Spring 2017

The Effects of Carrier Ligands on Cisplatin Binding to Cysteine and Methionine

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THE EFFECTS OF CARRIER LIGANDS ON CISPLATIN BINDING
TO CYSTEINE AND METHIONINE

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
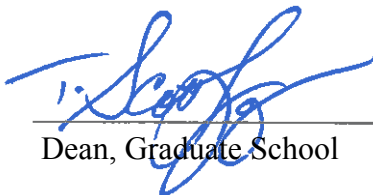Adam Christopher Raphael Smith

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THE EFFECTS OF CARRIER LIGANDS ON CISPLATIN BINDING
TO CYSTEINE AND METHIONINE

Date Recommended 4/13/17

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ACKNOWLEDGMENTS

I would first like to acknowledge my research advisor Dr. Kevin Williams, without whom this would not even been remotely possible. I would like to thank him for his kindness and seemingly endless patience in answering my questions about the research and writing this thesis. There is no one I would have rather had as my research advisor. I would also like to thank other members of the faculty and staff for their support and encouragement including Dr. Blairanne Williams, Dr. Darwin Dahl, Prof. Carnetta Skipworth, and Ms. Haley Smith.

On a more personal level I would like to thank my family, especially my parents David and Theresa Smith, for their love and support, for helping me become the man I am. I would like to thank all the Fathers of Mercy for all their prayers on my behalf and their patience and advice over the years. I would like to thank all the friends I have made along the way. Without them I would have gotten discouraged and given up long ago. Among them I would especially like to thank Kristen Griffin, Nate Puckett, and Mara Krempel.

I would like to thank Matthew Mercer, Marisha Ray, Liam O'Brien, Taliesin Jaffe, Laura Bailey, Travis Willingham, Sam Riegel, Ashley Johnson, and Orion Acaba for keeping me entertained and sane for these past years.

Lastly, I would like to thank the Lord God for His love and guidance throughout my life.

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THE EFFECTS OF CARRIER LIGANDS ON CISPLATIN BINDING
TO CYSTEINE AND METHIONINE

Adam Smith   May 2017   19 pages

Directed by: Dr Kevin Williams, Dr. Darwin Dahl, and Dr. Eric Conte

Department of Chemistry   Western Kentucky University

We have reacted several derivatives of the anticancer drug cisplatin with N-acetyl-L-cysteine (N-AcCys) and N-acetyl-L-methionine (N-AcMet), which are two of the primary amino acid targets of platinum. NMR spectroscopy was used to monitor the reactions and determine the effect the different ligands would have on the platinum reactivity. Several of the platinum compounds were tested at pH of 4 and 7, and with platinum:amino acid ratios of 1:1, 2:1 and 1:2. Competition reactions between cysteine and methionine were done to confirm which would react with the platinum compound first.

$[Pt(dien)(NO_3)]^+$ reacts faster with methionine than with cysteine at both pH 4 and 7 at a 1:1:1 ratio. $[Pt(N,N,N',N',N''-pentamethyldiethylenetriamine)(NO_3)]^+$ reacts with methionine faster at pH 4 but with cysteine faster at pH 7. This is most likely due to the thiol in the cysteine starting to deprotonate around pH 7. $[Pt(Me_4en)(NO_3)_2]$ (Me_4en = N,N,N',N'-tetramethylethylenediamine) forms several products with N-AcCys at both pH 4 and 7, with the amounts of the products varying depending on the ratio of platinum and Cys. Mass spectrometry indicated one product as $\{[Pt(Me_4en)(H_2O)]_2(N-AcCys)\}^{2+}$, with two platinum compounds coordinated to a single cysteine. Lastly $Pt[(en)(NO_3)_2]$

when reacting with N-AcCys at a ratio of 1:1 will coordinate with 2 different Cys molecules. With an excess of Pt the complex prefers to bind to only 1 Cys.

I.   Introduction.

1.1   Brief history of Cisplatin.

Cisplatin is an anticancer drug that was discovered in the early 1960s used mainly on testicular and ovarian cancers. When added to the anticancer regimen at the time it raised the success rates of patients from 14 to 50%. After some more adjustments to the regimen and an increase of the cisplatin dosage the rate increases to 78%. With modern surgery techniques, the rate increases further to ≈95%. When treating ovarian cancers cisplatin has a success rate of 27-29%, when combined with other drugs it increases to 73%[1]. Cisplatin and other platinum complexes like carboplatin and oxaliplatin are useful in many types of cancer, such as testicular, ovarian, head and neck, cervical and lung cancer. The use of these drugs can be rather limited due to the body's natural ability to build a resistance to them and due to the somewhat severe side effects. Potential side effects include nephrotoxicity (toxic buildup or damage in the kidneys), emetogenisis (vomiting), and neurotixicity (toxic buildup or damage to the neural cells). The nephrotoxicity and emetogenisis can be effectively controlled using hydration, diuretics, and serotonin-receptor antagonists. The neurotoxicity is one of the main reasons that cisplatin is dose-limiting, it can cause peripheral neuropathy (weakness or numbness in hands and/or feet), tinnitus (a frequent or constant buzzing/ringing in the ear), and the loss of the ability to hear high frequencies[2].

Cisplatin is the first generation of the Platinum (II) class of drugs, but due to its side effects the second generation was made, carboplatin and oxaliplatin. These three are the only Pt drugs currently used with FDA approval but there are others that are in clinical trials. The later generations were made in an effort to reduce the side effects

while keeping a similar effectiveness in killing cancer cells. On average the later

generations did indeed reduce the side effects somewhat but also reduced the

effectiveness against all types of cancer[3,4].

Figure 1.1[3] The Lewis structures of the 1st and 2nd generation Pt drugs.

