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The Reaction of Methionine with a Non-C2-Symmetrical Platinum (II) Diamine Compound

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THE REACTION OF METHIONINE WITH A NON-C_2-SYMMETRICAL PLATINUM (II) DIAMINE COMPOUND

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By

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ABSTRACT

The research that we have conducted has allowed us to discover that the amino acids, methionine (Met) and N-acetyl methionine (N-AcMet), will react in a 1:1 and 1:2 molar ratio with platinum complexes containing bulky diamine ligands. Previous research has allowed us to gain a plethora of information on the experimentation and results of specific bulky diamine ligands such as N,N,N',N'-tetramethylethylenediamine and N,N-diethylethylenediamine. In the current study, we have investigated the bulky diamine ligand of N,N-dimethylethylenediamine, or Me_2en. With our focus on the bulk of the Me_2en ligand, we have been able to synthesize a platinum complex in $Pt(Me_2en)Cl_2$ and react it with Met and N-AcMet. Through analysis using NMR spectroscopy and LC/MS, we have been able to observe the formation of a S,O-chelate formation in the reaction of the platinum complex with N-AcMet. This implies that a second methionine is not able to coordinate to the position that is *cis* to the bulk.

Keywords: Cisplatin, Methionine, Nuclear Magnetic Resonance, Ligand, Chelate

Dedicated to my professors, friends, and family.

VITA

May 15, 1990.....Born- Raleigh, North Carolina

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Major: Chemistry

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CHAPTER 1

INTRODUCTION

As time has progressed, a vast amount of diseases and illnesses have become apparent in society. However, with time, a plethora of treatments and scientific advances have made way into the pharmaceutical industry to counteract these diseases and illnesses. One of the most significant diseases in the world today is cancer. There are many different forms of cancer that specialize in attacking different tissues and organs of the body. These different forms of cancer tend to have such diverse symptoms on the body, with the most significant being death. These devastating symptoms have led to many scientists experimenting with a wide array of anticancer treatments ranging from new radiation and chemotherapy techniques to development of novel anticancer drugs. These new developments eventually led way to research surrounding platinum (II) compounds and their affect on suppressing cancer. This breakthrough in platinum chemistry established the foundation for this research project concerning the reaction of bulky platinum (II) complex's with amino acids.

One of the most significant anticancer drugs used today is a platinum based drug known as cisplatinum, cisplatin, or cis-diamminedichloroplatinum. The discovery of cisplatin and all of its anticancer attributes was a great breakthrough in the field of oncology. Not only did cisplatin revolutionize how cancer could be treated, but it also led to the development and research of other derivatives of platinum compounds and their significance on anticancer activity. Oxaliplatin and carboplatin were two platinum compounds discovered from cisplatin that have been determined to act as anticancer agents.

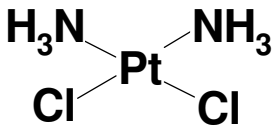

Figure 1. Illustration of cisplatinum, cisplatin, or cis-diamminedichloroplatinum.

Cisplatin is an incomparable drug in that it is not complex in structure, yet has the ability to target and fight certain cancers in the body. Structurally speaking, cisplatin, or $Pt(NH_3)_2Cl_2$, consists of two ammine ligands and two chloride ligands in ideal biological conditions as depicted in Figure 1. It maintains a square planar geometry[1]. Platinum (II) tends to have a high affinity for nitrogen and sulfur ligands, which are commonly found in proteins and nucleotides in DNA[2]; this is a result of platinum being known as a "soft" acid and bonding strongly as described by the Hard Soft Acid Base theory[5,6]. This platinum complex can be found in two conformations: *cis*, the commonly used anticancer drug,

and *trans*, the inactive form[5,6]. These features of cisplatin allow it to function flawlessly in the body.

Originally the compound has been known since 1845, but its anticancer activity was not discovered until 1970[1]. Cisplatin, formely known as Peyrone's chloride, was synthesized by Michael Peyrone in 1845[1,8]. The drug's activity was discovered by Dr. Barnett Rosenberg at Michigan State University in which he was able to experiment with how platinum complexes could lead to inhibition of cell proliferation[1,5,6]. Ironically, Rosenberg was focusing on bacterial growth's effects from electric fields using platinum electrodes; this eventually led to the origin of cisplatin[1]. Rosenberg was then able to test cisplatin on tumors in mice, which showed the effectiveness of the drug[1]. Cisplatin was eventually approved by the United States Food and Drug Administration and became licensed to Bristol-Myers Squibb in 1977[7]. It is the main component in the drug Platinol® and its derivative, carboplatin, is known as Paraplatin®[7]. The purpose of cisplatin was to be used in chemotherapy in order to target ovarian, testicular, stomach, lung, and bladder cancers[5,6]. The administering of cisplatin for testicular cancer treatments has led to a dramatic increase in survival rate from 10% to 80%[7].

