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Synthesis and Molecular Mechanics Study of Platinum Triamine Complex with N-Acetyl-L-Methionine and Guanosine 5'monophosphate

Nitin Tanajirao Katare
Western Kentucky University, nitintanajirao.katare782@topper.wku.edu

SYNTHESIS AND MOLECULAR MECHANICS STUDY OF PLATINUM TRIAMINE COMPLEX WITH N-ACETYL-L-METHIONINE AND GUANOSINE 5'-MONOPHOSPHATE

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SYNTHESIS AND MOLECULAR MECHANICS STUDY OF PLATINUM TRIAMINE COMPLEX WITH N-ACETYL-L-METHIONINE AND GUANOSINE 5'-MONOPHOSPHATE

Date Recommended March 31, 2020

Kevin Williams
Digitally signed by Kevin Williams
DN: cn=Kevin Williams, o=Western Kentucky University, ou=Department of Chemistry, email=kevin.williams@wku.edu, c=US
Date: 2020.06.19 07:00:43 -05'00'

Dr. Kevin Williams, Director of Thesis

Lawrence Hill
Digitally signed by Lawrence Hill
Date: 2020.06.19 07:26:26 -05'00'

Dr. Lawrence Hill

B. Blairanne Williams
Digitally signed by B. Blairanne Williams
Date: 2020.06.18 15:52:18 -05'00'

Dr. Blairanne Williams

Cheryl D Davis
Digitally signed by Cheryl
Date: 2020.06.19 10:11:15 -05'00'

Dean, Graduate School Date

I dedicate my thesis to three important persons in my life-

My parents Kalpana and Tanajirao Katare, who have always believed in my dreams and provided me the best possible education.

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SYNTHESIS AND MOLECULAR MECHANICS STUDY OF PLATINUM TRIAMINE COMPLEX WITH N-ACETYL-L-METHIONINE AND GUANOSINE 5'-MONOPHOSPHATE

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Directed By: Dr Kevin Williams, Dr Blairanne Williams and Dr Lawrence Hill

Department of Chemistry Western Kentucky University

Cisplatin and its analogues have been used and tested over decades for cancer treatment, but because of the serious side effects, it is important to find ways to overcome those side effect by synthesizing a novel platinum molecule. We have synthesized triamine complex and conducted its reaction with biomolecules such as 5'GMP and Methionine in the lab as well as conducted molecular mechanics (MM). MM methods are used for predicting and designing new structures and properties, and to model geometries of small molecules. MM and dynamics calculations have been used to study the 9 Ethylguanine and N-Ac-L-methionine complexes with platinum triamine compound. The AMBER force field has been supplemented with previous modifications and has been further modified to include parameters for platinum bound to 2-(4-Methyl-1,4-diazepan-1-yl)ethanamine. By utilizing the modifications made previously to the AMBER force field, we were able to model the Chloro[2-(4-Methyl-1,4-diazepan-1-yl)ethanamine]platinum(II) complex with methionine and 9 Ethylguanine adducts. All possible rotamers were generated with the chiral centers in the molecule, minimum energy structures were calculated for all possible rotamers of target molecules. For the methionine adducts, we found that certain orientations of the methionine residue led to much lower energy structures, though no steric clashes have been observed for all rotamers, the differences in minimum energy structures of rotamers could help explain the experimental observation that an initial

methionine product can be replaced by a more stable product. We modeled 9-ethylguanine products in which 9-EtG displaced either the chloride only or both the chloride and one nitrogen atom of the triamine ligand, as experimental results suggested both types of guanine products are possible. MM studies also suggested the reason for the molecule's inclination towards the ring opening. Our synthesized platinum analogues showed high reactivity towards both the biomolecules.

CHAPTER I: Introduction

A. Cancer

The human body is made of trillions of cells, these cells grow, divide and die orderly to maintain metabolic activity. Cancer is a condition of cells in which its growth becomes unsystematic and results in the accumulation of uncontrolled grown cells. Accumulated cells tend to spread in surrounding tissues as malignant tumors. The major cause of cancer is an alteration of genes which can be a result of genetic mutations, environmental factors, unhealthy lifestyle and exposure to certain radiations.[1]

Mutations within cells' DNA prohibit the apoptosis and/or cause faster replication. The new cells formed by the replication still contain a similar mutation ultimately cause neoplasm. The neoplastic cells are considered as benign if they remain a lump of abnormal cells. However, when the mutated cells start to invade neighboring tissue, spreads through the circulatory system and develops the tumor considered as malignant cancer.[2] There are nearly 200 types of cancers have been detected, each with different metabolic functions, growth and spread rate. Some categories of cancer depend on their cell origin, for instance, carcinoma originates from epithelial cells, leukemia originates from bone-marrow.[3]

Cancer is one of the leading health problems worldwide and in the United States. It is ranked as the second most likely cause for death after heart disease in the United States. The National Center for Health Statistics estimated cancer will surpass death rates of heart disease within the next 5 years. However, cancer death rates have been declined by 22% in the last two decades. The available therapy for cancer is chemotherapy, radiation,

and surgery.[4] The resistance for drugs and cancer recurrence has been observed in a few treated patients that are mainly caused by the activation of different signaling pathways. Different therapeutic approaches and combinations are used to stop the cancer recurrence.[5]

B. Platinum Based Anticancer Drugs and Mechanism of Action

A family of platinum complex drugs represents the first line of defense for several kinds of cancer.[6] The discovery of the first platinum anticancer drug, cisplatin, was an accident. Cisplatin was synthesized by Michael Peyrone in 1845 but its anticancer activity was discovered in 1965 when Barnett Rosenberg's research team at Michigan State University found platinum complexes from platinum electrodes inhibit the binary fission in *Escherichia coli*. The interruption of cell division by platinum complex suggested its possible use as anti-carcinogenic agent.[7] Later on, cisplatin is considered as a first-generation anticancer platinum complex which was most prominent against testicular and ovarian cancer. However, its side effects such as nephrotoxicity, neurotoxicity, and development of resistance limit its use.[8]

Limitation of cisplatin resulted in several platinum analog syntheses, among which FDA approved drugs are cisplatin, carboplatin, and oxaliplatin (Figure 1).[9] Carboplatin,

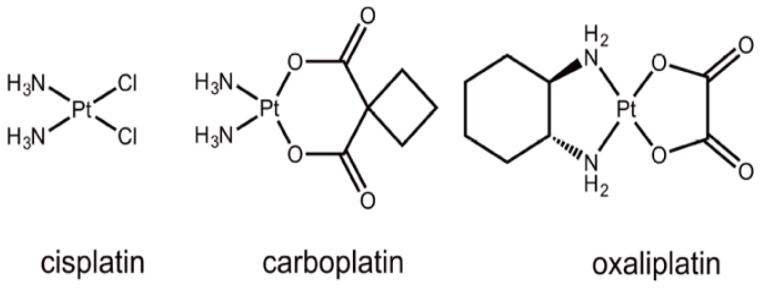

Figure 1: Chemical Structures of US FDA approved platinum complexes (Adapted from ref. 9)

Cis-Diammine(1,1-cyclobutandicarboxylato)platinum(II), known as second-generation platinum compound, was discovered in the 1980s and approved by US FDA in 2003. The carboplatin showed its efficacy against the lung and ovarian cancer. However, it showed a similar side effect as cisplatin such as emetogenesis, nephrotoxicity, and neurotoxicity.[10] Moreover, to reduce the side effects the several synthetic attempts were made. Among those, Oxaliplatin was approved for the treatment of colorectal cancer. It was approved by the US FDA in 2002. In Oxaliplatin, single bidentate ligand replaced two ammine ligands. It has a different spectrum of activity than cisplatin, however, its clinical success was limited.[11]

Platinum based drugs act by two modes of cell death, necrosis, and apoptosis. Necrosis is characterized by cytosolic swelling, whereas, apoptosis is a condensation of chromatin, DNA fragmentation, and shrinkage of a cell. Apoptosis occurs at a low concentration of platinum drugs and necrosis at high concentrations. However, the major neoplastic activity of platinum complexes leads by apoptosis.[12] Platinum drugs act by binding to DNA at N7 position of 5'-GMP, causing a cytotoxic lesion. Bonding with DNA can be monofunctional or bifunctional (Figure 2).

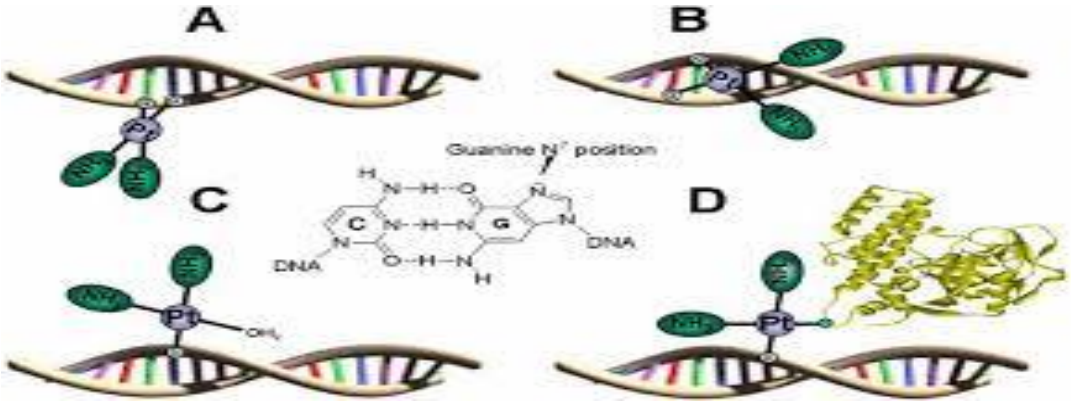

Figure 2: Binding of platinum complex with guanine at N7 position of 5'-GMP; A) Intra-strand cross-link B) Inter-strand cross-link C) Mono-functional adduct D) Protein DNA cross link (Adapted from ref. 13)

