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Synthesis, DNA-binding and photocleavage studies of Ru(II) complexes of phenyl-(4,5,9,14-tetraaza-benzo[*b*]triphenylen-1,1-*yl*)-methanone

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Abstract. A novel polypyridyl ligand phenyl-(4,5,9,14-tetraaza-benzo[*b*]triphenylen-1,1-*yl*)-methanone (PTBM) and its complexes $[Ru(phen)_2(PTBM)]^{2+}$ (**1**) (phen = 1,10-phenanthroline) and $[Ru(bpy)_2(PTBM)]^{2+}$ (**2**) (bpy = 2,2′-bipyridine) have been synthesized and characterized by elemental analysis, mass spectroscopy, and ^1H NMR. The DNA-binding properties of the two complexes were investigated by spectroscopic and viscosity measurements. The results indicate that both complexes bind to DNA via an intercalative mode and the DNA-binding affinity of complex **1** is greater than that of complex **2**. When irradiated at 365 nm, complex **1** was found to be a more-effective DNA-cleaving agent than complex **2**.

Keywords. Ruthenium(II) complex; DNA-binding; photocleavage.

1. Introduction

During the last decade, the interaction between transition metal complexes and DNA has been extensively studied.[1–14] Binding studies of small molecules to DNA are very important in the development of new therapeutic reagents and DNA molecular probes.[15–18] Polypyridyl ruthenium(II) complexes can bind to DNA in a non-covalent interactions fashion such as electrostatic binding, groove binding,[19] intercalative binding and partial intercalative binding.[20] Many useful applications of these complexes require that the complexes bind to DNA through an intercalative mode. Therefore, the vast majority of such studies have been focused on the interaction of those complexes containing the fully planar ligands,[21–28] and investigations of such complexes with ligands containing substituent as DNA-binding reagents have been relatively few. In fact, some of these complexes also exhibit interesting properties on binding to DNA.[29–31] Varying substitutive group or subsistent position in the intercalative ligand can create some interesting differences in the space configuration and the electron density distribution of Ru(II) polypyridyl complexes, which will result in some differences in spectral properties and the DNA-binding behaviours of the complexes, and will be helpful to more clearly understand the binding mechanism of Ru(II) polypyridyl complexes to DNA. Therefore, further studies using different structural ligands to evaluate and understand the factors that determine the DNA-binding mode are necessary. Thus it is of interest to delineate the effects of the planarity of the intercalative ligand on interaction and the binding mode of the complexes to DNA.

We report here the synthesis and characterization of a new polypyridyl ligand PTBM (PTBM = Phenyl-(4,5,9,14-tetraaza-benzo[*b*]triphenylen-1,1-*yl*)-methanone) and its Ru(II) complexes $[Ru(phen)_2(PTBM)]^{2+}$ (**1**) (phen = 1,10-phenanthroline) and $[Ru(bpy)_2(PTBM)]^{2+}$ (**2**) (bpy = 2,2′-bipyridine) (scheme 1). The DNA-binding properties of the two Ru(II) complexes were explored by spectroscopic methods and viscosity measurements. Their photocleavage behaviours toward pBR 322 were also investigated. We hope the results to be of value in further understanding the DNA-binding and efficiency of DNA recognized and cleaved by Ru(II) complexes, as well as laying the foundation for the rational design of new photoprobes and photonucleases of DNA.

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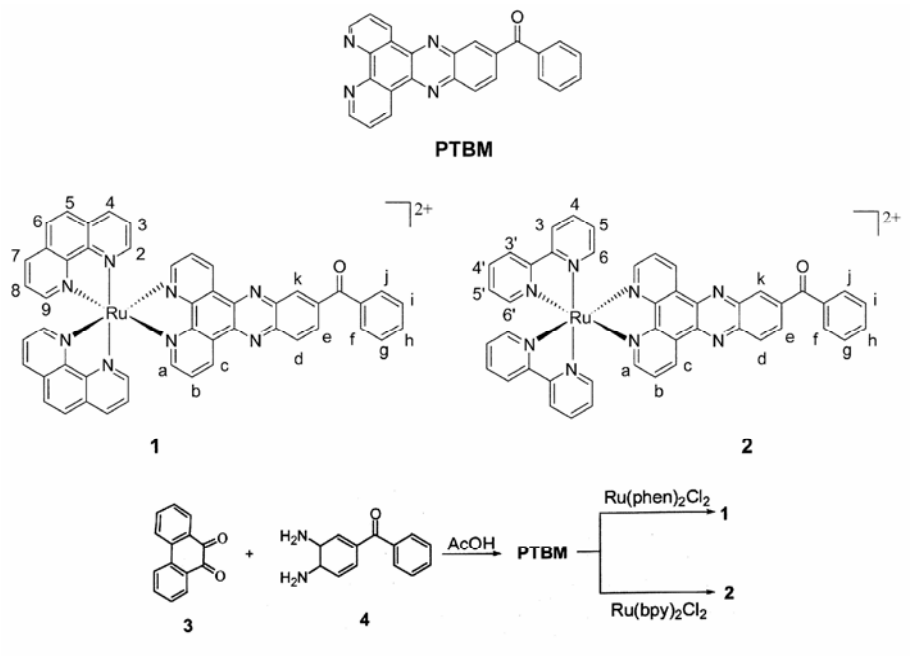

Scheme 1. Syntheses of the ligand PTBM and its Ru complexes **1** and **2**.