Not only is cisplatin, and its derivatives, notable for its ability to be an ideal treatment for most cancers, but it is also one of a few cancer drugs based from inorganic complexes; most cancer drugs used today

are complex in structure due to the compilation of many organic molecules. The structure of cisplatin causes it to alter the repair mechanism of the cell by binding to the DNA and eventually leading to the suppression of cancer cells from proliferating[1,2]. Once the drug has entered the cell, it undergoes hydrolysis in which a charged platinum complex forms while one of the chlorine ligands is displaced due to it being replaced by a water molecule[1,2]. The platinum complex is coordinated to DNA. The platinum complex eventually coordinates to a second nucleotide, resulting because of further hydrolysis and displacement of the second chloride ligand[1,2]. This newly formed adduct eventually leads to a kink in the DNA helix[1,2]. The formed complexes have a tendency to react with both protein and DNA[1,2].

Cisplatin's main component, platinum, tends to have a great affinity for binding sites on proteins containing sulfur molecules. Sulfur atoms are only available in two kinds of amino acid residues, one being cysteine, and the other being of interest, methionine. The sulfur on the methionine contains a slightly partial positive charge and is available as a thioether. The interaction between methionine and cisplatin can lead to the formation of monodentate and bidentate compounds.

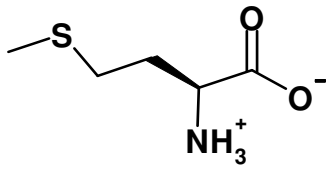

Figure 2. Depiction of the amino acid, methionine.

The proposed research project focuses on the $Pt(Me_2en)Cl_2$ complex, where the Me_2en ligand is N,N-dimethyethylenediamine, and its interactions with L-Methionine (Met) and N-Acetyl Methionine (N-AcMet). It was noted that Met and N-AcMet reacts in a 1:1 and 1:2 molar ratio with platinum complexes containing bulky diamine ligands. Previous research has allowed us to gain a plethora of information on the experimentation and results of specific bulky diamine ligands such as N,N,N',N'-tetramethylethylenediamine (Me_4en) and N,N-diethylethylenediamine (Et_2en)[3,4]. The current Me_2en ligand does appear to have sufficient bulk to prevent coordination of a second Met or N-AcMet to the coordination position *cis* to the bulk of the methyl groups. The sulfur atom of the first methionine is able to coordinate to the *trans* position, however, a sufficient amount of bulk is still surrounding the *cis* position in relation to the methyl groups on the ligand. This leads to the oxygen of the first methioine coordinating to the *cis* position and forming a S,O- chelate instead of a sulfur atom of a second methionine coordinating to that positions. The displacement of the Me_2en ligand occurred and the formation of a mono product was detected through NMR spectroscopy.

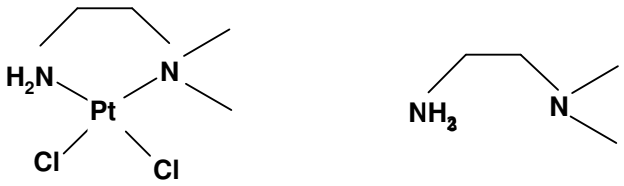

Figure 3. Structure of $Pt(Me_2en)Cl_2$ and Me_2en ligand.

This research allows an in-depth perspective of the reaction of bulky platinum complexes with L-Methionine and N-Acetyl Methionine and how this data can lead to a better understanding of cleavage reactions involved in proteins[3,4].

CHAPTER 2

METHODOLOGY

Synthesis of $Pt(Me_2en)Cl_2$:

In order to properly exhibit the formation of any product through the coordination of a peptide, the $Pt(Me_2en)Cl_2$ complex needed to be synthesized. Potassium tetrachloroplatinate was dissolved in H_2O. Dimethylethylenediamine was combined with methanol. This solution was then added drop-wise to the previous solution. The combined solution stirred for 24 hours (developed a "peach" color). Upon completion of stirring, the solution was placed on the rotary evaporator in order for the sample to be cooled and a product be precipitated; this process yielded a final product of $Pt(Me_2en)Cl_2$.

