Interaction of $[Ru(dmp)_2(dppz)]^{2+}$ and $[Ru(dmb)_2(dppz)]^{2+}$ with DNA: Effects of the Ancillary Ligands on the DNA-Binding Behaviors

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Introduction

Binding studies of small molecules with DNA are very important in the development of new therapeutic reagents and DNA molecular probes.[1,2] The interactions of polypyridyl ruthenium complexes with DNA have been well documented;[3−10] however, the binding mechanism of this kind of complex still remains to be fully understood, and there is little consensus regarding the orientation and/or the location (major or minor groove) of the enantiomers binding with DNA.[7−10]

Dipyridophenazine (dppz) complexes of Os(II),[11] Ru(II),[12−14] and Re(I)[15] provide sensitive luminescent probes for double-stranded DNA in solution. $[Ru(L)_2(dppz)]^{2+}$ (L = bipyridine (bpy), phenanthroline (phen)) show no photoluminescence in aqueous solution at ambient temperature but luminesce brightly upon binding intercalatively with the dppz ligand between adjacent DNA base pairs, displaying the characteristic of molecular light switches. The classical intercalative binding of these complexes with DNA has been unambiguously confirmed by ^{1}H NMR,[16] resonance Raman,[17] and linear dichroism spectra,[18] as well as viscosity measurements.[19] The emission profile of these dppz complexes is sensitive to solvent accessibility, which makes them also excellent molecular probes to probe hydrophobic matrixes in aqueous solution such as Nafion films[20] and SDS micelles.[21] A series of derivatives of $[Ru(phen)_2(dppz)]^{2+}$ has been synthesized through substitution on the dppz ligand,[22] and it has been found that modification in the structure of the intercalating ligand caused changes in the emission bound to DNA and in the orientation of the intercalated complex. The influence of the ancillary ligands on the redox, optical, and DNA-binding properties of these complexes, however, is very limited. The complex $[Ru(NH_3)_4(dppz)]^{2+}$ has been reported to exhibit no luminescence either in the absence

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or in the presence of double-helical DNA.[23] Recently, $[Ru(IP)_2(dppz)]^{2+}$ (IP = imidazo[4,5-*f*][1,10]phenanthroline) has been found to bind more avidly to DNA than does the parent complex $[Ru(bpy)_2(dppz)]^{2+}$ and described as a sensitive luminescent reporter for double-helical DNA in solution.[13] The effect of structure changes in the ancillary phen or bpy ligands, which may result in different orientation of the ancillary ligands, on the interactions of dppz ruthenium complexes with DNA has yet to be reported. We report herein the synthesis of $[Ru(dmp)_2(dppz)](PF_6)_2$, **1** (dmp = 2, 9-dimethyl-1,10-phenanthroline), and $[Ru(dmb)_2(dppz)](PF_6)_2$, **2** (dmb = 4,4′-dimethyl-2,2′-bipyridine), along with the crystal structure of **1** and examine their different DNA-binding behaviors.

Experimental Section

Instrumentation and Materials. All reagents and solvents were purchased commercially and used without further purification unless otherwise noted. Solutions of calf thymus DNA (CT-DNA) in 50 mM NaCl/5 mM Tris-HCl (pH = 7.2) 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.[24] The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm.[25] Dialysis membranes (molecular weight cutoff of 8000−10000) were obtained from Union Carbide Co., Ltd. and were treated as described previously before use.[7a] Doubly distilled water was used to prepare buffers.

Physical Measurements. Microanalysis (C, H, and N) was carried out with a Perkin-Elmer 240Q elemental analyzer. ^{1}H NMR spectra were recorded on a Varian-500 spectrometer. All chemical shifts were given relative to tetramethylsilane (TMS). UV/vis spectra were recorded on a Shimadzu MPS-2000 spectrophotometer, and emission spectra were recorded on a Shimadzu RF-5000 luminescence spectrometer at room temperature. The circular dichroism (CD) spectra were measured on a JASCO-J20C or JASCO-J715 spectropolarimeter.

Cyclic voltammetry was performed on an EG & G PAR 273 polarographic analyzer. The electrochemical measurements were made in dried MeCN solutions with n-Bu_4PF_6 (0.1 M) as the supporting electrolyte. All samples were purged with N_2 prior to measurements. A standard three-electrode system was used comprising a Pt microcylinder working electrode, Pt-wire auxiliary electrode and a saturated calomel reference electrode (SCE).

