Inorg. Chem. **2004**, *43*, 1799–1806

The pH-Induced Emission Switching and Interesting DNA-Binding Properties of a Novel Dinuclear Ruthenium(II) Complex

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Received September 20, 2003

A novel dinuclear Ru(II) complex, $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2](ClO_4)_4$, where bpy = 2,2′-bipyridine and ebipcH$_2$ = *N*-ethyl-4,7-bis([1,10]-phenanthroline[5,6-*f*]imidazol-2-yl)carbazole, has been newly synthesized. The pH effects on UV–vis absorption and emission spectra of the complex are studied, and ground- and excited-state ionization constants of the complex are derived. The binding of the complex to calf thymus (ct) DNA is investigated with absorption and luminescence titrations, steady-state emission quenching, and viscosity measurements. The complex acts as a pH-induced "on–off" emission switch between pH 8.0 and pH 10.0 with a maximum on–off ratio of ~100 which is favorably compared with the other imidazole-containing Ru(II) complex congeners, and a strong ct-DNA intercalator with an intrinsic binding constant of $1.31(\pm 0.08) \times 10^6$ M^{-1} in buffered 50 mM NaCl.

Introduction

Ruthenium(II) polypyridyl complexes have attracted much interest as light absorbers, photoluminescent sensors or switches, and intramolecular energy and electron transfer agents.[1] Dinuclear and polynuclear ruthenium(II) complexes bridged by bis bidentate and tridentate ligands have received special attention in recent years in connection with the design of molecular electronic devices.[2] The studies have revealed that intramolecular electron transfer events can be governed by several factors, including the donor−acceptor electronic coupling, the free-energy change of the reaction, and the polarity of the solvent.[3] The interaction between donor and acceptor in polynuclear Ru complexes is strongly dependent on the bridging ligand. Thus, the design of the bridging ligand is one of the key steps in realizing molecular electronic devices based on polynuclear Ru complexes. Pyridine-, pyrazine-, and pyrimidine-containing ligands have relatively low-lying π^* orbitals, and therefore they acted as good π-acceptors.[3e,4] In contrast, the imidazole-containing ligands

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10.1021/ic035109x CCC: $27.50 © 2004 American Chemical Society
Published on Web 02/11/2004

such as bis(imidazole) are poorer π-acceptors and better π-donors. The advantages of using imidazole-containing ligands could be also seen by the appreciable ability to control orbital energies by proton transfer.[5] These complexes with imidazole rings coordinated to the metal ion are usually nonemissive or weakly emissive in fluid solution at room temperature;[5a−g] those with imidazole rings uncoordinated to the Ru(II) center were, however, recently demonstrated to be good emitters with interesting proton induced on−off emission switching characteristics but low on−off ratios.[5h−5k]

On the other hand, the potential of substitution-inert metal complexes as photochemical structural and stereoselective probes of nucleic acids has been explored extensively over the past decades.[6] 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. All the studies[7−14] revealed that modification of the ligands would lead to subtle or substantial changes in the binding modes, location, and affinities, giving chances to explore various valuable conformation- or site-specific DNA probes and potential chemotherapeutical agents. However, much less attention has been focused on the interaction of dinuclear or polynuclear ruthenium(II) complexes with DNA[15−21] even though the dinuclear Ru(II) complexes are of varied shapes and sizes, and more specificity. We wish to report here on a novel imidazole- and phenanthroline moiety-containing dinuclear Ru(II) complex which showed impressive on−off emission switching and strong binding to calf thymus DNA.

Experimental Section

Materials. *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$,[22] 1,10-phenanthroline-5,6-dione,[23] 1-ethylcarbazole,[24] and 4,7-diformyl-1-ethylcabazole[25] were synthesized according to the literature methods. Solvents were purified and dried according to standard methods.[26] The other materials were obtained from commercial sources and used without further purification.

Preparation of ebipc$H_2\cdot$DMF$\cdot H_2O$. This compound was synthesized by following the procedure reported before.[27] The crude product was purified by recrystallization with *N*,*N*-dimethylformamide−diethyl ether. Yield: 83%. Anal. Calcd for $C_{40}H_{25}N_9\cdot$DMF$\cdot H_2O$: C, 71.45; H, 4.74; N, 19.38. Found: C, 70.96; H, 4.79; N, 19.28. IR: ν_{max} (KBr, cm^{-1}): 3429 (N−H), 1654 (C═N), 1605 (ring), 1441 (ring). ^{1}H NMR (500 MHz, Me_2SO-d_6, 298 K): 9.24 (s, 2H), 9.03 (m, 8H), 8.48 (d, 2H, $J = 8.4$), 7.90 (m, 6H), 4.61 (q, 2H, $J = 6.85$), 1.38 (t, 3H, $J = 6.85$).

