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Kinetic and Structural Studies on the Intermediates Formed in the Reactions of 5′-Adenosine Monophosphate and 5′-Guanosine Monophosphate with *cis*-Dichlorodiammineplatinum(II) Using ^{1}H and ^{195}Pt Magnetic Resonance Spectroscopy

G. M. Clore* and A. M. Gronenborn*

Contribution from the Division of Molecular Pharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA, United Kingdom. *Received August 21, 1981*

Abstract: The kinetics of the reactions of 5′-AMP and 5′-GMP with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of KCl at pH* 6.5 have been investigated by ^{1}H NMR and analyzed by nonlinear numerical integration and optimization techniques. The species formed have been further characterized by ^{195}Pt NMR. The reaction with 5′-AMP involves a branching pathway: 5′-AMP reacts with *cis*-[Pt(NH$_3$)$_2$Cl$_2$] to form either species IA or IIA; species IA and IIA then react with *cis*-[Pt(NH$_3$)$_2$Cl$_2$] to form species IIIA, the final product of the reaction. Species IA, IIA, and IIIA arise from Pt binding in a monodentate mode to N7, N1, and both N1 and N7 of the adenine ring, respectively. The reaction with 5′-GMP proceeds as follows: 5′-GMP reacts with *cis*-[Pt(NH$_3$)$_2$Cl$_2$] to form species IG and IIG either simultaneously or in sequence (the latter being unlikely on structural grounds); species IG and IIG are in equilibrium which is rapid in terms of the overall reaction, such that the ratio of the concentrations of species IG to that of species IIG is constant throughout the entire course of the reaction, but slow on the ^{1}H NMR scale as two distinct signals are seen in the ^{1}H NMR spectrum, separated by only 10 Hz at the spectrometer frequency of 270 MHz. The final step of the reaction involves the conversion of species IG and IIG into species IIIG. From the kinetic data, one cannot discern whether species IIIG is formed directly from species IG and indirectly from species IIG via species IG, or vice versa. We propose that species IG and IIG are rotamers with Pt binding in a monodentate mode at N7 of the guanine ring, rotation occurring about the Pt–N7 bond, and that species IIIG is a chelate in which the Pt atom forms a bridge between N7 and the 6-keto group.

Introduction

The platinum(II) complex *cis*-[Pt(NH$_3$)$_2$Cl$_2$] has been the subject of intense investigation[1,2] owing to its potent antitumor activity.[3] There is considerable evidence that DNA is the primary site of action of *cis*-[Pt(NH$_3$)$_2$Cl$_2$], binding occurring preferentially to guanine followed by adenine.[4-7] Spectroscopic studies, including ^{1}H NMR,[8-13] ^{13}C NMR,[14] UV[15] and Raman[12,13] of complexes formed on the reaction of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] with nucleotides have shown that the main binding sites on the bases are N1 and N7 for adenine, N7 for guanine, and N3 for cytosine. In addition, bidentate chelation between N7 and the 6-NH$_2$ group in adenosine, and between N7 and the 6-keto group in guanine has been proposed on the basis of UV[15] and X-ray photoelectron spectroscopy,[16,17] respectively.

On the monomer level, the apparent association constants for the binding of aquated *cis*-[Pt$^{\mathrm{II}}$(NH$_3$)$_2$] with guanosine, adenosine, and cytidine are approximately equal ($\log K = 3.7$, 3.6, and 3.7, respectively).[18] No observable reaction occurs with thymidine.[15] However, at ratios of aquated *cis*-[Pt$^{\mathrm{II}}$(NH$_3$)$_2$] to nucleotide of $\lesssim 0.2$, the reaction with a mixture of all four natural nucleotides shows a larger than expected preferential fixation to 5′-guanosine monophosphate (5′-GMP) relative to 5′-adenosine monophosphate (5′-AMP), and little reaction with the pyrimidine nucleotides.[19] On the basis of this observation it was suggested that the selectivity of aquated *cis*-[Pt$^{\mathrm{II}}$(NH$_2$)$_2$] toward nucleotides is kinetically controlled.[19]

In order to extend our knowledge of the interaction of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] with purine nucleotides, we have undertaken a ^{1}H NMR study of the kinetics of the reactions of 5′-AMP and 5′-GMP with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] and further characterized the intermediates formed by ^{195}Pt NMR.

Experimental Section

cis-[Pt(NH$_3$)$_2$Cl$_2$] was obtained from Aldrich Chemicals and 5′-AMP and 5′-GMP were from Sigma Chemicals, all used without further purification. The *cis*-[Pt(NH$_3$)$_2$(GMP)$_2$] complex was prepared exactly as described by Kong and Theophanides.[9] All other chemicals used were of the highest purity commercially available.

Samples for NMR were D$_2$O solutions containing 50 mM potassium phosphate buffer pH* 6.5 (meter reading uncorrected for the isotope effect on the glass electrode), 1 mM dioxan, and 500 mM KCl. KCl and buffer were used to ensure that no hydrolysis of the Pt complex occurred and that *cis*-[Pt(NH$_3$)$_2$Cl$_2$] was the only reactive species present.[20]

^{1}H NMR spectra were recorded at 25 °C on a Bruker WH270 spectrometer operating at 270 MHz with a spectral width of 4200 Hz, using 8K data points and an acquisition time of 0.974 s; 500 transients were averaged for each spectrum. All chemical shifts were measured from dioxan as internal standard (3.71 ppm downfield from that of

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sodium dimethylsilapentane-5-sulfonate (DSS)). Intensity measurements performed on these spectra were verified by recording several spectra with a long pulse delay (5 s), and the intensities obtained were found to be identical within experimental error.

^{195}Pt NMR spectra were recorded at 25 °C on a Bruker WM200 wide-bore spectrometer operating at 42.91 MHz with a spectral width of 50 000 Hz, using 16K data points and an acquisition time of 0.164 s; 90 000 transients were averaged for each spectrum. All chemical shifts were measured upfield from $[PtCl_6]^{2-}$ as external standard.

