaction is of great importance in medical diagnosis for the determination of the hormonally active catecholamine adrenaline, noradrenalin and dopa [11]. The synthesis and investigation of functional model complexes for metalloenzymes with oxidize or oxygenase activity is therefore of great promise for the development of new and efficient catalysts for oxidation reactions [12]. Further, compounds showing the property of cleaving double stranded DNA under physiological conditions are of importance since these could be used as diagnostic agents in medicinal and genomic research [13]. Several efficient cleaving agents have been developed over the course of time, however, most of the studies deal with macrocyclic mononuclear Cu(II) complexes [14–16]. It has been noticed that macrocyclic binuclear copper(II) complexes show greater DNA cleaving efficiency than mononuclear complexes [17,18]. For these reasons, we aimed to design new polyaza macrocyclic binuclear Cu(II) complexes, which are suitable to

* Corresponding author. Tel./fax: +91 44 22300488.
E-mail address: mkands@yahoo.com (M. Kandaswamy).

0277-5387/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.poly.2008.09.022

cleave DNA significantly. As a consequence, the design of the binucleating ligand has to satisfy a number of conditions: metal–metal distance, steric, electronic and bridging ligand features, etc. A new aromatic tetraldehyde is used to function as a precursor in the preparation of polydentate ligands which can form binuclear complexes. The above features can be altered by condensing the precursor compound with various amines. The tetraldehyde is readily condensed with aliphatic α,ω-diamines and subsequent reduction of the intermediate imine compounds provides many macrocyclic ligands [19]. In this paper, we report the synthesis and characterization of new symmetrical macrobicyclic ligands and their dicopper(II) complexes. We describe here two macrocyclic binucleating ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ (Scheme 1) by employing the new precursor compound, 2-[2-(2,6-diformyl-4-methylphenoxy)propoxy]-5-methylisophthalaldehyde and α,ω-diamines.

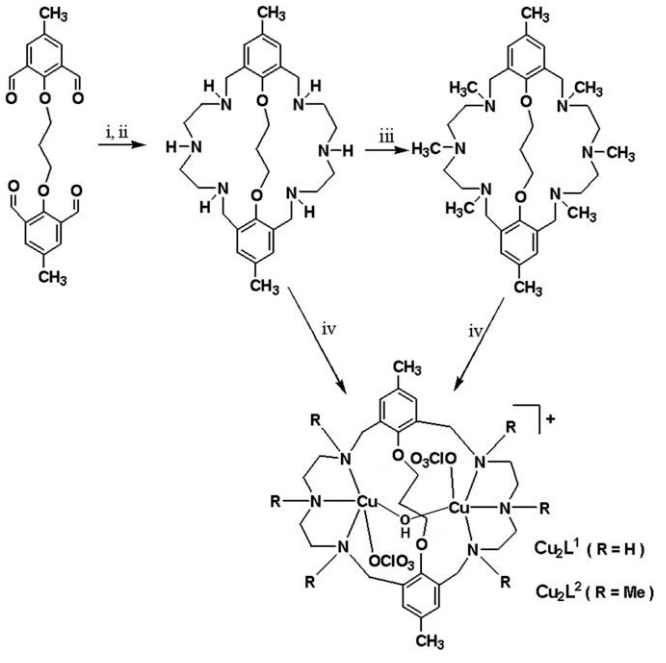
2. Experimental

2.1. Materials

2,6-Diformyl-4-methylphenol [20] and the binucleating macrobicyclic ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ were prepared by a modified procedure of the literature methods [21]. Tetra(*n*-butyl)ammonium perchlorate (TBAP), used as the supporting electrolyte in electrochemical measurements, was purchased from Fluka and recrystallized from hot methanol (Caution! All perchlorate salts are potentially explosive; hence, care should be taken in handling TBAP and complexes containing the perchlorate anion). All the solvents were purified by reported procedures [22]. The supercoiled pBR322 DNA was purchased from Bangalore Genei (India). Superoxide dismutase (SOD), ethidium bromide (EB) and L-histidine were obtained from Sigma (USA). Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared by using deionized, sonicated triple distilled water.

2.2. General methods

Elemental analysis was carried out on a Carlo Erba model 1106 elemental analyzer. FT-IR spectra were obtained on a Perkin Elmer FTIR spectrometer with the samples prepared as KBr pellets. UV–Visible spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer operating in the range 200–1400 nm with quartz cells and ε values are given in $M^{-1}\,cm^{-1}$. NMR spectra were recorded in $CDCl_3$, using TMS as an internal standard, on a BRUKER 300 instrument. EI mass spectra were recorded on a JEOL DX-303 EI mass spectrometer. Electrospray ionization mass spectral measurements were done using a Thermo Finnigan LCQ-6000 Advantage Max-ESI mass spectrometer. Cyclic voltammograms were obtained on a CH11008 Electrochemical analyzer using a three-electrode set-up comprising of a glassy carbon working, platinum wire auxiliary and a saturated Ag/AgCl reference electrode under oxygen free conditions. The concentration of the complexes was 10^{-3} M. TBAP (Tetra(butyl)ammonium perchlorate) (10^{-1} M) was used as the supporting electrolyte. The FAB mass spectra of the complexes were recorded on a JOEL SX 102/DA 6000 mass spectrometer using 3-nitrobenzyl alcohol as the matrix solvent. EPR spectra were recorded on powdered samples of **1** and **2** using a JEOL TES 100 ESR spectrometer. Low temperature measurements were made using a liquid nitrogen Dewar.



Scheme 1. (i) $NH(CH_2CH_2NH_2)_2$, CH_2Cl_2–EtOH(high dilution); (ii) $NaBH_4$; (iii) HCHO, HCOOH; (iv) $Cu(ClO_4)_2 \cdot 6H_2O$.

2.3. Synthesis

2.3.1. Synthesis of the precursor compound (2-[2-(2,6-diformyl-4-methylphenoxy)propoxy]-5-methylisophthalaldehyde)

A mixture of 2,6-diformyl-4-methylphenol (3 g, 18.2 mmol), 1,3-dibromopropane (1.73 g, 9.1 mmol) and K_2CO_3 (1.26 g, 9.1 mmol) was stirred in dry DMF at 90 °C for 2 h. The resulting solution was cooled to room temperature and poured onto crushed ice. A white solid separated by filtration and was dried. Colorless crystals suitable for XRD analysis were obtained by slow evaporation of a dichloromethane solution of this solid. Yield: 5.5 g (81%). Mp 210 °C. Elemental *Anal.* Calc. for $C_{21}H_{20}O_6$ (L^1) C, 68.5; H, 5.4. Found: C, 68.4; H, 5.5%. EI Mass: $[M^+ + 1] = 369$. ^1H NMR ($CDCl_3$, 300 MHz): 2.36 ($2CH_3$, s, 6H), 2.43 (CH_2, quintet, 2H), 4.28 ($2CH_2$, t, 4H, J = 6.3 Hz), 7. 84 (Ar H, s, 4H), 10.28 (CHO, s, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): 20.5 (CH_3), 29.6 (CH_2), 75.9 (CH_2), 129.8, 136.0 (ArH), 188.5 (CHO). IR (cm^{-1}, KBr pellet): 2925 (CH stretching), 1669 (CO stretching), 1435 (O–CH_2).

