Molecular Light Switches for Calf Thymus DNA Based on Three Ru(II) Bipyridyl Complexes with Variations of Heteroatoms

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The three Ru(II) complexes of $[Ru(bpy)_2(bipp)](ClO_4)_2$ (**1**), $[Ru(bpy)_2(bopp)](ClO_4)_2$ (**2**), and $[Ru(bpy)_2(btpp)](ClO_4)_2$ (**3**) (where bpy = 2,2′-bipyridine, bipp = 2-benzimidazoyl-pyrazino[2,3-*f*] [1,10]phenanthroline, bopp = 2-benzoxazolyl-pyrazino[2,3-*f*] [1,10]phenanthroline, and btpp = 2-benzthiazolyl-pyrazino[2,3-*f*] [1,10]-phenanthroline) with variations in heteroatoms of NH (**1**), O(**2**), and S(**3**), have been synthesized and characterized. These complexes have been shown to act as promising calf thymus DNA intercalators and a new class of DNA light switches for the DNA, as evidenced by UV−visible and luminescence titrations, steady-state emission quenching by $[Fe(CN)_6]^{4-}$, DNA competitive binding with ethidium bromide, reverse salt titrations, viscosity measurements, and DNA melting experiments.

1. Introduction

$[Ru(bpy)_2(dppz)]^{2+}$ (bpy = 2,2′-bipyridine, dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine) was first reported by Barton et al. to be almost nonemissive in water but with its luminescence "switched on" in the presence of DNA and was termed as "light switch" for DNA.[1a] Since then, the synthesis of new light-switch Os(II)[2] Ru(II),[1] Rh(III),[3] and Re(I),[4] complexes for DNA, light switching mechanism studies,[1a,c,5] and applications of transition-metal complexes of this family have gained keen interest.[6] The widespread applications of $[Ru(bpy)_2(dppz)]^{2+}$and $[Ru(phen)_2(dppz)]^{2+}$ (phen = 1,10-phenanthroline), which have distinguished these complexes as "star molecules" in bioinorganic chemistry, include luminescent probes for DNA structures[7] and DNA base mismatches[8] as well as other hydrophobic matrixes such as Nafion films[9] and SDS micelles,[10] enantioselective binding to DNA,[11] various switching devices,[12] signaling probes for DNA-protein binding,[13] determination of nucleic acids[14] and Aptamer-based ATP assay,[15] studies for long-range fast electron-transfer that is mediated by the stacked bases of DNA,[16] and potential phototherapeutic and DNA-targeting anticancer drugs.[11a,17]

As far as the molecular light-switch Ru(II) complexes (see the Supporting Information) for DNA reported are concerned, the main efforts have been made on modified dppz systems,[1b,5a,7e] but often they gave less effective light-switching properties than the prototypical light switches of $[Ru(bpy)_2(dppz)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$, as they usually exhibited residual emission in DNA-free water solution. However, the emission intensity of the DNA-bound $[Ru(phen)_2(tppz)]^{2+}$ (tppz = tetrapyrido-[3,2-*a*:2′,3′-*c*:3″,2″-*h*:2″,3″-*j*]phenazine) was reported to possess a 2.5-fold larger quantum yield than DNA-bound $[Ru(bpy)_2(dppz)]^{2+}$ at a similar binding ratio and concentrations; therefore, $[Ru(phen)_2(tppz)]^{2+}$ functions as a "molecular light switch" more effectively in this regard.[18] tppz contains a phenanthroline-like coordination site at its periphery where Cu^{2+} complexation can occur, thus flipping the DNA molecular light switch off.[18a] Recently, chemical cycling the molecular light switch on and off has also been achieved by Dunbar and Turro by successive addition of Co^{2+} and EDTA.[18b] Interestingly, although structurally dinuclear Ru(II) complexes containing tppz as bridging ligand, $[Ru(bpy)_2(tppz)Ru(bpy)_2]^{4+}$ and $[(phen)_2Ru(tppz)Ru(phen)_2]^{4+}$, seem not to be capable of an intercalative interaction with DNA, a very recent study carried out by Haq and Thomas et el. has revealed that addition of ct-DNA into 20 mM NaCl solutions of $[(bpy)_2Ru(tppz)Ru(bpy)_2]^{4+}$ and $[(phen)_2Ru(tppz)Ru(phen)_2]^{4+}$ results in more than 60-fold emission enhancements.[19] These two complexes were shown to potentially bind to the DNA intercalatively with large binding constants $(3.1-6.0) \times 10^6\ M^{-1}$ at a high ion strength (200 mM KCl), but a small ΔT_m value of 5.6 °C obtained by thermal denaturation experiment and a small increases in relative viscosity of the DNA upon addition of the complexes seem inconsistent with classic intercalative binding. On the contrary, an analogous complex $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (tatpp = tetraazatetrapyrido[3,2-*a*:2′,3′-*c*:3″,2″-*l*:2‴,3‴-*n*]pentacene) does not show evidence of emission, even in the presence of excessive duplex DNA.[20] Mesmaeker reported a novel light switch for DNA, $[Ru(phen)_2(phehat)]^{2+}$ (phen = 1,10-phenanthroline; phehat = 1,-10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene), in which the ligand phehat not only induces intercalation but also makes the complex oxidizing in the excited state, and thus photoreacting with DNA.[21]

The effect of ancillary ligand on DNA light switching properties is also an interesting subject. Although the additions of ct-DNA can induce the photoluminescence enhancements of $[Ru(bpy)_2(taptp)]^{2+}$ and $[Ru(phen)_2(taptp)]^{2+}$ (taptp-4,5,9,18-tetraazaphenanthreno[9,10-*b*]triphenylene), but the phen-containing complex has a negligible luminescence in DNA-free water buffer contrast to the strong luminescence for the bpy-containing complex.[22] As the ancillary ligand is ip (ip = imidazole[4,5-*f*][1,10]phenanthroline), the complex $[Ru(ip)_2(dppz)]^{2+}$ shows a small emission enhancement factor of ~12 with evident residual emission for the aqueous solution of the free complex.[23] The DNA light-switching properties are also elusive, and Ji and co-workers have found that neither $[Ru(bpy)_2(pztp)]^{2+}$ nor $[Ru(phen)_2(pztp)]^{2+}$ (pztp = 3-(pyrazin-2-

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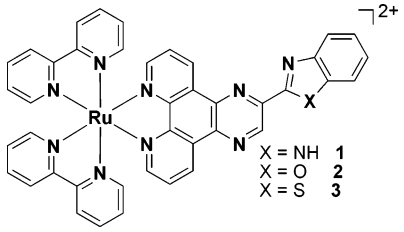
SCHEME 1: Molecular Structures of $[Ru(bpy)_2(bipp)]^{2+}$(1), $[Ru(bpy)_2(bopp)]^{2+}$ (2), and $[Ru(bp)_2(btpp)]^{2+}$ (3)



yl)-as-triazino[5,6-*f*]-1,10-phenanthroline) exhibit enhanced luminescence in the presence of DNA,[24a] but it is interesting to note that replacement of a bpy or a phen by a pztp in the above complexes, namely complexes of $[Ru(bpy)(pztp)_2]^{2+}$ and $[Ru(phen)(pztp)_2]^{2+}$, shows light switching properties for DNA with emission quantum yields of 0.07% and 0.35% for DNA bound complexes, respectively.[24b] It is noteworthy that significant progress has recently been made by Barton et al. on using the light-switch complex $[Ru(bpy)_2(tactp)]Cl_2$ (tactp = 4,5,9,18-tetraazachryseno[9,10-*b*]-triphenylene) for binding preferentially to a CC base pair mismatch, although this complex is luminescent in aqueous solution at micromolar concentrations and exhibits an only 12-fold enhancement in luminescence in the presence of DNA.[8a]

Dipyrido[2,2-d:2′,3′-*f*]quinoxaline (dpq) is structurally related to but less conjugate than dppz. It is noteworthy that dpq-related ligand-containing complexes which have so far been reported are very limited. $[Ru(phen)_2(dcdpq)]^{2+}$ and $[Ru(phen)(dcdpq)_2]^{2+}$ (dcdpq = 6,7-dicyanodipyrido[2,2-*d*:2′,3′-*f*]quinoxaline) was shown by Ambroise and Maiya to have emission enhancement factors at saturation being approximately 16 and 8 upon successive addition of ct-DNA to buffered aqueous solutions containing these two complexes.[25] The Ru(II) complexes with dpq and methyl substituted dpq (Medpq), $[Ru(phen)_2dpq]^{2+}$ and $[Ru(phen)_2Medpq]^{2+}$ (Medpq = 2-methyldipyrido[3,2-*f*:2′,3′-*h*]-quinoxaline), do not exhibit DNA molecular light switching properties, being strongly luminescent in the absence and presence of DNA,[26] but an amide functionalized dpq-containing Ru(II) complex, $[Ru(phen)_2dpqa]^{2+}$ (dpqa = 2-pentylamidodipyrido[3,2-*f*:2′,3′-*h*]-quinoxaline), was shown by Kelly and Kruger to be a molecular light switch for DNA, although no defined emission enhancement factor was reported.[26b] During the course of our studies on the effects of structural variations of Ru(II) complexes on DNA-binding properties,[27] we have very recently discovered a novel DNA light-switch $[Ru(bpy)_2(bopp)]^{2+}$ in which the key ligand is synthesized by grafting benzoxazolyl to dpq.[27f] We have also found that the light switching properties still remain strong as the heteroatom oxygen in the benzoxazolyl group in the Ru(II) complex is replaced by NH and S (Scheme 1). We would like to report related results in this paper.