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 28.0 ± 0.1 °C in a thermostatic bath. DNA samples approximately 200 base pairs in average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility.[26] Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Data were presented as $(\eta/\eta^0)^{1/3}$ versus binding ratio,[27] where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA alone.

Equilibrium dialyses were conducted at room temperature with 5 cm^3 of calf thymus DNA (1.0 mM) sealed in a dialysis bag and 10 cm^3 of the complexes (50 μM) outside the bag with the solution stirring for 48 h.

Synthesis and Characterization. Dipyridophenazine (dppz),[28] *cis*-$[Ru(dmp)_2Cl_2]\cdot 2H_2O$,[29] *cis*-$[Ru(dmb)_2Cl_2]\cdot nH_2O$,[30] and $[Ru(bpy)_2(dppz)](PF_6)_2$[31] were synthesized according to literature methods.

$[Ru(dmp)_2(dppz)](PF_6)_2$, 1. A mixture of *cis*-$[Ru(dmp)_2Cl_2]\cdot 2H_2O$ (0.298 g, 0.5 mmol) and dppz (0.145 g, 0.5 mmol) was heated to reflux in ethylene glycol (30 mL) for 2 h under argon atmosphere, after which the solution was cooled, diluted with water (50 mL), and treated with an excess amount of ammonium hexafluorophosphate. The precipitated complex was dried, dissolved in a small amount of acetonitrile, and purified by chromatography over alumina using acetonitrile/toluene (1:1, v/v) as an eluent. Yield: 0.38 g (70%). Halide salt was prepared from the hexafluorophosphate by precipitation in acetone solution with tetra-*n*-butylammonium chloride. Found: C, 50.8; H, 3.4; N, 10.4%. Calcd for $C_{46}H_{34}N_8F_2P_2Ru$: C, 50.7; H, 3.1; N, 10.3%. λ_{max}, nm (ϵ, M^{-1} cm^{-1}) (H_2O): 270 (52 400), 372 (10 100), 450 (7400). ^{1}H NMR (ppm, DMSO-d_6): 9.44 (d, 2H, J = 8.0 Hz), 8.72 (s, 2H), 8.91 (d, 2H, J = 8.0 Hz), 8.46 (d, 2H, J = 8.5 Hz), 8.42 (m, 4H), 8.25 (d, 2H, J = 8.5 Hz), 8.14 (q, 2H), 7.97 (d, 2H, J = 8.5 Hz), 7.61 (q, 2H), 7.51 (d, 2H, J = 6.0 Hz), 7.40 (d, 2H, J = 8.0 Hz), 1.96 (s, 6H), 1.85 (s, 6H).

$[Ru(dmb)_2(dppz)](PF_6)_2\cdot 0.5H_2O$, 2. This complex was obtained by a procedure similar to that described above. Yield: 0.37 g (65%). Found: C, 47.8; H, 3.6; N, 10.6%. Calcd for $C_{42}H_{34}N_8F_{12}P_2Ru\cdot 0.5H_2O$: C, 47.9; H, 3.3; N, 10.6%. λ_{max}, nm (ϵ, M^{-1} cm^{-1}) (H_2O): 282 (70 700), 355 (17 300), 372 (13 300), 445 (12 400). ^{1}H NMR (ppm, DMSO-d_6): 9.61 (d, 2H, J = 8.5 Hz), 8.72 (s, 2H), 8.69 (s, 2H), 8.52 (q, 2H), 8.23 (d, 2H, J = 5.5 Hz), 8.19 (q, 2H), 8.0 (q, 2H), 7.64 (d, 2H, J = 5.5 Hz), 7.56 (d, 2H, J = 6.0 Hz), 7.42 (d, 2H, J = 6.0 Hz), 7.18 (d, 2H, J = 7.0 Hz), 2.56 (s, 6H), 2.48 (s, 6H).

X-ray Crystallography. A crystal of **1** suitable for single-crystal X-ray diffraction with a size of 0.26 mm × 0.22 mm × 0.20 mm was selected. Data were measured on a Simens P4 diffractometer in ω scans with graphite-monochromated Mo Kα (λ = 0.710 73 Å) radiation. The structure was solved by direct methods and refined with a full-matrix least-squares technique using the SHELXL-93 programs.[32] All hydrogen atoms were generated geometrically (C−H, 0.96 Å). Crystal parameters and details of the data collection and refinement are given in Table 1. Selected bond lengths (Å) and bond angles (deg) are given in Table 2.

