Synthesis, DNA Binding, and DNA Photocleavage of the Ruthenium(II) Complexes $[Ru(bpy)(btip)]^{2+}$ and $[Ru(dmb)(btip)]^{2+}$ (bpy = 2,2′-Bipyridine; btip = 2-Benzo[*b*]thien-2-yl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline; dmb = 4,4′-Dimethyl-2,2′-bipyridine)

by **Li-Feng Tan***[a]), **Fang Wang**[a]), and **Hui Chao**[b])

[a]) College of Chemistry, Xiangtan University, Xiangtan 411105, P. R. China (fax: 86-732-8292477; email: lfwyxh@yahoo.com.cn)

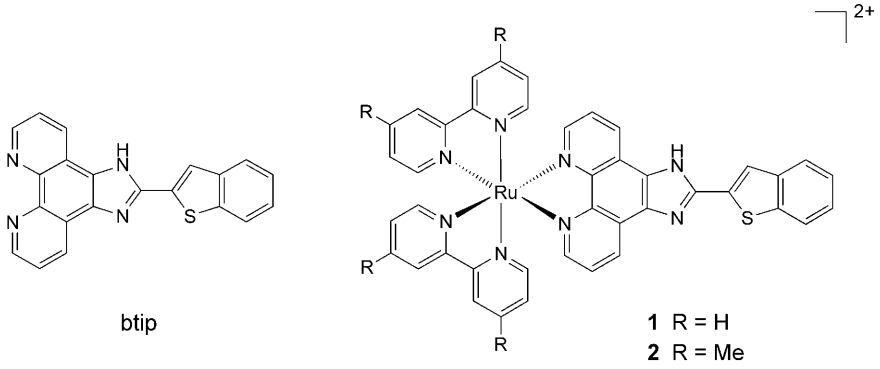
[b]) State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, P. R. China

The new polypyridyl ligand btip (= 2-benzo[*b*]thien-2-yl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline) and its Ru^{II} complexes $[Ru(bpy)_2(btip)]^{2+}$ (**1**; bpy = 2,2′-bipyridine) and $[Ru(dmb)_2(btip)]^{2+}$ (**2**; dmb = 4,4′-dimethyl-2,2′-bipyridine) were synthesized and characterized by elemental analysis, MS, and ^{1}H-NMR. The DNA-binding properties of the two complexes to calf-thymus DNA (CT-DNA) were investigated by different spectrophotometric methods and viscosity measurements. The results suggest that both complexes bind to CT-DNA through intercalation. Also, when irradiated at 400 nm, the two complexes promote the photocleavage of plasmid pBR-322 DNA. Thereby, under comparable experimental conditions, complex **1** cleaves DNA more effectively than complex **2** does. Mechanistic studies reveal that singlet oxygen ($^1O_2$) and hydroxyl radicals ($OH^•$) play a significant role in the photocleavage.

Introduction. – During the last decade, the interaction between transition-metal complexes and DNA has been extensively studied [1–5]. Binding studies of small molecules to DNA are very important in the development of new therapeutic agents and DNA molecular probes [6–18]. Polypyridyl ruthenium(II) complexes can bind to DNA by non-covalent interactions such as electrostatic binding, groove binding [19], intercalative binding, and partial intercalative binding [20]. The useful application of such complexes generally requires that they bind to DNA through intercalation. Therefore, the vast majority of studies has been focused on the interaction of transition-metal complexes containing fully planar ligands [21–28]. In contrast, investigations with substituted ligands as DNA-binding reagents have been relatively few. However, some of these less-planar complexes also exhibit interesting DNA-binding properties [29]. Varying types and/or positions of substituents in intercalative ligands may create interesting differences in the spatial configuration and electron-density distribution of ruthenium(II)–polypyridyl complexes. This also results in differences in the spectroscopic properties and the DNA-binding potentials of such complexes, and will be helpful to more clearly understand the binding mechanism of these complexes to DNA. Therefore, further studies with ligands differing both in functionalization and degree of planarity are necessary.

