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# Effect of Leaving Ligands of Platinum(II) Diamine Complexes on DNA and Protein Residues

Ramya Kolli

Western Kentucky University, ramya.kolli549@topper.wku.edu

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EFFECT OF LEAVING LIGANDS OF PLATINUM(II) DIAMINE COMPLEXES ON  
DNA AND PROTEIN RESIDUES

A Thesis  
Presented to  
The Faculty of the Department of Chemistry  
Western Kentucky University  
Bowling Green, Kentucky

In Partial Fulfillment  
Of the Requirements for the Degree  
Master of Science

By  
Ramya Kolli

May 2013

EFFECT OF LEAVING LIGANDS OF PLATINUM(II) DIAMINE COMPLEXES ON  
DNA AND PROTEIN RESIDUES

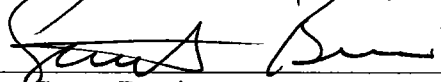
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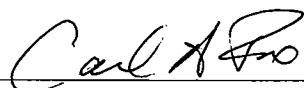
Dr. Kevin Williams, Director of Thesis



Dr. Rajalingam Dakshinamurthy



Dr. Stuart Burris



Dean, Graduate Studies and Research

5-6-13

Date

I would like to dedicate my thesis to my research advisor, Dr. Kevin Williams, for his support all through my time in Western Kentucky University. I also would like to dedicate this work to my parents, Madhusudhan Rao Kolli, Venkata Lakshmi Akkineni, and my brother, Naveen Kolli, for being supportive during my challenging times.

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# EFFECT OF LEAVING LIGANDS OF PLATINUM (II) DIAMINE COMPLEXES ON DNA AND PROTEIN RESIDUES

Ramya Kolli

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Directed by: Kevin M. Williams, Rajalingam Dakshinamurthy, and Stuart Burris

Department of Chemistry

Western Kentucky University

Platinum compounds are widely used drugs in cancer treatments. Although DNA is the biological target, reaction of platinum compounds with proteins is also potentially significant. Our objective is to study the effects of leaving ligands on the relative reactivity between 5'-GMP (guanosine 5' phosphate), a key DNA target, and N-Acetyl - L-Methionine (N-AcMet), a key protein target. We have used NMR spectroscopy to monitor reactions with N-AcMet and 5'-GMP added to a platinum complex to see which products are formed preferentially. Previous research showed that both a non-bulky complex such as  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{2+}$  [en=ethylenediamine], and a bulky complex such as  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{2+}$  [Me<sub>4</sub>en= N, N, N', N'-tetramethylethylenediamine] react more quickly with 5'-GMP than with N-AcMet. To improve the activity of platinum compounds in our current research, oxalates as leaving ligands are used. The results suggest that  $[\text{Pt}(\text{en})(\text{Ox})]$  [Ox= oxalate] reacts faster with N-AcMet than with 5'-GMP. Also,  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  reacts slowly with 5'-GMP without N-AcMet and the reaction favors N-AcMet when both ligands are added simultaneously. Interestingly, the formation of the sulfur-oxygen chelate is slow enough to be observable in the oxalate reaction; but the mono product is not independently observed in the dinitrate complex.

## I. INTRODUCTION

### A. History

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems. There are over 200 different known cancers that effect humans. Many things may elevate the risk of cancer, including external factors such as chemicals, radiation, environmental pollutants as well as internal factors like mutations, hormones, and immune system conditions. Genetics account for approximately 5-10% of cancers. The current treatment options are surgery, radiation, chemotherapy, hormone therapy, biological therapy, and targeted therapy.<sup>1</sup>

The anticancer activity of cisplatin  $[\text{PtCl}_2(\text{NH}_3)_2]$  was first discovered by Barnett Rosenberg in 1960. It is estimated that about 90% of testicular cancers are cured by cisplatin and considered to play vital roles in treatment of ovarian, neck, bladder, and cervical cancers.<sup>2</sup> The clinical success of this drug initiated the use of metal compounds for cancer.<sup>1</sup> Following this, preparation and evaluation of thousands of platinum compounds was started by scientists. However, only a few compounds have actually entered into clinical use.<sup>1</sup> Carboplatin  $\{\text{cis}-(\text{Pt}(\text{NH}_3)_2(\text{CBDCA}))\}$ , CBDCA= 1,1-cyclobutanedicarboxylic acid} and oxaliplatin  $\{[\text{Pt}(\text{oxalate})(1R,2R\text{-chxn})], \text{chxn}=\text{cyclohexane-1,2-diamine}\}$  were a few of the drugs that were discovered, found to display anticancer activity complementary to cisplatin.

The anticancer activity of platinum compounds were first investigated by Rosenberg while he was studying electric field effects on bacterial growth. The

electrodes that were made of platinum electrolyzed during the experiment and released a platinum complex that caused inhibition of cell division in the bacterial rods. This experiment was first carried out in *Escherichia coli* (*E. coli*) using ammonium chloride buffer as a growth medium. Current was passed through inert platinum electrodes immersed in the buffer.<sup>3,4</sup> The result was that the *E. coli* cells began appearing long and filamentous, similar to spaghetti, instead of their original sausage shape.<sup>4</sup> From the filamentation assay, the scientists hypothesized that the charged platinum complexes were bactericidal and that this inhibition of cell division was not due to the electrical current. It instead was due to the platinum hydrolysis products formed from the platinum electrodes.<sup>3</sup> Rosenberg also reported that the cis form of platinum (IV) complex  $[\text{PtCl}_4(\text{NH}_3)_2]$  was responsible for inhibition, whereas the trans form is ineffective. An experiment was also carried out on swiss white mice injected with *cis*- $[\text{Pt}(\text{Cl}_4(\text{NH}_3)_2)]$ , a platinum (IV) complex, and a platinum(II) complex *cis*- $[\text{Pt}(\text{Cl}_2(\text{NH}_3)_2)]$ ; the result was that the large solid tumors in the mice were reduced in size and the mice survived.<sup>3,4</sup> The first clinical trials in 1972 gave very promising results. The experiments established cisplatin's efficacy as an anticancer drug, and the final approval of this drug in the United States was in 1979. Since then, it has become one of the leading and most widely used anticancer drugs. Better knowledge of the mechanism may lead to better administration procedures and derivatives or analogues with superior properties.<sup>3</sup>

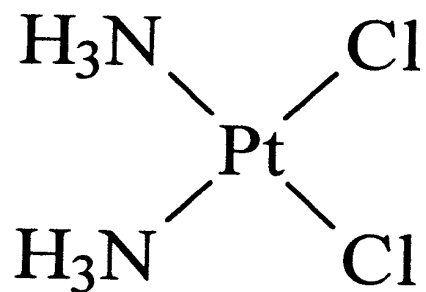


Figure 1. Structure of Cisplatin

## B. Synthesis of Cisplatin

The synthesis of cisplatin was carried out in 1845 by Michel Peyrone.<sup>1</sup> Initially the synthesis of cisplatin was unreliable, often producing impure products. Alterations were made for improved purity of the product.<sup>1,4,5</sup> The synthetic procedure of cisplatin is explained in a schematic diagram in Fig 2. First, it involves potassium tetrachloroplatinate ( $\text{K}_2[\text{PtCl}_4]$ ) which is then converted to the tetraiodo analog  $\text{K}_2[\text{PtI}_4]$  by the addition of potassium iodide (KI). The ammonia is added, forming a yellow compound that is dried. After that, addition of an aqueous solution of silver nitrate ( $\text{AgNO}_3$ ) to *cis*- $[\text{PtI}_2(\text{NH}_3)_2]$  causes insoluble AgI to precipitate. This is then filtered off. Finally potassium chloride (KCl) is added to attain product of cisplatin *cis*- $[\text{PtCl}_2(\text{NH}_3)_2]$ .<sup>1,4</sup>

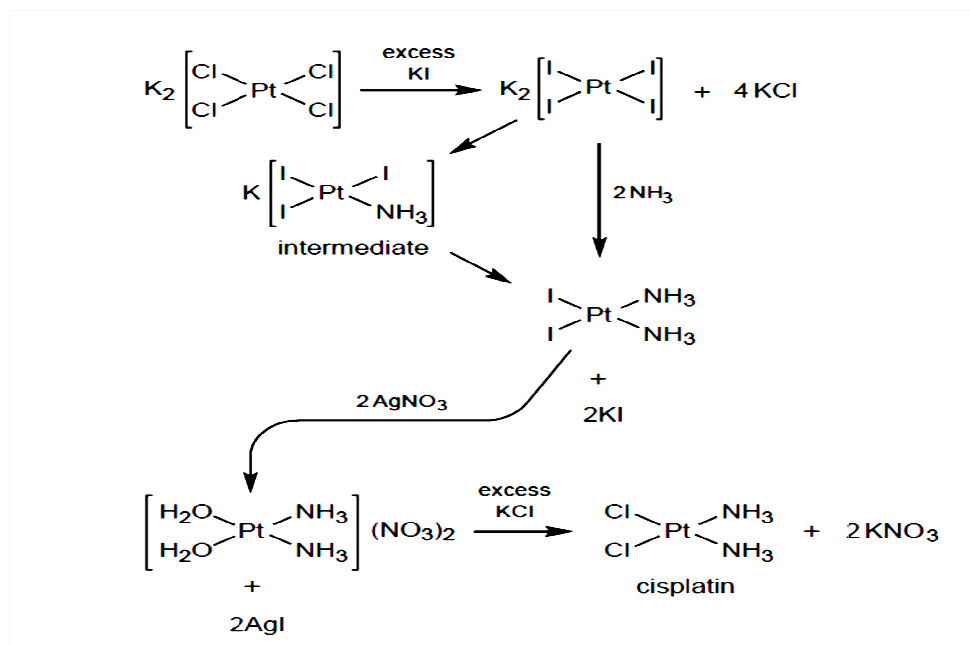


Figure 2. Synthetic pathway of Cisplatin

### C. Rate of Ligand-Exchange Reactions of Platinum Compounds

Ligand-exchange kinetics play an important role in determining the anticancer activity.<sup>6,7</sup> The platinum ligand bond, having thermodynamic strength of a typical coordination bond, is weaker than C-C, C-N or C-O, single, and double bonds. The ligand-exchange behavior provides a reaction from minutes to days to allow to reach reaches the target site despite various competing interactions. Certain ligand-exchange reactions on platinum occur faster than others.<sup>3</sup> The trans-effect determines the relative reaction velocities of platinum compounds. This concept was first introduced by Chernyaev in the year 1926.<sup>1</sup> It states that, ligands in positions opposite (trans) to a certain ligand in a platinum(II) compound are more or less rapidly exchanged compared to others. This results in sequences of reactivity. For example, ligands trans to the

following compounds in the sequence are more easily replaced:  $\text{CN}^- > \text{phosphine} > \text{NO}_2^- > \text{I}^- > \text{Br}^- > \text{Cl}^- > \text{NH}_3 > \text{H}_2\text{O}$ .<sup>1,3,4</sup>

When the intermediate triiodo species  $\text{K}[\text{PtI}_3(\text{NH}_3)]$  reacts with the second ammonia group there are two options as illustrated in Fig. 2, they are either displacement of the iodo ligand that is trans to an ammonia ligand or displacement of the iodo ligand that is trans to another iodo ligand. With an increase of the trans directing influence of iodo ligand relative to ammonia ligand, the ligand trans to iodide is labile and can be easily displaced to give cis configuration of the final product.<sup>1</sup> Therefore ligands, in the platinum compounds, determine the reactivity. In addition to the trans effect rule, Pt-amine ligand bonds such as  $\text{Pt}(\text{NH}_3)_2^{+2}$  are usually very stable and Pt- $\text{H}_2\text{O}$  bonds and Pt-Cl bonds are labile.<sup>3</sup> Based on this concept, different platinum compounds are synthesized by modifying their ligands to change their reactivity.<sup>3,4</sup>

#### D. Structure and Activity Relationship

Although cisplatin has clinical success, there are side-effects associated with the cisplatin treatment such as nephrotoxicity, nausea, ototoxicity, and severe anemia.<sup>1,6</sup> For a drug to gain clinical approval it needs to have at least one unique clinical advantage over cisplatin, such as reduced toxic side-effects. There are certain structural criteria for the platinum compounds to show the antitumor activity (i.e. there is a relationship between structure and activity).<sup>1</sup>

- It is necessary that the compound should have two leaving groups that are cis with respect to each other.<sup>8,9</sup> Leaving groups are those that are most easily lost. The ease with which these leaving groups are lost will affect the activity as well as toxicity of the compound.<sup>8,9</sup>



- The compound should be neutral with fewer alkyl substituents on the amine ligands.<sup>1</sup>
- The two amine groups should have cis geometry. The trans geometry will make these compounds inactive for antitumor activity.<sup>1,3</sup>
- Two cis-monodentate or one bidentate leaving group is required.<sup>9</sup>
- The geometry of the complexes is either square planar or octahedral.<sup>9</sup>
- The complexes should have at least one N-H group, which is required for hydrogen bonding with the biological target.<sup>3,9</sup>
- The leaving groups must be approximately 3.4 Å apart on the molecule.<sup>9</sup>
- The rates of exchange of these groups should fall into restricted region.

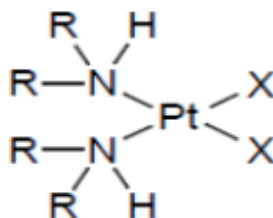


Figure 3. General Structure of Platinum Compounds with Anti-Tumor Activity

X is the leaving group such as two chloro groups or a bidentate malonate ligand;  
R=H or an alkyl substituent. (Fig.3)

The majority of the platinum compounds that are clinically successful have adhered to this structure and activity criteria.<sup>1</sup> Based on these structure activity relationship, other platinum compounds with improved efficacy and less toxicity are designed. Second and third generation platinum drugs such as carboplatin and oxaliplatin, respectively to overcome cisplatin resistance have been introduced.