Preparation of $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2](ClO_4)_4\cdot 2CH_3OH\cdot H_2O$. The synthetic route to the dinuclear Ru(II) complex is described in Scheme 1. The synthetic details are given as follows. The reaction of $[Ru(bpy)_2Cl_2]\cdot 2H_2O$ (0.1562 g, 0.3 mmol) with ebipcH_2 (0.0983 g, 0.156 mmol) in ethylene glycol (3 cm^3) under N_2 for 8 h at 100 °C to give a clear red solution was followed by precipitation by 4-fold excess of a saturated aqueous $NaClO_4$ solution. (**Caution**! *All the perchlorate salts are potentially explosive and therefore should be handled in small quantity with care*.) The red precipitate was filtered and purified by column chromatography on alumina using CH_2Cl_2−MeOH (10:1,v/v) as the eluent and dried in vacuo. Yield: 0.214 g, 77%. Anal. Calcd for $C_{80}H_{57}N_{17}Cl_4O_{16}\ Ru_2\cdot 2CH_3OH\cdot H_2O$: C, 51.23; H, 3.51; N, 12.39. Found: C, 51.43; H, 3.81; N, 12.23. IR: ν_{max} (KBr, cm^{-1}): 3427 (N−H), 1602 (C═N), 1092 (ClO_4^-). ^{1}H NMR (500 MHz,

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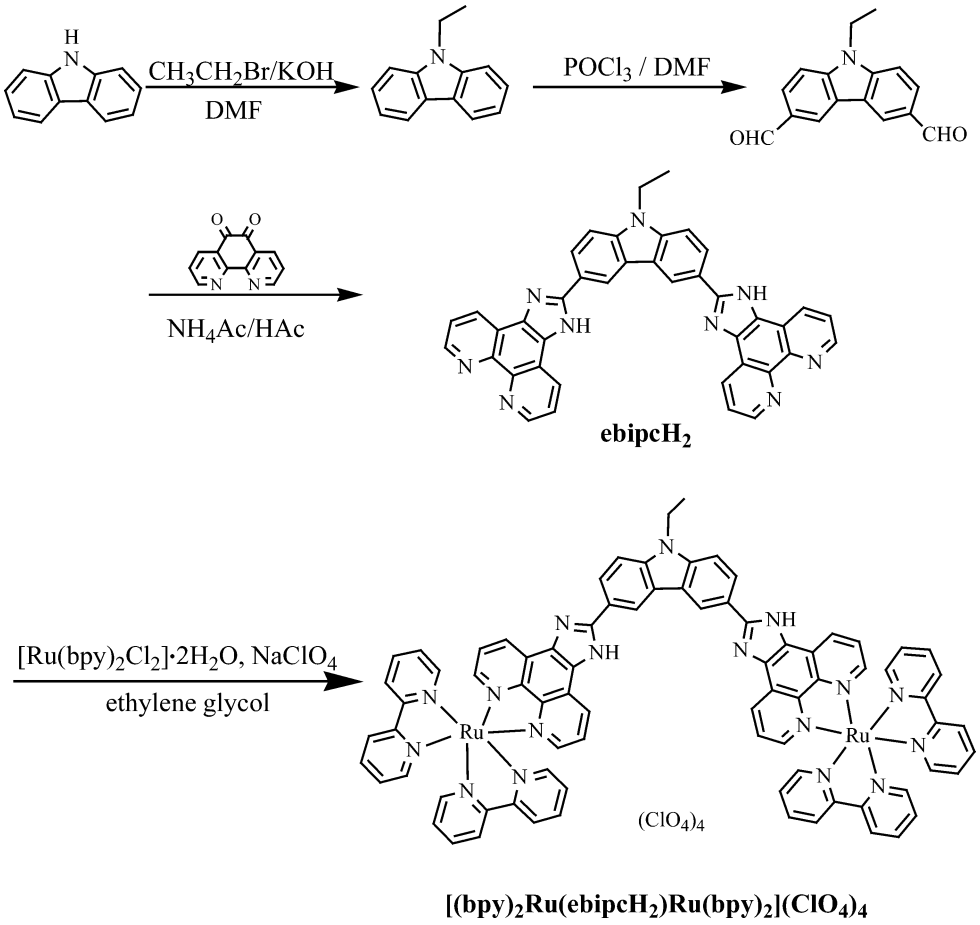
Scheme 1. The Synthetic Route to the Dinuclear Ru(II) Complex



Me_2SO-d_6, 298 K): δ 9.34 (s, 2H), 9.16 (m, 4H), 8.88 (dd, 9H), 8.58 (d, 2H, J = 7.625), 8.22 (t, 5H, J = 7.56), 8.12 (t, 5H, J = 7.52), 7.80 (m, 12H), 7.63 (m, 9H), 7.38 (t, 4H, J = 6.69), 4.60 (q, 2H), 1.46 (t, 3H, J = 6.55).