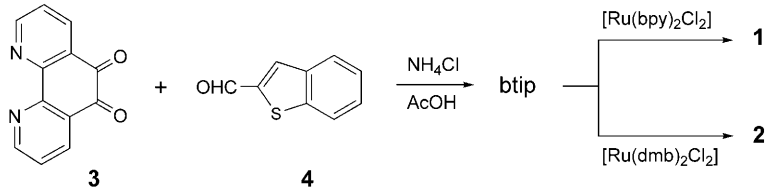
Herein, we report the synthesis and characterization of 2-benzo[*b*]thien-2-yl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline (btip), a new polypyridyl ligand, and its RuII complexes $[Ru(bpy)_2(btip)]^{2+}$ (**1**; bpy = 2,2′-bipyridine) and $[Ru(dmb)_2(btip)]^{2+}$ (**2**;

dmb = 4,4′-dimethyl-2,2′-bipyridine). The DNA-binding properties of the two complexes were explored by spectroscopic and viscosity measurements, and their photocleavage behavior toward pBR-322 DNA were investigated. We hope that our results will aid in the understanding of DNA recognition and binding by Ru^{II} complexes, as well as laying the foundation for the rational design of new photoprobes and photonucleases for DNA.



Results and Discussion. – 1. *Synthesis and Characterization*. An outline of the synthesis of the Ru^{II} complexes **1** and **2** with the new btip ligand is presented in the *Scheme* below. The btip ligand was prepared through condensation of 1,10-phenanthroline-5,6-dione (**3**) with benzo[*b*]thiophene-2-carbaldehyde (**4**) on the basis of the method for the preparation of imidazole rings according to *Steck* and *Day* [30]. The complexes $[Ru(bpy)_2(btip)]^{2+}$ (**1**) and $[Ru(dmb)_2(btip)]^{2+}$ (**2**) were then prepared in relatively good yield (71 and 64%, resp.) by reaction of btip with *cis*-$[Ru(bpy)_2Cl_2]\cdot 2\,H_2O$ and *cis*-$[Ru(dmb)_2Cl_2]\cdot n\,H_2O$, 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 mass spectra of **1** and **2**, the $[M-ClO_4]^+$, $[M-2\,ClO_4-H]^+$ and $[M-2\,ClO_4]^{2+}$ were observed, and the determined molecular weights were consistent with expected values.

Scheme



Both the complexes **1** and **2** gave well-resolved ^{1}H-NMR spectra, permitting unambiguous identification and assessment of purity. The ^{1}H-NMR chemical shifts were assigned with the aid of ^{1}H,^{1}H-COSY experiments, and by comparison with the values of similar compounds [29a] [31]. Due to the shielding influences of the adjacent btip and bpy (or dmb) moieties, the bpy (or dmb) H-atoms of **1** and **2** exhibit two distinct

sets of signals. In addition, the NH resonance of btip was not observed due to rapid H^+ exchange between the two N-atoms of the imidazole ring. A similar case has been reported previously [31].

The UV/VIS absorption spectra of **1** and **2** showed three well-resolved bands in the range 200–600 nm, characterized by intense $\pi \rightarrow \pi^*$ ligand transitions in the UV, as well as by metal-to-ligand charge-transfer (MLCT) transitions in the VIS. The broad MLCT absorption bands appeared at 460 and 468 nm for **1** and **2**, respectively, and are attributed to $Ru(d\pi) \rightarrow btip(\pi^*)$ transitions. These bands were bathochromically shifted relative to those of $[Ru(bpy)_3]^{2+}$ (452 nm) [32], in accord with the extension of the corresponding π framework. The signal below 400 nm was assigned to internal $\pi \rightarrow \pi^*$ transition of the ligands, by comparison with the spectra of $[Ru(bpy)_3]^{2+}$.

2. *DNA Binding*. 2.1. *Viscosity Measurements.* Hydrodynamic measurements sensitive to length changes, as reflected in viscosity and sedimentation, are regarded as the least-ambiguous and most-critical tests of a binding model in solution in the absence of crystallographic data [29a][33]. A classical intercalation model demands that the DNA helix lengthens as base pairs are separated to accommodate the binding ligand, which, in turn, leads to an increase in the viscosity of DNA [29a][33].

In *Fig. 1*, the change in viscosity on rod-like DNA is shown in the presence of complexes **1** and **2**, $[Ru(bpy)_3]^{2+}$, and ethidium bromide (EB). Whereas EB, a well-known DNA intercalator, gave rise to a strong change in DNA viscosity upon complexation, $[Ru(bpy)_3]^{2+}$, which binds by electrostatic interactions only, exerted essentially no such effect. As can be seen from *Fig. 1*, upon increasing the amounts of **1** or **2**, the relative viscosity of DNA increased steadily, similar as in the case of EB. The increase in relative viscosity, expected to correlate with the compounds DNA-intercalating potential, followed the order EB > **1** > **2**. These results suggest that complexes **1** and **2** both bind to DNA through intercalation, the differences in binding strength probably being caused by the different ancillary ligands. The four additional Me groups in **2** relative to **1** exert some steric hindrance. Therefore, complex **1** is probably more deeply intercalated and more tightly bound to adjacent DNA base pairs than complex **2**.

