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Characterization and Reaction of an Analog of the Anticancer Drug Oxaliplatin

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CHARACTERIZATION AND REACTION OF AN ANALOG OF THE ANTICANCER DRUG OXALIPLATIN

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By

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ABSTRACT

Oxaliplatin is an anticancer drug that reacts with DNA, RNA, and proteins both in vitro and in vivo. Our research focuses on synthesizing analogs of oxaliplatin and understanding how bulky ligand groups affect reaction with amino acids. (R,R)-N,N'-dimethyl-1,2-diaminocyclohexane platinum(II) oxalate or Pt(Me_2dach)(ox) varies from oxaliplatin or Pt(dach)(ox) in that it has one methyl group attached to each platinum coordinated nitrogen. Nuclear Magnetic Resonance (NMR) spectroscopy has shown that the reactions of *N*-Acetylmethionine (*N*-AcMet) with Pt(Me_2dach)(ox) and Pt(dach)(ox) proceed at similar rates suggesting that the methyl groups of Pt(Me_2dach)(ox) have little effect on the initial reaction. Whereas the reaction of Pt(dach)(ox) and *N*-AcMet can form 1:1 or 1:2 complexes, Pt(Me_2dach)(ox) with *N*-AcMet can form only 1:1 products. Depending on the Pt:*N*-AcMet ratios, Pt(dach)(ox) has the potential to form either a [Pt(dach)(*N*-AcMet-*S*)$_2$] or [Pt(dach)(*N*-AcMet-*S*,*N*)] complex. The bis product is not found in Pt(Me_2dach)(ox) reactions because the formation of a [Pt(Me_2dach)(*N*-AcMet-*S*,*O*)]$^+$ product hinders the coordination of a second *N*-AcMet. It has therefore been deduced that the additional methyl groups of Pt(Me_2dach)(ox) limit reaction with *N*-AcMet to a 1:1 molar product.

Keywords: Oxaliplatin, Cancer, Platinum, Nuclear Magnetic Resonance, Proteins

Dedicated to those trying to connect the dots.

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PUBLICATIONS

1. Williams, K.M.; Poynter, A.D.; Hendrie, J.D.; Jackson, D.C.; Martin, V.K. "Comparison of N-acetylmethionine reactivity between oxaliplatin and an oxaliplatin derivative with chiral (S,S) amine nitrogen atoms", Inorg. Chim. Acta, 2013, 401, 64-69.

FIELDS OF STUDY

Major Field: Biochemistry

Minor Field: Biophysics

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CHAPTER 1

INTRODUCTION

Cisplatin, (SP-4-2)-Diamminedichloroplatinum, *cis*-DDP is an anticancer drug that was first synthesized by Michael Peyrone in 1845. However, the chemotherapeutic activity of the drug wasn't discovered until more than a century later, in 1970, when Barnett Rosenberg and his research team inadvertently stumbled upon it. The research team was studying the growth of *Escherichia coli*, a type of bacteria, in the presence of an electric current. Platinum electrodes were used to establish electric fields, due to their inertness, and the *E. coli* did not divide, but rather elongated. It was determined that this anomaly was caused by the reaction of the platinum electrodes with agents in the bacterial solution, rather than by the electric current [1].

The drug gained popularity as studies showed that cisplatin was a multidimensional anti-carcinogenic agent inhibiting the metastasis of testicular, ovarian, lung, and stomach cancers. During clinical research, cisplatin displayed negative side effects including but not limited to nephrotoxicity, neurotoxicity, and low blood mineral levels [1-2]. This discovery prompted investigation into safer cisplatin analogs with similar anticancer activity. Whereas cisplatin displays chemotherapeutic activity, its stereoisomer transplatin does not, for reasons discussed later.

The second generation anticancer drug carboplatin, cis-Diammine(1,1-cyclobutanedicarboxylato)platinum(II), was discovered in the late 1980s. Following FDA approval in 2003, the drug entered the market under the trade name Paraplatin. The drug was found effective in fighting ovarian and non-small cell lung cancers [3]. Side effects of using carboplatin were greatly reduced in comparison to its parent cisplatin [4].

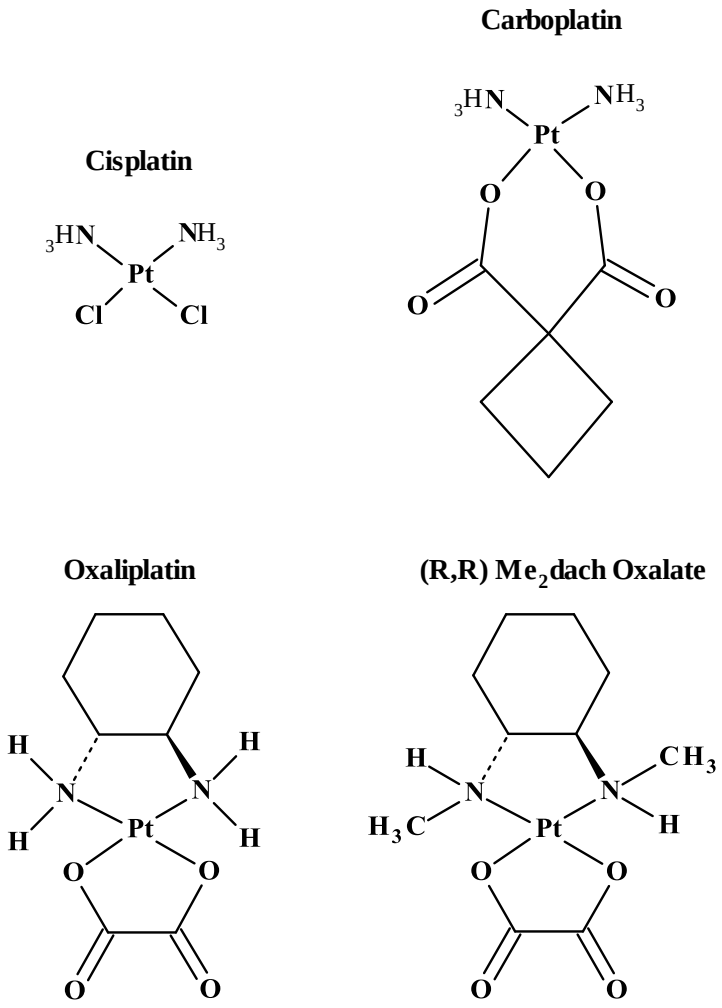

Figure 1.1. Skeletal Structures of Platinum Compounds.

