Synthetic Strategies for the Design of Platinum Anticancer Drug Candidates

by

Justin Jeff Wilson

B.S. Chemistry
University of California, Berkeley, 2008

Submitted to the Department of Chemistry in Partial Fulfillment of the Requirement for the
Degree of

DOCTOR OF PHILOSOPHY IN INORGANIC CHEMISTRY

at the
Massachusetts Institute of Technology

June 2013

© Massachusetts Institute of Technology, 2013
All Rights Reserved

Signature of Author:

Department of Chemistry
April 25, 2013

Certified by:

Arthur Amos Noyes Professor of Chemistry
Thesis Supervisor

Accepted by:

Chairman, Departmental Committee on Graduate Students
This doctoral thesis has been examined by a committee of the Department of Chemistry as follows:

Christopher C. Cummins
Professor of Chemistry
Committee Chairperson

Stephen J. Lippard
Arthur Amos Noyes Professor of Chemistry
Thesis Supervisor

Elizabeth M. Nolan
Pfizer Laubach Career Development Assistant Professor of Chemistry
Abstract

Chapter 1. The Synthetic Chemistry of Platinum Anticancer Agents
Since the inception of cisplatin as a clinically approved anticancer agent, a large number of platinum compounds have been synthesized with the aim of finding new, improved drugs. As a result of these efforts, only two additional platinum-based drugs received FDA approval for the treatment of cancer. Nevertheless, fundamental advancements in the synthetic chemistry of platinum arose from these research endeavors. This chapter presents a comprehensive review of synthetic methods for the preparation of classic and non-conventional platinum compounds with therapeutic potential.

Chapter 2. Platinum(II) Complexes Bearing Fluorescent Di-2-Pyridylmethane Ligands
A strategy to investigate the cellular uptake and localization of platinum anticancer agents is to label them with a fluorescent ligand. In pursuit of this strategy, three new platinum(II) complexes with modified di-2-pyridylmethane (dpm) ligands, two of which are fluorescent, were prepared. These new ligands contain either a non-fluorescent tosyl group (Ts-dpm) or fluorescent NBD or dansyl group (NBD-dpm and dansyl-dpm). The photophysical and solution conformational properties of the complexes [Pt(dpm')Cl₂], where dpm' is one of the three ligands described
above, are presented. The thermal and photolytic decomposition products and the hydrogen peroxide oxidation products were investigated.

Chapter 3. Outer-Sphere Amide Bond Coupling Reactions for the Preparation of a Fluorescent Platinum(IV) Redox Sensor

Because of the poor solubility, stability, and lack of structural similarity to clinically used platinum anticancer agents of the complexes described in Chapter 2, synthetic strategies were devised to prepare alternative fluorescent platinum compounds. The dangling carboxylic acid groups of the platinum(II) complexes \([\text{Pt(edma)C}_2]\) and \([\text{Pt(edda)C}_2]\), where edma = ethylenediamine-\(N\)-acetic acid and edda = ethylenediamine-\(N,N\)\(^{\prime}\)-diacetic acid, were functionalized by amide bond coupling using benzyl amine. For \([\text{Pt(edda)C}_2]\), the resulting product was a mixture of diastereomers owing to chirality at both coordinating nitrogen atoms. Only \([\text{Pt(edma)C}_2]\) was further modified by coupling it to dansyl ethylenediamine to form \([\text{Pt(edDS)C}_2]\), where edDS is the dansyl ethylenediamine-containing ligand. Upon oxidation of this complex with iodobenzene dichloride, the emission of the dansyl fluorophore was substantially quenched. By reducing the oxidized product \([\text{Pt(edDS)C}_4]\) with an excess of the biological reducing agents glutathione, cysteine, and ascorbic acid in aqueous buffer, a 6.3-fold turn-on in emission intensity was observed. This turn-on response suggests that \([\text{Pt(edDS)C}_4]\) and its analogues may serve as fluorescent redox sensors to monitor the reduction of platinum(IV) in living cells.

Chapter 4. In Vitro Anticancer Activity of Platinum(II) Complexes with \(\beta\)-Diketonate Leaving Group Ligands

To investigate the role of the leaving group ligand on the anticancer activity of platinum(II) complexes, five compounds with the general formula \([\text{Pt(NH}_3)_2(\beta\text{-diketonate})]^+\) were prepared
and characterized. The β-diketonate ligands were chosen to tune the lipophilicity and electrophilicity of the resulting complexes. Three general synthetic protocols for preparing such complexes were established. These compounds were tested for anticancer activity in a panel of four different cell lines. Structure-activity relationships were derived, correlating high cytotoxicity with increasing lipophilicity and decreasing donor strength of the β-diketonate.

Chapter 5. Acetate-Bridged Dinuclear Platinum(III) Complexes Derived from Cisplatin

The oxidation chemistry of a previously reported acetate-bridged dinuclear cis-diammineplatinum(II) complex, cis-[Pt\(\text{II}\)(NH\(\text{3}\))\(\text{2}\)(μ-OAc)\(\text{2}\)Pt\(\text{II}\)(NH\(\text{3}\))\(\text{2}\)](NO\(\text{3}\))\(\text{2}\), was explored. Treatment of this complex with either PhICl\(\text{2}\) or Br\(\text{2}\) afforded the 2-electron oxidized halide-capped dinuclear complexes, cis-[XPt\(\text{III}\)(NH\(\text{3}\))\(\text{2}\)(μ-OAc)\(\text{2}\)Pt\(\text{III}\)(NH\(\text{3}\))\(\text{2}\)X](NO\(\text{3}\))\(\text{2}\), where X is either bromine or chlorine. The platinum(II) and platinum(III) complexes were fully characterized by X-ray crystallography and multinuclear NMR spectroscopy. The oxidation of cis-[Pt\(\text{II}\)(NH\(\text{3}\))\(\text{2}\)(μ-OAc)\(\text{2}\)Pt\(\text{II}\)(NH\(\text{3}\))\(\text{2}\)](NO\(\text{3}\))\(\text{2}\) with PhI(O\(\text{2}\)CF\(\text{3}\))\(\text{2}\) and XeF\(\text{2}\) was also explored. The use of PhI(O\(\text{2}\)CF\(\text{3}\))\(\text{2}\) gave the unexpected amido-bridged tetranuclear platinum(III) complex, cis-[(O\(\text{2}\)CF\(\text{3}\))Pt\(\text{III}\)(NH\(\text{3}\))\(\text{2}\)(μ-OAc)\(\text{2}\)Pt\(\text{III}\)(NH\(\text{3}\))(μ-NH\(\text{2}\))]\(\text{2}\)(NO\(\text{3}\))\(\text{4}\), which was characterized structurally by X-ray crystallography. From the analogous reaction using XeF\(\text{2}\) instead of PhI(O\(\text{2}\)CF\(\text{3}\))\(\text{2}\), yellow crystals were obtained, and the crystal structure revealed an infinite chain of acetate-bridged dinuclear platinum units.


The reaction of cis,cis,trans-[Pt(NH\(\text{3}\))\(\text{2}\)Cl\(\text{2}\)(OH)\(\text{2}\)] with alkyl and aryl isocyanates (RNCO) in DMF afforded dicarbamate complexes of the general formula cis,cis,trans-[Pt(NH\(\text{3}\))\(\text{2}\)Cl\(\text{2}\)(O\(\text{2}\)CNHR)\(\text{2}\)]. The resulting complexes were fully characterized by X-ray
crystallography, multinuclear NMR spectroscopy, and cyclic voltammetry. The anticancer activities of these complexes were assessed in human lung cancer (A549) and human lung fibroblast (MRC-5) cell lines. Although no clear structure-activity relationships could be delineated, the complexes exhibited activity on the same order of magnitude as that of the clinically established drug cisplatin. Therefore, the reaction of cis,cis,trans-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$] with isocyanates provides a powerful new synthetic pathway to functionalize platinum(IV) anticancer agents.

**Appendix A. Aqueous Electrochemistry of a Platinum(IV) Prodrug**

Electrochemical studies of cis,cis,trans-[Pt(NH$_3$)$_2$Cl$_2$(OAc)$_2$] in aqueous media were carried out. Cyclic voltammetry in pH 7.4 phosphate-buffered saline with glassy carbon and Pt disk working electrodes gave substantially different peak potentials for the irreversible reduction feature. Under these conditions, the glassy carbon electrode was plated with platinum metal derived from the platinum(IV) complex, as determined by cyclic voltammetry and chronoamperometry experiments. The bulk electrolysis of cis,cis,trans-[Pt(NH$_3$)$_2$Cl$_2$(OAc)$_2$] in aqueous solution at a carbon felt working electrode was investigated by $^1$H NMR spectroscopy. These studies indicate ligand loss upon reduction from both axial and equatorial sites of the platinum(IV) complex.

**Appendix B. Targeting the Mitochondria with Platinum Anticancer Agents using Mitochondria-Penetrating Peptides**

Early results of a collaborative effort with the lab of Professor Shana O. Kelley at the University of Toronto to deliver platinum anticancer agents to the mitochondria are presented. Succinylacetone (Hsuccac) was used as a leaving group ligand for a cis-diammineplatinum(II) complex. The complex [Pt(succac)(NH$_3$)$_2$](NO$_3$), which contains a terminal, uncoordinated carboxylic acid functional group, was prepared and fully characterized. This complex was
conjugated to a mitochondria-penetrating peptide (MPP) using standard solid-phase coupling chemistry. The anticancer activity of the Pt-MPP construct was tested in both wild-type and cisplatin-resistant ovarian cancer cell lines, A2780 and A2780CP70. Although less potent than cisplatin, the construct is equally toxic to both cell lines, thereby indicating that targeting the mitochondria provides a viable strategy for circumventing resistance to platinum drugs.

Appendix C. Synthesis and Characterization of Several Novel Platinum Complexes

Throughout the course of this thesis work, several platinum complexes were synthesized and characterized, but ultimately not fully pursued as potential anticancer agents. These species include platinum compounds with dichloroacetate, 2,2'-bis(1-methylimidazolyl)phenylmethoxymethane (BIPhMe), nitrogen mustard-containing, and nitroimidazole-derivatized ligands. The syntheses and characterization of these compounds are reported. Crystal structures are described for several of them.

Thesis Supervisor: Stephen J. Lippard
Title: Arthur Amos Noyes Professor of Chemistry
ACKNOWLEDGEMENTS

I believe that there is an old proverb that says, "It takes a village to write a thesis." Maybe that's not how it actually goes, but I wouldn't know otherwise because I just spent the last five years of my life studying chemistry, not ancient proverbs. In any case, I think this "proverb" is certainly applicable to my experience here at MIT. With that said, there are numerous individuals that must be thanked for their assistance, both direct and indirect, in the successful completion of this thesis.

Before thanking individuals, I want to acknowledge the generous financial support that has enabled the construction of this thesis. All of the work presented here was made possible by funding from the National Cancer Institute under grant number CA034992. Additionally, during my final year, I was supported by a David H. Koch Graduate Student Fellowship, of which I am immensely grateful. I was able to attend a couple of conferences by funding assistance from the Society of Biological Inorganic Chemistry and the Morse family through their continuing sponsorship of the Morse Travel Grant program for the Chemistry Department of MIT.

My thesis supervisor, Professor Stephen J. Lippard, as the proverbial "village leader", deserves my immense gratitude. Steve expects only the best out of his group members. In trying to meet his expectations, I have developed a rigorous attention to detail in my writing, science, and everyday life. Steve has given me a lot of freedom to move my thesis project in different directions. In doing so, I have gained confidence as an independent researcher. Thanks Steve; it has been a good five years, and I have learned very much from you.

I would like to thank my thesis committee chairperson, Professor Kit Cummins. The advice and feedback that I received from Kit throughout our various interactions has always been insightful and valuable. I immensely enjoyed his class on main group chemistry, which exposed me to various aspects of inorganic chemistry that I would have otherwise missed out on.

I also owe thanks to the third member of my thesis committee, Professor Liz Nolan. Liz and I have met on several occasions to discuss research progress and career goals. These meetings have always been very fruitful, and I am appreciative of her valuable insight as an alumnus of the Lippard group.

The rest of the inorganic chemistry faculty have also contributed to my growth as a scientist. I have taken classes taught by Dan Nocera, Dick Schrock, and Jonas Peters, which broadened my knowledge base. I have interacted with Mircea Dincă on several occasions (not counting those back in the Long group), and he has given me valuable advice on electrochemistry.

Rich Girardi always keeps things in the Lippard group running as smoothly as possible and is always available to assist in any sort of administrative manner. I thank Allison Kelsey so much for not only introducing cookies from Flour to the Inorganic Chemistry Seminar Series, but for also letting me take the stragglers. I would feel sick after those seminar days, but that is probably be expected after eating several cookies from Flour within an hour. I learned most of what I know about crystallography from Peter Müller. His class on structure refinement is exceptional and incredibly valuable as an inorganic chemist. Furthermore, I thank Peter for organizing the Bruker Symposium, which I have always immensely enjoyed.

From U.C. Berkeley, I thank Professor Jeff Long for having me in his research group. The Long group was an incredibly fun place to do science. In the Long group, I was fortunate to interact with a number of graduate students and postdocs who are now professors at highly esteemed universities. In particular, I want to thank Professor (then postdoc) Bart Bartlett. I was
working in the organic chemistry stockroom when Bart came up, showed me a picture of his most recent crystal structure, and convinced me to work with him in the Long group. I am incredibly grateful for his mentorship, which is the basis for my scientific career. Professor John Arnold, who taught my first bona fide inorganic chemistry class, was also a formative figure in developing my research interests.

Over the last couple of years, I have interacted with many postdocs and graduate students in the Lippard Group. Several of these postdocs helped me out immensely when I was a first year student that wandered around the lab with an expression on my face analogous to that of a deer looking into headlights. Elisa Tomat fielded my naive questions about column chromatography with patience and clarity. Zach Tonzetich gave me practical advice on most aspects of lab work, and his excitement for chemistry was contagious. Joel Rosenthal was an invaluable resource regarding photochemistry. Mike Pluth shared his expansive knowledge about NMR spectroscopy and engaged me in fruitful discussions regarding many different aspects of our chemistry. Daniela Buccella gave me much invaluable crystallographic and all-around general chemistry advice. I am happy to have worked with Maksim Royzen and Yang Li on several projects. In particular, they gave me the opportunity to solve more complex crystal structures. I also worked with Ulf-Peter Apfel. In addition to being an awesome chemist, he had one heck of a volleyball serve (50% of the time). Speaking of volleyball, Shawn Lu aka Pikachu, Pablo River Fuentes, and Wei Lin were key members to our summer volleyball team. Amit Majumdar was too; apparently you are supposed to rub his belly for good luck. Robert Radford is a tremendous scientist and mentor, and a so-so volleyball player. We have collaborated on a project together (see Appendix B) that has been very exciting so far.

Within the platinum subgroup, Wee Han Ang, Guangyu Zhu, and Nora Graf were all kind and helpful resources. Nora was the official lab ice cream trip organizer. Unfortunately, I ate much less ice cream after she left. Patricio Marques-Gallego is an experienced platinum chemist who has given me valuable input on many occasions. She, too, was a key member of our volleyball team. Ga Young Park is kind and extremely hard working; I am proud to have co-authored a manuscript with her. Ying Song and I have had many discussions about chemistry and life. It has been fun to talk science with Yaorong Zheng; his project using supramolecular cages for platinum drug delivery has really turned out cool. Sami Osman and Rama Suntharalingam are relatively new postdocs to the subgroup that I have had enjoyable interactions with.

I would like to thank all of the grad students in our lab for support as we traversed the Ph.D program together. Loi Do was a great, careful researcher who was incredibly hard-working and inspiring. I remember coming in early on Sundays (7 AM) to find Loi already diligently manipulating his Schlenk line. Julia Kozukh is a fellow Berkeley alum, and we therefore spent many hours reminiscing about California. Usually, these conversations occurred during snow storms in winter, when the temperature on the west coast was in the 80s. Eric Victor provided hilarity to the lab, not because he is funny looking, but because he tells really good stories. Tim Johnstone, a fellow platinum subgroup member, is incredibly smart. We have had many enduring conversations about all sorts of miscellaneous chemistry topics. Additionally, he is my go-to partner-in-crime when it comes to finding free food around campus. For that, I am very appreciative. Ali Liang accompanied Tim and me on some of our food adventures as well. Usually, her accompaniment would result in the acquisition of even more food. Mik Minier and I have had many valuable discussions about our research. He is frequently in trouble with Lt. Dan Stevens though. Sean Yoo is a new graduate student who is just beginning his Ph.D journey. All in all, I am grateful to the current crop of graduate students, which is very cohesive. They have
been a good support system for the bad days and the good. In addition to thanking fellow grad students, I also need to thank the Muddy Charles pub and the Cambridge Brewing Company.

I have had the privilege to mentor two talented undergraduate students. I thank both Jennifer Hope and Maria Chan for their hard work and dedication. I have probably learned just as much from them as they have from me.

I need to acknowledge family, as well. I want to thank all of them for being so understanding about not being able to see me more often. Thank you Dad, Madelyn, Woody, Lynn, Al, Cathy, Niki, and Jesse. Thanks to Grandma for the phone calls, occasional delivery of cookies and chocolates, and continuous love and support.

Finally, I thank Julie. I convinced her to leave her warm California climate and come out with me to the east coast. After the first blizzard, she did not leave. After the second, third, and fourth she stayed. By the fifth, I thought she would leave for sure. But she didn't! That is true love and dedication! Now we are both happily headed towards the next stages of our lives, together.
Preface

Medicinal chemistry has long been the domain of the organic chemist. The seminal discovery by Barnett Rosenberg in 1969 that cis-diamminedichloroplatinum(II), or cisplatin, inhibits tumor growth in mice demonstrated the utility of metal compounds in medicine and inspired subsequent generations of inorganic chemists to investigate medicinal applications of their field. Several factors have contributed to the dominance of organic molecules in medicine. One is the concern held by general public of toxicity associated with heavy metal ions, given experience with mercury and lead, for example. Research has shown that the careful choice of ligands can ameliorate toxic side effects of many metal ions. Another factor is the lack of general synthetic methodologies for rationally preparing new metal complexes. Libraries of compounds with minor structural differences are often necessary for medicinal chemistry studies where such variations can have profound effects on the biological activity of a compound. Whereas hundreds of reactions are at the disposal of an organic chemist for preparing a compound library, the inorganic chemist is essentially limited to ligand substitution and redox reactions. A unifying theme of the work in this thesis is the invention of general synthetic routes to anticancer platinum drug candidates through variations of classical ligand substitution and redox strategies.

In Chapter 1 appears a comprehensive review of the different synthetic protocols for preparing platinum complexes with known anticancer activity. This chapter classifies the different types of ligands that have successfully been used in platinum anticancer complexes. These ligands can be categorized as the leaving groups, non-leaving groups, and axial. The leaving group ligands, typically halides or carboxylates, are those that are displaced from the platinum coordination sphere and ultimately substituted by DNA, the biological target of cisplatin and its analogues. The non-leaving group ligands, usually amines or N-heterocycles, are those that remain within the platinum coordination sphere after binding to DNA. Axial ligands are present only in octahedral complexes of platinum in the +3 or +4 oxidation state and are situated orthogonal to the coordination plane composed of leaving and non-leaving group ligands. As described below, the work in this thesis covers variations of all three ligand types.

Chapters 2 and 3 report platinum(II) complexes with modified non-leaving group ligands containing fluorescent labels. In Chapter 2, variants of the di-2-pyridylmethane ligand were prepared and purified using standard organic chemistry methodologies prior to coordination to dichloroplatinum(II) centers. In contrast, the chemistry described in Chapter 3 allows for modifications of the non-leaving group ligand while it remains coordinated to the platinum(II) center. The platinum(II) complexes [Pt(cheda)Cl₂] and [Pt(edma)Cl₂], where cheda = N,N'-ethylenediamine diacetic acid and edma = N-ethylenediamine monoacetic acid, which both contain free carboxylic acid groups, could be readily functionalized by amide bond coupling chemistry. The utility of this method was demonstrated by attaching a dansyl fluorophore to a platinum complex. An interesting feature of the dansylated platinum complex was that the emission of the fluorophore was modulated by the oxidation state of the platinum center. The straightforward and predictable amide coupling chemistry employed here enables the preparation of a large number of variants, which may exhibit improved biological properties and be useful as mechanistic probes.

Chapter 4 describes how modifications of the leaving group ligands of platinum(II) anticancer complexes affect their biological activities. In particular, β-diketonates were selected as leaving group ligands. Three different synthetic protocols for the preparation of cis-diammine(β-diketonato)platinum(II) complexes are reported. The protocols were optimized to
accommodate different solubilities of the resulting platinum complexes and the β-diketonate ligands. The β-diketonates were selected because most are commercially available and contain functional groups with different lipophilicities and electron-withdrawing properties. Structure-activity relationships discovered here indicate that both properties are important in determining the anticancer efficacy of the resulting cis-diammine(β-diketonato)platinum(II) complexes.

In the final two chapters, modifications of axial ligands in higher-valent platinum complexes are investigated. Dinuclear platinum(III) compounds with metal-metal bonds are the focus of Chapter 5. Because of the limited stability of these complexes to reducing agents, this chapter primarily explores synthetic inorganic chemistry. It may be desirable, however, to use dinuclear platinum(III) compounds as anticancer agents if they could be sufficiently stabilized to tolerate biological conditions. Chapter 6 investigates an under-explored reactivity pathway for the preparation of kinetically stable platinum(IV) complexes. In particular, the reaction of cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂] with aryl and alkyl isocyanates was investigated. The nucleophilic hydroxo ligand of cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂] readily attacks the electrophilic isocyanates, forming stable platinum(IV) dicarbamates. As demonstrated in this chapter, such compounds are cytotoxic. Therefore, this facile chemistry can be used to prepare a large number of derivatives and attach platinum(IV) to various delivery or targeting devices via carbamate linkages.

Appendices A, B, and C report the results of tangential work. Appendix A reports the aqueous electrochemistry of a common platinum(IV) prodrug. Appendix B describes synthetic methodologies for attaching peptides to leaving group ligands of platinum(II) complexes. Appendix C contains synthetic protocols for and characterization of some miscellaneous platinum complexes prepared during the course of this thesis, but not otherwise thoroughly investigated.

Cisplatin has been in clinical use for over 40 years. During that period, many aspects regarding its mechanism of action have been determined. New effective platinum-based drugs should be optimized for increased cellular uptake, tumor selectivity, and transcription inhibitory properties. The lack of chemical methods to prepare such constructs, however, is often a limiting factor in the process of platinum-based drug development, despite the long history of platinum coordination chemistry. The research presented in this thesis details several new and general synthetic protocols for synthesizing a wide range of platinum compounds. As new synthetic methodologies for platinum coordination compounds are unveiled, novel platinum anticancer drug candidates with enhanced properties become available with the ultimate goal of improving the treatment of patients and saving lives.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title Page</td>
<td>1</td>
</tr>
<tr>
<td>Signature Page</td>
<td>2</td>
</tr>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>8</td>
</tr>
<tr>
<td>Preface</td>
<td>11</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>13</td>
</tr>
<tr>
<td>List of Figures</td>
<td>18</td>
</tr>
<tr>
<td>List of Charts</td>
<td>21</td>
</tr>
<tr>
<td>List of Schemes</td>
<td>22</td>
</tr>
<tr>
<td>List of Tables</td>
<td>24</td>
</tr>
<tr>
<td>List of Equations</td>
<td>25</td>
</tr>
<tr>
<td><strong>Chapter 1. The Synthetic Chemistry of Platinum Anticancer Agents</strong></td>
<td>26</td>
</tr>
<tr>
<td>1.1. Introduction</td>
<td>27</td>
</tr>
<tr>
<td>1.2. Synthesis of Platinum(II) Complexes</td>
<td>31</td>
</tr>
<tr>
<td>Synthesis of cis- and trans-[PtL₂X₂] Complexes</td>
<td>31</td>
</tr>
<tr>
<td>Synthesis of cis- and trans-[PtLL'X₂] Complexes with Mixed Am(m)ine Ligands</td>
<td>40</td>
</tr>
<tr>
<td>Synthesis of Monofunctional Platinum(II) Complexes</td>
<td>48</td>
</tr>
<tr>
<td>Platinum(II) Complexes Synthesized by Ligand-Based Reactivity</td>
<td>55</td>
</tr>
<tr>
<td>1.3. Synthesis of Platinum(IV) Complexes</td>
<td>65</td>
</tr>
<tr>
<td>Oxidation of Platinum(II)</td>
<td>66</td>
</tr>
<tr>
<td>Outer-Sphere Ligand-Based Reactivity</td>
<td>78</td>
</tr>
<tr>
<td>Ligand Substitution Reactions</td>
<td>88</td>
</tr>
<tr>
<td>1.4. Concluding Remarks</td>
<td>91</td>
</tr>
<tr>
<td>1.5. References</td>
<td>92</td>
</tr>
<tr>
<td><strong>Chapter 2. Platinum(II) Complexes Bearing Fluorescent Di-2-Pyridylmethane Ligands</strong></td>
<td>104</td>
</tr>
<tr>
<td>2.1. Introduction</td>
<td>105</td>
</tr>
<tr>
<td>Chapter 5. Acetate-Bridged Dinuclear Platinum(III) Complexes Derived from Cisplatin</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>5.1. Introduction</td>
<td>201</td>
</tr>
<tr>
<td>5.2. Experimental Methods</td>
<td>202</td>
</tr>
<tr>
<td>5.3. Results and Discussion</td>
<td>210</td>
</tr>
<tr>
<td>Synthesis</td>
<td>210</td>
</tr>
<tr>
<td>Description of Crystal Structures</td>
<td>212</td>
</tr>
<tr>
<td>Multinuclear NMR Spectroscopy</td>
<td>216</td>
</tr>
<tr>
<td>Electrochemistry</td>
<td>221</td>
</tr>
<tr>
<td>DFT Calculations</td>
<td>224</td>
</tr>
<tr>
<td>Synthesis and Characterization of the Tetranuclear Complex $<a href="%5Ctext%7BNO%7D_3">4</a>_4$</td>
<td>228</td>
</tr>
<tr>
<td>Reaction of $<a href="%5Ctext%7BNO%7D_3">1</a>_2$ with XeF$_2$</td>
<td>234</td>
</tr>
<tr>
<td>5.4. Summary and Conclusions</td>
<td>236</td>
</tr>
<tr>
<td>5.5. References</td>
<td>237</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1. Introduction</td>
<td>242</td>
</tr>
<tr>
<td>6.2. Experimental Methods</td>
<td>243</td>
</tr>
<tr>
<td>6.3. Results and Discussion</td>
<td>255</td>
</tr>
<tr>
<td>Synthesis and Characterization</td>
<td>255</td>
</tr>
<tr>
<td>X-Ray Crystal Structures</td>
<td>260</td>
</tr>
<tr>
<td>Cyclic Voltammetry</td>
<td>264</td>
</tr>
<tr>
<td>Theoretical Calculations</td>
<td>268</td>
</tr>
<tr>
<td>Biological Properties</td>
<td>273</td>
</tr>
</tbody>
</table>
6.4. Summary and Conclusions

