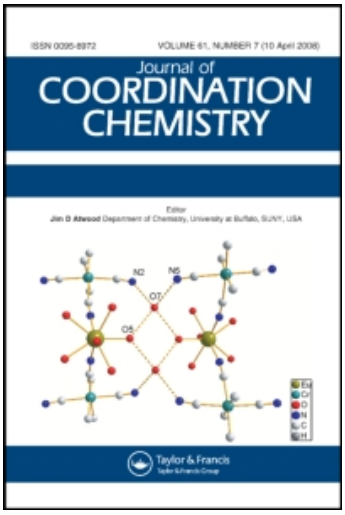


Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Effect of substituents on DNA-binding behaviors of ruthenium(II) complexes: $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$

YI Liu[a]; Yun-Jun Liu[a]; Jun-Hua Yao[b]; Wen-Jie Mei[a]; Fu-Hai Wu[c]
[a] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, P.R. China [b] Instrumentation Analysis and Research Center, Sun Yat-Sen University, Guangzhou, P.R. China [c] School of Public Health, Guangdong Pharmaceutical University, Guangzhou, P.R. China

To cite this Article Liu, YI , Liu, Yun-Jun , Yao, Jun-Hua , Mei, Wen-Jie and Wu, Fu-Hai(2009) 'Effect of substituents on DNA-binding behaviors of ruthenium(II) complexes: $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$', Journal of Coordination Chemistry, 62: 10, 1701 — 1708
To link to this Article: DOI: 10.1080/00958970802649984
URL: http://dx.doi.org/10.1080/00958970802649984

Journal of Coordination Chemistry
Vol. 62, No. 10, 20 May 2009, 1701–1708

Taylor & Francis
Taylor & Francis Group

Effect of substituents on DNA-binding behaviors of ruthenium(II) complexes: $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$

YI LIU†, YUN-JUN LIU*†, JUN-HUA YAO‡, WEN-JIE MEI† and FU-HAI WU*§

†School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, P.R. China
‡Instrumentation Analysis and Research Center, Sun Yat-Sen University, Guangzhou, P.R. China
§School of Public Health, Guangdong Pharmaceutical University, Guangzhou, P.R. China

(*Received 9 June 2008; in final form 5 August 2008*)

Two Ru(II) complexes $[Ru(dmb)_2(dtmi)](ClO_4)_2$ (**1**) (dmb = 4, 4′-dimethyl-2, 2′-bipyridine, dtmi = 3-(pyrazin-2-yl)-as-triazino[5, 6-f]-5-methoxylisatin) and $[Ru(dmb)_2(dtni)](ClO_4)_2$ (**2**) (dtni = 3-(pyrazin-2-yl)-as-triazino[5, 6-f]-5-nitroisatin) have been synthesized and characterized by elemental analysis, ES-MS, and ^1H NMR. DNA-binding behaviors of these complexes have been investigated by spectroscopic titration, viscosity measurements, and thermal denaturation. The results indicate that the two complexes interact with calf thymus DNA by intercalation.

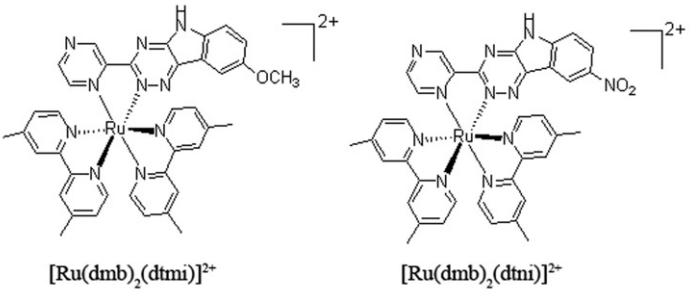
Keywords: Ru(II) complex; DNA; Thermal denaturation

