Electronic Supplementary Material (ESI) for RSC Advances

Supplementary Information

Electrochemical, phosphate hydrolysis, DNA binding and DNA cleavage properties of new polyaza macrobicyclic dinickel(II) complexes†

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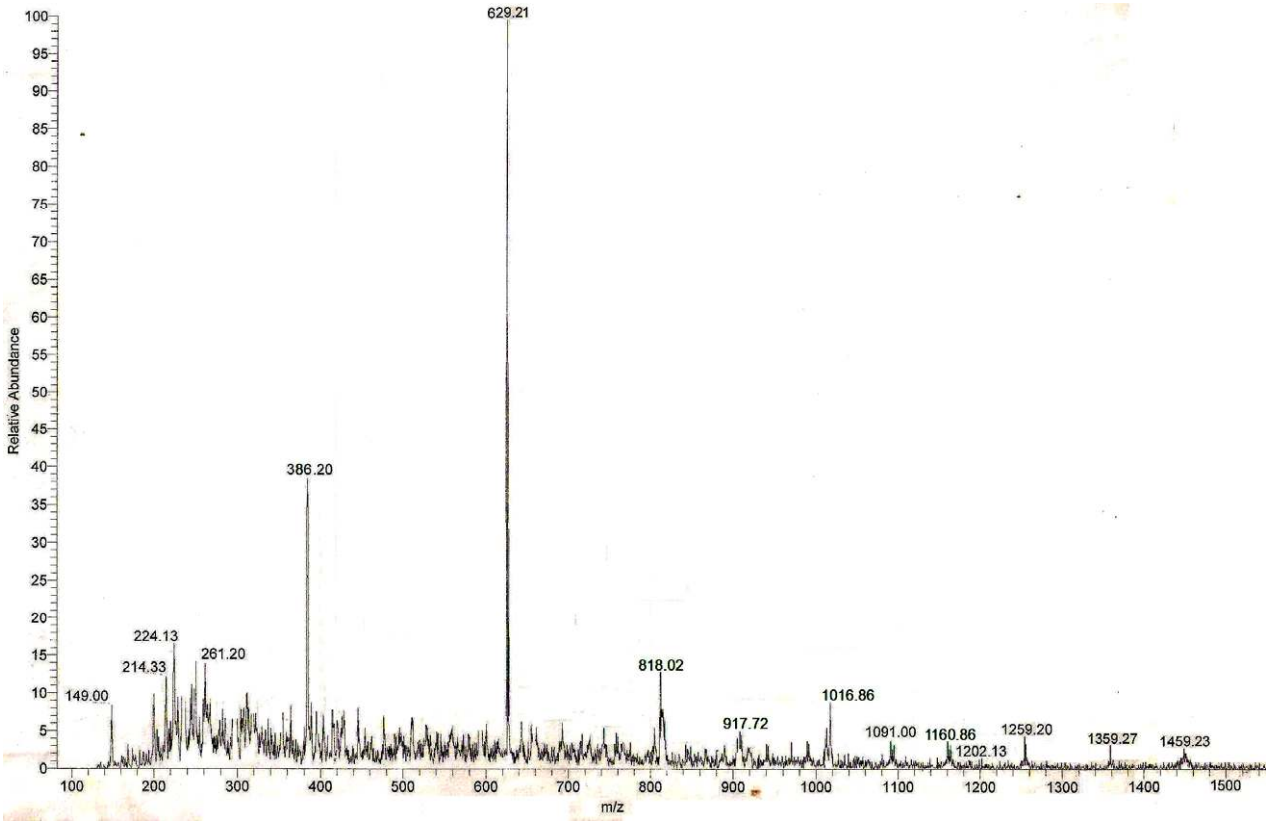

Figure S1. ESI-MS spectrum of complex $[Ni_2L^1(phen)_2(H_2O)_4](ClO_4)_4$ (3) showing the parent ion peak in MeCN. The peak corresponds to $[M–2ClO_4]^{2+}$ species.

