Partial displacement of a triamine ligand from a platinum(II) complex after reaction with N-acetylmethionine

Kevin M. Williams^{a,*}, Morgan Gruner,^a Julia Gensheimer,^a Alexandra Wright,^a Morgan Blair,^a Shane A. Autry,^b and Nathan I. Hammer^b

^aDepartment of Chemistry, Western Kentucky University, 1906 College Heights Blvd #11079, Bowling Green, KY 42101-1079

^bDepartment of Chemistry and Biochemistry, University of Mississippi, Oxford, MS 38655.

* Corresponding author: Kevin M. Williams, Department of Chemistry, Western Kentucky University, 1906 College Heights Blvd #11079, Bowling Green, KY 42101-1079, Phone: 270-745-8899, Fax: 270-745-5361, e-mail: kevin.williams@wku.edu

Abstract

The compound $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]\text{Cl}$, where Et_2dien = N,N-diethyldiethylenetriamine, has been synthesized and reacted with N-acetylmethionine (N-AcMet) and guanosine 5'-monophosphate (5'-GMP). Reaction with 5'-GMP at pH 4 leads to formation of $[\text{Pt}(\text{Et}_2\text{dien})(5'\text{-GMP})]^+$, as expected. Reaction with N-AcMet occurs more rapidly than with 5'-GMP; when the reaction with N-AcMet occurs at low pH values (~ 2), one set of resonances is observed initially but a second set of resonances appears over several days as the initial set decreases. The initial set of resonances disappears slowly (several days) at pH 2 but disappears much faster (minutes to hours) if the pH is 4 or higher; the initial resonances do not re-appear even if the pH is lowered. Reaction of the $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ with a mixture of N-AcMet and 5'-GMP at pH 4 leads to formation of a unique set of NMR resonances that does not correspond to the resonances of either the 5'-GMP or the N-AcMet products; mass spectrometry identifies a new product corresponding to $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})(5'\text{-GMP})]$, in which the Et_2dien ligand is bidentate. Such a complex is also observed if 5'-GMP is added to a sample of $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$ that has been kept at low pH, but it is not observed when N-AcMet is added to a sample of $[\text{Pt}(\text{Et}_2\text{dien})(5'\text{-GMP})]^+$. We propose that the additional set of resonances observed for $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$ at low pH is due to a less stable conformation of the complex and the addition of 5'-GMP when this less stable conformation exists leads to displacement of one of the nitrogen atoms.

Keywords

Platinum; methionine; guanine; nuclear magnetic resonance; molecular mechanics; Raman

Abbreviations

Et_2dien , N,N-diethyldiethylenetriamine; Me_5dien , N,N,N',N',N''-pentamethyldiethylenetriamine;
N-AcMet, N-acetylmethionine

1. Introduction

Because of the cytotoxic activity of cisplatin and its derivatives, the reactions of platinum complexes with amino acid and nucleotide targets has been studied for many years. Reaction with DNA, primarily at guanine residues, is widely accepted to be responsible for cytotoxicity; however, reaction with proteins is also important. Methionine is one of the primary amino acid targets; as methionine and cysteine residues are a key part of the copper transporter CTR1,¹ which may be partly responsible for cisplatin uptake,^{2,3} it is important to understand how platinum complexes react with both methionine and guanine.

Reactions of platinum(II) triamine complexes with N-acetylmethionine (N-AcMet) and guanosine 5'-monophosphate (5'-GMP) have been studied as simple models systems of cisplatin with only one leaving ligand. $[\text{Pt}(\text{dien})\text{Cl}]^+$ has been shown to react faster with thioethers such as S-methylglutathione and methionine than with 5'-GMP.^{4,5} $[\text{Pt}(\text{dien})\text{Cl}]^+$ has therefore represented a simple model system in which only one biological ligand coordinated to the platinum complex. The faster reactivity of thioethers relative to 5'-GMP of this simple model system is consistent with observations that cisplatin formed more adducts with proteins than with DNA.⁶

The sterically hindered $[\text{Pt}(\text{Me}_5\text{dien})(\text{H}_2\text{O})]^{2+}$ complex slowed reaction with N-AcMet more than with 5'-GMP so that the latter was kinetically favored.⁷ This was the first report of a platinum(II) triamine complex with a significant kinetic preference for 5'-GMP over N-AcMet. Because the 5'-GMP ligand is planar at the site of Pt reaction (N7) and the N-AcMet is not, the steric hindrance affected the methionine reaction more. Later studies with $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{2+}$ and $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{2+}$ indicated that the steric hindrance in the latter slowed reaction with N-AcMet by a factor of ~16, indicating that having a tertiary amine nitrogen atom *cis* to the coordination site significantly affected the kinetics of the reaction.⁸

