4-1-2010

The Reaction of a Water Soluble Platinum Compound with Methionine and Derivatives

Yueh Ying Liao
Western Kentucky University, carol.liao@wku.edu

THE REACTION OF A WATER SOLUBLE PLATINUM COMPOUND WITH METHIONINE AND DERIVATIVES

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
Yueh Ying Liao

May 2010

THE REACTION OF A WATER SOLUBLE PLATINUM COMPOUND WITH METHIONINE AND DERIVATIVES

Date Recommended 4/23/2010

Kevin Williams
Director of Thesis

Cathleen J. Webb

Lester Pesterfield

Dean, Graduate Studies and Research Date

ACKNOWLEDGEMENTS

I would like first to thank my research advisor, Dr. Kevin Williams, for his research guidance, knowledge and patience assisting me on the research. He has given me knowledge and information about the pharmaceutical field, inspired the way to research and has given me the motivation to strive for anti cancer drug research. I appreciated all his input, suggestions and corrections of my thesis to be more influential, novel and fluent.

I would like to express appreciation and thanks to Dr. Cathleen J. Webb for correcting my thesis and being there for me when I needed her. Dr. Webb always encourages me to not give up on my research, always makes time to correct my thesis when she is busy, and has a friendly face and ear to listen to my ideas about my thesis. I would like to thank Dr. Lester Pesterfield who gave me the knowledge for inorganic chemistry, and taught me how to operate the NMR and how to handle the problems.

I would like to thank the international office for helping me stay in the United States to finish my research. I would like to thank the Lord for supporting me with strength and courage throughout my life. My family has been my financial support and instrumental in my personal growth. My friends continue to support me and give me a lot of ideas about being a wonderful graduate student. The Chemistry Department and the entire faculty at WKU gave me the opportunity to enjoy my Western study life.

Finally, I dedicate my thesis to my parents for their patience through my education, and who supported me during any challenges at Western Kentucky University.

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THE REACTION OF A WATER SOLUBLE PLATINUM COMPOUND WITH METHIONINE AND DERIVATIVE

Yueh Ying Liao May 2010 40 Pages

Directed by: Dr. Kevin Williams

Department of Chemistry Western Kentucky University

Water soluble platinum complexes are a recent area of emphasis of cisplatin chemistry. The water soluble complexes could have a reduced toxicity compared with cisplatin. Oxaliplatin, which has an oxalate leaving group, has previously been shown to have less nephro-toxicity and higher water solubility than cisplatin. [Pt(en)(oxalate)] (en = ethylenediamine) has been prepared from $Pt(en)Cl_2$ and silver oxalate. This complex has been reacted with methionine and N-acetylmethionine at different molar ratios. At high Pt: methionine ratios, chelates with the sulfur and nitrogen atoms of the methionine are dominant; at lower Pt: methionine ratios, a bis-methionine product is formed. The en ligand is displaced by methionine but not N-acetylmethionine.

I. INTRODUCTION

1. History:

Cisplatin, [*cis*-diamminedichloroplatinum(II)] (CAS 15663-27-1), is widely used in the chemotherapy of solid tumors, testicular cancer, ovarian cancer, and lung cancer as well as neck tumors and head cancer[1]. Many more oral derivatives of cisplatin are being synthesized every year. There are over 3000 cisplatin analogs that have been tested, with 28 that are currently awaiting clinical trials. Typically, it is estimated that more than 10,000 compounds need to be screened in order to obtain a new, effective anticancer drug[1].

Cisplatin was first described by M. Peyrone in 1839[1] and is historically called Peyrone's salt. Transplatin and cisplatin are isomeric compounds, and the molecular structure differences between the two complexes were resolved by Werner in 1845[2]. In the 1960s, Barnett Rosenberg and Van Camp found that use of a platinum electrode inhibited binary fision in *E. coli* bacteria when an electric field was created. While the bacteria grew to 300 times their normal length, cell division would fail[3].

Rosenberg conducted a series of experiments to test the effects of platinum compounds and coordination complexes on sarcomas. The key research was done at Michigan State University; specifically, cisplatin and other platinum complexes were tested on tumors artificially implanted in rats[3].

Many studies have used mouse leukemia L1210/0 cells to react with cisplatin, which increases DNA excision repair capacity and causes DNA defect mutagen hypersensitivity[4]. Cisplatin was tested and established as a potential drug with curative

ability in the treatment of testicular cancer. The Food and Drug Administration (FDA) approved cisplatin for metastatic ovarian tumors for a Phase I clinical trial in 1971. Commercially, cisplatin was named Platinol[5].

2. Types of platinum compounds:

Platinum has two dominant charge states, +2 and +4[6]. The +2 charge state forms square planar complexes and the +4 forms octahedral complexes. The earliest synthesized anti-tumor drugs were cis-$[Pt^{II}(NH_3)_2Cl_2]$ and cis-$[Pt^{IV}(NH_3)_2Cl_4]$. Most of the well-known platinum anticancer complexes have a +2 charge. The general formula of the anticancer complexes is cis-$[Pt^{II}X_2(NHR_2)_2]$, in which R = organic fragment and X = leaving group such as chloride, chelating bis-carboxalate or oxalate.

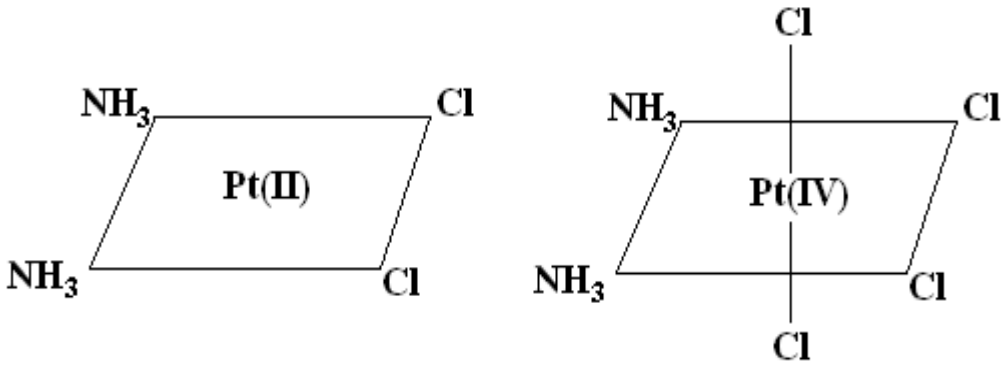


Figure 1. cis-dichlorodiammineplatinum(II) and cis-tetrachlorodiammineplatinum(IV)[7].

Cisplatin has a 90% curative rate for ovarian and testicular cancers. Although transplatin and cisplatin are geometric isomers, trans-species suppress growth at higher concentration and are inactive at low concentrations (ppm). Trans-

diamminedichloroplatinum(II), (trans-DDP, or transplatin), is therefore clinically ineffective [7].

In Figure 2, the structures of cisplatin and other platinum anti cancer drugs are shown: (1) Cisplatin; (2) Carboplatin; (3) Nedaplatin; (4) Oxaliplatin; (5) JM-216; bis-acetato-ammine-dichlorocyclohexylamine platinum(IV); (6) AMD473; cis-Amminedichloro (2-methylpyridine) platinum(II); (7) Dinuclear complex; and (8) Trinuclear complex.

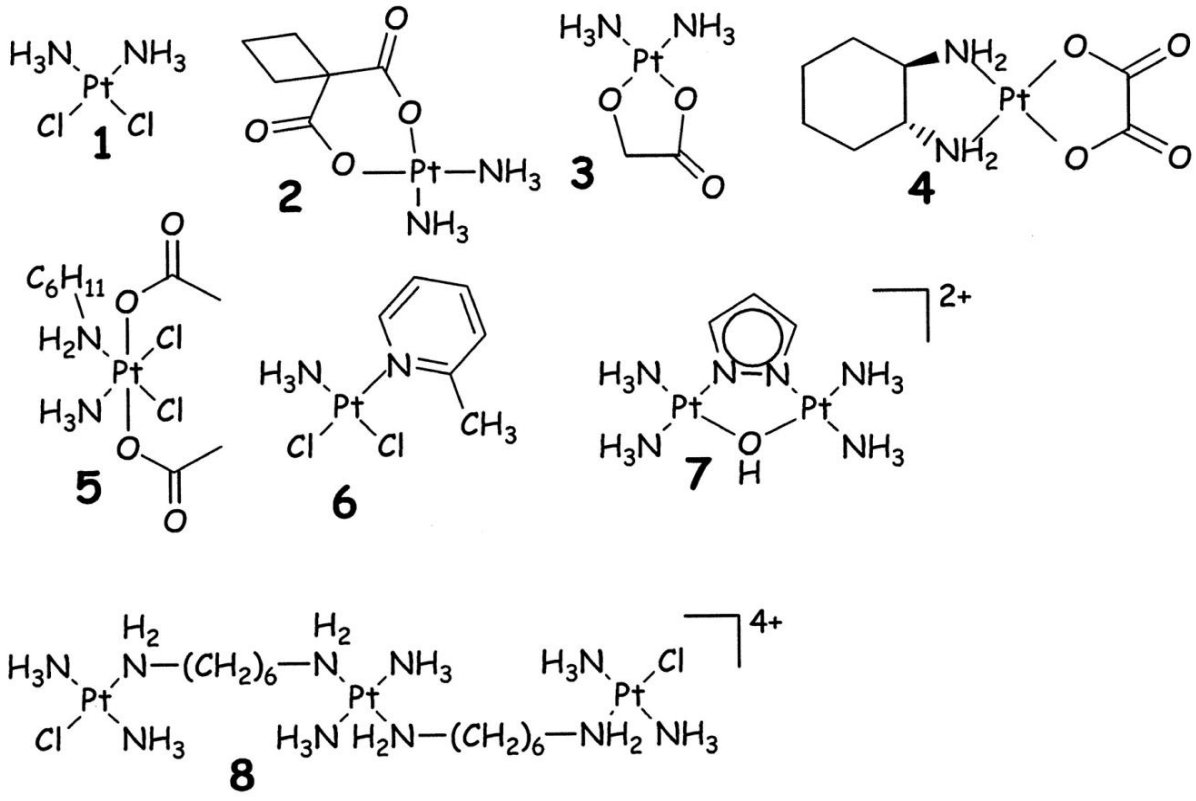


Figure 2. Cispaltin Family: (1) Cisplatin; (2) Carboplatin; (3) Nedaplatin; (4) Oxaliplatin; (5) JM-216; bis-acetato-ammine-dichlorocyclohexylamine platinum(IV); (6) AMD473; cis-Amminedichloro (2-methylpyridine) platinum(II); (7) Dinuclear complex; and (8) Trinuclear complex.