Table 1. Crystal Data and Structure Refinement for **1**

fw	1089.82
cryst syst	monoclinic
space group	*C*2/*c*
T (K)	293(2)
unit cell dimensions	
a (Å)	26.667(5)
b (Å)	24.686(5)
c (Å)	18.820(4)
β (deg)	125.63(3)
V (Å^3)	10 070(4)
Z	8
D_c (g/cm^3)	1.438
abs coeff (mm^{-1})	0.460
F(000)	4384
θ range for data collection (deg)	2.19−25.60
index ranges	$-32 < h < 24$, $0 < k < 29$, $0 < l < 22$
reflns collected	7938
independent reflns	7938 (R_{int} = 0.0000)
abs correction	SEMIEMPIRICAL
max and min transm	1.102, 0.788
refinement method	full-matrix least-squares on F^2
data/restraints/params	7938/45/691
GOF on F^2	1.175
final *R* indices [$I > 2\sigma(I)$][a]	R1 = 0.0672, wR2 = 0.1831
R indices (all data)	R1 = 0.0739, wR2 = 0.1880
largest diff peak and hole (e Å^{-3})	0.574, −0.402

[a] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$, $wR2 = [\sum w(F_o^2 - |F_c^2|)^2/\sum w(F_o^2)^2]^{1/2}$.

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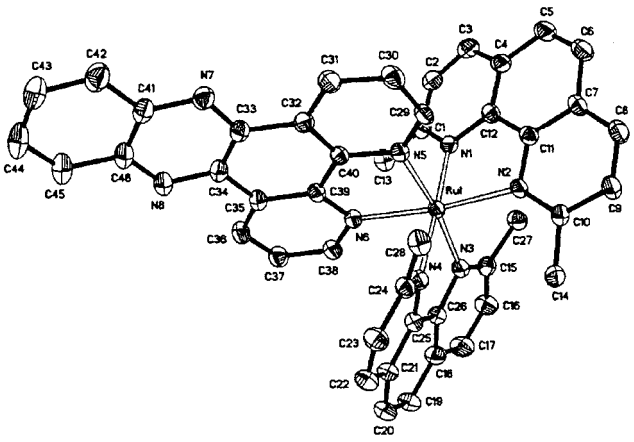

Figure 1. An ORTEP drawing of **1** and the atom numbering.

Table 2. Select Bond Lengths [Å] and Angles [deg] for **1**

Ru(1)−N(6)	2.094(2)	Ru(1)−N(1)	2.096(2)
Ru(1)−N(5)	2.098(2)	Ru(1)−N(3)	2.102(2)
Ru(1)−N(2)	2.109(2)	Ru(1)−N(4)	2.125(2)
N(6)−Ru(1)−N(1)	97.98(9)	N(6)−Ru(1)−N(5)	78.86(9)
N(1)−Ru(1)−N(5)	84.24(9)	N(6)−Ru(1)−N(3)	93.76(9)
N(1)−Ru(1)−N(3)	100.78(9)	N(5)−Ru(1)−N(3)	171.66(9)
N(6)−Ru(1)−N(2)	171.45(8)	N(1)−Ru(1)−N(2)	79.41(9)
N(5)−Ru(1)−N(2)	92.73(9)	N(3)−Ru(1)−N(2)	94.74(9)
N(6)−Ru(1)−N(4)	79.82(9)	N(1)−Ru(1)−N(4)	177.72(9)
N(5)−Ru(1)−N(4)	95.87(9)	N(3)−Ru(1)−N(4)	78.85(9)
N(2)−Ru(1)−N(4)	102.85(9)	C(1)−N(1)−C(12)	117.1(2)

