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# Reactions of Platinum(II) Compounds with Selenium Containing Amino Acids

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REACTIONS OF PLATINUM(II) COMPOUNDS  
WITH SELENIUM CONTAINING AMINO ACIDS

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  
Stephanie Robey

May 2013

REACTIONS OF PLATINUM(II) COMPOUNDS  
WITH SELENIUM CONTAINING AMINO ACIDS

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I first dedicate this thesis to Dr. Kevin Williams for his time and effort in helping me succeed and for not giving up on me, when I wanted to give up on chemistry. I also dedicate this thesis to my mom, Anita Milburn, if it was not for her, I would not be where I am today. I additionally want to dedicate this thesis to my dad, Cliff Robey, if it was not for the encouragement and support, I would not have succeeded as far as I have come. I would also like to dedicate this thesis to my fiancé, Ben Spitler, for his time, support, love, and encouragement. Lastly, I dedicate this thesis to all my family and friends for all of the love, support and encouragement to keep going when times got tough.

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# REACTIONS OF PLATINUM(II) COMPOUNDS WITH SELENIUM CONTAINING AMINO ACIDS

Stephanie Robey

May 2013

45 Pages

Directed by: Dr. Kevin Williams, Dr. Lester Pesterfield and Dr. Darwin Dahl

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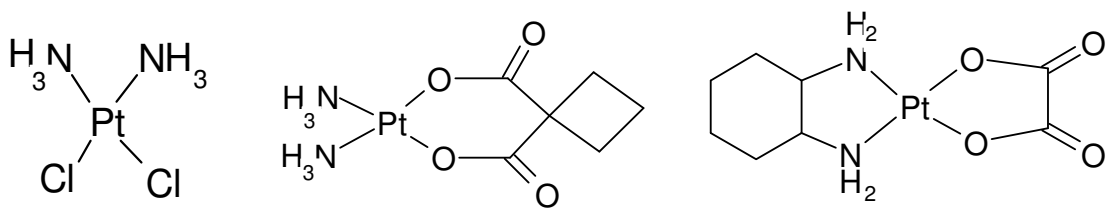
Platinum(II) anticancer medications essentially react with DNA forming kinks in the double helix of DNA and causing apoptosis. It has also been noted that these anticancer medications react with methionine and cysteine in the body. With the new discoveries of selenium containing amino acids including selenomethionine and selenocysteine, new research is ongoing to see what types of products can be formed from these amino acids. Our research reacts  $[\text{Pt}(\text{Met-S,N})\text{Cl}_2]^{2+}$  with selenomethionine to determine what types of products are produced. Monochelates including  $[\text{Pt}(\text{SeMet-Se,N})\text{Cl}_2]^{2+}$  have formed two isomers, as well as other products that insinuate both selenomethionine and methionine binding with the platinum to form various  $[\text{Pt}(\text{SeMet-Se,N})(\text{Met-S,N})]^{2+}$  products. When initially reacting 6 mM  $[\text{Pt}(\text{Met-S,N})\text{Cl}_2]^{2+}$  with 3 mM SeMet, the monochelates of both are produced without forming any free methionine which would suggest that there is free platinum in our solution creating the SeMet monochelate. When adding additional SeMet to the solution the same products are formed that are created when reacting 6 mM  $[\text{Pt}(\text{Met-S,N})\text{Cl}_2]^{2+}$  and 6 mM SeMet. The  $^1\text{H}$  NMR spectrum for these products imply a product of  $[\text{Pt}(\text{SeMet-Se,N})(\text{Met-S,N})]^{2+}$ . Also, reactions with  $[\text{Pt}(\text{en})(\text{ox})]^{2+}$  and SeMet were conducted and produced various products at two different pH's. A  $[\text{Pt}(\text{SeMet-Se,N})_2]^{2+}$  product was formed at lower pH and produced free ethylenediamine, however at a higher pH only  $[\text{Pt}(\text{en})(\text{SeMet-Se,N})]^{2+}$  was produced.

## I. INTRODUCTION

### A. History

According to the National Cancer Institute, around 1,638,910 human beings would be diagnosed with cancer, and 577,190 of them would die from cancer in the United States in 2012.<sup>1</sup> On January 1, 2009, there were approximately 12,553,337 men and women alive that have had some form of cancer, which included people with cancer at that time or that had been cured of cancer at a previous point in their life.<sup>1</sup> Odds are that most everyone in the U.S. population either knows someone directly or indirectly that has been affected by cancer. Current anti-cancer medications have delivered results to patients that help them cope with the symptoms or allow the patient to go into remission. One field of anti-cancer medications that is being studied is platinum(II) compounds; currently there are three platinum-containing FDA approved medications on the market for patients. The first to ever be approved is Cisplatin, (*cis*-diamminedichloroplatinum(II)). Carboplatin, (*cis*-diammine-cyclobutanedicarboxylatoplatinum(II)), is a second generation platinum-containing anti-cancer medication and was developed with fewer side effects than that of cisplatin. Oxaliplatin, ((*1R,2R*-diamminocyclohexane) oxalatoplatinum(II)) is a third generation medication and works for patients that are resistant to cisplatin and carboplatin.

Figure 1.1)



Structures of Cisplatin, Carboplatin and Oxaliplatin<sup>3</sup> contains the FDA approved platinum structures of Cisplatin, (*cis*-diamminedichloroplatinum(II)), Carboplatin, (*cis*-diammine-cyclobutanedicarboxylatoplatinum(II)) and Oxaliplatin, ((*1R,2R*-diamminocyclohexane) oxalatoplatinum(II)).

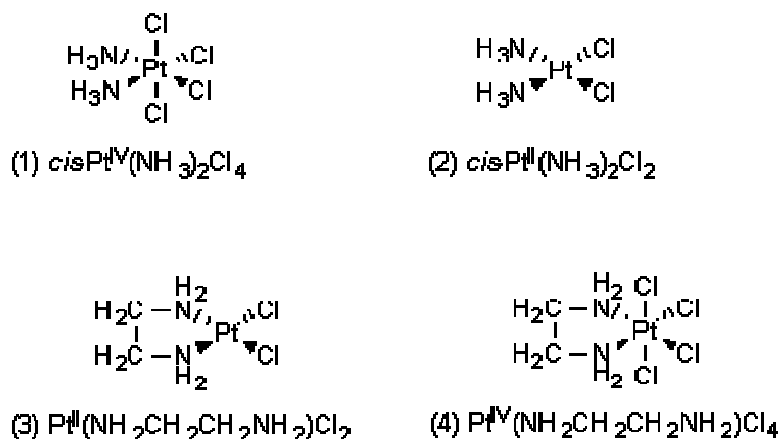
The cisplatin compound was actually first synthesized in 1845 by Michael Peyrone.<sup>3</sup> Its anti-cancer activity wasn't discovered until the 1960's, and clinical trials intravenously in humans were completed by mid-1972.<sup>4</sup> The clinical trials showed major advantages in testicular cancers, but also had produced renal toxicity in patients.<sup>4</sup> There were other toxicities that developed in other patients including ototoxicity, hematological toxicity, neuropathy and seizures. Ultimately, the studies led to the 1978 FDA approval of cisplatin for the treatment of testicular and ovarian cancer. The drug also plays an important role in the treatment of other cancers, including, head and neck cancers, melanoma, bladder cancer and cervical cancers and many more other types of cancers.<sup>5</sup>

During 1967 at Michigan State University, Dr. Barnett Rosenberg published an article in the Journal of Bacteriology stating that the platinum salt, ammonium hexachloroplatinate(II), was responsible for inhibiting cell division.<sup>2</sup> A study was done in Dr. Rosenberg's lab trying to see how an electric field would affect the growth process of bacteria, specifically *E. coli*.<sup>2</sup> It was then found that a platinum salt that was being produced in the electric field inhibited cell division but had no effects on cell growth.<sup>2</sup>

The platinum salt was identified to be  $[\text{PtCl}_6]^{2-}$ ; the media containing the platinum salt and free ammonia that had also been exposed to ultraviolet radiation actually produces three different ligand platinum compounds, all with different effects on the bacteria.<sup>2</sup> Ammonium hexachloroplatinate(II) was one of the compounds that acted as a disinfectant and killed the bacteria.<sup>2</sup> The second compound was  $[\text{PtCl}_5\text{NH}_3]^-$  and seemed to have no effect on the bacteria.<sup>2</sup> The last platinum compound formed was  $[\text{PtCl}_4(\text{NH}_3)_2]$  and was the most stable species; this compound seemed to have an inhibition on cell division, but did not affect the cell growth.<sup>2</sup> It was then decided that platinum salts should be studied to determine its biological effects.

When it became obvious to Dr. Rosenberg's lab that platinum metal has more than one oxidation state and there were different configurations being produced, *cis* and *trans*, four compounds were studied in mammalian cells. Success was achieved with the  $[\text{PtCl}_4(\text{NH}_3)_2]$  in the +2 oxidation state and with a *cis* configuration. The thought was that the platinum compounds would inhibit cell growth in tumor cells but have no effect on the host animal. There were four different platinum compounds that were tested in mice, shown below in Figure 1.2.

Figure 1.2)



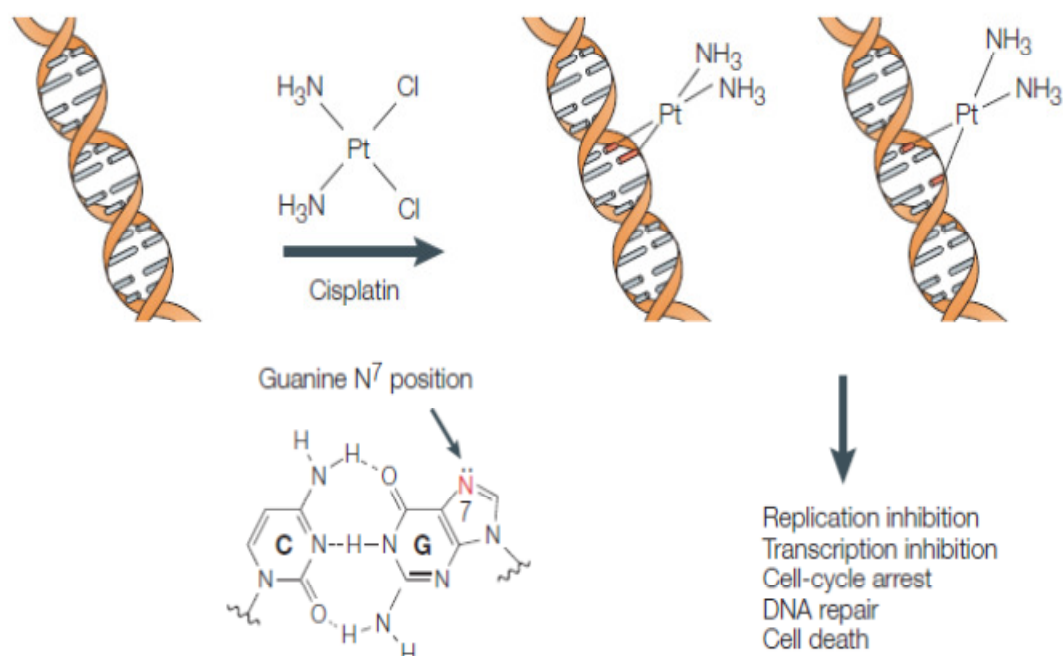
Structures of Platinum Compounds: (1)  $cis\text{-Pt(IV)(NH}_3)_2\text{Cl}_4$ ; (2)  $cis\text{-Pt(II)(NH}_3)_2\text{Cl}_2$ ; (3)  $\text{Pt(II)(NH}_2\text{CH}_2\text{CH}_2\text{NH}_2)\text{Cl}_2$ ; and (4)  $\text{Pt(IV)(NH}_2\text{CH}_2\text{CH}_2\text{NH}_2)\text{Cl}_4$ .

The compounds that showed the most successful anti-cancer activity were compounds 2 and 4.<sup>5</sup> They were tested against Sarcoma-180 tumors in mice, and the tumors actually had shrunk in size due to the platinum compounds.<sup>5</sup> The mice survived the treatment, and after 6 months of being treated they still showed no signs of cancer.<sup>5</sup> Based on these results, this was when compound two entered clinical trials in humans and showed promising results.