2.2. *UV/VIS Titration*. The application of electronic-absorption spectroscopy in DNA-binding studies is one of the most-useful techniques [34]. Complex binding with DNA through intercalation usually results in hypochromism and bathochromism, due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and DNA base pairs. The extent of hypochromism commonly parallels the intercalative binding strength.

In *Fig. 2*, the absorption spectra of the Ru^{II} complexes **1** and **2** (at constant concentration) are shown in the absence and presence of calf-thymus (CT)-DNA. As can bee seen for complex **1**, upon increasing the CT-DNA concentration, the hypochromism at 462 nm (MLCT band) reached 29.4%, with a red shift of 8 nm at a [DNA]/[Ru] ratio of 3.06. For complex **2**, under the same experimental conditions, the MLCT band at 471 nm showed hypochromism by *ca.* 25%, and a red shift of 2 nm at a [DNA]/[Ru] ratio of 2.55. Comparing the hypochromism of **1** or **2** with that of the parent complex $[Ru(phen)_3]^{2+}$ (12% hypochromism for MLCT band at 445 nm, 2-nm red shift) [11a], which interacts with DNA through semi- or quasi-intercalation [35], and considering that the absorption spectrum of $[Ru(bpy)_3]^{2+}$, a typical electrostatic DNA-binding complex, was demonstrated to be unchanged upon addition of CT-DNA [19], the observed

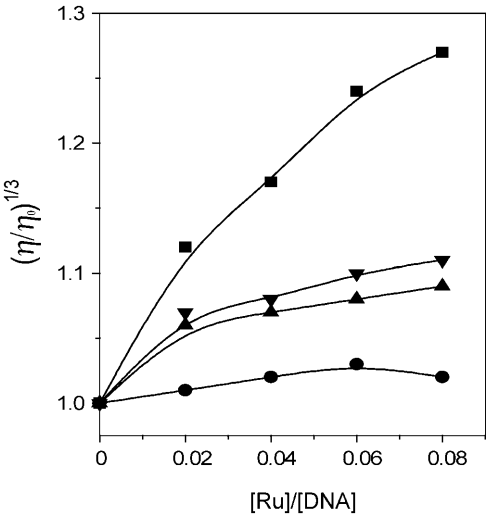


Fig. 1. *Effect of increasing amounts of ethidium bromide* (■), *[Ru(bpy)$_3$]$^{2+}$* (●), **1** (▼), *and* **2** (▲) *on the relative viscosity of CT-DNA*. Total DNA concentration: 0.5 mM, $T=30\pm0.1°$.

spectroscopic characteristics clearly suggest that **1** and **2** interact with DNA most likely through a mode that involves a stacking interaction between the aromatic chromophore and the DNA base pairs. The spectra also indicate that complex **1** binds stronger to DNA than complex **2** does.

To quantitatively compare the binding strengths of their two complexes, their intrinsic binding constants K_b were determined by UV/VIS titration. This was done by monitoring the change in absorbance at 462 nm for complex **1**, and at 471 nm for complex **2**, with increasing concentration of DNA, using *Eqn. 1* [36]:

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

Here, [DNA] is the concentration of DNA in base pairs, and ε_a, ε_f, and ε_b are the extinction coefficients of the apparent, free, and bound metal complexes, respectively. When plotting $[DNA]/(\varepsilon_a-\varepsilon_f)$ *vs.* [DNA], K_b is given by the ratio of the slope to the intercept. The intrinsic binding constants K_b of complexes **1** and **2** were, thus, determined as 2.85×10^4 M^{-1} and 2.05×10^4 M^{-1}, respectively. This result indicates that, as the ancillary ligand varies from bpy to dmb, the DNA-binding affinity of the Ru^{II} complexes declines. For comparison, the intrinsic binding constants K_b of typical 'intercalative-type' Ru^{II} complexes is in the range of 1.1×10^4–4.8×10^4 M^{-1} [37], whereas that of the parent complex [Ru(phen)$_3$]$^{2+}$ is 5.5×10^3 M^{-1} [37a]. Hence, complexes **1** and **2** clearly bind to DNA by intercalation, **1** having a higher affinity than **2**, in accord with the above viscosity studies.