The kinetics of the reactions of 5′-AMP and 5′-GMP with excess *cis*-$[Pt(NH_3)_2Cl_2]$ were studied at 80 °C, a temperature at which the reaction is relatively rapid (completion being achieved in 8 to 10 h), and at which a reasonably high concentration of *cis*-$[Pt(NH_3)_2Cl_2]$ in solution can be obtained. The concentrations of nucleotide and *cis*-$[Pt(NH_3)_2Cl_2]$ used were 2.88 and 30 mM, respectively. The reaction was initiated by incubating the nucleotide–*cis*-$[Pt(NH_3)_2Cl_2]$ reaction mixture at 80 °C for a given period of time, after which the reaction mixture was rapidly cooled to 25 °C, a temperature at which the reaction proceeds extremely slowly and most of the *cis*-$[Pt(NH_3)_2Cl_2]$ complex comes out of solution (the solubility at 25 °C is ca. 2.5 mg/mL[21]), and a ^{1}H NMR spectrum recorded. The sample was then heated up rapidly to 80 °C to allow the reaction to proceed for a further set period of time; it was cooled down again to 25 °C and a ^{1}H NMR spectrum recorded. This procedure was repeated until the reaction was complete. The intensities of the H2 and H8 resonances for each species, determined by integration with an error of 5–10%, were normalized with respect to their sum over all the species (i.e., the normalized intensity K_{ji} of the jth resonance of the ith species is given by $K_{ji} = I_{ji}/\sum_{i=1}^{i=N} I_{ji}$ where I_{ji} is the absolute intensity). By normalizing the intensities in this way, alterations in the absolute intensities by, for example, progressive deuteration during the course of the reaction, as in the case of the H8 proton, do not affect the relative intensities.

The kinetic data were analyzed by the numerical integration and optimization procedures developed by Clore and Chance.[22,23] We minimize the residual sum squares (RSQ) given by

$$\mathrm{RSQ} = \sum_{i=1}^{n}\sum_{j=1}^{m} R_{ij}^2 = \sum_{i=1}^{n}\sum_{j=1}^{m} [(v_{ij} - u_{ij})/\sigma_i]^2 \qquad (1)$$

where j identifies the time point and i the data curve, R_{ij} are the residuals, v_{ij} the observed values, u_{ij} the corresponding calculated values, and σ_i the standard error of curve i. At the minimum, RSQ is equal to the normalized χ^2. From the RSQ we calculate the overall standard deviation (SD) of the fit

$$\mathrm{SD} = \phi[\mathrm{RSQ}/(d - p)]^{1/2} \qquad (2)$$

where d is the total number of experimental points, p the number of optimized parameters, and ϕ the overall standard error of the data given by

$$\phi = \sum_{i=1}^{n} \sigma_i r_i \Big/ \sum_{i=1}^{n} r_i \qquad (3)$$

where r_i is the range of curve i. The nature of the distribution of residuals is examined using the mean absolute correlation index, $\bar{C}$, given by

$$\bar{C} = \frac{1}{n}\sum_{i=1}^{n}\left|\sum_{i=1}^{m} R_{ij}/\sqrt{\mathrm{RSQ}_i}\right| \qquad (4)$$

A value of $\bar{C} \lesssim 1$ (the expected root-mean-square value of $\bar{C}$ if the residuals were all independent random variables of zero mean and the same variance) indicates a random distribution of residuals; a value of $\bar{C} >> 1$ indicates that the departures between calculated and observed values are systematic.[22] The overall standard error of the data for both the 5′-AMP and 5′-GMP reactions was 7.5 ± 2.5%.

Results

Kinetics of the Reaction of Excess *cis*-$[Pt(NH_3)_2Cl_2]$ with 5′-AMP in the Presence of KCl. Figure 1 shows the progress of the reaction of 5′-AMP with excess *cis*-$[Pt(NH_3)_2Cl_2]$ in the presence of KCl at 80 °C and pH* 6.5 monitored by ^{1}H NMR. Signals arising from H2 and H8 protons were easily distinguished using 5′-AMP-d_8 and their intensities as a function of time are shown in Figure 2. It can readily be seen that three species are formed during the reaction: species IA with signals at 4.593 (H2) and 5.345 ppm (H8), species IIA with signals at 4.931 (H2) and 4.817 ppm (H8), and species IIIA with signals at 5.079 (H2) and 5.456 ppm (H8). These compare to free 5′-AMP with signals at 4.498 (H2) and 4.768 ppm (H8).

As the concentration of free 5′-AMP decreases, so the concentrations of species IA and IIA rise simultaneously to plateaus of 38 and 12%, respectively, of the total 5′-AMP concentration. Species IA and IIA are then converted simultaneously into species

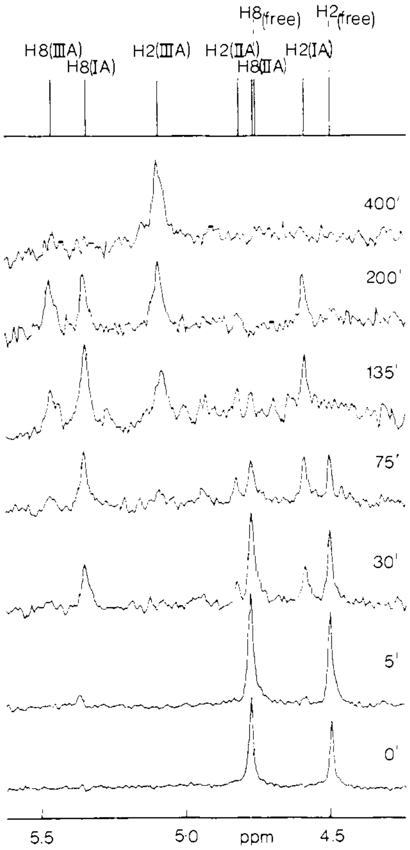

Figure 1. Progress of the reaction of 5′-AMP with excess *cis*-$[Pt(NH_3)_2Cl_2]$ in the presence of KCl at 80 °C and pH* 6.5 monitored by ^{1}H NMR. Spectra are recorded at 25 °C and only the aromatic portion of the spectrum is shown. It should be noted that the H8 resonance of species IIIA is no longer detectable in the spectrum taken 400 min after the initiation of the reaction owing to deuteration during the course of the reaction. The experimental conditions are: 2.88 mM 5′-AMP, 30 mM *cis*-$[Pt(NH_3)_2Cl_2]$, 50 mM potassium phosphate buffer pH* 6.5, 500 mM KCl, and 1 mM dioxan (as internal standard) in D_2O.

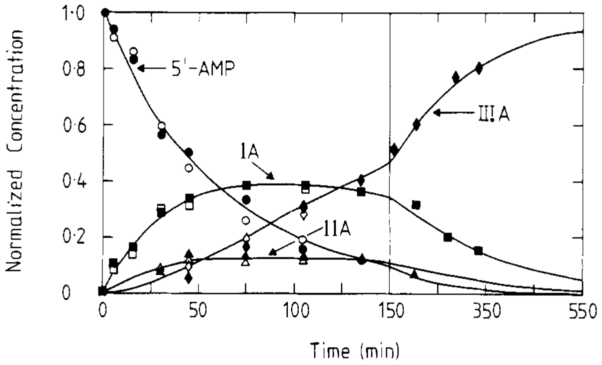

Figure 2. Comparison of the experimental and computed kinetics of the reaction of 5′-AMP with excess *cis*-$[Pt(NH_3)_2Cl_2]$ in the presence of KCl at 80 °C and pH* 6.5. The experimental intensities of the H2 and H8 protons are shown as: ● (H2), ○ (H8) for 5′-AMP; ■ (H2), □ (H8) for species IA; ▲ (H2), △ (H8) for species IIA; and ◆ (H2), ◇ (H8) for species IIIA. The computed best fit curves for Scheme I are shown as continuous lines. The optimized values of the rate constants are given in Table I. The overall standard deviation of the fit is 4.8% which is within the standard error of the data (5–10%) and the distribution of residuals is random ($\bar{C} < 1.0$). The experimental conditions are given in the legend to Figure 1.