1. Introduction

Metal complexes, which bind to DNA, have been studied extensively with emphasis on understanding the photophysical, photochemical, and redox perturbations that are imposed through interaction with the DNA strand [1]. The interaction of transition metal polypyridyl complexes with DNA has attracted considerable attention stemming from developing attractive candidates as DNA secondary structure probes and photocleavage reagents [2, 3]. Many studies show that Ru(II) polypyridyl complexes bind to DNA in a noncovalent interaction such as electrostatic binding, groove binding [4, 5], intercalative binding, and partial intercalative binding [6–8]. Many useful applications of these complexes require binding to DNA through intercalation. One of our current interests focuses on the design and syntheses of Ru(II) polypyridyl complexes in that octahedral Ru(II) complexes are particularly suitable for these applications, because they are coordinatively saturated and inert to substitution. When associating with DNA, hypochromic or hyperchromic effects on electronic absorptions can be indicative of association [9–14]. Many factors influence the interaction of complex with DNA, including substituent effects. In this article, we report the synthesis,

*Corresponding authors. Email: lyjche@163.com; fuhaiwu@163.com

ISSN 0095-8972 print/ISSN 1029-0389 online © 2009 Taylor & Francis
DOI: 10.1080/00958970802649984



Scheme 1. The structures of $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$.

characterization, and DNA-binding behaviors of $[Ru(dmb)_2(dtmi)](ClO_4)_2$ (**1**) (dmb = 4, 4′-dimethyl-2, 2′-bipyridine, dtmi = 3-(pyrazin-2-yl)-as-triazino[5, 6-f]-5-methoxylisatin) and $[Ru(dmb)_2(dtni)](ClO_4)_2$ (**2**) (dtni = 3-(pyrazin-2-yl)-as-triazino-[5, 6-f]-5-nitroisatin (Scheme 1). The DNA-binding behaviors of these complexes have been investigated by spectroscopic titration, viscosity measurements and thermal denaturation. The results show **1** and **2** interact with calf thymus DNA (CT-DNA) by intercalation. The DNA-binding affinities follow the order **2** > **1**.

2. Experimental

2.1. *Material and methods*

Calf thymus DNA was obtained from the Sino-American Biotechnology Company. Doubly-distilled water was used to prepare buffers (5 mM tris(hydroxymethylaminomethane)-HCl, 50 mM NaCl, pH = 7.2). A solution of CT-DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of ca 1.8–1.9 : 1, indicating that the DNA was sufficiently free of protein [15]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($6600\ M^{-1}\,cm^{-1}$) at 260 nm [16].

2.2. *Physical measurements*

Microanalyses (C, H, and N) were carried out with a Perkin–Elmer 240Q elemental analyzer. Electrospray mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA) using methanol as mobile phase. The spray voltage, tube lens offset, capillary voltage, and capillary temperature were set at 4.50 kV, 30.00 V, 23.00 V, and 200°C, respectively, and the quoted m/z values are for the major peaks in the isotope distribution. ^{1}H NMR spectra were recorded on a Varian-500 spectrometer. All chemical shifts were given relative to tetramethylsilane (TMS). UV/vis spectra were recorded on a Shimadzu UV-3101PC spectrophotometer at room temperature.

2.3. *Syntheses of complexes*

2.3.1. $[Ru(dmb)_2(dtmi)](ClO_4)_2$ (1). A mixture of *cis*-$[Ru(dmb)_2Cl_2]\cdot 2H_2O$ [17] (0.288 g, 0.5 mmol) and dtmi [18] (0.139 g, 0.5 mmol) in EtOH (30 cm^3) was refluxed under argon for 8 h to give a clear red solution. Upon cooling, a brown-red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on neutral alumina with CH_3CN-toluene (3:1, v/v) as eluent. The brown-red band was collected, solvent removed under reduced pressure and a brown-red powder obtained. Yield: 64%. Anal. Found: C, 48.3; H, 3.7; N, 14.7; Calcd for $C_{38}H_{34}N_{10}Cl_2O_9Ru$: C, 48.2; H, 3.6; N, 14.8%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 14.6 (s, 1H), 8.71 (d, 2H, $J = 8.6$ Hz), 8.60 (d, 2H, $J = 8.8$), 8.29 (s, 1H), 8.18 (d, 1H, $J = 8.5$), 8.14 (t, 1H, $J = 7.6$ Hz), 7.76 (t, 2H, $J = 7.5$ Hz), 7.55 (t, 2H, $J = 7.7$ Hz), 7.38 (d, 2H, $J = 8.3$ Hz), 7.34 (d, 1H, $J = 8.2$), 7.30 (d, 2H, $J = 8.4$ Hz), 7.07 (d, 1H, $J = 8.0$ Hz), 6.87 (s, 1H), 3.77 (s, 3H), 3.28 (s, 6H), 2.31 (s, 6H). ES-MS (CH_3OH): m/z 746.3 ($[M–2ClO_4–H]^+$), 373.3 ($[M–2ClO_4]^{2+}$).