2. Experimental Section

2.1. Materials. *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$[28] (bpy = 2,2′-bipyridine) and 1,10-phenanthroline-5,6-dione[29] were prepared by the literature methods. The ligands bipp, bopp, and btpp were synthesized by condensing pyrazino[2,3-*f*][1,10]phenanthroline-2-carboxylic acid[30] with *o*-phenylenediamine, 2-aminophenol, and 2-aminothiophenol in a molar ratio of 1:1 in syrupy phosphoric acid at 200 °C, respectively. Upon cooling to room temperature, the reaction mixture was poured into 400 mL of cold water under vigorous stirring. The solution was neutralized to pH = 7.0 with concentrated NH_3 solution. The crude products were then recrystallized twice from MeOH. Yields 55% for bipp, 25% for bopp, and 58% for btpp. ^{1}H NMR for bipp (500 Hz, Me_2SO-d_6): δ 13.52 (s, 1H), 9.82 (s, 1H), 9.81 (d, 1H), 9.35 (d, J = 8.0 Hz, 1H), 9.22 (d, J = 3.3 Hz, 1H), 9.19 (d, J = 3.1 Hz, 1H), 7.96 (dd, J_1 = 7.7 Hz, J_2 = 4.2 Hz, 1H), 7.88 (dd, J_1 = 8.0 Hz, J_2 = 4.3 Hz, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.38 (t, J = 7.0 Hz, 1H), 7.31 (t, J = 7.1 Hz, 1H). The ligands of bopp and btpp are sparingly soluble in common organic solvents, so they were not characterized by ^{1}H NMR.

2.2. Syntheses of $[Ru(bpy)_2(bipp)](ClO_4)_2$ (1), $[Ru(bpy)_2(bopp)](ClO_4)_2$ (2), and $[Ru(bpy)_2(btpp)](ClO_4)_2$ (3). The complexes were prepared by the same procedures as described below for $[Ru(bpy)_2(bopp)](ClO_4)_2$. A suspension of $[Ru(bpy)_2Cl_2]\cdot 2H_2O$ (1.1 mmol) and bopp(1.0 mmol) in 50 mL of EtOH/H_2O (v/v, 4:1) was refluxed under nitrogen for 6 h. After most of the solvent was removed under reduced pressure, a red precipitate was then obtained by dropwise addition of a 4-fold excess of saturated aqueous $NaClO_4$ solution. The purification was carried out by column chromatography on silica gel with CH_3CN-H_2O-saturated aqueous KNO_3 (60:6:1, v/v/v) as eluent followed by reprecipitation with a saturated $NaClO_4$aqueous solution. Caution: Perchlorate salts of metal complexes with organic ligands are potentially explosive. Although no detonation tendencies have been observed, caution is advised, and handling of only small quantities is recommended.

$[Ru(bpy)_2(bipp)](ClO_4)_2\cdot 3H_2O$. Yield: 53%. Anal. Calcd for $C_{41}H_{28}N_{10}Cl_2O_8Ru\cdot 3H_2O$: C, 48.53; H, 3.377; N 13.81. Found: C, 48.93; H, 3.499; N, 13.72. ^{1}H NMR (500 MHz, Me_2SO-d_6): δ 13.75 (br s, 1H), 10.11 (s, 1H), 10.01 (d, J = 8.2 Hz, 1H), 9.58 (d, J = 8.2 Hz, 1H), 8.91 (d, J = 8.2 Hz, 2H), 8.87 (d, J = 8.2 Hz, 2H), 8.29 (d, J = 5.3 Hz, 2H), 8.26 (td, J_1 = 8.0 Hz, J_2 = 1.4 Hz, 2H), 8.14 (m, 3H), 8.05 (dd, J_1 = 5.4 Hz, J_2 = 4.1 Hz, 1H), 7.85 (t, J = 5.0 Hz, 2H), 7.75 (m, 4H), 7.62 (t, J = 6.0 Hz, 2H), 7.38 (m, 4H). IR (KBr, cm^{-1}): 3430, 3073, 1603, 1557, 1465, 1445, 1423, 1399, 1370, 1317, 1090, 766, 729, 623. MALDI-TOF MS: m/z 778.82 ($[M-2ClO_4^- + H_2O - H^+]^+$), 760.80 ($[M-2ClO_4^- - H^+]^+$), 605.04 ($[M-2ClO_4^- - bpy-H^+]^+$). UV−vis in H_2O: λ/nm ($\epsilon \times 10^4$ M^{-1} cm^{-1}): 285 (6.7); 370 (3.2); 449 (1.7).

$[Ru(bpy)_2(bopp)](ClO_4)_2$. Yield: 43%. Anal. Calcd for $C_{41}H_{27}N_9Cl_2O_9Ru$: C, 49.36; H, 3.132; N, 12.63. Found: C, 49.06; H, 3.435; N, 12.61. ^{1}H NMR (500 MHz, Me_2SO-d_6): δ 10.16 (s, 1H), 9.66 (d, J = 8.0 Hz, 1H), 9.61 (d, J = 8.1 Hz, 1H), 8.89 (dd, J_1 = 17.0 Hz, J_2 = 8.2 Hz, 4H), 8.33 (m, 2H), 8.25 (t, J = 7.9 Hz, 2H), 8.15 (m, 2H), 8.06 (m, 4H), 7.85 (d, J = 5.5 Hz, 2H), 7.74 (m, 2H), 7.62 (m, 4H), 7.38 (t, J = 6.6 Hz, 2H). IR (KBr, cm^{-1}): 3076, 1603, 1465, 1405, 1375, 1089, 762, 730, 622. MALDI-TOF MS: m/z 862.04 ($[M-ClO_4^-]^+$), 763.08 ($[M-2ClO_4^-]^{2+}$). UV−vis in H_2O: λ/nm ($\epsilon \times 10^4$ M^{-1} cm^{-1}): 284 (7.9); 361 (3.6); 447 (1.8).

$[Ru(bpy)_2(btpp)](ClO_4)_2\cdot 3H_2O$. Yield: 87%. Anal. Calcd for $C_{41}H_{27}N_9Cl_2O_8RuS\cdot 3H_2O$: C, 47.73; H, 3.220; N, 12.22. Found: C, 47.97; H, 3.563; N, 12.06. ^{1}H NMR (500 MHz, Me_2SO-d_6): δ 10.13 (s, 1H), 9.59 (t, J = 7.1 Hz, 2H), 8.90 (d, J = 8.0 Hz, 2H), 8.87 (dd, J_1= 8.1 Hz, J_2 = 3.3 Hz, 2H), 8.30 (m, 4H), 8.24 (t, J = 8.0 Hz, 2H), 8.13 (m, 3H), 8.06 (dd, J_1= 8.2 Hz, J_2 = 5.4 Hz, 1H), 7.86 (br, 2H), 7.76 (d, J = 5.6 Hz, 1H), 7.72 (d, J = 5.6 Hz, 1H), 7.68 (m, 4H), 7.38 (m, 2H). IR (KBr, cm^{-1}): 3446, 1603, 1446, 1384, 1087, 764, 625. MALDI-TOF MS: m/z 878.06 ($[M-ClO_4^-]^+$), 779.11 ($[M-2ClO_4^-]^{2+}$). UV−vis in H_2O: λ/nm ($\epsilon \times 10^4$ M^{-1} cm^{-1}): 284 (7.8); 368 (3.4); 450 (1.8).

2.3. Physical Measurements. Infrared spectra were recorded with a Nicolet Avtar 360FT-IR spectrometer as KBr disks. ^{1}H NMR spectra were collected with a Bruker DRX-500 NMR spectrometer with $(CD_3)_2SO$ as solvent at room temperature. All shifts were given relative to TMS. Microanalyses (C, N, and H) were performed with a Vario EL elemental analyzer. Matrix-assisted laser desorption inoization mass spectra (MALDI-TOF MS) were run on a API Q-star pulsar (Applied Biosystems) mass spectrometer. UV−visible absorption spectra were recorded with a GBC Cintra 10e UV−visible spectrophotometer. Emission spectra were obtained on a Shimadzu RF-5301PC spectrofluorimeter. The emission quantum yields were calculated by comparison with $[Ru(bpy)_3]^{2+}$ ($\varphi = 0.033$)[31] in aerated aqueous solution at room temperature using eq 1

$$\varphi = \varphi_{std}(A_{std}/A)(I/I_{std}) \quad (1)$$

where φ and φ_{std} are the quantum yields of unknown and the standard sample, A and A_{std} are the absorbances at the excitation wavelength, and I and I_{std} are the integrated emission intensities.