Separately, reactivity of the highly cytotoxic phenanthriplatin and the less cytotoxic pyriplatin were compared, and the larger size of the ligand of the former was found to slow reaction with N-AcMet more than with 5'-GMP.⁹ This observation is consistent with the interpretation from other studies that steric hindrance due to nonleaving ligands has a greater effect on N-AcMet than on 5'-GMP. Unlike the traditional platinum anticancer drugs, phenanthriplatin does not bend the DNA but instead arrests transcriptional machinery. Thus, as the heterocyclic ligands of a series of platinum compounds increased in size, the cytotoxicity increased as well. Other monofunctional compounds with steric hindrance have also shown significant antitumor activity as well.¹⁰

Phenanthriplatin has three nitrogen containing ligands and a single leaving Cl and thus has some similarities to the dien-based triamine complexes; however, only one of the ligands *cis* to the leaving position (the phenanthridine) is sterically hindering, and thus neither the dien nor the Me₅dien ligand have a similar steric pattern to phenanthriplatin though the steric hindrance is on an sp³ rather than an sp² nitrogen atom. The current study focuses on the reactivity of a triamine complex with steric hindrance on only one side of the coordination plane.

[Pt(Et₂dien)Cl]Cl, where Et₂dien = N,N-diethyldiethylenetriamine, has been prepared using a method similar to that reported for [Pt(dien)Cl]Cl previously.⁴ The Et₂dien ligand has significant steric hindrance on one of the two nitrogen atoms *cis* to the coordination site (see Scheme 1).

Both [Pt(dien)(Met)]²⁺ and [Pt(Me₅dien)(N-AcMet)]⁺ complexes are prone to slow displacement of the methionine residue when 5'-GMP is added to the solution.^{5,7} Thus, the 5'-GMP products are thermodynamically favored even though in the former case the methionine product is kinetically favored. In contrast to the displacement of the methionine residues in these previous studies, the [Pt(Et₂dien)(N-AcMet)]⁺ complex in the present study shows a unique reactivity with 5'-GMP.

2. Materials and Methods

Potassium tetrachloroplatinate (Sigma-Aldrich), N-acetylmethionine (Acros), Guanosine 5'-monophosphate (Sigma-Aldrich), N,N-diethyldiethylenetriamine (Sigma-Aldrich), and 2-hydroxy-4-(methylthio)butyrate (Sigma-Aldrich) were used as received. [Pt(dien)Cl]Cl was synthesized by a previously published procedure.⁴

2.1. Synthesis of [Pt(Et₂dien)Cl]Cl. [Pt(Et₂dien)Cl]Cl was synthesized by a method similar to that used for [Pt(dien)Cl]Cl⁴. Potassium tetrachloroplatinate (1 g) was dissolved in 30 mL of DI water, and 1 mL of N,N-diethyldiethylenetriamine was added. The pH of the solution was lowered to ~3 with HCl, and the solution was refluxed for at least 6 hours. The volume of the solution was reduced, and the resulting precipitate was collected and washed with ethanol.

2.2. Reaction of [Pt(Et₂dien)Cl]Cl. Typically, 10 mM stock solutions of [Pt(Et₂dien)Cl]Cl, 5'-GMP, and/or N-AcMet were prepared in D₂O; the pH of each solution was adjusted as necessary. Aliquots of the stock solutions were mixed to give solutions of the appropriate final concentrations.

2.3. NMR spectroscopy. ¹H and ¹⁹⁵Pt NMR data were collected on a JEOL 500 MHz NMR instrument. Spectra were referenced to the residual HOD signal (¹H) or K₂PtCl₆ (¹⁹⁵Pt).

2.4. Mass spectrometry. Mass spectra were acquired on a Varian LC/MS 500 Ion Trap instrument in positive ion mode using an m/z range from 0-1100. The samples were injected via direct infusion.

