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# Synthesis and the Effect of a Peripheral *N*-Arylcarbazole Moiety on the Acid-Base and DNA Binding Properties of a Novel $Ru^{II}$ Complex

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A novel $Ru^{II}$ complex of $[Ru(bpy)_2(cpipH)](ClO_4)_2$ (where bpy = 2,2′-bipyridine, cpipH = 2-[4-(9*H*-carbazol-9-yl)phenyl]-1*H*-imidazol[4,5-*f*][1,10]phenanthroline) was synthesized. The binding of the complex to calf thymus DNA was investigated with UV/Vis absorption and luminescence titrations, steady-state emission quenching by $[Fe(CN)_6]^{4-}$, DNA competitive binding with ethidium bromide, and thermal denaturation. The pH effects on the UV/Vis absorption and emission spectra of the complex were also studied, and the ground- and excited-state ionization constants were derived. The results indicated that the complex intercalatively bound to the DNA, with an intrinsic binding constant of $(8.2 \pm 0.8) \times 10^5\ M^{-1}$ in buffered 50 mM NaCl, is stronger than the parent complex, $[Ru(bpy)_2(pip)]^{2+}$ (pip = 2-phenylimidazo[4,5-*f*][1,10]phenanthroline), and its excited states are 0.7–0.8 p$K_a$ units more basic than those of the ground states.


## Introduction

Over the past two decades ruthenium(II) polypyridyl complexes have attracted much attention with regard to DNA interaction studies because of their potential applications as nonradioactive structural probes of nucleic acids, in DNA cleaving, and as chemotherapeutic agents.[1,2] The results have shown that the subtle changes in the molecular structures of $Ru^{II}$ complexes might bring about substantial effects on binding modes, sites, and affinities, and provide a chance to explore valuable information on conformation- or site-specific DNA probes. The various factors of ligand planarity, the charge carried on the complex, enantioselectivity, the formation of intramolecular hydrogen bonding, and the hydrophobicity of ancillary ligands, etc., have been effectively explored, but the new $Ru^{II}$ complexes are still needed to establish the DNA binding property-structure relationships.[1,2] On the other hand, the $Ru^{II}$ complexes that append protonatable/deprotonatable groups, e.g. hydroxy, carboxyl and amino groups may respond sensitively to the changes in environmental pH, providing a chance to make pH sensing or switching molecular devices,[3] and to greatly modulate their biological functionalities.[4] The ruthenium(II) complexes containing imidazole groups have been well studied with respect to their interaction with DNA[5–8] or reversible acid-base interconversion.[6a,9–12] However, studies of the $Ru^{II}$ complexes with both the DNA-binding and acid-base interconversion properties remain relatively scarce. We have been interested in the syntheses and studies of the acid-base and DNA binding properties of both mononuclear and dinuclear imidazole-containing $Ru^{II}$ complexes.[6,13]

In this paper we wish to report on a novel $Ru^{II}$ complex, $[Ru(bpy)_2(cpipH)](ClO_4)_2$, in which cpipH was synthesized by grafting an *N*-substituted carbazole group to 2-phenylimidazo[4,5-*f*][1,10]phenanthroline (pip). Attention was paid to the effects of the peripheral carbazole group on the acid-base and DNA binding properties of this complex by comparison with the parent complex, $[Ru(bpy)_2(pip)]^{2+}$. 2,2′-Bipyridine (bpy) was chosen as the ancillary ligand for the complex since the "parent" complex $[Ru(bpy)_3]^{2+}$ was reported to bind extremely weakly to double-stranded DNA.[14] Hence, it is reasonable for us to examine the "inspected" ligand on the DNA binding properties of the complex. We demonstrate here the interesting findings regarding this novel $Ru^{II}$ complex, $[Ru(bpy)_2(cpipH)]^{2+}$, intercalated to the DNA with a binding constant of $(8.2 \pm 0.8) \times 10^5\ M^{-1}$ in buffered 50 mM NaCl, which is greater than that for $[Ru(bpy)_2(pip)]^{2+}$. The excited states of this new complex are 0.7–0.8 p$K_a$ units more basic than those of the ground states.

## Results and Discussion

### Synthesis and Common Spectral Characteristics

The synthetic route to the complex is summarized in Figure 1. CpipH was synthesized by coupling 1,10-phenanthroline-5,6-dione with 4-(carbazol-9-yl)benzaldehyde ac-

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cording to protocols reported by Steck and Day.[15] The complex $[Ru(bpy)_2(cpipH)](ClO_4)_2$ was synthesized by direct reaction of $Ru(bpy)_2Cl_2{\cdot}2H_2O$ with cpipH in EtOH/$H_2O$ (4:1, v/v), and purified by column chromatography on silica gel with $CH_3CN/H_2O$/saturated aqueous $KNO_3$ (400:7:1, v/v/v) as eluent. The complex was obtained in satisfactory purity, which was verified by elemental analysis and $^1H$ NMR spectroscopy.

