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Mono- and bi-nuclear ruthenium(II) complexes containing a new asymmetric ligand 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]1,10-phenanthroline: synthesis, characterization and DNA-binding properties

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A novel asymmetric ligand 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]1,10-phenanthroline (pztp) and its mono- and bi-nuclear complexes $[Ru(bpy)_2(pztp)]^{2+}$ **1** and $[(bpy)_2Ru(pztp)Ru(bpy)_2]^{4+}$ **2** (bpy = 2,2′-bipyridine) have been synthesized and characterized by UV/VIS, IR, ^{1}H NMR and mass spectra. The electrochemical behaviours of complexes **1** and **2** were observed and displayed oxidation potentials at 1.37 and 1.39 and 1.60 V *vs*. saturated calomel electrode, respectively. The binding of the two complexes with calf thymus DNA has also been investigated by a spectrophotometric method and viscosity measurements. The results indicated that the two complexes interact with DNA in sharply different ways. The mononuclear complex **1** intercalates into DNA base pairs despite its small hypochromicity value, while the binuclear complex **2** binds to DNA *via* electrostatic interaction. The circular dichroism signals of the dialysates of the racemic complexes against calf thymus DNA are discussed.

The potential of substitution-inert metal complexes as photochemical structural and stereoselective probes of nucleic acids has been explored extensively over the past decade.[1] Despite a considerable amount of reported materials, however, to the best of our knowledge the nature of the binding of these complexes to DNA and their binding geometries has remained relatively modest. The binding mode of the parent complex $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) is still ambiguous.[2] On the other hand, there is a consensus about classical intercalative binding of recently developed complexes, such as $[Ru(bpy)_2(dppz)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), in which the dppz ligand intercalates between the base pairs of double helical DNA.[3–5] Therefore, the binding of ruthenium(II) polypyridyl complexes to DNA has initiated vigorous interest and many new structural analogues based on the prototype $[Ru(phen)_3]^{2+}$ have been also synthesized and investigated.

However, it is noticed that most of those reported complexes contain only symmetrical or essentially symmetrical aromatic ligands, and investigations of polypyridyl ruthenium(II) complexes with asymmetric ligands as DNA-binding reagents have been very limited.[6] In fact, molecular shape, among the various factors that contribute to stabilizing the metal complex on the DNA helix, appears to be the most significant.[7] In addition, much less attention has been focused on the interaction of bi- and/or poly-nuclear ruthenium complexes and DNA.[8] We have now designed and synthesized a new asymmetric ligand 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]1,10-phenanthroline (pztp), its mononuclear complex $[Ru(bpy)_2(pztp)][ClO_4]_2\cdot 3H_2O$ **1** and binuclear complex $[(bpy)_2Ru(pztp)Ru(bpy)_2][ClO_4]_4\cdot 4H_2O$ **2** and characterized them by spectral and electrochemical methods. The DNA binding behaviours of the ruthenium(II) complexes have also been observed by a spectrophotometric method and viscosity measurements. The circular dichroism of the dialysates of the racemic complexes against calf thymus DNA has been measured and discussed. In both complexes two bpy are used as co-complexation ligands because bpy has been previously considered to be only minimally efficient at inducing intercalative binding with DNA.[7,9]

Experimental

Synthesis

1,10-Phenanthroline-5,6-dione (phendione),[10] *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$[11] and $[Ru(bpy)_2(phendione)][ClO_4]_2$[12] were prepared according to the literature procedures, and other chemicals were commercially available.

Pyrazine-2-carboxamide hydrazone. The compound was synthesized using the method described by Case[13] and confirmed by NMR spectra. ^{1}H NMR (DMSO-d_6): δ 9.11 (s, 1 H), 8.53 (d, 2 H), 5.74 (s, 2 H) and 5.63 (s, 2 H).

pztp. A mixture of pyrazine-2-carboxamide hydrazone (0.356 g, 2.6 mmol) and phendione (0.525 g, 2.5 mmol) was refluxed with stirring in ethanol. In a few minutes much yellow precipitate appeared. After 3 h stirring the insoluble material was removed by filtration while hot, washed with ethanol (3 × 5 cm^3), then dried at 50 °C *in vacuo*. Yield: 0.544 g, 70% (Found: C, 65.62; H, 3.20; N, 31.70. Calc. for $C_{17}H_9N_7$: C, 65.57; H, 2.92; N, 31.51%). ^{1}H NMR (CD_2Cl_2): δ 10.11 (s, 1 H), 9.85 (d, 1 H, J = 8.4), 9.76 (d, 1 H, J = 8.0), 9.43 (d, 1 H, J = 4.5), 9.40 (d, 1 H, J = 4.5), 8.97 (d, 1 H, J= 2.7), 8.87 (d, 1 H, J = 2.4 Hz), 7.94 (q, 1 H) and 7.91 (q, 1 H). FAB-MS: m/z = 312 (M^+).