2.5 Molecular mechanics and dynamics calculations. Molecular mechanics and dynamics calculations were performed using HyperChem 7 (Hyper, Inc.) for the PC. The AMBER forcefield with previously described modifications to include platinum bonded to guanine¹¹ and

methionine¹² were included. Charges were set as described previously¹². Molecular dynamics were run at 300 K for 250 ps, saving structures every 1 ps. The generated structures were subjected to 1000 cycles of steepest descents and 10,000 cycles of Fletcher-Reeves conjugate gradient minimization until the gradient was <0.01 kcal/mol Å.

2.6 Quantum mechanics calculations. The B3LYP density functional method was used with the 6-311G(d,p) basis set to optimize the molecular geometries and calculate the vibrational frequencies and Raman intensities using the Gaussian 09 suite of programs. Raman spectra were simulated by summing Lorentzian lineshapes of each frequency.

2.7 Raman Spectroscopy. Raman spectra were acquired using a 785 nm laser line and a Horiba Scientific LabRAM HR Evolution Raman Spectroscopy system.

3. Results

3.1 Comparison of [Pt(Et₂dien)Cl]⁺ and [Pt(dien)Cl]⁺. A comparison between [Pt(dien)Cl]⁺ and the analogous [Pt(Et₂dien)Cl]⁺ structure was studied using electronic structure theory and Raman spectroscopy in order to determine possible differences in the physical characteristics exhibited by the two ethyl ligand groups. When comparing the experimental Raman data there is an observed red shift in the [Pt(Et₂dien)Cl]⁺ spectrum when compared to the [Pt(dien)Cl]⁺ spectrum (Figure 1).

The two structures of interest were both optimized to their lowest energy states, and frequency calculations were generated in order to obtain simulated Raman data. Both experimental and theoretical Raman spectra showed a vibrational red shift of the [Pt(Et₂dien)Cl]⁺ relative to the [Pt(dien)Cl]⁺ structure. Also, a strain on the bond angle of the Cl-Pt-N(Et₂) was observed theoretically. The bond angle for the Cl-Pt-N was calculated to be ~96°, and the Cl-Pt-N(Et₂) bond angle was calculated to be ~102°.

3.2. Reaction of [Pt(Et₂dien)Cl]Cl with 5'-GMP. When [Pt(Et₂dien)Cl]Cl was reacted with 5'-GMP, a new set of signals appeared over time that was consistent with the formation of [Pt(Et₂dien)(5'-GMP)]⁺ (Figure 2). At pH 4, a broad feature is seen at ~8.5 ppm, a chemical shift indicative of coordination of Pt to N7; this feature grows over time, and even after several days the reaction is not complete. When the temperature of the sample is raised, two distinct, broad resonances are observed in the H8 region. Because the Et₂dien ligand is non-C₂-symmetrical, two rotational isomers are possible; the H8 atom of the guanine base could be on the same side or on the opposite side as the hydrogen of the *trans* (central) NH group of the dien ligand. Interconversion of these rotational isomers would require rotation of the GMP around the Pt-N7 bond; since one of the nitrogen atoms of the Et₂dien ligand has steric hindrance, interconversion of these rotational isomers on the NMR timescale would likely be slow, consistent with the observation of multiple H8 signals.

The reaction of 5'-GMP at pH 7 is somewhat slower than the pH 4 reaction according to NMR observations (Figure 2); only one H8 resonance is observed for the product at ~8.6 ppm, though two H1' signals are observed. The pK_a of platinum-coordinated water molecules are typically ~5.5-6.5,¹³ and thus slower reaction at pH 7 may be due to the conversion of [Pt(Et₂dien)Cl]⁺ into [Pt(Et₂dien)(OH)]⁺. Reaction of 5'-GMP at pH 2, in contrast, occurs very slowly because the N7 atom would be largely protonated at this pH.¹⁴

3.3. Reaction of [Pt(Et₂dien)Cl]Cl with N-AcMet. When [Pt(Et₂dien)Cl]⁺ is reacted with N-AcMet at pH 2.0, broad NMR signals are observed for the reaction products; the initial product formation occurs with a half-life of approximately 2 h at room temperature, which is considerably faster than the reaction with 5'-GMP at any pH. Broad resonances are observed at room temperature, but at 60 °C there is a sharper resonance at ~2.45 ppm corresponding to an S-

CH₃ resonance of a sulfur-coordinated N-AcMet. After one day, the resonance at 2.45 ppm decreases and a resonance at 2.6 ppm appears and increases over time; however the 2.45 ppm resonance is still visible after 8 days (Figure 3). If the reaction pH is raised to 4 or higher, the peak at ~2.45 ppm disappears within ~1 h; also, if the initial reaction pH is 4 or higher, the resonance at 2.45 ppm is never prominent (Figure 3). Once the resonance at 2.45 ppm disappears, it does not return even if the pH of the sample is lowered again.