### E. Analogs of Cisplatin

**Carboplatin:** Introduced in the year 1980, carboplatin is the second generation platinum drug with lesser side-effects when compared to cisplatin; it is used in combination therapy.<sup>6</sup> It is also called cis-diammine (1.1-cyclobutanedicarboxylato) platinum(II) and is mainly used in ovarian carcinoma, lung, head and neck cancer.<sup>10-12</sup>

The cyclobutanedicarboxylate (CBDCA) ring on carboplatin replaces the chloride leaving group of cisplatin. This replacement reduces the toxicity of carboplatin when compared to cisplatin as the rate of conversion to active species is slowed down.<sup>1,6</sup> These drugs bond with the thiol sulfur atoms of proteins and amino groups in DNA. The only difference between cisplatin and carboplatin is that the carboplatin is a bigger molecule with a dicarboxylate ligand which reduces the breakdown of agent metabolically and lowers the formation of toxic byproducts. Carboplatin is considered markedly less toxic to the kidneys and nervous system than cisplatin and caused less nausea and vomiting retaining equivalent anti-tumor activity.<sup>12</sup>

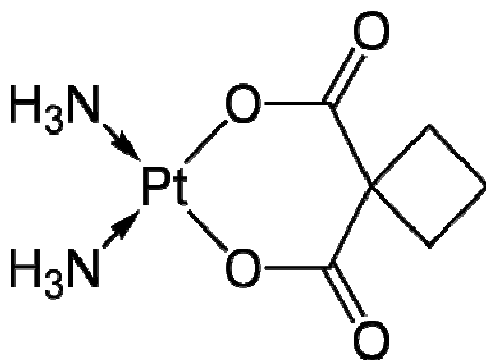


Figure 4. Structure of Carboplatin

**Oxaliplatin:** Oxaliplatin is a third generation platinum anticancer drug with a diaminocyclohexane (dach) carrier ligand and an oxalate leaving group.<sup>11</sup> Known also as trans-R, R-1, 2-diaminocyclohexane oxalate platinum(II), it has decreased resistance in

cisplatin-resistant tumors. Oxaliplatin is the FDA approved drug with greater efficacy in treating colorectal cancer. Although it has similar DNA binding specificity to cisplatin, the differences in cellular activity may be due to the differential recognition of DNA adducts. These differences may be due to the differences in uptake. Oxaliplatin produces the similar type of inter and 1,2-GG intrastrand cross links as cisplatin, but has a different spectrum of activity, mechanisms of actions, and resistance than cisplatin and carboplatin. However, the steric characteristics of non-hydrolysable diaminocyclohexane platinum adduct results in absence of cross resistance with cisplatin and carboplatin. The main side-effects of this drug are neurotoxicity, hematological toxicity, gastrointestinal tract toxicity, and tubular necrosis.<sup>12</sup>

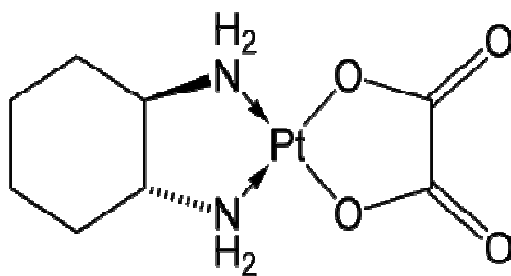


Figure 5. Structure of Oxaliplatin

#### F. Hydrolysis Reactions of Cisplatin

After administration of the cisplatin drug through injection or infusion in the blood stream, a variety of chemical reactions occur. A high concentration of chloride ions (100 mM) suppresses hydrolysis to maintain the cisplatin in a neutral state.<sup>13</sup> The cisplatin that remains intact enters the tumor cells by diffusing through the cell membrane. With intracellular chloride concentration in the blood plasma being relatively low (i.e. 4-20 mM), a chloro ligands of cisplatin is replaced by water to form a reactive,

positively charged species that cannot readily leave the cell.<sup>13</sup> The aqua ligand in *cis*  $[\text{PtCl}(\text{H}_2\text{O})(\text{NH}_3)_2]^+$  is easily displaced. This allows the platinum atoms to bind to all kinds of molecules inside the cell like DNA, RNA, proteins.<sup>3</sup> *In vitro*, studies have shown that this monoaquated platinum species is responsible for at least 98 percentage of platinum binding to DNA within the cell nucleus.<sup>1</sup> Of the DNA bases, guanine is the most preferred.<sup>14</sup>

It is generally accepted that chloride hydrolysis is considered as a rate determining step in the reaction of cisplatin with DNA.<sup>4</sup> Significant losses of platinum occur due to 50-70% of the administered platinum being excreted within 24 hours.<sup>4</sup>

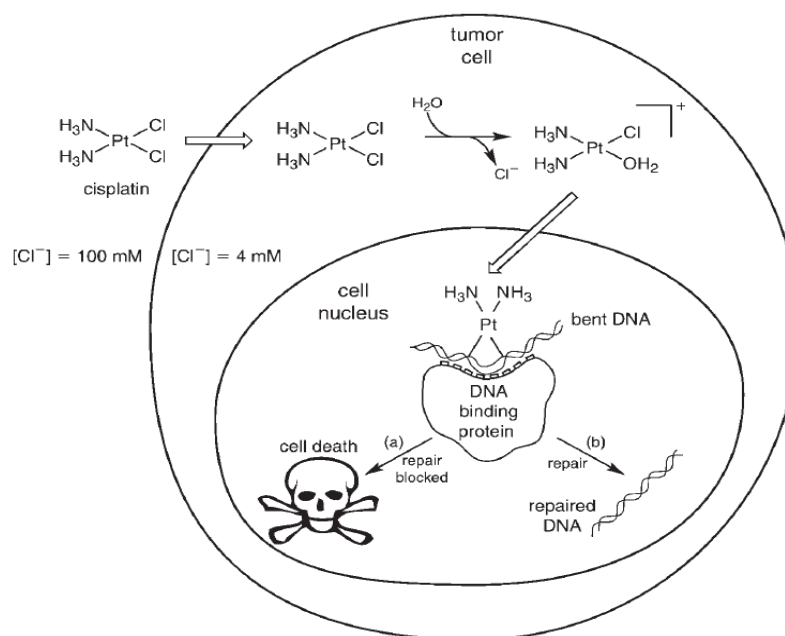
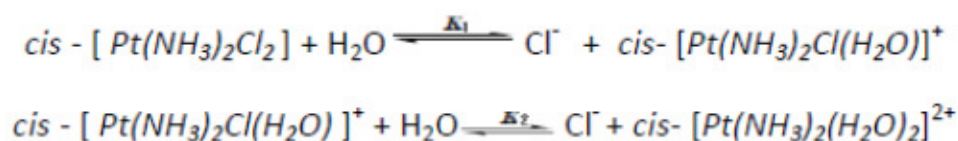


Figure 6. Hydrolysis Reactions of Cisplatin

## G. Mechanism of Action of Platinum Anti-Tumor Drugs

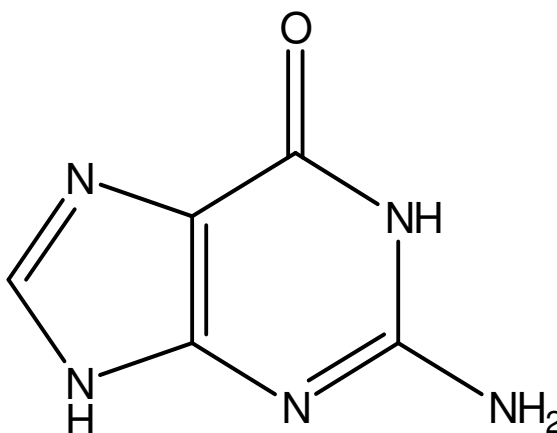


Figure 7. Guanine Residues of DNA

### DNA as the Target

Specific interaction of cisplatin with DNA causes cell death.<sup>4</sup> Aquated form of cisplatin is considered more reactive than neutral cisplatin. Earlier studies have shown that cisplatin preferentially binds at the nitrogen atoms of the nucleobases.<sup>4</sup> To understand this, studies have been conducted on binding preferences of nucleobases adenine (A), guanine (G), and cytidine (C). In guanine, binding is possible at N7 and deprotonated N1 under alkaline conditions. Of all these binding modes those at N7 atoms of adenine and guanine seem most compatible to DNA. This is thought to happen because the other sites of A, G, and C are involved in the Watson-Crick base pairing of double helix.<sup>4</sup> The kinetic and competition studies conducted a decade ago have shown that guanine N7 has a strong kinetic preference. Recent investigations have shown a Pt-N7 guanine bond is

stable.<sup>3,4</sup> The cis-Pt unit has two reactive sites and after binding to one guanine N7 a second reaction with a nearby guanine can occur.<sup>4</sup>

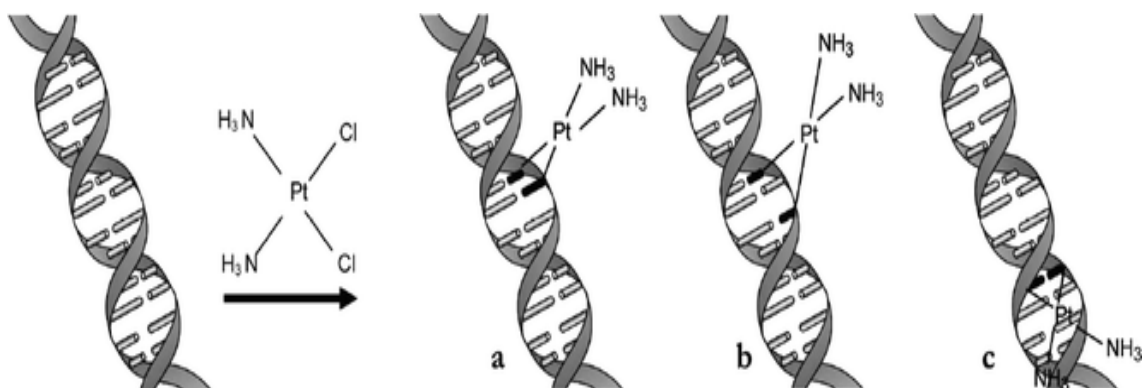


Figure 8. Cisplatin Interaction with DNA

In carboplatin and oxaliplatin, the interactions with DNA occur at the position where cyclobutane-1, 1-dicarboxylate (CBDCA) and oxalate ligands are attached to platinum.<sup>15,16</sup> This binding leads to a distortion of double helical structure of DNA thus impairing its processing. The 1, 2 intra strand cross-links bend the DNA towards the major groove exposing it to a wide shallow minor groove surface to which several proteins bind. High mobility group proteins, or the HMG proteins, are the proteins which are bound and cause cell cycle arrest, cell death, replication inhibition, and transcription inhibition.<sup>13</sup>

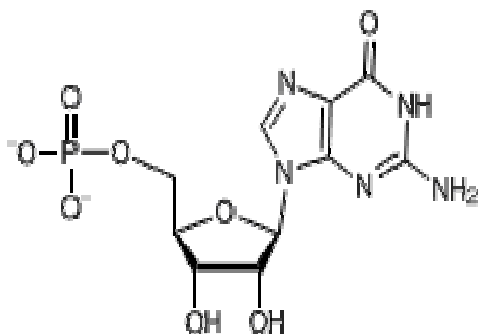


Figure 9. Guanosine 5'-Monophosphate

## H. Preference of Platinum(II) Complexes for Guanine over Adenine

Previous results from the experimental observations suggest that the guanine is the preferred reactant for platination.<sup>17</sup> The N7 platination of guanine is both thermodynamically and kinetically more favorable. A strong hydrogen bond between the hydrogen of ammine ligand on platinum and the oxo group at C6 position of guanine stabilize the platinum-guanine adduct. In comparison to the platinum-adenine adduct, this results in 50% of the differential energy when the Pt-chloroaqua complex is the reactant. The Pt-adenine adduct serves as the hydrogen bond donor and is energetically unfavorable compared to an isomer where the amino functionality on the C6 position acts as hydrogen donor to the chlorine ligand. If the diaqua complex is used as platination agent, both the kinetic and thermodynamic preferences for guanine are increased dramatically to 9.6 and 7.4 Kcal/mol respectively.<sup>17</sup> Therefore guanine is preferred with higher selectivity if diaqua complex is the active platinum (II) complex to bind.

## I. Reaction of Platinum Complexes with Proteins

DNA is not the only target for platinum complexes; binding to proteins also occurs.<sup>3</sup> Studies have shown that one day after cisplatin administration, 65 to 98% of the platinum in blood plasma is protein bound and significant protein adducts are formed.<sup>18,19</sup> The reaction of platinum complexes with proteins results in formation of protein adducts, which may be responsible for side-effects and drug resistance. In amino acids, sulfur donor residues (Cysteine, Methionine) are primary targets owing to the relative softness of platinum.<sup>21</sup> Platinum also binds to the lone pairs of nitrogen atoms which occur in amino acids in the absence of S-donor ligands.<sup>20</sup>

Due to cisplatin's affinity for sulfur ligands, reaction with peptides and proteins at cysteine and methionine residues is common.<sup>18</sup> Many studies have utilized the amino acid N-Acetyl –L-Methionine (N-AcMet), which has an amide nitrogen and is the representative of internal methionine residue in peptide or protein to study the reactivity of platinum complexes.<sup>21-22</sup> Research has been conducted to study the intermolecular and intramolecular competition reactions to verify the binding preference of platinum in presence of both sulfur groups and N7 atoms of nucleobases.<sup>23-25</sup> It was shown that platinum reaction with methionine and related ligands is kinetically favored whereas the reaction of platinum with guanine is thermodynamically favored.<sup>18,24,25</sup> Sulfur group reactivity with platinum complexes may act as a reservoir for platinum to form active intermediates which result in platination of N7 position of DNA.<sup>9</sup> It is, therefore, important to better understand the interaction of platinum complexes with DNA and proteins and to determine what factors affect the rates of reaction.<sup>18</sup>

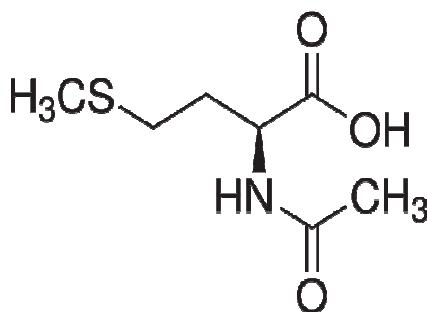


Figure 10. Structure of N-Acetyl-L-Methionine (N-AcMet)

Previous studies were focused on  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{+2}$  ( $\text{Me}_4\text{en}=\text{N}, \text{N}, \text{N}', \text{N}'$ -tetramethylethylenediamine) as a bulky platinum diamine complex.  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$  ( $\text{en}=\text{ethylenediamine}$ ) a less bulky ligand, was used to represent the diamine ligands of cisplatin and carboplatin. NMR spectroscopy monitored reactions where N-AcMet and 5'-GMP are added to platinum complexes at pH 4. It was analyzed from the studies that



the  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$  reacts more quickly than  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{+2}$  as expected.<sup>18</sup>

Although both complexes showed good reactivity with 5'-GMP,  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$  complex favors reaction with 5'-GMP because of hydrogen bonding of the en ligand with the 5'-phosphate. In contrast, reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{+2}$  is disfavored with N-AcMet due to steric clashes.<sup>16</sup> Because the bulk had little effect on N-AcHis (N-Acetylhistidine), His residues may be favored reaction over methionine residues in protein reactions<sup>18</sup>

Present research focuses on modifying the platinum(II) complexes with oxalates as a leaving groups and ethylenediamine as ligands.  $[\text{Pt}(\text{en})(\text{Ox})]$  represents a less bulkier complex where as  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  is used as bulkier complex. Both compounds feature a square planar coordination complex and are classified as alkylating agents and a coordination complex.

