



Binding sites of $cis\text{-Ru}^{\text{II}}\text{Cl}_2(\text{DMSO})_4$ to mononucleotides: ^1H and ^{31}P NMR evidence

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(Received 30 July 1996; accepted 28 October 1996)

Abstract—The binding modes of $cis\text{-Ru}^{\text{II}}\text{Cl}_2(\text{DMSO})_5(\text{DMSO})$ ($cis\text{-RDT}$, three out of the four DMSO's are S-bonded to the Ru, while the last one is O-bonded) with 5'-GMP and 5'-AMP in aqueous solution at physiological pH value were investigated by high-resolution ^1H and ^{31}P NMR. A ^1H NMR recognition probe, $[\text{trans-}\eta\text{-Os}(\eta\text{-H}_2)](\text{CF}_3\text{SO}_3)_2$, was used in order to verify further the results. 5'-GMP with $cis\text{-RDT}$ formed two products, clearly indicating that the guanine N_7 and phosphate group bind to Ru^{II} and form a chelate to the metal center; but binding of 5'-AMP to $cis\text{-RDT}$ may only occur *via* the phosphate group. © 1997 Elsevier Science Ltd. All rights reserved.

Keywords: antitumor compound; ruthenium complex; mononucleotides; binding mode; NMR recognition probe; NMR spectra.

Among the various anticancer metal compounds, ruthenium complexes have shown good antitumor activity [1–3] and a remarkably low toxicity [4], which makes them interesting for possible clinical use [5]. After the discovery of the mutagenic properties [6] and good antineoplastic activity [4] of $cis\text{-RDT}$, which suggested that one target of the complex *in vivo* is DNA, many efforts have been made to understand its mechanism of action and to investigate its binding sites to DNA [7]. Despite the success observed with the change of the CD spectrum of poly(dGdC) and poly(dAdT) with $cis\text{-RDT}$ [8], the binding sites suggested, N_7 of guanine and N_7 of adenine, are only a deduction.

In this paper we present the reaction of $cis\text{-RDT}$ with 5'-GMP and 5'-AMP in aqueous solution under physiological conditions using the NMR method. In contrast to previous investigations [9,10], we have demonstrated that 5'-GMP can coordinate to the initially achiral $cis\text{-RDT}$ antitumor agent to form, predominantly, two isomers which have an opposite chirality at Ru^{II} . In a diastereomeric pair of isomers the nucleotide chelates to Ru by N_7 and a phosphate

oxygen. We have also demonstrated that 5'-AMP can coordinate to $cis\text{-RDT}$ through the phosphate group, but the binding of 5'-AMP's N_7 with $cis\text{-RDT}$ was not observed.

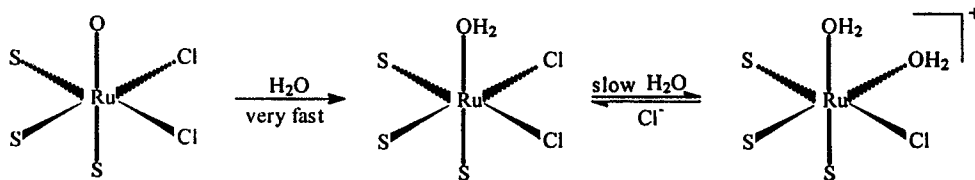
EXPERIMENTAL

$cis\text{-RDT}$ was synthesized as already reported [11] and recrystallized from a DMSO/acetone mixture.

The nucleotides, adenosine-5'-monophosphate (AMP), guanosine-5'-monophosphate (GMP) and 2'-deoxyguanosine-5'-monophosphate (dGMP) were purchased from Sigma Chemical Company, purified by precipitation from an aqueous solution and purity was verified with ^1H and ^{31}P NMR spectroscopy. The probe, $[\text{trans-}\eta\text{-Os}(\eta\text{-H}_2)](\text{CF}_3\text{SO}_3)_2$, was a generous gift from Professor Henry Taube and Dr Zeiwei Li, Stanford University, CA, and its purity was checked by ^1H NMR spectroscopy.

Proton and phosphorus NMR were recorded on a VXR-300 MHz NMR spectrometer in D_2O , proton chemical shifts are referenced to DSS as internal standard, and phosphorus chemical shifts are referenced to 85% H_3PO_4 as external standard. The probe, $cis\text{-RDT}$ and the mononucleotide were mixed as 1 : 1 : 1

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Scheme 1.