2.3. *Fluorescence Quenching*. In the absence of DNA, complexes **1** and **2** are luminescent in *Tris* buffer at ambient temperature, with a fluorescence maximum at 613 and 623 nm, respectively. As shown in *Fig. 3*, upon addition of CT-DNA, the fluorescence-emission intensities of **1** and **2** increased by a factor of *ca*. 2.45 and 2.08, respectively.

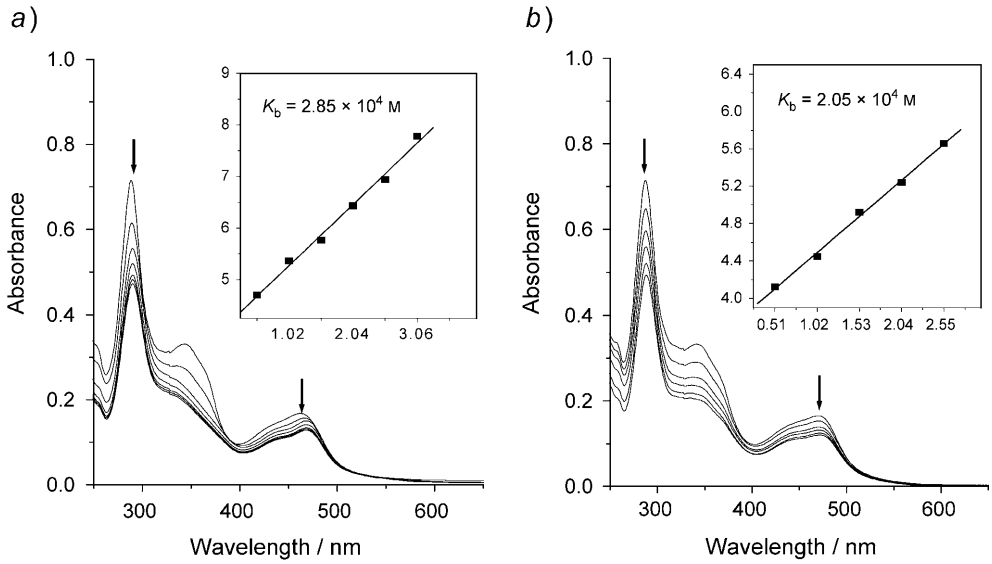


Fig. 2. *UV/VIS Absorption spectra of* **1** (*a*) *and* **2** (*b*) *in aq.* Tris·*HCl buffer upon addition of CT-DNA*. [Ru] = 1×10^{-5} M, [DNA] = $0-10.98 \times 10^{-5}$ M. Arrows show absorbance changes upon increasing the DNA concentration. Inset: plot of $10^{-9} \times [DNA]/(\varepsilon_a - \varepsilon_f)$ (in M^2 cm) *vs.* [DNA] (in M) for the titration of DNA with the complex for the determination of the binding constant K_b.

This indicates that both complexes strongly interact with DNA, which efficiently 'protects' them, since the hydrophobic environment inside the DNA helix reduces the accessibility of solvent H_2O to the complex, and because complex mobility is restricted at the binding site, factors that results in a decrease of the vibrational modes of relaxation and, thus, in higher emission intensity.

Steady-state fluorescence-quenching experiments with $[Fe(CN)_6]^{4-}$ as quencher can provide some information about complexes binding to DNA, but do not indicate binding modes. We decided to perform some experiments at ambient temperature, using a similar method as that described by *Satyanarayana et al.* [38]. In experiments at constant ionic strength, KCl was added along with $K_4[Fe(CN)_6]$. As illustrated in *Fig. 4*, the fluorescence-quenching curves at constant ionic strength were non-linear. For complexes **1** and **2**, the final fluorescence intensities were originally 76.6 and 69.3%, respectively. These results, thus, further confirm that **1** binds to DNA more strongly than **2** does.

2.4. *Enantioselective DNA 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 [39], 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. Thus, racemic solutions of the two complexes were dialyzed against CT-DNA for 36 h, and then subjected to circular-dichroism (CD) analysis. In *Fig. 5*, the CD spectra in the UV region of the dialysates of **1** and **2** are shown. The dialysate of **1** (solid line) shows two CD signals with a positive peak at 277 nm and a negative

