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Registry No. d(TCTCGGTCTC), 88904-80-7; d(GAGACCGAGA), 88904-81-8; d(TCTCGGTCTC)-d(GAGACCGAGA), 88904-82-9; *cis*-$Pt(NH_3)_2Cl_2$, 15663-27-1; $Pt(en)Cl_2$, 14096-51-6; *cis*-$Pt(MeNH_2)_2Cl_2$, 15273-32-2; *cis*-$Pt(NH_3)_2$[d(TCTCGGTCTC)-N7(5),N7(6)], 98478-95-6; Pt(en)[d(TCTCGGTCTC)-N7(5),N7(6)], 121808-76-2; *cis*-$Pt(MeNH_2)_2$[d(TCTCGGTCTC)-N7(5),N7(6)], 121808-77-3; *cis*-$Pt(NH_3)_2$[d(TCTCGGTCTC)-N7(5),N7(6)]-d(GAGACCGAGA), 110975-19-4.

Supplementary Material Available: Tables of temperature dependence of ^{31}P NMR signals and UV melting data and a contour plot of a ^1H-detected ^1H-^{31}P 2D *J* correlation map of *cis*-$Pt(NH_3)_2$[d(TCTCGGTCTC)-N7(5),N7(6)] (4 pages). Ordering information is given on any current masthead page.

Novel Diastereomers with Opposite Chirality at Ruthenium Formed by N7,α-PO_4 Chelation of 5′-dGMP to the Antimetastatic Agent *trans*-$RuCl_2(DMSO)_4$: NMR and CD Evidence

Enzo Alessio,*,† Yinghai Xu,‡ Sabina Cauci,§ Giovanni Mestroni,† Franco Quadrifoglio,§ P. Viglino,§ and Luigi G. Marzilli*,‡,⊥

Contribution from the Department of Chemical Sciences, University of Trieste, 34127 Trieste, Italy, Department of Chemistry, Emory University, Atlanta, Georgia 30322, and Institute of Biology, University of Udine, 33100 Udine, Italy. *Received December 14, 1988*

Abstract: A novel diastereomeric pair of isomers was discovered in an initial study of the interaction of the antimetastatic agent *trans*-$RuCl_2(DMSO)_4$ with nucleic acid components. In particular, 5′-dGMP forms two products that have characteristic features clearly indicating that the guanine N7 and the α-phosphate group form a chelate to the metal center. These features include (a) a pronounced downfield shift of the ^{31}P NMR signals of the chelates; (b) a downfield shift of the H8 ^1H NMR signals of the guanine in the chelates—this downfield shift persists in monodentate N7 coordinated forms favored by protonation of the phosphate group at acid pH; and (c) characteristic changes in the shifts and coupling constants of the deoxyribose ^1H NMR signals. This unusual chelation mode of binding has been characterized by NMR spectroscopy in only two previous studies. Interestingly, these studies involved other classes of metalloanticancer agents, namely Pt(II) and metallocene drugs. However, in these latter classes of drugs, only one isomer was possible. In the Ru derivatives studied here, the octahedral configuration leads to the possibility of diastereomers. After separation of the chelates by HPLC, the isomers were found to have nearly identical UV absorption spectra and a weak visible band at ~410 nm. However, the CD spectra have bands that have opposite signs but similar intensities and positions. Thus, the compounds are isomers that differ principally by having an opposite chirality at ruthenium. Analysis of several types of experiments demonstrated that the isomers have the composition $[Ru^{II}Cl(H_2O)(DMSO)_2(5'\text{-dGMP})]^-$. In contrast to the widely studied Pt(II) anticancer agents, the Ru drug does not readily bind to two 5′-dGMP at neutral pH. Thus, in addition to stereochemical differences between the octahedral Ru(II) and square-planar Pt(II) drugs, the Ru(II) compounds may not easily form the N7,N7 GpG crosslink characteristic of the DNA adducts formed by Pt anticancer drugs. Should the Ru(II) drugs form such a crosslink form, however, two diastereomers, with opposite chirality at Ru, are possible.

