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Experimental and theoretical study on DNA-binding and photocleavage properties of chiral complexes Δ- and Λ-[Ru(bpy)$_2$L] (L = *o*-hpip, *m*-hpip and *p*-hpip)†

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In order to systematically perform an experimental and theoretical study on DNA-binding and photocleavage properties of chiral complexes Δ- and Λ-[Ru(bpy)$_2$L] (L = *o*-hpip, *m*-hpip and *p*-hpip) on the basis of reported Δ- and Λ-[Ru(bpy)$_2$(*o*-hpip)] (Δ-**1** and Λ-**1**), a series of novel enantiomerically pure polypyridyl ruthenium(II) complexes, Δ- and Λ-[Ru(bpy)$_2$(*m*-hpip)](PF$_6$)$_2$ (Δ-**2** and Λ-**2**; bpy = 2,2′-bipyridine, *m*-hpip = 2-(3-hydroxyphenyl)-imidazo[4,5-*f*][1,10]phenanthroline), and Δ- and Λ-[Ru(bpy)$_2$(*p*-hpip)](PF$_6$)$_2$ (Δ-**3** and Λ-**3**, *p*-hpip=2-(4-hydroxyphenyl)-imidazo[4,5-*f*][1,10]phenanthroline), have been synthesized and characterized by elemental analysis, ^{1}H NMR, ESI-MS and CD spectra. The DNA-binding properties of these complexes have been investigated with UV-Vis, emission spectra, CD spectra and viscosity measurements. It is experimentally found that (1) both complexes [Ru(bpy)$_2$(*m*-hpip)]$^{2+}$ **2** and [Ru(bpy)$_2$(*p*-hpip)]$^{2+}$ **3** can bind to DNA with intercalation; (2) for complexes **2** and **3**, a subtle but detectable difference was observed in the interaction of these isomers with CT-DNA. The DNA-binding of the Δ-isomer is stronger than that of Λ-isomer, whereas that of Λ-isomer is swifter. (3) Under irradiation with UV light, Ru(II) complexes **2** and **3** can promote almost complete conversion of pBR322 DNA from form I to form II at concentrations of 0.5, 1.0, 0.5, 1.0 (× 10^{-4}M) for Δ-**2**, Λ-**2**, Δ-**3** and Λ-**3**, respectively. On the other hand, theoretical calculations for these three isomer complexes have been carried out applying the density functional theory (DFT) method on the level of the B3LYP/LanL2DZ basis set. Some frontier molecular orbital energies and stereographs, as well as a schematic diagram of the energies and related ^{1}MLCT transitions of [Ru(bpy)$_2$L]$^{2+}$ are presented, and applied to reasonably explain the obtained experimental regularities or trends in the DNA-binding strength or binding constants (K_b) and some spectral properties of the complexes.

Introduction

The interaction of ruthenium(II) polypyridine complexes with double-strand DNA has been studied for many years.[1–3] This interaction can be attributed to the special redox properties, and special photophysical and photochemical properties of ruthenium complexes, and their potential utility as DNA probes, molecular light switches, in chemotherapy and photodynamic therapy (PDT).[4–11] Therefore, a great deal of interest is concentrated on the functional molecular design of ruthenium complexes binding to DNA, the binding mechanism and electron transfer between the complexes in DNA medium. Since each octahedral polypyridyl Ru(II) complex is formed from a central metal ion and three polypyridyl ligands with conjugated π bonds, in which there are two N atoms as coordination points in each ligand, the whole complex is a very large conjugated molecule,[12] and thus modifying the polypyridine ligands or changing subsitituent sites on the main ligands can create some interesting differences in the properties of the resulting complexes. Recently, many new Ru(II) polypyridine complexes and their substituted derivatives have been designed, synthesized and characterized.[13–19] Some types of DNA-binding modes have been proposed and further improved.[20–24]

On the other hand, Ru(II) polypyridine complexes have attracted many theoretical chemists' attention. In order to correlate experimental findings with theoretical predictions, more and more theoretical computations, in particular, computations applying the DFT method[25–28] on Ru(II) and other transition metal complexes have been reported,[29–39] because DFT can better consider electron correlation energies, obviously reduce the computation expenses and suit complexes in the singlet state.[32–35] Recently, Rillema and coworkers suggested that the HOMO and LUMO distributions for Ru(II) two-ring diimine complex cations from DFT calculations support the idea that the lowest energy transitions are metal-to-ligand charge transfer and that the LUMO for the mixed ligand complexes is located on the ligands.[29] Zhang *et al.* reported hydrolysis theory for cisplatin and its analogues based on density functional studies.[30] Kurita and Kobayashi further reported density functional MO calculations for stacked DNA base-pairs with backbones.[31] We also reported the studies on disubstitution effects, electron structures and related properties in some Ru(II) polypyridyl complexes with the DFT method.[32–35] These direct theoretical efforts on the level of molecular electronic structures of the complexes are very significant in guiding experimental work.

In order to systematically clarify the effect of the hydroxyl group on different positions (*ortho*, *meta* and *para*) on aromatic heterocyclic ligands on the interaction of complexes with DNA, we newly synthesized the Ru(II) complexes [Ru(bpy)$_2$(*m*-hpip)]$^{2+}$ **2** and [Ru(bpy)$_2$(*p*-hpip)]$^{2+}$ **3** on the basis of reported [Ru(bpy)$_2$(*o*-hpip)]$^{2+}$ (Δ-**1** and Λ-**1**).[40] UV-Vis and emission spectra, together with CD spectra and viscosity experiments were carried out to determine the binding affinity and binding mode of these complexes to CT-DNA. The photocleavage of pBR322 DNA in the presence of **2** and **3** was also investigated. At the same time, the theoretical calculations for these three isomer complexes [Ru(bpy)$_2$L]$^{2+}$ (L = *o*-hpip, *m*-hpip and *p*-hpip) have been carried out applying the density

† Electronic supplementary information (ESI) available: electronic spectra and photocleavage diagrams. See http://www.rsc.org/suppdata/dt/b2/b212443b/

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functional theory (DFT) method on the level of the B3LYP/LanL2DZ basis set. Some frontier molecular orbital energies and stereographs, as well as the schematic diagram of the energies and related ^{1}MLCT transitions of $[Ru(bpy)_2L]^{2+}$ are presented, and applied to explain the obtained experimental regularities or trends in the DNA-binding strength or binding constants (K_b) and some spectral properties of the complexes.