2. Experimental

2.1 *Materials*

All materials were commercially available and of reagent grade, and were used without further purification unless otherwise noted. The compounds 1,10-phenanthroline-5,6-dione,[32] *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ and *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$[33] were synthesized according to the literature methods. *Tris*–HCl buffer (5 mM *Tris*–HCl, 50 mM NaCl, pH 7·2, *Tris* = Tris(hydroxymethyl)methylamine) solution was prepared using doubly distilled water. The dialysis membrane was purchased from Union Carbide Co. and treated by means of general procedure before use. CT–DNA was obtained from the Sino-American Biotechnology Company.

2.2 *Synthesis of PTBM*

A mixture of (3,4-Diimino-cyclohexa-1,5-dienyl)-phenyl-methanone (425 mg, 2 mmol), 1,10-phenanthroline-5,6-dione (420 mg, 2 mmol), and glacial acetic acid (20 mL) was heated at reflux with stirring for 2 h. The cooled solution was filtered, diluted with H_2O and neutralized with concentrated aqueous ammonia. The yellow solid was collected and purified by column chromatography on alumina with ethanol–toluene (6 : 1, v/v) as eluent to give the title compound as amorphous yellow solid. Yield: 660 mg (85%).

Anal. Calc. for $C_{25}H_{14}N_4O$: C, 77·71; H, 3·65; N, 14·50. Found: C, 77·53; H, 3·82; N, 14·40. FAB-MS: *m/z* 387·6 [M+1].

2.3 *Synthesis of* $[Ru(phen)_2(PTBM)](ClO_4)_2\cdot 2H_2O$ **(1)**

A mixture of *cis*-$[Ru(phen)_2(Cl)_2]\cdot 2H_2O$ (114 mg, 0·2 mmol), PTBM (77·6 mg, 0·2 mmol), and ethylene glycol (15 mL) was thoroughly deoxygenated. The purple mixture was heated for 8 h at 150°C under argon atmosphere. When the solution finally turned salmon pink, it was cooled to room temperature, and an equal volume of saturated aqueous sodium perchlorate solution was added under vigorous stirring. The salmon pink solid was collected and washed with small amounts of H_2O, EtOH, and Et_2O, dried under vacuum, and purified by CC (neutral Alox; MeCN/toluene 1 : 1) to afford 209 mg (39%) of the title compound.

UV (ε/dm^3 mol^{-1} cm^{-1}, MeCN): 440 (47190), 376 (31050), 281 (238860), 264 (251730), ^{1}H NMR (400 MHz, DMSO-d_6 (dimethyl sulfoxide); *d*, doublet; *s*, singlet; *t*, triplet; *m*, multiplet): 9·64 (*q*, 2H), 8·81 (*t*, 4H), 8·72 (*s*, 1H), 8·68 (*d*, 1H), 8·48 (*d*, 1H), 8·42 (*s*, 4H), 8·30 (*q*, 2H), 8·23 (*q*, 2H), 8·08 (*d*, 2H), 7·96 (*d*, 2H), 7·91 (*q*, 2H), 7·78–7·86 (*m*,

2H), 7·81 (*m*, 3H), 7·69 (*t*, 4H). ESI–MS (MeCN): *m/z* 963·4 ($[M–ClO_4]^+$), 864·1 ($[M–2ClO_4–H]^+$), 432·3 ($[M–2ClO_4]^{2+}$). Anal. Calc. for $C_{45}H_{34}N_8Cl_2O_{11}Ru$: C, 54·34; H, 3·14; N, 10·35. Found: C, 54·44; H, 2·99; N, 10·37.

2.4 *Synthesis of [Ru(bpy)$_2$(PTBM)](ClO$_4$)$_2$·2H$_2$O* (**2**)

Prepared analogous to **1**, but from *cis*-$[Ru(bpy)_2(Cl)_2]·2H_2O$ (104 mg, 0·2 mol). Yield: 299 mg (44%).

UV (ε/dm^3 mol^{-1} cm^{-1}, MeCN): 442 (38010), 363 (41695), 264 (288110). ^{1}H NMR (400 MHz, DMSO-d_6): 9·67 (*q*, 4H), 8·89 (*t*, 4H), 8·73 (*s*, 1H), 8·68 (*d*, 1H), 8·49 (*d*, 1H), 8·29 (*t*, 2H), 8·25 (*t*, 2H), 8·16 (*t*, 2H), 8·07 (*t*, 2H), 8·03 (*q*, 1H), 7·97 (*d*, 2H), 7·85 (*d*, 2H), 7·81 (*t*, 2H), 7·70 (*t*, 2H), 7·62 (*t*, 2H), 7·42 (*t*, 2H). ESI–MS (MeCN): *m/z* 920·6 ($[M–ClO_4]^+$), 820·9 ($[M–ClO_4\ H]^+$), 410·6 ($[M–2ClO_4]^{2+}$). Anal. Calc. for $C_{49}H_{34}N_8Cl_2O_{11}Ru$: C, 52·22; H, 3·28; N, 10·83. Found: C, 52·32; H, 3·12; N, 10·85.