Two important recent topics in metal–nucleic acid and metal–nucleotide chemistry have been the following: (a) the elucidation of the mechanism of action of metalloantineoplastic agents[1] and (b) the exploitation of metal complex chirality in exploring nucleic acid structure and biochemistry.[2] Although chiral metal anticancer drugs could bind more selectively to DNA, the likely cellular target, only modest success has been achieved thus far in such endeavors.[3] Most chiral anticancer agents studied have been pseudosquare-planar platinum compounds.[1,3] Since the chirality is centered on the nonleaving ligand, the effects of chirality are expected to be less important in square-planar complexes than in pseudooctahedral complexes,[2] where the chirality is centered on the metal.

The great success of the drug *cis*-$PtCl_2(NH_3)_2$ (cisplatin) has encouraged the search for anticancer agents with other transition metals.[4] We have shown that *cis*- and *trans*-$Ru^{II}Cl_2(DMSO)_4$ have good antimetastatic activity against several murine metastasizing tumors.[5,6] Moreover, the complexes were found to possess

† University of Trieste.
‡ Emory University.
§ University of Udine.
⊥ L.G.M. is also a member of the Winship Cancer Center.

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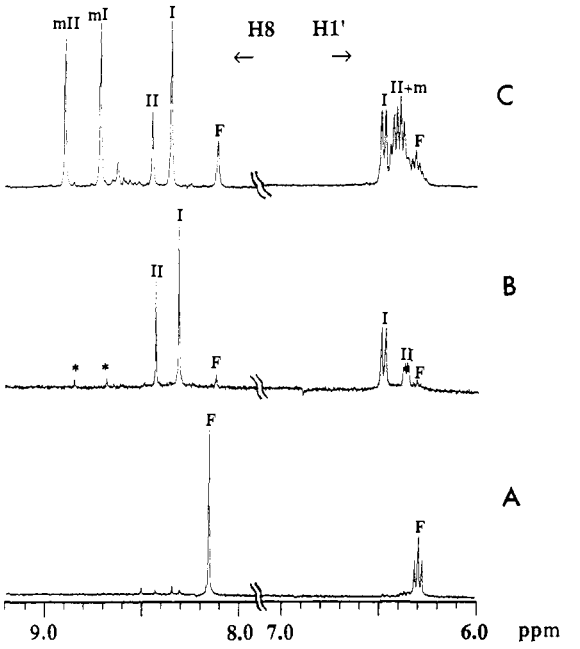

Figure 1. ^1H NMR spectra in D_2O at 22 °C of H8 and H1' signals of (A) 5'-dGMP (F), pH 6.4; (B) chelates I and II, pH 6.4 [Note that trace amounts of free 5'-dGMP (F) and monodentate species are present (signals with *).]; and (C) 0.5 h after lowering the pH to 4.5 of a solution of I and II similar to that in (B) but in the presence of a slight excess of 5'-dGMP (not at equilibrium).

mutagenic properties and to interact both in vivo and in vitro with DNA, with preferential attack at the guanine bases.[6]

In this report, we demonstrate that 5'-dGMP can coordinate to the initially achiral *trans*-$RuCl_2(DMSO)_4$ antitumor agent to form predominantly two isomers which have an opposite chirality at Ru. In both isomers, the nucleotide chelates by N7 and an α-phosphate oxygen, a binding mode clearly identified only very recently and, interestingly, in two other classes of anticancer drugs, namely Pt(II)[7] and metallocenes.[8] Such metal-centered chirality could also occur in chelates formed with DNA, but is not possible with the Pt(II) and metallocene drugs and, thus, is reported for the first time here.

Experimental Section

trans-$RuCl_2(DMSO)_4$ and *trans*-$RuBr_2(DMSO)_4$ were synthesized and recrystallized according to reported procedures.[9] 5'-dGMP and 5'-dIMP were purchased from Aldrich and Sigma, respectively, and used without further purification.

Reactions were carried out in 5-mm NMR tubes, and in all cases reported pH values were obtained directly from the NMR sample with a 0.3 mm × 20 cm pH electrode (Ingold); the pH was adjusted with ~1 M DNO_3 and NaOD.