$[Ru(bpy)_2(pztp)][ClO_4]_2\cdot 3H_2O$ 1. The complex $[Ru(bpy)_2(phendione)][ClO_4]_2$ (0.260 g, 0.3 mmol) was dissolved in acetonitrile (50 cm^3) and pyrazine-2-carboxamide hydrazone (0.048 g, 0.35 mmol) in ethanol (10 cm^3) was added dropwise. The mixture was refluxed under argon for 3 h to give a red solution. The solvent was removed by rotory evaporation, the product collected and purified by column chromatography on alumina with acetonitrile as eluent and dried *in vacuo*. Yield: 0.180 g, 64.7% (Found: C, 45.12; H, 2.69; N, 15.81. Calc. for $C_{37}H_{31}Cl_2N_{11}O_{11}Ru$: C, 45.39; H, 3.16; N, 15.75%). λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})(water): 441 (17900), 284 (89300) and 254 (55200). ^{1}H NMR (CD_3CN): δ 10.11 (s, 1 H), 9.87 (d, 1 H, J = 8.4), 9.78 (d, 1 H, J = 8.4), 9.01 (d, 1 H, J = 2.4), 8.95 (d, 1 H, J = 2.4), 8.63 (d, 2 H, J = 8.8), 8.59 (d, 2 H, J = 8.7), 8.38

(d, 1 H, $J = 5.4$), 8.34 (d, 1 H, $J = 5.3$), 8.19 (t, 2 H), 8.09 (t, 2 H), 8.03 (q, 1 H), 8.01 (m, 1 H), 7.92 (d, 2 H, $J = 4.8$), 7.78 (d, 2 H, $J = 4.7$ Hz), 7.54 (m, 2 H) and 7.34 (m, 2 H). FAB-MS: $m/z = 824$ ($M - ClO_4$) and 725 ($M - 2ClO_4$).

$[(bpy)_2Ru(pztp)Ru(bpy)_2][ClO_4]_4 \cdot 4H_2O$ 2. A mixture of $[Ru(bpy)_2Cl_2] \cdot 2H_2O$ (0.35 g, 0.67 mmol) and the bridging ligand pztp (0.100 g, 0.32 mmol) in ethanol–water (2:1 v/v, 60 cm^3) was refluxed for 5 h, during which time the solution turned dark red. After most of the ethanol was removed by rotary evaporation, a dark red precipitate was obtained by dropwise addition of aqueous $NaClO_4$ solution. The product was purified by column chromatography on alumina with acetonitrile–ethanol (9:1 v/v) as eluent. Yield: 0.411 g, 80.0% (Found: C, 42.40; H, 2.68; N, 12.80. Calc. for $C_{57}H_{49}Cl_4N_{15}O_{20}Ru_2$: C, 42.54; H, 3.04; N, 13.05%). λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})(water): 476 (24000), 410 (sh), 283 (116100) and 245 (60900). ^1H NMR (CD_3CN): δ 10.20 (s, 1 H), 9.83 (d, 1 H, $J = 8.4$ Hz), 8.85 (d, 1 H), 8.7–8.5 (m), 8.45–8.35 (m), 8.34–8.2 (m), 8.19–7.95 (m), 7.9–7.55 (m), 7.54–7.45 (m), 7.36 (t) and 7.26 (m).

CAUTION: perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of the material should be prepared and handled with great care.

Measurements

Elemental analyses (C, H and N) were performed on a Perkin-Elmer 240Q elemental analyser. Infrared spectra were obtained on a Nicolet 170SX-FTIR spectrometer as KBr discs, UV/VIS spectra on a Shimadzu MPS-2000 spectrophotometer, and ^1H NMR spectra on a Bruker ARX-300 NMR spectrometer with CD_2Cl_2 (for ligand) or CD_3CN (for complex) as solvent at room temperature and $SiMe_4$ as an internal standard. The two-dimensional NMR experiments were carried out with the standard program.