2.5 *Physical measurements*

Microanalyses (C, H and N) were carried out on a Perkin-Elmer 240Q elemental analyzer. ^{1}H NMR spectra were recorded on an Avancev-400 spectrometer with DMSO-d_6 (dimethyl sulfoxide) as the solvent at room temperature and TMS (tetramethylsilane) as the internal standard. UV-Vis (UV-visible) spectra were recorded on a Perkin-Elmer Lambda-25 spectrophotometer, and emission spectra were recorded on a Perkin-Elmer LS-55 luminescence spectrometer at room temperature. Electrospray mass spectra (ES–MS) were recorded on a LQC system (Finngan MAT, USA) using CH_3CN as the mobile phase. Circular dichroism (CD) spectra were measured on a JASCO-J715 spectropolarimeter.

All spectroscopic titrations were carried out in Tris–HCl buffer at room temperature. A solution of CT–DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of 1·8–1·9 : 1, indicating that the DNA was sufficiently free of protein.[34] The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm.[35] Stock solutions were stored at 4°C and used within 4 days. Titration experiments were performed by using a fixed Ru(II) complex concentration, The complex-DNA solutions were allowed to equilibrate for 5 min before spectra were recorded.

Viscosity measurements were carried out with an Ubbelodhe viscometer maintained at a constant temperature of 26·0 ± 0·1°C in a thermostated bath. DNA samples of ca. 200-bp average length were prepared by sonication.[36] The flow time was measured with a digital stopwatch, and each sample was tested three times to get an average calculated flow time. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio,[37] where η is the viscosity of DNA in the presence of complex, η_0 being the viscosity of free DNA.

For the gel-electrophoresis experiments, supercoiled pBR-322 DNA (0·1 μg) was treated with **1** or **2** in buffer Tris–HCl (50 mM Tris-acetate, 18 mM NaCl buffer, pH 7·2). After pre-incubation of the solutions in the dark for 1 h, the samples were irradiated for 60 min inside the sample chamber of the spectrofluorimeter (λ_{ex} = 365 ± 5 nm, slit-width = 5 nm). The samples were then analysed by gel-electrophoresis for 30 min at 75 V in Trisacetate buffer containing 1% agarose gel. The gel was stained with ethidium bromide (EB; 1 μg/mL^{-1}) and photographed under UV light.

3. Results and discussion

3.1 *Synthesis and characterization*

An outline of the synthesis of Ru(II) complexes **1** and **2** with the new PTBM ligand are presented in scheme 1. The ligand PTBM was prepared through condensation of 1,10-phenanthroline-5,6-dione (**3**) with 3,4-diaminobenzophenone (**4**) on the basis of the method for dppz ring according to literature methods.[38] Complexes **1** and **2** were then prepared in yields of 39 and 44%, respectively, by direct reaction of PTBM with *cis*-$[Ru(phen)_2Cl_2]2H_2O$ and *cis*-$[Ru(bpy)_2Cl_2]·2H_2O$, respectively, in the appropriate molar ratios, using ethylene glycol as solvent. The desired Ru(II) complexes were isolated as the corresponding perchlorates, and were purified by column chromatography. In the ESI-MS spectra of both complexes **1** and **2**, the signals of ($[M–ClO_4]^+$), ($[M–2ClO_4–H]^+$), and ($[M–2ClO_4]^{2+}$) were observed, and the determined molecular weights were consistent with the expected values.

Both complexes **1** and **2** give well-defined ^{1}H NMR spectra (figure 1), permitting unambiguous identification and assessment of purity. The ^{1}H NMR chemical shifts were assigned by the aid of ^{1}H, ^{1}H–COSY experiments, and by comparison with

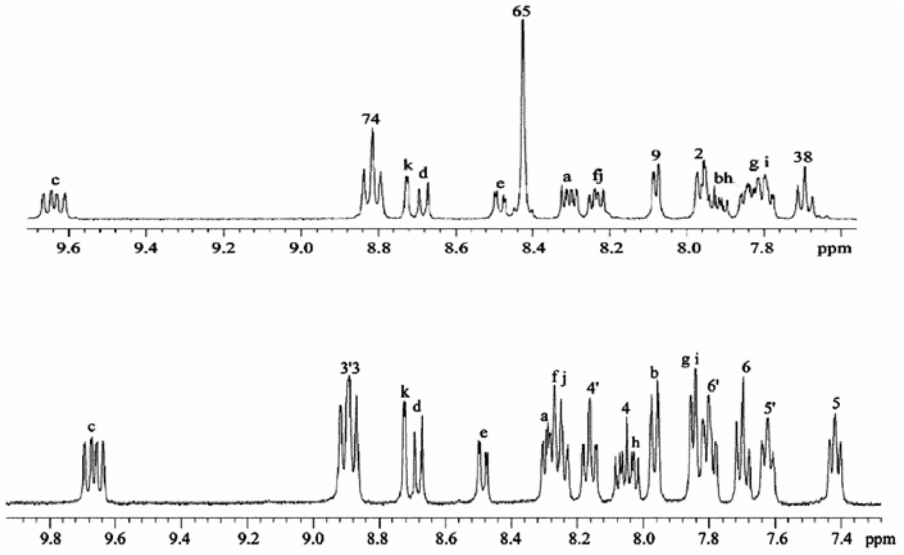

Figure 1. ^{1}H NMR spectra of complexes **1** (top) and **2** (bottom).

the values of similar compounds.[21,39] Due to the shielding influences of the adjacent PTBM and phen (or bpy) moieties, H-atoms of complexes **1** and **2** exhibit two distinct sets of signals.