^1H NMR spectroscopy was performed on a Nicolet 360-MHz spectrometer. Spectra were typically recorded on samples dissolved in 99.8% D_2O with the following parameters: 90° pulse; presaturation of HOD; 16 K data points; 0.1 Hz line broadening; 32 scans. Reported chemical shifts were based on TSP (trimethylsilyl propionate) as an internal reference.

^{31}P NMR spectroscopy was performed at 80.96 MHz with an IBM WP-200 SY spectrometer and 0.01% TMP (trimethylphosphate) in D_2O as an external reference. Typical instrumental conditions: 1.0 Hz line broadening; 4 K data points; 2 s relaxation delay; 30° pulse; sweep width 4000 Hz; broad band proton decoupling; ~500–3000 scans.

A Jasco J500A circular dichroism spectropolarimeter equipped with a thermostated cell holder was used for the CD measurements. Absorption spectra were obtained with a Cary 2200 spectrophotometer.

HPLC separations were performed with a Jasco BIP-I liquid chromatograph equipped with a TSKG 2000 PW (7.5 × 600 mm) column.

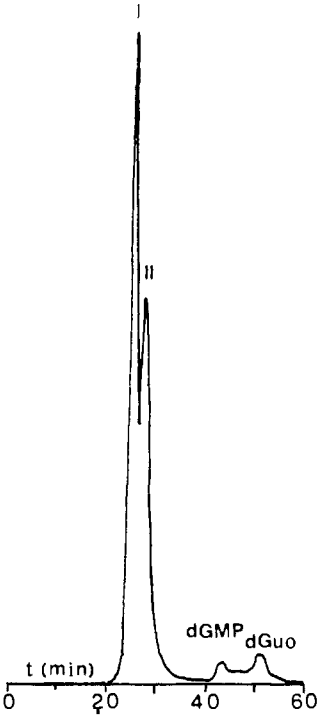

Figure 2. HPLC chromatogram monitored at 278 nm of the reaction mixture of equimolar (15 mM) amounts of *trans*-$RuCl_2(DMSO)_4$ and 5'-dGMP after 2 h of incubation at 37 °C in water. Elution was carried out with 100 mM $NaClO_4$, 10 mM phosphate buffer, pH 7.2, with a flow rate of 1 mL/min.

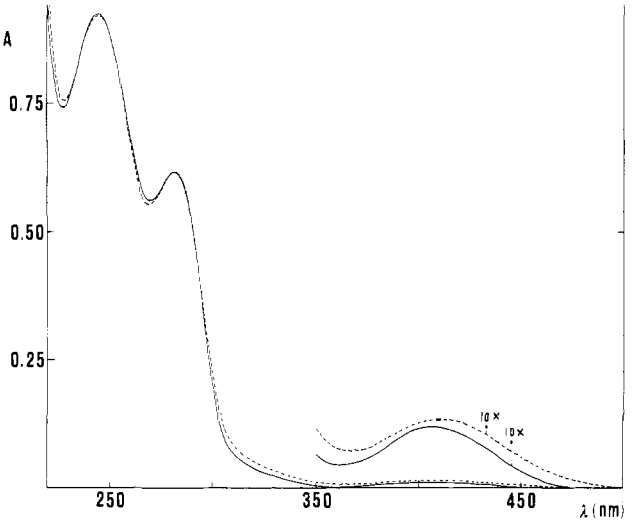

Figure 3. Absorption spectra of products I (—) and II (--) after HPLC separation.

Results and Discussion

Upon dissolution in water, *trans*-$RuCl_2(DMSO)_4$ releases two cis DMSO molecules quickly and one Cl slowly.[9] Examination of stoichiometric reaction mixtures containing 5'-dGMP by NMR or HPLC (Figures 1 and 2) reveals only two major species after several hours at pH 6–7. The 5'-dGMP is nearly consumed. These results suggest that the ratio of 5'-dGMP to Ru in the products is 1:1 (monomers), 2:2 (dimers), etc. After HPLC separation, the two products have nearly identical UV spectra and a similar weak visible band at ~405 nm for I (first band eluted) and at