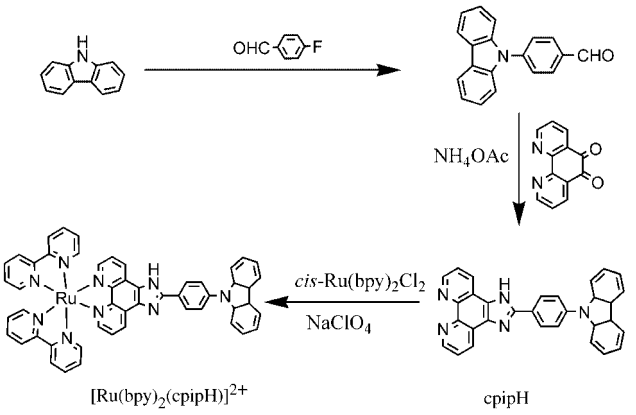


Figure 1. The synthetic route to the $Ru^{II}$ complex.

The complex, in aqueous solution, showed the lowest-energy metal-ligand charge transfer (MLCT) transition absorption band at 462 nm. The energy in the MLCT band increased in the order: $[Ru(bpy)_2(cpipH)]^{2+}$ (462 nm) < $[Ru(bpy)_2(pip)]^{2+}$ (458 nm) < $[Ru(bpy)_2(ip)]^{2+}$(455 nm).[5a] The high-energy absorption bands at 285 and 338 nm are attributed to the intraligand (IL) π-π* transition by comparison with those of $[Ru(bpy)_3]^{2+}$.[16] The order of energies in the IL bands parallels that of the MLCT bands: $[Ru(bpy)_2(cpipH)]^{2+}$ (285 nm) < $[Ru(bpy)_2(pip)]^{2+}$ (283 nm) < $[Ru(bpy)_2(ip)]^{2+}$(280 nm), indicating enhanced π delocalization upon grafting carbazole to $[Ru(bpy)_2(pip)]^{2+}$. The complex, in TRIS buffer at room temperature, was strongly emitting under visible light excitation at 465 nm with emission maxima occurring at 608 nm, which is characteristic of MLCT luminescence,[17] and is assigned to the $^3$MLCT [dπ(Ru) → π* (ligand)] state. An emission quantum yield for the complex in aerated $H_2O$ was determined to be 0.0497 by comparison with $[Ru(bpy)_3]^{2+}$ ($\varphi_{std}$ = 0.033) in aerated aqueous solution.[18,19] The MLCT emission maxima were bathochromically shifted on going from $[Ru(bpy)_2(cpipH)]^{2+}$ to $[Ru(bpy)_2(ip)]^{2+}$: $[Ru(bpy)_2(cpipH)]^{2+}$ ($\lambda_{em}$ = 608 nm) < $[Ru(bpy)_2(pip)]^{2+}$ ($\lambda_{em}$ = 615 nm) < $[Ru(bpy)_2(ip)]^{2+}$($\lambda_{em}$ = 625 nm).[5a]

## DNA-Binding Studies

### *UVIVis Spectra*

The electronic absorption spectra traces of the complex that was titrated with DNA are given in Figure 2. With increasing DNA concentrations, the hypochromisms for the bands at 285, 338 and 462 nm were found to be 54.2%, 37.3% and 23.7%, respectively, and the bathochromisms were found to be 4, 2 and 6 nm, respectively. The hypochromism of 23.7% for the MLCT band for the complex is larger than 15.5% for $[Ru(bpy)_2(ip)]^{2+}$ and 21.9% for $[Ru(bpy)_2(pip)]^{2+}$.[5a] The hypochromism observed for the IL transition of 54.2% (285 nm) for our complex appreciably prevailed over the 3.8% (280 nm) observed for $[Ru(bpy)_2(ip)]^{2+}$ and 20% (283 nm) for $[Ru(bpy)_2(pip)]^{2+}$.[5a] The spectroscopic changes suggest that the complex has a stronger interaction with DNA than $[Ru(bpy)_2(ip)]^{2+}$ and $[Ru(bpy)_2(pip)]^{2+}$.

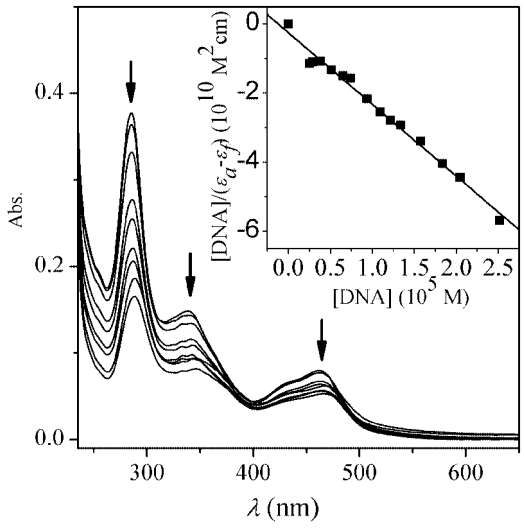


Figure 2. UV/Vis absorption spectra of the $Ru^{II}$ complex (4.6 μM), in the absence and presence of increasing concentrations of ct-DNA (0–25 μM).

To further illustrate the binding strength of the complex quantitatively, the intrinsic DNA binding constant of the complex was determined by monitoring the changes in absorbance at 285 nm with increasing concentrations of DNA, according to Equation (1),[20] where [DNA] is the concentration of DNA in base pairs; the apparent absorption coefficients $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ correspond to the extinction coefficients at a given DNA concentration, the free complex in solution and the complex that is fully bound to DNA, respectively; and $K_b$ is given by the ratio of the slope to the intercept.