The UV/VIS absorption spectra of **1** and **2** showed well-resolved bands in the range 200–700 nm, characterized by intense $\pi \rightarrow \pi^*$ ligand transitions in the UV, as well as by metal-to-ligand charge transfer (MLCT) transition in the VIS. The broad MLCT absorption bands appear at 440 nm (for complex **1**) and 442 nm (for complex **2**), respectively, which are attributed to Ru(dπ) $\rightarrow$ PTBM (π^*) transitions.

3.2 *Electronic absorption titration*

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques.[40] Complex binding with DNA through intercalation usually result in hypochromism and bathchromism, due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The extent of the hypochromism commonly parallels the intercalative binding strength. The absorption spectra of complexes **1** and **2** in the absence and presence of CT–DNA (at a constant concentration of complex) are given in figure 2. As the concentration of DNA is increased, for complex **1**, the hypochromism reaches about 25·2% at 267 nm with a red shift of 4 nm at a [DNA]/[Ru] ratio of 5·06. The MLCT band at 443 nm shows hypochromism by about 25·7% and a red shift of 4 nm under the same conditions. For complex **2**, on addition of DNA, the IL band at about 288 nm exhibit hypochromism of 31·2% with a 2 nm red shift at a [DNA]/[Ru] ratio of 3·62. The MLCT band at 443 nm shows hypochromism by about 16·9% and a red shift of 2 nm under the same conditions. Comparing the hypochromism of the two complexes with that of their parent complex $[Ru(phen)_3]^{3+}$ (hypochromism in MLCT band at 445 nm of 12% and red shift of 2 nm (15), which interacts with DNA through a semi-intercalation or quasi-intercalation (19(b)) and considering that the absorption spectrum of $[Ru(bpy)_3]^{2+}$, a typical electrostatic binding complex, was demonstrated to be unchanged on the addition of the DNA,[18] these spectral characteristics obviously suggest that both **1** and **2** in our paper interact with DNA most likely through a mode that involves a stacking interaction between the tail-end phenyl chromophore and the base pairs of DNA.

In order to compare quantitatively the binding strength of the two complexes, the intrinsic binding constants K_b of the two complexes with DNA were obtained by monitoring the changes in absorbance at 267 nm for complex **1** and 288 nm for complex **2** with increasing concentration of DNA using the following equation:[41]

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

wherein [DNA] is the concentration of DNA in base pairs, ε_a, ε_f, and ε_b are the apparent-, free- and