Experimental

Chemicals

Solutions of DNA in 5 mM Tris-HCl buffer (pH 7.2), 50 mM NaCl 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.[41] The concentration of calf thymus DNA was determined spectrophotometrically using the molar absorption 6600 M^{-1} cm^{-1} (260 nm).[42]

CT-DNA was purchased from the Sino-American Biotechnology Company and pBR322 DNA from the Sangon (Canada) Biotechnology Company. All reagents and solvents were purchased commercially and used without further purification unless specially noted, and doubly distilled water was used to prepare buffer solutions.

Synthesis and characteristics

cis-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$, *cis*-$[Ru(bpy)_2(py)_2]Cl_2$ and Δ/Λ-[Ru(bpy)$_2$(py)$_2$][*o*,*o*′-dibenzoyl-D-tartrate]·$12H_2O$ were prepared and characterized according to the literature.[43,44]

Microanalyses were carried out on an Elemental Vario EL elemental analyser. ^{1}H NMR spectra were recorded on a Bruker ARX-300 spectrometer. All chemical shifts were given relative to TMS. Electrospray experiments were carried out with a Thermo Finnigan LCQ DECA XP ion trap mass spectrometer, equipped with an ESI source.

2-(3-Hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline (*m*-hpip). The ligand 2-(2-hydroxyphenyl)imidazo [4,5-*f*][1,10]-phenanthroline (*m*-hpip) was prepared by a similar method as in ref. 45, and with some modification.

A solution of phenanthraquinone (525 mg, 2.5 mmol), ammonium acetate (3.88 g, 50 mmol) and 3-hydroxyphenylaldehyde (431 mg, 3.5 mmol) in 10 ml glacial acetic acid was refluxed for 2 h. The cooled deep red solution was diluted with 25 ml water, and neutralized with ammonium hydroxide. Then the mixture was filtered and the precipitates were washed with water and acetone, then dried and purified by chromatography over 60–80 mesh SiO_2 using absolute ethanol as eluent, and the obtained yield was 760 mg (83%). Calc. for $C_{19}H_{12}N_4O\cdot 3H_2O$: C: 62.3; H: 4.95; N: 15.3. Found: C: 63.0; H: 4.96; N: 15.3%.

2-(4-Hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline (*p*-hpip). *p*-hpip was synthesized by using the same method as above, but with phenanthraquinone (525 mg, 2.5 mmol) and 4-hydroxyphenylaldehyde (431 mg, 3.5 mmol), and the obtained yield was 724 mg (79%). Calc. for $C_{19}H_{12}N_4O\cdot 3H_2O$: C: 62.3; H: 4.95; N: 15.3. Found: C: 62. 6; H: 4.72; N: 15.4%.

Δ-[Ru(bpy)$_2$(*m*-hpip)](PF$_6$)$_2$·2H$_2$O(Δ-2)Δ-[Ru(bpy)$_2$(*m*-hpip)]-$(PF_6)_2\cdot 2H_2O$ was synthesized as described in the literature[46] with slight modification.

Δ-[Ru(bpy)$_2$(py)$_2$][*o*,*o*′-dibenzoyl-D-tartrate]·$12H_2O$ (260 mg, 0.2 mmol) and *m*-hpip (180 mg, 0.56 mmol) were added to 20 ml ethylene glycol–water (9 : 1, v/v). The mixture was refluxed for 6 h under an argon atmosphere. The cooled reaction mixture was diluted with water (40 ml) and filtered to remove solid impurities. The complex was then separated from soluble impurities by precipitation with NH_4PF_6. The precipitated complex was dried, dissolved in a small amount of acetonitrile, and purified by chromatography over alumina, using MeCN–toluene (2 : 1, v/v) as eluent, yield: 154 mg, 73%. Calc. for $C_{39}H_{28}F_{12}N_8OP_2Ru\cdot 2H_2O$: C: 44.5; H: 3.07; N: 10.7. Found: C: 44.4; H: 3.21; N: 10.4%. ^{1}H NMR (DMSO-d$_6$): δ 9.07 (1H, s); 9.06 (1H, d); 8.85 (4H, 2d); 8.21 (2H, t); 8.15 (2H, d); 8.10 (2H, t), 8.02 (2H, d); 7.91 (2H, q); 7.84 (2H, d); 7.59 (3H, t); 7.56 (1H, s); 7.34 (2H, t); 7.22 (1H, s); 7.02 (2H, d); 6.44 (1H, s); ESI-MS: *m*/*z* 725 (M − 2PF$_6$ − H), 363 (M − 2PF$_6$/2); CD (CH_3CN, λ_{max}/nm): 294 (−).

Λ-[Ru(bpy)$_2$(*m*-hpip)](PF$_6$)$_2$·2H$_2$O (Λ-**2**) was similarly obtained, yield: 176 mg, 84%. Calc. for $C_{39}H_{28}F_{12}N_8OP_2Ru\cdot 2H_2O$: C: 44.5; H: 3.1; N: 10.6. Found: C: 45.9; H: 2.86; N: 10.9%. ESI-MS: *m*/*z* 725.3 (M − 2PF$_6$ − H), 363.3 (M − 2PF$_6$/2). CD (λ_{max}/nm): 294 (+).

