ЖУРНАЛ СТРУКТУРНОЙ ХИМИИ

*2008. Том 49, № 6* *Ноябрь – декабрь* *С. 1086 – 1091*

UDC 541.49

# STUDY ON THE INTERACTION OF A PALLADIUM COMPLEX WITH DNA

© 2008 **E.J. Gao[1]*, H.X. Yin[1], M.C. Zhu[1], Y.G. Sun[1], X.F. Gu[1], Q. Wu[1], L.X. Ren[2]**

[1]*Laboratory of Coordination Chemistry, Shenyang Institute of Chemical Technology, Shenyang 110142, China*
[2]*College of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110015, China*

*Received November, 26, 2007*

The complex $[Pd(bipy)Cl_2]$ (**1**) (bipy = 2,2′-bipyridyl) has been synthesized and characterized by NMR spectroscopy, elemental analysis and X-ray diffraction method. The first step hydrolysis reaction kinetics for the complex was studied by UV-absorption spectroscopy; the speed constant ($k_1$) was found to be $3.0\times10^{-4}$ $s^{-1}$. The fluorescence spectra have been collected to investigate the interaction of complex (**1**) with fish sperm DNA (FS-DNA) and the results indicate that the complex (**1**) has an effective intercalation within DNA. The reaction of complex (**1**) with adenine in ethanol/water results in the compound $[Pd_2(bipy)_2(ade)_2]Cl_2\cdot3H_2O$ (**2**) (ade = adenine) whose crystal structure was determined by X-ray diffraction method. The structure is orthorhombic, *Pmmn*, $a = 12.993(4)$, $b = 14.512(5)$, $c = 9.837(3)$ Å, $V = 1854.8(11)$ Å$^3$, $Z = 2$ ($C_{30}H_{30}Cl_2N_{14}O_3Pd_2$), final $R_1 = 0.0675$. The palladium complex is a binuclear cation, where two ade ligands bridge two Pd(II) centers, while each Pd(II) is also chelated by one bipy ligand.

**K e y w o r d s:** palladium(II) complex, adenine, interaction mechanism, kinetics, crystal structure.

## INTRODUCTION

Over the past decades, the studies on the interaction of metal complexes with deoxyribonucleic acid (DNA) were of interest and the research on the metal complexes had attracted much attention due to their potential applications as anticancer medications and stereoselective probes of nucleic acid structures [ 1—3 ]. As is well-known, *cis*-diamminedichloroplatinum(II) (Cis-DDP, Cisplatin) was approved for use in the 1970 s for the treatment of a selection of human cancers, but its development was limited because of its toxic side effects and resistance [ 4 ]. In recent years, researchers were focused on the palladium complexes owing to their similar to platinum complexes coordination geometry and character [ 5—8 ].

Our team is interested in the preparation and anticancer activity of palladium complexes with mixed ligands and has obtained some valuable conclusions about their anticancer mechanism [ 9—15 ]. In a previous paper we reported the synthesis and crystal structure of the compound $[Pd_2(phen)_2(ade)_2]Cl_2\cdot4.5H_2O$ (phen = 1,10-phenanthroline; ade = adenine) [ 16 ] which provided important information for understanding the activity mechanism of active palladium complexes with DNA. DNA is the primary intracellular target of platinum-based anticancer drugs and the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells, and result in the cells death [ 17—19 ].

There are four kinds of nucleobases in DNA, adenine, guanine, cytosine and thymine, which may provide coordination sites for active metal complexes interacting with DNA [ 20 ]. It is well known that platinum complexes can react with DNA, by coordinating to N7 of guanine, forming the classical intrastrand and interstrand Pt-DNA cross-links that are responsible for the cellular damage [ 21—24 ].