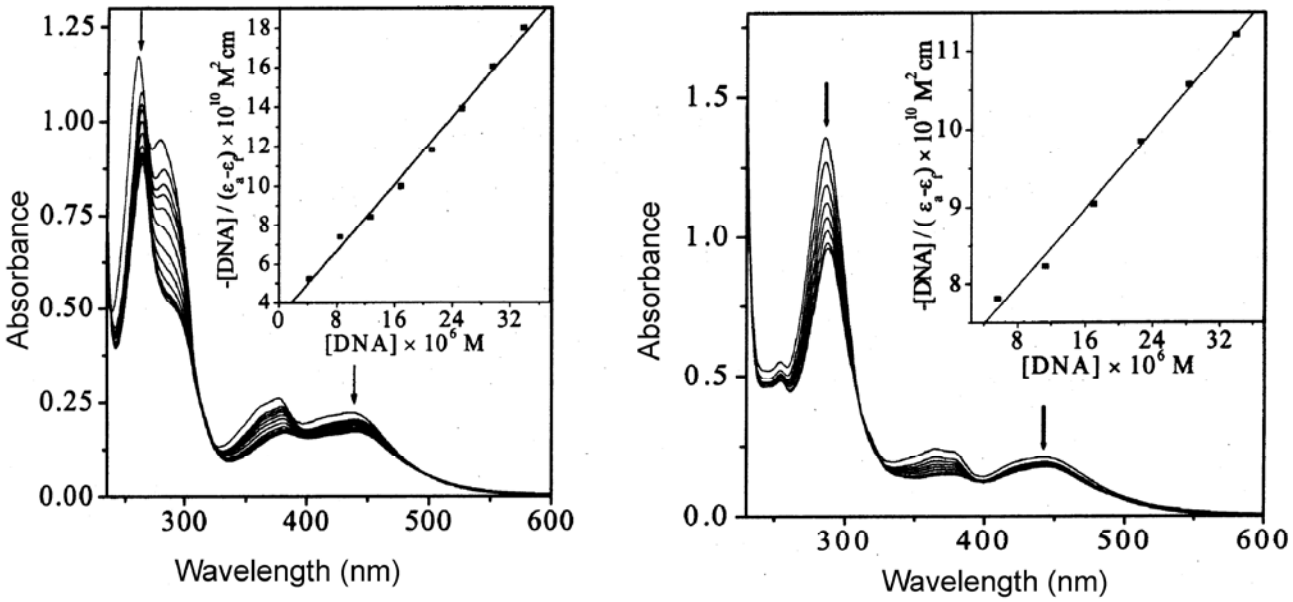

Figure 2. Absorption spectra of complex **1** (left) and **2** (right) in *Tris*–HCl buffer upon addition of CT–DNA. Left: [Ru] = 1×10^5 M, [DNA] = (0–5·06) $\times 10^{-5}$ M. Right: [Ru] = 1·25 $\times 10^5$ M, [DNA] = (0–3·62) $\times 10^{-5}$ M. Arrow shows the absorbance changing upon increasing DNA concentrations. Inset: plots of [DNA]/(ε_a–ε_f) vs [DNA] for the titration of DNA with the complex.

bound-metal-complex extinction coefficients respectively. K_b is the equilibrium binding constant (in M^{-1}) of complex binding to DNA. When plotting [DNA]/(ε_a–ε_f) vs [DNA], K_b is obtained by the ratio of the slope to the intercept. The intrinsic binding constants K_b of complexes **1** and **2** were (1·30 ± 0·05) $\times 10^5$ M^{-1} and (4·54 ± 0·05) $\times 10^4$ M^{-1}, respectively The value is smaller than those of $[Ru(phen)_2(dppz)]^{2+}$ (>10^6 M^{-1}),[11] $[Ru(bpy)_2(dppz)]^{2+}$ (dppz = 7,8-dimethyldipyridophenazine, 8·8 $\times 10^6$ M^{-1}),[42] $[Ru(phen)_2(phehat)]^{2+}$ (phehat = 1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene, 2·5 $\times 10^6$ M^{-1}).[43] However, the value is comparable to those of some other reported Ru(II) complexes with extended, planar ligands, $[Ru(bpy)_2(dpq)]^{2+}$ (dpq = dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline) and $[Ru(bpy)_2(dpqc)]^{2+}$ (dpqc = dipyrido-6,7,8,9-tetrahydrophenazine) show much low affinity for DNA (5·9 $\times 10^4$ M^{-1} and 8·5 $\times 10^4$ M^{-1}) than $[Ru(bpy)_2(dppz)]^{2+}$ due to less -conjugated aromatic area;[44] $[Ru(bpy)_2(taptp)]^{2+}$ (taptp = 4,5,9,18-tetraazaphenanthreno[9,10-*b*]triphenylene) and $[Ru(bpy)_2(atatp)]^{2+}$ (atatp = acenaphtheno[1,2-*b*]-1,4,8,9-tetraazatriphenylene) also show smaller DNA affinity (1·7 $\times 10^5$ M^{-1} and 7·6 $\times 10^4$ M^{-1}) than $[Ru(phen)_2(dppz)]^{2+}$, the reason is proposed that the size of the ligands are too wide.[5] This data indicates that the size and the shape of the intercalated ligand has a significant effect on the strength of DNA binding, and the most suitable intercalating ligand leads to the highest affinity of complexes with DNA. The different DNA-binding properties of complexes **1** and **2** are due to the ancillary ligand. On going from bpy to phen, the plane area and hydrophobicity increased, which led to a greater binding affinity to DNA.

3.3 *Luminescence titration studies*

In organic solvents such as MeCN, MeOH, and EtOH, complexes **1** and **2** emit luminescence. The results likely suggest that the more polar the solvent is, the smaller relative intensities are observed. This phenomenon has also been found with the Ru(II) dppz complexes under the similar conditions.[45] At the same time, it is noteworthy that the luminescence of complexes **1** and **2** in these organic solvents are very sensitive to the presence and concentration of water, being almost quenched completely in the presence of 10% water (v/v). Thus, the lack of luminescence in water for both complex may be due to a mechanism similar to that for $[Ru(phen)_2(dppz)]^{2+}$.

In the absence of DNA, complexes **1** and **2** emit weak luminescence in Tris buffer at room temperature, with a maximum appearing at 613 and 607 nm,

respectively. However, on addition of CT–DNA, the emission intensities of complexes **1** and **2** increase slightly (figure 3). The emission intensities of complexes **1** and **2** increase to around 1·56 and 1·40 times larger than the original, respectively. This implies that both complexes can interact with DNA and be protected by DNA. Comparing **1** with **2**, it has been found that complex **1** can be protected by DNA more effectively, which is due to the stronger binding affinity of the complex with DNA.