Cisplatin   Carboplatin   Oxaliplatin
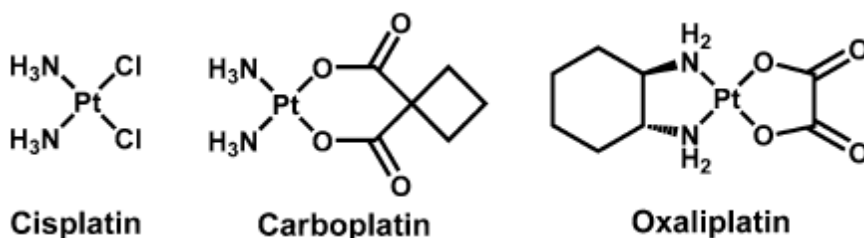
1.2   Platinum (II) compound chemistry

The Platinum (II) compounds are composed of a central Pt atom with 4 ligands. Those of

the Cisplatin family have 2 carrier ligands and 2 leaving ligands. The leaving ligands

typically would leave the Pt atom very quickly when placed in an aqueous

environment[5,6], though the ligands on Oxaliplatin and Carboplatin tend to stay attached

until removed by the biological targets. When the ligands leave it creates a positive

charge on the Pt which allows it to bind to its preferred targets, this usually occurs once

the Pt compound enters the cell.[3,5] The carrier ligands (which are typically some variety

of nitrate molecule) help the Pt atom to move to the targets, if they are bulky then the

carrier ligands can also help stop the cell's DNA Ligase from repairing the damage

caused by the Pt drug. Once the Pt compound enter the nucleus, through either active or

passive transport, its favored target is the N7 positions of 2 Guanine molecules, forming

either a 1,2 or a 1,3 intrastrand crosslink. This crosslink will create a "kink" in the DNA

strand that prevents the cell from repairing or copying the DNA strand, eventually

leading to apoptosis[2,7,8].

Figure 1.2[2,3] Graphic of Cisplatin binding to DNA
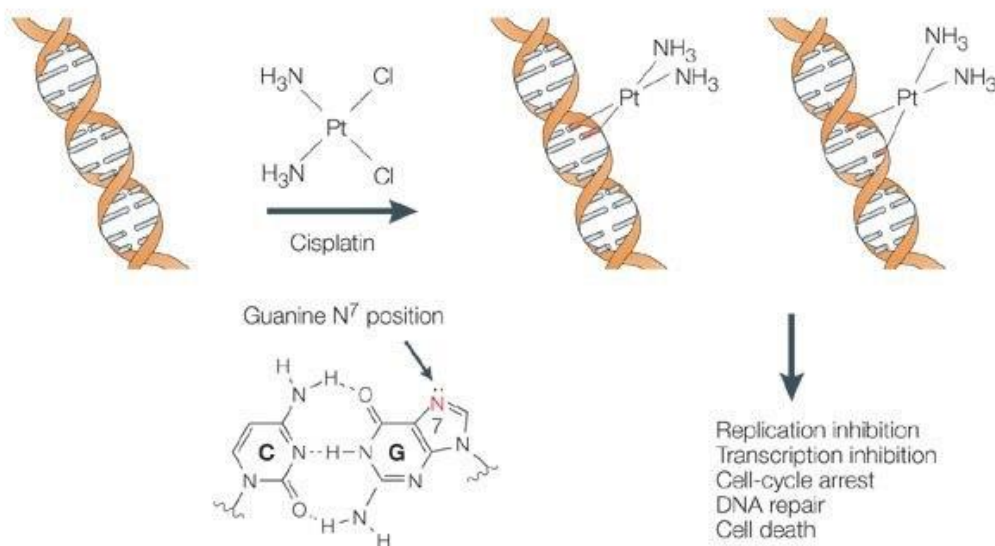
Cisplatin and most of the related Pt compounds can exist in both the *cis-* and *trans-*

conformations, but only the *cis-* conformations exhibits the anti-cancer activity. There

are other potential targets for the Pt compound to bind to however, including Sulfur

found in Methionine and Cysteine, Selenium found in Selenomethionine and

Selenocysteine, or the Nitrogen in Histidine[9–15]. These binding tendencies can be

explained using the Hard/Soft Acid/Base Theory, Pt is acting as a soft acid and is

attracted to soft bases in the cell, like S and Se, therefor its favored targets would be

molecules that contain a soft base and is not too allosterically constrained to bind to[3,16–20].

1.3   Previous Research done by the Williams lab.

There has been previous work done by the Williams research lab pertaining to the

binding tendencies of Pt compounds. $[Pt(en)NO_3]_2$ with a relatively small diamine ligand

will react with Met, N-AcMet, 5'-GMP, and Guanosine very well and N-AcHis very

slowly. When reacting with Met with excess platinum it binds at the N terminus and

forms S,N chelates. This is similar to what has been seen with oxaliplatin with Met and

Cys[6,21,22]. If coordinated with N-AcMet the product can be displaced by 5'GMP.

[Pt(en)Cl]Cl interacts with the 5'-GMP phosphate via H^+ bonding through the NH_2

groups on the en ligand and the lack of steric hindrance on the en ligand allows the

complex to bind unimpeded[23,24]. [Pt(Me$_4$en)(NO$_3$)$_2$] is very similar in structure but has

the added bulk of 4 methyl groups, it reacts in much the same way as the Pt(en)(NO$_3$)$_2$

but much more slowly. The rate of reaction with N-AcMet is affected much more than

with guanosine, indicating that steric hindrance affects the Met ligand more than guanine.

The only tested target whose binding rate remains unchanged is N-AcHis, implying that

His targets may be unaffected by the steric hindrance of the carrier ligands. When

coordinating with N-AcMet [Pt(Me$_4$en)(NO$_3$)$_2$] will form S,O chelates, specifically with

the O of the carboxyl group. The bulk of the carrier ligands only allows binding

stoichiometry to be 1:1and also prevents coordination of the amide nitrogen atom[23,24].

For [Pt(Et$_2$en)(D$_2$O)$_2$] the diamine ligand bulk will prevent the coordination of 2 N-

AcMet and prevent the chelation of the amide N of a coordinated N-AcMet. The

chelation of the O is observed but it occurs slowly.[25] Together, these studies indicate that

steric hindrance on a diamine ligand affects N-AcMet more than it affects 5'-GMP.