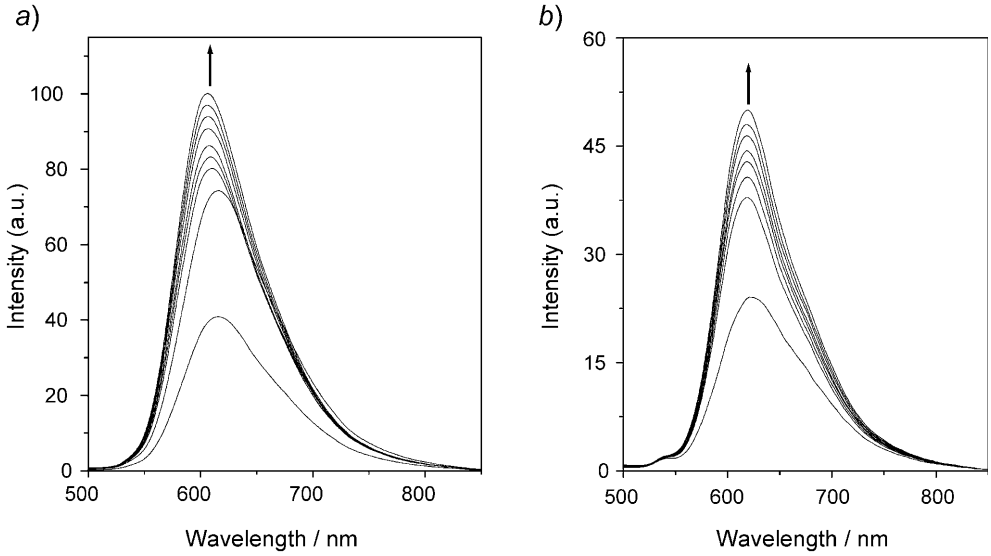


Fig. 3. *Fluorescence spectra of* **1** (*a*) *and* **2** (*b*) *in aq*. Tris·*HCl buffer at 298 K in the presence of CT-DNA*. Arrows indicate intensity changes upon increasing the DNA concentration. [Ru] = 2×10^{-6} M, [DNA]/[Ru] = 18.11 and 20.70 for **1** and **2**, resp.

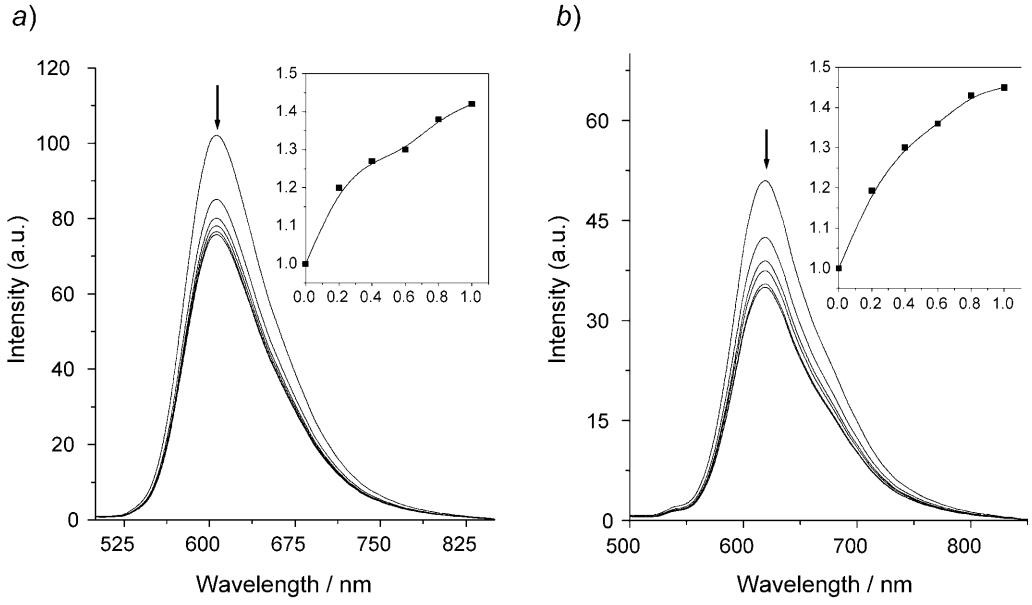


Fig. 4. *Fluorescence quenching* of **1** (*a*) *and* **2** (*b*) *by* $[Fe(CN)_6]^{4-}$. [Ru] = 2×10^{-6} M, [DNA]/[Ru] = 40, $[K^+] = 4\times10^{-3}$ M, $[Fe(CN)_6]^{4-} = 0-1.0$ mM. Inset: plot of I_0/I *vs.* $[Fe(CN)_6]^{4-}$ (in mM), where I_0 and I are the fluorescence intensities in the absence and presence of the quencher, resp.

peak at 293 nm, while complex **2** (doted line) shows weak CD signals with a positive peak at 279 nm and a negative one at 298 nm, respectively. Although neither of the complexes was resolved into the pure enantiomers, and although we could 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** suggests a large DNA-binding discrimination between its two antipodes.