Cisplatin, carboplatin, and oxaliplatin abundantly show bifunctional binding where two leaving groups interact with two N7 positions of 5'-GMP. It can form 1,2-intrastand cross-link, inter-strand cross link or protein-DNA cross link.[13] These Platinum-DNA linkage leads to the various responses from cells like replication arrest, transcription inhibition, cell-cycle arrest, and apoptosis.[14] Platinum complexes interact with different proteins, which may result in toxic side effects or the development of resistance in platinum based chemotherapy.[15] The uptake of platinum based-complexes, their intracellular transport, and efflux play indispensable roles in the biochemical mechanism of platinum complexes. These factors determine the extent of the effect of platinum based drugs on the cell.[16]

C. Platinum Complexes: Side Effects and Development of Resistance

Platinum complexes can kill the cancer cells, but its cytotoxicity affects normal cells as well. While being transported, these complexes lead to toxic effects by interfering with proteins and by interfering in multiple signaling pathways. General cell-damaging effects cause nausea and vomiting, decreased blood cell and platelet count, decreased response to infection. Whereas its severe side effects include kidney damage, hearing loss, and damage of neurons.[17]

Cisplatin and its analogs experience resistance caused by various mechanisms, which is a major complication in cancer chemotherapy. It accounts for the major failure in curing cancer patients. Development of resistant tumor cells occurs because of one or more reasons which are increased repair of cisplatin adducts, reduced cell accumulation by the increase in efflux, increased cisplatin adducts tolerance and failure of apoptotic pathways

and inactivation of cisplatin by glutathione, metallothionein or other sulfur-containing molecules.[18,19]

D. Molecular Mechanics and Molecular Dynamics

The constant work is being done to synthesize new platinum complexes with less toxicity and higher efficacy. The computational molecular mechanics modeling can be embedded in designing of these compounds and study of these complexes with proteins and DNA adduct. The modeling can aid to narrow down the potentially useful compounds and save time, money and resources which would have been used in trials. Moreover, molecular modeling calculations can be used to interpret experimental data.[20] The molecular modeling is based on the consideration that all the molecular properties such as stabilities and reactivities are based on molecular structure. Before carrying out any computational study on molecular properties a molecular model needs to be established. The basis of the model is taking estimated account of all the forces between atoms, and calculate them using a mechanical approach.[21]

The purpose of molecular modeling is to find the minimum energy structure of a molecule. The minimum energy will be achieved when a slight alteration in conformation does not lead to lowering the total energy of the system. Molecular mechanics use an empirical, algebraic, atomistic energy function for a chemical system to find a minimum energy structure. This is done by a force field, a programmed parameter set. Force field is used to calculate the molecular system's total energy as a sum of bonded and non-bonded terms. Bonded energy accounts for the bond length, bond angle, and dihedral. Whereas, the non-bonded energy is a summation of electrostatic and Van der Waals forces. The modeling program uses the force field to find a minimum energy conformation. These

force fields vary depending on the empirical formula, energy functional form, and simulation target. For instance, the optimized potential for liquid simulations (OPLS) force field was developed for the simulation of organic liquids and proteins. Some other examples of force field are AMBER and MMFF.[22] Molecular dynamics were first developed in the late 1970s to reduce the computational complexity. It is a technique where atoms are allowed to interact within a timeframe and energy is calculated by solving Newton's laws of motion for that system. Force fields describe the contribution of various atomic forces that regulate molecular dynamics.[23]

Weiner developed the AMBER (Assisted Model Building with Energy Refinement) force field to model nucleic acids. AMBER is a collection of codes designed to work together, and its force field is widely used for biomolecular simulation.[24] AMBER has been used to model metal complexes, hence the research group at Emory University in 1994 selected the AMBER to develop an acceptable force field for DNA-Platinum adduct. For the optimization of the force field, new atom types were created. The crystallographic information of platinum complexes was used to select the equilibrium values for the force field and to asses the scale for the bending and torsional angle of these complexes. Six new atom types were used to describe the right angle between *cis* and coaxial angle between *trans.* These new atom type used to implement the square planar geometry of four platinum complex.[25]

The introduced force field with new atom types of platinum compounds included changes in bond length, angle bend, torsional angle deformation, out-of-plane deformation, Van der Waals radius for platinum, hydrogen bonding parameters and atomic charges. Some of the changes in parameters are adopted and some are the result of new calculations and

experimental data. The AMBER was initially optimized for platinum by Yao *et al* but my research group further added parameters for chlorine and methionine bound to the platinum atom.[26,27] With this modified force field for platinum complex and DNA, the more accurate results can be achieved.[28]

E. Literature Review and Previous Lab Research

Side effects and drug resistance are the prime factors for a lack of clinical success with platinum compounds. To overcome these challenges new compounds have been synthesized with adding bulky ligands, for example, phenanthriplatin, a derivative of pyriplatin. It shows higher toxicity than cisplatin, the explanation for the higher toxic effect is the bulky ligand protects the platinum center from deactivation by non-nucleoside nucleophiles such as cysteine and glutathione. The bulky group adds the lipophilicity which also aids platinum complexes in crossing the cellular lipid bilayer.[29]

The choice of rational design of bidentate or tridentate platinum(II) complexes by adding bulky steric groups provides greater efficacy. These ligands add stability to cationic platinum complexes by decreasing undesired substitution reactions.[30] Various approaches can be used to discover promising platinum complexes, for instance, Cis-methylpyridine dichloroplatinum(II) was designed to develop steric hindrance with glutathione and other cellular thiols which intercept and sequester platinum drugs before they can reach to the genome. This new approach has resulted in improved in vitro activity in compare to cisplatin.[31]

In the Williams lab, we mainly focus on the study of the binding patterns and affinities of platinum complexes with protein and DNA nucleobases. The platinum drug toxicity is

known due to its binding to proteins, specifically, methionine and cysteine residues. Research shows that sulfur atom present in methionine and cysteine is the major target of platinum but N7 position of 5'-GMP is a competitor to be attached by platinum. When both biomolecules compete for the one coordination site at platinum complex co-ordination of methionine usually occur first and eventually replaced by the 5'-GMP.[32]

In one project, it was hypothesized that the bulky amine ligands can lead to a different level of steric clashes for 5'-GMP and methionine. This can be confirmed with the molecular mechanics' calculation for the stabilities of modeled platinum complexes with these biomolecules. Results suggested that $[Pt(Me_4en)(D_2O)_2]^{2+}$ does not form 2:1 methionine to platinum complex stoichiometry but can form 2:1 complex with guanine.[33] In contrast, $[Pt(Me_5dien)(D_2O)_2]^{2+}$ showed faster reactivity with 5'-GMP than with N-Acetylmethionine. This was the result of higher steric clashes between methyl groups of (Me_5dien) and N-Acetylmethionine.[34]

F. Our Approach

We designed and synthesized the platinum compound to study the bulky ligand effect on the reactivity of the platinum complex with biomolecules. The idea behind the conducted research was to study the reactivity of complex towards relevant biomolecules such as methionine and guanine. We chose N-Acetylmethionine because methionine is a major protein target and proteins play a major role in the influx and efflux of the compound whereas the guanine base on DNA is the primary target for platinum complexes to cause cytotoxicity.

We have also conducted molecular mechanics study on the platinum triamine complex previously synthesized in our lab. The plan was to model the platinum complex with methionine and guanine to find out the minimum energy structures, to support the experimental data. The complex was synthesized and analyzed by the ^{1}H and ^{195}Pt NMR spectroscopies and mass spectrometry.

CHAPTER II: Synthesis and Analysis of Platinum Triamine Complex

1. Methods and Materials

1.1 Reagents

The following chemicals were used to synthesize and analyze the platinum complex: Potassium tetrachloroplatinate(II) (K_2PtCl_4), 1-[2-(Dimethylamino)ethyl]piperazine, 99.9% Deuterium oxide (D_2O), Guanosine 5'-monophosphate, N-Acetyl-L-methionine, Hydrochloric acid (HCl).

1.2 Synthesis of Platinum Complex

500 mg potassium tetrachloroplatinate was mixed, in a round bottom flask, with 15 mL of DI water and 500 mg of 1-[2-(Dimethylamino)ethyl]piperazine. Hydrochloric acid was used to adjust the pH to 3. The reaction was stirred under the heat and refluxed for 20 minutes. The white precipitate was observed which converted to black color within the next 10 minutes. The black color precipitate was removed by filtration. The heat was continued for the next 30 minutes with reflux and stirring. When the peach color precipitate appeared, the flask kept on stirring overnight. The precipitate was collected with gravity-filtration and air-dried. A similar synthesis was carried out at pH 2 and pH 5 while keeping all the conditions the same as the above stated procedure. The filtered compound was scraped off from the filter paper and stored in Eppendorf safe lock tube.

1.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

The proton NMR spectra were obtained on a JOEL Eclipse 500 MHz Nuclear Magnetic Resonance Spectrometer. The prepared sample NMR tubes were loaded into the sample

holder by removing the reference tube. Samples were monitored by running several single pule scans. After running the sample by giving commands on NMR software JOEL Delta v5.0.4, the sample was unloaded, and the reference solution tube was reloaded.

1.4 Preparation of Samples to Obtain ^{1}H NMR

2 mg of the synthesized compound was dissolved in either 500 µL of deuterium oxide or dimethyl sulfoxide to obtain the ^{1}H NMR. For the reaction of the compound with 5'GMP or N-Acetyl-L-methionine, the 1:1 proportion were used in 700 µL of solvent in 5 mm NMR tubes.

2. RESULTS

2.1 Characterization of the platinum complexes $[Pt(L)Cl]^+$

Synthesis of platinum complexes resulted in 1 intermediate and the three different color complexes at three different pH. Color of intermediate was white, final product color at pH 2, 3 and 5 was blue, peach and pale yellow respectively. The name of proposed synthesized compound is Chloro{1-[2-(dimethylamino)ethyl]piperazine} platinum(II) chloride (figure 3). The structure of complex shows there are three nitrogen atoms, six

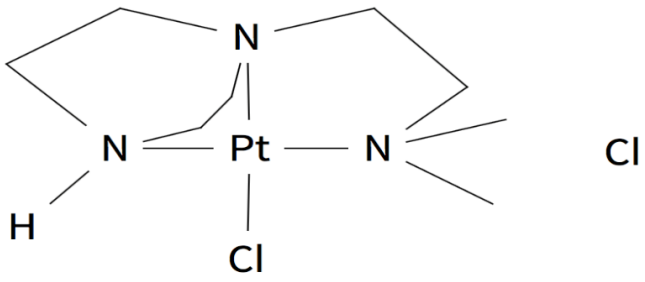

Figure 3: Proposed chemical structure of synthesized platinum compound

membered heterocyclic ring and a chloride attached to the platinum center. Platinum, three nitrogen and the chloride lie within the same geometric plane.