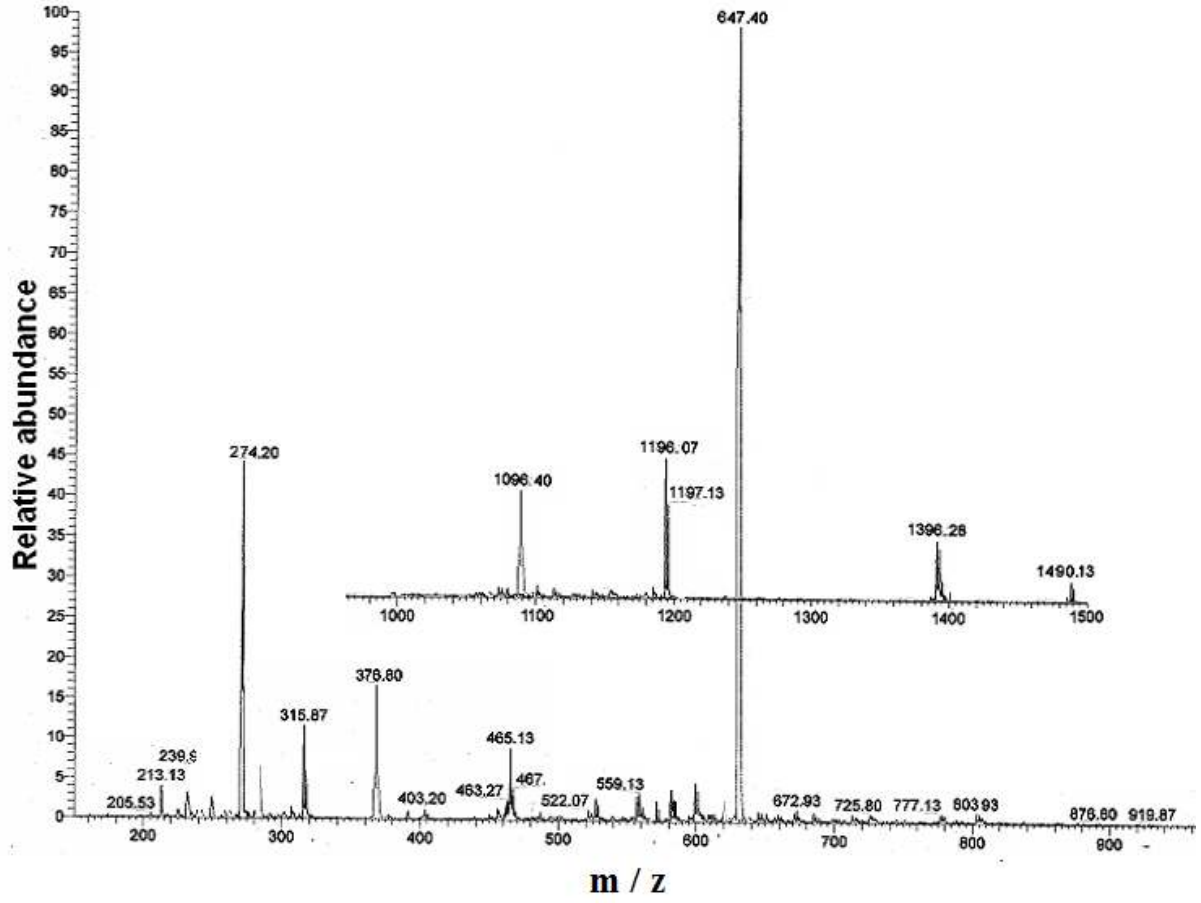

Figure S2. ESI-MS spectrum of complex $[Ni_2L^1(bpy)_2(H_2O)_4](ClO_4)_4$ (5) showing the parent ion peak in MeCN. The peak corresponds to $[M–2ClO_4]^{2+}$ species.