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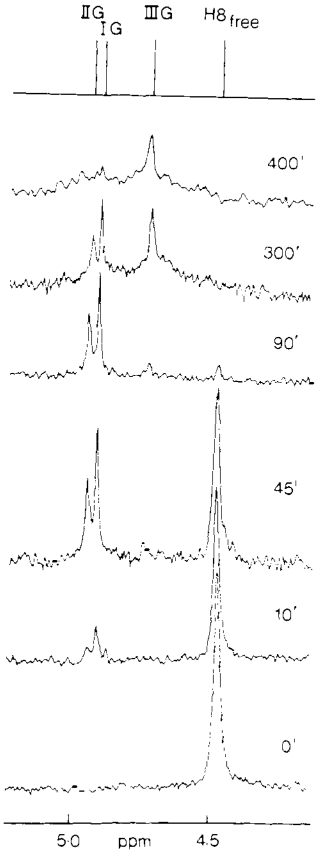
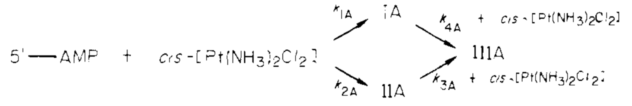


Figure 3. Progress of the reaction of 5'-GMP with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of KCl at 80 °C and pH* 6.5 monitored by ^{1}H NMR. Spectra are recorded at 25 °C and only the aromatic portion of the spectrum is shown. The experimental conditions are: 2.88 mM 5'-GMP, 30 mM *cis*-[Pt(NH$_3$)$_2$Cl$_2$], 50 mM potassium phosphate buffer pH* 6.5, 500 mM KCl, and 1 mM dioxan (as internal standard) in D$_2$O.

Scheme I



IIIA which is the final product of the reaction. Species IIIA shows an induction period in its formation with a slow rise to its final plateau value (100% of the total 5'-AMP concentration), characteristic of the product of a multistep reaction sequence. It should be noted that the ratio [IA]/[IIA] is not constant during the entire course of the reaction.

The simplest kinetic scheme required to account for the data is a branching one given by Scheme I. It should be noted we cannot ascertain from the kinetic data alone whether the conversion of species IA and IIA to species IIIA is a first- or second-order process as the experiments were carried out under essentially pseudo-first-order conditions ([*cis*-[Pt(NH$_3$)$_2$Cl$_2$]]$_0$ > [5'-AMP]$_0$), and the concentration of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] was not varied. However, we present below unambiguous evidence from ^{195}Pt NMR that in species IA and IIA only one Pt atom is bound and in species IIIA two Pt atoms are bound, so that the conversion of species IA and IIA to species IIIA is probably a second-order process.

The best fit curves, obtained by fitting the computed time courses of the intermediates, calculated by numerical integration of the ordinary differential equations derived for Scheme I, to all the experimental data simultaneously by nonlinear optimization of the four unknown constants, are shown in Figure 2. The overall standard deviation of the fit is 4.8% which is within the standard error of the data (5–10%), and the distribution of residuals is random ($\bar{C}$ < 1.0). The values of the optimized rate constants are given in Table I.

Table I. Optimized Values of the Rate Constants Obtained by Fitting Scheme I to the Data in Figure 2 for the Reaction of 5'-AMP with Excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] at 80 °C in the Presence of KCl

rate constant, M^{-1} s^{-1}	optimized value (±std dev)
k_{1A}	7.7×10^{-3} ($\pm 0.3 \times 10^{-3}$)
k_{2A}	2.5×10^{-3} ($\pm 0.3 \times 10^{-3}$)
k_{3A}	4.8×10^{-3} ($\pm 0.3 \times 10^{-3}$)
k_{4A}	4.8×10^{-3} ($\pm 0.3 \times 10^{-3}$)

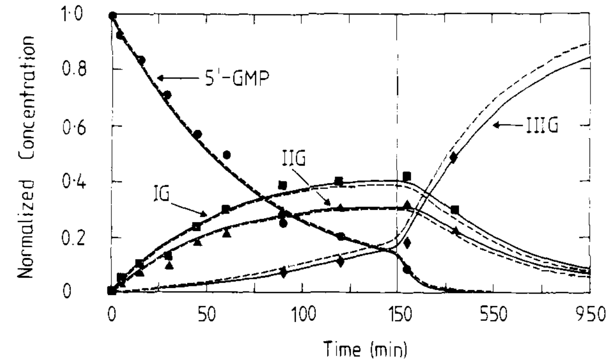
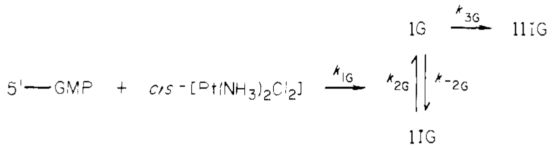
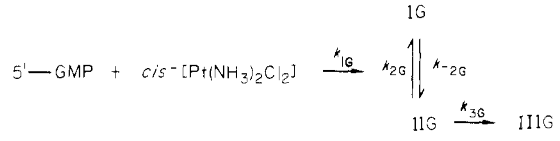


Figure 4. Comparison of the experimental and computed kinetics of the reaction of 5'-GMP with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of KCl at 80 °C and pH* 6.5. The experimental intensities of the H8 protons are shown as: ●, 5'-GMP; ■, species IG; ▲, species IIG; ◆, species IIIG. The computed best fit curves for Schemes II and III are shown as continuous (—) and interrupted (---) lines, respectively. The optimized values of the rate constants are given in Table II. The overall standard deviation of the fit for both Schemes II and III is 6.3% which is within the standard error of the data (5–10%), and the distribution of residuals is random in both cases ($\bar{C}$ < 1.0). The experimental conditions are given in the legend to Figure 3.