The third generation antineoplastic drug oxaliplatin was discovered in Japan in 1976 by Kidani [6]. It became FDA approved in 2002 taking on the trade name Eloxatin [7]. Eloxatin is used in combination with 5-fluorouracil and leucovorin to treat colorectal cancer [8].

These platinum based drugs are alkylating-like agents subcategorized as metallic salts that exhibit anticancer activity by targeting cells with expedited replication. Due to the influx of agents in rapidly dividing cells, chemotherapy drugs are often uptaken; platinum based drugs bind to DNA and bend it thereby inhibiting transcription and further replication. They are cell cycle non-specific meaning they affect cells at rest [9]. Cisplatin, carboplatin, and oxaliplatin each react by the same mechanism, and are administered via intravenous (IV) injection. Once in the blood these compounds circulate in their inert forms – chlorides on cisplatin, biscarboxylate on carboplatin, oxalate on oxaliplatin – with the potential to react with proteins and other agents [10]. Platinum(II) compounds have a high kinetic affinity for binding proteins, and these interactions may play critical roles in oxidative stress [11], cellular uptake [12], or resistance pathways [13]. There is currently no certainty as to how these cytotoxic agents enter a cell; one suggestion is that these drugs passively diffuse into cells or get uptaken by the human

1,2 – Diaminocyclohexane Pt(II) Dichloride	Dach Dichloride
1,2 – Diaminocyclohexane Pt(II) Oxalate	Dach Oxalate or Oxaliplatin
N,N' – Dimethyl – 1,2 –Diaminocyclohexane Pt(II) Dichloride	Me_2dach Dichloride
N,N' – Dimethyl – 1,2 –Diaminocyclohexane Pt(II) Oxalate	Me_2dach Oxalate
N-Acetylmethionine	N-AcMet
Deoxyribonucleic Acid	DNA
Ribonucleic Acid	RNA

copper transporter 1 (hCTR1) [14].

CTR1 is a transmembrane hydrophilic copper transporter. It forms as a homotrimer leaving a pore composed of cysteines, methionines, and histidines through which copper passes. Methionines 150 and 154, and histidine 139 transport Cu(I) down a concentration gradient via transchelation reactions [15]. Platinum based drugs may be uptaken using the same mechanism, and the fact that platinum based compounds are attracted to the nucleophilic sulfur sites of cysteine and methionine residues strengthens this idea[16]. The attraction of sulfur and platinum can also be explained using Hard-Soft Acid-Base theory, which states that hard acids prefer to interact with hard bases and soft acids with soft bases. Platinum, being a 5d transition metal is considered a soft acid while sulfur is a known as a soft base, hence the strong interaction. Whereas lack of CTR1 expression leads to resistance of cisplatin, carboplatin, and oxaliplatin in mammalian and yeast cells, overexpression enhances cellular recognition of their toxic effects thereby increasing influx of these agents [17]. This demonstrates a direct link between CTR1 and the anticancer activity of these platinum drugs.

Once inside the cell, these compounds become aquated e.g., cisplatin forming $[Pt(NH_3)_2(H_2O)Cl]^{1+}$ and ultimately $[Pt(NH_3)_2(H_2O)_2]^{2+}$. Once they become positively charged and incapable of crossing the hydrophobic membrane, they are trapped within the cell and passively diffuse into the nucleus. The two most nucleophilic regions of DNA base pairs are the N7-positions of guanine and adenine. It is here where platinum complexes forms interstrand or intrastrand cross bridges with DNA. Potential cross-links include 1,2-intrastrand, 1,3-intrastrand, 1,2-interstrand, and DNA-protein adducts.

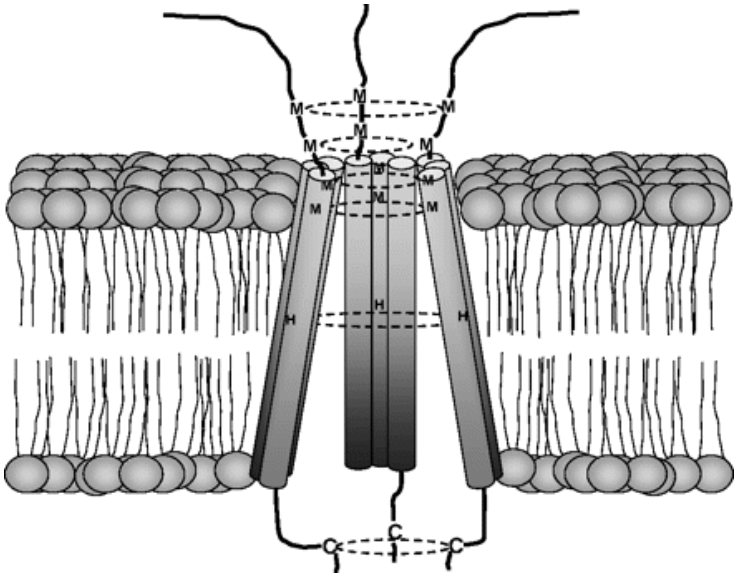

Figure 1.2. Visual Depiction of the Copper Transporter 1 homotrimer [14]

1,2-intrastrand cross-links form 90^{o} angles between two adjacent guanine or adenine residues and the platinum atom, which bends the DNA duplex towards the major groove. This exposes the minor groove of the DNA strand to which multiple classes of proteins can bind, such as the damage recognition proteins human upstream binding factor (UBF), TATA box-binding proteins (TBP), and high-mobility group box proteins (HMGB1), and mismatch-repair proteins [14]. HMG-domain proteins have a high affinity for platinum-guanine, guanine adducts [18] and binding leads to inhibition of nucleotide excision repair, transcription, and replication, consequentially warranting apoptosis [14]. This leads to chemotherapeutic activity and tumor reduction.

