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DNA-binding and photocleavage studies of ruthenium(II) complexes containing asymmetric intercalative ligand

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ABSTRACT

A novel asymmetric ligand 2-(pyridine-2-yl)-1-H-imidazo[4,5-i]dibenzo[2,3-a:2′,3′-c]phenazine (pidbp) and its ruthenium complexes $[Ru(L)_2(pidbp)]^{2+}$ (L = bpy (2, 2′- bipyridine), phen (1, 10 – phenanthroline)), have been synthesized and characterized by elemental analysis, ES-MS, ^{1}H NMR. Various methods support the conclusion that both Ru(II) complexes can intercalate into DNA base pairs. Complex $[Ru(bpy)_2(pidbp)]^{2+}$ **4** exhibits its DNA "molecular light switch" properties. Furthermore, the two complexes are efficient DNA-photocleavers under irradiation at 365 nm, and complex **5** exhibits a stronger DNA-photocleavage efficiency than complex **4**. The mechanism of DNA cleavage is an oxidative process by generating singlet oxygen.

1. Introduction

There has been tremendous interest recently in the design of novel Ru(II) polypyridyl complexes due to their unique photophysical properties and potential applications in stereoselective probes of nucleic acid structures, molecular "light switches", DNA-photocleavage reagents, catalysts for chemical reactions, photodynamic therapy (PDT) agents and solar energy utilizations[1–16]. Of these, research of molecular "light switches" has received a lot of attentions, as one of the hot topics of superamolecular chemistry. Up to now, Ru(II) polypyridyl complex exhibits its "light switch" effect mainly through the following four channels: (i) "off-on" light switches effect based on DNA binding; such as $[Ru(bpy)_2(dppz)]^{2+}$ (L = bpy (2,2′-bipyridine); dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), which does not emit luminescence in aqueous solution but displays strong photoluminescence in DNA solution after binding to DNA in intercalation mode [9]; (ii) chemical cycling the DNA light switch on and off based on DNA binding and modulation by metal ions and EDTA, such as $[Ru(bpy)_2(tpphz)]^{2+}$ (tpphz = tetrapyrido[3,2-*a*:2′,3′-*c*:3″,2″-*h*:2‴,3‴-*j*]phenazine, which exhibits this phenomenon through the successive addition of Co^{2+} and EDTA after binding to DNA[17,18]; (iii) "electro-photo switch" based on the redoxation of ligand, such as the redox couple $[Ru(bpy)_2(qdppz)]^{2+}$/$[Ru(bpy)_2(hqdppz)]^{2+}$ (qddpz = naphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine-5,18-dione; hqdppz = 5,18-dihydroxynaphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine) [19]; (iv) pH-induced molecular light switches, such as $[Ru(bpy)_2(btppz)]^{2+}$ (btppz = btppz = benzo[*h*]tripyrido[3,2-*a*:2′,3′-*c*:2″,3″-*j*]phenazine, which exhibits "on-off" emission switch in water solutions with various pH values[20]. Among these systems, DNA "light switches" have attracted particular attention, owing to their possible applications such as detection of DNA base mismatches [21], molecular-scale logic gates, DNA sensing, the signaling of DNA protein binding [22–25] and luminescent probes of DNA structure [16]. The requirements for these applications are the DNA-binding and steady-state photophysical properties of Ru(II) polypyridyl complexes. Recently, there has been a great interest on the binding of ruthenium(II) polypyridyl complexes with DNA, because it may provide important information for new cancer therapeutic agents and potential probes of DNA structure and conformation. In general, Ru(II) complexes can interact with DNA through three non-covalent modes such as electrostatic binding, groove binding, or intercalation. Among these interactions, intercalative binding mode is one of the most important DNA binding modes, which is related to DNA "light switches" and the antitumor activity of the complexes.

In addition, more recently, many ruthenium(II) complexes with symmetric intercalative ligand have been synthesized and applied to DNA-binding studies. However, the DNA-binding investigations of such complexes containing asymmetric ligands have attracted much less attention. In fact, these Ru(II) complexes with asymmetric ligands also exhibit interesting properties upon binding to

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DNA[26–29]. A great number of reports have shown that varying the shape of the intercalative ligand can create some interesting difference in the space configuration and the electron density distribution of Ru(II) polypyridyl complexes, this will result in the changes in the DNA-binding properties. Therefore, an extensive study of some new complexes with asymmetric ligands is necessary for further understanding the DNA-binding mechanism and guiding the design of new Ru(II) complexes with an excellent bioactivity.

The motivation of this research is stimulated by DNA "light switch" effect of $[Ru(bpy)_2(dppz)]^{2+}$ reported by Hartshorn and Barton [10]. $Ru(bpy)_2(dppz)]^{2+}$ exhibits its DNA "light switch" effect, because the intercalative ligand dppz possesses phenazine ring, and DNA protected the phenazine nitrogen atoms from interaction with water upon intercalation compared to free in aqueous solutions [10]. In this work, we report the synthesis and characterization of a novel asymmetric ligand (pidbp = 2-(pyridine-2-yl)-1-H-imidazo[4,5-i]dibenzo[2,3-a:2′,3′-c]phenazine) containing phenazine ring and its ruthenium complexes $[Ru(L)_2(pidbp)]^{2+}$ (L = bpy (2,2′-bipyridine), phen (1, 10 – phenanthroline)). $[Ru(bpy)_2(pidbp)]^{2+}$ is demonstrated here to act as a DNA "molecular light switch" with the luminescence enhancement factor of 10.23 on binding to DNA. To the best of our knowledge, it is the first example of Ru(II) complexes with asymmetric ligand, as DNA "molecular light switch". Furthermore, the DNA-binding and DNA-photocleavage properties of the two novel Ru(II) complexes were carefully studied.

2. Experimental

All materials were commercially available and used without further purification. Calf thymus DNA (CT-DNA) was obtained from Sigma (St. Louis, MO, USA). Supercoiled pBR 322 DNA was purchased from MBI Fermentas. Doubly distilled water was used to prepare buffer. All DNA-binding experiments were carried out in buffer A (50 mM NaCl, 5 mM Tris–HCl, pH = 7.2). For DNA photocleavage experiments, samples were treated in buffer B (50 mM Tris, 18 mM NaCl, pH = 7.8). Solutions of CT-DNA in buffer A gave a ratio of UV–vis absorbance of 1.8–1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [30]. The concentration of DNA was determined spectrophotometrically ($\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$) [31]. The complexes *cis*-$[RuCl_2(bpy)_2]\cdot 2H_2O$, *cis*-$[RuCl_2(phen)_2]\cdot 2H_2O$ [32], methyl pyridine-2-carboximidate [33,34], **1,2-diamino-4,5-(p-toluenesulfamidobenzene)** [35,36], were prepared by the literature methods.