Cyclic voltammetry was performed on an EG&G PAR 273 polarographic analyser and 270 universal programmer. The supporting electrolyte was 0.1 mol dm^{-3} tetraethylammonium perchlorate in acetonitrile freshly distilled from phosphorus pentaoxide and deaerated by purging with nitrogen. A standard three-electrode system was used comprising a platinum microcylinder working electrode, platinum-wire auxiliary electrode and a saturated calomel reference electrode (SCE).

Equilibrium dialyses were conducted at room temperature with 5 cm^3 of calf thymus DNA (1.06 mmol dm^{-3}) sealed in a dialysis bag and 10 cm^3 of the complex (0.6 mmol dm^{-3}) outside the bag and the system agitated on a shaker bath. After 48 h the circular dichroism (CD) spectrum of the dialysate outside the bag was measured on a JASCO J-715 spectropolarimeter.

All the experiments involving the interaction of the complexes with DNA were carried out in aerated buffer (5 mmol dm^{-3} Tris–HCl, 50 mmol dm^{-3} NaCl, pH 7.0). Solutions of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of *ca.* 1.9:1, indicating that the DNA was sufficiently free of protein.[14] The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 dm^3 mol^{-1} cm^{-1}) at 260 nm.[15]

Viscosity experiments were carried on an Ubbelodhe viscometer, immersed in a thermostatted water-bath maintained at 32.7 ±0.1 °C. DNA Samples approximately 200 base pairs in average length were prepared by sonication in order to minimize complexities arising from DNA flexibility.[16] Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the concentration of ruthenium(II) complexes, where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions ($t > 100$ s) corrected for the flow time of buffer alone (t_0), $\eta = t - t_0$.[4b]

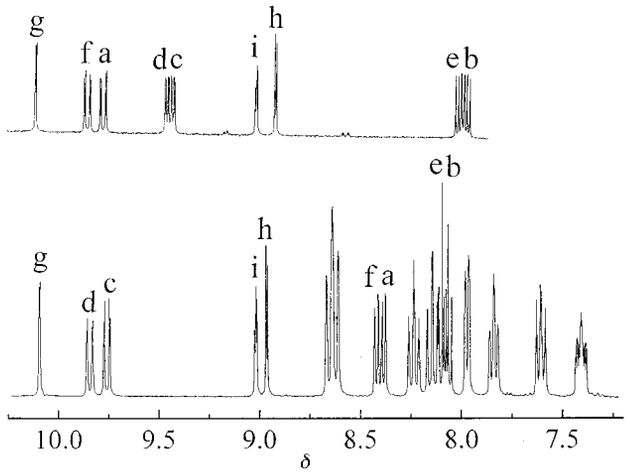
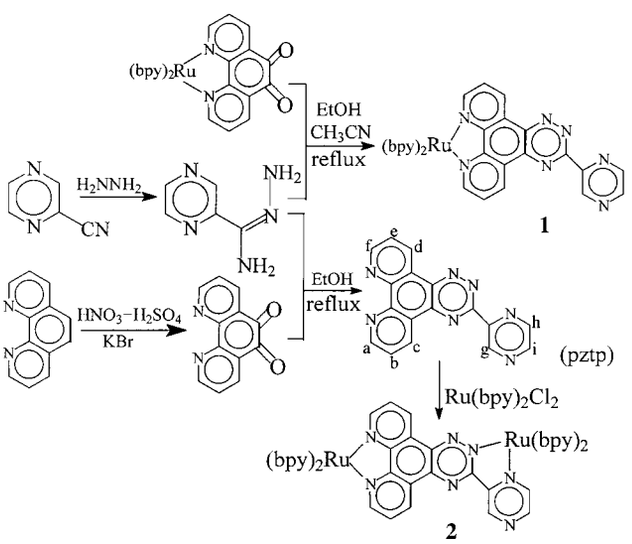


Fig. 1 The ^1H NMR spectra of the ligand pztp in CD_2Cl_2 (top) and its mononuclear complex **1** in CD_3CN (bottom).