Δ-[Ru(bpy)$_2$(*p*-hpip)](PF$_6$)$_2$·2H$_2$O(Δ-3).Δ-[Ru(bpy)$_2$(*p*-hpip)]-$(PF_6)_2\cdot 2H_2O$ was prepared by the same method as above but starting from Δ-[Ru(bpy)$_2$(py)$_2$][*o*,*o*′-dibenzoyl-D-tartrate]·$12H_2O$ (260 mg, 0.2 mmol) and *p*-hpip (180 mg; 0.6 mmol), yield: 161 mg, 77%. Calc. for $C_{39}H_{28}F_{12}N_8OP_2Ru\cdot 2H_2O$: C: 44.5; H: 3.07; N: 10.65. Found: C: 44.4; H; 3.12; N; 10.6%. ^{1}H NMR (DMSO-d$_6$): δ 9.08 (2H, d); 8.87 (4H, 2d); 8.21 (2H, t); 8.12 (2H, d); 8.08 (2H, t); 8.05 (2H, d); 8.04 (2H, s), 7.85 (2H, d); 7.59 (4H, m); 7.34 (2H, t); 7.04 (2H, d). ESI-MS: *m*/*z* 725.3 (M − 2PF$_6$ − H), 363.3 (M − 2PF$_6$/2). CD (λ_{max}/nm): 294 (−).

Λ-[Ru(bpy)$_2$(*p*-hpip)](PF$_6$)$_2$·2H$_2$O (Λ-**3**) was similarly obtained, yield: 147 mg, 70%. Calc. for $C_{39}H_{28}F_{12}N_8OP_2Ru\cdot 2H_2O$: C: 44.5; H: 3.07; N: 10.6. Found: C: 44.2; H: 3.14; N; 10.5%. ESI-MS: 725.3 (M − 2PF$_6$ − H), 363.3 (M − 2PF$_6$/2). CD (λ_{max}/nm): 294 (+).

Physical measurements

Emission spectra were carried out on a Shimadzu RF-5000 spectrofluorophotometer with excitation at 470 nm and circular dichroism (CD) spectra on a Jasco J-7115 spectropolarimeter. Electronic spectra were recorded on a Shimadzu UVPC-3000 spectrophotometer, and the binding constants K_b of the complexes were determined according to eqn. (1),[47] through a plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [DNA].

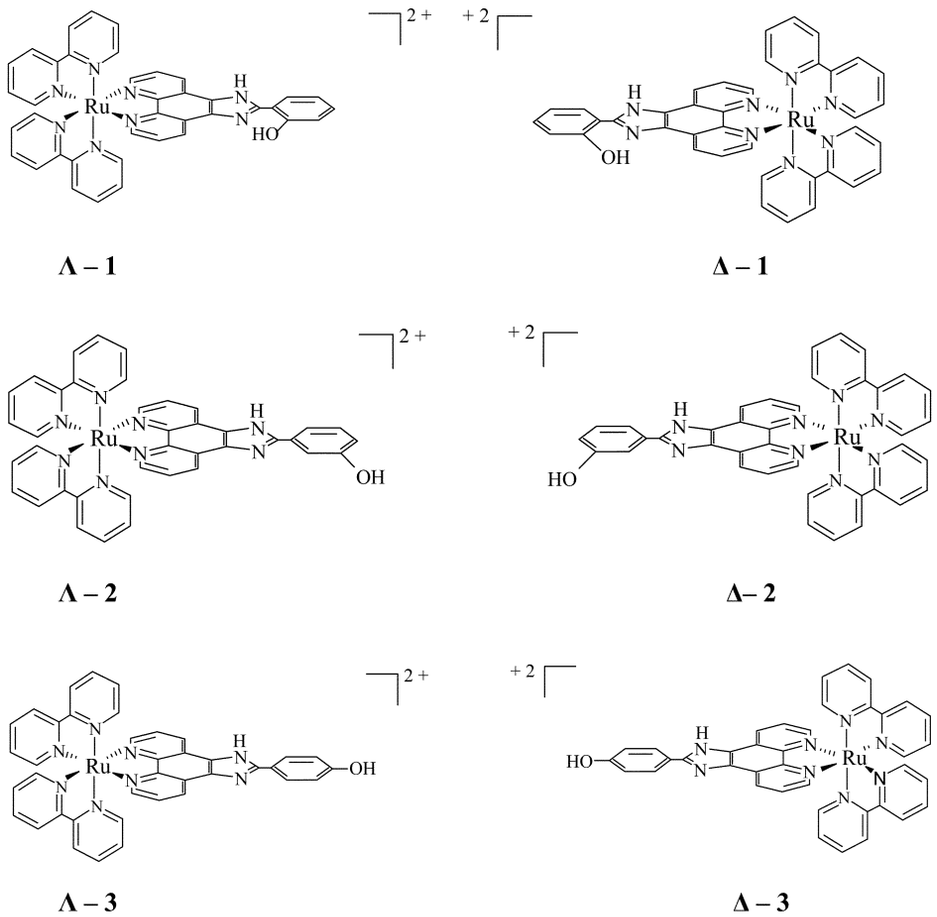
$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_b - \varepsilon_f} + \frac{1}{k_b(\varepsilon_b - \varepsilon_f)} \quad (1)$$

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_f and ε_b are, respectively, the apparent extinction coefficient (A_{obs}/[M]), the extinction coefficient for free metal (M) complex and the extinction coefficient for the metal (M) complex in the fully bound form. In plots of [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [DNA], K_b is given by the ratio of the slope to the intercept.

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 32 (± 0.1) °C in a thermostatic bath. The DNA samples contained approximately 200 base pairs. Flow times were measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated. Data are presented as $(\eta/\eta^0)^{1/3}$ *vs.* binding ratio,[48,49] where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA in the absence of complex.

Equilibrium dialysis was conducted at room temperature with 5 ml of calf thymus DNA (1.0 mM) sealed in a dialysis bag and 10 ml of the complex (50 μM) outside the bag. The circular dicroism spectrum of the dialyzate was measured on a Jasco J-7115 spectropolarimeter.