Reaction of $Pt(Me_2en)Cl_2$ with L-Methionine and N-Acetyl Methionine:

The $Pt(Me_2en)Cl_2$ complex needed to be reacted with various proportions of amino acids and monitored for periods of times to observe any displacement of the Me_2en ligand and formation of any product complexes. Approximately 3.5 mg of the platinum complex was dissolved in 1.0 mL of deuterium oxide (D_2O) and reacted with L-Methionine and N-acetyl methionine (2 and 4 mg); these samples were adjusted to a pH

of 4. This allowed us to compare the reactions at a 1:1 and 1:2 molar ratio and monitor the samples at various time intervals through NMR spectroscopy.

NMR Spectroscopy:

The reactions of the platinum complexes with proportions of the peptides were analyzed using ^{1}H NMR spectroscopy. This data was attained on a JOEL Eclipse 500MHz NMR instrument. Through NMR spectroscopy and analysis, we can determine when there may be a displacement of the Me_2en ligand, when the major products are forming, and find any correlation between the different ratios of the samples.

LC/MS

A 5mM concentration of the platinum complex was reacted with a 10mM concentration of Met and N-AcMet and then analyzed by liquid chromatography/ mass spectrometry. The data was attained on a Varian LC/MS 500 Ion Trap. This instrumentation helps in distinguishing various compounds from each other using a mass-to-charge ratio; this is useful for this research in detecting any possible chelate formations.

CHAPTER 3

RESULTS

Based on the intensity and shift of the peak signals in relation to the other signals, we can determine the presence of any product complexes, as well as the possible structure/coordination of the product. It can be inferred that displacement of the bulky diamine ligand typically will only occur when both coordination positions *trans* to the diamine ligand are occupied by sulfur atoms that originated from the amino acids.

However, upon conducting experiments utilizing LC/MS, the experiment involving the reaction of the platinum complex with N-AcMet suggested that a sulfur atom coordinated to the *trans* position in relation to the methyl groups on the ligand, and the oxygen atom on the same methionine coordinated to the *cis* position—this formed a S,O chelate. This was determined from the LC/MS by a peak at 473.3 mass-to-charge ratio.

Previous studies with $Pt(Me_4en)(NO_3)_2$ complexes have indicated that only one Met or N-AcMet could possibly coordinate because of steric clashes[3,4]. Together, these results have been able to suggest that two

methyl groups on one amine nitrogen are sufficient to prevent coordination of a sulfur atom from a second methionine.

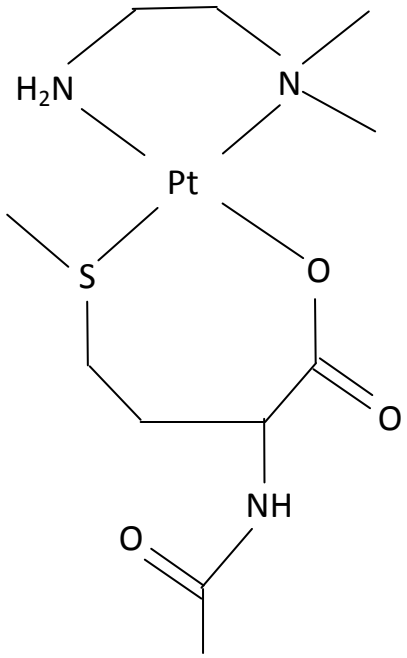

Figure 4. Structure of Pt(Me_2en)(NAcMet-S,O).

Reaction of $Pt(Me_2en)Cl_2$ with L-Methionine and N-Acetyl Methionine [1:1 ratio]:

$Pt(Me_2en)Cl_2$ was dissolved in 1mL of deuterium oxide and reacted with 4mg of L-Methionine. This reaction was observed by NMR analysis and data was collected at time intervals of 1, 4, and 24 hours.

