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$[Ru(dipn)(tpy)](ClO_4)_2$ 的合成、表征、DNA 结合性质及与系列化合物之比较

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摘要:合成了 1 个含脂肪多胺的钌配合物$[Ru(dipn)(tpy)](ClO_4)_2$ (**1**)(tpy 和 dipn 分别代表 2,2′:6′2″-三联吡啶和二丙三胺),用元素分析、电喷雾质谱、紫外-可见光谱及循环伏安进行了表征。采用电子吸收光谱滴定、EB 竞争荧光光谱及粘度实验研究了该化合物与小牛胸腺 DNA 作用的性质,并与其系列化合物$[Ru(dipn)(dptp)](ClO_4)_2$ (**2**)和$[Ru(dipn)(pat)](ClO_4)_2$ (**3**)(dptp=5,6-二苯基-3-(2-邻菲咯啉基)-1,2,4-三嗪;pat=3-(2-邻菲咯啉基)-1,2,4-三嗪并[5,6-f]苊)作了对比。结果表明,3 个化合物 **1**、**2**、**3** 分别以静电吸引、部分插入及插入作用的模式与 DNA 作用,与 DNA 的亲和力的相对大小为:**1**<**2**<**3**。

关键词:钌配合物; DNA 作用; 多吡啶; 脂肪多胺
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Synthesis, Characterization and DNA-Binding Properties of $[Ru(dipn)(tpy)](ClO_4)_2$ Compared with Those of Its Series Compounds

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Abstract: A new ruthenium(Ⅱ) complex containing aliphatic polyamine $[Ru(dipn)(tpy)](ClO_4)_2$ (**1**), (tpy and dipn stand for 2,2′:6′ 2″-terpyridine and *N*-(3-aminopropyl)propane-1,3-diamine, respectively) was synthesized and characterized by EA, ESMS, UV-Vis and CV. The DNA-binding behaviors of this complex were studied by absorption titration, EB-competition fluorescence spectra and viscosity measurements, compared with those of it′s series compounds $[Ru(dipn)(dptp)](ClO_4)_2$ (**2**) and $[Ru(dipn)(pat)](ClO_4)_2$ (**3**) (dptp=2-(5,6-diphenyl-1,2,4-triazin-3-yl)-1,10-phenanthroline; pat=9-(1,10-Phenanthrolin-2-yl) acenaphtho[1,2-e][1,2,4]triazine). The results indicate that the three complexes interact with DNA in different mode: electrostatic attraction, partial intercalation and intercalation for **1**, **2** and **3**, respectively. Their DNA affinities are in order: **1** < **2** < **3**.

Key words: Ruthenium complex; DNA binding; polypyridine; aliphatic polyamine

Ruthenium(Ⅱ) polypyridine complexes have been more extensively studied over past decades, due to their abundant photophysical, photochemical and outstanding redox properties, which can be conveniently regulated via changing ligands or substituents on them[1-3]. These in combination arise researchers′ vast interesting to exploit Ru(Ⅱ) complexes as functional reactants with DNA such as DNA-cleavage agents[4-7], DNA structural probes[8-10], medicines and antitumor drugs[11-14]. It is well documented that Ru(Ⅱ)) polypyridyl complexes can bind DNA not only through covalent interaction like Pt(Ⅱ) complexes but also through non-convalent interaction[15],