Results and discussion

Syntheses

The outline of the syntheses of the ligand and its complexes is presented in Scheme 1. The ligand pztp was prepared through



Scheme 1 Synthetic routes for the preparation of the ligand and complexes.

condensation of phendione with pyrazine-2-carboxamide hydrazone using a similar method to that described by Case.[13] This method provides a very convenient way to prepare variously aryl-substituted asymmetric compounds containing a 1,2,4-triazine ring. The pztp is an asymmetric ligand which can co-ordinate to metal ions *via* two different sites, one is the nitrogen atoms of 1,10-phenanthroline and the other is composed of one of the pyrazyl ring and one of the 1,2,4-triazine ring. Indeed, treatment of 1 equivalent of pztp ligand with 2 equivalents of $[Ru(bpy)_2Cl_2] \cdot 2H_2O$ provided a new asymmetric binuclear ruthenium(II) complex **2** in high yield. Obviously, there can be two isomers involving N^2 and N^4 of the triazine ring. However in this case it is obvious that the co-ordination is at N^2 rather than at N^4 from the viewpoint of the geometry of the ligands. Therefore, to prepare the mononuclear complex **1** we have chosen condensation of pyrazine-2-carboxamide hydrazone with the preco-ordinated phendione complex $[Ru(bpy)_2(phendione)]^{2+}$ to avoid the formation of binuclear complex and the other mononuclear complex co-ordinated by pyrazine and triazine (Scheme 1). This can also be identified by NMR spectra (see below). In fact, treatment of the ligand

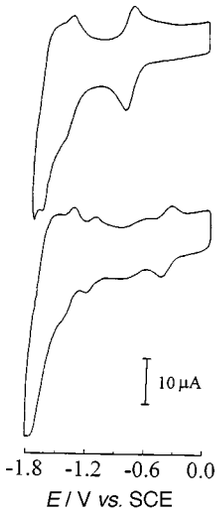


Fig. 2 Cyclic voltammograms of complexes **1** (top) and **2** (bottom) in CH_3CN in the range −1.8 to 0 V (0.1 mol dm^{-3} NEt_4ClO_4, scan rate 200 mV s^{-1}).

pztp and $[Ru(bpy)_2Cl_2]\cdot 2H_2O$ in a ratio of 1:1, still gave the binuclear complex as the main product and only very little mononuclear complex was obtained.

^{1}H NMR spectra

The ^{1}H NMR spectrum of complex **1** in comparison with that of its asymmetric ligand pztp is shown in Fig. 1. The proton chemical shifts are assigned according to ^{1}H–^{1}H COSY experiments and comparison with those of similar compounds. The protons of H_g and H_h in free pztp show downfield shifts of about 0.58 and 0.14 ppm, respectively, but H_i remains nearly unchanged in comparison with the corresponding protons of free 2,2′-bipyrazine.[17] The protons of H_a, H_f, H_b and H_e in free pztp all experience downfield shifts of 0.40–0.65 ppm while H_c and H_d show much larger ones of 0.84 ppm in comparison with the corresponding protons of free phen.[18] These pronounced shifts may be attributed to the extensive π-electron shared system of the electron-deficient fused *as*-triazine ring and the substituted pyrazyl ring. The chemical shifts of H_d, H_e and H_f are considered to be downfield in comparison with those of H_a, H_b and H_c respectively. The triazine ring is an electron-withdrawing group, and N^1 and N^2 are close to the H_d–H_f ring. That is to say, the electron-withdrawing effect of the triazine ring on the H_d–H_f ring is stronger than that of the H_a–H_c ring. A similar example has been reported.[19]

It is easy to assign the chemical shifts of the protons of the ligand pztp in complex **1** bearing in mind the free pztp data. The chemical shifts of H_g, H_h and H_i on the pyrazine ring remain almost unchanged on co-ordination; this also indicates that the nitrogen atom of the pyrazinyl ring does not co-ordinate. However, the protons on the phen ring all experience much larger shifts: H_c and H_d show 0.38 and 0.40 ppm downfield shifts, H_b and H_e about 0.1 ppm, while H_a and H_f experience surprising upfield shifts of 1.42 and 1.47 ppm, respectively, in comparison with those of free pztp. The dramatic upfield shifts of H_a and H_f may be due to the effect of the ring current of the bpy ligands.

In the ^{1}H NMR spectrum of complex **2** the downfield signals at δ 10.20, 9.83 and 8.85 can easily be assigned to H_g, H_d and H_h respectively, in comparison with those of the pztp ligand and complex **1**. The resonance peaks of H_c and H_i were shifted upfield and overlap with other peaks, due to the effect of the ring currents of the pyrazinyl and bpy rings, respectively. In complex **1** the pyrazinyl ring can be rotated to a suitable angle to relieve the steric interaction between it and the phen rings. However in **2** the nitrogen atoms of the pyrazinyl and triazine