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

An intrinsic binding constant was derived to be $(8.2 \pm 0.8) \times 10^5$ M$^{-1}$ for $[Ru(bpy)_2(cpipH)]^{2+}$, and ca. twice that for $[Ru(bpy)_2(pip)]^{2+}$ ($4.7 \times 10^5$ M$^{-1}$).[20] The hypochromism ($H$%) and the intrinsic DNA binding constant of $[Ru(bpy)_2(cpipH)]^{2+}$ are compared with those reported for representative DNA intercalators in Table 1. The $K_b$ value of the complex is larger than those for most of the DNA intercalators collected in Table 1, while smaller than those for the strong DNA intercalators of Δ-$[Ru(phen)(dppz)]^{2+}$ ($3.2 \times 10^6$ M$^{-1}$), Λ-$[Ru(phen)(dppz)]^{2+}$ ($1.7 \times 10^6$ M$^{-1}$)[21] and $[Ru(bpy)_2(ebipcH_2)(bpy)_2Ru]^{2+}$ ($1.3 \times 10^6$ M$^{-1}$).[6a] The $H$% value listed in Table 1 for $[Ru(bpy)_2(cpipH)]^{2+}$ is even larger than those for the typical DNA intercalators mentioned above. The large hypochromism and clear red shifts, as well

  

Table 1. A comparison of DNA binding data for $[Ru(bpy)_2(cpipH)]^{2+}$ with those for analogous Ru(II) complexes.

| Complex[a] | Hypochromism[b] $H^b$/% ($\lambda_{max}$/nm) | $K_b \times 10^5$ [$M^{-1}$] | $R$[c] | Ref. |
|---|---|---|---|---|
| $[Ru(bpy)_2(ip)]^{2+}$ | 15.5 (455) | – | 0.25 | [5a] |
| $[Ru(bpy)_2(pip)]^{2+}$ | 21.9 (458) | 4.7 | 0.051 | [5a,20a] |
| $[Ru(bpy)_2(taptp)]^{2+}$ | 34.8 (248) | 1.7 | ≈0 | [20a] |
| $[Ru(bpy)_2(ipbp)]^{2+}$ | 17.4 (458) | 0.42 | 3.43 | [20b] |
| $[Ru(bpy)_2(cpipH)]^{2+}$ | 54.2 (285) | 8.2 | 0.0093 | this work |
| $[Ru(bpy)_2(ebipcH_2)(bpy)_2Ru]^{2+}$ | 36.6 (288) | 13.1 | ≈0 | [15] |
| $\Delta$-$[Ru(phen)_2(dppz)]^{2+}$ | 32.1 (372) | 32 | – | [21] |
| $\Lambda$-$[Ru(phen)_2(dppz)]^{2+}$ | 29.8 (372) | 17 | – | [21] |

[a] ip = imidazo[4,5-*f*][1,10]phenanthroline; pip = 2-phenylimidazo[4,5-*f*][1,10]phenanthroline; taptp = 4,5,9,18-tetraazaphenanthreno[9,10-*b*]triphenylene; ipbp = 3-(1*H*-imidazo[4,5-*f*][1,10] phenanthrolin-2-yl)-4*H*-1-benzopyran-4-one; $ebipcH_2$ = *N*-ethyl-4,7-bis([1,10]phenanthroline[5,6-*f*]imidazo-2-yl)carbazole; dppz = dipyrido[3,2-*a*:2′,3′-*c*]-phenazine. [b] The maximum hypochromism selected from the UV/Vis absorption peaks. [c] *R* is the ratio of the Stern–Volmer quenching constant by using $[Fe(CN)_6]^{4-}$ as the quencher, and obtained from the ratio of the presence of DNA to that obtained in the absence of DNA.

as a relatively large $K_b$ value observed for the interaction of the $Ru^{II}$ complex with the DNA, indicate that complex $[Ru(bpy)_2(cpipH)]^{2+}$ might bind to the DNA by intercalation.

### *Luminescence Studies*

Steady-state emission quenching experiments for the complex, using $[Fe(CN)_6]^{4-}$ as the quencher, further support the intercalation interaction. As illustrated in Figure 3, in the absence of DNA, the emission of $[Ru(bpy)_2(cpipH)]^{2+}$ is efficiently quenched by $[Fe(CN)_6]^{4-}$, resulting in a slope of 92.6 for a linear Stern–Volmer plot, but in the presence of DNA the slope of the quenching plot decreases remarkably to 0.49. The positively charged free complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$, but when bound to DNA, the complex can be protected from the quencher because the highly negatively charged $[Fe(CN)_6]^{4-}$ would be repelled by the negative DNA phosphate backbone, hindering quenching of the emission of the bound complex. A ratio (*R*) of Stern–Volmer quenching constants, using $[Fe(CN)_6]^{4-}$ and obtained from the ratio of the presence of DNA to that of the absence of DNA, can be taken as a measure of the binding affinity. A large *R* value corresponds to a poorer protection and a weaker binding to DNA. As anticipitated, an *R* value of 0.0093 found for the complex we studied in this paper, is smaller than a value 0.051 reported for $[Ru(bpy)_2(pip)]^{2+}$, and 0.25 for $[Ru(bpy)_2(ip)]^{2+}$,[5a] providing further evidence that the binding affinity to the DNA is in the order of $[Ru(bpy)_2(cpipH)]^{2+} > [Ru(bpy)_2(pip)]^{2+} > [Ru(bpy)_2(ip)]^{2+}$,[5a] and the interaction mode between the complex and the DNA is intercalative.