Both the 2.45 and 2.6 ppm signals in the ¹H NMR spectrum are in a range that is expected for the S-CH₃ resonance of a methionine residue that is coordinated to platinum via the sulfur atom. Both of these sets of resonances observed at pH 2 are independent of the relative ratios of the Pt and the N-AcMet that are reacted together, as both can be observed regardless of whether there is excess platinum or excess N-AcMet. Furthermore, ¹⁹⁵Pt NMR data for both complexes show chemical shifts near -3300 ppm, which is a shift range characteristic of a PtN₃S environment.

To determine whether one complex could have the methionine residue coordinated via both the sulfur and amide nitrogen atoms (partially displacing the Et₂dien ligand), we also looked at the reaction of [Pt(Et₂dien)Cl]⁺ with 2-hydroxy-4-(methylthio)butyrate, which is a methionine analog with an –OH group replacing the –NH₃⁺ of methionine. When the reaction was conducted at a pH of ~2, similar ¹H NMR resonances were observed (~2.45 and 2.6 ppm) to those seen in the analogous reaction with N-AcMet (Figure 4); furthermore, ¹⁹⁵Pt NMR data showed chemical shifts again characteristic of a PtN₃S environment, indicating that only the sulfur atom of the incoming ligand was coordinated to the platinum center and that the Et₂dien ligand remained tridentate. Thus, the species corresponding to the 2.45 ppm resonance is most likely a less stable form of [Pt(Et₂dien)(N-AcMet)]⁺ (the charge of +1 is when the methionine carboxyl group is deprotonated) that converts into a more stable form (corresponding to the 2.6 ppm resonance).

3.4 *Molecular mechanics calculations of [Pt(Et₂dien)(Met)]*. In order to better understand the differences between the intermediate and final products formed in the reaction of [Pt(Et₂dien)Cl]⁺ with N-AcMet, we used molecular mechanics and dynamics models of the methionine complex. The sulfur atom of such complexes is chiral, and molecular dynamics simulations usually do not invert this chirality in the 250 ps simulated time. Likewise, the central (*trans*) nitrogen atom of the dien is also chiral. Thus, calculations were performed on all chirality combinations of the sulfur atom and nitrogen atom. Also, the methionine was rotated to different starting positions prior to beginning the dynamics; general starting positions were labeled *syn* and *anti*, where *syn* designates positions in which the improper torsion angle between the carbon of the S-CH₃ group and the hydrogen of the dien NH was ~0° (i.e. the carbon and hydrogen are on the same side of the coordination plane). Although there is possible rotation around the Pt-S bond, during the course of the dynamics and minimization the interconversion between the *syn* and *anti* forms is usually not observed due to the steric hindrance between the methionine and the ethyl groups of the Et₂dien ligand.

We noted that the minimum energy of the complex was strongly dependent on the orientation of the methionine (Table 1). For each combination of chirality at the N and S atoms, there was a particular methionine orientation favored (the improper torsion would be mostly *anti* for R,R and S,S; mostly *syn* for R,S or S,R); the favored methionine orientation in each case was at least ~3.5 kcal/mol more stable than the other orientation. In each of these four favored methionine orientations, the carbon atoms directly bonded to the sulfur were oriented away from the ethyl groups of the Et₂dien to reduce the interligand clashes (Figure 5). The two lowest energy structures of those four were those with the *anti* relationship of the NH and the S-CH₃ groups. A key difference in the energies was due to angle strain; specifically, in the lowest energy structure (R,R; *anti*), the S-Pt-N(Et₂) bond angle was ~6° lower than in the highest energy structure (R,R; *syn*). Thus, the two [Pt(Et₂dien)(N-AcMet)]⁺ forms observed

experimentally may consist of one more strained structure (2.45 ppm ^1H NMR resonance) forming kinetically and a more stable structure that eventually forms from the kinetic product (2.6 ppm resonance).