2.3.2. $[Ru(dmb)_2(dtni)](ClO_4)_2$ (2). This complex was synthesized by using the same procedure as described for **1** with dtni [18] in place of dtmi. Yield: 63%. Anal. Found: C, 46.3; H, 3.2; N, 16.1; Calcd for $C_{37}H_{31}N_{11}Cl_2O_{10}Ru$: C, 46.2; H, 3.3; N, 16.0%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 10.0 (s, 1H), 8.80 (d, 2H, $J = 8.5$ Hz), 8.60 (d, 2H, $J = 8.70$ Hz), 8.31 (d, 2H, $J = 8.64$ Hz), 8.15 (d, 2H, $J = 8.35$ Hz), 7.74–7.80 (m, 2H), 7.60 (t, 2H, $J = 7.5$ Hz), 7.40 (d, 2H, $J = 8.22$ Hz), 7.30 (d, 2H, $J = 8.40$ Hz), 7.24 (d, 2H, $J = 8.61$ Hz), 3.30 (s, 6H), 2.07 (s, 6H). ES-MS (CH_3OH): m/z 761.20 ($[M–2ClO_4–H]^+$), 381.20 ($[M–2ClO_4]^{2+}$).

Caution! Perchlorate salts of metal complexes with organic ligands are potentially explosive, and only small amounts of the material should be prepared and handled with great care.

2.4. *DNA-binding studies*

The absorption titrations of Ru(II) complexes in buffer were performed by using a fixed ruthenium concentration to which increments of the DNA stock solution were added. Ruthenium complex solutions employed were 20 μM in concentration and CT-DNA was added to a ratio of 10 : 1 of [DNA]/[Ru]. Ruthenium–DNA solutions were allowed to incubate for 5 min before absorption spectra were recorded. The intrinsic binding constants K of Ru(II) complexes to DNA were derived from equations (1) and (2) [19–21]:

$$\frac{\varepsilon_a - \varepsilon_f}{\varepsilon_b - \varepsilon_f} = \frac{\sqrt{(b - (b^2 - 2K^2C_t[DNA]/s}}{2KC_t} \quad (1)$$

$$b = 1 + KC_t + K[\mathrm{DNA}]/2s \quad (2)$$

where [DNA] is the concentration of CT-DNA in base pairs, the apparent absorption coefficients ε_a, ε_f, and ε_b correspond to A_{obsed}/[Ru], the absorbance for the free ruthenium complex, and the absorbance for the ruthenium complex in fully bound

form, respectively, K is the equilibrium binding constant, C_t is the total metal complex concentration in nucleotides, and s is the binding site size.

Luminescence spectra of Ru(II) complexes (10 μM) were obtained in the absence and presence of varying concentrations of DNA in Tris buffer. From the luminescence measurements, the intrinsic binding constants were determined using equations (3) and (4):

$$C_F = C_T(I/I_0 - P)/(1 - P) \tag{3}$$

where C_T is the concentration of probe added, C_F is the concentration of the free probe, and I_0 and I are the fluorescence intensities in the absence and presence of DNA, respectively; P is the ratio of the observed fluorescence quantum yield of the bound probe to that of the free probe. The value of P was obtained from a plot of I/I_0 *versus* 1/[DNA], such that it is the limiting fluorescence yield given by the y-intercept [22]. The amount of bound probe (C_B) at any concentration was equal to $C_T - C_F$. A plot of r/C_F *versus* r, where r is equal to C_B/[DNA], was constructed according to the modified Scatchard equation (4)

$$r/C_F = K(1 - nr)[(1 - nr)/(1 - (n - 1)r)]^{n-1} \tag{4}$$

by McGhee and Von Hippel [23]. In equation (4), K is the intrinsic binding constant and n is the binding site size in base pairs. The binding data were fitted to equation (4) using nonregression analysis. The K and n were obtained from the best fit of the data to the equation.