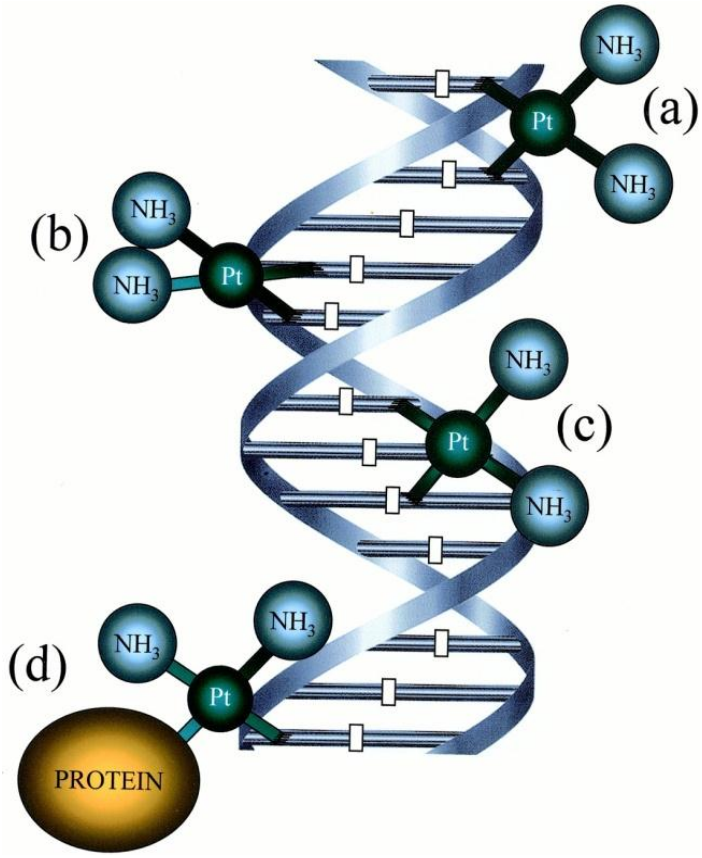

Figure 1.3. Potential cross-links of platinum drugs: (a) 1,2-interstrand (b) 1,2-intrastrand (c) 1,3-intrastrand (d) DNA-protein adduct

As previously described, these drugs have the ability to bind with DNA and/or proteins [19]. Our lab focuses on the reactions of oxaliplatin and its derivatives with different amino acids found within select proteins. Previous research has shown that platinum based chemotherapeutic drugs react with proteins located in the blood, cytosol, or embedded in membranes. Research is aimed at identify how much bulk can be added to oxaliplatin analogs before slowing down such reactions. It is speculated that bulk amount and anticancer activity are inversely related. The current analog we are studying,

(R,R)-N,N'-dimethyl-1,2-diaminocyclohexane platinum(II) oxalate or Pt(Me_2dach)(ox) is being studied and characterized upon reaction with *N*-Acetylmethionine (*N*-AcMet). It differs from oxaliplatin in that it has one methyl group attached to each of the platinum coordinating nitrogen atoms.

We have found that the additional bulk of Pt(Me_2dach)(ox) does slow reaction with N-AcMet, but minimally. Pt(dach)(ox) has the ability to coordinate to two amino acids in a 1:2 Pt:*N*-AcMet ratio, but the bulk of Pt(Me_2dach)(ox) limits reaction to a 1:1 product in the form of a S,O-chelate. This could either prevent the compound from entering the cell by hindering transchelation reactions within the hCTR1 or block the molecule from forming Pt-DNA complexes.

CHAPTER 2

METHODOLOGY

A JEOL Eclipse 500 MHz NMR Spectrometer was used to obtain ^{1}H and ^{195}Pt NMR spectra. Deuterium Oxide (D_2O) was used as a standard reference.

Several syntheses were common throughout this research in order to obtain:

Silver Oxalate

Silver oxalate was synthesized by mixing 350 mg oxalic acid ($C_2H_2O_4$) with 1 g of silver nitrate ($AgNO_3$) in 20 mL DI H_2O (1.5 mM $AgNO_3$: 1 mM $C_2H_2O_4$) in an amber vial. After stirring for approximately 30 minutes the milky white precipitate was run through a vacuum filtration apparatus for 5-10 minutes. The vial was rinsed with ethanol to get all $Ag_2C_2O_4$ out and once dry, the solid was collected. Yield = 798.8 mg.

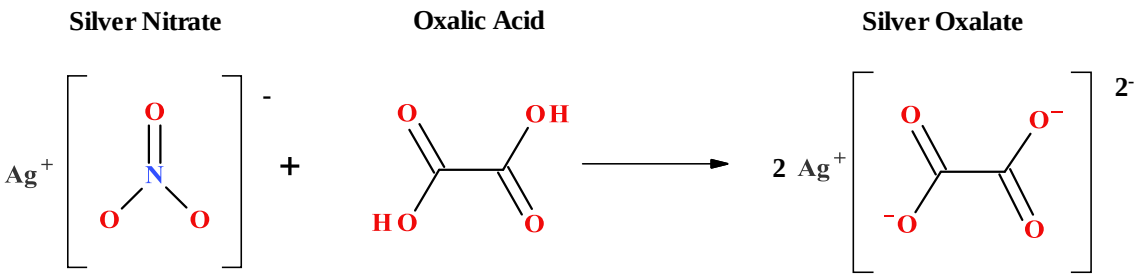

FIGURE 2.1. Synthesis of silver oxalate.