* E-mail: ejgao@yahoo.com.cn

It has been reported that the interaction mechanism of palladium complexes with DNA is different from that of cisplatin [ 25, 26 ]. In this paper, we report on the synthesis of complex $[Pd(bipy)Cl_2]$ (**1**) (bipy = 2,2′-bipyridyl), which crystal structure has already been reported [ 27 ], and investigate its interaction with DNA using fluorescence spectra. The complex reveals strong intercalation action with DNA. In order to investigate the interaction mechanism further, we designed an experiment illustrated below (see Chart). Also, we synthesized compound $[Pd_2(bipy)_2(ade)_2]Cl_2 \cdot 3H_2O$ (**2**) (ade = adenine) and studied its crystal structure by single crystal X-ray diffraction.

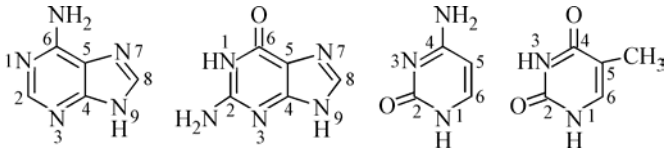

*Chart.* General process scheme studied

## EXPERIMENTAL

**Reagents and spectroscopy measurements.** $PdCl_2$, bipy and KCl were of analytical grade; ade, fish sperm DNA (FS-DNA) were biochemical reagents and were used without further purification. To increase the solubility of Pd(II), $K_2[PdCl_4]$ was prepared. $^1$H NMR spectra were recorded on a Mercury-300 (300 MHz) spectrometer (a selection of data is given in Table 1). Elemental analyses (C, H, N) were performed on a Finnigan EA 1112 instrument. The atomic absorption spectra were recorded on an AA2600 atomic absorption spectrophotometer; UV-visible absorption spectra were recorded on a UV-2550 spectrometer; emission spectra, on a Perkin-Elmer LS55 fluorescence spectrofluorometer.

**Synthesis of complexes.** Complex (**1**). $K_2[PdCl_4]$, aqueous solution (10 mM, 10 ml), and bipy, ethanol solution (10 mM, 20 ml) were mixed with stirring. Then a KOH solution (0.5 M) was added to adjust pH until the turbid solution became clear at pH = 7.12. The mixture was stirred for over two hours at 40 °C and then the solution was left to evaporate at room temperature. After two weeks primrose yellow transparent crystals were obtained.

Complex (**2**). An aqueous solution (20 ml) of ade (0.5 mmol) was added dropwise to an ethanol/water solution of complex $[Pd(bipy)Cl_2]$ with continuous stirring. Then a KOH solution (0.5 M) was added to adjust pH to pH = 8.31. After filtering off a small amount of insoluble material, the resulting brown transparent solution was left to evaporate at room temperature. After one month brown crystals were isolated. Anal.: Found, %: C 39.29, H 3.23, N 21.22, Pd 23.17. Calcd, %: C 39.23, H 3.29, N 21.34, Pd 23.18.

**X-ray diffraction experiments.** Single crystals of complexes (**1**) and (**2**) were examined by X-ray diffraction method. The crystal structure of complex (**1**) turned out to be known [ 27 ] and is not discussed here.

A brown crystal of complex (**2**) with dimensions of 0.20×0.18×0.14 mm was mounted on a glass fiber. A total of 10414 reflections in the range of $4.14° < 2\theta < 52.8°$ were collected using ω-2θ scan on a Bruker Smart CCD X-ray single crystal diffractometer with Mo$K_\alpha$ radiation ($\lambda = 0.71073$ Å) at 293(2) K. 2076 observed reflections ($R_{int} = 0.0453$) were used in the structure determination and refinement.

The structure was solved by direct methods. All calculations and drawings were performed by Siemens SHELXTL crystallographic software package. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were found from the difference Fourier map and refined isotropically. Compound (**2**) forms orthorhombic crystals, space group *Pmmn*. Crystal structure data and refinement parameters are listed in Table 2. Selected interatomic distances and angles are given in Table 3. Full crystallographic data for complex (**2**) have been deposited with Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk), CCDC deposition number 653543. These data may be obtained free of charge via www.ccdc.cam.ac.uk/data_request.cif.

