

$[trans-en_2Os(\eta-H_2)]^{2+}$ as an 1H NMR probe for the coordination of some anticancer metal complexes to deoxyguanotide*

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Abstract The molecular hydrogen complex of osmium, $[trans-en_2Os(\eta-H_2)]^{2+}$ (en, entylendiamine) is a new 1H NMR recognition probe for biomolecules. By using this probe, the coordination between deoxyguanylic acid (dGMP) and four metal anticancer complexes, titanocene dichloride, $(C_2H_5)_2SnCl_2(phen)$, $(C_2H_5)_2SnCl_2$ and cisplatin are studied in D_2O under the condition near the physiological one. The results show that the former three complexes mainly and strongly coordinate with the phosphate oxygen of dGMP, while cisplatin mainly coordinates with N(7) of dGMP.

Keywords: molecular hydrogen complex of osmium, 1H NMR probe, metal anticancer complex, deoxyguanylic acid.

The molecular hydrogen complex of osmium, $[trans-en_2Os(\eta-H_2)]^{2+}$ (see fig. 1) is a new 1H NMR recognition probe. Its advantages in recognition of biomolecules, such as nucleotides, RNA, amino acids, peptides and phospholipids, have been shown in a previous report^[1]. The sixth ligand L of $[trans-en_2Os(\eta-H_2)L]$ is a liable ligand, which can be replaced by a variety of biomolecules, and this makes Os directly coordinate with the O, N and S sites of biomolecules. Each coordination form (different biomolecular ligands or different coordination sites of the same ligand) leads to a characteristic 1H NMR chemical shift for the molecular hydrogen ligand of $[trans-en_2Os(\eta-H_2)L]$ that appears in a spectral window in the range $\delta = 0 - 20$, which can be easily distinguished. Therefore, $[trans-en_2Os(\eta-H_2)]^{2+}$ is an effective recognition probe for biomolecules. However, the application of this probe has not been widely developed. In this paper, we apply the probe to the detection of the coordination between metal anticancer complexes and nucleotide in aqueous solution.

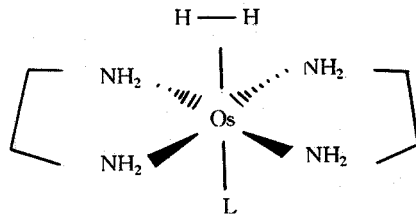


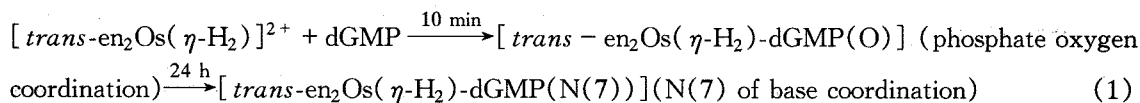
Fig. 1. Structure of the probe $[trans-en_2Os(\eta-H_2)L]$.

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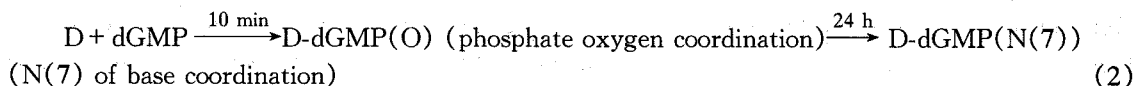
Many anticancer metal complexes function via the interaction with the DNA in its target cells. Therefore elucidating the coordination between the complex and deoxy-mononucleotides, the basic unit of DNA, especially the deoxyguanylic acid, is an important step in expounding the interaction mode between the complex and DNA. Cisplatin, titanocene dichloride and organotin complexes are three important anticancer complexes. The anticancer activity of cisplatin is mainly due to the coordination of Pt with N(7) of deoxyguanylic acid in DNA^[2,3]. It is inferred that the anticancer activities of titanocene dichloride^[4] and organotin complexes^[5] are also due to the interaction between each of the two complexes and DNA. Because of the high instability of titanocene dichloride and organotin complexes in aqueous solution, the coordination modes between either of the two and nucleotides in aqueous solution are still unknown^[6,7]. In this paper, using [*trans*-en₂Os(η -H₂)]²⁺ as an ¹H NMR probe, we study the aqueous coordination between deoxy-gaunotide and the above-mentioned three anticancer metal complexes, and try to provide evidence for the interaction modes between titanocene dichloride, organotin complexes and nucleotides.