Scheme II



Scheme III



Kinetics of the Reaction of Excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] with 5'-GMP in the Presence of KCl. Figure 3 shows the progress of the reaction of 5'-GMP with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of KCl at 80 °C and pH* 6.5 monitored by ^{1}H NMR. As in the case of the reaction of 5'-AMP with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$], it can readily be seen that three species are formed during the course of the reaction. The chemical shifts of the H8 proton in free 5'-GMP, species IG, species IIG, and species IIIG are 4.449, 4.874, 4.912, and 4.700 ppm, respectively. The intensities of these signals as a function of time are shown in Figure 4. The kinetics of the reaction of 5'-GMP with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] follows the same general pattern as that of 5'-AMP. However, the two reactions differ in one important aspect, namely, that in the reaction with 5'-GMP the ratio [IG]/[IIG] is constant throughout the entire course of the reaction whereas in the reaction with 5'-AMP the ratio [IA]/[IIA] is not. This implies that species IG and IIG are in equilibrium, and that the equilibrium is fast in terms of the overall reaction but slow on the NMR scale as two distinct signals are seen. The alternative possibility that species IG and IIG are formed and react further at exactly the same rates without being in equilibrium is highly unlikely and can be excluded on the basis of the observation that species IG and IIG are directly

Table II. Optimized Values of the Unknown Parameters Obtained by Fitting Schemes II and III to the Data in Figure 4 for the Reaction of 5′-GMP with Excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] at 80 °C in the Presence of KCl

parameter	optimized values (±std dev)	
	Scheme II	Scheme III
k_{1G} (M^{-1} s^{-1})	8.1×10^{-3} ($\pm 0.6 \times 10^{-3}$)	8.0×10^{-3} ($\pm 0.5 \times 10^{-3}$)
k_{3G} (M^{-1} s^{-1})	3.8×10^{-3} ($\pm 0.4 \times 10^{-3}$)	5.7×10^{-3} ($\pm 0.6 \times 10^{-3}$)
k_{2G}/k_{-2G}[a]	1.3 (±0.1)	1.3 (±0.1)

[a] In the optimization ($k_{2G} + k_{-2G}$) was set equal to 0.2 s^{-1}, and the ratio k_{2G}/k_{-2G} was optimized. The value of k_{2G}/k_{-2G} applies to the temperature at which the ^{1}H NMR spectra were recorded, namely, 25 °C; in contrast, the values for the rate constants k_{1G} and k_{3G} apply to the temperature at which the reaction was allowed to proceed, namely, 80 °C. The temperature dependence of the ratio k_{2G}/k_{-2G} over the temperature range 10-65 °C is shown in Figure 5.

interconvertible (see below). The simplest schemes required to account for the data are given by Schemes II and III. The separation of the H8 signals in species IG and IIG is 10 Hz so the following condition must be satisfied:

$$k_{1G}[cis\text{-}[\mathrm{Pt(NH_3)_2Cl_2}]],\ k_{3G} << k_{2G} + k_{-2G} << 2\pi \times 10\ \mathrm{s^{-1}} \sim 60\ \mathrm{s^{-1}} \quad (5)$$

As a result, it is impossible to tell under the experimental conditions employed whether species IG and IIG are formed sequentially or simultaneously, as in the case of the corresponding species IA and IIA in the reaction with 5′-AMP. If species IG and IIG are formed simultaneously then

$$k_{1G} = k^{\mathrm{I}}_{1G} + k^{\mathrm{II}}_{1G} \quad (6)$$

where k^{I}_{1G} and k^{II}_{1G} are the rate constants for the formation of species IG and IIG respectively from 5′-GMP and *cis*-[Pt(NH$_3$)$_2$Cl$_2$]. As the experiments were carried out under essentially pseudo-first-order conditions (i.e., [*cis*-[Pt(NH$_3$)$_2$Cl$_2$]]$_0$ >> [5′-GMP]$_0$) and the concentration of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] was not varied, one cannot ascertain from the kinetic data whether the conversion of species IG or IIG into species IIIG is a first- or second-order process. However, evidence is presented below using ^{195}Pt NMR that only one platinum atom is bound to 5′-GMP in species IIIG, so that the conversion of species IG or IIG into species IIIG is a first-order process.

In order to fit the experimental data to schemes II and III we set ($k_{2G} + k_{-2G}$) equal to 0.2 s^{-1} and varied k_{1G}, k_{3G}, and the ratio k_{2G}/k_{-2G}. It should be noted that the value of k_{2G}/k_{-2G} obtained from the data in Figure 4 applies to the temperature at which the ^{1}H NMR spectra were recorded, namely, 25 °C, whereas the values of k_{1G} and k_{3G} apply to the temperature at which the reaction was allowed to proceed, namely 80 °C.

All the experimental data were fitted simultaneously. The best fit curves are shown in Figure 4 and the values of the optimized parameters are given in Table II. The overall standard deviation of the fit is 6.3% for both Schemes II and III which is within the standard error of the data (5–10%), and the distribution of residuals is random in both cases ($\bar{C}$ < 1.0).

Temperature Dependence of the Equilibrium between Species IG and IIG. The temperature dependence of the equilibrium constant $K_{\mathrm{IG/IIG}}$ for the interconversion of species IG and IIG over the temperature range 10–65 °C as measured by ^{1}H NMR is shown in Figure 5 ($K_{\mathrm{IG/IIG}} = k_{2G}/k_{-2G}$ = [IG]/[IIG]). Below 30 °C species IG is the predominant species, whereas above 30 °C species IIG predominates. The linear dependence of log ($K_{\mathrm{IG/IIG}}$) on $1/T$ clearly demonstrates that species IG and IIG are directly interconvertible with a value for $\Delta H_{\mathrm{IG-IIG}}$ of ~−174 J mol^{-1}. At 25 °C, $\Delta G_{\mathrm{IG-IIG}}$ has a value of ~−650 J mol^{-1} and $\Delta S_{\mathrm{IG-IIG}}$ of 1.6 J mol^{-1} K^{-1}.

^{195}Pt NMR of Species Formed in the Reaction of 5′-AMP with Excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the Presence of KCl. Figure 6 shows the ^{195}Pt NMR spectrum and corresponding ^{1}H NMR spectrum obtained on reacting 15 mM 5′-AMP with 40 mM *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of KCl for 4.5 h at 80 °C. The proportions of species IA, IIA, and IIIA from the ^{1}H NMR spectrum are 0.32, 0.13, and 0.55, respectively. In the ^{195}Pt NMR spectrum, however, only two bound ^{195}Pt resonances are seen at −2353.8 and −2397.7 ppm with relative intensities of 0.57 and 0.43. The ratio ([IA] + [IIIA])/([IIA] + [IIIA]) from the ^{1}H NMR data is 1.33, and the ratio of the low- to the high-field bound ^{195}Pt resonances is 1.3. At the end stage of the reaction when only species IIIA is present, the two bound ^{195}Pt resonances are of equal intensity. The determination of intensities in the ^{1}H NMR spectra is straightforward since a long pulse delay (5 s) was employed to ensure correct intensities. The intensities in the ^{195}Pt NMR spectra, however, may reflect a contribution from the spin–lattice relaxation time of the observed Pt atom since the pulse interval (0.164 s) is short compared to the spin–lattice relaxation times of ^{195}Pt which lie in the range 0.32–1.28 s.[24] On the assumption that the Pt atom in species IA and IIA has approximately the same spin–lattice relaxation time which is reasonable in view of their