Results and Discussion

Synthesis and Structure. Complexes **1** and **2** were prepared using standard synthetic methods. An ORTEP[33] diagram of the cation of **1** with the atomic numbering scheme is shown in Figure 1. As expected, Ru(II) possesses a distorted octahedral coordination sphere as a consequence of the small bite angles of the bidentate ligand, with a bite angle of 79.0° averaged over the three bidentate ligands. The mean Ru−N bond length is 2.104 Å, which is somewhat larger than that found in [Ru(phen)-(dpq)]$^{2+}$ (2.064 Å, dpq = dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline).[8b] The dppz and dmp ligands are planar to within 0.10 Å. The dihedral angle between the two dmp planes, plane I (N1, N2, C1−C4) and plane II (N3, N4, C15−C28), is 67.8°. Between planes I and III (N5−N8, C29−C46), the dppz plane, the dihedral angle is 80.9°, while this angle is only 50.2° between the planes II and III, which is much smaller than that found in the analogous octahedral Ru(II) compound.[6b,8b]

Absorption and Emission. The electronic absorption spectra of **1** and **2** in water are characterized by intense ligand-centered transitions in the UV region and a metal-to-ligand charge transfer (MLCT) transition in the visible region. The peaks at 372 nm in both compounds are characteristic of the $\pi-\pi^*$ transition of the dppz ligand. The low-energy bands at 450 nm (ϵ = 7400 M^{-1} cm^{-1}) and 445 nm (ϵ = 12 400 M^{-1} cm^{-1}) for compounds **1** and **2,** respectively, are assigned as MLCT Ru(dπ) → dppz-(π^*) transitions. Compound **1** exhibits no photoluminescence at room temperature in water or in any organic solvent examined. This similar phenomenon has also been found with our previous prepared complex [Ru(dmp)$_2$(atatp)]$^{2+}$.[6d] Compound **2** does not show any photoluminescence at room temperature in water but emits intensively in acetonitrile with an emission maximum at 617 nm, displaying the high sensitivity to solvent.

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Table 3. Electrochemical Potentials for Ruthenium(II) Complexes

		reduction[a] $E_{1/2}$ (V)		
complex	oxidation[a] $E_{1/2}$ (V)	I	II	III
[Ru(dmb)$_2$(dppz)]$^{2+}$	1.20	−1.0	−1.52	−1.73
[Ru(bpy)$_2$(dppz)]$^{2+}$ [b]	1.24	−1.02	−1.44	−1.67
[Ru(dmp)$_2$(dppz)]$^{2+}$	1.32	−0.98	−1.46	−1.64
[Ru(phen)$_2$(dppz)]$^{2+}$ [c]	1.36	−1.0		
[Ru(IP)$_2$(dppz)]$^{2+}$ [d]	1.42	−1.03	−1.24	−1.57

[a] Redox potentials were quoted vs SCE in 0.1 M TBAH−CH_3CN. Scan rate = 200 mV s^{-1}. [b] From ref 31. [c] From ref 11b. [d] From ref 13.

Table 4. Electronic Absorption Data upon Addition of CT-DNA

	hypochromism, H (%)[a]		
compound	IL (372 nm)	MLCT	K_b (M^{-1})
Ru(IP)$_2$dppz^{2+}	46.3	40.4	2.1×10^7
Ru(dmb)$_2$dppz^{2+}	38.5	15.6	4.5×10^6
Ru(dmp)$_2$dppz^{2+}	31.1	11.2	2.3×10^6
Ru(bpy)$_2$dppz^{2+}	40.1	14.5	5.0×10^6

[a] Conditions were as follows: [Ru] = 20 μM, [DNA] = 200 μM in 50 mM Tris-HCl and 50 mM NaCl buffer (pH 7.2).

Electrochemistry. Cyclic voltammetry of compounds **1** and **2** in acetonitrile shows one oxidation and three reduction waves in the sweep range from −1.85 to +1.70 V (vs SCE). The results along with relative data for [Ru(L)$_2$(dppz)]$^{2+}$ (L = bpy, phen, IP) are listed in Table 3. The electrochemical behavior of the ruthenium(II) polypyridyl complex has been rationalized in terms of a metal-based oxidation and a series of reductions which are ligand-based occurring in a stepwise manner for each π^* system.[36] As expected, the incorporation of electron-donating methyl groups on the ancillary phen or bpy ligands shifts the Ru(II)/Ru(III) oxidation to the negative. These data are consistent with the electron donors stabilizing the Ru(III) state via raising the absolute energy of the highest occupied molecular orbital (HOMO).[37] The first reduction, which is usually controlled by the ligand having the most stable lowest unoccupied molecular orbital (LUMO), is assigned to a reduction centered on the dppz ligand.