All experiments dealing with the interaction of the Ru(II) complexes with calf thymus DNA (ct-DNA) were carried out in 5 mM Tris-HCl buffer (pH 7.1, 50 mM NaCl). A solution of ct-DNA gave ratios of UV absorbance at 260 and 280 nm of about 1.9:1, indicating that the DNA was sufficiently free of protein. The polynucleotide phosphate concentration of the DNA was determined spectrophotometrically by assuming $\epsilon_{260} = 6600$ M^{-1} cm^{-1}. The molar extinction coefficients of the Ru(II) complexes were determined by linear fit of the plots of the solution absorbance versus the concentrations below 10.0 μM. The UV−vis absorption and emission spectrophotometric titrations of the Ru(II) complexes with calf thymus (ct) DNA were performed by keeping the concentrations of the Ru(II) complex constant while varying the DNA concentrations. For the presentations and data treatments of the UV−vis absorption spectrophotometric titrations with the DNA, the absorption of the DNA has been subtracted. The experiments of DNA thermal denaturation were performed on a UV−visible spectrophotometer in buffer consisting of 1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4, and 0.25 mM Na_2EDTA. Using the thermal melting program, the temperature of the cell containing the cuvette was ramped from 50 to 90 °C and the absorbance at 260 nm was measured every 0.5−1 °C. Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 32.10 ± 0.02 °C in a thermostatic bath. DNA samples for viscosity measurements were prepared by sonicating in order to minimize complexities arising from DNA flexibility. Flow time was measured, and each sample was measured at least three times, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus [Ru]/[DNA], where η is the viscosity of DNA in the presence of the Ru complex and η_0 is the viscosity of DNA alone.

3. Results and Discussion

3.1. UV−Vis Absorption Spectra. The UV−vis spectra of three complexes in water all exhibit a bpy-centered $\pi-\pi^*$ transition band at ~285 nm, and an L-delocalized (L = bipp, bopp, and btpp) $\pi-\pi^*$ transition band at 370 nm for **1**, 363 nm for **2**, and 367 nm for **3**, as well as an overlapped Ru → bpy and Ru → L metal-to-ligand charge transfer (MLCT) transition band at 452 nm for **1**, 448 nm for **2**, and 449 nm for **3**. These assignments are made on the basis of the comparisons of the spectra of **1**−**3** with that for $[Ru(bpy)_3]^{2+}$.[32] Upon increasing concentrations of DNA, all of the absorption bands of the complexes displayed clear hypochromicities and red shifts. As an example, the spectral changes of **3** upon addition of ct-DNA are shown in Figure 1a, and those of **1** and **2** are shown in Figures S1 and S2 (in the Supporting Information), respectively. The peaks can be seen to shift progressively toward a limit which represents a spectrum of the complex in a fully complexed form. The addition of ct-DNA resulted in hypochromism H% ($H\% = 100(A_{free} - A_{bound})/A_{free}$) for the $\pi-\pi^*$ absorption at ~370 nm of 47% for **1**, 31% for **2**, and 36% for **3** with bathochromic shifts of 12, 9, and 10 nm, respectively; 28% for **1**, 25% for **2**, and 17% for **3** at the $\pi-\pi^*$ absorption at ~285 nm with bathochromic shifts of <2 nm; and 30% for **1**, 18% for **2**, and 15% for **3** at the MLCT absorption of ~450 nm with small red-shifts of <2 nm. The large hypochromism and red-shifts observed for the L-based $\pi-\pi^*$ absorption at ~370 nm are comparable to or larger than those observed for typical DNA intercalators,[5c] e.g., 32.1% (372 nm) for Δ-$[Ru(phen)_2(dppz)]^{2+}$ and 29.8% (372 nm) for Λ-$[Ru(phen)_2(dppz)]^{2+}$. These facts indicate that the complexes strongly bound to the DNA with bipp, bopp, and btpp moieties insert into the adjacent base pair of the DNA via a probable intercalative mode, since bpy was previously demonstrated to be at best only minimally efficient at inducing intercalative binding with DNA.[11b,d,7a] The values of the intrinsic binding constant K_b illustrating the binding strength of the complexes with ct-DNA were determined from eq 2 through a plot of $(\epsilon_a - \epsilon_f)/(\epsilon_b - \epsilon_f)$ vs [DNA][7e,33], where [DNA] is the concentration of DNA in base pairs

$$(\epsilon_a - \epsilon_f)/(\epsilon_b - \epsilon_f) = [b - (b^2 - 2K_b{}^2C_t[\mathrm{DNA}]/n)^{1/2}]/(2K_bC_t) \quad (2)$$

$$b = 1 + K_bC_t + K_b\,[\mathrm{DNA}]/2n$$

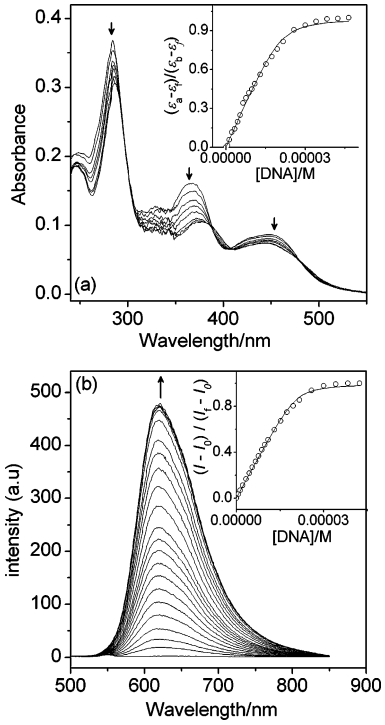


Figure 1. (a) Changes in UV−vis absorption spectra of $Ru(bpy)_2(btpp)^{2+}$ (4.7 μM) upon addition of ct-DNA (0.0−50.0 μM). Inset: plot of $(\epsilon_a - \epsilon_f)/(\epsilon_b - \epsilon_f)$ vs [DNA], and the nonlinear fit curve. (b) The changes in emission spectra ($\lambda_{ex} = 467$ nm) of $Ru(bpy)_2(btpp)^{2+}$ (4.7 μM) upon addition of ct-DNA (0.0−50.0 μM). Inset: plot of $(I_a - I_f)/(I_b - I_f)$ vs [DNA], and the nonlinear fit curve.

TABLE 1: Comparisons of Percentage of Hypochromicities *H*%, Bathochromic Shift $\Delta\lambda$, Binding Constant K_b, and *n* Values for Some Ru(II) Complexes Binding to ct-DNA

compound[a]	*H*/%(λ_{max}/nm)[b]	$\Delta\lambda$/nm(λ_{max}/nm)	K_b/(M^{-1})	binding mode	*n*
Λ-$[Ru(phen)_2(dppz)]^{2+}$	29.8(372)	8(372)	1.7×10^5	intercalation	3
Δ-$[Ru(phen)_2(dppz)]^{2+}$	32.1(372)	8(372)	3.2×10^5	intercalation	2
$[Ru(bpy)_2(dpt)]^{2+}$	18.1(474)	5(474)	6.3×10^4	intercalation	
$[Ru(phen)_2(hdppz)]^{2+}$	13.1(450)	4(450)	4.4×10^5	intercalation	0.36
1	28(285);47(370);30(452)	12(370)	3.8×10^6	intercalation	1.5
2	25(284);31(363);18(448)	9(363)	2.4×10^6	intercalation	1.7
3	17(284);36(367); 15(449)	10(367)	4.0×10^6	intercalation	2.1

[a] bpy = 2,2′-bipydidine; phen = 1,10-phenanthroline; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine; dpt = 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]phenanthrene; hdppz = 6-hydroxyl-dipyrido[3,2-*a*:2′,3′-*c*]phenazine. [b] $H\% = 100(A_{free} - A_{Bound})/A_{free}$.

The apparent absorption coefficients ϵ_a, ϵ_f, and ϵ_b correspond to A_{obsd}/[Ru], the extinction coefficient for the free ruthenium complex and the extinction coefficient for the ruthenium complex in the fully bound form, and *n* is the binding site size. The K_b and *n* values of the complexes were derived to be $K_b = (3.8 \pm 0.3) \times 10^6$ M^{-1}, $n = 1.5 \pm 0.1$ for **1**, $K_b = (2.4 \pm 0.3) \times 10^6$ M^{-1}, $n = 1.7 \pm 0.1$ for **2**, $K_b = (4.0 \pm 0.2) \times 10^6$ M^{-1}, $n = 2.1 \pm 0.1$ for **3** as shown in the inset in Figure 1a (for **1** and **2** see the Supporting Information) by monitoring the decay in L-based (L = bipp, bop, btpp) $\pi \rightarrow \pi^*$ absorption bands. The K_b values derived for the complexes **1**−**3** are of the same order of magnitude as 1.7×10^6 M^{-1} for Λ-$[Ru(phen)_2(dppz)]^{2+}$, 3.2×10^6 M^{-1} for Δ-$[Ru(phen)_2(dppz)]^{2+}$ [5c,f] and 200 times greater than those for the analogues: 3.0×10^4 M^{-1} for $Ru(phen)_2(dicnq)^{2+}$,[25] 6.3×10^4 M^{-1} for $Ru(bpy)_2(dpt)^{2+}$,[34] and 5.9×10^4 M^{-1} for the parent complex $[Ru(bpy)_2(dpq)]^{2+}$ [26] under the same experimental conditions (see Table 1). We ascribe the remarkable enhancements in the binding affinities of **1**−**3** with respect to $[Ru(bpy)_2(dpq)]^{2+}$ to the synergistic contributions by extension of π-conjugated systems and increased hydrophobicity upon the grafting of the benzoazoles (benzimidazoyl, benzoxazolyl, and benzothiazolyl) to dpq. The spectral characteristics of large hypochromism and clear red-shifts as well as the large K_b values observed may strongly suggest that the complexes most likely intercalatively bind to DNA, involving a strong stacking interaction between the aromatic chromophore and the base pairs of the DNA.