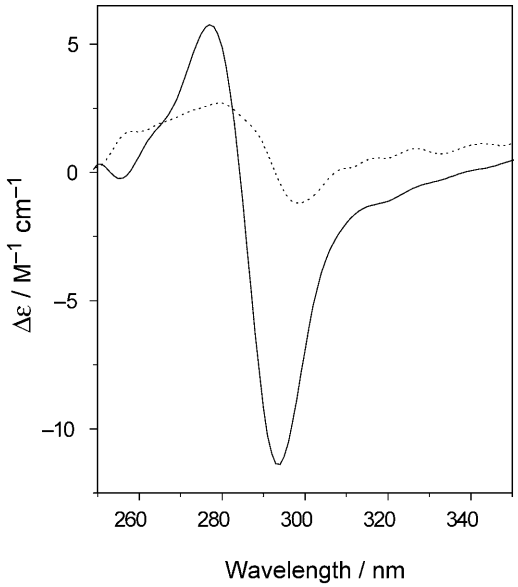


Fig. 5. *CD Spectra of* **1** (—) *and* **2** (···) *after 36 h of dialysis against CT-DNA in stirred aq. solution*

2.5. *Photo-Activated Cleavage of pBR-322 DNA*. The cleavage of plasmid DNA can be monitored by agarose-gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively fast migration is generally observed for the intact supercoiled form (I). When scission occurs on one strand (nicking), the supercoil relaxes to generate a slower-moving, open-circular form (II). When both strands are cleaved, a linear form (III) is generated, migrating between type-I and type-II DNA [40].

In *Fig. 6*, the gel-electrophoresis pattern of pBR-322 DNA is shown after incubation with **1** or **2** and irradiation at 400 nm. No DNA cleavage was observed for negative controls (lane *0*). With increasing concentration of the Ru^{II} complexes (lanes *1–4*), the amount of type-I pBR-322 DNA was gradually diminished, whereas type-II DNA increased. At a concentration of 40 μM of **1** (lane *4*), complete conversion of DNA from type-I to type-II was observed, in contrast to complex **2**, where only partial conversion was observed at this concentration. These results indicate that **1** is more effective in clearing DNA than **2**. These different cleavage efficiencies parallel the observed DNA-binding affinities of the two complexes, as has been reported before in other cases [27][41].

It is of interest to note that $[Ru(phen)_3]Cl_2$ has been reported to involve a singlet-oxygen ($^1O_2$)-mediated DNA-photocleavage mechanism [42]. To identify the nature

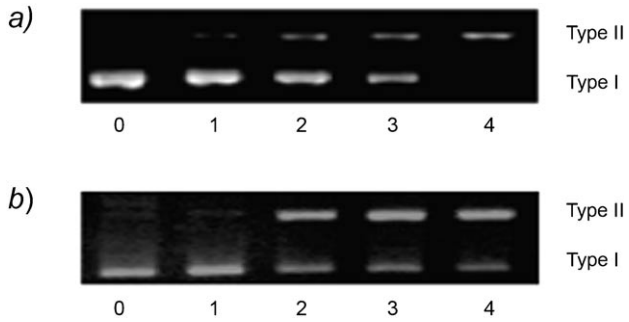


Fig. 6. *Photo-activated cleavage of pBR-322 DNA in the presence of* **1** (*a*) *or* **2** (*b*) *after 60 min of irradiation at 400 nm*. Lane *0*, DNA alone; lanes *1–4*, at 10, 20, 30, and 40 μM, Ru^{II} complex, resp.

of the reactive species responsible for photo-activated cleavage of plasmid DNA, we further investigated the influence of different potentially inhibiting agents. In the case of complex **1**, studies with the $^1O_2$ quencher histidine (His) were carried out (*Fig. 7*). Indeed, plasmid-DNA cleavage by **1** was inhibited in the presence of His (lane *2*), which indicated that $^1O_2$ acts as a competing cleavage agent. In contrast, in the presence of superoxide dismutase (SOD), a facile superoxide-anion-radical ($O_2^{\bullet-}$) quencher, no inhibition was observed (lane *3*), indicating that $O_2^{\bullet-}$ was not the reactive species. Furthermore, in the presence of mannitol, a facile hydroxyl-radical (OH$^\bullet$) scavenger, no obvious inhibition of the photo-induced cleavage of the plasmid was observed (lane *4*). However, in the presence of sodium formate (lane *5*) and DMSO (lane *6*), two other OH$^\bullet$ scavengers, different degrees of inhibition were found. This indicates that, in the case of **1**, hydroxyl radicals play a significant role in the photocleavage mechanism; photoreduction of Ru^{II} complexes with concomitant oxidation of OH is an important step in DNA cleavage [43]. Similar data (not shown) were also obtained for complex **2**.