There are a few different mechanisms proposed for the anti-cancer activity of cisplatin, but the one that is most widely accepted proposes the drug reacts with DNA and causes apoptosis.<sup>5</sup> When cisplatin enters the blood stream via intravenous exposure; the chloride ligands remain attached due to the high concentration of chloride in blood plasma.<sup>5</sup> Once inside the cell the chloride ligands are replaced with water due to the concentration difference; the chloride concentration inside the cell is only 4 mM as opposed to 10 mM in blood plasma.<sup>5</sup> Replacement of the chlorides creates a positively

charged compound that is now in its active form, and this form cannot readily leave the cell. From here the platinum compound enters the cell nucleus and reacts with DNA; usually two adjacent guanine residues on the double helix.<sup>5</sup> Cisplatin can also bind to adjacent adenine-guanine residues. Binding to adjacent residues causes the double helix to form a kink and become bent, which allows for the DNA binding protein to attach and either repair the DNA or initiate cell death.<sup>5</sup> When the platinum compound attaches to DNA via guanine residues, specifically at the N7 position of the guanine residue, it typically forms a 1, 2 G-G intra-strand crosslink.<sup>5</sup> Other possible products are formed including, 1, 2 A-G, and 1, 3 G-G intra-strand crosslinks, 1,2 and 1,3 G-G inter-strand crosslinks, and DNA-protein crosslinks.<sup>6</sup> Below is a depiction showing the mechanism of binding with cisplatin and DNA.

Figure 1.3)

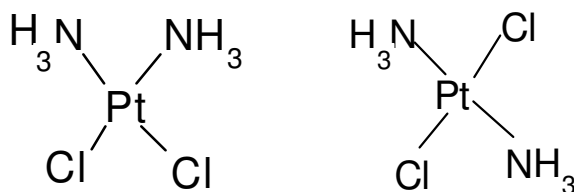


Mechanism of Action for Cisplatin: cisplatin reacting with the double helix and forming the various crosslinks causing apoptosis.<sup>3</sup>

## B. Chemistry of Platinum Compounds

Around the early 1900's cisplatin's geometry became the debate and topic for Alfred Werner's theory of coordination chemistry.<sup>5</sup> Werner's theory showed two isomers that were formed, a *cis* and a *trans*, hence the name *cisplatin* and *transplatin*.<sup>5</sup> He also suggested the square planar configuration correctly, and won the Nobel Prize for Chemistry in 1913.<sup>5</sup>

Figure 1.4)



Structure of *Cisplatin* and *Transplatin*.

Years later, scientists had studied how the structure contributed to the compounds anticancer activity. Cleare and Hoeschele, developed the “structure-activity relationships,” and suggested what should be structurally required of a compound, as listed below:<sup>5</sup>

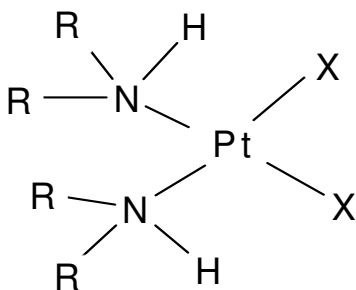
- The compound should contain two amine groups in a *cis* position, because the *trans* geometry is inactive.
- It should contain two leaving groups, also with a *cis* geometry relative to each other.
- The ability of the leaving groups to be lost, contribute to the toxicity and activity of the compound. It's suggested that the leaving groups be easily removed.
- The compound should also not have an overall charge.



- The greater the activity of the compound, the fewer the alkyl substituents on the amine ligands, and each ligand should contain at least one proton.

The general structure for these compounds should have a similar configuration to the structure in Figure 1.5:

Figure 1.5)



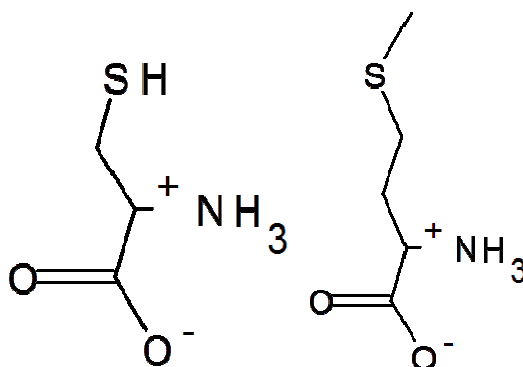
General Structure for Platinum(II) Compounds: X=leaving groups (two chloro groups or a bidentate malonate), and ligand R=H or an alkyl substituent.<sup>5</sup>

Any new platinum-containing anti-cancer drug to be considered for clinical trials must possess one of the following characteristics: to be delivered as an oral medication, generate reduced toxic side effects, and/or have activity against cancers with intrinsic or acquired resistance to cisplatin treatment.<sup>5</sup> Any type of platinum compound that has deviated from the general structure will change the reactivity of the compound and how it reacts with DNA. For example steric effects such as bulky amine ligands slow the reaction with DNA.<sup>7</sup> A bulky amine ligand on a square planar platinum complex reduces the rate of the reaction with DNA and also slows down the binding to thiol containing proteins, which potentially reduces side effects and toxicity.<sup>7</sup>

Since the drug must travel many places in the body before it ultimately reaches cellular DNA, there are many other prospective biomolecules with which the compound has the ability to react. Platinum compounds have a high affinity for sulfur-containing

compounds, which is explained by the HSAB (Hard Soft/Acid Base) theory. The HSAB theory states that “soft acids” tend to favor “soft base,” and likewise with hard acids and bases; therefore, the “soft” sulfur atom will donate a ligand to the “soft” platinum compound. Potential targets that contain the sulfur atom are proteins that contain the amino acids methionine (Met) and cysteine (Cys). In theory, these reactions of platinum and the amino acids would be thermodynamically and kinetically favored, because of basic coordination chemistry knowledge.

Figure 1.6)



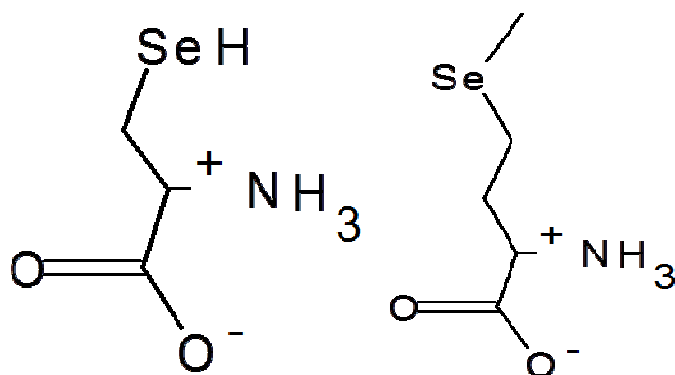
Structure of Cysteine and Methionine

The HSAB theory helps explain why platinum(II) molecules react with certain amino acids and proteins. The theory was developed in the 1960's by Ralph Pearson, and also known as the donor-acceptor theory.<sup>9</sup> Pearson categorizes acids and bases upon their ability to polarize, their size and valence electrons. Lewis acids and bases are easily polarizable, larger in size, and typically have a lower charge (+1, +2).<sup>10</sup> All Lewis soft acids are electron acceptors, and soft bases are electron donors.<sup>10</sup> A few examples of soft acids include Cu<sup>+</sup>, Au<sup>+</sup>, Ag<sup>+</sup>, Hg<sup>2+</sup>, and Pb<sup>2+</sup>; and for soft bases this includes, S<sup>2-</sup>, I<sup>-</sup>,

$\text{Br}^-$ , and  $\text{RSe}^-$ .<sup>10</sup> Hard acids and bases are typically not polarizable, smaller in size and have a higher charge ( $\geq +3$ ).<sup>10</sup> Examples of hard acids include the *s*, and *f* blocks of the periodic table,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$ .<sup>10</sup> Some examples of hard bases include specific compounds that include oxygen and fluorine, such as  $\text{O}^{2-}$ ,  $\text{F}^-$ ,  $\text{OH}_2$ ,  $\text{CO}_3^{2-}$ , and  $\text{PO}_4^{3-}$ .<sup>10</sup> Therefore, when reacting a soft acid, it will prefer a soft base; likewise hard acids are preferential to hard bases.

The donor-acceptor theory has been useful in helping researchers understand why metal ions in the body typically bind with some types of sulfur containing proteins. Cisplatin and other platinum(II) containing anti-cancer drugs react with proteins containing methionine and/or cysteine. The platinum anti-cancer drug is a soft acid, and the sulfur biomolecules are soft bases. The sulfur will donate its electrons to the platinum, which is the electron acceptor. There are other biomolecules that contain selenium in place of sulfur, selenomethionine (SeMet) and selenocysteine (SeCys), and donate electrons to platinum anti-cancer drugs.

Figure 1.7)



Structure of Selenocysteine and Selenomethionine

### C. Reactions of Platinum Anti-Cancer Agents with Biomolecules

After the platinum(II) drug is given intravenously to the patient, the drug enters the blood stream where it enters the cell, passes into the nucleus of the cell and reacts with DNA. Before the medication enters the cell, there are many proteins available to react with the drug, and the reaction with DNA is thought to lead to cytotoxicity. A question researchers have been debating is why does platinum react with sulfur residues, but ultimately ends up reacting with DNA. One thought to the question is that the reaction of these platinum drugs with the amino acids cause toxicity and resistance.<sup>7</sup> However, it should be noted that Pt-S bonding also has positive effects in the body; examples include preventing side effects, as well as using the Pt-S bond as optimal transport for the anti-cancer medication throughout the body.<sup>7</sup> Platinum drugs can react with thiol residues of cysteine-containing proteins, or the thioether residues of methionine-containing complexes.<sup>8</sup>

Thioether residues are responsible for serving as a drug reservoir for platination at DNA inside the cell. This is due to the concentration difference of the residues inside the cell which are much higher than outside the cell.<sup>8</sup> As a drug reservoir this gives two new possible reaction pathways for DNA platination; either the instantaneous release of the platinum from sulfur then the reaction with DNA occurs, or the guanine N-7 group on DNA directly displaces the platinum from the sulfur.<sup>7</sup> Thioether reactions serve as intermediates and are kinetically favored.<sup>8</sup> However, the thioether bond can be broken by a thiol residue, the resulting bond is thermodynamically stable. Yet the bond is not easily broken, and contributes to toxicity and resistance of the anticancer medication.<sup>8</sup>

When the platinum binds to thiol containing groups, the Pt-S (thiol) bond contributes to the occurrence of toxicities with platinum(II) complexes as anticancer agents.<sup>8</sup> However, the Pt-S bond can be broken with the use of “rescue agents”, which are strong nucleophiles also containing sulfur.<sup>8</sup> “Rescue agents” or “protecting agents” are sulfur containing compounds, administered with the Pt(II) anticancer medication. A few of these rescue agents are sodium thiosulfate (STS), sodium diethyldithiocarbamate (Naddtc), thiourea, glutathione (GSH), cysteine (Cys), methionine (Met), N-acetylcysteine, biotin and sulfathiazole.<sup>7</sup> These compounds either prevent or terminate the Pt-S adducts in proteins and do not lower the anticancer activity of the Pt(II) complex.<sup>7</sup> More research has been ongoing to determine the effects of these rescue agents and their potential uses. Positive uses of these rescue agents include preventing side effects as well as optimal transport.<sup>7</sup> For example, it was known that GSH is present in the cell has many functions, including detoxification of chemotherapeutic agents. A clinical study with glutathione and cisplatin reported significantly reduced toxicity in ovarian cancer cases with cisplatin as treatment.<sup>8</sup>

Other types of bonds can be formed with platinum(II) anticancer compounds, such as selenium containing amino acids. Glutathione peroxidase and thioredoxin reductase (TrxR) are two enzymes that contain selenocysteine, and have been shown to react with platinum anticancer medications.<sup>11</sup> Thioredoxin reductase contains a selenocysteine residue in its active site which is an easy target for platinum anticancer medications.<sup>13</sup> This enzyme has been shown to be inhibited due to reaction of platinum with selenocysteine; the uninhibited enzyme plays important roles in maintaining redox systems in the body.<sup>13</sup> When the enzyme is inhibited, an increase of oxidized thioredoxin

(Trx) occurs, creating cellular conditions for stimulation of apoptosis.<sup>13</sup> Selenocysteine is deliberately added to proteins, as opposed to selenomethionine which is arbitrarily inserted into proteins in place of methionine.<sup>11</sup> Selenocysteine has a low pK<sub>a</sub> value and is a strong nucleophile, which makes it difficult for the amino acid to exist freely in cells, but is highly reactive when incorporated into proteins.<sup>14</sup> Selenium containing compounds have been studied for their antioxidant effects as protecting agents for toxicity and resistance.<sup>11</sup> Selenomethionine and S-methylselenocysteine showed increased survival rates in mice when given a toxic dose of cisplatin and oxaliplatin.<sup>11</sup>

Disulfide bonds of oxidized glutathione (GSSG) have been studied for their participation in the redox systems in the body and are known to form during protein folding.<sup>16</sup> Cysteine has the ability to partake in acid/base chemistry to form the disulfide bonds, either as a catalytic redox component or to convey structural stability.<sup>16</sup> Selenogluthathione (GSeSeG) was found to be more stable than GSSG and the selenocysteine residues were capable of oxidizing cysteine residues in unfolded proteins, which helps regain protein conformational stability upon folding.<sup>16</sup>

Previous studies have shown that natural diselenide bonds have been created and in comparison to the disulfide bonds, diselenide bonds have a low redox potential.<sup>15</sup> A similar study showed that the diselenide bond in selenocystine was studied for its reactivity with platinum anticancer drugs. The results concluded that the diselenide bond could be split via the platinum structures, however, this meant that somehow redox reactions and/or reducing agents were used to complete this.<sup>14</sup> One structure used to determine the ability to cleave the diselenide bond was [Pt(Met)Cl<sub>2</sub>]<sup>2+</sup>.<sup>14</sup> Results indicated that [GS-Sec]<sup>-</sup> and [GSSG]<sup>-</sup> were formed indicating that platinum drugs could

potentially interfere with the electron transfer process from reduced glutathione to glutathione peroxidase through the altering of the selenocystine residue therefore reducing the antioxidant ability of glutathione peroxidase.<sup>14</sup> These studies are important to determine more information regarding these new selenium containing amino acids and their antioxidant effects in regards to platinum anticancer medications.