3.2. Emission Spectra. The luminescence has been examined in water and in acetonitrile. All of the complexes luminesce brightly in acetonitrile at ∼620 nm but weakly in water at ∼623 nm. The intensities for complexes **1**−**3** in the former solvent are about 13, 43, and 22 times larger than that in the latter solvent, respectively. Comparing with $[Ru(bpy)_2(dpq)]^{2+}$ which exhibits a strong luminescence,[26] the quenching of emission in water for **1**−**3** might reasonably be explained by a hydrogen-bonding interaction of the solvent with the imidazole/oxazole/thiazole groups on **1**−**3** in ^{3}MLCT states.[1d,26b] Luminescence reviviscence is apparent on binding of the complexes to the DNA. The enhancements in the emission intensities for **3** with increasing DNA concentrations are shown in Figure 1b (for **1** and **2** see the Supporting Information). In the absence of the DNA, the complexes all emit negligible luminescence in Tris buffer at room temperature. Upon addition of ct-DNA, the emission intensities increased sharply: enhancements of a factor 50 for **1**, 90 for **2**, and 180 for **3** are observed with corresponding emission quantum yields being 4.9% ($\lambda_{em} = 621$ nm), 0.48% ($\lambda_{em} = 639$ nm), and 1.3% ($\lambda_{em} = 646$ nm), respectively. These emission enhancements are very effective, upon comparisons of the quantum yields for DNA-bound **1**−**3** at saturation with those (1.3%−1.7%) observed in aerated CH_3CN ($\lambda_{em} = 616$, 621, and 620 nm, respectively). Complexes **1**−**3** can be regarded as a new class of light-switches as the molecular light switching properties for the DNA exhibited by **1**−**3** are caused by the grafting of benzimidazoyl, benzoxazolyl, and benzothiazolyl substituents to $[Ru(bpy)_2(dpq)]^{2+}$. It is noteworthy that the DNA-induced luminescence enhancement factors observed for **1**−**3** are favorably compared with those observed for $[Ru(phen)_2phehat]^{2+}$,[21] $[Ru(phen)_2dpq(CN)_2]^{2+}$,[25] $[Ru(bpy)_2tpphz]^{2+}$,[18] $[Ru(phen)_2dppx]^{2+}$,[5a] and $[Ru(phen)_2dpqa]^{2+}$[5a] but much less than a factor of $\sim 10^4$ reported for $[Ru(bpy)_2dppz]^{2+}$ and $[Ru(phen)_2dppz]^{2+}$.[1a] It was reported that $[Ru(bpy)_2(dpq)]^{2+}$ emits in water in the absence of the DNA and displays only 2-fold luminescence enhancements upon addition of the DNA.[26a] Unlike the nitrogen atoms of phenazine in $[Ru(bpy)_2(dppz)]^{2+}$ or $[Ru(phen)_2(dppz)]^{2+}$, the nitrogen atoms of quinoxaline in $[Ru(bpy)_2(dpq)]^{2+}$ probably do not strongly interact with water both in the absence and presence of the DNA. Since $[Ru(bpy)_2(dpq)]^{2+}$ does not show DNA light switching behavior, the complexes **1**−**3** that we studied here, which are the linkages of benzoazoles (benzimidazole, benzoxazole, and benzothiazole) to $[Ru(bpy)_2(dpq)]^{2+}$, represent a new class of DNA light switches. Substantial enhancements are normally observed for intercalation of the complexes into the DNA base pairs. Usually, the DNA light switching behaviors observed are due to the following three possibilities resulting from a noncovalent intercalative binding mode: (i) the protection of the intercalative moieties from interacting with water which was facilitated by formation of intermolecular hydrogen bonds, or excited proton-transfer reaction; (ii) more hydrophobic environment; (iii) a higher rigidity of the local environments of the complexes, decreasing nonradiative vibrational modes of relaxation.[1−2,5e,6e,11a]

The K_b values of the complexes could also be obtained by the luminescence titration data. By fitting the fractional changes in emission intensities, $(I - I_0)/(I_f - I_0)$, as a function of DNA concentrations according to the Bard−Torp−Murphy equation,[6f] the K_b and *n* values are derived to be $K_b = (1.1 \pm 0.3) \times 10^6$ M^{-1} and $n = 1.4 \pm 0.1$ for **1**, $K_b = (4.6 \pm 0.1) \times 10^6$ M^{-1} and $n = 2.2 \pm 0.05$ for **2**, and $K_b = (9.4 \pm 0.8) \times 10^6$ M^{-1} and $n = 2.2 \pm 0.1$ for **3** (inset in Figure 2b and the Supporting Information). They are in good agreement with the values derived from UV−vis spectral titration data and are comparable to 2.1×10^6 M^{-1} ($n = 2.4$) previously derived from the same binding model for the DNA intercalator, $[Ru(phen)_2(dppz)]^{2+}$.[6f] Further investigation is clearly needed to establish the exact nature of the complexes binding to DNA. However, NMR studies on the related complex $[Ru(phen)_2(dpq)]^{2+}$ have established that binding in the minor groove of DNA occurs via intercalation of the dpq moiety.[26d,e] Intercalation is a possible mode of binding for **1**−**3** as each of them bears a more extending aromatic plane than dpq.

3.3. EB Competition Assay. The competitive binding experiments with a well-established quenching assay based on the displacement of the intercalating drug ethidium bromide (EB) from ct-DNA may give further information regarding the DNA binding properties of **1**−**3** to DNA. If **1**−**3** would be DNA intercalators, the successive additions of **1**−**3** to the DNA

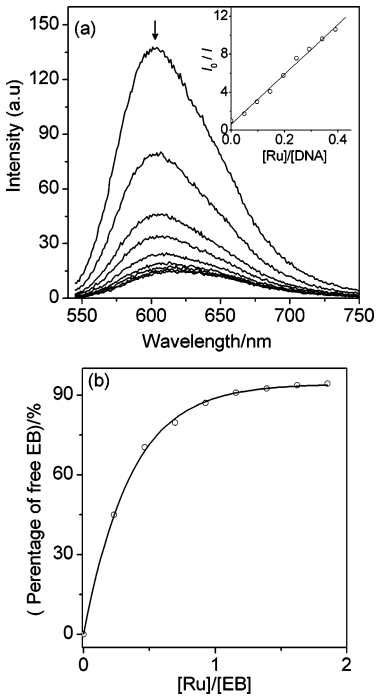


Figure 2. (a) Emission spectra of EB bound to DNA in the presence of $[Ru(bpy)_2(btpp)]^{2+}$ (0.0−40.0 μM). The arrows show the intensity changes upon increasing concentrations of the complex. Inset: florescence quenching curve of DNA-bound EB by the complex. (b) Plot of percentage of free EB vs [Ru]/[EB]. [EB] = 20.0 μM, [DNA] = 100.0 μM, λ_{ex} = 537 nm.

pretreated with EB would cause the removal of EB molecules inserted between the base pairs, resulting in the sharp decreases in EB emission intensities. This is because the free EB molecules were much less fluorescent than the bound EB molecules because of the surrounding water molecules which quenched its fluorescence[16c] and **1**−**3** and DNA-bound **1**−**3** are also negligibly weakly emissive as excited at λ_{ex} = 537 nm. For the case of **3**, the changes in emission spectra characteristic for EB emission as excitation into EB at λ_{ex} = 537 nm upon successive addition of the EB−DNA system is shown in Figure 2a (for **1** and **2** see Figures S3 and S4 in the Supporting Information). Upon addition of **1**−**3** to the EB−DNA system, appreciable reduction in emission intensities of EB by 76.4% for **1**, 74.6% for **2**, and 90.4% for **3** relative to that observed in the absence of **1**−**3**. The groove DNA binders were reported to be also capable of causing the reduction in EB emission intensities, but only moderately.[41a] As shown in the inset of Figure 2a for **3**, the quenching plots of I_0/I vs [Ru]/[DNA] are in good agreement with the linear Stern−Volmer equation with K_D = 8.4, 25.3, and 40.0 for **1**−**3**, respectively. To further illustrate the DNA binding strength of **1**−**3**, a competitive binding model can be used to calculate the apparent binding constants from the competition experiments using eq 3[35]

$$K_{app} = K_{EB}[EB]_{50\%}/[Ru]_{50\%} \quad (3)$$

where K_{app} is the apparent DNA binding constant of the Ru(II) complex, K_{EB} is the DNA binding constant of EB, and $[EB]_{50\%}$ and $[Ru]_{50\%}$ are the EB and Ru(II) complex concentrations at 50% fluorescence. In the plots of percent of free EB, [EB]/([EB] + [EB-DNA]) vs [Ru]/[EB], we can see that 50% of EB molecules were replaced from DNA-bound EB at a concentration ratio of [Ru]/[EB] = 0.39, 0.22, and 0.28 (Figure 2b and the Supporting Information) for **1**−**3**, respectively. The DNA binding constants of EB reported vary considerably,[36] and the

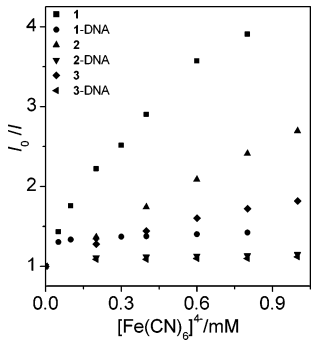


Figure 3. Emission quenching of 5.0 μM $Ru(bpy)_2(bipp)^{2+}$ (**1**), 4.7 μM $Ru(bpy)_2(bopp)^{2+}$ (**2**), and 5.1 μM $Ru(bpy)_2(btpp)^{2+}$ (**3**) with increasing concentrations of $[Fe(CN)_6]^{4-}$ (0.0−1.00 mM) in the absence and presence of 55.0 μM DNA in 5 mM Tris buffer at pH 7.0. **1**(■); **1**−DNA (●); **2** (▲); **2**−DNA (▼); **3** (◆); **3**−DNA (left-pointing solid triangle).

displacement does not follow a 1:1 stoichiometry, both of which complicate the use of a competitive binding model for establishing DNA binding constants. The most accurate usage likely would employ the ct-DNA binding constant of K_{app} (4.94×10^5 M^{-1})[37] for EB that represents an average binding constant, yielding $K_{app} = 1.3 \times 10^6$, 2.2×10^6, and 1.8×10^6 M^{-1} for **1**−**3**.