(each 0.01 mol L⁻¹, pD 7.0) in oxygen-free D₂O, D₂O was thoroughly saturated with nitrogen before use. pH measurements were carried out with an American Scientific pH 1 meter and a Wilmad combination pH electrode. All reported pD values in D₂O are corrected pH readings (pD = pH + 0.44).

RESULTS AND DISCUSSION

The octahedral complex *cis*-RDT dissolved in water shows the dissociation mechanism reported [8] in Scheme 1.

As shown above, *cis*-RDT, once dissolved in water, immediately releases the O-bonded dimethyl sulfoxide molecule, this step is followed by the slow dissociation of a Cl⁻ anion to give the cationic species.

The NMR spectroscopic data for *cis*-RDT with 5'-GMP and 5'-AMP are shown in Table 1.

When *cis*-RDT (0.01 mol L⁻¹) was incubated with 5'-GMP in a 1 : 1 molar ratio in D₂O at 25 °C, examination of reaction mixtures by ¹H NMR (Fig. 1c) revealed two new major species (**I** and **II**) after 12 h at pD7.0; species **I** and **II** exist in nearly equal amounts and 5'-GMP is nearly all consumed. These suggest that the ratio of 5'-GMP to Ru in the products is 1 : 1. The downfield position of the H₈ signals, δ 8.00 for **I** and 8.20 for **II**, and the downfield position of the H₁, resonance of the sugar, δ 5.78 for **I** and δ 5.97 for **II**, suggests N₇ coordination [12].

The ³¹P NMR spectrum also reveals consumption of the 5'-GMP, but, more importantly, an unusually far downfield position of the two signals (δ11.29 for **I** and 12.90 for **II**) establishes phosphate group coordination [10,13] (Fig. 1d). The above results are very similar to those for 5'-GMP with *trans*-RDT. A comparison of *cis*-RDT-5'-GMP with *trans*-RDT-5'-GMP is shown in Table 2, and suggests that **I** and **II** are chelate complexes with the N₇ and phosphate

group of 5'-GMP bound to the same Ru^{II} center, and that the two species are diastereomers with the same Ru coordination environment, differing primarily in the orientation of the chelate moiety, as shown in Scheme 2.

When *cis*-RDT (0.01 mol L⁻¹) was incubated with 5'-AMP under the same conditions as 5'-GMP, an upfield shift of *ca* 0.1 ppm for H₈ and *ca* 1.7 ppm for ³¹P signals, but almost unchanged H₂ signals, were observed (Table 1). Protonation of the base function of a nucleotide should cause a downfield shift of the nucleobase proton signals, whilst protonation on the phosphate group should cause an upfield shift of the nucleobase proton signals [14]. Also, diamagnetic metallation of a nucleoside produces effects quite similar to protonation [15]. So, the slight upfield shift of H₈ could be a consequence of the direct phosphate coordination to ruthenium.

In order to further examine the sites of *cis*-RDT at 5'-GMP and 5'-AMP, we used [*trans*-en₂Os(η-H₂)]²⁺ (Scheme 3) as a ¹H NMR recognition probe. The probe, as previously demonstrated [16], binds readily to a variety of biomolecules such as nucleotides, with its sixth ligand, L, substituted by these biomolecules, which results in the direct coordination of Os^{II} to the donor atoms (such as N, O) of the biomolecules. In each case the binding leads to a characteristic ¹H NMR spectrum for the dihydrogen unit that appears in a spectral window in the range δ = 0 to -20.

When 5'-dGMP was added to a solution of the probe in D₂O at 25°C each solute at 0.01 mol L⁻¹, the phosphate oxygen and N₇ of dGMP both coordinated to the probe, the former being dynamically preferred, and the interaction was complete in 10 min. The equilibrium quotient (*K*) for the formation of Os^{II}-RPO₄ (dGMP) is 3 × 10², while the latter is thermodynamically preferred, the binding of Os^{II}-N₇(dGMP) is complete in 24 h, and the value of *K* is

Table 1. NMR spectroscopic data (ppm) for *cis*-RDT with 5'-GMP and 5'-AMP (pD 7.0)

Compound	H ₈	H ₂	P
5'-GMP	7.97		3.14
5'-GMP- <i>cis</i> -RDT	8.20, 8.00		12.90, 11.29
5'-AMP	8.39	8.02	3.76
5'-AMP- <i>cis</i> -RDT	8.29	8.04	2.06

*¹H chemical shift *vs* DSS and ³¹P chemical shift *vs* 85% H₃PO₄.

