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Oxaliplatin and Oxaliplatin Derivatives: Synthesis, Characterization, and Reactivity with Biologically Relevant Ligands

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OXALIPLATIN AND OXALIPLATIN DERIVATIVES: SYNTHESIS, CHARACTERIZATION, AND REACTIVITY WITH BIOLOGICALLY RELEVANT LIGANDS

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Honors College Graduate Distinction at Western Kentucky University

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ABSTRACT

Oxaliplatin is a third generation anticancer drug that has proven to be successful in fighting ovarian and testicular cancer. We are interested in determining how oxaliplatin and oxaliplatin derivatives interact with proteins, as well as how that interaction is affected by the size and shape of these platinum compounds. We have synthesized oxaliplatin as it is used in cancer treatment, as well as similar platinum compounds with the same diaminocyclohexane ligand as oxaliplatin but with additional bulk added to the nitrogen atoms. We are reacting oxaliplatin with key amino acids, including methionine, and will be comparing the kinetics of this reaction, as well as the adducts formed in the reaction, with the bulkier oxaliplatin derivative.

Keywords: oxaliplatin, platinum (II) compounds, anti-cancer drugs, cisplatin, nuclear magnetic resonance

To Dr. Williams, for being endlessly patient with and supportive of the girl who broke the sink her first day in his lab. Without his confidence in me and his encouragement, I would not have had the opportunity to present my research at local and national meetings or gain confidence in my abilities as a developing scientist. My growth as a scientist has been exponential since working with him and it is to him I attribute a great deal of my professional growth.

I would also like to dedicate this to each and every chemistry professor with whom I had class, as well as Dr. Conte, who gave me the opportunity to have the most amazing summer of my life doing research in Taiwan. Through my classes and my research opportunities, I have learned more than I could have ever imagined possible. Though it was never easy, a sense of pride comes with knowing I *earned* every grain of knowledge I have in the field of chemistry.

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CHAPTER 1

INTRODUCTION

With almost 12,000,000 people in the United States having been diagnosed with some form of cancer, as of 2008, there is really no question as to why cancer treatment has been a very popular field of research in many scientific disciplines (*1*). Cancer encompasses more than 200 diseases, each with different detection capabilities, growth rates, and abilities to spread. It is caused by genetic alterations due to genetics, environmental factors and lifestyle choices (*2*). Current methods of treating cancer include surgery, radiation therapy, and chemotherapy, which is treatment using "anti-cancer drugs." One of the first and most widely-used anti-cancer drugs was cisplatin, or *cis*-diamminedichloroplatinum(II).

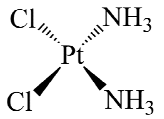


Figure 1. *cis*-diamminedichloroplatinum(II), or cisplatin.

The cancer-fighting ability of platinum(II)-containing compounds was first discovered by accident in 1965 by the Rosenberg group at Michigan State University, when it was observed that the electrolysis of platinum of electrodes generated a platinum complex that inhibited binary fission in Escherichia coli (E. coli) bacteria. This platinum complex was found to be *cis* $PtCl_2(NH_3)_2$, or cisplatin. The complex was then tested and

proved greatly effective in reducing sarcomas in rats(*3*). As a result of this serendipitous finding, cisplatin was developed and approved by the FDA for use in the treatment of cancer in December 1978 (*4*). While cisplatin was found to be very successful in treating testicular and ovarian cancer, there are many disadvantageous side effects associated with the drug and often led to nausea and vomiting, as well as peripheral neuropathy experienced by patients In addition, high levels of nephrotoxicity are associated with cisplatin(*5*). These high levels of toxicity are thought to be a result of the formation of adducts between platinum and DNA, as well as adducts between platinum and proteins (*6*). In response to these harmful side effects, scientists attempted to synthesize platinum(II) compounds with the same therapeutic effects of cisplatin but with lower toxicity. As a result, the second generation anti-cancer drug carboplatin, or cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) was developed. Carboplatin is shown to react much more slowly than cisplatin and is known to form complexes with a longer stability-- two conditions to which its lower nephrotoxicity is attributed (*7*).

The third generation anti-cancer drug oxaliplatin and an analog of the drug are the focus of the current research described throughout this paper. Oxaliplatin, or (R,R')-1,2-diaminocyclohexaneplatinum(II) oxalate, was approved by the FDA for treatment of colorectal cancer and cisplatin-resistant tumors (*8*). The compound consists of a highly stable diaminocyclohexane (dach) ligand that causes the compound to show a behavior that highly contrasts that of cisplatin or carboplatin, both of which also have ammine ligands but different leaving ligands. The reason for this is the inability for the dach ligand to be released from the platinum atom due to its higher stability caused by chelate

effect (*7*); the trans amine ligand in cisplatin and carboplatin can be displaced by the coordination of a sulfur atom.