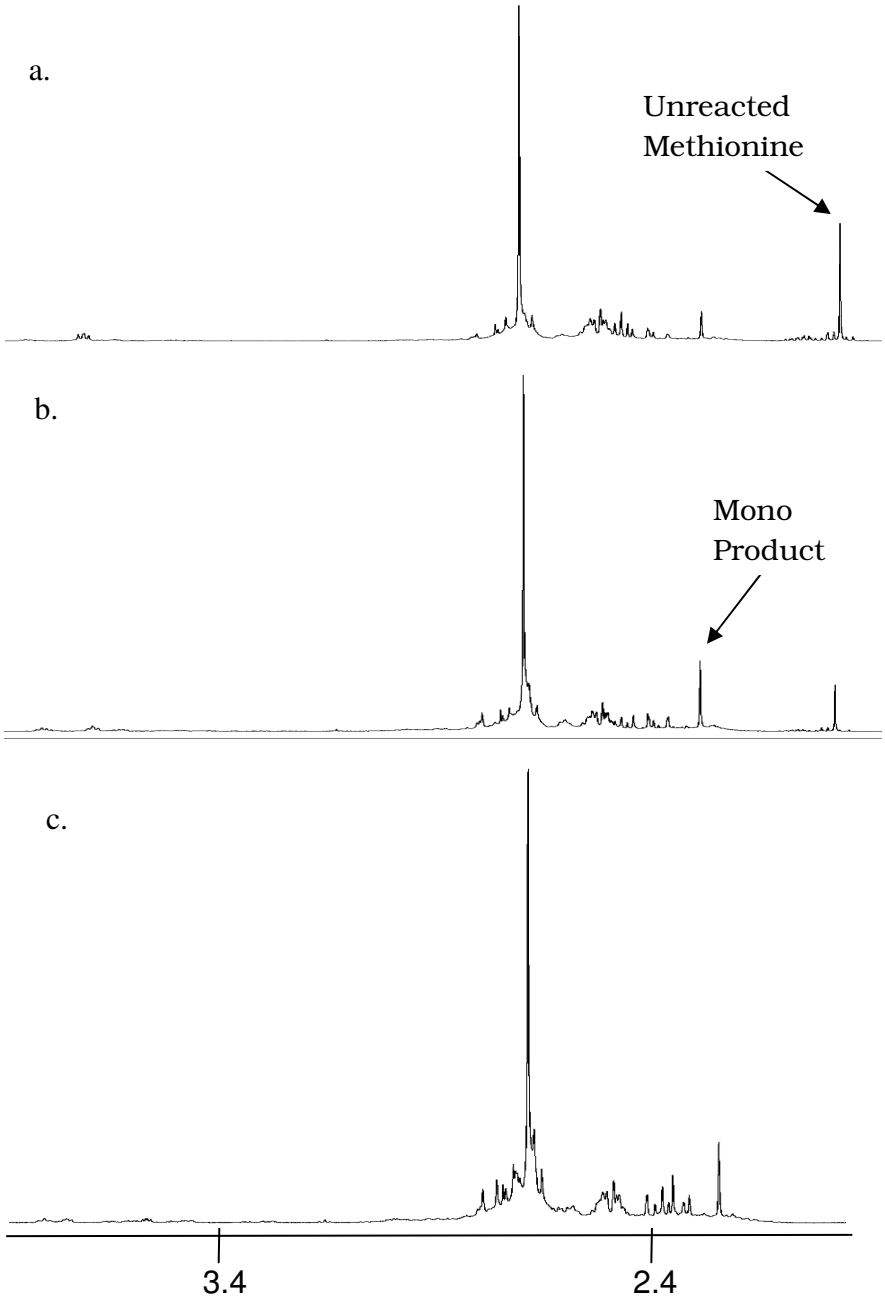

Figure 5. Reaction of Methionine and $Pt(Me_2en)Cl_2$ at a 1:1 molar ratio; monitored at (a) 1 hr (b) 4 hrs (c) 24 hrs.

$Pt(Me_2en)Cl_2$ was dissolved in 1mL of deuterium oxide and reacted with 4mg of N-Acetyl Methionine. This reaction was observed by NMR analysis and data was collected at time intervals of 1, 4, and 24 hours.