Thermal denaturation studies were carried out with a Perkin–Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (±0.1°C). The absorbance at 260 nm was continuously monitored for solutions of CT-DNA (80 μM) in the absence and presence of Ru(II) complexes. The temperature of the solution was increased by 1°C min^{-1}.

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at 28.0 (±0.1)°C in a thermostatic bath. DNA samples approximately 200 base pairs in average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility [24]. Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio [25], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

3. Results and discussion

3.1. *Absorption spectroscopic studies*

The absorption spectra of the complexes are shown in Supplementary Material and characterized by intense $\pi \rightarrow \pi^*$ ligand transitions in the UV and metal-to-ligand charge transfer (MLCT) transition in the visible region. Bands below 300 nm are attributed to intraligand (IL) $\pi \rightarrow \pi^*$ transitions, and the lowest energy bands at 433 or 462 nm are assigned to MLCT transitions by comparison with spectra of other polypyridyl Ru(II) complexes [7, 26, 27].

Figure S1 in Supplementary Material exhibits the electronic spectral trace of the complexes titrated. With the addition of DNA concentration, the hypochromism in the MLCT band reaches as high as 15.7% at 433 nm and 19.8% at 462 nm for **1** and **2**, respectively. Comparing with the hypochromism of $[Ru(phen)_3]^{2+}$ (12% hypochromism of the MLCT band at 445 nm) [28], which interacts with DNA through a semi-intercalation or quasi-intercalation [29], these spectral characteristics obviously suggest that **1** and **2** interact with DNA most likely through a mode that involves a stacking interaction between the aromatic chromophore and the base pairs of DNA.

In order to further illustrate the binding strength of the complexes, the binding constants K were determined by monitoring the changes of absorbance in the MLCT band with increasing the concentration of DNA, the binding constants K of **1** and **2**, $2.63 \times 10^4\,M^{-1}$ ($s = 1.56$) and $8.65 \times 10^4\,M^{-1}$ ($s = 1.67$) indicate these complexes bind to DNA with moderate strength. These values are comparable to $[Ru(bpy)_2L]^{2+}$ [L = ptdb ($1. \times 10^4$), ptda(3.1×10^4) and ptdp(5.9×10^4)] [25] and $[Ru(bzimpy)_2]^{2+}$ (1.8×10^4) [30], but smaller than that observed for $[Ru(bpy)_2(dppz)]^{2+}$ ($>10^6$) [31] and $[Ru(bpy)_2(ppd)]^{2+}$ ($1.3 \times 10^6\,M^{-1}$) [32]. The different DNA-binding properties of **1** and **2** are due to substituents in the intercalative ligands. The electron-withdrawing substituent ($-NO_2$ in dtni) on the intercalative ligand can improve the DNA-binding affinity, whereas the electron-pushing substituent ($-OCH_3$ in dtmi) decreases the DNA affinity. Such a trend suggests that the DNA-binding affinity of the complex can be effectively controlled by substituents.

3.2. *Luminescence spectroscopic studies*

The results of emission titrations for $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$ with CT-DNA are illustrated in figure 1. Upon addition of CT-DNA, the complexes emit luminescence in Tris buffer at ambient temperature, the emission intensities of **1** and **2** increase to about 6.08 and 6.87 times larger than the original. This implies that $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$ interact with CT-DNA and are protected by DNA efficiently. The hydrophobic environment inside the DNA helix reduces the accessibility of water to the complex and complex mobility is restricted at the binding site, leading to decrease of the vibrational modes of relaxation. Scatchard plots for