The fluorescence of ethidium bromide (EB) itself in aqueous solution is very weak, but when it intercalates between adjacent DNA base pairs of double-stranded DNA ($K_b = 1.4 \times 10^6$ $M^{-1}$),[22] the fluorescence is greatly enhanced. If a second DNA intercalator is added to the EB–DNA system, it will compete with EB for the DNA binding site, and the fluorescence of the EB–DNA system will be quenched.[23,24] So, the quenching extent of fluorescence of the EB–DNA system can reflect the DNA binding strength of the second molecule. Otherwise, if the second added molecule is not a DNA intercalator, its addition will not

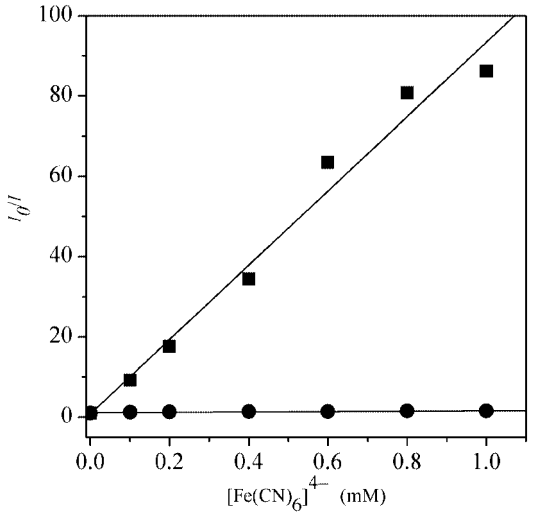


Figure 3. Emission quenching of the $Ru^{II}$ complex with increasing concentrations of $[Fe(CN)_6]^{4-}$ in the absence (■) and presence (●) of ct-DNA:[Ru] = 4.6 μM, [DNA]/[Ru] = 9.

cause the evident reduction in emission intensities of the EB–DNA system. The changes in the emission spectra of the EB–DNA system in the absence and presence of the complex are shown in Figure 4. The emission of the EB–DNA system was efficiently quenched by the complex, resulting in a linear Stern–Volmer plot according to Equation (2), where $I_0$ and $I$ represent the fluorescence intensities in the absence and presence of complex, respectively; $K$ is a linear Stern–Volmer quenching constant dependent on the ratio of the bound concentration of EB to the concentration of DNA; and $r$ is the ratio of the total concentration of the complex to that of DNA ([Ru]/[DNA]).

$$I_0/I = 1 + Kr \quad (2)$$

As shown in the inset of Figure 4, the Stern–Volmer plot has of a slope of $K = 6.17$, which is larger than 2.72 for $[Ru(dmp)_2(obpip)]^{2+}$ (dmp = 2,9-dimethyl-1,10-phenanthroline; obpip = 2-(2-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline), and 5.98 for $[Ru(dmp)_2(pbip)]^{2+}$(pbip =2-(4-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline),[25] but smaller than a *K* value of 19.3 for [Ru(bpy)-

  

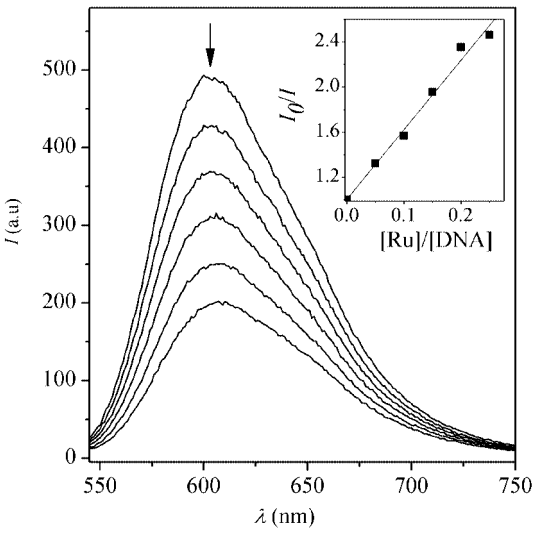


Figure 4. Emission quenching of the EB–DNA system with increasing concentration of the $Ru^{II}$ complex. [EB] = 20 μM, [DNA] = 100 μM, [Ru]:[DNA] = 0–0.25.

$(pp[2,3]p)_2]^{2+}$ (bpy = 2, 2′-bipyridine, pp[2,3]p = pyrido[2′,3′:5,6]pyrazino[2,3-*f*][1,10]phenanthroline), and 31.2 for $[Ru(phen)(pp[2,3]p)_2]^{2+}$ (phen = 1,10-phenanthroline).[13a] From the data in Figure 4, we also know that 50% of EB was replaced by $[Ru(bpy)_2(cpipH)]^{2+}$ from the DNA bound EB at a concentration ratio of [Ru]/[EB] ≈ 0.8. Hence, an apparent DNA binding constant of $1.8 \times 10^6$ M$^{-1}$ was derived [$K_b$(EB)/0.8].