6.5. References

Appendix A. Aqueous Electrochemistry of a Platinum(IV) Prodrug

A.1. Introduction

A.2. Experimental Methods

A.3. Results

Cyclic Voltammetry in Aqueous Solution

Chronoamperometry

Bulk Electrolysis and Characterization of Reduction Products

A.4. Discussion

A.5. Summary and Conclusions

A.6. References

Appendix B. Targeting the Mitochondria with Platinum Anticancer Agents using Mitochondria-Penetrating Peptides

B.1. Introduction

B.2. Experimental Methods

B.3. Results and Discussion

Synthetic Strategy

Synthesis and Characterization of [Pt(succac)(NH₃)₂](NO₃)

Preparation of Pt-MPP and Pt-MPP(TAMRA)

Cytotoxicity Studies

Cell Imaging Studies

B.4. Conclusions and Ongoing Work

B.5. References

Appendix C. Synthesis and Characterization of Several Novel Platinum Complexes

C.1. Introduction

Complexes with DCA Ligands

Using BIPhMe for Platinum Chemistry

Amide Coupling to Functionalize Platinum(II) Complexes

C.2. Experimental Methods

C.3. Results and Discussion
New Analogues of Mitaplatin 333
Preparation of [Pt(BIPhMe)Cl₂] 336
Nitroimidazole- and Nitrogen Mustard-Platinum Conjugates 338

C.4. Summary 338
C.5. References 339

Biographical Note 341
Curriculum Vitae 342
List of Figures

Chapter 1
Figure 1.1. Schematic diagram showing the different components of a platinum anticancer agent. 30

Chapter 2
Figure 2.1. Solid-state molecular structures of Ds-dpm and NBD-dpm. 117
Figure 2.2. Solid-state molecular structures of 1–3. 119
Figure 2.3. Depiction of the solid-state exo/endo disorder of 3. 121
Figure 2.4. Variable-temperature $^1$H NMR spectra of 1. 123
Figure 2.5. $^1$H–$^1$H NOESY NMR spectrum of 1. 125
Figure 2.6. $^{195}$Pt NMR spectra of 1–3. 126
Figure 2.7. Optical absorption spectra. 128
Figure 2.8. Normalized emission spectra. 129
Figure 2.9. $^1$H NMR spectra monitoring the thermolysis of 3. 132
Figure 2.10. Solid-state molecular structures of $1_{ax}$ and $3_{ax}$. 135

Chapter 3
Figure 3.1. $^1$H NMR spectrum of 1 and 4. 154
Figure 3.2. Solid-state molecular structures of 1 and 3. 156
Figure 3.3. Depiction of $\lambda$ and $\delta$ chelate ring isomers. 157
Figure 3.4. Solid-state molecular structures of 4 and 6. 158
Figure 3.5. Molecular orbital diagram for 2 and 5. 162
Figure 3.6. Emission spectra of 5 before and after addition of 10 equiv glutathione. 163

Chapter 4
Figure 4.1. Solid-state molecular structures of 3 and 4. 182
Figure 4.2. Bar graph of log $P$ values and graph of log $P$ versus calculated ligand log $P$. 184
Figure 4.3. Bar graph chart of $IC_{50}$ values for cancer cell lines. 187
Figure 4.4. Plot complex log $P$ versus cellular uptake in HeLa cells. 189

Chapter 5
Figure 5.1. Solid-state molecular structure of [1]$^{2+}$. 213
Figure 5.2. Solid-state molecular structures of [2]$^{2+}$ and [3]$^{2+}$. 215
Figure 5.3. $^1\text{H}$ NMR spectra $[1]^{2+} - [3]^{2+}$.  
Figure 5.4. $^{14}\text{N}\{^1\text{H}\}$ NMR spectra of $[1]^{2+} - [3]^{2+}$.  
Figure 5.5. $^{195}\text{Pt}\{^1\text{H}\}$ NMR spectra of $[1]^{2+} - [3]^{2+}$.  
Figure 5.6. Cyclic voltammogram of $[1]^{2+}$.  
Figure 5.7. Cyclic voltammograms of $[2]^{2+}$ and $[3]^{2+}$.  
Figure 5.8. Frontier molecular orbitals of $[1]^{2+} - [3]^{2+}$.  
Figure 5.9. Solid-state molecular structure of $[4]^{4+}$.  
Figure 5.10. Variable temperature NMR spectra of $[4]^{4+}$.  
Figure 5.11. NMR spectra of $[4]^{4+}$ before and after the addition of excess NaTFA.  
Figure 5.12. Cyclic voltammograms of $[4]^{4+}$.  
Figure 5.13. Crystal structure of yellow crystals obtained from XeF$_2$ and $[1]^{2+}$.

Chapter 6

Figure 6.1. Variable-temperature $^1\text{H}$ and $^{195}\text{Pt}$ NMR spectra of 5.  
Figure 6.2. Solid-state molecular structures of 4–7.  
Figure 6.3. Solid-state molecular structures of 8–11.  
Figure 6.4. Depiction of intramolecular hydrogen bonding patterns in the crystal structures.  
Figure 6.5. Cyclic voltammograms of 1–11.  
Figure 6.6. Molecular orbital diagrams for 1–3.  
Figure 6.7. Molecular orbital diagrams for 6, 7, and 11.  
Figure 6.8. Plot of computed adiabatic electron affinity versus reduction peak potential.

Appendix A

Figure A.1. Cyclic voltammograms of 1 with a glassy carbon electrode in PBS.  
Figure A.2. Cyclic voltammograms of 1 with a Pt electrode in PBS.  
Figure A.3. Cyclic voltammograms of pure pH 7.4 PBS with a glassy carbon and Pt electrode.  
Figure A.4. Cyclic voltammograms of a glassy carbon electrode modified by 1.  
Figure A.5. Constant potential current versus time traces in PBS with different electrodes.  
Figure A.6. $^1\text{H}$ NMR spectra of bulk electrolysis products of 1.
Appendix B

Figure B.1. Solid-state molecular structures of succinylacetone and [Pt(succac)(NH$_3$)$_2$](NO$_3$).

Figure B.2. $^{195}$Pt NMR spectra of Pt-MPP and Pt-MPP(TAMRA).

Figure B.3. $^1$H NMR spectrum of Pt-MPP(TAMRA).

Figure B.4. Dose-response curves for treatment of A2780 and A2780CP70 cells with Pt-MPP.

Figure B.5. Fluorescent images of A2780 cells treated with Pt-MPP(TAMRA).

Figure B.6. Fluorescent images of A2780CP70 cells treated with Pt-MPP(TAMRA).

Appendix C

Figure C.1. Solid-state molecular structure of cis-[Pt(NH$_3$)$_2$(DCA)$_2$].

Figure C.2. Solid-state molecular structure of cis-[Pt(NH$_3$)$_2$(DCA)$_4$].

Figure C.3. Solid-state molecular structure of [Pt(BIPhMe)Cl$_2$].
## List of Charts

### Chapter 1

<table>
<thead>
<tr>
<th>Chart</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1.</td>
<td>Structures of clinically used platinum anticancer drugs.</td>
<td>28</td>
</tr>
<tr>
<td>1.2.</td>
<td>Structures of mixed amine platinum(II) complexes that have undergone clinical trials.</td>
<td>40</td>
</tr>
<tr>
<td>1.3.</td>
<td>Structures of several monofunctional platinum(II) complexes with anticancer activity.</td>
<td>50</td>
</tr>
<tr>
<td>1.4.</td>
<td>Structures of the different isomers of [Pt(iminoether)₂Cl₂].</td>
<td>56</td>
</tr>
<tr>
<td>1.5.</td>
<td>Structures of clinically investigated platinum(IV) complexes.</td>
<td>65</td>
</tr>
</tbody>
</table>

### Chapter 2

<table>
<thead>
<tr>
<th>Chart</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.</td>
<td>Structures of newly reported platinum(II) complexes in this chapter.</td>
<td>106</td>
</tr>
</tbody>
</table>

### Chapter 3

<table>
<thead>
<tr>
<th>Chart</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.</td>
<td>Structures of newly reported platinum complexes in this chapter.</td>
<td>142</td>
</tr>
</tbody>
</table>

### Chapter 4

<table>
<thead>
<tr>
<th>Chart</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.</td>
<td>Structures of cisplatin, carboplatin, and β-diketonate platinum(II) complexes reported in this chapter.</td>
<td>170</td>
</tr>
</tbody>
</table>

### Chapter 6

<table>
<thead>
<tr>
<th>Chart</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1.</td>
<td>Structures of possible isomers of platinum(IV) dicarbamate complexes.</td>
<td>260</td>
</tr>
</tbody>
</table>

### Appendix B

<table>
<thead>
<tr>
<th>Chart</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.1.</td>
<td>Schematic drawing of Pt-MPP and Pt-MPP(TAMRA).</td>
<td>311</td>
</tr>
</tbody>
</table>

### Appendix C

<table>
<thead>
<tr>
<th>Chart</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.1.</td>
<td>Structures of newly reported platinum complexes in this appendix.</td>
<td>326</td>
</tr>
</tbody>
</table>
**List of Schemes**

**Chapter 1**

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Synthesis of cisplatin by the method of Dhara.</td>
<td>33</td>
</tr>
<tr>
<td>1.2</td>
<td>Reactions associated with the Kurnakow test.</td>
<td>35</td>
</tr>
<tr>
<td>1.3</td>
<td>Synthesis of transplatin.</td>
<td>37</td>
</tr>
<tr>
<td>1.4</td>
<td>Different synthetic routes for modifying leaving group ligands.</td>
<td>39</td>
</tr>
<tr>
<td>1.5</td>
<td>Synthesis of cis-[Pt(NH₃)Cl₂] from [Pt(NH₃)Cl₃]⁻.</td>
<td>44</td>
</tr>
<tr>
<td>1.6</td>
<td>Synthesis of cis-[PtL'LCl₂] from [PtL'I(μ-I)]₂.</td>
<td>46</td>
</tr>
<tr>
<td>1.7</td>
<td>Synthesis of mixed amine complexes with a chelating leaving group ligand.</td>
<td>47</td>
</tr>
<tr>
<td>1.8</td>
<td>Synthesis of trans-[PtLL'Cl₂].</td>
<td>48</td>
</tr>
<tr>
<td>1.9</td>
<td>Synthesis of [Pt(NH₃)₂Cl]Cl.</td>
<td>50</td>
</tr>
<tr>
<td>1.10</td>
<td>Synthetic routes for the preparation of cis-[Pt(L₂)(RR'SO)Cl]Cl.</td>
<td>51</td>
</tr>
<tr>
<td>1.11</td>
<td>Synthesis of cis-[Pt(NH₃)₂LCl(NO₃)].</td>
<td>54</td>
</tr>
<tr>
<td>1.12</td>
<td>Synthesis of monofunctional, thiourea platinum(II) complexes.</td>
<td>55</td>
</tr>
<tr>
<td>1.13</td>
<td>Synthesis of cis- and trans-[PtCl₂(iminoether)₂].</td>
<td>57</td>
</tr>
<tr>
<td>1.14</td>
<td>Synthesis of platinum(II) amidine complexes.</td>
<td>60</td>
</tr>
<tr>
<td>1.15</td>
<td>Synthesis of monofunctional platinum(II) amidine complexes.</td>
<td>61</td>
</tr>
<tr>
<td>1.16</td>
<td>Condensation reactions involving coordinated ammonia ligands.</td>
<td>63</td>
</tr>
<tr>
<td>1.17</td>
<td>Outer-sphere ligand-based reactivity of platinum(II) complexes.</td>
<td>64</td>
</tr>
<tr>
<td>1.18</td>
<td>Oxidation of platinum(II) with chlorine and hydrogen peroxide.</td>
<td>68</td>
</tr>
<tr>
<td>1.19</td>
<td>Peroxide oxidations of platinum(II) in acidic solvents.</td>
<td>72</td>
</tr>
<tr>
<td>1.20</td>
<td>Ring-closing oxidation reactions of platinum(II).</td>
<td>73</td>
</tr>
<tr>
<td>1.21</td>
<td>Oxidation of platinum(II) with alternative oxidizing agents.</td>
<td>76</td>
</tr>
<tr>
<td>1.22</td>
<td>Oxidation of platinum(II) with hypervalent iodine reagents.</td>
<td>78</td>
</tr>
<tr>
<td>1.23</td>
<td>Synthetic methods for the preparation of platinum(IV) carboxylates.</td>
<td>81</td>
</tr>
<tr>
<td>1.24</td>
<td>Synthesis of trans hydroxo-carboxylato platinum(IV) complexes.</td>
<td>84</td>
</tr>
<tr>
<td>1.25</td>
<td>Reactivity of platinum(IV) hydroxides with electrophiles.</td>
<td>85</td>
</tr>
<tr>
<td>1.26</td>
<td>Outer-sphere reactivity of platinum(IV) complexes.</td>
<td>87</td>
</tr>
<tr>
<td>1.27</td>
<td>Ligand substitution reactions of platinum(IV) under acidic conditions.</td>
<td>89</td>
</tr>
<tr>
<td>1.28</td>
<td>Ligand substitution reactions of platinum(IV) under basic conditions.</td>
<td>91</td>
</tr>
</tbody>
</table>
Chapter 2
Scheme 2.1. Synthesis of modified di-2-pyridylmethane (dpm) ligands. 116
Scheme 2.2. Synthesis of 1–3. 118
Scheme 2.3. Depiction of conformational exo and endo isomers. 120
Scheme 2.4. Proposed photolytic decomposition pathway of 2. 131
Scheme 2.5. Thermolytic and photolytic decomposition pathways of 3. 133

Chapter 3
Scheme 3.1. Synthesis of 1–3. 152
Scheme 3.2. Oxidation of 1–3 with iodobenzene dichloride. 153

Chapter 4
Scheme 4.1. Synthesis of cis-diammine(β-diketonate)platinum(II) complexes. 180

Chapter 5
Scheme 5.1. Synthesis of [1](NO₃)₂. 211
Scheme 5.2. Synthesis of [2](NO₃)₂ and [3](NO₃)₂. 212
Scheme 5.3. Synthesis of [4](NO₃)₄. 228

Chapter 6
Scheme 6.1. Reductive activation of platinum(IV) prodrugs. 242
Scheme 6.2. Synthesis of cis,cis,trans-[Pt(NH₃)₂Cl₂(O₂CNHR)₂]. 256

Appendix A
Scheme A.1. Possible reduction pathways for a platinum(IV) prodrug. 281
Scheme A.2. Electrochemical reduction pathways of 1. 290

Appendix B
Scheme B.1. Synthesis of [Pt(succac)(NH₃)₂](NO₃). 308
### List of Tables

#### Chapter 2

| Table 2.1 | X-Ray Crystallographic Data Collection and Refinement Parameters for Ds-dpm and NBD-dpm. | 112 |
| Table 2.2 | X-Ray Crystallographic Data Collection and Refinement Parameters for 1–3. | 114 |
| Table 2.3 | Space Group and Unit Cell Parameters for 1ox and 3ox. | 115 |
| Table 2.4 | Selected Interatomic Distances and Angles of 1–3. | 120 |
| Table 2.5 | Selected 1H and 195Pt NMR Chemical Shifts for 1–3. | 124 |
| Table 2.6 | Thermodynamic Parameters for Exo-Endo Interconversion. | 127 |
| Table 2.7 | Photophysical Properties of Ligands and 1–3. | 128 |

#### Chapter 3

| Table 3.1 | X-Ray Crystallographic Data Collection and Refinement Parameters for 1-DMF, 3, 4-DMF, and 6. | 149 |
| Table 3.2 | Selected Interatomic Distances and Angles of 1 and 3. | 157 |
| Table 3.3 | Selected Interatomic Distances and Angles of 4 and 6. | 159 |
| Table 3.4 | Photophysical Properties of 2, 5, and Ds-en. | 160 |

#### Chapter 4

| Table 4.1 | X-Ray Crystallographic Data Collection and Refinement Parameters for 3 and 4. | 175 |
| Table 4.2 | Selected 1H, 13C, 19F, and 195Pt NMR Chemical Shifts. | 181 |
| Table 4.3 | Selected Interatomic Distances and Angles of 3 and 4. | 182 |
| Table 4.4 | Measured and Computed log P Values. | 183 |
| Table 4.5 | IC50 Values in HeLa, A549, U2OS, and MCF-7 Cell Lines and Cellular Uptake in HeLa Cells. | 186 |
| Table 4.6 | Results of COMPARE Analysis of 4. | 188 |
| Table 4.7 | Intracellular DNA Platination Levels. | 190 |

#### Chapter 5

| Table 5.1 | X-Ray Crystallographic Data Collection and Refinement Parameters. | 209 |
| Table 5.2 | Relevant Structural Features of [1]2+–[3]2+ and Related Complexes. | 216 |
| Table 5.3 | Multinuclear NMR Spectroscopic Data. | 217 |
Table 5.4. Comparison of Geometric Parameters Obtained Experimentally and from DFT Calculations. 225
Table 5.5. DFT-Computed Isotropic $^4$H NMR Chemical Shifts and EFG Parameters. 228
Table 5.6. Selected Structural Features of [4]$^{4+}$. 230

Chapter 6
Table 6.1. X-Ray Crystallographic Data Collection and Refinement Parameters for 4–7. 252
Table 6.2. X-Ray Crystallographic Data Collection and Refinement Parameters for 8–11. 253
Table 6.3. Selected $^1$H and $^{195}$Pt NMR Chemical Shifts. 258
Table 6.4. Selected Interatomic Distances and Angles. 261
Table 6.5. Peak Reduction Potentials Measured by Cyclic Voltammetry. 267
Table 6.6. Computed Adiabatic Electron Affinities and Ligand Bond Distance Changes Upon One-Electron Reduction. 272
Table 6.7. IC$_{50}$ Values in A549 and MRC-5 Cells. 274

Appendix B
Table B.1. X-ray Crystallographic Data Collection and Refinement Parameters for Succinylacetone and [Pt(succac)(NH$_3$)$_2$(NO$_3$)]. 305
Table B.2. IC$_{50}$ Values and Resistance Factors in A2780 and A2780CP70 Cells. 314
Table B.3. Pearson’s Correlation Coefficients for Colocalization of Pt-MPP(TAMRA) and Organelle Dyes. 317

Appendix C
Table C.1. X-Ray Crystallographic Data Collection and Refinement Parameters for cis-[Pt(NH$_3$)$_2$(DCA)$_2$]$\cdot$H$_2$O, cis-[Pt(NH$_3$)$_2$(DCA)$_4$]$_3$$\cdot$6H$_2$O, and [Pt(BIPhMe)Cl$_2$]$_2$$\cdot$H$_2$O. 332

List of Equations
Chapter 5
Equation 5.1. 227

Appendix A
Equation A.1. 280
Chapter 1

The Synthetic Chemistry of Platinum Anticancer Agents
1.1. Introduction

The demonstration in the 1960's that cis-diamminedichloroplatinum(II), or cisplatin, inhibits cellular division of E. coli\(^1\) led to the subsequent discovery that this simple coordination compound is also an effective antitumor agent in mouse models.\(^2\) Continuing studies validated cisplatin as an effective anticancer agent in humans as well,\(^3\)\(^-\)\(^7\) and FDA approval of cisplatin for the treatment of metastatic ovarian and testicular cancers was granted in 1978.\(^8\) Its inception as a chemotherapeutic agent significantly improved the survival outlook for many cancer patients; the cure rate for testicular cancer before the approval of cisplatin was less than 10%, significantly lower than the 90% cure rate attained with modern platinum chemotherapy.\(^9\)\(^,\)\(^10\)

Cisplatin kills cancer cells primarily by cross-linking DNA and inhibiting transcription.\(^11\) The chemical origin of this process begins when cisplatin enters the cell and undergoes an aquation reaction via loss of one or both chloride ligands. The resulting platinum(II) aqua complexes are potent electrophiles that readily react with a number of biological ligands with loss of the bound water molecules. The purine bases of nucleic acids are strongly nucleophilic at the \(N7\) position. Thus, cisplatin binds readily to DNA, forming primarily bifunctional adducts with loss of both chloride ligands. The major cisplatin-DNA adduct is the intrastrand 1,2-d(GpG) lesion, which accounts for 60–65% of the bound platinum.\(^12\) The resulting Pt-DNA adducts, which distort and bend the DNA structure,\(^13\)\(^-\)\(^15\) impede transcription.\(^16\) The downstream effects of transcription inhibition ultimately lead to cell death.

Despite its great curative success in testicular cancer, cisplatin is not universally effective in other cancer types and induces a number of toxic side effects.\(^17\)\(^-\)\(^19\) Additionally, certain cancers are resistant to cisplatin therapy. This resistance is either intrinsic or developed during prolonged treatment.\(^20\)\(^,\)\(^21\) To circumvent these problems, new platinum complexes have been pursued and
investigated for their antitumor properties. Although well over a thousand complexes have been prepared and tested thus far, only two other platinum drugs are approved for clinical use worldwide, and three additional compounds are approved for regional use in Asia. These complexes, displayed in Chart 1.1, operate with a mechanism of action similar to that of cisplatin, which involves DNA binding and transcription inhibition.

![Chemical structures of the clinically used platinum-based anticancer drugs.](image)

**Chart 1.1.** Chemical structures of the clinically used platinum-based anticancer drugs. The top three complexes, cisplatin, carboplatin, and oxaliplatin, are approved for use worldwide. The bottom three complexes, nedaplatin, heptaplatin, and lobaplatin, are approved for use in Japan, Korea, and China, respectively.

In designing a new platinum anticancer agent, several structural features can be strategically modified. As shown in Figure 1.1, three different ligand types generally comprise a platinum anticancer complex. The non-leaving group ligands are typically nitrogen donors. These are referred to as “non-leaving group” ligands because they form thermodynamically stable bonds with platinum and are retained in the final platinum-DNA adduct. Modifications of
these ligands directly affect the nature of the resulting platinum-DNA adducts\textsuperscript{24-26} and, accordingly, the manner by which cellular repair pathways respond to those adducts. Complexes that contain amine ligands different from those of cisplatin usually exhibit different spectra of activity in cancer cell lines and are usually not cross-resistant with cisplatin.\textsuperscript{27} Oxaliplatin, with its chelating and chiral diamine ligand \textit{trans-1R,2R-DACH} (DACH = diaminocyclohexane), for example, fits into this category. Modifications of the leaving group ligands, so named because they are lost upon DNA-binding, can alter the overall reaction and aquation kinetics for a platinum anticancer complex. Complexes that react quickly, such as those with labile nitrate ligands, are generally more toxic because of their indiscriminate binding to off-target biological nucleophiles.\textsuperscript{28} Carboplatin, on the other hand, contains a relatively stable chelating CBDCA (CBDCA = 1,1-cyclobutanedicarboxylato) ligand as its leaving group. By comparison to cisplatin, carboplatin can be administered at higher doses because of its lower toxicity profile.\textsuperscript{29,30} Although less toxic, carboplatin has a similar spectrum of activity and exhibits cross-resistance to cisplatin, which is a result of the same non-leaving group ammine ligands.\textsuperscript{27,31} A third ligand category comprises the axial ligands. Axial ligands are present only in higher valent platinum complexes, such as those of platinum(III) and platinum(IV). These ligands are ultimately lost after biological reduction of the platinum complex and provide convenient points for installation of tumor-targeting moieties or nanoparticle attachment units. Any of the three ligand types can be modified in order to alter the lipophilicity and water solubility of the resulting platinum complex. Both of these properties are important for the design of an effective drug. The stereochemistry and the number of each respective ligand type can be altered as well.
Figure 1.1. Different components of platinum anticancer agents. Additional factors that can be varied are the stereochemistry and the respective number of non-leaving and leaving group ligands.

In this chapter, an overview of known synthetic strategies for making platinum anticancer complexes is presented. Previous review articles have focused on the mechanistic details of platinum-based drugs at the cellular level, the chemistry of platinum under biological conditions, and new trends for the rational design of platinum anticancer agents. This chapter provides a summary of strategies for the synthesis and purification of platinum anticancer drug candidates, the subject that marks the main contribution of this thesis. There are two major sections that describe, respectively, the synthesis of platinum(II) and platinum(IV) complexes. These sections are further divided based on the nature and stereochemistry of the target complexes. In each section, a short overview is provided of the anticancer properties of these target complexes. Multinuclear platinum complexes, some of which are very good drug
candidates,\textsuperscript{47,48} have been omitted from this review to focus on single-site reactivity. The reaction schemes do not depict fully balanced chemical reactions, but rather show only the major platinum-containing products. Two generic ligand types, L and X, are utilized, with ligands symbolized by “L” representing either amine or N-heterocycle ligands. When “(L\textsubscript{2})” is used, the ligand is bidentate. Ligands depicted with “X” are monoanionic like halides or carboxylates.

1.2. Synthesis of Platinum(II) Complexes

All clinically used platinum drugs (Chart 1.1) contain this element in the +2 oxidation state where square-planar coordination geometries are almost exclusively observed. The major reaction pathways for platinum(II) and other square-planar d\textsuperscript{8} complexes involve associative ligand substitution. These reactions proceed through five-coordinate trigonal bipyramidal intermediates. The stereochemistry of the resulting products is dictated by the relative trans effect of the ligands within the complex. Synthetic strategies discussed in the following sections, therefore, rely heavily on the trans effect principle. For more detailed summaries of substitution reactions of platinum(II) and other d\textsuperscript{8} complexes, as well as the trans effect, the reader is referred elsewhere.\textsuperscript{49-51} An early review on the synthesis of monodentate amine complexes of platinum(II) is also available.\textsuperscript{52}

Synthesis of \textit{cis-} and \textit{trans-}[PtL\textsubscript{2}X\textsubscript{2}] Complexes. Cis- and transplatin, \textit{trans-}diamminedichloroplatinum(II), the trans stereoisomer of cisplatin, are representative members of the class of complexes having the general formula, \textit{cis-} and \textit{trans-}[PtL\textsubscript{2}X\textsubscript{2}] where L is an am(m)ine or N-heterocycle and X is a halide or other labile ligand. Both cis- and transplatin were first prepared over 100 years ago by Peyrone and Reiset, respectively,\textsuperscript{53,54} and were commonly known as Peyrone’s chloride and Reiset’s second chloride. Cisplatin and transplatin, both yellow
solids, were recognized to be isomers of Magnus' green salt, \([\text{Pt(NH}_3\text{)}_4][\text{PtCl}_4]\). The structural differences between these three species helped validate Werner's theory of coordination chemistry.\(^{55}\)

Since Peyrone's initial preparation of cisplatin, several different synthetic routes have been described. The common starting material for these procedures is \(\text{K}_2[\text{PtCl}_4]\), a water-soluble salt, which can be prepared directly from platinum metal in two steps.\(^{56}\) As with Peyrone's initial synthesis, several protocols for the synthesis of cisplatin involve the direct action of aqueous ammonia on the tetrachloroplatinate ion.\(^{57,58}\) This reaction results inevitably in the formation of Magnus' green salt and the trans isomer as undesired byproducts, which both must be removed by additional purification steps.\(^{57,58}\) Recently, the use of microwave irradiation for the synthesis of cisplatin directly from \(\text{K}_2\text{PtCl}_4\) and \(\text{NH}_4\text{OAc}\) was reported.\(^{59}\) The adaptation of this method with flow chemistry techniques enables cisplatin to be synthesized on the gram scale in one step with no impurities from Magnus' salt or transplatin.