Physical Measurements. Elemental analyses were performed on a Vario EL elemental analyzer. Infrared spectra were recorded on a Nicolet Avtar 360FT-IR spectrometer as KBr disks. UV−vis spectra were obtained on a GBC Cintra 10e UV−visible spectrometer. NMR spectra were collected on a Bruker DRX-500 NMR spectrometer with Me_2SO-d_6 as solvent. Emission spectra were obtained on a Shimadzu RF-5301PC spectrofluorimeter at room temperature. UV−vis and emission pH spectroscopic titrations were carried out in aqueous solution with a Britton−Robinson buffer and 0.2 mol/L NaCl to keep a constant ion strength. All the experiments involving the interaction of the complex with ct-DNA were carried out in aerated buffer (5 mmol·dm^{-3} Tris-HCl, pH 7.1) containing NaCl. Viscosity experiments used an Ubbelohde viscometer, immersed in a thermostated water-bath maintained at 29.4 ± 0.1 °C. DNA samples approxmately 200 base pairs in average length were prepared by sonication in order to minimize complexities arising from DNA flexibility.[28] Data were presented as $(\eta/\eta_o)^{1/3}$ versus the ratio of the concentration of the ruthenium(II) complex to that of the DNA, where η and η_o are the viscosities of DNA solutions in the presence and absence of complex, respectively. Viscosity values were calculated from the observed flow time of DNA containing solutions (t) corrected for that of buffer alone (t_0), $\eta = t - t_0$.[29]

Results and Discussion

Electronic Absorption Spectra. UV−vis spectral pH titrations were carried out over the pH range 0.99−12.95, and the spectral changes with pH were reversible. The electronic spectra of the complex in aqueous solution mainly consist of three well-resolved bands: the two bands at ∼288 and ∼370 nm which are assigned to the intraligand $\pi-\pi^*$ transitions, and one broad band at ∼470 nm which can be attributed to the metal-to-ligand charge transfer (MLCT) upon comparison with those of $[Ru(bpy)_3]^{2+}$.[1]

The UV−vis spectra of the aqueous solution of the complex as a function of pH are shown in Figure 1. It is clear from Figure 1 that the complex underwent four successive deprotonation processes over the pH range 1.46−12.80. Upon increasing pH from 1.46 to 3.20, the band at 287 and 378 nm decreased slightly in the intensities and one isosbestic point at 473 nm appeared; the spectral changes observed here are due to the concurrent dissociation of the two protons on protonated imidazole rings. The second deprotonation step, which took place over pH 3.90−6.90, is assigned to the single-proton dissociation of protonated alkylcarbazole moiety, resulting in the following spectral changes: all the absorption intensities for the bands at 288, 368, and 473 nm became slightly increased. The third

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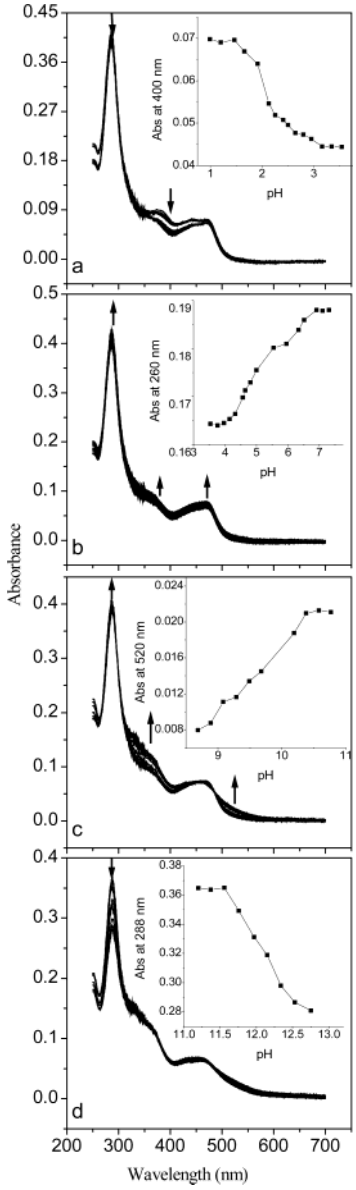

Figure 1. The changes of UV−vis spectra of the Ru(II) complex upon raising the pH: (a) pH = 0.99−3.54; (b) pH = 3.35−7.30; (c) pH = 8.69−10.99; (d) pH = 11.20−12.95.

deprotonation process, which was assigned to the deprotonation of the proton on one of two neutral imidazole rings, occurred between pH 8.60 and 10.40, accompanying the following spectral features: the wave valley at 526 nm increased slightly, the band at 368 nm increased, and two new isosbestic points appeared at 454 and 485 nm. As the pH increased from 11.50 to 12.80, the last deprotonation of the other neutral imidazole ring occurred and was characterized by decreases in the intensities for the band at 288 nm.

It is clear from the discussion above that the complex $[(bpy)_2Ru(ebipcH_5)Ru(bpy)_2]^{7+}$ underwent four successive deprotonation processes as shown in Scheme 2 upon raising the pH from 1.46 to 12.80. The changes in absorbance at different wavelengths as a function of pH are shown in the insets of Figure 1. By taking the pH_i (for single-proton deprotonation process) or $2pH_i$ (for two-proton deprotonation process),[30,33] where pH_i is the pH at the inflection points of these curves, four ground-state ionization constants of $pK_{a1} = 4.16 \pm 0.01$, $pK_{a2} = 5.07 \pm 0.01$, $pK_{a3} = 9.65 \pm 0.01$, and $pK_{a4} = 12.09 \pm 0.01$ were obtained. The comparison of pK_{a3} and pK_{a4} for deprotonation processes of the two protons on the imidazole rings on the dinuclear complex in this study, with those listed in Table 1 for corresponding deprotonations of analogue Ru(II) complexes, shows that $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2](ClO_4)_4$ is more basic than the other Ru(II) complex analogues due to the presence of the electron-donating carbazole moiety.