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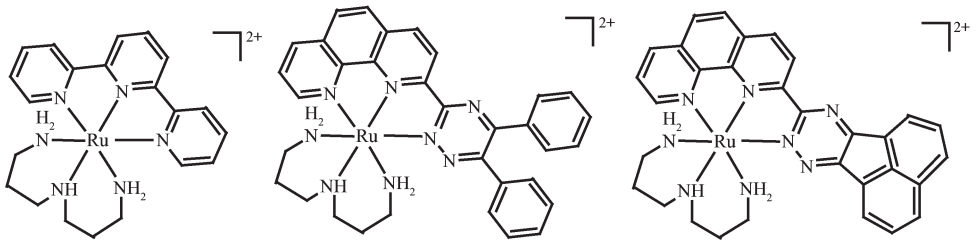
and the latter contains electrostatic attraction, partial and classical intercalation. However the vast majority of such studies have focused on tris-diimine complexes derived from the parent compound $[Ru(bpy)_3]^{2+}$ or $[Ru(phen)_3]^{2+}$, (bpy=2,2′-bipyridine, phen=1,10-phenanthroline)[16-17], very few tridendate complexes based on the prototype $[Ru(tpy)_2]^{2+}$, (tpy=2,2′:6′2″-terpyridine) have been studied until recently[18-19]. Meanwhile, most such compounds are homo-coordinated, the heteroleptic complexes have attracted much less attention, and the aliphatic polyamine-containing complexes remain largely untapped.

As a part of our systematic studies, in this contribution we have synthesized a new polyamine-containing ruthenium(Ⅱ) complex [Ru(dipn)(tpy)] $(ClO_4)_2$ (**1**), (dipn stands for *N*-(3-aminopropyl)propane-1,3-diamine), which structurally relates to the previous reported complexes [Ru(dipn)(dptp)] $(ClO_4)_2$ (**2**) and [Ru(dipn)(pat)] $(ClO_4)_2$ (**3**) (dptp=2-(5,6-diphenyl-1,2,4-triazin-3-yl)-1,10-phenanthroline; pat=9-(1,10-phenanthrolin-2-yl) acenaphtho [1,2-e][1,2,4]triazine)[20], their cation ions are shown in Scheme 1. All the three complexes **1**, **2** and **3** consist of a aromatic ligand and a flexible aliphatic polyamine dipn, so there are less steric interligand repulsions in them than in the corresponding homoleptic compounds Ru $(tpy)_2^{2+}$, Ru $(dptp)_2^{2+}$ and Ru $(pat)_2^{2+}$ [21-22]. These structure features offer us the chance for further investigating the differences in DNA-binding modes and affinities stemming from the aromatic ligand.



Scheme 1 Structures of the cation ions of (**1**), (**2**) and (**3**)

1 Experimental

1.1 Materials and general methods

All reagents and solvents were of reagent grade and obtained commercially, and the aqueous solution were prepared with doubly distilled water. Calf thymus DNA (CT-DNA, purchased from Sino-American Biotechnology Company) was dissolved in Tris-HCl buffer (5 mmol·L^{-1} Tris-HCl, 50 mmol·L^{-1} NaCl, pH 7.2, Tris=Tris(hydroxymethyl)aminomethane), this solution gave a ratio of UV absorbance at 260 and 280 nm of 1.8 ~1.9, being certain that the DNA was sufficiently free of protein[23]. The DNA concentration per nucleotide was determined by absorption coefficient (6 600 $L\cdot mol^{-1}\cdot cm^{-1}$) at 260 nm[24].

1.2 Physical measurements

Microanalysis (C, H and N) was carried out with a Perkin-Elmer 240Q elemental analyzer. Electrospray mass spectrum (ES-MS) were recorded on a LQC system (Finngan MAT, USA) using CH_3CN as mobile phase. UV-Vis spectra were made with a Shimadzu MPS-2000 spectrophotometer. Luminescence measurements were made on a Hitachi RF-2500 fluorescence spectrophotometer at room temperature. All the UV-Vis, Emission spectra measurements are using Tris-HCl buffer as solvent.

Cyclic voltammetry was performed on an EG&G PAR 273 polarographic analyzer and 270 universal programmer. A standard three-electrode system was used comprising a platinum microcylinder working electrode, platinum-wire auxiliary electrode and asaturated calomel reference electrode (SCE).

Viscosity measurements were carried out with an Ubbelodhe viscometer at (30.0±0.1) ℃. DNA samples ~ 200 base pairs in average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility[25]. Data were presented as $(\eta/\eta^0)^{1/3}$ vs binding ratio c_{Ru}/c_{DNA}, where η is the viscosity of DNA

in the presence of complex and η^0 is the viscosity of DNA alone [26]. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of buffer alone (t_0), $\eta=(t-t_0)/t_0$.