A sample for analysis was prepared with 2.0 mg complex and 700 µL of D_2O. The ^1H NMR of platinum complexes and the intermediate were collected. All the spectra of synthesized compounds show a sharp signal between 2.9 to 2.92 ppm which indicates platinum triamine complex (figure 4). The compound has two methyl group which are responsible for a sharp signal in the ^1H NMR that will always appear regardless of pH

change. This signal is a key correlation peak of Chloro{1-[2 (dimethylamino)ethyl]piperazine} platinum(II) chloride.

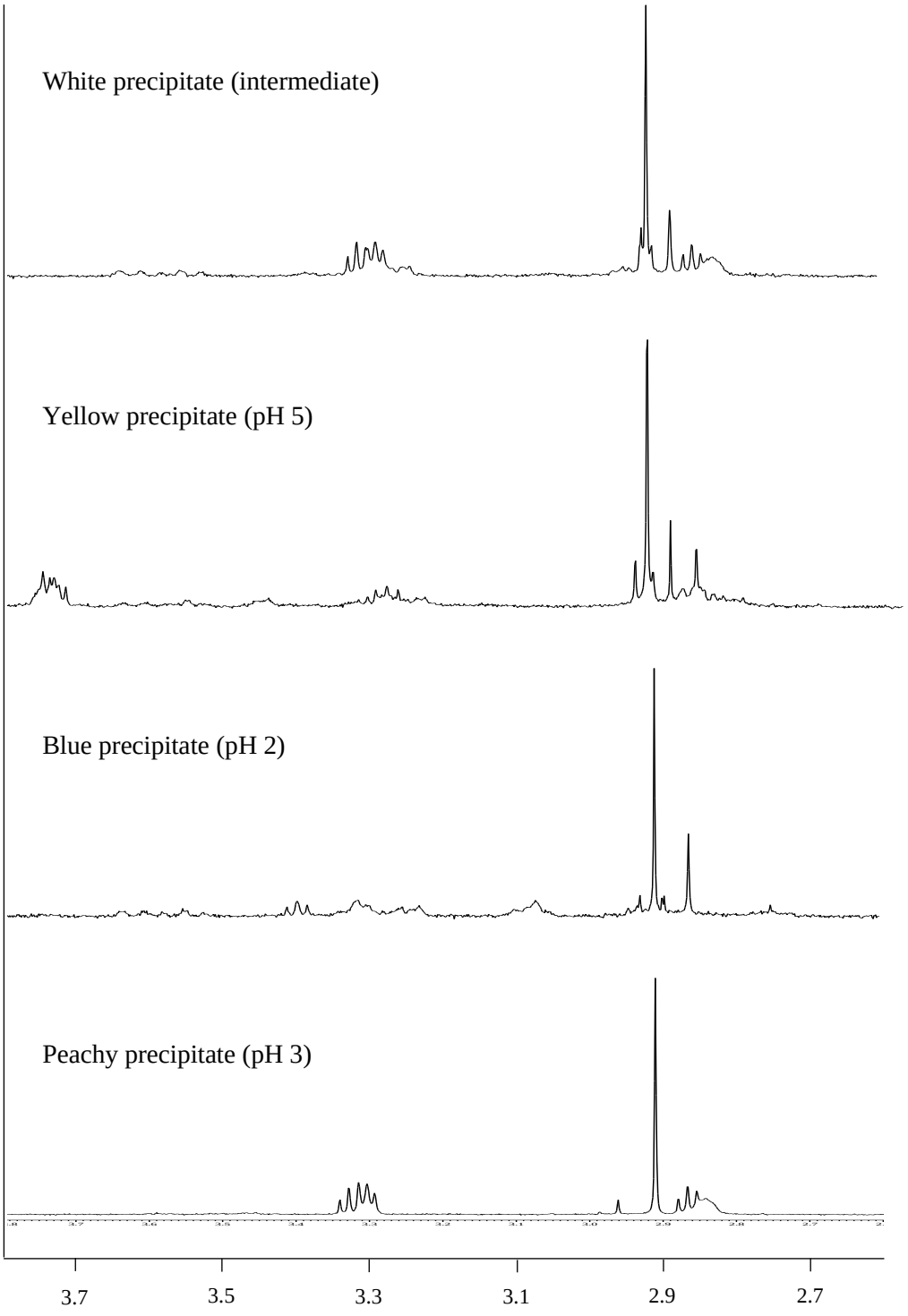

Figure 4: Proton NMR of intermediate and synthesized complexes and intermediate

2.2 ^{1}H NMR spectrum of platinum complexes with Guanosine 5’-monophosphate

Different samples were prepared with all four precipitates for analysis. 2 mg of 5’-GMP in 700 µL of D_2O to with precipitates in 1:1 ratio to check proton NMR at particular intervals to examine product formation. Figure 5 shows the ^{1}H NMR of white precipitate

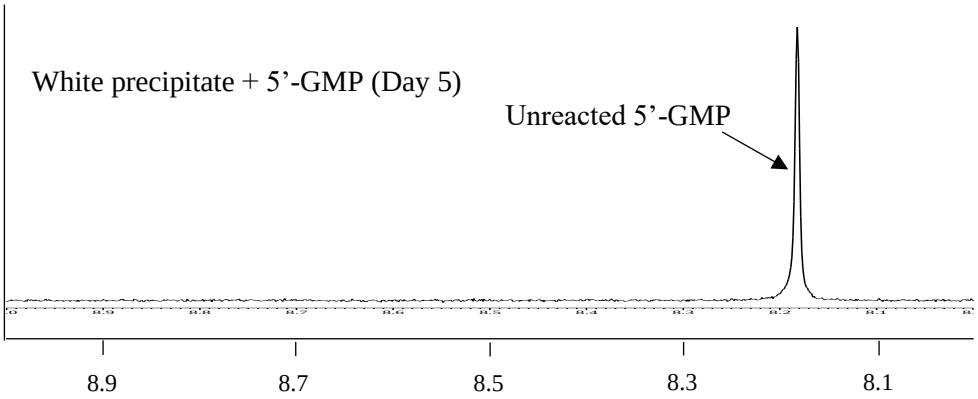

Figure 5: Proton NMR of intermediate white precipitate with 5'-GMP

which is intermediate with the 5’-GMP. The peak between 8.1 and 8.2 ppm indicates the H8 atom of 5’-GMP. The spectra were collected after every 24 hours, no product formation was observed with the intermediate after 5 days.

The ^{1}H NMR of the blue product shows no reaction within the 30 minutes of 5’-GMP addition, although it shows one prominent product peak at 8.4 ppm along with very small budding peaks in between 8.2 to 8.7 ppm after 24 hours (Figure 6). On the sixth day, several peaks have been observed between 8.3 to 9 ppm. The 5’-GMP peaks were observed throughout the analysis of blue product at all time intervals.

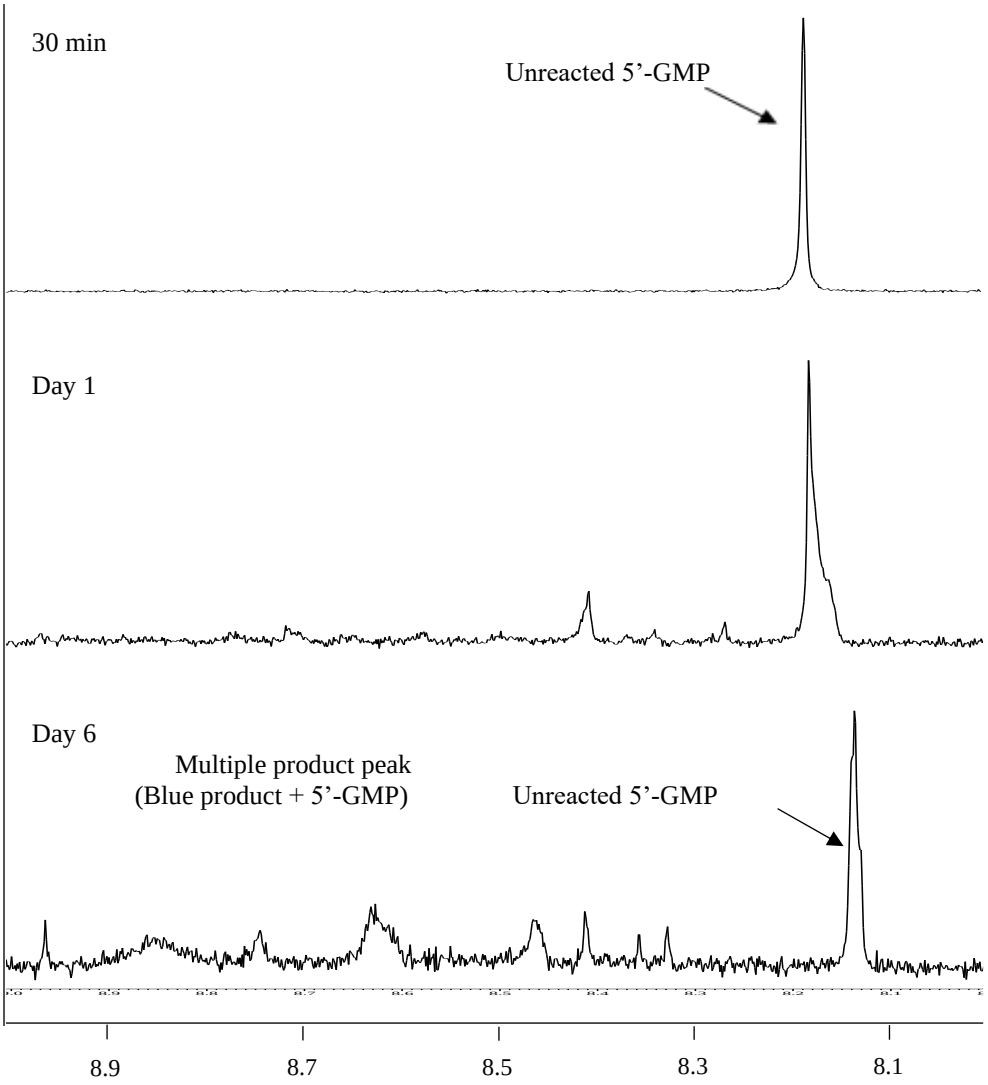

Figure 6: Proton NMR of Blue product + 5'-GMP at particular time intervals

Synthesis conducted at pH 5 resulted in the pale yellow color compound which was analyzed for its reactivity towards the 5’-GMP. The equivalent amount of both compounds was dissolved in 700 µL of D_2O and checked for proton NMR with particular time intervals. No product formation was observed within 30 minutes of addition (Figure 7). After the 24 hours, several peaks were observed between 8.3 to 9 ppm. After 10 days the signal from H8 of 5’-GMP was very small compared to the product signals.