Steady-state completive binding experiment using complex **1** or **2** as quencher may give further information about the binding of complexes **1** and **2** to DNA. Ethiduim bromide (EB) emits intense fluorescence light in the presence of DNA, due to its strong intercalation between adjacent DNA base pairs. It was previously reported that the enhanced fluorescence can be quenched, at least partially, by the addition of a second molecule.[46] The quenching extent of fluorescence of EB bound to DNA is used to determine the extent of binding of the second molecule to DNA. The emission spectra of EB bound to DNA

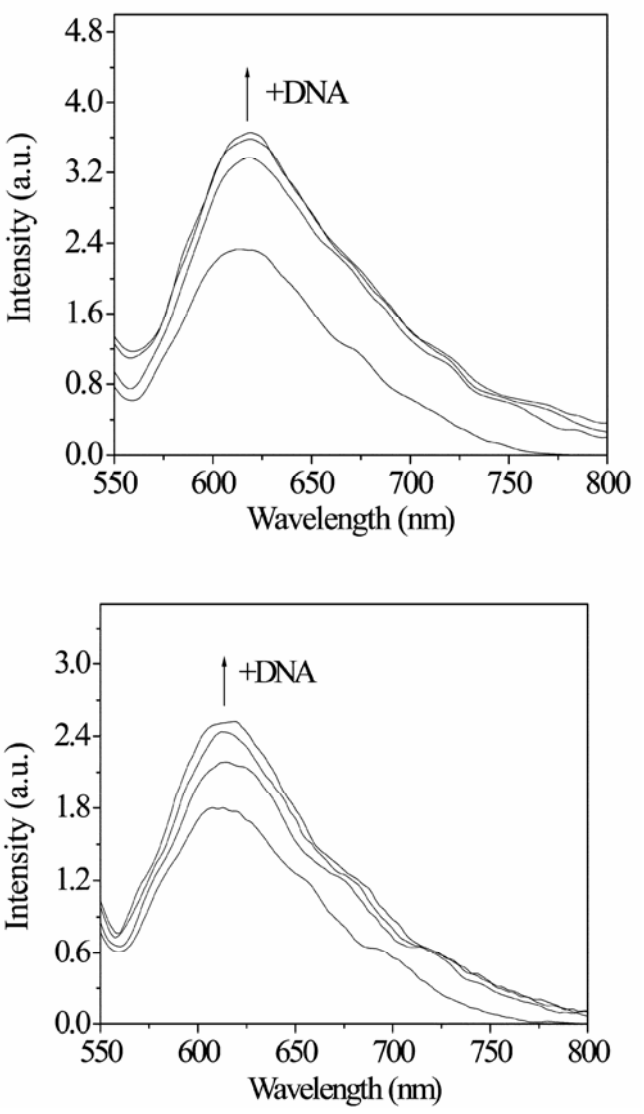

Figure 3. Emission spectra of complexes **1** (top) and **2** (bottom) in Tris–HCl buffer in the absence and presence of CT–DNA. Arrow shows the intensity change on increasing DNA concentrations.

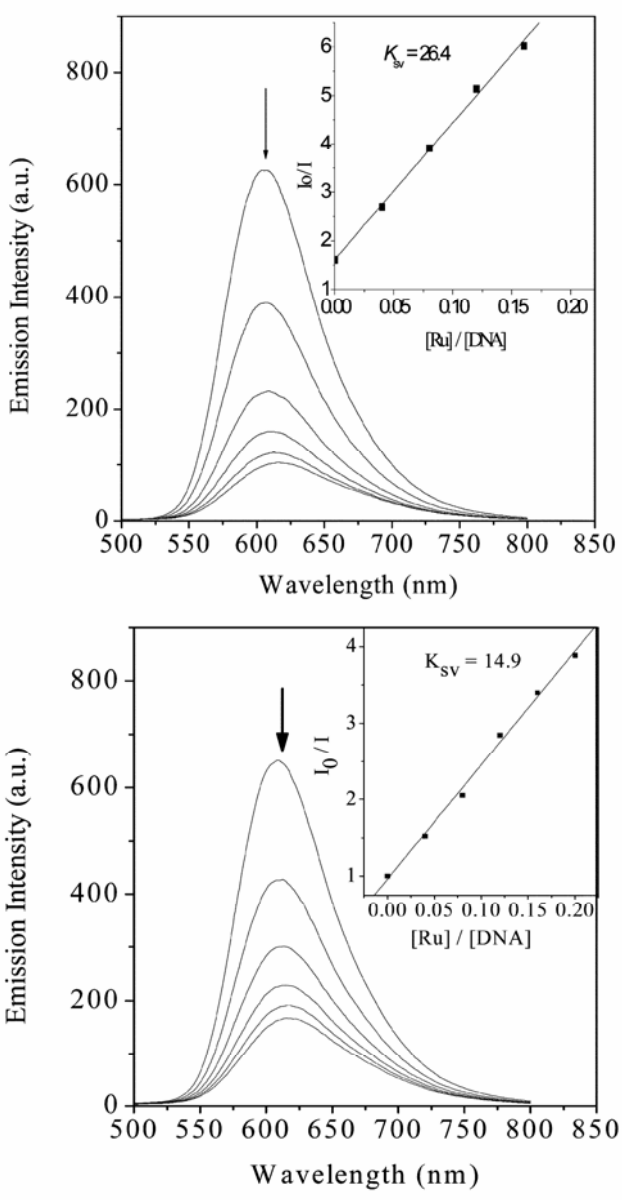

Figure 4. Emission spectra of EB bound to DNA in presence of the complexes **1** (top, λ_{ex} = 461 nm) and **2** (bottom, λ_{ex} = 458 nm). [EB] = 20 μM, [DNA] = 100 μM; [Ru]/[DNA] = 0·00, 0·04, 0·08, 0·12, 0·16. The arrows show the intensity changes on increasing concentrations of the complexes. Inset: plots of I_0/I vs [Ru]/[DNA].