The most widely used method for preparing cisplatin is that reported by Dhara in 1970.\(^{60}\) For this multistep reaction (Scheme 1.1), aqueous \([\text{PtCl}_4]^{2-}\) is first converted to \([\text{PtI}_4]^{2-}\) upon treatment with 4 equiv of \(\text{KI}\). The addition of ammonium hydroxide to the dark brown solution of \([\text{PtI}_4]^{2-}\) yields the yellow precipitate, \(\text{cis-}[\text{Pt(NH}_3\text{)}_2\text{I}_2]\). The removal of the iodide ligands of this complex with 2 equiv of \(\text{AgNO}_3\) in water gives the diaqua cation, \(\text{cis-}[\text{Pt(NH}_3\text{)}_2(\text{OH}_2)_2]^{2+}\), from which isomerically pure cisplatin can be isolated as a yellow solid following treatment with excess chloride ion. The absence of the trans isomer is attributed to the high trans effect of the iodide compared to that of the chloride ligand. The key intermediate in the formation of cisplatin from the tetrahaloplatinate ions is the monosubstituted complex, \([\text{Pt(NH}_3\text{)}X_3]^-\). When \(X\) is \(\text{I}\), the large trans effect ensures that the next \(\text{NH}_3\) ligand substitutes trans to an iodide to give the
desired cis isomer. When X is Cl, the lower trans effect of this ligand renders substitution trans to NH₃ kinetically competitive with substitution trans to chloride, thus yielding a small amount of the trans isomer. This method has been adapted to prepare cis complexes with other amine or N-heterocyclic ligands, cisplatin with ¹⁵N-labeled ammines, and radiolabeled Pt-cisplatin. When chelating diamines are used, this method is preferred as well. In cases where the desired amine or N-heterocyclic ligands are not water-soluble, an alternative synthetic route involves the action of two equiv of the amine ligand on K₂PtCl₄ in a solvent mixture comprising water and an alcohol at elevated temperatures. The use of DMF instead of ethanol or methanol as a cosolvent for this reaction has also been reported.

\[
\begin{align*}
\text{Scheme 1.1. Synthesis of cisplatin using the method of Dhara.}^{60} & \quad \text{All reactions steps are carried out in aqueous solution.}
\end{align*}
\]
Purification of cisplatin can be accomplished by recrystallization from hot water containing either 0.1 M HCl or 0.9% NaCl. The high chloride concentration inhibits the formation of platinum aqua or hydroxo complexes. The use of amide solvents to recrystallize cisplatin is also an effective means of purification. Dissolution of cisplatin in \(N,N\)-dimethylacetamide (DMA) to a concentration of 18 mg/mL, followed by the addition of three volume equiv of 0.1 N HCl and incubation in an ice bath, affords analytically pure compound. Alternatively, storing a concentrated solution (~22 mg/mL) of cisplatin in DMF at 3 °C overnight yields yellow cube-like crystals of a DMF solvate, cisplatin-DMF. Removal of DMF from the crystal lattice under vacuum gives solvent-free cisplatin of very high purity. Care should be taken when recrystallizing new complexes of the general formula, cis-[PtL2Cl2], from hot solvents. Although not observed for cisplatin, several other complexes of this formula isomerize upon recrystallization from hot acetone or DMF, giving instead the pure trans isomer. Thus, stereochemistry should be confirmed after each purification step.

A quick way to test for the presence of isomeric impurities in cis- and transplatin or related [PtL2Cl2] complexes is the Kurnakow test. This test is carried out by adding an excess of thiourea (tu) to an aqueous mixture of cis/trans-[Pt(NH3)2Cl2]. Transplatin transforms to the white, water-insoluble powder, trans-[Pt(NH3)2(tu)2]Cl2, whereas cisplatin becomes the yellow, soluble complex [Pt(tu)4]Cl2 (Scheme 1.2). The formation of different products from the two isomers is a consequence of the high trans effect of thiourea. The first equiv of thiourea displaces a chloride ligand. For cisplatin, this substitution places the thiourea trans to an ammine ligand, whereas for transplatin the thiourea binds trans to a chloride. The ammine of cisplatin is sufficiently labilized by the thiourea ligand such that it can be displaced. The ammines of transplatin are never found trans to the thiourea ligand and therefore remain coordinated. The use
of the Kurnakow test in conjunction with HPLC provides a powerful and sensitive method to detect isomeric impurities. In contrast to cis- and transplatin, the thiourea-coordinated products exhibit significantly different retention times and contain stronger UV-vis absorbance features, which make them amenable for HPLC analysis. NMR spectroscopy provides another potential tool to distinguish between isomers. Unfortunately, the $^{195}$Pt NMR chemical shifts of the cis and trans isomers are very similar. For example, $\delta = -2104$ ppm for cisplatin and $-2101$ ppm for transplatin. The evaluation of 3-bond Pt-H coupling constants ($^3J_{PtH}$) determined by $^1$H NMR spectroscopy, however, revealed that these values are 8–14 Hz greater for cis isomer, indicating that the magnitudes of these coupling constant can be used to distinguish the two isomers. A potential limitation to this method is the need for a low-field NMR spectrometer, which decreases chemical-shift anisotropy effects of the $^{195}$Pt nucleus that broaden resonances and obscure coupling.

Scheme 1.2. The use of the Kurnakow test to distinguish cis- and transplatin. Reactions with thiourea are carried out in sub-boiling water.
The discovery that transplatin lacks the biological activity of cisplatin influenced the early structure-activity relationships derived for platinum-based anticancer agents.\textsuperscript{28,86,87} In contradiction to these structure-activity relationships, which prescribe cis geometry for activity, it was later discovered that some trans complexes of general formula \([\text{PtCl}_2\text{L}_2]\) are cytotoxic when \(\text{L}\) is an \(N\)-heterocycle like pyridine or thiazole.\textsuperscript{88-90} Several methods have been described for the synthesis of these symmetric trans compounds from the \([\text{PtCl}_4]^{2-}\) ion. To prepare transplatin, excess ammonia is added to the \([\text{PtCl}_4]^{2-}\) anion to generate the complex cation, \([\text{Pt(NH}_3)_4]^{2+}\). \([\text{Pt(NH}_3)_4]\)\textsubscript{Cl}_2 forms colorless aqueous solutions from which transplatin can be precipitated as a yellow solid after the addition of hydrochloric acid (Scheme 1.3).\textsuperscript{57} This synthetic pathway also exemplifies the principles of the trans effect. After the treatment of \([\text{Pt(NH}_3)_4]\)\textsubscript{Cl}_2 with HCl, an ammine ligand is replaced by a chloride, forming \([\text{Pt(NH}_3)_3\text{Cl}]^+\). The trans effect of chloride is greater than that of ammonia. Hence, the next incoming chloride ion preferentially displaces the ammine trans to the coordinated chloride, selectively giving rise to the trans isomer. This general scheme is applicable to other amine ligands and \(N\)-heterocycles as well.\textsuperscript{90} Trans complexes can also be prepared directly from \([\text{PtL}_4]\)\textsubscript{X}_2 complexes without the use of hydrochloric acid. Upon heating a \([\text{PtL}_4]\)\textsubscript{X}_2 as a suspension in an organic solvent or as a solid under vacuum, the outer-sphere halides substitute the inner-sphere amine or \(N\)-heterocycle, giving the trans isomer exclusively.\textsuperscript{90-93} In an attempt to make \textit{trans-[PtL}_2\text{Cl}_2\] where \(L\) is imidazole, \([\text{PtL}_4]\)\textsubscript{Cl}_2 was treated with HCl. Instead of obtaining the expected product, \textit{trans-[PtL}_2\text{Cl}_2\], only platinum(IV) complexes were obtained.\textsuperscript{61} The reaction of \([\text{PtL}_4]\)\textsubscript{Cl}_2 with excess Et\textsubscript{4}NCl in refluxing DMF ultimately gave the desired trans complexes.\textsuperscript{61} Interestingly, for the analogous complex where \(L\) is pyrazole, \textit{trans-[PtL}_2\text{Cl}_2\] was obtained from \([\text{PtL}_4]\)\textsubscript{Cl}_2 and HCl without any reported difficulties.\textsuperscript{94}
Scheme 1.3. Synthesis of transplatin. Reactions are carried out in water at elevated (50-100 °C) temperatures.

As described in Section 1.1, the leaving group ligands can have profound effects on the biological properties of the resulting platinum complexes. The importance of this behavior is exemplified by the clinically used drugs, carboplatin and nedaplatin (Chart 1.1), which differ from cisplatin only by the substitution of the chloride leaving groups by chelating ligands. The chloride ligands of complexes of the general formula cis- and trans-[PtL2Cl2], where L is an amine or N-heterocyclic ligand, can readily be replaced by other desired ligands. A number of different synthetic routes are available for substitution of the halides with other ligands. For the synthesis of dicarboxylato species, these synthetic routes and their practical applications have been reviewed in great detail. Here, we summarize these reactions schemes and reiterate some practical aspects.

Typically, a water-soluble silver(I) salt, such as AgNO3, is allowed to react with a suspension of the platinum(II) complex in water. Silver chloride or iodide is formed as a white or yellow solid, respectively, and removed by filtration. The filtrate contains the substitution-labile complex [PtL2(OH2)2]2+. The protonation state and charge on the complex cation depend on the pH of the solution. At high pH, inert platinum(II) hydroxo compounds form. These species
readily oligomerize to form equally unreactive multinuclear platinum(II) complexes containing bridging hydroxide ligands. At slightly acidic pH, however, the aqua ligands are readily displaced by other nucleophiles. Treatment of \( \text{cis-}[\text{PtL}_2(\text{OH}_2)_2]^{2+} \) in water with sodium salts of anionic nucleophiles, \( \text{NaNu} \), forms the complexes \( \text{cis-}[\text{PtL}_2(\text{Nu})_2] \) (Scheme 1.4a). This reaction proceeds best when the resulting product is insoluble in water. The insoluble product can then be isolated by filtration without the need to evaporate the solution to dryness. In cases where the desired product is water soluble, contamination of the final product with \text{NaNO}_3 is a problem.

In addition to preparing diamine dicarboxylato platinum(II) complexes, this reaction has been used to synthesize platinum(II) diazido complexes, which are precursors to photoreactive platinum(IV) prodrugs. The platinum(II) dinitrato complexes, \( [\text{PtL}_2(\text{ONO}_2)_2] \), can also be prepared independently and isolated as solids. An efficient synthesis of \( \text{cis-}[\text{PtL}_2(\text{ONO}_2)_2] \) is accomplished by treating \( \text{cis-}[\text{PtL}_2] \) with \( \text{AgNO}_3 \) in acetone. The reaction proceeds substantially faster in acetone compared to water. Solid \( \text{cis-}[\text{PtL}_2(\text{ONO}_2)_2] \) dissolves in water with heating to form the diaqua species described above, which can then be used to install new leaving group ligands.

An alternative reaction for preparing water-soluble complexes with modified leaving groups utilizes a silver(I) salt of the desired new ligand. The silver(I) salts react directly with cisplatin and related diaminedihalidoplatinum(II) complexes in water, yielding insoluble \( \text{AgX} \) as the only byproduct (Scheme 1.4b). The resulting silver(I) halide can be removed by filtration, leaving the soluble product to be recovered from the filtrate by evaporation of the solvent. This strategy was employed for an optimized synthesis of radiolabeled \( ^{195m}\text{Pt-carboplatin} \). In cases where the silver(I) salt of a desired carboxylate ligand is not readily available, a one-pot strategy can be employed, in which the diaminedihalidoplatinum(II) complex, \( \text{Ag}_2\text{CO}_3 \), and the
carboxylic acid are mixed together. In another approach, the sulfate salt of \([PtL_2(OH_2)_{2}]^{2+}\), generated by the reaction of \(Ag_2SO_4\) with \([PtL_2X_2]\) in water, is used in conjunction with \(Ba(II)\) salts of the carboxylates, usually formed in situ from \(Ba(OH)_2\), to synthesize the desired platinum(II) carboxylate (Scheme 1.4c). The byproduct of this reaction, insoluble \(BaSO_4\), can be easily removed by filtration. This reaction has been used to attach \(\beta\)-diketone and sulfonate ligands to platinum(II). Another synthetic approach, which has not found widespread use, requires a platinum(II) oxalate complex and the calcium salt of a ligand. The mixture of these two species produces insoluble calcium(II) oxalate and the target complex, the latter of which remains in solution (Scheme 1.4d).

\[
\begin{align*}
(a) & \\
Pt_L^X & + 2AgNO_3 + H_2O \rightarrow [Pt_L^X(OH_2)]^{(NO_3)_2} + 2NaNu + 2NaNO_3 \\
(b) & \\
Pt_L^X & + 2AgNu + H_2O \rightarrow [Pt_L^X(Nu)]^{2+} \\
(c) & \\
Pt_L^X & + Ag_2SO_4 + H_2O \rightarrow [Pt_L^X(OH_2)]^{(SO_4)} + Ba(Nu)_2 + BaSO_4 \\
(d) & \\
Pt_L^X & + Ca(Nu)_2 + H_2O \rightarrow [Pt_L^X(Nu)]^{2+} - Ca(oxalate) \\
\end{align*}
\]

Scheme 1.4. Different synthetic routes to replace halide leaving group ligands.

Various other interesting leaving groups have also been attached to platinum to generate anticancer drug candidates. Platinum(II) diamine complexes with squarate, selenite,
tellurate, ascorbate, and methyl sulfinyl carboxylate leaving groups have been prepared using the protocols described above. Additionally, the complex, \([\text{Pt}(\text{trans}-1R,2R-\text{DACH})(\text{B}_2\text{O}_5\text{H}_2)]\) with a chelating borate ligand, was prepared from \([\text{Pt}(\text{trans}-1R,2R-\text{DACH})(\text{OH})_2]\) and a mixture of boric acid and tetraborate in water. These novel complexes demonstrate the synthetic versatility of the methods described above for preparing new platinum compounds with different leaving group ligands, some of which may have therapeutic potential.

**Synthesis of cis- and trans-[PtLL'X₂], Complexes with Mixed Am(m)ine Ligands.**

Platinum(II) complexes that bear two different amine or N-heterocyclic ligands have gained importance in recent years as a new class of anticancer agents. The complexes \(\text{cis-[Pt(NH}_3)(2\text{-picoline})\text{Cl}_2]\) (picoplatin) and \(\text{cis-[Pt(NH}_3)(\text{cyclopentylamine})(\text{hydroxybutanedioato})]\) (cycloplatam) (Chart 1.2), for example, have undergone clinical trials. Cis-[Pt(NH₃)(cyclohexylamine)Cl₂] (JM-118) (Chart 1.2), an active metabolite of the clinically investigated platinum(IV) complex satraplatin, is another member of this class of compounds. Trans platinum complexes of mixed amine or N-heterocyclic ligands are also of importance. Some members of these “trans planar amine (TPA)” compounds with the general formula \(\text{trans-[Pt(NH}_3)LX}_2\), where L is an N-heterocycle, exhibit potent anticancer activity and are not cross-resistant with cisplatin. Moreover, they serve as precursors for the preparation of phototoxic platinum(IV) diazido complexes.

![Chart 1.2. Structures of mixed amine platinum(II) complexes that have undergone clinical trials.](chart.png)
The most obvious synthetic route to mixed amine platinum(II) complexes with cis stereochemistry involves the use of the [PtLCl$_3$]$^-$ anion as an intermediate. Treating this ion with another amine or $N$-heterocycle, L', is expected to yield the complex cis-[PtLL'X$_2$]. A limitation to this approach, however, is the difficulty in preparing am(m)inetrichloridoplatinate(II) ions. Initial reports of the syntheses of K[Pt(NH$_3$)Cl$_3$] (Cossa's salt) appeared over a century ago. Since then, researchers have focused on finding straightforward, high yielding protocols for obtaining this and related ions. The treatment of K$_2$PtCl$_4$ with one equiv of L more readily generates 0.5 equiv of the highly insoluble cis-[PtL$_2$Cl$_2$] complex rather than the soluble salt K[PtLCl$_3$]. An early, inefficient preparation of K[Pt(NH$_3$)$_3$] involved treatment of cisplatin with hydrochloric acid in the presence of a catalytic amount of Pt metal at elevated temperatures. After removing unreacted cisplatin and Pt metal by filtration, the [Pt(NH$_3$)$_4$]$^{2+}$ cation was added to precipitate the salt [Pt(NH$_3$)$_4$][Pt(NH$_3$)Cl$_3$]. This salt was then treated with K$_2$PtCl$_4$, giving insoluble Magnus' salt, [Pt(NH$_3$)$_4$][PtCl$_4$], and Cossa's salt, K[Pt(NH$_3$)$_3$Cl], in the filtrate. A typical yield was not reported for this method, but the large quantity of undesired platinum-containing byproducts makes this method expensive and undesirable. The reaction was later optimized to obtain Cossa's salt in 60% yield from cisplatin. In this case, the salt was isolated by anion-exchange chromatography and therefore did not require formation of the platinum double salts. With careful control of temperature and reaction time, the ammonium salt of [Pt(NH$_3$)Cl$_3$]$^-$ was reportedly isolated in 90% yield, similarly by the action of hydrochloric acid on cisplatin, but in the absence of a Pt metal catalyst.

An alternative, more commonly used route for the preparation of the [Pt(NH$_3$)Cl$_3$]$^-$ anion employs $N$,$N$-dimethylacetamide (DMA) as the solvent. At high temperatures (100 °C) with a stream of nitrogen gas bubbling through the DMA solution, the direct reaction between an
excess amount of tetraethylammonium chloride and cisplatin affords the desired anion, which can be subsequently precipitated from an aqueous solution as the PPh₄⁺ salt.¹²⁷ The use of other cis-[PtL₂Cl₂] complexes as starting materials in this reaction generally affords the corresponding [PtLCl₃]⁻ ions. This transformation, however, is only successful if the leaving amine ligand is sufficiently volatile, driving the reaction by the evaporation of the amine. Because of this limitation, the anion [Pt(cyclohexylamine)Cl₃]⁻ could not be prepared via this route.¹²⁷ The high temperatures employed for this method, which result in the eventual decomposition of cisplatin to insoluble platinum black, restrict the overall yield and efficiency of the reaction. The introduction of substoichiometric amounts of NH₄Cl (0.25 mol%) prevents formation of platinum black (Scheme 1.5).¹²⁹ The reported yield in this case was 58%, but unreacted cisplatin could be recovered for later use.¹²⁹ Water-soluble forms of the [Pt(NH₃)Cl₃]⁻ ion as either its sodium or potassium salt can be isolated using an ion-exchange resin,¹²⁸,¹²⁹ or metathesis with either KPF₆¹³⁰ or NaBPh₄.¹³¹ An HPLC method was described recently to assess the purity of the potassium salt.¹³² To prepare the analogous anions where L is an N-heterocycle instead of an amine, direct reaction between K₂PtCl₄ and one equiv of L in DMF at 75 °C can be used. This reaction also produces some of the disubstituted neutral product cis-[PtL₂Cl₂].¹³³,¹³⁴ The amount of this undesired species formed depends on the steric bulk of the incoming heterocycle.¹³⁴ For example, much higher yields of the anion were afforded with 2,4-lutidine than with pyridine. Presumably, the steric properties of the ortho methyl groups of 2,4-lutidine disfavor simultaneous coordination of two such ligands to a single platinum center. The use of steric effects to synthesize of [PtLCl₃]⁻ directly from K₂PtCl₄ in water, where L is an aliphatic amine, has also been described.¹³⁵ The sterically crowded amines, isopropyl and tert-butyl amine, substantially retard the formation of cis-[PtL₂Cl₂], enabling isolation of K[PtLCl₃] in 16-36%
yields. For either reaction, the desired soluble anion can be separated from the insoluble disubstituted complex by extraction into water.

The \([\text{PtLCl}_3]^-\) ions are suitable precursors for the synthesis of mixed ligand complexes of the type \(\text{cis-}[\text{PtLL'}\text{X}_2]\). The reaction between \(L'\) and \([\text{PtLCl}_3]^-\) in water or DMF gives the mixed amine complex with the expected cis stereochemistry. Under these conditions, however, a small amount of \(\text{cis-}[\text{PtL'}\text{Cl}_2]\) can also be formed, presumably arising from \([\text{PtCl}_4]^{2-}\) impurities in the starting material. By analogy to Dhara’s method for the synthesis of cisplatin, the preparation of cis mixed amine complexes was improved by first treating the \([\text{PtLCl}_3]^-\) anion with two equiv of NaI or KI in water (Scheme 1.5). Multinuclear NMR spectroscopic studies verified that the addition of two equiv of iodide, when \(L = \text{NH}_3\), forms primarily the ion \(\text{trans-}[\text{PtI}_2\text{Cl}(\text{NH}_3)]^+\), resulting from substitution of two chloride ligands. The large trans effect of the iodide favors the amine substitution reaction. The addition of an amine to \(\text{trans-}[\text{PtI}_2\text{Cl}(\text{NH}_3)]^+\) readily yields the mixed halide species \(\text{cis-}[\text{PtLL'}\text{ClI}]\). In this case, some impurity resulting from \([\text{PtCl}_4]^{2-}\) is present in the form of \(\text{cis-}[\text{PtL'}\text{Cl}_2]\) is present. This impurity, however, can be readily removed from the desired product by its dissolution in acetone. The mixed halide intermediate can then be converted to the dichloride by the removal of the iodide ligand with \(\text{Ag}^+\) and the addition of \(\text{Cl}^-\) to the resulting platinum aqua complex (Scheme 1.5). In the case where both \(L\) and \(L'\) are quinoline derivatives, the direct reaction \([\text{PtLCl}_3]^-\) and \(L'\) in mixed aqueous and organic solvent directly afforded the desired compound in its pure form without the need to proceed through the mixed halide species.
Scheme 1.5. Synthesis of cis-[Pt(NH₃)LCl₂] starting from cisplatin using the [Pt(NH₃)Cl₃]⁻ ion as an intermediate.¹²⁵,¹²⁹

The other commonly used route for the synthesis of mixed cis amine platinum(II) complexes utilizes iodido-bridged dimers, [PtL₂I₂], as intermediates. The reaction of cis-[PtL₂] with perchloric acid forms these species, which are generally insoluble and brown in color (Scheme 1.6).¹⁴¹ The scope of this reaction extends to a range of aliphatic¹⁴² and aromatic amines,¹⁴³ and N-heterocycles.¹⁴⁴ For sterically hindered amines, such as tert-butyl amine, the analogous iodido-bridged dimers form directly upon their reaction with K₂PtI₄; formation of the expected product, cis-[PtL₂I₂], does not proceed, presumably due to the large steric hindrance of the bulky amine ligands.¹⁴² The perchloric acid acts to remove an amine ligand from cis-[PtL₂I₂] by protonolysis. The vacant coordination site of the platinum(II) center is then filled by an iodide
ligand of another complex. Because both the starting material and products are poorly soluble in water, the reaction can take an exceedingly long time to reach completion; for cyclopropyl amine, a reaction time of three weeks was necessary to achieve full conversion. Furthermore, the lack of solubility of both species makes it difficult to gauge the extent of the reaction. For reactions that have not gone to completion, the final product may be contaminated by starting material. The iodido-bridged dimers exist in two isomeric forms, syn and anti (Scheme 1.6), depending on the disposition of the two amine ligands about the Pt–Pt vector. Solution NMR spectra display resonances from both isomers, but in the solid-state only anti isomers have been observed by X-ray crystallography.