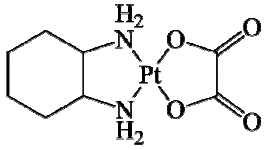


Figure 2. (R,R')-1,2-diaminocyclohexaneplatinum(II) oxalate , or oxaliplatin

Platinum(II) compounds are known to form adducts with nucleobases of DNA and form crosslinks between adjacent nucleobases. These compounds are known to bind to the N7 of guanine in particular. In addition, platinum(II) compounds have a high affinity for sulfur groups, which are often used as "rescue agents" in chemotherapy, meaning they reduce binding to certain biomolecules and therefore reduce certain toxic side effects (*7*, *9*). The cytotoxicity of these anti-cancer compounds is thought to be caused by reactions between the platinum compounds and proteins and peptides, which leads to the formation of Pt-DNA adducts on adjacent guanines. This adduct formation is shown to activate DNA damage recognition proteins, which eventually leads to apoptosis, or programmed cell death (*8*). Since cisplatin reacts more with proteins than with DNA, it is essential to study the reactivity of the compounds with sulfur-containing proteins and peptides. Because platinum has a high affinity for sulfur and methionine is one of two sulfur-containing amino acids, methionine is the main target for platinum interactions with proteins.

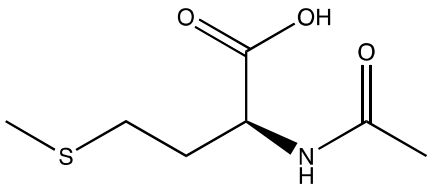


Figure 3. N-Acetyl-Methionine, a derivative of the amino acid methionine

It is for this reason that a study of the reactivity of both oxaliplatin and the analog (R,R')-1,2-dimethyl-diamminocyclohexaneplatinum(II) with the amino acid methionine, which contains a thioether group and is one of two amino acids containing a sulfur atom. An area of interest for future work is the reaction between the two compounds and glutathione, a sulfur-containing peptide.

In a study done by Heudi et al. on the reactions of oxaliplatin and carboplatin with L-methionine, in contrast to cisplatin, it was shown that carboplatin formed the same five methionine-platinum adducts, which is suggestive of carboplatin transforming into cisplatin in the presence of sodium chloride. Since oxaliplatin contains the dach ligand, a different amine ligand, this same transformation to cisplatin does not occur (*6*).

It is thought that oxaliplatin's lower toxicity can be attributed to its preferential reaction with L-methionine rather than guanosine. In addition, the diaminocyclohexane ligand of oxaliplatin is highly stable and has the ability to form a wide range of conformations, including the boat, twist, and chair conformations due to the presence of the flexible cyclohexane ring, with the chair conformation being the most stable and primary form. After oxaliplatin coordinates to DNA, the diamine ligand remains attached to the molecule rather than being released such as in cisplatin and carboplatin.

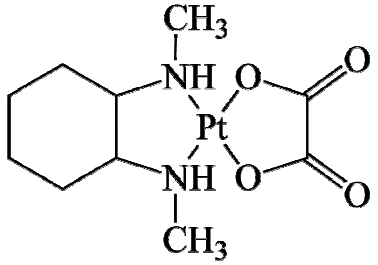


Figure 4. The structure of (R,R)-N,N'-dimethyl-1,2-diaminocyclohexaneplatinum oxalate [Pt(Me_2dach)(ox)].

In our studies, we have used a variety of different techniques to arrive at our results, including inorganic synthesis, Nuclear Magnetic Resonance (NMR) spectroscopy for characterization and kinetics studies, and High Performance Liquid Chromatography (HPLC) for purification of the oxaliplatin analog.

NMR spectroscopy is an essential tool in studying the structure of a molecule. In the research project described, ^{1}H NMR spectroscopy is used to characterize the synthesized compounds, as well as monitor the kinetics of experiments with methionine. ^{1}H NMR spectroscopy gives a separate signal for each hydrogen atom in a compound, allowing for an accurate assignment of position for each hydrogen atom based on known chemical shifts characteristic of a certain proton interaction. ^{1}H NMR spectroscopy is also very useful in studying the kinetics of an experiment, as a spectrum can show you signals of hydrogens from both the reactants and the products of a reaction. Any signals displayed in a spectrum that are not characteristic of the reactant are product signals—as such, you can observe the decrease in the concentration of reactants and subsequent increase in the concentration of product. The rate of reaction can be monitored by examining the decrease of the signal of the reactant and the increase of the product signal.