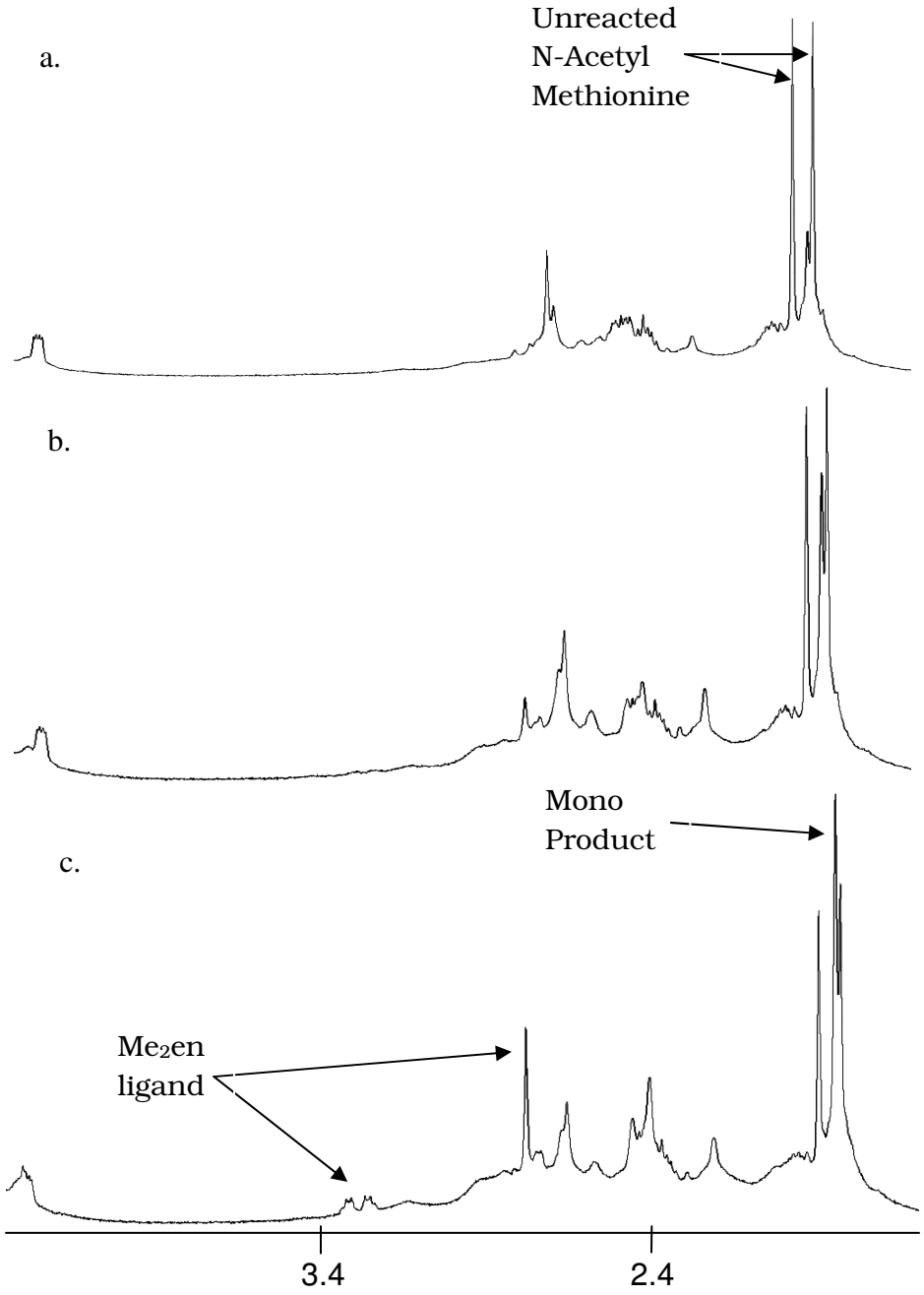

Figure 6. Reaction of N-Acetyl Methionine with $Pt(Me_2en)Cl_2$ at a 1:1 molar ratio; monitored at (a) 1 hr (b) 4 hrs (c) 24 hrs.

Reaction of $Pt(Me_2en)Cl_2$ with L-Methionine and N-Acetyl Methionine [1:2 ratio]:

$Pt(Me_2en)Cl_2$ was dissolved in 1mL of deuterium oxide and reacted with 2mg of L-Methionine. This reaction was observed by NMR analysis and data was collected at time intervals of 1, 4, and 24 hours.