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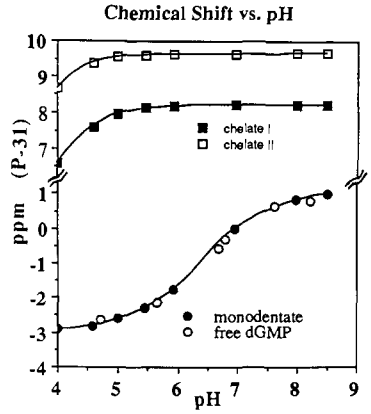

Figure 4. Chemical shift vs pH for ^{31}P NMR signals at 25 °C. Chelates I and II and the overlapping signal of the two species mI and mII were recorded for a solution with $[5'\text{-dGMP}]_0 = [\text{Ru drug}]_0 = 40$ mM. Data for free 5′-dGMP (40 mM) were recorded for a separate solution.

Table I. Selected ^1H NMR Results for 5′-dNMP and Metal Complexes

	pH	H1′ (ppm)	$^3J_{1'2'}$ (Hz)	$^3J_{1'2''}$ (Hz)	ref
free 5′-dGMP	6.6	6.30	6.8	6.8	*a*
chelate I	6.4	6.48	~0	7.5	*a*
chelate II	6.4	6.37	2.2	6.1	*a*
monodentate I	4.5	6.42	6.1	6.1	*a*
monodentate II	4.5	6.39	6.5	6.5	*a*
chelate I_{Br}	6.5	6.48	~0	7.9	*a*
chelate II_{Br}	6.5	6.37	1.8	5.4	*a*
Mo chelate (5′-dAMP)	~7.3		4.6	6.6	8
Pt chelate (5′-dGMP, 5′-dIMP)	6.4		<0.2	6.9~7.4	7

[a] This work.

~415 nm for II (Figure 3). These similar spectra strongly imply that the species are isomers. HPLC monitoring demonstrated that, at first, I and II exist in nearly equal amounts, but, with time, II converts into I to a significant extent. Likewise, one set of ^1H NMR signals decreases with a concomitant increase in the other set of signals (see below). Thus, the ^1H NMR signals for I and II are readily identified.

The 1:1 or 2:2, etc., stoichiometry of the products is confirmed by the 12:1 integration of the coordinated DMSO methyl signals vs the H8 signals of the coordinated 5′-dGMP (not shown). The downfield position of the H8 signals, 8.31 ppm for I and 8.43 ppm for II, suggests N7 coordination.[10]

The ^{31}P NMR spectrum also reveals consumption of the 5′-dGMP, but, more importantly, the very far downfield position of the two signals (8.21 ppm for I and 9.69 ppm for II) establishes PO_4 coordination.[7,11] Our evidence suggests that I and II are chelate complexes with the N7 and PO_4 group of 5′-dGMP bound to the same Ru(II) center. In Figure 4, we plot the ^{31}P NMR shift for these chelates as a function of pH. The ^{31}P signal shifts upfield starting at ~pH 5. The pH dependence is characteristic of a chelate.[11] In contrast, the plots of the ^{31}P NMR shifts for free 5′-dGMP and monodentate N7 5′-dGMP (see below) exhibit typical sigmoidal behavior with a pK_a of ~6.3.

Such N7,α-PO_4 coordination necessitates an altered nucleotide conformation with characteristic changes in the ^1H NMR shifts and coupling patterns of the deoxyribose moiety.[7,8,11] For example, the H1′ signal is a sharp doublet at 6.48 ppm for chelate I, while it is a doublet of doublets at 6.37 ppm for chelate II (Figure 1). These H1′ NMR patterns, which are a consequence of small $J_{1'2'}$,

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Table II. Values of K_1 and K_2 Calculated at pH = 4.5 with the Assumption That I and II Are Monomers Compared with Calculations Based on Dimers

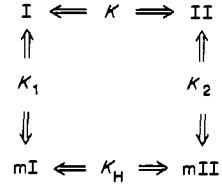
concentration[a] (mM)	K	if monomers K_1[b]	if monomers K_2[c]	if dimers (mM) "K_1"[b]	if dimers (mM) "K_2"[c]
10	0.20	1.0	2.7	3.5	6.7
20	0.19	0.9	2.5	7.8	17.0
40	0.21	0.8	2.4	12.5	24.1
40	0.23	0.8	2.1	11.0	20.4