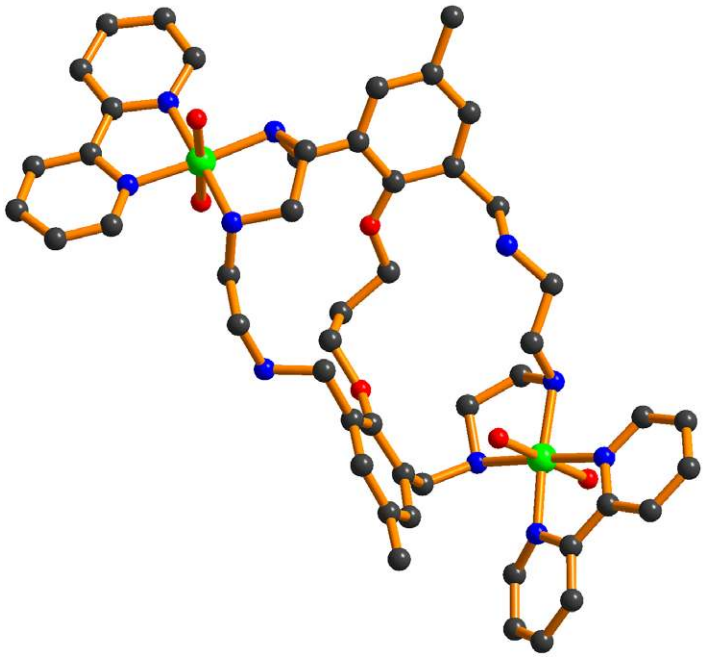

Figure S3. Energy minimized structure of 2,2'–bipyridine coordinated macrobicyclic dinickel(II) complex **2**. Color code; Zn = green, O = red, N = blue, C = grey.

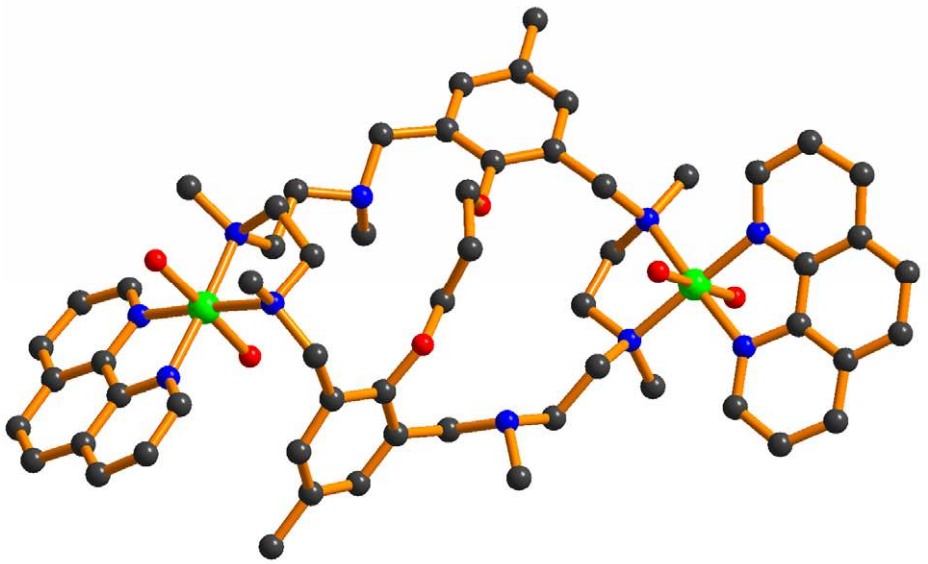

Figure S4. Energy minimized structure of 1,10–phenanthroline coordinated macrobicyclic dinickel(II) complex **6**. Color code; Zn = green, O = red, N = blue, C = grey.

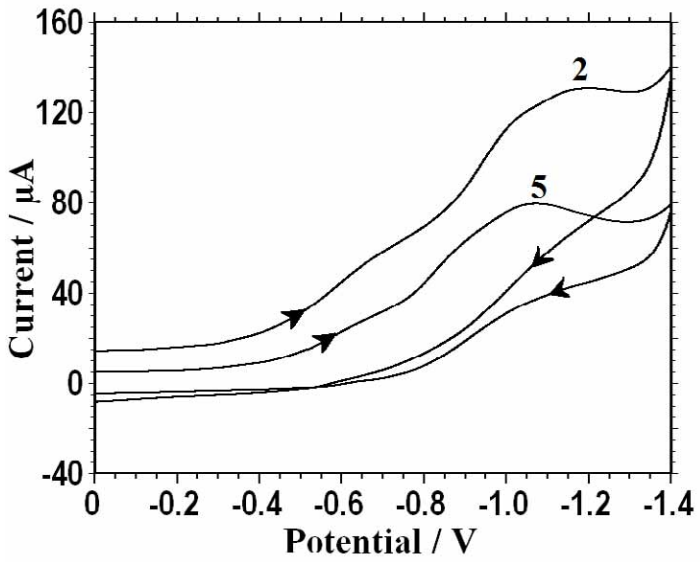

Figure S5. Cyclic voltammograms of 2,2'–bipyridine coordinated macrobicyclic dinickel(II) complexes **2** and **5** in CH_3CN (cathodic region).