High Performance Liquid Chromatography is a common technique for the separation and purification of compounds based on polarity. A reverse-phase HPLC column, as used in the described studies to separate isomers, consists of a non-polar stationary phase and a polar mobile phase. A non-polar compound will require more time to elute from the column, whereas a polar compound will elute more quickly. For cases, such as this, a concentration gradient is used in which both water and a polar

solvent are used as the mobile phase, gradually increasing the concentration of the polar solvent in order to gradually elute the more polar compounds from the column.

CHAPTER 2

EXPERIMENTAL METHODS

Synthesis and characterization of Pt(dach)(ox). In order to synthesize oxaliplatin, a mixture of 64 mg liquid diaminocyclohexane ligand and 5 mL of methanol were added dropwise to a mixture of 232 mg potassium tetrachloroplatinate and 5 mL deionized water until a color change to yellow was absorbed and a precipitate was formed. The reaction yielded 128.8 mg of $Pt(dach)Cl_2$. 64.8 mg of the $Pt(dach)Cl_2$ was then reacted with 51.8 mg of silver oxalate to yield 27.9 mg of oxaliplatin, the identity of which was confirmed by ^1H NMR in D_2O using a 500 MHz JEOL Eclipse instrument.

Synthesis and characterization of Pt(Me_2dach)(ox). $Pt(Me_2dach)(Cl_2)$ was then synthesized by adding dropwise 56.8 mg Me_2dach in 5.0 mL methanol to 166 mg potassium tetrachloroplatinate in 5.0 mL deionized water and stirring for a minimum of 2 hours. The product was then converted to $Pt(Me_2dach)(ox)$ by reacting 100 mg of $Pt(Me_2dach)(Cl_2)$ with 75 mg silver oxalate in 35 mL deionized water. The reaction stirred overnight and was then filtered and dried. The reaction yielded 72.2 mg of $Pt(Me_2dach)(ox)$, which was confirmed by NMR using the same method as the Pt(dach)(ox) . NMR and mass spectrometry also confirmed a mixed isomer form of the product.

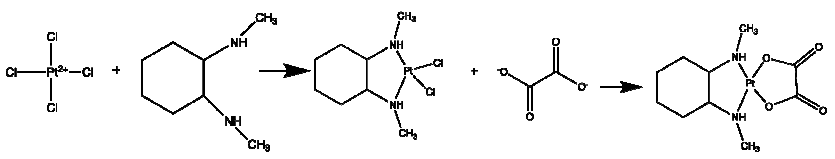


Figure 5. Reaction mechanism for synthesis of Pt(Me_2dach)(ox).

Purification of Pt(Me_2dach)(ox). The mixed isomer form of the Pt(Me_2dach)(ox) compound was separated using HPLC. The separation was first tested using an analytical column and it was found that the isomers could be separated using a gradient of water and methanol. The separation of the compound was then performed on an apHera C18 Polymer HPLC column (25 cm x 4.6 mm x 5 μm) using a Hitachi Lachrom Elite system. A concentration gradient of water and methanol was used in 80:20 water:methanol for 1-20 min., 40:60 for 20-21 min., and 80:20 for 22 minutes and beyond, each at a flow rate of 0.500 mL/min.

Reaction of Pt(dach)(ox) and N-Ac-Met. The kinetics of the reaction of Pt(dach)(ox) and N-Ac-Met were monitored in a 2:1 N-Ac-Met:Pt(dach)(ox) ratio in D_2O using a 500 MHz JEOL Eclipse instrument. The reaction was monitored over a 12-hour period and sporadically throughout the week to ensure no further activity had occurred.

Reaction of Pt(Me_2dach)(ox) and N-Ac-Met. The kinetics of the reaction of a mixed isomer form of Pt(Me_2dach)(ox) and N-Ac-Met were monitored in the presence of excess N-Ac-Met using the same method as the reaction with Pt(dach)(ox). Excess N-Ac-Met was used due to uncertainty regarding the stoichiometry of the impure isomer. In

the near future, reactions of N-Ac-Met and a pure isomer of Pt(Me_2dach)(ox) will be studied.

CHAPTER 3

RESULTS

The NMR spectra of the Pt(dach)(ox) and Pt(Me_2dach)(ox) compounds that were synthesized confirmed the correct identity of the product. The NMR spectrum of Pt(Me_2dach)(ox) is shown below in Figure 6.