**DNA binding experiment.** The studies on the interaction of the metal complexes with DNA were carried out in a doubly distilled water buffer containing tris(hydroxymethyl)aminomethane (Tris)

T a b l e 1

*Chemical shifts* (ppm) *for* $H_a$—$H_d$ *protons in* $^1$H NMR *spectra of bipy and complex* (**1**)

| Compound | $H_a$ | $H_b$ | $H_c$ | $H_d$ |
|---|---|---|---|---|
| bipy | 8.714 | 8.417 | 7.968 | 7.470 |
| complex (**1**) | 9.115 | 8.585 | 8.360 | 7.810 |

T a b l e 2

*Crystal data and structure refinement parameters for complex* (**2**)

| Compound | $[Pd_2(bipy)_2(ade)_2]Cl_2 \cdot 3H_2O$ |
|---|---|
| Gross formula | $C_{30}H_{30}Cl_2N_{14}O_3Pd_2$ |
| $M$ | 459.19 |
| Crystal system | orthorhombic |
| Space group | *Pmmn* |
| Unit cell dimensions | |
| $a$, $b$, $c$, Å | 12.993(4), 14.512(5), 9.837(3) |
| $V$, Å$^3$ | 1854.8(11) |
| $Z$ | 2 |
| $D_{calc}$, g/cm$^3$ | 1.644 |
| $T$, K | 293(2) |
| $\mu(MoK_\alpha)$, mm$^{-1}$ | 1.166 |
| Observed unique reflections | 2076 |
| $[I > 2\sigma(I)]$ | |
| $R_{int}$ | 0.0453 |
| $R_1$ [reflections with $I > 2\sigma(I)$] | 0.0675 |
| $R_1$ [all data] | 0.0947 |
| CCDC deposition N | 653543 |

T a b l e 3

*Selected bond lengths* (Å) *and angles* (°) *for complex* (**2**)

| | | | |
|---|---|---|---|
| Pd(1)—N(2) | 2.020 | N(2)—Pd(1)—N(3) | 94.59 |
| Pd(1)—N(3) | 2.123 | N(2)—Pd(1)—N(4) | 81.32 |
| Pd(1)—N(4) | 2.020 | N(3)—Pd(1)—N(9A) | 89.40 |
| Pd(1)—N(9A) | 1.861 | N(4)—Pd(1)—N(9A) | 94.59 |

(5 mM) and sodium chloride (50 mM) and adjusted to pH = 7.2 with hydrochloric acid. The fluorescence spectra of the intercalation of the complex with FS-DNA have been assayed by previous procedures [ 28 ].

## RESULTS AND DISCUSSION

**$^1$H NMR spectra.** The $^1$H NMR spectral experimental data of complex (**1**) and bipy are compared in Table 1. It can be seen that the chemical shift of $H_a$, $H_b$, $H_c$ and $H_d$ (for labeling see Figure 1) in bipy are 8.714, 8.417, 7.968 and 7.470 ppm, as compared to 9.115, 8.585, 8.360 and 7.810 ppm for the corresponding protons in complex (**1**). The $H_a$—$H_d$ signals in complex (**1**) experience large shifts with respect to those in free bipy ligand showing that N-atoms of bipy are coordinated to Pd(II). It can be found from $^1$H NMR spectra that chemical shift for $H_9$ of ade is 12.872 ppm but this proton resonance peak disappeared in the complex (**2**) spectrum. These data are in good agreement with X-ray diffraction analysis showing that Pd(II) is coordinated with N9 of ade.