1 Principle

The coordination between probe [*trans*-en₂Os(η -H₂)]²⁺ and dGMP has been studied in detail^[1]. The phosphate oxygen and N(7) of dGMP can both coordinate with the probe. The former coordination is of dynamic preference, the interaction is complete in 10 min after mixing, and the equilibrium quotient for the formation of the probe-RPO₄²⁻ complex is 3×10^2 , the characteristic peak appears at $\delta = -13.57$. The latter is of thermodynamic preference, the formation of the probe-N(7) (dGMP) is complete in 24 h after mixing and the equilibrium quotient is 2.9×10^3 , the characteristic peak appears at $\delta = -9.72$. Eq. (1) expresses the above interaction:



Suppose that anticancer metal complex (D) could bind dGMP via a similar process:



If a metal anticancer drug is added to the mixture of dGMP and the probe, the drug will compete the binding site (phosphate oxygen site or base N(7) site) of dGMP with the probe. The coordination of the drug with phosphate oxygen of dGMP will dispossess the complex [*trans*-en₂Os(η -H₂)-dGMP(O)] and cause the ¹H NMR peak at $\delta = -13.57$ to diminish or disappear. The coordination of the drug to N(7) of dGMP will dispossess the complex [*trans*-en₂Os(η -H₂)-dGMP(N)]²⁺ and lead to the NMR peak at $\delta = -9.72$ to diminish or disappear. Therefore, according to the changes of the two peaks, the site of drug bound to dGMP can be determined.

2 Materials and methods

The probe [*trans*-en₂Os(η -H₂)] [CF₃SO₃] was synthesized according to ref. [8]. Cp₂TiCl₂, Et₂SnCl₂ and Et₂SnCl₂(Phen) were prepared and identified according to literature methods^[9-11]. Cisplatin was purchased from Shandong Qilu Pharmacy Company, and 2'-deoxyguanosine-5'-

monophosphate (dGMP) was from the Sigma Company. The other reagents are of A. R. grade.

The probe, drug and dGMP were mixed at the ratio of 1:1:1 (each being 0.01 mol/L, $\text{pD} = 7.0$) in D_2O in the absence of O_2 . ^1H NMR spectra of the mixture were recorded at certain intervals on a Bruke AM-500 MHz NMR spectrometer, using DSS as the internal standard.

3 Results and discussion

3.1 Coordination between probe and dGMP

The interaction process between the probe and dGMP was similar to that described in an earlier report^[1]. Ten minutes after mixing, the probe mainly coordinates with the phosphate group of dGMP and gives a characteristic doublet at $\delta = -13.56$ and -13.57 . Meanwhile, two small peaks at $\delta = -9.72$ and $\delta = -13.18$ are already discernible after 10 min, which are assigned to N(7)-binding and D_2O binding, respectively. 24 h later, the coordination of the probe with N(7) of dGMP is complete, and only two peaks at $\delta = -9.72$ and $\delta = -7.74$ exist in the range from 0 to -20 , the latter is assigned to the *cis* form of N(7)-binding.

3.2 Coordination between TDC and dGMP

It can be seen from fig. 2 that the original peak at $\delta = -13.57$, corresponding to the singal of phosphate binding to the probe, disappears completely 10 min after mixing of the three species; while the signals of N(7)-binding and D_2O -binding change little. Meanwhile, a new peak at $\delta = -13.21$ appears, which is attributed to Cl^- as the coordinate species¹⁾. The NMR signals 24 h after mixing are almost the same as those after 10 min. The above results indicate that the phosphate oxygen site of dGMP is occupied by TDC, therefore it cannot coordinate with the probe. The ^1H NMR signal corresponding to the probe coordination with N(7) of dGMP is not affected, suggesting N(7) of dGMP still coordinates with the probe but not with TDC. The peak at $\delta = -13.21$, which is ascribed to the probe coordination with Cl^- , was due to Cl^- -hydrolysed from TDC.