1.3 Synthesis

The compounds Ru(tpy)Cl_3[27], [Ru(dipn)(dptp)]$(ClO_4)_2$ and [Ru(dipn)(pat)]$(ClO_4)_2$ were synthesized according to literature methods[20].

[Ru(tpy)(dipn)]$(ClO_4)_2$ (**1**): A mixture of Ru(tpy)Cl_3 (0.1 g, 0.227 mmol), dipn (0.044 6 g, 0.340 mmol) and triethylamine (1 mL) in ethanol-water (1∶1, V/V, 50 mL) was refluxed for 6 hours, till the mixture turned to red-purple solution. After being cooled to room temperature, the most solvent was removed off under reduced pressure followed by adding saturated $NaClO_4$ aqueous solution. The resulting solid product was desiccated in vacuum drier with P_2O_5 in it. The purification was achieved by column chromatography using alumina as the column support and acetonitrile-ethynol 100∶1(V/V) as eluent. The dark purple band was recovered from the alumina column, and the product was obtained by rotary evaporation. Yield: 43%. Anal. Calcd. for $C_{21}H_{28}N_6Cl_2O_8Ru$(%): C, 38.01; H, 4.24; N, 12.46. Found(%): C, 37.95; H, 4.21; N, 12.65. ESMS: m/z 565.1 ([Ru(dipn)(tpy)(ClO_4)]$^+$) and 233.2 ([Ru(dipn)(tpy)]$^{2+}$).

2 Results and discussion

2.1 Synthesis and characterization

The complex **1** can be synthesized with the literature method, and readily isolated from the reaction mixture as the perchlorates and purified by column chromatography. It is worth noting that complex **1** has relatively strong affinity to the column support Al_2O_3 resulting from the polar functional group NH or NH_2, adding 1% ethanol to the eluent is necessary. The elemental analyses of **1** are in agreement with the molecular formula.

Electrospray mass spectrometry (ES-MS) has recently been shown to be a powerful tool for measuring the molecular mass of non-volatile and thermally unstable compounds[28]. In the ES-MS spectrum (Fig.1), only the signals of [Ru(dipn)(tpy)(ClO_4)]$^+$ and [Ru(tpy)(dipn)]$^{2+}$ were observed, and the blow up of base peak region isotope distribution (m/z=233.2) is well consistent with the simulation of the stable isotope patterns for [Ru(dipn)(tpy)]$^{2+}$.

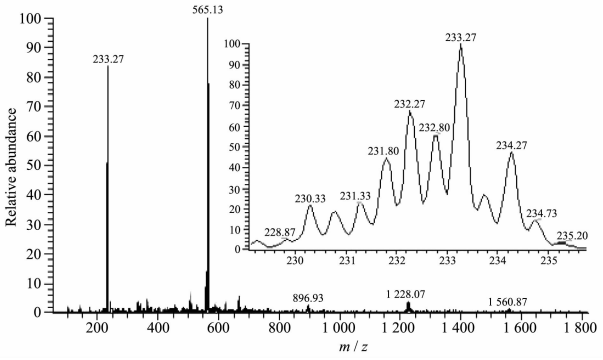


Fig.1　ES-MS spectrum of complex **1** and the base peak region isotope distribution (insert part)

The absorption spectra of **1** in CH_3CN with those of **2** and **3** [20] for comparison were shown in Fig.2, the maxium absorption wavelength λ_{max} were also lilisted in it. The three complexes showed fine structure spanning from 220 to 320 nm like their corresponding uncoordinated ligands which is characteristic the π-π^* transition of the ligands and the broad peaks between 490 and 560 nm which can be assigned as MLCT bands[20-22]. With the increase of the extent of π delocalization of the ligands tpy < dptp < pat, the energy of the MLCT excited-state decreases. Comparing to the [Ru(tpy)$_2$]$^{2+}$, [Ru(dptp)$_2$]$^{2+}$ and [Ru(pat)$_2$]$^{2+}$ the corresponding heteroleptic complexes of **1**, **2** and **3** have the lower MLCT excited state, this can be reasonablely attributed to the electron donating effect of the nitrogine of the aliphatic polyamine, causing the electron density on Ru(Ⅱ) center increasing, consequently Ru(Ⅱ) state become unstable and MLCT shift to lower energy[20-22].