For the gel electrophotolysis experiments, supercoiled pBR 322 DNA (0.1 μg) was treated with enantiomers in 50 mM Tris-HCl buffer (pH 8.3), 18 mM NaCl, and the solution was then irradiated at room temperature with a UV lamp (302 nm, 10 W) for 60 min. The samples were analysed by electrophotolysis for 40 min at 60 V on a 1% agarose gel in Tris-boracic acid–EDTA



Scheme 1 Structures of the chiral Ru(II) complexes $[Ru(bpy)_2L]^{2+}$ (L = *o*-hpip, *m*-hpip and *p*-hpip).

buffer. The gel was stained with 1 μg ml^{-1} ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide) and photographed under UV light.

Theoretical section

Each of the octahedral complexes $[Ru(bpy)_2L]^{2+}$ (L = *o*-hpip, *m*-hpip, *p*-hpip) forms from Ru(II) and one main ligand L or intercalated ligand and two co-ligands (bpy). There is no symmetry in these complexes. The full geometry optimization computations were performed for these complexes applying the DFT-B3LYP method[25–28] and LanL2DZ basis set.[28,50] The structural models of the studied compounds are shown in Scheme 1, but only one chiral complex in every pair of Δ- and Λ- chiral isomers was computed and the singlet state was assumed.[51] All computations were performed with the G98 quantum chemistry program-package.[52] In order to vividly depict the detail of the frontier molecular orbital interactions, the stereographs of some related frontier MO of the complexes were drawn with the Molden v3.6 program[53] based on the obtained computational results.

Results and discussion

Electronic spectra

The electronic spectra of these Ru(II) complexes in water (0.1% DMSO) are characterized by an intense ligand-centered transition (IL) in the UV region, at 284 and 285 nm for **2** and **3**, respectively, and a metal-to-ligand charge transfer (MLCT) in the visible region, at 457 and 458 nm for **2** and **3**, respectively. The half-weight band widths ($w_{1/2}$) for Δ-**2**, Λ-**2**, Δ-**3** and Λ-**3** are 84, 73, 86 and 84 nm, respectively. When calf thymus DNA (CT-DNA) is added into the solution, large hypochromism is observed for both IL and MLCT transition absorptions, as shown in Table 1. For Δ-**2**, the MLCT absorption shifts from 458 to 463 nm ($\Delta\lambda$ = 5 nm), with 22% hypochromism (H). For Λ-**2**, Δ-**3** and Λ-**3**, the MLCT transition bands exhibit hypochromism of about 20, 21 and 17%, and red shifts of 5, 4 and 4 nm, respectively. The determined binding constants for Δ-**2**, Λ-**2**, Δ-**3** and Λ-**3** are about 1.5 (± 0.2), 1.0 (± 0.3), 1.0 (± 0.2) and 0.7 (± 0.2) × 10^5 M^{-1}, respectively. The binding constants of Δ-**2** and Δ-**3** are higher than those of Λ-**2** and Λ-**3**, respectively. This can be explained as follows: the Λ enantiomer does not intercalate as deeply as the Δ enantiomer, as also found for complex **1**, as indicated by ^{1}H NMR spectroscopy.[54] Complex **2** binds more strongly to CT-DNA than complex **3**, as theoretically explained below.

For comparison, the intrinsic binding constants of chiral $[Ru(bpy)_2(o\text{-hpip})]^{2+}$ to DNA were also determined to be 6.8 × 10^5 (Δ-isomer) and 5.3 × 10^5 M^{-1} (Λ-isomer), respectively. The binding strength of these chiral complexes is comparable with that of their racemic complexes **1** $[Ru(bpy)_2(o\text{-hpip})]^{2+}$ (6.5 × 10^5 M^{-1}).[40] These data indicate that the binding affinity of complexes **2** and **3** is not as strong as that of $[Ru(bpy)_2(o\text{-hpip})]^{2+}$. The latter contains an intramolecular hydrogen bond between the nitrogen atom of the imidazole ring and the 2-phenolic group on the *o*-hpip ligand and such a hydrogen bond additionally stabilizes to the DNA–complex adduct.

Steady-state emission spectra

At room temperature, these isomers emit luminescence in range of 500–700 nm, with the maximum at 598 and 591 nm for **2** and **3**, respectively. Upon the addition of CT-DNA, an obvious enhancement of emission intensity was observed for both enantiomers **2** and **3** (Fig. 1).

At [DNA]/[Ru] ≈ 24 : 1, the emission intensity increases by about 2.4 and 2.2× for Δ-**2** and Λ-**2**, respectively, while for Δ-**3**

Table 1 Absorption spectra λ_{max} (nm) and DNA-binding constants K_b ($\times 10^5$ M^{-1}) of Ru(II) complexes and comparison between experimental ΔE and computed $\Delta\varepsilon$ (eV) (^{1}MLCT) values

	Experiment					Computation				
Compound	λ_{max} (free)	λ'_{max} (bound)	$\Delta\lambda$/nm	H (%)	$K_b/10^5$ M^{-1}	ΔE/eV	$\Delta\varepsilon$/eV	X^a/eV	$\lambda''_{max}{}^b$	Transition
1 (*o*-hpip)	458	464	6	−26	6.8 (Δ)	2.707	3.363	0.656	453	H-2 to L
	458	464	6	−24	5.3 (Λ)					
2 (*m*-hpip)	458	463	5	−22	1.5 (Δ)	2.713	3.347	0.634	455	H-2 to L
	457	462	5	−20	1.0 (Λ)					
3 (*p*-hpip)	458	462	4	−21	1.0 (Δ)	2.707	3.336	0.629	457	NH to L
	458	462	4	−17	0.7 (Λ)					

[a] $X = \Delta\varepsilon_{L-NH}$ or $\Delta\varepsilon_{L-(H-2)} - \Delta E(^1\text{MLCT})$. [b] The predicted wavelength is calculated, applying the experimental λ value (452 nm) and $\Delta\varepsilon_{L-H} = 0.1239$ au of the parent complex $[Ru(bpy)_3]^{2+}$ and $\Delta\varepsilon_{L-NH}$ (or $\Delta\varepsilon_{L-(H-2)}$) of the corresponding complexes obtained by the DFT method.