Although several recent publications have reported that some of these iodido-bridged dimers exhibit anticancer activity, their primary use is for the synthesis of cis mixed amine complexes. In this context, it should be noted that these iodido-bridged dimers can also serve as precursors for the [PtLCl$_3$]$^-\text{anion}$ discussed above. Treatment of [PtLI(μ-I)$_2$] with excess AgNO$_3$ in water, followed by the addition of excess KCl, provides another route to the [PtLCl$_3$]$^-\text{ion}$. More useful, however, is the direct reaction of these dimers with another amine or N-heterocycle to form the mononuclear mixed amine complexes, cis-[PtLL'$_2$] (Scheme 1.6). Despite the presence of both anti and syn isomers in the iodido-bridged dimer starting material, only cis-[PtL$_2$L$_2$] is obtained from this reaction. The iodide ligands can be exchanged for other halides using an appropriate silver(I) salt as described above. Analogous chlorido-bridged dimers can be synthesized by the photolysis of trans-[Pt(ethylene)LCl$_2$]. Cleavage of these chlorido-bridged dimers with another amine ligand, however, leads to formation of both the trans and cis isomers of the mixed amine complex, rendering this procedure less useful.
When a bidentate oxygen ligand is desired as the leaving group instead of monodentate halides, a different synthetic route can be used to access the mixed amine complex. The first step in this pathway requires the synthesis of cis-[Pt(DMSO)₂(O₂Chel)], where O₂Chel is a typically anionic chelating ligand with oxygen donor atoms. This intermediate is prepared by the reaction of cis-[Pt(DMSO)₂Cl₂], which itself is obtained from commercially available K₂PtCl₄ and DMSO, and the disilver salt of the chelating ligand in water (Scheme 1.7). The first DMSO ligand of cis-[Pt(DMSO)₂(O₂Chel)] can be substituted by an amine L at 40 °C in water to form isolable complexes of the type cis-[PtL(DMSO)(O₂Chel)]. The addition of a different amine, L', to this complex at higher temperatures (100 °C) in water enables substitution of the second DMSO ligand to afford the mixed amine complex cis-[PtLL'(O₂Chel)] (Scheme 1.7). The difficulty in removing the second DMSO ligand is emphasized by the fact that even the use of chelating diamine ligands requires heating to 100 °C to enforce bidentate coordination. For bidentate N-heterocycles like 2,2'-bipyridine, lower temperatures (refluxing methanol) can be used to substitute both DMSO ligands. The concentrations of the reactants are also important because, when high concentrations of chelating diamine ligands are used, both the DMSO...
ligands and oxygen chelate get displaced, forming \([\text{Pt}(L_2)_2]^{2+}\).\textsuperscript{153} Despite the apparent utility of this method, it has not been widely applied.\textsuperscript{95,154-156}

![Scheme 1.7. Synthesis of mixed amine complexes with a chelating oxygen donor leaving group.\textsuperscript{151}]

The processes for preparing mixed amine complexes with trans stereochemistry are more straightforward than those discussed above for cis complexes. These compounds, having the general formula \(\text{trans-}[\text{PtLL'}\text{Cl}_2]\), are of interest because many of them exhibit in vitro anticancer activity superior to that of cisplatin, despite their trans stereochemistry.\textsuperscript{157-162} Additionally, they exhibit no cross-resistance with cisplatin.\textsuperscript{157,158,160} Their preparation begins with the complex \(\text{cis-}[\text{PtL}_2\text{Cl}_2]\), the synthesis of which has been described earlier. The addition of greater than two equiv of \(L'\) to a suspension of this complex in boiling water typically gives rise to a colorless solution containing the salt, \(\text{cis-}[\text{PtL}_2L'_2]\text{Cl}_2\) (Scheme 1.8). The solubility and color can vary.
slightly, depending on the hydrophobicity and electronic properties of the amine or N-heterocycle ligands. Addition of concentrated hydrochloric acid to this salt at elevated temperatures leads to substitution of one L and one L’ ligand by chloride, forming $\text{trans-}[\text{PtLL’Cl}_2]$ (Scheme 1.8). The stereochemistry of the product is dictated by the kinetic trans effect. The first chloride substitution can yield either of the intermediates, $[\text{PtL}_2\text{L’Cl}]$ or $[\text{PtLL’}_2\text{Cl}_2]$. The larger trans effect of chloride compared to those of amines or N-heterocycles ensures that the second chloride substitutes trans to the first chloride. Further substitution to form $[\text{PtLCl}_3]$ or $[\text{PtCl}_4]$ is impeded by the low solubility of $\text{trans-}[\text{PtLL’Cl}_2]$, which precipitates from solution as a yellow solid immediately upon its formation.

$$\begin{align*}
\text{L} & \text{Pt} \text{Cl} \quad \text{L} \quad \text{Cl} \\
+ \quad \text{x} \quad \text{L’} & \quad \text{H}_2\text{O}, \text{90-100 °C} & \quad \text{Cl}_2 \\
\text{[L} & \text{Pt[PtL’Cl]} \quad \text{L’} \quad \text{Cl}_2 \quad \text{conc. HCl, 90-100 °C} & \quad \text{L} \quad \text{Pt} \quad \text{Cl} \quad \text{L’} \\
\end{align*}$$

**Scheme 1.8.** Synthesis of $\text{trans-}[\text{PtLL’Cl}_2]$.157

**Synthesis of Monofunctional Platinum(II) Complexes.** Platinum(II) complexes containing only one substitution-labile ligand are described as monofunctional in contrast to cisplatin and carboplatin, both of which contain two substitution-labile coordination sites and are referred to as bifunctional. The earliest monofunctional platinum(II) complexes investigated for their potential anticancer activity were $[\text{Pt(dien)Cl}]\text{Cl}$ (dien = diethylenetriamine) and $[\text{Pt(NH}_3)_2\text{Cl}]\text{Cl}$. The observation that these two complexes are inactive helped establish the traditional structure-
activity relationships for platinum therapeutics, which state that, among other requirements, charge neutrality and bifunctionality are necessary for activity.\textsuperscript{28,163} In spite of their lack of anticancer properties, these two monofunctional complexes have found use in modeling the reactions of platinum anticancer agents with biologically relevant nucleophiles because the presence of only one ligand exchange site simplifies the interpretation of results.\textsuperscript{164-167}

Optimized synthetic routes to these salts have been reported.\textsuperscript{168,169} The synthesis of \([\text{Pt(NH}_3\text{)}_3\text{Cl}]\text{Cl}\) commences by treatment of transplatin with one equiv of KI to form the mixed halide complex, \textit{trans-}[\text{Pt(NH}_3\text{)}_2\text{Cl}]\text{Cl}. The addition of one equiv of AgNO\textsubscript{3} in water selectively precipitates AgI and gives \textit{trans-}[\text{Pt(NH}_3\text{)}_2\text{Cl(}\text{OH}_2\text{)}](\text{NO}_3)\text{.} Aqueous ammonia readily replaces the labile aqua ligand, yielding \([\text{Pt(NH}_3\text{)}_3\text{Cl}]\text{Cl}\) as a colorless to pale yellow solid after precipitation from the aqueous solution with a mixture of ethanol and diethyl ether (Scheme 1.9).\textsuperscript{168} The aqua intermediate, \textit{trans-}[\text{Pt(NH}_3\text{)}_2\text{Cl(}\text{OH}_2\text{)}](\text{NO}_3)\text{, could also conceivably used as a synthon for complexes of the general type, \textit{trans-}[\text{Pt(NH}_3\text{)}_2\text{LCl}](\text{NO}_3)\text{.} The optimized preparation of \([\text{Pt(dien)Cl}]\text{Cl}\) utilizes the reaction of either \textit{cis/trans-}[\text{PtCl}_2(\text{SMe}_2)]\textsuperscript{170} or \textit{cis-}[\text{Pt(DMSO)}_2\text{Cl}_2]\textsuperscript{150} with dien in refluxing methanol. Isolation of \([\text{Pt(dien)Cl}]\text{Cl}\) as a white solid in yields of >90\% is accomplished by the addition of either CHCl\textsubscript{3} or CH\textsubscript{2}Cl\textsubscript{2} to the resulting methanolic reaction mixture.\textsuperscript{169}
More recently, a number of monofunctional platinum(II) complexes have been discovered that break the traditional structure-activity relationships by exhibiting anticancer properties. The most thoroughly investigated members of this class are complexes of the general formula cis-[PtL₂L'Cl]⁺, where L is a monodentate or bidentate amine and L' is either an N-heterocycle, a sulfoxide, or a thiourea derivative (Chart 1.3).

Chart 1.3. Examples of cationic monofunctional platinum(II) complexes that exhibit anticancer activity.
For the sulfoxide and thiourea complexes, chelating diamines are used exclusively because the strong trans effect of these ligands labilizes monodentate amines, leading to their dissociation. Although we define the sulfoxide compounds, \( \text{cis-}[\text{Pt}(L_2)(RR'SO)Cl]^+ \), as monofunctional because of the presence of only one labile Pt–Cl bond, these complexes form bifunctional DNA-adducts.\(^{179}\) Rapid substitution of the chloride for water or nucleobases is followed by the slow substitution of the sulfoxide ligand. The kinetics of the sulfoxide substitution reaction are effectively modulated by the steric bulk of the sulfoxide.\(^{172,176,179}\) The synthesis of these complexes proceeds either by the addition of the chelating amine ligand to \( \text{cis-[PtCl}_2(\text{RR'SO})_2] \) or the addition of one equiv of sulfoxide to \( \text{cis-[Pt}(L_2)\text{Cl}_2] \) (Scheme 1.10). The latter reaction pathway is the preferred route; the use of \( \text{cis-[PtCl}_2(\text{RR'SO})_2] \) as the starting material in the former pathway gives variable amounts of \( \text{cis-[Pt}(L_2)\text{Cl}_2] \) as an undesired byproduct.\(^{172}\)

\[
\text{R-S S R'} \text{Pt} + \text{NH}_2 \text{NH}_2 \xrightarrow{\text{MeOH, rt}} \begin{array}{c}
\text{R-} \\
\text{NH}_2 \\
\text{Cl}
\end{array}
\]

\[
\begin{array}{c}
\text{Cl} \\
\text{Pt} \\
\text{NH}_2
\end{array}
\text{Cl'} + \begin{array}{c}
\text{O} \\
\text{S} \\
\text{R'}
\end{array}
\xrightarrow{\text{MeOH, rt}} \begin{array}{c}
\text{R} \\
\text{S} \\
\text{Cl}
\end{array}
\text{Pt} \begin{array}{c}
\text{NH}_2 \\
\text{Cl'}
\end{array} \begin{array}{c}
\text{O} \\
\text{S} \\
\text{R'}
\end{array} \text{Cl'}
\]

Scheme 1.10. Two different synthetic routes for the preparation of \( \text{cis-[Pt}(L_2)(\text{RR'SO})\text{Cl}]^+ \).\(^{172}\)

Complexes of the type \( \text{cis-[Pt(NH}_3)_2\text{LCl}]^+ \), where \( \text{L} \) is an N-heterocycle, have attracted significant attention since the initial discovery of their antitumor properties in 1989.\(^{171}\) In contrast to the sulfoxide complexes discussed above, these cations bind to DNA and nucleobases
in monofunctional manner with no indication of ammonia or N-heterocycle loss to form
d bifunctional adducts. Although the monofunctional lesions do not significantly bend
DNA, they still manage to effectively destabilize the structure of the double helix and
impede DNA replication and transcription. The characteristic monofunctional DNA
adducts may be responsible for the different spectrum of activity observed for these compounds
in comparison to those for clinically used bifunctional platinum drugs. A number of
derivatives of cis-[Pt(NH$_3$)$_2$LCl]* have been synthesized, with L being a wide range of different
N-heterocycles. These N-heterocycles include derivatives of pyridine with different
substituents and fused aromatic rings, pyrimidines, a fluorescently labeled pyridine, steroid functionalized pyridines, imidazothiazoles, 9-aminoacridine, the antimalarial
drug chloroquine, ethidium, the anticancer drug 5-fluorouracil, and several antiviral
agents. Of these drug candidates, the complex utilizing phenanthridine as its N-heterocyclic
ligand, termed phenanthriplatin, exhibits in vitro cytotoxicity greater than that of cisplatin across
a wide range of cell lines. As phenanthriplatin illustrates, the judicious choice of the N-
heterocycle can give rise to very potent monofunctional complexes.

The synthesis of monofunctional complexes can be accomplished by stirring a mixture of
cisplatin and one equiv of the N-heterocycle in water at 50–60 °C for several days. This
reaction proceeds by direct substitution of the chloride ligand for the N-heterocycle. This
method, however, typically gives low yields of impure compounds. The major impurity is the
disubstituted species, cis-[Pt(NH$_3$)$_2$L$_2$]Cl$_2$, which arises from substitution of both chloride
ligands. Pretreatment of cisplatin with one equiv of AgNO$_3$ in water to generate the reactive
monoaqua complex, cis-[Pt(NH$_3$)$_2$Cl(OH$_2$)]$, followed by the addition of another ligand has also
been reported. This method gave rise to a substantial portion of unreacted cisplatin and
unidentified byproducts. The reaction mixture containing one equiv AgNO₃ and cisplatin in water has been analyzed by $^{195}$Pt NMR spectroscopy. The monoaqua complex, cis-\([\text{Pt(NH}_3\text{)}_2\text{Cl(OH}_2\text{)}]^+\), comprises 57% of the total platinum in solution, whereas the diaqua complex, cis-\([\text{Pt(NH}_3\text{)}_2\text{OH}_2\text{)}^2+\), and unreacted cisplatin account for 39 and 4%, respectively.\(^{171}\) The large quantity of the diaqua complex should lead to the formation of an equally large proportion of the undesired disubstituted complex. Treating cisplatin with one equiv of AgNO₃ in DMF, however, gives a much more favorable product distribution; 79-86% of the total platinum is in the monosolvated form cis-\([\text{Pt(NH}_3\text{)}_2\text{Cl(solv)}]\), where “solv” is either DMF or nitrate, whereas only 9% and 9-12% of the platinum comprises disolvated species and unreacted cisplatin, respectively.\(^{171,194}\) The preferred synthetic route to monofunctional complexes, therefore, involves the reaction of cisplatin with one equiv of AgNO₃ in DMF followed by the addition of the N-heterocyclic ligand (Scheme 1.11).\(^{171,199}\) Care must be taken to purify the final monofunctional product from unreacted cisplatin and disubstituted byproducts. Because cisplatin is not soluble in methanol, it can be removed by filtration after dissolving the crude product in this solvent. A final recrystallization step from either dilute HCl or methanol is necessary to separate the monofunctional complex from disubstituted byproducts. The mononitrato species, cis-\([\text{Pt(NH}_3\text{)}_2\text{Cl(NO}_3\text{)}]\), has reportedly been isolated as a solid by evaporation of an aqueous solution containing a 1:1 mixture of cisplatin and AgNO₃.\(^{198}\) This complex was then used as a precursor for new monofunctional complexes. Only CHN analyses were presented as characterization for cis-\([\text{Pt(NH}_3\text{)}_2\text{Cl(NO}_3\text{)}]\); it is most likely that the isolated solid comprised the mixture of species found in solution by $^{195}$Pt NMR spectroscopic studies, as discussed above.
Another important class of monofunctional platinum anticancer agents contains complexes of the general formula cis-[Pt(L₂)Cl(tu)]⁺, where L₂ is a chelating diamine ligand and tu is a derivative of thiourea, coordinated through the sulfur atom (Chart 1.3, right side). Although the first generation analogues of these complexes with underivatized thiourea ligands exhibit only poor to moderate in vitro cytotoxicity, the second generation compounds with acridine-functionalized thioureas are typically more cytotoxic than cisplatin. These Pt-acridinylthiourea, or PT-ACRAMTU, conjugates form hybrid DNA adducts; the Pt center binds preferentially to N7 of guanine bases and the acridine intercalates between base pairs. In contrast to the monofunctional sulfoxide complexes discussed above, the thiourea ligand does not get displaced by nucleobases and therefore only monofunctional covalent DNA adducts are formed. The unique hybrid DNA-binding motif of these complexes gives rise to a profile of activity in a wide range of cancer cells substantially different from those of cisplatin and related platinum anticancer drugs. The synthesis of PT-ACRAMTU complexes follows a protocol similar to that used for the monofunctional cis-diammine complexes. A 1:1 mixture of cis-[Pt(L₂)Cl₂] and AgNO₃ are stirred in DMF, and the filtrate is treated with one equiv of the

\[
\text{Cl} \begin{array}{c}
\text{Pt} \\
\text{Cl} \\
\text{NH}_3
\end{array} + \text{AgNO}_3 \rightarrow \text{DMF, 50-60 °C} \rightarrow \text{Solv} \\
\text{Cl} \begin{array}{c}
\text{Pt} \\
\text{Cl} \\
\text{NH}_3
\end{array} \text{(NO}_3\text{)} \\
\text{Cl} \begin{array}{c}
\text{Pt} \\
\text{Cl} \\
\text{NH}_3
\end{array} \text{(NO}_3\text{)} \\
\text{Cl} \begin{array}{c}
\text{Pt} \\
\text{Cl} \\
\text{NH}_3
\end{array} \text{(NO}_3\text{)} \\
\text{Cl} \begin{array}{c}
\text{Pt} \\
\text{Cl} \\
\text{NH}_3
\end{array} \text{(NO}_3\text{)}
\]

Scheme 1.11. Synthesis of cis-[Pt(NH₃)₂Cl]NO₃.¹⁷¹,¹⁸⁸
thiourea ligand (Scheme 1.12). This reaction fails when monodentate amine ligands are used; the addition of thiourea induces full substitution of the monodentate amine ligands, forming \([Pt(tu)_4]^{2+}\), consistent with the expected results of the Kurnakow test, as discussed above.

![Scheme 1.12. Synthesis of monofunctional, thiourea platinum(II) complexes.](image)

**Platinum(II) Complexes Synthesized by Ligand-Based Reactivity.** The synthetic strategies discussed in the previous sections all rely on ligand substitution reactions at the platinum(II) center. Alternative synthetic pathways involve the use of outer-sphere, ligand-based reactivity. These reactions are facilitated by the ability of transition metal ions to activate coordinated ligands, making them more susceptible to certain reactivity pathways that would otherwise be inaccessible to them in the unbound form. This section covers several examples of these reactions that have been utilized to synthesize novel platinum anticancer drug candidates.

Iminoether complexes of platinum(II) of general formula \([Pt(\text{iminoether})_2\text{Cl}_2]\) display potent anticancer activity both in vitro and in vivo. As for traditional platinum anticancer agents, this activity is proposed to arise from DNA binding. For the trans isomers, monofunctional adducts and protein-DNA cross-links are invoked as the predominant cytotoxic lesions.
The trans isomers tested initially proved to be more active than their cis congeners, although some recent studies have reported the opposite observation for new members of this class of compounds. The cis and trans structure-activity relationships for these compounds, therefore, depends on the exact chemical nature of the iminoether ligand. In addition to the stereochemistry at the square-planar platinum center, the iminoether ligands can exist in either $E$ or $Z$ configuration, depending on the orientation of the substituents about the C–N double bond. As a result, six isomers exist for $[\text{Pt(iminoether)}_2\text{Cl}_2]$ complexes, neglecting rotational isomers involving the Pt–N vector (Chart 1.4). For both cis and trans isomers, the stereochemistry of the ligand had a substantial effect on the biological activity of the complex. Therefore, precise synthetic control over the total stereochemistry of the final complex is important for further biological applications.

**Chart 1.4.** Depiction of the six different stereoisomers of $[\text{PtCl}_2(\text{iminoether})_2]$. The terms cis and trans refer to the stereochemistry at the platinum(II) center, whereas $E$ and $Z$ denote the stereochemistry at the C–N double bond of the iminoether ligands.
The nitrile complexes, cis- and trans-[PtCl₂(NCR)₂], are precursors for the synthesis of cis- and trans-[PtCl₂(iminoether)₂] complexes. The reaction of cis- or trans-[PtCl₂(NCR)₂] in alcohols²¹⁵ or alcohol-dichloromethane mixtures²¹⁶ with a catalytic amount of KOH affords the iminoether complexes by nucleophilic attack of the alkoxide on the coordinated nitrile ligand (Scheme 1.13). Formation of the iminoether occurs with retention of the starting stereochemistry of the platinum complex, but generally a mixture of E and Z ligand-based isomers are obtained. Formation of the Z iminoether is kinetically preferred; isomerization to the E isomer occurs in the presence of catalytic amounts of base, which is present under the reaction conditions.²¹⁷ Carrying the reaction out at low temperature (0 °C) also significantly impedes isomerization to the E isomer, giving predominantly Z isomers.²¹⁸ The ZZ, EZ, and EE isomers can be separated on the basis of solubility differences by fractional crystallization or silica gel column chromatography.²¹⁷

![Scheme 1.13. Synthesis of cis- (top) and trans-[PtCl₂(iminoether)₂] (bottom).²¹¹,²¹⁷](image-url)
Mixed iminoether-ammine platinum(II) complexes of formula \([\text{PtCl}_2(\text{NH}_3)(\text{iminoether})]\) also exhibit in vitro and in vivo anticancer activity.\(^{218,219}\) These complexes are prepared from mixed ammine-nitrile complexes by the attack of an alkoxide on the coordinated nitrile ligand under conditions similar to those employed for the bis(iminoether) complexes described above. The synthetic protocols for the precursor complexes, \(\text{cis- and trans-}[\text{PtCl}_2(\text{NH}_3)(\text{NCR})]\), are similar to those described for mixed amine platinum(II) complexes described above. To prepare \(\text{trans-}[\text{PtCl}_2(\text{NH}_3)(\text{NCR})]\), \(\text{cis-}[\text{Pt}(\text{NH}_3)_2]\) is treated with two equiv of \(\text{AgNO}_3\) in water to form the diaqua complex, from which \(\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{NCR})_2]\) can be formed by addition of a large excess of nitrile at 70 °C. Excess aqueous KI displaces two ligands; the large trans effect of iodide enforces trans stereochemistry in the final product, \(\text{trans-}[\text{PtI}_2(\text{NH}_3)(\text{NCR})]\).\(^{219}\) The iodide ligands can be replaced by chlorides via sequential treatment with \(\text{AgNO}_3\) and KCl. The synthesis of \(\text{cis-}[\text{PtCl}_2(\text{NH}_3)(\text{NCR})]\) is accomplished more simply by action of a large excess of nitrile on Cossa’s salt, \(\text{K}[\text{Pt}(\text{NH}_3)\text{Cl}_3]\).\(^{218}\)

In addition to alcohols and alkoxides, platinum(II) nitriles are also activated for nucleophilic attack by amines to form amidine complexes. Amidine complexes of formulas \(\text{cis- and trans-}[\text{PtCl}_2(\text{amidine})_2]\), \(\text{cis- and trans-}[\text{PtCl}_2(\text{NH}_3)(\text{amidine})]\), and \(\text{trans-}[\text{Pt}(\text{amine})_2(\text{amidine})_2]\text{Cl}_2\) have been investigated for anticancer potential, both in vitro and in vivo.\(^{220-225}\) Like their iminoether analogues, these complexes generally show good activity in both cisplatin-sensitive and -resistant cell lines. Additionally, both metal- (cis or trans) and ligand-based (\(\text{EE, EZ, or ZZ}\)) stereoisomers are possible, as for the related iminoether complexes. The syntheses of \(\text{cis- and trans-}[\text{PtCl}_2(\text{amidine})_2]\), like those for the analogous iminoether complexes, begin with \(\text{cis- and trans-}[\text{PtCl}_2(\text{NCR})_2]\). Low temperature reactions (−10 °C) of the bisacetonitrile complexes with secondary or primary amines in \(\text{CH}_2\text{Cl}_2\) affords the amidine
complexes (Scheme 1.14).\textsuperscript{226} For the cis isomer, five equiv of the amine are required, whereas for the trans isomer 50 equiv of amine are required for complete conversion to the diamidine product.\textsuperscript{227} The metal stereochemistry is initially retained in both cases, but in pure water these complexes isomerize, forming an equilibrium mixture of cis and trans species.\textsuperscript{228} This isomerization is prevented in the presence of 100 mM NaCl.\textsuperscript{228} The stereochemical outcome of the amidine ligand ($E$ vs $Z$) appears to be dictated by the nature of the coordinated nitrile ligand and the nucleophilic amine. In the reaction with the bisacetonitrile or bis(benzylcyanide) platinum complexes, primary amines selectively give $ZZ$ isomers and secondary amines give $EE$ isomers.\textsuperscript{222,229} When the coordinated nitrile is benzonitrile, a mixture of $EE$ and $ZZ$ isomers is obtained.\textsuperscript{230} Preparative TLC can be used to separate and isolate some of these isomers.\textsuperscript{220}

The action of ammonia on both cis- and trans-[PtCl$_2$(NCR)$_2$] has also been investigated.\textsuperscript{221,231} For cis dinitrile complexes, addition of aqueous ammonia in THF affords the expected diamidine species.\textsuperscript{221} When gaseous ammonia is bubbled through a CH$_2$Cl$_2$ solution of the trans dinitrile complex at $-10\,^{\circ}\mathrm{C}$, the major product is trans-[PtCl(NH$_3$)(amidine)$_2$]Cl, which results from substitution of a chloride ligand by ammonia in addition to the expected attack on the coordinated nitrile.\textsuperscript{231} This complex is also the major product when the trans dinitrile complex is treated with aqueous ammonia in THF.\textsuperscript{221} At room temperature and with 24 h reaction times, both chlorides of trans-[PtCl$_2$(NCR)$_2$] can be substituted by aliphatic amines or ammonia to form the salts, trans-[Pt(amine)$_2$(amidine)$_2$]Cl$_2$ (Scheme 1.14),\textsuperscript{232} which also exhibit anticancer activity.\textsuperscript{224} The greater tendency of the trans dinitrile complex to lose its chloride ligands in comparison to that of the cis is proposed to be a consequence of the greater solubility of the intermediate diamidine complex, trans-[PtCl$_2$(amidine)$_2$], which makes it susceptibility to
further reactivity with the nucleophilic amines. For the cis complex, the diamidine complex precipitates from solution, thus impeding further substitution reactions.

Scheme 1.14. Synthesis of cis- (top) and trans-[PtCl₂(amidine)₂] (middle). Room temperature conditions and extended reaction times lead to the formation of primarily trans-[Pt(NH₂R')₂(amidine)₂] from trans-[PtCl₂(NCR)₂] (bottom).