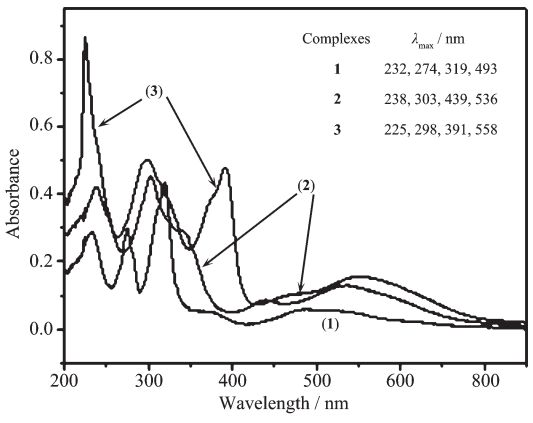


Fig.2　Absorption spectra of **1**, **2** and **3** in CH_3CN

The electrochemical behavior of the complex **1** was studied in MeCN by cyclic voltammetry (CV) in the sweep range between −2.0 and 1.6 V. It exhibits one metal center oxidation peak at 0.97 V (Ru^{+2}/Ru^{+3}) and two successive ligand-based reduction (tpy^0/tpy^{-1} and tpy^{-1}/tpy^{-2}) at −1.33 and −1.58 V. The oxidation potential based on metal-center is less than those of [Ru(dipn)(dptp)]$(ClO_4)_2$ (1.06 V) and [Ru(dipn)(pat)]$(ClO_4)_2$ (1.18 V)[20], implying the both dptp and pat can better stabilize the Ru(Ⅱ) state than tpy, which is consistent with the stronger electron-withdrawing ability of dptp and pat. Mean-while, the oxidation potential of **1** is less positive than that of the corresponding compound $[Ru(tpy)_2]^{2+}$ (1.27 V)[21], this can be attributed to the stronger electron-donor capacity of dipn than that of tpy, thus increasing the electron density at the Ru(Ⅱ) center, and conse-quently promoting the oxidation of Ru(Ⅱ) to Ru(Ⅲ).

2.2 DNA binding properties

2.2.1 UV-Vis absorption titration

The absorption spectra of complex **1** in the absence and presence of CT-DNA at various complex concentrations was made (shown in Fig.3). The intrinsic binding constants to CT-DNA were obtained by monitoring the changes in the absorbance at 493 nm with increasing concentration of DNA, and the following equation was applied[29]:

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

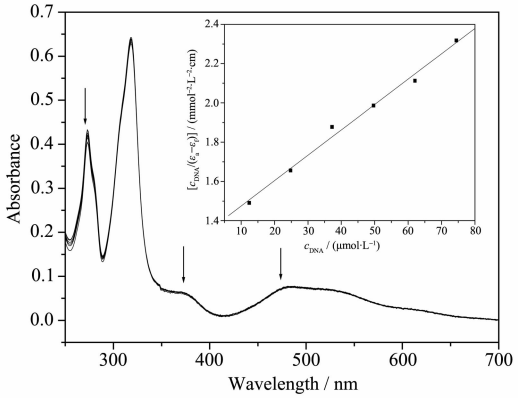


Fig.3 Absorption spectra of **1** in the presence of increasing amounts of DNA (c_{Ru}=20 μmol·L^{-1}, c_{DNA}=0~160 μmol·L^{-1}); Arrows show the absorbance changes on increasing DNA concentration. Insert part is linear fitting plot of $c_{DNA}/(\varepsilon_a-\varepsilon_f)$ vs c_{DNA}