2.1. Physical measurement

C, H, and N analyses were carried out with a Perkin–Elmer 240Q elemental analyzer. Electrospray mass spectra were recorded on a LQC system (Finngan MAT, USA) using CH_3CN as mobile phase. Fast atomic bombardment mass spectra (FAB-MS) were obtained on a VG ZAB-HS spectrometer. 1H NMR spectra were recorded on a Bruker ARX-500 spectrometer with $(CD_3)_2SO$ for the complexes at 500 MHz at room temperature. Absorption spectra were recorded with a Shimadzu UV-2450 spectrophotometer and emission spectra on a Hitachi F-2500 spectrofluorophotometer at room temperature.

2.2. DNA-binding and photocleavage experiments

The absorption titrations of Ru(II) complexes in buffer A were performed by using a fixed ruthenium concentration (20 μM), to which increments of the DNA stock solution were added. Ruthenium–DNA solutions were allowed to incubate for 5 min before the absorption spectra were recorded.

DNA viscosities were measured using an Ubbelohde viscometer maintained at a constant temperature of 30.0 ± 0.1 °C in a thermostatic bath. The DNA samples for viscosity measurement were prepared by sonication in order to minimize complexities arising from DNA flexibility [37]. Every sample was measured at least three times and an average flow time was calculated. The DNA viscosity was calculated according to $\eta_i = (t_i - t_0)/t_0$, where η_i is the corresponding values of DNA viscosity; t_i is the flow time of the solutions in the presence or absence of the complex; and t_0 is the flow time of buffer alone. Data were presented as $(\eta/\eta_0)^{1/3}$ vs. binding ratio [38], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

The competitive binding experiments was conducted by adding increasing amounts of Ru(II) complex directly into the samples containing 5 μM ethidium bromide (EB) and 100 μM DNA in buffer A. Emission spectra were recorded in the region 500–700 nm, and samples were excited at 515 nm.

For the gel electrophoresis experiments, supercoiled pBR322 DNA (0.1 μg) was treated with Ru(II) complexes in buffer B (50 mM Tris, 18 mM NaCl, pH = 7.8), and the solutions were incubated for 1 h in the dark, then irradiated at room temperature with an UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 2 h at 75 V in TBE buffer C (89 mM Tris, 89 mM boric acid, 2 mM EDTA) containing 1% agarose gel. The gel was stained with 0.5 μg/ml ethidium bromide and then photographed under UV light.

2.3. Synthesis

2.3.1. 5,6-(p-Toluenesulfonamide) -2-(pyridine-2-yl)-1H-benzimidazole(**1**)

A solution of methyl pyridine-2-carboximidate (ca. 0.4 mmol), 1,2-diamino-4,5-(p-toluenesulfamidobenzene) 0.178 g (0.4 mmol) in 10 ml glacial acetic acid was refluxed under argon for 4 h. The cooled solution was diluted with water and neutralized with concentrated aqueous ammonia. The khaki precipitate was collected and dried under vacuum. Yield: 0.195 g, 91.3%. Anal (%): (Found: C, 58.37; H, 4.23; N, 12.92%. Calcd for $C_{26}H_{23}N_5O_4S_2$: C, 58.52; H, 4.35; N, 13.13%). FAB-MS: 534 $[M+1]^+$

2.3.2. 2-(Pyridine-2-yl)-5,6-diamino-1H-benzimidazole (**2**)

5,6-(p-Toluenesulfonamide) -2-(2′-pyridineyl)-1H-benzimidazole(**1**) 0.533 g (1.0 mmol) and 4 ml concentrated sulfuric acid were heated at 100 °C for 4 h. The dark violet solution was then added dropwise to ice water. Treatment of the resulting solution with a saturated Na_2CO_3 solution gave a clear green solution. And the solution was extracted with dichloromethane (3 ml × 100 ml). The combined extracts were dried over $MgSO_4$. Removal of the solvent at reduced pressure gave the product as a yellow solid. Yield: 0.089 g, 39.6%. Anal (%): (Found: C, 63.11; H, 4.99; N, 30.82%. Calcd for $C_{12}H_{11}N_5$: C, 63.99; H, 4.92; N, 31.09%). FAB-MS: m/z =226 $[M+1]^+$.

2.3.3. 2-(Pyridine-2-yl)-1-H-imidazo[4,5-i]dibenzo[2,3-a:2′,3′-c]phenazine(pidbp) (**3**)

A mixture of 2-(pyridine-2-yl)-5,6-diamino-1H-benzimidazole (**2**) 0.067 g (0.3 mmol) and phenanthrenequinone 0.062 g (0.3 mmol) was refluxed for 2 hours in methanol (30 ml). The cooled solution was poured into water. The resulting yellow precipitate was filtered and washed with water, and then dried *in vacuo*. Yield: 0.098 g, 82.1%. Anal (%): (Found: C, 78.42; H, 3.89; N,

17.46%. Calcd for $C_{26}H_{15}N_5$: C, 78.56; H, 3.81; N, 17.63%). ES-MS (CH_3CH_2OH): m/z = 398.0 ($[M+H]^+$).

2.3.4. $[Ru(bpy)_2(pidbp)](ClO_4)_2{\cdot}H_2O$ **(4)**

A mixture of pidbp 0.120 g (ca. 0.3 mmol), $[Ru(bpy)_2Cl_2]{\cdot}2H_2O$ (0.156 g, 0.3 mmol), and ethylene glycol (10 ml) was deoxygenated with argon. The mixture was heated at 140 °C under argon for 6 h. When the solution was cooled to room temperature, the resulting clear red solution was diluted with water (ca. 60 ml), then treated with a saturated aqueous solution of $NaClO_4$. The orange precipitate was collected and washed with small amounts of water and diethyl ether, then dried under vacuum. The crude product was purified by column chromatography on a neutral alumina with acetonitrile–toluene (3:1, v/v) as an eluent. Yield: 0.209 g, 68.9%. Anal (%): (Found: C, 54.55; H, 3.16; N, 12.42%. Calcd for $C_{46}H_{31}N_9O_8RuCl_2$: C, 54.72; H, 3.09; N, 12.48%). ES-MS (CH_3CN): m/z = 810.1 ($[M-2ClO_4^- - H]^+$), 405.5 ($[M-2ClO_4^-]^{2+}$). ^{1}H NMR (500 MHz, ppm, DMSO-d6): 9.28 (d, 1H, J = 7.5 Hz), 9.04 (d, 1H, J = 8.0 Hz), 8.95 (d, 1H, J = 8.0 Hz), 8.85 (dd, 2H, J = 4.5 Hz, J = 7.5 Hz), 8.65 (d, 1H, J = 7.5 Hz), 8.51 (s, 1H), 8.46 (t, 1H, J = 8.0 Hz), 8.19 (t, 1H, J = 8.0 Hz), 8.15 (t, 1H, J = 8.0 Hz), 8.10 (t, 1H, J = 7.5 Hz), 8.02 (t, 1H, J = 8.0 Hz), 7.97 (t, 1H, J = 5.5 Hz), 7.92 (d, 1H, J = 5.0 Hz), 7.79 (m, 6H), 7.70 (d, 1H, J = 5.0 Hz), 7.58 (t, 1H, J = 6.5 Hz), 7.53 (m, 2H, J = 5.5 Hz), 7.48 (t, 1H, J = 6.5 Hz), 6.38 (s, 1H).