Other studies have looked at how bulky amine ligands react with selenium containing amino acids, as well as competition studies of sulfur vs. selenium with the bulky platinum complexes. Our investigation of cisplatin analogs with SeMet and Met showed the displacement of amine ligands due to the *trans* effect of selenium and sulfur ligation.<sup>11</sup> In the same study when bidentate or tridentate ligands were used, the displacement of amine ligands was less prevalent.<sup>11</sup> One study showed that with the use of an ethylenediamine (en) ligand on a platinum complex, it reacts with SeMet and produces a bis-product of two selenomethionine residues joining the Pt(en) species forming  $[\text{Pt}(\text{en})(\text{SeMet-Se})_2]^{2+}$ .<sup>11</sup> Chelation eventually occurs and displaces the en ligand to form a bis-chelate product,  $([\text{Pt}(\text{SeMet-Se}, \text{N})_2]^{2+})$ , and the remaining nitrogen on SeMet residues coordinate to platinum forming a six-membered ring.<sup>11</sup>

The research that is presented in this thesis will report the effects of using different leaving group ligands on bulky platinum(II) complexes and their reaction with selenomethionine and selenocysteine variants. Particular amino acids that will be used are L-Methionine, Seleno-L-Methionine, Seleno-L-Cystine, and Se-(Methyl)selenocysteine. Our research will consist of the following platinum compounds: ethylenediamine platinum(II) (oxalate and dinitrate groups)  $([\text{Pt}(\text{en})(\text{ox})]^{2+})$  or  $[\text{Pt}(\text{en})(\text{NO}_3)_2]^{2+}$ . These studies will also lead us to react the previous made  $[\text{Pt}(\text{Met-}$

$S,N)Cl_2]^{2+}$  with selenomethionine to determine its reactivity. We will use NMR spectroscopy, LC/MS, and HPLC to characterize our results. An attempt to react the platinum compounds with the selenium-containing amino acids will be made at different pH ranges as well as with different stoichiometric variations.

## II. EXPERIMENTAL

### A Materials Used

The following items were purchased from Sigma Aldrich: Sodium Chloride (NaCl), Potassium Tetrachloroplatinate(II) ( $K_2PtCl_4$ ), Silver Nitrate ( $AgNO_3$ ), Dichloro(ethylenediamine)platinum(II) ( $(H_2NCH_2CH_2NH_2)PtCl_2$ ), Deuterium Oxide ( $D_2O$ ), and Se-(Methyl)selenocysteine Hydrochloride. From Acros the following were purchased: Anhydrous Oxalic Acid, L-Methionine, L-Selenocystine, and L-Selenomethionine.

### B NMR

A JEOL Eclipse 500MHz Nuclear Magnetic Resonance was used to characterize products using deuterium oxide as a solvent. When adjusting the pH of the different solutions made, 1.0% deuterated nitric acid and 1.0% sodium deuterioxide were used. Typically only a proton ( $^1H$ ) NMR scan was utilized; however, occasionally other scans were used including a HMQC (Heteronuclear Multiple Quantum Coherence), and a COSY (COrrrelation SpectroscopY).



## C LC/MS

An Agilent 1100 HPLC coupled with a MSD (SL) ion-trap electrospray ionization spectrometer system was used to acquire the mass spectrometry data for these experiments. The sample was introduced into the mass analyzer by the mobile phase solution of Methanol:Water (80:20) and injected at a flow rate of  $200\ \mu\text{L min}^{-1}$ . The voltage used for the electrospray needles was 3.5 kV, the capillary temperature was  $350\ ^\circ\text{C}$  and recorded in negative ion mode. The isotopic distribution pattern for each complex was simulated with the Isopro 3.0 program.

## D Synthesis of Platinum(II) Methionine Dichloride $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$

$[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  was made according to the method described by Norman et al.<sup>12</sup> A solution containing potassium tetrachloroplatinate(II) (10.5 mg,  $25.3\ \mu\text{mol}$ ), sodium chloride (4.5 mg,  $77\ \mu\text{mol}$ ), and L-Methionine (3.5 mg,  $23.3\ \mu\text{mol}$ ) were dissolved in 0.6 mL of deuterium oxide. The solution was mixed in an amber vial and heated in a water bath at 353 K for 10 minutes. This produced a clear yellow solution with a pH of 1.6. A 6 mM solution of  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  was made as described by Liu et al.<sup>13</sup> The solution was made by obtaining  $142\ \mu\text{L}$  of the  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  and diluted with  $858\ \mu\text{L}$  of deuterium oxide.

## E Synthesis of Platinum(II) Selenomethionine Dichloride $[\text{Pt}(\text{SeMet})\text{Cl}_2]^{2+}$

Two solutions of  $[\text{Pt}(\text{SeMet})\text{Cl}_2]^{2+}$  were similarly made to that of  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$ . The first solution was made using an amber vial and 10.5 mg of potassium tetrachloroplatinate, 4.9 mg of selenomethionine and 4.5 mg of sodium chloride in 0.6 mL of deuterium oxide. The solution was heated for ten minutes in a water bath at 353

K, and produced a yellow solution with a pH of 1.5. To yield a 6 mM solution, 142  $\mu\text{L}$  of the original sample was mixed with 858  $\mu\text{L}$  of deuterium oxide. The second solution was made the exact same way except there was no sodium chloride added, and the pH initially was 1.1, adjusting the pH to 9-10, using 1% sodium deuteroxide, the reactants finally dissolved in solution. Again, 142  $\mu\text{L}$   $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  was mixed with 858  $\mu\text{L}$  of deuterium oxide and adjusted the pH to 7.0 to obtain a final 6 mM solution.

#### F Preparation of Selenomethionine Solutions

For a 6 mM solution of selenomethionine, 2.4 mg of the amino acid was dissolved in 1.0 mL of deuterium oxide and pH was adjusted to 8. Typically for a 1:2 ratio of platinum:SeMet, 500  $\mu\text{L}$  of each solution was mixed together. For a 3 mM solution of selenomethionine, 0.6 g of the amino acid was dissolved in 1.0 mL of deuterium oxide. Usually the pH of these solutions would eventually be adjusted either to around 3-4 or 7-8, using 1.0% sodium deuteroxide and/or 1.0% deuterated nitric acid.

#### G Preparation of Methionine, Selenomethionine and Platinum Compound

This solution was made as two separate mixtures and then combining the mixtures together. For the first vial, 2.35 mg of selenomethionine was added to 1.8 mg of methionine in 1.0 mL of  $\text{D}_2\text{O}$  and stirred for around 2 hours. The second solution was made with 10.5 mg (25.3  $\mu\text{mol}$ ) of potassium tetrachloroplatinate(II), 4.5 mg of sodium chloride in 1.0 mL of  $\text{D}_2\text{O}$  and also stirred for about 2 hours. Both mixtures were then combined into one vial having an initial pH of 1.8, then adjusted to 6.5. After the pH was adjusted, the solution was left in an 80°C heat bath overnight. All solutions were mixed at an equimolar ratio of 25.3  $\mu\text{mol}$ .

#### H Preparation of [Pt(en)ox]<sup>2+</sup>

This solution was made with 50 mg of silver oxalate, 50 mg of dichloro(ethylenediamine)platinum(II) in 30 mL of water and stirred for a few hours to ensure the reaction complete. Next the solution was syringe filtered and placed on a rotovap and the ending product weight was 18.9 mg.

#### I Reactions with [Pt(en)ox]<sup>2+</sup> with Selenomethionine

For a solution containing excess selenomethionine by weight, 2.0 mg of [Pt(en)ox]<sup>2+</sup> was mixed in 1.0 mL of D<sub>2</sub>O, and in another vial 1.0 mg of selenomethionine was dissolved in 1.0 mL of D<sub>2</sub>O. Both solutions were allowed to stir separately and then a 500  $\mu$ L aliquot of [Pt(en)ox]<sup>2+</sup> was added to the selenomethionine solution and resulted in a pH of 4.6. Another sample with similar quantities of reagents was prepared except with this solution, the pH of both samples were raised to 7.0 before mixing and then after mixing samples together the pH was adjusted to 6.9.

A sample containing excess platinum was mixed using 4.0 mg of [Pt(en)ox]<sup>2+</sup> in 1.0 mL of D<sub>2</sub>O in one vial, and in a separate vial 1.0 mg of SeMet was mixed in 1.0 mL of D<sub>2</sub>O. Both solutions were allowed to stir separately overnight and then the next day both solutions were mixed together and allowed to stir for around 3 hours and resulted in a pH of 5.0.

### III. RESULTS

Initially this project began by replicating Liu et al. paper, by trying to reproduce the selenocystine cleavage that they had accomplished.<sup>14</sup> We were unable to do so, which lead us to reactions with selenomethionine. Having previously worked with selenomethionine, products are simpler to assign using <sup>1</sup>H NMR. Using various

platinum(II) compounds with ammine ligands, such as  $[\text{Pt}(\text{en})(\text{D}_2\text{O})_2]^{2+}$  (en=ethylenediamine) and  $[\text{Pt}(\text{Me}_4\text{en})(\text{D}_2\text{O})_2]^{2+}$ , (Me<sub>4</sub>en= N,N,N',N'-tetramethylethylenediamine) and having reacted with SeMet, typically the less bulky ammine ligand will displace from platinum allowing the formation of bischelate products.<sup>11</sup> For example, the ethylenediamine ligand does displace from platinum forming various products, while the Me<sub>4</sub>en ligand does not become displaced and allows for only the formation of monochelate products.<sup>11</sup> With these results and the results of Liu et al. paper, using selenomethionine and  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  were reacted at various ratios forming several products.

#### A Reactions with $[\text{Pt}(\text{Met})\text{Cl}_2]$ and Selenomethionine

To begin, using a method previously described by Norman et al., a 1:1 mixture of  $\text{K}_2\text{PtCl}_4$  was mixed with methionine to form  $[\text{Pt}(\text{Met-}S,N)\text{Cl}_2]$ . To confirm our results LC/MS was used and a mass of 413 was observed in negative ion mode, which is comparable to that obtained via Liu et al. paper. The following  $^1\text{H}$  NMR spectrum shows the two major isomers formed, following the two structures for both isomers:

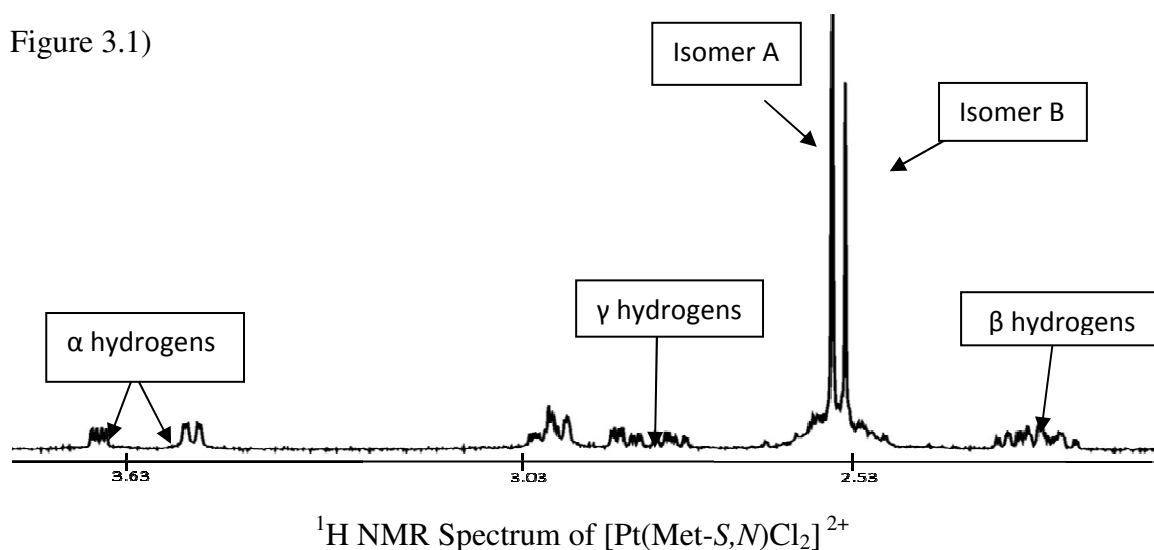
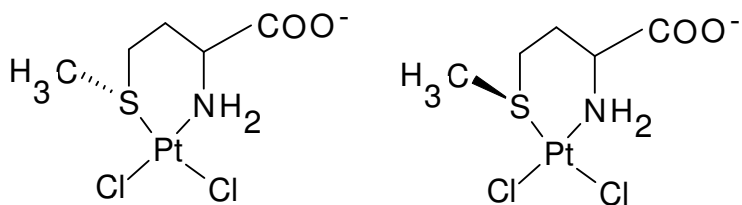
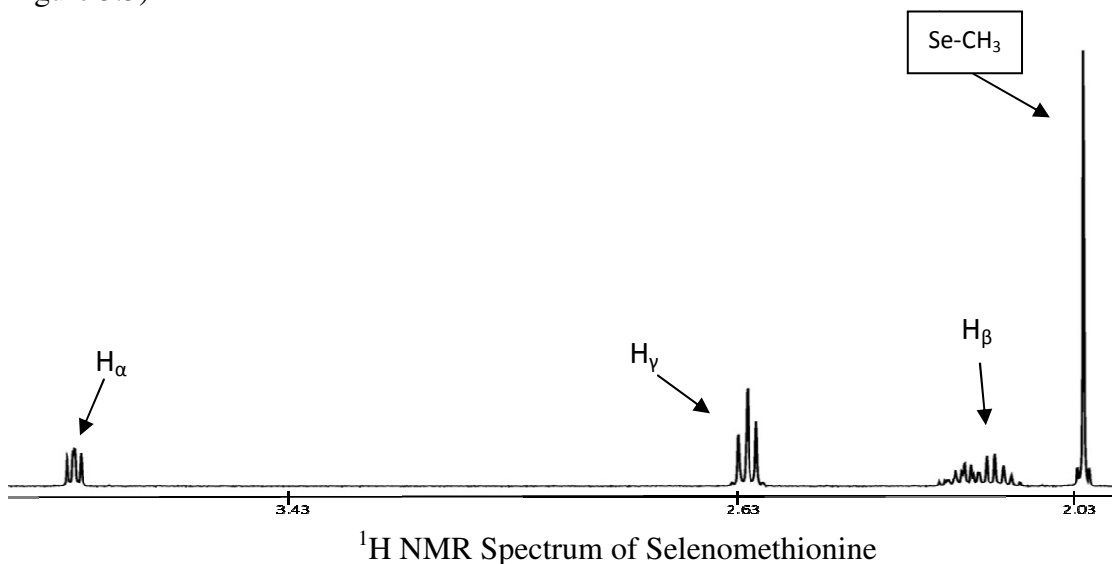


Figure 3.2)



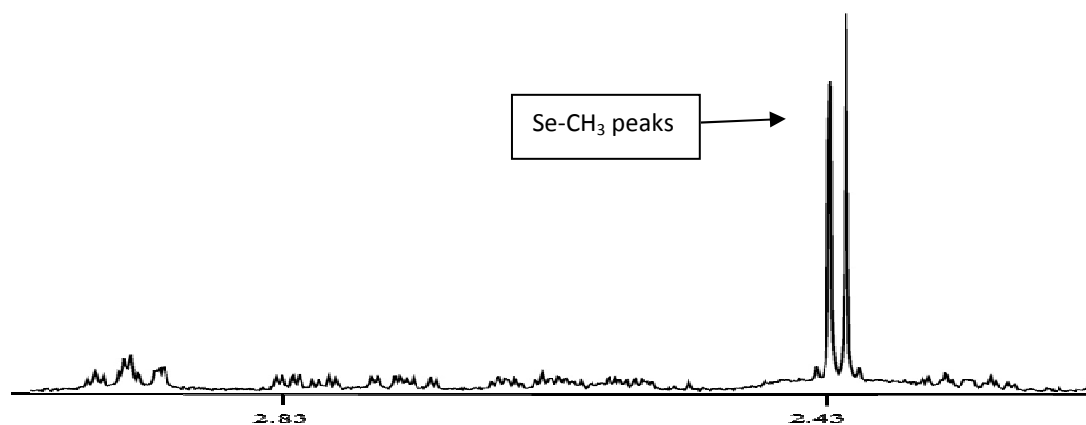
Structures of  $[\text{Pt}(\text{Met-S,N})\text{Cl}_2]^{2+}$  The left structure is isomer A (*R* configuration at S, envelope ring conformation) and the right structure is isomer B (*S* configuration at S,

Figure 3.3)



To determine the possible products that could be potentially produced, solutions containing plausible products were made. This included making  $[\text{Pt}(\text{SeMet})\text{Cl}_2]^{2+}$  products, adapting Norman et al. method of making a 1:1 solution of  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$ . Mixing 10.5 mg of potassium tetrachloroplatinate(II), 4.9 mg of selenomethionine and 4.5 mg of sodium chloride in 0.6 mL of deuterium oxide in an amber vial and heating it in a heat bath at 80°C for ten minutes gave a yellow solution with a pH of 1.5. Using 142  $\mu\text{L}$  of said sample and mixing it with 848  $\mu\text{L}$  of deuterium oxide produced the following spectrum:

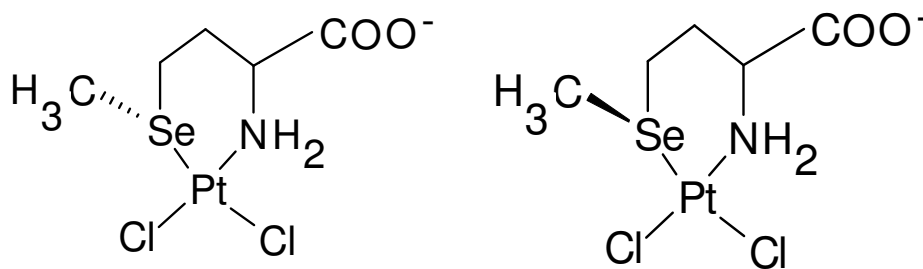
Figure 3.4)



$^1\text{H}$  NMR Spectrum of  $[\text{Pt}(\text{SeMet-}i{Se,N})\text{Cl}_2]^{2+}$

The monochelate products have two isomers possible, depending on which way the methyl group is pointing. This proton NMR spectrum clearly depicts two major isomers forming in solution. The figure below shows two possible structures for the SeMet monochelates:

Figure 3.5)



Structures of  $[\text{Pt}(\text{SeMet-}i{Se,N})\text{Cl}_2]^{2+}$

To further characterize products, an attempt to make the selenomethionine bischelate was made similarly to Norman et al. Using 27.0 mg of potassium tetrachloroplatinate, 26.1 mg of selenomethionine (133  $\mu\text{M}$ ), and 6.0 mg of sodium

chloride was mixed with 0.6 mL of D<sub>2</sub>O in a heat bath at 343 K for two minutes and produced a clear yellow solution with a pH of 1.6. The pH was adjusted to 6.5 and the solution turned colorless, the following spectrum shows the products produced:

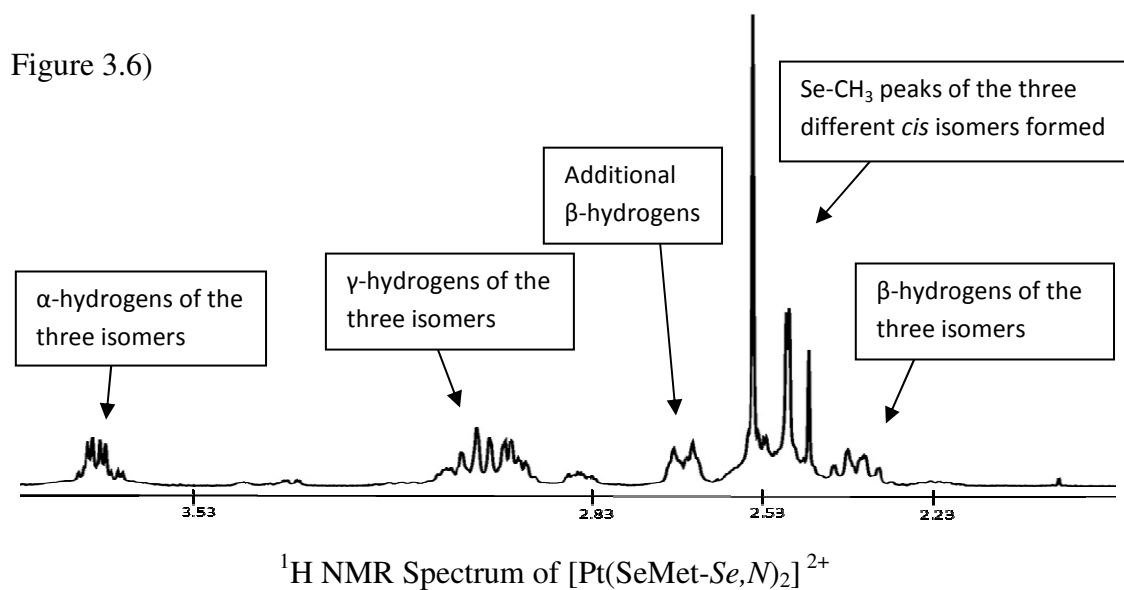
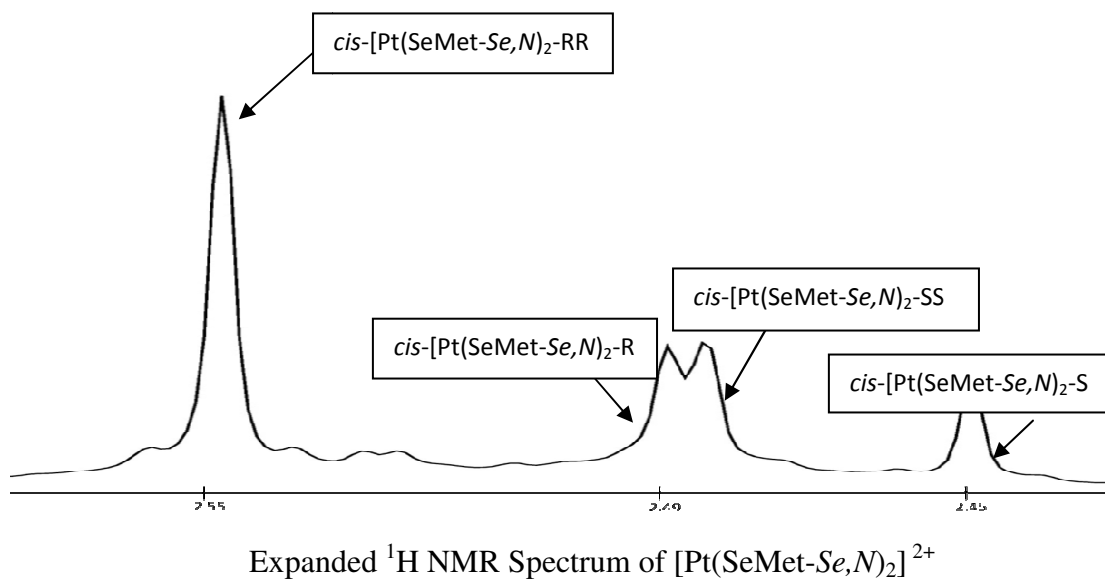


Figure 3.7)

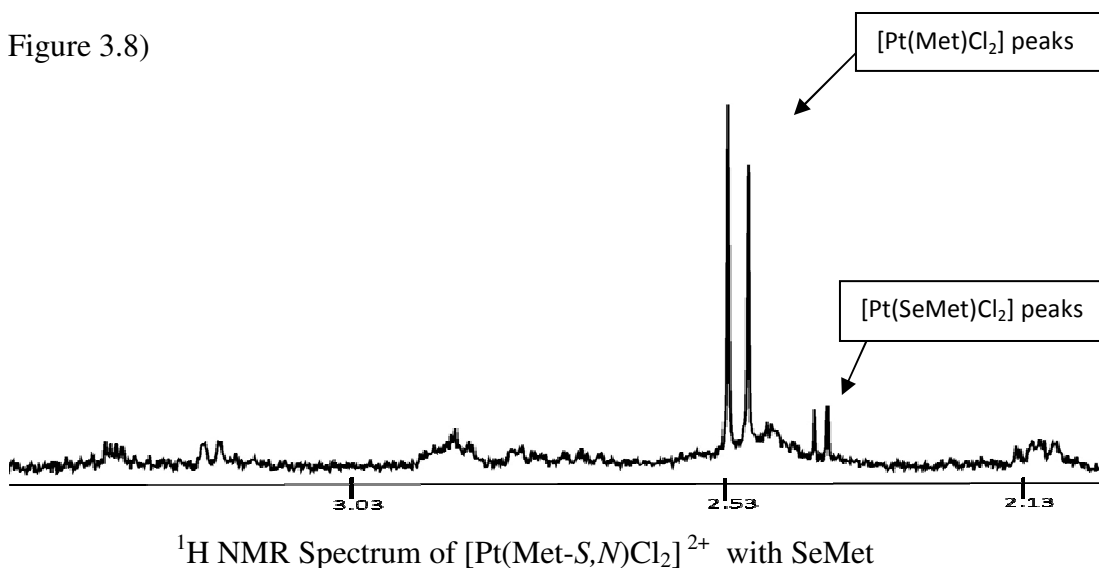


When assigning peaks to each of the isomers, we compared our spectra to those assigned previously.<sup>11</sup> It has been previously noted that SeMet chelates have a stronger  $\pi$ -accepting ability when compared to sulfur-containing chelates, and that a *cis* geometry is favored over a *trans* geometry.<sup>11</sup> Typically, *trans* S-containing chelates have much broader signals in <sup>1</sup>H NMR than Se-containing chelates. Also, using LC/MS a mass of 585 was recorded when using negative ion mode, which also corresponds to the bischelate product. Other masses recorded was 621 which corresponds to [Pt(SeMet-*Se,N*)(SeMet-*Se,Cl*)], and also a little less abundant was a mass of 460 which corresponds to the [Pt(SeMet-*Se,N*)(Cl<sub>2</sub>)], the monochelate.