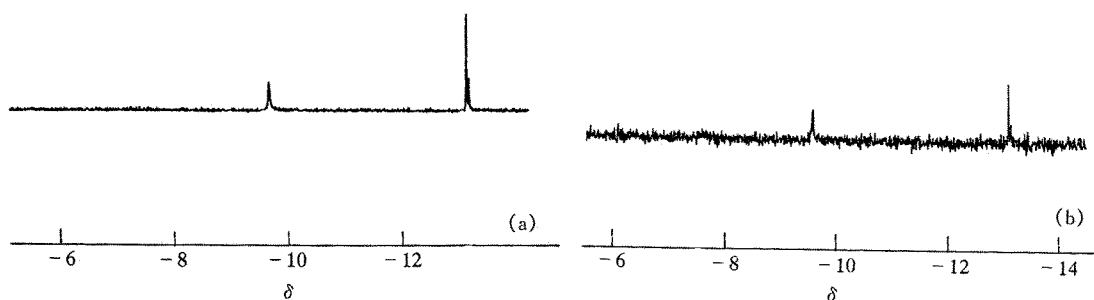


Fig. 2. ^1H NMR spectra of the probe with TDC and dGMP in D_2O . (a) 10 min, (b) 24 h.

1) Unpublished results of our laboratory.

3.3 Coordination between $\text{Et}_2\text{SnCl}_2(\text{phen})$ and dGMP

As shown in fig. 3, the original peak at $\delta = -13.56$ diminishes obviously in intensity in contrast to that of the mixture of dGMP and the probe 10 min after the mixing of the three species, and disappears after 6 h. A new peak at $\delta = -13.15$ grows after 10 min. This is because, in the mixed system, the drug and the probe competitively coordinate with dGMP. Since Sn(IV) forms a hard Lewis acid, while Os(II) does a soft Lewis acid, the coordination affinity to oxygen for Sn(IV) is stronger than that for Os(II) , the replacement of binding of Os-phosphate by Sn-phosphate causes the NMR peak at -13.56 to disappear 6 h later. The appearance of the new peak at $\delta = -13.15$ is due to the coordination of Cl^- with Os(II) . This confirms that the chloride atom of $\text{Et}_2\text{SnCl}_2(\text{phen})$ is replaced in the interaction. Compared with the probe-dGMP system, in the mixed system, the peak at $\delta = -9.67$ corresponding to the N(7)-binding of the probe seems not to be affected by $\text{Et}_2\text{SnCl}_2(\text{phen})$ during the reaction process. The new peak at $\delta = -7.85$ appearing 24 h later is assigned to the *cis* form of the probe-dGMP (N(7)), suggesting that the binding of probe-dGMP(N(7)) is not disturbed by $\text{Et}_2\text{SnCl}_2(\text{phen})$. The above results indicate that Sn(IV) is bound to the phosphate oxygen of dGMP but not to N(7) of dGMP.

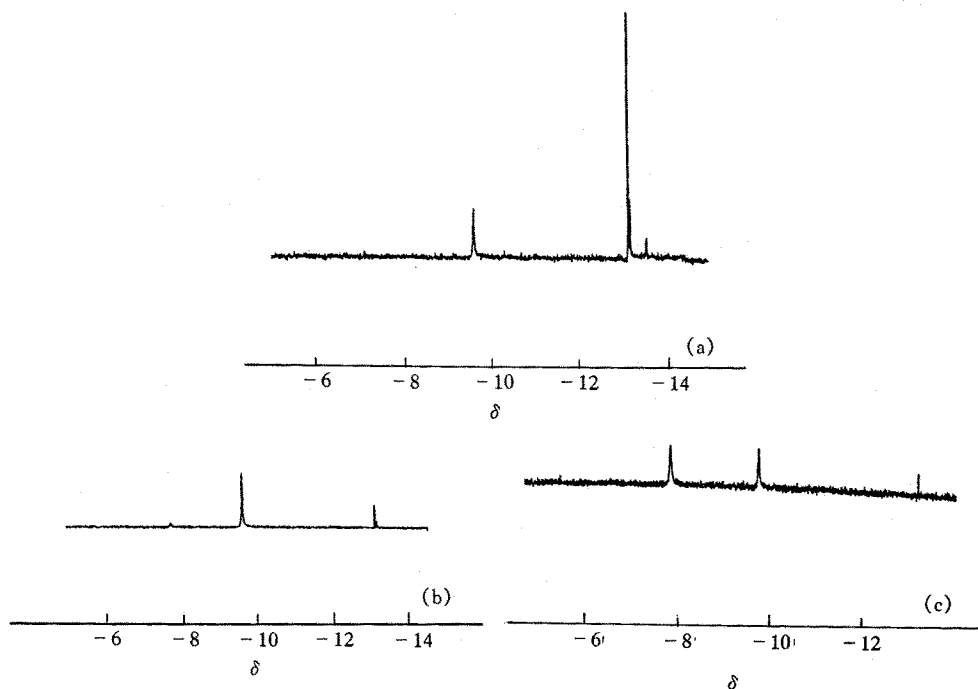


Fig. 3. ^1H NMR spectra of the probe with $\text{Et}_2\text{SnCl}_2(\text{phen})$ and dGMP in D_2O . (a) 10 min, (b) 6 h, (c) 24 h.