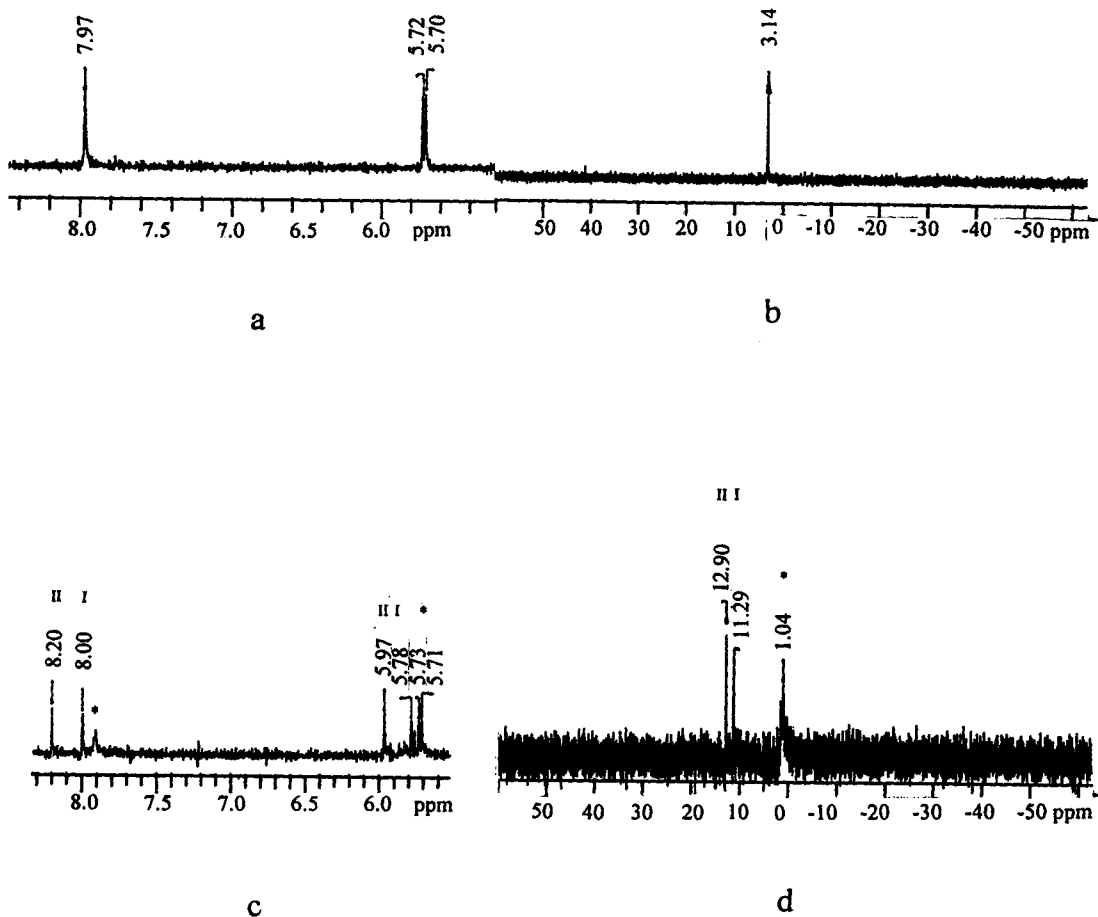
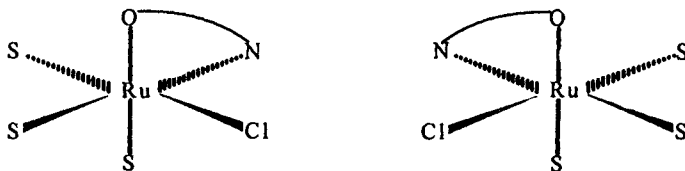