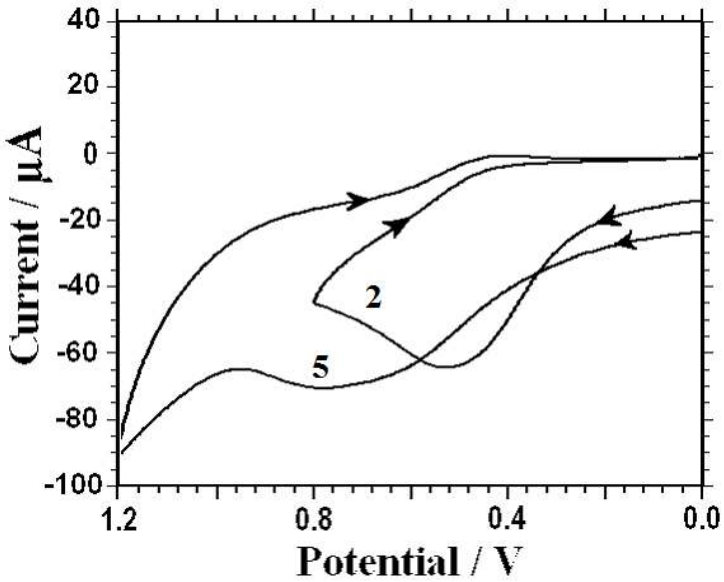

Figure S6. Cyclic voltammograms of 2,2'–bipyridyl coordinated macrobicyclic dinickel(II) complexes **2** and **5** in CH_3CN (anodic region).

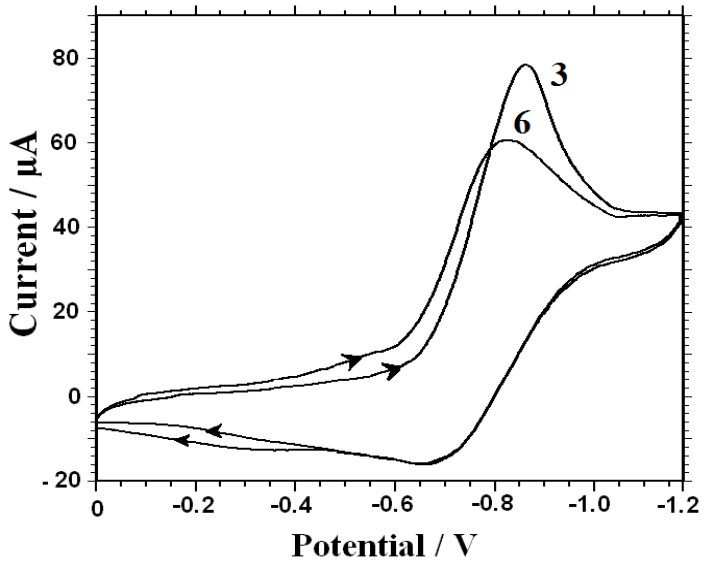

Figure S7. Cyclic voltammograms of 1,10–phenanthroline coordinated macrobicyclic dinickel(II) complexes **3** and **6** in CH_3CN (cathodic region).

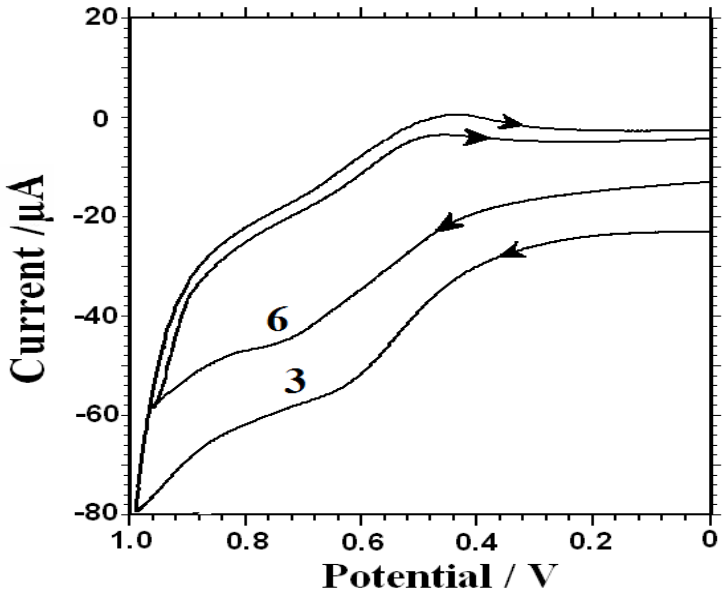

Figure S8. Cyclic voltammograms of 1,10–phenanthroline coordinated macrobicyclic dinickel(II) complexes **3** and **6** in CH_3CN (anodic region).