3.4 Coordination between Et_2SnCl_2 and dGMP

As shown in fig. 4, the original peak at $\delta = -13.56$, corresponding to the signal of phosphate bound to the probe disappeared after 10 min. This suggests that probe-dGMP(O) is replaced by Sn-dGMP(O). Similar to that of $\text{Et}_2\text{SnCl}_2(\text{phen})$ -dGMP system, a new peak at $\delta =$

-13.17 appeared 10 min later, indicating that the chloride atom of Et_2SnCl_2 has been hydrolyzed. Moreover, the peak at $\delta = -9.68$ corresponding to the N(7)-binding of the probe is affected little by Et_2SnCl_2 during the reaction process, suggesting no coordination of Sn(IV) with N(7) of dGMP.

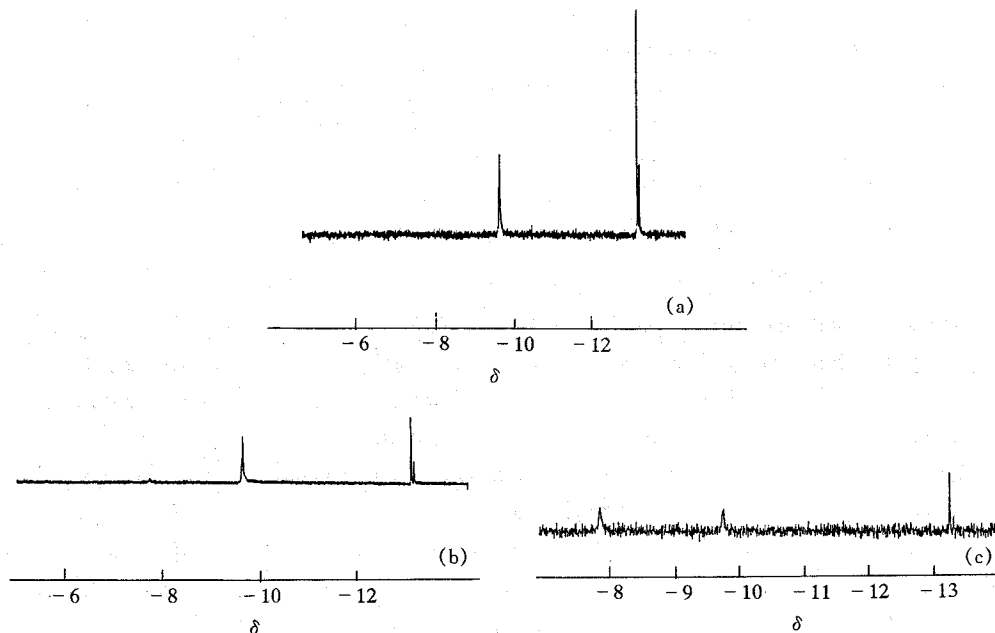


Fig. 4. ^1H NMR spectra of the probe with Et_2SnCl_2 and dGMP in D_2O . (a) 10 min, (b) 6 h, (c) 24 h.

3.5 Coordination between cisplatin and dGMP

The coordination between cisplatin and dGMP or GMP in aqueous solution has been studied widely^[12,13]. The results show that platinum mainly coordinated with N(7) of guanosine. A main goal of this section is to test and verify the method used in this paper. Fig. 5 shows the ^1H NMR spectra of the interaction among cisplatin-probe-dGMP. It can be seen from fig. 5 that the peaks at $\delta = -13.57$ (corresponding to probe-dGMP(O)) and $\delta = -13.18$ (corresponding to probe- D_2O) were affected little after 10 min of mixing; while the peak at $\delta = -9.74$ (corresponding to probe-dGMP (N(7))) was obviously diminished. This indicates that the competition between cisplatin and the probe lies in binding to the N(7) site of dGMP but not to the phosphate oxygen site. 24 h later, the peak of N(7) binding was still very small, and the characteristic peak at $\delta = -7.75$ of *cis*-probe-dGMP(N(7)) did not appear yet. This implies that cisplatin inhibits the coordination of the probe with N(7) of dGMP. The above results show that cisplatin binds to N(7) but not to phosphate oxygen of dGMP, which is the same as the results of refs. [12, 13], confirming the reliability of methods being used in this paper.