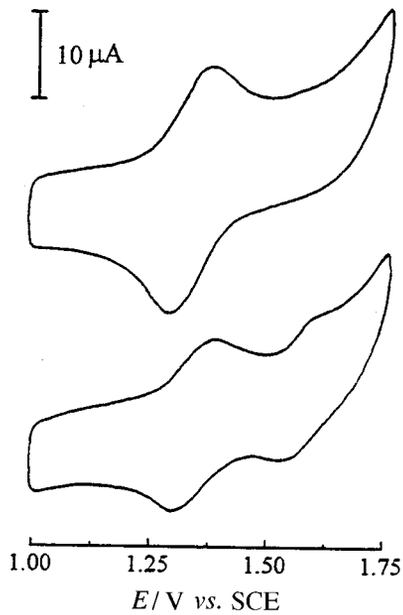


Fig. 3 Cyclic voltammograms of complexes **1** (top) and **2** (bottom) in CH_3CN in the range of 1.0 to 1.75 V (details as in Fig. 2).

rings co-ordinate to Ru^{II}, therefore, the pyrazinyl ring cannot rotate freely. The other resonance peaks are too complicated (two metal centers are bridged asymmetrically) to be assigned.[20] Complex **2** can also be identified by its absorption spectrum and electrochemistry (see below).

Electrochemistry

The cyclic voltammograms of complexes **1** and **2** are given in Figs. 2 and 3, and consistent with one or two metal-based oxidations and reductions at positive and negative potentials, respectively. This pattern is common to most d^6 metal polypyridyl complexes where the redox orbitals are localized on the individual ligands.[21] For the mononuclear complex **1** dissolved in CH_3CN three reduction potentials are observed at −0.87, −1.47 and −1.72 V (*vs.* SCE), respectively. A series of waves are observed for the binuclear complex **2**, occurring at −0.48, −0.83, −1.23, −1.46 and −1.76 V. The mononuclear complex exhibits one reversible oxidation at +1.37 V while the binuclear complex exhibits two at +1.39 and +1.60 V (see Fig. 3), respectively.

For both the mononuclear and binuclear complexes the first reduction potential may be assigned to the pztp ligand. Since the redox and spectroscopic orbitals in these complexes tend to be similar, the lowest energy excited state involves promotion of an electron from the metal to the pztp ligand. For the binuclear complex, introduction of a second $Ru(bpy)_2^{2+}$ moiety at the remote bidentate site of $[Ru(bpy)_2(pztp)]^{2+}$ results in a 0.39 V shift in the reduction potential of the pztp ligand, reflecting a net stabilization of the π* orbital of the bridging relative to the terminal pztp ligand. The second reduction of the binuclear complex occurs at a potential −0.83 V comparable to that of the first reduction of the mononuclear complex (−0.87 V). This is very similar to those of mononuclear $[Ru(bpy)_2(2,3\text{-dpp})]^{2+}$ and binuclear $[(bpy)_2Ru(2,3\text{-dpp})Ru(bpy)_2]^{4+}$ [dpp = 2,3-bis(2-pyridyl)pyrazine].[22]

The difference in the potentials of the first oxidation of the mononuclear and the binuclear complex is not significant (*ca.* 20 mV), indicating a fairly weak metal–metal interaction in the binuclear complex. This shift has been attributed to electrostatic effects of the second metal center and the shared π system.[23] The second oxidation potential of the binuclear complex at +1.60 V is similar to that observed for the binuclear complex $[(bpy)_2Ru(tpt)Ru(bpy)_2]^{4+}$ at +1.55 V [tpt = 2,4,6-tris(2-pyridyl)triazine].[24]

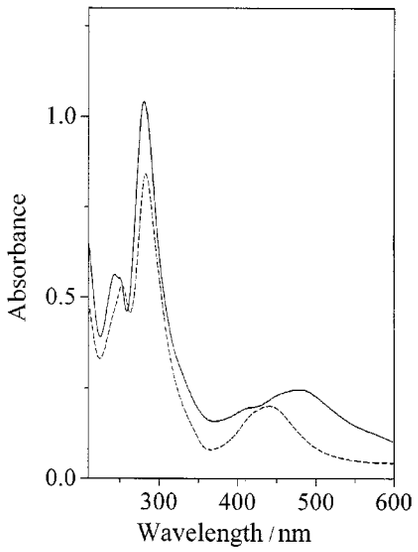


Fig. 4 Absorption spectra of the mononuclear complex **1** (---) and of binuclear complex **2** (——) in water at the same concentration of 10 μmol dm^{-3}.