in the absence and presence of **1** or **2** to DNA, pretreated with EB, causes appreciable reduction in emission intensity of 83·4% for **1** and 74·3% for **2**, respectively, at a [Ru]/[EB] ratio of 1 : 1. The quenching plot (inset in figure. 4) illustrates that the removal of EB bound to DNA by complex **1** or **2** is in good agreement with the linear Stern–Volmer equation. The Stern–Volmer constants *K* (*K* is a liner Stern–Volmer quenching constant dependent on the ratio of the bound concentration of ethidium bromide to the concentration of DNA) of complexes **1** and **2** were, thus, determined as 26·4 and 14·9, respectively, which is consistent with the UV/Vis-titration results (see above). These results suggest that *t* complex **1** intercalated more strongly than complex **2**.

3.4 *Viscosity measurements*

The measurement of viscosity of DNA is regarded as the least ambiguous and the most critical test of a DNA binding model in solution in the absence of Crystallographic structural data[47,48] and provides stronger arguments for inter-calative DNA binding mode. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to the increase of the viscosity of the DNA solution.[47,48] However, a partial and/or non-classical intercalation of ligand may bend (or link) DNA helix, resulting in the decrease of its effective length and its viscosity.

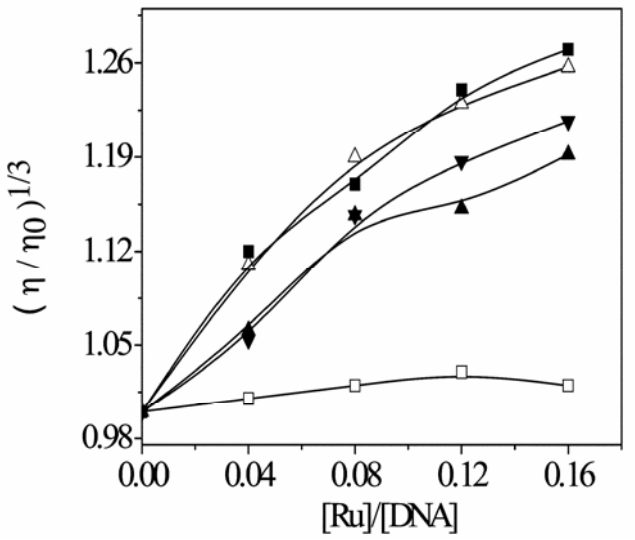

Figure 5. Effect of increasing amounts of ethidium bromide (■), $[Ru(bpy)_3]^{2+}$ (□), $[Ru(bpy)_2dppz]^{2+}$ (Δ), complex **1** (▼) and complex **2** (▲) on the relative viscosity of CT–DNA at 26 ± 0·1°C. The total concentration of DNA is 0·5 mM.

The effects of complexes **1** and **2**, together with $[Ru(bpy)_3]^{2+}$, $[Ru(bpy)_2dppz]^{2+}$ and ethidium bromide (EB) on the viscosity of rod-like DNA, are shown in figure 5. As expected, EB increases the relative specific viscosity for the lengthening of the DNA double helix resulting from intercalation. For complex $[Ru(bpy)_3]^{2+}$, which has been known to bind with DNA in electrostatic mode, it exerts essentially no effect on DNA viscosity. However, on increasing the amounts of complexes **1** and **2**, the relative viscosity of DNA increases steadily. The incorporation of benzoyl group into the dppz ligand may cause steric hindrance when complexes **1** and **2** containing ligand PTBM interact with DNA. Therefore, $[Ru(bpy)_2dppz]^{2+}$ could intercalate more deeply and tightly into adjacent DNA base pairs than complexes **1** and **2**, Thus, the increased degree of viscosity, which may depend on its affinity to DNA follow the order of EB > $[Ru(bpy)_2dppz]^{2+}$ > **1** > **2** > $[Ru(bpy)_3]^{2+}$. These results suggest that complexes **1** and **2** both bind to DNA through intercalation, and the difference in binding strength probably being caused by the different ancillary ligands.

3.5 *Enantioselective binding*

Equilibrium-dialysis experiments offer the opportunity to examine the enantioselectivity of complexes binding to DNA. According to the proposed binding model by Barton and co-workers,[49] the Δ enantiomer of the complex, a right-handed propeller-like structure, displays a greater affinity than the Λ enantiomer with the right-handed CT–DNA helix due to more appropriate steric matching. We, thus, decided to test if the racemic complexes could be (partly) resolved in the presence of chiral CT–DNA. To this end, racemic solutions of the two complexes were dialyzed against CT–DNA for 42 h, and then subjected to circular-dichroism (CD) analysis. In figure 6, the CD spectra in the UV region of the dilysates of **1** or **2** are shown. The dilysates of **1** (dotted line) shows two CD signals with a positive peak at 269 nm and a negative peak at 291 nm, while complex **2** (solid line) shows weak CD signals with a positive peak at 273 nm and a negative peak at 289 nm, respectively.