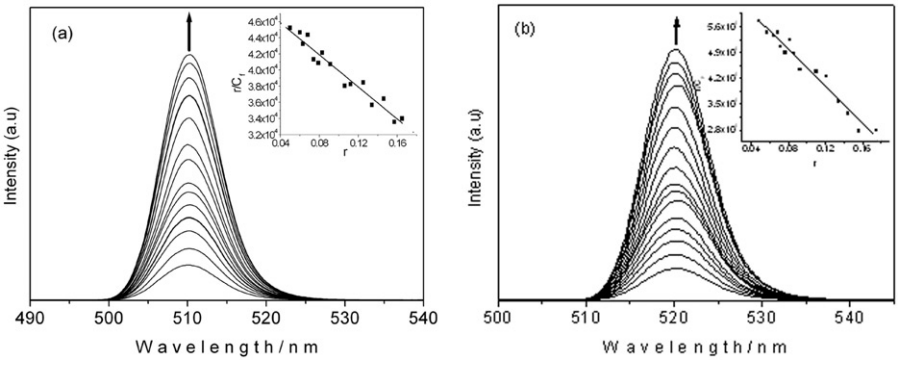


Figure 1. Emission spectra of complexes **1** (a) and **2** (b) in Tris-HCl buffer in the absence and presence of CT-DNA. Arrow shows the intensity change upon increasing DNA concentrations.

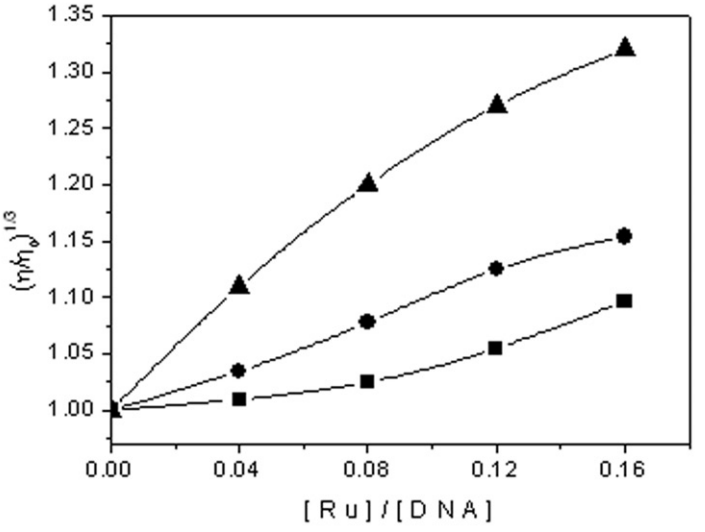


Figure 2. Effect of increasing amounts of EB (▲), complex **1** (■) and **2** (●) on the relative viscosity of CT-DNA at 28 (±0.1)°C. [DNA] = 0.5 mM.

complexes have been constructed from luminescence spectra and binding constants and binding site size were 3.73×10^4 and 2.03 for **1**, 8.46×10^4 and 2.42 for **2**, respectively. The binding constants obtained from both the absorption titration and luminescence spectra are consistent and within the error limits.

3.3. *Viscosity measurements*

Viscosity measurements of DNA are regarded as the least ambiguous and most critical test of a DNA-binding model in solution, and provide strong arguments for intercalative DNA-binding mode [33, 34]. A classical intercalation model results in lengthening the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to increase of DNA viscosity. In contrast, a partial, nonclassical intercalation of ligand could bend (or kink) the DNA helix and reduce its effective length, and concomitantly, its viscosity [33, 34]. In order to clarify the interaction between the complex and DNA, the viscosity measurements were performed in the presence of **1** and **2**; increasing the amounts of complexes increases the viscosity of DNA solution steadily (figure 2), which is similar to that of the classical intercalative compound ethidium bromide (EB). The results show that the two complexes interact with CT-DNA through intercalation.

3.4. *Thermal denaturation behaviors*

Thermal denaturation of DNA provides further evidence for intercalation of the Ru(II) complex into the helix. Intercalation of small molecules into the double helix increases the helix melting temperature. In the presence of complex, the thermal behavior of DNA can give insight into conformational changes when the temperature is raised, and