3.4. Luminescence Quenching by $[Fe(CN)_6]^{4-}$. Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as the quencher were also used to gauge the DNA binding properties of **1**−**3**. Anionic quenchers such as $[Fe(CN)_6]^{4-}$, very efficiently quench the emission of ruthenium complexes which are free in solution due to ion pairing[11c] but poorly quench the emission of ruthenium complexes which are closely bound to the DNA polyanion. Figure 3 shows Stern−Volmer plots for the emission quenching of **1**−**3** by $[Fe(CN)_6]^{4-}$ in the absence and presence of ct-DNA. In these plots, the steeper slope (K_Q) reflects more efficient quenching (less protection). The larger ratio R of the quenching constant in the absence to that in the presence of the DNA reflects less accessibility of the Ru(II) complex to $[Fe(CN)_6]^{4-}$. As illustrated in Figure 3, in the absence of DNA, sufficiently low quencher concentrations are utilized to yield linear dependences on quencher concentrations, the values of K_Q were derived to be 2690, 1660, and 800 M^{-1} for **1**−**3**, respectively. However, on addition of DNA, the slopes of the Stern−Volmer plots decline drastically, with K_Q values of 110, 55, and 36 M^{-1} **1**−**3**, respectively. The corresponding R values of 25, 30, and 22 for **1**−**3**, respectively, are close to an R value of 20 for proven DNA intercalator $[Ru(bpy)_2(pip)]^{2+}$,[38] indicating that **1**−**3** were effectively protected from accessibility of $[Fe(CN)_6]^{4-}$, probably **1**−**3** bind into the adjacent base pairs of the DNA deeply, leaving the negatively charged DNA phosphate backbone out to electrostatically repeal the approaching of $[Fe(CN)_6]^{4-}$.[11b,c]

3.5. Reverse Salt Effect. At neutral pH, the Ru complex has a dipositive charge, and it may therefore be expected that the interaction between the Ru(II) complex and nucleic acids would be influenced by such factors as the presence of other cations or the ionic strength of the solution.[39a] The sensitivity to ionic strength is expected to decrease in the order of the binding modes: electrostatic > groove-binding > intercalative. Therefore, these results may be used in both a qualitative and a quantitative manner (dissection of DNA binding free energy into electrostatic and nonelectrostatic components, Table 2) to give information on the DNA binding mode. The effects of the ionic strength on the emission yields of **1**−**3** were tested by the addition of NaCl. In the absence of DNA, the addition of NaCl

TABLE 2: Thermodynamic Parameters for the Binding of Ru(II) Complexes and Other Ligands to ct-DNA[a]

DNA binder[b]	$K_b/(10^3M^{-1})$	ΔG_{obs}/(kJ/mol)	*SK*	*Z*	ΔG_{pe}/(kJ/mol)	ΔG_t ($\Delta G_t/\Delta G_{obs}$)	ref
ethidium bromide	494	−32.2	0.75	0.85	−5.0	−27.2(85%)	37
daynomycin	4900	−37.7	0.84	0.95	−5.9	−31.8(84%)	37
$Ru(bpy)_2(Me_2bpy)^{2+}$	9.1	−10.5	1.5	1.70	−10.1	−0.4	39b
$Ru(bpy)_2(phen)^{2+}$	0.55	−15.6	1.6	1.82	−11.6	−4.0(26%)	39c
$Ru(phen)_2(dppz)^{2+}$	3200	−37.2	1.9	2.15	−13.8	−23.4(63%)	39c
$[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$	1310	−34.3	2.5	2.84	−18.1	−16.2(47%)	27b
$[(bpy)_2Ru(Mebpy)(CH_2)_7(Mebpy)Ru(bpy)_2]^{4+}$	780	−23.5	2.2	2.50	−16.0	−7.5(32%)	39b
1	2450	−36.5	1.7	1.93	−12.6	−23.9(66%)	
2	3500	−37.4	1.3	1.48	−9.7	−27.7(74%)	this work
3	6700	−39.0	1.2	1.36	−8.9	−30.1(77%)	

[a] In 50 mM NaCl, 5 mM Tris-HCl buffer at room temperature. δG_{obs} (kJ mol^{-1}) is the binding free energy change caculated by eq 5. The parameter *SK* is the absolute value of the slope obtained from the plots of inset in Figure 5. δG_{pe} and δG_t (kJ mol^{-1}) are the electrostatic and the nonelectrostatic contributions, respectively. The electrostatic contribution was calculated using eq 6 and evaluated at $[Na^+] = 50$ mM. The nonelectrostatic portion of the free energy change was calculated by the difference. The values in parentheses for δG_t correspond to the percentage of the nonelectrostatic contributions to the overall binding free energy changes (δG_{obs}). [b] Me_2bpy = 4,4′-dimethyl-2,2′-bipydidine; Mebpy = 4-methyl-2,2′-bipyridine; $ebipcH_2$ = *N*-ethyl-4,7- bis([1,10]phenanthroline[5,6-*f*]imidazol-2-yl)carbazole.

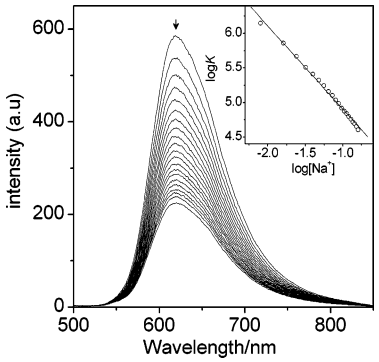


Figure 4. Changes in emission spectra of $Ru(bpy)_2(btpp)^{2+}$−DNA upon addition of NaCl. [Ru] = 5.3 μM; [DNA] = 22.0 μM; [NaCl] = 0−100 mM. Inset: salt dependence of binding constant (K_b) for the binding of the complex to ct-DNA. The slope of this plot corresponds to the *SK*.

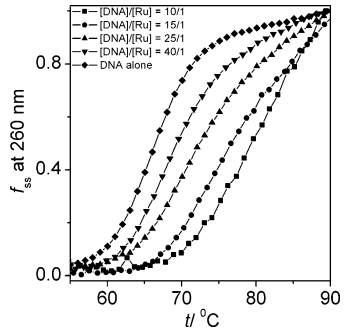


Figure 5. Thermal denaturnation curves of ct-DNA (50.0 μM) at different concentrations of $Ru(bpy)_2(btpp)^{2+}$. [DNA]/[Ru] = 10/1 (■); 15/1 (●); 25/1 (▲); 40/1 (▼); DNA alone (◆).

from 0.005 to 0.100 M had little or no effect on the emission yields of the complexes. However, in the presence of ct-DNA, successive addition of NaCl gradually quenches the emission of DNA bound **1**−**3** (Figure 4 and Figure S5 in the Supporting Information). The estimated K_{SV} values by plotting I_0/I vs the salt concentrations were 9.9, 13.7, and 9.6 M^{-1} for **1**−**3**, respectively. Since the emission from the free complexes was not quenched by NaCl, it is evident that the emission quenching of **1**−**3** in the presence of DNA was due to the release of the complexes from the DNA bound assemblies into the bulk aqueous solution, where they can undergo rapid nonradiative deactivation. We may conclude from these results that the ionic concentrations of the medium are important factors in the interaction of **1**−**3** with the DNA. The effects of the ionic strength on the DNA binding strength of **1**−**3** have generally been explained by postulating the existence of secondary sites to which the drug can bind with a lower affinity which is similar to EB.[40a] In order to evaluate the relative importance of electrostatic and nonelectrostatic contributions, polyelectrolyte theory developed by Record et al.[39a] was used. The binding constants (K_{obs}) at each salt concentration were obtained over the concentration range 0.005−0.100 M NaCl. The salt concentrations of 0.005−0.100 M were selected in this study because the polyelectrolyte theories which will be used for subsequent analysis are based on limiting laws that are strictly applicable to salt concentrations of lower than 0.100 M. It has been reported that the dependence of K_{obs} on salt concentration becomes nonlinear at higher concentrations of salt.[11h,37] The plot of log[Na^+] against log K_{obs} for the binding of **3** to ct-DNA is given in Figure 4 (for **1**−**2** see Figure S5 in the Supporting Information). It is clear from the plots that the binding constants decrease with increasing salt concentrations. This is due to the stoichiometry release of sodium ion following the binding of the Ru(II) complexes to the DNA, suggesting that electrostatic interaction is involved in the DNA-binding event. The slope of the linear fitting of Figure 5 is equal to *SK* in the following equation:

$$SK = -Z\psi = \delta \log K_{obs}/\delta \log [Na^+] \quad (4)$$

where *Z* is the charge on the Ru(II) complexes and ψ is the fraction of counterions associated with each DNA phosphate ($\psi = 0.88$ for double-stranded B-form DNA). The binding free energy can be calculated based on the standard Gibbs relations

$$\Delta G_{obs} = -RT \ln K_{obs} \quad (5)$$

$$\Delta G_{pe} = (SK)RT \ln [Na^+] \quad (6)$$

$$\Delta G_t = \Delta G_{obs} - \Delta G_{pe} \quad (7)$$

Electrostatic (ΔG_{pe}) and nonelectrostatic (ΔG_t) portions of the free energy can be calculated from eqs 6 and 7, respectively. The *SK* values of −1.7, −1.3, and −1.2 were obtained from Figure 4 and the Supporting Information, so the charges *Z* of 1.9, 1.5, and 1.4 on the complexes **1**−**3** are thus obtained and less than two positive charges carried by **1**−**3** which is quite common for metal complexes containing phen and its derivatives.[37,39] This may be caused by the factors such as the release of the counteranion of **1**−**3**, the hydration changes of **1**−**3** or DNA, and the structural features upon binding. ΔG_{pe} and ΔG_t values along with those for other complexes are summarized in Table 2. ΔG_t and ΔG_{pe} values were derived to be −23.9 and

TABLE 3: Comparison of ΔT_m of ct-DNA upon Binding to Some Ru(II) Complexes and Other Ligands

DNA binder[a]	[DNA]/[Ru]	ΔT_m/°C	ref
EB	10	13	40b
$Ru(bpy)_3^{2+}$	10	<2	42a
$Ru(phen)_3^{2+}$	12	18	42a
$Ru(phen)_2(dicnq)^{2+}$	25	5	25
Δ-$[Ru(phen)_2(dppz)]^{2+}$	1	16	8c
Λ-$[Ru(phen)_2(dppz)]^{2+}$	1	5	8c
$Ru(cyclam)_2(phi)^{2+}$	1	>5	42b
$Ru(cyclam)_2(bqdi)^{2+}$	1	1	42b
AMAC	1	5	42c
1	10	15.7	
2	10	15.0	this work
3	10	13.8	

[a] For the abbreviations, see Table 1 and 2, footnote. dicnq = 6,7-dicyanodipyrido[2,2-*d*:2′,3′-*f*]quinoxaline; phi = 9,10- phenanthroquinonediimine; bqdi = *o*-benzoquinonediimine; cyclam = 1,4,8,11-tetraazacyclotetradecane; AMAC = (9- anthrylmethyl)ammonium chloride.

-12.6 kJ·mol^{-1} for **1**, -27.7 and -9.7 kJ·mol^{-1} for **2**, and -30.1 and -8.9 kJ·mol^{-1} for **3** in 50 mM NaCl, respectively. The nonelectrostatic energies of the complexes are comparable to or more than those of the proven intercalators, such as $[Ru(phen)_2(dppz)]^{2+}$ and EB, and much more than those of semi-intercalator $[Ru(phen)_3]^{2+}$[39c] and electrostatically dominating $[(bpy)_2Ru(Mebpy)(CH_2)_7(Mebpy)Ru(bpy)_2]^{4+}$.[39b] These tendencies are consistent with the intercalative binding mode of **1**−**3**.

3.6. DNA Melting Experiments. The melting of DNA can be used to distinguish between the molecules that bind by intercalation and those that bind externally with DNA, i.e., electrostatically. Intercalation of small molecules into the double helix is known to increase the helix melting temperature, the temperature at which the double helix denatures into single stranded DNA. The extinction coefficient of DNA bases at 260 nm in the double-helical form is much less than in the single strand form; hence, melting of the helix leads to an increase in the absorption at this wavelength.[41] Thus, the helix-to-coil transition temperature can be determined by monitoring the absorbance of the DNA bases at 260 nm as a function of temperature. As shown in Figure 5 and the Support Information, the T_m of ct-DNA was 66.9 °C in the absence of the Ru(II) complex and was successively increased upon increasing the concentrations of the complexes. The melting points were increased by 15.7, 15.0, and 13.8 °C for complexes **1**−**3** at a concentration ratio of [Ru]/[DNA] = 1:10, respectively. On comparison with some DNA binding complexes (Table 3), the largely increased ΔT_m values suggest an intercalative binding mode of the complexes to DNA. The values of the binding constant K for the complexes to ct-DNA at T_m were determined by McGee's equation[43a]

$$1/T_m^0 - 1/T_m = (R/\Delta H_m)\ln(1 + KL)^{1/n} \quad (8)$$

where T_m^0 is the melting temperature of ct-DNA alone, T_m is the melting temperature in the presence of the complex, ΔH_m is the enthalpy of DNA melting (per bp), ΔH_m = 6.9 kcal mol^{-1},[43b] R is the gas constant, L is the free Ru(II) complex concentration (approximated by the total complex concentration at T_m), and n is the binding site size. K was derived to be 1.5 × 10^5 M^{-1} at 82.6 °C, 2.2 × 10^5 M^{-1} at 81.9 °C, and 2.6 × 10^5 M^{-1} at 80.7 °C for **1**−**3** by taking n = 1.5, 2.0, and 2.2 bp (approximated by the n values at 298 K) for complexes **1**−**3**. Theses K values indicate that the complexes still display binding affinity at the melting point of DNA. This observation was similar to those for $[Ru(phen)_3]^{2+}$ and $[Ru(cyclam)(phi)]^{2+}$ (cyclam = 1,4,8,11-tetraazacyclotetradecane, phi = 9,10-phenanthroquinonediimine) but different from that of AMAC (AMAC = (9-anthrylmethyl)ammonium chloride) which showed no binding to the single-strand DNA or to the phosphate backbone. The change of standard enthalpy was determined according to van't Hoff's equation[43c] (eq 9) and changes of standard free energy and standard entropy of the binding of the Ru(II) complex to DNA according to eqs 10 and 11

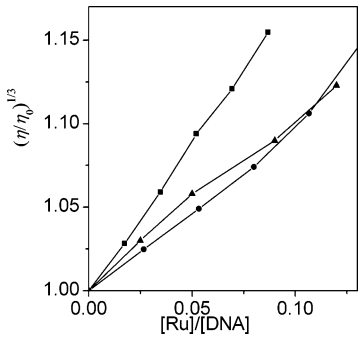


Figure 6. Changes in viscosities of ct-DNA (0.45 mM) upon addition of **1**, **2**, and **3** shown as a function of the concentration ratios of [Ru]/[DNA]. **1**(▲); **2** (■); **3** (●).

$$\ln(K_1/K_2) = (\Delta H^0/R)[(T_1 - T_2)/T_1T_2] \quad (9)$$

$$\Delta G_T^0 = -RT\ln K \quad (10)$$

$$\Delta G_T^0 = \Delta H^0 - T\Delta S^0 \quad (11)$$

where K_1 and K_2 are the DNA binding constants of the complex at temperatures T_1 and T_2, respectively. ΔG_T^0, ΔH^0, and ΔS^0 are the changes of the standard free energy, standard enthalpy, and standard entropy of binding of the complex to ct-DNA, respectively. For example, for complex **1**, a K_1 value of 2.5 × 10^6 M^{-1} (T_1 = 298 K) and a K_2 value of 1.5 × 10^5 M^{-1} (T_2 = 356 K) were used in eq 9, and ΔH^0 was thus derived to be -42.5 kJ mol^{-1}. By substituting K_1 = 2.5 × 10^6 M^{-1} (T_1 = 298 K) and $\Delta H^0 = -42.5$ kJ mol^{-1} into eqs 10 and 11, ΔG_{298K}^0 = -36.5 kJ mol^{-1} and $\Delta S^0 = -20.2$ J mol^{-1} K^{-1} for **1** at 25 °C were derived. The values of ΔG_{298K}^0, ΔS^0, and ΔH^0 were thus derived to be -37.4 kJ mol^{-1}, -17.7 J mol^{-1}, and -42.6 kJ mol^{-1} for **2**, respectively, and to be -39.0 kJ mol^{-1}, -41.2 J mol^{-1}, and -51.3 kJ mol^{-1} for **3**, respectively. The negative binding free energy implies that the sum of the free energies of free complexes and DNA is higher than that of the adduct, and the binding of the Ru(II) complexes to ct-DNA is energically highly favorable at 298 K, and the binding reaction was driven enthalpically. Although the large negative entropy change indicates that the complexes were very restricted in freedom upon binding to DNA, it is unfavorable for binding. Upon intercalation at the unwound site, there is a substantial structural overlap between the base pairs and the intercalator. The intercalator becomes rigidly held and oriented with the planar moiety perpendicular to the helical axis. Intercalation produces an extension, unwinding, and stiffening of the DNA helix. These changes are a consequence of the untwisting of the base pairs and helical backbone needed to accommodate the intercalator.