**Hydrolysis kinetics of complex (1).** The hydrolysis of complex (**1**) was studied by UV-visible absorption spectroscopy according to a literature method [ 29 ]; the spectra are illustrated in Figure 2. The hydrolyzation reaction proceeds in two steps:

$$[Pd(bipy)Cl_2] + H_2O \leftrightarrow [Pd(bipy)(Cl)(H_2O)]^+ + Cl^- \quad k_1$$

$$[Pd(bipy)(Cl)(H_2O)]^+ + H_2O \leftrightarrow [Pd(bipy)(H_2O)_2]^{2+} + Cl^- \quad k_2$$

Only the first step of the hydrolyzation reaction ($k_1$) was studied. The two chlorides of complex (**1**) are active groups prone to be gone; the active species $[Pd(bipy)(Cl)(H_2O)]^+$ must combine with a target molecule once being produced, so the rate of hydrolyzation could be an important factor in the binding the active complex with DNA. The study on the hydrolyzation reaction of this kind of palladium complex would help to understand the interaction mechanism with DNA from dynamic point of view. The hydrolyzation of $[Pd(N,N)L_2]$ [(N,N) = diamine] coordination complex, suppression dy-

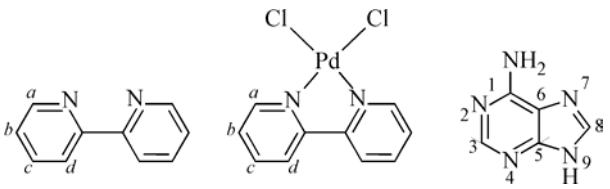


*Fig. 1.* Atom labeling schemes for bipy, complex (**1**) and complex (**2**)

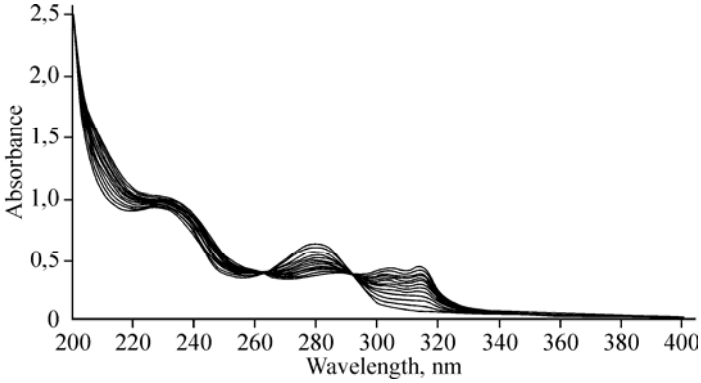


*Fig. 2.* The hydrolyzation reaction UV spectrum for complex (**1**)

namics of coordination saturation complex $[Pd(en)_2]^{2+}$ (or $[Pd(bpy)(en)]^{2+}$, $[Pd(4,4'\text{-}Me_2bpy)(en)]^{2+}$, $Pd(N,N'\text{-}Me_2en_2)]^{2+}$) have been extensively studied [ 30, 31 ]. The hydrolyzation reaction equation is $\ln(A_\infty - A_t) = \ln(A_\infty - A_0) - K_{obs}t$ ($A_t$, $A_0$ and $A_\infty$ are absorbencies when time is $t$, 0 and infinity, respectively). For complex (**1**), the relation between $\ln(A_\infty - A_t)$ and $t$ is linear and the speed constant ($k_1$) is $3.0 \times 10^{-4}$ s$^{-1}$. This hydrolyzation reaction rate indicates that $[Pd(bipy)(H_2O)_2]^{2+}$ is able to target a DNA molecule, blocking the division of DNA.

**DNA-binding study of complex (1).** The fluorescence spectra of complex (**1**) interacting with FS-DNA were studied according to previously developed procedures [ 32 ]. The spectra are shown in Figure 3.