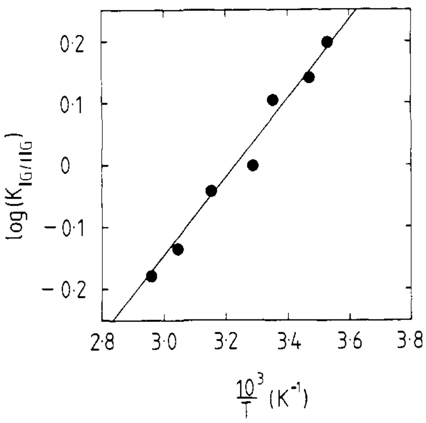


Figure 5. Temperature dependence of the equilibrium constant $K_{\mathrm{IG/IIG}}$ for the interconversion of species IG and IIG over the temperature range 10–65 °C. 5′-GMP was treated with excess *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of KCl at pH* 6.5 for 100 min at 80 °C and then cooled down to 10 °C. ^{1}H NMR spectra were then recorded over the temperature range 10–65 °C, and the ratio of the intensities of the signals of species IG to IIG was measured. Experimental conditions are given in the legend to Figure 3.

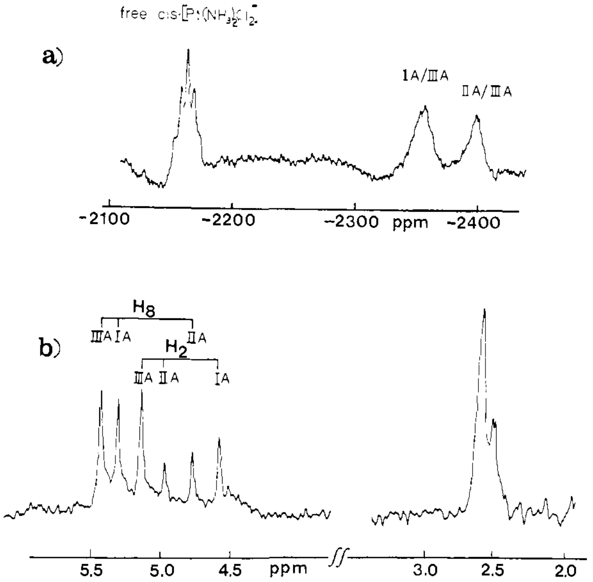


Figure 6. ^{195}Pt (a) and ^{1}H (b) NMR spectra of the complexes formed after treating 15 mM 5′-AMP with 40 mM *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of KCl for 4.5 hr at 80 °C and pH* 6.5. All spectra are recorded at 25 °C. Details of the experimental procedure are given in the Experimental Section. (The rise in the base line at the low field end of the ^{195}Pt NMR spectrum is an instrumental artifact due to the large sweep width employed.)

Table III. ^1H and ^{195}Pt Chemical Shifts of the Species Formed in the Reactions of 5′-AMP and 5′-GMP with Excess *cis*-[$Pt(NH_3)_2Cl_2$] in the Presence of KCl, and of the *cis*-[$Pt(NH_3)_2(GMP)_2$] Complex Together with the ^1H Chemical Shifts of 5′-AMP and 5′-GMP, and the ^{195}Pt Chemical Shifts of *cis*-[$Pt(NH_3)_2Cl_2$], $K_2[PtCl_4]$, and [$Pt(NH_3)_4$]Cl_2

species	^1H chemical shift (ppm downfield from dioxane)		^{195}Pt chemical shift (ppm upfield from $PtCl_6^{2-}$)	proposed structure
	H_2	H_8		
5′-AMP	4.498[a]	4.768[a]		
IA	4.593	5.345	2353.8	one Pt atom bound to N7 of the adenine ring
IIA	4.931	4.817	2397.7	one Pt atom bound to N1 of the adenine ring
IIIA	5.079	5.456	2353.8, 2397.7	one Pt atom bound to N7 and one Pt atom bound to N1 of the adenine ring
5′-GMP		4.449[a]		
IG		4.874	2302.0	rotamers: one Pt atom bound to N7 of the guanine ring
IIG		4.912	2302.0	
IIIG		4.700	2346.2	one Pt atom bridging N7 and keto group of guanine ring
cis-[$Pt(NH_3)_2(5'\text{-GMP})_2$]		4.931[b]	2451.3	one Pt atom forming a bridge between two 5′-GMP molecules binding to N7 of the guanine ring of each 5′-GMP molecule
cis-[$Pt(NH_3)_2Cl_2$]			2161.4[c]	
$K_2[PtCl_4]$			1650[d]	
[$Pt(NH_3)_4$]Cl_2			2560[d]	

[a] The ^1H chemical shifts of the H2 and H8 protons of free 5′-AMP and of the H8 proton of free 5′-GMP show a considerable concentration dependence due to stacking; the shift reported here are for a concentration of 2.88 mM. [b] The ^1H chemical shift of the H8 proton of *cis*-[$Pt(NH_3)_2(GMP)_2$] reported by Marcellis et al.[26] is 4.95 ppm (downfield from dioxan). [c] The ^{195}Pt resonance of *cis*-[$Pt(NH_3)_2Cl_2$] is a quintet with a 1:2:3:2:1 intensity distribution due to quadrupolar coupling to the two equivalent directly bonded ^{14}N atoms, each with $I = 1$. The measured coupling constant, $^1J(^{195}Pt,^{14}N)$ is 220 ± 10 Hz. [d] From ref 24.

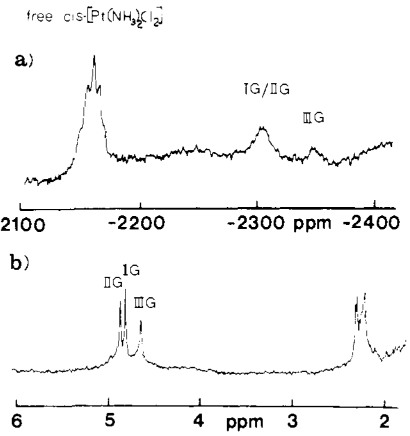


Figure 7. ^{195}Pt (a) and ^1H (b) NMR spectra of the complexes formed after reacting 15 mM 5′-GMP with 40 mM *cis*-[$Pt(NH_3)_2Cl_2$] in the presence of KCl for 105 min at 80 °C and pH* 6.5. All spectra are recorded at 25 °C. Details of the experimental procedure are given in the Experimental Section. (The rise in the base line at the high-field end of the ^{195}Pt NMR spectrum is an instrumental artifact due to the large sweep width employed.)

very similar chemical structure (one Pt atom bound per molecule of 5′-AMP), we can assign the resonance at −2353.8 ppm to species IA and IIIA, and the resonance at −2397.7 ppm to species IIA and IIIA.