Absorption spectra

Absorption spectra of complexes **1** and **2** were obtained in water (Fig. 4). The spectrum of complex **1** consists of three well resolved bands at 441, 284 and 254 nm in range 200 to 700 nm, similar in shape to those of $[Ru(bpy)_3]^{2+}$. The bands at 284 and 254 nm are attributed to intraligand π–π* transitions by comparison with the spectrum of $[Ru(bpy)_3]^{2+}$. The lowest energy band at 441 nm is assigned to metal–ligand charge transfer (MLCT) transition. This band is blue shifted in comparison with that of $[Ru(bpy)_3]^{2+}$, which can be attributed to the increased π delocalization and thus π-acceptor capacity of the pztp ligand, resulting in decreased electron density on the Ru and in turn stabilization of the metal d_π orbital.[25]

For the binuclear complex **2** three bands at 476, 283 and 245 nm are observed. That at 476 nm is assigned to MLCT transition. Generally in a MLCT absorption process an electron is removed from the metal orbital (d_π) to an empty orbital of the ligand (π*). Going from the mononuclear complex **1** to the binuclear complex **2**, the absorption maximum of the MLCT transition shifts to longer wavelength by about 35 nm. This indicates that the co-ordination of the second ruthenium(II) ion stabilizes the π* level of the pztp ligand and the weak metal–metal interaction in **2**,[26] which is consistent with the electrochemistry results. Similar cases were observed between $[Ru(dmb)_2(bbdb)]^{2+}$ and $[(dmb)_2Ru(bbdb)Ru(dmb)_2]^{4+}$ [dmb = 4,4′-dimethyl-2,2′-bipyridine, bbdb = 1,4-bis(4′-methyl-2,2′-bipyridin-4-yl)buta-1,3-diene].[27] Generally, in a complex containing a bis-bidentate bridging ligand, the molar absorptivity of the symmetrical binuclear complex is approximately twice that of its mononuclear complex. However, in our case, the molar absorptivity of complex **2** is about 1.4 times that of **1**, which may be explained by the asymmetry of the binuclear complex which decreases the overlap of the $Ru(d_\pi) \rightarrow$pztp (π*) transitions and broadens the MLCT band.[28]

Electronic absorption spectroscopy has been applied widely to study the binding of ruthenium polypyridyl complexes and DNA.[1] In general, the extent of hypochromism in the visible MLCT band parallels the intercalative strength. For instance, in the presence of DNA, the hypochromism in MLCT bands of complexes $[Ru(bpy)_2(ip)]^{2+}$ (ip = imidazo[*f*]1,10-phenanthroline) and $[Ru(bpy)_2(pip)]^{2+}$ (pip = 2-phenylimidazo[*f*]1,10-phenanthroline) was about 15.5 and 21.9%,[29] and $[(bpy)_2Ru(dpb)Ru(bpy)_2]^{4+}$ [dpb = 2,3-bis(2-pyridyl)benzoquinoxaline] and $[(H_3N)_4Ru_4(dpb)Ru(NH_3)_4]^{4+}$ about 0 and 29.0%,[8b] respectively. Therefore the latter intercalated more strongly than the former. The visible absorption spectra of complexes **1** and **2** in the absence and the presence of calf thymus DNA are illustrated in Fig. 5. The hypochromism in MLCT bands of complexes **1** and **2** is about 12.0 and 5.0%, respectively. For **1** there were two isosbestic points at about 365 and 490 nm in the range from 350 to 600 nm when different concentrations of DNA were added. For **2** no obvious isosbestic point was observed in the above range. It is not surprising that the MLCT band of complex **2** is just slightly affected on the addition of DNA because it contains two $[Ru(bpy)_3]^{2+}$-like units. The hypochromism of **2** is only slightly larger than that of $[Ru(bpy)_3]^{2+}$,[6b] indicating that the electrostatic interaction is the main factor inducing the hypochromism effect between complex **2** and DNA. For the mononuclear complex **1** the pyrazine ring in the potential intercalative ligand pztp is basically coplanar with the *as*-triazine moiety which is fused to phenanthroline (although no crystal structural data are available, there is no hydrogen atom on the *as*-triazine ring and interaction involving the hydrogen atom thus can be avoided). So it seems that complex **1** can intercalate its substituted pyrazine moiety into the DNA base pairs. The hypochromism of **1** is much bigger than that of **2**, indicating that they may bind to DNA in different ways. However, the hypochromism of the mononuclear complex is much smaller than those of many other so-called intercalative complexes such as $[Ru(bpy)_2(pip)]^{2+}$,[29] $[Ru(bpy)_2(bpbpy)]^{2+}$ [bpbpy = 4,4′-bis(4-pyridyl)-2,2′-bipyridine][8c] and $[Ru(bpy)_2(phi)]^{2+}$ (phi = 9,10-phenanthrenequinone diimine),[7] even smaller than that of the complex $[Ru(NH_3)_4(dppz)]^{2+}$ which was considered on the border between simple electronic association and intercalation with the helix DNA.[30]

Unfortunately, no luminescence was observed for both complexes **1** and **2** upon excitation in the MLCT bands above 350 nm either in aqueous solution or in the presence of calf thymus DNA. The detailed mechanism is unclear.