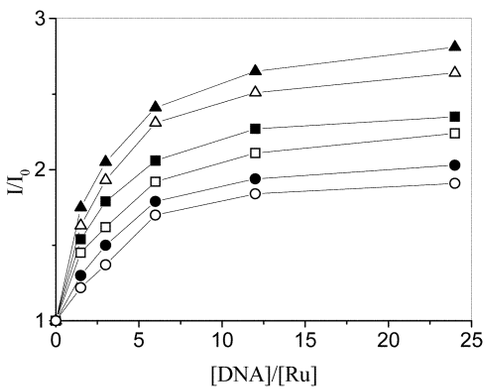


Fig. 1 Plots of relative emission intensity *vs.* the [DNA]/[Ru] ratio for Ru(II) complexes Δ-**1** (▲), Λ-**1** (△), Δ-**2** (■), Λ-**2** (□), Δ-**3** (●) and Λ-**3** (○) in 5 mM Tris-HCl buffer (pH 7.2), 50 mM NaCl, [Ru] = 1.0×10^{-5} M.

and Λ-**3**, the enhancements of emission intensity under the same conditions are 2.0 and 1.9×, respectively, as shown in Fig. 1. It is apparent that more luminescence enhancement occurs for $[Ru(bpy)_2(o\text{-hpip})]^{2+}$ **1** than for **2** or **3**. Such a trend is consistent with that in their electronic spectra, and can be also explained by the intramolecular hydrogen bond between the *ortho* phenolic group of *o*-hpip and the nitrogen atom of the imidazole ring. This results in the planar enlargement of the intercalating ligand, and thus higher binding affinity to CT-DNA.[40]

Viscosity experiments

The changes of relative viscosity of rod-like CT-DNA in the presence of Ru(II) complexes are shown in Fig. 2. The relative viscosity of rod-like CT-DNA is increased in the presence of Δ-**2** or Λ-**2**, and it is similar to Δ-**3** or Λ-**3**. For comparison, the changes of relative viscosity of DNA in presence of complex Δ-**1** and Λ-**1** are also given in Fig. 2. The observed increase in relative viscosity occurs as a result of a length increase of the duplex due to the intercalation of the complex.[55]

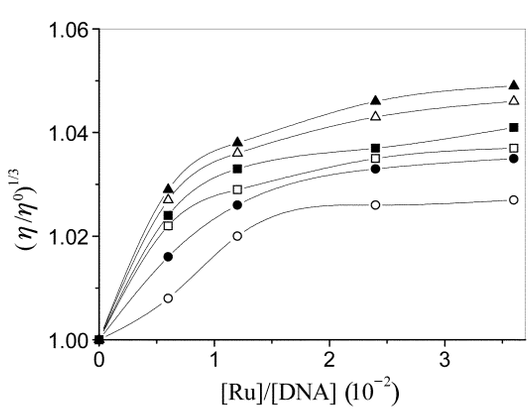


Fig. 2 Effect of increasing amount of Ru(II) complexes Δ-**1** (▲), Λ-**1** (△), Δ-**2** (■), Λ-**2** (□), Δ-**3** (●) and Λ-**3** (○) on the relatively viscosity of CT-DNA in 5 mM Tris-HCl buffer (pH 7.2), 50 mM NaCl at 32 (± 0.1) °C, [DNA] = 5.0×10^{-4} M.

Circular dichroism spectra

The CD spectra were compared between the free and fully bound enantiomer in 5 mM Tris-HCl buffer (pH 7.2), 50 mM NaCl, as shown in Fig. 3.

The CD spectrum of free Δ-**2** is characterized by a negative band around 293 nm. Upon the addition of CT-DNA, the peak position shifts to 294 nm with an increase of ellipticity from −12.8 to −10.8 mdeg. For Λ-**2**, the peak position shifts from 292.2 to 292.6 nm with a decrease of ellipticity from +12.5 to +11.3 mdeg. For complex **3**, the CD spectrum undergoes a change similar to complex **2** upon the addition of CT-DNA, as shown in Fig. 3. This may be the result of perturbations of either the geometric or electronic structure.[56–58]

Equilibrium dialysis

Equilibrium dialysis has been performed to observe the enantioselectivity of the Ru(II) complexes and results are shown in Fig. 4.

The CD signals of the dialyzate of complex **3** changed during the dialysis process. From the beginning to 18 h, the CD signal increased from zero to a maximum, and then decreased. Eventually, the CD signal of **3** disappeared (Fig. 4). For the CD signals of dialyzate for complex **3** at 18 h, two CD signals were observed with a positive peak around 278 nm, and a negative peak around 293 nm. These phenomena were also observed for complex **2**, and are consistent with those of $[Ru(bpy)_2(o\text{-hpip})]^{2+}$.[59] This may be explained from the different binding rates of the isomers of Ru(II) complexes to DNA, the diastereomeric orientation effects of the chromophores and the different penetrations upon binding to the double helix.

Photocleavage of pBR 322 DNA by Ru(II) complexes

Generally, transition metal complexes as DNA-intercalators can photocleave plasmid DNA to form II or III. This is likely due to the formation of singlet $^1O_2$ arising from energy transfer from the excited complex to ground state oxygen in solution,[60] and the degree of cleavage closely correlates to the binding affinity and concentration.[61–63] The photocleavage of pBR 322 DNA in the absence and presence of Ru(II) complex was carried out in 50 mM Tris-HCl buffer (pH = 8.3), 18 mM NaCl under UV-irradiation.