2.3.2. Synthesis of the macrobicyclic binucleating ligand ($\mathbf{L^1}$)

The precursor compound (3.68 g, 10 mmol) in chloroform (100 ml) and diethylenetriamine (2.06 g, 20 mmol) in ethanol were added simultaneously over a period of 8 h to a mixture of chloroform (300 ml) and ethanol (100 ml). After complete addition, the reaction mixture was stirred at room temperature for 24 h. The chloroform was then removed at reduced pressure and a solution of sodium borohydride (3.03 g, 80 mmol) in ethanol (100 ml) was added. After stirring at room temperature for 2 h, the reaction mixture was acidified to pH 1 with conc. hydrochloric acid and the resulting colorless suspension was evaporated to dryness. Water (100 ml) and chloroform (200 ml) were added to the residue, the pH of the aqueous phase was adjusted to 13 with 3 M aqueous potassium hydroxide, and the heterogeneous mixture was stirred vigorously for 1 h. The aqueous phase was extracted with chloroform and dried over sodium sulfate. Evaporation of the solvent gave $\mathbf{L^1}$ as a colorless foamy solid. Yield: 4.3 g (84%). M.p. 173 °C. Elemental *Anal.* Calc. for $C_{29}H_{46}N_6O_2$ ($\mathbf{L^1}$) C, 68.2; H, 9.0; N, 16.5. Found: C, 68.1; H, 9.2; N, 16.7%. ESI-MS in CH_3OH: *m*/*z* 511.47 $[M+H]^+$. ^1H NMR ($CDCl_3$, 75 MHz): 2.09 (CH_3, s, 6H), 2.29 (–CH_2–, t, 2H, J = 6.6 Hz), 2.69 (–CH_2–, s, 16H), 3.67 (–CH_2–, s, 8H), 4.18 (–CH_2–, t, 4H, J = 6.6 Hz), 4.54 (NH, s, 2H), 4.72 (NH, br s, 4H), 6.90 (ArH, s, 4H). ^{13}C NMR $CDCl_3$, 75 MHz): 19.6 ($2CH_3$), 30.6 (CH_2), 48.1 ($4CH_2$), 48.5 ($4CH_2$), 71.5 ($2CH_2$), 129.7, 132.0, 132.5, 153.4 (ArC). IR (cm^{-1}, KBr pellet): 3235 (NH stretching), 2916 (CH stretching), 1517 (NH bending), 1215 (O–C stretching).

2.3.3. Synthesis of the macrobicyclic binucleating ligand (L^2)

The binucleating macrobicyclic ligand L^1 (5.1 g, 10.0 mmol) was dissolved in formic acid (20 mL). To the above warm solution, aqueous formaldehyde (37%, 20 mL) was added. The reaction mixture was heated under reflux for 24 h, then concentrated in vacuo to about 10 mL. Water (50 mL) and dichloromethane (100 mL) were added to the sticky residue, the pH of the aqueous phase was adjusted to 13 by the addition of 3 M aqueous potassium hydroxide solution and the heterogeneous mixture was stirred vigorously for 30 min. The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 100 mL). The combined organic fractions were dried over K_2CO_3 and filtered. Methanol (100 mL) was added to the clear solution. On evaporation of the dichloromethane, the permethylated macrocycle **L^2** was obtained as colorless crystals. Yield: 5.2 g (87%). M.p. 120 °C. Elemental *Anal.* Calc. for $C_{35}H_{58}N_6O_2$ (**L^2**) C, 70.7; H, 9.8; N, 14.1. Found: C, 70.8; H, 9.5; N, 14.3%. ESI-MS in CH_3OH: *m/z* 595.53 $[M+H]^+$. ^1H NMR ($CDCl_3$, 300 MHz): 2.24 (N–CH_3, s, 18H), 2.28 (Ar–CH_3, s, 6H), 2.76 (–CH_2–, quintet, 2H), 2.66 (8CH_2, s, 16H), 3.52 (–CH_2–, s, 8H), 4.32 (–CH_2–, t, 4H, J = 6.6 Hz), 6.99 (ArH, s, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): 20.7 (2CH_3), 31.7 (1CH_2), 42.0 (4N–CH_3), 54.8 (CH_2), 55.2 (8CH_2), 57.5 (8CH_2), 72.18 (2CH_2), 131.2, 132.2, 132.4, 155.11 (Ar-C). IR (cm^{-1}, KBr pellet): 2946 (CH stretching), 1224 (O–C stretching).

*2.3.4. Synthesis of the binuclear Cu(II) complex (**1**) [Cu_2L^1 (μ-OH)(ClO_4)$_2$] (ClO_4)*

The binuclear Cu(II) complex **1** was synthesized by refluxing a methanolic solution of the ligand **L^1** (1 g, 1.96 mmol) and a methanolic solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (1.45 g, 3.92 mmol). A blue colored solid was separated on evaporating the solution at room temperature and the resulting compound was washed with cold methanol and dried. Yield: (77%). Elemental *Anal.* Calc. for $C_{29}H_{47}Cl_3Cu_2N_6O_{15}$ (**1**) C, 36.5; H, 4.9; N, 8.8. Found: C, 36.2; H, 4.6; N, 8.3%. FAB-MS in NBA: *m/z* 837 $[L^1 + 2Cu + 2ClO_4 + H]^+$; 738 $[L^1 + 2Cu + ClO_4 + H]^+$. Λ_M, S $m^2\,M^{-1}$ in CH_3CN at 25 °C: 81. IR (cm^{-1}, KBr pellet): 3431, 3221w ν(N–H), 2926w, 2879 ν(C–H), 1635m, 1458w, 1209s (C–O), 1105vs, 1085vs $\nu(ClO_4)$, 624m, 420s. UV–Visible [λ/nm ($\epsilon/M^{-1}\,cm^{-1}$)] in CH_3CN: 633 (380), 272 (84,000). g = 2.081. μ_{eff}, μ_B at 298 K: 1.43.