[Pt(dien)NO$_3$]NO$_3$ when reacted with GMP and AcMet, will react with Met faster[14].

[Pt(Me$_5$dien)NO$_3$]NO$_3$ will coordinate with GMP much faster, it will react with Met only

very slowly. If already coordinated with Met and GMP is added to the solution the GMP

will displace the Met[26]. The preference for 5'-GMP over N-AcMet is so far unique to the

Me$_5$dien ligand; this was the first example of a platinum compound reacting significantly

faster with 5'-GMP than with a methionine ligand.

Finally, Pt(dach)(ox) will coordinate with 2 AcMet with little steric hindrance in the presence of excess N-AcMet. Pt(Me$_2$dach)(ox) with the additional bulk of 2 methyl groups on the carrier ligands will readily coordinate with one AcMet and will prevent the coordination of a second[10]. However, the rate of reaction with the first N-AcMet is not affected by the presence of one methyl group on each nitrogen atom.

The binding tendencies of the Pt compounds are affected by several different factors. The major factors being pH, the nature of the target site and the size of the carrier ligands of the Pt compounds. The research done by the Williams lab seem to suggest that the greatest factor is the size of the carrier ligands. This project, was focused on trying to see if there was a combination of factors that could be used to cause a large carrier ligand Pt compound to prefer to bind to the larger AcCys molecule rather than the smaller AcMet. As well as to reaffirm the importance of carrier ligand steric hindrance on binding tendencies.

II.   Experimental.

2.1   Purchased Materials

The following items were purchased from Sigma-Aldrich: Silver Nitrate, Potassium

Iodide, Acetic Acid, Guanine, L-Histidine, N,N,N',N' Tetraethylenetriamine, Dichloro

(ethylenediamine) platinum (II), cis-Diamminedichloro platinum (II), Sodium Acetate,

Deuterium Oxide. N-acetyl-L-Cysteine and Ethylenediamine were purchased from

Acros. N-acetyl-L-Methionine was purchased from Fluka.

2.2   NMR

A 500 mHz JEOL NMR (Nuclear Magnetic Resonance) was used to characterize the

results of the reactions. All samples were dissolved in Deuterium Oxide. The ^{1}H NMR

spectra were referenced to the residual water signal, adjuster for temperature. Most of the

reactions were monitored using several single pulse scans. For several of the reactions a

kinetics run was used instead. The kinetics run was set to start a 12 single pulse scans,

one every hour for 12 hours.

2.3   LC/MS

A Varian 500 ion trap mass spectrometer with a Varian 212 HPLC system was used to

characterize the products of one reaction. For the experiment 20 µl aliquots of the

samples were injected with a flow rate of 0.2 ml/min. Solvent A is 0.1% formic acid in

water, solvent B is acetonitrile. At time zero the gradient is 95% A and 5% B, held for 5

min, pumps were linearly programmed to reach 50% A and 50% B at 50 min, 10% A

and 90% B 60 min, 95% A and 5% B 70 min.

2.4   Synthesis of [Pt(Me$_5$dien)I]$_2$(Pt$_2$I$_6$)

.509g K$_2$PtCl$_4$ and .820g KI were mixed in 5 ml Deionized water. The mixture was

stirred for 30 minutes, after which 250μl Me$_5$dien was added, it was then left to stir

overnight. After filtering and leaving the product to air dry 592.4 mg of product was

collected. 299.2 mg of the product was mixed with 97.4mg of AgNO$_3$ in 20 ml deionized

water in a 50ml beaker, it covered with aluminum foil and left to stir for 16 hrs. The

mixture was then vacuum filtered through celite and the filtrate dried using the roto

evaporator. 20 ml of diethyl ether was added to the product which stirred for 8 days and

air dried.

2.5   Synthesis of Platinum (II) diethylenetriamine [Pt(dien)(NO$_3$)]NO$_3$

Starting with 1.0024g Potassium Tetrachloroplatinate dissolved in 30 ml of water and 1

ml diethylenetriamine, adjusted to pH 3.05 with concentrated Hydrochloric acid. The

solution is then stirred with a magnetic stir for 7 hours, after stirring for 30 min it was

thawed to a boil for 6.5 hrs.   The remaining solution was evaporated in a roto evaporator

until roughly 4 ml and chilled overnight. It was then evaporated to roughly 3 ml and

chilled for another night until precipitation had occurred. Lastly the precipitate was

vacuum filtered, washed with 2 ml of ethanol, and left to air dry.

2.6   Synthesis of Platinum (II) N, N, N', N' Tetramethylethylenediaminedinitrate,

Pt(Me$_4$en)(NO$_3$)$_2$

100.9 mg Pt (Me$_4$en)I$_2$ was dissolved in 110 ml of acetone in a 500 ml round bottom

flask. Then 60.3 mg AgNO$_3$ was added, the flask was then sealed with parafilm and

stirred with a magnetic stir for 5 hours. The solution was gravity filtered twice and the

solute was evaporated to dryness using a roto evaporator. This yielded 62.6 mg of Pt $(Me_4en)(NO_3)_2$, confirmed via NMR scan.

2.7   Previously made compounds

The following compounds were previously synthesized by other members of the Williams lab; [Pt(dien)Cl]Cl, [Pt(dien)(NO3)]NO3, Pt(en)(NO3)2, [Pt(Et4dien)Cl]Cl, [Pt(Me5dien)(NO3)](NO3), Pt(Me_4en)I_2, Me_5dien.

2.8   Preparation of Samples.

In all 1:1 reactions the reactants were prepared separately in 1 ml of D_2O at a concentration of 10 mM. The pH was then adjusted to either pH 4 or 7 as required by the experiment. 0.25 ml of each reactant was then mixed together in 0.5 ml of D_2O; a 0.7 ml aliquot was then put in an NMR tube and scanned accordingly. In competition reactions, the Cysteine and Methionine would be mixed together prior to being mixed with the Platinum compound. In 1:2 reactions the reactants were prepared like the 1:1 method, except that instead of double the concentration of the "2" compound we halved the concentration of the "1" compound.