3.7. Viscosity Measurements. A critical test for a binding model in solution in the absence of crystallographic structural data is hydrodynamic measurements, which are more sensitive to the length changes of nucleic acids.[43a,44] Of the hydrodynamic measurements, viscosity measurements provide a tool for the

study of metal complex−DNA interactions since optical studies provide necessary but not sufficient clues to support the binding model. Under appropriate conditions, intercalation of drugs like EB causes a significant increase in viscosity of DNA solution due to an increase in the separation of base pairs at intercalation sites and, hence, an increase in overall DNA contour length. By contrast, drug molecules that bind exclusively in the DNA groove (e.g., netropsin and distmycine) under the same conditions typically cause less pronounced (positive or negative) or no change in DNA solution viscosity.[45] Here, the viscosities of ct-DNA increased with increasing concentrations of **1**−**3** (Figure 6). Such a trend is typical of intercalators.

4. Conclusions

Three Ru(II) complexes of $[Ru(bpy)_2(bipp)](ClO_4)_2$ (**1**), $[Ru(bpy)_2(bopp)](ClO_4)_2$ (**2**), and $[Ru(bpy)_2(btpp)](ClO_4)_2$ (**3**) with variations in heteroatoms of NH(1), O(2), and S(3) have been synthesized and characterized. These complexes have been shown to act as a new class of ct-DNA light switches. The interaction of **1**−**3** with the DNA has resulted in large hypochromisms of 31%−47% at ∼270 nm, bathochromic shifts of 9−12 nm for ∼270 nm absorption peaks, large DNA binding constants at 10^6 M^{-1} orders of magnitude, largely enhanced emission, effective protection of the complexes from emission quenching by $[Fe(CN)_6]^{4-}$, favorably competitive binding to the DNA with EB, dominant nonelectrostatic binding free energy relative to the electrostatic binding one revealed by reverse salt effect, increased melting temperatures of 13.8−15.7 °C at [Ru]/[DNA] = 1:10, and evident increases in viscosities of the DNA. These results lead to a self-consistent set of conclusions concerning the mode and efficiency of binding of these complexes to ct-DNA: the complexes avidly bound to ct-DNA in intercalative mode in buffered 50 mM NaCl.

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Supporting Information Available: Scheme of DNA light switches based on ruthenium complexes reported, data of UV−vis and luminescence spectroscopic titrations, DNA competitive binding with EB and salt-dependent experiments for **1**−**2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Friedman, A. E.; Chambron, J. C.; Sauvage, J. P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960. (b) Liu, J. G.; Zhang, Q. L.; Shi, X. F.; Ji, L. N. *Inorg. Chem.* **2001**, *40*, 5045. (c) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809. (d) Olson, E. J. C.; Hormann, A.; Jonkman, A.; Arkin, M. R.; Stemp, E. D. A.; Barton, J. K.; Barbara, P. F. *J. Am. Chem. Soc.* **1997**, *119*, 11458. (e) Liu, J. G.; Ye, B. H.; Li, H.; Ji, L. N.; Li, R. H.; Zhou, J. Y. *J. Inorg. Biochem.* **1999**, *73*, 117. (f) Arounaguiri, S.; Maiya, B. G. *Inorg. Chem.* **1999**, *38*, 842. (g) Brennaman, M. K.; Alstrum-Acevedo, J. H.; Fleming, C. N.; Jang, P.; Meyer, T. J.; Papanikolas, J. M. *J. Am. Chem. Soc.* **2002**, *124*, 15094.

(2) (a) Holmlin, R. E.; Barton, J. K. *Inorg. Chem.* **1995**, *34*, 7. (b) Holmin, R. E.; Yao, J. A.; Barton, J. K. *Inorg. Chem.* **1999**, *38*, 174. (c) Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 5236.

(3) (a) Chow, C. S.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 2839. (b) Sitlani, A.; Barton, J. K. *Biochemistry* **1994**, *33*, 12100. (c) Lim, A. C.; Barton, J. K. *Bioorg. Med. Chem.* **1997**, *5*, 1131. (d) Sitlani, A.; Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 12589. (e) Krotz, A. H.; Hudson, B. P.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 12577.

(4) (a) Stoeffler, H. D.; Thornton, N. B.; Temkin, S. L.; Schanze, K. S. *J. Am. Chem. Soc.* **1995**, *117*, 7119. (b) Yam, V. W.-W.; Lo, K. K.-W.; Cheung, K.-K.; Kong, R. Y.-C. *J. Chem. Soc., Chem. Commun*, **1995**, 1191.

(5) (a) Hartshorn, R. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 5919. (b) Tuite, E.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.* **1997**, *119*, 239. (c) Hiort, C.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 3448. (d) Erikkssion, M.; Leijon, M.; Hiort, C.; Norden, B.; Graslund, A. *Biochemistry* **1994**, *33*, 5031. (e) Bernnaman, M. K.; Meyer, T. J.; Panpanikolas, J. M. *J. Phys. Chem. A* **2004**, *108*, 9938. (f) Phillips, T.; Haq, I.; Meijer, A. J. H. M.; Adams, H.; Soutar, I.; Swanson, L.; Sykes, M. J.; Thomas, J. A. *Biochemistry* **2004**, *43*, 13657.

(6) (a) Xiong, Y.; Ji, L. N. *Coord. Chem. Rev.* 1999, *185*−*186*, 711. (b) Pichot, F.; Beck, J. H.; Ellliott, M. *J. Phys. Chem. A* **1999**, *103*, 6263. (c) Liu, Y.; Duan, Z. Y.; Zhang, H. Y.; Jiang, X. L.; Han, J. R. *J. Org. Chem.* **2005**, *70*, 1450. (d) Anzenbacher, P., Jr.; Tyson, D. S.; Jursikova, K.; Castellano, F. N. *J. Am. Chem. Soc.* **2002**, *124*, 6232. (e) Gholamkhass, B.; Koike, K.; Negishi, N.; Hori, H.; Takeuchi, K. *Inorg. Chem.* **2001**, *40*, 756. (f) Nair, R. B.; Murphy, C. J. *J. Inorg. Biochem.* **1998**, *69*, 129.

(7) (a) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res.* **1991**, *19*, 2595. (b) Onfelt, B.; Gostring, L.; Lincoln, P.; Norden, B.; Onfelt, A. *Mutagenesis* **2002**, *17*, 317. (c) Dupureur, M.; Barton, J. K. *Inorg. Chem.* **1997**, *36*, 33. (d) Greguric, I.; Aldrich-Wright, J. R.; Collins, J. G. *J. Am. Chem. Soc.* **1997**, *119*, 3621. (e) Nair, R. B.; Teng, E. S.; Kirkand, S. L.; Murphy, C. J. *Inorg. Chem.* **1998**, *37*, 139.

(8) (a)Ruba, E.; Hart, J. R.; Barton, J. K. *Inorg. Chem.* **2004**, *43*, 4570. (b) Bichenkova, E. V.; Yu, X.; Bhadra, P.; Heissigerova, H.; Pope, S. J. A.; Coe, B. J.; Faulkner, S.; Douglas, K. T. *Inorg. Chem.* **2005**, *44*, 4112. (c) Petitjean, A.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 14728. (d) Zeglis, B. Z.; Barton, J. K. *J. Am. Chem. Soc.* **2006**, *128*, 5654. (e) Schatzschneider, U.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 8630. (f) Jackson, B. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 12986. (g) Jackson, B. A.; Barton, J. K. *Biochemistry* **2000**, *39*, 6176. (g) Xiong, Z.-H.; Yang, P. *J. Mol. Struc. (Theochem)* **2002**, *582*, 107.

(9) Sabatani, E.; Nikol, H. D.; Gray, H. B.; Norden, B.; Chowdhry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc.* **1996**, *118*, 1158.

(10) Chambron, J. C.; Sauvage, J. P. *Chem. Phys. Lett.* **1991**, *182*, 603.

(11) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518. (c) Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 2081. (d) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1991**, *111*, 3051. (e) Barton, J. K.; Basile, L. A.; Danishefsky, A.; Alexandrescu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1961. (f) Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777. (g) Moucheron, C.; Krisch-DeMesmaeker, A.; Kelly, J. M. *Phtotchem. Photobiol. B* **1997**, *40*, 91. (h) Haq. I.; Lincoln, P.; Suh, D. C.; Norden, B.; Chowdhry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc.* **1995**, *117*, 4788. (i) Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* **1994**, *116*, 10286. (j) Yun, B. H.; Kim, J. O.; Lee, B. W.; Norden, B.; Kim, J. M.; Kim, S. K. *J. Phys. Chem. B* **2003**, *107*, 9858.

(12) (a) Marian, I.; Enzo, A.; Marzilli, L. G. *Inorg. Chem.* **1996**, *35*, 2384. (b) Goulle, V.; Harriman, A.; Lehn, J. M. *J. Chem. Soc., Chem. Commun.* **1993**, 1034.

(13) (a) Lasey, R. C.; Banerji, S. S.; Ogawa, M. Y. *Inorg. Chim. Acta* **2000**, *300*−*302*, 822. (b) Novakova, O.; Kasparkova, J.; Bursova, V.; Hofr, C.; Vojtiskova, M.; Chen, H.; Sadler, P.; Brabec, V. *Chem. Biol.* **2005**, *12*, 121.

(14) (a) Pyle, A. M.; Morii, T.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 9432. (b) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*. 2303. (c) Campisi, D.; Morii, T.; Barton, J. K. *Biochemistry* **1994**, *33*, 4130.

(15) Wang, J.; Jiang, Y.; Zhou, C.; Fang, X. *Anal. Chem.* **2005**, *77*, 3542.