In the original paper by Liu et al., to obtain the various products a 6 mM, [Pt(Met)Cl<sub>2</sub>]<sup>2+</sup> was reacted with 3 mM selenocystine. Our project used similar ratios, 6 mM [Pt(Met)Cl<sub>2</sub>]<sup>2+</sup> with 3 mM SeMet. First, a solution of the [Pt(Met)Cl<sub>2</sub>]<sup>2+</sup> was made and a separate solution of the SeMet was mixed, taking a 500  $\mu$ L aliquot of both solutions, they were mixed and left overnight in a heat bath of around 70°C. The initial pH of the solution after mixing was 2.4, after leaving it in the heat bath overnight, not much had changed of the proton NMR peaks, so the pH was raised to 6.3 and put back into the heat bath for another night. The reaction produced the following <sup>1</sup>H NMR spectrum:

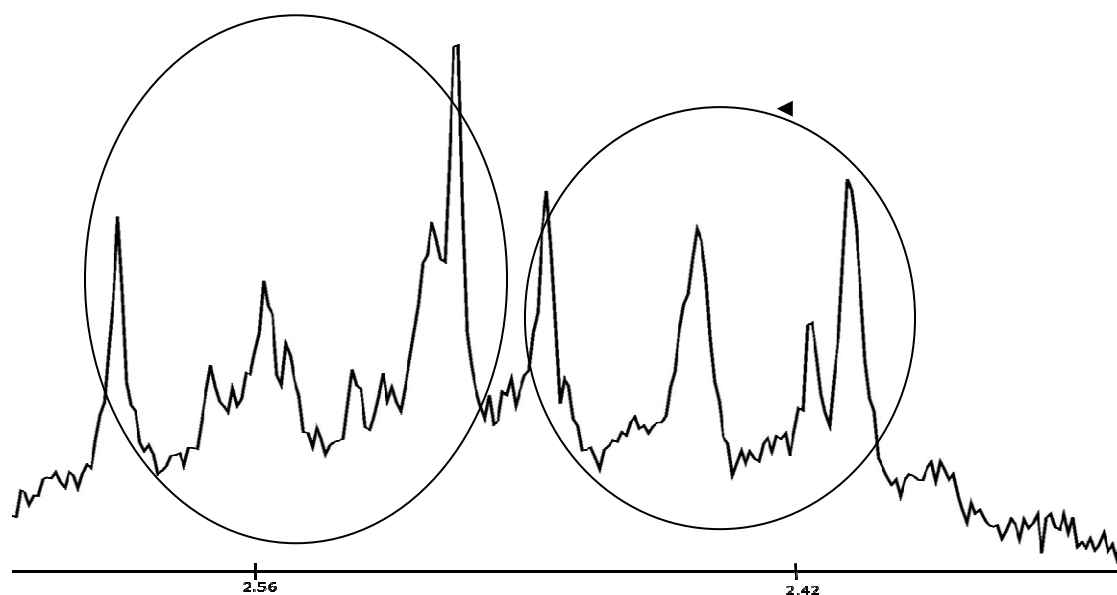


Figure 3.8)



When comparing  $[\text{Pt}(\text{Met-S,N})\text{Cl}_2]^{2+}$  S-CH<sub>3</sub> peaks to Se-CH<sub>3</sub>  $[\text{Pt}(\text{SeMet-Se,N})\text{Cl}_2]^{2+}$  peaks, selenium is typically upfield of sulfur peaks, and comparing to that of the Norman et al. paper we determined the two small peaks around 2.38 ppm were the selenomethionine monochelates. We expected the selenomethionine chelates to form and the methionine monochelate would be knocked off and free methionine would be visible; however, from our spectrum we do not see any free methionine. This leads us to believe that there may be free potassium tetrachloroplatinate(II) in our  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  solution, which would not show up in a proton NMR spectrum. Next, 0.3 mg of selenomethionine was added to the solution to see if the monochelate peaks would become more prominent. Allowing around 2 hours for the reaction to finish there was still free selenomethionine in solution. The reaction was heated to 80°C in a water bath overnight, and the pH had dropped the next day to around 2.8. It was noted that all of the selenomethionine had been used up and produced the following spectrum:

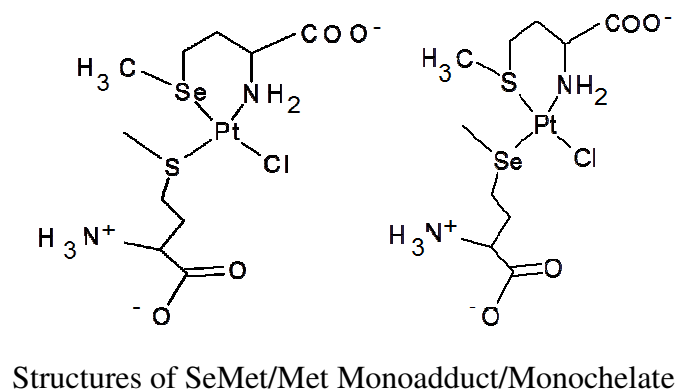
Figure 3.9)



$^1\text{H}$  NMR Spectrum after additional SeMet added

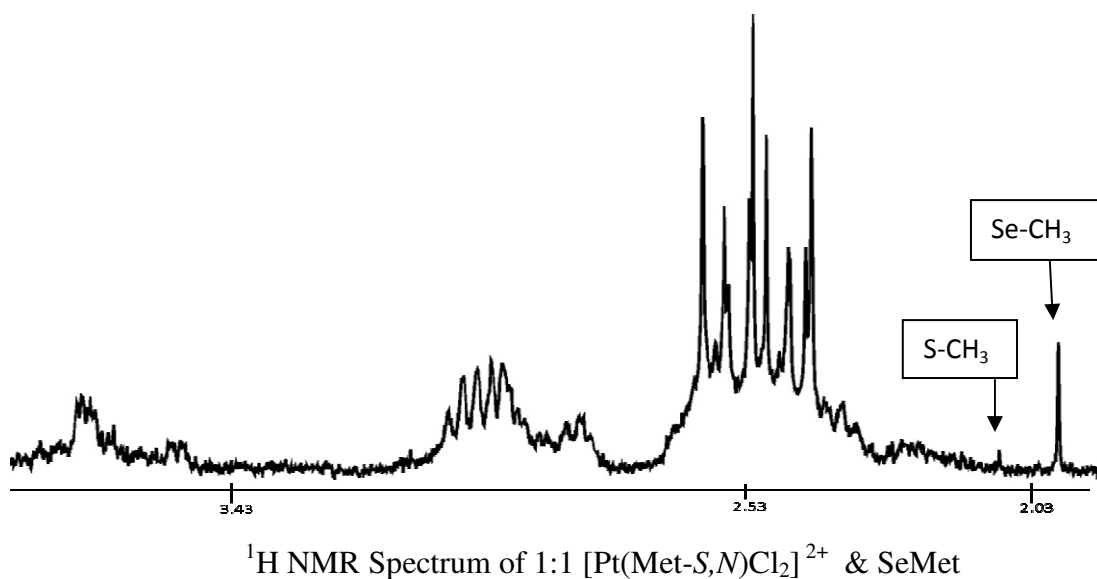
The pH of the solution had dropped to 2.8, and the previous peaks that were visible had now turned into two different sets of three peaks. The first black circle around the first three peaks refers to one product, while the second black circle around the other set of three peaks refers to another product. Typically, when these types of peaks are formed, this suggests the formation of both Met and SeMet attaching to the platinum to form bischelates as  $[\text{Pt}(\text{SeMet-Se},N)(\text{Met-S},N)]$ . After performing LC/MS on the sample a mass of 574 (M-1) was observed which corresponds to either SeMet or Met chelating while the other is only a monoadduct and a  $\text{Cl}^-$  attached to the platinum, thus forming something like  $[\text{Pt}(\text{SeMet-Se},N)(\text{Met-S})\text{Cl}]$  or  $[\text{Pt}(\text{Met-S},N)(\text{SeMet-Se})\text{Cl}]$ . The following figure depicts possible structures produced in the reaction:

Figure 3.10)



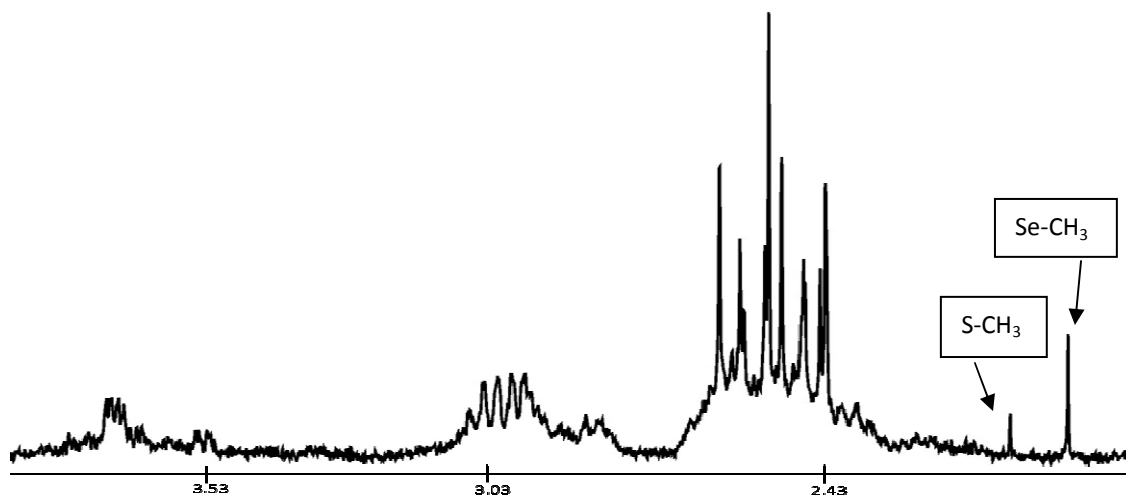
Subsequently, a reaction consisting of 6 mM  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  and 6 mM SeMet was mixed and the pH was adjusted up to 6.5 and produced the following spectrum:

Figure 3.11)



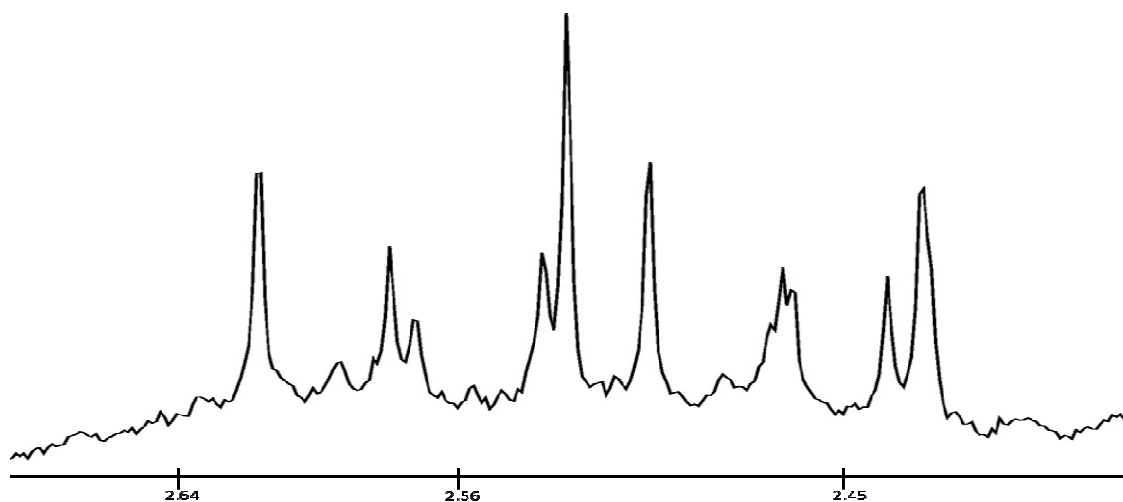
The reaction was placed in an 80°C water bath overnight and the pH had dropped to 6.1, spectrum obtained after two weeks is shown in the following figure:

Figure 3.12)



$^1\text{H}$  NMR Spectrum of 1:1  $[\text{Pt}(\text{Met-S,N})\text{Cl}_2]^{2+}$  & SeMet after 2 weeks

Figure 3.13)

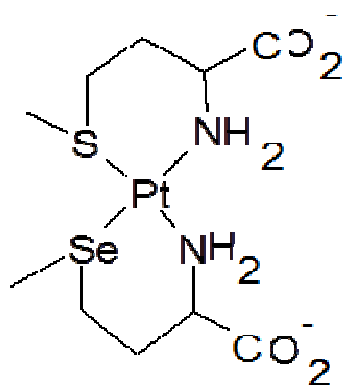


Expanded  $^1\text{H}$  NMR Spectrum of  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$  & SeMet

After 2 weeks of the reaction, it still seems there is a little free methionine available, which would insinuate that the selenomethionine is displacing the methionine, allowing selenomethionine to bind. Expanding the spectrum, two sets of three peaks are

very similar to each other and also comparable to what the previous reaction turns into after extra selenomethionine is added to the reaction. This again implies that both selenomethionine and methionine are binding to the platinum to form  $[\text{Pt}(\text{SeMet-Se,N})(\text{Met-S,N})]$ . After running LC/MS a mass of 538 (M-1) is observed which corresponds to both methionine and selenomethionine being chelated to the platinum. The following figure depicts the structure of the plausible products formed:

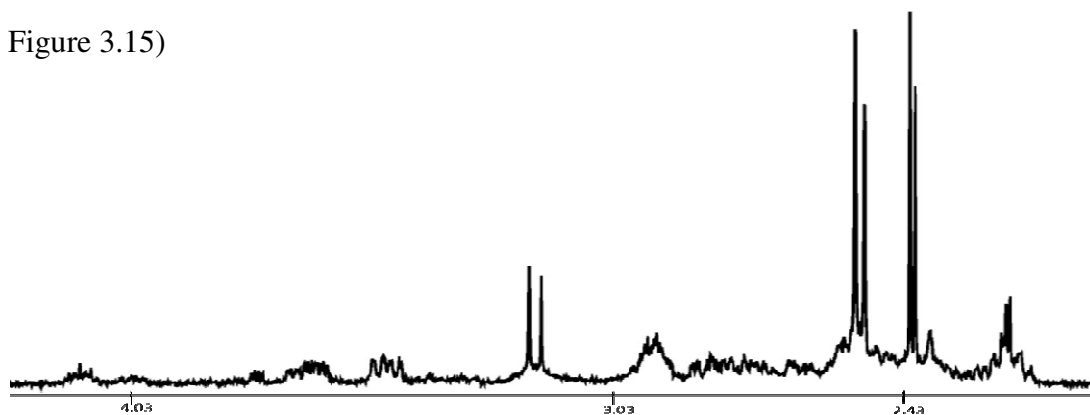
Figure 3.14)



Structure of  $[\text{Pt}(\text{SeMet-Se,N})(\text{Met-S,N})]^{2+}$

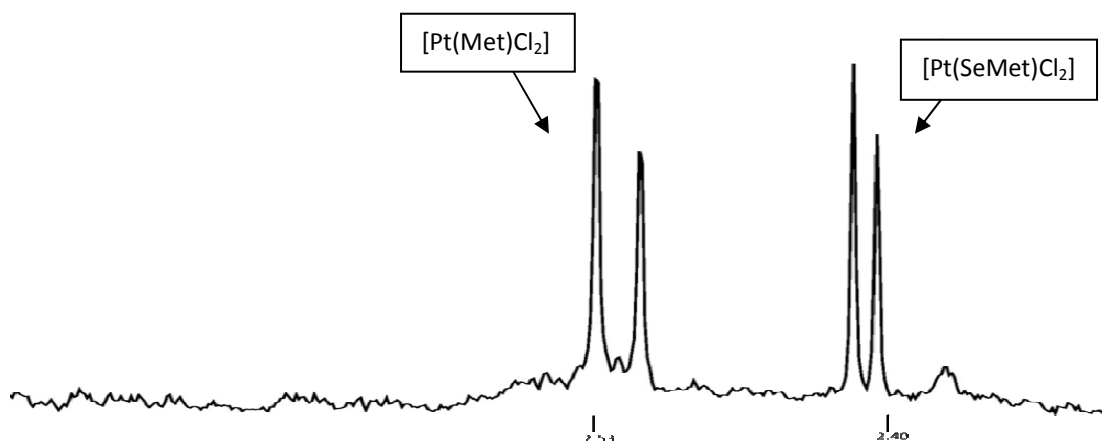
The succeeding result was formed by mixing SeMet and Met in one vial while  $\text{K}_2\text{PtCl}_4$  and NaCl were mixed in a separate vial, after said solutions were dissolved both vials were mixed all together. Potassium tetrachloroplatinate and sodium chloride were mixed as one 12 mM solution, a separate solution of 12 mM selenomethionine and 12 mM methionine were mixed. The two solutions were mixed together resulting in a pH of 1.8. The pH of the mixture was adjusted to 6.5 and put in an 80°C heat bath overnight. The next day the pH had dropped to 4.0 and produced the following  $^1\text{H}$  NMR spectra:

Figure 3.15)



$^1\text{H}$  NMR Spectrum of Competition Reaction with SeMet & Met

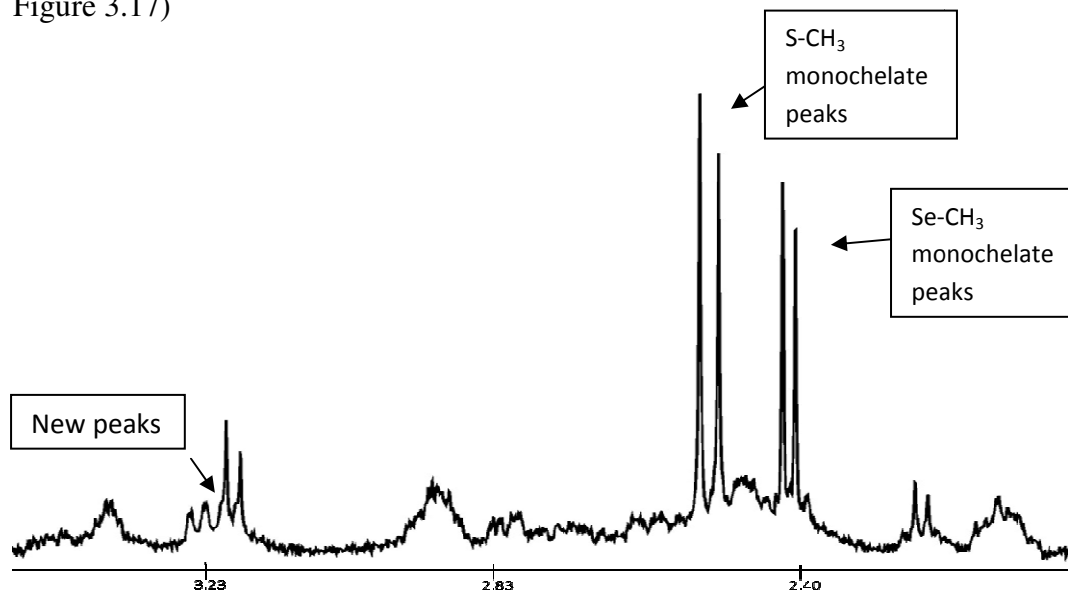
Figure 3.16)



Expanded  $^1\text{H}$  NMR Spectrum of Competition Reaction

The previous spectra show the monochelates formed by SeMet and Met giving  $[\text{Pt}(\text{SeMet})\text{Cl}_2]^{2+}$  and  $[\text{Pt}(\text{Met})\text{Cl}_2]^{2+}$ . To ensure that the reaction was complete, the mixture was put back into the  $80^\circ\text{C}$  heat bath again overnight, and the pH had not changed significantly. The reaction observed for the next week and the final spectrum of the reaction is shown in the following figure:

Figure 3.17)



$^1\text{H}$  NMR Spectrum of Competition Reaction after 1 week

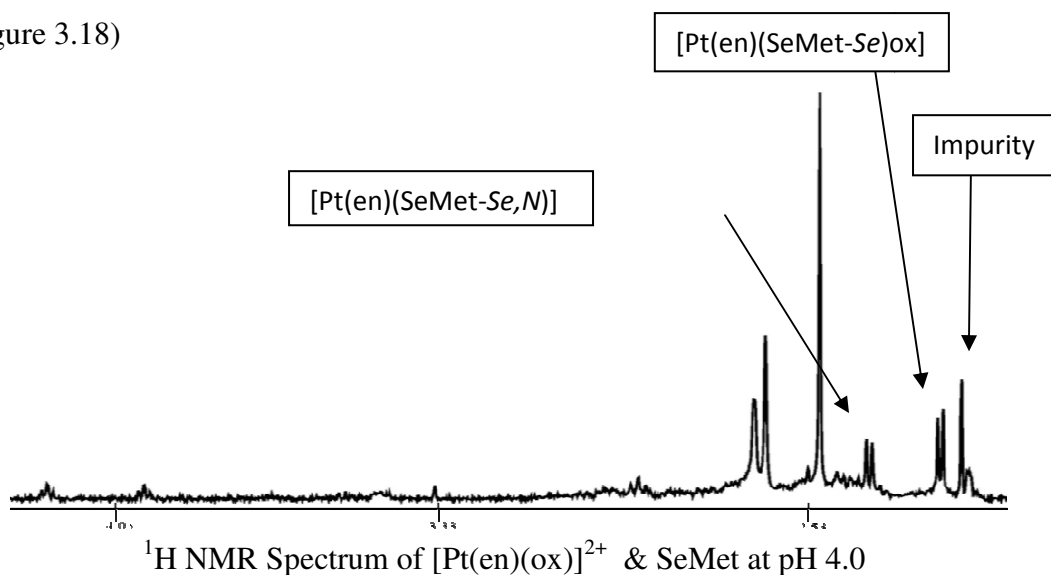
#### B Reactions with $[\text{Pt}(\text{en})\text{ox}]^{2+}$ and Selenomethionine

Oxaliplatin was a third generation platinum(II) anticancer medication approved that contains a DACH ligand (DACH=1,2-diaminocyclohexane) giving the compound a higher stability which leads to different reaction pathways than cisplatin and carboplatin.<sup>17</sup> According to Küng et al., the *S,N* chelate of methionine is produced with the DACH ligand still coordinated to the platinum, when the oxalate is the leaving group. When reacting 5'-GMP (GMP=guanosine monophosphate), with methionine and oxaliplatin, not only is the monochelate of methionine produced, but a species containing two monoadducts of GMP-N7 are also produced, but Met and GMP do not displace one another, which insinuates a competitive reaction for the platinum between the two.

An oxaliplatin analog containing an ethylenediamine ligand and an oxalate leaving group was reacted in our research with selenomethionine. These reactions took place at pH 4.0 and 7.0 with excess SeMet or excess platinum, and formed various products.