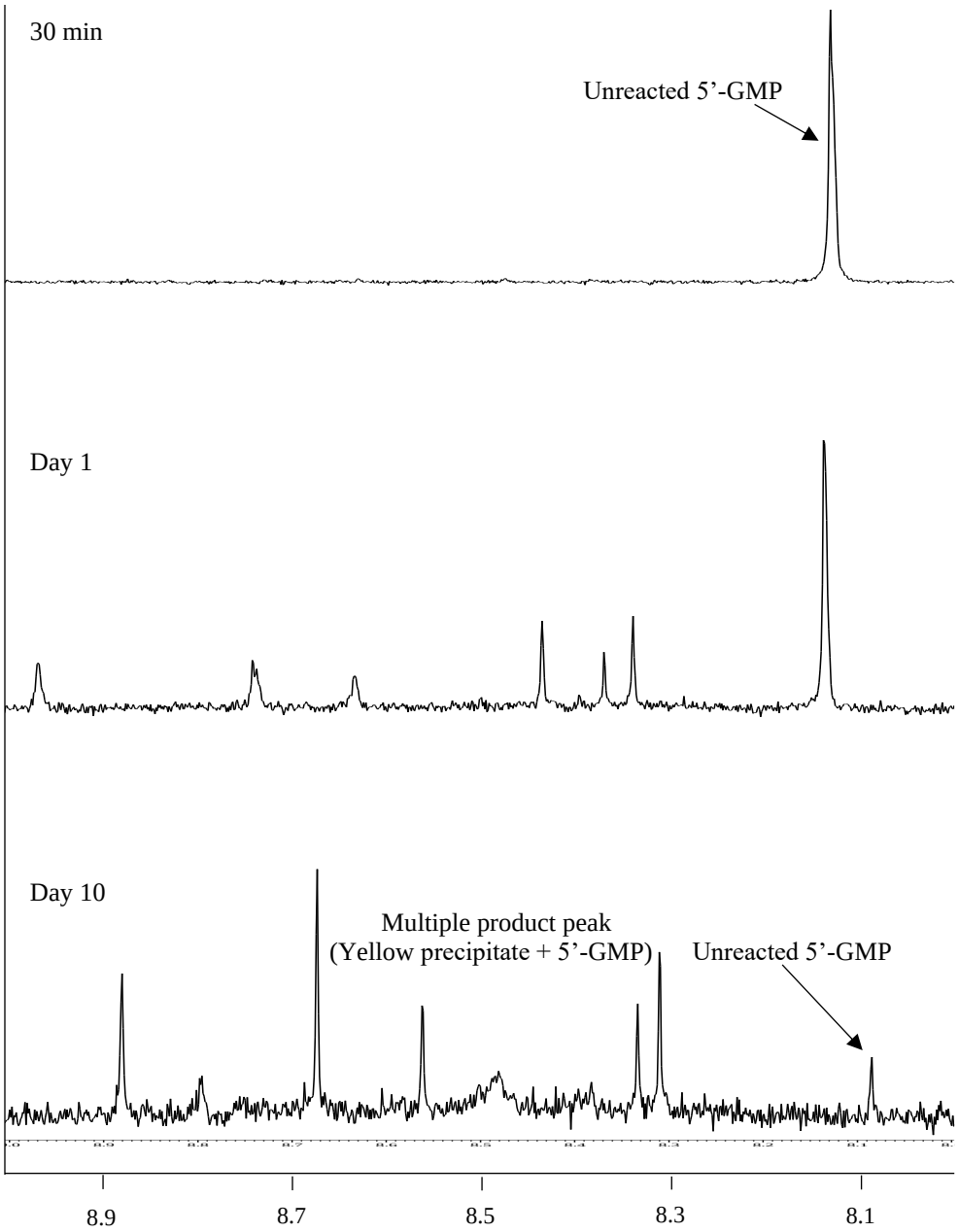

Figure 7: Proton NMR of yellow precipitate + 5'-GMP at particular time intervals

Synthesis of triamine complex at pH 3 resulted in a peach color product which was reacted with 5'-GMP in 1:1 ratio in D_2O. This complex showed high reactivity, and a couple of product peaks were observed within 30 minutes (Figure 8). After 24 hours all the 5'-GMP was consumed and several product peaks were observed. Additional 5'-GMP were added as the same amount of first addition. On day 6 the three prominent product

peaks were observed which eventually goes off by leaving a single peak at 8.52 ppm. The product peak on day 10 was larger than the 5'-GMP peak.

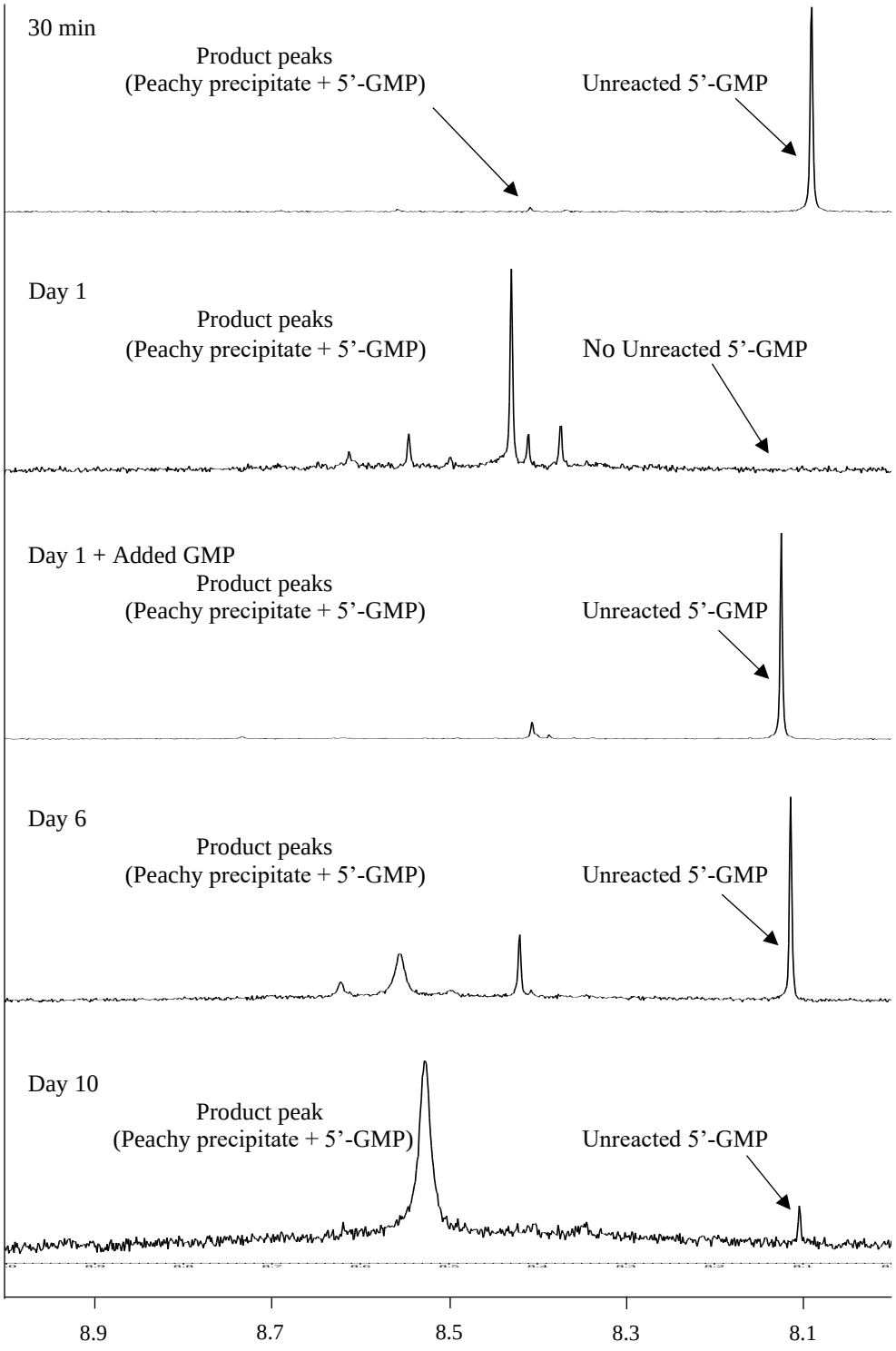

Figure 8: Proton NMR of peach color product with 5'-GMP at particular time intervals

2.3 ^{1}H NMR spectrum of $[Pt(L)Cl]^{+}$ complexes with N-AcMet

A sample of the yellow complex with an equivalent amount of N-acetylmethionine (N-AcMet) was prepared in D_2O. ^{1}H NMR was collected to analyze the reactivity of complex towards N-AcMet. The peak at 2.03 and 2.1 ppm represent the unreacted N-AcMet. Whereas, peaks at 2.04 and 2.61 ppm correspond to product (Figure 9). The product peak of complex and N-AcMet grows with time.