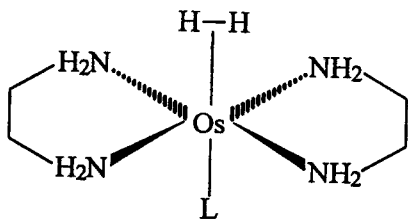
Fig. 1. 2'-Guanosine-5'-monophosphate (5'-GMP) with *cis*-RDT in D₂O as monitored by 300 MHz ¹H and ³¹P NMR spectroscopy. Asterisk(*) represent signals of free 5'-GMP. (a) ¹H NMR of free 5'-GMP. (b) ³¹P NMR of free 5'-GMP. (c) ¹H NMR of 5'-GMP-*cis*-RDT. (d) ³¹P NMR of 5'-GMP-*cis*-RDT.

Table 2. A comparison of NMR chemical shift data (ppm) for *cis*-RDT and *trans*-RDT with 5'-GMP

Compound	H ₈	P	Ref.
<i>cis</i> -RDT-5'-GMP	8.20, 8.00	12.90, 11.29	This work
<i>trans</i> -RDT-5'-GMP	8.43, 8.31	9.69, 8.21	9



Scheme 2.



Scheme 3.

2.9×10^3 . Figure 2 clearly shows the reaction process. The peaks at $\delta -13.38$ and -13.76 grow in 10 min. The second peak is due to phosphate oxygen binding. Meanwhile, a peak at $\delta -9.93$, already discernible after 10 min (Fig 2a), continues to grow at the expense of the others. This peak is assigned to the N_7 binding. There is a competing reaction between D_2O binding, phosphate binding and N_7 binding, which is not in complete equilibrium in 10 min. The affinity of Os^{II} for

the D_2O and phosphate is not high and the conversion from D_2O binding and phosphate binding to N_7 binding is almost complete after 24 h (Fig. 2b). Usually the binding of an antitumor metallic agent to dGMP is also *via* N_7 or the phosphate group of dGMP; therefore, if an antitumor metal agent is added to the probe-dGMP binary system, it will compete for these binding sites of dGMP with the probe, and the characteristic peaks due to binding of the probe with phosphate or N_7 of dGMP will change. According to these changes, the binding sites of the antitumor metal complexes to dGMP can be determined.

When dGMP, *cis*-RDT and the probe were mixed at the same time at $25^\circ C$, both of the peaks due to the phosphate binding and N_7 binding diminished obviously in their intensity after 10 min (Fig. 2c) compared with those of the dGMP-probe binary system. After 24 h the peaks for D_2O binding were still present, indicating that N_7 binding of *cis*-RDT has restrained the conversion from D_2O binding to N_7 binding of the