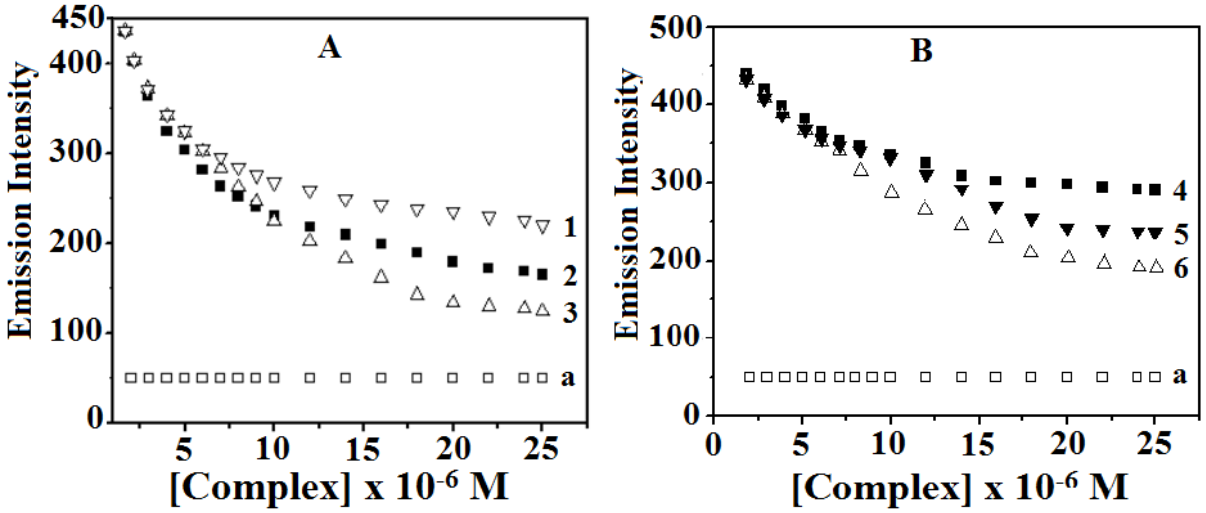

Figure S9. The effect of addition of complexes **1–6** on the emission intensity of 40 μM CT DNA–bound EB in Tris–HCl/NaCl buffer (50 mM, pH 7.5) at 25 °C; (**a**) on the emission intensity of the EB in absence of CT DNA but at different concentrations of complexes (**1–6).**

Table S1. The electrochemical data of the dinickel(II) complexes **1**−**6**

	Reduction process		Oxidation process			
Complex	E_{pc} (V)	E_{pa} (V)	E_{pa} (V)	E_{pc} (V)	$E_{1/2}$ (V)	ΔE (mV)
1	–1.13	–1.03	+0.85	+0.68	+0.76	170
2	–1.18	–0.93	+0.52	+0.43	+0.48	090
3	–0.86	–0.73	+0.62	+0.46	+0.54	160
4	–1.05	–0.93	+0.92	+0.75	+0.83	170
5	–1.08	–0.78	+0.74	+0.38	+0.56	360
6	–0.81	–0.72	+0.78	+0.46	+0.62	320

Experimental methods

Phosphate hydrolysis reaction

The hydrolysis of 4-nitrophenyl phosphate (4–NPP) was run on the Perkin Elmer UV spectrophotometer in the quartz cell in kinetic analysis mode following the increase in absorption at 397 nm due to d[4–nitrophenolate]/d*t*. The effect of pH on the reaction rate for the hydrolysis of 4–NPP promoted by complexes **1–6** was determined over the pH range 3.9–10.5. Reactions were performed using the following conditions: 3 mL of freshly prepared buffer aqueous solution (50 mM, 0.1 mM KCl, buffer: acetato (pH 3.9, 4.6 and 5.2), MES (pH 5.8 and 6.2), Bis-Tris propano (pH 7.2, 7.8 and 8.5), CHES (pH 8.98 and 10.25) and 1 mL of 5×10^{-4} M complex solution [acetonitrile–water (2.5% (v/v))] were added to a 1 cm path length at 25 °C. To correct for the spontaneous 4-NPP hydrolysis, each reaction was measured against a reference cell that was identical to the sample cell in composition except in the absence of dinickel(II) complexes.