*2.3.5. Synthesis of the binuclear Cu(II) complex (**2**) [Cu_2L^2 (μ-OH)(ClO_4)$_2$] (ClO_4)*

The complex **2** was prepared by following the above method using ligand **L^2** (1 g, 1.68 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.24 g, 3.36 mmol), which resulted in a bluish green colored solid. Yield: (56%). Elemental *Anal.* Calc. for $C_{35}H_{59}Cl_3Cu_2N_6O_{15}$ (**2**) C, 40.5; H, 5.7; N, 8.1. Found: C, 40.9; H, 5.4; N, 8.4%. FAB-MS in NBA: *m/z* 921 $[L^2 + 2Cu + 2ClO_4 + H]^+$; 822 $[L^2 + 2Cu + ClO_4 + H]^+$. Λ_M, S $m^2\,M^{-1}$ in CH_3CN at 25 °C: 85. IR (cm^{-1}, KBr pellet) 3462br, 2931m, 2860m ν(C–H), 1685, 1637, 1463, 1212s ν(C–O), 1105vs, 1088vs $\nu(ClO_4)$, 627m, 420s. UV–Visible [λ/nm ($\epsilon/M^{-1}\,cm^{-1}$)] in CH_3CN: 657 (260), 283 (3,90,000). g = 2.102. μ_{eff}, μ_B at 298 K: 1.46.

2.4. X-ray data collection and reduction

Crystals of the precursor compound and ligand **L^2** were colorless and could be sorted using a polarizing microscope (Leica DMLSP). Crystals were cut to a suitable size (less than the collimator cross section diameter) and mounted on a Kappa Apex 2 CCD diffractometer equipped with graphite monochromatic Mo(Kα) radiation (λ = 0.71073 Å). The unit cell parameters were obtained using 36ω-frames of 0.5° width and scanned from three different zones of the reciprocal lattice. The intensity data were collected using ω and φ scans with a frame width of 0.5°. The frame integration and data reduction were performed using Bruker SAINT-PLUS (Version 7.06a) software [23]. The multi-scan absorption corrections were applied to the data using the SADABS [24] program. Both samples were stable at room temperature. The structures were solved using SIR92 [25]. Full matrix least squares refinement was performed using SHELXL-97 [26] programs. All the non-hydrogen atoms were refined with anisotropic displacement parameters. All the hydrogen atoms could be located in the Difference Fourier map. However, they were relocated at chemically meaningful positions and were given riding model refinement with following restraints: tertiary CH_3 groups (C–H = 0.96 Å, U_{iso} = 1.5U_{eq} of parent carbon), secondary CH_2 group (C–H = 0.97 Å, U_{iso} = 1.2U_{eq} of the parent carbon), aromatic C–H group (C–H = 0.93 Å U_{iso} = 1.2U_{eq} of the parent carbon). Selected crystallographic data of the precursor compound and ligand **L^2** are depicted in Table 1.

2.5. Kinetic studies of oxidation of catechol (catecholase activity)

The catalytic oxidation of 4,5-ditertiarybutyl catechol (DTBC) to the corresponding *o*-quinone (DTBQ-ditertiary butyl quinone) was studied using 10^{-3} M of the copper(II) complexes as a biomimetic catalyst in acetonitrile solution. The reaction was followed spectrophotometrically by choosing the strongest absorbance of the product, DTBQ, around 395 nm and monitoring the increase in the absorbance at this wavelength over time intervals of 10 min. Initial rates (d*c*/d*t*) (<2% conversion of DTBC into DTBQ) were determined by a spectrophotometric method. The initial reaction rate values were obtained from the slope of the trace at 395 nm during the first 30 min of the reactions, when the absorption at 395 nm increases linearly.

2.6. DNA cleavage experiments

The cleavage of supercoiled pBR322DNA by the complexes was studied by agarose gel electrophoresis. The gel electrophoresis experiments were performed as follows: pBR322DNA (0.2 μg, 33 μM) was treated with the copper(II) complexes **1** and **2** in 50 mM Tris–HCl/NaCl buffer (pH 7.2) and H_2O_2 followed by dilution with Tris–HCl buffer to a total volume of 18 μL. All the samples were incubated for 30 min at 37 °C followed by their addition to

Table 1
Selected crystallographic data of the precursor compound and the ligand **L^2**.

Parameters	Precursor compound	L^2
Empirical formula	$C_{21}H_{20}O_6$	$C_{35}H_{58}N_6O_2$
Formula weight	368.37	594.87
T (K)	293(2)	293(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	monoclinic	triclinic
Space group	*C*2	$P\bar{1}$
a (Å)	15.8317(11)	9.2656(3)
b (Å)	4.1407(3)	13.1391(4)
c (Å)	14.3460(11)	14.9542(5)
α (°)	90	81.354(2)
β (°)	103.443(5)	84.839(2)
γ (°)	90	76.139(2)
V (A^3)	914.73(12)	1744.69(10)
Z	2	*2*
D_{calc} (Mg/m^3)	1.337	1.132
Absorption coefficient (mm^{-1})	0.098	0.071
Crystal size (mm)	0.26 × 0.16 × 0.16	0.16 × 0.12 × 0.12
θ Range for (data collection (°)	3.37–25.00	2.65–19.34
Maximum and minimum transmission	0.9749–0.9845	0.988–0.9915
Data/restraints/parameters	2636/1/137	2972/0/390
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0498 (2170)	R_1 = 0.0688 (2392)
R indices (all data)	wR_2 = 0.1525 (2636)	wR_2 = 0.2127 (2972)

the loading buffer containing 25% bromophenol blue, 0.25% Xylene cyanol, 30% glycerol (3 μL). The solution was finally loaded on 0.8% agarose gel containing ethidium bromide (1 μg/mL^{-1}). Electrophoresis was done for 1 h at 50 V in TBE (45 mM Tris, 45 mM H_3BO_3, 1 mM-EDTA, pH 8.3) buffer. Bands were visualized by UV light and photographed. Quantification of closed circular and nicked DNA was made by densitometric analysis of ethidium bromide containing agarose gels. Quantification was performed by fluorescence imaging by the use of a Gel-Doc 1000 (BioRad) and data analysis with MULTIANALYSIS software (version 1.1) provided by the manufacturer, using the volume quantification method. In all cases, background fluorescence was subtracted by reference to a lane containing no DNA. A correction factor of 1.47 was used for supercoiled DNA since the ability of ethidium bromide to intercalate into supercoiled DNA (form I) decreased relative to the nicked circular (form II). The fraction of each form of DNA was calculated by dividing the intensity of each band by the total intensities of all the bands in the lane. All results were obtained from experiments that were performed at least in triplicate.