### *Thermal Denaturation Studies*

The thermal melting study of the DNA is a technique used to evaluate the stability of a DNA double helix. When a complex intercalates into DNA base pairs, the base stacking will be more stable and hence the melting temperature of the double-stranded DNA will rise. So the DNA melting temperature ($T_m$) is useful in establishing the extent of intercalation.[26] The melting curves of ct-DNA in the absence and presence of the complex are presented in Figure 5. $T_m$ of ct-DNA was found to be 66.3 °C. In the presence of the complex at a concentration ratio [Ru]/[DNA] = 1:10, $T_m$ of ct-DNA was raised to 73.5 °C. The large increase of 7.2 °C in $T_m$ is comparable to that observed for a classical DNA intercalator.[22–29]

The DNA intrinsic binding constant at 73.5 °C can be obtained from the McGhee equation [Equation (3)], where $T_m^0$ is the melting temperature of ct-DNA alone, $T_m$ is the melting temperature in the presence of the $Ru^{II}$ complex, $\Delta H$ is the enthalpy of DNA (per base pair), $R$ is the gas constant, $K$ is the DNA binding constant at $T_m$, $L$ is the free complex concentration (approximated at the $T_m$ by the total complex concentration), and $n$ is the size of the binding site.

$$1/T_m^0 - 1/T_m = (R/\Delta H)\ln(1 + KL)^{1/n} \quad (3)$$

For the ct-DNA used in these studies, under identical solution conditions, a melting enthalpy of 6.9 kcal mol$^{-1}$ was determined by differential scanning calorimetry.[30]

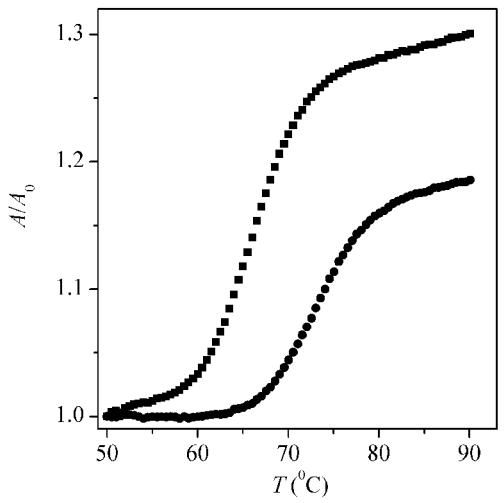


Figure 5. Melting curves of ct-DNA in the absence (■) and presence (●) of the $Ru^{II}$ complex. [Ru] = 5.8 μM, [DNA] = 58 μM.

And on the basis of the neighbor exclusion principle the $n$ value for the complex was assumed to be 2.0 base pairs. As a result $K$ was calculated to be $9.14 \times 10^4$ M$^{-1}$ at 73.5 °C. As the complex binds to ct-DNA, the changes in standard enthalpy, standard entropy and standard free energy can be determined according to Equations (4), (5), and (6), where $K_1$ and $K_2$ are the DNA intrinsic binding constants of the complex at temperature $T_1$ and $T_2$, respectively; $\Delta G°$, $\Delta H°$, and $\Delta S°$ are the corresponding standard free energy change, standard enthalpy change and standard entropy change, respectively.

$$\ln(K_1/K_2) = (\Delta H°/R)(T_1 - T_2)/T_1T_2 \quad (4)$$

$$\Delta G_T^\circ = -RT\ln K \quad (5)$$

$$\Delta G_T^\circ = \Delta H° - T\Delta S° \quad (6)$$

By substituting $K_1 = 8.21 \times 10^5$ M$^{-1}$ ($T_1$ = 298 K) and $K_2 = 9.14 \times 10^4$ M$^{-1}$ ($T_2$ = 346.5 $K$) into Equations (4)–(6), the values of $\Delta H°$, $\Delta G_{298}^\circ$ and $\Delta S°$ were found to be –38.9 kJ mol$^{-1}$, –33.8 kJ mol$^{-1}$ and –17.1 J mol$^{-1}$ K$^{-1}$ at 25 °C, respectively. The negative $\Delta G°$ value suggests that the energy of the adduct is lower than the sum of the energies of the free complex and DNA. The negative $\Delta H°$ suggests that the binding of the complex to DNA at 25 °C is exothermic and enthalpically driven.