Luminescence Spectroscopic Studies. The complex in aerated aqueous solutions at room temperature emitted strongly with emission maxima at 624 nm, which is characteristic of MLCT luminescence[31,32] and is assigned as derived from the ^{3}MLCT ($d\pi(Ru) \rightarrow \pi^*$ (ligand)) state,[1,33] upon visible light excitation.

The emission spectral changes in aqueous solution as a function of pH are shown in Figure 2. We can see that the emission spectra are sensitive to pHs. Upon pH being increased from 0.90 to 3.5, the emission maxima blue-shifted from 632 to 606 nm and the intensities decreased by about 5.6%. From pH 5.1 to 7.1, new spectral characteristics were observed: the emission intensities decreased slightly and the emission maxima were almost unchanged at 606 nm. The sharp decreases in the emission intensities were observed upon further increasing pH from 7.8 to 10.2, and the emission maxima were slightly blue-shifted from 606 at pH = 7.8 to 602 nm at pH = 10.2. On the contrary, increasing pH from 11.20 to 12.34 resulted in a slight increase in the emission intensities without a shift in the emission maximum of 602 nm. Clearly, the emission spectral changes discussed above are associated with four excited-state deprotonation processes, and each of the processes dealt with the same proton/protons as UV−vis spectral titrations. The changes of relative intensities vs pHs are shown in the insets of Figure 2. Clearly, the complex acted as an excellent on−off emission switch with a maximum on−off ratio of ~100 upon changing pH over a narrow range of 8.0−10.0. It is noteworthy that this on−off ratio is favorably compared to those (1.7−16, see Table 1) reported for imidazole-containing Ru(II) complex congeners.

Excited-state ionization constants, pK_a^*, could be roughly evaluated on the basis of the Förster cycle,[34] which correlates pK_a^* with pK_a thermodynamically by eq 1, in which ν_B and ν_{HB} are pure 0−0 transitions in cm^{-1} for the basic and acidic species, respectively. In practice, ν_B and ν_{HB} are often

$$pK_a^* = pK_a + (0.625/T)(\nu_B - \nu_{HB}) \qquad (1)$$

difficult or even impossible to obtain. A good approximation is to use the emission maxima for ν_B and ν_{HB} since

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Scheme 2. The Acid−Base Equilibria of the Complex Ion $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$

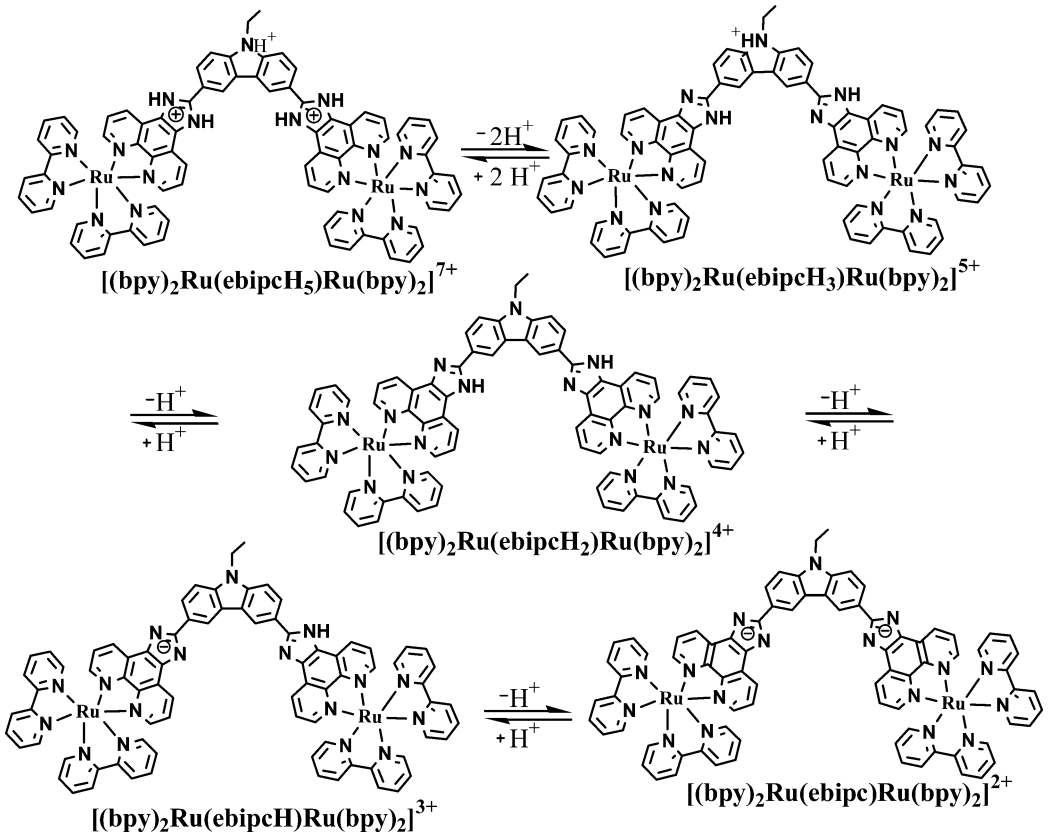

Table 1. The Comparison of Ground- and Excited-State pK_a of the Complex and the On−Off Ratio for Emission Switch with Those of Representative Ru(II) Analogue Complexes