Using the abovementioned amidine-forming reactions, new analogues of the monofunctional PT-ACRAMTU complexes, discussed above, were prepared. These complexes have the general formula, cis-[PtCl₂(L₂)(amidine)](NO₃), where L₂ is either two ammine ligands or a chelating diamine, and the amidine ligand is tethered to an intercalating acridine unit. These complexes exhibit excellent activity against non-small cell lung cancer (NSCLC) cell lines without displaying cross-resistance to cisplatin. Furthermore, compared to first generation thiourea analogues, these amidine complexes react less readily with cysteine.
sulfur, yet bind more rapidly to DNA. By comparison to cisplatin, these complexes display a significantly larger degree of intracellular accumulation and DNA platination. The syntheses of such species is accomplished by first treating cis-[PtL₂(NCR)Cl]Cl with one equiv of AgNO₃ to exchange the outer-sphere chloride with a nitrate counterion, followed by the addition of an acridinyl amine to a DMF solution of the complex at -10 °C (Scheme 1.15). Acidic workup with HNO₃ yields the product in its protonated form. The low temperature conditions employed during the addition of the amine is presumably necessary to prevent it from displacing the chloride ligand. The mononitrile precursor complex, cis-[PtL₂(NCR)Cl]Cl, is synthesized by refluxing a mixture of cis-[PtL₂Cl₂] and excess nitrile in dilute HCl (Scheme 1.15). This straightforward, amidine-forming reaction has recently been applied to combinatorially screen a number of new platinum complexes that differ in the starting nitrile ligand and the acridinyl amine. This method was used to delineate some structure-activity relationships for this new class of platinum anticancer complexes.

\[
\begin{align*}
\text{L} \text{Pt} \text{L} + \text{xs RCN} & \xrightarrow{\text{dil. HCl (pH 4), 60 °C}} \text{L} \text{Pt} \text{L} \text{Cl} \text{NCR} ^{\text{Cl}} \\
\left[ \text{L} \text{Pt} \text{L} \text{Cl} \text{NCR} ^{\text{Cl}} \right] & \xrightarrow{\text{DMF, rt, AgNO}_3} \text{L} \text{Pt} \text{L} \text{Cl} \text{NCR} ^{\text{NO}_3} \\
\left[ \text{L} \text{Pt} \text{L} \text{Cl} \text{NCR} ^{\text{NO}_3} \right] & \xrightarrow{\text{DMF, -10 °C, Acidic work-up}} \text{L} \text{Pt} \text{L} \text{NCR} ^{\text{(NO}_3)_2}
\end{align*}
\]

Scheme 1.15. Multi-step synthetic scheme for the preparation of monofunctional platinum-amidine complexes.
In addition to nitriles, amines and ammonia are also activated for novel reactivity pathways when coordinated to platinum. Such ligands can engage in condensation reactions to form coordinated imines or iminates. For example, reactions of \([\text{Pt(NH}_3\text{)}_6]\)^{4+} and \([\text{Pt(en)}_3]\)^{4+} with acetylacetone afford \(\beta\)-diketiminate complexes.\(^{239,240}\) Additionally, both cis and transplatin react with 2-pyridinecarbaldehyde to form \([\text{Pt(pmpa)}\text{Cl}]\) where pmpa = \(N\)-(2-picoly)picolinamide), presumably by condensation of the coordinated NH\(_3\) group with the aldehyde (Scheme 1.16).\(^{241}\)

Amine insertion into a carbon-carbon double bond of the axial ligand of a platinum(IV) complex has also been observed.\(^{242}\) Acetonimine complexes of platinum(II), cis and trans-\([\text{PtX}_2(\text{acetonimine})_2]\) where \(X = \text{Cl}\) or I, were synthesized by the reaction of cis and trans-\([\text{Pt(NH}_3\text{)}_2X]\) with acetone in the presence of KOH (Scheme 1.16).\(^{243}\) These complexes are of therapeutic interest because they display good in vitro anticancer activity against a panel of human cancer cell lines without exhibiting cross-resistance to cisplatin.\(^{243}\) Cis- and transplatin react more slowly with acetone than their corresponding iodide analogues, and the complexes of cis stereochemistry are more reactive than the trans complexes. Based on these observations, the ligand trans to the amines is proposed to modulate the condensation reactivity, which occurs first by deprotonation of the amine to form a nucleophilic amido ligand. Higher trans effect ligands lower the ammine pK\(_a\) by stabilizing the anionic amido ligand. Another route to bis(acetonimine) platinum(II) complexes was also reported; direct ligand substitution reaction of \([\text{PtL}_2\text{Cl}_2]\) (L in this case is a phosphine) by \([\text{Ag(acetonimine)}_2]\)\(\text{ClO}_4\) affords such species.\(^{244}\)
Scheme 1.16. Condensation reactions involving the coordinated ammine ligands of cis- and transplatin, as well as their diiodo analogues.\textsuperscript{241,243}

Ligand-based reactivity does not necessarily require activation by platinum coordination. If the ligand has a functional group that is not in direct interaction with the platinum ion, this functional group can display its typical reactivity, provided that the reaction conditions or byproducts do not lead to decomposition of, or ligand dissociation from, the platinum complex. The platinum(II) complexes \([\text{Pt(edma)}\text{Cl}_2]\) and \([\text{Pt(edda)}\text{Cl}_2]\), where edma = ethylenediaminemonoaetic acid and edda = ethylenediamine-\(N,N'\)-diacetic acid, can engage in reactions associated with their free carboxylic acid groups (Scheme 1.17). The reaction of \([\text{Pt(edma)}\text{Cl}_2]\) with thionyl chloride in methanol converts the acid to a methoxy ester group, presumably through an intermediate acid chloride.\textsuperscript{245} Furthermore, the carboxylic acids of both \([\text{Pt(edma)}\text{Cl}_2]\) and \([\text{Pt(edda)}\text{Cl}_2]\) can be converted to amides after activation with 1,1'-
carbonyldiimidazole (CDI) and treatment with an amine. In both cases, the platinum coordination sphere remains unaffected. Platinum(II) complexes of a chelating diamine ligand having a pendant azide have also been synthesized. The azide functional group was employed for the Cu(I)-catalyzed click reaction with terminal alkynes. This chemistry was used to attach a number of different groups to the platinum complex (Scheme 1.17). Notably, the coordination sphere of the platinum(II) core remained intact in the presence of the Cu(I) catalyst.

Platinum(II) complexes with thiol-reactive maleimide derivatives attached to both the non-leaving and leaving group ligands were prepared. As expected, the maleimide moiety readily reacted with thiols. This reaction was used to link carboplatin derivatives to human serum albumin for improved tumor delivery.

Scheme 1.17. Outer-sphere ligand-based reactivity pathways of several platinum(II) complexes.
1.3. Synthesis of Platinum(IV) Anticancer Complexes

Several platinum(IV) complexes have undergone clinical trials, but to date none has been approved for use. Examples include iproplatin, tetraplatin, and satraplatin (Chart 1.5). An advantage of platinum(IV) complexes over their platinum(II) analogues is their six-coordinate octahedral coordination geometry. The introduction of two additional ligands allows for further tuning of the properties and confers the ability to attach functional or targeting groups. Moreover, being complexes of d⁶ octahedral metal ions, platinum(IV) compounds are substantially more inert than those of platinum(II). Thus, undesirable side reactions with proteins or intracellular thiols can generally be avoided using platinum(IV) complexes. The kinetic inertness of satraplatin is most likely the reason why it was deemed suitable for oral administration in contrast to all other platinum drugs, which are delivered intravenously.

![Chart 1.5. Examples of several clinically investigated platinum(IV) anticancer agents.](image)

Because platinum(IV) complexes are inert, they usually undergo reduction to platinum(II) before binding to their ultimate intracellular target, DNA. Reduction of platinum(IV) occurs with loss of two ligands, giving a square-planar geometry for the platinum(II) product. It has generally been assumed that the two ligands lost upon reduction are
located trans to each other and that both derive from the axial positions. This longstanding notion has been challenged by number of recent studies, which show that the composition of the reduced platinum(II) products depends on the nature of the reducing agent. Furthermore, the kinetics of intracellular platinum(IV) reduction depend both on the cell type and the ligands that define the coordination sphere of the complex. The precise synthetic control over new platinum(IV) anticancer drug candidates is critical for discovering new therapeutic agents and for further elucidating structure-activity relationships.

**Oxidation of Platinum(II).** A common synthetic route to complexes of platinum(IV) proceeds via two-electron oxidation of an appropriate platinum(II) precursor. For potential platinum(IV) anticancer agents, the most widely used oxidizing agents are hydrogen peroxide and chlorine. These two molecules react with platinum(II) to give trans oxidative addition products (Scheme 1.18). The equatorial ligands of the resulting platinum(IV) complex are generally retained with the stereochemistry of the starting platinum(II) compound. For some diamine dicarboxylato platinum(II) complexes, however, the action of chlorine leads to undesired displacement of the carboxylate groups to form diaminetetrachloroplatinum(IV) compounds.

Platinum(IV) dihydroxo compounds, obtained by oxidation of platinum(II) complexes in water, are important starting materials for the synthesis of further derivatized platinum(IV) compounds (vide infra). The treatment of a yellow-orange suspension of cisplatin in water with 10–100 fold excess of H₂O₂ at 50 °C gives rise to a pale-yellow suspension comprising cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂]. This reaction is general for related cis and trans platinum(II) complexes. When isolated directly from the mother liquor, this complex crystallizes with a molecule of hydrogen peroxide that forms hydrogen bonds with the hydroxo ligands. Hydrogen peroxide is also retained in the crystal lattice of the related complex, cis,cis,trans-
The observation that these complexes are able to cleave DNA is a consequence of hydrogen peroxide in the lattice rather than the platinum(IV) complex. The hydrogen peroxide can be removed by recrystallization from pure water. For the all trans isomer, trans,trans,trans-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$], which does not contain lattice-bound hydrogen peroxide, recrystallization from water leads to isomerization, giving trans,cis,cis-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$]. Diphenyl phosphate (DPP) can also be incorporated into the crystal lattice of trans-platinum(IV) hydroxo compounds. In these crystals, the diphenyl phosphate forms strong hydrogen bonds with the coordinated hydroxide or water ligands. The DPP adducts exhibit improved aqueous solubility. Thus, the use of an appropriate co-crystallization agent may enhance the pharmacological properties of the complex. The abovementioned results point to a large degree of complexity for the H$_2$O$_2$ oxidation of anticancer platinum(II) complexes. Care should be taken to avoid co-crystallized molecules that themselves might have biological activity. Additionally, isomerization may impede purification attempts.
Scheme 1.18. Oxidation of a platinum(II) complex with chlorine (top)\textsuperscript{261} and hydrogen peroxide\textsuperscript{263} (bottom). The products obtained for the hydrogen peroxide oxidation are dependent on the solvent used.\textsuperscript{270,271}

The mechanism of platinum(II) oxidation by hydrogen peroxide and chlorine has important implications for the composition of the isolated products. Studies of hydrogen peroxide oxidation of platinum(II) complexes using \textsuperscript{18}O-labeled water revealed that only one of the hydroxide ligands on the platinum(IV) complex originates from hydrogen peroxide; the other comes from water.\textsuperscript{272} Recently, the oxidation of \([\text{Pt}(\text{cis-1,4-DACH})\text{Cl}_2]\) (DACH = diaminocyclohexane) by chlorine gas was investigated in several different solvents.\textsuperscript{273} By NMR spectroscopy, an intermediate corresponding to the addition of one solvent molecule and one chloride ligand, \(\text{fac-[Pt(cis-1,4-DACH)(solv)Cl}_3]\), was detected. The remaining outer-sphere chloride counterion then substitutes the labile solvent molecule, giving the expected tetrachlorido species.\textsuperscript{273,274} Further support for this mechanism comes from the crystal structure of a mixed
trans-chloridoaqua complex obtained by the aqueous chlorine oxidation of a platinum(II) oxazole complex. Some early studies report the observation of a transient red or orange color upon oxidation of cisplatin or [Pt(en)Cl₂] with chlorine. The color then changes to the characteristic yellow of the tetrachloride complexes. In our lab, we observed similar behavior following the oxidation of cisplatin and related platinum(II) complexes with hypervalent iodine reagents. The transient red color has been tentatively proposed to arise from dinuclear metal-metal bonded platinum(III) complexes, some of which are also red and readily form tetrachloroplatinum(IV) complexes by disproportionation. These dinuclear intermediates probably only occur at high platinum concentrations.

The use of coordinating solvents enables isolation of mixed trans oxidative addition products. For example, oxidation of platinum(II) in alcohols with H₂O₂ gives trans hydroxo-alkoxo complexes (Scheme 1.18). An optimized protocol for the synthesis of cisplatin analogues of these species, cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)(OR)], utilizes high dilution conditions in neat alcohol and oxidation with 50% aqueous H₂O₂. The use of 50% rather than 30% H₂O₂ presumably minimizes the amount of water in solution, which can compete with the alcohol for coordination to platinum. Addition of hydrogen peroxide to a platinum(II) complex with a 9-fluorenylidenepropanedioate (fpd) ligand in refluxing methanol afforded a complex with two axial methoxide ligands instead of the expected mixed hydroxo-methoxo complex. The formation and stability of the dimethoxo complex is proposed to arise from an intramolecular stabilizing interaction between the methoxide and fpd ligands, and therefore this reaction is probably not general for most platinum(II) complexes. The use of ethyleneglycol as a solvent for the hydrogen peroxide oxidation of platinum(II) affords a trans hydroxo-ethyleneglycolato complex. The terminal alcohol group of the ethyleneglycolate ligand can
presumably be further functionalized by electrophiles, but such reactions have not yet been investigated.

The hydrogen peroxide oxidation of platinum(II) complexes in the presence of carboxylic acids has also been explored. The oxidation of [Pt(CBDCA)(DPDA)] (DPDA = 2,2-dimethyl-1,3-propanediamine) in acetic acid with hydrogen peroxide gave the unexpected cis diacetate complex, cis-[Pt(CBDCA)(DPDA)(OAc)₂]. In contrast, the use of acetic anhydride mixed with a small amount of acetic acid, present due to hydrolysis of the anhydride, as a solvent selectively afforded the trans diacetate complex, trans-[Pt(CBDCA)(DPDA)(OAc)₂] (Scheme 1.19). The mechanistic details and reasons for the isomeric preferences of these reaction products have not yet been elucidated. The oxidation of the platinum(II) dihydroxo compound, [Pt(DPDA)(OH)₂], with hydrogen peroxide in carboxylic acids at room temperature gave an unexpected species of general formula and stereochemistry, fac-[Pt(DPDA)(OH)(O₂CR)₃] (Scheme 1.19). In our lab, we also explored the oxidation of cisplatin with hydrogen peroxide in neat formic acid. The major product obtained is the diformate complex, cis,cis,trans-[Pt(NH₃)₂Cl₂(O₂CH)₂] (Scheme 1.19).

Recently, the oxidation of oxaliplatin with hydrogen peroxide in the presence of greater than 40 equiv of carboxylic acid in a minimum volume of THF was reported. The major products observed were the monocarboxylato species, trans-[Pt(trans-1,2-DACH)(oxalate)(OH)(O₂CCR)]. When bromoacetic acid was used, however, a large quantity of the dicarboxylato species was obtained. This observation was rationalized based on the lower pKₐ of bromoacetic acid (2.9) compared to those of the other acids screened (≈ 4.8). With its greater acidity, the bromoacetic acid can protonate the second hydroxo ligand and displace it, as discussed below. Unlike hydrogen peroxide oxidations carried out in alcohols where one
alkoxide and hydroxide are added, there is a greater tendency to add at two carboxylate ligands when the oxidation is performed in organic acids. The species initially formed are most likely mixed hydroxo-carboxylato complexes, analogous to the mixed chloride-solvento species observed in chlorine oxidations. The highly acidic conditions, not present in alcohol solutions, lead to protonation and subsequent substitution of the hydroxide ligand by a carboxylate, as discussed below. The relative pKₐ values of the hydroxo ligands and carboxylic acids most likely dictate whether the major product will be the mono or dicarboxylato complex. Another strategy to prepare mixed hydroxo-acetato complexes employs a different oxidant and solvent mixture. Oxaliplatin and trans-[PtLL'C₂] complexes, suspended in a 1:1:1 mixture of DMF, CH₂Cl₂, and acetic acid, can be oxidized with tert-butyl hydroperoxide (in decane) to afford trans-[Pt(trans-1,2-DACH)(oxalate)(OH)(OAc)] and trans,trans,trans-[PtLL'C₂(OH)(OAc)]. The use of entirely non-aqueous solvent and peroxide for these reactions prevent formation of the dihydroxo compound. These complexes can serve as precursors for the synthesis of mixed dicarboxylato species.
Oxidation with hydrogen peroxide can also be used to increase the denticity of a ligand. Platinum(II) complexes with the formulas, [Pt(edma)Cl₂], [Pt(edda)Cl₂], and [Pt(edta)Cl₂] contain one, two, and four uncoordinated carboxylic acid groups, respectively. Upon oxidation of [Pt(edma)Cl₂] with H₂O₂, the carboxylate coordinates and the ligand binds in a facial, tridentate...
manner, forming fac-[Pt(edma)Cl₂(OH)] (Scheme 1.20). Similarly, both carboxylates of [Pt(eddd)Cl₂] coordinate upon oxidation with H₂O₂. For [Pt(edta)Cl₂], only two of the four free carboxylates bind upon oxidation. The remaining two carboxylates can potentially be functionalized by standard amide-bond coupling chemistry, as for the platinum(II) analogues described above. These ring-closing reactions provide a general synthetic route to stable platinum(IV) complexes with multidentate ligands. The design of ligands for platinum(II) complexes with strategically placed donors can facilitate such reactions upon oxidation.

Scheme 1.20. Ring-closing oxidation reactions of [Pt(edma)Cl₂] (top), [Pt(eddd)Cl₂] (middle), and [Pt(edta)Cl₂] (bottom).
Apart from chlorine and hydrogen peroxide, few other oxidants have been explored for the synthesis of platinum(IV) anticancer agents. One such alternative oxidant is the dithiobis(formamidinium) cation. The dichloride salt of the dithiobis(formamidinium) cation oxidized cisplatin, adding a thiourea and a chloride to the axial positions (Scheme 1.2).\textsuperscript{288} The oxidation of transplatin with this reagent afforded initially the all trans isomer, \textit{t},\textit{t},\textit{t}-\([\text{PtCl}_2(\text{NH}_3)_2(\text{tu})\text{Cl}]\), but over time it isomerized to give the same product obtained by the oxidation of cisplatin, \textit{c},\textit{c},\textit{c},\textit{t}-\([\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{tu})\text{Cl}]\).\textsuperscript{289} Notably, the tetrafluoroborate salt of the dithiobis(formamidinium) cation does not give any oxidation products, thus highlighting the important role of the coordinating chloride counterion in facilitating the oxidative addition.\textsuperscript{288,289}

Bromine, like chlorine, also oxidizes diamine platinum(II) complexes, generally forming trans dibromo complexes (Scheme 1.2).\textsuperscript{290} The bromine oxidation of several dichloro platinum(II) complexes, however, failed to give the expected trans dibromo products; instead, a mixture of complexes with different ratios of chloride and bromide ligands was obtained.\textsuperscript{247,291} For diphosphine complexes, this halide scrambling reaction is initiated by light.\textsuperscript{292} Oxidation of cisplatin with \(\text{KMnO}_4\)\textsuperscript{293} or ozone\textsuperscript{294} in water reportedly both lead to the formation of the dihydroxo compound, \textit{c},\textit{c},\textit{c},\textit{t}-\([\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{OH})_2]\), which is also obtained by oxidation with hydrogen peroxide. The use of peroxydisulfate, \(\text{S}_2\text{O}_8^{2-}\), as an oxidant, primarily gives trans hydroxo-sulfato platinum(II) complexes.\textsuperscript{293,295} The reaction of diamine amidate complexes of platinum(II) with \(\text{NaOCl}\) yields the corresponding trans hydroxo-chloro platinum(IV) complexes (Scheme 1.2).\textsuperscript{296} The tetrachloroaurate ion, \([\text{AuCl}_4]^-\), can also oxidize platinum(II). The complex \textit{c}-\([\text{Pt}(\text{NH}_3)_2(1\text{-methylthymine})_2]\) was oxidized by \(\text{NaAuCl}_4\) to afford \textit{c},\textit{c},\textit{t}-\([\text{Pt}(\text{NH}_3)_2(1\text{-methylthymine})_2(\text{OH})(\text{OH}_2)]\).\textsuperscript{297} The addition of two ligands originating from water was unexpected and rationalized on the basis of steric crowding at the axial sites by the 1-
methylthymine ligands. In contrast, the oxidation of a platinum(II) terpyridine complex by 
$\text{AuCl}_4^-$ added two chloride ligands to the resulting platinum(IV) coordination sphere.\textsuperscript{298} Nitrogen
dioxide gas can also oxidize cisplatin in an aqueous solution containing one equiv of KCl
(Scheme 1.21).\textsuperscript{299} The product, $[\text{PtCl}_3(\text{NO}_2)(\text{NH}_3)]$, which could not be isolated as analytically
pure material, comprised primarily the facial isomer, as determined by NMR spectroscopy.
Analysis by X-ray diffraction revealed a disordered mixture of facial and meridional isomers.\textsuperscript{299}
Potassium dichromate, $\text{K}_2\text{Cr}_2\text{O}_7$, and potassium chlorochromate, $\text{KCrO}_3\text{Cl}$, can also oxidize
cisplatin.\textsuperscript{300} On the basis of spectroscopic data, the products were proposed to be a dimetallic
heteronuclear Pt–Cr complex and a trimetallic Pt–Cr\textsubscript{2} complex, respectively, with oxo ligands
bridging the metal centers (Scheme 1.21).\textsuperscript{300} Similarly, cisplatin was oxidized by iron(III)
complexes of the general formula, $\text{cis-}[\text{Fe(bpy')}_2(\text{CN})_2](\text{NO}_3)$ where bpy' is derivative of 2,2'-
bipyridine, to form trinuclear cyanide-bridged complexes (Scheme 1.21).\textsuperscript{301,302} The
photochemistry of these complexes was explored, and in some cases the release of aquated
cisplatin as a photoproduct occurred,\textsuperscript{301,302} thus signifying the potential use of such complexes as
photoactivated anticancer agents.
Hypervalent iodine species are a class of powerful oxidizing agents with utility in organic synthesis. Iodobenzene dichloride (PhICl₂), which can be isolated as a crystalline solid, acts as
an easy-to-handle surrogate for chlorine gas. It efficiently converts cisplatin and related diaminedichloridoplatinum(II) complexes to their corresponding tetrachloridoplatinum(IV) analogues (Scheme 1.22).^246,247 A recent report describes the action of PhICl$_2$ on an organoamide platinum(II) complex in a mixture of acetone and basic water.\[304\] A mixed trans hydroxo-chlorido platinum(IV) complex is obtained (Scheme 1.22),\[304\] consistent with a solvent-assisted mechanism, similar to that observed for oxidations by chlorine and hydrogen peroxide.

The oxidations of cis- and trans-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$], where NH$_2$Cy is cyclohexylamine, with PhI(OAc)$_2$ in dichloromethane have been investigated.\[305\] The major products derive from the oxidative addition of two acetate ligands in a cis orientation. For cis-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$], the major product is cis,cis,cis-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$(OAc)$_2$], whereas, when the starting material is trans-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$], the major product is cis,trans,cis-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$(OAc)$_2$] (Scheme 1.22). In addition to the major product, minor products mer-[Pt(NH$_3$)(NH$_2$Cy)Cl(OAc)$_3$] and fac-[Pt(NH$_3$)(NH$_2$Cy)(OAc)Cl$_3$] also form upon PhI(OAc)$_2$ oxidation of cis-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$], indicative of intermolecular ligand substitution reactions. The apparent limitations of using PhI(OAc)$_2$ in contrast to PhICl$_2$ are the formation of products without predictable stereochemistry and unexpected ligand stoichiometries. Given the large number of hypervalent iodine complexes in the literature, further investigations of their reactivity with platinum(II) complexes is certainly warranted, for they may lead to new, valuable synthetic routes to novel platinum(IV) complexes.
Outer-Sphere Ligand-Based Reactivity. Outer-sphere ligand-based reactivity pathways for the preparation of new platinum(IV) complexes is a valuable synthetic route because their kinetic inertness limits the utility of ligand substitution reactions. A reaction of key importance in this regard is that of Pt(IV)-hydroxo compounds with electrophiles. The coordinated hydroxide ligand of platinum(IV) complexes is sufficiently nucleophilic to enable such transformations. The acetylation of \( \text{cis,cis,trans-}[Pt(\text{PrNH}_2)_2\text{Cl}_2(\text{OH})_2] \) with trifluoroacetic anhydride to form \( \text{cis,cis,trans-}[Pt(\text{PrNH}_2)_2\text{Cl}_2(\text{O}_2\text{CCF}_3)_2] \) was first demonstrated in 1983.\(^{306}\) This chemistry was
further expanded to include a broader range of acid anhydrides, pyrocarbonates, and isocyanates as electrophiles to afford dicarboxylate, dicarbonate, and dicarbamate platinum(IV) complexes, respectively. In all cases, retention of the stereochemistry of the starting dihydroxo platinum(IV) compound is observed. For acetylation of trans,trans,trans-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$(OH)$_2$] with acetic anhydride, however, light-induced isomerization of the initial product, trans,trans,trans-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$(OAc)$_2$], to trans,cis,cis-[Pt(NH$_3$)(NH$_2$Cy)Cl$_2$(OAc)$_2$] occurred.

Since the initial report, a wide variety of trans platinum(IV) dicarboxylate complexes have been synthesized in this manner. The carboxylate ligands have significant effects on both the lipophilicity and reduction potentials of the resulting platinum(IV) complexes. Furthermore, the ubiquitous nature of the carboxylate functional group in many different organic molecules enabled the synthesis of platinum(IV) complexes bearing biologically active ligands, attached via the carboxylate. Different protocols have called for the use of excess anhydride, either neat, or together with acetone, dichloromethane, acetonitrile or DMSO as the solvent (Scheme 1.23). Similarly, tetracarboxylato platinum(IV) complexes, some of which exhibit anticancer activity when administered orally, can be synthesized from cis-[PtL$_2$(OH)$_4$] and excess anhydride in dichloromethane (Scheme 1.23). This reaction demonstrates that equatorial cis hydroxide ligands also display nucleophilic properties. Acyl chlorides also react with trans dihydroxo platinum(IV) complexes to form dicarboxylates. A difficulty in this reaction, noted early on, is the formation of hydrochloric acid as a byproduct, which can remove the hydroxo ligands of platinum(IV) by protonation. Optimized reaction conditions utilize refluxing acetone as a solvent and an excess of pyridine as a base to...
sequester the HCl that is formed (Scheme 1.23). Aromatic carboxylate ligands can be
installed on platinum(IV) with this method as well.