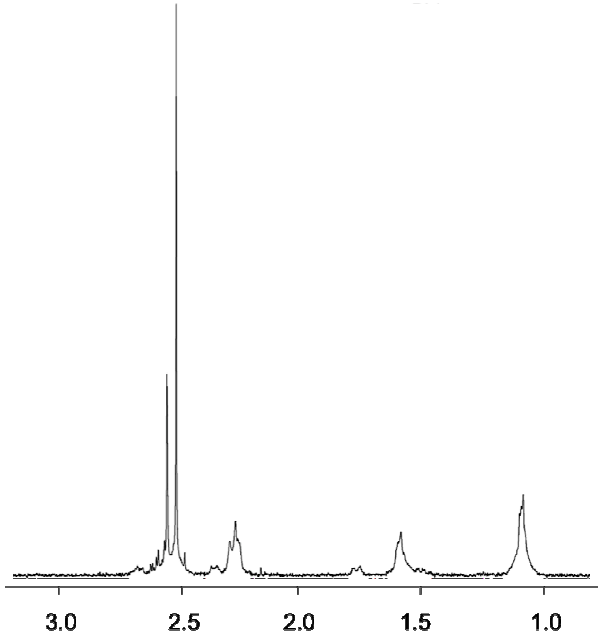


Figure 6. **^{1}H** NMR spectra of Pt(Me_2dach)(ox).

NMR results suggest that the synthesis of Pt(Me_2dach)(ox) yielded at least two isomers, as noted by the two singlet peaks occurring at approximately 2.5 ppm. The synthesis of Pt(Me_2dach)(ox) is capable of yielding multiple isomers, because the

addition of the methyl groups to the amine nitrogens in the dach ligand creates two chiral centers, which allows for the possibility of several different stereochemistries. In this case, the Pt(Me_2dach)(ox) can be in (S,R,R,S), (S,R,R,R), or (R,R,R,R) configurations. These configurations arise due to the chirality of the compounds and describe the stereochemistry occurring at the amine nitrogens and the carbons at N, C, C, and N, respectively. In order to accurately test the kinetics of Me_2DachOx, it is necessary that an isomerically pure form of the compound be obtained. To achieve this, the compound was separated using HPLC chromatography. Figure 7 shows the HPLC data for the separation of the Pt(Me_2dach)(ox) compound.

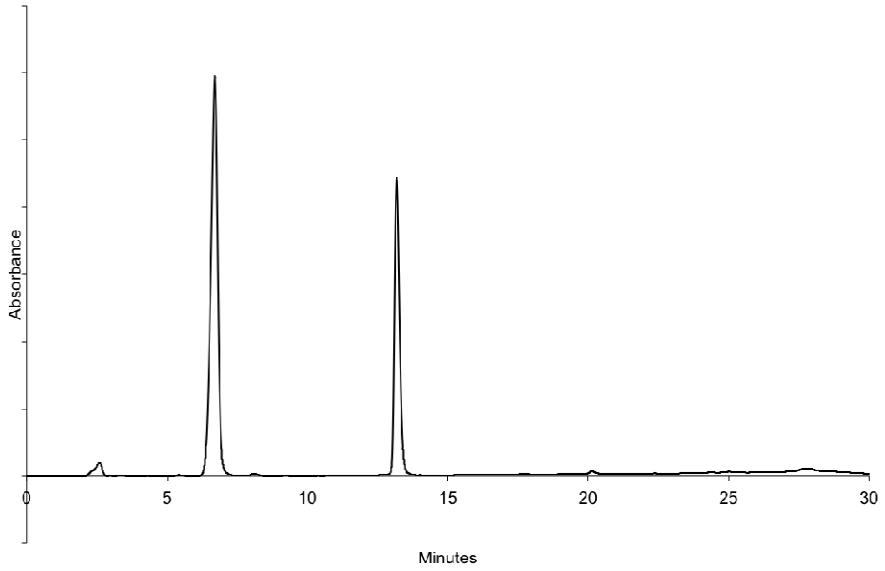


Figure 7. HPLC chromatogram of Pt(Me_2dach)(ox) .

As evident in the HPLC spectra, there was a very clear resolution between the two peaks, which suggest the elution of two isomers with different polarities, with approximately 7 minutes between the elution of each potential isomer. The isomers were then collected in separate vials until enough was obtained for experimental use (approximately 3 mg) and then a small sample was characterized by NMR spectroscopy to determine which isomer was the most pure and thus the best for reaction with N-Ac-Met. The NMR spectra of each HPLC peak is shown in Figures 8 and 9 below.