3. Result and discussion

The precursor compound and the ligand **L^1** were prepared by using a modified literature procedure of Clark et al. [27]. We synthesized two macrocyclic binucleating ligands by employing the new precursor compound, 2-[2-(2,6-diformyl-4-methylphenoxy)propoxy]-5-methylisophthalaldehyde and α,ω-diamines. The macrocycle **L^1** was obtained by a (2+1) condensation between the precursor compound and bis(aminoethyl)amine in ethanol-dichloromethane, followed by reduction with $NaBH_4$ (Scheme 1). Reductive methylation of **L^1** with formaldehyde and formic acid under Eschweiler–Clarke conditions gave the permethylated derivative **L^2**. The structure of **L^2** was derived from that of ligand **L^1** by replacing the six NH hydrogen atoms by methyl groups. Synthetic pathways for the complexes are shown in Scheme 1. The molar conductivity measurements of the copper(II) complexes **1** and **2** in CH_3CN resulted in Λ_M values of 81 and 85 S m^2 M^{-1} respectively, which indicate that the complexes are 1:1 electrolytes [28]. The FAB mass spectrum of complex 1, $[Cu_2L^1(\mu\text{-}OH)(ClO_4)_2]$ (ClO_4), showed a peak at $m/z = 837$, corresponding to the $[L^1 + 2Cu + 2\text{-}ClO_4 + H]^+$ ion, and the complex **2**, $[Cu_2L^2(\mu\text{-}OH)(ClO_4)_2]$ (ClO_4), showed a peak at $m/z = 920$, corresponding to the $[L^2 + 2Cu + 2\text{-}ClO_4 + H]^+$ ion. The FAB mass spectral data of the copper(II) complexes confirm the proposed formula of the complexes.

3.1. Crystal structures

The precursor compound crystallizes in the monoclinic system with the space group C2 and half of the molecule in the asymmetric unit. The molecule has twofold symmetry with a two fold axis passing through at C11. The tetraldehyde molecule and its twofold equivalents are linked through C10–H10B···O2 hydrogen bonds (2.58 Å, 143.25°, Symm: 1.5 − x, ½ + y, −z). The hydrogen bonding extends through a-translation to form a one dimensional chain. The packing is further stabilized through Van der Waals interactions. The ORTEP diagram of the precursor compound is given in Fig. 1. The permethylated macrocyclic ligand **L^2** crystallizes in the triclinic space group $P\bar{1}$ with one molecule in the asymmetric unit. The molecules are stabilized by Van der Waals interactions, weak intramolecular C–H···O, C–H···N bonds and intermolecular C–H (π) interactions. Fig. 2 represents the ORTEP diagram of the macrocyclic ligand **L^2**.

3.2. Spectral studies

The IR spectra of the two complexes showed a strong band in the region around 1000–1100 cm^{-1} and a sharp band in the region around 626 cm^{-1}, which could be due to the antisymmetric stretch and antisymmetric bend of perchlorate ions, respectively. The perchlorate peak around 1100 cm^{-1} splits in to two peaks around 1105 and 1085 cm^{-1}, indicating that the perchlorate ions present in the complexes are coordinated to the Cu(II) ions. Complex **1** has another prominent feature of a sharp but weak band at 3221 cm^{-1}, a frequency typical for the N–H stretching vibration of a coordinated secondary amine group. The disappearance of the band around 3220 cm^{-1} and the increasing intensity of the band at 2925 cm^{-1} for complex **2** confirms the N-methylation of the ligand **L^2**. The important strong band observed at around 3460 cm^{-1} for both of the macrocyclic Cu(II) complexes are assignable to OH vibrations. The electronic spectra of an acetonitrile solution of complexes **1** and **2** show three intense intraligand bands in the UV region. In the visible region the copper(II) complexes **1** and **2** exhibit absorption maxima at 633 and 657 nm (Fig. 3), respectively. This strongly suggests that the coordination geometry around the metal ion might be distorted square pyramidal [29]. An increase in λ_{max} (red shift) of complex **2** may be due to the weaker field strength of ligand **L^2** and larger distortion of the coordination geometry around the Cu(II) ion than for complex **1**. The EPR spectra of the solid com-

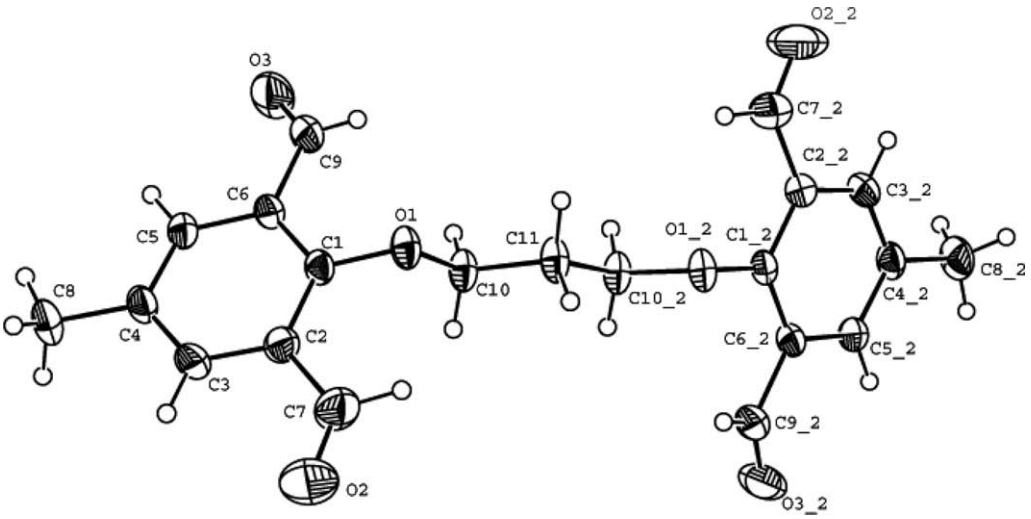


Fig. 1. ORTEP diagram of precursor compound **I**. Displacement ellipsoids are drawn at the 50% probability level.