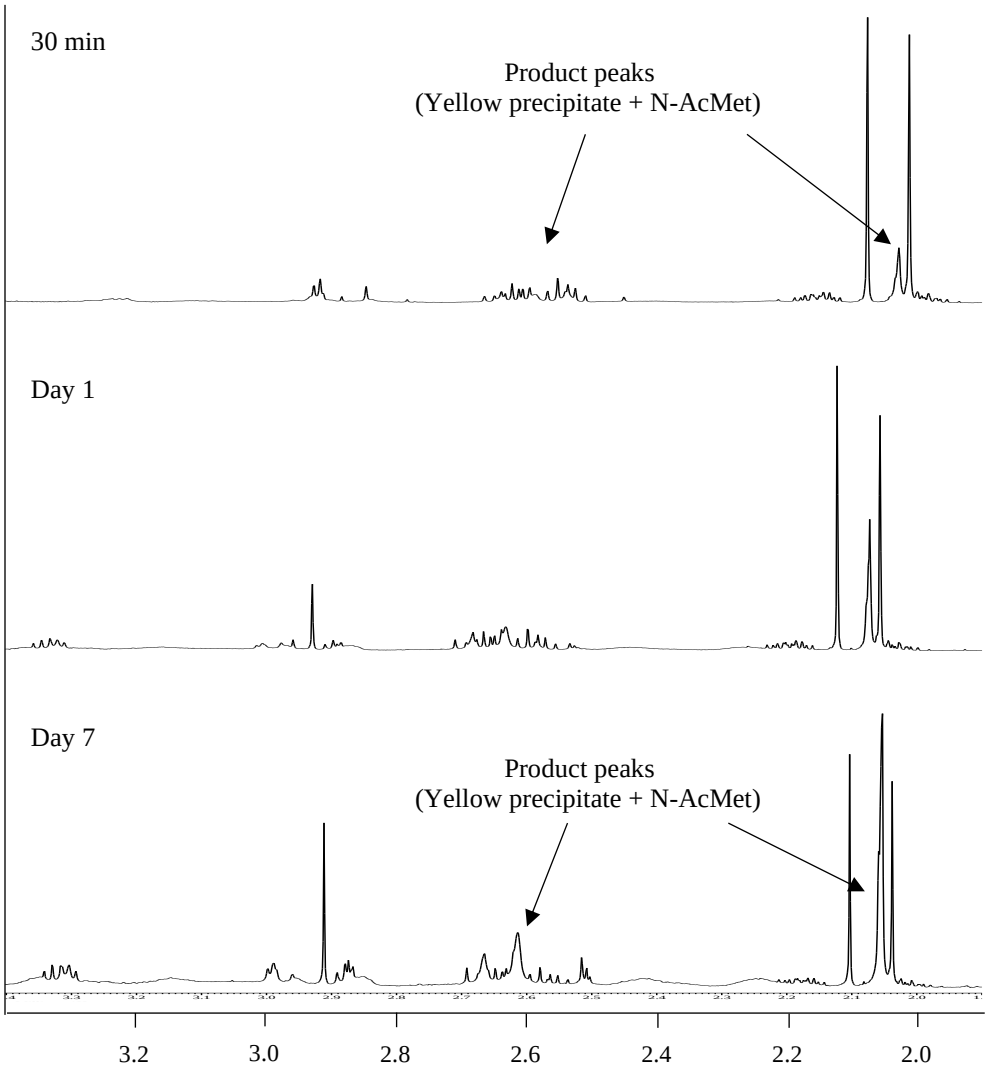

Figure 9: ^{1}H NMR of yellow platinum complex with N-AcMet at a particular time interval

Similarly, the reactivity of the peach color triamine complex was checked with N-AcMet. The proton NMR pattern for the complex was similar to the yellow color complex. It shows the product peak at 2.05 and 2.61 ppm (Figure 10). Signals from unreacted N-AcMet decreases in size as the platinum complex reacts to form a product.

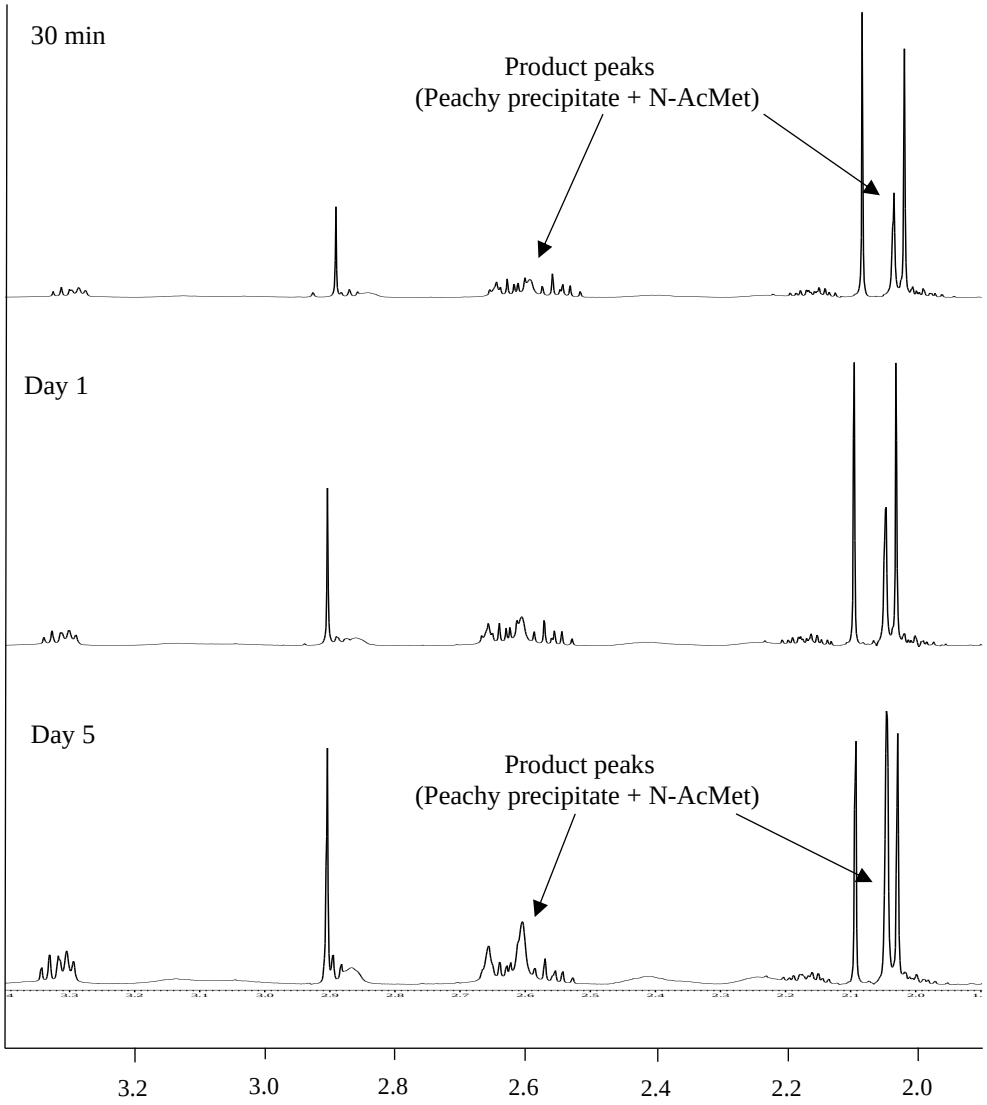

Figure 10: ^{1}H NMR of Peach color platinum complex with N-AcMet at a particular time interval

3. DISCUSSION

Since the finding of the anticancer property of the platinum compound, various analogs of platinum were synthesized with different ligand attached to the platinum center. Chloride is the preferred leaving group in a platinum complex which causes the binding of the platinum compound with biological targets.[30] Phenanthriplatin is a prototype of platinum compounds, with three amine groups attached to the platinum center, which shows higher cytotoxicity than the cisplatin. The hydrophobic nature of amine ligands aids in the biological transportation of compounds to the target site compared to cisplatin. Platinum compounds have a high affinity towards the sulfur-containing proteins than nucleobases. However, this preference can be reversed with the strong inter ligand strain or bulkiness of amine ligand.[9]

The precise mechanism of platinum compound transportation and the source of the cellular drug resistance is unclear, it is important to continually investigate various platinum derivatives.[35] Based on the previous research we chose the bulky ligand for our platinum complex synthesis. The synthesis conducted at different pH resulted in different products including the intermediate, which at first, we suspected as our product. The compounds had different color: pH 2 synthesis ended up with blue color, pH 3 peach color, pH 5 with the yellow color compound. To analyze these complexes, we gathered the ^{1}H NMR spectrum. The spectrum had signs of ligand-platinum coordination but was not much of use as all the spectrum almost had a similar pattern of signals.

We had two ways to analyze the compounds, explore the structure of each compound and then check the reactivity with the biomolecules or check the reactivity first and focus on

the compounds which show significant results. We went for the latter approach as it can save our time to be more productive.

Firstly, we reacted to our intermediate (white precipitate) with the 5'-GMP and collected proton NMR for 5 consecutive days. We chose 5'-GMP because it is a target nucleobase of platinum complexes. No product peak has appeared after allowing 5 days of reaction time. It indicated that this is not the right anticancer candidate. Secondly, we reacted the compound synthesized at pH 2 (blue precipitate) with 5'-GMP. As the reaction proceeded the several product peaks have been appeared in between 8.3 to 9 ppm but no significant consumption of 5'-GMP has been observed. Thirdly, the synthesis at pH 5 (yellow color) was reacted with the 5'-GMP and ^{1}H NMR spectrum were analyzed. The spectrum showed several product peaks after 24 hours of reaction which was promising as it indicates the reaction proceeding fast. On the tenth day unreacted 5'-GMP peak was very small compared to the product peaks. The monofunctional product, ideally, should have only 1 to 2 product peaks. These multiple product peaks suggest the possibility of bis-product formation. These results can be correlated with the study conducted by Anddreponte *et al*. which revealed that the bulk of ligand can cause Pt-N bond weakening to such extent which leads to the conversion of tridentate ligand to the bi-dentate ligand.

Lastly, the synthesis at pH 3 (peach color) was analyzed for its activity towards 5'-GMP. This compound showed product peak within 30 minutes after the addition of product and 5'-GMP in D_2O. After 24 hours, there was no peak for unreacted 5'-GMP; this suggests the compound completely reacted with the 5'-GMP. The more 5'-GMP was added which was equivalent to first addition, now the ratio of compound to 5'-GMP was 1:2. The proton NMR showed that there were three product signals eventually two of them

reduced and the only one signal was present. The product signal was much larger than the unreacted 5'-GMP which indicates high reactivity of the compound with 5'-GMP.