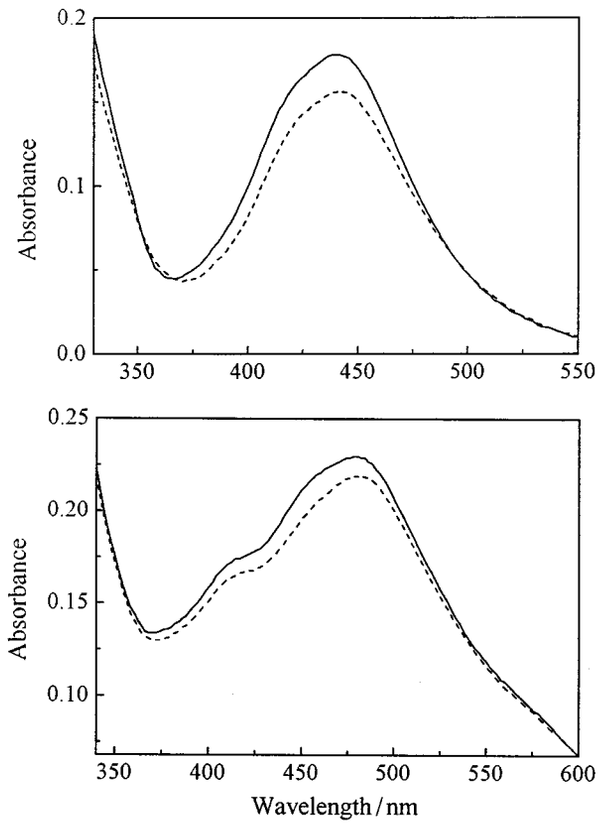


Fig. 5 Visible absorption spectra of complexes **1** (top, [DNA]/[Ru] = 8) and **2** (bottom, [DNA]/[Ru] = 10) in the absence (——) and presence (---) of calf thymus DNA with subtraction of the DNA absorbance (5 mmol dm^{-3} Tris–HCl, pH 7.0, 50 mmol dm^{-3} NaCl, [Ru] 10 μmol dm^{-3}).

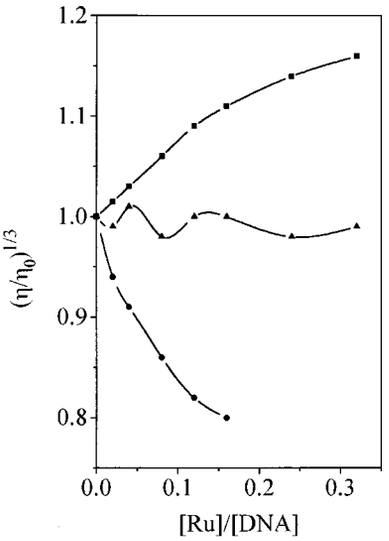


Fig. 6 Effect of increasing amounts of $[Ru(bpy)_3]^{2+}$ (▲), $[Ru(bpy)_2(pztp)]^{2+}$ (■) and $[(bpy)_2Ru(pztp)Ru(bpy)_2]^{4+}$ (●) on the relative viscosities of calf thymus DNA at 32.7 ±0.1 °C.

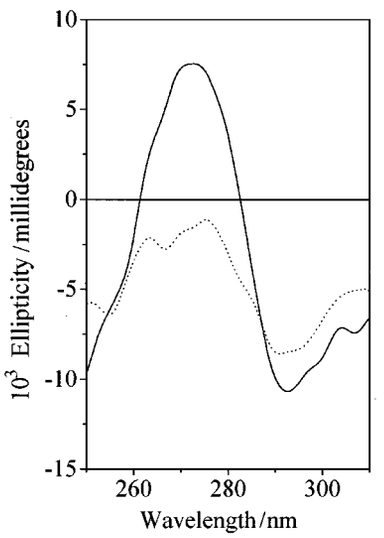


Fig. 7 Circular dichroism spectra of the dialysates of $[Ru(bpy)_2(pztp)]^{2+}$ (——) and $[(bpy)_2Ru(pztp)Ru(bpy)_2]^{4+}$ (---) after 48 h dialysis against calf thymus DNA ([Ru] = 0.6, [DNA] = 1.06 mmol dm^{-3}).