Carboplatin (cyclobutane-1,1-dicarboxylic acid), also called Azanide, (41575-94- 4) is one of many third generation platinum compounds that have been approved for clinical usage in the United States. Carboplatin was discovered and developed at the Insitute of Cancer Research in London. In 1989, Carboplatin, still under the brand name Paraplatin, was approved by the FDA (Food and Drug Administration). Starting in October 2004, generic versions of the drug became available. Carboplatin is a chemotherapy drug used against some forms of cancer (mainly ovarian carcinoma, lung, head and neck cancers) [9]. Carboplatin has less nephrotoxicity, neurotoxicity, ototoxicity and emetogenesis and is more stable than cisplatin. The platinum-based antineoplastics have a broad spectrum of antitumor activity in the treatments of carcinomas of ovarian, testicular, bladder, small and non–small cell lung, head and neck carcinoma, and seminoma.

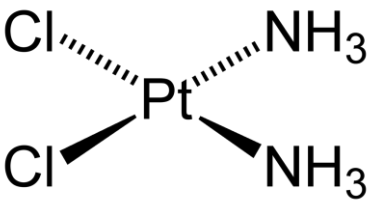
Oxaliplatin, {(R, R)-1,2-diaminocyclohexane(ethanedioate-O,O)platinum(II)} (63121-00-6) has an oxalate ligand as a leaving group. It is typically administered with fluorouracil and leucovorin in a combination used in the treatment of colorectal cancer. Oxaliplatin is a platinum-based cancer chemotherapy drug, classified as an alkylating agent. The alkylating agents are not capable of actually adding alkyl groups to DNA, but simply work by a similar mechanism[10]. It was discovered in 1976 at Nagoya City University by Professor Yoshinori Kidani, and was subsequently in-licensed by Debiopharm. Oxaliplatin was developed as an advanced colorectal cancer treatment[11]. It is known that a diaminocyclohexane group contributes greater cytotoxicity than cisplatin and carboplatin. Oxaliplatin is not generally cross-resistant to cisplatin or carboplatin. All are capable of forming platinum complexes producing inter- and intrastrand DNA cross

links with neighboring guanine residues, resulting in DNA-mismatch repair (MMR) activity in the cancer cells.

II. PROPERTIES OF CISPLATIN

1. Physical properties[12]:

Table 1: Physical properties of cis-diamminedichloroplatinum(II)

Formal Name	cis-diamminedichloroplatinum(II) CDDP	 (CAS 15663-27-1)
Common/ Commercial Names	Cisplatin/Platinol	
Agent	Anti-neoplastic	
Molecular Formula	$Cl_2H_6N_2Pt$	
Molecular Weight	300.1	
Normal State	Crystalline solid	
Color	Deep yellow (crystalline solid) & Clear (reconstituted solution)	
Structure	Tetragonal (square) planar	
Symmetry	C_{2V}	
Melting Point	Decomposes at 270°C to give chlorine gas and nitrogen oxides	
Solubility	Soluble in water and saline	
Insolubility	Ethanol, Hexane, Benzene and Acetone	

2. Chemical properties:

a) Aqueous solubility:

Cisplatin is water soluble. The chloride ligands undergo slow displacement with a water (an aqua ligand) molecule. The aqua ligand in the resulting $[PtCl(H_2O)(NH_3)_2]^+$ is easily displaced, allowing cisplatin to coordinate to a basic site of DNA. Subsequently, the platinum cross-links a second base via displacement of the other chloride ligands[13].

$$cis\text{-}[Pt(NH_3)_2Cl_2] + H_2O \xrightleftharpoons{K_1} Cl^- + cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+$$

$$cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ + H_2O \xrightleftharpoons{K_2} Cl^- + cis\text{-}[Pt(NH_3)_2(H_2O)_2]^{2+}$$

Figure 3. Mechanism for aquation of cisplatin[14].

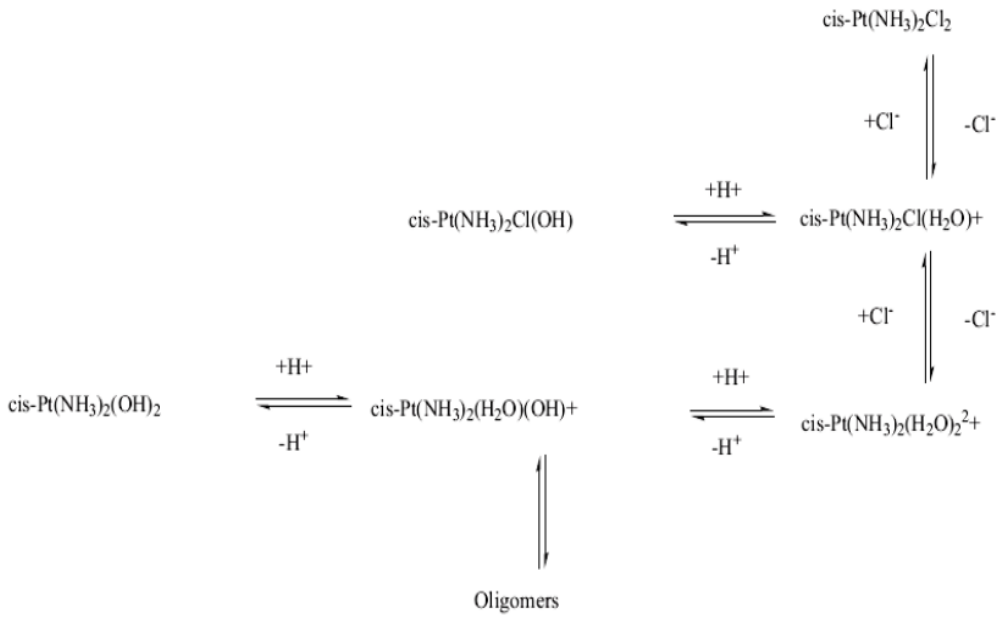


Figure 4. Aquation and deprotonation of cisplatin[15].

b) Chemical activity:

- Stability

Lyophilized Cisplatin powder should be stored under refrigeration (2-8 °C) and kept from light. Cisplatin solution (Platinol-Bristol) should be stored above freezing and below 25°C, and protected from light. The half life of a di-aqua complex is about 5 hours at 30°C at pH 7. The chloride ligands of cisplatin may also be substituted by a variety of other nucleophiles like –OH.

- Stability in solution

Cisplatin is hydrolyzed in water, losing a chloride ion and gaining a water molecule to form the monohydrated complex (MHC). MHC in its protonated form, monoaqua cisplatin, is positively charged and highly reactive with a pK_a of 6.56 which is quite close to physiological pH[16]. At physiological pH (7.4) 85% of the MHC will be in its deprotonated form, the much less reactive monohydroxo cisplatin. The important cisplatin equilibria are affected by both pH and chloride concentrations of administration solution and body fluid.

III. SYNTHESIS AND BIOLOGICAL ACTIVITY

1. Synthetic procedures:

   Cisplatin can be synthesized by many procedures, including:

   - Addition of ammonia to ammonium tetrachloroplatinum(II)
   - Heating of tetraammineplatinum(II) chloride at 250 °C
   - Reaction of ammonium carbonate with tetrachloroplatinic(II) acid

The following preparation is a modification of Ramberg and Peyrone respectively, and involves a minimum number of side reactions which serves which serves to maximize yield.

a) Synthesis equation[17]:

$$2K_2[PtCl_6] + N_2H_4 \cdot 2HCl \longrightarrow 2K_2[PtCl_4] + N_2 + 6\,HCl$$

$$K_2[PtCl_4] + 2\,NH_3 \xrightarrow{NH_4Cl} \textit{cis}\text{-}[Pt(NH_3)_2Cl_2] + 2KCl$$

b) Overall reaction scheme:

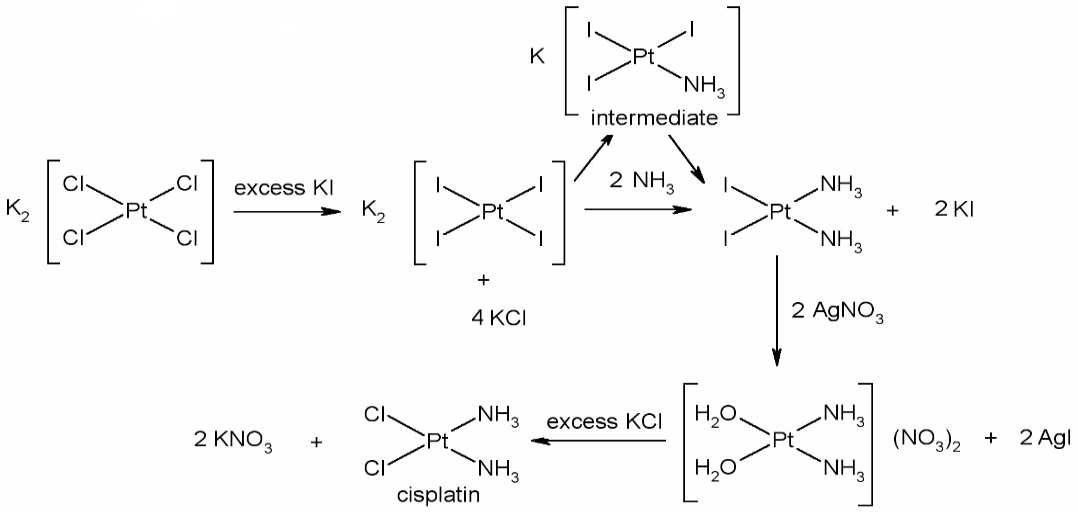


Figure 5. Synthetic scheme for the synthesis of cisplatin[18].

2. Biological activity of cisplatin

a) Cisplatin reaction with DNA

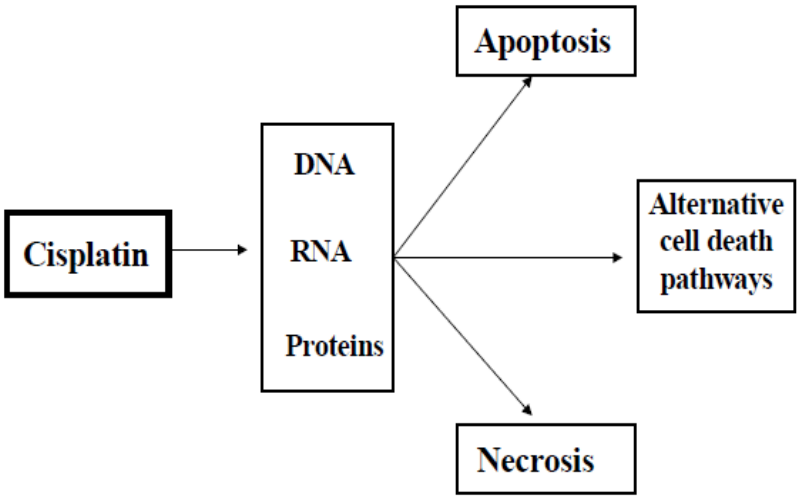


Figure 6. Cisplatin reacts with DNA and Proteins[19].

The platinum in cisplatin cross-links a second base via displacement of the second chloride ligand[18]. Cisplatin cross-links DNA in several different ways, interfering with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible.