The fluorescence intensity of ethidium bromide (EtBr, classical fluorescence probe [ 31 ]) itself is very faint, but it was shown to emit intense fluorescence light in the presence of DNA due to its intercalation into DNA between base pairs. Upon addition of a second molecule, which binds to DNA more strongly than EtBr, the DNA-induced EtBr emission would be quenched [ 34, 35 ], indicating the replacement of EtBr by the second molecule intercalated into DNA [ 36, 37 ]. The emission spectrum of EtBr bound to DNA in the absence and presence of complex (**1**) is given in Figure 3. It is clear from the Figure that the addition of complex (**1**) to DNA pretreated with EtBr causes appreciable reduction in the emission intensity. The higher the concentration of complex (**1**), the weaker the emission. When the ratio of $C_M/C_{DNA}$ was 0.3, the fluorescence intensity of EtBr·DNA dropped below ~50 % of the intensity observed in the absence of complex (**1**) indicating that complex (**1**) interacted with DNA strongly.

**Interaction of complex (1) with adenine.** In order to investigate the interaction of complex (**1**) with a DNA nucleobase, the crystal stucture of complex (**2**) was studied. The molecular structure of complex (**2**) is shown in Figure 4, together with atom numbering scheme. In the binuclear complex

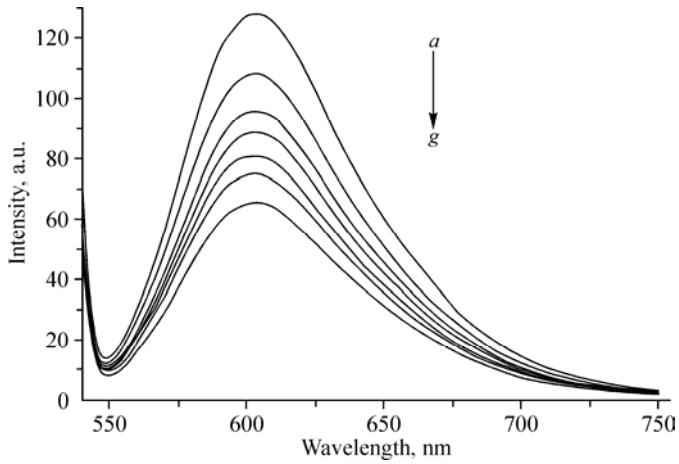


*Fig. 3.* Emission spectrum of EtBr bound to DNA in the absence and presence of complex (**1**) ($C_{EtBr} = 5\times10^{-5}$ M, $C_{DNA} = 5\times10^{-5}$ M, a → g: $C_{Complex}/C_{DNA} = 0$, 0.05, 0.1, 0.15, 0.20, 0.25, 0.30, $\lambda_{ex} = 527$ nm)

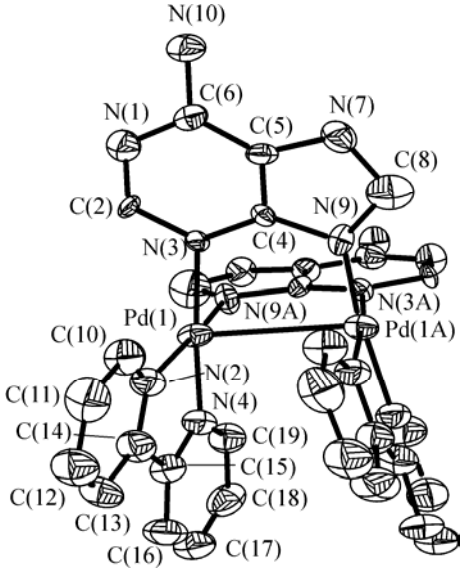


*Fig. 4.* Molecule structure of complex cation in (**2**)

cation, each Pd(II) is chelated by one bipy ligand (coordinated by N1, N4), while the two Pd(II) cations are bridged by two ade ligands (one coordinated by N3, N9A, another, by N9, N3A). From Table 3 it can be seen that Pd(II) has a square-planar (distorted to rectangular) coordination geometry. The distance Pd1—Pd1A is 3.061 Å, indicating chemical metal—metal interaction [ 38 ]. The distances for the corresponding C…C contacts between the two bipy moieties are 4.258, 4.713, 4.551, 3.883, 3.460, and 3.640 Å, the average distance being 4.084 Å, that may suggest intramolecular π—π stacking interaction which is slightly weaker than that (3.954 Å) previously observed in the complex $[Pd_2(phen)_2(ade)_2]Cl_2 \cdot 4.5H_2O$ [ 16 ].