complex[a]	pK_a	pK_a* based on Förster cycle	on−off ratio	ref
$[Ru(bpy)_2(pidbH_2]^{2+}$	1.97, 3.75, 10.56	−, 4.22, 10.71	16	5h
$[(bpy)_2Ru(mbpibH_2)Ru(bpy)_2]^{4+}$	3.2, 9.3	−, −	1.7	5i
$[(bpy)_2Ru(bpibH_2)Ru(bpy)_2]^4$	4.11, 7.84	4.34, 7.46	5	5j
$[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$	4.16, 5.07, 9.65, 12.09	4.54, 5.07, 9.76, 12.09	100	this work

[a] $pidbH_2$ = (1-[1,10]-phenanthroline[5,6-*d*]imidazo-2-yl)-4-*N*,*N*-dimethylbenzene. $mbpibH_2$ = 1,3-bis([1,10]-phenanthroline[5,6-*d*]imidazo-2-yl)benzene. $bpibH_2$ = 1,4-bis([1,10]-phenanthroline[5,6-*d*]imidazo-2-yl)benzene.

protonation equilibrium is almost certainly established between the ^{3}MLCT states.[35] If the either protonated species or its conjugate base is nonluminescent, a further approximation can be made by employing MLCT absorption maxima for ν_B and ν_{HB}.[36]

By using the emission band maxima of both protonated and deprotonated forms of the ruthenium complex studied for ν_B and ν_{HB} in eq 1, four pK_a* values of pK_{a1}* = 4.54 ± 0.01, pK_{a2}* = 5.07 ± 0.01, pK_{a3}* = 9.76 ± 0.01, and pK_{a4}* = 12.09 ± 0.01 were obtained. The acidities of various species for the dinuclear Ru(II) complex in the ground state are therefore only slightly more basic or equal to those in the excited state, showing that the excited electron was delocalized on ancillary bpy rather than $ebipcH_2$-related species.

Binding Studies with Calf Thymus DNA

Absorption Spectroscopic Studies. The interaction of the complex with calf thymus DNA was investigated by absorption spectroscopic titration of $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2](ClO_4)_4$ with the DNA at room temperature, in 5 mmol of Tris buffer at pH 7.1 at a complex concentration of 2.59 μM and calf thymus DNA added from 0 to 21 μM. As shown in Figure 3, the electronic absorption spectra of the Ru(II) complex upon titration with calf thymus DNA showed no shift and little changes in the intensities for the charge transfer band at 475 nm. However, the addition of DNA clearly yielded absorbance hypochromism of 36.6% for the UV band at 288 nm. The large hypochromism observed may support an intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pair of DNA. This result is different from observations on the interaction of DNA with some Ru(II) complexes reported[20,37] which gave simultaneous decreases in absorption for both UV and visible (MLCT) bands. The little disturbance of the MLCT band upon interaction of $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2](ClO_4)_4$ with

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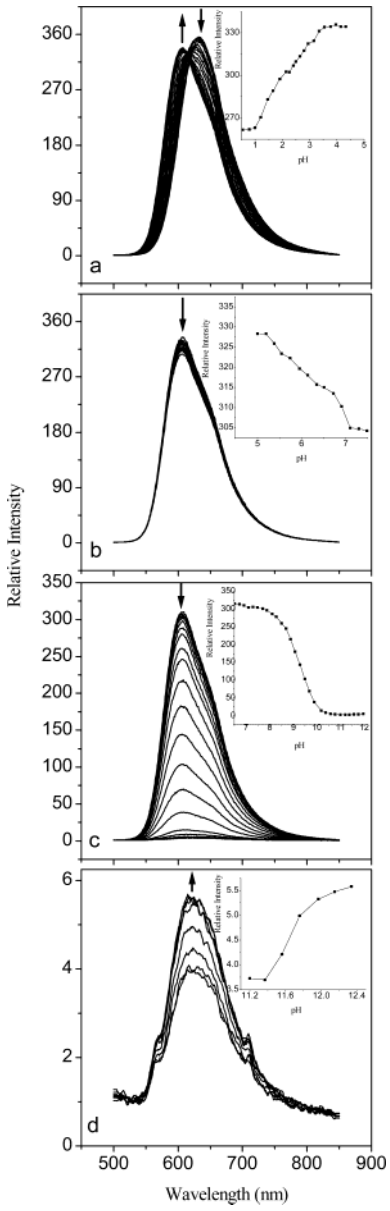

Figure 2. The changes of emission spectra of the Ru(II) complex upon raising the pH: (a) pH = 0.55−4.81; (b) pH = 4.81−7.10; (c) pH = 7.10−1120; (d) pH = 11.20−12.34.