^{195}Pt NMR of Species Formed in the Reaction of 5′-GMP with Excess *cis*-[$Pt(NH_3)_2Cl_2$] in the Presence of KCl. Figure 7 shows the ^{195}Pt NMR spectrum and corresponding ^1H NMR spectrum obtained after reacting 15 mM 5′-GMP with 40 mM *cis*-[$Pt(NH_3)_2Cl_2$] in the presence of KCl for 105 min at 80 °C. The proportion of species IG, IIG, and IIIG from the ^1H NMR spectrum are 0.40, 0.31, and 0.29, respectively. In the ^{195}Pt NMR spectrum, however, only two bound ^{195}Pt resonances are seen at −2303.0 and −2346.2 ppm with relative intensities of 0.70 and 0.30, respectively. At the end stage of the reaction (ca. 400min) when only species IIIG is seen in the ^1H NMR spectrum, only one bound ^{195}Pt resonance at −2346.2 ppm remains, which therefore has to be attributed to species IIIG. Thus species IG and IIG show an identical ^{195}Pt chemical shift of −2302.0 ppm.

^1H and ^{195}Pt NMR of the *cis*-[$Pt(NH_3)_2(5'\text{-GMP})_2$] Complex. ^1H and ^{195}Pt NMR spectra of the *cis*-[$Pt(NH_3)(5'\text{-GMP})_2$] complex (prepared as described in ref 9) were also obtained at pH* 6.5. The *cis*-[$Pt(NH_3)_2(5'\text{-GMP})_2$] complex exhibits a single resonance for the H8 proton at 4.931 ppm and a single ^{195}Pt resonance at 2451.3 ppm. The H1′ proton resonances for *cis*-[$Pt(NH_3)_2(5'\text{-GMP})_2$] are at 2.143 ppm, 0.02 ppm upfield from that of free 5′-GMP (2.163 ppm). For comparison, the H1′ proton resonances of species IG, IIG, and IIIG are at ca. 2.28 ppm.

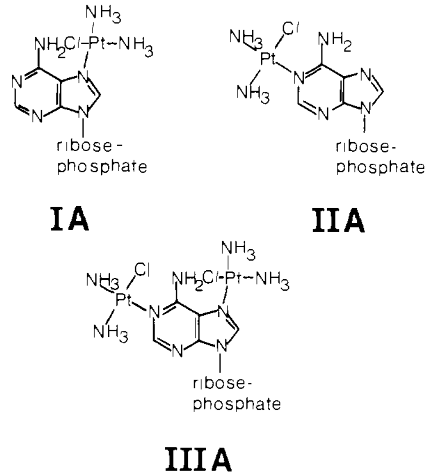


Figure 8. Proposed structures of species IA, IIA, and IIIA formed in the reaction of 5′-AMP with excess *cis*-[$Pt(NH_3)_2Cl_2$] in the presence of KCl at pH* 6.5 (see text for detailed discussion).

Discussion

Structural Assignments of Species IA, IIA, and IIIA. The ^1H and ^{195}Pt NMR parameters for species IA, IIA, and IIIA formed in the reaction of 5′-AMP with excess in *cis*-[$Pt(NH_3)_2Cl_2$] in the presence of KCl are summarized in Table III, and their proposed structures are given in Figure 8.

5′-AMP has four potential sites, N1, N3, N7, and the 6-NH_2 group, for monodentate binding of metal ions, and two potential sites for bidentate binding between N1 and the 6-NH_2 group, and N7 and the 6-NH_2 group. The pK_a (N1) of 5′-AMP is 3.62–4.15, and over the pH range 1–10 no other nitrogen atoms are protonated.[25] Binding to the 6-NH_2 group of 5′-AMP is assumed

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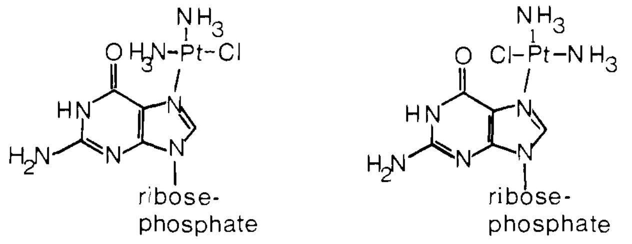
(24) J. J. Pesek and W. R. Mason, *J. Magn. Reson.*, **25**, 519 (1977).



IG **IIG**

Figure 9. Proposed structures of species IG and IIG formed in the reaction of 5′-GMP with excess *cis*-[Pt(NH_3)$_2$$Cl_2$] in the presence of KCl at pH* 6.5 (see text for detailed discussion).

to be negligible unless the 6-NH_2 group loses a proton in the process; this could not be demonstrated.[15] Binding to the N3 of 5′-AMP has not been reported for any metal ion.[15,25] In the present series of experiments, the pH* of the reaction was maintained at 6.5 so that none of the ring nitrogen atoms is protonated. Our ^1H NMR data show that *cis*-[Pt(NH_3)$_2$$Cl_2$] has to bind to the adenine ring in a very similar manner to [Pt(dien)Cl]Cl, namely, in a monodentate mode, since the ^1H chemical shifts for the H2 resonances of complexes between adenosine and [Pt(dien)Cl]$^+$ [10] are almost identical with those for the complexes between 5′-AMP and *cis*-[Pt(NH_3)$_2$$Cl_2$] reported here; binding of a Pt atom in a monodentate mode to N1, N7, and both N1 and N7 leads to ^1H chemical shifts for the H2 proton of 8.60, 8.25, and 8.90 ppm, respectively, with [Pt(dien)Cl]$^+$ compared to 8.64, 8.31, and 8.79 ppm, respectively, with *cis*-[Pt(NH_3)$_2$$Cl_2$] ($\delta$ values from DSS). On the basis of these data we therefore consider it highly unlikely that binding of *cis*-[Pt(NH_3)$_2$$Cl_2$] to N1 or N7 of 5′-AMP occurs in a bidentate mode with the 6-NH_2 group, replacing the second Cl atom. This is further supported by the results from ^{195}Pt NMR studies which show that only two ^{195}Pt resonances arise from the three species formed in the reaction: species IA gives rise to the resonance at −2358.8 ppm, species IIA to the resonance at −2397.7 ppm, and species IIIA exhibits two resonances of equal intensity at positions identical with those for species IA and IIA. The positions of these ^{195}Pt resonances are reasonably close to the expected ^{195}Pt chemical shift of −2333 ppm for a Pt(II) complex coordinated to three NH_3 groups and one Cl atom given by