Under UV-light irradiation, no DNA cleavage was observed for controls in which neither complex nor light irradiation was present, and a slight DNA cleavage was observed for controls in which the complex was absent. However, the concentration of nicked form II gradually increases with increasing amount of complexes. At a concentration of 0.5×10^{-4} M, isomer Δ-**2** promotes almost the complete conversion of DNA from form I

Table 2 Some frontier molecular orbital energies (ε_i/au) and total energies (E_{total}/au) of the complexes

Compound	H-3	H-2	NH	HOMO	LUMO	NL	L + 2	ε_{L-H}	ε_{L-NH}	$\varepsilon_{L-(H-2)}$	E_{total}
1 (*o*-hpip)	−0.4056	−0.4002	−0.3830	−0.3573	−0.2766	−0.2732	−0.2694	0.0807	0.1064	0.1236	−2109.5835
2 (*m*-hpip)	−0.4016	−0.3963	−0.3779	−0.3614	−0.2733	−0.2701	−0.2642	0.0881	0.1046	0.1230	−2109.5747
3 (*p*-hpip)	−0.3997	−0.3991	−0.3953	−0.3565	−0.2727	−0.2695	−0.2628	0.0838	0.1226	–	−2109.5767

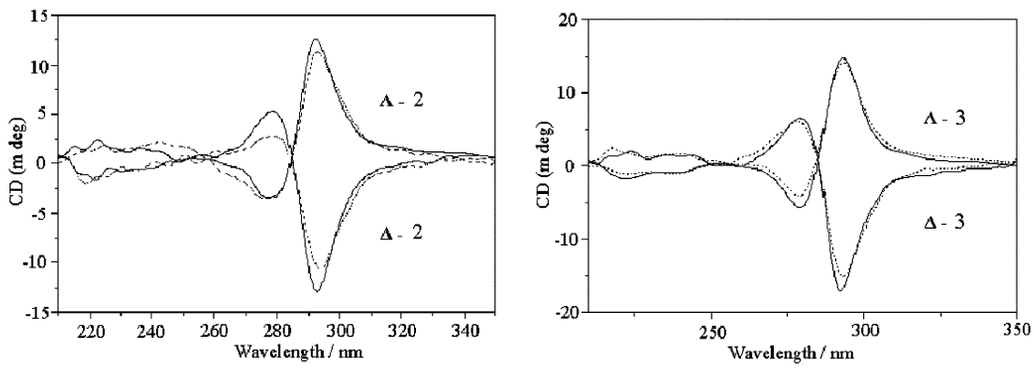


Fig. 3 CD spectra of Δ-**2**, Λ-**2**, Δ-**3** and Λ-**3** in 5 mM Tris-HCl buffer (pH 7.2), 50 mM NaCl in the absence (—) and in the presence (---) of CT-DNA. [Ru] = 1.0×10^{-5} M; [DNA] = 1.5×10^{-4} M; path length 1.0 cm. The CD spectrum of CT-DNA was subtracted from those of the mixtures.

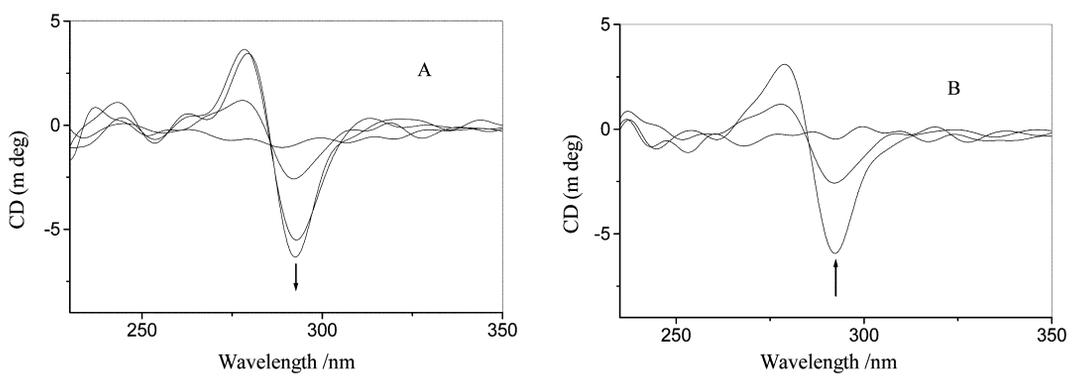


Fig. 4 CD spectra of the dialyzates of **3** against CT-DNA for A, t = 0, 6, 12, 18 h, and for B, t = 18, 24, 48 h, respectively, with stirred solutions; [Ru] = 5.0×10^{-5} M, [DNA] = 1.0×10^{-3} M.

to II after irradiating for 60 min, and this was also observed for Λ-**2**, Δ-**3** and Λ-**3** at concentrations of 1.0, 0.5 and 1.0×10^{-4} M, respectively. With increasing irradiation time, the amount of nicked form II gradually increases, while form I gradually decreases. This is the result of single-stranded cleavage of pBR 322 DNA. The cleavage difference between the Δ- and Λ-isomer may originate from different degrees of intercalation between base pairs.[55] The photocleavage mechanism of DNA in the presence of Ru(II) complexes is a very controversial subject and further investigation in this field is being actively pursued.

Theoretical explanation of trends in DNA-binding and spectral properties of the complexes

The above-mentioned trends in DNA-binding and some spectral properties can be well explained by our theoretical computations using the DFT method. Some frontier molecular orbital energies and total energies, the schematic diagram of the energies and related ^{1}MLCT transitions, and the molecular orbital stereographs of $[Ru(bpy)_2L]^{2+}$ are given in Table 2, Fig. 5 and Fig. 6, respectively, based on the computed results.