A comparison between the binding modes of the four anticancer complexes to dGMP shows that the modes of the former three are similar; they all bind to dGMP by the metal center coordinating with the phosphate oxygen, with the chloric atoms left. But cisplatin binds to N(7) of dGMP with the chloric atoms leaving. The central metals of the former three complexes with Ti

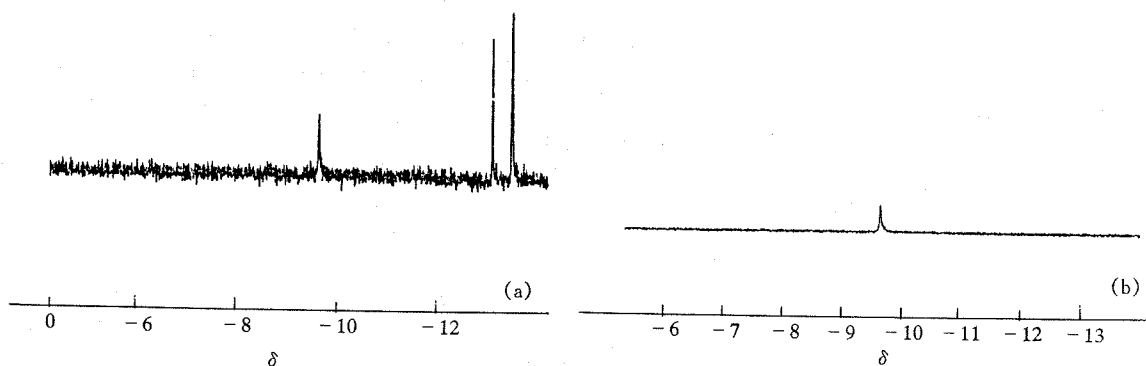


Fig. 5. ^1H NMR spectra of the probe with cisplatin and dGMP in D_2O . (a) 10 min, (b) 24 h.

and Sn being +4 valence form typical hard Lewis acids. They have high affinities to phosphate oxygen, a typical hard Lewis base. So the interactions between titanocene dichloride or Et_2SnCl_2 and dGMP are very fast and the coordination are completed in 10 min, while the interaction between $\text{Et}_2\text{SnCl}_2(\text{phen})$ and dGMP is quite slow, the coordination is complete in 6 h. This may be due to the fact that the hard Lewis acid activity of Sn(IV) in $\text{Et}_2\text{SnCl}_2(\text{phen})$ becomes softer than that in Et_2SnCl_2 because of the coordination of phenanthroline with Et_2SnCl_2 . Therefore, the affinity of Sn(IV) in $\text{Et}_2\text{SnCl}_2(\text{phen})$ to the dinegative phosphate is not as high as that in Et_2SnCl_2 . In addition, the planar phenanthroline may still coordinate with Sn(IV) after the formation of Sn-dGMP; whereas the central metal in cisplatin is of +2 valence, a typical soft Lewis acid, it may easily bind to N(7), a soft Lewis base. So it binds to N(7) in a competition with the probe, while it has few effects on the probe-dGMP(O) binding.

The above results show that it is feasible to use $[\text{trans-}en_2\text{Os}(\eta\text{-H}_2)]^{2+}$ as an ^1H NMR probe to study the binding modes between metal anticancer agents and nucleotides. The equilibrium quotient for phosphate oxygen binding is 3×10^2 and that for N(7) binding is 2.9×10^3 , and the former shows preference to a dynamic interaction. It is easy to identify the characteristic signals between phosphate binding and N(7) binding. And the probe has a good solubility in water. All the above merits of the probe make it a useful aqueous recognition ^1H NMR probe. By now, there has been still no other effective methods for detecting the coordination modes between the phosphate oxygen of nucleotide and metal ions. Although ^{31}P NMR provide a path, some metals coordinating with phosphate oxygen of nucleotide cause only a little change in ^{31}P NMR chemical shift. In some cases, the changes of chemical shift are less than 1, and it is hard to ascribe such a change to which one of the following: the change in H-bonds, the nucleotide conformational change or the metal coordinating with nucleotides. So ^{31}P NMR has its limitation^[14,15]. But it is easy to identify this coordination by means of the probe.