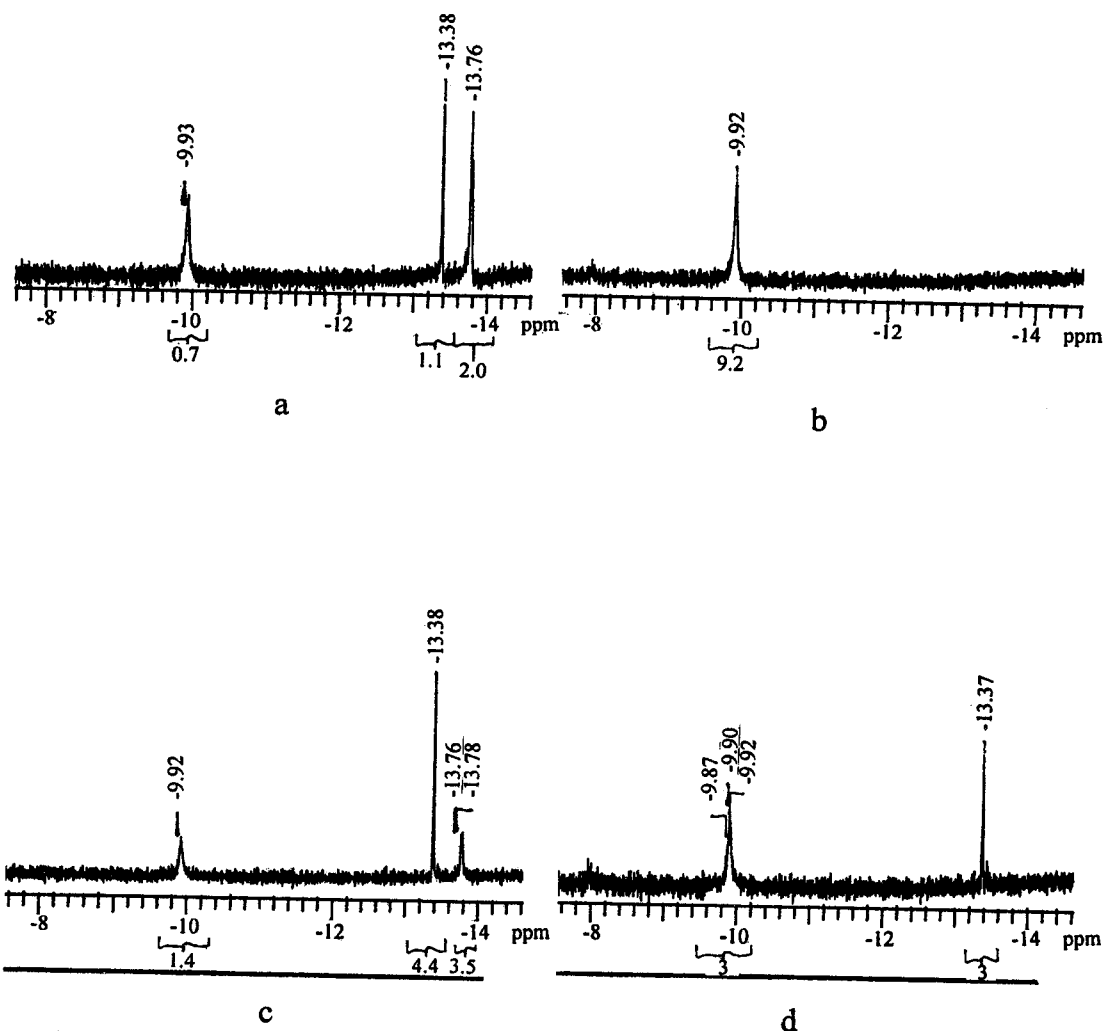


Fig. 2. 1H NMR spectra (300 MHz) of 5'-dGMP mixed system. (a) Probe with dGMP after 10 min. (b) Probe with dGMP after 24 h. (c) Probe, dGMP and *cis*-RDT after 10 min. (d) Probe, dGMP and *cis*-RDT after 24 h.

probe (Fig. 2d). This result provides further evidence of N₇ and phosphate group coordination.

The interaction process between the probe and AMP is shown in Fig. 3a and b. Because the *K* value for the formation of Os^{II}-PO₄-AMP is 1.9×10^2 , much higher than the corresponding *K* for the formation of Os^{II}-N₇-AMP, which is 1.2×10 [16], the dominant signal in the AMP-probe system is that of phosphate binding, which appears at $\delta -13.77$, while the signal for N₇ binding is too weak to be observed. It can be seen from Fig. 3c that *cis*-RDT mixed into the AMP-probe system results in the loss of the peak at $\delta -13.77$ after 10 min in contrast to the AMP-probe binary system. After 24 h, the peak due to the

phosphate binding has disappeared completely (Fig. 3d). These results suggest that *cis*-RDT can bind to AMP *via* the phosphate oxygen.

³¹P NMR is a commonly used method to investigate the direct coordination of metal ions to mononucleotides *via* phosphate group [17]. In particular, the case of direct binding leads to a large ³¹P NMR shift. However, in some cases, the binding of metal ions to phosphate group of mononucleotides, owing to the possible conformation changes or H bond changes in the mononucleotides caused by metal ions, only leads to a small ³¹P NMR shift (less than 1 ppm) [8,19]. It is difficult to determine which form mentioned above should be responsible for so little ³¹P NMR changes