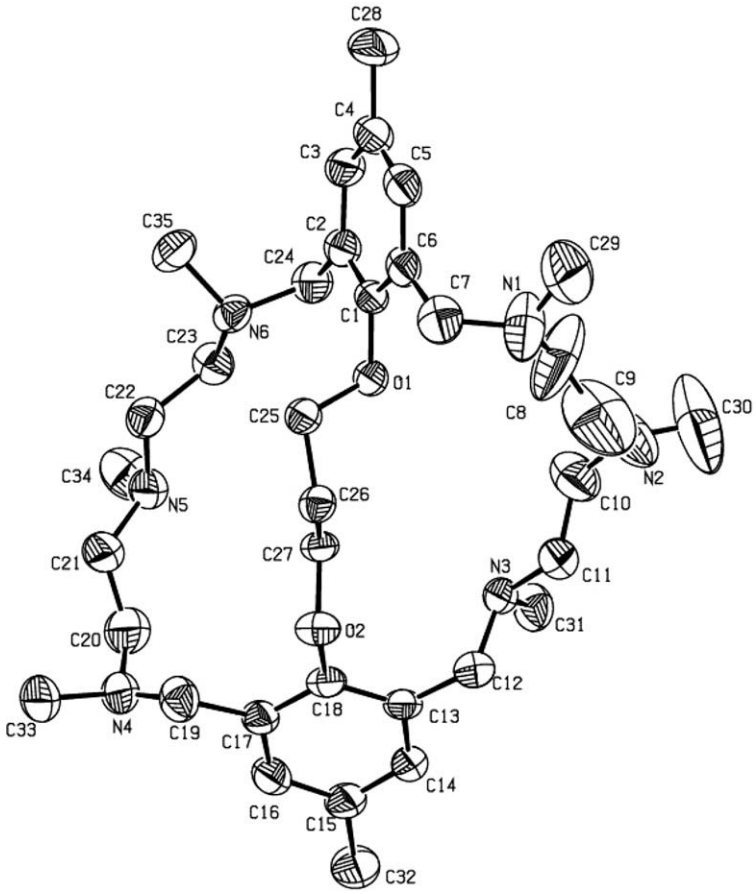


Fig. 2. ORTEP diagram of ligand L^2. Displacement ellipsoids are drawn at the 50% probability level.

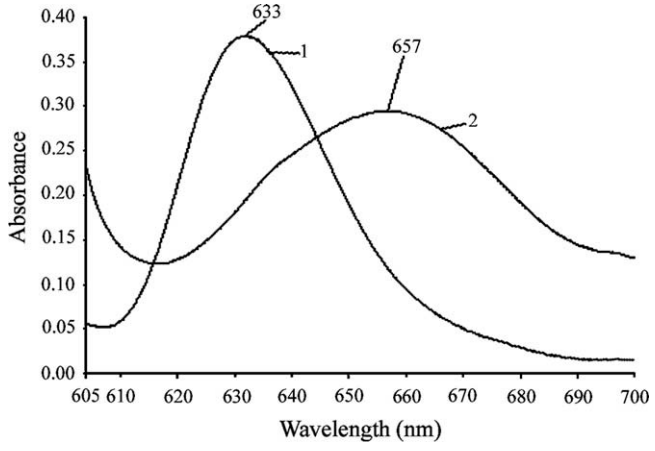


Fig. 3. Electronic spectra of complexes **1** and **2** in CH_3CN.

plexes **1** and **2** were recorded at liquid nitrogen temperature (LNT). Both the binuclear complexes showed a single broad-band resonance at ca. $g = 2.06–2.10$, with the half field signal at 1500G ($M = \pm 2$), which suggest magnetic interactions between the two copper ions through the hydroxyl bridge (Fig. 4). The probe room temperature magnetic studies of the binuclear copper(II) complexes **1** and **2** resulted in magnetic moment values of 1.43 and 1.46 BM, respectively, which suggest the presence of an antiferromagnetic interaction between the two Cu(II) ions.

3.3. Electrochemistry

The cyclic voltammograms of complexes **1** and **2** (Fig. 5) were recorded in CH_3CN solution. Both complexes display in the negative region of potentials a quasireversible redox wave followed by an irreversible cathodic peak at a lower potential. The first reduction potentials are observed at (E^1_{pc}) −0.59 V and −0.55 V for complexes **1** and **2**, respectively. Coulometric titrations by potentiostatic exhaustive electrolysis performed at 100 mV more negative to the first reduction wave consumed approximately one electron ($n \approx 0.96$). This indicates that each process corresponds to a single electron transfer process, and this can be assigned to the redox couple $Cu^{II}Cu^{II}/Cu^{II}Cu^{I}$. The second reduction potentials for complexes **1** and **2** are irreversible and are observed at $E^2_{pc} = -1.07$ and −1.02 V versus Ag/AgCl, respectively. These can be attributed to the transformation $Cu^{II}Cu^{I} \rightarrow Cu^{I}Cu^{I}$. The fact that this process is completely irreversible for both complexes suggests a fast decomposition of the $Cu^{I}Cu^{I}$ species, as evidenced by Neves et al. [7]. Complex **2** reduces at less negative potentials than the complex **1**. This suggests that the geometry around the copper ions with ligand $\mathbf{L^2}$ is more distorted than the copper ions with ligand $\mathbf{L^1}$. The ligands are structurally different in that the amine donor nitrogens of $\mathbf{L^2}$ contain bulky methyl groups, whereas the ligand $\mathbf{L^1}$ has hydrogen atoms. The deviation of the metal from its coordination plane should be larger for the $\mathbf{L^2}$ complex in order to destabilize the Cu(II) oxidation state. Thus, the electron density on the copper ions of complex **2** is less and the copper coordination geometry may also be more distorted due to the steric effect of the bulky methyl group substituents. It has been suggested [30,31] that reduction in electron density on the copper ions and distortion in

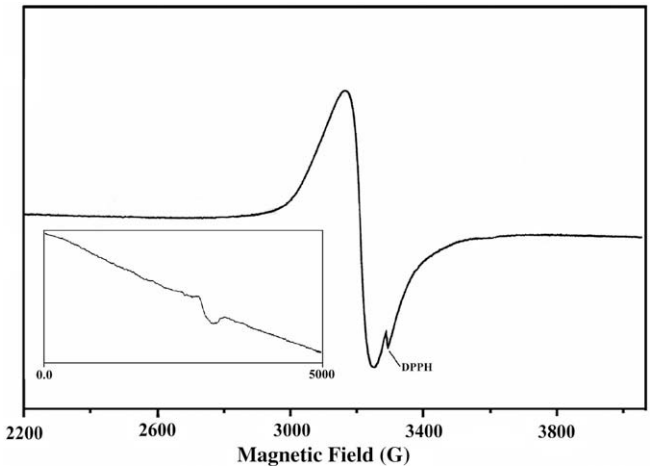


Fig. 4. X- band EPR spectrum of frozen solid (**1**) at 77 K. Inset gives the EPR showing the $\Delta M_s = \pm 2$ transition.