As is well established, there are π–π interactions in the DNA-binding of these complexes in intercalation mode. According to frontier molecular orbital theory,[64,65] for a reaction controlled by orbital interactions between reactant molecules, a higher HOMO energy of one reactant molecule and a lower LUMO energy of the other are more advantageous to the reaction between the two molecules, because electrons more easily transfer from the HOMO of one reactant to the LUMO of the other

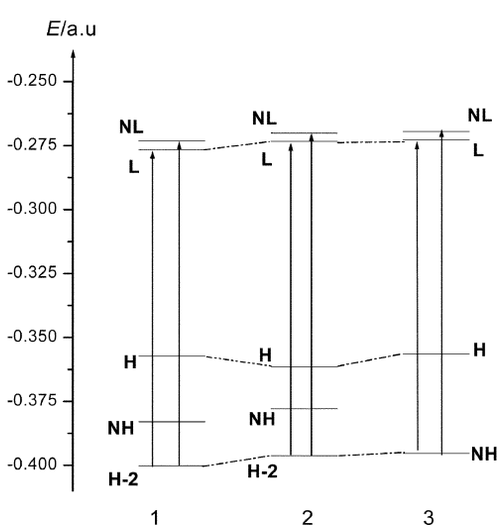


Fig. 5 Schematic diagram of some frontier MO energies and the related ^{1}MLCT transitions of $[Ru(bpy)_2L]^{2+}$.

in the orbital interaction. A simple calculation model and computed results by the DFT method for stacked DNA base-pairs with backbones have been reported by Kurita and Kobayashi.[31] It should be a better simplified approximation model for DNA, and thus should be useful and feasible for such a purpose. The reported HOMO and NHOMO (NH) energies of the DNA section model with base pairs are much higher (−1.27 and

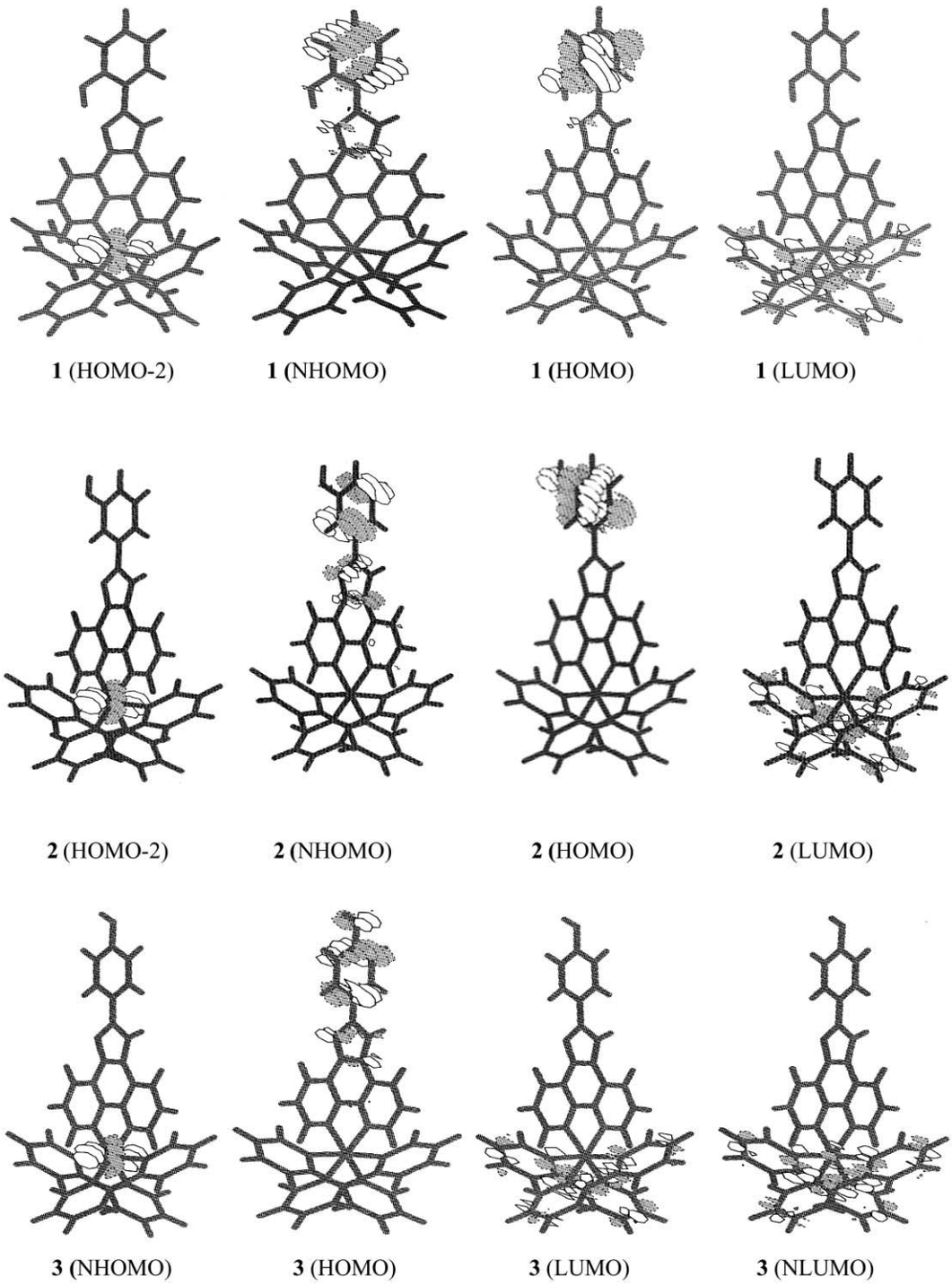


Fig. 6 Some related frontier MO stereographs of $[Ru(bpy)_2L]^{2+}$.