Oxalates are mainly preferred as they are very good water soluble leaving ligands and are used by oxaliplatin. The features for the bidentate ligand, ethylenediamine, would be less steric bulk and symmetry with two monodentate ammine ligands of cisplatin.

Proton NMR spectroscopy has been utilized to monitor the competition reactions in which N-Acetyl-L-Methionine (N-AcMet) and Guanosine-5'-mono-phosphate (5'-GMP) are added to platinum complexes to see which products are formed at pH-4. Different ratios of 5'-GMP and N-AcMet are reacted with platinum to maintain the constant ratio of platinum (II) complexes at pH 4.

## II. MATERIALS AND METHODS

### A. Materials Used

The following items were purchased from Sigma Aldrich: Potassium tetrachloroplatinate (II) ( $\text{K}_2\text{PtCl}_4$ ), silver nitrate ( $\text{AgNO}_3$ ), dichloro (ethylenediamine) platinum(II) ( $\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ ) $\text{PtCl}_2$ ), deuterium oxide ( $\text{D}_2\text{O}$ ), N-Acetyl -L-Methionine (N-AcMet), ( $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}(\text{NHCOCH}_3)\text{CO}_2\text{H}$ ), guanosine 5'- mono-phosphate dehydrate (5'-GMP), ( $\text{C}_{10}\text{H}_{14}\text{N}_5 \text{O}_8\text{P}$ ), oxalic acid (anhydrous) ( $\text{C}_2\text{H}_2\text{O}_4$ ), silver oxalate ( $\text{Ag}_2\text{C}_2\text{O}_4$ ).

### B. Nuclear Magnetic Resonance Spectroscopy (NMR)

A JEOL Eclipse 500 MHz Nuclear Magnetic Resonance spectrometer was utilized.  $^1\text{H}$  NMR spectroscopy was used to characterize the products and to analyze the rate of the product formation.

### C. Synthesis of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$

In this synthesis, 83 mg of potassium tetrachloroplatinate(II) (98%) is added to 5 mL of water in a round bottom flask. 30  $\mu\text{L}$  of N, N, N', N' - tetramethylethylenediamine and 5 mL of methanol were combined in a beaker. This mixture is added drop-wise to the potassium tetrachloroplatinate mixture, and allowed to stir for 24 hours. The obtained solid is collected through gravity filtration and then dried.

#### Preparation of Silver Oxalate

A sample of 450 mg of oxalic acid and 1690 mg of silver nitrate are added in an amber vial. A 20 mL deionized water was added and allowed to stir for one hour. The insoluble silver oxalate was collected by vacuum filtration.

#### The Final Step in the Synthesis of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$

Silver oxalate is combined with the dried product in an equal molar ratio in ~30 mL of water and stirred overnight. After this, the mixture is syringe filtered to remove the silver chloride. The aqueous solution is evaporated to dryness to obtain the product.

Weight of silver oxalate to be added =

Obtained product of N, N, N, N tetramethylethylenediamine  $\times$  Mass of silver Oxalate

---

Weight of  $\text{Pt}(\text{Me}_4\text{en})\text{Cl}_2$

#### D. Second Synthetic Method for Preparation of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$

56.5 mg of  $\text{Pt}(\text{Me}_4\text{en})\text{I}_2$  and 30 mg of silver oxalate is added to 30 ml of deionized water. The product was dried in a rotovap to obtain  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$ .

#### E. Synthesis of $[\text{Pt}(\text{en})(\text{Ox})]$

65.2 mg of platinum(II) ethylenediamine dichloride and 60 mg of silver oxalate are added to 35 mL of water and allowed to stir for 24 hours. The product was gravity filtered to remove any excess silver oxalate and dried in a rotovap to obtain  $[\text{Pt}(\text{en})(\text{Ox})]$ .

#### F. Reactivity of $[\text{Pt}(\text{en})(\text{Ox})]$ with 5'- GMP and N-AcMet

The reaction between the water soluble platinum compound  $[\text{Pt}(\text{en})(\text{Ox})]$ , N-AcMet and 5'-GMP was monitored at mole ratios of 1:1, 1:2 individually and competitively at mole ratios of 1:2:1 and 1:4:1. Additionally 1.5 mL of  $\text{D}_2\text{O}$  was added to the amber vial. After keeping the pH around 4-5, the solution was transferred to an NMR tube for NMR spectroscopy.

#### G. Reactivity of [Pt(Me<sub>4</sub>en)(Ox)] with 5'- GMP and N-AcMet

The reaction between [Pt(Me<sub>4</sub>en)(Ox)], 5'-GMP and N-AcMet was followed at mole ratios of 1:1, 1:2 individually and competitively at mole ratios of 1:2:1 and 1:1:2. After keeping the pH around 4-5, the solution was transferred to an NMR tube for NMR spectroscopy.

### III. RESULTS

The competition reactions between N-AcMet and 5'-GMP with  $[\text{Pt}(\text{en})(\text{Ox})]$  and similarly with  $[\text{Pt}(\text{Me}_4\text{en})\text{Ox}]$  are studied quantitatively using  $^1\text{H}$  NMR at pH 4 at varying concentrations.

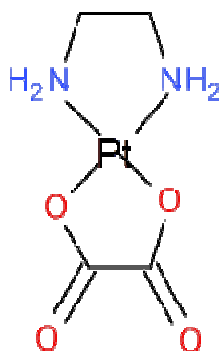


Figure 11. Structure of  $[\text{Pt}(\text{en})(\text{Ox})]$

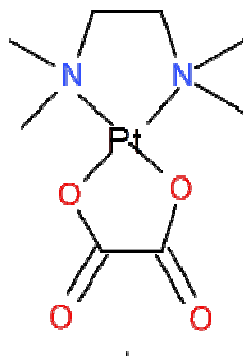


Figure 12. Structure of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$

To begin, 10 mM  $[\text{Pt}(\text{en})(\text{Ox})]$  is added with 10 mM 5'-GMP. The pH is determined and adjusted to 4. This reaction produced the following  $^1\text{H}$  NMR spectra (Fig.13). The downfield shift of H8 reactivity signals at  $\sim 8.5$  ppm, indicates the significant reactivity of  $[\text{Pt}(\text{en})(\text{Ox})]$  with 5'-GMP via N7.

#### A. Reactivity of [Pt(en)(Ox)] with 5'-GMP at 1:1 Ratio

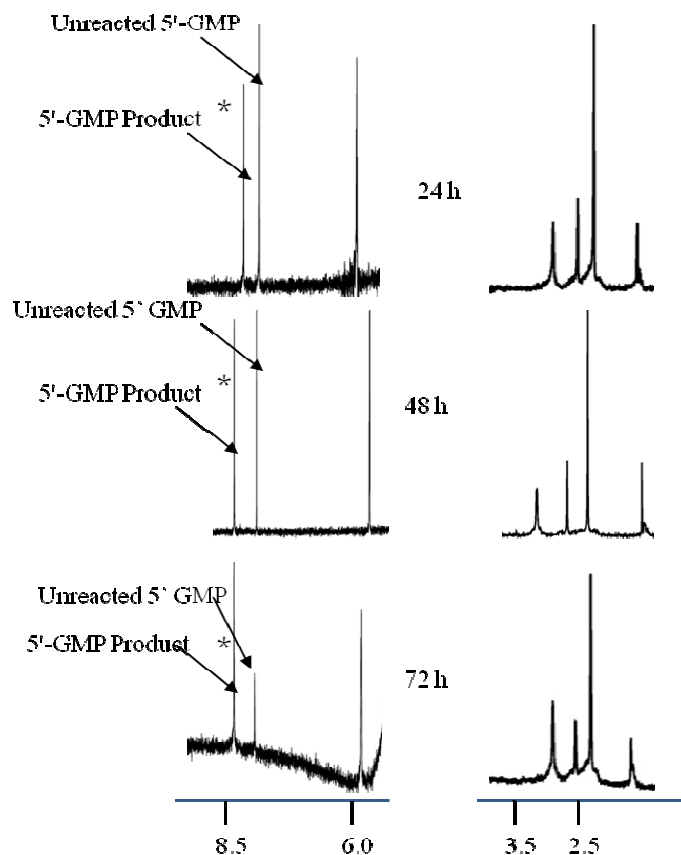


Figure 13.  $^1\text{H}$  NMR spectrum of [Pt(en)(Ox)] with 5'-GMP at 1:1 ratio

Each side of the NMR spectrum has the y-axis scaled to the largest peak to make the signals easier to observe (Fig. 13). The 5'-GMP product signals are indicated by an asterisk. The initial concentrations of each reactant are 10 mM.

When [Pt(en)(Ox)] and 5'-GMP at 1:1 ratio were combined at pH 4, key NMR resonances were observed within 24 hours. The 5'-GMP H8 signals shifted downfield to ~8.5 ppm, indicating coordination of the platinum complex with 5'-GMP via N7 atom of the guanine. Previously, it was shown that the  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$  reactivity with 5'-GMP results in two possible steps. One is the reaction of diaqua complexes with the first guanine ligand. The second is the reaction of the mono-product with a second guanine

ligand.<sup>18</sup> Here, in this reaction, when oxalates as a leaving ligand for Pt(en) are utilized, only [Pt(en)(5'-GMP)<sub>2</sub>] is observed. The unreacted [Pt(en)(Ox)] indicates that the bis-products are formed predominantly with the second 5'-GMP addition being more rapid than the first 5'-GMP.

#### B. Reactivity of [Pt(en)(Ox)] and 5'-GMP at 1:2 Ratio

We considered reactions between [Pt(en)(Ox)] and 5'-GMP at 24 hours, 48 hours, and 72 hours. 500  $\mu$ L aliquots of 10 mM [Pt(en)(Ox)] and 20 mM of 5'-GMP are combined together at pH 4. (Fig.14) The reactivity of 5'-GMP with [Pt(en)(Ox)] was observed within 24 hours. The downfield shift of H8 signals at 8.5 ppm indicates the coordination of platinum complex with 5'-GMP via N7.

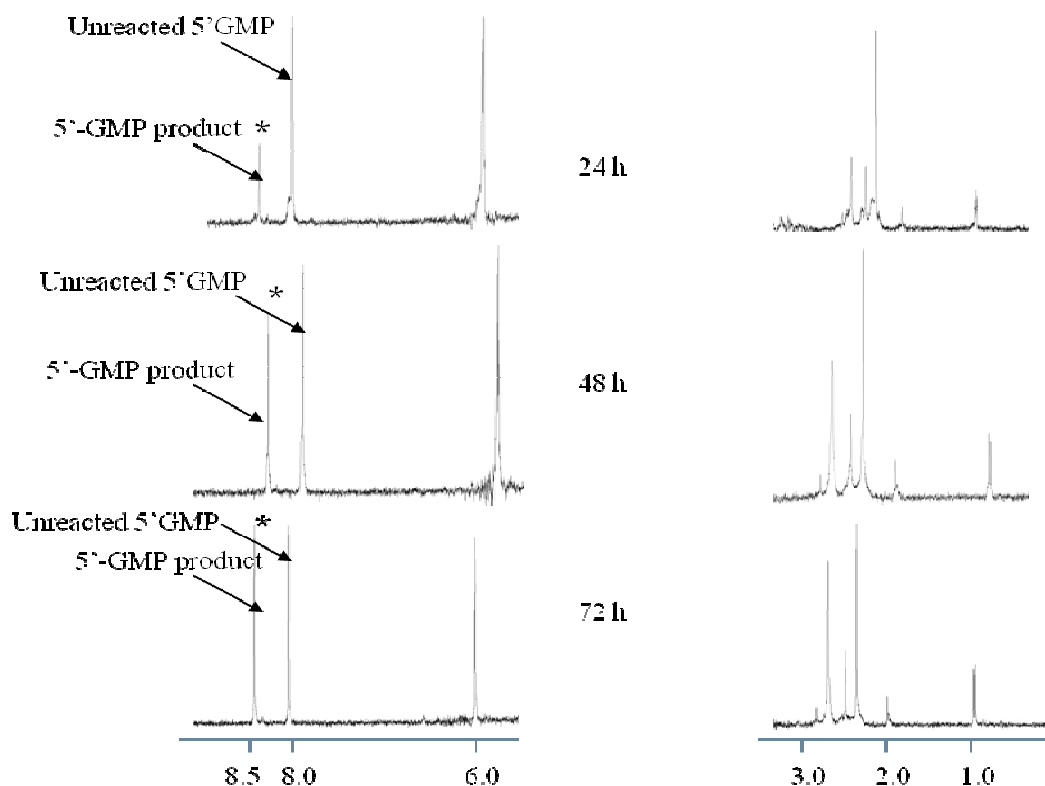


Figure 14. <sup>1</sup>H NMR spectrum of reactivity of [Pt(en)(Ox)] with 5'-GMP at 1:2 ratio.

The 1:2 products formed from the reaction have key resonances at ~8.5 ppm. Each side of the NMR spectrum has the y-axis scaled to the largest peak to make the signals easier to observe. The product signals are indicated by an asterisk.

The 5'-GMP H8 signals shifted downfield at ~8.5 ppm indicates the coordination of the platinum complex with 5'-GMP via N7 atom of the guanine. The predominant bis-products are formed indicating the formation of the complex  $[\text{Pt}(\text{en})(\text{Ox})(5'\text{-GMP})_2]$ .

For both the molar ratios of 1:1 and 1:2 the  $[\text{Pt}(\text{en})(\text{Ox})]$  reacts with 5'-GMP. The downfield chemical shift at ~8.5 ppm indicates the  $[\text{Pt}(\text{en})(\text{Ox})]$  reacts with 5'-GMP, resulting in the formation of bis-products with two 5'-GMP substituted in a platinum compound.