2.3.5. $[Ru(phen)_2(pidbp)](ClO_4)_2$ **(5)**

This complex was synthesized by a similar procedure as that described for complex **4**, with $[RuCl_2(phen)_2]{\cdot}2H_2O$ (0.170 mg, 0.3 mmol) in place of $[Ru(bpy)_2]Cl_2{\cdot}2H_2O$. Yield: 0.173 g, 54.6%. Anal (%): (Found: C, 56.53; H, 3.08; N, 11.76%, Calc for $C_{50}H_{31}N_9O_8RuCl_2$: C, 56.77; H, 2.95; N, 11.92%). ES-MS (CH_3CN): m/z = 858.4 ($[M-2ClO_4^- - H]^+$), 429.5 ($[M-2ClO_4^-]^{2+}$). ^{1}H NMR (500 MHz, ppm, DMSO-d6): 9.22 (d, 1H, J = 8.0 Hz), 9.00 (d, 1H, J = 8.0 Hz), 8.95 (d, 1H, J = 7.5 Hz), 8.76 (m, 2H), 8.67 (d, 4H, J = 8.0 Hz), 8.46 (s, 2H), 8.41 (t, 3H, J = 9.0 Hz), 8.38 (s, 1H), 8.29 (d, 1H, J = 8.5 Hz), 8.21 (m, 2H), 8.13 (t, 1H, J = 7.5 Hz), 8.02 (t, 1H, J = 7.0 Hz), 7.94 (t, 1H, J = 7.0 Hz), 7.87 (t, 1H, J = 6.5 Hz), 7.79 (m, 5H), 7.67 (d, 1H, J = 5.5 Hz), 7.38 (t, 1H, J = 6.5 Hz), 6.13 (s, 1H).

Each of the above ClO_4 salts was dissolved in the minimum amount of acetone, and a saturated TBACl (Tetrabutylammonium chloride) in acetone was added dropwise until precipitation was complete. The water-soluble chloride salts were filtered off and washed thoroughly with acetone, and then dried under vacuum (yield ~92% in each case).

3. Results and discussion

3.1. Synthesis and characterization

The synthetic pathway for the asymmetric ligand pidbp and its two complexes is shown in Scheme 1. The precursor compound **1** was obtained on the basis of the method for imidazole ring preparation established by Schaefer and Peters [33,34,39,40]. Deprotection of the tosyl groups was carried out in concentrated sulfuric acid to give **2**. Condensation of phenanthrenequinone with the precursor diamine compound **2** in refluxing methanol gave the asymmetric ligand **3** in good yields. The corresponding ruthenium(II) complexes **4** and **5** were prepared by direct reaction of ligand with the appropriate mol ratios of the precursor complexes in ethylene glycol. All these complexes were purified for DNA-binding and photocleavage experiments, by chromatography on a neutral alumina column using acetonitrile-toluene (3:1 and 2:1, respectively) as eluents, and characterized by element analysis, ES-MS and ^{1}H NMR. In the ES-MS spectra for the two complexes **4** and **5**, only the signals of $[M-2ClO_4^- - H]^+$ and $[M-2ClO_4^-]^{2+}$ were observed. In both case, the doubly charged species appeared as major peak. The measured molecular weights were consistent with expected values.