III.   Results

3.1 $[Pt(dien)(NO_3)]^+$ Competition at pH 4

The sample was prepared as detailed in the methodologies section for competition reactions. This reaction was done to test which amino acid the Pt compound reacts with at a faster rate.

Figure 3.1. [Pt(dien)(NO$_3$)]$^+$ Competition at pH 4 at both 19 minutes and 2 days, the "*" marks Methionine products and the "‡" marks the Cysteine products.
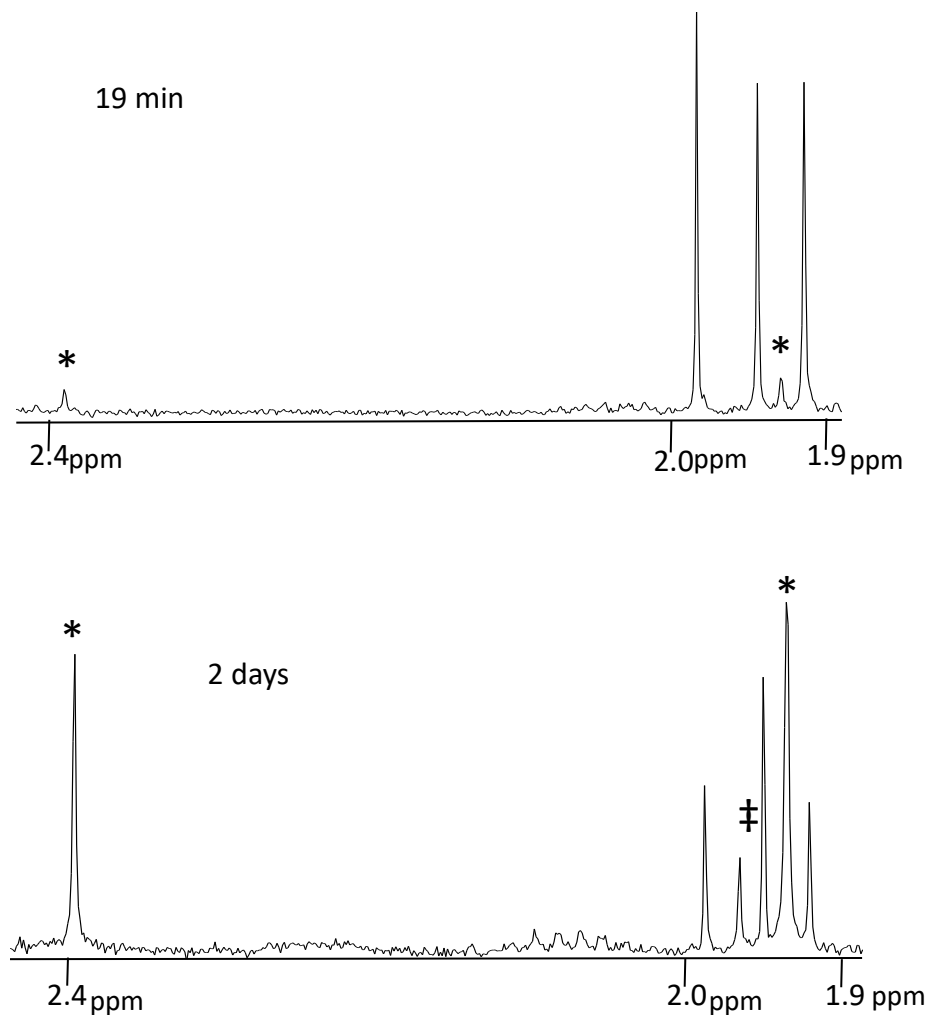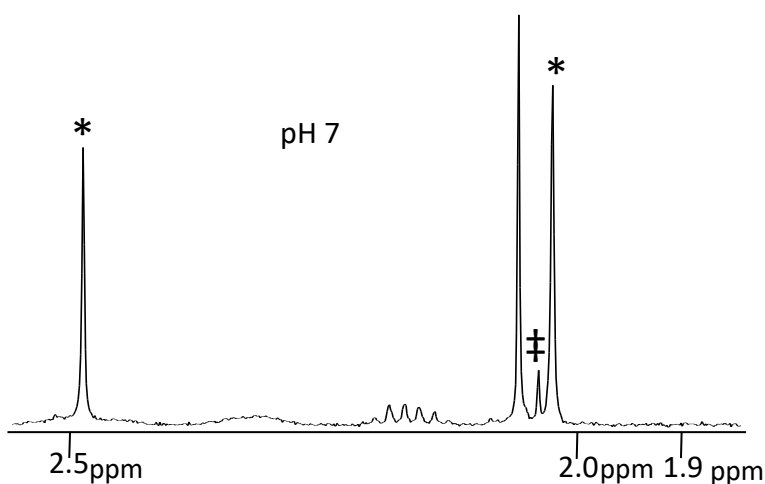

The characteristic peaks for Methionine are the peaks closest to 2.0 and 1.9 ppm. The peak for unreacted Cysteine is at 1.95 ppm. The Methionine product peaks are starting to be detected as early as 19 min (marked with the "*") while the Cysteine product peak has yet to form. After 2 days of reaction time the free Methionine peaks are substantially smaller and the reacted Methionine peaks are much larger. The Cysteine product peak

has started to form around 1.97 ppm (marked with the "‡") but is approximately one third

the size of the Methionine product peaks. This show that under the 1:1:1, pH 4

conditions Methionine will react faster than Cysteine with $[Pt(dien)(NO_3)]^+$.

3.2 $[Pt(dien)(NO_3)]^+$ Competition at pH 7

Figure 3.2. $[Pt(dien)(NO_3)]^+$ Competition at pH 7 after a period of 2 days, the "*" marks

Methionine products and the "‡" marks the Cysteine products.

After a period of 2 days (to insure the reaction had reached completion) the characteristic

peaks for the Methionine products have formed in similar amounts to the pH 4

experiment. The Cysteine product peak formed but in only a small amount. This suggests

that in a competition at pH 7 Methionine will react with $[Pt(dien)(NO_3)]^+$ faster than

Cysteine.