Viscosity measurements

For further clarification of the interaction between the two complexes and DNA, viscosity measurements were carried out. Optical photophysical probes provide necessary, but not sufficient, clues to support a binding model.

Hydrodynamic measurements that are sensitive to length change (*i.e.* viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data.[2a,31] A classical intercalation model results in lengthening the DNA helix as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial and/or non-classical intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity.[2a,31] Here we present the first example of a dramatic decrease of DNA viscosity just caused by electrostatic interaction between DNA and complex.

The effects of the complexes **1**, **2** and $[Ru(bpy)_3]^{2+}$ on the viscosity of rod-like DNA are shown in Fig. 6. The viscosity of DNA remains almost unchanged upon addition of $[Ru(bpy)_3]^{2+}$, which is consistent with an electrostatic association. With complex **1** the viscosity of DNA increases dramatically and nearly linearly at low complex concentration ([Ru]/[DNA] < 0.15), the slope being about 0.83 which is just slightly smaller than that of ethidium (3,8-diamino-5-ethyl-6-phenanthridinium) (0.91).[31] The result strongly indicates that complex **1** intercalates into DNA base pairs deeply despite its much smaller hypochromism in absorption spectra compared with those of the complexes $[Ru(bpy)_2(ppz)]^{2+}$ (ppz = 4′,7′-phenanthrolino-5′,6′ : 2 : 3-pyrazine)[8c] and $[Ru(bpy)_2(pip)]^{2+}$.[29] However, with complex **2** the viscosity of DNA decreases dramatically. To the best of our knowledge, this is the first example of a pronounced decrease of viscosity of DNA because of electrostatic interaction between DNA and a complex. There are two $[Ru(bpy)_3]^{2+}$-like units in complex **2**, so it cannot intercalate between the base pairs of DNA even partially. Similar examples of Δ-$[Ru(phen)_3]^{2+}$,[31] *rac*-$[Ru(bpy)_2(cip)]^{2+}$ {cip = 2-(2-chlorophenyl)imidazo[4,5-*f*][1,10]phenanthroline} and *rac*-$[Ru(bpy)_2(nip)]^{2+}$ {nip = 2-(2-nitrophenyl)-imidazo[4,5-*f*][1,10]phenanthroline}[32] have been observed which could decrease the viscosity of DNA because they only intercalated partially into the DNA base pairs just like a "wedge" and thus caused a bend or kink in the helix. The contrasting results from the viscosity measurements also indicate the obviously different interactions between the two complexes and calf thymus DNA. It seems that the binuclear complex binds to DNA by electrostatic interaction while its mononuclear counterpart can intercalate into DNA base pairs deeply despite its small change in absorption spectrum on addition of DNA.

Enantioselective binding

According to the proposed binding model,[33] the Δ enantiomer of the complex, a right-handed propeller-like structure, will display a greater affinity than the Λ enantiomer with the right-handed calf-thymus DNA helix, due to the appropriate steric matching. Therefore, the enantiospecific binding of complex to DNA can be observed clearly from circular dichroism spectra.

The CD spectra in the UV region of complexes **1** and **2** after their racemic solutions had been dialysed against calf thymus DNA are shown in Fig. 7. The presence of CD signals indicates enrichment of the isomer binding less favorably to the DNA. The dialysate for complex **1** (solid line) shows two strong CD signals with a positive peak at 273 nm and a negative peak at 292 nm, while **2** shows CD signals with negative peaks at 276 and 291 nm, respectively. In addition, the CD signals of **1** are much stronger than those of **2**, which may be explained by the different binding modes of the complexes. The former binds to DNA by intercalation while the latter is just a electrostatic binder, so the steric hindrance has a more evident effect on the former.

The CD spectra results indicate that complex **1** can be a good candidate as an enantioselective binder to DNA.

Conclusion

The new asymmetric binuclear ligand pztp and its mono- and bi-nuclear complexes were synthesized and characterized. The interactions of the two complexes and calf thymus DNA were observed by absorption spectra, viscosity measurement and CD spectra. The results indicate that the mononuclear complex **1** intercalates into the base pairs of DNA and the binuclear complex **2** binds to DNA by electrostatic interaction.

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