The first experiment began with excess platinum in solution relative to selenomethionine at a pH of around 4.0. This experiment produced the following  $^1\text{H}$  NMR spectrum:

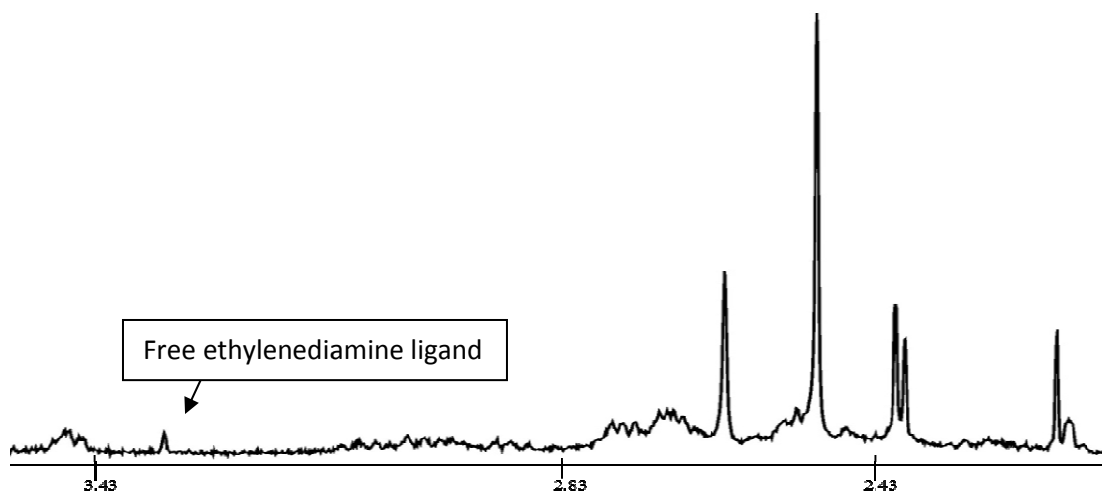
Figure 3.18)



Initially we see peaks corresponding to the monoadduct of selenomethionine and the oxalate ligand, as well as the Se,N monochelate with the en ligand still attached as well. There is also an impurity around 2.2 ppm from the deuterium oxide used. Five days later another spectrum was produced



Figure 3.19)

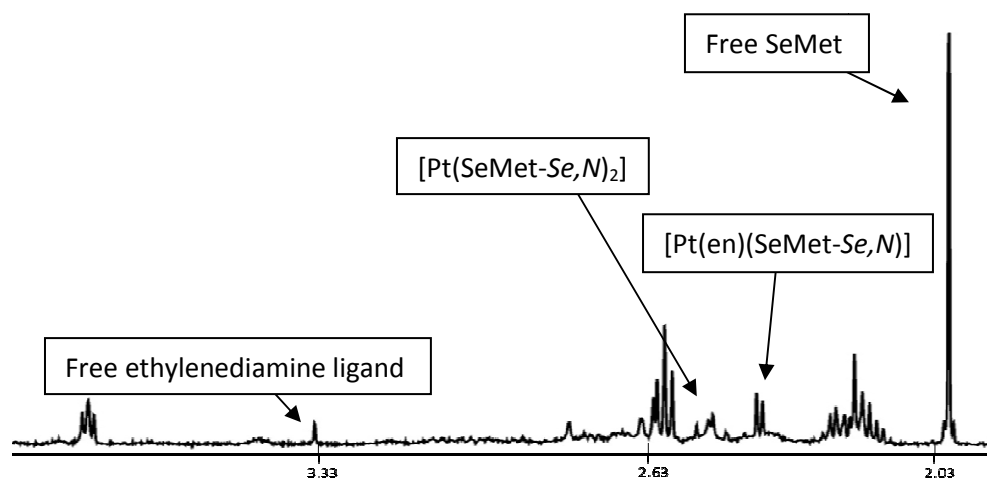


$^1\text{H}$  NMR Spectrum of  $[\text{Pt}(\text{en})(\text{ox})]^{2+}$  & SeMet after 5

After five days the monoadducts of SeMet have now turned into the monochelate forming  $[\text{Pt}(\text{en})(\text{SeMet-}Se,N)]^{2+}$ , as well as the remaining impurities. We can also note that around 3.3 ppm, a small peak has begun showing which corresponds to the free ethylenediamine ligand. This reaction had initially begun at a pH of 5.0 and after 5 days the pH had dropped to 3.9, due in part to the deprotonation of the amine nitrogen upon coordination to the platinum.

The next reaction consisted of excess SeMet and had an initial pH of 4.6 and produced the following spectra after four hours of the reaction being mixed:

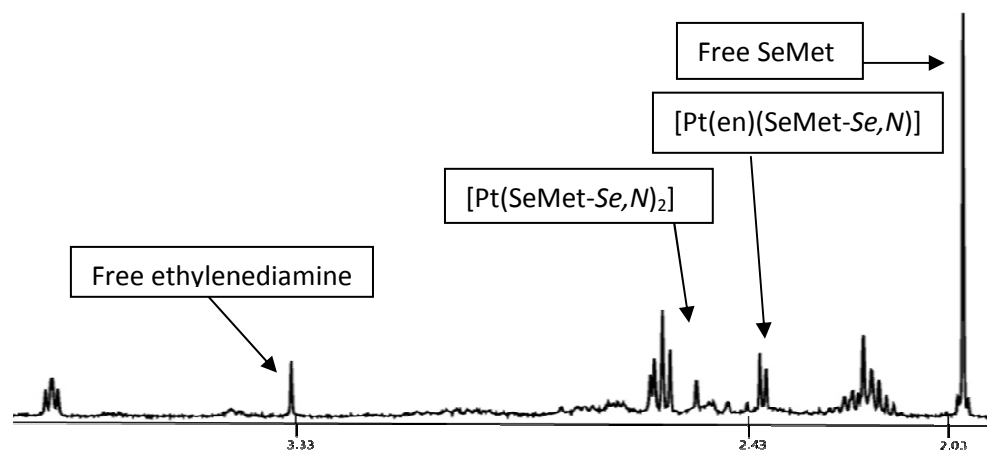
Figure 3.20)



$^1\text{H}$  NMR Spectrum of  $[\text{Pt}(\text{en})(\text{ox})]^{2+}$  with excess SeMet at pH 4.0

At pH 4.0 with excess selenomethionine, it is obvious that there is more free ethylenediamine produced initially here than with excess platinum at the same pH. We also can see a SeMet bischelate having been produced here as well. Another spectrum was produced after approximately 20 hours of the initial reaction been mixed shown in the following figure:

Figure 3.21)

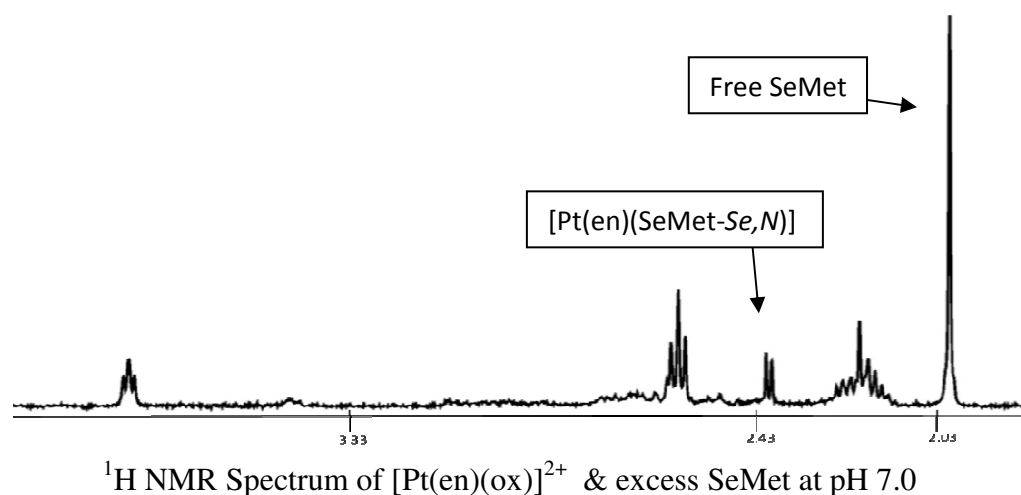


$^1\text{H}$  NMR Spectrum of  $[\text{Pt}(\text{en})(\text{ox})]^{2+}$  & excess SeMet at pH 4.0 after 20 hours

In the above NMR spectrum we see the peak for the free ethylenediamine has grown and there is still free selenomethionine. The SeMet monochelate is still being produced as well as the SeMet bischelate. We can attribute the additional free ethylenediamine to the pH difference when chelation occurs. When SeMet is chelating it loses a hydrogen atom causing the pH to drop.

The next experiment consisted of the same conditions as the prior experiment except the pH was adjusted to 7.0. For this reaction 2.0 mg of  $[\text{Pt}(\text{en})\text{ox}]^{2+}$  was mixed in 1.0 mL of  $\text{D}_2\text{O}$  and the pH was adjusted to 7.0, while in a separate vial 1.0 mg of SeMet was mixed in 1.0 mL of  $\text{D}_2\text{O}$  and the pH was also adjusted to 7.0. Both solutions were allowed to stir for 2 hours separately and then a 500  $\mu\text{L}$  aliquot of the platinum solution was mixed with the entire SeMet solution. Again the solution was allowed to stir for a few hours and the pH was again adjusted to 7.0 and produced the following NMR spectrum:

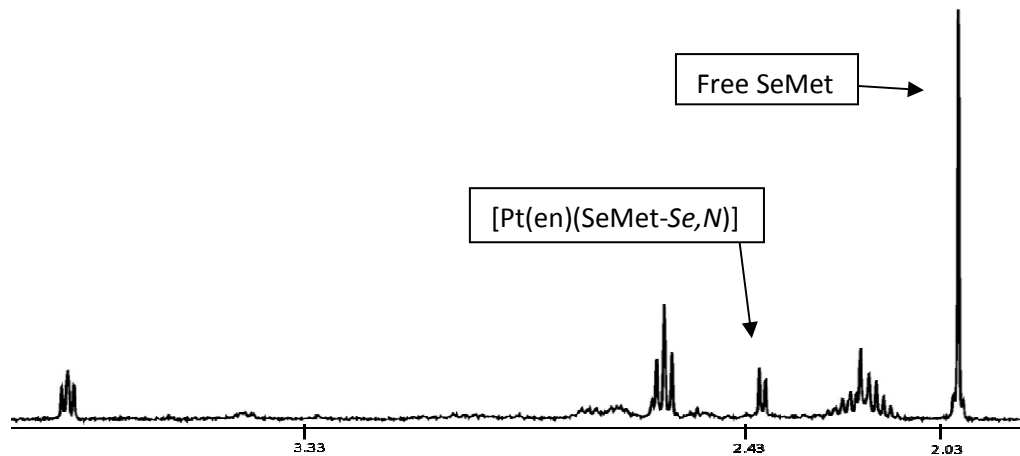
Figure 3.22)



At a higher pH there are quite a few differences in the spectrum when compared to that at a lower pH. For instance, there is no free ethylenediamine ligand available,

although the SeMet monochelate is still being produced. This again would make sense due to the SeMet favoring monochelate formation at higher pH. After 12 hours of reaction the spectrum still looks the same with no free ethylenediamine being produced and only the SeMet monochelate being produced as shown in the following figure:

Figure 3.23)



<sup>1</sup>H NMR Spectrum of [Pt(en)(ox)]<sup>2+</sup> & excess SeMet at pH 7.0 after 5 days

Even after only a few days the pH had only dropped from 6.9 to 5.3, which again insinuates that only the SeMet bischelate can be formed under these conditions at a low pH of around 4.0, due to the SeMet becoming deprotonated at a high pH.

#### IV. DISCUSSION

Confirmed metabolites of patients taking cisplatin include the methionine monochelate, so potentially selenomethionine could be a potential metabolite of patients since platinum kinetically prefers reactions with selenium rather than sulfur.<sup>12,11</sup> After injecting rats with cisplatin, Pt(II)-methionine complexes have been reportedly produced

and relatively quickly, and potentially selenomethionine complexes would be produced as well.<sup>12</sup>

This research was typically monitored via  $^1\text{H}$  NMR spectroscopy, and to confirm our results we used LC/MS. Once the results from the Liu et al. paper could not be replicated we determined to study selenomethionine, as products were much easier to assign and we had previously studied reactions with selenomethionine and platinum.

Using Norman et al. method, we were able to reproduce the methionine monochelate, and we used a similar strategy to form the selenomethionine monochelates as well. Reacting potassium tetrachloroplatinate(II) and selenomethionine with sodium chloride at a 1:1 ratio produces the selenomethionine monochelate. When reacting the same reagents but at a 1:2 ratio, there are three different isomers of the bischelate formed; *RR*, *RS*, and *SS*. In order to confirm our results we used LC/MS and obtained a mass of 585 which corresponded to the  $[\text{Pt}(\text{SeMet-Se},N)_2]^{2+}$ , and the monochelate produced a mass of 460 (M-1) giving the product  $[\text{Pt}(\text{SeMet-Se},N)\text{Cl}_2]^{2+}$ . When comparing the SeMet monochelate to that of Norman et al. Met monochelate, the ratio of isomers is relatively similar.<sup>12</sup> Also, when comparing the SeMet and Met bischelates, the most stable isomer formed is the *cis* product with both Met and SeMet bischelates, while only small traces of *trans* isomers were produced only in the Met bischelate.<sup>11,12</sup> This is of interest because a complex conveyed as the *trans* isomer of  $[\text{Pt}(\text{Met-S},N)_2]^{2+}$  was isolated in patients treated with cisplatin, but the *cis* isomer could be potentially explained due to selenium having an increased  $\pi$ -accepting ability over sulfur.<sup>11,12</sup>

When trying to react 6 mM  $[\text{Pt}(\text{Met-}i>S,N)\text{Cl}_2]^{2+}$  with 3 mM SeMet, various products are formed. When both monochelates of Met and SeMet were formed with this solution, this would suggest that there was free potassium tetrachloroplatinate available for the SeMet to react with thus forming the monochelate. In this reaction there was never any free methionine produced, which would also suggest that there is free platinum for the SeMet to react with. Free platinum from  $\text{K}_2\text{PtCl}_4$  would not show up in a proton NMR, and having previously checked the  $[\text{Pt}(\text{Met-}i>S,N)\text{Cl}_2]^{2+}$  sample, there wasn't any free methionine, so it was assumed that everything was done reacting. However, the results suggested otherwise by the formation of both monochelates.