## Spectrophotometric pH Titrations

### *UV/Vis Absorption Spectra*

As shown in Figure 6, $[Ru(bpy)_2(cpipH)]^{2+}$ underwent two successive deprotonation processes upon increasing the pH from –0.08 to 11.23. When the pH was increased from 0.10 to 3.73, the bands at 286 and 342 nm increased in intensity and an isosbestic point appeared at 360 nm. These spectral changes resulted from the dissociation of the proton on the protonated imidazole ring. The second depro-

  

tonation step, which takes place over the pH range of 7.00–10.45, is assigned to the deprotonation of the proton on the neutral imidazole ring, accompanying the following spectral features: the band at 342 nm and the wave valley at 490 nm increase, and the band at 286 nm is red-shifted from 286 nm to 289 nm without any changes in the intensities. The changes in absorbance at the fixed wavelengths as a function of pH are shown in the insets of Figure 6. Two ground-state ionization constants of $pK_{a1} = 0.31 \pm 0.06$, $pK_{a2} = 8.24 \pm 0.03$ were obtained by nonlinear sigmoidal fits of the data, shown in the insets of Figure 6(a) and Figure 6(b). As compared with the two ionization constants of the protonated imidazole ring on some representative $Ru^{II}$ analog complexes, the complex we studied in this paper is more acidic than $[Ru(bpy)_2(ip)]^{2+}$ ($pK_{ai}$ = 1.97, 10.46) and $[Ru(bpy)_2(ebipcH_2)(bpy)_2Ru]^{2+}$ {$ebipcH_2$ = *N*-ethyl-4,7-bis([1,10]phenanthroline[5,6-*f*]imidazo-2-yl)-carbazole} ($pK_{ai}$ = 4.16, 9.65).[6a,10]

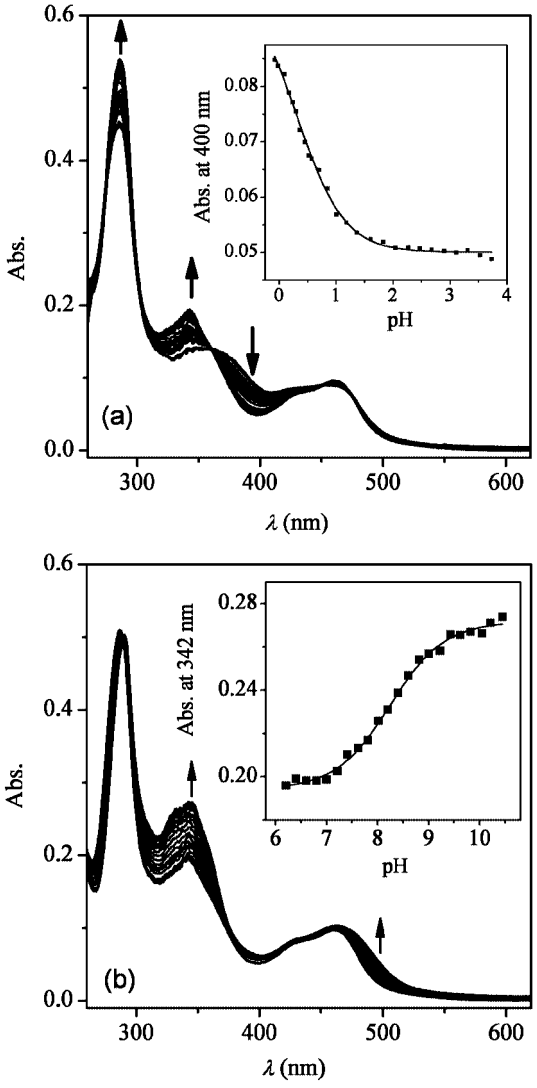


Figure 6. The changes of electronic absorption spectra of the $Ru^{II}$ complex upon raising the pH: (a) pH = –0.08 to 3.73; (b) pH = 6.21 to 10.45.

### *Luminescence Spectroscopic Studies*

As shown in Figure 7, the emission spectra of the complex are sensitive to pH. When the pH is increased from 0.10 to 3.31, the emission maxima are blue-shifted from 628 to 613 nm and the intensities decrease by about 13.9%. As the pH increases from 6.21 to 10.45, the emission maxima become red-shifted from 610 to 622 nm, and the intensities decrease by about 38.1%. The insets of Figure 7, which are the changes of relative emission intensities vs. pH, clearly show that the profiles consist of two sigmoidal curves of opposite gradients, indicative of two deprotonation processes. This is the same result as that described in the section on UV/Vis absorption spectral titrations. The protonation/deprotonation processes are summarized in Figure 8.