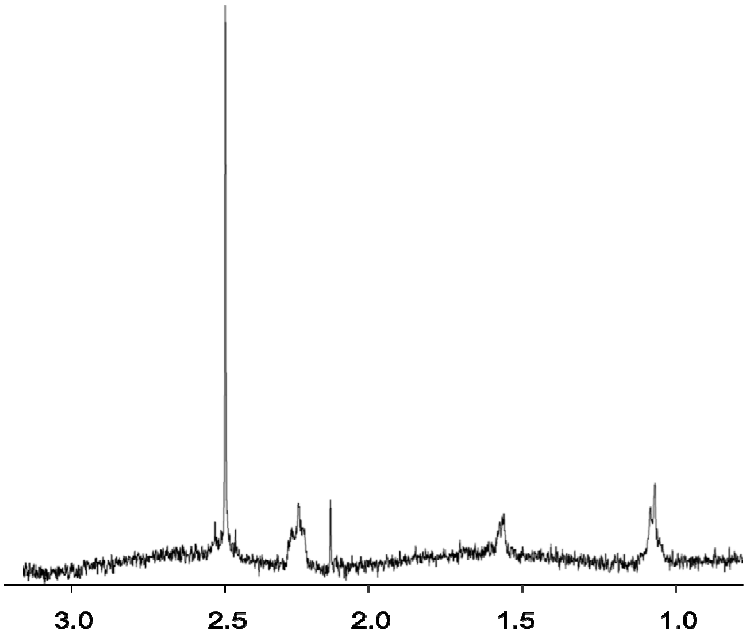


Figure 8. ^{1}H NMR spectrum of first peak obtained from HPLC separation of Pt(Me_2dach)(ox).

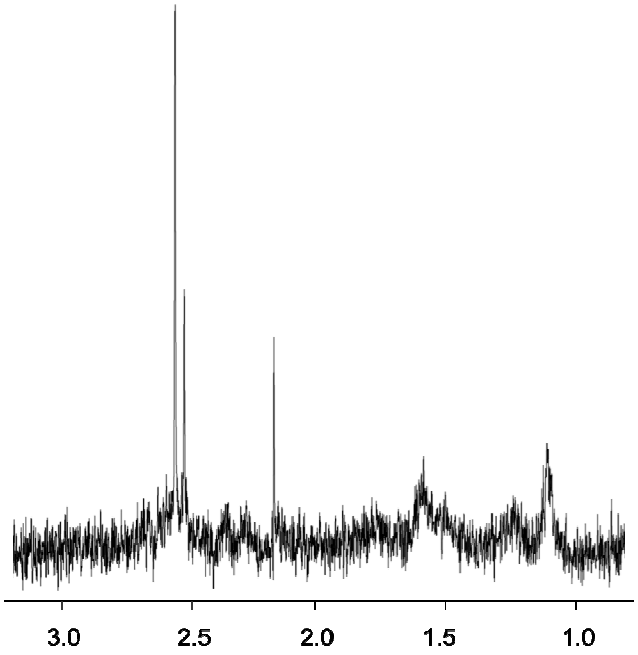


Figure 9. ^{1}H NMR spectrum of second peak obtained from HPLC separation of Pt(Me_2dach)(ox).

The NMR spectra of the product collected from the major peak, occurring at approximately 7 minutes in the HPLC chromatogram, suggests that the collection from the first elution is the most pure, due to the presence of only one singlet signal at

approximately 2.5 ppm, and is thought to be of the (S,R,R,S) configuration. The NMR spectrum of the second peak eluted at approximately 13 minutes shows two singlet signals at approximately 2.5 ppm, suggesting a mixed isomer form. It was intended that this product then be used to study the kinetics of the reaction between Pt(Me_2dach)(ox) and N-Ac-Met, however mechanical issues with the NMR equipment prevented the experiment from taking place. Prior to the purification, a kinetics experiment was performed with impure Pt(Me_2dach)(ox) and excess N-Ac-Met for preliminary results. The NMR study of the reaction is shown below in Figure 10.