3.3   Pt(Me$_5$dien)(NO$_3$)]$^+$ with Methionine at pH 4 and pH 7 Comparison

Figure 3.3.   Pt(Me$_5$dien)(NO$_3$)]$^+$ with Methionine at pH 4 and pH 7 after a period of 9 hours, the "*" marks Methionine products.
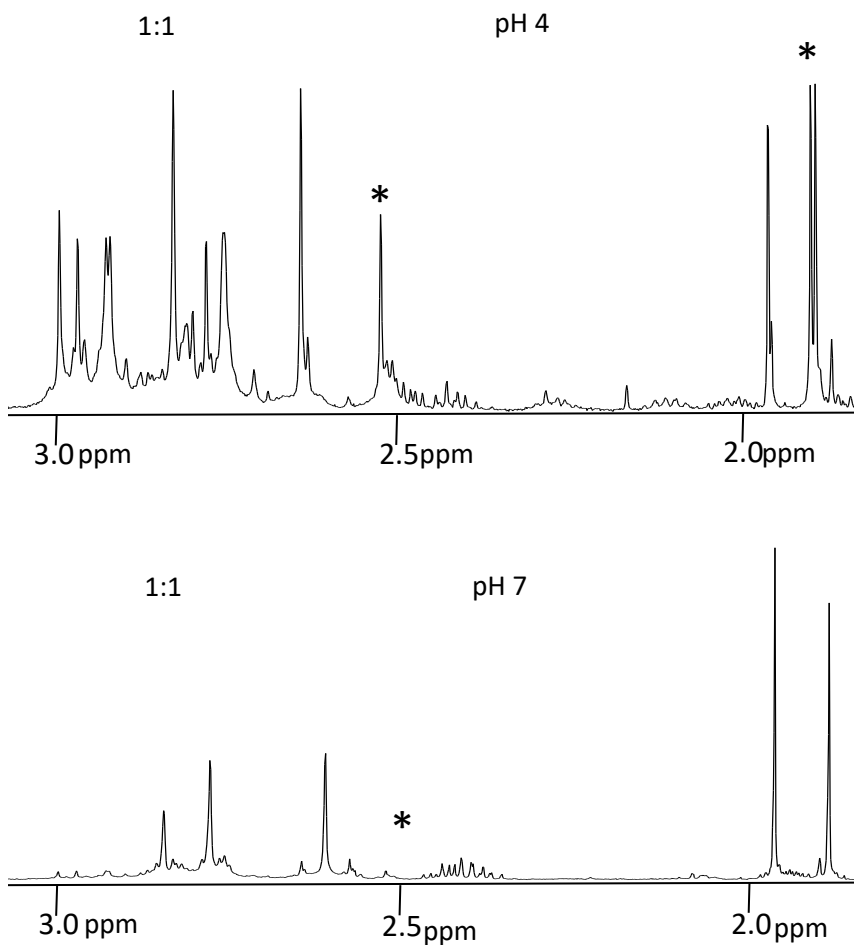
After a period of 9 days, pH 4, and at a 1:1 ratio the Methionine product peaks formed well. At pH 4 Methionine will react readily with Pt(Me$_5$en)(NO$_3$)]$^+$. However, after 8 days and at pH 7 very little of the Methionine product peaks have formed. This suggests that at pH 7 Methionine will not react well with Pt(Me$_5$en)(NO$_3$)]$^+$.

3.4   [Pt(Me₅dien)(NO₃)]⁺ pH 4 and 7 with Cys Comparison

Figure 3.4. [Pt(Me₅dien)(NO₃)]⁺ with Cys at pH 4 and 7 over a period of 2 days, the "‡"

marks the Cysteine products.
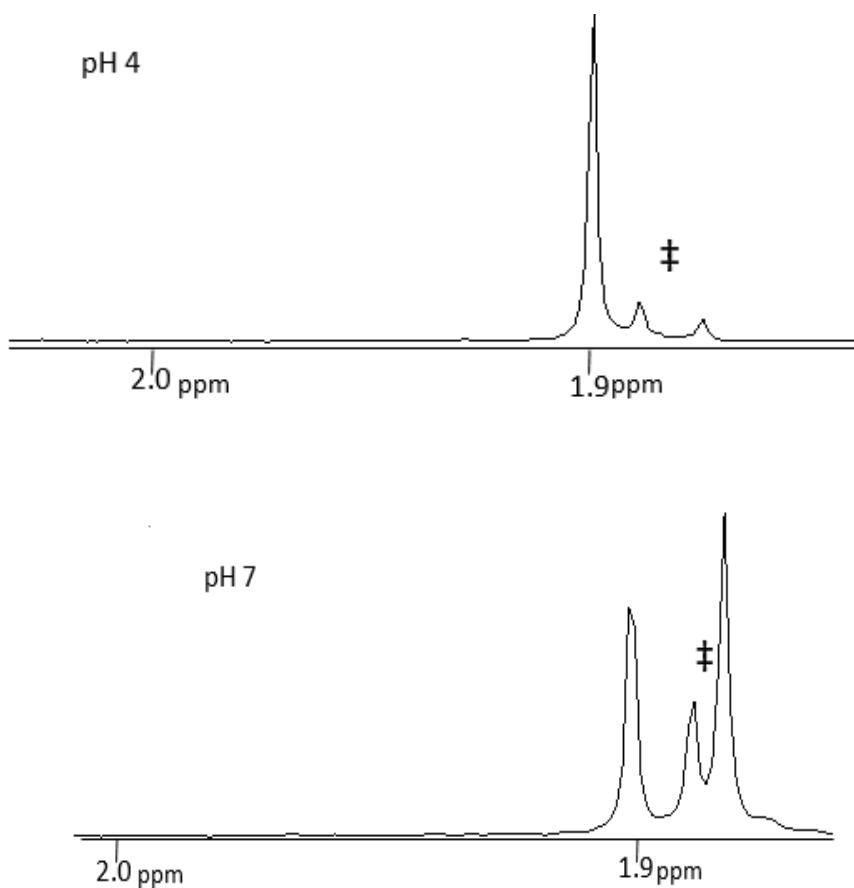
At pH 4 very little of the expected Cysteine product peaks formed suggesting that very

little reaction had occurred under these conditions. At pH 7 the Cysteine product peaks

are much larger than the pH 4. This suggests that Cysteine will react well at pH7 but not

pH 4.