A third route to trans platinum(IV) dicarboxylates utilizes the ring opening reactions of
the platinum(IV) hydroxo complexes with cyclic anhydrides. Succinic, maleic, glutaric,
phthalic, and naphthalic anhydrides have all been used in this reaction, together with the
traditional Chinese medicine cantharidin. Early protocols for this reaction involved
the treatment of a platinum(IV) hydroxo compound with the cyclic anhydride in
refluxing dichloromethane for two days. Currently, the most commonly used method
utilizes either DMF or DMSO as solvent at 50–80 °C for 6–24 h (Scheme 1.23). Activating
carboxylic acids with an appropriate coupling reagent has also been an effective means of
forming trans dicarboxylate complexes. For example, the reaction of a platinum(IV) analogue of
oxaliplatin, trans-[Pt(trans-1,2-DACH)(oxalate)(OH)₂], with 3 equiv of carboxylic acid,
triethylamine (TEA), and the coupling reagent O-benzotriazol-1-yl-N,N,N',N'-
tetramethyluronium tetrafluoroborate (TBTU) readily yields the dicarboxylato complex, trans-
[Pt(trans-1,2-DACH)(oxalate)(O₂CR)₂] (Scheme 1.23). This one pot procedure may provide a
useful alternative to the abovementioned methods because it avoids the need to prepare
anhydrides or acyl chloride as intermediates.
Scheme 1.23. Synthetic methods for the preparation of platinum(IV) carboxylates.
The synthesis of mixed platinum(IV) carboxylate complexes can introduce greater molecular complexity and provide a means of installing different functional or targeting groups. The reaction of a platinum(IV) dihydroxo complex with a mixture of different anhydrides gives rise to a statistical mixture of symmetric and asymmetric platinum(IV) dicarboxylates that can be separated by silica gel chromatography. Similarly, mixed tetracarboxylates were synthesized by the reaction of a platinum(IV) tetrahydroxo complex with different ratios of anhydrides. The resulting products required purification by either silica gel chromatography or preparative reverse-phase HPLC. Another, more elegant pathway to mixed platinum(IV) dicarboxylates requires the isolation of a trans mixed hydroxo-carboxylato platinum(IV) complex. The remaining hydroxo ligand could then be derivatized with a different carboxylate ligand. The synthesis of several mixed hydroxo-carboxylato complexes by the peroxide oxidation of platinum(II) in the presence of an excess of carboxylic acid was described above, as shown in Scheme 1.19. These complexes can react with another acid anhydride to yield a mixed-dicarboxylate complex. The selective acetylation of a single hydroxo ligand is another potential strategy. One way to accomplish this selective reaction is with steric control. Initially, the acetylation of a platinum(IV) analogues of sterically hindered picoplatin, cis,cis,trans-[Pt(NH\textsubscript{3})(2-pic)Cl\textsubscript{2}(OH)\textsubscript{2}], with acetic anhydride was reported to be unsuccessful because the ortho methyl group of the picoline ligand impedes the hydroxo nucleophilic attack. It was later shown, however, that the acetylation of this complex can be successfully executed with a number of different anhydrides to give symmetric dicarboxylate complexes. Thus the steric effects of the 2-picoline ligand did not successfully favor formation of the monohydroxo complex. The platinum(IV) complex cis,cis,trans-[Pt(en')Cl\textsubscript{2}(OH)\textsubscript{2}], where en' is N,N-dimethylethlenediamine, is selectively acetylated at only one of the hydroxo ligands in the
presence of excess succinic anhydride (Scheme 1.24). The inability to form the dicarboxylate was attributed to steric repulsion induced by the additional methyl groups on the ethylenediamine ligand. Although this reaction demonstrates that steric effects can be used to selectively obtain mixed hydroxo-carboxylato complexes, it is unlikely, because of those same steric effects, that this complex could be further acetylated with an additional anhydride to form a mixed dicarboxylate complex. The isolation of other mixed hydroxo-carboxylato platinum(IV) complexes as synths for mixed dicarboxylates was described recently. These complexes were obtained by careful control of the reaction conditions. The room temperature reactions of platinum(IV) dihydroxo complexes with an acid anhydride in DMSO were used to increase the yield of the desired monocarboxylate complexes with respect to that of the dicarboxylate species (Scheme 1.24). These reaction conditions were employed to prepare complexes with axial aromatic carboxylates and succinate. In general, the desired mixed hydroxo-carboxylato complexes are insoluble in acetone, thereby enabling the undesired dicarboxylate complexes to be removed by dissolution in this solvent. An alternative procedure utilizes a carboxylic acid preactivated by N,N'-dicyclohexylcarbodiimide (DCC). The one pot reaction of a trans-[Pt(trans-1,2-DACH)(oxalate)(OH)\textsubscript{2}] with only slightly greater than one equiv of DCC and a carboxylic acid in DMF at rt preferentially gave the monocarboxylato species (Scheme 1.24).
In addition to acid anhydrides and acid chlorides, several other electrophiles react with platinum(IV) hydroxo complexes. The reactivity of platinum(IV) hydroxides with pyrocarbonates and isocyanates to form platinum(IV) carbonates and carbamates has been known for over 15 years.\textsuperscript{128} The reactions of cis,cis,trans-[Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(OH)\textsubscript{2}] with both alkyl and aromatic isocyanates were recently investigated in detail,\textsuperscript{333} as described in Chapter 6. The optimal conditions utilized 4 equiv of the isocyanate and DMF as the solvent at room temperature for several hours (Scheme 1.25). The action of trimethylsilyl chloride (TMSCl) on the tetrahydroxo complex, cis-[Pt(DPDA)(OH)\textsubscript{4}] has also been explored.\textsuperscript{320} At room temperature in THF with TEA as a base, the main reaction product is the trans disiloxide complex, cis,trans,cis-[Pt(DPDA)(OSiMe\textsubscript{3})\textsubscript{2}(OH)Cl], whereas refluxing conditions afforded cis,trans,cis-
The substitution of the hydroxide ligands most likely arises from the HCl byproduct, which acts as the source of protons to labilize the hydroxide ligands and as the source of the nucleophilic chloride ligands.

Scheme 1.25. Reactivity of platinum(IV) hydroxides with electrophiles.\cite{ref1, ref2, ref3}

The additional axial ligands available in platinum(IV) but not platinum(II) complexes can be selected for introducing reactive organic functionalities. For example, the ring-opening
reaction of platinum(IV) hydroxides with cyclic acid anhydrides yields platinum(IV) complexes with free terminal carboxylic acids. The terminal carboxylic acids can undergo amide and ester coupling reactions with the octahedral platinum(IV) center remaining intact (Scheme 1.26). These reactions have been used to covalently modify platinum(IV) complexes with estrogen, peptides, and nano-delivery devices. Additionally, a wide variety of amines and alcohols have been coupled to such platinum(IV) complexes in order to systematically adjust their lipophilicities. For coupling reactions carried out in DMF, N,N'-diisopropylcarbodiimide (DIPC), O-(7-azabenzotriazol-1-yl)-N,N,N',N-tetramethyluronium hexafluorophosphate (HATU), and 1,1'-carbonyldiimidazole (CDI) were all used with success, whereas for aqueous coupling reactions the combination of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) works well. Two platinum(IV) complexes with axial ligands containing aldehyde and ketone functional groups have also been reported recently. The reaction of these functional groups with organic hydrazines and hydroxylamines leads to the formation of hydrazones and oximes, providing a new convenient method to conjugate platinum(IV) to different units (Scheme 1.26). This strategy has already been employed to attach platinum(IV) to a short peptide and a polymeric nanoparticle. A platinum(IV) complex containing an axial carboxylate ligand with a pendant azide has also recently been described. In the presence of Cul, the azide ligand reacts with an alkyne to form the expected triazole, and the intact platinum(IV) complex can be isolated by preparative HPLC in approximately 50% yield (Scheme 1.26). A possible limitation of this reaction for use in the preparation of other platinum(IV) complexes could be undesired reduction of the platinum(IV) center by the Cu(I) ion. In another recent report, a thiol-reactive maleimide
functional group was installed in the axial positions of two platinum(IV) complexes via carbamate linkages. The maleimide was used to attach the platinum(IV) complex to human serum albumin at its single exposed cysteine residue (Scheme 1.26).

Scheme 1.26. Outer-sphere reactivity of platinum(IV) complexes with organic functional groups.
**Ligand Substitution Reactions.** Because of the inert nature of platinum(IV) complexes, direct ligand substitution reactions are generally very slow and require harsh conditions. As a result, such reactions are rarely employed to prepare anticancer platinum(IV) prodrugs. Complexes of the type \([\text{Pt}(L_2)\text{Cl}_4]\) where \(L_2\) is a chelating diamine ligand, however, can be synthesized by the reaction of the \([\text{PtCl}_6]^{2-}\) anion with \(L_2\) in water, usually under refluxing conditions.\(^{354-358}\) For more complicated structures, different strategies can be utilized to facilitate ligand substitution of platinum(IV), as discussed below.

Hydroxide ligands bound to platinum(IV) complexes can be substituted under acidic conditions. A popular synthetic route to \(\text{cis}-[\text{Pt(NH}_3)_2\text{Cl}_4]\) and other diamine tetrachlorido complexes utilizes \(\text{cis,cis,trans-[Pt(NH}_3)_2\text{Cl}_2(OH)_2}\) as a platinum(IV) starting material. Treatment of this compound with hydrochloric acid affords the tetrachloride (Scheme 1.27).\(^{307}\) This reaction most likely proceeds via protonation of the hydroxo ligands, converting them to labile water ligands, followed by their displacement by the nucleophilic chloride ion. This reaction also works for hydrobromic acid to substitute bromide for hydroxide ligands.\(^{359}\) When hydrochloric acid is added to the tetrahydroxo compound, \([\text{Pt(trans-1,2-DACH)}(\text{OH})_4]\), all four hydroxides are replaced by chloride ligands.\(^{360}\) In contrast, suspending \([\text{Pt(trans-1,2-DACH)}(\text{OH})_4]\) in neat carboxylic acid affords the trisubstituted species, \(\text{fac-[Pt(trans-1,2-DACH)}(\text{OH})(\text{O}_2\text{CR})_3]\) (Scheme 1.27).\(^{360}\) It is hypothesized by the authors of these studies that the \(pK_a\) of the last hydroxide ligand is too low to be protonated by a carboxylic acid (\(pK_a \approx 5\)), but not lower than that of the stronger acid HCl. This hydroxo ligand, however, still retains its nucleophilic character as it can be acetylated with other carboxylic anhydrides to rationally make mixed carboxylate complexes.\(^{361}\) When trifluoroacetic anhydride is added to these tricarboxylate complexes in the absence of a base, the unexpected product \(\text{cis,cis,cis-[Pt(trans-1,2-DACH)}(\text{OH})(\text{O}_2\text{CR})_3]\)
DACH)(O₂CR)₂(O₂CCF₃)₂ is obtained (Scheme 1.27).³⁶² The hydroxide is most likely initially acetylated by trifluoroacetic anhydride, releasing the strong acid, trifluoroacetic acid, as a byproduct. Trifluoroacetic acid can then protonate and release a coordinated carboxylate ligand and bind to the platinum(IV) center. Consistent with this hypothesis is the observation that adding a base with the trifluoroacetic anhydride gives rise only to the expected complex, fac-[Pt(trans-1,2-DACH)(O₂CCF₃)(O₂CR)₃], presumably by neutralizing the trifluoroacetic acid byproduct.³⁶²

Just as acidic conditions favor ligand substitution reactions in platinum(IV) complexes by protonolysis, basic conditions can also induce ligand substitution, albeit by a different mechanism. Under basic conditions, platinum amine complexes can undergo ligand substitution via the base hydrolysis mechanism.³⁶³-³⁶⁵ In general, these reactions result in the net substitution
of a chloride by a solvent ligand. The first step is the deprotonation of an amine ligand to form an amido ligand. The high trans effect of the amido ligand favors dissociation of a trans chloride ligand, yielding a five-coordinate intermediate. The addition of a solvent molecule to this five-coordinate intermediate results in the observed product. In the realm of platinum-based anticancer agents, this reaction mechanism has been proposed, albeit without supporting kinetic data, for the formation of several new derivatives of satraplatin.\textsuperscript{305,366} The reaction of \textit{cis,cis,trans-}[Pt(NH\textsubscript{3})(NH\textsubscript{2}Cy)Cl\textsubscript{2}(O\textsubscript{2}CC\textsubscript{3}H\textsubscript{7})\textsubscript{2}] with sodium methoxide in methanol resulted in substitution of the chloride trans to the cyclohexylamine for a methoxide ligand, forming \textit{cis,cis,trans-}[Pt(NH\textsubscript{3})(NH\textsubscript{2}Cy)Cl(OMe)(O\textsubscript{2}CC\textsubscript{3}H\textsubscript{7})\textsubscript{2}] (Scheme 1.28).\textsuperscript{366} Similarly, in basic water \textit{cis,cis,trans-}[Pt(NH\textsubscript{3})(NH\textsubscript{2}Cy)Cl\textsubscript{2}(O\textsubscript{2}CCH\textsubscript{3})\textsubscript{2}] converts to the monohydroxide complex, \textit{cis,cis,trans-}[Pt(NH\textsubscript{3})(NH\textsubscript{2}Cy)Cl(OH)(O\textsubscript{2}CCH\textsubscript{3})\textsubscript{2}], where the hydroxide ligand is also trans to the cyclohexylamine (Scheme 1.28).\textsuperscript{305} In DMA containing 2 M LiCl, the addition of TEA as a base to \textit{cis-[Pt(NH\textsubscript{3})(NH\textsubscript{2}Cy)(OAc)\textsubscript{4}]} afforded the monochloro complex \textit{mer-[Pt(NH\textsubscript{3})(NH\textsubscript{2}Cy)Cl(OAc)\textsubscript{3}]} (Scheme 1.28).\textsuperscript{305} In this case, the base hydrolysis route was able to substitute an acetate ligand rather than a chloride. This reaction also demonstrates that, if a non-coordinating solvent is used, other ligands besides solvent can be added to the platinum(IV) complex.
Scheme 1.28. Ligand substitution reactions facilitated by basic conditions, presumably through a base hydrolysis mechanism.

1.4. Concluding Remarks

The chemistry of cisplatin and its isomers was first explored over one hundred years ago. The discovery of its anticancer properties in 1969 motivated further exploration into its coordination chemistry and that of related species, with the ultimate goal of finding new complexes with improved therapeutic properties. The development of new synthetic methodologies can give access to new platinum complexes with novel structures and possibly novel modes of biological activity. This chapter summarizes the known reactivity patterns and synthetic strategies for a range of platinum anticancer complexes. Notably, much of this chemistry has only been developed within the last 20 years. Thus, as long as the need for new platinum anticancer agents persists, new chemistry will be developed and investigated in order to
obtain such compounds. The following chapters in this thesis address these needs by surveying new synthetic methods for the preparation of novel platinum anticancer complexes.

1.5. References

Kawai, K.; Tanaka, Y.; Nakano, Y.; Ehrlich, W.; Akaboshi, M.


97


Chapter 2

Platinum(II) Complexes Bearing Fluorescent Di-2-Pyridylmethane Ligands

2.1. Introduction

There is still much uncertainty regarding the mechanism of uptake and cellular
distribution of cisplatin and related platinum-based anticancer drug candidates.\textsuperscript{1-3} Reliable
methods to image cisplatin in living cells will most likely aid in the understanding of these
processes. Synchrotron x-ray spectroscopic techniques have been used to visualize cisplatin and
its derivatives in cells.\textsuperscript{4-8} These methods, however, are limited
by the availability of synchrotron radiation and the resolution of the images that they produce. An alternative strategy is the use of
fluorescence microscopy, a relatively non-invasive technique, which can be used to image living
cells with high resolution.\textsuperscript{9} Unfortunately, most clinically relevant platinum anticancer agents are
not intrinsically fluorescent, thereby preventing use of this technique.

To make fluorescence microscopy a viable method for investigating platinum anticancer
agents, many researchers have attached fluorescent ligands to platinum complexes that are
structurally similar to the established drugs.\textsuperscript{10-12} A potential problem with this strategy, however,
is that the fluorophore rather than the platinum ion may dictate the localization and uptake
properties of the complex, as has been observed for other metal-fluorophore conjugates.\textsuperscript{13} The
design of entirely new platinum structural motifs that are both cytotoxic and fluorescent is
another area of research.\textsuperscript{14-19} As new drug entities, these types of complexes can be imaged
without the concern of altered localization properties induced by an external fluorophore.

In this chapter, we report the preparation of three new ligands based on di-2-
pyridylmethane. Two of these ligands contain the fluorescent dansyl and 7-nitro-1,2,3-
benzoxadiazole (NBD) units. The initial goal of this research was to prepare new anticancer
platinum complexes with intrinsic fluorescent properties. As demonstrated here, however, the
resulting platinum(II) dichloride complexes of these ligands (1–3, Chart 2.1) are not soluble in
water, are thermally and photolytically unstable, and poorly emissive. In spite of these shortcomings, full synthetic details and characterization of these complexes is reported in this chapter. Additionally, some preliminary studies involving the oxidation of these complexes are described as well.

Chart 2.1. Structures of \([\text{Pt(Ts-dpm)Cl}_2]\) (1), \([\text{Pt(Ds-dpm)Cl}_2]\) (2), and \([\text{Pt(NBD-dpm)Cl}_2]\) (3), the syntheses and characterizations of which are presented in this chapter.

2.2. Experimental Methods

General Considerations. Di-2-pyridylmethanamine was prepared from commercially available di-2-pyridyl ketone by a reported method.\(^{20}\) \(\text{K}_2\text{PtCl}_4\), purchased from Strem Chemicals, was used to prepare \(\text{cis-}[\text{Pt(DMSO)}_2\text{Cl}_2]\).\(^{21}\) \([\text{Pt(dpk)}\text{Cl}_2]\)^{22} and 4-amino-7-nitro-2,1,3-benzoxadiazole (NBD-NH\(_2\))\(^{23}\) were also prepared by literature procedures. Other reagents were used as received from commercial vendors. Methanol (MeOH) and acetonitrile (CH\(_3\)CN) were used without prior degassing or drying. Tetrahydrofuran (THF) was saturated with argon and purified by passage over two columns of Al\(_2\)O\(_3\) prior to use.

Physical Measurements. NMR measurements were recorded on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility. \(^1\text{H}\) and \(^{13}\text{C}\) NMR
spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane (SiMe$_4$; $\delta = 0$ ppm). $^{195}$Pt NMR spectra were referenced externally using a standard of $\text{K}_2\text{PtCl}_4$ in D$_2$O ($\delta = -1628$ ppm). Optical absorption spectra were recorded with a Cary 1E spectrophotometer. Emission spectra were obtained with a Photon Technology International QM-4/2003 fluorimeter. Quantum yields for emission were measured using either fluorescein in 0.1 M NaOH ($\Phi = 0.95$)$^{24}$ for NBD-containing compounds or quinine sulfate in 0.1 M H$_2$SO$_4$ ($\Phi=0.58$)$^{25}$ for dansyl-containing compounds as references and by exciting samples at their wavelengths of maximum absorbance. Electrospray ionization mass spectrometry (ESI-MS) measurements were acquired on an Agilent Technologies 1100 series LC-MSD trap. Elemental analyses were carried out by a commercial analytical laboratory.

**Synthesis of N-(Di-2-pyridylmethyl)tosylamide (Ts-dpm).** To a mixture of di-2-pyridylmethanamine (0.706 g, 3.80 mmol) and Na$_2$CO$_3$ (1.21 g, 11.4 mmol) in 25 mL of CH$_3$CN was added dropwise a solution of tosyl chloride (0.703 g, 3.70 mmol) in 5 mL of CH$_3$CN. The resulting mixture was stirred at room temperature for 12 h and then filtered. The colorless filtrate was concentrated in vacuo to a volume of approximately 10 mL, at which point Ts-dpm separated from the solution as a white crystalline solid. This solid was collected by vacuum filtration. An additional crop was collected by further concentration of the filtrate to yield 0.551 g (44%) of Ts-dpm. Mp: 143–144 °C. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 8.41 (2H, d, $J = 4.7$ Hz), 7.59 (2H, d, $J = 8.0$ Hz), 7.50 (2H, t, $J = 7.6$ Hz), 7.28 (3H, m), 7.08 (4H, m), 5.68 (1H, d, $J = 5.8$ Hz), 2.28 (3H, s). $^{13}$C{$^1$H} NMR (100 MHz, CDCl$_3$): $\delta$ (ppm) 158.1, 148.9, 143.2, 137.1, 137.0, 129.5, 127.4, 122.8, 122.3, 62.1, 21.6. ESI-MS: $m/z$ 340.0 ([M+H]$^+$, calcd. 340.1), 338.0 ([M–H]$^-$, calcd. 338.1). Anal. Calcd. for Ts-dpm, C$_{18}$H$_{17}$N$_3$O$_2$S: C, 63.70; H, 5.05; N, 12.38. Found: C, 63.71; H, 5.06; N, 12.55.
Synthesis of \(N\)-(Di-2-pyridylmethyl)danslyamide (Ds-dpm). To a mixture of dansyl chloride (3.60 g, 13.4 mmol) and Na\(_2\)CO\(_3\) (7.10 g, 67.0 mmol) in 20 mL of CH\(_3\)CN was added dropwise di-2-pyridylmethanamine (2.75 g, 14.8 mmol) in 5 mL of CH\(_3\)CN. The resulting mixture was stirred at room temperature for 12 h and then filtered. The orange filtrate was concentrated to dryness under vacuum to give a thick oil, which was purified by silica gel column chromatography, eluting with 9:1 CH\(_2\)Cl\(_2\)/MeOH. After chromatography, the product was initially obtained as a thick yellow-green oil. Upon recrystallization with 1:1 MeOH/\(\text{Et}_2\)O, pure Ds-dpm was obtained as a pale-yellow crystalline solid (4.20 g, 75%). Mp: 101-104 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ (ppm) 8.34 (1H, dt, \(J=\) 8.7 and 0.8 Hz), 8.30 (1H, dt, \(J=\) 8.4 and 1.0 Hz), 8.23 (2H, m), 8.12 (1H, dd, \(J=\) 7.3 and 1.3 Hz), 7.50 (1H, app t), 7.40 (1H, d, \(J=\) 6.5 Hz), 7.34-7.30 (3H, m), 7.11 (2H, d, \(J=\) 7.2 Hz), 7.07 (1H, dd, \(J=\) 7.32 and 0.8 Hz), 6.93-6.90 (2H, m), 5.57 (1H, d, \(J=\) 6.5 Hz), 2.78 (6H, s). \(^{13}\)C \(^1\)H) NMR (100 MHz, CDCl\(_3\)): δ (ppm) 157.8, 151.7, 148.7, 136.6, 134.9, 130.3, 130.0, 129.64, 129.62, 128.3, 123.1, 122.5, 122.0, 119.5, 115.1, 62.5, 45.6. ESI-MS: \(m/z\) 419.1 ([M+H]\(^+\), calcd. 419.2); 441.0 ([M+Na]\(^+\), calcd. 441.1); 859.2 ([2M+Na]\(^+\), calcd. 859.3). Anal. Calcd. for Ds-dpm, C\(_{23}\)H\(_{22}\)N\(_4\)O\(_2\)S: C, 66.01; H, 5.30; N, 13.39. Found: C, 65.80; H, 5.16; N, 13.44.

Synthesis of \(N\)-(Di-2-pyridylmethyl)-7-nitro-2,1,3-benzoxadiazole-4-amine (NBD-dpm). To a mixture of di-2-pyridylmethanamine (0.802 g, 4.33 mmol) and K\(_2\)CO\(_3\) (3.00 g, 22.0 mmol) in 15 mL of THF was added dropwise a 10 mL THF solution of 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl; 0.900 g, 4.50 mmol). The mixture was stirred at room temperature for 12 h and then filtered. The dark-brown filtrate was concentrated to dryness in vacuo, and the resulting residue was partitioned between 50 mL of CH\(_2\)Cl\(_2\) and 50 mL of 1 M HCl. The organic layer was separated, and the aqueous layer was further extracted with two 25-mL portions of
CH$_2$Cl$_2$. The organics were combined, washed with 50 mL of saturated NaCl, and dried with MgSO$_4$. Pure NBD-dpm was obtained after silica gel chromatography (5% MeOH in CH$_2$Cl$_2$) as a brown solid (0.714 g, 46%). Mp: 188–192 °C (dec). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 9.15 (1H, bs), 8.65 (2H, d, $J = 4.8$ Hz), 8.35 (1H, d, $J = 8.6$ Hz), 7.65 (2H, t, $J = 7.7$ Hz), 7.40 (2H, d, $J = 7.9$ Hz), 7.26 (2H, t, $J = 6.1$ Hz, overlaps with residual CHCl$_3$ peak), 6.13 (1H, d, $J = 8.6$ Hz), 6.02 (1H, bs). $^{13}$C{H} NMR (100 MHz, CDCl$_3$): $\delta$ (ppm) 157.1, 149.4, 144.8, 144.1, 142.5, 137.9, 136.5, 124.7, 123.7, 122.0, 100.9, 63.2. ESI-MS: $m/z$ 349.1 ([M+H]$^+$, calcd. 349.1); 370.9 ([M+Na]$^+$, calcd. 371.2). Anal. Calcd. for NBD-dpm, C$_{17}$H$_{12}$N$_6$O$_3$: C, 58.62; H, 3.47; N, 24.13. Found: C, 58.13; H, 3.44; N, 24.02.

Synthesis of [Pt(Ts-dpm)Cl$_2$] (1). Ts-dpm (0.270 g, 0.800 mmol) in 10 mL of MeOH was added to a suspension of cis-[Pt(DMSO)$_2$Cl$_2$] (0.330 g, 0.780 mmol) in 10 mL of MeOH. After stirring for 12 h at room temperature, the reaction mixture was filtered to collect 1 as a white solid. The solid was washed sequentially with 10 mL of MeOH and 10 mL of Et$_2$O before being dried in vacuo to obtain 0.417 g (88%) of 1. $^1$H NMR (400 MHz, DMF-$d_7$), endo conformer: $\delta$ (ppm) 9.54 (1H, d, NH), 9.23 (2H, d), 8.13 (2H, t), 7.90 (2H, d), 7.60 (2H, t, overlapped by exo conformer peaks), 7.78 (2H, d), 7.34 (2H, d, overlapped by exo conformer peaks), 6.15 (1H, d, py$_2$CHNHR), 2.39 (3H, s, CH$_3$); exo conformer: $\delta$ (ppm) 10.15 (1H, d, NH), 9.13 (2H, d), 8.19 (2H, t), 7.87-7.84 (4H, m), 7.60 (2H, t), 7.36 (2H, d), 6.79 (1H, d, py$_2$CHNHR), 2.34 (3H, s, CH$_3$). $^{195}$Pt NMR (86 MHz, DMF-$d_7$): $\delta$ (ppm) −2082 (endo), −2198 (exo). ESI-MS: $m/z$ 603.9 ([M−H]$^-$, calcd. 604.0). Anal. Calcd. for 1, C$_{18}$H$_{17}$Cl$_2$N$_3$O$_2$SPt: C, 35.71; H, 2.83; N, 6.94. Found: C, 35.71; H, 2.87; N, 6.96.

Synthesis of [Pt(Ds-dpm)Cl$_2$] (2). By following the procedure described for 1 using 1.14 g (2.69 mmol) of cis-[Pt(DMSO)$_2$Cl$_2$] and 1.50 g (3.58 mmol) of Ds-dpm, 2 was obtained as a
yellow solid (1.75 g, 95%). \(^1\)H NMR (400 MHz, DMF-\(d_7\)), exo + endo: \(\delta\) (ppm) 10.40 (d, \(NH\), exo), 9.65 (d, \(NH\), endo), 9.14 (d, o-py, endo), 9.08 (d, o-py, exo), 8.62 (d, exo), 8.53 (d, endo), 8.47 (d, exo), 8.36 (d, exo), 8.20 (d, endo), 8.02–7.98 (m, exo + endo), 7.75–7.48 (m, exo + endo), 7.29 (d, exo), 7.18 (d, endo), 6.82 (d, \(\text{py}_2\text{CHNHR}\), exo), 6.30 (d, \(\text{py}_2\text{CHNHR}\), endo), 2.85 (s, N(CH\(_3\))\(_2\), endo), 2.83 (s, N(CH\(_3\))\(_2\), exo). \(^{195}\) Pt NMR (86 MHz, DMF-\(d_7\)): \(\delta\) (ppm) –2105 (endo), –2199 (exo). ESI-MS: \(m/z\) 682.8 ([M–H]\(^–\), calcd. 683.0). Anal. Calcd. for 2, C\(_{23}\)H\(_{22}\)Cl\(_2\)N\(_4\)O\(_2\)SPt: C, 40.36; H, 3.24; N, 8.19. Found: C, 40.40; H, 3.36; N, 8.30.