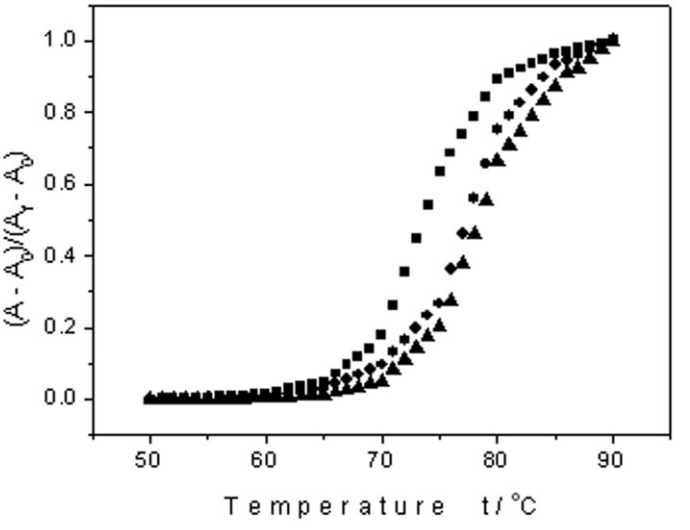


Figure 3. Thermal denaturation of CT-DNA in the absence (■) and presence of complex **1** (●) and **2** (▲), [Ru] = 20 μM, [DNA] = 80 μM.

information about the interaction strength of complex with DNA. When complexes intercalate into the DNA base pairs, the DNA melting temperature (T_m) rises and the change of T_m is consistent with the binding strength of complexes to DNA. The melting temperature T_m, which is defined as the temperature where half of the total base pairs are unbonded, is determined from the thermal denaturation curves of DNA. The intercalation of natural or synthesized organic compounds and metallointercalators [22, 35, 36] generally results in considerable increase in T_m. The thermal denaturation carried out for DNA in the absence of any added complex gave a T_m of $73.7 \pm 0.5^\circ$C under our experimental conditions. In the presence of Ru(II) complex, the thermal denaturation experiment (figure 3) revealed increase $\Delta T_m = 3.7$ and 4.8°C for **1** and **2**, respectively. The change in the T_m of DNA observed here in the presence of **1** and **2** is indicative of weak interaction of the complexes with DNA. This is also reflected in the binding constant values obtained spectroscopically.

4. Conclusion

Two Ru(II) polypyridyl complexes $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$ with asymmetric intercalative ligands have been synthesized and characterized. The DNA binding has been studied by spectroscopic titration, viscosity measurements, and thermal denaturation. The results show that $[Ru(dmb)_2(dtmi)]^{2+}$ and $[Ru(dmb)_2(dtni)]^{2+}$ intercalate into the DNA base pairs with DNA-binding constant K of **2** ($K = 8.65 \times 10^4\,M^{-1}$) larger than that of **1** ($K = 2.63 \times 10^4\,M^{-1}$). The experimental results suggest that the DNA-binding strength of **1** and **2** is moderate.

Acknowledgments

We are grateful to the Nature Science Foundation of Guangdong Province (No. 0711220400121), the Science and Technology Foundation of Guangdong Province (No. 2004B33301029), and Guangdong Pharmaceutical University for financial support.