DNA Binding Properties. Absorption and Steady-State Emission of the Complexes in the Presence of DNA. Figure 2 shows the absorption spectra of **1** and **2** in the presence of increasing concentrations of DNA. Like their parent complex, the association of these two compounds with DNA results in a significant perturbation in the $\pi-\pi^*$ transition on the dppz, consistent with preferential intercalation of this ligand into the base stack of the DNA helix. With increasing DNA concentration, the percent hypochromism increases and eventually reaches saturation at $R_{\mathrm{nucl-Ru}} \approx 20$.

It is useful to compare the percent hypochromism in the $\pi-\pi^*$ transition on the dppz for related Ru(II) compounds in the presence of DNA at saturation (Table 4). Complex **1**, with substituents on the 2- and 9-positions of the phen ancillary ligand, exhibits less hypochromism than do other related compounds, which is likely to reflect levels of penetration of

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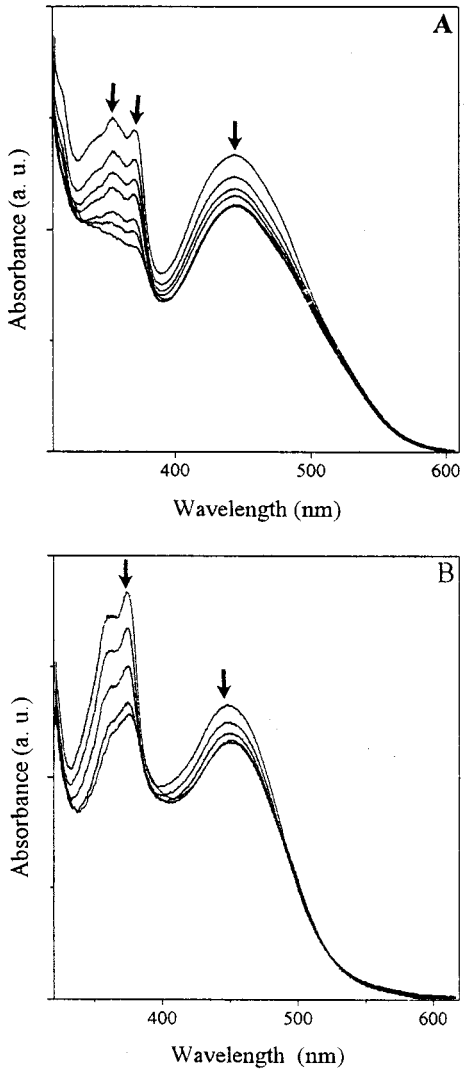

Figure 2. Absorption spectra of complexes **1** (B) and **2** (A) in 50 mM Tris-HCl and 50 mM NaCl buffer (pH 7.2) in the presence of increasing amounts of DNA ([Ru] = 20 μM, [DNA] = 0−200 μM).

the dppz ligand into the DNA base stack. To compare quantitatively the binding strength of the compounds, the intrinsic binding constants K_b with CT-DNA are determined from the decay of the absorbance at 372 nm with increasing concentration of DNA using the equation[23,38]

$$(\epsilon_a - \epsilon_f)/(\epsilon_b - \epsilon_f) = (b - (b^2 - 2k^2C_t[\mathrm{DNA}]/s)^{1/2})/(2kC_t)$$

$$b = 1 + kC_t + k[\mathrm{DNA}]/(2s)$$

where ϵ_a, ϵ_f, and ϵ_b are the extinction coefficients of the complex at a given DNA concentration, the complex free in solution, and the complex fully bound to DNA, respectively, K is the equilibrium binding constant, C_t is the total metal complex concentration, [DNA] is the DNA concentration in nucleotides, and s is the binding site size. The intrinsic binding constant, K, of **1** and **2** for DNA was determined to be $(2.3 \pm 0.2) \times 10^6$ M^{-1} ($s = 0.9$) and $(4.5 \pm 0.4) \times 10^6$ M^{-1} ($s = 0.7$), respectively. The values of K we obtained in the absorption titration are similar to what we determined by equilibrium dialysis ($(1.8 \pm 0.2) \times 10^6$ and $(4.8 \pm 0.4) \times 10^6$ M^{-1} for **1** and **2**, respectively.) For comparison, we determined the binding constant of $[Ru(bpy)_2(dppz)]^{2+}$ to calf thymus DNA to be $(5.0 \pm 0.4) \times 10^6$ M^{-1} ($s = 0.7$ [39]), which is comparable to the values found by others ($K > 10^6$ M^{-1} [12a] and 4.9×10^6 M^{-1} [40]).