Many studies have been reported dealing with the binding mechanism of platinum complexes to DNA [ 39 ] and recently a class of palladium complexes containing bipy ligand has been reported [ 40, 41 ] but the mechanism of its action is not completely understood. In this paper the study on the hydrolysis kinetics of complex (**1**), synthesis and characterization of complex (**2**) provide more insight into the binding mechanism of these palladium complexes with DNA.

## CONCLUSION

The palladium complex $[Pd(bipy)Cl_2]$ (**1**) has been synthesized, characterized and examined for the DNA-binding properties with the use of fluorescence spectra. The results support the fact that the complex binds to DNA in an intercalation mode. The reaction of complex (**1**) with ade yields $[Pd_2(bipy)_2(ade)_2]Cl_2 \cdot 3H_2O$ (**2**) whose crystal structure was determined by single crystal X-ray diffraction method. This study on the synthesis and crystal structure of complex (**2**) provides important information which could help to understand the mechanism of activity of the palladium complexes interacting with DNA.

## ACKNOWLEDGMENTS

We gratefully acknowledge the National Natural Science Foundation of China (No. 20671064), the Natural Science Foundation of Liaoning Province (No. 20052014), Foundation of Educational Department of Liaoning Province (No. 20060679), and Foundation of Liaoning Bai Qian Wan Talents Program (No. 2008921054).

*REFERENCES*

1. *Amo-Ochoa P., Alexandre S.S., Pastor C., Zamora F.* // J. Inorg. Biochem. – 2005. – **99**, N 11. – P. 2226.
2. *Zhao G.H., Lin H.K.* // Current Medicinal Chemistry — Anti-Cancer Agents. – 2005. – **5**, N 2. – P. 137.
3. *Liu X.-W., Li J., Li H. et al.* // J. Inorg. Biochem. – 2005. – **99**, N 12. – P. 2372.
4. *Collins G.G., Wheate N.J.* // Ibid. – 2004. – **98**, N 10. – P. 1578.
5. *Zhao G.H., Lin H.K., Yu P. et al.* // Ibid. – 1999. – **73**, N 3. – P. 145.
6. *Zuideveld M.-A., Swennenhuis B.H.-G., Boele M.D.-K. et al.* // Dalton Trans. – 2002. – N 11. – P. 2308.
7. *Onoa G.B., Moreno V., Freisinger E., Lippert B.* // J. Inorg. Biochem. – 2002. – **89**, N 3-4. – P. 237.
8. *Quiroga A.-G., Pérez J.-M., López-Solera I. et al.* // Ibid. – 1998. – **69**, N 4. – P. 275.
9. *Gao E.-J., Sun Y.-G., Liu Q.-T., Duan L.-Y.* // J. Coord. Chem. – 2006. – **59**, N 11. – P. 1295.
10. *Gao E.-J., Zhao S.-M., Zhan D., Liu Q.-T.* // Chin. J. Chem. – 2005. – **23**, N 1. – P. 54.
11. *Gao E.-J., Wang K.-H., Gu X.-F. et al.* // J. Inorg. Biochem. – 2007. – **101**, N 10. – P. 1404.
12. *Gao E.-J., Liu Q.-T.* // Acta Chim. Sinica. – 2002. – **60**, N 4. – P. 674.
13. *Gao E.-J., Zhao S.-M., Liu Q.-T., Xu R.* // Ibid. – 2004. – **62**, N 6. – P. 593.
14. *Gao E.-J., Zhao S.-M., Liu Q.-T.* // Chin. J. Inorg. Chem. – 2004. – **20**, N 2. – P. 191.
15. *Gao E.-J., Li H.-Y., Liu Q.-T.* // Acta Chim. Sinica. – 2005. – **63**, N 13. – P. 1225.
16. *Gao E.-J., Liu Q.-T.* // Ibid. – 2005. – **63**, N 8. – P. 725.
17. *Li V.S., Choi D., Wang Z. et al.* // J. Amer. Chem. Soc. – 1996. – **118**, N 10. – P. 2326.
18. *Zuber G., Quada Jr. J.C., Hecht S.M.* // Ibid. – 1998. – **120**, N 36. – P. 9368.
19. *Hecht S.M.* // Journal of Natural Products. – 2000. – **63**, N 1. – P. 158.
20. *Perrin L.C., Prenzler P.D., Cullinane C. et al.* // J. Inorg. Biochem. – 2000. – **81**, N 1-2. – P. 111.
21. *Fichtinger-Schepman A.M.J., Van der Veer J.L., Den Hartog J.H.J. et al.* // Biochemistry. – 1985. – **24**, N 3. – P. 707.
22. *Yang D.Z., Van Boom S.S.G.E., Reedijk J. et al.* // Ibid. – 1995. – **34**, N 39. – P. 12912.
23. *Malinge J.-M., Giraud-Panis M.-J., Leng M.* // J. Inorg. Biochem. – 1999. – **77**, N 1-2. – P. 23.