The most notable reaction of cisplatin reacting with DNA is the formation of 1, 2-intrastrand cross-links on the purine bases adenine (A) and guanine (G). A dominating preference for initial attacks at N7-position of guanine has been established experimentally. Cisplatin binding to purines is preferred with guanine favored over adenine[20]. Following the thermodynamics and kinetics for the binding of the antitumor drug cisplatin, the monofunctional platination of guanine requires less energy than that of adenine. Cisplatin-G has a stronger molecular orbital interaction than the hydrogen bond

between the chloride ligand of cisplatin and the H_2N-C6 group of adenine. These cross-links include 1,2-intrastrand d(GpG) adducts, 1,2-intrastrand d(ApG), and 1,3-intrastrand d(GpXpG). Almost 90% of adducts are the 1,2- d(GpG) adducts. Much less common is the 1,2-intrastrand d(ApG) adducts. 1,3-intrastrand d(GpXpG) adducts occur but are readily excised by the nucleotide excision repair (NER) mechanism.

Furthermore, cisplatin also interacts with cellular proteins, particularly HMG (High Mobility Group) domain proteins, which can bind specifically to cisplatin-modified DNA and be a more advanced mechanism of interfering with mitosis, although this is probably not its primary method of action[21].

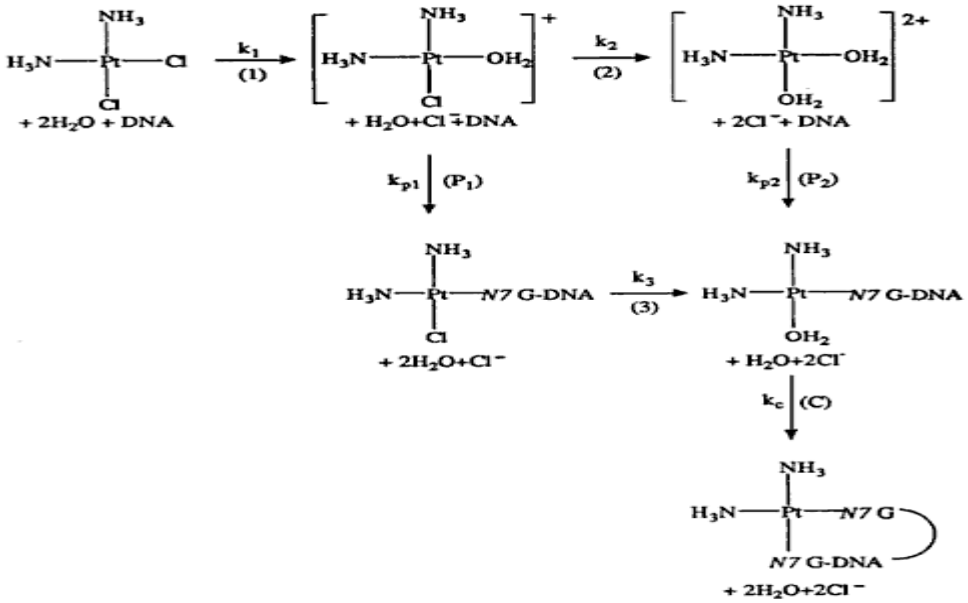


Figure 7. Mechanism of formation of cisplatin-DNA adducts.

(k_1, k_2, k_3 are equation-rate constants, k_1=1.9 ×10^{-4} s^{-1}, k_2= 2.3 ×10^{-4} s^{-1}, k_{p1} and k_{p2} are platination-rate constants, k_c is the chelation-rate constant[22]).

b) Cisplatin reaction with protein

Cisplatin not only reacts with DNA, but also binds to a number of extra- and intracellular proteins. The reaction with amino acids is most common with the sulfur donors, Cys and Met, also with the nitrogen donor to His[23]. The high affinity of platinum compounds for sulfur atoms could lead to platination for proteins[24]. Those proteins could serve as a drug reservoir for the nucleus of the cell. There are many studies that report the intermediate platinum binding on O- and N- donor atoms[25]. The intra-molecular competition of cisplatin with SAH (S-adenosyl-L-homocysteine), and SGH (S-guanosyl-L-homocysteine), between a sulfur-containing amino acid moiety and the nitrogen atom of a nucleobase, however, shows no coordination, either to the adenine N1 or to the N7 atom. From this competition reaction, the Pt-sulfur interaction is kinetically preferred, while the Pt with guanine-N7 is often thermodynamically favored[20, 26].

c) Cisplatin side effects

The substantial body of literature documents the side effects of platinum compounds. The side effects associated with cisplatin (at single doses $\geq$ 50 mg/m^3) includes nausea, vomiting, nephrotoxicity, neuropathy, ototoxicity and myelosuppression. The nephrotoxicity of the parent compound, cisplatin, almost led to its abandonment, but more novel platinum drugs developed for reduced side effects. The development for novel platinum anticancer drug is a driving force behind both the search for less toxic analogues and for more effective treatments. The hydrated cisplatin compounds were found to reduce side effects and prevent acute renal failure[27]. The novel platinum

compound analogues, carboplatin and oxaliplatin, which are less toxic than cisplatin, are better hydrated and more easily replaced on the chelate.

$[Pt(en)(H_2O)_2]^{2+}$, a carboplatin analogue, can react with methionine-containing di- and tri-peptides (met-Hgly, met-gly-Hgly) in the pH range of 2.5-11.0. Different pH leads to different chelation with N(amino), S(thioether) or N(amino), and N'(amide)[28].

d) Cisplatin, Carboplatin and Oxaliplatin

Advances in pharmaceutical research are crucial for improving the design of compounds to reduce toxic side effects by increasing the water solubility of anti-cancer drugs. Carboplatin is an analogous of cisplatin that is more easily administered and reduced the side effect at standard doses. Carboplatin has a bidentate dicarboxylate ligand as its leaving group instead of the more labile chloride ligands. It has good water solubility, exhibits excellent activity against a variety of leukemias and solid tumors with low nephrotoxicity and pharmacokinetic stability[29]. The other toxicities of carboplatin are generally milder and better tolerated than those of cisplatin. This is due to a different pharmacokinetic profile resulting from the substitution of a more stable leaving group.

Oxaliplatin[30] has less nephrotoxicity than cisplatin, presumably related to it is more slowly hydrolyzed leaving group. The leaving group, oxalate, is the divalent anion $C_2O_4^{2-}$ or $(COO)_2^{2-}$. It is an excellent ligand for metal ions, and usually binds as a bidentate ligand forming a 5-membered MO_2C_2 ring. The drug, oxaliplatin, exhibits improved water solubility relative to cisplatin, and avoids the dose-limiting side-effect of nephrotoxicity.

Our current study focuses on $[Pt(en)ox]^{31}$, oxalate platinum ethylenediamine, where en is ethylenediamine and ox is oxalate. The compound features a square planar coordination complex and is classified as an alkylating agent and a coordination complex.

Oxalate, is a good leaving ligand and similar to oxaliplatin, which is water soluble, and easily be replaced. The features for the bidentate ligand, ethylenediamine, would be less steric bulk and symmetry with two monodentate ammine ligand.

IV. MATERIALS AND METHODS

1. Materials

Table 2. Oxalic acid (anhydrous)

Company name:	ARCOS ORGANICS
C.A.S No:	C.A.S 144-62-7
Molecular formula	$C_2H_2O_4$
Molecular weight	MW= 90.04 g/mol

Table 3. Silver nitrate, 99+%

Company name:	Sigma –Aldrich A.C.S reagent
C.A.S No:	C.A.S 7761-88-8
Molecular formula	$AgNO_3$
Molecular weight	MW= 169.88 g/mol

Table 4. Silver oxalate

Chemical Name:	Silver oxalate
Chemical form	$Ag_2C_2O_4$
Molecular weight	MW= 303.75 g/mol

Table 5. Dichloro(ethylenediamine) platinum(II) 99%

Company name:	Sigma –Aldrich
C.A.S No:	C.A.S 14096-51-6
Molecular formula	$C_2H_8Cl_2N_2Pt$
Molecular weight	MW= 326.1 g/mol

Table 6. Methionine (Met)

Company name:	ACROS Organics
C.A.S No:	C.A.S 63-68-3
Molecular formula	$C_5H_{11}NO_2S$
Molecular weight	MW= 149.21 g/mol

Table 7. N-Acetyl-L- Methionine (N-AcMet)

Company name:	ACROS Organics
C.A.S No:	C.A.S 65-82-7
Molecular formula	$C_7H_{13}NO_3S$
Molecular weight	MW= 191.25 g/mol

Table 8. Deuterium oxide, 99.9%

Company name:	Sigma –Aldrich
C.A.S No:	C.A.S 7789-20-0
Molecular formula	D_2O
Molecular weight	MW= 20.03 g/mol

2. Methods

1) Synthesis of Silver Oxalate

A sample of 450 mg of oxalic acid and 1690 mg of silver nitrate was prepared and to an amber vial. A 20 mL sample of deionized water were added and the mixture was stirred for at least one hour. The insoluble silver oxalate was collected by vacuum filtration. The yield is approximately 95%.

2) Synthesis of Pt(en)ox

Dichloride platinum(II)-ethylenediamine [Pt(en)Cl_2],was reacted with silver oxalate in an amber vial for at least 18 hours at room temperature. As the reaction processed, the chloride ion of the platinum compound was replaced by the oxalate, and AgCl formed as a white precipitate. The solution was filtered through celite powder, and the solution was placed on the rotary evaporator to dry. The resulting solid is [Pt(en)ox].

3) Nuclear Magnetic Resonance, NMR[32]

NMR spectra were collected on a JEOL Nuclear Magnetic Resonance Spectrometer Eclipse 500 MHz in D_2O. The chemical shift reference was set to HOD at 4.76 ppm.

3. Reactions:

1) Reactions of Pt(en)ox and Methioine:

The reaction between the water soluble platinum compound, [Pt(en)ox] (339.09g/mol), and Methionine (149.21 g/mol) was monitored at mole ratios of 2:1, 4: 1 and 1:1, and 1:2. Additionally 1.5 mL of D_2O was added to the amber vial. The solution was transferred to an NMR tube, after keeping the pH around 4.0-5.0. The solution was checked with NMR spectroscopy between 4 hours and 24 hours.

2) Reaction of Pt(en)ox and N-acetylmethioinine:

The reaction between [Pt(en)ox] and N-acetylmethionine was followed at a mole ratios of 2:1, 1:1 and 1:2, at the pH value. The NMR spectrum was checked between 4 hours and 24 hours.

V. RESULTS

1. Reaction of [Pt(en)ox] with N-Acetylmethionine (N-AcMet):

   a) [Pt(en)ox] reacted with N-Acetylmethionine (N-AcMet) at a 2:1 molar ratios

The reaction was monitored by ^{1}H NMR spectroscopy at 4 and 24 hours (Figure 8 and 9). The signal after 2.5 ppm is assigned to the en ligand of the unreacted platinum compound. Signals around 2.5 ppm are due to the S-CH_3 resonance at the products. The original S-CH_3 signal of unreacted N-AcMet is around 2.0 ppm, but the signal shifts to 2.4 ppm due to the reaction with the platinum compound. The predicted product would only have one N-AcMet coordinated via sulfur and nitrogen atoms.