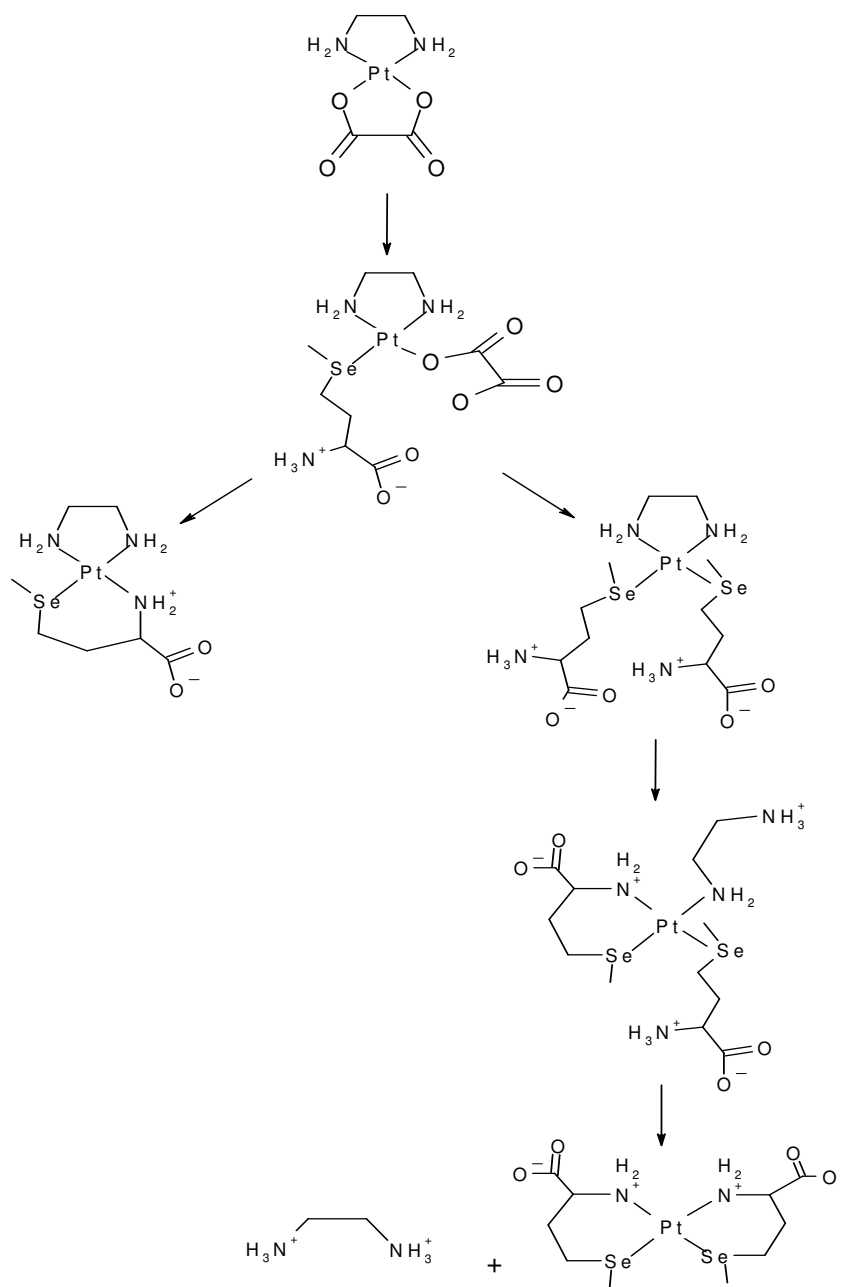
After adding additional SeMet to the solution, similar peaks were produced when compared to the reaction of 6 mM  $[\text{Pt}(\text{Met-}i>S,N)\text{Cl}_2]^{2+}$  and 6 mM SeMet. The equimolar solution produced various peaks, producing two sets of three peaks and the pairs of peaks were extremely similar to one another suggesting that some sort of species consisting of a methionine, selenomethionine bonding to a platinum atom being produced. The peaks from the 6 mM  $[\text{Pt}(\text{Met-}i>S,N)\text{Cl}_2]^{2+}$  and 3 mM SeMet with the extra addition of SeMet after a few days resulted in comparable peaks to that of the equimolar ratio solution which would again suggest a species containing both methionine and selenomethionine joining a platinum atom. Using LC/MS to help confirm our results after adding the additional selenomethionine a mass of 574 was produced which is consistent with a species consisting of both methionine and selenomethionine having been connected to one platinum atom, because either SeMet or Met is chelated to platinum while the other is only a monoadduct. With LC/MS one cannot determine specifically which is chelated and which is not. However, we tried to compare alpha hydrogens of both a methionine

monochelate and a selenomethionine monochelate, but the resolution from the NMR spectrum is not good enough to come to a definitive conclusion on which is chelated and not.

When both amino acids were mixed together in one vial, while the platinum and sodium chloride were mixed in a separate vial and later both vials mixed together, it was never thought that only monochelates would form. After looking at the NMR spectrum and noticing only monochelates developing, it was observed that one isomer was formed preferentially. The methionine monochelate in previous papers has already been determined that isomer A (Figure 3.2) is favored due to the methyl group that is pointed away from the rest of the structure creating less steric hindrance. When looking at the monochelates formed by selenomethionine, it could be determined that one isomer is also favored, and could be concluded that it is isomer B (Figure 3.2). This could also be concluded due to the methyl group having less steric hindrance when pointed away from the rest of the structure.

When reacting selenomethionine with  $[\text{Pt}(\text{en})\text{ox}]^{2+}$  various products are formed and some products are not formed at a higher pH. When using excess SeMet at a pH of 4.0 the SeMet bischelate is formed through a  $[\text{Pt}(\text{en})(\text{SeMet-Se})_2]^{2+}$  intermediate and then releases free ethylenediamine. This would mean that the second SeMet is competing with the amine group from the first bonded SeMet, and would insinuate that the amine is less protonated making the *Se* a better ligand rather than the amine ligand. A reaction scheme below shows the formation of the SeMet bischelate:

Figure 4.1)



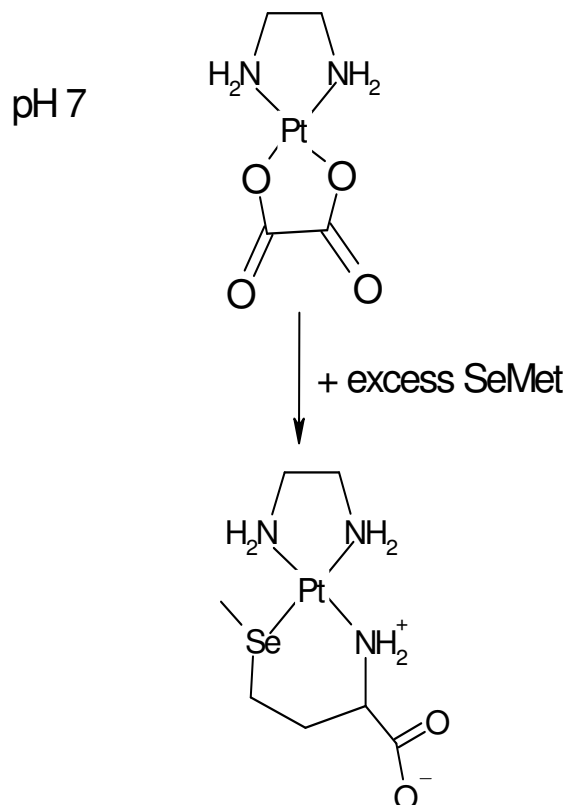
Reaction Scheme forming SeMet bischelate at pH 4.0

However, using the same conditions and only changing the pH up to 7.0, there is no free ethylenediamine produced and no SeMet bischelate formed, only the SeMet monochelate is produced. This would suggest again that at a higher pH the amine group



on the first bonded SeMet is more deprotonated making it a better ligand this time rather than a second *Se* of SeMet.

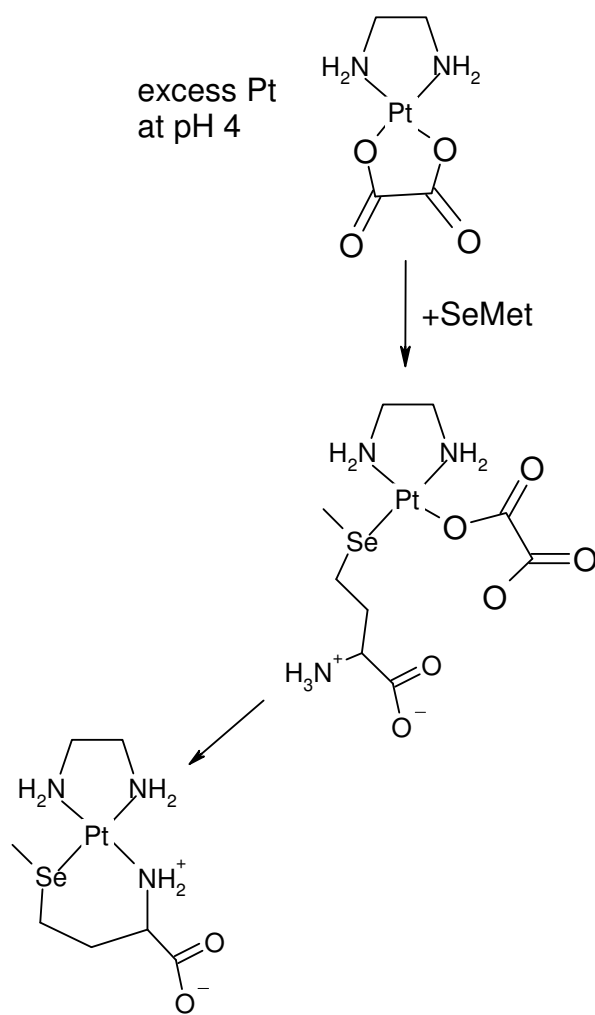
Figure 4.2)



Reaction Scheme forming SeMet monochelate at pH 7.0

Also, when using excess platinum but at a low pH, a small amount of ethylenediamine is available, but a SeMet monochelate is produced, and the SeMet monoadduct is formed as well. The following reaction scheme depicts the products formed at a pH 4.0 with excess platinum:

Figure 4.3)



Reaction Scheme forming SeMet monochelate at pH 4.0

Since the SeMet bischelate is formed at a low pH when using  $[\text{Pt}(\text{en})\text{ox}]^{2+}$ , this can be explained due to the SeMet becoming deprotonated when adding another SeMet to the platinum releasing a hydrogen in relatively acidic conditions and additionally dropping the pH even further allowing chelation to occur. At a pH of 4.0 this occurs relatively quickly, and doesn't occur at all in pH of 7.0. When using excess of platinum at a low pH the SeMet bischelate does not form and there is very little free

ethylenediamine ligand available when compared to using excess SeMet at pH 4.0.

While many papers have reported a  $[\text{Pt}(\text{dach})(\text{Met-}S,N)]^{2+}$  product, a bischelate using the ligand DACH has not been produced, and may could be due to the previous studies being done at a pH of around 7.0, while it seems that the bischelate can be produced at a lower pH instead of a higher pH.

## V. CONCLUSION

These metabolites are interesting as both selenomethionine and methionine are found in the body and once a patient takes a platinum(II) anticancer medication, these results could be similar to ones formed inside the body. The fact that a sulfur and selenium containing amino acid can bind to potentially the same platinum forming similar products at relatively similar rates is intriguing and what would happen with these products in the body are interesting.

These results show that a methionine and selenomethionine can bind to the same platinum molecule. The selenomethionine monochelate is also formed when reacting at a 1:1 ratio; the bischelate was not formed with any of these reactions when methionine and selenomethionine are both present in solution.

There is still further work to be done with these results such as confirming which amino acid is chelated versus which is not and which isomers are formed. Using these results and trying similar experiments using selenocystine or cysteine would be another plausible area to explore.

In concluding the results with the reactions of  $[\text{Pt}(\text{en})\text{ox}]^{2+}$  and SeMet a bischelate is formed at a lower pH and is not formed at a higher pH. This would suggest that at a higher pH the monochelate is only formed due to the amine nitrogen on the first

coordinating SeMet adduct being more deprotonated and making a better ligand rather than another SeMet being coordinated and then forming the bischelate. While at a lower pH it is just the opposite and suggests a competition for the amine group of the first coordinated SeMet adduct and the second SeMet-Se, making the Se of the second SeMet a better ligand than the amine group of the first SeMet.

Many of these results are of interest due to the known metabolites that have been produced in patients taking platinum(II) medications. In summary many new products have been synthesized and formed at different pH's which gives new insight into trying various platinum compounds at lower pH's to see if bischelates can be formed.

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