#### C. Reactivity of $[\text{Pt}(\text{en})(\text{Ox})]$ with N-AcMet at 1:1 Ratio

N-AcMet was initially chosen because it has been utilized in similar studies and because the methyl signal from the acetyl group provides another NMR signal that is readily observable. 10 mM  $[\text{Pt}(\text{en})(\text{Ox})]$  and 10 mM N-AcMet at 1:1 molar ratio are combined. The pH is determined and adjusted to 4. The reaction was monitored at intervals 1 hour, 24 hours, and 48 hours. The signal at ~2.5 ppm is a typical chemical shift for the S-CH<sub>3</sub> resonance of sulfur coordinated N-AcMet.<sup>26</sup> The N-AcMet connects to the  $\text{Pt}(\text{en})$  via sulfur.

Fig. 15 shows the <sup>1</sup>H NMR spectrum of  $[\text{Pt}(\text{en})(\text{Ox})]$  and N-AcMet. The intensity of the signals corresponding to a chemical shift of ~2.2 ppm and ~2.5 ppm increased as the time of the reactivity increased.



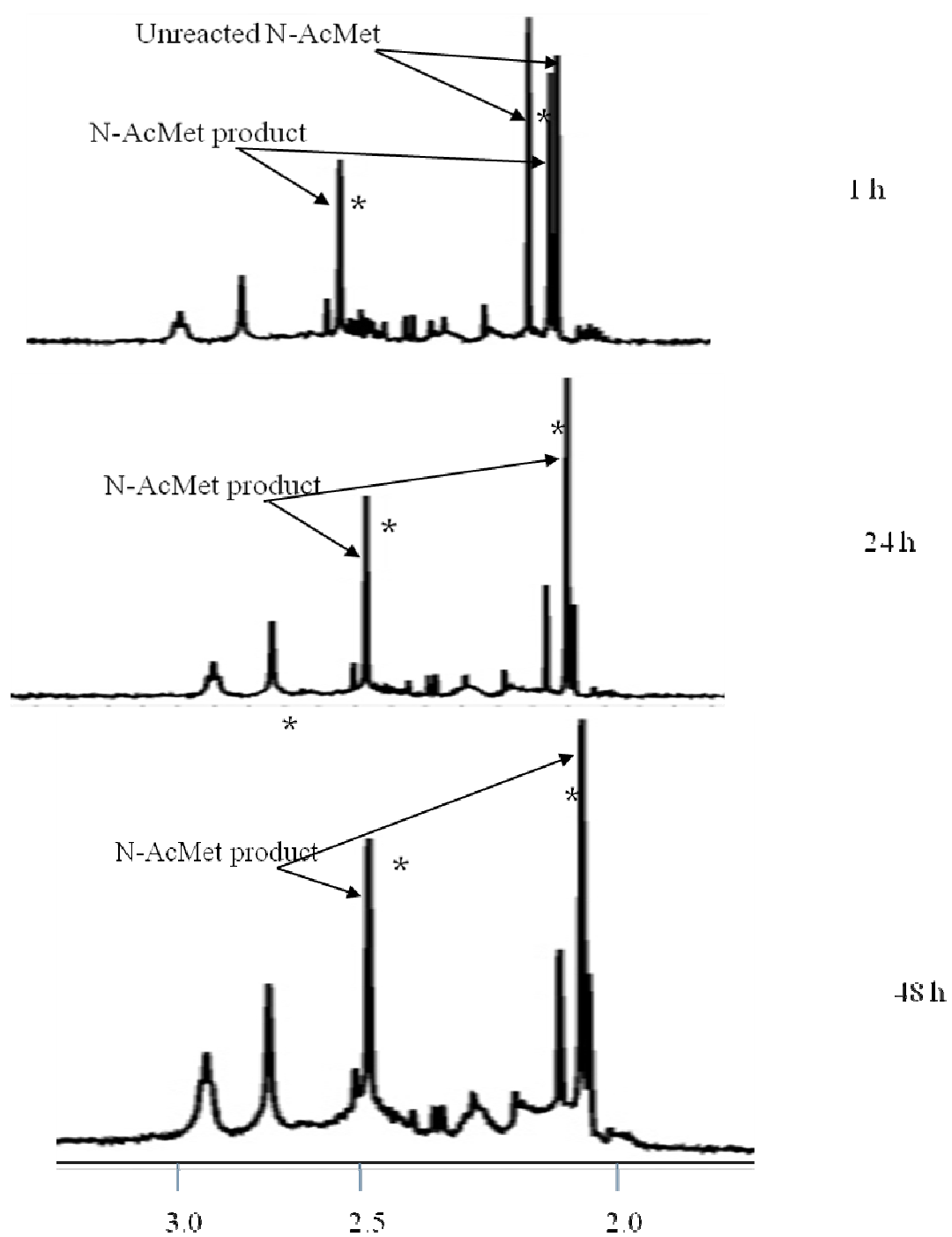


Figure 15.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{en})(\text{Ox})]$  with N-AcMet at 1:1 ratio

The signal at ~ 2.2 ppm, ~2.5 ppm is a typical chemical shift for the S-CH<sub>3</sub> resonance of a sulfur coordinated N-AcMet.<sup>18,26</sup> The signals from ~ 2.1 ppm and ~2.3 ppm are from unreacted N-AcMet. The amount of unreacted N-AcMet decreases only slightly from 24 to 48 hours indicating the reaction between platinum and N-AcMet is mostly complete after 24 hours.

Comparing the reactivity of N-AcMet with [Pt(en)(Ox)] to the reactivity of [Pt(en)(Ox)] with 5'-GMP, the reaction of N-AcMet with [Pt(en)(Ox)] is faster and shows the reactivity at 1 hour and is mostly complete within 24 hours. The reactivity with 5'-GMP is only partially complete after 24 hours. The results from Kung et al., indicated a competitive situation between 5'-GMP and L-Methionine for coordinating oxaliplatin and a favored coordination of L-Methionine.<sup>27</sup> Consistent with the current study, [Pt(en)(Ox)] reactivity with N-AcMet is faster when compared to the reactivity with 5'-GMP. The oxalates are easily displaced with that of methionine residues and thus product formation was observed quickly in hours rather than days as in the case of reaction of [Pt(en)(Ox)] with 5'-GMP.

#### D. Reactivity of [Pt(en)(Ox)] with N-AcMet at 1:2 Ratio

10 mM [Pt(en)(Ox)] and 20 mM N-AcMet at 1:2 molar ratio were reacted at pH 4. The reaction was monitored at 1 hour, 24 hours, and 48 hours. The signals at ~2.2 ppm and ~2.5 ppm are typical chemical shifts for the S-CH<sub>3</sub> resonance of a sulfur coordinated N-AcMet.<sup>26</sup> The N-AcMet connects to the pt(en) via sulfur.

Fig. 16 shows the partial <sup>1</sup>H NMR spectrum of 10 mM of [Pt(en)(Ox)] and 20 mM of N-AcMet at pH 4.0 after 1 hour and 24 hours. The products formed from the reaction with [Pt(en)(Ox)] and N-AcMet has key resonances at ~2.2 ppm and ~2.5 ppm

indicating the sulfur of methionine coordination with  $[\text{Pt}(\text{en})(\text{Ox})]$  and thus formation of products.

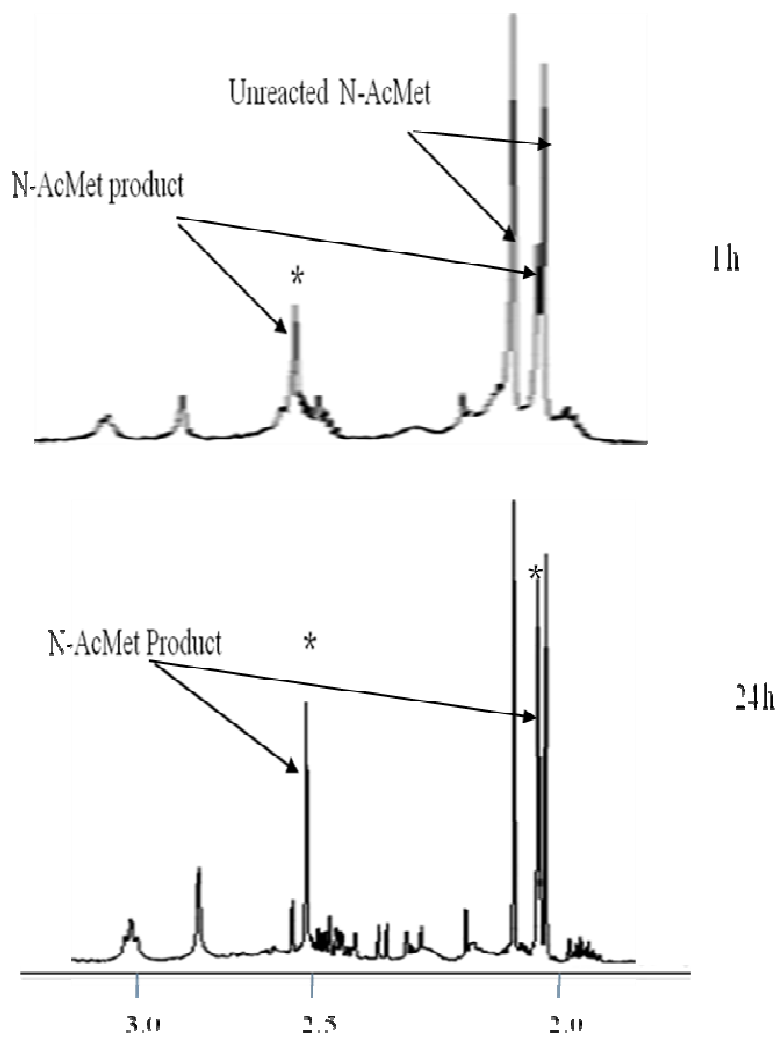


Figure 16.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{en})(\text{Ox})]$  with N-AcMet at 1:2 ratio.

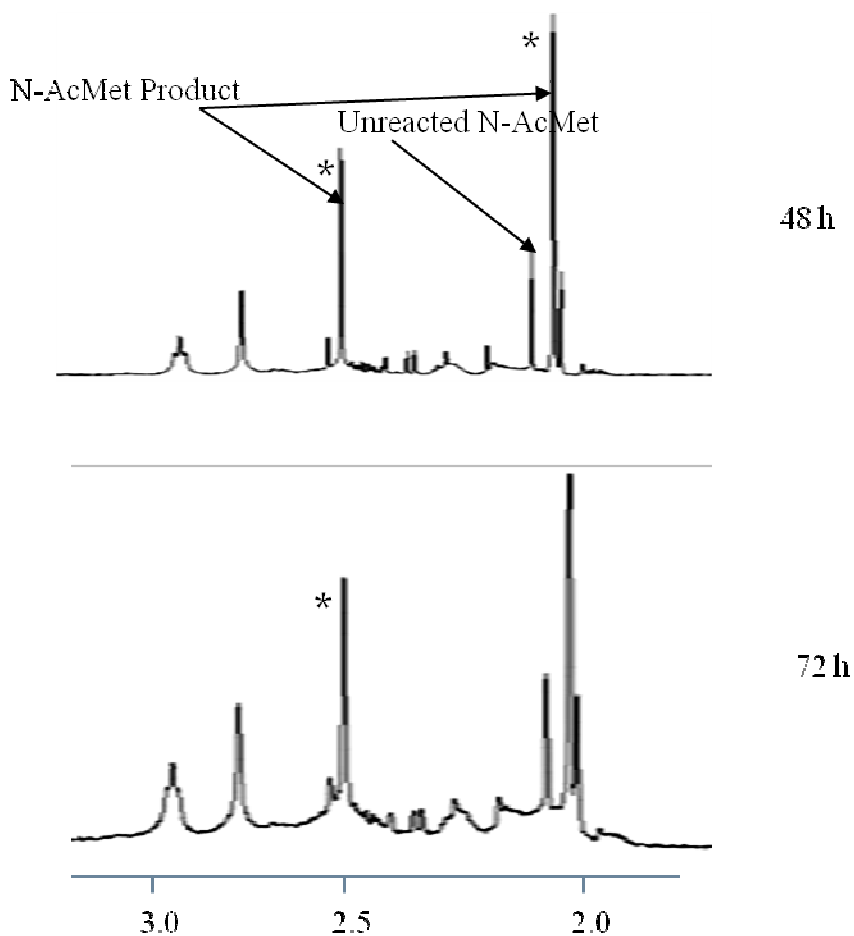


Figure 17.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{en})(\text{Ox})]$  with N-AcMet at 1:2 ratio

Fig. 17 highlights the N-AcMet S-CH<sub>3</sub> signal shifted downfield to ~2.5 ppm indicating the coordination of sulfur atom of methionine to platinum complexes. The original S-CH<sub>3</sub> signals of unreacted N-AcMet are around ~2.3 ppm, but the signal shifts to ~2.5 ppm due to the reaction with the platinum compound. On the basis of the previous results<sup>18, 26</sup> we assigned the new sets of resonances that appeared to be  $[\text{Pt}(\text{en})(\text{N-AcMet-S})_2]$ .<sup>18</sup>

From the molar ratio 1:1-1:2  $[\text{Pt}(\text{en})(\text{Ox})]$  reacts with N-AcMet. The signal intensity at 2.5 ppm, indicates the S-CH<sub>3</sub> connect with platinum and have two N-AcMet

react with the platinum. The one major signal that is formed at ~2.5 ppm results in the formation of bis-products  $[\text{Pt}(\text{en})(\text{N-AcMet-S})_2]$ . (Fig.18)

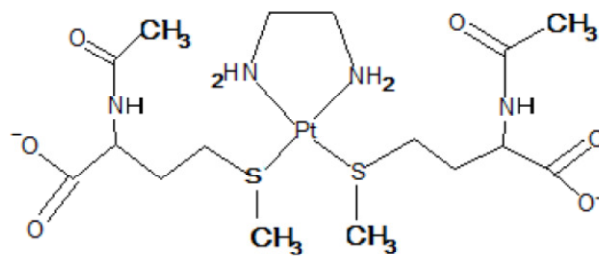


Figure 18. Predicted Structure of  $\text{Pt}(\text{en})(\text{N-AcMet-S})_2$  complex.