The synthesis at pH 3 and pH 5 showed promising results, hence we conducted their reactivity with the N-AcMet, a key protein binding site of Ctr1. This plays a major part in cellular uptake and efflux of the platinum complex. The products reacted with the N-AcMet and the proton NMR was obtained at specific intervals. Both the products reacted with N-AcMet and produced two product peak which was grown with time. Considering a mono-product should have only 1 to 2 product peak we can say the N-AcMet was able to replace the chloride ion and coordinated to platinum center. No signals have appeared which can suggest the replacement of one of the nitrogen, coordinated with platinum, by N-AcMet.

In summary, the platinum triamine complex synthesized at pH 3 and pH 5 showed the significant reactivity with 5'-GMP and the N-AcMet. These complexes further can be studied to explore the reason behind mono and bis product formation with 5'-GMP by complex synthesized at pH 3 and pH 5 respectively. The other interesting study will be a competition reaction of 5'-GMP and N-AcMet, to see which one reacts faster with these synthesized complexes. Furthermore, these compounds can be used in a biological assay to study uptake and the cytotoxicity against the cancer cell lines.

CHAPTER III. Molecular Mechanics and Dynamics Calculation of Platinum Triamine Complex and its Product with Biologically Relevant Molecules

1. Experimental Procedure

The modified AMBER89 force field was used in this study, which includes the new molecular types suitable for platinum complex study. Preexisting parameters were used for all the calculations from the published research articles by Williams and Yao research groups. These parameters include the bond lengths for bindings of guanine and methionine to the platinum.[25,35]

Molecular mechanics (MM) calculations were performed on the previously synthesized platinum triamine complex by our research group.[36] All MM calculations performed using HyperChem 7 program by Hypercube, Incorporated on a Dell OptiPlex computer running Windows 7. Firstly, the structure of the Chloro[2-(4-methyl-1,4-diazepan-1-yl)ethanamine] platinum(II) ($[Pt(L)Cl]^+$, where L = 2-(4-Methyl-1,4-diazepan-1-yl)ethanamine), platinum complex with guanine and methionine were constructed. Secondly, atomic charge modification was calculated. Atom directly connected to platinum from the ligand, guanine or methionine were categorized as primary, atoms connected to primary labeled as secondary and atoms connected to secondary considered as tertiary. The charge was distributed to atoms in a 10:3:1 ratio. The geometric optimization was performed on the models until it reaches its minimum energy.

All possible conformers were created before structures subject to energy minimization. Molecular dynamics were typically run at a simulated temperature of 300 Kelvin for 250 picoseconds. Structures were saved every 1 picosecond, and all the 250 structures were

subjected to 1000 cycles of steepest descents and 10,000 cycles of Fletcher-Reeves conjugate gradient minimization or until the gradient was <0.01 Kcal/mol Å. Lastly, the log files were created to report the strain energies of these minimized molecules. The file will be generated showing all the computations performed on the molecules. The strain energies of the conformers calculated by summing up the bond, angle, dihedral and, Van der Waals energies and reported into the log file.

2) Results

2.1 Minimum energy structure of complex

The minimum energy structure for the complex was determined by the modified AMBER force field after the modification of charges on the ligand (Figure 11). The structure includes two chiral centers because of it two, R,R and S,S chiralities of the ligand are possible but these two conformations are equal in energy if no other chiral centers are present. One noticeable feature was the bond angle between the chloride, platinum and the nitrogen of 7-membered ring was 98.06^{o} and the angle between the chloride, platinum and, non-7-membered ring nitrogen was 89.46^{o}. The first angle is wider than the expected 90^{o} and may indicate strain in the structure.

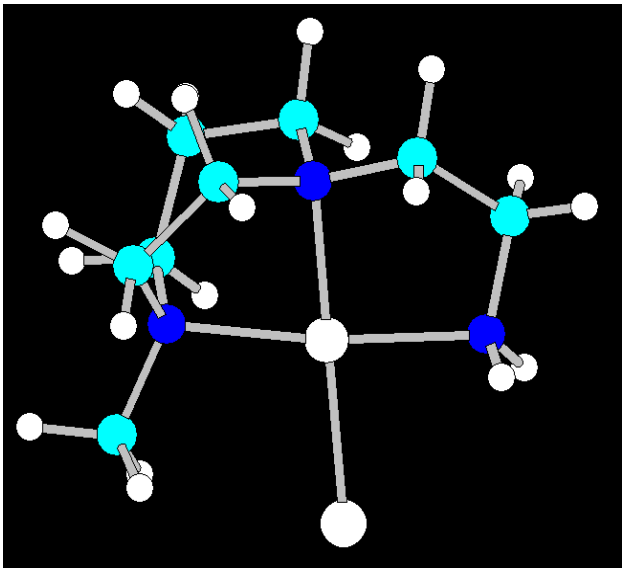

Figure 11: Minimum energy complex $[Pt(L)Cl]^{+}$ where L = 2-(4-Methyl-1,4-diazepan-1-yl)ethanamine)

2.2 Modelling of the $[Pt(L)Cl]^+$ complex with one 9-Ethylguanine replacing chloride ion

The minimized energy structure of the platinum triamine complex was used to model with the 9-ethylguanine. The model contains two chiral centers which are the two nitrogen atoms of the 7-membered ring. R,R and S,S configuration are possible when both nitrogen atoms are connected to the platinum center. Syn and anti conformation are based on the orientation of the H8 hydrogen of the 9-EtG. If the H8 lies on the side of three carbon chains of the 7-membered ring it was designed as 'syn'. Whereas, H8 lies on the two-carbon chain of a 7-membered ring designed as "anti". Table 1 shows the minimum energy structure of both conformers. Provided strain energies of the conformers are the sum of the bond, angle, dihedral, and Van der Waals energies.

Table 1: Minimum energy conformers of $[Pt(L)Cl]^+$ with 9-EtG replacing chloride ion

Sr. no.	Conformation	Minimum energy (Kcal/mol)	Strain energy (Kcal/mol)
1	R_Syn_9EtG	-8.4326	23.5169
2	R_Anti_9EtG	**-12.4903**	21.9133

2.3 Modeling of the $[Pt(L)Cl]^+$ complex with two 9-Ethylguanine

Additional structures were created by replacing chloride ion and one nitrogen of the 7-membered ring with the one 9-ethylguanine (9-EtG). Four possible conformers were created, and the nomenclature was adopted from the literature. The nomenclature depends on the orientation of the H8 molecule of the 9-EtG. If both the H8 atoms are on the same side of the three-carbon of 7-membered ring it is designed as head to head up (HHu), if towards the two carbon of the 7-membered ring head to head down (HHd).

Rotamers with H8 in opposite directions designated as head to tail (HT), if the slope of the imaginary line between atoms O6 is positive it labeled as delta head to tail (ΔHT) and if the slope is negative lambda head to tail (ΛHT)(Figure 12).

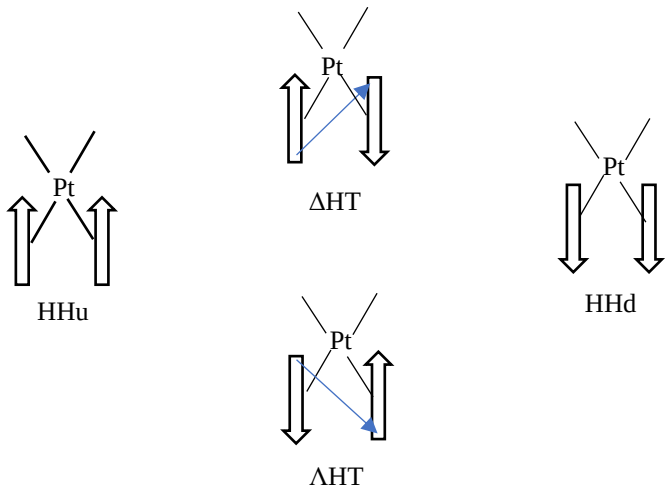

Figure 12: Schematic representation of possible rotamers of the complex with 9-EtG

The energy minimization results of the modeled complex with the two 9-EtG are given in table 2 with its calculated strain energy.

Table 2: Minimum energy and strain energy of $[Pt(L)Cl]^+$ model with two 9-EtG

Sr. No.	Conformation	Minimum energy (Kcal/mol)	Strain energy (Kcal/mol)
1	HHu	**-53.5485**	16.4711
2	HHd	-45.5226	17.3529
3	LamdaHT	-49.3475	19.4442
4	DeltaHT	-51.6452	16.3107

2.4 Modeling of the $[Pt(L)Cl]^+$ complex with methionine replacing the chloride ion

The model possesses three chiral centers, one sulfur of methionine and the nitrogen of the 7-membered ring. The two nitrogen atoms are bonded to platinum exhibits the same

chirality for all the conformers which gives the 8 possible conformations. Syn and anti are based on the orientation of the methyl group on sulfur of the methionine. If methyl group is on the same side of 3 nitrogen of 7 membered ring or on the side of 2 nitrogen of 7 membered ring it denoted as syn or anti respectively. The minimum energy and strain energy for all structures are listed in table 3.

Table 3: Minimum energy and strain energy of $[Pt(L)Cl]^+$ model with methionine replacing chloride ion (First alphabet represents nitrogen chirality, second alphabet represent chirality of sulfur, syn and anti notations represent the orientation of methyl group on sulfur)

Sr. No.	Conformation	Minimum energy (Kcal/mol)	Strain energy (Kcal/mol)
1	S_S_Syn	34.0766	45.1709
2	S_S_Anti	30.8561	41.3984
3	S_R_Syn	**26.0181**	39.2085
4	S_R_Anti	46.0396	43.1596
5	R_S_Syn	27.35	37.8055
6	R_S_Anti	33.7505	45.9597
7	R_R_Syn	33.1556	45.3236
8	R_R_Anti	27.9436	41.3597

2.5 Modeling of the $[Pt(L)Cl]^+$ complex with methionine replacing nitrogen of 7-membered ring

The model of the platinum complex with methionine replacing nitrogen of the 7-membered ring while the chloride is coordinated with platinum has three chiral centers. In this model chirality of both nitrogen can be different as the result of ring-opening and sulfur of methionine is also a chiral center, it gives 16 conformers. The calculated energies are shown in table 4. The conformers which changed their chirality of atoms during energy minimization are listed on the table.