Fig. 7. *Photo-activated cleavage of pBR-322 DNA by* $[Ru(bpy)_2(btip)]^{2+}$ (**1**; 20 μM) *in the presence of different inhibitors after irradiation at 400 nm for 60 min*. Lane *0*, without **1**; lane *1*, without inhibitor; lane *2*, with His (1.2 mM); lane *3*, with superoxide dismutase (1000 U/ml), lane *4*, with mannitol (50.0 mM); lane *5*, with sodium formate (100.0 mM); lane *6*, with DMSO (10.0 mM).

3. Conclusions. – In summary, two novel complexes, $[Ru(bpy)_2(btip)]^{2+}$ (**1**) and $[Ru(dmb)_2(btip)]^{2+}$ (**2**) have been synthesized and characterized. Spectroscopic studies, viscosity measurements, and equilibrium dialysis in combination with CD spectroscopy showed that both **1** and **2** bind (enantioselectively) to CT-DNA through intercalation. When irradiated at 400 nm, complex **1** was found to be a more-effective DNA-cleaving agent than **2**. Thereby, singlet oxygen ($^1O_2$) and hydroxyl radicals (OH$^\bullet$) were found to be the reactive species responsible for the cleavage of plasmid DNA.

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Experimental Part

General. All reagents and solvents are commercially available and used without further purification, unless noted otherwise. Doubly distilled H_2O was used to prepare buffers. CT-DNA was obtained from *Sino-American Biotechnology Co.*, and 1,10-phenanthroline-5,6-dione (**3**) [44], *cis*-$[Ru(bpy)_2Cl_2]\cdot 2\ H_2O$ and *cis*-$[Ru(dmb)_2Cl_2]\cdot n\ H_2O$ [45] were prepared according to literature procedures. All other materials were commercially available and of reagent grade. UV/VIS Spectra: *Perkin-Elmer Lambda-25* apparatus; λ_{max} in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. Fluorescence Spectra: *Perkin-Elmer LS-55* spectrophotometer, at r.t. CD Spectra: *JASCO-J715* spectropolarimeter. ^{1}H-NMR Spectra: *Bruker Avance-400* apparatus, at 400 MHz in (D_6)DMSO at r.t.; δ in ppm rel. to Me_4Si, J in Hz. FAB-MS: *VG ZAB-HS* mass spectrometer, 3-nitrobenzyl alcohol (NBA) matrix. ESI-MS: LQC system (*Finnigan MAT*), with MeCN as mobile phase; spray voltage 4.50 KV, tube-lens offset 30.00 V, capillary voltage 23.00 V, and capillary temp. 200°; in m/z. Elemental analyses (C, H, N): *Perkin-Elmer 240Q* elemental analyzer.

*2-Benzo[*b*]thien-2-yl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline* (*btip*). A mixture of **3** (0.16 g, 0.5 mmol), **4** (0.07 g, 1.0 mmol), ammonium acetate (1.54 g, 20 mmol), and glacial AcOH (10 ml) was heated at reflux with stirring for 1 h. The cooled soln. was filtered, diluted with H_2O, and neutralized with conc. aq. NH_3. The yellow precipitate was collected and purified by column chromatography (CC) (*Alox*; EtOH/toluene 5:1) to afford btip (0.15 g, 85%). Amorphous, yellow solid. FAB-MS: 353.1 ($[M+1]^+$). Anal. calc. for $C_{21}H_{12}N_4S$: C 71.57, H 3.43, N 15.90; found: C 71.35, H 3.57, N 15.74.