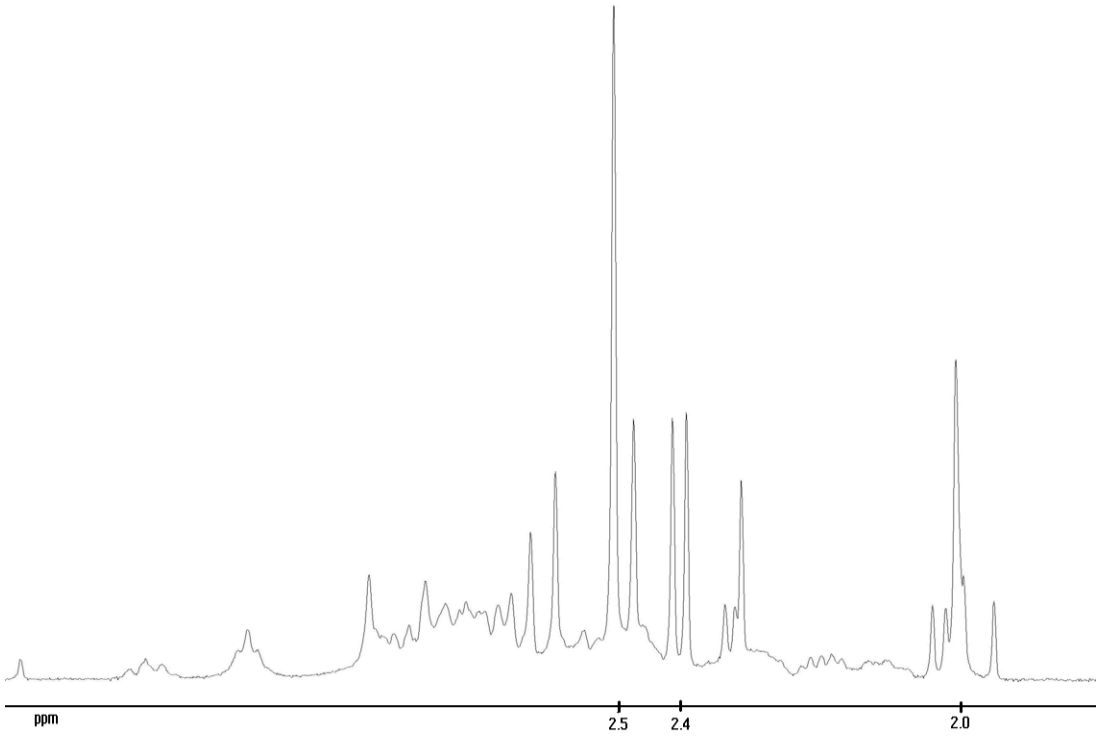


Figure 8. ^{1}H NMR spectrum for Pt(en)ox: N-AcMet, molar ration 2: 1, in D_2O 4 hours.

Comparing Figure 8 and Figure 9, the peak at 2.5ppm is slightly different between 4 and 24 hours, the signal on 2.5 ppm on the 24 hours is getting stronger[33]. The reaction at the same molar ratio would be more activity by following the time.

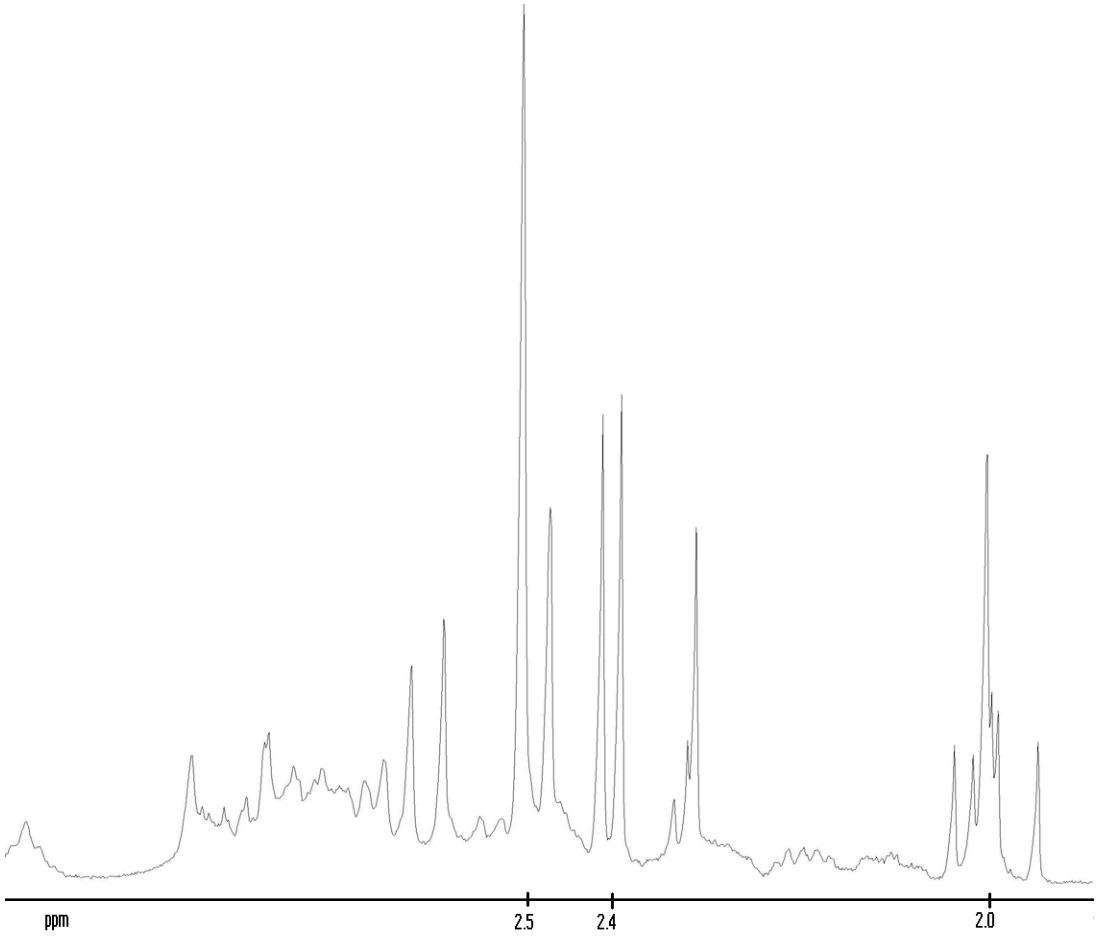


Figure 9. ^{1}H NMR spectrum for Pt(en)ox: N-AcMet, molar ration 2: 1, in D_2O 24 hours.

The predicted compound for mono adduction, the [Pt(en)(N-AcMet-*S*, *N*)] is:

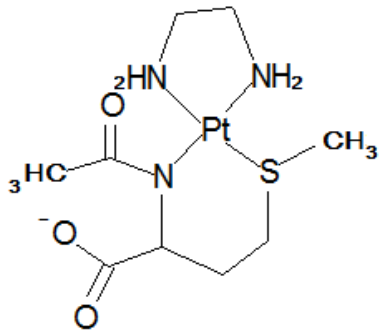


Figure 10. Pt(en)(N-AcMet-S,N), platinum chelate with N-AcMet.

b) [Pt(en)ox] reacted with N-Acetylmethionine (N-AcMet) at a 1:1 molar ratio

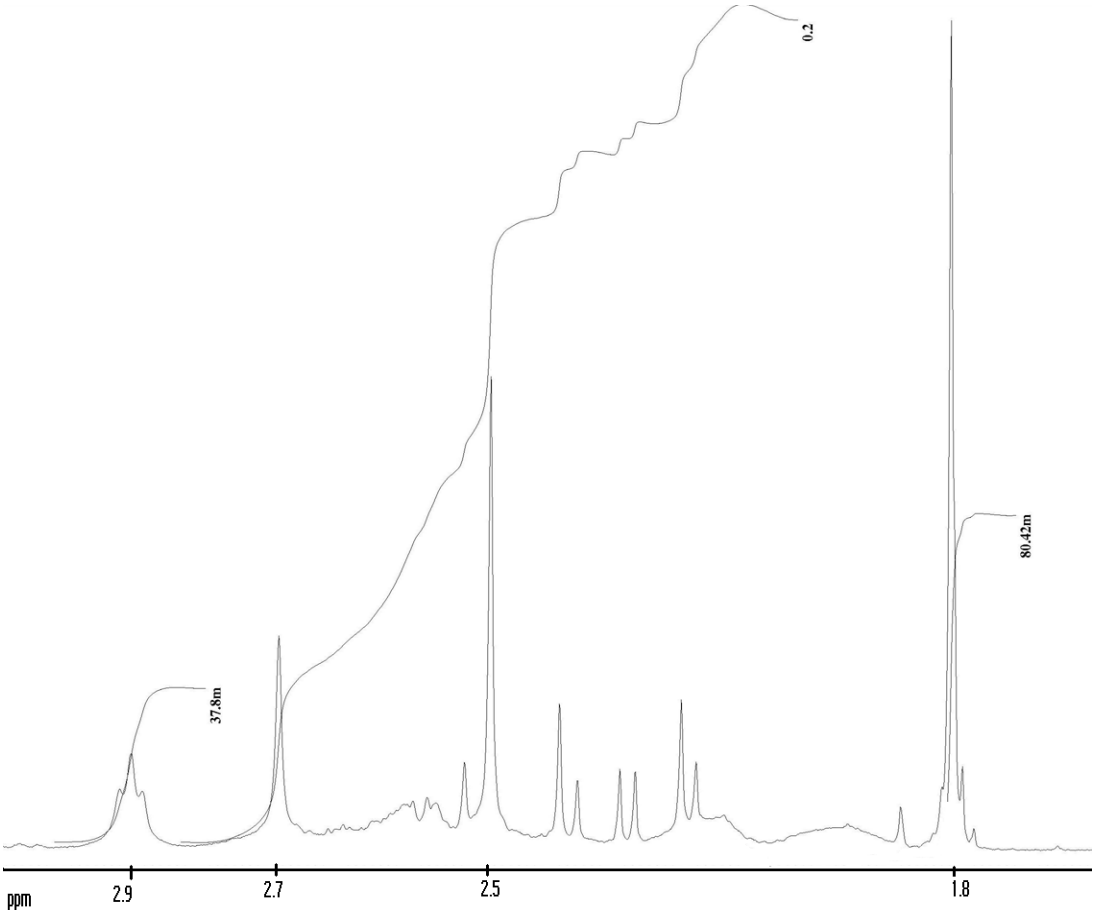


Figure 11. ^{1}H NMR spectrum for Pt(en)ox: N-AcMet, molar ration 1: 1, in D_2O 4 hours.