Table 4: Minimum energy and strain energy of $[Pt(L)Cl]^+$ model with methionine replacing nitrogen of the 7-membered ring (first, second and third alphabet represent nitrogen of 7-membered ring coordinated with platinum, other nitrogen of 7-membered ring and Sulphur, syn and anti notations represent the orientation of methyl group on sulfur)

Sr. No.	Conformation	Conformation Converted to	Minimum energy (Kcal/mol)	Strain energy (Kcal/mol)
1	R_R_R_Syn	R_S_R_Syn	27.2939	38.428
2	R_R_R_Anti		36.6731	48.7276
3	R_R_S_Syn		37.6963	48.6608
4	R_R_S_Anti	R_S_S_Anti	27.4042	39.6247
5	R_S_S_Syn		40.8905	40.9307
6	R_S_S_Anti	R_R_S_Anti	27.2053	39.6414
7	R_S_R_Syn		27.3771	39.217
8	R_S_R_Anti		29.9062	34.1954
9	S_S_S_Syn	S_R_S_Syn	26.8449	39.4093
10	S_S_S_Anti	S_R_S_Anti	37.4097	45.4986
11	S_S_R_Syn		33.714	45.8716
12	S_S_R_Anti	S_R_R_Anti	**26.2711**	38.3411
13	S_R_S_Syn		26.8109	39.2971
14	S_R_S_Anti	S_S_S_Anti	37.4456	46.136
15	S_R_R_Syn		33.7144	45.6291
16	S_R_R_Anti		**26.2716**	38.3499

2.6 Modeling of the $[Pt(L)Cl]^+$ complex with methionine replacing non 7-membered ring nitrogen

The model has three chiral centers, but the two nitrogen of the 7-membered ring has the same chirality which leads us to 8 possible conformers. Table 5 shows the calculated energies of these conformers.

Table 5: Minimum energy and strain energy of $[Pt(L)Cl]^+$ model with methionine replacing a non 7-membered ring nitrogen (First alphabet represents nitrogen chirality, second alphabet represent chirality of Sulphur, syn and anti represent the orientation of methyl group on sulfur)

Sr. No.	Conformation	Minimum energy (Kcal/mol)	Strain energy (Kcal/mol)
1	S_S_Syn	**32.0569**	42.1367
2	S_S_Anti	42.8444	49.8963
3	S_R_Syn	40.2806	51.959
4	S_R_Anti	32.2604	44.1291
5	R_S_Syn	33.9171	34.5474
6	R_S_Anti	35.6049	44.6969
7	R_R_Syn	34.4273	44.5443
8	R_R_Anti	42.9627	53.9275

2.7 Modeling of the platinum complex with methionine and guanine

The complex modeled with the methionine and the guanine adduct. Two different models were constructed one in which methionine replaced the nitrogen of the 7-membered ring and guanine replaced the chloride ion. Other constructed with guanine replacing the 7-membered ring and methionine replaced the chloride ion. Both the model consists of three chiral centers and the chirality of two nitrogen can be different in this model, it leads to 32 conformations for each model. Syn and anti are based on the H8 atom of the guanine and methyl group on Sulfur of methionine. The minimum energies are provided in tables 6 and 7 along with their strain energies. The conformers which changed their chirality of atoms are listed on the table.

Table 6: Minimum energy and strain energy of $[Pt(L)Cl]^+$ model with methionine replacing the nitrogen of the 7-membered ring and guanine replacing chloride ion. (First to fifth notation of conformers represent chirality of nitrogen coordinated to platinum, nitrogen of the opened ring, sulfur of the methionine respectively, methyl group of methionine and H8 of guanine sequentially)

Sr. No.	Conformation	Conformation Converted to	Minimum energy (Kcal/mol)	Strain energy (Kcal/mol)
1	R_R_R_Syn_Syn	R_S_R_Syn_Syn	-3.6995	41.6858
2	R_R_R_Syn_Anti	R_S_R_Syn_Anti	-5.2481	40.0573
3	R_R_R_Anti_Syn	R_S_R_Anti_Syn	5.3741	52.8252
4	R_R_R_Anti_Anti	R_S_R_Syn_Anti	-5.2491	40.2996
5	R_R_S_Syn_Syn	R_S_S_Syn_Syn	0.1604	46.4041
6	R_R_S_Syn_Anti	R_S_S_Syn_Anti	2.3857	46.0846
7	R_R_S_Anti_Syn	R_S_S_Anti_Syn	**-6.9114**	40.9816
8	R_R_S_Anti_Anti	R_S_S_Anti_Anti	0.2326	46.5034
9	R_S_R_Syn_Syn	R_R_R_Syn_Syn	-6.7027	40.3936
10	R_S_R_Syn_Anti		-5.2583	40.1161
11	R_S_R_Anti_Syn		5.3893	52.6836
12	R_S_R_Anti_Anti	R_R_R_Anti_Anti	-5.2594	40.1429
13	R_S_S_Syn_Syn		0.179	46.7696
14	R_S_S_Syn_Anti		2.348	46.4316
15	R_S_S_Anti_Syn		**-6.9225**	40.9131
16	R_S_S_Anti_Anti		0.2312	46.6864
17	S_S_S_Syn_Syn		1.2128	49.1418
18	S_S_S_Syn_Anti		-6.4045	41.6013
19	S_S_S_Anti_Syn	S_R_S_Anti_Syn	5.4979	49.2733
20	S_S_S_Anti_Anti	S_R_S_Anti_Anti	0.3355	46.5886
21	S_S_R_Syn_Syn	S_R_R_Anti_Syn	-4.8795	40.5672
22	S_S_R_Syn_Anti		4.5935	52.7014
23	S_S_R_Anti_Syn	S_R_R_Anti_Syn	-4.5735	41.1956
24	S_S_R_Anti_Anti	S_R_R_Anti_Anti	-4.9955	41.1957
25	S_R_S_Syn_Syn		1.1631	48.2621
26	S_R_S_Syn_Anti		-6.3699	41.6688
27	S_R_S_Anti_Syn		2.2278	45.9244
28	S_R_S_Anti_Anti	S_S_S_Anti_Anti	0.5431	47.5952

29	S_R_R_Syn_Syn	S_S_R_Syn_Syn	6.8213	49.0422
30	S_R_R_Syn_Anti		1.9224	48.417
31	S_R_R_Anti_Syn		-4.8844	40.4439
32	S_R_R_Anti_Anti		-5.8398	40.9199

Table 7: Minimum energy and strain energy of $[Pt(L)Cl]^+$ model with guanine replacing the nitrogen of the 7-membered ring and methionine replacing chloride ion. (First to fifth notation of conformers represent chirality of nitrogen coordinated to platinum, nitrogen of the opened ring, sulfur of the methionine respectively, methyl group of methionine and H8 of guanine sequentially)

Sr. No.	Conformation	Conformation Converted to	Minimum energy (Kcal/mol)	Strain energy (Kcal/mol)
1	R_R_R_Syn_Syn		-0.0746	42.3107
2	R_R_R_Syn_Anti	R_S_R_Syn_Anti	-8.5409	40.4723
3	R_R_R_Anti_Syn		-7.8111	40.2084
4	R_R_R_Anti_Anti	R_S_R_Anti_Anti	-1.9728	41.5842
5	R_R_S_Syn_Syn	R_S_R_Syn_Syn	-3.5188	41.3293
6	R_R_S_Syn_Anti	R_S_S_Syn_Anti	**-10.0383**	39.5857
7	R_R_S_Anti_Syn	R_S_S_Anti_Syn	-7.3319	39.8416
8	R_R_S_Anti_Anti	R_S_S_Anti_Anti	-2.0724	41.2597
9	R_S_R_Syn_Syn	R_R_R_Syn_Syn	-1.2067	43.4514
10	R_S_R_Syn_Anti		-8.5441	40.6975
11	R_S_R_Anti_Syn		-6.3539	37.1248
12	R_S_R_Anti_Anti	R_R_R_Anti_Anti	-3.2626	40.604
13	R_S_S_Syn_Syn		-3.4982	41.2004
14	R_S_S_Syn_Anti		**-10.0402**	39.6213
15	R_S_S_Anti_Syn	R_R_S_Syn_Anti	-2.1464	41.7484
16	R_S_S_Anti_Anti	R_R_S_Anti_Anti	-2.0665	41.0879
17	S_S_S_Syn_Syn	S_R_S_Syn_Syn	-2.9485	40.9763
18	S_S_S_Syn_Anti	S_R_S_Syn_Anti	-6.8551	40.3551
19	S_S_S_Anti_Syn	S_R_S_Anti_Syn	-9.5681	39.6941
20	S_S_S_Anti_Anti	S_R_S_Anti_Anti	-3.1864	41.1603
21	S_S_R_Syn_Syn	S_R_R_Syn_Syn	-4.0406	40.3267
22	S_S_R_Syn_Anti	S_R_R_Syn_Anti	-8.9811	38.4555
23	S_S_R_Anti_Syn	S_R_R_Anti_Syn	-7.0738	40.9986
24	S_S_R_Anti_Anti	S_R_R_Anti_Anti	-1.0438	42.4721
25	S_R_S_Syn_Syn		-2.9435	40.9424

26	S_R_S_Syn_Anti		-8.1424	38.8854
27	S_R_S_Anti_Syn		-9.5638	39.7196
28	S_R_S_Anti_Anti		-0.2022	38.5362
29	S_R_R_Syn_Syn		-4.039	40.32
30	S_R_R_Syn_Anti		-8.9699	38.3377
31	S_R_R_Anti_Syn		-7.9611	40.6557
32	S_R_R_Anti_Anti		-1.0476	42.3128

3. Discussion

Molecular mechanics (MM) calculations of platinum compounds with guanine or methionine adduct have been utilized extensively. MM calculations provide insights regarding the favored conformations of the platinum compound adduct with the guanine/amino acid and the ligands of the platinum.[37,38] We chose to study MM on the platinum complex and its products with biologically relevant molecules such as methionine and guanine, which play a major role in transportation and cytotoxicity respectively.