3.5. Reaction of $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]\text{Cl}$ with N-AcMet and 5'-GMP. Experimentally, we reacted $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ with a mixture of N-AcMet and 5'-GMP at pH 4 (Figure 6). After ~2 days, there were NMR resonances visible that were not consistent with any of the previously observed products with N-AcMet or 5'-GMP. A set of four broad H8 resonances were observed in the 8.8-8.9 ppm region, which indicate coordination of Pt to N7 but are significantly downfield of what was observed for the GMP product. Broad resonances were also observed around 2.3 ppm, indicative of Pt-S coordination but upfield of those seen for the N-AcMet products. The ^{195}Pt NMR gave a broad resonance at -3240 ppm, slightly downfield of the signals around -3300 seen for the N-AcMet adducts. When the sample was heated, the H8 resonances in the ^1H NMR spectrum eventually coalesced into two resonances by 80 °C, suggesting the different resonances are due to different conformations of a single product. When the pH of the sample was raised to 7 after the reaction had completed, no significant change in the signals was observed. However, if the reaction began and remained at pH 7, the unique resonances (8.8-8.9 and ~2.3 ppm) were never observed.

Mass spectrometry of the sample that was reacted at pH 4 (Figure 7) showed significant peaks at 353.2 ($[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+ - \text{HCl}$), 389.2 ($[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$), 544.3 ($[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$), and 907.5. The latter mass is consistent with $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})(5'\text{-GMP})]^+$. This unique product has N-AcMet, 5'-GMP, and the Et_2dien ligand all coordinated to the platinum center; thus, the Et_2dien ligand is bidentate rather than tridentate in this case.

We could also observe this product by NMR spectroscopy by adding 5'-GMP to a sample of $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$ that had reacted and remained at pH 2 prior to the addition of 5'-GMP;

however, we did not see evidence of this product when N-AcMet was added to a sample of $[\text{Pt}(\text{Et}_2\text{dien})(5'\text{-GMP})]^+$ or if 5'-GMP were added to a sample of $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$ that was at a pH >4 or had been previously raised to pH >4.

4. Discussion

The reactions of $[\text{Pt}(\text{dien})\text{Cl}]^+$ and $[\text{Pt}(\text{dien})(\text{D}_2\text{O})]^{2+}$ with thioethers and 5'-GMP have been well-studied; in these reactions, the thioether reacts faster than the 5'-GMP,^{4,5,7} reflecting the preference of platinum for soft ligands. However, steric hindrance on the amine ligands of platinum diamine and triamine complexes tends to slow the reaction of methionine more than it slows reaction with 5'-GMP. In the case of $[\text{Pt}(\text{Me}_5\text{dien})(\text{H}_2\text{O})]^{2+}$, reaction with 5'-GMP is significantly faster than with N-AcMet.⁷

Monofunctional platinum(II) compounds containing two NH_3 ligands and one heterocyclic ligand were found to have significant toxicity to various cancer cell lines.⁹ Two of these compounds, pyriplatin and phenanthriplatin, were compared in their reactivity with 5'-GMP and N-AcMet; phenanthriplatin's larger heterocyclic ligand slowed reactivity with N-AcMet more than with 5'-GMP.

The $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ represents somewhat of an intermediate between $[\text{Pt}(\text{dien})\text{Cl}]^+$, which has little interligand steric hindrance, and $[\text{Pt}(\text{Me}_5\text{dien})(\text{H}_2\text{O})]^{2+}$, which has hindrance possible from both nitrogens that are *cis* to the available coordination site. Also, the steric hindrance on only one *cis* N to the incoming site is similar to phenanthriplatin's steric profile. Raman spectroscopy indicated that the $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ had interligand strain that broadened the N(Et_2)-Pt-Cl angle (Figure 1). The $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ complex reacts significantly faster with N-AcMet than with 5'-GMP (Figures 2 and 3), with the former reacting within hours and the latter reacting within days. Thus, the preference of 5'-GMP over N-AcMet appears to be unique to complexes

such as $[\text{Pt}(\text{Me}_5\text{dien})(\text{D}_2\text{O})]^{2+}$ that have steric hindrance at both nitrogen atoms that are *cis* to the leaving ligand.