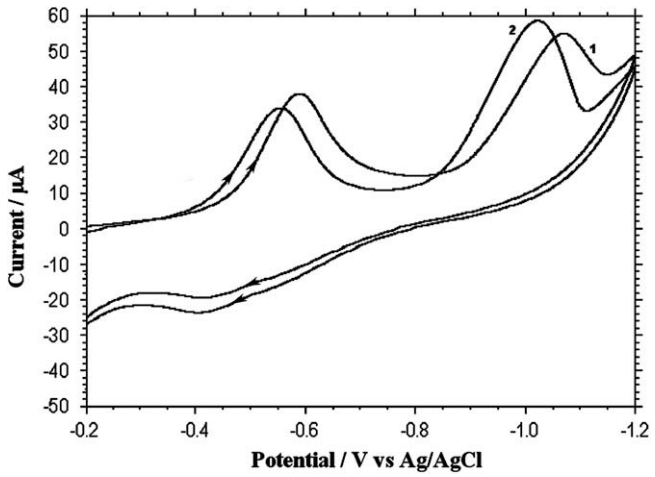


Fig. 5. Cyclic voltammograms of complexes **1** and **2** in CH_3CN.

geometry favors the reduction process ($Cu^{II} \rightarrow Cu^{I}$) at less negative potentials, as observed in the complex of the ligand $\mathbf{L^2}$ relative to the ligand $\mathbf{L^1}$.

3.4. Catalytic studies

The catecholase activity of the polyaza binuclear copper(II) complexes **1** and **2** has been studied, with the help of electronic spectroscopy, by monitoring the appearance of the absorbance maximum of the substituted quinone [(DTBQ) ditertiary butyl quinone] (λ_{max} = 395 nm). The time dependent formation of DTBQ in the presence of **1** and **2** is shown in Fig. 6. The initial rate values for the binuclear copper(II) complexes **1** and **2** are 1.15×10^{-4} and $1.66 \times 10^{-4}\,Ms^{-1}$ respectively. The oxidation of catechol to *o*-quinone requires the presence of two metal ions in close proximity; hence, the initial rate values for binuclear complexes are comparatively high. The intrinsic flexibility of the ligand $\mathbf{L^2}$ makes the metal ion reduce easily and bind with the substrate; this facilitates the better catalytic activity of complex **2** compared to complex **1**. It has been assumed that the geometry around the copper ions and the intermetallic distance are the two key factors that determine the catalytic activity of the complexes. The Cu(II) ions may be in close proximity when coordinated by the ligand $\mathbf{L^2}$, and the bridging catechol coordination is compatible with the distance between

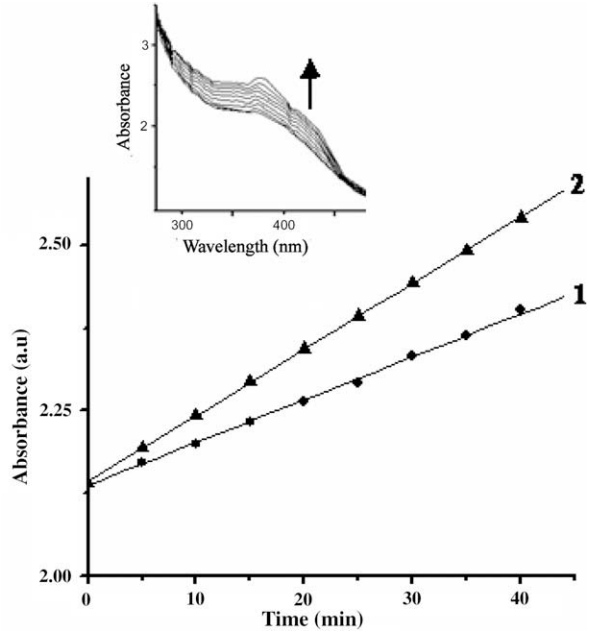


Fig. 6. Time-dependent formation of DTBQ in the presence of complexes **1** and **2**. Inset shows oxidation of 3,5-DTBC by the binuclear copper(II) complex **2**.

the two *o*-diphenol oxygen atoms [32]. The presence of the bulky *N*-methyl group of ligand $\mathbf{L^2}$ makes the coordination geometry around the copper(II) ions more distorted than with the macrocycle ligand $\mathbf{L^1}$. It is evident from the literature [33,34] that the initial rate values for more distorted complexes are higher than those of less distorted complexes.

3.5. DNA cleavage studies

As evident from the literature, complexes of polyamine ligands play an important role due their good nuclease activity [35,36]. In order to assess any cleavage of DNA as a result of the binding of complexes **1** and **2**, agarose gel electrophoresis has been performed with the supercoiled form of plasmid DNA. This method using supercoiled pBR322 DNA in the presence of the macrocyclic Cu(II) complexes **1** and **2** was carried out in a medium of 50 mM Tris–HCl/NaCl Buffer (pH 7.2). Both complexes showed remarkable cleavage in the presence of H_2O_2 (40 μM) as an oxidizing agent. Fig. 7a shows the gel electrophoretic results of the interaction of complexes **1** and **2** with plasmid pBR322 DNA. Under similar conditions, no cleavage of pBR322 DNA occurred for free H_2O_2 (Fig. 7b; lane 2) or free complex (lane 4). The control DNA (lane 1) itself shows two bands [Form I, SC $\geqslant$ 90%; Form II, NC $\leqslant$ 10%]. The Cu(II) complex/H_2O_2 treated DNA also results in two bands, which are exactly parallel to the bands of lane 1, and the band intensity corresponding to Form I (SC DNA) is diminished whilst the band

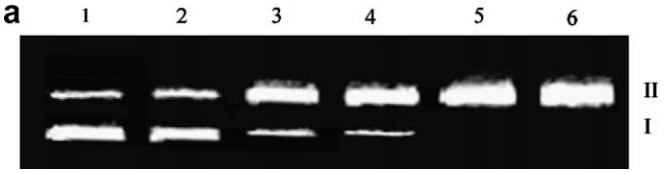


Fig. 7a. Cleavage of SC pBR322 DNA (0.2 μg, 33 μM) by the Cu(II) complexes **1** and **2** (30–50 μM) in the presence of reducing agent H_2O_2 (40 μM) in 50 mM Tris–HCl/NaCl buffer (pH 7.2). Lane 1, DNA control; Lane 2, DNA + 40 μM H_2O_2; Lanes 3–4, DNA + 40 μM H_2O_2 + **1** and **2** (30 μM), respectively; Lanes 5–6, DNA + 40 mM H_2O_2 + **1** and **2** (50 μM), respectively.