[a] $[5'\text{-dGMP}]_0 = [\text{Ru}]_0$. [b] Based on integrated intensity. [c] Based on peak height due to small concentration of II at pH 4.5.

closely resemble those observed in N7,α-PO_4 chelates with Pt(II)[7] and molybdocenes[8] (Table I). The sugar is conformationally flexible, and the small $J_{1'2'}$ values suggest, especially in the case of I, a major increase in the percentage of the N-type conformation.[7,11]

The strained configuration required by N7,α-PO_4 coordination of nucleotides[7,11] limits the pH range under which such chelates can be observed for Pt(II) complexes. At high pH (> ~7), OH^- displaces the phosphate group.[7] At low pH, the phosphate group is protonated, following its dissociation to favor monodentate N7-coordinated species.

In contrast to the Pt(II) chelates,[7] I and II are stable at pH > 7. However, just as for Pt(II),[7] monomeric species with N7-coordinated monodentate 5′-dGMP (mI, mII) are favored by phosphate group protonation at low pH. The principal processes that occur can be described by the following equilibria:



The equilibrium constant $K = [\text{II}]/[\text{I}]$ has a value of ~0.2 (Table II). The values for $K_1 = [\text{mI}]/[\text{I}]$ and $K_2 = [\text{mII}]/[\text{II}]$ are ~0.9 and 2.4, respectively (Table II). These values were obtained from solutions with a total Ru(II) concentration of 10–40 mM, each with 1 equiv of 5′-dGMP added and at pH 4.5 and 22 °C. An alternative possibility for nucleotide complexes in which both phosphate groups and endocyclic N donors are coordinated is the formation of dimers. In Table II, we also treat our results assuming that I and II are dimers. In contrast to the relatively constant values for K_1 and K_2 that have been calculated based on the monomer assumption, the values calculated by assuming that I and II are dimers increase by a factor of 3 to 4. In another experiment (not shown), we used equimolar 5′-dGMP and 5′-dIMP, a nucleotide which behaves analogously to 5′-dGMP in forming species such as I and II. In this mixed nucleotide experiment, we observed no new mixed nucleotide complexes; this result is consistent with chelates and inconsistent with dimers.[7]

Another feature of these equilibria is worthy of note. At pH = 4.5, the value of K_H (the equilibrium constant between the two monodentate forms, $[\text{mII}]/[\text{mI}]$) is ~0.6, and ΔG is ~0.5 kJ/mol. The value of K of ~0.2 reveals an ~4 kJ/mol difference in free energy between the chelates. This greater difference in stability for the chelate isomers vs the monodentate isomers could arise in part from the slightly different deoxyribose conformations in the chelates suggested by the different coupling constants in Table I.

The ratio of I:II is independent of added Cl^-, and both isomers are formed more readily when the Ru solution is pretreated with 1 equiv of $AgNO_3$. Addition of more than 1 equiv of $AgNO_3$ did not significantly alter the results, suggesting that one chloride is tightly bound. On the other hand, chelates closely analogous but slightly different from I and II (I_{Br}: H8, 8.52 ppm; ^{31}P, 8.40 ppm. II_{Br}: H8, 8.65 ppm; ^{31}P, 9.90 ppm) are formed from *trans*-$RuBr_2(DMSO)_4$. Therefore, the coordination environment of the Ru(II) contains one halide along with one H_2O, two DMSO, and the 5′-dGMP chelate. Twelve isomers are possible, if the DMSO