(16) (a) Fukuzumi, S.; Tanaka, M.; Nishimine, M.; Ohkubo, K. *J. Photochem. Photobiol. A* **2005**, *175*, 79. (b) Westerlund, F.; Pierard, F.; Eng, M. P.; Norden, B.; Lincoln, P. *J. Phys. Chem. B* **2005**, *109*, 17327. (c) Ortmans, I.; Elias, B.; Kelly, J. M.; Moucheron, C.; Kirsch-DeMesmaeker, A. *Dalton Trans.* **2004**, 668. (d) Boon, E. M.; Jackson, N. M.; Wrightman, M. D.; Shana, O.; Hill, M. G.; Barton, J. K. *J. Phys. Chem. B* **2003**, *107*, 11805.

(17) (a) Murphy, C. J.; Barton, J. K. *Methods Enzymol.* **1993**, *226*, 576. (b) Jackson, B. A.; Alekseyev, V. Y.; Barton, J. K. *Biochemistry* **1999**, *38*, 4655. (c) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731. (d) Dandlier, P. J.; Holmlin, R. E.; Barton, J. K. *Science* **1997**, *274*, 1465. (e) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295.

(18) (a) Tysoe, S. A.; Kopelman, R.; Schelzig, D. *Inorg. Chem.* **1999**, *38*, 5196. (b) Liu, Y.; Chouai, A.; Degtyareva, N. N.; Lutterman, D. A.; Dunber, K. R.; Turro, C. *J. Am. Chem. Soc.* **2005**, *127*, 10796.

(19) Rajput, C.; Rutkaite, R.; Swanson, L.; Haq, I.; Thomas, J. A. *Chem. Eur. J.* **2006**, *12*, 4611.

(20) Konduri, R.; de Tacconi, N. R.; Rajeshwar, K.; MacDonnell, F. M. *J. Am. Chem. Soc.* **2004**, *126*, 11621.

(21) (a) Boisdenghien, A.; Moucheron, C.; Kirsch-De Mesmaeker, A. *Inorg. Chem.* **2005**, *44*, 7678. (b) Leveque, J.; Elias, B.; Moucheron, C.; Kirsch-De, Mesmaeker, A. *Inorg. Chem.* **2005**, *44*, 393. (c) Moucheron, C.; Kirsch-De Mesmaeker, A.; Choua, S. *Inorg. Chem.* **1997**, *36*, 584.

(22) (a) Zhen, Q. Y.; Ye, B. H.; Zhang, Q. L.; Liu, J. G.; Li, H.; Ji, L. N. *J. Inorg. Biochem.* **1999**, *76*, 47. (b) Li, J.; Chen, J. C.; Xu, L. C.; Zheng, K. C.; Ji, L. N. *J. Organomet. Chem.* **2007**, *692*, 831.

(23) Liu, J. G.; Ye, B. H.; Li, H.; Ji, L. N.; Zhou, R. H.; Jian, Y. *J. Inorg. Biochem.* **1999**, *73*, 117.

(24) (a) Zou, X. H.; Ye, B. H.; Li, H.; Liu, J. G.; Xiong, Y.; Ji, L. N. *J. Chem. Soc., Dalton Trans.* **1999**, 1423. (b) Zou, X. H.; Ye, B. H.; Li, H.; Zhang, Q. L.; Chao, H.; Liu, J. G.; Ji, L. N.; Li, X. Y. *J. Biol. Inorg. Chem.* **2001**, *6*, 143.

(25) Ambroise, A.; Maiya, B. G. *Inorg. Chem.* **2000**, *39*, 4264.

(26) (a) O'Donoghue, K. A.; Penedo, J. C.; Kelly, J. M.; Kruger, P. E. *Dalton Trans.* **2005**, 1123. (b) O'Donoghue, K. A.; Kelly, J. M.; Kruger, P. E. *Dalton Trans.* **2004**, 13. (c) Daleney, S.; Pascaly, M.; Bhattacharya, P. K.; Han, K.; Barton, J. K. *Inorg. Chem.* **2002**, *41*, 1966. (d) Collins, J. G.; Sleeman, A. D.; Aldrich-Wright, J. R.; Greguric, I.; Hambley, T. W. *Inorg. Chem.* **1998**, *37*, 3133. (e) Greguric, I.; Aldrich-Wright, J. R.; Norden, B. *J. Am. Chem. Soc.* **1997**, *119*, 3621.

(27) (a) Bai, G. Y.; Wang, K. Z.; Duan, Z. M.; Gao, L. H. *J. Inorg. Biochem.* **2004**, *98*, 1017. (b) Liu, F. R.; Wang, K. Z.; Bai, G. Y.; Zhang, Y. A.; Gao, L. H. *Inorg. Chem.* **2004**, *43*, 1799. (c) Han, M. J.; Gao, L. H.; Lu, Y. Y.; Wang, K. Z. *J. Phys. Chem. B* **2006**, *110*, 2364. (d) Han, M. J.; Gao, L. H.; Wang, K. Z. *New. J. Chem.* **2006**, *30*, 208. (e) Lu, Y. Y.; Gao, L. H.; Han, M. J.; Wang, K. Z. *Eur. J. Inorg. Chem.* **2006**, 430. (f) Hao, Q.; Duan, Z. M.; Han, M. J.; Zheng, S. Z.; Lu, Y. Y.; Wang, K. Z. *Chem. J. Chinese Universities* (in Chinese) **2006**, *27*, 1217.

(28) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334.

(29) Bolger, L.; Goarden, A.; Ishow, E.; Launay, J. P. *Inorg. Chem.* **1996**, *35*, 2937.

(30) Delgadillo, A.; Romo, P.; Leiva, A. M.; Loeb, B. *Helv. Chim. Acta* **2003**, *86*, 2110.

(31) Reichmann, M. E.; Rice, S. A.; Thomas, C. A.; Doty, P. *J. Am. Chem. Soc.* **1954**, *76*, 3047.

(32) Ji, Z.; Huang, S.; Guadalupe, A. R. *Inorg. Chim. Acta* **2000**, *305*, 127.

(33) (a) Cater, M. T.; Rodriguez, M.; Bard, A. J. *J. Am. Chem. Soc.* **1989**, *111*, 8901. (b) Smith, S. R.; Neyhart, G. A.; Karlsbeck, W. A.; Thorp, H. H. *New. J. Chem.* **1994**, *18*, 397.

(34) Deng, H.; Cai, J.; Xu, H.; Zhang, H.; Ji, L. N. *Dalton Trans.* **2003**, 325.

(35) Boger, D. L.; Fink, B. E.; Brunette, S. R.; Tse, W. C.; Hedrick, M. P. *J. Am. Chem. Soc.* **2001**, *123*, 5878.

(36) Baguley, B. C.; Falkenhaug, E. M. *Nucleic Acids Res.* **1978**, *5*, 161.

(37) Mudasir, Wijaya, K.; Wahyuni, E. T.; Yoshioka, N.; Inoue, H. *Biophys. Chem.* **2006**, *121*, 44.

(38) (a) Xiong, Y.; He, X. F.; Zou, X. H.; Wu, J. Z.; Chen, X. M.; Ji, L. N. Li, R. H.; Zhou, J. Y.; Yu, K. B. *J. Chem. Soc., Dalton Trans.* **1999**, 19. (b) Ji, L. N.; Liu, J. G.; Ye, B. H. *Mater. Sci. Eng.* C **1999**, *10*, 51.

(39) (a) Record, M. T.; Lohman, T. M.; De Haseth, P. *J. Mol. Biol.* **1976**, *107*, 145. (b) O'Reilly, F. M.; Kelly, J. M. *J. Phys. Chem. B* **2000**, *104*, 7206. (c) Pierard, F.; Guerzo, A. D.; Kirsch-De Mesmaeker, A.; Demeunynck, M.; Lhomme, J. *Phys. Chem. Chem. Phys.* **2001**, *3*, 2911.

(40) (a) Wang, M. J. *J. Mol. Biol.* **1965**, *13*, 269. (b) Cory, M.; Mckee, D. D.; Kagan, J.; Henry, D. W.; Miller, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 2528.

(41) (a) Patel, D. J. *Acc. Chem. Res.* **1979**, *12*, 118. (b) Patel, D. J.; Canuel, L. L. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3343. (c) Berman, H. M.; Young, P. A. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87.

(42) (a) Kelly, J. M.; Tossi, A. B.; MeConnell, D. J.; OhUigin, C. *Nucleic Acids Res.* **1985**, *13*, 6017. (b) Chan, H. L.; Liu, H. Q.; Tzeng, B. C.; You, Y. S.; Peng, S. M.; Yang, M.; Che, C. M. *Inorg. Chem.* **2002**, *41*, 3161. (c) Kumar, C. V.; Asuncion, E. H. *J. Am. Chem. Soc.* **1993**, *115*, 8547.

(43) (a) McGhee, J. D. *Biopolymers* **1976**, *15*, 1345. (b) Satyanarayana. S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573. (c) Leng, F.; Chairs, J. B.; Waring, M. J. *Nucleic Acids Res.* **2003**, *21*, 6191.

(44) Satyanarayana, S.; Dabrowiak, J. C.; Chairs, J. B. *Biochemistry* **1992**, *31*, 9319.

(45) Lerman, L. *J. Mol. Biol.* **1961**, *3*, 18.