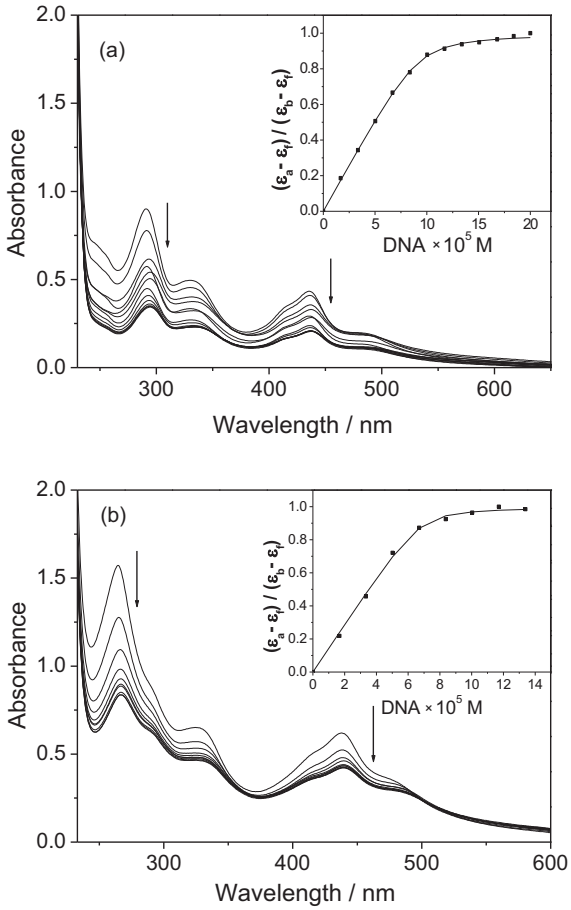


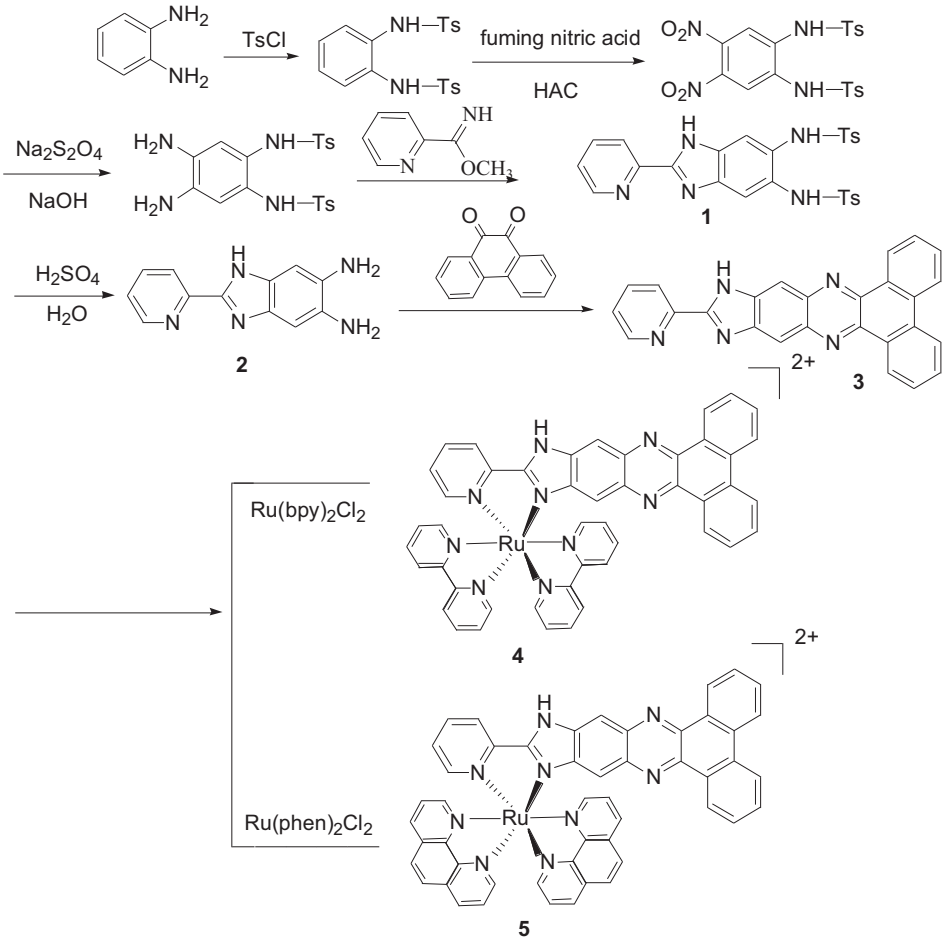
Fig. 1. Absorption spectra of complexes **4** (a) and **5** (b) in buffer A upon the addition of CT-DNA, [Ru] = 20 μM, [DNA] = 0 – 200 μM. Arrow shows the absorbance changing upon the increase in DNA concentration. Inset: plots of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ vs. [DNA] for the titration of DNA to Ru(II) complexes.

The two Ru(II) complexes $[Ru(bpy)_2(pidbp)]^{2+}$ and $Ru(phen)_2(pidbp)]^{2+}$ gave well-defined ^{1}H NMR spectra (Fig. S1). The proton chemical shifts were assigned via comparison with those of similar Ru(II) complexes with asymmetric ligand [26–29]. The chemical shifts of all the protons in aromatic region were presented in Section 2.

3.2. Electronic absorption titration

The DNA binding properties of the complex is usually characterized through electronic absorption titration. Complex bound to DNA through intercalation usually results in different extents of hypochromism and red shift (bathochromism), due to the intercalation mode involving a strong π–π stacking interaction between aromatic chromophore and the base pairs of DNA. The extent of the hypochromism in the visible ^{1}MLCT band is commonly consistent with the intercalative binding strength [10].

Fig. 1 shows that the absorption spectra of complexes $[Ru(bpy)_2(pidbp)]^{2+}$ and $Ru(phen)_2(pidbp)]^{2+}$ at a constant complex concentration (20 μM) in the absence and presence of calf-thymus (CT) DNA. With increasing concentration of CT-DNA, the UV–vis spectra of the two complexes showed a clear hypochromism in absorption bands. For complex **4,** the decreases in the ^{1}MLCT transitions reach as high as 28.7% at 436 nm at a ratio of [DNA]/[Ru] = 10.1. For complex **5**, upon addition of DNA, the MLCT band at 438 nm exhibits hypochromism of about 32.6% at a ratio of



Scheme 1. The synthetic routes of the ligand and its Ru(II) complexes $[Ru(bpy)_2(pidbp)]^{2+}$ **4** and $Ru(phen)_2((pidbp))]^{2+}$ **5**.

[DNA]/[Ru] = 6.7, respectively. No obvious red shift was observed for both complexes. The DNA binding-induced hypochromism suggest that both complexes bind to DNA with high affinity.

In order to evaluate quantitatively the DNA-binding affinities of the complexes, the intrinsic binding constants K of these two complexes to DNA were determined by monitoring the changes of the ^{1}MLCT absorbance at 436 nm for complex **4** and at 438 nm for complex **5** using Eq. (1) [41].

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

$$b = 1 + KC_t + \frac{K[DNA]}{2s} \quad (1b)$$

where [DNA] is the concentration of DNA in M (nucleotide), the apparent absorption coefficient ε_a, ε_b and ε_f correspond to the extinction coefficient observed for the ^{1}MLCT absorption band at a given DNA concentration, the extinction coefficient of the complex in the absence of DNA, and the extinction coefficient of the complex fully bound to DNA. 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 binding constants K were obtained by fitting the absorption titration data using a non-linear least-square method.

The values of intrinsic binding constants K were $1.68 \pm 0.2 \times 10^6\ M^{-1}$ ($s = 2.33$) and $3.09 \pm 0.1 \times 10^6\ M^{-1}$ ($s = 1.71$) for complex **4** and **5**, respectively. The values are smaller than that of those Ru(II) complex reported in the literature, such as DNA intercalator $[Ru(phen)_2(dppz)]^{2+}$ ($K_b = 5.1 \times 10^6\ M^{-1}$ [42]), but are much larger than that of Ru(II) complexes with asymmetric ligand, $[Ru(bpy)_2PYNI]^{2+}$ (PYNI = 2-(2′-pyridyl)naphthoimidazole) ($3.81 \times 10^4\ M^{-1}$) [26], $[Ru(bpy)_2PZNI]^{2+}$ (PZNI = 2-(pyrazin-2-yl)naphthoimidazole) ($3.42 \times 10^4\ M^{-1}$)[27], $[Ru(phen)_2PZNI]^{2+}$($5.86 \times 10^4\ M^{-1}$)[27], and $[Ru(dmb)_2(pdta)]^{2+}$($2.37 \times 10^5\ M^{-1}$) [28]. A possible explanation for these facts may be due to the different planarity of the intercalative ligand. From the results, we could deduce that the two complexes bind to DNA with high affinities. In addition, complex **5** exhibits a stronger DNA-binding affinity than complex **4** due to the different plane area and hydrophobicity of the ancillary ligands. However, the DNA binding mode cannot be determined exclusively using optical method, since surface aggregation leads to similar results. Further investigation is needed to determine the DNA-binding mode.