#### E. Reactivity of $[\text{Pt}(\text{en})(\text{Ox})]$ with 5'-GMP and N-AcMet at 1:1:2 Ratio

500  $\mu\text{l}$  aliquots of 10 mM of  $[\text{Pt}(\text{en})(\text{Ox})]$ , 10 mM 5'-GMP, and 20 mM N-AcMet are combined and the pH is adjusted to 4. The reactivity is interpreted by the  $^1\text{H}$ NMR spectra after 24 hours and 48 hours. (Fig.19)

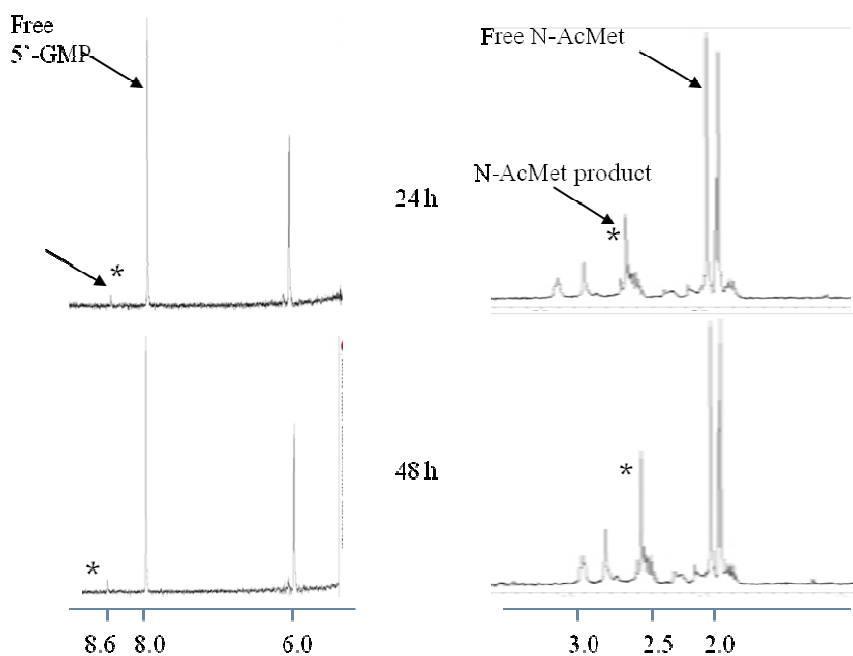


Figure 19.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{en})(\text{Ox})]$  with 5'-GMP and N-AcMet

The products formed from the reaction with 5'-GMP and N-AcMet have key resonances at ~8.6 ppm and ~ 2.5 ppm respectively. (Fig.19).

The reactivity of [Pt(en)(Ox)] with 5'-GMP was slow after 24 hours and remained similar in its intensity even after 48 hours. Product signals at ~8.6 ppm were observed corresponding to [Pt(en)(N-AcMet-S)(5'-GMP-N7)] which indicates the reaction of the platinum complex with guanine via N7 residues at 24 hour time. There was significant amount of product formed for N-AcMet initially after 24 hours and increased dramatically even after 48 hours. Also, there were more intense peaks observed at ~2.5 ppm indicating the downfield shift of methionine with [Pt(en)(Ox)] at 48 hours. The S-CH<sub>3</sub> signal of [Pt(en)(N-AcMet-S)] occurs at ~2.5 ppm. As Fig. 19 indicates, more products are initially formed with N-AcMet than with 5'-GMP. Throughout the reaction, the amount of N-AcMet reacted exceeds the amount of 5'-GMP reacted. Within ~48 hours, signals due to [Pt(en)(N-AcMet-S)<sub>2</sub>] were observed. On the basis of previous results,<sup>18,26</sup> the final product from the studies are [Pt(en)(N-AcMet-S)(5'-GMP-N7)] and a bis coordinated N-AcMet product [Pt(en)(N-AcMet-S)<sub>2</sub>]. Compared to the previous results,<sup>18</sup> the [Pt(en)(ox)] reacted quickly with N-AcMet than 5'-GMP.

#### F. Reactivity of [Pt(en)(Ox)] with 5'-GMP and N-AcMet at 1:4:1 Ratio

We modified the reactivity by adding 10 mM of [Pt(en)(Ox)] to 40 mM 5'-GMP and 10 mM N-AcMet. 500 µl aliquots of each were mixed together and the pH was determined. The reactivity is interpreted by <sup>1</sup>H NMR spectra after 24 hours and 48 hours. (Fig. 20).

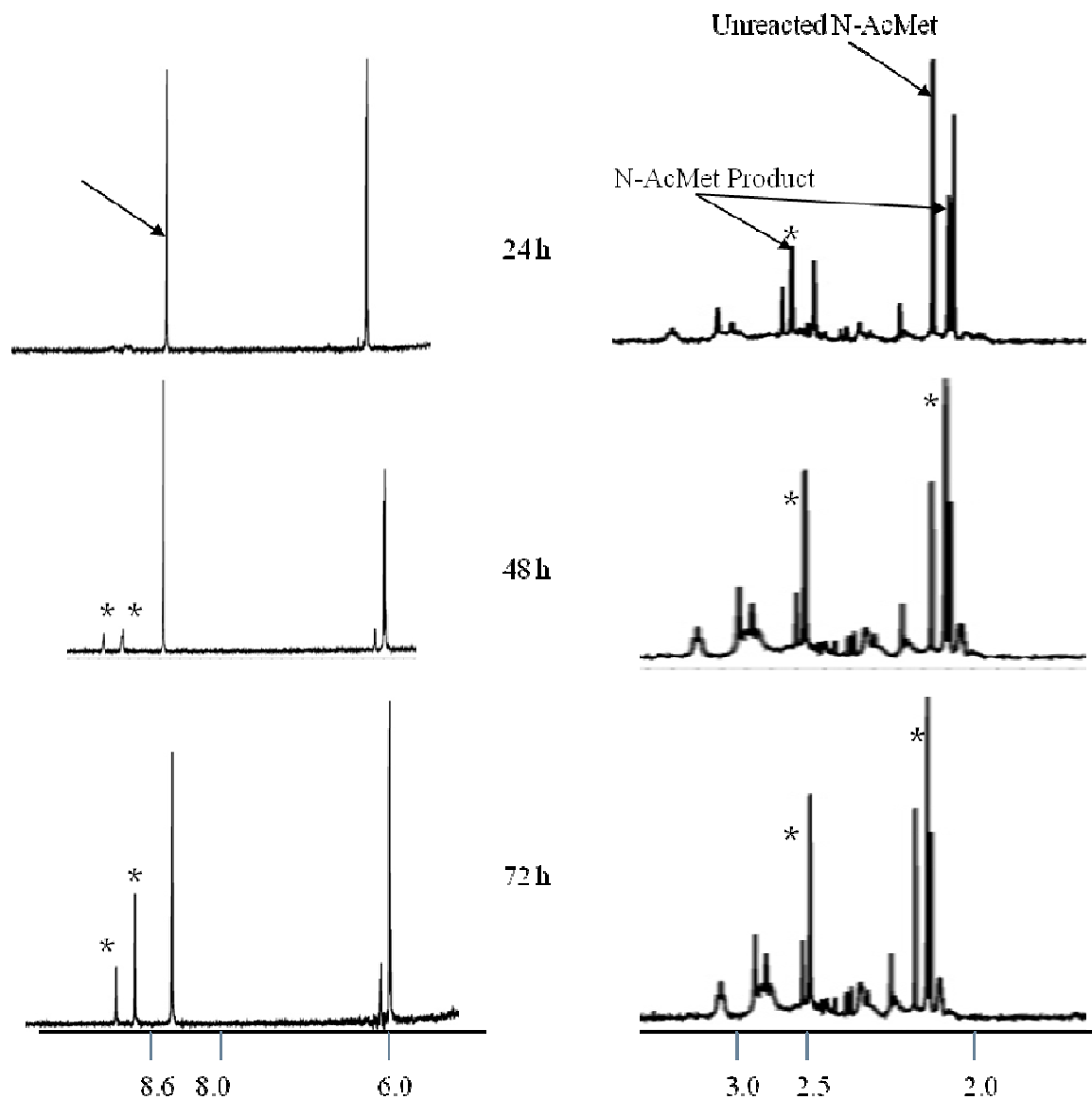


Figure 20.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{en})(\text{Ox})]$  with 5'-GMP and N-AcMet

The 1: 4 products formed from reaction with 5'-GMP and N-AcMet have key resonances at  $\sim 8.5$  ppm,  $\sim 8.6$  ppm, and  $\sim 2.5$  ppm respectively. (Fig. 20).

Four equivalents of 5'-GMP were added to one equivalent of  $[\text{Pt}(\text{en})(\text{Ox})]$  and two equivalents of N-AcMet. When the reaction was monitored after 24 hours, 48 hours, and 72 hours, two key resonances at  $\sim 8.5$  ppm and  $\sim 8.6$  ppm were observed. The significant downfield shift of H8 atoms indicates N7 coordination of 5'-GMP. On the

basis of the previous results,<sup>18,26</sup> the products' peaks can be interpreted. The intensity of signals for N-AcMet increased from a 24 hour time period to a 72 hour time period. The downfield shift of H8 signals at ~2.5 ppm corresponds to the N-AcMet bis-product [Pt(en)(N-AcMet-S)<sub>2</sub>], which is due to a sulfur coordination with methionine. There are also two products in the GMP range. At least one of the products that is left is due to [Pt(en)(N-AcMet-S)(5'-GMP-N7)], whereas the other signal which corresponds to bis 5'-GMP products on the right side is due to [Pt(en)(5'-GMP)<sub>2</sub>].

In comparison to the previous results,<sup>26</sup> the products formed when four equivalents of 5'-GMP were added to the one-to-one mixture of [Pt(en)(Ox)] and N-AcMet are [Pt(en)(N-AcMet-S)<sub>2</sub>], [Pt(en)(N-AcMet-S)(5'-GMP-N7)] and [Pt(en)(5'-GMP)<sub>2</sub>].

### Bulky Compounds

The competition reactions between N-AcMet and 5'-GMP with [Pt(Me<sub>4</sub>en)(Ox)] are studied quantitatively using <sup>1</sup>H NMR at pH 4 at varying concentrations.

### G. Reactivity of [Pt(Me<sub>4</sub>en)(Ox)] with 5'-GMP at 1:1 Ratio

The reactivity of [Pt(Me<sub>4</sub>en)(Ox)] with 5'-GMP is studied by the <sup>1</sup>H NMR spectroscopy. 500 µL aliquots of 10 mM of [Pt(Me<sub>4</sub>en)(Ox)] with 10 mM of 5'-GMP are combined together and pH is adjusted to 4. The reactivity is interpreted by <sup>1</sup>H NMR spectra after 24 hours, 48 hours, and 72 hours respectively.



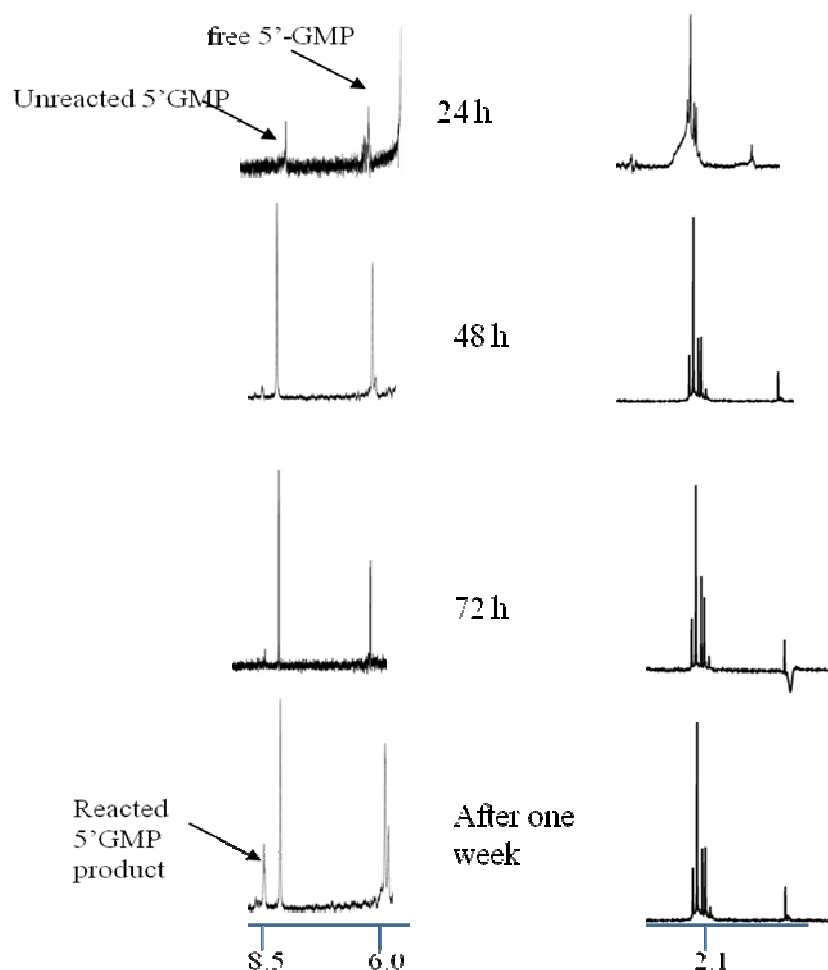


Figure 21.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP at 1:1 ratio

The reactivity of 5'-GMP with  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  is very slow. After 24 hours, there was no significant amount of product signals formed from the reaction indicating that there is no reactivity of 5'-GMP and hence no observance of new H8 signals. After one week, there were product signals formed, indicating the reactivity of 5'-GMP. The downfield shift of H8 signals at  $\sim 8.3$  ppm indicates the coordination of platinum complex with 5'-GMP via N7.  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  is the bulky compound, and its reactivity with 5'-GMP was observed after one week forming a predicted bis-products with two substituted 5'-GMP's. The results which show the slow reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP were consistent with the previous study which was conducted on  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{+2}$ .

Analysis of rate constants of  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{+2}$  and  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$  with 5'-GMP's from the previous papers have shown that  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$  reacts about 13 times faster than  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{+2}$ .<sup>16</sup> This could be due to several factors including the steric bulk of  $\text{Me}_4\text{en}$  ligand, hydrogen bonding to the  $\text{NH}_2$  groups in  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$ , and possible trans effect differences due to reaction trans to an  $\text{NH}_2$  vs. an  $\text{N}(\text{CH}_3)_2$ . We conclude that the presence of bulky  $\text{Me}_4\text{en}$  ligand slows the reaction with 5'-GMP's. Thus, the bulk factor hinders the reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP quickly.