**Synthesis of [Pt(NBD-dpm)Cl\(_2\)] (3).** By following the procedure described for 1 using 0.106 g (0.250 mmol) of cis-[Pt(DMSO)\(_2\)Cl\(_2\)] and 0.100 g (0.290 mmol) of NBD-dpm, 3 was obtained as an orange-brown solid (0.109 g, 71%). \(^1\)H NMR (400 MHz, DMF-\(d_7\)), endo conformer: \(\delta\) (ppm) 10.40 (1H, d, \(NH\)), 9.31 (2H, d), 8.63 (1H, d), 8.40–8.35 (4H, m), 7.73 (2H, t), 7.17 (1H, d, \(\text{py}_2\text{CHNHR}\)), 7.00 (1H, d); exo conformer: \(\delta\) (ppm) 10.44 (1H, d, \(NH\)), 9.28 (2H, d), 8.77 (1H, d), 8.31 (2H, t), 8.18 (2H, d), 7.70 (2H, t), 7.60 (1H, br, \(\text{py}_2\text{CHNHR}\)), 7.13 (1H, d). \(^{195}\) Pt NMR (86 MHz, DMF-\(d_7\)): \(\delta\) (ppm) –2057 (endo), –2196 (exo). ESI-MS: \(m/z\) 612.8 ([M–\(\text{H}\)]\(^–\), calcd. 613.0). Anal. Calcd. for 3, C\(_{17}\)H\(_{12}\)Cl\(_2\)N\(_6\)O\(_3\)Pt: C, 33.24; H, 1.97; N, 13.68. Found: C, 33.28; H, 2.04; N, 13.44.

**Preliminary Oxidation Chemistry of 1.** To a suspension of 1 (0.050 g, 0.083 mmol) in 10 mL of glacial acetic acid was added 30% \(\text{H}_2\text{O}_2\) (66 \(\mu\)L, 0.58 mmol). The resulting suspension was stirred at room temperature for 12 h and then filtered to collect 0.029 g of an off-white solid, which was washed with 10 mL of Et\(_2\)O. The \(^1\)H NMR spectrum of this material freshly dissolved in DMSO-\(d_6\) revealed the presence of a single compound formulated to be the Cs-symmetric meso-[Pt(\(\kappa^3\)-Ts-dpm)Cl\(_2\)(OH)], a result inconsistent with the lack of symmetry observed in the
isolated crystal, $I_{\text{o}}$. After several days standing in solution, the $^1$H NMR spectrum revealed the presence of at least two different compounds.

**Preliminary Oxidation Chemistry of 3.** To an orange suspension of 3 (0.020 g, 0.043 mmol) in 5 mL of glacial acetic acid was added 30% H$_2$O$_2$ (34 μL, 0.30 mmol). After stirring at room temperature for 12 h, acetic acid was removed under reduced pressure from the resulting red suspension. A 10-mL portion of Et$_2$O was added to the red residue, and the dark-red solid that resulted was collected by filtration and washed with 20 mL of Et$_2$O. The yield was 0.022 g. The $^1$H NMR spectrum of this material indicated the presence of at least two different compounds. No attempts were made to isolate and characterize these compounds.

**Thermal and Photochemical Reactions.** Photochemical reactions were performed using a 1000 W high-pressure Hg/Xe arc lamp (Oriel). Compounds 2 and 3 were irradiated in standard borosilicate NMR tubes. Because of the low absorptivity of 1 in the visible region, it was irradiated in a quartz NMR tube to allow better UV light penetration. Thermal reactions were conducted by placing NMR tubes covered with aluminum foil in a temperature-controlled oil bath. The temperature was maintained between 60 and 65 °C. Dioxygen- and water-free samples were prepared in a nitrogen-filled glovebox with dimethylformamide (DMF)-d$_7$ that had been dried over 4 Å molecular sieves for 48 h. NMR tubes were sealed with rubber septa and electrical tape.

**X-ray Crystallographic Studies.** Single crystals were mounted in paratone oil on a cryoloop and frozen under a 110 or 100 K KRYO-FLEX nitrogen cold stream. Data were collected on a Bruker APEX CCD X-ray diffractometer with graphite-monochromated MoKα radiation ($\lambda = 0.71073$ Å) controlled by the APEX2 software package. Empirical absorption corrections were applied using SADABS. The structures were solved using direct methods and refined on $F^2$. 

111
with the SHELXTL-97 software package. Structures were checked for higher symmetry using PLATON. All non-hydrogen atoms were located and refined anisotropically. In general, all hydrogen atoms were placed in idealized locations and given isotropic thermal parameters equivalent to either 1.5 (methyl hydrogen atoms) or 1.2 times the thermal parameter of the atom to which they were attached. For NBD-dpm and 2, the amine and sulfonamide hydrogen atoms (H3N) were located on the difference Fourier map and refined with constrained H–N bond distances (0.88 Å) and isotropic thermal parameters (1.2 times the thermal parameter of the attached nitrogen atom).

**Table 2.1. X-Ray Crystallographic Data Collection and Refinement Parameters for Ds-dpm and NBD-dpm.**

<table>
<thead>
<tr>
<th></th>
<th>Ds-dpm</th>
<th>NBD-dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>formula</td>
<td>C23H22N4O2S</td>
<td>C17H12N6O3</td>
</tr>
<tr>
<td>fw</td>
<td>418.51</td>
<td>348.33</td>
</tr>
<tr>
<td>space group</td>
<td>Pbca</td>
<td>C2/c</td>
</tr>
<tr>
<td>a, Å</td>
<td>10.4517(9)</td>
<td>13.9662(10)</td>
</tr>
<tr>
<td>b, Å</td>
<td>15.8232(13)</td>
<td>10.6609(8)</td>
</tr>
<tr>
<td>c, Å</td>
<td>24.852(2)</td>
<td>21.2035(15)</td>
</tr>
<tr>
<td>α, deg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β, deg</td>
<td></td>
<td>101.7580(10)</td>
</tr>
<tr>
<td>γ, deg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, Å³</td>
<td>4109.9(6)</td>
<td>3090.8(4)</td>
</tr>
<tr>
<td>Z</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>(ρ_{calc}), g/cm³</td>
<td>1.353</td>
<td>1.497</td>
</tr>
<tr>
<td>T, °C</td>
<td>-163(2)</td>
<td>-163(2)</td>
</tr>
<tr>
<td>μ (Mo Kα), mm⁻¹</td>
<td>0.186</td>
<td>0.108</td>
</tr>
<tr>
<td>θ range, deg</td>
<td>1.64–26.37</td>
<td>1.96–26.70</td>
</tr>
<tr>
<td>total no. of data</td>
<td>55468</td>
<td>31855</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>4194</td>
<td>3998</td>
</tr>
<tr>
<td>no. of params</td>
<td>276</td>
<td>238</td>
</tr>
<tr>
<td>completeness to Θ (%)</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>(R^2) (%)</td>
<td>4.41</td>
<td>4.53</td>
</tr>
<tr>
<td>wR(^2) (%)</td>
<td>9.45</td>
<td>10.93</td>
</tr>
<tr>
<td>GOF(^c)</td>
<td>1.033</td>
<td>1.024</td>
</tr>
<tr>
<td>max, min peaks, e/A³</td>
<td>0.312, -0.378</td>
<td>0.438, -0.318</td>
</tr>
</tbody>
</table>

\(a R1 = Σ|F_o| - |F_c|/Σ|F_o|\). \(b wR2 = \{Σ[w(F_o^2 - F_c^2)^2]/Σ[w(F_o^2)^2]\}^{1/2}\). \(c GOF = \{Σ[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}\) where \(n\) is the number of data and \(p\) is the number of refined parameters.
X-ray-quality crystals of the ligands Ds-dpm and NBD-dpm were grown by evaporation from 1:1 MeOH/Et₂O and CH₂Cl₂ solutions, respectively. X-ray-quality crystals of the platinum complexes 1–3, 1ₒx, and 3ₒx were grown by vapor diffusion of diethyl ether into DMF solutions. Compound 2 crystallized with a well-ordered molecule of DMF in the asymmetric unit. Compound 3 crystallized with half a molecule of disordered diethyl ether in the asymmetric unit, with the central oxygen atom occupying a crystallographic inversion center. This molecule of diethyl ether was refined anisotropically with the help of similarity restraints on bond distances (SADI) and anisotropic thermal parameters (SIMU and DELU). In addition, 3 crystallized as a disordered mixture of the exo and endo conformers. Restraints to enforce planarity (FLAT) and similarity (SAME, SIMU, and DELU) were used in the refinement of the disordered NBD rings. The thermal parameters of C₆ and its disordered component C₆A were constrained to be identical with the use of the EADP command. Both 1ₒx and 3ₒx afforded only small, low-quality crystals. Refinement suffered from several unreasonable thermal ellipsoids. The ellipsoid of the oxygen atom of the hydroxo ligand in 1ₒx failed the Hirschfeld rigid-bond test.¹¹ The Pt–O bond separation was around 2.1 Å, which is 0.1 Å longer than most Pt-hydroxo bond lengths. These two results suggest some occupational disorder between a chloride and an oxygen atom. A hydrogen-bonding interaction is present between the sulfoxide oxygen (O₂) and the coordinated oxygen (O₃, 2.96 Å) suggesting that the modeled stereochemistry is most likely the most prevalent in the crystal structure. The thermal ellipsoid of C₆ in 3ₒx became nonpositive definite upon the initial anisotropic refinement and was therefore subsequently constrained to equal that of its neighbors. Additionally, large solvent-accessible voids that contained heavily disordered electron density were present in both cases, thus affecting the overall weighting factor of the refinement. Because of these problems, only the probable atomic connectivity is discussed for
these structures. A summary of the crystallographic data and refinement information for the ligands and 1–3 is presented in Tables 2.1 and 2.2, respectively. Because the crystal structures of 1_{ox} and 3_{ox} were not refined to completion, only the space group and unit cell parameters are given in Table 2.3.

### Table 2.2. X-Ray Crystallographic Data Collection and Refinement Parameters for 1–3.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2·DMF</th>
<th>3·0.5Et_{2}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>formula</td>
<td>C_{18}H_{17}Cl_{2}N_{3}O_{2}Pt</td>
<td>C_{26}H_{29}Cl_{2}N_{5}O_{3}PtS</td>
<td>C_{19}H_{17}Cl_{2}N_{6}O_{3.50}</td>
</tr>
<tr>
<td>fw</td>
<td>605.40</td>
<td>757.59</td>
<td>651.38</td>
</tr>
<tr>
<td>space group</td>
<td>Cc</td>
<td>P1</td>
<td>P1</td>
</tr>
<tr>
<td>a, Å</td>
<td>11.4122(12)</td>
<td>9.3238(12)</td>
<td>8.5263(4)</td>
</tr>
<tr>
<td>b, Å</td>
<td>13.2503(14)</td>
<td>10.2881(13)</td>
<td>11.3987(6)</td>
</tr>
<tr>
<td>c, Å</td>
<td>13.7957(19)</td>
<td>16.284(2)</td>
<td>11.9663(6)</td>
</tr>
<tr>
<td>α, deg</td>
<td>74.290(2)</td>
<td>86.0360(10)</td>
<td></td>
</tr>
<tr>
<td>β, deg</td>
<td>112.456(2)</td>
<td>79.220(2)</td>
<td>85.1820(10)</td>
</tr>
<tr>
<td>γ, deg</td>
<td>65.939(2)</td>
<td>69.5230(10)</td>
<td></td>
</tr>
<tr>
<td>V, Å³</td>
<td>1927.9(4)</td>
<td>1367.8(3)</td>
<td>1084.67(9)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ρ_{calc}, g/cm³</td>
<td>2.086</td>
<td>1.839</td>
<td>1.994</td>
</tr>
<tr>
<td>T, °C</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-163(2)</td>
</tr>
<tr>
<td>μ (Mo Kα), mm⁻¹</td>
<td>7.683</td>
<td>5.439</td>
<td>6.752</td>
</tr>
<tr>
<td>θ range, deg</td>
<td>2.47—28.28</td>
<td>1.30—26.51</td>
<td>1.71—26.37</td>
</tr>
<tr>
<td>total no. of data</td>
<td>18845</td>
<td>24234</td>
<td>19570</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>4712</td>
<td>5667</td>
<td>4436</td>
</tr>
<tr>
<td>no. of params</td>
<td>246</td>
<td>350</td>
<td>435</td>
</tr>
<tr>
<td>completeness to Θ</td>
<td>100.0</td>
<td>99.5</td>
<td>100.0</td>
</tr>
<tr>
<td>R^{a} (%)</td>
<td>2.24</td>
<td>2.87</td>
<td>2.50</td>
</tr>
<tr>
<td>wR^{2} (%)</td>
<td>4.46</td>
<td>6.22</td>
<td>5.16</td>
</tr>
<tr>
<td>GOFc</td>
<td>1.006</td>
<td>1.055</td>
<td>1.092</td>
</tr>
<tr>
<td>max, min peaks,</td>
<td>1.424, -0.767</td>
<td>1.450, -1.758</td>
<td>1.086, -0.606</td>
</tr>
</tbody>
</table>

---

1. R1 = Σ|F_{o}|-|F_{c}|/Σ|F_{o}|
2. wR2 = (Σ[w(F_{o}^{2} - F_{c}^{2})^{2}]/Σ[w(F_{o}^{2})^{2}])^{1/2}
3. GOF = (Σ[w(F_{o}^{2} - F_{c}^{2})^{2}]/(n - p))^{1/2} where n is the number of data and p is the number of refined parameters.
Table 2.3. Space Group and Unit Cell Parameters of 1_{ox} and 3_{ox}.

<table>
<thead>
<tr>
<th></th>
<th>1_{ox}</th>
<th>3_{ox}</th>
</tr>
</thead>
<tbody>
<tr>
<td>space group</td>
<td>PI</td>
<td>Pbca</td>
</tr>
<tr>
<td>a, Å</td>
<td>7.4999(5)</td>
<td>14.3561(9)</td>
</tr>
<tr>
<td>b, Å</td>
<td>11.6437(7)</td>
<td>15.0452(9)</td>
</tr>
<tr>
<td>c, Å</td>
<td>13.9301(9)</td>
<td>23.5886(15)</td>
</tr>
<tr>
<td>α, deg</td>
<td>95.2240(10)</td>
<td></td>
</tr>
<tr>
<td>β, deg</td>
<td>95.0050(10)</td>
<td></td>
</tr>
<tr>
<td>γ, deg</td>
<td>92.5160(10)</td>
<td></td>
</tr>
<tr>
<td>V, Å³</td>
<td>1205.15(13)</td>
<td>5094.9(5)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>T, °C</td>
<td>-163(2)</td>
<td>-163(2)</td>
</tr>
</tbody>
</table>

2.3. Results and Discussion

Ligand Synthesis and Characterization. The three ligands were prepared in moderate to good yields from di-2-pyridylmethanamine and the corresponding sulfonyle (Ts-dpm and Ds-dpm) or aryl (NBD-dpm) chloride in the presence of a carbonate salt as a base to neutralize the hydrochloric acid byproduct, as shown in Scheme 2.1. Di-2-pyridylmethanamine is a convenient starting material for the synthesis of these new fluorescent ligands. The primary amine group is readily functionalized by a number of amine-specific fluorescent labeling reagents, while the two pyridyl arms remain available for metal coordination. This property is demonstrated here by our use of dansyl and NBD, both well-known fluorophores. Because platinum complexes containing similar ligands display cytotoxic activities similar to that of cisplatin,\textsuperscript{32-34} we hypothesized that platinum complexes utilizing our modified dpm ligands would similarly be cytotoxic.
Scheme 2.1. Syntheses of modified di-2-pyridylmethane (dpm) ligands.

The three ligands were characterized by $^1$H and $^{13}$C NMR spectroscopy and by ESI-MS. Both Ds-dpm and NBD-dpm were structurally characterized by X-ray diffraction as well. The NMR spectra of the ligands are consistent with the structures depicted in Scheme 2.1. The $^{13}$C NMR spectra displayed characteristic resonances for the methine carbon atoms (py$_2$CHNHR) at 62–63 ppm for all three ligands. The proton resonance of this methine carbon atom appeared as a doublet near 5.6 ppm in the $^1$H NMR spectra of Ts-dpm and Ds-dpm. In NBD-dpm, this resonance was a broad singlet at 6 ppm.

The molecular structures of Ds-dpm and NBD-dpm as determined by X-ray crystallography are shown in Figure 2.1. Both structures reveal an intramolecular hydrogen bond between N2 of the pyridine ring and N3 of the sulfonamide. The N···N distance is 2.73 Å for Ds-
dpm and 2.60 Å for NBD-dpm. Ds-dpm exhibits no other noteworthy intra- or intermolecular interactions in the solid state. In contrast, NBD-dpm engages in a long range intermolecular π-stacking interaction along the crystallographic c axis. This stacking interaction occurs in a BBABB-type pattern, where the pyridine ring (A) is flanked on either side by two consecutively stacked NBD heterocycles (B). The interplanar distance between the NBD heterocycles is 3.37 Å, and the spacing between the NBD heterocycle and pyridine ring is 3.23 Å.

Figure 2.1. Solid-state molecular structures of Ds-dpm (left) and NBD-dpm (right). Ellipsoids are drawn at the 50% probability level. Unlabeled grey ellipsoids and green open circles represent carbon and hydrogen atoms, respectively.

Syntheses and X-Ray Crystal Structures of the Platinum Complexes. The platinum complexes 1–3 were prepared by treatment of a suspension of cis-[Pt(DMSO)_2Cl_2] in MeOH with a methanolic solution of the ligand, as shown in Scheme 2.2. This general synthetic route for the preparation of diiminedichloroplatinum(II) compounds has been used previously. The platinum complexes were isolated as analytically pure solids in yields of greater than 70%. These
compounds were characterized both in the solid state by X-ray diffraction and in solution by NMR spectroscopy.

\[
cis-[\text{Pt(DMSO)}_2\text{Cl}_2] + \text{RNH-dpm} \xrightarrow{\text{MeOH, rt}} \ 1, \ R = \text{Ts} \\
2, \ R = \text{Ds} \\
3, \ R = \text{NBD}
\]

**Scheme 2.2.** Syntheses of platinum(II) complexes 1–3.

The molecular structures of 1–3 are shown in Figure 2.2. Selected interatomic distances and angles are displayed in Table 2.4. As anticipated, the ligands adopt a bidentate coordination mode through the nitrogen atoms of the pyridine rings. The typical square-planar geometry for platinum(II) is observed for 1–3 with only minor deviations from the ideal 90° L–Pt–L interatomic angles. These values range from 87.6° to 92.0° in the three complexes. The Pt–L bonds have the expected distances of about 2 Å for Pt–Npy and 2.3 Å for Pt–Cl. In the solid state, these compounds are asymmetric. For 1 and 2, the tosyl and dansyl groups are positioned over a coordinating pyridine ring. In 3, the NBD heterocycle is tilted to one side, thus destroying a potential mirror plane containing Pt1, C6, and N3.
Figure 2.2. Solid-state molecular structures of 1–3. Ellipsoids are drawn at the 50% probability level. Unlabeled grey ellipsoids and green open circles represent carbon and hydrogen atoms, respectively. Solvent molecules and the disorder of 3 is omitted for clarity.

Unlike the related diimine ligands 2,2′-bipyridine and 1,10-phenanthroline, which form five-membered planar chelate rings, dpm and the analogues studied here give rise to six-membered chelate rings that are not planar. As observed in the structures of 1–3, the chelate rings adopt a boat-like conformation. Because the bridging methine carbon atom is bound to two inequivalent substituents in addition to the pyridine rings, two different conformational isomers exist. These conformers, exo and endo, are depicted in Scheme 2.3.
Table 2.4. Selected Interatomic Distances (Å) and Angles (°) for 1–3.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2·DMF</th>
<th>3·0.5Et\textsubscript{2}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt1—Cl1</td>
<td>2.2980(13)</td>
<td>2.2917(9)</td>
<td>2.2851(10)</td>
</tr>
<tr>
<td>Pt1—Cl2</td>
<td>2.2903(11)</td>
<td>2.2943(9)</td>
<td>2.3044(9)</td>
</tr>
<tr>
<td>Pt1—N1</td>
<td>2.032(4)</td>
<td>2.015(3)</td>
<td>2.015(3)</td>
</tr>
<tr>
<td>Pt1—N2</td>
<td>2.020(3)</td>
<td>2.018(3)</td>
<td>2.009(3)</td>
</tr>
<tr>
<td>N1—Pt1—N2</td>
<td>87.93(14)</td>
<td>87.71(11)</td>
<td>87.64(11)</td>
</tr>
<tr>
<td>N1—Pt1—Cl1</td>
<td>178.33(10)</td>
<td>178.70(8)</td>
<td>177.48(8)</td>
</tr>
<tr>
<td>N1—Pt1—Cl2</td>
<td>90.90(10)</td>
<td>90.39(8)</td>
<td>90.47(8)</td>
</tr>
<tr>
<td>N2—Pt1—Cl1</td>
<td>90.65(11)</td>
<td>91.09(8)</td>
<td>89.92(9)</td>
</tr>
<tr>
<td>N2—Pt1—Cl2</td>
<td>178.73(11)</td>
<td>177.41(8)</td>
<td>178.11(9)</td>
</tr>
<tr>
<td>Cl1—Pt1—Cl2</td>
<td>90.52(4)</td>
<td>90.83(3)</td>
<td>91.98(4)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Numbers in parentheses are the estimated standard deviations of the last significant figures. Atoms are labeled as indicated in Figure 2.2.

Scheme 2.3. Depiction of the two conformational isomers of 1–3.

Compound 1 crystallizes exclusively as the endo conformer, 2 as the exo conformer, and 3 as a disordered mixture of the two. The occupancy factors of the disordered components of 3 were allowed to refine freely, and a final value of 66\% was obtained for the exo conformer. The two disordered components of 3 are shown in Figure 2.3.
Figure 2.3. Disordered components of 3. Both exo (left) and endo (right) isomers were present in the crystal lattice.

Another noteworthy feature in the structures of 1–3 is a close Pt···H interaction observed for the hydrogen atoms of the methine carbon (C6) in the exo conformers and the hydrogen atom of amine or sulfonamide nitrogen (N3) of the endo conformers. For 1 and the endo conformer of 3, the hydrogen atom of N3 is 2.88 and 2.90 Å from the Pt center, respectively. The corresponding N–H–Pt angles are 102.4° and 110.9°. These values, however, should be interpreted with caution because the hydrogen atoms were placed at calculated positions rather than being freely refined. The hydrogen atoms could not be freely refined because they were not located on the difference map. Similar Pt···H distances are observed in the exo conformers. For the exo conformer of 3, the hydrogen atom of C6 is at distances of and 2.67 Å from the platinum atom with a C–H–Pt angle of 97.4°. These values also are derived from a hydrogen atom placed at a calculated location. For 2, which crystallizes in the exo conformer, the hydrogen atom of the methine carbon could be located on the difference map and freely refined. In the final model, however, this hydrogen atom was placed at a calculated position. The Pt···H distance for the freely refined model is 2.92(4) Å, whereas for the model with the calculated position it is 2.79 Å.
The corresponding C–H–Pt angles are 87.7 and 95.5° for the freely refined and calculated models, respectively. The disparity in these values emphasizes the potential shortcomings in using X-ray diffraction data to interrogate distances between heavy metals and hydrogen atoms.

The nature of similar axial M···H interactions of less than 3 Å in d^8 square-planar complexes has previously been investigated by us and by others. These studies examined whether such interactions should be considered agostic. Authentic agostic interactions are characterized as covalent 3-center-2-electron interactions. In all of the studies, these particular d^8 square-planar interactions were characterized as purely electrostatic in nature and therefore not agostic. In the cases of 1–3, the close Pt···H distances are most likely a consequence of the ligand-binding geometry. However, the NMR data discussed below are consistent with the presence of weak electrostatic interactions.

**NMR Spectroscopic Characterization of 1–3.** The three platinum complexes are insoluble in nonpolar organic solvents and water. The compounds are very soluble in DMSO and DMF. Given the propensity of DMSO to interact with platinum(II) by ligand substitution reactions, DMF-d_7 was chosen as the solvent for conducting NMR spectroscopic studies of 1–3. The ^1H NMR spectra of 1–3 in DMF-d_7 at 20 °C displayed two distinct sets of resonances of unequal intensity corresponding to the inequivalent exo and endo conformers. At temperatures above 65 °C, the signals coalesce as interconversion rapidly occurs and the different conformers become indistinguishable on the NMR time scale, as shown in Figure 2.4 for 1. Similar behavior was observed for 2 and 3. This process is reversible through several temperature cycles. However, prolonged exposure of these compounds to elevated temperatures does eventually lead to decomposition (vide infra).
Proton resonances were assigned using 2D NMR spectroscopic techniques. Selected $^1$H and $^{195}$Pt NMR chemical shifts are displayed in Table 2.5. The resonances of the protons ortho to the coordinating nitrogen atoms of the pyridine rings fall between 9.0 and 9.3 ppm for the three complexes in both conformations. These resonances are 0.6-1.0 ppm downfield from the corresponding values in the free ligands, consistent with coordination to the Pt center. In the $^1$H-$^{13}$C HSQC spectra, the amine and sulfonamide protons could be identified as the only resonances not correlated to a carbon nucleus. These proton chemical shifts are farthest downfield, with values ranging from 9.5 to 10.4 ppm. The large downfield shifts of these protons are attributed in
part to hydrogen-bonding interactions with the highly polar DMF solvent. The methine proton resonances (C6 in Figure 2.2) were identified based on coupling to the NH resonances (observed by $^1$H-$^1$H COSY) and by coupling to carbon atoms near 65 ppm (observed by $^1$H-$^{13}$C HSQC). These resonances occur between 6.3 and 7.2 ppm in complexes 1–3.