Nevertheless, unique reactivity of the $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ with N-AcMet is observed, especially when the pH is kept low. Two different sets of resonances are observed in NMR spectra of $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$ (Figure 3); the differences are not due to chelation of the amide nitrogen, as a methionine analog without a nitrogen atom shows a similar pattern of signals (Figure 4). Furthermore, ^{195}Pt NMR spectra and reactions at different Pt:N-AcMet ratios indicate that only one methionine is coordinated. One set of signals slowly disappears at pH 2 but disappears much more rapidly when the pH is >4 ; the disappearance of this set of resonances is irreversible, as lowering the pH does not result in their reappearance. Reaction of N-AcMet *cis* to a nitrogen with two ethyl groups would be expected to be sterically hindered; for example, when N-AcMet was added to $[\text{Pt}(\text{Et}_2\text{en})(\text{D}_2\text{O})_2]^{2+}$, which has steric hindrance *cis* to one of the available coordination sites, reaction with N-AcMet occurred exclusively *cis* to the unhindered nitrogen and no product with two N-AcMet's coordinated was observed.¹⁵ However, this reaction of N-AcMet with $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ is significantly faster than the reaction of N-AcMet with $[\text{Pt}(\text{Me}_5\text{dien})(\text{H}_2\text{O})]^{2+}$ despite the latter having a weaker leaving ligand.⁷

Molecular mechanics results suggest that the energy of the $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$ complex would be highly dependent on the orientation of the methionine (Table 1, Figure 5), and rotation around the Pt-S bond would likely be sterically hindered; furthermore, two chiral centers (the nitrogen atom of the dien NH group and the sulfur of the N-AcMet) could slowly change chiralities. Inversion of the chirality of nitrogen atoms was previously observed for platinum complexes of Me_3dien ¹⁶ and Me_2dap ¹⁷, the latter occurring much faster as the pH was raised. The pH-dependence of the slow conversion of the less stable $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^+$ complex to the more stable form suggests that inversion of the nitrogen chirality is one necessary part of the interconversion between the experimentally observed forms, as conformational changes and

sulfur inversion would not be expected to show a significant pH dependence. However, the MM calculations suggest that the inversion of the nitrogen would not be sufficient for changing to a more stable conformation, as the relative positions of the methionine residue and the ethyl groups would be unchanged (for example, inversion of the nitrogen chirality of R,R,*syn* would produce S,R,*anti*; this is also a structure with a relatively high strain energy, see Table 1). The exchange of the NH group may lead to additional conformational flexibility that permits inversion of the sulfur chirality or rotation around the Pt-S bond.

The [Pt(Et₂dien)(N-AcMet)(5'-GMP)] product observed when N-AcMet and 5'-GMP are both added at a pH of ~4 is unusual because a tridentate ligand is partially displaced by a monodentate ligand (GMP). Formation of the product requires N-AcMet to react first, followed by reaction of GMP; if 5'-GMP is reacted first and then N-AcMet is added later, no reaction with the N-AcMet occurs (Figure 8, bottom left). Further, the reaction only occurs if the pH of the sample remains low (pH ~4). Thus, consistent with our model of a less stable conformation of [Pt(Et₂dien)(N-AcMet)]⁺ forming initially, if GMP is present in the sample when the complex is in its less stable form (Figure 8, top middle), then displacement of one nitrogen of the Et₂dien ligand is possible because such a displacement would relieve the interligand strain. However, once the complex converts to its stable form (Figure 8, top right), GMP cannot displace the Et₂dien ligand; thus, the displacement only occurs on the initial (strained) N-AcMet complex (identifiable by the ~2.45 ppm singlet in the NMR spectrum) and not the final (stable) N-AcMet complex (~2.6 ppm singlet in the NMR spectrum). Conversion of the reactive form to the stable form occurs slowly (over several days) at pH 2 but more rapidly (~1 hour) at pH 4. Consistent with this model, if both N-AcMet and 5'-GMP are added simultaneously at pH 4, the 5'-GMP is able to displace part of the Et₂dien ligand before the more stable N-AcMet adduct forms (Figure 6). However, if N-AcMet reacts at pH 4 and 5'-GMP is added several days later, the 5'-GMP does not react.

Because of the broadness of the signals and the large number of signals in the ~2.5-3.0 ppm (including not only the dien CH₂ groups but also ribose hydrogens from the GMP), it was not possible to assign the signals of the dien CH₂ groups to potentially identify which nitrogen of the Et₂dien was displaced. A bidentate dien ligand with the central nitrogen uncoordinated has been characterized previously;¹⁸ however, this complex was formed from reduction of a six-coordinate platinum(IV) complex in which the dien ligand had been tridentate. The most likely nitrogen atom of the Et₂dien ligand that was displaced in this case is the one with the two ethyl groups attached, as interligand strain between that atom and the N-AcMet would be the most severe.