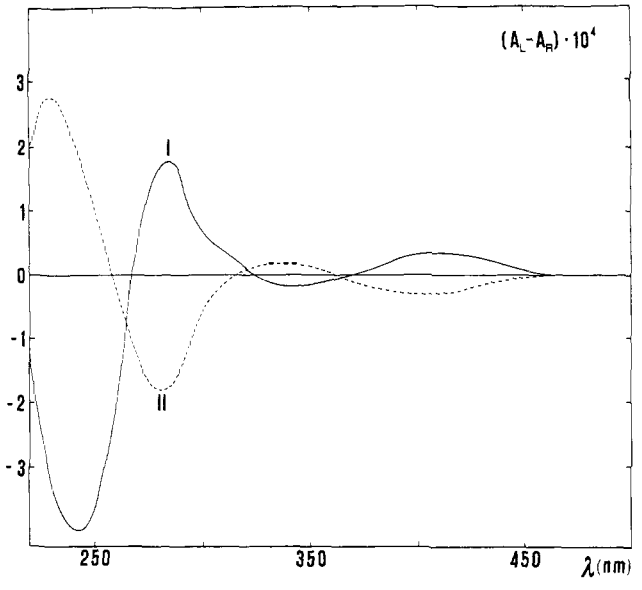
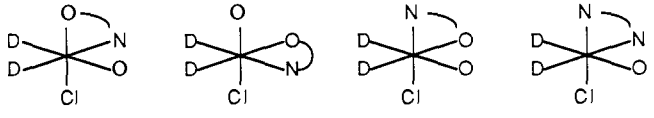


Figure 5. CD spectra of products I (—) and II (--) after HPLC separation. The measurements were made on the same solutions for which absorption spectra are reported in Figure 3.

ligands remain bound by S. Evidence that the DMSO ligands are bound by S is found in the small difference in shifts of the liganded DMSO for $[Ru^{II}Cl_2(D_2O)_2(DMSO)_2]$ (3.35 ppm) from those for I (3.39, 3.36, 3.32, 3.26 ppm) and II (3.40, 3.36, 3.29, 3.24 ppm). The similar values for I and II (including the overlap at 3.36 ppm) are consistent with isomers and demonstrate that all four DMSO methyl groups in each complex are inequivalent.

Insights into the overall geometry can be found by examining the CD spectra of I and II (Figure 5) recorded after HPLC resolution. The almost identical UV spectra (Figure 3) and nearly opposite CD spectra demonstrate that the two species are diastereomers with the same Ru coordination environment, differing primarily in the orientation of the chelate moiety. Several pairs of isomers are consistent with all our results. Three pairs of isomers maintain the facial relationship of the Cl and the two *cis*-DMSO ligands (*A*, *B*, *C* below and the diastereomers; D = DMSO, O = H_2O, N–O = 5'-dGMP). *C* can be excluded since both diastereomers lead to the same monodentate form. We favor *A* since the opposite CD spectra are more readily understood; the novelty of these compounds, however, gives us no literature precedent upon which to distinguish among isomers.



A *B* *C* *D*

Interestingly, the relatively favorable coordination of the phosphate group precludes formation of bis nucleotide complexes at pH 7. Even with a 2-fold *excess* of 5'-dGMP, the ^1H NMR spectra (not shown) demonstrate that half the added 5'-dGMP had not reacted. In contrast, *excess* Pt(II) drug is required to prevent formation of bis purine nucleotide complexes.[10-12] The Pt drugs form N7,N7-crosslinked GpG adducts.[1] Our findings raise the possibility that, if *trans*-$RuCl_2(DMSO)_4$ formed such intrastrand N7,N7-crosslinked GpG adducts, diastereomers (*D*, N–N = GpG) analogous to *A* could be formed.

Although the different patterns of anticancer activity found for *trans*-$RuCl_2(DMSO)_4$ and cisplatin could have many different explanations (biodistribution, enzymatic repair of lesions, etc.) in addition to the nature of the DNA lesion, the failure of the Ru(II) species to bind a second 5'-dGMP at pH 7 suggests that an alternative type of lesion may be formed with DNA. On the other hand, the phosphodiester groups in DNA should bind to Ru(II) much less strongly than the phosphate monoester groups in nucleotides.[7] Studies with oligonucleotides will be needed to resolve these issues.

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Registry No. *trans*-$RuCl_2(DMSO)_4$, 72904-47-3; *trans*-$RuBr_2(DMSO)_4$, 72904-46-2; $[Ru^{II}Cl(H_2O)(DMSO)_2(5'\text{-dGMP})]^-$, 121844-74-4; 5'-dGMP, 902-04-5.

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