3.5   [Pt(Me₅dien)(NO₃)]⁺ pH 7 Competition

Figure 3.5. [Pt(Me₅dien)(NO₃)]⁺ with Cys and Met at pH 7 after a period of about 2

days, the "*" marks Methionine products and the "‡" marks the Cysteine products.
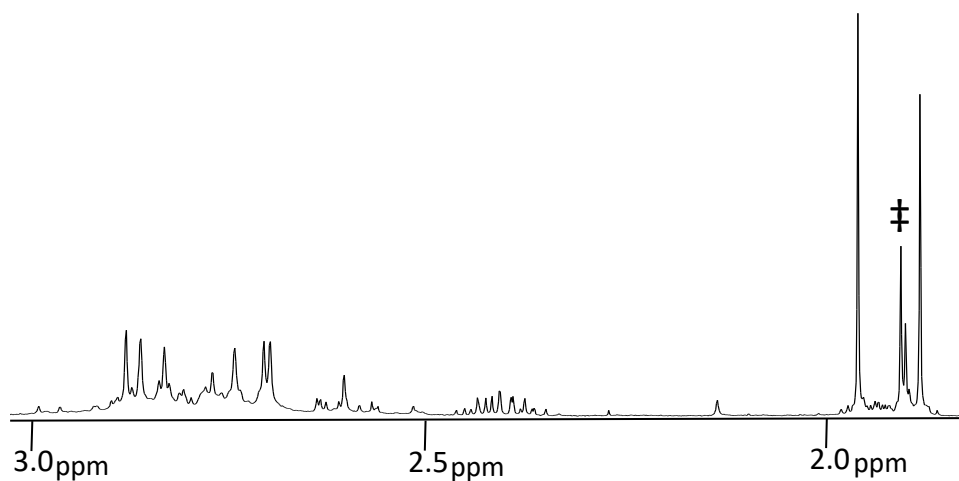
When put in competition at pH 7, a strong Cysteine product signal forms but little to no

Methionine product peaks are seen. This would suggest that at pH 7 Methionine will

have little if any reaction with [Pt(Me₅en)(NO₃)]⁺ but Cysteine will react readily.

3.6   [Pt(Me₄en)(NO₃)₂] pH 4 with Cys

Figure 3.6. [Pt(Me₄en)(NO₃)₂] with Cys pH 4 after a period of 2 days and at a ratio of

1:1, "‡" marks the Cysteine products.
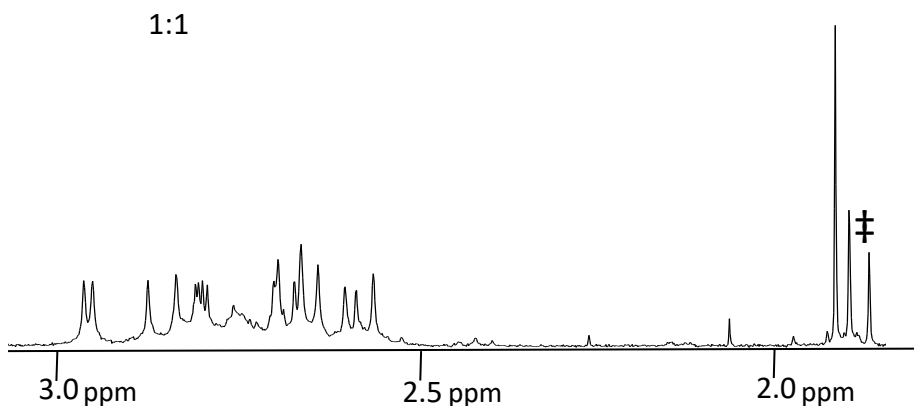
Upon completion, the product peaks associated with Cysteine are clearly seen near 1.9 ppm. The peaks between 3.0 and 2.5 ppm we did not characterize the product peaks but they are likely due to the Me_4en ligand and the CH_2 of the Cys.

3.7   [Pt(Me_4en)(NO_3)$_2$] pH 4 with Cys 2:1 and 1:2

Figure 3.7. [Pt(Me_4en)(NO_3)$_2$] with Cys pH 4 after a period of 2 days and at ratios of 2:1 and 1:2, "‡" marks the Cysteine products.
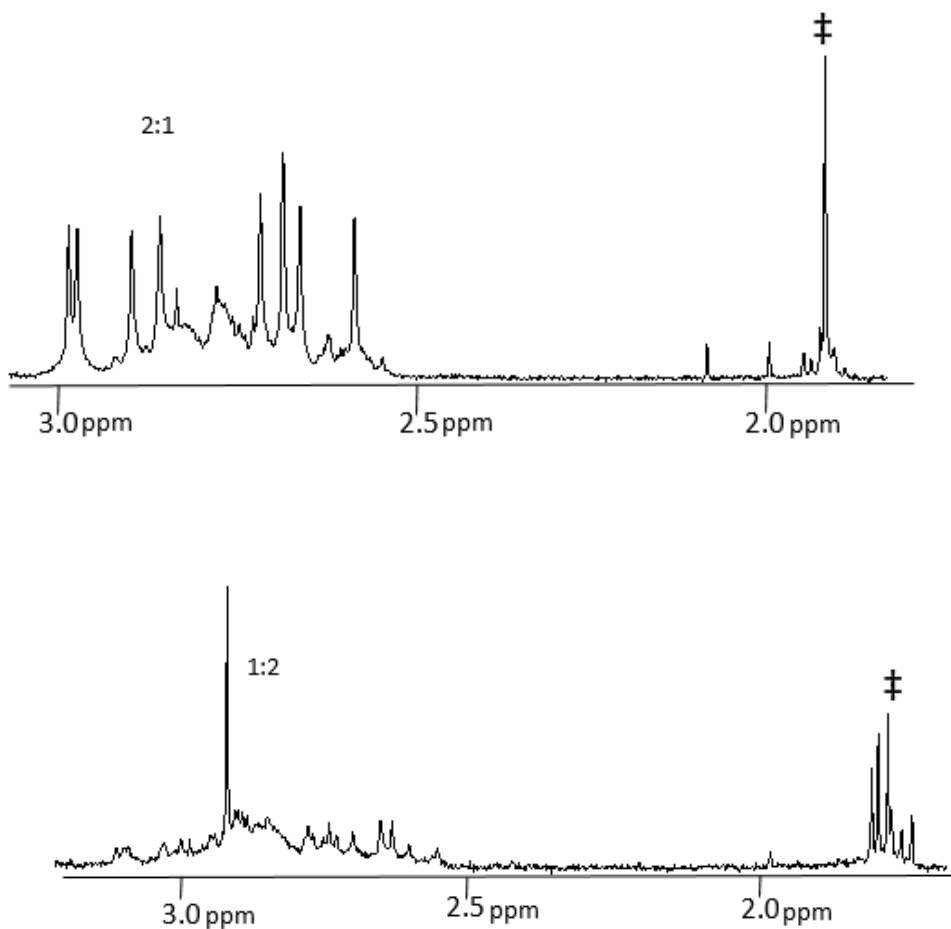