where c_{DNA} is the concentration of DNA in base pairs, ε_a, ε_f and ε_b correspond to the apparent absorption coefficient A_{obsd}/c_{Ru}, the extinction coefficient for the free ruthenium complex and the extinction coefficient for the ruthenium complex in the fully bound form, respectively. In plots of $c_{DNA}/(\varepsilon_a-\varepsilon_f)$ versus c_{DNA}, intrinsic binding constant K_b is given by the ratio of slope to the intercept. The K_b value obtained for complex **1** is 1.01×10^3 L·mol^{-1}, which is smaller than those observed for [Ru(dipn)(dptp)]$(ClO_4)_2$ (7.61×10^3 L·mol^{-1}) and [Ru(dipn)(pat)]$(ClO_4)_2$ (4.63×10^4 L·mol^{-1}).

When the amount of DNA was increased, a absorbance decrease of 3.47% in the MLCT transition is found for complex **1**, much smaller than 8.08% and 25.08% for complexes **2** and **3** respectively. It can be infered that the complex **1** binds DNA with much weaker affinity[30], and DNA-binding ability is following the order **1** <**2** <**3**, congruent with the gradual enlargement of π-conjugated aromatic area of the ligands from tpy, dptp to pat.

2.2.2 Luminescence spectra

Ethidium bromide (EB) is a well probing agent for DNA, its fluorescence could be quenched by the addition of a second molecule. The quenching extent of fluorescence of EB binding to DNA is used to determine the extent of binding between the second molecule and DNA. In order to comparing the quenching effect of the three complexes, the mixture Tris-solution of DNA and EB, prepared one day in advance, was added dosingly a certain concentration of Ru(Ⅱ) complex solution and monitoring the changes of emission spectra (Fig.4). The result indicates that the three complexes at different degrees reduce the emission intensity resulting from the EB disassociation from the DNA by the binding of complex to DNA in place of EB. The replacement ratio $(I_0-I_b)/I_0$, where I_0, I_b respectively denote the initial and final fluorescence intensity, is 90%, 70% and 15% for $[Ru(dipn)(pat)]^{2+}$, $[Ru(dipn)(dptp)]^{2+}$ and $[Ru(dipn)(tpy)]^{2+}$, respectively.

According to the classical Stern-Volmer equation[31]: $I_0/I=1+Kr$ where I_0 and I are the fluorescence intensities in the absence and the presence of complexes, respectively, K is a linar Stern-Volmer quenching

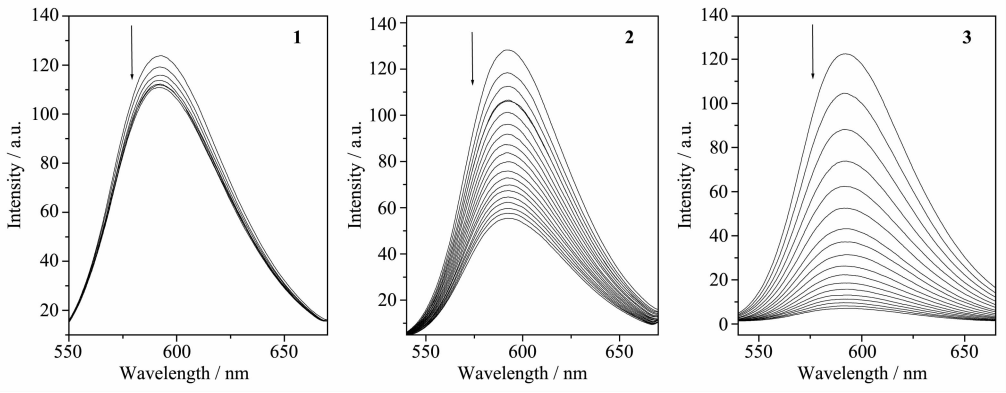


Fig.4　Emission spectra of EB bound to DNA in the presence of complexes **1**, **2** and **3** (c_{EB}=5 μmol·L^{-1}, c_{DNA}=25 μmol·L^{-1}, c_{Ru}=0~5 μmol·L^{-1}, λ_{ex}=340 nm); Arrows show the intensity changes on increasing the complex concentrations

constant and r is the ratio of the total concentration of complex to that of DNA.