-1.33 eV)[31] than our computated LUMO and NLUMO (NL) energies (~ -7.0 eV) of the complexes $[Ru(bpy)_2L]^{2+}$ (L = *m*-hpip, *p*-hpip and *o*-hpip). We believe that such a trend in the relative energies will be retained in our DNA system, because the attraction of metal complex cations with high positive charges for electrons in MOs is much stronger than that of DNA, and thus the electron must easily transfer from the HOMO of base pairs of DNA to the LUMO of the complexes intercalating to DNA. From Table 2 and Fig. 5, we can see that ε_L (**1**) < ε_L (**2**) < ε_L (**3**) in this series of complex isomers. This energy order suggests that the trend in DNA-binding constants (K_b) should be K_b(**1**) > K_b(**2**) > K_b(**3**). This is in satisfactory accord with the above experimental results.

According to the DFT computation results, we can clearly see that for $[Ru(bpy)_2(o\text{-hpip})]^{2+}$ and $[Ru(bpy)_2(m\text{-hpip})]^{2+}$, the λ_{max} singlet metal to ligand charge-transfer (^{1}MLCT) bands should correspond to the electron transitions from the metal HOMO − 2 (H − 2) orbitals to LUMOs, whereas that for $[Ru(bpy)_2(p\text{-hpip})]^{2+}$ should correspond to the electron transition from its NHOMO to the LUMO (see Fig. 6). This assignment is supported by our spectral experiments. The experimental spectral data and the computed related $\Delta\varepsilon$ are collected in Table 1. Comparing the results between theory calculations and experiments, we can see that: the energy differences ($\Delta\varepsilon_{L-(H-2)}$ or $\Delta\varepsilon_{L-NH}$) from DFT calculations are greater than the corresponding absorption spectral energies (ΔE) by about 0.63–0.65 eV (almost constant) for all of these Ru(II) complexes. This fact suggests that such an error may be attributed to a systemic error arising from theory calculations and environment factors, *e.g.*, molecular solvation, geometrical changes and polarization in aqueous solution. The differences (X) between computed and spectral experimental data reflect

these effects, and show that these effects for various Ru(II) polypyridyl complexes are always similar. Therefore, such a systemic error can be obviously reduced by using a standard sample correction method to obtain more accurate calculated results in good agreement with the experiments as shown in Table 1. When the computations are performed using the parent complex $[Ru(bpy)_3]^{2+}$ as a standard sample (experimental λ_{max} = 452 nm, $\Delta\varepsilon_{L-H}$ = 0.1239 au [32]) and $\Delta\varepsilon_{L-H}$ data of the complexes in Table 2, the computed λ_{max} ^{1}MLCT absorption bands are at 453, 455 and 457 nm, *cf.* the experimental data at 458, 457 and 458 nm in $[Ru(bpy)_2L]^{2+}$ (L = *o*-hpip, *m*-hpip and *p*-hpip), respectively, as shown in Table 1.

Table 1 also lists the wavelengths of complexes binding to calf thymus DNA (third column). Since in absorption and emission spectra of the Ru(II) complexes, the corresponding energy change between the presence and absence of DNA is small, and no special pattern changes in the absorption or emission spectra of Ru(II) complexes in the presence of DNA have been detected, except an increase in the luminescence intensity and hypochromism in the absorption intensity, we consider that there is not a great effect on the HOMO and LUMO and $\Delta\varepsilon_{L-H}$ for the complexes binding to DNA. This further shows that the interaction between the series of complexes and DNA is weak, so that the λ_{max} electron transition band in these Ru(II) complexes binding to DNA can be assigned to ^{1}MLCT. At the present time, we are not yet able to calculate the frontier molecular orbital energies of the whole hyper-molecule system formed from these complexes and DNA by the DFT method, and therefore, DFT studies on some trends in electronic structures and related properties for such complexes should be very significant.

In addition, the trend in total energies of these complex isomers, E_{total}(**1**) < E_{total}(**3**) < E_{total}(**2**), can be also explained as follows: The total energy of **1** is the lowest among the three isomers because of intramolecular H-bond bonding, while E_{total}(**3**) < E_{total}(**2**) can be explained according to the law of polarity alternation [66] and the idea of polarity interference.[32,67] That is to say, there are some constructive (the same direction) polarity interferences in the main-ligand of **3**, whereas there are some destructive (reverse direction) polarity interferences in the main-ligand of **2**.

Conclusions

The following have been learnt from this study:

(1) On the basis of reported Δ- and Λ-$[Ru(bpy)_2(o\text{-hpip})]$ (Δ-**1** and Λ-**1**), a series of novel enantiomerically pure polypyridyl Ru(II) complexes, Δ- and Λ-$[Ru(bpy)_2(m\text{-hpip})](PF_6)_2$ (Δ-**2** and Λ-**2**), and Δ- and Λ-$[Ru(bpy)_2(p\text{-hpip})](PF_6)_2$ (Δ-**3** and Λ-**3**) have been synthesized and characterized. The binding properties of these complexes to CT-DNA have been investigated by UV-visible and emission spectra, together with viscosity experiments. It is found that complexes **2** and **3** bind to DNA *via* an intercalative mode.

(2) The binding affinity of **2** or **3** to CT-DNA is weaker than that of $[Ru(bpy)_2(o\text{-hpip})]^{2+}$, because an intramolecular hydrogen bond exists for the ligand *o*-hpip. The investigation of CD spectra of **2** and **3** in the absence and presence of CT-DNA indicates that there are different intercalating geometries for the DNA-binding of these complexes. A subtle but detectable difference was observed in the interaction of the different enantiomers with CT-DNA. The DNA-binding of the Δ-isomer is stronger than that of the Λ-isomer, whereas that of the Λ-isomer is swifter.

(3) Under UV light irradiation, enantiomers **2** and **3** can cleave pBR322 DNA, with the degree of cleavage closely correlating to the binding affinity and concentration of the complexes.

(4) The theoretical calculation results for these three isomer complexes $[Ru(bpy)_2L]^{2+}$ (L = *o*-hpip, *m*-hpip and *p*-hpip) applying the density functional theory (DFT) method on the level of the B3LYP/LanL2DZ basis set, can be used to reasonably explain the obtained experimental regularities or trends in the binding strength or binding constants (K_b) and some spectral properties of the complexes.

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