3.3. Viscosity properties

In order to further clarify the exact nature of both complexes binding to DNA, DNA viscosity measurements were carried out on CT-DNA by increasing the concentration of Ru(II) complexes. The DNA viscosity measurement is an useful means of determining whether a complex intercalate into DNA, which is sensitive to length change of DNA (i.e. viscosity and sedimentation) and regarded as the least ambiguous and most critical tests for the DNA-binding mode in the absence of crystallographic structural data [43,44]. When a complex intercalate into DNA, the DNA helix lengthens as base pairs are separated to accommodate the bound

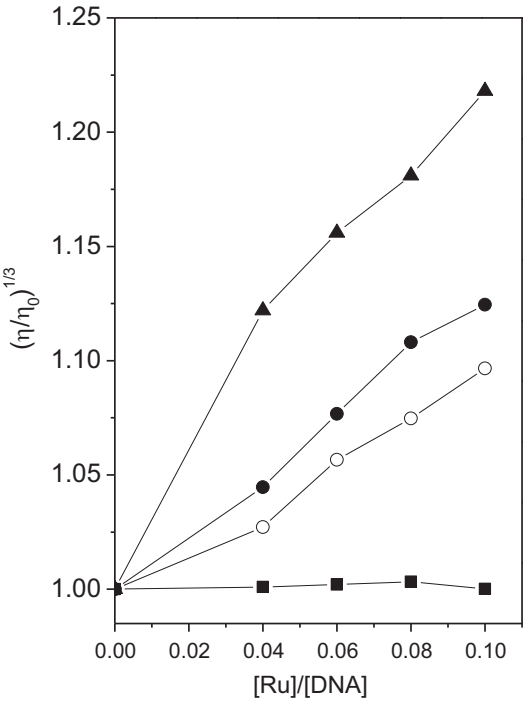


Fig. 2. Effects of the increase in amounts of EB (▲), complex **4** (○), **5** (●) and $[Ru(bpy)_3]^{2+}$ (■) on the relative viscosity of CT-DNA at 30 (±0.1) °C, respectively. The total concentration of DNA is 0.25 mM.

ligand, which result in the increase of DNA viscosity. In contrast, the complex which interacts with DNA by a partial, non-classical intercalation could bend (or kink) the DNA helix, reducing its length and, concomitantly, its viscosity [45]. In addition, electrostatic binding mode has little effect on DNA viscosity.

The changes in DNA viscosity upon addition EB, complex **4**, **5** and $[Ru(bpy)_3]^{2+}$ are shown in Fig. 2. As is well known, ethidium bromide (EB) interacts with DNA through intercalative binding mode, and can increase the relative DNA viscosity for lengthening of the DNA double helix; while complex $[Ru(bpy)_3]^{2+}$, which bind to DNA in an electrostatic binding mode, has little effect on DNA viscosity. On increasing the concentrations of Ru(II) complexes **4** and **5**, the relative viscosities of CT-DNA increase steadily, similarly to the behavior of EB. The increased degree of viscosity, which may depend on the DNA-binding mode and affinity, follows the order of **EB** > **5** > **4** > $[Ru(bpy)_3]^{2+}$. The results suggest that these two complexes could interact with DNA through a classical intercalative binding mode. Due to the more hydrophobic ability of co-ligand phen, complex **5** can intercalate into DNA base pairs deeper and thus show stronger DNA-binding affinity than complex **4**. The experiment results are consistent with the above spectroscopic results.

3.4. Steady-state emission studies

Emission spectroscopy is one of the most common and sensitive ways to investigate the interaction between complex and DNA. As shown in Fig. 3, in the absence of DNA, complex **4** showes negligible luminescence in buffer A at ambient temperature; while complex **5** can emit weak luminescence with maximum appearing at 578 nm. Upon the addition of CT-DNA, an obvious increase in emission intensity was observed for the two complexes, and a red shift of 11 nm was also observed for complex **5**. The emission intensity increases steadily to ca. 10.23 times of the original for complex **4**, and 1.96 times for complex **5** (Fig. 3). From Fig. 3(a), we can see that the complex $[Ru(bpy)_2(pidbp)]^{2+}$ emits negligible luminescence in Tris buffer at room temperature in the absence of DNA, and upon the addition of DNA, the 10.23-fold increase in emission intensity was observed. Obviously, complex $[Ru(bpy)_2(pidbp)]^{2+}$ can act as DNA "molecular light switch". Other examples of DNA "molecular light switch" have been reported previously, such as $[Ru(bpy)_2(dppz)]^{2+}$, $[Ru(bpy)_2(tpphz)]^{2+}$ and $[Ru(bpy)_2(btppz)]^{2+}$ [9,17,18,20] et al. These complexes function as "molecular light switches" in aqueous solution, exhibiting negligible luminescence in the absence of DNA and strong luminescence upon addition of DNA. The luminescence enhancement may be due to the protection of the phenazine nitrogens from solvent. To the best of our knowledge, it is the first example of Ru(II) complexes with asymmetric ligand, as DNA "molecular light switch". Although in most cases, the extent of the luminescence enhancement of Ru(II) complexes upon binding to DNA commonly (but not absolutely) parallels the intercalative binding strength, here, $[Ru(phen)_2(pidbp)]^{2+}$ with higher DNA affinities exhibits the lower luminescence enhancement compared to $[Ru(bpy)_2(pidbp)]^{2+}$. A possible explanation of this fact is due to the difference of the background luminescence between the two complexes. This observation is similar to that of $[Ru(phen)_2dppz]^{2+}$ and $[Ru(bpy)_2dppz]^{2+}$. The luminescence enhancement was found to be 3000 for $[Ru(phen)_2dppz]^{2+}$ with CT-DNA [46]. However, the enhancement factor was > 10^4 for $[Ru(bpy)_2dppz]^{2+}$ upon binding to DNA [10]. In addition, the titration results also indicate that two complexes can bind to DNA with high affinities and be protected by DNA efficiently, since the hydrophobic environment inside the DNA helix reduces the accessibility of solvent water molecules to the complex and the complex mobility is restricted at the binding site, leading to the decrease of vibration modes of relaxation.

Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher may provide further information about the DNA-binding properties of the two complexes and DNA. As shown in Fig. 4, in the absence of DNA, the emission of complex **5** were efficiently quenched by $[Fe(CN)_6]^{4-}$, complex **4** cannot be studied by emission quenching experiment in absence of DNA due to its negligible luminescence. However, in the presence of DNA, the emission of the two complexes were difficult to be quenched by $[Fe(CN)_6]^{4-}$. This may be explained by repulsion of the highly negative $[Fe(CN)_6]^{4-}$ from the DNA polyanion backbone which hinders access of $[Fe(CN)_6]^{4-}$ to the DNA-bound complexes [47]. The curvature reflects different extent of protection, a larger slope for the Stern–Volmer curve parallels poorer protection and lower binding. The results suggest that complex **5** binds to DNA more strongly than complex **4,** which is consistent with the results observed by electronic absorption titration (Fig. 1).