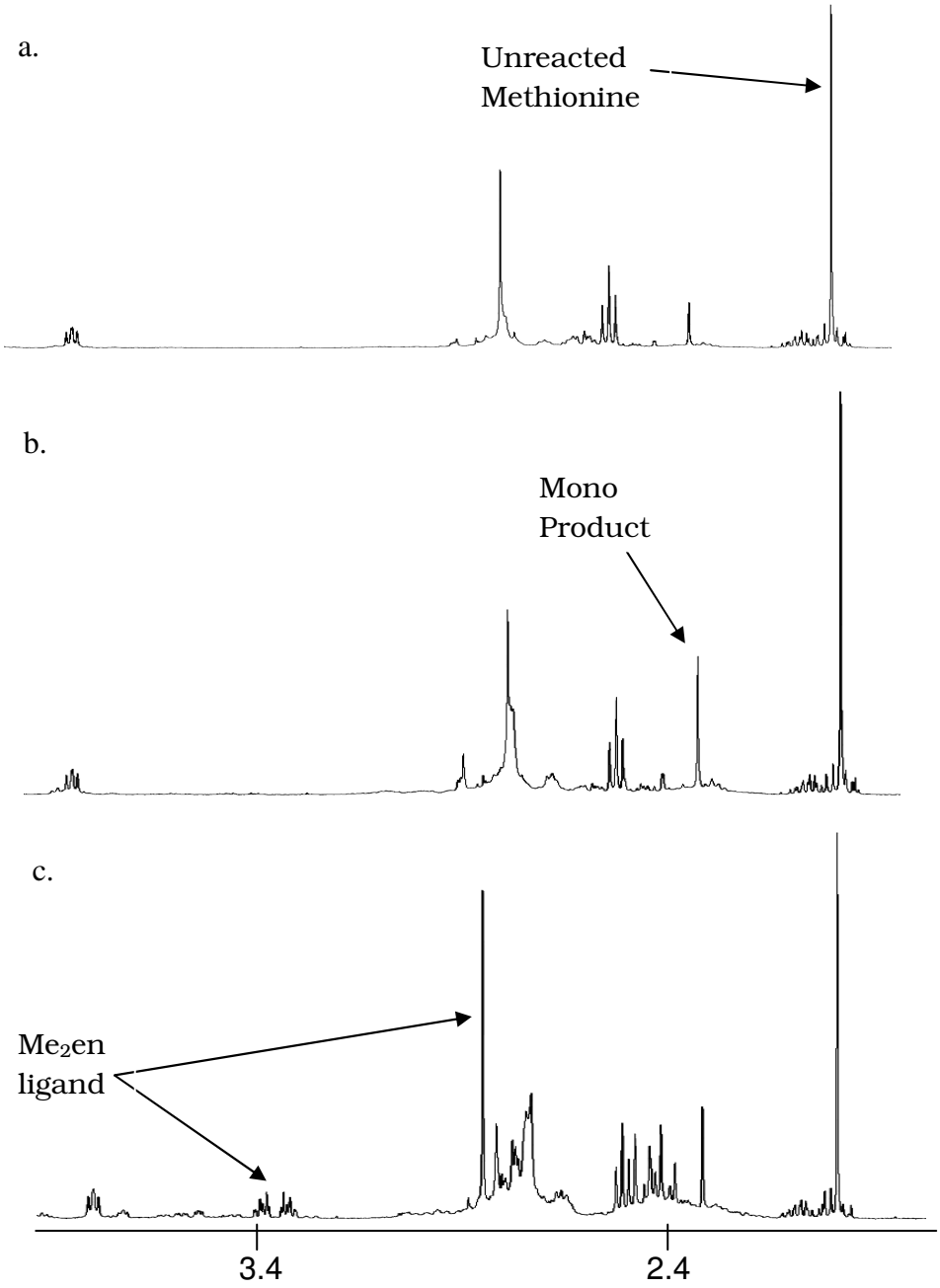

Figure 7. Reaction of L-Methionine with $Pt(Me_2en)Cl_2$ at 2:1 ratio; monitored at (a) 1hr (b) 4hrs (c) 24 hrs.

$Pt(Me_2en)Cl_2$ was dissolved in 1mL of deuterium oxide and reacted with 2mg of N-Acetyl Methionine. This reaction was observed by NMR analysis and data was collected at time intervals of 1, 4, and 24 hours.