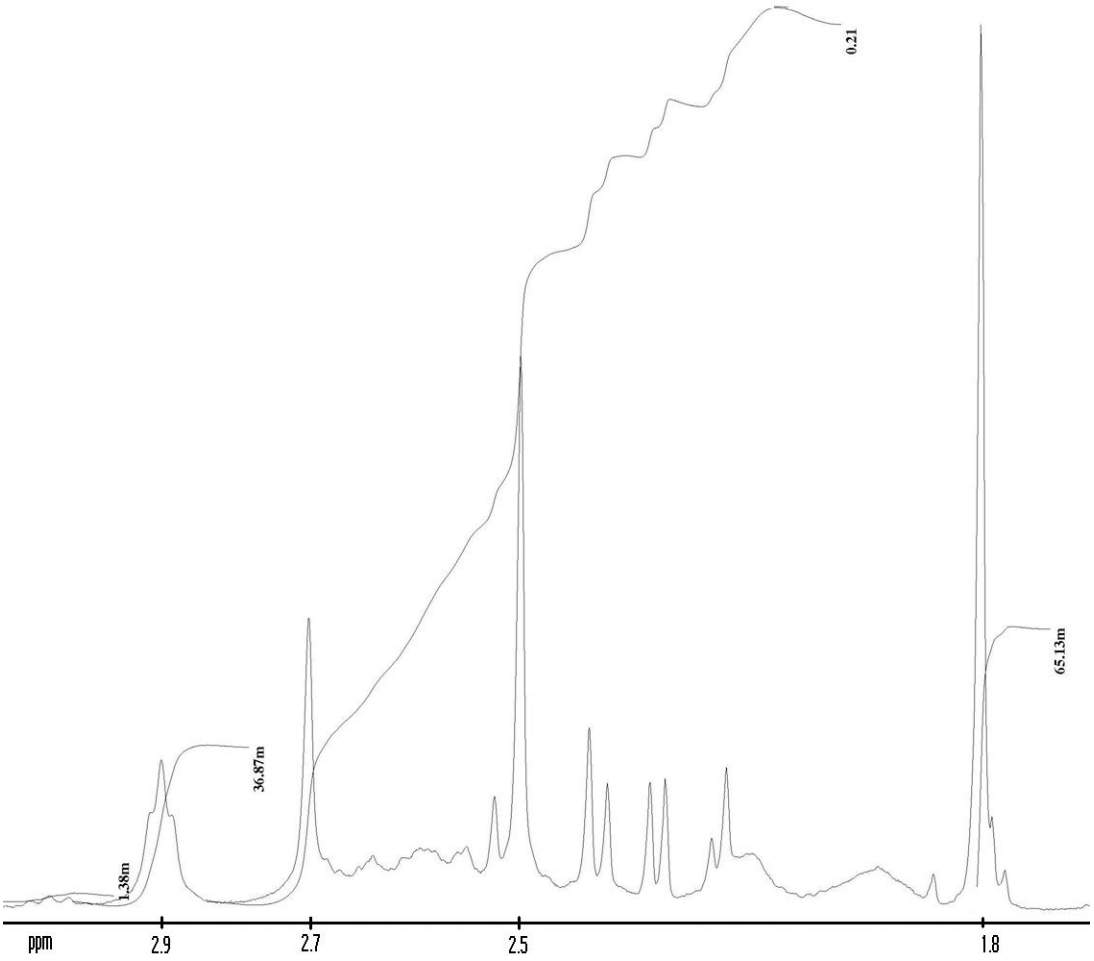


Figure 12. ^{1}H NMR spectrum for Pt(en)ox: N-AcMet, molar ration 1: 1, in D_2O 24 hours.

The reaction of [Pt(en)ox] with N-AcMet at mole ratio of 1:1 was monitored at 4 hours and 24 hours (Figures 11, and 12). The signal at 2.5 ppm[33] is a typical chemical shift for the S-CH_3 resonance of a sulfur-coordinated N-AcMet. The N-AcMet connects to the Pt(en) via sulfur. The signal at 2.5 ppm, 2.7 ppm and 2.9 ppm is from the en ligand, which reacts with platinum and signal shift. These signals intensity is can indicate the complex of the product has C_2 symmetry. That means two N-AcMet have reacted with platinum by the sulfur side. The small signals at 1.8 ppm and 1.7 ppm are from unreacted

N-AcMet and decrease over time. The amount of the unreacted N-AcMet decreases only slightly with time indicating the reaction between platinum and N-AcMet is quite complete in 4 hours.

c) [Pt(en)ox] reacted with N-acetylmethionine (N-AcMet) at 1:2 molar ratio

The reaction for [Pt(en)ox] with N-AcMet at a mole ratio of 1: 2 was monitored at 4 hour and 24 hours (Figures 13. and 14). Signals at 2.5 ppm, 2.7 ppm and 2.9 ppm as compared to those at a 1:1 molar ratio did not change significantly, except that more intense signals from unreacted N-AcMet were observed.

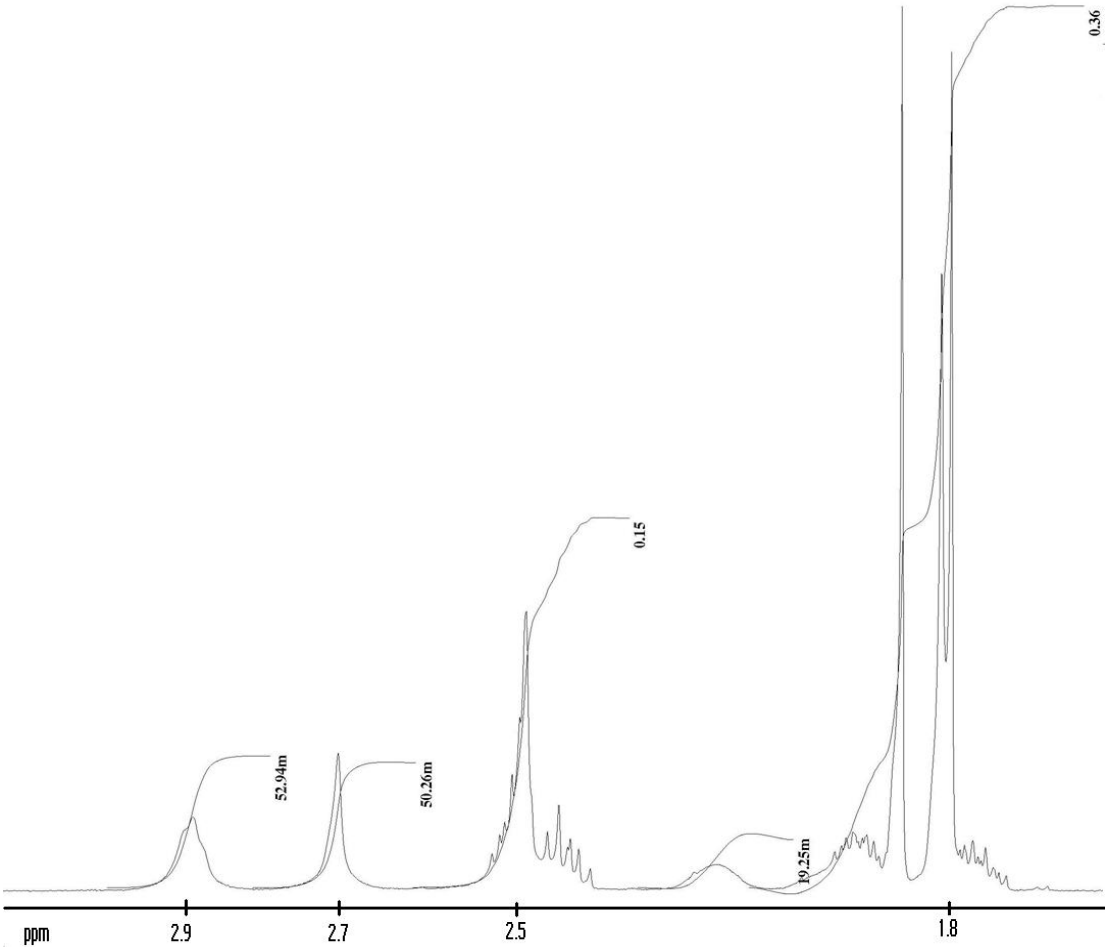


Figure 13. ^{1}H NMR spectrum for Pt(en)ox: N-AcMet, molar ration 1: 2, in D_2O 4 hours.

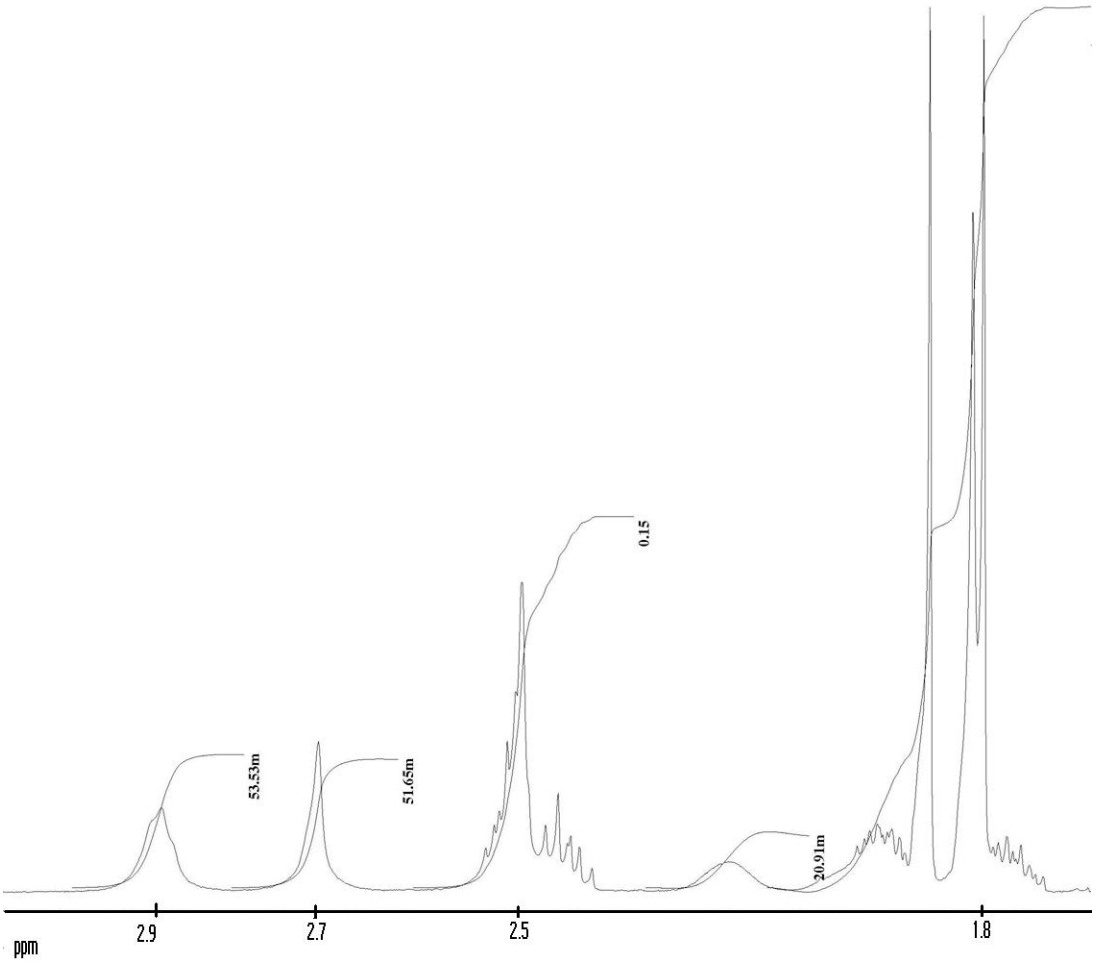


Figure 14. ^{1}H NMR spectrum for Pt(en)ox: N-AcMet, molar ration 1: 2, in D_2O 24 hours.