(R,R)-1,2-Diaminocyclohexane Platinum(II) Dichloride

The synthesis of Dach Dichloride required making two separate solutions. Solution 1 consisted of 64 mg dach ligand and 5 mL of methanol in a 10 mL beaker. Solution 2 involved mixing 232 mg (equi-molar amount) of potassium tetrachloroplatinate (K_2PtCl_4) and 5 mL of DI H_2O in an amber vial. Solution 2 was added drop-wise to solution 1 while stirring. After stirring for 2-3 hours, the precipitate was collected by vacuum filtration and rinsed with water, ethanol, and ether. Yield = 149 mg yellow solid.

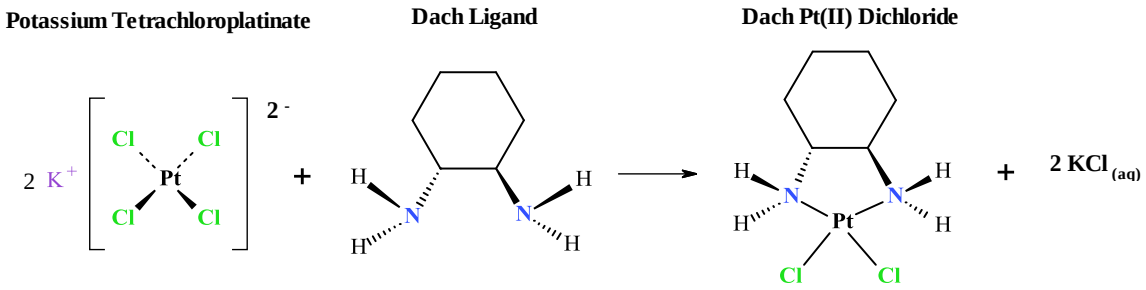

FIGURE 2.2. Synthesis of Dach Dichloride

(R,R)-1,2-Diaminocyclohexane Platinum(II) Oxalate

In an amber vial, equimolar amounts of Dach Dichloride and silver oxalate were dissolved in 35 mL of DI H_2O (100 mg Dach Dichloride and 80 mg silver oxalate was standard). They sample was put on a stir plate for two days or until completely mixed. Once properly mixed, the sample was filtered via syringe and a 0.2 micron non-disposable filter into a 50 mL round bottom flask to remove the $AgCl_{(s)}$ precipitate. At this point, a laser pointer was used to check for undissolved suspension. If clear, the solution was dehydrated using a rotary evaporator and collected. Yield = 78.1 mg.

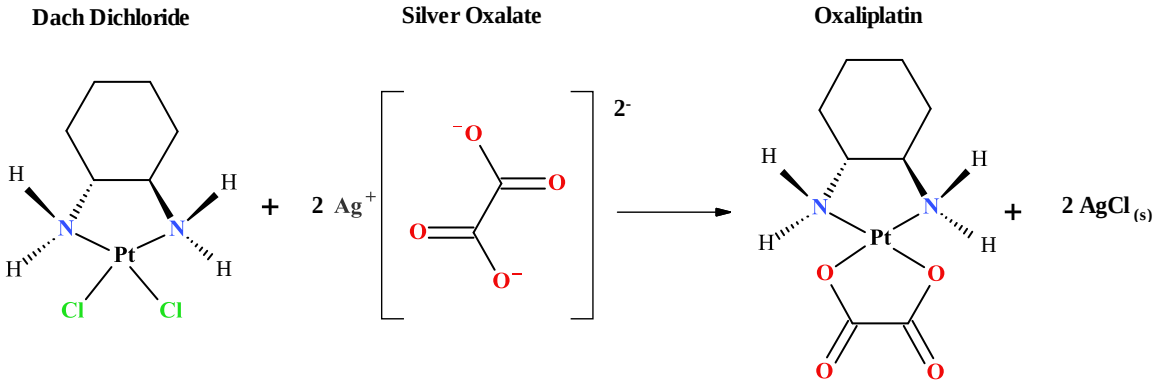

FIGURE 2.3. Synthesis of oxaliplatin.

(R,R)-N,N'-Dimethyl-1,2-Diaminocyclohexane Platinum(II) Dichloride

The synthesis of Me_2dach Dichloride required making two separate solutions. Solution 1 consisted of 56.8 mg Me_2dach ligand and 5 mL of methanol in a 10 mL beaker. Solution 2 involved mixing 166 mg (equi-molar amount) of potassium tetrachloroplatinate (K_2PtCl_4) and 5 mL of DI H_2O in an amber vial. Solution 2 was added drop-wise to solution 1 while stirring. After stirring for 2-3 hours, the yellow solution was run through a vacuum filtration apparatus and rinsed with water, ethanol, and ether. Yield = 124.5 mg.

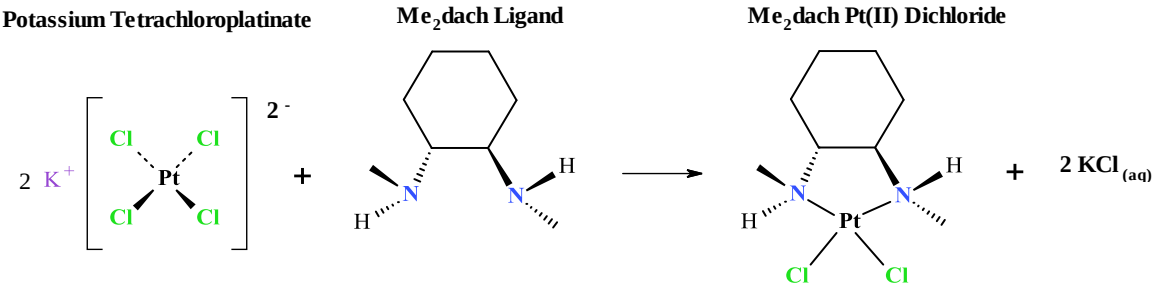

FIGURE 2.4. Synthesis of Me_2dach Dichloride.