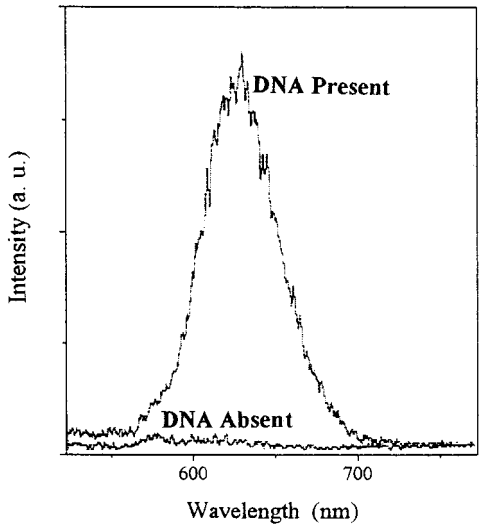

Figure 3. Emission spectra of **2** in the absence and presence of DNA ([DNA]/[Ru] = 10.0).

Among the compounds examined, **1** shows the least binding strength to double-helical DNA. As revealed by the complex crystal structure analysis, substitution on the 2- and 9-positions of the ancillary phen ligands may cause severe steric constraints near the core of Ru(II) when the complex intercalates into the DNA base pairs. The methyl groups may come into close proximity of the base pairs at the intercalation site. These steric clashes then prevent the complex from intercalating effectively, which causes a diminution of the intrinsic binding constant. Such steric clashes would not be present with substitution on the 4- and 4′-positions of the ancillary bpy ligands,[41] although other steric clashes between the methyl groups and the wall of the groove may arise.

The emission spectra of **2** in the absence and presence of DNA are shown in Figure 3. No detectable luminescence is observed for the complex in buffer solution. Upon addition of DNA, however, luminescence with the emission maximum at 628 nm is apparent. The emission yield in the presence of DNA is reduced ($\sim$1/3) compared with that of the parent $[Ru(bpy)_2(dppz)]^{2+}$ under similar conditions. This observation has been made previously for $[Ru(4,7\text{-dmp})_2(dppz)]^{2+}$, which has a reduced quantum yield for emission in the presence of DNA compared with that of $[Ru(phen)_2(dppz)]^{2+}$.[42] Such an observa-

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(39) The values for the binding size obtained are $s < 1$, which has also been found previously in refs 13, 23, and 40. The values of s may reflect a thermodynamic average binding parameter rather than structurally the size of the molecule bound at any individual site on the helix. See: Pyle, A. M.; Rehmann, J. P.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051−3058.

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(41) The crystal data for **2** is as follows: $C_{42}H_{35}F_{12}N_8O_{0.5}P_2Ru$, M = 1050.79, triclinic crystal system, space group $P1$, $Z = 8$, $a = 15.043(12)$ Å, $b = 24.346(18)$ Å, $c = 27.97(2)$ Å, $\alpha = 73.06(7)°$, $\beta = 82.77(8)°$, $\gamma = 82.44(6)°$, $V = 9680(14)$ Å^3, $D_c = 1.442$ g/cm^3, $F(000) = 4232$, $\mu = 0.476$ mm^{-1}, λ (Mo Kα) = 0.710 73 Å, $T = 298(2)$ K. Due to the severe disorder of PF_6^- in the structure, the final least-squares refinement ($I > 2.00\,\sigma(I)$) converged to rather high R values, R1 = 0.107 and wR2 = 0.3101. Each of the dmb ligands makes a dihedral angle of 90.5° and 90.6°, respectively, with the dppz ligand and an angle of 92.1° with each other.