$$\delta Na_2[Pt(Cl_4)] + 0.75(\delta[Pt(NH_3)_4]Cl_2 - \delta[PtCl_4])$$

on the basis of the linear relationship between the ^{195}Pt chemical-shift increment and substitution step number.[26] This suggests that the electronic influence of a ring nitrogen atom of the adenine ring on the Pt atom is similar to that of an NH_3 group. If binding of *cis*-[Pt(NH_3)$_2$$Cl_2$] occurred in a bidentate manner between either N1 and the 6-NH_2 group, or N7 and the 6-NH_2 group in any of the species, one would expect that species to exhibit a ^{195}Pt chemical shift similar to that for [$Pt^{II}(NH_3)_4$]$^{2+}$ of −2560 ppm,[24] which is, however, 162 ppm to higher field than our observed highest resonance. We therefore conclude that binding of *cis*-[Pt(NH_3)$_2$$Cl_2$] to both N1 and N7 of 5′-AMP occurs in a monodentate manner.

The observation that the three species IA, IIA, and IIIA each exhibit only a single H2 and a single H8 resonance implies either free rotation about the Pt–N bond which has to be fast on the NMR time scale, or fixation of the plane about the Pt atom in space. The latter is unlikely as rotation about Pt–N bonds is known to be relatively easy,[27] and fixation of the Pt–N bond is unlikely to occur in the absence of chelation.

Structural Assignments of Species IG, IIG, and IIIG. The ^1H and ^{195}Pt NMR parameters for species IG, IIG, and IIIG formed in the reaction of 5′-GMP with excess *cis*-[Pt(NH_3)$_2$$Cl_2$] in the presence of KCl are summarized in Table III, and the proposed structures of species IG and IIG are given in Figure 9. 5′-GMP has three potential sites, N1, N3, and N7, for monodentate binding of metal ions, and two potential sites for bidentate binding between N1 and the 6-keto group, and N7 and the 6-keto group. The pK_a(N1) and pK_a(N7) of 5′-GMP are 9.24–9.94 and 2.3, respectively,[25] which implies that under the conditions of our experiments, namely pH* 6.5, N1 will be protonated and is therefore unlikely to react with a Pt atom. As in the case of 5′-AMP, no reaction of metal ions with N3 has been demonstrated for 5′-GMP.[15]

Figure 3 shows the progress of the reaction of 5′-GMP with *cis*-[Pt(NH_3)$_2$$Cl_2$] monitored by ^1H NMR. Two signals for the H8 proton, separated by only 0.038 ppm, appear simultaneously in the first stages of the reaction at 4.874 and 4.912 ppm. The ratio of the intensities of the high-field to low-field resonance is constant throughout the entire course of the reaction and has a value of 1.3 at 25 °C (see Table II). We attribute these two signals to two rotamers of the complex between 5′-GMP and *cis*-[Pt(NH_3)$_2$$Cl_2$] in which the Pt atom is bound to N7 of the guanine ring. Binding at N7 is in agreement with the findings of Marcellis et al.[28] who reported the formation of a new species on addition of KCl to a solution of the *cis*-[Pt(NH_3)$_2$5′-GMP(OH)]$^+$ complex, in which the Pt atom is bound to N7, with an H8 proton resonance at 8.64 ppm which is very close to the positions we find for the H8 proton resonances of 8.58 and 8.62 ppm (δ values from DSS). (The fact that Marcellis et al.[28] only observe one resonance is probably due to the low-field strength (100 MHz) and the lower resolution of their spectrum.) The observation that the two signals attributed to the two rotamers are not of equal intensity implies that one of the rotamers has to represent a preferred form. Considering the two possibilities with the Cl atom either directed away from the 6-keto group (species IG) or toward the 6-keto group (species IIG), we suggest that species IG will be favored at low temperatures (<30 °C; see Figure 5) owing to the formation of a possible hydrogen bond between the NH_3 ligand coordinated to the Pt atom and the 6-keto group, while in species IIG, the two highly electronegative atoms, Cl and O, would tend to repel each other. We therefore attribute the signal at 4.874 ppm to species IG and the one at 4.912 ppm to species IIG. Species IG and IIG exhibit a single identical ^{195}Pt resonance at 2302.0 ppm (see Table III), thus confirming that these two species cannot arise from two complexes with the Pt atom bound in one case to N7 and in the other to N1. This is in marked contrast to the structures of species IA and IIA formed in the reaction of 5′-AMP with *cis*-[Pt(NH_3)$_2$$Cl_2$] (Figure 8).

As the reaction of 5′-GMP with excess *cis*-[Pt(NH_3)$_2$$Cl_2$] proceeds, a new signal, attributed to the final product of the reaction, species IIIG, arises in the ^1H and ^{195}Pt NMR spectra coupled with a decrease in the resonances attributed to species IG and IIG. The H8 proton resonance of species IIIG (4.700 ppm) is to high field of those of species IG and IIG (see Table III), and has a greater line width than these. Evidence that species IIIG is not a complex formed by the binding of a second *cis*-[Pt(NH_3)$_2$$Cl_2$] molecule to N1 of the guanine ring comes from the ^{195}Pt NMR data. Only a single resonance at −2346.2 ppm is observed at the end of the reaction (ca. 400 min); if species IIIG were a di-Pt complex, as in the case of species IIIA, two ^{195}Pt resonances of equal intensity should have remained in the ^{195}Pt NMR spectrum. We therefore suggest that species IIIG may represent the chelate with the Pt atom bridging N7 and the 6-keto group.

In addition to the sharp signal of species IIIG, we note that there are possibly some very broad resonances underlying the ^1H NMR spectrum taken at 400 min (Figure 3). These are probably due to polynuclear complex formation, as suggested by the appearance of the sample which showed some degree of precipitation at this stage of the reaction. In this respect we note that broad ^1H NMR signals have been reported for 1:1 *cis*-[Pt(NH_3)$_2$$X_2$]

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inosine (Ino) and 5′-GMP complexes such as $[(NH_3)_2Pt(InoH_{-1})]^+$ and $[(NH_3)_2Pt(GMPH_{-1})]^-$; there were attributed to polynuclear complex formation.[12,13]

Furthermore, there is clear evidence that none of the species formed during the reaction is a Pt complex with two 5′-GMP molecules bound via N7 of the guanine ring to the Pt atom from both ^1H and ^{195}Pt NMR data of the *cis*-$[Pt(NH_3)_2(5'\text{-}GMP)_2]$ complex. The latter exhibits a H8 proton resonance at 3.931 ppm and a ^{195}Pt resonance of −2451.3 ppm which is considerably shifted to high field from the ^{195}Pt resonances of species IG/IIG and IIIG (see Table III). Further, the resonances for the H1′ protons in the *cis*-$[Pt(NH_3)_2(5'\text{-}GMP)_2]$ complex are at 2.143 ppm, 0.020 ppm to high field of the corresponding resonance in free 5′-GMP (2.163 ppm), while in species IG, IIG, and IIIG the H1′ signals are at ca. 2.28 ppm which is to low field of the corresponding free signal.