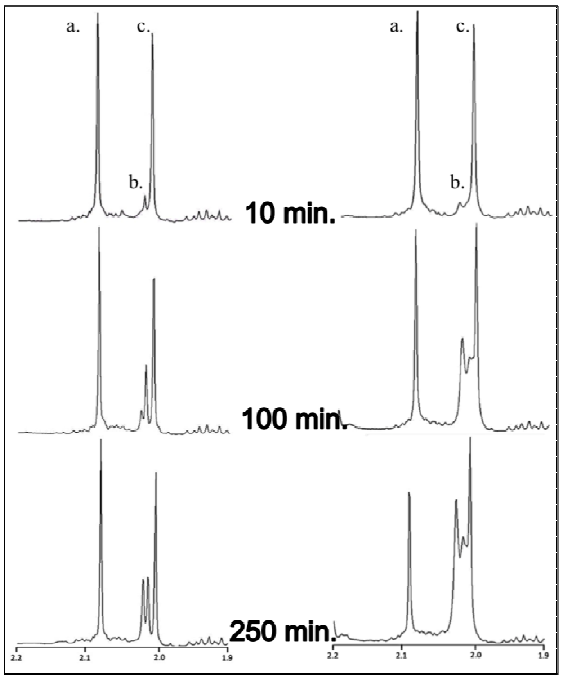


Figure 10. Column 1 shows the reaction of oxaliplatin with N-Ac-Met over the span of 10, 100, and 250 minutes, respectively where a) unreacted S-CH_3 of N-Ac-Met, b) acetyl-methyl product, and c) unreacted N-Ac-Met. Column 2 shows the reaction of Pt(Me_2dach)(ox) with N-Ac-Met over the span of 10, 100, and 250 minutes, respectively where a) unreacted S-CH_3 of N-Ac-Met, b) acetyl-methyl product, and c) unreacted N-Ac-Met.

Though the data obtained from the experiment is not as conclusive as results yielded from a pure isomer, the data does suggest that no significant change in the kinetics of the reaction after the addition of bulk (a larger, more sterically hindered

group) to the amine nitrogens of oxaliplatin. The NMR spectra above show the reaction of oxaliplatin with N-Ac-Met and the reaction of Pt(Me_2dach)(ox) with N-Ac-Met at 10, 100, and 250 minutes, respectively. The NMR shows that the increase of the acetyl-methyl product signal, labeled b. and subsequent decrease of unreacted N-Ac-Met do not vary greatly between the two reactions. Both reactions completed within approximately three hours. The NMR spectra does suggest that the reaction of N-Ac-Met with Pt(Me_2dach)(ox) is faster, however this is due to the reaction with Pt(Me_2dach)(ox) being in the presence of excess N-Ac-Met. More conclusive data will be obtained once the reaction is performed with an isomerically pure form of Pt(Me_2dach)(ox).

The NMR spectra above for the kinetics experiment of oxaliplatin and N-Ac-Met do not show the signal for S-CH_3 resonance anticipated in the product. As the amount of product in the sample increased, the S-CH_3 signal should shift due to the coordination of the sulfur atom to the platinum atom of oxaliplatin. Since this was not observable, we suspected that our sample had multiple conformations in intermediate exchange, which broadened the NMR signal. Increasing the temperature of the sample can circumvent the effect of intermediate exchange, because the rate of exchange increases as temperature increases. In order to confirm that the product yielded the anticipated S-CH_3 peak, a variable temperature experiment was a ^{1}H NMR spectra of the product was taken at 40°C, 60°C, and 80°C and the results are shown below in Figures 11, 12, and 13, respectively.

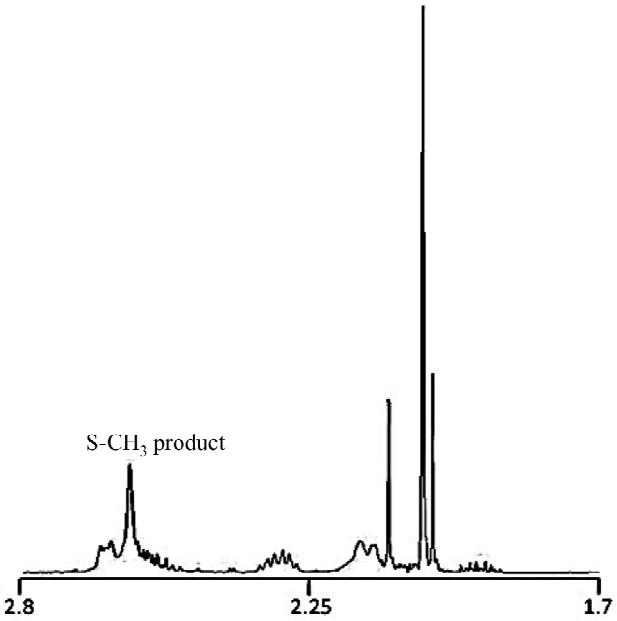


Figure 11. ^{1}H NMR spectrum of product of kinetics reaction of N-Ac-Met and oxaliplatin at 40°C.