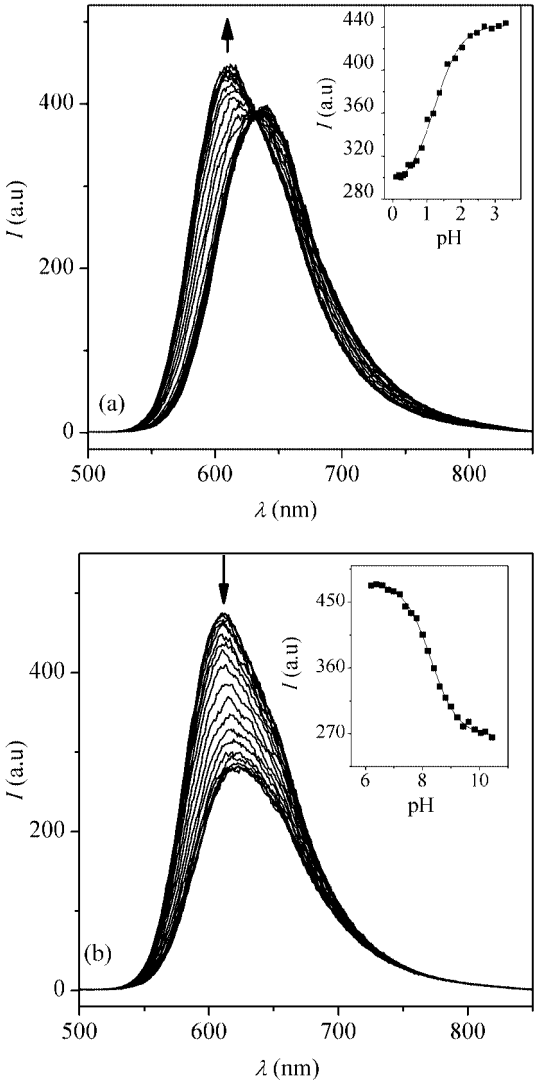


Figure 7. The changes in the emission spectra of the $Ru^{II}$ complex upon raising the pH: (a) pH = 0.10–3.31; (b) pH = 6.21–10.45.

Excited-state ionization constants, $pK_a^*$, could be roughly evaluated on the basis of the Förster cycle,[3] which thermodynamically correlates $pK_a^*$ with $pK_a$ according to

  

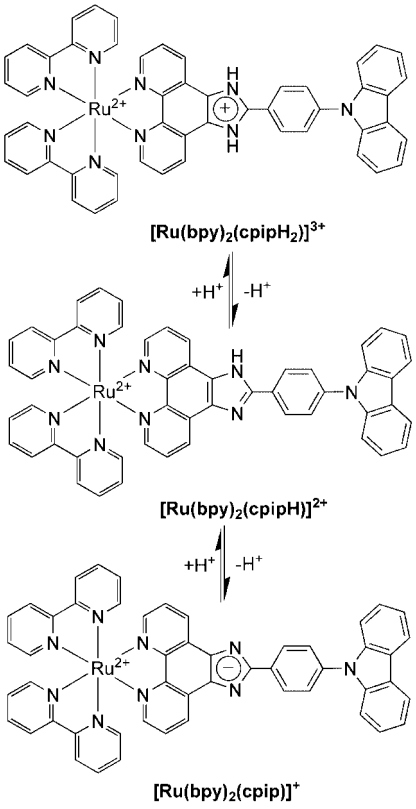


Figure 8. The acid–base equilibria of the $Ru^{II}$ complex.

Equation (7), where $\nu_B$ and $\nu_{HB}$ are pure 0–0 transitions in $cm^{-1}$ for the basic and acidic species, respectively.

$$pK_a^* = pK_a + (0.625/T)(\nu_B - \nu_{HB}) \quad (7)$$

In practice, $\nu_B$ and $\nu_{HB}$ are often difficult or even impossible to obtain. A good approximation is obtained by using the emission maxima for $\nu_B$ and $\nu_{HB}$, since protonation equilibrium is almost certainly established between the $^3$MLCT states.[18]

By using the emission band maxima from both the protonated and deprotonated forms of the ruthenium complex for $\nu_B$ and $\nu_{HB}$ in Equation (7), two $pK_a^*$ values of $pK_{a1}^* = 1.1 \pm 0.1$ and $pK_{a2}^* = 8.9 \pm 0.1$, were obtained. The excited state $pK_a^*$ values are 0.7–0.8 $pK_a$ units more basic than the respective ground-state ones, indicating that the excited electron was delocalized on the cpipH moiety rather than on that of the bpy moiety.

## Conclusions

In summary, a newly synthesized $Ru^{II}$ complex of $[Ru(bpy)_2(cpipH)](ClO_4)_2$ was demonstrated to be a DNA intercalator by evident hypochromism and clear bathochromic shifts of the band at 286 nm, and protection from emission quenching by $[Fe(CN)_6]^{4-}$ upon binding to the DNA, as well as comparable competitive binding to the DNA with the proven DNA intercalator EB and the large increase of 7.2 °C in the melting temperature of the double-stranded DNA at a concentration ratio [Ru]/[DNA] = 1:10. Its interesting DNA-binding and pH responsive spectroscopic properties make it attractive for applications involving molecular optical devices.

## Experimental Section

**Physical Measurements:** Elemental analyses were performed with a Vario EL elemental analyzer. Infrared spectra were measured with a Nicolet–Avatar 360 FT-IR spectrometer using KBr disks. $^1$H NMR spectra were obtained with a Bruker DRX-500 spectrometer. UV/Vis absorption spectra were determined with a GBC Cintra 10e UV/Vis spectrophotometer. Emission spectra were recorded with a Shimadzu RF-5301PC spectrofluorimeter. The interaction of the complex with DNA was conducted in buffer A (5 mM TRIS, 50 mM NaCl, pH = 7.1). A solution of ct-DNA gave ratios of UV absorbance at 260 and 280 nm of about 1.8–1.9:1 indicating that the DNA was sufficiently free of protein. The DNA per nucleotide was determined spectrophotometrically by assuming $\varepsilon_{260}$ = 6600 $M^{-1}cm^{-1}$. Thermal denaturation experiments of the DNA were performed on a GBC Cintra 10e UV/Vis spectrophotometer in Buffer B (1.5 mM $Na_2HPO_4$, 0.5 mM $NaH_2PO_4$, 1 mM $Na_2EDTA$). Using the thermal melting program, the temperature of the cell containing the cuvette was increased from 50 °C to 90 °C and the absorbance at 260 nm was measured every 0.5 °C. The data were smoothed at 0.5 °C intervals with a filter of 5, and the derivative of the resulting curve was taken at a data interval of 0.5 °C. The pH effects on the UV/Vis and emission spectra of the complex were carried out in DMF/Britton–Roberson buffer (4 mM $H_3BO_3$, 4 mM $H_3PO_4$, 4 mM $CH_3COOH$) (1:1, v/v).