From the structures of species IG/IIG given in Figure 9 in which the Pt atom is bound to N7 of the guanine ring, one would expect the ^{195}Pt chemical shift of species IG/IIG to be given approximately by the mean of the ^{195}Pt chemical shifts of *cis*-$[Pt(NH_3)_2Cl_2]$ and *cis*-$[Pt(NH_3)_2(5'\text{-}GMP)_2]$; this has a value of −2306.4 ppm which is in excellent agreement with the measured value of −2302.0 ppm.

Comparison of the Kinetics of the Reactions of 5′-AMP and 5′-GMP with Excess *cis*-$[Pt(NH_3)_2Cl_2]$ in the Presence of KCl. A comparison of the kinetics of the reactions of 5′-AMP and 5′-GMP with excess *cis*-$[Pt(NH_3)_2Cl_2]$ in the presence of KCl at 80 °C leads to the following observations. Tables I and II show the relationship $k_{1G} \sim k_{1A} > k_{3A} = k_{4A} > k_{2A}$ for the second-order association rate constants, which implies that the dissociation of a Cl atom from *cis*-$[Pt(NH_3)_2Cl_2]$ is not rate limiting. Considering only the 5′-AMP reaction with *cis*-$[Pt(NH_3)_2Cl_2]$, we observe that the association rate constant for binding to N7 (k_{1A}) is larger than that for binding to N1 (k_{2A}), a finding which correlates inversely with the smaller p*K* of N7 compared to N1 of the adenine ring.[25] However, once a Pt atom is bound to either N1 or N7, the association rate constants (k_{3A} and k_{4A}) for binding to the remaining site are equal. This implies an electronic redistribution upon binding of a Pt atom to either N1 or N7, such that the reactivity toward *cis*-$[Pt(NH_3)_2Cl_2]$ of the remaining available site is less than that of N7 but greater than that of N1 in free 5′-AMP. Considering the schemes for the reaction of 5′-GMP with excess *cis*-$[Pt(NH_3)_2Cl_2]$, it is clear that species IG and IIG could either be formed simultaneously (as in the case of species IA and IIA in the 5′-AMP reaction) or in sequence. Given our proposed structures for species IG and IIG (Figure 9), the latter seems unlikely. Moreover, it is likely that the association rate constants for the formation of the two rotamers, IG and IIG, would be equal and given by $k_{1G}/2$. Assuming this assumption is correct, we note the relationship $k_{1A} > k_{1G}/2 > k_{2A}$ for the second-order association rate constants for the binding of the first molecule of *cis*-$[Pt(NH_3)_2Cl_2]$ to 5′-AMP and 5′-GMP which is inversely correlated with the known relationship $pK_a(N7)_{5'\text{-}AMP} < pK_a(N7)_{5'\text{-}GMP} < pK_a(N1)_{5'\text{-}AMP}$ for the protonation of ring nitrogen atoms.[25]

1,2,5-Thiadiazole 1-Oxides. 3. An Experimental and Theoretical Investigation of the Inversion Barrier

Joseph S. Amato, Sandor Karady,* Robert A. Reamer, H. Bernhard Schlegel,*† James P. Springer, and Leonard M. Weinstock*

Contribution from the Merck Sharp & Dohme Research Laboratories, Division of Merck & Company, Inc., Rahway, New Jersey 07065. *Received June 29, 1981*

Abstract: The 1,2,5-thiadiazole 1-oxide system was synthesized via cyclocondensation of diethyl oxalimidate or dimethyl thiooxalimidate with thionyl chloride. The alkoxy and alkylthio group in these products are replaced by amines, e.g., pyrrolidine, which produced 3-ethoxy-4-(1-pyrrolidinyl)-1,2,5-thiadiazole 1-oxide from the diethoxy analogue at room temperature. Examination of this product by ^{13}C NMR in the presence of a chiral shift reagent showed two isomers, indicative of a stable pyramidal sulfoxide structure. Reaction of 3,4-diethoxy-1,2,5-thiadiazole 1-oxide with optically active amines, e.g., *l*-ephedrine, produces readily separable diastereoisomeric mixtures. The diastereoisomers undergo inversion only at elevated temperature, $\Delta G^{*}_{120} = 33$ kcal mol^{-1}, compared to only ca. 14.8 kcal mol^{-1} for a thiophene 1-oxide and 36 kcal mol^{-1} for diaryl sulfoxides. X-ray analysis of 3,4-bis(methylthio)-1,2,5-thiadiazole 1-oxide demonstrates that the ring is essentially nonaromatic and confirms the pyramidal sulfoxide structure. Interaction between the sulfur lone pair and the diene is small, the C_3–C_4 bond length lying closer to that of cyclopentadiene than of thiophene or thiadiazole. Theoretical calculations indicate that aromaticity effects lower the inversion barrier nearly equally in the thiophene and thiadiazole sulfoxides by stabilizing the planar transition state and destabilizing the nonaromatic pyramidal structure. The reduction of the barrier in the thiadiazole, however, is counteracted by the effect of the electronegative nitrogen atoms, thus raising the inversion barrier back to the range of normal sulfoxides.

1,2,5-Thiadiazole (**1**) is a planar aromatic ring system with molecular parameters similar to thiophene. The aromaticity of **1** is supported by theoretical calculations, physical measurements, and chemical reactivity.[1] The 1,1-dioxides of 1,2,5-thiadiazoles **2**,[2] on the other hand, do not appear to exhibit aromatic properties and are much less stable[1] thermally and more electrophilic than the nonoxidized form. Little is known, however, about the corresponding monoxides (**3**). In fact, the literature contains very few detailed accounts of the chemistry of sulfoxides derived from thioaromatic systems. Mock[3] prepared 2,5-di-*tert*-octylthiophene 1-oxide (**4**) and showed spectroscopically that two forms of the pyramidal sulfur can be observed at −10 °C, while at room temperature the sulfoxide undergoes rapid inversion. This low inversion barrier (e.g., ca. 20 kcal mol^{-1} less than diaryl sulfoxides) was attributed by Mock to be a consequence of either a delo-

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† Department of Chemistry, Wayne State University, Detroit, MI 48202.

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