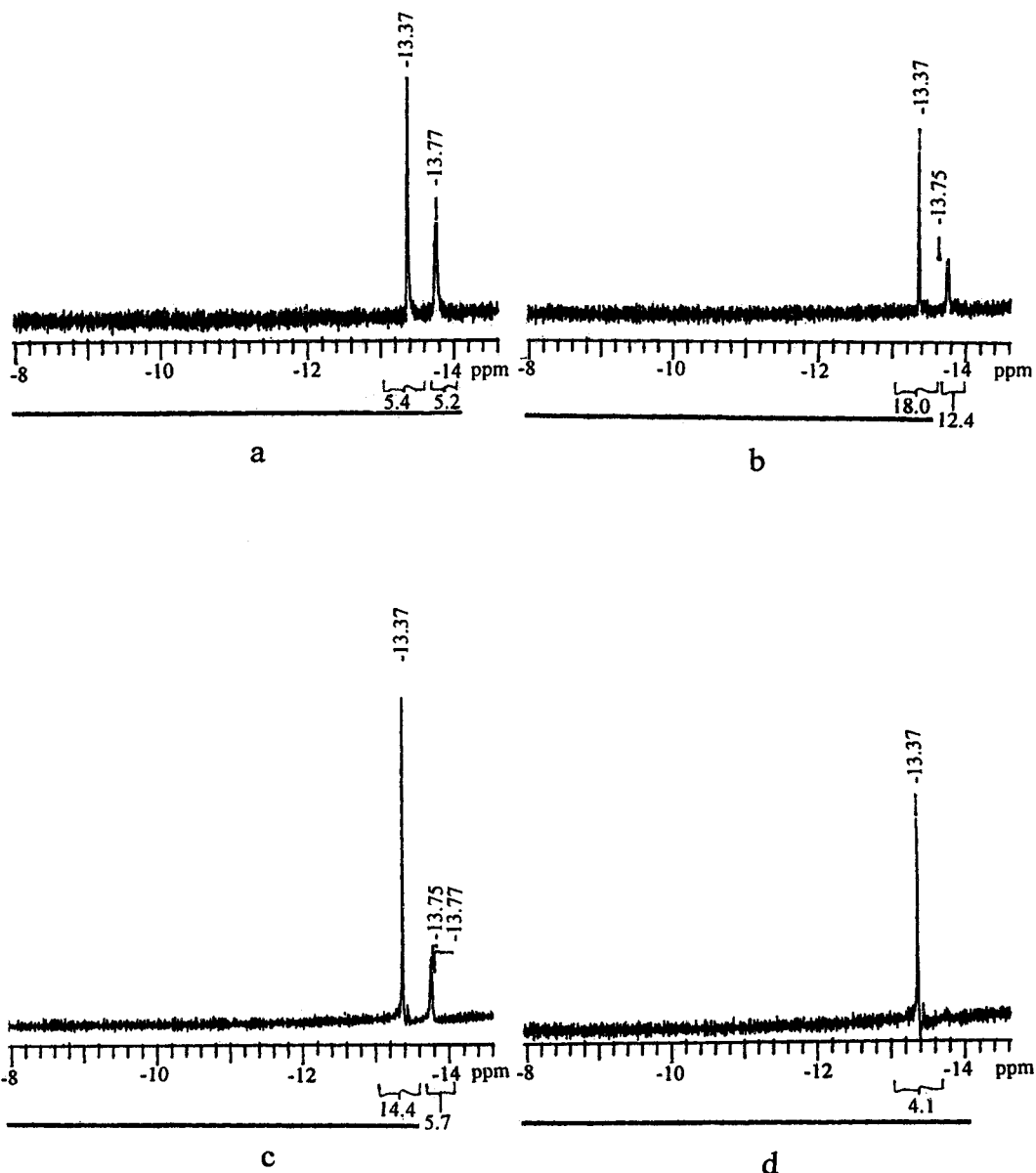


Fig. 3. ³¹P NMR spectra (300 MHz) of 5'-AMP mixed system. (a) Probe with AMP after 10 min. (b) Probe with AMP after 24 h. (c) Probe, AMP and *cis*-RDT after 10 min. (d) Probe, AMP and *cis*-RDT after 24 h.

in the case of *cis*-RDT with 5'-AMP, whereas, as demonstrated above, it is easy to distinguish the direct coordination *via* phosphate group using the probe.

As discussed above, the binding mode of *cis*-RDT to AMP is different from that of *cis*-RDT to GMP. The coordination sites are both the phosphate group and N₇ between *cis*-RDT and GMP, but the coordination sites between *cis*-RDT and AMP may be only the phosphate group.

Acknowledgements—The financial support of the National Natural Science Foundation of China and the National Research Laboratories of Natural and Biomimetic Drugs are gratefully acknowledged. We wish to thank Professor Henry Taube and Dr Zaiwei Li for their generous gift of the probe.

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