References

[1] A. Kirsch-De Mesmaeker, J.P. Lecomte, J.M. Kelly. *Top. Curr. Chem.*, **26**, 177 (1996).
[2] L.-N. Ji, X.-H. Zou, J.-G. Liu. *Coord. Chem. Res.*, **216**, 513 (2001).
[3] A. Hergueta-Bravo, M.E. Jimenez-Hernandez, F. Montero, E. Oliveros, G. Orellana. *J. Phys. Chem. B*, **106**, 4010 (2002).
[4] G. Yang, J.-Z. Wu, L. Wang, L.-N. Ji, X. Tian. *J. Inorg. Biochem.*, **66**, 141 (1997).
[5] G. Yang, L. Wang, L.-N. Ji. *J. Inorg. Biochem.*, **67**, 289 (1997).
[6] J.-Z. Wu, H. Li, T.-X. Zeng, L.-N. Ji, J.-Y. Zhou, R.-H. Li. *Polyhedron*, **16**, 103 (1997).
[7] J.-Z. Wu, B.-H. Ye, L. Wang, L.-N. Ji, J.-Y. Zhou, R.-H. Li, Z.-Y. Zhou. *J. Chem. Soc., Dalton Trans.*, 1395 (1997).
[8] Y. Xiong, X.-F. He, X.-H. Zou, J.-Z. Wu, X.-M. Chen, L.-N. Ji, R.-H. Li, J.-Y. Zhou, K.-B. Yu. *J. Chem. Soc., Dalton Trans.*, **33**, 19 (1999).
[9] L.-F. Tan, H. Chao. *Inorg. Chim. Acta*, **360**, 2016 (2007).
[10] D.Z. Coogan, I.S. Haworth, P.J. Bates, A. Robinson, A. Rodger. *Inorg. Chem.*, **38**, 4486 (1999).
[11] I.S. Haworth, A.H. Elcock, A. Rodger, W.G. Richards. *J. Biomol. Struct. Dyn.*, **9**, 553 (1991).
[12] X.-W. Liu, J. Li, H. Li, K.-C. Zheng, H. Chao, L.-N. Ji. *J. Inorg. Biochem.*, **99**, 2372 (2005).
[13] D. Lawrence, V.G. Vaidyanathan, B.U. Nair. *J. Inorg. Biochem.*, **100**, 1244 (2006).
[14] L.-F. Tan, H. Chao, K.-C. Zheng, J.-J. Fei, F. Wang, Y.-F. Zhou, L.-N. Ji. *Polyhedron*, **26**, 5458 (2007).
[15] J. Marmur. *J. Mol. Biol.*, **3**, 208 (1961).
[16] M.F. Reichmann, S.A. Rice, C.A. Thomas, P. Doty. *J. Am. Chem. Soc.*, **76**, 3047 (1954).
[17] B.P. Sullivan, D.J. Salmon, T.J. Meyer. *Inorg. Chem.*, **17**, 3334 (1978).
[18] Y.-J. Liu, X.-Y. Wei, F.-H. Wu, W.-J. Mei, L.-X. He. *Spectrochim. Acta, Part A*, **70**, 171 (2008).
[19] J.K. Barton, J.J. Dannenberg, A.L. Raphael. *J. Am. Chem. Soc.*, **106**, 2172 (1984).
[20] S.R. Smith, G.A. Neyhart, W.A. Karlsbeck, H.H. Thorp. *New J. Chem.*, **18**, 397 (1984).
[21] M.T. Carter, M. Rodriguez, A.J. Bard. *J. Am. Chem. Soc.*, **111**, 8901 (1989).
[22] C.V. Kumar, E.H. Asuncion. *J. Am. Chem. Soc.*, **115**, 8547 (1993).
[23] J.D. McGhee, P.H. von Hippel. *J. Mol. Biol.*, **86**, 469 (1974).
[24] J.B. Chaires, N. Dattagupta, D.M. Crothers. *Biochemistry*, **21**, 3933 (1982).
[25] G. Cohen, H. Eisenberg. *Biopolymers*, **8**, 45 (1969).
[26] Q.-X. Zhen, B.-H. Ye, Q.-L. Zhang, J.-G. Liu, H. Li, L.-N. Ji, L. Wang. *J. Inorg. Biochem.*, **76**, 47 (1999).
[27] H. Deng, J. Li, K.-C. Zheng, Y. Yang, H. Chao, L.-N. Ji. *Inorg. Chim. Acta*, **358**, 3430 (2005).
[28] Y. Xiong, L.-N. Ji. *Coord. Chem. Rev.*, **185**, 711 (1999).
[29] P. Lincoln, B. Nordén. *J. Phys. Chem. B*, **102**, 9583 (1998).
[30] V.G. Vaidyanathan, B.U. Nair. *J. Inorg. Biochem.*, **91**, 405 (2002).
[31] J.E. Coury, J.R. Anderson, L. McFail-Isom, L.D. Williams, L.A. Bottomley. *J. Am. Chem. Soc.*, **119**, 3792 (1997).
[32] F. Gao, H. Chao, F. Zhou, Y.-X. Yuan, B. Peng, L.-N. Ji. *J. Inorg. Biochem.*, **100**, 1487 (2006).
[33] S. Satyanarayana, J.C. Dabroniak, J.B. Chaires. *Biochemistry*, **31**, 9319 (1992).
[34] S. Satyanarayana, J.C. Dabroniak, J.B. Chaires. *Biochemistry*, **32**, 2573 (1993).
[35] J.M. Kelly, A.B. Tossi, D.J. McConell, C. OhUigin. *Nucleic Acids Res.*, **13**, 6017 (1985).
[36] G.A. Neyhart, N. Grover, S.R. Smith, W.A. Kalsbeck, T.A. Fairly, M. Cory, H.H. Thorp. *J. Am. Chem. Soc.*, **115**, 4423 (1993).