DNA binding and cleavage experiments

Absorption spectral studies

The DNA binding experiments were performed in Tris–HCl / NaCl buffer (50 mM Tris HCl / 1 mM NaCl buffer, pH 7.5) using dimethyl formamide (DMF) (10%) solution of the complexes **1–6**. The concentration of CT DNA was determined from the absorption intensity at 260 nm with a ε value[1] of 6600 $M^{-1}cm^{-1}$. Absorption titration experiments were made using different concentration of CT DNA, keeping the complex concentration as constant. Due correction was made for the absorbance of the CT DNA. Samples were equilibrated before recording each spectrum. For the complexes **1–6** the intrinsic binding constant K was determined from the spectral titration data using the following equation.[2]

$$(\varepsilon_a - \varepsilon_f) / (\varepsilon_b - \varepsilon_f) = (b - (b^2 - 2K^2C_t\,[DNA] / s)^{1/2} / 2KC_t \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(a)}$$

$$b = 1 + KC_t + K\,[DNA\,]/ 2s \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(b)}$$

where; [DNA] is the concentration of CT-DNA in base pairs, the apparent absorption coefficients ε_a, ε_f and ε_b correspond to A_{obsd}/[Complex], the absorbance for the free copper(II) complex, and the absorbance for the copper(II) complex in the fully bound form, respectively. K is the equilibrium binding constant in M^{-1}, C_t is the total metal complex concentration, and s is the binding site size. The non-linear fit analysis was done using Origin Lab, version 6.1.

CD spectral studies

A circular dichoric (CD) spectrum of the CT DNA was measured using a JASCO J–715 spectropolarimeter equipped with a Peltier temperature control device at 25 ± 0.1 °C. All experiments were done using a quartz cell of 1 cm pathlength. Each CD spectrum was recorded after averaging over at least 5 accumulations using a scan speed of 100 nm min^{-1}.

Fluorescence spectral studies

The fluorescence spectral method using ethidium bromide (EB) as a reference to determine the relative DNA binding properties of the complexes **1**–**6** to CT DNA in 50 mM Tris–HCl / 1 mM NaCl buffer, pH 7.5. Fluorescence intensities of EB at 735 nm with an excitation wavelength of 530 nm were measured at different complex concentrations. Reduction in the emission intensity was observed with addition of the complexes. The relative binding tendency (K_1) of the complexes to CT DNA was determined from a comparison of the slopes of the lines in the fluorescence intensity versus complex concentration plot. The apparent binding constant (K_{app}) was calculated using the equation K_{EB}[EB] / K_1, where the complex concentration was the value at a 50% reduction of the fluorescence intensity of EB and K_{EB} = 1.0 x 10^7 M^{-1} ([EB] = 3.3 μM).[3]

Viscosity measurements

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature of 25.0 ± 0.1 ºC in a thermostatic bath. DNA samples approximately 200 bp in length were prepared by sonication in order to minimize complexities arising from DNA flexibility.[4] The flow time was measured with a digital stopwatch, and each sample was tested three times to get an average calculated time. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio,[5] where η is the viscosity of DNA in the presence of complex, η_0 is the viscosity of free DNA.

DNA cleavage studies

The cleavage of plasmid DNA was determined by agarose gel electrophoresis. The supercoiled pBR322 DNA (0.020 mg/mL^{-1}) was treated with dicopper(II) complexes **1**–**6** (30 μM) in 50 mM Tris–HCl/NaCl buffer (pH 7.2) at 25 ± 0.2 ºC. The concentration of the complexes in water or the additives in buffer corresponded to the quantity in 2 μL stock solution after dilution to the 18 μL final volume using Tris–HCl buffer. All the samples were incubated for 1 h at 37 ºC followed by its addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 μL). All the samples were finally loaded on 0.8% agarose gel containing EB (1 μg/mL^{-1}). Electrophoresis was carried out at 50 V for 1 h in TBE buffer (45 mM Tris, 45 mM H_3BO_3, 1 mM–EDTA, pH 8.3). Resulting bands were visualized under UV light and photographed. Quantification of closed circular and nicked DNA was made by densitometric analysis of EB containing agarose gels. Quantification was performed by fluorescence imaging by using 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 EB to intercalate into supercoiled DNA (form I) decreased relative to nicked (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.

References

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