The competitive binding experiments were carried out using a molecular fluorophore ethidium bromide (EB) as a probe. The EB competitive binding experiments is a well-established assay based on the displacement of the intercalating drug EB from CT-DNA, and may provide more information about the DNA-binding mode and the DNA affinities of the complex. EB can emit strong fluorescence in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. If a complex could replace EB from DNA-bound EB, the fluorescence of the solutions would be efficiently quenched as free EB shows no apparent emission intensity in buffer A because of solvent quenching. Fluorescence quenching spectra of DNA-bound EB by Ru(II) complexes are shown in Fig. 5. On the excitation at 515 nm, both the two Ru(II) complexes in the absence and presence of DNA and free EB emit negligible fluorescence, therefore their emission has little influence on EB competitive binding experiment. As shown in Fig. 5, additions of the complexes to EB-DNA system resulted in sharp decreases in EB emission intensities. In the plot of percentage of quenching fluorescence, $(I_0 - I)/I_0$ versus [Ru]/[EB], we can see that 50% EB molecules were displaced from adjacent DNA base pairs at a concentration ratio of [Ru]/[EB] = 4.26

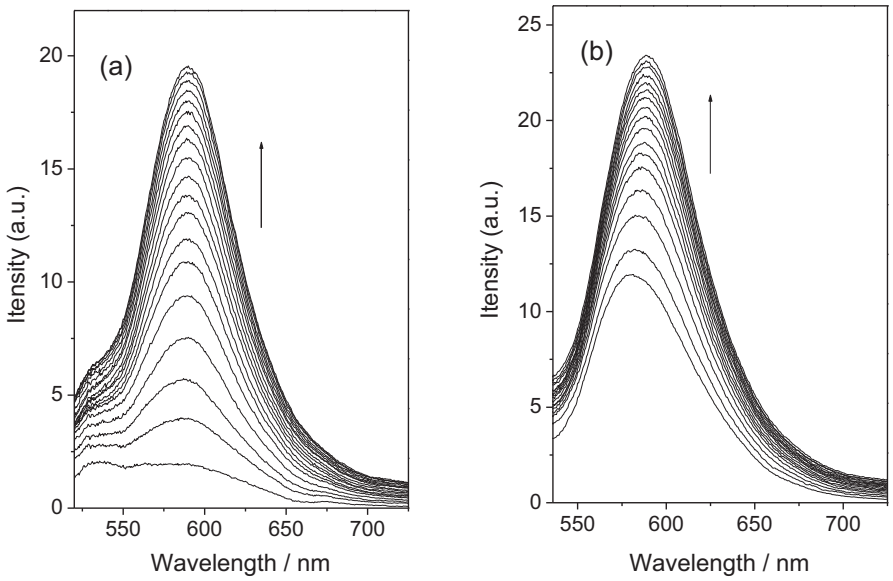


Fig. 3. Emission spectra of Ru(II) complexes (5 μM) **4** (a) and **5** (b) in Tris–HCl buffer at 298 K in the absence and the presence of CT-DNA. Arrow shows the intensity change upon the increase in DNA concentration.

for complex **4**, and 3.31 for complex **5**. By taking the DNA binding constant of $1.4 \times 10^6\ M^{-1}$ for EB [48,49], the apparent DNA binding constants K_{app} values of the two complexes were calculated according to Eq. (2) [50].

$$K_{app} = K_{EB}\left(\frac{[EB]_{50\%}}{[Ru]_{50\%}}\right) \quad (2)$$

where K_{app} is the apparent DNA binding constant of the Ru(II) complex, K_{EB} is the DNA binding constant of EB, and $[EB]_{50\%}$ and $[Ru]_{50\%}$ are the EB and Ru(II) complex concentrations at 50% fluorescence, respectively. The values are $3.29 \times 10^5\ M^{-1}$ for complex **4**, and $4.23 \times 10^5\ M^{-1}$ for complex **5**, respectively, which is slightly

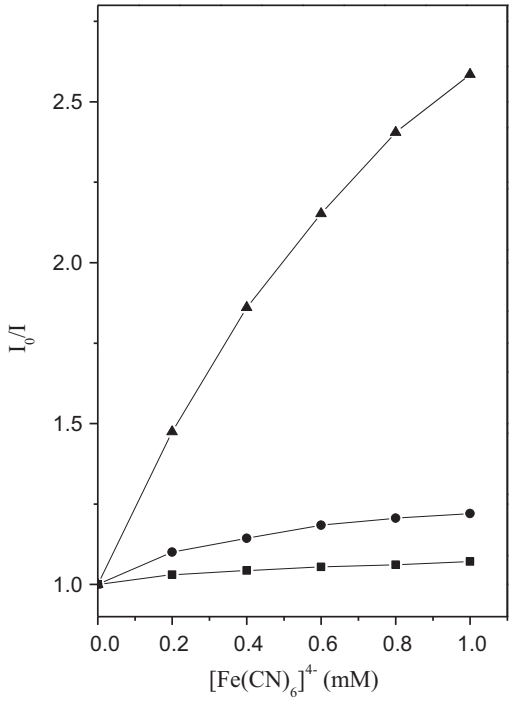


Fig. 4. Emission quenching curves of the complexes **4** + DNA (●), **5** (▲), **5** + DNA (■) with increasing concentration of $[Fe(CN)_6]^{4-}$. [Ru] = 5 μM, [DNA]/[Ru] = 40.

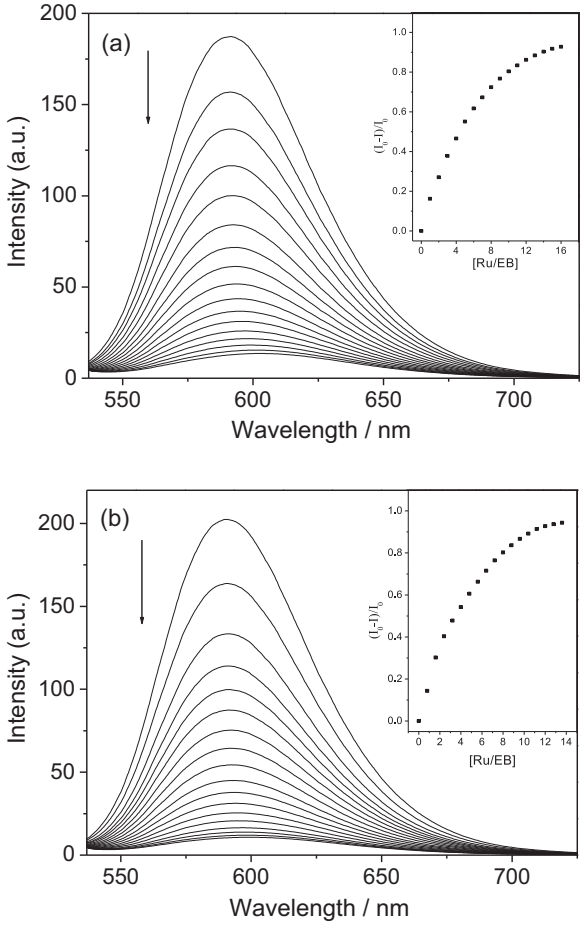


Fig. 5. Fluorescence quenching spectra of EB bound to DNA by Ru(II) complexes **4** (a) and **5** (b), [DNA] = 100 μM, [EB] = 5 μM. Arrow shows the intensity change upon increasing Ru(II) complexes concentration. Inset: plots of relative integrated fluorescence intensity vs. [Ru]/[EB].