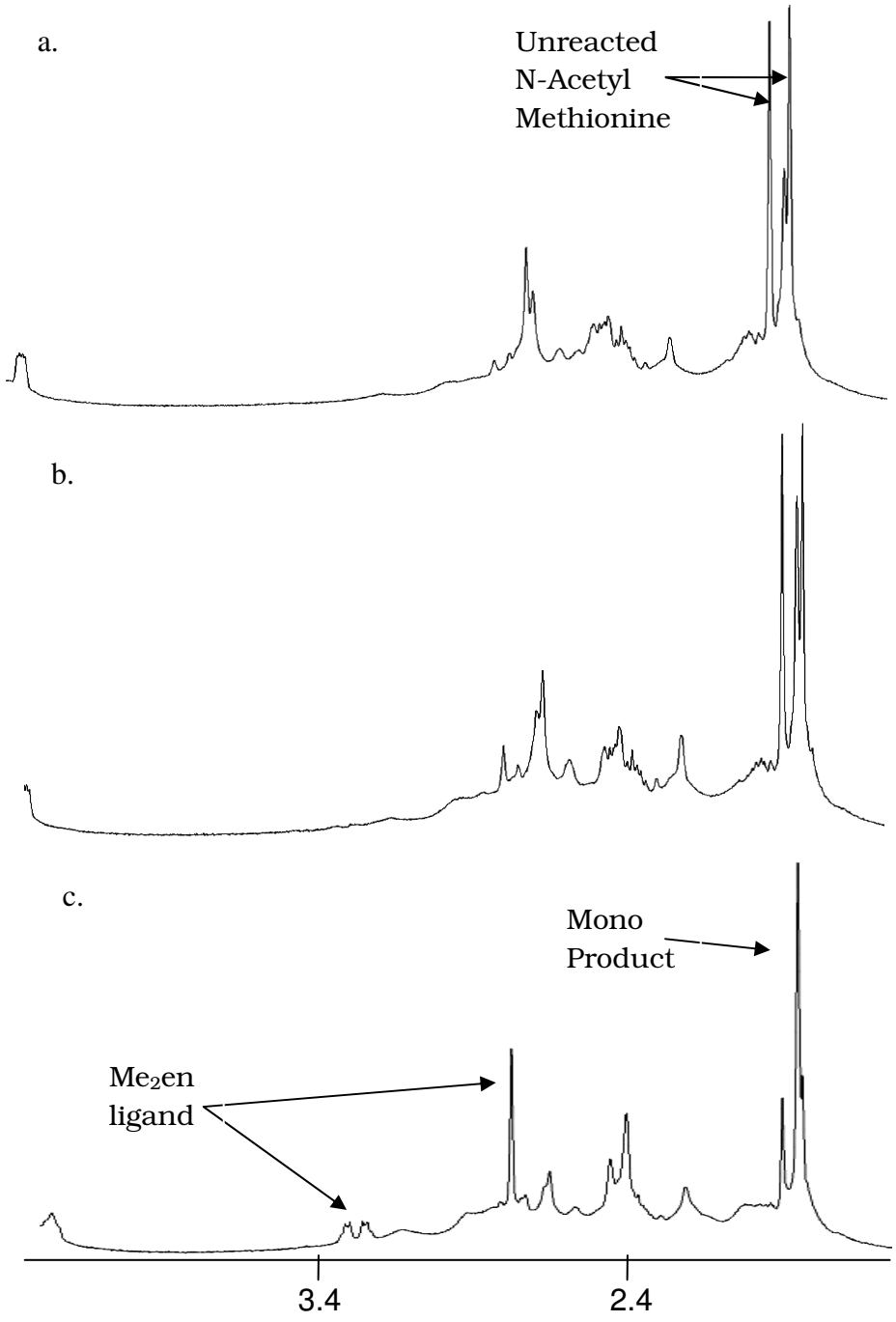

Figure 8. Reaction of N-Acetyl Methionine with $Pt(Me_2en)Cl_2$ at a 2:1 ratio; monitored at (a)1hr (b) 4hrs (c) 24 hrs.

Peak signals at ~3.0ppm and ~3.4ppm correspond to displacement of the Me_2en ligand. This is attained through the coordination of the methionine.

Reaction of 5mM $Pt(Me_2en)Cl_2$ with 10mM N-AcMet (LC/MS):