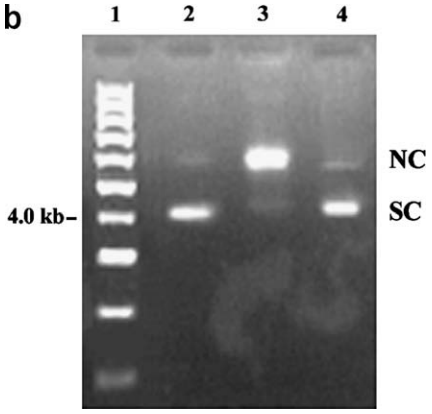


Fig. 7b. Lane 1, Molecular weight markers (1 kb); lane 2, DNA control, lane 3, DNA + 40 μM H_2O_2 + Complex **1** (50 μM); lane 4, DNA + Complex **1** (50 μM). SC – supercoiled, NC – Nicked circular.

intensity of Form II (NC DNA) is increased (lanes 3–4), confirming the formation nicked DNA. At a slightly higher concentration of the binuclear complexes (50 μM), the cleavage is found to be much more efficient in lane 5 and 6. The amount of Form I (supercoiled) decreases, whereas that of Form II (nicked circular) increases with the increase in complex concentration. It is clear that the cleavage of pBR322 DNA is highly dependent on the concentration of the complexes. The cleavage mechanism of pBR322 DNA induced by complexes **1** and **2** was investigated (Fig. 8) and clarified in the presence of a hydroxyl radical scavenger, 1 mM DMSO (lanes 1–2), superoxide scavenger (SOD) (lanes 3–4) and singlet oxygen quencher L-histidine (lanes 5–6) [37]. Only a slight inhibition of DNA cleavage was observed in the presence of the scavengers, which suggests that hydroxyl radical, superoxide and singlet oxygen might not occur in the reaction. The binuclear Cu(II) complexes were found to be highly active in cleaving DNA in the presence of hydrogen peroxide. The H_2O_2 is coordinated to the copper ions of the complexes, affording a peroxo-dicopper species [38]. This coordinated peroxide ion attacks the DNA phosphate bond via a nucleophilic mechanism and hydrolyzes the P–O bond [39–41]. The polyamine groups of the ligand also have the ability to promote the performance of the metal complex in the cleavage of DNA under physiological conditions [17]. The reactions of SC (Form I) plasmid DNA with complex **1** were carried out in the presence of H_2O_2. Typical results from this set of experiments are shown in Fig. 9a and 9b. The disappearance of SC versus time followed pseudo first-order kinetics. The reaction profile for the complex **1** mediated reaction displayed approximately pseudo-first-order kinetic behavior (Fig. 9b), with $k_{obs} \sim 0.07\ min^{-1}$ and $R^2 = 0.984$. On comparison of the cleavage property with some reported results [42,43] of Cu(II) complexes, we note that a lesser amount of oxidizing agents (here hydrogen peroxide) and complex are required in our case for maximum cleavage of DNA. This is well reflected from the cleavage data which show more than 80% of cleavage occurs with a very low

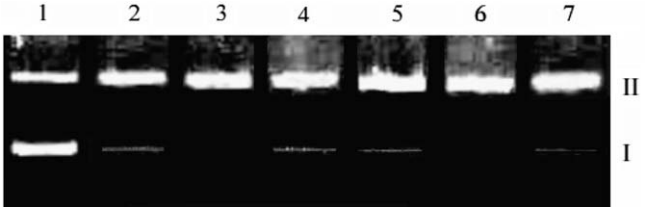


Fig. 8. Lane 1, DNA control; lanes 2–3, DNA + 40 μM H_2O_2 + DMSO (0.1 mM) + **1** and **2** (50 μM), respectively; lanes 4–5, DNA + 40 μM H_2O_2 + SOD (4 units) + **1** and **2** (50 μM) respectively; lanes 6–7, DNA + 40 μM H_2O_2 + L-histidine (0.21 mM) + 1 and 2 (50 μM), respectively.

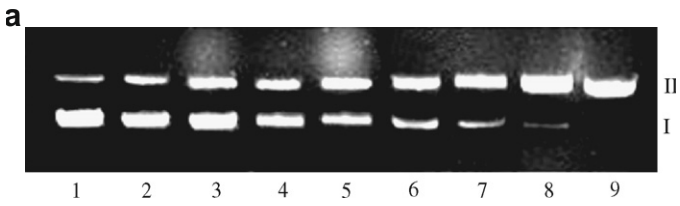


Fig. 9a. Cleavage activity of **1** monitored by 0.8% agarose gel electrophoresis, where [DNA] 0.2 μg, 33 μM (complex 1) 50 μM, and [H_2O_2] 40 μM. Time course measured in 10 mM Tris buffer, pH 7.4, 37 °C, showing the disappearance of supercoiled DNA (I) at (1) 0 min, (2) 2 min, (3) 4 min, (4) 6 min, (5) 10 min, (6) 15 min, (7) 20 min, (8) 25 min, (9) 30 min. (Gel image showing supercoiled (Form I) and circular relaxed (Form II) DNA).

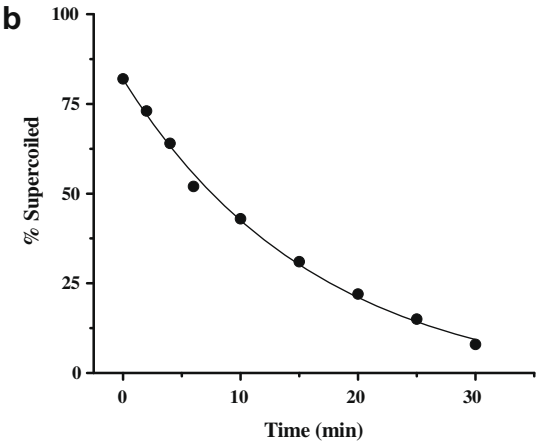


Fig. 9b. Reaction curve, showing a pseudo-first-order kinetic profile (R^2) 0.974, $k_{obs} \sim 0.04\ min^{-1}$.

amount of complex concentration (50 μM). Meanwhile, due to the cyclic nature of the ligands, the two Cu(II) ion centers display an appropriate distance and angle in the cleavage process, so a synergistic effect might exist in the system. Further studies on the macrocyclic ring size effect on DNA molecules as well as on the sequence selectivity of the copper(II) complexes are in progress.

4. Conclusions

Two symmetrical polyaza macrobicyclic binuclear Cu(II) complexes have been synthesized from the new macrocyclic ligand $\mathbf{L^1}$ and its permethylated derivative ligand $\mathbf{L^2}$ and subsequently characterized. Both ligands were prepared using a new tetraldehyde as a precursor compound. Both complexes show good catalytic activity for the catechol oxidation reaction and remarkably promote the hydrolytic cleavage of supercoiled plasmid DNA under physiological conditions in the presence of H_2O_2 at pH 7.2 and 37 °C.