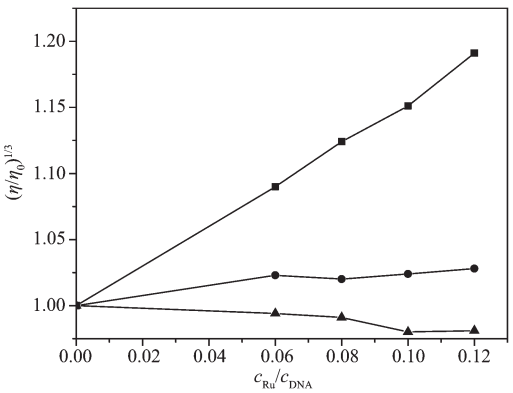
The fluorescence quenching plots (I_0/I versus r, not be shown here) illustrate that the fluorescence quenching of EB binding to DNA by the three complexes are in good agreement with the linear Stern-Volmer equation, implying the interations between the Ru(Ⅱ) complexes and DNA. K can be obtained by the ratio of the slope to intercept in the plot of I_0/I versus $c_{complex}/c_{DNA}$. The K values for **1**, **2**, **3** are 4.50, 15.93 and 59.77, respectively. These data suggest that the interaction of **3** with DNA is the strongest, followed by **2**, and then **1**, which is consistent with the above absorption spectral results.

2.2.3　Viscosity studies

To further explore the binding of the present complexes, viscosity measurements were carried out on calf thymus DNA by varying the concentration of the added complexes. Optical photophysical probes generally provide necessary but not sufficient clues to support a binding model. Viscosity measurements that are sensitive to DNA length changes are regarded as the least ambiguous and the most critical tests of binding model in solution in the absence of crystallographic structural data or NMR[32].

It is popularly accepted that a classical intercalation mode results in lengthening the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial and/or non-classical intercalation of ligand could bend (or kink) the DNA helix, act as a "wedge" to pry apart one side of a base-pair stack, reduce its effective length and, concomitantly, its viscosity[33-34].

The results of viscosity measurements for complexes **1**, **2** and **3** are given in Fig.5. As previously reported, the viscosity of DNA lightly decreases upon increasing the concentration of **2** and dramatically increases upon increasing the concentration of **3**[20]. However, when the amount of **1** is increased, the relative viscosity of DNA almost has no changes, implying that it binds DNA through the eletrostatic mode, which is similar to the behavior of complex $[Ru(bpy)_3](PF_6)_2$[35]. It can be reasonably concluded that the three complexes are interaction with DNA in different mode: electrostatic attraction, partial intercalative and intercalative mode for **1**, **2** and **3**, respectively.



c_{DNA}=0.5 mmol·L^{-1} and c_{Ru}/c_{DNA}=0.04, 0.06, 0.08, 0.10, 0.12

Fig.5　Effect of increasing amounts of complexes **1** (●), **2** (▲) and **3** (■) on the relative viscosities of DNA at (30.0±0.1) ℃

3 Conclusion

A new ruthenium(Ⅱ) complex containing aliphatic polyamine [Ru(dipn)(tpy)]$(ClO_4)_2$ (**1**) was synthesized and characterized. The DNA-binding behaviors of this complex are different from those of its series compounds [Ru(dipn)(dptp)]$(ClO_4)_2$ (**2**) and [Ru(dipn)(pat)]$(ClO_4)_2$ (**3**). The three complexes are interaction with DNA in different mode: electrostatic attraction, partial intercalative and intercalative mode for **1**, **2** and **3**, respectively. Their DNA affinities are in the order: **1** < **2** < **3**.

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