The aqueous coordination chemistry between nucleotides and titanocene dichloride or organotin complexes is still unknown due to the high instability in aqueous solution of the metal complexes. This paper provides for the first time the evidence for the coordination between the complex and dGMP. The results show that Ti or Sn mainly coordinates with the phosphate oxygen of nucleotide in a stable and powerful manner. Unlike that of cisplatin, it is difficult for Ti or Sn to coordinate with N(7). But the results in this paper do not exclude the possibility of weak

coordination between N(7) and Ti or Sn. It needs to be further investigated whether the stable coordination between phosphate oxygen of dGMP and Ti or Sn contributes to the anticancer activity of these agents.

References

- 1 Li, Z. W., Taube, H., Use of a dihydrogen osmium complex as a versatile ^1H NMR recognition probe, *Science*, 1992, 256; 210.
- 2 Sundquist, W. I., Lippard, S. J., The coordination chemistry of platinum anticancer drugs and related compounds with DNA, *Coord. Chem. Rev.*, 1990, 100; 293.
- 3 Umaphathy, P., The chemical and biochemical consequences of the binding of the antitumor drug cisplatin and other platinum group metal complexes to DNA, *Coord. Chem. Rev.*, 1989, 95; 129.
- 4 Koepf-Maier, P., Koepf, H., Non-platinum-group metal antitumor agents: history, current status and perspectives, *Chem. Rev.*, 1987, 87; 1137.
- 5 Saxena, A. K., Organotin compounds and cancer chemotherapy, *Coord. Chem. Rev.*, 1989, 95; 109.
- 6 Toney, J. H., Marks, T. J., Hydrolysis chemistry of the metallocene dichlorides $\text{M}(\eta\text{-C}_5\text{H}_5)_2\text{Cl}_2\text{Cl}_2$, $\text{M} = \text{Ti, V, Zr}$: Aqueous kinetics, equilibria, and mechanistic implications for a new class of antitumor agents, *J. Am. Chem. Soc.*, 1985, 107; 947.
- 7 Kuo, L. Y., Kanatzidis, M. G., Sabat, M. *et al.*, Metallocene antitumor agents: Solution and solid-state molybdenocene coordination chemistry of DNA constituents, *J. Am. Chem. Soc.*, 1991, 113; 9027.
- 8 Li, Z. W., Taube, H., Modulation of physical and chemical properties of $\eta\text{-H}_2$ complexes of osmium amines by facile substitution, *J. Am. Chem. Soc.*, 113; 8946.
- 9 Wilkinson, G., Birningham, J. M., Bis-cyclopentadienyl compounds of Ti, Zr, V, Nb and Ta, *J. Am. Chem. Soc.*, 1954, 76; 4281.
- 10 Crowe, A. J., Smith, P. J., The synthesis and tin-119m Mössbauer spectra of some diorganotin dihalide and dipseudohalide complexes with nitrogen- and oxygen-donor ligands, *J. Organomet. Chem.*, 1982, 224; 223.
- 11 Crowe, A. J., Smith, P. J., Atassi, G., Investigation into the antitumor activity of organotin compounds (I): Diorganotin dihalides and dipseudohalide complexes, *Chem-Bio. Interact.*, 1980, 32; 171.
- 12 Inagaki, K., Dijt, F. J., Lempers, E. L. M. *et al.*, Bulky ligand substituent effect on the reaction of 5'-GMP with $\text{Pt}(1, 3\text{-diamine})$: Rotation of 5'-GMP about the Pt-N bond and kinetic effects, *Inorg. Chem.*, 1988, 27; 382.
- 13 Okamoto, K., Behnam, V., Phanviet, M. T. *et al.*, FT-IR and ^1H NMR spectroscopic studies of C2'-endo, C3'-endo sugar ring conformations in 5'-GMP and 3'-GMP nucleotides and their platinum complexes, *Inorg. Chim. Acta*, 1986, 123; L3.
- 14 Marzilli, L. G., Reily, M. D., Heyl, R. B. *et al.*, Evidence for similar structural changes on binding of platinum anti-tumor agents to DNA and nucleosomes, *FEBS*, 1984, 176; 389.
- 15 Toney, J. H., Brock, C. P., Marks, T. J., Aqueous coordination chemistry of vanadocene dichloride, $\text{V}(\eta\text{-C}_5\text{H}_5)_2\text{Cl}_2\text{Cl}_2$, with nucleotides and phosphoesters; Mechanistic implication for a new class of antitumor agents, *J. Am. Chem. Soc.*, 1986, 108; 7263.