We performed MM study to verify the experimental results of $[Pt(L)Cl]^+$ where L = 2-(4-Methyl-1,4-diazepan-1-yl)ethanamine) and its reactivity with guanine and methionine.[36] The complex was synthesized and analyzed by the previous member of our lab. The minimum energy complex showed the stretched angle between the nitrogen of seven membered ring, platinum and chloride. This leads to increased strain on the molecule and may cause breaking of nitrogen-platinum bond when a bulky group such as guanine replace the chloride. This bond break can covert tridentate ligand to bidentate ligand.

The MM calculations conducted on $[Pt(L)Cl]^+$ with one guanine adduct replacing chloride ion shows no steric clashes. The experimental NMR and LC-MS results also support these results. Whereas, the experimental results also suggest the possibility of bis-product of the platinum complex with guanine with multiple product peaks. LC-MS of 5'-GMP and platinum complex shows the formation of mono-product as well as bis-product (Figure 13).

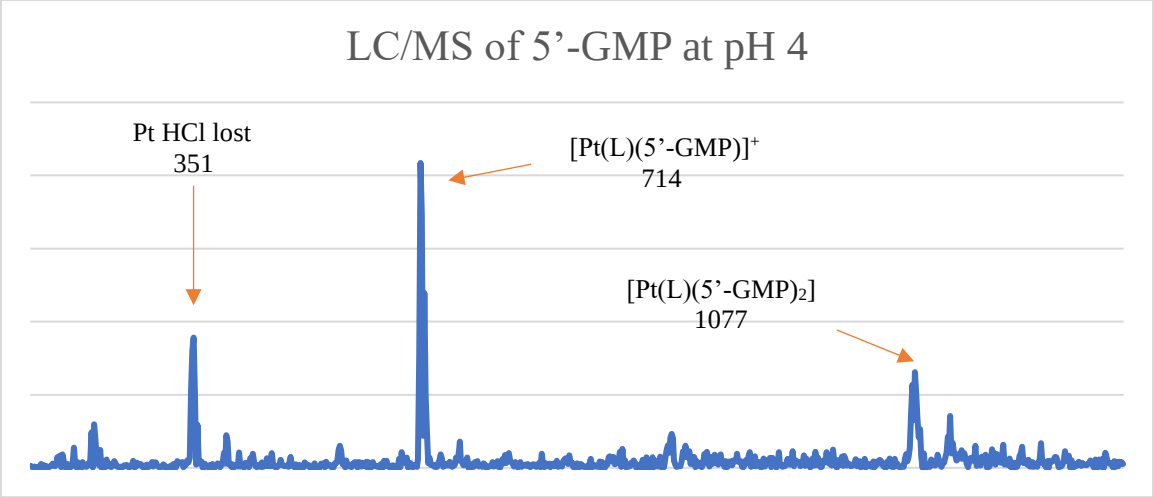

Figure 13: Mass spectrum of $[Pt(L)Cl]^+$ with 5’-GMP at pH 4

The performed MM calculations on platinum complex adduct with two guanines also seems to be energetically favorable with no severe steric clashes. The head to head conformation was most energetically favorable where the opened ring moves away from the guanine adduct and both guanines lie parallel to each other.

The MM calculations were performed on the platinum complex and methionine adduct at three different positions. Firstly, calculations were performed on methionine adduct replacing the chloride ion which suggested the complex is energetically favored with no steric clashes, these energies cannot be compared to the other two calculations of methionine adduct as the number and type of atoms in these calculations are different. The experimental results comply with these findings, nevertheless, they suggest two mono-product formations which causes the two different product peaks at 2.4 and 2.6 ppm. Eventually 2.4 coverts to 2.6 ppm peak. LC-MS also shows the methionine causing the ring opening of the complex (Figure 14). We performed MM calculations on the

methionine adduct replacing nitrogen of the 7-membered ring and non-7-membereed nitrogen to check which platinum-nitrogen bond was broken.

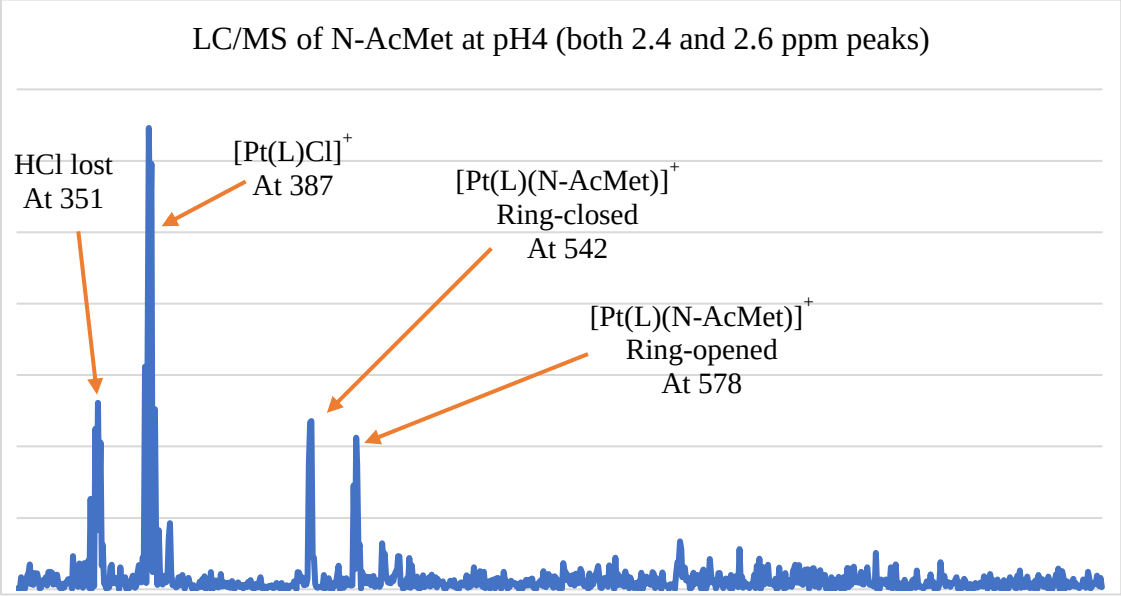

Figure 14: Mass spectrum of $[Pt(L)Cl]^+$ with N-AcMet at pH 4

The minimum energy calculated on the methionine adduct replacing nitrogen suggests that the structure with methionine replacing nitrogen of the 7-membered ring is energetically more favored (26.27 Kcal/mol) then the guanine replacing nitrogen of the non 7-membered ring (32.05 Kcal/mol). These two energies can be compared because the number of atoms and the types of atoms is equal to each other. These calculations clarify that the nitrogen of the 7-membered ring was replaced by the methionine. This data explains the two product peaks formation one by replacing the chloride ion and other by replacing nitrogen of the 7-membered ring. Although comparing these two energies was not possible, experimental data suggests 2.6 ppm peak is from the replacement of chloride ion.

The competition reaction of N-AcMet and guanine was conducted with platinum complex. According to the LC-MS result, there were three product peaks; one with single N-AcMet, one with single 5'-GMP, and the third one with both N-AcMet and 5'-GMP bonded to the platinum complex (Figure 15).

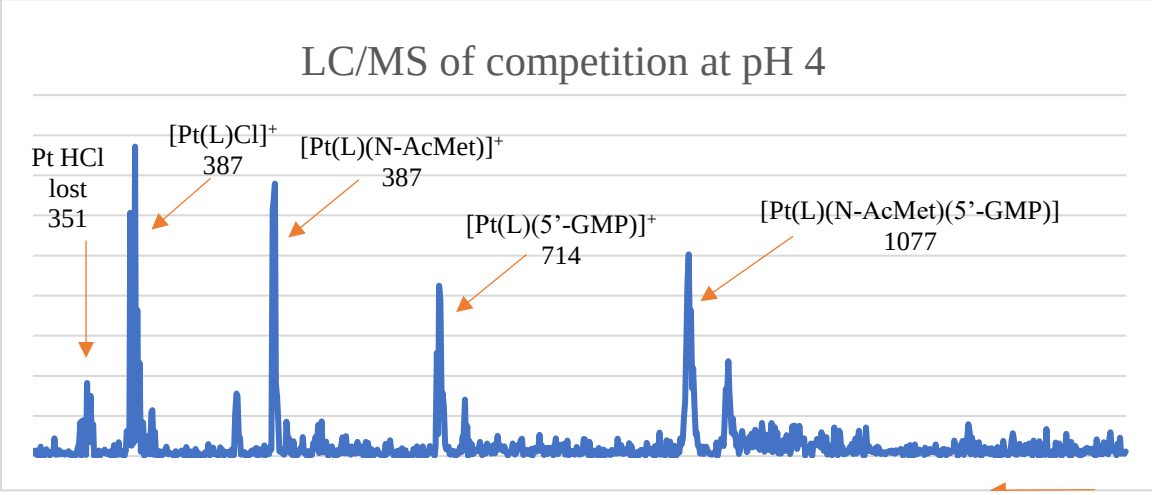

Figure 15: Mass spectrum of competition reaction of N-AcMet and 5'-GMP with platinum compound

The molecular mechanics calculations done by the two adducts one with N-AcMet and one with guanine show no severe steric hindrance in the formation of this product molecule. The interest of conducting the MM calculations was to find out which adduct replaces the chloride and which replaces the nitrogen of the 7-membered ring. All possible conformers were modeled with these two biomolecules and subjected to energy minimization. The results manifest that the minimum energy of the platinum complex with guanine replacing the nitrogen of 7-membered ring and methionine replacing chloride ion was -6.91 Kcal/mol. Whereas for the complex where methionine replaced the nitrogen of 7-membered ring and the guanine replace the chloride ion was -10.04 Kcal/mol. Later complex is energetically more favored than the prior one.

In summary, the MM calculations conducted on platinum complex and its product with these biomolecules show consistency with experimental results. The platinum complex adducts with one 5'-GMP, two 5'-GMP, one N-AcMet is possible. With these pieces of evidence, we can conclude that methionine reacts faster than the 5'-GMP with the platinum compound. The methionine adduct with platinum center weakens the platinum-nitrogen bond which ultimately replaced by the 5'-GMP to form a bifunctional product.

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