At a ratio of 2:1 Pt:Cys there is a large product peak that are likely associated with 2 Pt bound to the S of a single Cysteine molecule. At a ratio of 1:2 the Cysteine product peaks

are smaller and there is a large product peak around 2.8 ppm. This peak could be due to the prevalence of 2 Cys binding to a single Pt as the major product.

3.8 [Pt(Me₄en)(NO₃)₂] Mass Spectroscopy
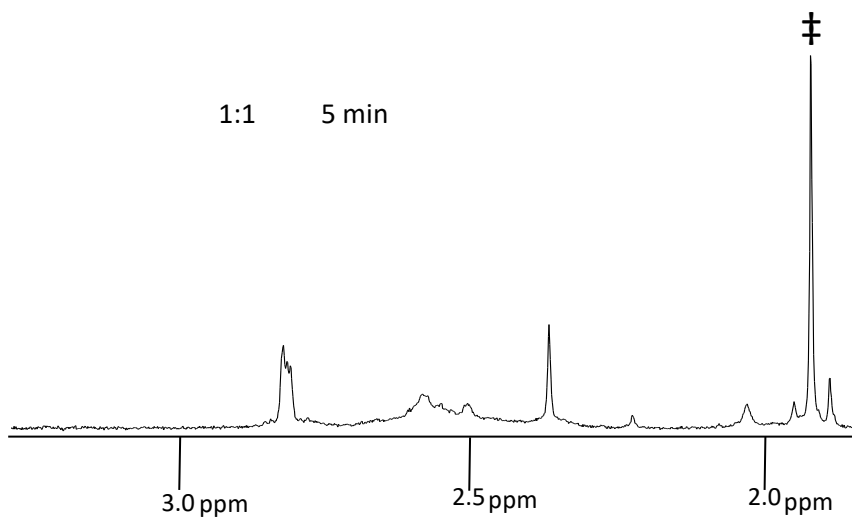
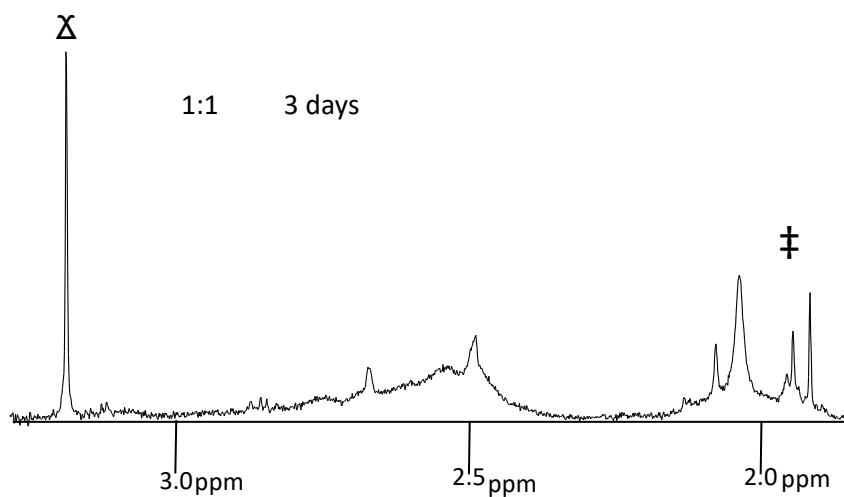Figure 3.8. Mass spectra and proposed Lewis structure of the 2:1 [Pt(Me₄en)(NO₃)₂] : Cys product at pH 4.
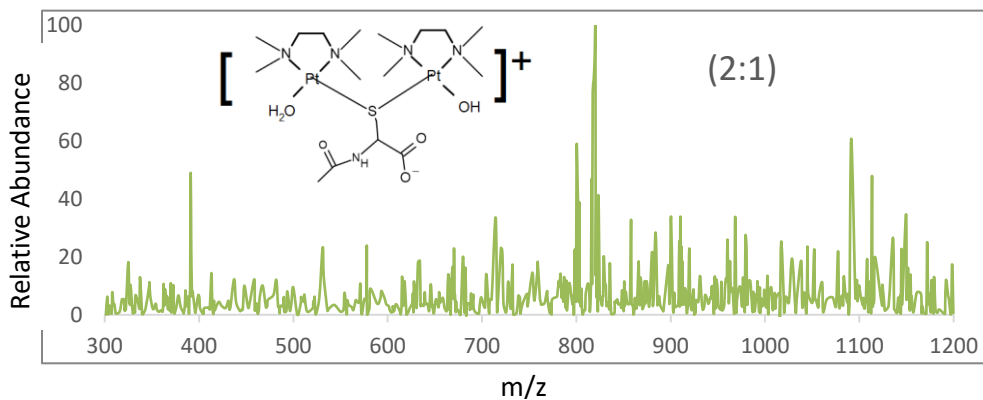
The largest peak in the Mass Spectrum is roughly 862 g/ml, which matches the weight of the 2 Pt to 1 Cys product.