In Figure 13 and Figure 14, the partial spectra from 2.0~3.0 ppm, indicates the product could be either mono or bis chelate complex[34]. From Figure 10, a molar 1:1 ratio with the signals between the 2.0~2.5 ppm shows that there is only one of S-CH_3 signal shift. For Figures 12, and 14, the signals from the 2.5~3.0 ppm is muti-signal indicating two S-CH_3 be coordinated together.

From the molar ratio 1:1 to 1:2, the [Pt(en)ox] reacts with N-AcMet. The signals intensity at 2.5 ppm, 2.7 ppm and 2.9 ppm indicate the S-CH_3 connect with platinum and have two N-Ac Met react with the platinum. The final product is symmetric predicted to be:

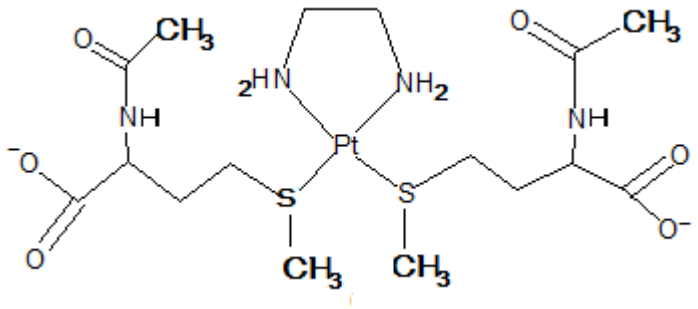


Figure 15. Predicted structure of Pt(en)(N-AcMet-S)$_2$ complex.

2. Reaction of [Pt(en)ox] with Methionine (Met):

a) [Pt(en)ox] reaction with Methionine (Met) at 2:1 molar ratio

The reaction of [Pt(en)ox] with Met at a molar ratio of 2:1 was monitored at 4 hours and 24 hours. In Figure 7, the signals at 2.3 ppm and 2.7 ppm are assigned to the H in S-CH_3 and the en of the product. Two Mets react with [Pt(en)ox] and replace the oxalate to form a bis-Met product. The signal at 2.5 ppm is from unreacted en. The signal at 3.2 ppm is from free ethylenediamine, which means the en ligand on the Pt(en) compound was replaced by Met. The platinum compound reacts with two methionine residues connecting on the nitrogen and sulfur atoms, and becomes [$Pt(Met)_2$]. Comparing the intensity at 24 hours, the signals at 3.2 ppm and 2.3 ppm increase with time as the product [$Pt(en)(Met)_2$] decrease and the free en ligand is displaced. The tiny signal at 1.9 ppm is unreacted Met, and the signal reduces with time.

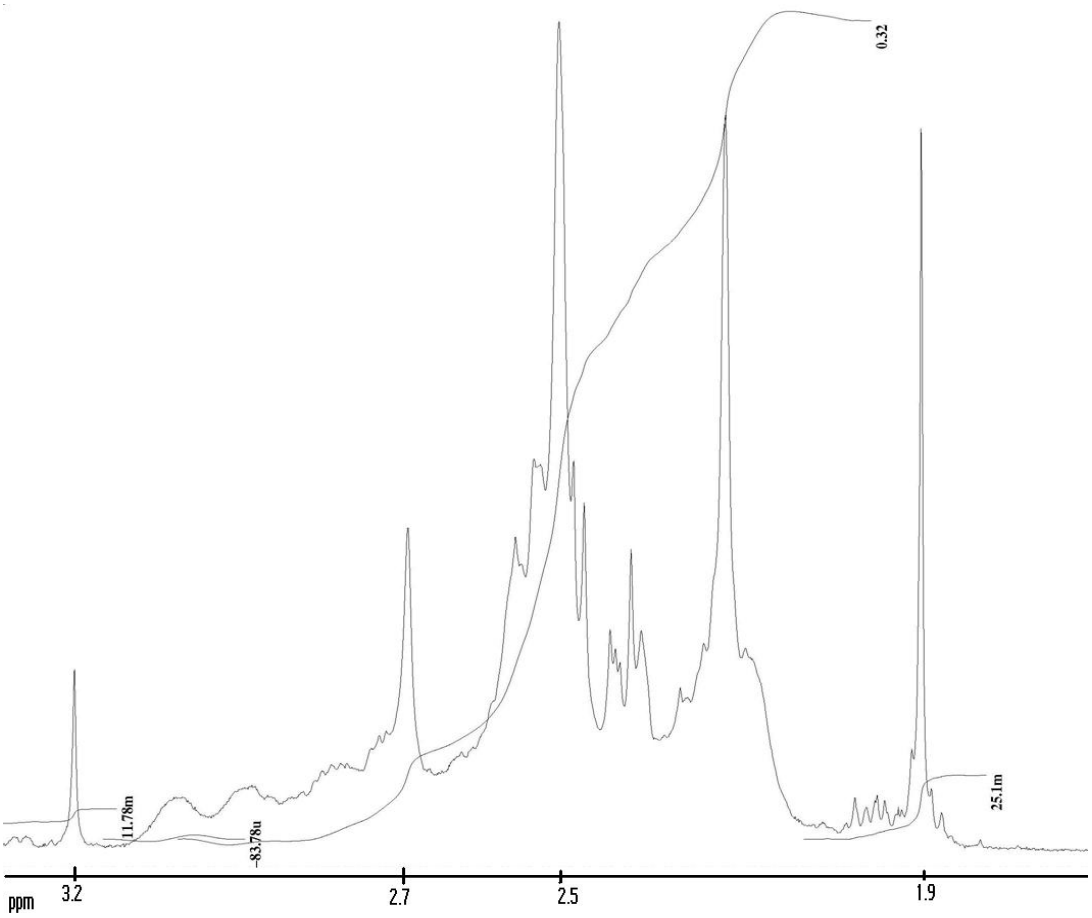


Figure 16. ^{1}H NMR spectrum for Pt(en)ox: N-Met, molar ration 2: 1, in D_2O 4 hours.

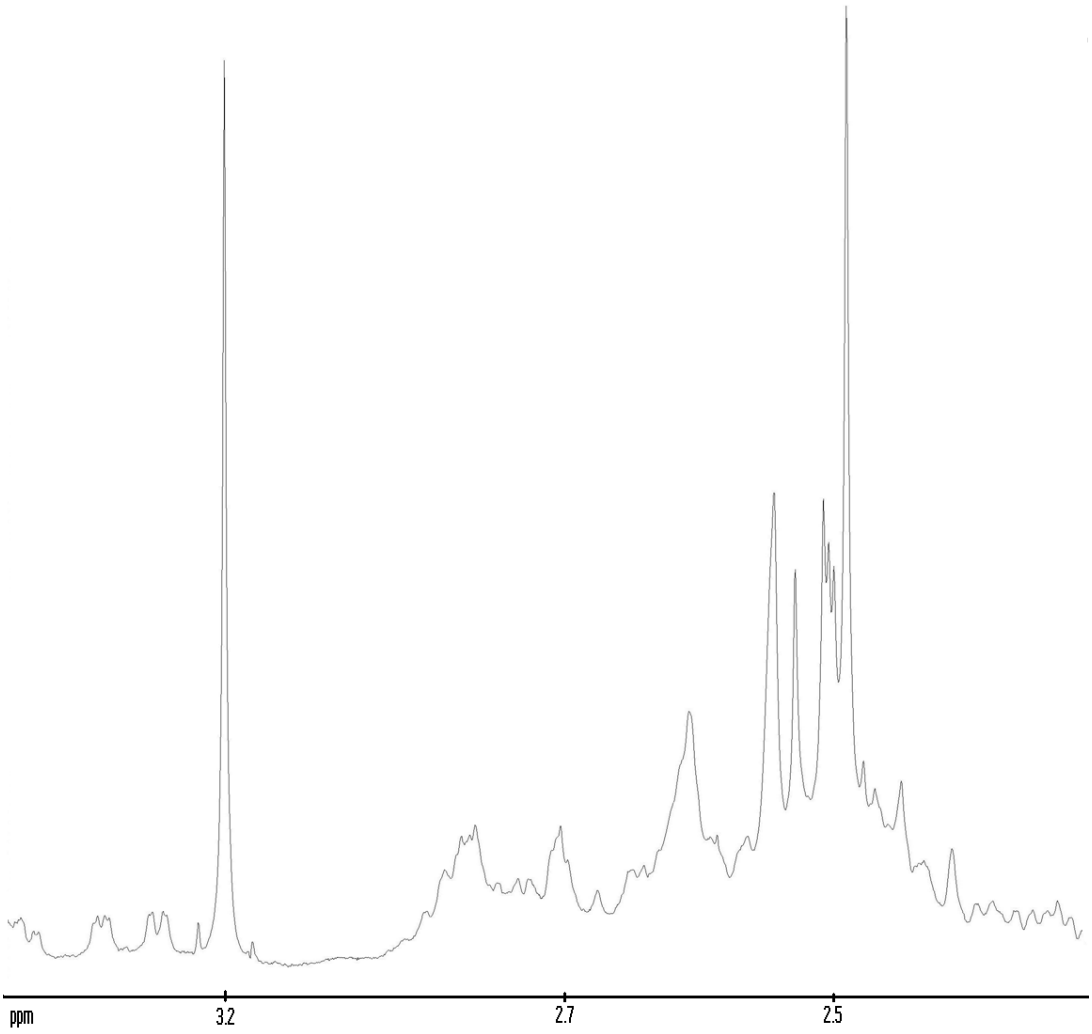


Figure 17. ^{1}H NMR spectrum for Pt(en)ox: Met, molar ration 2: 1, in D_2O 24 hours.

The predicted structures for the methionine reacted with [Pt(en)ox] to form bis-chelate, $Pt(Met\text{-}S,N)_2$ complex and $[Pt(en)(Met\text{-}S)_2]^{2+}$ complex:

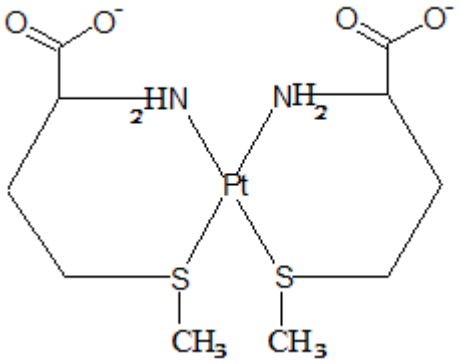


Figure 18. Bis-chelate for $Pt(Met\text{-}S,N)_2$ complex.