Acknowledgement

The authors gratefully acknowledge DST-FIST, India for financial assistance.

Appendix A. Supplementary data

CCDC 673462 and 688536 contain the supplementary crystallographic data for compounds **I** and $\mathbf{L^2}$. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

References

[1] E. Kimura, Tetrahedron 48 (1992) 6175.
[2] B. Sarkar, P. Mukhopadhyay, P.K. Bharadwaj, Coord. Chem. Rev. 236 (2003) 1.
[3] R.M. Izatt, K. Pawlak, J. Bradshaw, S. Bruening, Chem. Rev. 91 (1991) 1721.
[4] M.H. Klingle, G. Steinfeld, B. Kersting, Z. Naturforsch. 56b (2001) 901.
[5] (a) M. Wu, D. Stoermer, T.D. Tullius, C.A. Townsend, J. Am. Chem. Soc. 122 (2000) 12884;
(b) M.C.B. Oliveira, M.S.R. Couto, P.C. Severino, T. Foppa, G.T.S. Martins, B. Szpoganicz, R.A. Peralta, A. Neves, H. Terenzi, Polyhedron 24 (2005) 495.
[6] H.A. Avila, D.S. Sigman, L.M. Cohen, R.C. Millikan, L. Simpson, Mol. Biochem. Parasitol. 48 (1991) 211.
[7] N.A. Rey, A. Neves, A. Bortoluzzi, C.T. Pich, H. Terenzi, Inorg. Chem. 46 (2007) 348.
[8] M.R. Malachowski, B.R. Dorsey, M.R. Parker, M.E. Adams, R.S. Kelly, Polyhedron 17 (1998) 1289.
[9] M.S. Niasari, A. Amiri, J. Mol. Catal. A: Chem. 235 (2005) 114.
[10] M. Tremolieres, J.B. Bieth, Phytochemistry 23 (1984) 501.
[11] D. Meiwes, B. Ross, M. Kiesshauer, K. Cammann, H. Witzel, M. Knoll, M. Borchardt, C. Sandermaier, Lab. Med. 15 (1992) 24.
[12] P. Gentschev, N. Moller, B. Krebs, Inorg. Chim. Acta 300–302 (2000) 442.
[13] D. Koushik, J. Ratha, M. Manassero, X-Y. Wang, S. Gao, P. Banerjee, J. Inorg. Biochem. 101 (2007) 95.
[14] E.L. Hegg, J.N. Burstyn, Inorg. Chem. 35 (1996) 7474.
[15] E.L. Hegg, K.A. Deal, L.L. Kiessling, J.N. Burstyn, Inorg. Chem. 36 (1997) 1715.
[16] Y.G. Fang, J. Zhang, S.Y. Chen, N. Jiang, H.H. Lin, Y. Zhang, X.-Q. Yu, Bioorg. Med. Chem. 15 (2007) 696.
[17] M.C.B. Oliveira, M.S.R. Couto, P.C. Severino, T. Foppa, G.T.S. Martins, B. Szpoganicz, R.A. Peralta, A. Neves, H. Terenzi, Polyhedron 24 (2005) 495.
[18] K.G. Ragunathan, H.-J. Schneider, Angew. Chem., Int. Ed. 35 (1996) 1219.
[19] B. Kersting, G. Steinfeld, Chem. Commun. (2001) 1376.
[20] N.V. Verani, E. Rentschler, T. Weyhermuller, E. Bill, P. Chaudhuri, J. Chem. Soc., Dalton Trans. (2000) 251.
[21] G. Siedle, B. Kersting, Z. Anorg. Allg. Chem. 629 (2003) 2083.
[22] M. Thirumavalavan, P. Akilan, M. Kandaswamy, Inorg. Chem. 42 (2003) 3308.
[23] Bruker-Nonius (2004). APEX-II and SAINT-Plus (Version 7.06a), Bruker AXS Inc., Madison, Wisconsin, USA.
[24] G.M. Sheldrick, SADABS, University of Göttingen, Germany, 2001.
[25] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, J. Appl. Crystallogr. 26 (1993) 343.
[26] G.M. Sheldrick, Acta Crystallogr., Sect. A 64 (2008) 112.
[27] B.P. Clark, J.R. Harris, G.H. Timms, Tetrahedron Lett. 36 (1995) 3889.
[28] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81.
[29] A.B.P. Lever, Inorganic Electronic Spectroscopy, second ed., Elsevier, Amsterdam, 1984.
[30] J. Zubieta, J. C. Hayes, K. D. Karlin, Copper coordination chemistry, biochemical and inorganic perspective (eds) (1983) 97.
[31] H.B. Gray, B.G. Malstrom, Inorg. Chem. 5 (1983) 203.
[32] C.T. Yang, M. Vetrichelvan, X. Yang, B. Moubaraki, K.S. Murray, J.J. Vittal, J. Chem. Soc., Dalton Trans. (2004) 113.
[33] P. Zanello, S. Tamburini, P.A. Vigato, A. Mazzocchin, Coord. Chem. Rev. 77 (1987) 165.
[34] B. Bosnich, Inorg. Chem. 38 (1999) 2554.
[35] Q.L. Li, J. Huang, Q. Wang, N. Jiang, C.Q. Xia, H.H. Lin, J. Wu, X.Q. Yu, Bioorg. Med. Chem. 14 (2006) 4151.
[36] Q.X. Xiang, J. Zhang, P.Y. Liu, C.Q. Xia, Y.Z. Zhou, R.G. Xie, X.Q. Yu, J. Inorg. Biochem. 99 (2005) 1661.
[37] N. Grover, N. Gupta, P. Singh, H.H. Thorp, Inorg. Chem. 31 (1992) 2014.
[38] N. Kitajima, K. Fujisawa, Y. Moro-oka, J. Am. Chem. Soc. 111 (1989) 8975.
[39] L.M.T. Schnaith, R.S. Hanson, L. Que Jr., Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 569.
[40] W.E. Gao, G. Bu, D. Yang, Z. Liao, S. Jiang, G. Yan, J. Way, J. Chem. Soc., Dalton Trans. (2000) 1431.
[41] S. Nishinoa, T. Kobayashia, H. Matsushimab, T. Tokiib, Y. Nishida, Z. Naturforsch. 56c (2001) 138.
[42] R. Cejudo, G. Alzuet, M.G. Alvarez, J.L.G. Gimenez, J. Borras, M.L. Gonzalez, J. Inorg. Biochem. 100 (2006) 70.
[43] M.G. Alvarez, G. Alzuet, J. Borras, B. Macıas, A. Castineiras, Inorg. Chem. 42 (2003) 2992.