Although neither of the complexes was resolved into the pure enantiomers, and we can not determine

which enantiomer binds preferentially to CT–DNA, it is evident that both **1** and **2** interact enantioselectively with CT–DNA. The stronger CD signals of complex **1** suggest a large DNA-binding discrimination between its two antipodes. Since the intercalative ligands of **1** and **2** are the same, the difference should, again, be attributable to the ancillary ligands. These results, thus, clearly indicate that ancillary ligands can have a significant effect on the DNA-binding discrimination.

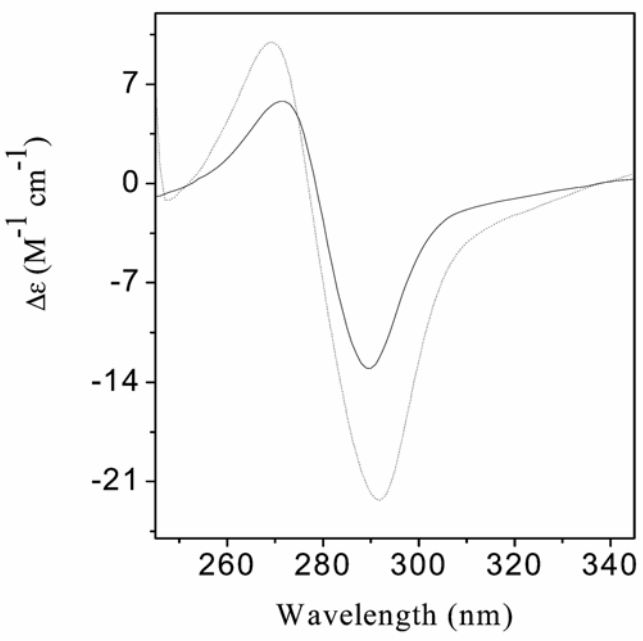

Figure 6. CD spectra of the dialyzate of complex **1** (dotted line) and **2** (solid line) after 42 h of dialysis against CT–DNA with solution stirred.

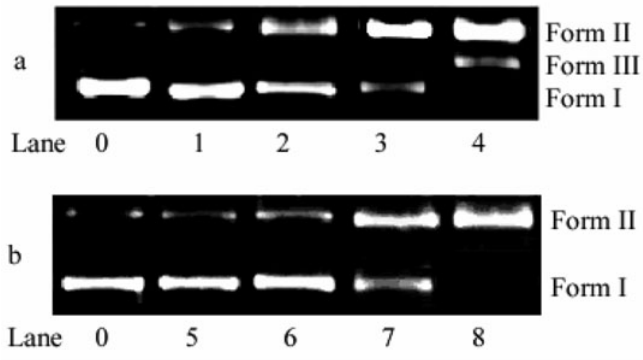

Figure 7. Photo-activated cleavage of pBR 322 DNA in the presence of complexes **1** and **2**, light after 30 min irradiation at 365 ± 5 nm. Lanes 0, DNA alone; lanes 1–4 in the different concentrations of complex **1**: (1) 5; (2) 10; (3) 15; (4) 20 μM; lanes 5–8, in the different concentrations of complex **2**: (1) 5; (2) 10; (3) 15; (4) 20 μM.

3.6 *Photoactivated cleavage of pBR-322 DNA by Ru(II) complexes* **1** *and* **2**

The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively, fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower-moving open circular form (Form Π). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form Π will be generated.[50]

In figure 7, the gel electrophoresis pattern of pBR 322 DNA is shown after incubation with complex **1** or **2** and irradiation at 365 ± 5 nm. No DNA cleavage was observed for the control in which metal complex was absent (lane 0). With increasing concentration of the Ru(II) complexes (lanes 1–4, 5–8), the amount of Form I pBR 322 DNA was diminished gradually, whereas Form-II increases and Form-III is also produced (lane 4). Under comparable experimental conditions, complex **1** exhibits more effective DNA cleavage activity than complex **2**. This may be related to the molecular structure of these complexes, which has been testified in other case.[51] Although DNA photocleavage by $[Ru(phen)_3]Cl_2$ has been reported to involve an $^1O_2$-based mechanism,[52] the nature of the reactive intermediates involved in the efficient DNA photocleavage by complexes **1** and **2** observed here is not clear. Further studies in detail are currently underway to clarify the cleavage mechanism.

4. Conclusions

Two new Ru(II) complexes $[Ru(phen)_2(PTBM)]^{2+}$ and $[Ru(bpy)_2(PTBM)]^{2+}$ have been synthesized and characterized. Spectroscopic studies and viscosity experiments supported that both complexes can intercalate into DNA via PTBM ligand. The CD signals of both complexes indicate that different structural ancillary ligands and binding affinity have important effects on the enantioselectivity of binding to DNA of polypyridyl Ru(II) complexes. When irradiated at 365 nm, complex **1** was found to be a more-effective DNA-cleaving agent than complex **2**.

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