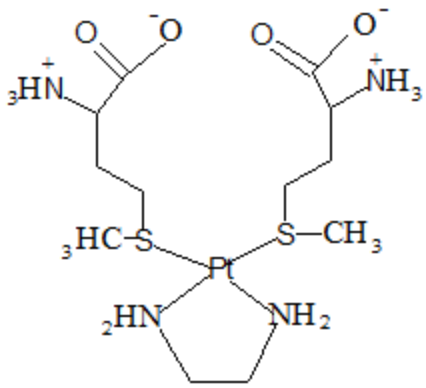


Figure 19. $[Pt(en)(Met\text{-}S)_2]^{2+}$ complex.

b) [Pt(en)ox] reaction with Methionine (Met) at 1:1 molar ratio

In Figure 20, ^{1}H NMR spectroscopy was used to monitor the reaction of [Pt(en)ox] and Met at a molar ratio 1:1. The signal at 2.4 ppm is assigned to the H of the S-CH_3 from the product, which is [Pt(en)(Met-S)$_2$]. The signal at 3.2 ppm is the free ethylenediamine and indicates the en ligand was replaced by Met. The signal at 2.0 ppm is the unreacted methionine.

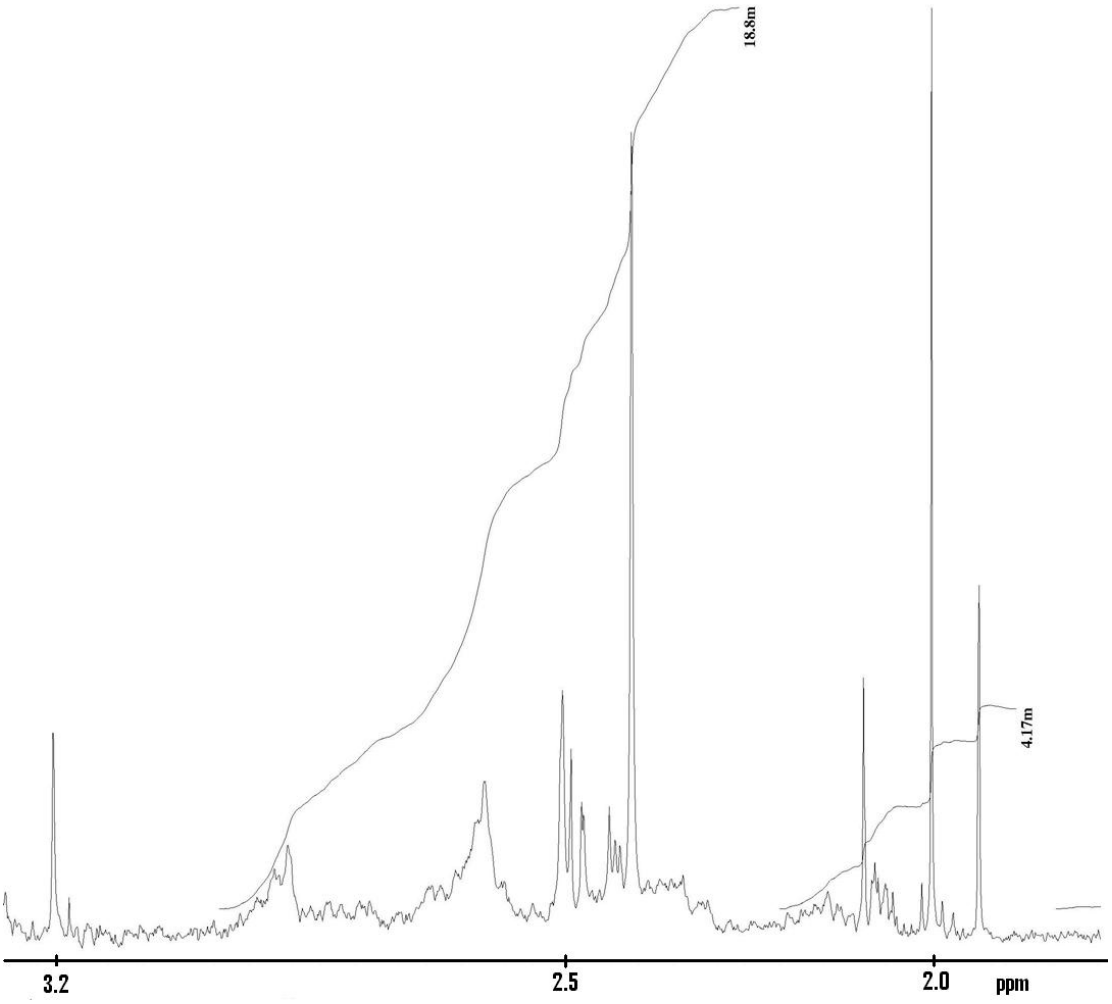


Figure 20. ^{1}H NMR spectrum for Pt(en)ox: Met, molar ration 1: 1, in D_2O 24 hours.

c) [Pt(en)ox] reaction with Methionine (Met) at 1:2 molar ratio

The reaction of [Pt(en)ox] with Met at a molar ratio of 1:2 after 4 hours in D_2O is shown in Figure 21. The signal at 2.4 ppm is from the H of S-CH_3 of [Pt(en)(Met-$S)_2$]. The unreacted Met has a signal at 2.0 ppm. As the reaction proceeds with time, the signal at 3.2 ppm increases from 4 hours to 24 hours; while more of the en ligand was replaced by the Met. The signal at 2.7 ppm was displaced for the 24 hr reaction, which means [Pt(Met-N,$S)_2$], was the final product.

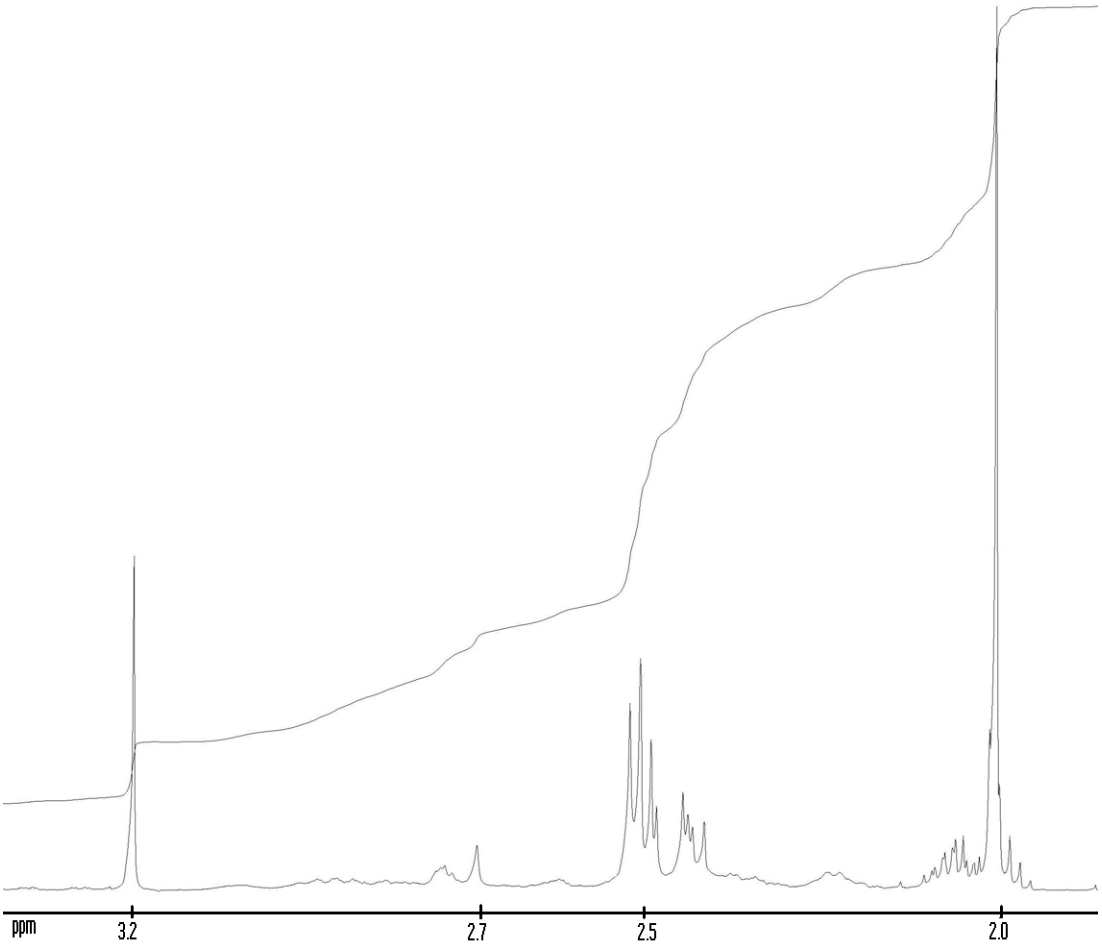


Figure 21. ^{1}H NMR spectrum for Pt(en)ox: Met, molar ration 1: 2, in D_2O 4 hours.

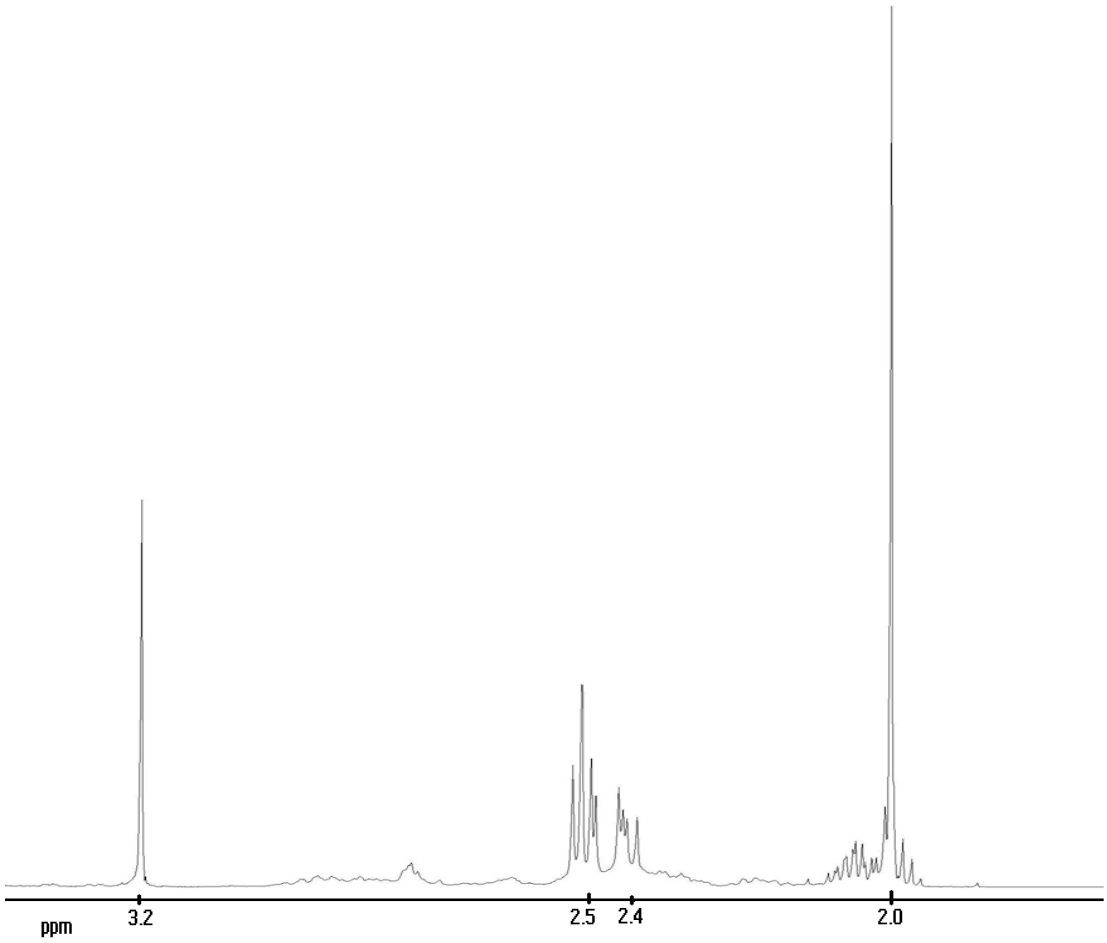


Figure 22. ^{1}H NMR spectrum for Pt(en)ox: Met, molar ration 1: 2, in D_2O 24 hours.

d) [Pt(en)ox] reaction with Methionine (Met) at a 1:4 molar ratio

The reaction of [Pt(en)ox] with Met at a molar ratio 1:4 is shown in Figures 23 and 24. The signal at 2.4 ppm is the hydrogen of the Met of [Pt(en)(Met-S)$_2$] bis-product. The unreacted Met is at 2.0 ppm. The reaction shows the [Pt(en)ox] reaction with Met, with products bis-chelate, [Pt(Met-N,S)$_2$], and bis [Pt(en)(Met-S)$_2$].