**Materials:** 4-(Carbazol-9-yl)benzaldehyde,[31] 1,10-phenanthroline-5,6-dione[32] and *cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$[33] were prepared according to literature methods.

**2-[4-(9*H*-Carbazol-9-yl)phenyl]-1*H*-imidazol[4,5-*f*][1,10]phenanthroline (cpipH):**[34] A solution of 1,10-phenanthroline-5,6-dione (0.21 g, 1 mmol), 4-(carbazol-9-yl)benzaldehyde (0.27 g, 1 mmol), and ammonium acetate (1.62 g, 21 mmol) dissolved in acetic acid (30 mL) was refluxed at 110 °C for 5 h under nitrogen. The solution was cooled to room temperature, and $H_2O$ (60 mL) was added. The suspension was then neutralized with concentrated aqueous ammonia. The yellow precipitate that formed was washed with water, dichloromethane and ethanol, and then dried at 100 °C in vacuo. Yield 0.185 g (40%). IR (KBr): $\tilde{\nu}_{max}$ = 3435 (s), 1610 (m), 1525 (m), 1482 (m), 1452 (s), 1352 (m), 739 (s), 720 (m) $cm^{-1}$. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 13.95 (s, 1 H), 9.07 (s, 2 H), 8.98 (d, $J$ = 7.7 Hz, 2 H), 8.59 (d, $J$ = 8.4 Hz, 2 H), 8.30 (d, $J$ = 7.8 Hz, 2 H), 7.94 (d, $J$ = 8.4 Hz, 2 H), 7.88 (m, 2 H), 7.56 (d, $J$ = 8.2 Hz, 2 H), 7.50 (t, $J$ = 7.3 Hz, 2 H), 7.35 (t, $J$ = 7.2 Hz, 2 H) ppm. $C_{31}H_{19}N_5 \cdot 0.2C_2H_5OH$ (470.7): calcd. C 80.13, H 4.29, N 14.87; found C 81.90, H 4.61, N 14.63.

**Caution!** *All the perchlorate salts are potentially explosive and therefore should be handled in small quantities with care.*

**[Ru(bpy)$_2$(cpipH)](ClO$_4$)$_2$:** A mixture of *cis*-$[Ru(bpy)_2Cl_2] \cdot 2H_2O$ (0.052 g, 0.1 mmol), $cpipH \cdot 0.2C_2H_5OH$ (0.046 g, 0.98 mmol), ethanol (8 mL), and $H_2O$ (2 mL) was refluxed at 100 °C for 9 h under nitrogen. The solution was cooled to room temperature and was filtered to remove the insoluble substance. An orange precipitate was obtained by dropwise addition of a fourfold excessive of aqueous $NaClO_4$ solution. The product was purified by column chromatography on silica gel with $CH_3CN/H_2O$/saturated aqueous $KNO_3$ (400:7:1, v/v/v) as eluent followed by reprecipitation with aqueous $NaClO_4$ solution. Red crystals were obtained. Yield

  

0.064 g (60%). $C_{51}H_{35}Cl_2N_9O_8Ru$ (1073.9): calcd. C 57.04, H 3.28, N 11.92; found C 56.60, H 3.55, N 11.86. IR (KBr): $\tilde{\nu}_{max}$ = 3431 (s), 1624 (m), 1603 (m), 1479 (m), 1447 (s), 1359 (w), 1121 (s), 1090 (s), 754 (m), 726 (m), 623 (s) $cm^{-1}$. $^1H$ NMR ([D$_6$]DMSO): $\delta$ = 14.55 (s, 1 H), 9.15 (t, $J$ = 9.1 Hz, 2 H), 8.90 (d, $J$ = 8.2 Hz, 2 H), 8.87 (d, $J$ = 8.2 Hz, 2 H), 8.62 (d, $J$ = 8.4 Hz, 2 H), 8.32 (d, $J$ = 7.8 Hz, 2 H), 8.24 (t, $J$ = 7.9 Hz, 2 H), 8.13 (t, $J$ = 7.8 Hz, 2 H) 8.11 (d, $J$ = 5.2 Hz, 2 H), 8.02 (d, $J$ = 8.4 Hz, 2 H), 7.97 (m, 2 H), 7.87 (d, $J$ = 5.4 Hz, 2 H), 7.66 (s, 2 H), 7.60 (m, 4 H), 7.51 (t, $J$ = 7.6 Hz, 2 H), 7.37 (m, 4 H) ppm.

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