(R,R)-N,N'-Dimethyl-1,2-Diaminocyclohexane Platinum(II) Oxalate

In an amber vial equimolar amounts of Me_2dach Dichloride and silver oxalate were dissolved in 35 mL of DI H_2O (84.8 mg Me_2dach Dichloride and 61.7 mg silver oxalate was standard). They were then put on a stir plate for two days or until completely mixed. Once properly mixed, the sample was filtered via syringe using a 0.2 micron non-disposable filter into a 50 mL round bottom flask to remove $AgCl_{(s)}$ precipitate. At this point, a laser pointer was used to check for undissolved suspension. If clear, the solution was dehydrated using a rotary evaporator. Yield = 75.1 mg.

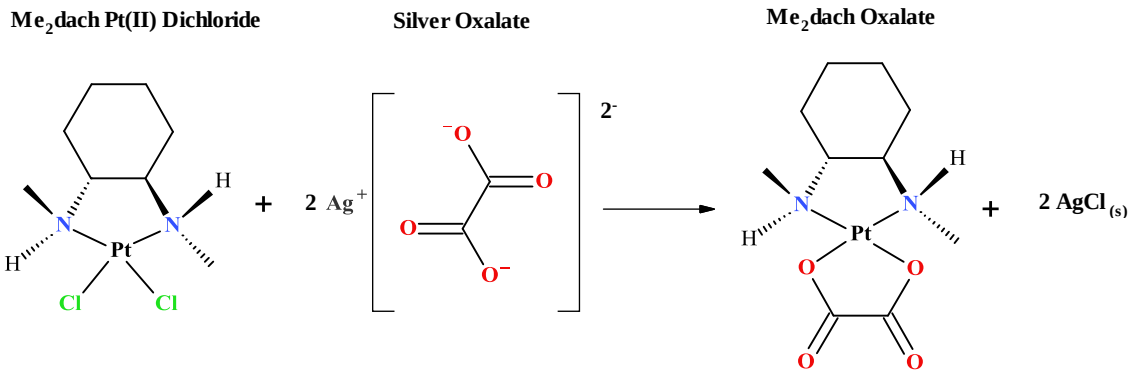

FIGURE 2.5. Synthesis of Me_2dach Oxalate

These syntheses were run and reacted with *N*-AcMet in the following solutions to observe product formations and reaction kinetics. Using the results, we have characterized these reactions and shown that Pt(Me_2dach)(ox) is limited to the formation of a mono-product, whereas Pt(dach)(ox) can form two products.

*Solution A = Dissolved 3.4 mg Me_2dach Oxalate in 1.2 mL D_2O.
*Solution B = Dissolve 3.8 mg *N*-AcMet in 1.0 mL D_2O.

600 µL NMR samples were prepared for various Pt:*N*-AcMet ratios:

1:1 Molar Ratio	1:2 Molar Ratio	5:1 Molar Ratio
300 µL Solution A	300 µL Solution A	750 µL Solution A
200 µL D_2O	100 µL D_2O	200 µL D_2O
100 µL Solution B	200 µL Solution B	50 µL Solution B

CHAPTER 3

RESULTS

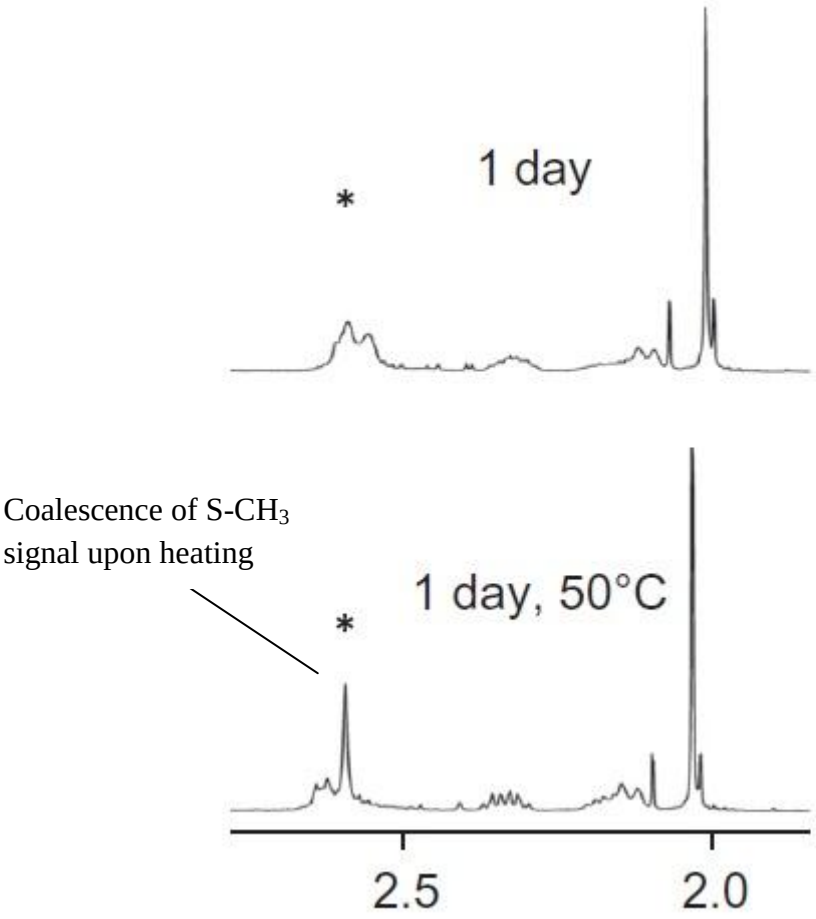

FIGURE 3.1. Partial ^{1}H NMR Spectra of 3.33 mM Pt(dach)(ox) and 6.66 mM N-AcMet (1:2). The Y-axes are scaled to the tallest peak and the * indicates the S-CH_3 interaction.