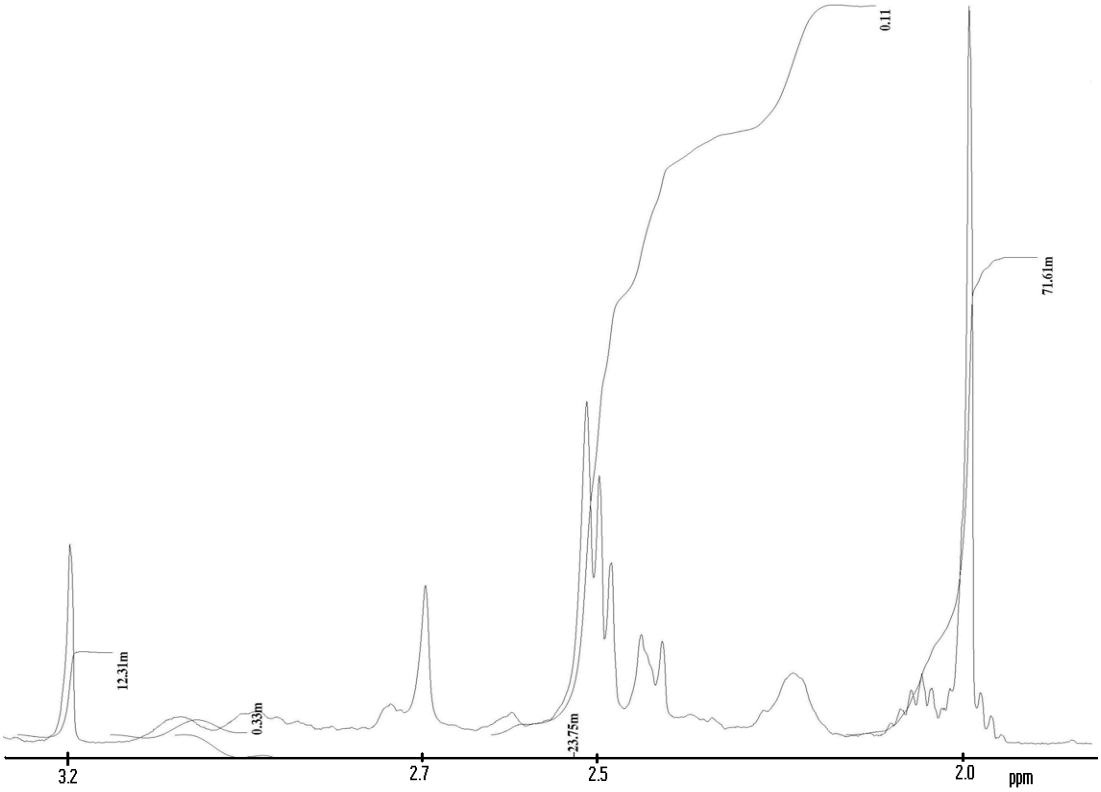


Figure 23. ^{1}H NMR spectrum for Pt(en)ox: Met, molar ration 1: 4, in D_2O 4 hours.

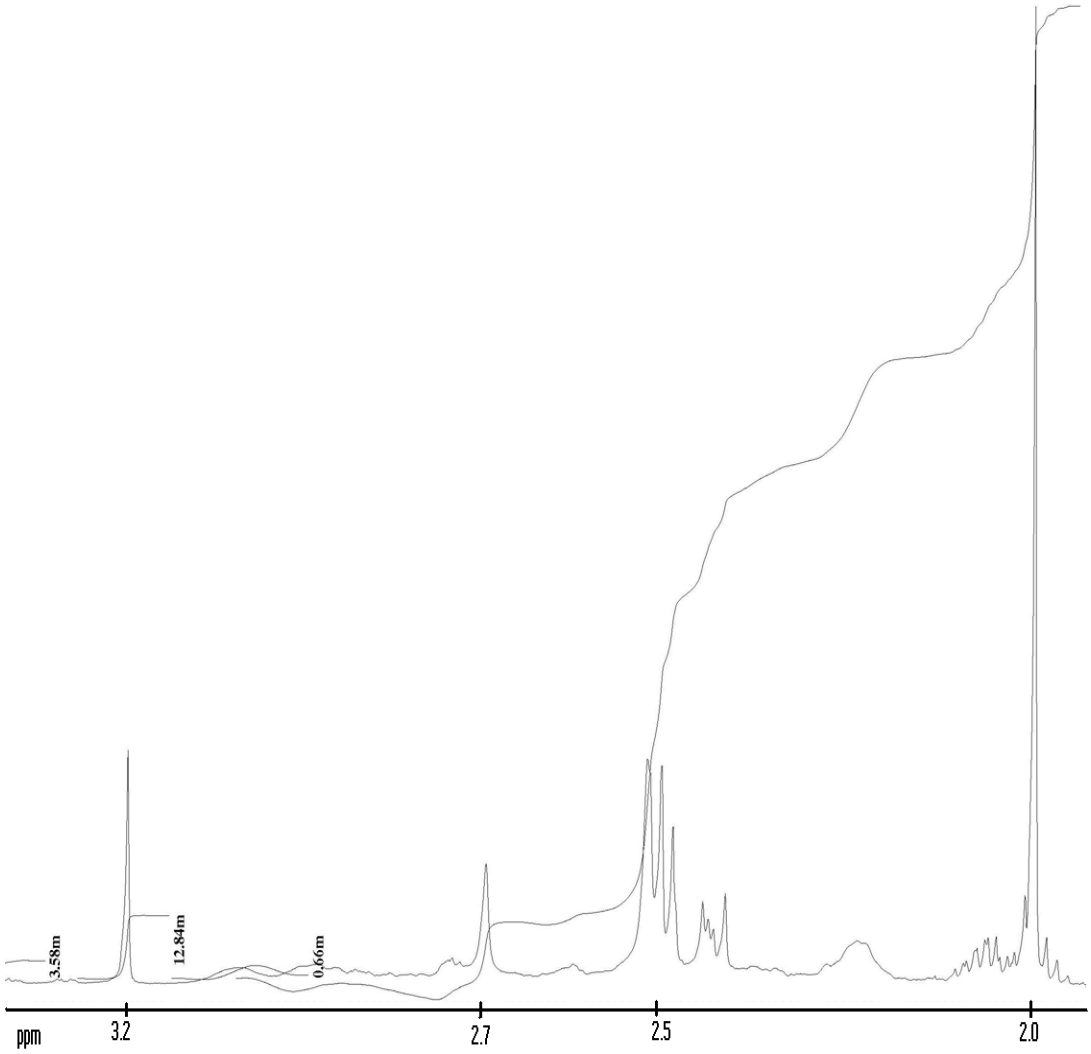


Figure 24. ^{1}H NMR spectrum for Pt(en)ox: Met, molar ration 1: 4, in D_2O 24 hours.

The methoinine connects with platinum compound on the sulfur and nitrogen, that product becomes the bis complex. Figure 24, the signals around 2.5 ppm are from the product. But the signal at the 3.2 ppm is the free en ligand, which indicates the en was replaced, and platinum would bond with the methionine at nitrogen.

VI. DISCUSSION

It was found that the [Pt(en)ox] complex reacts with methionine (Met) and residue N-acetylmethionine (N-AcMet) to form mono (Pt-S) adducts and bis (Pt-S-S) adducts. Typically, [Pt(en)ox] reacts with Met and the Met replaces the en ligand and makes [Pt(Met-S, $N)_2$].

Platinum connecting with sulfur is favored over connecting with oxygen and nitrogen. Because the platinum is of low electronegativity, and has low-energy lying LUMO, which could be the soft Lewis acid to accept electron. Comparing sulfur with nitrogen and oxygen, the sulfur has a large atomic radius, high polarizability, and low electronegativity, that sulfur could be soft base better than nitrogen and oxygen. Following HSAB theory, Lewis soft acids reacts faster and form stronger bonds with soft bases. Compared with sulfur, the affinity of the nitrogen and oxygen, a electron donor, is more suitable for a low polarizable and high electronegativity metal ion, which is strong Lewis acid[35]. Platinum connects with sulfur faster than with nitrogen and oxygen.

In the reaction of the platinum compound reacting with methionine at a the molar ratio of 2:1, the en ligand was replaced and the new product is, [Pt(Met-S,$N)_2$]. The new product has a six ring-membered chelate, compared to five membered ring for [Pt(en)(Met-$S)_2$].

The cisplatin analog $[Pt(en)(H_2O)_2]^{2+}$ (en=ethane-1,2-diamine) reacting with complex containing methionine (met) di- and tri- peptides would have N(amino), S(thiother) and N(amino) and N"(amide) chelation modes. The reaction at pH <8.7 have the N, S complexes, but the reaction at pH >7.4 has the N, N" complexes dominate for

these bioligands[36]. On the reaction with one Met and one N-AcMet, the pH is around 4.6~ 6.0 and the products are mono, and bis chealte products connecting via the sulfur and nitrogen. In the future, changing the solution pH value could make the platinum compound connect with amino acid on nitrogen or sulfur.

The product of [Pt(en)ox] with excess methionine is Pt connected to two methionines via S and N; the en ligand is replaced. In the future, studies will focus on the product of reaction at different pH and time differences. [Pt(en)ox] reacting with methionine should have a ring-opened intermediate.

The reactions with N-AcMet and Met both form the bis-products, but for the reaction with Met the en ligand replaced. The N of the N-AcMet is sp^2 hybridized, so the lone pair of nitrogen is, therefore, not available for coordination.

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