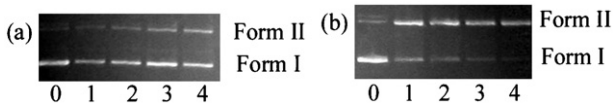


Fig. 6. Photoactivated cleavage of pBR322 DNA in the presence of Ru(II) complexes after 2 h irradiation at 365 nm. Lane 0, DNA alone; Lanes 1–4: complex **4** (a) and **5**(b) at 10, 20, 40 and 80 μM.

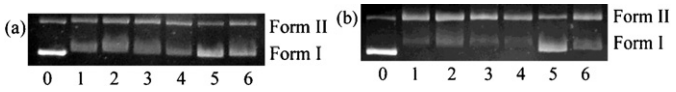


Fig. 7. Agarose gel showing Cleavage of pBR322 DNA incubated with Ru(II) complex **4** (a), **5**(b) and different inhibitors after 2 h irradiation at 365 nm, [Ru] = 80 μM. Lane 0:DNA alone, lane 1: DNA + Ru, lanes 2–6: DNA + Ru + 1 M DMSO, 100 mM mannitol, 1000 U ml^{-1} SOD, 25 mM NaN_3, 1.2 mM histidine.

smaller than the K_b values derived from the absorption spectral studies.

3.5. Photocleavage of pBR 322 DNA by Ru(II) complexes

Many Ru(II) complexes with polypyridyl ligands have been shown to cleave DNA under irradiation. Most of them, commonly known as "DNA photocleavers", are activated by light, and generate singlet oxygen, thus induce single-strand or double-strand cleavage of DNA [51]. Upon irradiation, the effective cleavage activity is attributed to the well-behaved redox-active and photochemical properties.

The abilities of the present complexes to cleavage DNA were studied by gel electrophoresis using supercoiled pBR322 DNA in TBE buffer (pH = 7.8). In general, when circular plasmid DNA is subjected to gel electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicked circulars), the supercoil will relax to generate a slower-moving nicked circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated [52].

Fig. 6 shows the results of the gel electrophoresis experiments carried out with supercoiled pBR322 DNA cleavage induced by various concentrations of the Ru(II) complexes under irradiation at 365 nm. No obvious DNA cleavage was observed for controls in the absence of the complex (Fig. 6: lane 0). The two complexes induced efficient DNA cleavage under irradiation as evidenced by the conversion of supercoiled to nicked circular form DNA. With increasing concentration of the Ru(II) complex **4** (Fig. 6 (a)) and **5** (Fig. 6 (b)), the amount of Form I of pBR322 DNA is decreased, whereas that of Form II is increased. And the increase in the amounts of nicked DNA was associated with the increase in the concentration of both complexes. Notably, under the same experimental conditions, when the concentration reached 80 μM, complex **5** can almost promote the complete conversion of DNA from Forms I to II. The DNA cleavage results show that both complexes **4** and **5** can cleave DNA upon irradiation and complex **5** exhibits a higher efficiency in DNA-photocleavage than complex **4**.

In order to determine the reactive species responsible for the DNA photocleavage of the two Ru(II) complexes, the mechanism experiments were performed in the presence of hydroxyl radical ($OH^{\bullet}$) scavengers [30,31,38] (DMSO and mannitol), singlet oxygen ($^1O_2$) scavengers [53] (NaN_3 and histidine), and a superoxide anion radical ($O_2^{\bullet -}$) scavenger (SOD). As shown in Fig. 7, NaN_3 and histidine (lanes 5, 6) efficiently inhibited the DNA cleavage activity of the two complexes, which suggest that singlet oxygen ($^1O_2$) is likely to be the cleaving agent. In the presence of other scavengers DMSO, mannitol or SOD, little inhibition was observed. These results indicated that superoxide anion radical ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$) were not indeed in the DNA cleavage of the Ru(II) complexes under irradiation, the mechanism of DNA cleavage is an oxidative process by generating singlet oxygen. Similar cases are found in other Ru(II) complexes [54,55].

4. Conclusions

In this work, a novel asymmetric ligand **3** and its Ru(II) complexes $[Ru(bpy)_2(pidbp)]^{2+}$ **4** and $[Ru(phen)_2(pidbp)]^{2+}$ **5** have been synthesized and characterized as potential complexes for DNA "light switch" and photocleavers. Various methods support the conclusion that both Ru(II) complexes can bind to DNA in an intercalative mode. Complex $[Ru(bpy)_2(pidbp)]^{2+}$ **4** exhibits it DNA "molecular light switch" properties. Furthermore, the two complexes are efficient DNA-photocleavers under irradiation at 365 nm, and complex **5** exhibits a stronger DNA-photocleavage efficiency than complex **4**. The mechanism experiments indicated that the singlet oxygen may play an important role in the DNA photocleavage of the two Ru(II) complexes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.saa.2011.11.014.