The signals at 2.56 and 2.58 ppm coalesce upon heating to 50^{o}C and represent the sulfur-methyl hydrogens upon Pt(dach)(*N*-AcMet-S_2)]formation. The peak at 2.02 ppm indicates the Ac-CH_3 resonances.

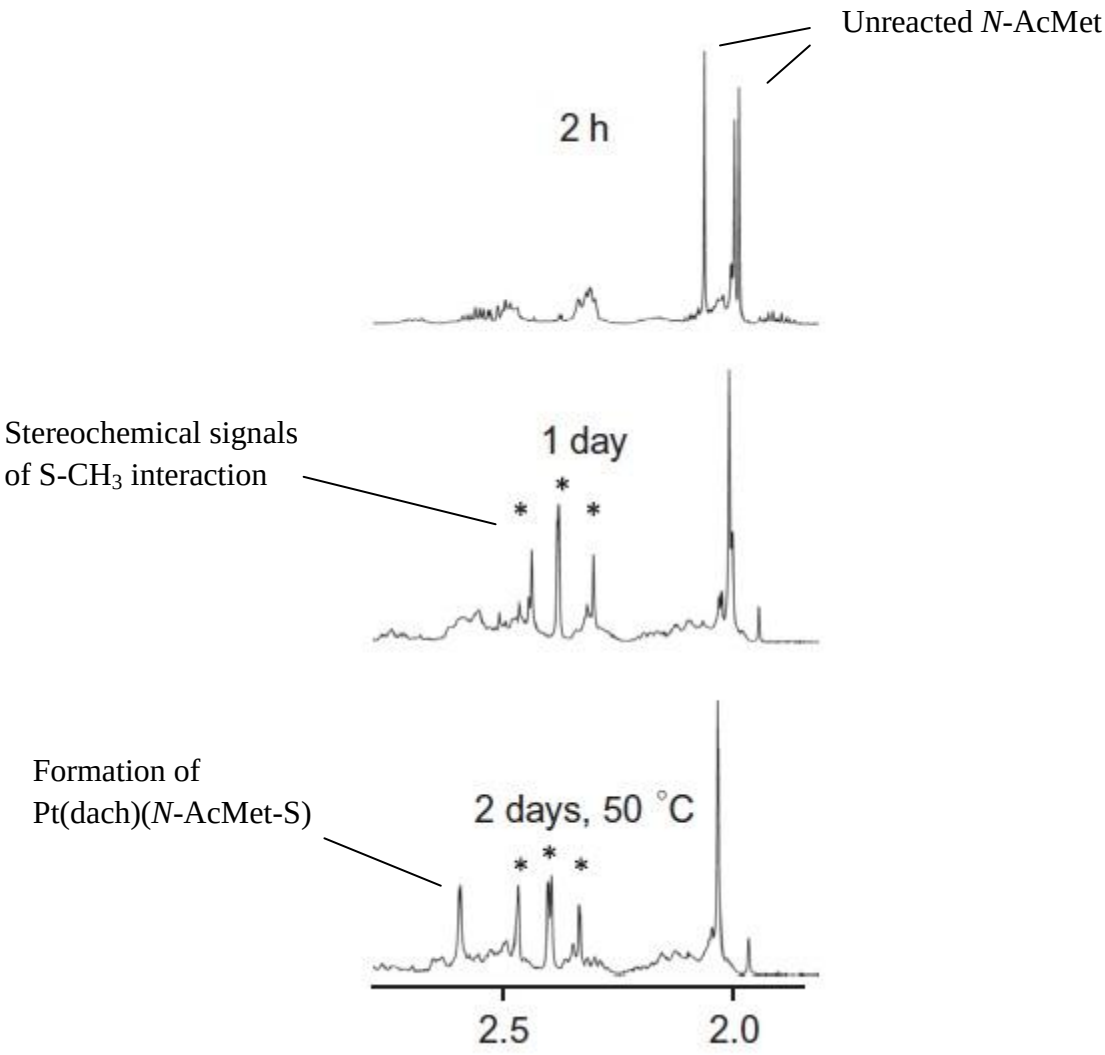

FIGURE 3.2. Partial ^{1}H NMR spectra of 3.33 mM Pt(dach)(ox) and 3.33 mM *N*-AcMet (1:1). Y-axes are scaled to the tallest peak and the * indicates the S-CH_3 signals of a S,N-chelate.

The three signals at 2.30, 2.37, and 2.43 ppm indicate the sulfur-methyl hydrogens after formation of a [Pt(dach)((*N*-AcMet-S,N)] complex, while the signal at 2.02 ppm respresents the overlap of Ac-CH_3 resonances [20]. Additionally, the sharpened bis-product signal appears upon heating reaction, suggesting a mixture of products for an equimolar ratio reaction.