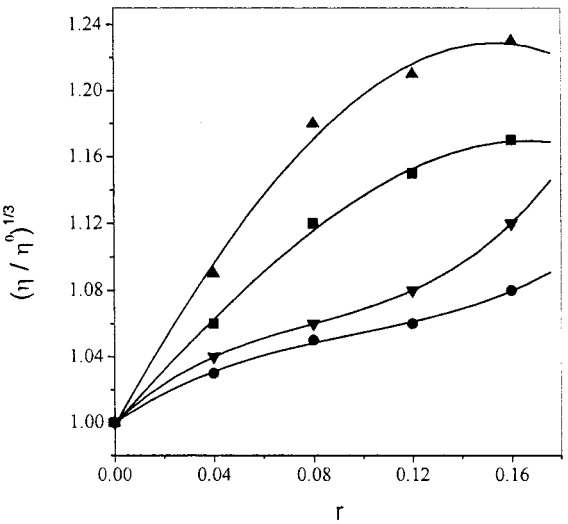

Figure 4. Effects of increasing amounts of the complexes of **1** (●), **2** (■), $[Ru(bpy)_2(dppz)]^{2+}$ (▲), and $[Ru(IP)_2(dppz)]^{2+}$ (▼) on the relative viscosities of calf thymus DNA at 28.0 ± 0.1 °C ([DNA] = 0.5 mM and r = [Ru]/[DNA]).

tion is the result of an increase in vibrational deactivation brought about by the flexibility of bpy derivatives.[43]

We detected no emission for **1** bound to DNA, despite the observation of ~30% hypochromism in the dppz $\pi-\pi^*$ transition band. Barton[12c] and co-workers have suggested that $[Ru(phen)_2(dppz)]^{2+}$ has two MLCT states closely spaced in energy, and one of these, which has a rapid nonradiative decay pathway, corresponds to a species in which the dppz has more readily accepted hydrogen bonds from the solvent. In water, the light switch effect would be due to this state being lower in energy than the "regular" red-emitting MLCT state. Given that compound **1** does not show any photoluminescence at room temperature in any organic solvent examined or in the absence and/or presence of double-helical DNA, we attribute this observation to the existence of low-lying dd states in this complex because of the distortion of the coordination sphere. (Each of the dmp ligands makes an angle of 50.2° and 80.9°, respectively, with the dppz ligand and an angle of 67.8° with each other, displaying a severe deviation from octahedral geometry. As a comparison, in the crystal structure of $[Ru(phen)_2(dpq)]^{2+}$, each of the phen ligands makes an angle of close to 90° with the dpq ligand and an angle of 79° with each phen ligand.[8b] In $[Ru(dmb)_2(dppz)]^{2+}$, the dihedral angles between the three planes, two dmb planes and one dppz plane, demonstrate that they are nearly perpendicular to each other.[41]) Such a "light switch" that is permanently off has also been found previously with the compounds $[Ru(NH_3)_4(dppz)]^{2+}$ [23]and $[Ru(acac)_2(dppz)]^{2+}$.[35]

Viscosity Measurements. Because the viscosity of a DNA solution is sensitive to the addition of organic drugs and metal complexes bound by intercalation, we examined the effect on the specific relative viscosity of DNA upon addition of complexes. Figure 4 shows the changes in viscosity upon addition of **1** and **2**, as well as the complexes $[Ru(bpy)_2(dppz)]^{2+}$ and $[Ru(IP)_2(dppz)]^{2+}$. The addition of both compounds increases the relative viscosity of the DNA solution consistent with intercalation. However, a much smaller extent of increase is observed for **1** compared with that for the parent complex.

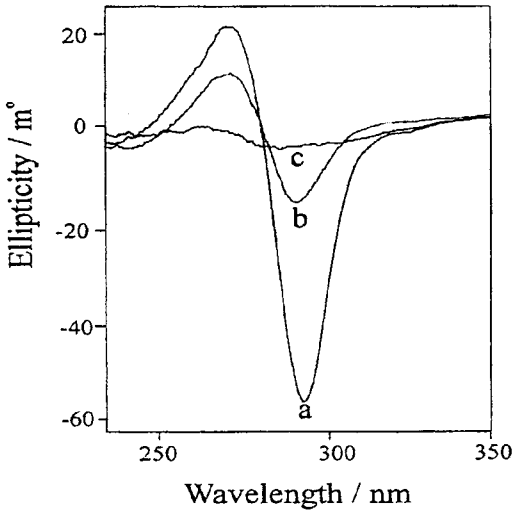

Figure 5. CD spectra of the dialyzate of **2** (a), $[Ru(bpy)_2(dppz)]^{2+}$ (b), and **1** (c) after 26 h of dialysis against CT-DNA with the solution stirred.