#### H. Reactivity of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$ with 5'-GMP at 1:2 Ratio

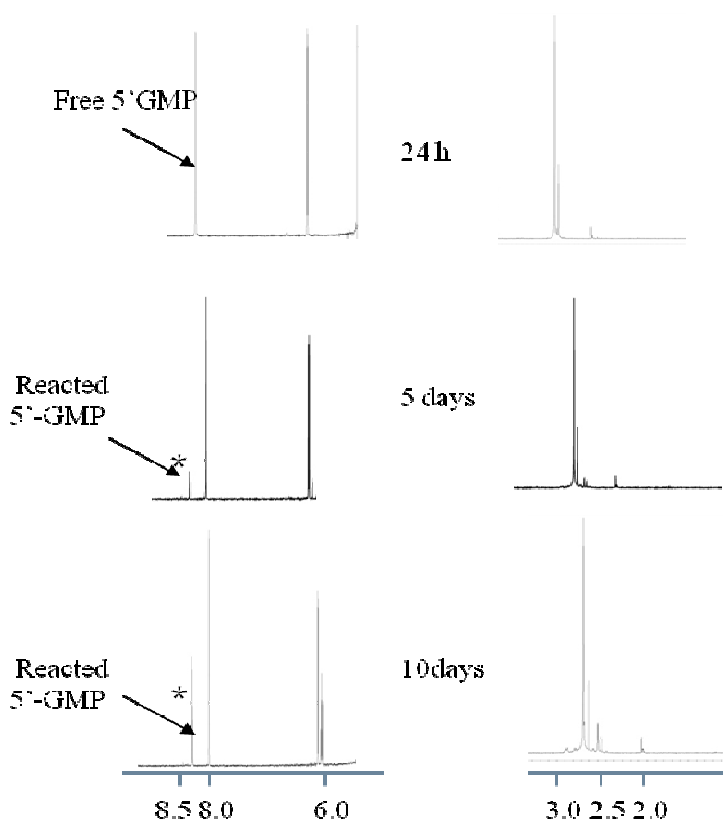


Figure 22.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP at 1:2 ratio

Fig. 22 shows the partial  $^1\text{H}$  NMR spectra from the reaction of 10 mM of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  and 20 mM of 5'-GMP at pH 4.0. The products formed from the reaction of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  and 5'-GMP have two key resonances at  $\sim 8.3$  ppm. The product signals for reactivity of 5'-GMP are not observed after one week. The above NMR spectrum scaled to the largest peak to make the signals easier to observe.

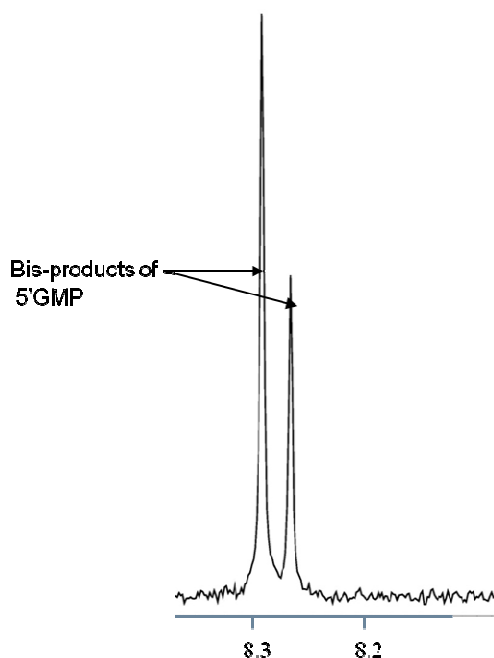


Figure 23.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP showing bis-products

Fig. 23 shows the partial  $^1\text{H}$  NMR spectra of bis-products' formation between  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  and 5'-GMP at 8.3 ppm. Product signals were observed after 5 days of reactivity.

We modified the previous reactivity with two equivalents of 5'-GMP and monitored from 24 hours to 10 days. H8 signals shifted downfield at  $\sim 8.3$  ppm indicating the N7 coordination of 5'-GMP after 5 days. Previously, it was found that when

$[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})]_2$  reacted with 5'-GMP results in two possible steps one is that the reaction of diaqua complexes with the first guanine ligand. The second is the reaction of the mono-product with a second guanine ligand.<sup>18</sup> Here, in this reaction when oxalates as a leaving ligands for  $\text{Pt}(\text{Me}_4\text{en})$  are utilized only  $[\text{Pt}(\text{Me}_4\text{en})(5'\text{-GMP})_2]$  is observed. The reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP was comparatively slow when compared to  $[\text{Pt}(\text{en})(\text{Ox})]$ . The  $\text{Me}_4\text{en}$  bulk slowed the reactivity with 5'-GMP and took days to complete the reaction. (Fig. 21), (Fig. 22).

#### I. Reactivity of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$ with N-AcMet at 1:2 Ratio

500  $\mu\text{L}$  aliquots of 10 mM of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 20 mM of N-AcMet were combined together, and reacted at pH 4; the reactivity is interpreted by the  $^1\text{H}$  NMR spectra after 1 hour, 24 hours, and 48 hours respectively.

A signal at  $\sim 2.5$  ppm was assigned to the S- $\text{CH}_3$  signal. The downfield shift of S- $\text{CH}_3$  signals indicates the sulfur coordination with methionine. (Fig 24). On the basis of comparison of previous results,<sup>20,26</sup> we assigned the new sets of resonances that appeared to be at  $\sim 2.5$  ppm which corresponds to  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S})(\text{ox-O})]$  (mono coordinated) and a signal at  $\sim 2.6$  ppm which corresponds to  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S}, \text{O})^+]$  (sulfur-oxygen chelate).

Formation of the complex  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S}, \text{O})^+]$  appeared to be significantly faster than the formation of 5'-GMP complex as evidenced by the extent of reaction of each at 24 hours. When N-AcMet is added to the bulky platinum complex the oxalates are easily displaced within 24 hours to 48 hours of reactivity. The N-AcMet product is formed at 1 hour and a significant increase in the intensity of product signals at 48 hours was observed.

The following  $^1\text{H}$  NMR spectrum shows the reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{ox})]$  with N-AcMet

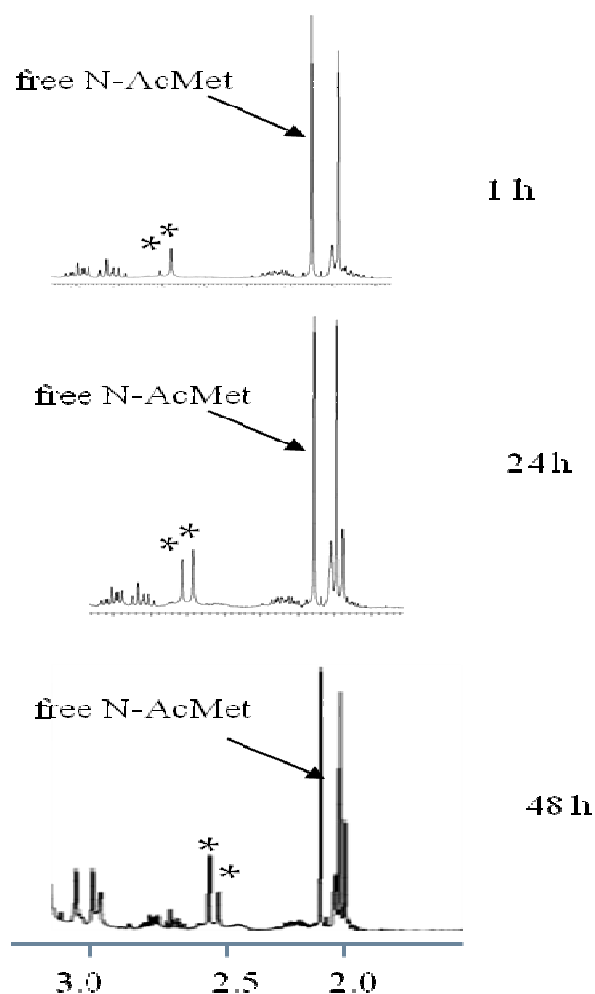


Figure 24.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with N-AcMet at 1:2

#### J. Reactivity of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$ and N-AcMet at 1:1 Ratio

500  $\mu\text{L}$  aliquots of 10 mM of  $[\text{Pt}(\text{Me}_4\text{en})(\text{ox})]$  with 10 mM of N-AcMet are combined together and pH is adjusted to 4. The reactivity is interpreted by the  $^1\text{H}$  NMR spectroscopy after 1 hour, 24 hours, and 48 hours respectively.

A signal at  $\sim 2.5$  ppm was assigned to the S- $\text{CH}_3$  signal; such a downfield chemical shift is similar to that observed at 1:2 ratio. On the basis of comparison of

previous results,<sup>20,26</sup> we assigned the new sets of resonances that appeared to be at ~2.5 ppm which corresponds to  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S})(\text{ox-O})]$  (mono-coordinated) and a signal at ~2.6 ppm corresponds to  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S}, \text{O})^+]$  (sulfur-oxygen chelate). The signals at ~2.0 ppm and ~2.1 ppm correspond to the unreacted N-AcMet. The intensity of product signals at ~2.5 ppm for S-CH<sub>3</sub> increases dramatically from 1 hour to 48 hours.

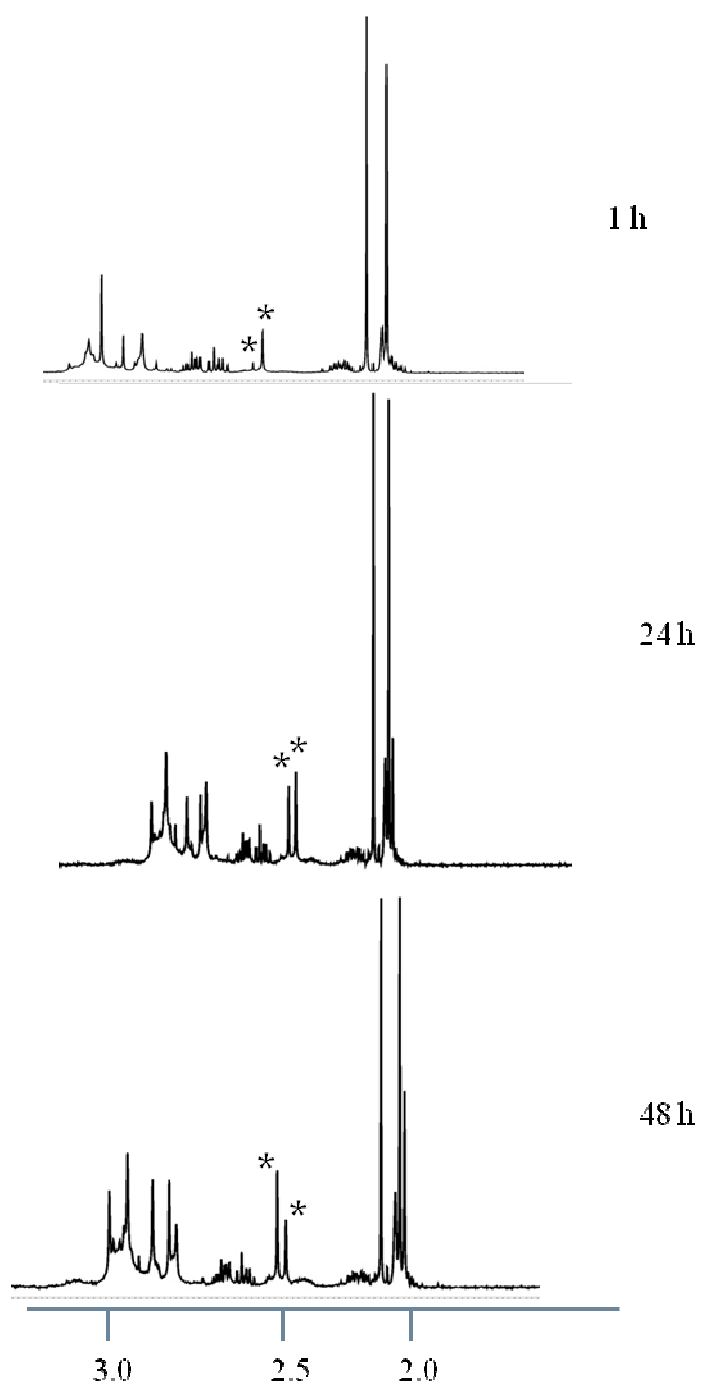


Figure 25.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP at 1:1 ratio.

### K. Reactivity of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$ With N-AcMet And 5'-GMP at 1:2:1 Ratio

500  $\mu\text{L}$  aliquots of 10 mM of  $[\text{Pt}(\text{Me}_4\text{en})(\text{ox})]$  with 20 mM of N-AcMet and 10 mM of 5'-GMP are combined together at pH 4. The reactivity is interpreted by the  $^1\text{H}$  NMR spectra.

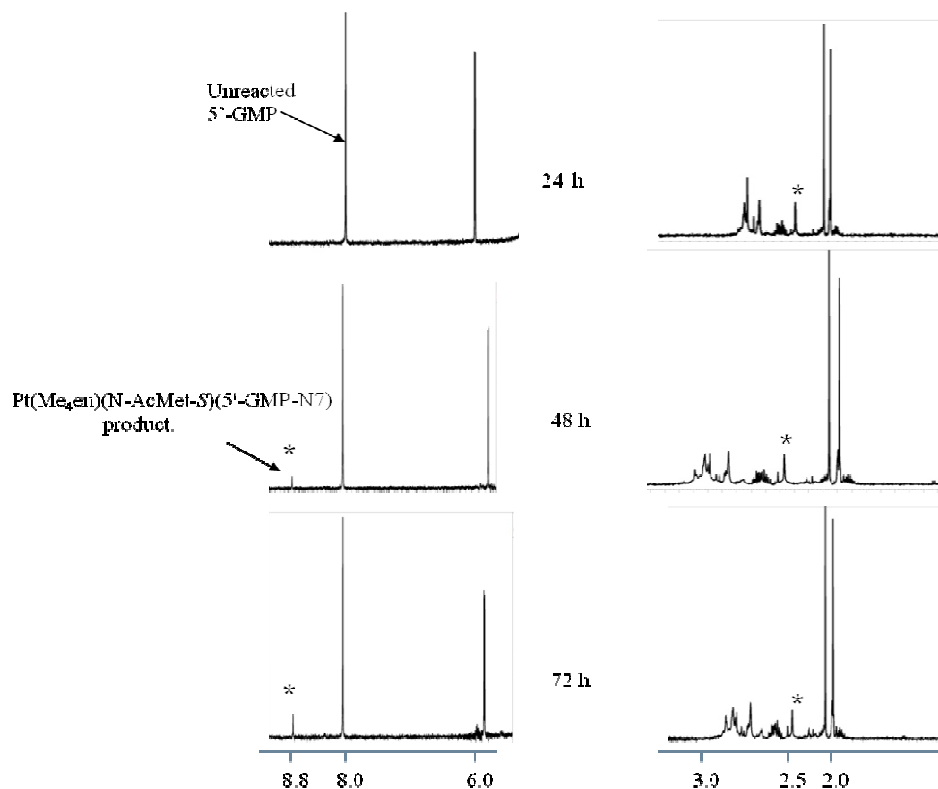


Figure 26.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP and N-AcMet.