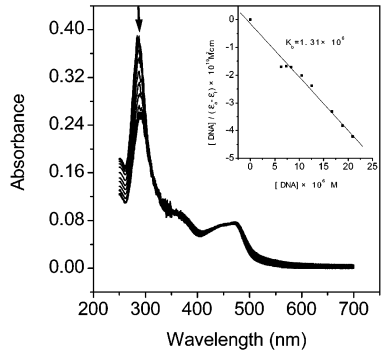

Figure 3. UV−vis spectra of the Ru(II) complex (2.59 μm), in the absence and presence of increasing amounts of DNA (0−21 μm) in buffered 50 mM NaCl,

the DNA may be due to the fact that the MLCT band is mainly Ru-to-bpy charge transfer in nature. Therefore the interaction of the dinuclear complex we studied with the

Table 2. The Comparison of the Interaction Parameters of the Ru(II) Complexes with ct-DNA in 50 mM NaCl Unless Noted Otherwise[a]

complex	hypochromism, H^b (%)	$K_b \times 10^{-4}$/ M^{-1}	ref
$[Ru(bpy)_2(ddt)]^{2+}$	9.5	2.1	39
$[Ru(bpy)_2(dpt)]^{2+}$	18.1	6.3	39
$[Ru(bpy)_2(taptp)]^{2+}$	24	17	40
Λ-$[Ru(phen)_2(dppz)]$	40	170	8c, 9c
Δ-$[Ru(phen)_2(dppz)]$	40	320	8c, 9c
$[(bpy)_2Ru(Me_2bpy)-(CH_2)_2-(bpyMe)Ru(bpy)_2]^{4+}$	10−19[c]	1.3	15c
$[(bpy)_2Ru(bdptb)Ru(bpy)_2]^{4+}$	33	76	41
$[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$	36.6	131	this work

[a] ddt = 3-(pyrazin-2-yl)-5,6-diphenyl-*as*-triazine. dpt = 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]phenanthrene. taptp = 4,5,9,18-tetraaza-phenthreno[9,10-*b*]triphenylene. bdptb = 2,2′-bis(5,6-diphenyl-1,2,4,-triazin-3-yl)-4,4′-bipyridine. Mebpy = 4-methyl-2,2′-bipyridine-4′-. [b] $H\% = 100(A_{free} - A_{bound})/A_{free}$. [c] Determined in 10 mM potassium phosphate buffer.

DNA is most probably by the mode of insertion of the bridging ligand $ebipcH_2$ moiety on the dinuclear complex between the base pair of the DNA. The interaction mode addressed above can also be inferred from the fact that the "parent" complex $[Ru(bpy)_3]^{2+}$ binds extremely weakly to double-stranded DNA.[6e−g] Hiort et al. ever deduced that the dppz in the $[Ru(phen)_2(dppz)]^{2+}$ intercalates into the DNA base pairs because the hypochromism of the intraligand transition of dppz is greater than that of MLCT.[8c]

The intrinsic binding constant, which illustrating the binding strength of the complex with ct-DNA, can be obtained by monitoring the changes in absorbance at 288 nm with increasing concentrations of DNA, according to the following equation:[38]

$$[DNA]/(\epsilon_a - \epsilon_f) = [DNA]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f) \quad (2)$$

where [DNA] is the concentration of DNA in base pairs, ϵ_a is the apparent absorption coefficient, which was obtained by calculating A_{abs}/[Ru], and ϵ_f and ϵ_b are the extinction coefficients for the free ruthenium complex and the ruthenium complex in the fully bound form, respectively. In a plot of $[DNA]/(\epsilon_a - \epsilon_f)$ versus [DNA], K_b is given by the ratio of the slope to the *y* intercept. An intrinsic binding constant of $1.31(\pm 0.08) \times 10^6$ M^{-1} was obtained in solutions containing 50 mM NaCl. The maximum hypochromicity and the intrinsic binding constant of $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$ with the DNA are compared in Table 2 with those reported for representative ruthenium complexes. The maximum hypochromicity for the Ru(II) complex in this study is close to those for DNA intercalators of Λ-$[Ru(phen)_2(dppz)]^{2+}$ and Δ-$[Ru(phen)_2(dppz)]^{2+}$,[9c] and the intrinsic binding constant is 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-$[Ru(phen)_2(dppz)]^{2+}$,[9c] and 1.25×10^6 M^{-1} for ethidium bromide[6i] as well as 0.76×10^6 M^{-1} for a dinuclear analogue $[(bpy)_2Ru(bdptb)Ru(bpy)_2]^{4+}$ in the same salt concentration.[41]