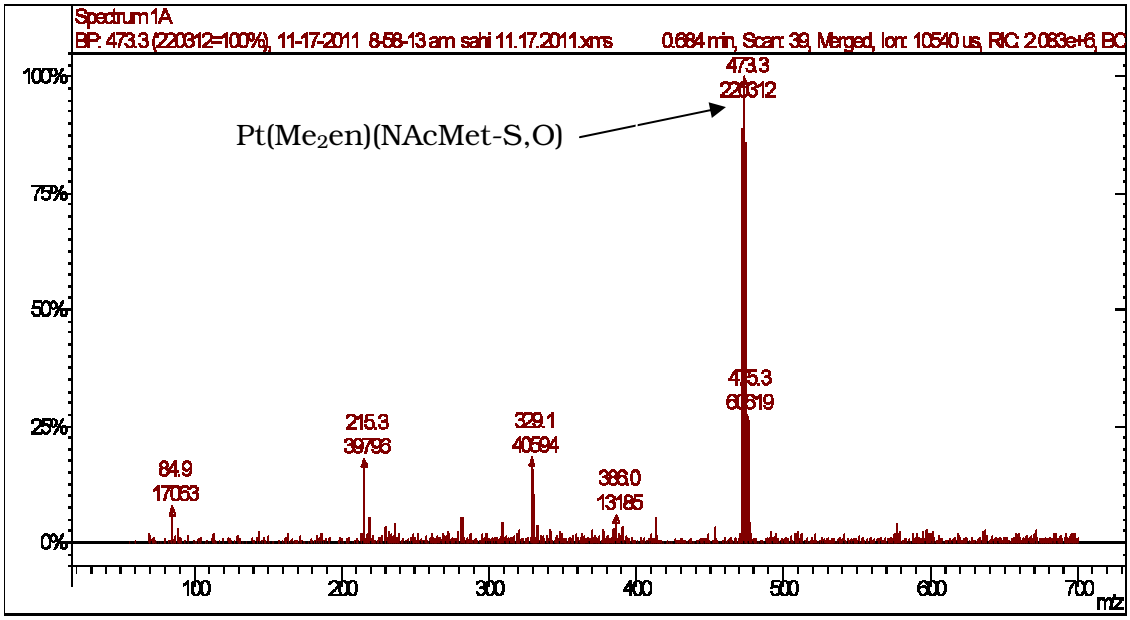

Figure 9. Mass spectrometry data of N-Acetyl Methionine with $Pt(Me_2en)Cl_2$.

CHAPTER 4

DISCUSSION

It was suspected that due to the sufficient bulk of the Me_2en ligand, there would not be coordination of a second L-Methionine or N-Acetyl Methionine to the platinum complex. Past research has shown that various bulky ligands, such as Et_2en and Me_4en, do not allow the sulfur atom of a second methionine to coordinate to the platinum complex[3-5]. The research surrounding these other bulky ligands were able to conclude that only one methionine would coordinate to the platinum complexes and result in the formation of a S,O-chelate. Hence, the objective of this research project was to determine the significance of bulkiness of this ligand and how the bulk on the platinum complex relates to the binding of methionine's found in proteins in the body. This research project, implementing NMR spectroscopy and LC/MS, allowed the retrieval of significant data leading towards the probability of the formation of a S,O-chelate.

Past research has proven that the sulfur atom of methionine will react rapidly with the platinum. So, it could be inferred that the sulfur atom would react at either the *cis* or *trans* coordination positions relative

to the nitrogen atom containing the two methyl groups. The sulfur atom has the greatest probability of coordinating to the *trans* position. The bulk of the Me_2en ligand, and specifically the bulky nitrogen atom, are showing that the sulfur cannot be in the *cis* position, and leads to the formation of [Pt(Me_2en)(N-AcMet-S,O)]. This chelate formation was verified by a peak at 473.3 mass-to-charge ratio under LC/MS analysis, as well as a doublet of doublets formation at ~5.4ppm through NMR spectroscopy. Research performed on the Et_2en and Me_4en ligand reported that the sulfur atom from methionine will coordinate to the *trans* position relative to the bulky nitrogen atom as well. However, that research also reported that the bulk of those ligands caused no coordination of another sulfur atom from methionine at the *cis* position. This research on the Me_2en ligand shows that this bulk still allows coordination of the carboxyl oxygen of N-Acetyl Methionine to the *cis* position, eventually resulting in a S,O-chelate. It is also to be noted that previous studies showed the formation of S,O-chelates involving the coordination of amino acids to platinum complexes.

The present study allowed the utilization of a platinum complex containing a non-C_2-symmetrical Me_2en ligand to determine if excessive bulk on one of the two nitrogens of the diamine ligand could inhibit coordination of the second methionine to the platinum complex and/or greatly affect the type of chelate that would form. This research helps to

understand to the basis of cleavage reactions found in proteins during the reaction of platinum complexes with methionine.

CHAPTER 5

FUTURE WORK

Further research will allow us to focus on the characterization of the products using NMR spectroscopy, high pressure liquid chromatography (HPLC), and mass spectrometry. Currently, more analysis is being done using 2D NMR and various kinetic NMR experiments. It is also essential to characterize the products formed in the reactions of $Pt(Me_2en)Cl_2$ with 5'-GMP (guanosine monophoshpate). Lastly, focus on the rates of reactions and molecular mechanics of $Pt(Me_2en)Cl_2$ with methionine and guanine targets is crucial in the continuation of this research.

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