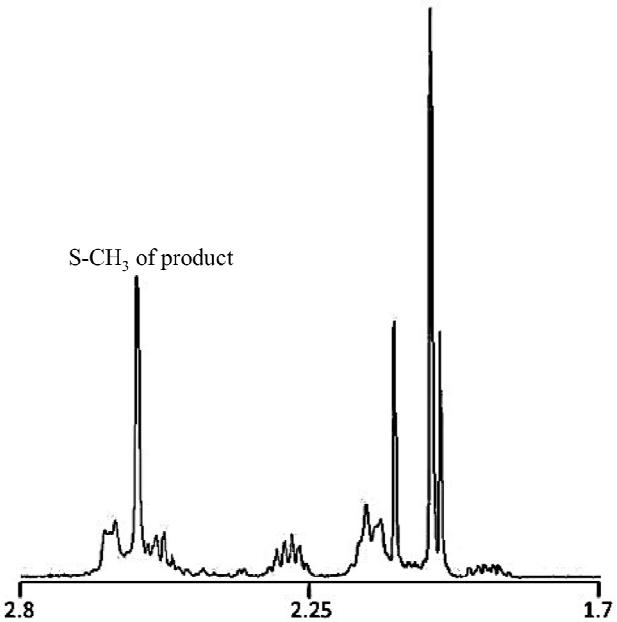


Figure 12. ^{1}H NMR spectrum of product of kinetics reaction of N-Ac-Met and oxaliplatin at 60°C.

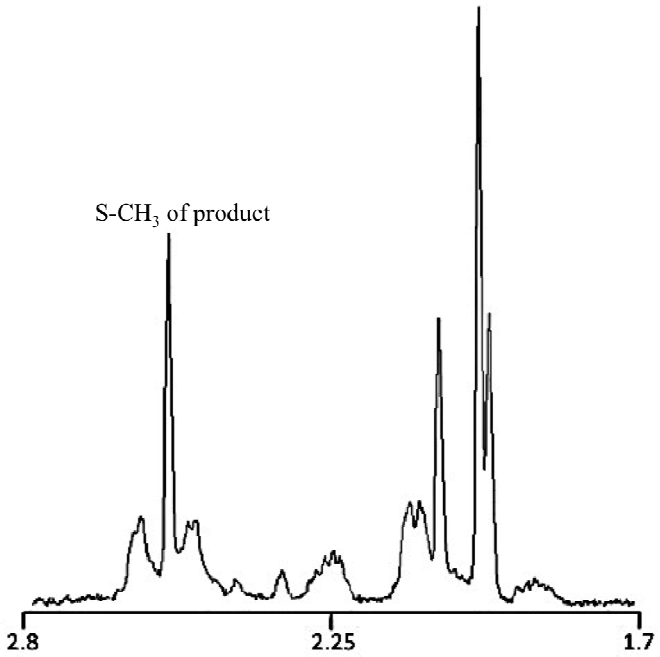


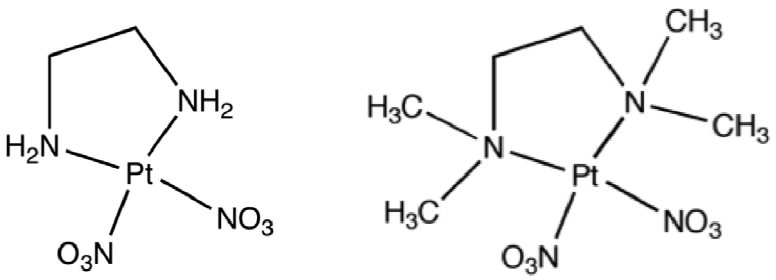
Figure 13. ^{1}H NMR spectrum of product of kinetics reaction of N-Ac-Met and oxaliplatin at 80°C.

The NMR spectra above clearly show that increasing the temperature of the sample sharpened the signal and revealed the S-CH_3 partner signal of the sample. The S-CH_3 signal shifted from approximately 2.1 ppm in the unreacted S-CH_3 peak as shown in Figure 10, peak a. to approximately 2.5 ppm, was suggests this is the S-CH_3 signal of the product.