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However, the intrinsic binding constant of $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$ with the DNA is much larger than those for $[Ru(bpy)_2(ddt)]^{2+}$, $[Ru(bpy)_2(dpt)]^{2+}$, and $[Ru(bpy)_2(taptp)]^{2+}$. This could not be easily understood by simply comparing the sizes of conjugate planes of the intercalative fragments on respective complexes. Kelly reported that the dinuclear Ru(II) complexes of $[(bpy)_2Ru(Me_2bpy)-(CH_2)_n-(bpyMe)Ru(bpy)_2]^{4+}$ ($n = 5$ and 7, Mebpy = 4-methyl-2,2′-bipyridine-4′-), which have predominantly electrostatic binding to DNA, have intrinsic binding constants more than 80−160 times as large as the mononuclear analogue.[15c] A dinuclear Ru(II) complex molecular staple designed by Nordén greatly stabilize its noncovalent interaction with DNA by a threading multi-intercalation.[16b] The dinuclear complex we studied is highly charged and "hairpin"-shaped; thus both the electrostatic effect and the threading interaction may make major contributions to the large intrinsic binding constant observed. In order to evaluate the relative importance of electrostatic and nonelectrostatic contributions, studies on the dependence of the binding constants on the concentrations of Na^+ were also carried out. The binding constants were found to decrease with increasing salt concentrations due to a stoichiometric amount of counterion release that accompanies the binding to a positively charged Ru(II) complex. According to the polyelectrolyte theory developed by Record et al.,[42] the observed binding constant K is a function of the charge on the cation (Z), the fraction of counterions associated with each DNA phosphate (Ψ), and the concentration of Na^+. Ψ is generally taken to be 0.8 for double-stranded B-form DNA. A slope in a plot of log K vs log $[Na^+]$ (Figure S1, Supporting Information) is equal to **SK** in the following equation: $\mathbf{SK} = \delta \log K/\delta \log [Na^+] = -Z\Psi$. The binding free energy can be calculated from $\Delta G_{obs} = -RT \ln K_{obs}$. Electrostatic ($\Delta G_{pe}$) and nonelectrostatic ($\Delta G_t$) portions of the free energy can be calculated from $\Delta G_{pe} = \mathbf{SK}\, RT \ln [Na^+]$ and $\Delta G_t = \Delta G_{obs} - \Delta G_{pe}$, respectively.[9c] An **SK** value of −2.5 for the interaction of the dinuclear Ru(II) complex with the DNA was obtained from Figure S1, so a charge Z of 3.1 on the Ru(II) complex obtained is less than four positive charges carried by the dinuclear complex. This may be caused by the "partial" intercalation between base pair by the bridging ligand moiety of the Ru(II) complex. A nonelectrostatic free energy ΔG_t was derived to be −16.2 kJ mol^{-1} in 50 mM NaCl, which is less than −20.1 kJ mol^{-1} for Λ-$[Ru(phen)_2(dppz)]^{2+}$,[9c] −23.4 kJ mol^{-1} for Δ-$[Ru(phen)_2(dppz)]^{2+}$,[9c] more than −13.0 kJ mol^{-1} for ethidium bromide,[6i] and −14.2 kJ mol^{-1} for Λ- or Δ-$[Ru(phen)_3]^{2+}$,[6h] and much more than electrostatically dominating $[(bpy)_2Ru(Me_2bpy)-(CH_2)_7-(bpyMe)Ru(bpy)_2]^{4+}$ ($\Delta G_t = -7.53$ kJ mol^{-1}).[15c] An electrostatic free energy ΔG_{pe} of −18.1 kJ mol^{-1} obtained is comparable to the ΔG_t value (−16.2 kJ mol^{-1}), and is more than the ΔG_{pe} values for Δ-$[Ru(phen)_2(dppz)]^{2+}$ (−13.8 kJ mol^{-1}), Λ-$[Ru(phen)_2(dppz)]^{2+}$ (−15.5 kJ mol^{-1}),[9c] ethidium bromide (−10.1 kJ mol^{-1}),[6i] Λ- or Δ-$[Ru(phen)_3]^{2+}$ (−9.2 kJ mol^{-1}),[6h] and even $[(bpy)_2Ru(Me_2bpy)-(CH_2)_7-(bpyMe)-Ru(bpy)_2]^{4+}$ (−15.9 kJ mol^{-1}).[15c] It is not surprising to have observed significant electrostatic contributions to the free energy upon considering that the dinuclear Ru(II) complex is highly and densely (two Ru(II) ions are close to each other) charged relative to all the other compounds mentioned above.

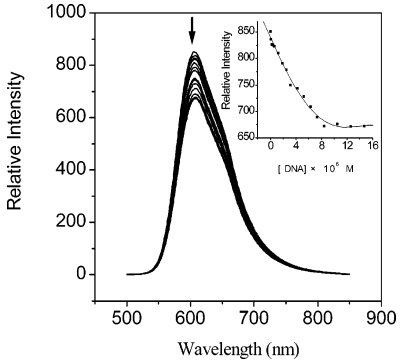

Figure 4. Emission spectra of the Ru(II) complex (2.59 μm), in the absence and presence of increasing amounts of DNA (0−15 μm) in buffered 50 mM NaCl.