We monitored the reactivity by conducting competition studies between  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$ , 5'-GMP, and N-AcMet and see if 5'-GMP could displace N-AcMet. We expected that the second Pt-O bond is easier to displace after the first Pt-O bond is broken which means N-AcMet reacts first to break the oxalate chelate then the 5'-GMP displaces the unidentate oxalate ligand; thus  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S})(5'\text{-GMP})]$  was a possible



product.<sup>26</sup> According to the previous studies conducted, the mixed ligand complexes would not have severe steric clashes.<sup>27</sup> The intensity of product peaks increased as the time increased resulting in the predicted product formation at ~8.8 ppm corresponding to  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet})(5'\text{-GMP-N7})]$  but is not due to  $[\text{Pt}(\text{Me}_4\text{en})(5'\text{-GMP})_2]$ .<sup>27</sup> The significant downfield shift of the H8 atom indicates N7 coordination of 5'-GMP. Likewise, the S-CH<sub>3</sub> signal at ~2.4 ppm which corresponds to  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})(\text{N-AcMet-S, O})]^+$ . The <sup>1</sup>H NMR signals did not disappear even after several days and  $[\text{Pt}(\text{Me}_4\text{en})(5'\text{-GMP})_2]$  signals appeared, indicating complete displacement of N-AcMet did not occur.

#### L. Reactivity of $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$ with 5'-GMP and N-AcMet at 1:1:2 Ratio

We modified the reactivity by adding one equivalent of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with one equivalent of N-AcMet and 2 equivalent of 5'-GMP. After 24 hours, new signals were observed in the NMR spectrum. A new resonance in the H8 region of the spectrum was observed at ~8.8 ppm suggesting it is due to  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet})(5'\text{-GMP})]^+$ . The significant downfield shift of the H8 atom indicates N7 coordination of 5'-GMP. Likewise, the S-CH<sub>3</sub> signals at ~2.4 ppm corresponds to  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S, O})^{+2}]$ . The <sup>1</sup>H NMR signals did not disappear even after several days and no  $[\text{Pt}(\text{Me}_4\text{en})(5'\text{-GMP})_2]$  signals appeared indicating the complete displacement of N-AcMet did not occur.

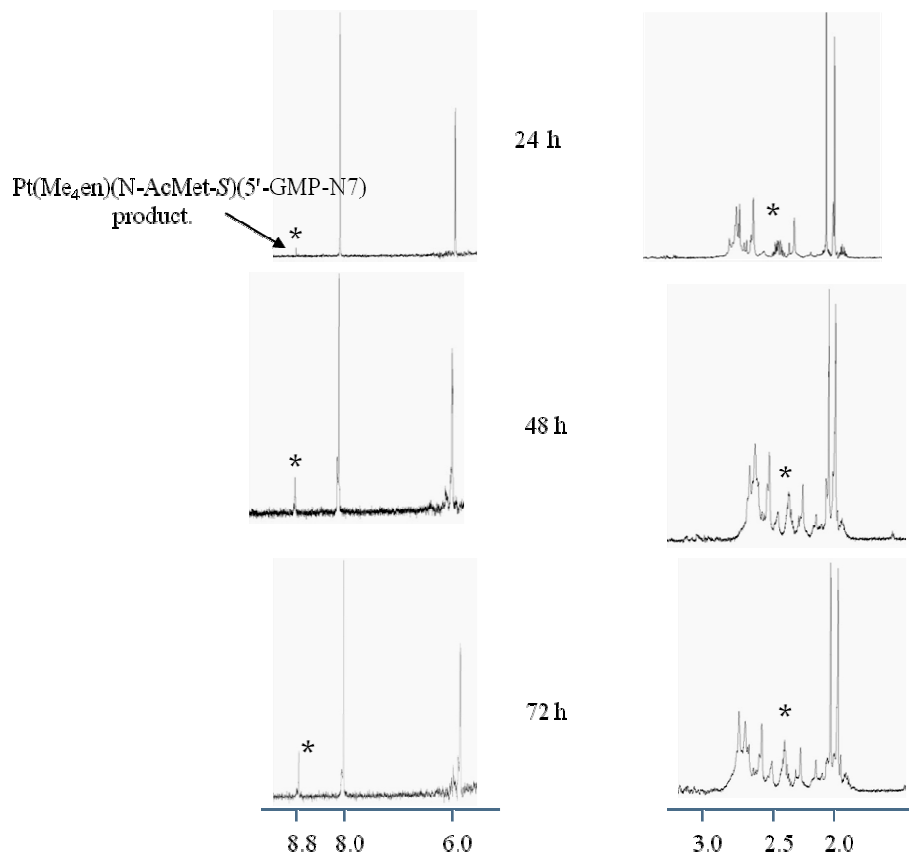


Figure 27.  $^1\text{H}$  NMR spectrum of reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP and N-AcMet

We conclude from Fig. 26 and Fig. 27 that the significant downfield shift of H8 atom indicates N7 coordination of 5'-GMP and the predicted possible product formed was  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet})(5'\text{-GMP})]^+$ .

#### IV. DISCUSSION

In the current research the main focus is to check the reactivity of platinum complexes with guanine residues of DNA and methionine residues of proteins. Although the reaction with DNA is thought to be responsible for the anticancer activity, the reaction of platinum complexes with proteins is significant. It is suggested that protein binding contributes to the toxicity of platinum anticancer drugs; however, the reactivity of platinum with biological thiols may be a pathway for detoxification and resistance.<sup>26</sup> Therefore, better understanding of the interactions of platinum complexes with DNA and protein targets is important. With platinum being a soft metal, the sulfur atoms of methionine and cysteine residues are the primary targets in proteins.<sup>26</sup>

Due to the high affinity of platinum(II) compounds to sulfur, sulfur containing molecules are frequently applied as rescue agents in cancer therapy. According to the previous investigations, mixed-ligand adducts formation is possible because of reversible methionine binding during the reaction of cisplatin with 5'-GMP in the presence of L-Methionine.<sup>28</sup> According to the Kung et al., the addition of L-Methionine at the physiological concentration results in an increase of the rate of complex formation at the initial step of the reaction and different adduct formation for the reaction with carboplatin,  $\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{HM})]$ , and  $\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{M})]$ .<sup>28</sup> Oxaliplatin reacts faster with 5'-GMP, and, contrary to the ammine ligands of the other complexes, the DACH ligand cannot be released.<sup>28,30</sup> Formation of the chelate is favored in the presence of equimolar amounts of 5'-GMP and L-Met and the displacement of L-Met by 5'-GMP cannot be observed.<sup>28</sup> These results indicate the correlation with our observations that 5'-GMP displaces oxalate ligands very slowly.

Platinum connectivity with sulfur is generally favored when compared to nitrogen and oxygen because platinum is a low electro negativity element and has a low energy LUMO. To differentiate sulfur from nitrogen and oxygen, sulfur has a large atomic radius, higher polarizability, and lower electro negativity than nitrogen and oxygen. Sulfur is also a soft base when compared to nitrogen and oxygen. According to HSAB theory, soft Lewis acids react faster and form stronger bonds with soft bases. Therefore, platinum connectivity with sulfur is more favored than with oxygen and nitrogen.

In the current study, reactivities of [Pt(en)(Ox)] and [Pt(Me<sub>4</sub>en)(Ox)] with 5'-GMP individually and in the presence of N-AcMet in 1:1 and 1:2 ratios at pH 4 are quantitatively studied using <sup>1</sup>H NMR spectroscopy. We used oxalates as leaving ligands because we wanted to see how the difference in the leaving ligand affected the reactivity of platinum complexes; the oxalate leaving ligand is utilized in the third generation anticancer drug oxaliplatin. We kept the pH at ~4 in order to keep the 5'-GMP phosphate group from completely deprotonating.

The reactivity of [Pt(en)(Ox)] with 5'-GMP and N-AcMet is faster when compared with the bulkier complex [Pt(Me<sub>4</sub>en)(Ox)]. Previously, it was shown that the [Pt(en)(D<sub>2</sub>O)]<sup>2+</sup> reacted 13 times faster with 5'-GMP than [Pt(Me<sub>4</sub>en)(D<sub>2</sub>O)]<sup>2+</sup>.<sup>18</sup> The possible reasons for the slow reactivity of [Pt(Me<sub>4</sub>en)(D<sub>2</sub>O)]<sup>2+</sup> were due to several factors including steric bulk of the Me<sub>4</sub>en ligand, hydrogen bonding to the NH<sub>2</sub> groups in [Pt(en)(D<sub>2</sub>O)]<sup>2+</sup>, and possible trans effect differences due to the reaction trans to an NH<sub>2</sub> vs. an N(CH<sub>3</sub>)<sub>2</sub> group. The current studies showed very slow reactivity of 5'-GMP with [Pt(Me<sub>4</sub>en)(Ox)]. After one week, two small downfield shifted H8 signals were observed, indicating the coordination of N7 residues with guanine. Thus, from these studies it can

be concluded the steric bulk of Me<sub>4</sub>en hindered the reactivity quickly when compared to en complex.

A previous study found that both platinum complexes [Pt(en)(D<sub>2</sub>O)<sub>2</sub>]<sup>+2</sup> and [Pt(Me<sub>4</sub>en)(D<sub>2</sub>O)]<sup>+2</sup> reacted faster with 5'-GMP. The possible explanation for its reactivity is due to the hydrogen bonding with a 5' phosphate and that the en ligand would not have any steric clashes with an incoming ligand.<sup>18</sup> The reactivity is explained in two possible steps: the reaction of the diaqua complexes with the first guanine ligand, and the reaction of the mono product with the second guanine ligand. Although the reaction conditions utilized the favored formation of a mono-product, a mechanism to account for further reactions to bis-products was used.<sup>18</sup> However, in the current study the reactions of the platinum complexes [Pt(en)(Ox)] and [Pt(Me<sub>4</sub>en)(Ox)] with 5'-GMP result in the formation of bis-products with no intermediates. It indicates the second 5'-GMP reacts faster than the first, since the first 5'-GMP has to break the oxalate chelate. The bis-products formed are [Pt(en(5'-GMP)<sub>2</sub>)] and [Pt(Me<sub>4</sub>en)(5'-GMP)<sub>2</sub>].

The scheme of the possible reactions of [Pt(en)(Ox)] and [Pt(Me<sub>4</sub>en)(Ox)] with 5'-GMP is explained in Fig 28 and Fig 29

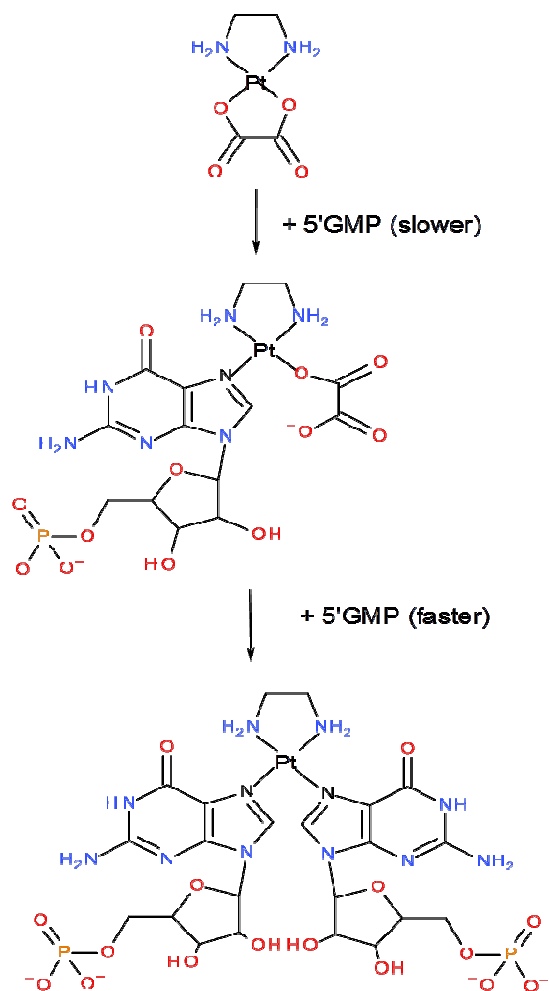


Figure 28. Scheme of reactions of [Pt(en)(Ox)] with 5'-GMP

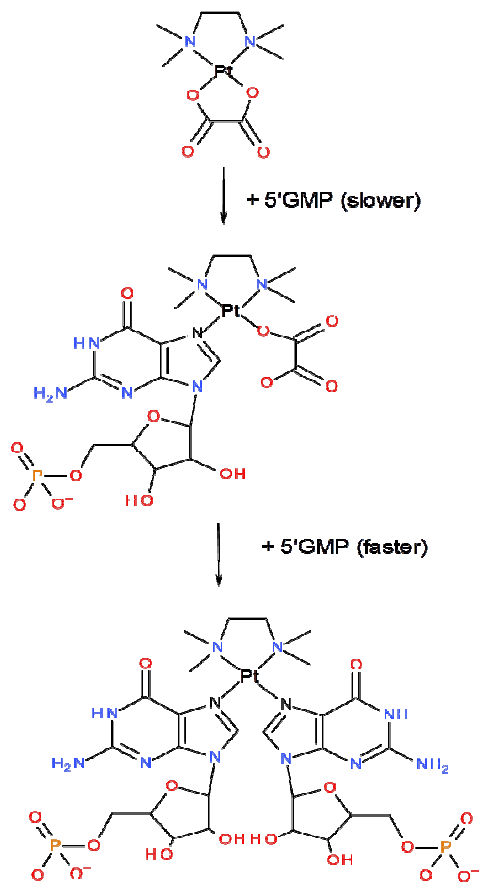


Figure 29. Scheme of reactions of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP

The  $[\text{Pt}(\text{en})(\text{Ox})]$  reactivity with N-AcMet was favored more quickly at a 1:2 molar ratio, and a new product,  $[\text{Pt}(\text{en})(\text{N-AcMet-S})_2]$ , is formed. Previously, during the reactions between  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{+2}$  and N-AcMet, several products were observed. In addition to the mono-substituted product, an intermediate  $[\text{Pt}(\text{en})(\text{N-AcMet-S})(\text{D}_2\text{O})]^+$  and bis-products  $[\text{Pt}(\text{en})(\text{N-AcMet-S})_2]$  are also formed.<sup>18</sup> When oxalates as leaving ligands are used only a bis substituted N-AcMet is formed with no intermediates.