CHAPTER 4

DISCUSSION

Initial results of the kinetics experiences comparing the reactivity of oxaliplatin and Pt(Me_2dach)(ox) with N-Ac-Met suggest that the addition of bulk in the form of two methyls to the amine nitrogen found in oxaliplatin has no significant effect on the kinetics of the reaction. Both the reaction of oxaliplatin and Pt(Me_2dach)(ox) with N-Ac-Met finished in approximately three hours. This is an interesting finding, considering previous research (*10*) has shown that the addition of bulk to similar platinum compounds has had a tremendous affect on the reactivity with sulfur-containing ligands. In this study, the addition of two methyls to the two amine nitrogens in platinum ethylenediame dinitrate resulted in a vast decrease in the rate constant in the reaction with N-Ac-Met and 5'-GMP, as well as a significant decrease in the rate constant with Guanosine compared to the original compound with no bulk added.



Pt(en)(NO_3)$_2$ **Pt(Me_4en)(NO_3)$_2$**

Figure 15. Platinum ethylenediamine dinitrate and tetramethylethylenediamine dinitrate.

Table 1. En/Me_4En rate constants for reactions with different sulfur-containing substrates.

	Ratio of En/Me_4En Rate Constants
N-Ac-Met	16
5'-GMP	13
Guanosine	5.4

This disparity in results between the experiments described above and the results of the previous experiment are at this time thought to be attributed to either the presence of a tertiary amine in the platinum ethylenediamine dinitrate as opposed to the secondary amine in Pt(Me_2dach)(ox) or perhaps the difference between the nitrate leaving groups instead of the oxalate leaving group of oxaliplatin and Pt(Me_2dach)(ox). It is known that the oxalate leaving group is not released as easily as other leaving ligands due to its higher stability and the chelate effect (*7*).

It is also interesting to note that in previous studies, experimental results suggested that the added bulk only coordinated to one methionine, suggesting the

formation of a mono product (*10*). Experimental results shown from our work adding bulk to oxaliplatin suggest that the bulk coordinates to two methionines, suggesting the formation of a bis product. A ^{195}Pt NMR experiment was performed using the sample from the kinetics reaction of Pt(Me_2dach)(ox) and methionine to determine whether a mono or bis product was formed. The ^{195}Pt NMR spectrum displayed a peak between -3600 and -3800 ppm, a known characteristic of the PtN_2S_2 coordination environment, suggesting the formation of a bis product. In the future, a second ^{195}Pt experiment with the reaction of pure Pt(Me_2dach)(ox) and N-Ac-Met will be performed to determine if a bis product is also formed in this case.

Future work includes continuing to purify Pt(Me_2dach)(ox) in quantities sufficient for kinetics studies with N-Ac-Met, as well as other sulfur-containing biological ligands, as well as using NMR to determine the conformation of the isomerically pure form of Pt(Me_2dach)(ox) and using ^{195}Pt NMR to determine whether a bis or mono product is formed in the reaction with N-Ac-Met.

REFERENCES

(*1*) Cancer Prevalence: How Many People Have Cancer? http://www.cancer.org/Cancer/CancerBasics/cancer-prevalence (accessed April 8).

(*2*) Panno, J., *Cancer: The Role of Genes, Lifestyle, and Environment, Revised Edition*. Facts On File, Inc.: New York, 2010.

(*3*) Rosenberg, B.; Vancamp, L.;Trosko, J.E.; Mansour, V.H. (1969). "Platinum Compounds: a New Class of Potent Antitumour Agents". *Nature* **222** (5191): 385–386.

(*4*) Carpenter, Daniel (2010). *Reputation and Power: Organizational Image and Pharmaceutical Regulation at the FDA*. Princeton: Princeton University Press.

(*5*) Tyagi, P.; Gahlot, P.; Kakkar, R. *Polyhedron* **2008**, *27*, 3567.

(*6*) Heudi, O.; Mercier-Jobard, S.; Cailleux, A.; Allain, P. *Biopharmaceutics & Drug Disposition* **1999**, *20*, 107.

(*7*) Kung, A.; Strickmann, D. B.; Galanski, M.; Keppler, B. K. *Journal of Inorganic Biochemistry* **2001**, *86*, 691.

(*8*) Ramachandran, S.; Temple, B. R.; Chaney, S. G.; Dokholyan, N. V. *Nucleic Acids Research* **2009**, *37*, 2434.

(*9*) Reedijik, J., Why Does Cisplatin Reach Guanine-N7 with Competing S-Donor Ligands Available in the Cell? *Chem. Rev.* **1999,** *99*, 2499-2510.

(10) Williams, K.M.; Rowan, C.; Mitchell, J. Effect of Amine Ligand Bulk on the Interaction of Methionine with Platinum(II) Diamine Complexes. *Inorg. Chem.* **2004**, 43, 1190-1196.