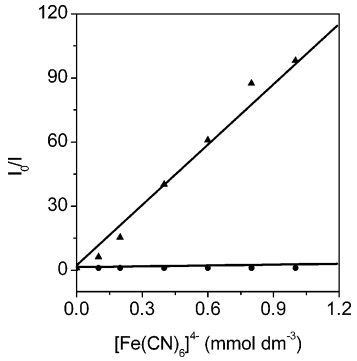

Figure 5. Emission quenching of the Ru(II) complex with increasing concentration of quencher $[Fe(CN)_6]^{4-}$ in the absence (▲) and presence (●) of DNA: [Ru] = 2.59 μM, DNA:Ru = 100:1.

Luminescence Studies. The results of the emission titration of the Ru(II) complex with DNA are illustrated in Figure 4. Upon addition of DNA the emission intensities decreased steadily by 16%. The quenching of the luminescent excited state of the Ru(II) complex is consistent with a photoelectron transfer from the guanine base of DNA to the ^{3}MLCT of the complex, as reported in the case of $[Ru(tap)_3]^{2+}$ (tap = 1,4,5,8-tetraazaphenanthrene) and $[Ru(bpz)_3]^{2+}$.[43,44]

Steady-state emission quenching experiments (Figure 5) using $[Fe(CN)_6]^{4-}$ as quencher can further support the intercalation interaction. In the absence of DNA, $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$ was efficiently quenched by the quencher, resulting in a strictly linear Stern−Volmer plot of a slope of 98, which is much larger than a slope of 2.2 for $[Ru(bpy)_2(cip)]^{2+}$ (cip = 2-(2-chlorophenyl)imidazo[4,5-*f*]-1,10-phenanthroline) and 0.86 for $[Ru(bpy)_2(pip)]^{2+}$ (pip = 2-phenylimidazo[4,5-*f*][1,10]phenanthroline).[45] In the presence of DNA the slope of the plot is remarkably decreased

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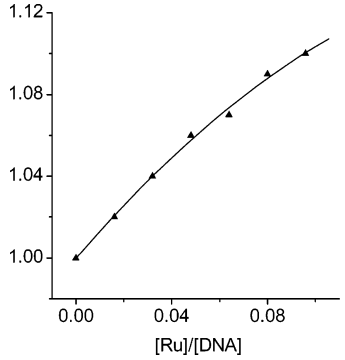

Figure 6. Effect of increasing amounts of the Ru(II) complex on the relative viscosities of calf thymus DNA in buffered 50 mM NaCl.

to near zero, which is much smaller than a slope of 0.86 for $[Ru(bpy)_2(cip)]^{2+}$ and comparable to that for $[Ru(bpy)_2(pip)]^{2+}$.[45] The ion $[Fe(CN)_6]^{4-}$ has been shown to be able to distinguish differently bound ruthenium(II) species.[46] Positively charged free complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$, when the complex bound to DNA can be protected from the quencher because of the high repulsion between the highly anionic $[Fe(CN)_6]^{4-}$ and the negative DNA phosphate backbone, hindering quenching of the emission of the bound complex. The slope can therefore be taken as a measure of binding affinity. A larger slope value corresponds to poorer protection and weaker binding to DNA. So $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$ bound tightly to the DNA.

Viscosity Measurements. 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.[9a,b] For further clarification of the interaction between the complex and DNA, viscosity measurement was carried out. 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 nonclassical intercalation of ligand could bend (or kink) the DNA helix, reducing its effective length and concomitantly its viscosity.[9a,b] The effects of the complex on the viscosity of ct-DNA are shown in Figure 6. The viscosity of DNA increased dramatically upon addition of the complex and nearly linearly at low complex concentrations. The slope is about 1.05, which is more than that of ethidium (3.8-diamino-5-ethyl-6-phenanthridium) (0.91).[9a] The result strongly indicated that the complex intercalated into DNA base pairs deeply.

Conclusions

A newly synthesized Ru(II) complex was demonstrated to be a sensitive pH emission switch with a maximum on−off ratio of ∼100. The large on−off ratio achieved over a narrow pH range of pH 8.0−10.0 by deprotonation of uncoordinated imdazole rings is unusual in comparison with other imidazole-containing Ru(II) complex congeners. Photophysical and viscosity measurements strongly supported intercalation of $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2]^{4+}$ into DNA efficiently. The interesting observation of significant hypochromism on the UV absorption with MLCT absorption almost undisturbed in the presence of ct-DNA makes a sharp contrast to many DNA intercalators reported, and may signify a different binding conformaton of insertion of the bridging moiety between the base pairs of the DNA. Further studies on tuning emission switches for biophysical pH response and DNA binding mechanism are in progress.

Acknowledgment. This project was supported by NNSFC (20071004 and 20371008), Beijing Talents Training Program and Scientific Research Foundation for the Returned Overseas Chinese Scholars (State Education Ministry, to K.W.).

Supporting Information Available: Figure S1 showing the dependence of intrinsic binding constants of the dinuclear Ru(II) complex with the DNA on $[Na^+]$. This material is available free of charge via the Internet at http://pubs.acs.org.

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