[Ru(bpy)$_2$(btip)](ClO$_4$)$_2\cdot H_2O$ (**1**). A mixture of *cis*-$[Ru(bpy)_2Cl_2]\cdot 2\ H_2O$ (94 mg, 0.18 mmol), btip (63 mg, 0.18 mmol), and ethylene glycol (10 ml) was thoroughly deoxygenated. The purple mixture was heated for 8 h at 120° under Ar atmosphere. When the soln. finally turned red, it was cooled to r.t., and an equal volume of sat. aq. $NaClO_4$ soln. was added under vigorous stirring. The red 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 2:1) to afford 130 mg (71%) of the title compound. UV/VIS (MeCN): 460 (23000), 351 (56000), 288 (110500). ^{1}H-NMR (400 MHz, (D_6)DMSO); 8.93 (*d*, $J=7.6$, 2 H); 8.87 (*d*, $J=8$, 2 H); 8.84 (*d*, $J=7.6$, 2 H); 8.20 (*t*, 2 H); 8.09 (*t*, 2 H); 8.00 (*s*, 1 H); 7.93 (*d*, $J=7.2$, 2 H); 7.87 (*t*, 2 H); 7.80 (*d*, $J=4.4$, 2 H); 7.74 (*t*, 2 H); 7.59 (*t*, 4 H); 7.30–7.39 (*m*, 4 H). ESI-MS (MeCN): 864.8 ($[M-ClO_4]^+$), 764.7 ($[M-2\ ClO_4-H]^+$), 383.1 ($[M-2\ ClO_4]^{2+}$). Anal. calc. for $C_{41}H_{30}Cl_2N_8O_9RuS$: C 50.11, H 3.08, N 11.40; found: C 49.97, H 3.21, N 11.36.

[Ru(dmb)$_2$(btip)](ClO$_4$)$_2\cdot H_2O$ (**2**). Prepared in analogy to **1**, but from *cis*-$[Ru(dmb)_2Cl_2]\cdot n\ H_2O$ (0.10 g, ca. 0.18 mmol). Yield: 0.12 g (64%). UV/VIS: (MeCN): 468 (7620), 347 (17600), 288 (36000). ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.97 (*d*, $J=7.6$, 2 H); 8.39 (*s*, 2 H); 8.34 (*s*, 2 H); 8.13 (*s*, 1 H); 7.90 (*d*, $J=5.2$, 4 H); 7.65 (*d*, $J=7.6$, 2 H); 7.63 (*d*, $J=4.8$, 2 H); 7.40 (*d*, $J=6.4$, 2 H); 7.37 (*d*, $J=1.6$, 2 H); 7.27 (*d*, $J=2.4$, 2 H); 7.03 (*d*, $J=6$, 2 H). ESI-MS (MeCN): 921.5 ($[M-ClO_4]^+$), 821.4 ($[M-2\ ClO_4-H]^+$), 411.7 ($[M-2\ ClO_4]^{2+}$). Anal. calc. for $C_{45}H_{38}Cl_2N_8O_9RuS$: C 52.02, H 3.69, N 10.79; found: C 51.99, H 3.78, N 10.65.

Viscosity Measurements. These experiments were carried out with an *Ubbelodhe* viscometer maintained at a const. temp. of 30.0 ± 0.1° in a thermostated bath. DNA samples of *ca.* 200-bp average length were prepared by sonication [46]. The flow time was measured with a digital stopwatch, and each sample was tested three times to get an average calculated flow time. Data are presented as $(\eta/\eta_0)^{1/3}$ *vs.* binding ratio [32], where η is the viscosity of DNA in the presence of complex, η_0 being the viscosity of free DNA.

UV/VIS Titrations. All experiments were carried out in buffer *A* (5 mM *Tris*·HCl, 50 mM NaCl, pH 7.2) at r.t. A soln. of CT-DNA in buffer *A* gave a ratio of UV absorbances at 260 and 280 nm of *ca.* 1.8:1 to 1.9:1, indicating that the DNA was sufficiently free of protein [47]. The concentration of CT-DNA was determined spectrophotometrically ($\varepsilon_{260}=6600\ cm^{-1}$) [48]. Stock solns. were stored at 4° and used within

4 d. Titration experiments were performed by using a fixed Ru^{II} complex concentration (10 μM), to which CT-DNA stock soln. was added up to a ratio of [DNA]/[Ru] 0.51:1. The complex–DNA solns. were allowed to equilibrate for 5 min before spectra were recorded.

Gel Electrophoresis. For the gel-electrophoresis experiments, supercoiled pBR-322 DNA (0.1 μg) was treated with **1** or **2** in buffer *B* (50 mM *Tris*·HCl, 18 mM NaCl, pH = 7.2). After pre-incubation of the solns. in the dark for 1 h, the samples were irradiated for 60 min inside the sample chamber of the spectrofluorimeter (λ_{ex} 400 ± 5 nm, slit-width 5 nm). The samples were then analyzed by gel electrophoresis over 30 min at 75 V in *Tris*-acetate buffer containing 1% agarose gel. The gel was stained with ethidium bromide (EB; 1 μg/ml^{-1}) and photographed under UV light.

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