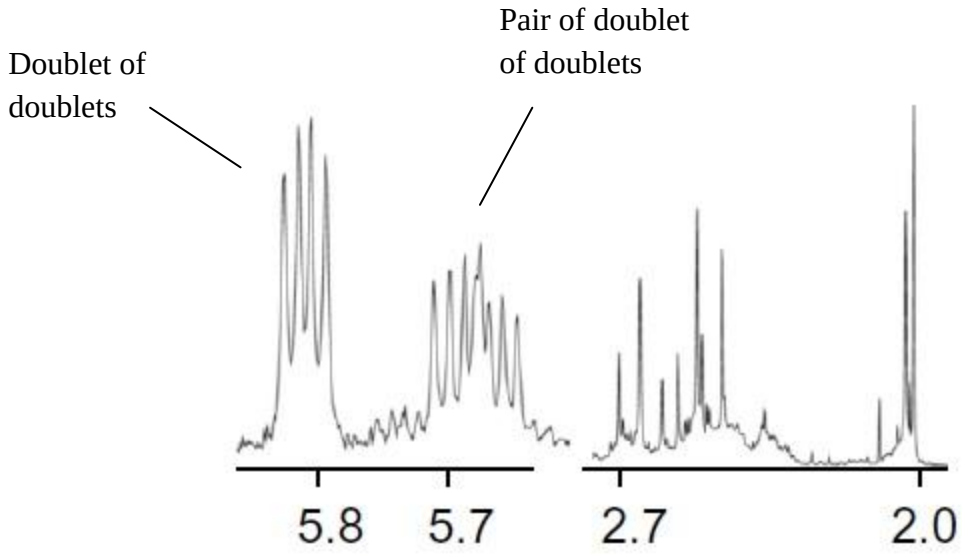

FIGURE 3.3. Partial ^{1}H NMR spectrum of 10 mM Pt(Me$_2$dach)(ox)and 10 mM *N*-AcMet (1:1). Y-axes are scaled to the tallest peak.

Three doublet of doublets appear between 5.7-5.9 ppm, which are characteristic of the *N*-AcMet alpha-hydrogen upon formation of the S,O-chleate [21].

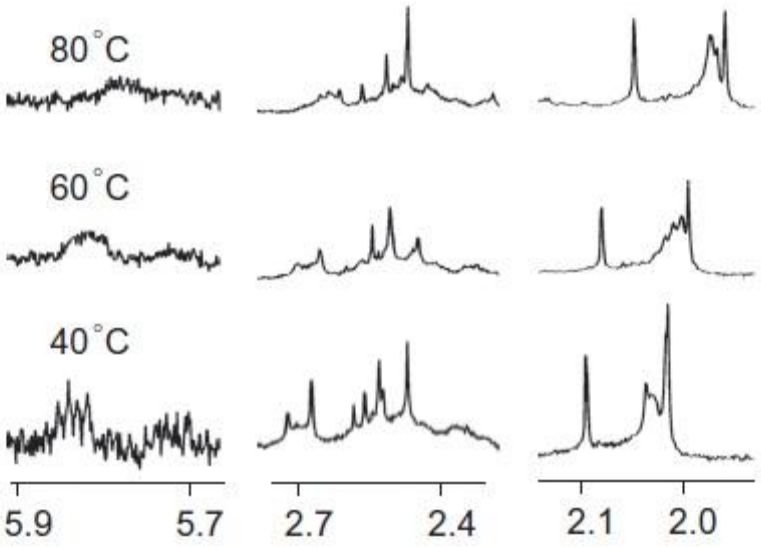

FIGURE 3.4. Partial ^{1}H NMR spectra of [Pt(Me$_2$dach)(*N*-AcMet-S,O)]$^{+}$ at varied temperatures.

The resonances of [Pt(Me$_2$dach)(*N*-AcMet-S,O)]$^{+}$ broaden with increased temperature, suggesting several species undergoing chemical exchange based on the chirality of the sulfur atom in the S,O-chelate.

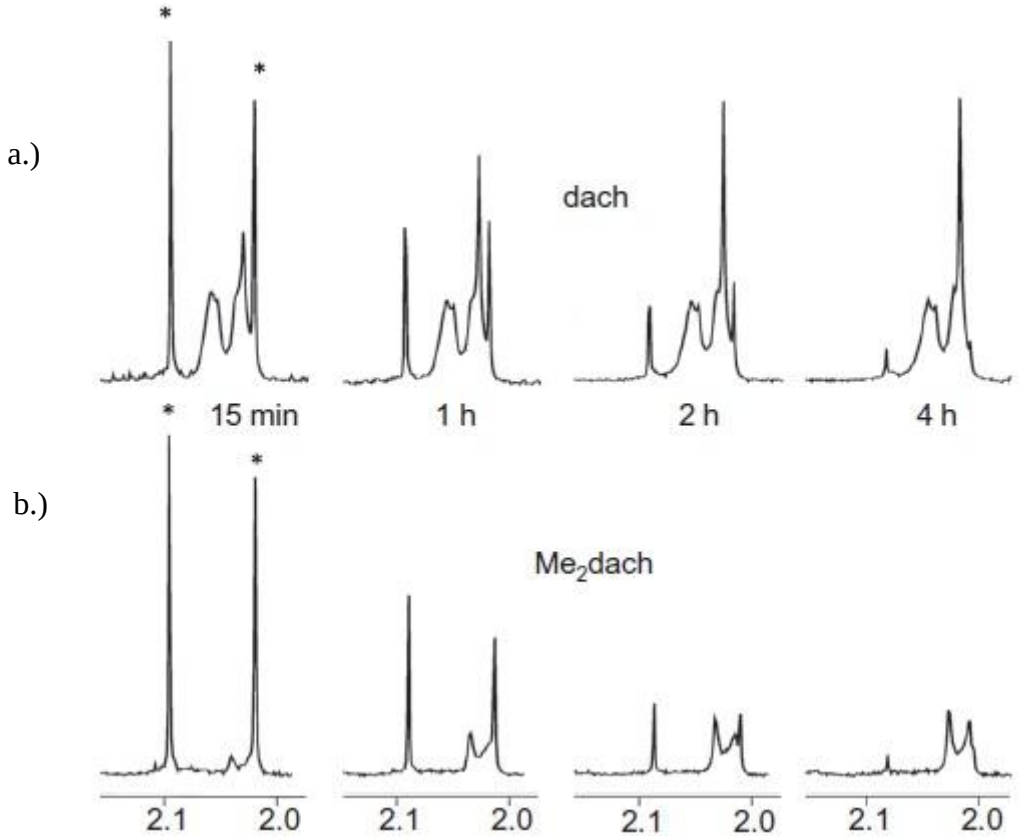

FIGURE 3.5. Partial ^{1}H NMR spectra of 5 mM a.) Pt(dach)(ox) and b.) Pt(Me$_2$dach)(ox) reacted with 1 mM of *N*-AcMet. Each compound's time points are scaled to the same factor and * represents unreacted *N*-AcMet.