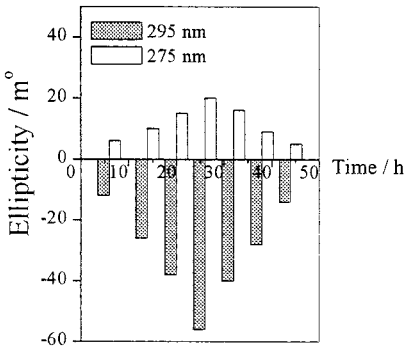

Figure 6. The ellipticities of the dialyzate of **2** at the band of 275 and 295 nm varying with the dialysis time ([Ru] = 50 μM, [DNA] = 1.0 mM).

The results may suggest that among the complexes examined, **1** is the least efficient intercalator.

Equilibrium Dialysis and CD Measurements. Equilibrium dialysis experiments may offer the opportunity to examine the enantioselectivity of complexes binding to DNA. Racemic solutions of **1** and **2** together with $[Ru(bpy)_2(dppz)]^{2+}$ were dialyzed against CT-DNA with stirring for 48 h and then subjected to CD analysis. Unfortunately, there is no CD signal that appeared for all of the complexes examined. Interestingly, during the dialysis process, we observed CD signals for the dialyzate of **2** and $[Ru(bpy)_2(dppz)]^{2+}$ but not for that of **1.** Figure 5 shows the results recorded with dialysis for 26 h. Very strong CD signals appeared for complex **2**, with a positive peak at 275 nm and a negative peak at 295 nm. In contrast, the CD signals for the dialyzate of complex **1** are too weak to be discerned from the background. It is likely that the structure differences between the complexes affect their DNA-binding behaviors. Figure 6 shows the ellipticities at the band of 275 and 295 nm of the dialyzate of **2** varying with the dialysis time. During the course of the dialysis, the CD signals started from none, increased to the maximum magnitude, then decreased, and at the end disappeared. A similar trend was also observed with the parent complex $[Ru(bpy)_2(dppz)]^{2+}$, except that the ellipticity observed was smaller than that of **2** under similar conditions.

From the equilibrium dialysis experiments, the results suggest that thermodynamically, there is no enantioselectivity in the interactions of both compounds **1** and **2** with CT-DNA.

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However, from the view of kinetic process, different kinetics of binding of enantiomers of **2** compared to **1** was obvious. The absence of chiral discrimination between isomers of ruthenium(II) complexes binding to DNA has been reported.[10a−c] During the dialysis experiments, the CD signals of the dialyzate of **2** experienced a disappearing−appearing−disappearing pattern, which may be ascribed to the different binding rates of the isomers. Compared with the CD spectra of the Δ-$[Ru(bpy)_2(dppz)]^{2+}$,[6g] it implies that the Λ isomer binds more rapidly than the Δ enantiomer. This behavior has been observed by NMR[16b] for enantiomers of $[Ru(phen)_2(dppz)]^{2+}$ binding with oligonucleotides and for isomers of other polypyridyl ruthenium compounds[6g] binding to CT-DNA. The different binding rates observed in our experiments may be the consequence of diastereomeric orientation effects of the chromophore and of different penetrations upon binding to the double helix, which has been illustrated by Nordén and co-workers.[18] It is likely that the Δ enantiomer may penetrate somewhat more deeply into the base pairs than the Λ enantiomer, and then a slower binding rate is observed. The absence of CD signal for the dialysate of **1** during the course of dialysis experiments may suggest a similar manner of binding of the isomers to the double helix.

Conclusions

By the incorporation of simple modifications on the ancillary ligands of phen and bpy, different DNA-binding behaviors of **1** and **2** were characterized. Different binding rates of the isomers of **2** with CT-DNA were observed through dialysis experiments but not with complex **1**. Substitution on the 4- and 4′-positions of the ancillary bpy ligands not only kept the DNA molecular light switch characteristic but also increased the difference of binding rates between the Δ and Λ isomers. Substitution on the 2- and 9-positions of the ancillary phen ligands may cause severe steric constraints near the core of Ru(II) when the complex intercalates into the DNA base pairs, as revealed by the very small dihedral angle between one of the dmp planes and the dppz plane. These steric clashes prevented the complex **1** from intercalating into the base pairs effectively. Subsequently, a diminution of the binding strength and the disappearance of the different binding rates between the isomers were observed.

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Supporting Information Available: X-ray crystallographic file in CIF format for the structure of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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