References

[1] A. Pyle, J.K. Barton, in: S.J. Lippard (Ed.), Progress in Inorganic Chemistry, vol. 38, 1990, pp. 413–475, New York.
[2] A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, vol. 33, Marcel Dekker, New York, 1996, pp. 177–252.
[3] L.N. Ji, X.H. Zou, J.G. Liu, Coord. Chem. Rev. 216–217 (2001) 513–536.
[4] B.M. Zeglis, V.C. Pierre, J.K. Barton, Chem. Commun. (2007) 4565–4579.
[5] J.C. Genereux, J.K. Barton, Chem. Rev. 110 (2010) 1642–1662.
[6] H.K. Liu, P.J. Sadler, Acc. Chem. Res. 44 (2011) 349–359.
[7] M.K. Nazeeruddin, C. Klein, P. Liska, M. Grätzel, M. Grätzel, Coord. Chem. Rev. 249 (2005) 1460–1467.
[8] S.S. Bhat, A.S. Kumbhar, P. Lönnecke, E. Hey-Hawkins, Inorg. Chem. 49 (2010) 4843–4853.
[9] C. Metcalfe, J.A. Thomas, Chem. Soc. Rev. 32 (2003) 215–224.
[10] R.M. Hartshorn, J.K. Barton, J. Am. Chem. Soc. 114 (1992) 5919–5925.
[11] S. Eyal, D.N. Hans, B.G. Harry, C.A. Fred, J. Am. Chem. Soc. 118 (1996) 1158–1163.
[12] K.A. O'Donoghue, J.M. Kelly, P.E. Kruger, Dalton Trans. (2004) 13–15.
[13] S. Shi, J. Liu, J. Li, K.C. Zheng, C.X.M. Huang, C.P. Tan, L.M. Chen, L.N. Ji, Dalton Trans. (2005) 2038–2046.
[14] J.H. Zhou, J.H. Liu, Y.Y. Feng, S.H. Wei, X.T. Gu, X.S. Wang, B.W. Zhang, Bioorg. Med. Chem. Lett. 15 (2005) 3067–3070.
[15] Y. Liu, A. Chouai, N.N. Degtyareva, S.A. Lutterman, K.R. Dunbar, C. Turro, J. Am. Chem. Soc. 127 (2005) 10796–10797.
[16] A.T. Steven, K. Roni, S. Dietrich, Inorg. Chem. 38 (1999) 5196–5197.
[17] A. Ambroise, B.G. Maiya, Inorg. Chem. 39 (2000) 4256–4263.
[18] Y.M. Chen, Y.J. Liu, Q. Li, K.Z. Wang, J. Inorg. Biochem. 103 (2009) 1395–1404.
[19] B.M. Zeglis, J.K. Barton, J. Am. Chem. Soc. 128 (2006) 5654–5655.
[20] E. Ruba, J.R. Hart, J.K. Barton, Inorg. Chem. 43 (2004) 4570–4578.
[21] A.E. Friedman, J.C. Chambron, J.P. Sauvage, N.J. Turro, J.K. Barton, J. Am. Chem. Soc. 112 (1990) 4960–4962.
[22] L.S. Ling, Z.H. He, G.W. Song, Y.E. Zeng, C. Wang, C.L. Bai, X.D. Chen, P. Shen, Anal. Chim. Acta 436 (2001) 207–214.
[23] L.S. Ling, Z.K. He, F. Chen, Y.-E. Zeng, Talanta 59 (2003) 269–275.
[24] A.P. DeSilva, N.D. McClenaghan, Chem. Eur. J. 10 (2004) 574–586.
[25] Y. Jiang, X. Fang, C. Bai, Anal. Chem. 76 (2004) 5230–5235.
[26] Y.J. Liu, H. Chao, L.F. Tan, Y.X. Yuan, W. Wei, L.N. Ji, J. Inorg. Biochem. 99 (2005) 700–708.
[27] Y.J. Liu, H. Chao, J.H. Yao, L.F. Tan, Y.X. Yuan, L.N. Ji, Inorg. Chim. Acta 358 (2005) 1904–1910.
[28] X.L. Hong, H. Li, C.H. Peng, J. Mol. Struct. 990 (2011) 197–203.

[29] X.H. Zou, B.H. Ye, H. Li, Q.L. Zhang, H. Chao, J.G. Liu, L.N. Ji, J. Biol. Inorg. Chem. 6 (2001) 143–150.
[30] J. Bolger, A. Gourdon, E. Ishow, J.P. Launay, Inorg. Chem. 35 (1996) 2937–2944.
[31] E. Ishow, A. Gourdon, J.P. Launay, Chem. Commun. (1998) 1909–1910.
[32] B.P. Sullivan, D.J. Salmon, T.J. Meyer, Inorg. Chem. 17 (1978) 3334–3341.
[33] F.C. Schaefer, G.A. Peters, J. Org. Chem. 26 (1961) 412–418.
[34] V.A. Milway, L. Zhao, T.S.M. Abedin, L.K. Thompson, Z.Q. Xu, Polyhedron 22 (2003) 1271–1279.
[35] G.W.H. Cheeseman, J. Chem. Soc. (1962) 1170–1176.
[36] F.E. Arnold, J. Polym. Sci. Part A 8 (1970) 2079–2089.
[37] J.B. Chaires, N. Dattagupta, D.M. Crothers, Biochemistry 21 (1982) 3933–3940.
[38] G. Cohen, H. Eisenberg, Biopolymers 8 (1969) 45–49.
[39] V.A. Milway, L. Zhao, T.S.M. Abedin, L.K. Thompson, Zh.Q. Xu, Polyhedron 22 (2003) 1271–1279.
[40] X.W. Liu, J.L. Lu, Y.D. Chen, L. Li, D.S. Zhang, Inorg. Chim. Acta 379 (2011) 1–6.
[41] J.D. McGhee, P.H. von Hippel, J. Mol. Biol. 86 (1974) 469–489.
[42] B.N. Rajesh, S.T. Emily, L.K. Shalawn, J.M. Catherine, Inorg. Chem. 37 (1998) 139–141.
[43] S. Satyanarayana, J.C. Dabroniak, J.B. Chaires, Biochemistry 31 (1992) 9319–9324.
[44] S. Satyanaryana, J.C. Daborusak, J.B. Chaires, Biochemistry 32 (1993) 2573–2584.
[45] J.K. Barton, J.M. Goldberg, C.V. Kumar, N.J. Turro, J. Am. Chem. Soc. 108 (1986) 2081–2088.
[46] C. Hiort, P. Lincoln, B. Norden, J. Am. Chem. Soc. 115 (1993) 3448–3454.
[47] C.V. Kumar, N.J. Turro, J.K. Barton, J. Am. Chem. Soc. 107 (1985) 5518–5523.
[48] M.J. Waring, J. Mol. Biol. 13 (1965) 269–282.
[49] J.B. LePecq, C. Paoletti, J. Mol. Biol. 27 (1967) 87–106.
[50] D.L. Boger, B.E. Fink, S.R. Brunette, W.C. Tse, M.P. Hedrick, J. Am. Chem. Soc. 123 (2001) 5878–5891.
[51] H.Y. Mei, J.K. Barton, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 1339–1343.
[52] J.K. Barton, A.L. Raphael, J. Am. Chem. Soc. 106 (1984) 2466–2468.
[53] F.R. Nilsson, P.B. Merkel, D.R. Kearns, Photochem. Photobiol. 16 (1972) 117–124.
[54] H.J. Yu, S.M. Huang, L.Y. Li, H.N. Ji, H. Chao, Z.W. Mao, J.Z. Liu, L.N. Ji, J. Inorg. Biochem. 103 (2009) 881–890.
[55] F. Gao, H. Chao, F. Zhou, Y.X. Yuan, B. Peng, L.N. Ji, J. Inorg. Biochem. 100 (2006) 1487–1494.