As seen, oxaliplatin and Me$_2$dach Oxalate react at a similar rate. Each of these react with a half-life of approximately one hour , while after 4 hours, *N*-AcMet has nearly completely reacted.

CHAPTER 4

DISCUSSION

We have found that Pt(dach)(ox) has the capability to form 1:1 and 1:2 Pt: *N*-AcMet products whereas our novel compound Pt(Me_2dach)(ox) is limited to a 1:1 molar ratio due to the formation of a [Pt(Me_2dach)(ox)(*N*-AcMet-S,O)]$^+$ complex. ***Figure* 3.1** shows the reaction of oxaliplatin with *N*-AcMet in a 1:2 molar ratio. As illustrated by the figure, we raised the temperature to 50^o C to speed up the reaction and found the formation of a [Pt(dach)(*N*-AcMet-S)$_2$] complex. The formation of this bis-product can be seen by the attenuation of the unreacted *N*-AcMet peaks and formation of product peaks, as well as the signals indicated by the asterisk. The asterisks represent the peaks that correspond to the S-CH_3 interactions of our bis-product. This was further characterized using ^{195}Pt NMR spectroscopy, which showed a peak at -3739 parts per million (ppm). This chemical shift falls in the range of -3600 to -3800 – the precedent for a PtN_2S_2 coordination environment [21, 22, 23].

FIGURE 3.2. shows the reaction of Pt(dach)(ox) with *N*-AcMet in a 1:1 molar ratio. We have deduced that this particular reaction forms two products – [Pt(dach)(*N*-AcMet-S,N)] and [Pt(dach)(*N*-AcMet-S_2)]. Again, we note the attenuation of the unreacted *N*-AcMet peaks around 1.95 and 2.1 ppm. After 24 hours we see the formation of three product peaks between 2.3-2.43 ppm and after 48 hours the same peak from

FIGURE 3.1. arises. As previously characterized by Barnham et al., three peaks within this range are indicative of the sulfur-methyl hydrogens upon formation of a S,N-chelate [20]. Furthermore, the ^{195}Pt spectrum showed signals at -3215 and -3260, which are characteristic of a PtN_3S coordination environment [21, 22].

FIGURE 3.3. is part of the ^{1}H NMR spectrum for the 1:1 Pt(Me_2dach)(ox):*N*-AcMet reaction. There are two doublets of doublets visible around 5.7 ppm and another doublet of doublets at 5.8 ppm that are characteristic of the alpha-hydrogen of a S,O-chelate [17, 23]. The series of peaks between 2.4-2.7 ppm signify the sulfur-methyl hydrogens from the various stereoisomeric intermediate products of the sulfur atom. ^{195}Pt NMR signals at -2870 and -2890 ppm are within the benchmark range of -2600 to -2900 ppm for a typical PtN_2SO coordination environment. After raising the temperature, as seen in **FIGURE 3.4.**, the resonances broadened suggesting multiple species undergoing chemical exchange, thus strengthening the conclusion drawn from the peaks at 2.4-2.7 ppm in **FIGURE 3.3.**.

Upon characterizing the reaction of Pt(Me_2dach)(ox) with *N*-AcMet in different molar ratios, we found our data to be consistent throughout. Therefore, we have deduced that the additional bulk or methyl groups of Pt(Me_2dach)(ox) limits the reaction to a 1:1 product in the form of [Pt(Me_2dach)(*N*-AcMet-S,O)]$^{+}$. This would prevent the platinum complex from binding with DNA or proteins, inhibiting some of its aforementioned functions.

In addition to characterizing the reactions of oxaliplatin and our anolog Me_2dach oxalate in different molar ratios with *N*-AcMet, we have also kinetically studied these

reactions. **FIGURE 3.5.** shows the reactions of both Pt(Me_2dach)(ox) and Pt(dach)(ox) with *N*-AcMet in 5:1 Pt:*N*-AcMet molar ratios over four hours. In each, we see the attenuation of initial peaks and the complete formation of product signals after 4 hours. Therefore, we have concluded that Pt(Me_2dach)(ox) and Pt(dach)(ox) react at similar rates, despite the fact that they form different products upon reaction with *N*-AcMet [24].

Our lab has previously studied another diamine compound pair – ethylenediamine platinum(II) dinitrate or Pt(en)$(NO_3)_2$ and *N*,*N*,*N*',*N*'-tetramethylethylenediamine platinum(II) dinitrate or Pt(Me_4en)$(NO_3)_2$. Similar to our present compound Pt(Me_2dach)(ox), Pt(Me_4en)$(NO_3)_2$ has additional methyl groups attached to the secondary amine atoms. Interestingly, the additional bulk of Pt(Me_4en)$(NO_3)_2$ limits product formation to a $[Pt(Me_4en)(N\text{-AcMet-}S,O)]^{2+}$. Conversely, Pt(en)$(NO_3)_2$ forms both bis-prodcuts and mono-products, similar to Pt(dach)(ox). As noted previously, the additional methyl groups of Pt(Me_2dach)(ox) did not have a significant effect on reaction kinetics; on the contrary the four methyl groups of Pt(Me_4en)$(NO_3)_2$ slow reaction nearly 16-fold in comparison to Pt(en)$(NO_3)_2$ [21]. These comparisons do not provide a conclusive relationship between substituent bulk and reaction rate, but demonstrate influence on product formation.

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