3.9 Pt[(en)(NO₃)₂] at pH 4 with Cysteine in ratios 1:1 and 2:1

Fig 3.9 Pt[(en)(NO₃)₂]at pH 4 with Cysteine at a 1:1 ratio at times 5 min and 3 days.

The signal that appears after 3 days at ≈3.5 (X) is representative of the ethylene diamine ligand being removed from the complex. This is indicative of both coordination sites being occupied by the S of 2 different N-AcCys, which then chelated with the platinum atom.
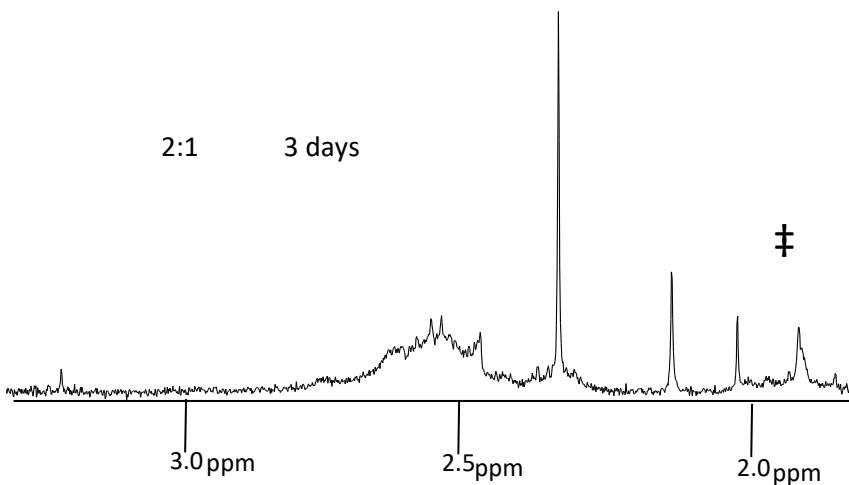
Fig. 3.10 Pt[(en)(NO_3)_2]at pH 4 with Cysteine at a 2:1 ratio at times 7 min and 3 days.

2:1   3 days

‡

3.0ppm   2.5ppm   2.0ppm
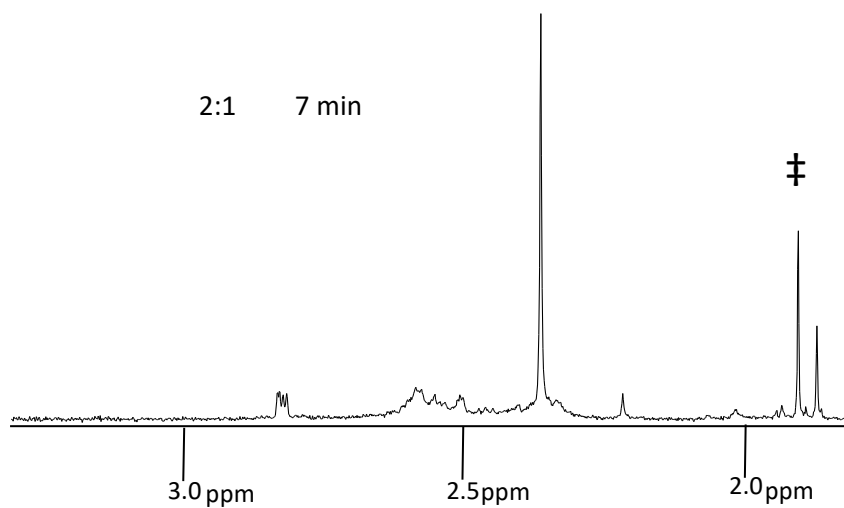
There is a large unreacted Pt signal present even after 3 days and no signal of displaced

ethylene diamine ligands. This implies that with excess Pt the complex preferers to bind

to only one N-AcCys. We have not characterized the product signals at this time.

IV.   Conclusion

The Me$_5$dien ligand has been previously been shown to react faster with Met than

guanosine. This research shows that at a pH 7, the Me$_5$dien ligand will preferentially bind

to the S of Cys. We think there are two reasons for this. The first is that the steric

hindrance caused by the ligand will clash with the methyl group on methionine's S atom

preventing the Pt from binding while Cys only has a thiol group and therefor has little

steric hindrance. The second is that the Pt will react well with Cys at pH 7 due to Cys

having a pI of 8.1. At pH 7 some of the Cys molecules are starting to deprotonate the

thiol groups, creating a charge on the S. Previously it has been shown that Me$_5$dien will

react faster with 5'-GMP than with methionine, implying that the Pt complex prefers to

react with DNA over proteins[26]. With the added information showing that it will also

react with cysteine faster than methionine, this implies that when reacting with proteins

Me$_5$dien will preferentially target cysteine residues. Potentially changing what proteins it will react with and the locations of the binding sites. Understand how the platinum compound ligands affect binding and under what conditions could help in understanding where they will preferentially bind to proteins and DNA.

The Me$_4$en ligand showed several different products. At a 1:1 ratio we characterized the products using LCMS. Based on the molecular weight of the major product, we believe 2 platinum compounds are bound to the sulfur of the same N-AcCys. This shows that the bulk of the Me$_4$en ligand does not cause enough steric hindrance to prevent 2 platinum complexes from binding to the same unhindered target site. This has already been seen in published literature with 2 [Pt(dien)]$^{2+}$ bound to the S of a single Glutatione[27].

The en ligand is small enough that when in a 1:1 ratio the dominant product is 2 Cys coordinated to the same platinum chelating and removing the en ligands. This implies that there is potential that Pt[(en)(NO$_3$)$_2$] can crosslink when reacting with proteins. It has already been shown to make crosslinks with DNA[23].

V. References

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