24. *Peleg-Shulman T., Katzhendler J., Gibson D.* // Ibid. – 2000. – **81**, N 4. – P. 313.
25. *Puthraya K.H., Srivastava T.S., Amonkar A.J. et al.* // Ibid. – 1986. – **26**, N 1. – P. 45.
26. *Khan B.T., Najmuddin K., Shansuddin S., Zakeeruddin S.M.* // Inorg. Chim. Acta. – 1990. – **170**, N 1. – P. 129.
27. *Canty A.J., Skelton B.W., Traill P.R., White A.H.* // Aust. J. Chem. – 1992. – **45**. – P. 417.
28. *Wang J., Zhang X.-D., Ling X. et al.* // J. Mol. Struct. – 2002. – **610**, N 1-3. – P. 151.
29. *Yang P., Gao F.* The Principles of Bioinorganic Chemistry. – 2002. – Science Press, Beijin. – P. 319.
30. *Cusumano M., Pietro M.L.D., Gianneto A. et al.* // J. Amer. Chem. Soc. – 2001. – **123**, N 9. – P. 1914.
31. *Bugarcic Z.D., Liehr G., Eldik R.* // Dalton Trans. – 2002. – **6**. – P. 951.
32. *Gao E.-J., Zhang D., Liu Q.-T.* // Acta Chim. Sinica. – 2003. – **61**, N 11. – P. 1834.
33. *Mansuri-Torshizi H., Mital R., Srivastava T.S. et al.* // J. Inorg. Biochem. – 1991. – **44**, N 4. – P. 239.
34. *Lakowicz, J.R., Weber G.* // Biochemistry. – 1973. – **12**, N 21. – P. 4161.
35. *Baguley B.C., LeBret M.* // Ibid. – 1984. – **23**, N 5. – P. 937.
36. *Liu C., Zhou J., Xu H.* // J. Inorg. Biochem. – 1998. – **71**, N 1-2. – P. 1.
37. *Hirohama T., Kuranuki Y., Ebina E. et al.* // Ibid. – 2005. – **99**, N 5. – P. 1205.
38. *Lai S.-W., Cheung T.-C., Chan M.C.W. et al.* // Inorg. Chem. – 2000. – **39**, N 2. – P. 255.
39. *Admiraal G., Alink M., Altona C. et al.* // J. Amer. Chem. Soc. – 1992. – **114**, N 3. – P. 930.
40. *Dorta R., Shimon L.J.W., Rozenberg H. et al.* // Inorg. Chem. – 2003. – **42**, N 10. – P. 3160.
41. *Yu S.-Y., Huang H.-P., Li S.-H. et al.* // Ibid. – 2005. – **44**, N 25. – P. 9471.