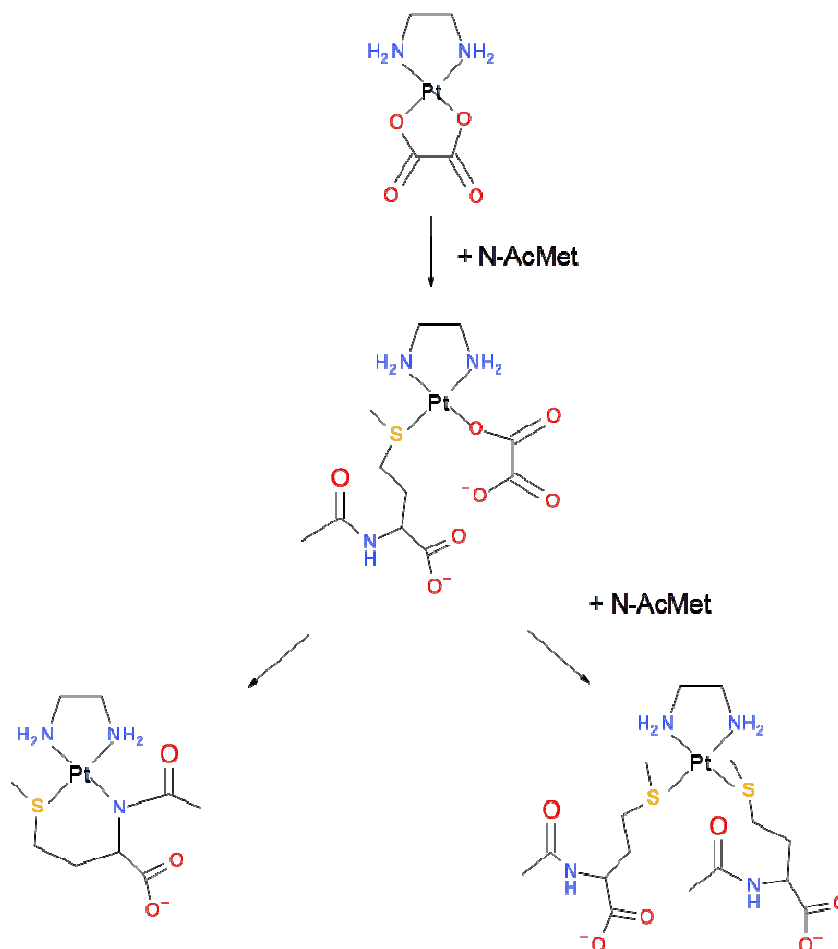


Figure 30. Scheme of reactions of  $[Pt(en)(Ox)]$  with N-AcMet

Our previous study found that the  $[Pt(Me_4en)(D_2O)_2]^{2+}$  complex reacted with only one N-acetyl methionine residue to form S, O chelates.<sup>18</sup> Molecular mechanics calculations suggested that a  $[Pt(Me_4en)(Met-S)_2]^{2+}$  complex would have severe steric clashes regardless of the chiralities of the sulfur atoms or the relative orientations of the methionine residues. Also, the bulk prevented coordination of the amide nitrogen of an N-acetyl methionine residue.<sup>18</sup> The possible explanation for the faster reactivity of  $[Pt(Me_4(en)Ox)]$  with N-AcMet than with 5'-GMP in the current study could be due to the easier replacement of oxalate ligands by methionine residues than by guanine



residues. The possible products formed when N-AcMet reacted with  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  are  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S}, \text{O})]^+$  as well as the mono-substituted N-AcMet product  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox-o})(\text{N-AcMet-S})]^+$

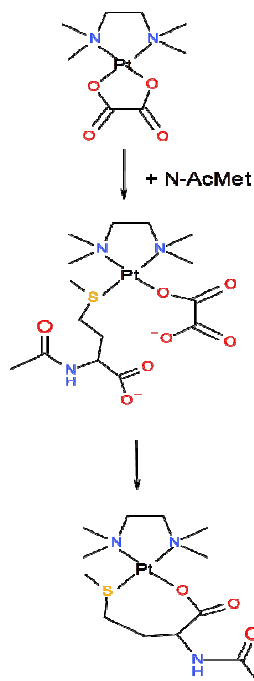


Figure 31. Scheme of reactions of  $[\text{Pt}(\text{Me}_4(\text{en})\text{Ox})]$  with N-AcMet

Previously, by using capillary electrophoresis-electrospray ionization-mass spectrometry (CE-ESI-MS), the binding behavior of oxaliplatin to 5'-GMP in the presence of the sulfur containing amino acid L-methionine was investigated.<sup>27, 31</sup> The results from Kung et al, indicated a competitive situation between 5'-GMP and L-Methionine for coordinating oxaliplatin and a favored coordination of L-Methionine.

Consistent with the current study,  $[\text{Pt}(\text{en})(\text{Ox})]$  reactivity with N-AcMet is faster when compared to the reactivity with 5'-GMP. The oxalates are easily displaced by methionine residues, and product formation was observed quickly in hours rather than

days as in the case of reaction with 5'-GMP. Also, when competitive studies between [Pt(en)(Ox)] with N-AcMet and 5'-GMP were investigated using  $^1\text{H}$  NMR, The predominant products formed were [Pt(en)(N-AcMet-S) $_2$ ], [Pt(en)(N-AcMet-S)(5'-GMP-N7)] and [Pt(en)(5'-GMP) $_2$ ].

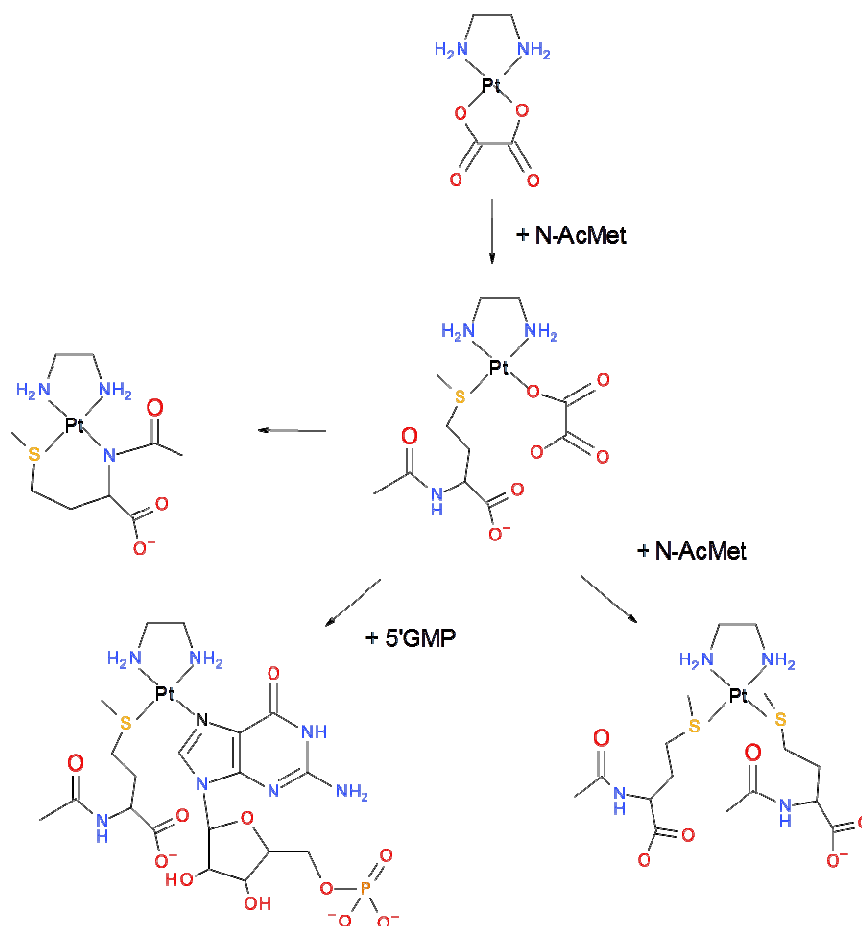


Figure 32. Scheme of reactions of [Pt(en)(Ox)] with N-AcMet and 5'-GMP

When [Pt(Me $_4$ en)(Ox)], N-AcMet, 5'-GMP were added together, a new resonance in the H8 region of the spectrum was observed at  $\sim 8.8$  ppm corresponding to [Pt(Me $_4$ en)(N-AcMet)(5'-GMP)] $^+$ .

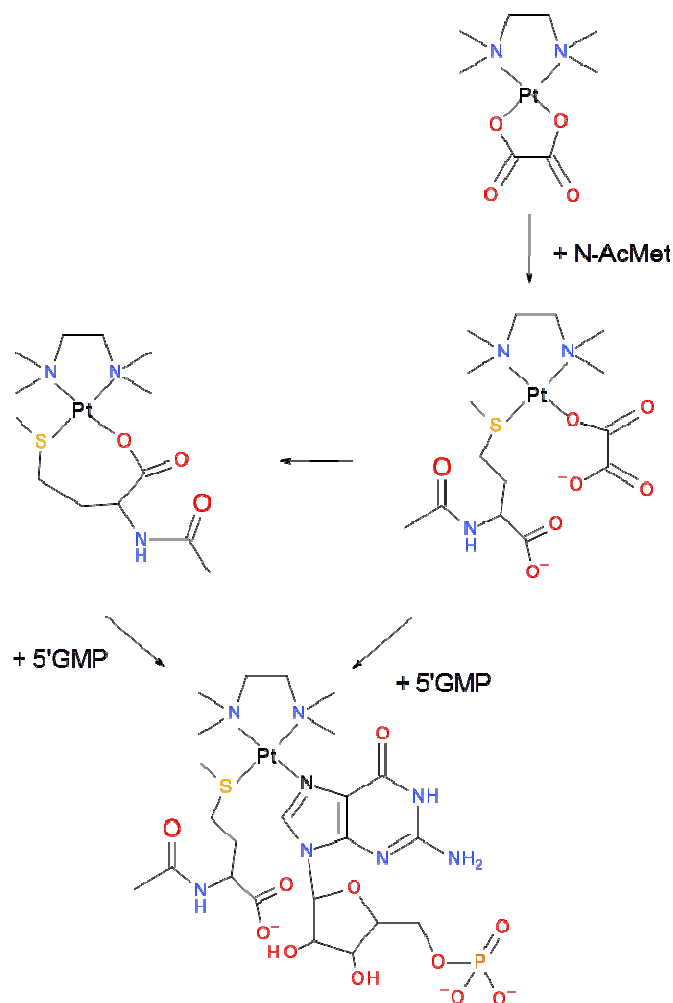


Figure 33. Scheme of reactions of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with N-AcMet and 5'-GMP

When  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  reacted with N-AcMet and 5'-GMP the predictable products formed were  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})(\text{N-AcMet-S})]^+$  and  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet})(5'\text{-GMP})]^+$ .

It was found that the oxalate leaving ligand slowed the reactivity more with 5'-GMP when compared to N-AcMet. The reaction rate with 5'-GMP was faster when N-AcMet was added simultaneously to the mixture of platinum complex and 5'-GMP. This

result suggests that the N-AcMet reacts first to break the oxalate chelate, then the 5'-GMP displaces the unidentate oxalate ligand.

In summary, we have utilized a  $^1\text{H}$  NMR spectroscopy to study the reactivity of platinum(II) diamine complexes [utilizing  $[\text{Pt}(\text{en})(\text{Ox})]$  as a non bulky complex and  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  as a bulky complex] with 5'-GMP and N-AcMet individually and competitively. The reactivity of  $[\text{Pt}(\text{en})(\text{Ox})]$  and  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP resulted in the bis-products with two substituted 5'-GMPs. The reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with 5'-GMP was very slow comparatively with  $[\text{Pt}(\text{en})(\text{Ox})]$ . Bis-products such as  $[\text{Pt}(\text{en})(\text{N-AcMet-S})_2]$  are predominantly formed when  $[\text{Pt}(\text{en})(\text{Ox})]$  reacts with N-AcMet. When  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  reacts with N-AcMet two products are possible: one is  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S}, \text{O})]^+$ , the other is  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox-o})(\text{N-AcMet-S})]^+$ . When competition studies of  $[\text{Pt}(\text{en})(\text{Ox})]$  with 5'-GMP and N-AcMet were conducted, the possible products formed were  $[\text{Pt}(\text{en})(\text{N-AcMet-S})(5'\text{-GMP-N7})]$  and  $[\text{Pt}(\text{en})(5'\text{-GMP})_2]$ . Reactivity of  $[\text{Pt}(\text{Me}_4\text{en})(\text{Ox})]$  with N-AcMet and 5'-GMP resulted in  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S})(5'\text{-GMP})]$ . The significant formation of  $[\text{Pt}(\text{en})(\text{N-AcMet-S})(5'\text{-GMP-N7})]$  and  $[\text{Pt}(\text{Me}_4\text{en})(\text{N-AcMet-S})(5'\text{-GMP})]$  product indicates that N-AcMet reacts first to break the oxalate chelate then the 5'-GMP displaces the unidentate oxalate ligand;

Future studies will focus on the product of the reaction at different pH levels and time differences. Focus on studying the reactivity of platinum (II) complexes with oxalates as leaving ligands at pH-7 is important as there is less chance of dimerization reactions. Study of the reactivity of platinum(II) complexes with other amino acids like histidine and cysteine will also be pursued.

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