Coordination of only two nitrogens of a diethylenetriamine ligand were observed in the synthesis of [Pd(Me₃dien)Cl₂] $\cdot$ HCl, though in solution the Me₃dien ligand became tridentate.¹⁹ Partial displacement of a dien ligand (from tridentate to bidentate) was previously observed in the presence of methionine, which has an amine nitrogen.²⁰ When the nitrogen-coordinated methionine complex had the pH lowered from 8 to 3, coordination of the sulfur displaced one of the dien nitrogens to form a long-lived intermediate. Also, the breaking of one Pt-N bond of en has been observed when chelation of a di- or tri-peptide occurs.²¹ Likewise, the displacement of one nitrogen of dien was concurrent with the formation of a S,N chelate.²⁰ We have previously observed the conversion of [Pt(en)(SeMet-Se)₂] into [Pt(SeMet-Se,N)₂] to completely dissociate a chelated en ligand.²² However, in each of these cases, the breaking of one chelate led to formation of another chelate. The breaking of one Pt-N bond of triamine ligand and replacement by a monodentate ligand (5'-GMP) observed in the present research is unusual. This displacement occurs at a pH of ~4; at a pH of 7, displacement is not observed and thus this displacement would not be biologically relevant for this particular complex. It is further interesting that the breaking of the chelate by 5'-GMP is observed at pH 4 but the chelate is not broken by excess N-AcMet at pH 4 or even pH 2; lower pH would be expected to increase the

likelihood of chelate displacement. The displacement by a second N-AcMet may be prevented by steric hindrance.

Recently, two 9-EtGua molecules coordinated to $[\text{Pt}(\text{di}-(6\text{-methyl-2-picolyl})\text{amine})\text{Cl}]^+$, with the second 9-EtGua displacing one of the picoline groups from the platinum atom.²³ Steric hindrance was concluded to be responsible for the weakening of the Pt-N bond of the picoline; however, this steric hindrance was likely inherent in the tridentate ligand, as displacement by chloride also was observed. When the less strained $[\text{Pt}(\text{N}-(6\text{-methyl-2-picolyl})-\text{N}-(2\text{-picolyl})\text{amine})\text{Cl}]^+$ was reacted with 9-EtGua,²⁴ the dominant product had only one 9-EtGua attached with ~20% conversion to the bis 9-EtGua adduct even after several weeks. Steric hindrance on only one side of the coordination plane in the latter was not sufficient for complete conversion to the bis adduct. We did not see any evidence of two 5'-GMP residues reacting with $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ to displace part of the Et_2dien ligand. If two 5'-GMP residues coordinated, the 5'-GMP residues would not be equivalent and the complex would likely have restricted rotation; this would result in several pairs of H8 resonances due to rotational isomers of the GMPs.^{23,25} Thus, the partial displacement of the Et_2dien ligand seems to be possible only after reaction of N-AcMet. Furthermore, excess N-AcMet did not lead to partial displacement of the triamine ligand, probably because steric hindrance would prevent a second methionine from coordinating, similar to sterically hindered platinum(II) diamine complexes that prevent a second methionine from coordinating.^{12,15,26}

When $[\text{Pt}(\text{Me}_5\text{dien})(\text{H}_2\text{O})]^{2+}$ was added to a mixture of 5'-GMP and N-AcMet, reaction with the former occurred almost exclusively.⁷ When N-AcMet was added first and 5'-GMP was added after the formation of $[\text{Pt}(\text{Me}_5\text{dien})(\text{N-AcMet})]^+$ was complete, slow displacement of the N-AcMet to form $[\text{Pt}(\text{Me}_5\text{dien})(5'\text{-GMP})]^+$ was observed; no formation of a product with both N-AcMet and 5'-GMP coordinated is observed even after two weeks at low pH. Reaction of $[\text{Pt}(\text{Me}_5\text{dien})(\text{H}_2\text{O})]^{2+}$ with N-AcMet only shows one set of NMR resonances even at low pH.

Furthermore, the reactions of N-AcMet and 5'-GMP with $[\text{Pt}(\text{dien})\text{Cl}]^+$ have been widely studied and there is no partial displacement of dien by GMP in these reactions. Thus, the Et_2dien ligand is unique among triamine ligands that have been previously studied in terms of the reactivity with N-AcMet. Since the ligand has steric hindrance concentrated on only one nitrogen atom, reaction with N-AcMet occurs reasonably fast initially but forms a product that is prone to partial displacement of the Et_2dien ligand.

Phenanthriplatin⁹ and complexes containing the 1-[2-(acridin-9-ylamino)-ethyl]-1,3-dimethylthiourea (ACRAMTU) ligand²⁷ have one leaving ligand (chloride) and one large nonleaving ligand *cis* to the leaving ligand; thus, sterically, the profile of these compounds has similarities to that of $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$. However, the Et_2dien ligand is a single tridentate ligand that has considerably less conformational flexibility. Furthermore, we note that the phenanthridine nitrogen is sp^2 -hybridized; an earlier study of chloride substitution by a nitrogen ligand was influenced not only by sterics but also by electronic contributions, with sp^2 donor nitrogens increasing the rate of displacement due to decreasing σ basicity.²⁸ Displacement of the phenanthridine or the ACRAMTU ligand by guanine would not be expected in those complexes even after reaction of methionine. However, for platinum(II) complexes having chelated ligands that produce significant interligand clashes near one side of an available coordination position, reaction of methionine at that coordination position could lead to a breaking of the chelate.

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Table 1. Minimum energies of structures from molecular mechanics and dynamics calculations of [Pt(Et₂dien)(N-AcMet)]. All energies are in kcal/mol. Structures are classified by the chirality of the dien NH, chirality of the sulfur atom of Met, and improper torsion angle of the hydrogen of the dien NH relative to the carbon of the S-CH₃.

Chirality at N	Chirality at S	Torsion	Energy
R	R	Syn	25.1
R	R	Anti	17.6
R	S	Syn	19.8
R	S	Anti	23.3
S	R	Syn	18.7
S	R	Anti	22.5
S	S	Syn	24.9
S	S	Anti	17.9

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Figure(s)

[Pt(dien)Cl]⁺

[Pt(Me₅dien)(NO₃)]⁺

[Pt(Et₂dien)Cl]⁺

Figure(s)

Figure 1. Experimental and theoretical Raman spectra for [Pt(Et₂dien)Cl] and [Pt(dien)Cl].

Figure 2. Partial NMR spectra of the reaction of $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ with excess 5'-GMP (indicated by *). Samples were mixed at the indicated pH to give a final concentration of 5 mM of each reactant and the reaction occurred for 6 days at room temperature prior to NMR spectrum collection. Bottom: pH 7. Middle: pH 4. Top: pH 4; spectrum acquired at 50 °C.

Figure 3. Reaction of excess N-AcMet (indicated by * in the top spectrum) with [Pt(Et₂dien)Cl]⁺. Bottom: After 45 minutes of reaction at pH 2. Middle: Different sample, after 8 days of reaction at pH 2. Top: Different sample, after 8 days of reaction at pH 4. All NMR spectra collected at 60 °C.

Figure 4. Partial NMR spectrum of the reaction of 2-hydroxyl-4-(methylthio)butyrate with $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$. Spectrum collected at 60°C .

Figure 5. Minimum energy structures of the anti (left) and syn (right) conformations of [Pt(Et₂dien)(Met)] with the chirality of the sulfur atom and the NH of the dien ring both R. The methionine residue is colored black and is in the front of the structure. The NEt₂ group is on the right.

Figure 6. NMR spectra of the reaction of equimolar N-AcMet and 5'-GMP with $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ at pH 4 after 5 days at room temperature.

Figure 7. Mass spectrum of the products of the reaction of $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]^+$ with N-AcMet and 5'-GMP at low pH.

Figure(s)

Figure 8. Proposed reaction scheme for $[\text{Pt}(\text{Et}_2\text{dien})\text{Cl}]$ with N-AcMet and 5'-GMP. Initially, N-AcMet reacts to form $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^*$, which is the form that can react with 5'-GMP to displace part of the Et_2dien ligand. Slowly at pH 2 or more rapidly at pH 4, the reactive $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]^*$ forms the more stable $[\text{Pt}(\text{Et}_2\text{dien})(\text{N-AcMet})]$, which cannot react with 5'-GMP.