Table 2.5. Selected $^1$H and $^{195}$Pt NMR Chemical Shifts for the Conformers of 1–3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\delta$ $^{195}$Pt, ppm</th>
<th>$\delta$ $^1$H, ortho-py, ppm</th>
<th>$\delta$ $^1$H, N-H, ppm</th>
<th>$\delta$ $^1$H, methine H, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>exo-1</td>
<td>-2198</td>
<td>9.13</td>
<td>10.15</td>
<td>6.79</td>
</tr>
<tr>
<td>endo-1</td>
<td>-2082</td>
<td>9.23</td>
<td>9.54</td>
<td>6.15</td>
</tr>
<tr>
<td>exo-2</td>
<td>-2199</td>
<td>9.08</td>
<td>10.40</td>
<td>6.82</td>
</tr>
<tr>
<td>endo-2</td>
<td>-2105</td>
<td>9.14</td>
<td>9.65</td>
<td>6.30</td>
</tr>
<tr>
<td>exo-3</td>
<td>-2196</td>
<td>9.28</td>
<td>10.44</td>
<td>7.60, broad</td>
</tr>
<tr>
<td>endo-3</td>
<td>-2057</td>
<td>9.31</td>
<td>10.40</td>
<td>7.17</td>
</tr>
</tbody>
</table>

$^1$H-$^1$H NOESY experiments identified the exo conformer as the major species in solution for all three compounds. Especially diagnostic is the presence of an NOE cross-peak between the NH proton and the protons at the 3 position of the pyridine rings only in the major set of signals. A similar cross-peak was observed between the methine proton and the protons at the 3 position of the pyridine ring in the minor set of signals, corresponding to the endo conformer. Negative phase cross-peaks between analogous protons of the exo and endo conformers were also observed, indicating that interconversion between the conformers occurs within the 400 ms mixing period of the experiment. Figure 2.5 shows a $^1$H-$^1$H NOESY NMR spectrum of 1 as a representative example.
Figure 2.5. $^1$H-$^1$H NOESY NMR spectrum of 1 in DMF-$d_7$ at 20 °C. Positive-phase cross peaks are in green and negative-phase cross-peak are in turquoise. Cross-peaks in squares are due to NOE interactions between the CH methine proton and the protons at the 3-position of the pyridine ring of the endo isomer. Cross-peaks in circles are due to NOE interactions between the sulfonamide NH proton and the protons at the 3-position of the pyridine ring of the exo isomer. The turquoise cross-peaks between NH and methine protons of the two isomers are indicative of chemical exchange within the 400 ms mixing time of the experiment. The cross-peak marked with a cross is an instrumental artifact due to folding over of the NMR spectrum.

The methine proton resonances of the exo conformers 1–3 occur about 1 ppm downfield from those in the endo conformers. This downfield shift is consistent with a weak electrostatic interaction between the methine proton and the Pt center, as observed in the crystal structures and discussed above. Agostic interactions, on the other hand, would shift these resonances upfield.44

$^{195}$Pt NMR spectra for 1–3 were recorded at 20 °C in DMF-$d_7$. As expected, two distinct resonances are observed for each compound, corresponding to the two conformers (Figure 2.6). The observed chemical shifts (−2057 to −2199 ppm) for these complexes are in the range...
expected for platinum(II) with an N₂Cl₂ coordination environment.⁴⁸-⁵⁰ For all three complexes, the exo conformer resonance occurs upfield from that in the endo conformer, indicating greater shielding of the Pt nucleus in the former. In the three complexes, the chemical shifts of the exo conformers all fall within 3 ppm of -2198 ppm. This similarity is remarkable given the large, 15,000 ppm window for the ¹⁹⁵Pt nucleus. The chemical shifts of the endo conformers span a wider range. The shifts are -2082, -2105, and -2057 ppm for 1-3 respectively. This wider range in shifts reflects the fact that the endo conformations position the different functional groups of the ligands closer to the Pt center than the exo conformations.

![Figure 2.6](image)

**Figure 2.6.** ¹⁹⁵Pt NMR spectra of 1–3 in DMF-d₇ at 20 °C. Signal are observed for the both the exo (major) and endo (minor) conformers.

From the VT-NMR experiments, thermodynamic data could be obtained by exploring how the relative ratios of the exo and endo conformers vary with temperature. From this data van't Hoff plots were constructed to afford values of ΔH⁰, ΔS⁰, and ΔG⁰ (at 298 K) for the
conversion of the exo conformer to the endo conformer. These parameters are collected in Table 2.6. The larger size of the dansyl group may influence the greater thermodynamic preference for the exo conformer in 2, relative to 1 and 3. In general, these energy differences are quite small, consistent with the presence of both conformers in the solid-state and solution.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \Delta H^\circ ), kcal mol(^{-1} )</th>
<th>( \Delta S^\circ ), cal K(^{-1})mol(^{-1} )</th>
<th>( \Delta G^\circ ) (298 K), kcal mol(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+1.38 ± 0.05</td>
<td>-2.4 ± 0.2</td>
<td>+2.09 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>+2.10 ± 0.07</td>
<td>-3.9 ± 0.2</td>
<td>+3.25 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>+1.31 ± 0.05</td>
<td>-2.5 ± 0.1</td>
<td>+2.06 ± 0.06</td>
</tr>
</tbody>
</table>

**Photophysical Properties.** Relevant photophysical properties for the ligands and compounds 1–3 in a DMF solution are summarized in Table 2.7, and optical absorption spectra are shown in Figure 2.7. Emission spectra of the compounds are provided in Figure 2.8. A pyridine-localized \( ^1\pi-\pi^* \) transition appears in all three ligands around 270 nm. For Ts-dpm, this is the only spectral feature observed. The band at 340 nm and the absorptions at 330 and 468 nm of Ds-dpm and NBD-dpm, respectively, can be assigned to \( ^1\pi-\pi^* \) transitions localized in the naphthalene ring system and fused heterocycle of the two ligands. Ts-dpm is nonemissive. Both Ds-dpm and NBD-dpm emit in the green region of the visible spectrum at 521 and 524 nm with quantum yields of 0.30 and 0.32, respectively. These values are similar to those reported for other compounds conjugated with dansyl\(^{51-53} \) and NBD\(^{54,55} \) in organic solvents. This result indicates that the pyridine rings do not induce significant quenching of the fluorophores.
Table 2.7. Electronic Absorption and Emission Data for Ligands and Platinum Complexes in DMF Solution.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorption $\lambda_{\text{max}}, \text{nm} (\varepsilon \times 10^3, \text{M}^{-1}\text{cm}^{-1})$</th>
<th>Emission $\lambda_{\text{em}}, \text{nm} (\Phi)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts-dpm</td>
<td>268 (5.04)</td>
<td></td>
</tr>
<tr>
<td>Ds-dpm</td>
<td>269 (18.7), 340 (4.90)</td>
<td>521 (0.30)$^a$</td>
</tr>
<tr>
<td>NBD-dpm</td>
<td>270 (8.80), 330 (6.30), 468 (22.0)</td>
<td>524 (0.32)$^b$</td>
</tr>
<tr>
<td>1</td>
<td>270 (10.00), 310 (5.10)</td>
<td>440 (0.019)$^a$</td>
</tr>
<tr>
<td>2</td>
<td>266 (16.30), 312 (8.50)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>270 (19.0), 388 (13.60), 476 (sh, 25.80), 496 (28.20)</td>
<td>545 (0.003)$^b$</td>
</tr>
</tbody>
</table>

$^a$Referenced to quinine sulfate ($\Phi = 0.58$ in 0.1 M H$_2$SO$_4$)$^b$ Referenced to fluorescein ($\Phi = 0.95$ in 0.1 M NaOH)

Figure 2.7. UV-vis absorption spectra for Ts-dpm (—), Ds-dpm (red ●), NBD-dpm (green ×), 1 (blue ■), 2 (maroon ●), and 3 (purple ○) in DMF at room temperature.
Figure 2.8. Room temperature normalized emission spectra of Ds-dpm (red •) and 2 (maroon *) (left), and NBD-dpm (green ×) and 3 (blue o) (right) in DMF. Spectra were obtained by exciting compounds at their wavelengths of maximum absorption.

Upon coordination to platinum(II), the absorption features of the ligands are altered. The pyridine-localized $^1\pi-\pi^*$ transitions occur at nearly the same wavelength but with a 2-fold increase in the extinction coefficients in the platinum complexes. For 1, a new band at 310 nm is observed that is not present in the free ligand. For 2, the band at 340 nm in the free ligand is shifted to higher energy, appearing at 312 nm. The major bands of NBD-dpm are red-shifted significantly to 388 and 496 nm when coordinated to platinum in 3.

Platinum coordination also affects the emission properties of the ligands (Figure 2.8). Like Ts-dpm, 1 is not emissive. The platinum complexes 2 and 3 exhibit broad, unstructured emission bands similar to those of the free ligands. The emission band of 2 is centered at 440 nm, which is 80 nm (3500 cm$^{-1}$) higher in energy than the free ligand. In contrast, the emission band of 3 is centered at 545 nm, 20 nm (740 cm$^{-1}$) lower in energy than the free ligand.

The measured quantum yields for fluorescence of 2 and 3 are 0.019 and 0.003, respectively, indicating large quenching of the ligand fluorescence. This effect most likely arises from the close proximity of the Pt center to the fluorophores. Heavy atoms can increase the rates
of spin-forbidden radiation-less transitions in aromatic systems, thus lowering the overall quantum yield of fluorescence.\textsuperscript{56,57} Fluorescence quenching of a dansyl fluorophore by platinum(II) occurs in a series of compounds with different-sized linkers between the metal atom and fluorophore.\textsuperscript{58} As expected, the fluorescence was quenched to a larger extent when the dansyl group was closer to the Pt center. The large quenching effect observed for 2 and 3 is undesirable for potential cellular imaging applications. For future ligand design, longer and more rigid alkyl chains as spatial linkers may be necessary to position the fluorophores farther away from the Pt center to avoid quenching by the heavy-atom effect.

**Thermal and Photochemical Reactivity.** Compounds 1–3 are stable for over 4 weeks in a DMF solution when kept in the dark at room temperature. To assess the stability of these compounds under elevated temperatures and exposure to light, controlled thermolytic and photolytic reactions were monitored by \textsuperscript{1}H and \textsuperscript{195}Pt NMR spectroscopy. At 60 °C in the dark, air-equilibrated solutions of 1 in DMF slowly decompose. Several new resonances appear in the \textsuperscript{1}H NMR spectrum, and the solution color changes gradually from almost colorless to pale yellow. The thermal reaction is slow; after 85 h at 60 °C, only 25\% of the starting compound is consumed. The decomposition products are numerous and were not conclusively identified. Irradiation of 1 at room temperature led to no observable changes in the \textsuperscript{1}H NMR spectrum.

An air-equilibrated solution of 2 in DMF is stable at 60 °C for at least 60 h, with no noticeable change in the \textsuperscript{1}H NMR spectrum or solution color. However, upon irradiation, the pale-yellow color of a solution of 2 turns dark red over the course of several days. The conversion is complete after 80 h, as monitored by \textsuperscript{1}H NMR spectroscopy. The \textsuperscript{1}H NMR spectrum of the photoproduc displays resonances consistent with the presence of a dansyl group and two equivalent pyridine rings. Neither the sulfonamide nor the methine proton was observed,
indicating their absence in this photoproduct. A signal at -2177 ppm was present in the $^{195}$Pt NMR spectrum. This value is within the range expected for platinum(II) having an $\text{N}_2\text{Cl}_2$ coordination environment.\textsuperscript{48-50} Attempts to crystallize the major photoproduct of 2 were unsuccessful. Additional attempts to characterize the compound by ESI-MS were also unsuccessful; no diagnostic peaks were observed in the MS spectrum in either positive- or negative-ion mode. Our current working hypothesis for the identity of this photoproduct is that a dichloroplatinum complex ligated by a sulfonylimine analogue of Ds-dpm forms, as illustrated in Scheme 2.4. This formulation is consistent with the lack of methine and NH protons in the $^1$H NMR spectrum.

\[
\begin{align*}
\text{H}_3\text{C} & . \text{N} . \text{CH}_3 \\
\text{O}_2\text{S} & \text{NH} \\
\text{N} & \text{Cl} \text{Pt} \cdot \text{Cl} \\
& \text{Pale Yellow}
\end{align*}
\]

\[
\begin{align*}
\text{H}_3\text{C} & . \text{N} . \text{CH}_3 \\
\text{O}_2\text{S} & \text{NH} \\
\text{N} & \text{Cl} \text{Pt} \cdot \text{Cl} \\
& \text{Dark Red}
\end{align*}
\]

\[
\begin{align*}
\text{hv}, \text{O}_2, \text{trace H}_2\text{O} & \rightarrow \\
\text{DMF} & \\
\end{align*}
\]

Scheme 2.4. Proposed photolytic decomposition pathway of 2.

Thermolysis of 3 at 60 °C for 80 h leads to its complete conversion to several new compounds, as monitored by $^1$H NMR spectroscopy. Two of the newly formed products were conclusively identified as [Pt(dpk)Cl$_2$] and NBD-NH$_2$. Figure 2.9 shows the $^1$H NMR spectrum of 3, its thermolysis products, and authentic samples of [Pt(dpk)Cl$_2$] and NBD-NH$_2$ in DMF-$d_7$.

The resonances marked with circles correspond to [Pt(dpk)Cl$_2$] and the resonances marked with crosses to NBD-NH$_2$. Further support for these assignments comes from $^{195}$Pt NMR
spectroscopy and ESI-MS. At 40 h into the thermolysis reaction, a $^{195}$Pt NMR spectrum was obtained of the reaction mixture. In addition to the two signals observed for the conformational isomers of 3, a new signal at $-1958$ ppm appeared. This value matches the $^{195}$Pt chemical shift of [Pt(dpk)Cl$_2$] measured independently, thus supporting its assignment in the $^1$H NMR spectrum.

ESI-MS studies of the reaction mixture revealed a peak at $m/z$ 178.9 in the negative-ion mode. This value matches that calculated for NBD-NH$^-$, $m/z$ 179.0. The doublets at 8.8 and 7.3 ppm and the broad peak at 7.8 ppm in the $^1$H NMR spectrum are not assigned but most likely correspond to another NBD-containing compound.

Figure 2.9. From bottom to top: $^1$H NMR spectra of 3, 3 after thermolysis at 60 °C for 80 h, NBD-NH$_2$, and [Pt(dpk)Cl$_2$] in DMF-$d_7$. Resonances marked with circles are assigned to [Pt(dpk)Cl$_2$], and resonances marked with crosses are assigned to NBD-NH$_2$. The doublets at 8.8
and 7.3 ppm and the broad singlet at 7.8 ppm are currently unassigned. The sharp singlet at 8 ppm in all spectra marked by an asterisk is from the solvent.

Decomposition of 3 to [Pt(dpk)Cl₂] and NBD-NH₂ also proceeds photochemically, as depicted in Scheme 2.5. Irradiation of 3 at room temperature produces the same changes in the ¹H NMR spectrum as those observed for the thermal reaction. The photochemical reaction, however, is much slower. After 85 h of irradiation at room temperature, only 50% conversion of 3 occurs.

Scheme 2.5. Thermolytic and photolytic decomposition pathways of 3.

For 1–3, the same thermal and photochemical conditions were applied under dioxygen- and water-free conditions by using samples prepared in a nitrogen-filled glovebox in septa-sealed NMR tubes. Neither irradiation with light nor heating at 65 °C for up to 72 h produced noticeable changes in the ¹H NMR spectra. It is therefore clear that dioxygen and at least trace amounts of water are necessary to promote the photochemical and thermal transformations reported here.

Preliminary Oxidative Reactivity Studies. Compounds 1 and 3 could be successfully oxidized to their platinum(IV) analogues by a procedure recently described for the oxidation of platinum(II) pyridylazido complexes. Suspensions of the complexes in acetic acid were treated
with H₂O₂ to afford the oxidized products. The anticipated products of these reactions were platinum(IV) hydroxoacetato species, but the observed products were generally more complex. In the case of 1, a ¹H NMR spectrum of the freshly isolated solid indicated the presence of a single compound. The ¹H NMR spectrum was consistent with a complex of C₄ symmetry containing no sulfonamide proton. Additionally, coupling to the ¹⁹⁵Pt nucleus was observed for protons ortho to the coordinating nitrogen atoms of the pyridine rings (J_{PtH} = 27.2 Hz) and, more surprisingly, to the methine (C6, Figure 2.2) proton (J_{PtH} = 20.4 Hz). Upon standing in solution, however, this compound undergoes chemical transformations to at least two new species over the course of several days, as monitored by ¹H NMR spectroscopy. The red material isolated from the oxidation reaction of 3 was also investigated by ¹H NMR spectroscopy. The ¹H NMR resonances reveal the presence of at least two different compounds.

Attempts to crystallize these oxidized products for structural characterization afforded small, weakly diffracting crystals from the reactions of both 1 and 3. Because several issues were encountered with data collection and refinement (see the Experimental Section), only the probable connectivity is considered here. The structures of these oxidized compounds, ¹ox and ³ox, are shown in Figure 2.10. The chemical formulas of ¹ox and ³ox, as postulated from the crystal structures are \textit{rac-}[Pt(κ³-Ts-dpm)Cl₂(OH)] and [Pt(κ³-NBD-dpm)Cl₃]. A common feature between the two structures is the change in the coordination mode of the dpm ligands from κ² to κ³ upon oxidation. A third binding atom of the dpm ligands is provided by the deprotonated anionic sulfonamide or amine nitrogen (N3, Figure 2.10). In this coordination mode, the methane proton is only three bonds from the Pt center, thus accounting for the Pt–H coupling observed in the ¹H NMR spectrum described above. It is also noteworthy that ¹ox lacks the pseudo-C₄
symmetry that is observed in 1–3 and in the initial oxidation product of 1 as observed by NMR spectroscopy, thus indicating that the complex isomerizes in solution from the meso isomer (both chlorides trans to pyridine) to the rac isomer. Also of interest are the three chloride ions in the coordination sphere of Pt in 3_{\text{ox}}. Given that no additional chloride source was present in the reaction mixture, the additional chloride ion of 3_{\text{ox}} must have been provided by another platinum complex. Although the characterization of these oxidation products is incomplete, these preliminary results demonstrate that the dpm-based ligands can bind in a tridentate manner.

![Figure 2.10. Ball-and-stick structures of 1_{\text{ox}} and 3_{\text{ox}} as determined by X-ray crystallography demonstrating atomic connectivity. Carbon and hydrogen atoms are not labeled. The hydrogen atom of O(3), a proposed hydroxide ligand, in 1_{\text{ox}} was not located on the difference map.](image)

2.4. Summary and Conclusions

The synthesis and characterization of three new ligands and their corresponding platinum complexes was reported. Two of the ligands are fluorescent. Use of di-2-pyridylmethanamine as a starting material provides access to fluorescent ligands by using any fluorescent amine-labeling reagent. These ligands can bind in either a bidentate or tridentate coordination mode, a property of relevance to their chemistry with platinum(IV) and other transition metals that form
octahedral complexes. Coordination of platinum to the ligands induces significant fluorescence quenching. Such quenching is undesirable for cellular imaging applications. The platinum complexes exhibit interesting oxygen-dependent photo- and thermochemical behavior. Although the products of these reactions for 1 and 2 have not been unambiguously characterized, the final products for such reactions of 3 were identified as [Pt(dpk)Cl₂] and NBD-NH₂. A combination of the lack of aqueous solubility, poor stability to light and heat, and low emission quantum yields of 1–3 precludes their use in biological or simulated biological studies.

2.5. References

Chapter 3

Outer-Sphere Amide Bond Coupling Reactions for the Preparation of a Fluorescent Platinum(IV) Redox Sensor

3.1. Introduction

The ability to systematically modify potential platinum anticancer agents by predictable and readily controlled chemistry is of value for the synthesis of new drug candidates having novel properties. In recent years, this principle has been applied to generate platinum(IV) prodrugs. For example, the free carboxylic acid groups of the platinum(IV) compound \(\text{cis,cis,trans-}[\text{Pt(NH}_3\text{)}_2\text{Cl}_2(\text{succinate})_2]\) can engage in amide bond coupling reactions.1 Functionalization of this compound via amide bond formation has led to the synthesis of many new platinum(IV) complexes that were tested for biological activity.1-8 This predictable chemistry has also been used to attach platinum(IV) prodrugs to peptides9-12 and various nano-delivery devices13-19 for improved anticancer efficacy.

As discussed in Chapter 1, platinum(IV) prodrugs must first be reduced intracellularly to form platinum(II) complexes that can bind to DNA.20 There remains some uncertainty about this process.21 It is not clear how quickly, by what means, and where such reduction occurs in living systems. X-ray fluorescence and absorption spectroscopy has been used to address these questions.22-25 These methodologies, however, are not applicable to living cells and generally require synchrotron radiation.

The Introduction of Chapter 2 described some advantages of using fluorescence microscopy as an imaging technique. Although fluorescence microscopy, unlike X-ray methods, cannot directly provide information about the oxidation state of metal ions, it is possible to install ligand systems in which the fluorescence intensity is modulated depending on the oxidation state of the coordinated metal.26,27 Such a system, involving the Pt(IV)/Pt(II) redox couple, has recently been described,28 in which analogous platinum(II) and platinum(IV) complexes were coordinated to fluorescent coumarin ligands by means of an aniline functional group on the dye.
The authors reported a 7-fold greater emission intensity for the platinum(II) compared to the platinum(IV) complex in DMF solution. Furthermore, confocal fluorescence microscopic imaging analyses revealed strong localization of the complexes in the cytosol and lysosomes. The free coumarin ligands localize in a manner similar to that of the complexes, thus raising the possibility that the coumarin ligands are displaced from platinum in the cell. Similar systems with strongly coordinated bidentate ligands may be able to provide information about platinum(IV) reduction in live cells.

In this chapter, outer-sphere amide bond coupling chemistry that is analogous to that described above for the platinum(IV) complex cis,cis,trans-[Pt(NH$_3$)$_2$Cl$_2$(succinate)$_2$] is reported for the platinum(II) complexes [Pt(eda)Cl$_2$] and [Pt(edma)Cl$_2$] where edda = ethylenediamine-$N,N'$-diacetic acid and edma = ethylenediamine-$N$-monoacetic acid, which contain two and one uncoordinated carboxylic acid groups, respectively. In both cases, benzylamine is used as a substrate for these reactions. For [Pt(edma)Cl$_2$], the fluorescent amine, dansyl ethylenediamine (Ds-en) is used as well to form the resulting fluorescent platinum(II) complexes [Pt(edDs)Cl$_2$]. The platinum(IV) analogue of this compound, [Pt(edDs)Cl$_4$], is substantially less emissive than [Pt(edDs)Cl$_2$]. Upon reduction of [Pt(edDs)Cl$_4$], a fluorescent turn-on is observed. Hence, the studies reported in this chapter describe both a modular synthetic route to new platinum(II) complexes, and the use of such chemistry to design a prototypical fluorescent platinum(IV) redox sensor. The compounds reported and investigated in this chapter are shown in Chart 3.1.
3.2. Experimental Methods

**General Considerations.** All reactions were carried out under normal atmospheric conditions. Solvents were used as received without additional drying or purification. The compounds, [Pt(edma)Cl$_2$], [Pt(edd)$_2$Cl$_2$], dansyl ethylenediamine (Ds-en), and iodobenzene dichloride were synthesized as previously described.

**Physical Measurements.** NMR spectra were recorded on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility at 20 °C. $^1$H and $^{13}$C NMR spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane, SiMe$_4$ (δ = 0 ppm). $^{195}$Pt NMR spectra were referenced externally to K$_2$PtCl$_4$ in D$_2$O (δ = −1628 ppm). Electrospray ionization mass spectra (ESI-MS) were acquired on an Agilent Technologies 1100 series LC MSD trap. Fourier transform infrared (FTIR) spectra were recorded with a ThermoNicolet Avatar 360 spectrometer running the OMNIC software. Samples were prepared as KBr disks. Optical absorption spectra were recorded with a Cary 1E spectrophotometer. Emission spectra were obtained with a Photon Technology International QM-4/2003 fluorimeter. Quantum yields for fluorescence were measured using quinine sulfate in...
0.1 M H$_2$SO$_4$ (Φ = 0.58) as the reference over a range of at least five different absorbance values. For measuring these values in phosphate buffered saline (PBS), the samples were diluted from DMF solutions to give aqueous solutions containing less than or equal to 1% DMF. For all photophysical measurements, the sample temperature was maintained at 25.0 ± 0.5 °C using a circulating water bath. Elemental analyses were carried out by a commercial analytical laboratory.

**Synthesis of [Pt(edBz)Cl$_2$] (I).** A solution of 1,1'-carbonyldiimidazole (CDI, 0.230 g, 1.42 mmol) in 10 mL of DMF was added to a solution of [Pt(edma)Cl$_2$] (0.535 g, 1.39 mmol) in 10 mL of DMF. The resulting mixture was heated at 60 °C for 10 min, and then sparged with N$_2$ for 5 min. Benzylamine (0.152 g, 1.42 mmol) in 15 mL of DMF was added dropwise to the solution containing the activated platinum complex. After stirring for 12 h, the solution was concentrated to 10 mL under reduced pressure and elevated temperature (60 °C), and then filtered through Celite. The addition of 10 mL of water afforded the desired compound as a pale yellow solid, which was isolated by filtration and washed sequentially with 5 mL of water, 2 x 5 mL of ethanol, and 2 x 5 mL of diethyl ether before being dried in vacuo. Yield: 0.268 g (40%). M.p. 298–300 °C (dec). $^1$H NMR (400 MHz, DMF-$d_7$): δ 8.60 (t, 1H), 7.35–7.26 (m, 5H), 6.15 (br s, 1H), 5.48 (br s, 2H), 4.43 (d, 2H), 4.27 (d, 1H), 3.71 (dd, 1H), 3.12–3.07 (br m, 1H), 2.82–2.72 (br m, 2H), 2.57–2.55 (br m, 1H). $^{13}$C($^1$H) NMR (100 MHz, DMF-$d_7$): δ 168.2, 139.4, 128.6, 127.7, 127.2, 57.8, 55.1, 47.3, 42.8. $^{195}$Pt($^1$H) NMR (86 MHz, DMF-$d_7$): δ −2339. IR (KBr, cm$^{-1}$): 3335 m, 3274 s, 3202 m, 2940 w, 1654 vs, 1576 m, 1545 s, 1455 w, 1436 m, 1425 m, 1392 vw, 1279 w, 1243 m, 1168 w, 1116 w, 1091 w, 1041 w, 967 w, 758 m, 702 s, 611 w, 572 w, 525 w. ESI-MS (negative-ion mode): m/z 509.4 ([M+Cl]$^-$, calcd. 508.0), 944.6 ([2M–H]$^-$, calcd.
Synthesis of [Pt(edDS)Cl₂] (2). A solution of CDI (0.206 g, 1.27 mmol) in 20 mL of DMF was added to a solution of [Pt(edma)Cl₂] (0.469 g, 1.21 mmol) in 16 mL of DMF, and the resulting mixture was stirred at 60 °C for 10 min. The solution was sparged with N₂ for 5 min, and then Ds-en (0.372 g, 1.27 mmol) in 20 mL of DMF was added dropwise. After stirring at rt for 12 h, the mixture was concentrated to 10 mL under vacuum and increased temperature (60 °C), and then filtered through Celite. Water (10 mL) was added to the filtrate to precipitate the desired compound. The pale yellow solid was isolated by filtration, and washed sequentially with water, ethanol, and diethyl ether. Yield: 0.471 g (59%). M.p. 258–262 °C (dec). ¹H NMR (400 MHz, DMF-d₇): δ 8.55 (d, 1H), 8.39 (d, 1H), 8.28 (t, 1H), 8.20 (d, 1H), 7.97 (br t, 1H), 7.68 (t, 1H), 7.62 (t, 1H), 7.30 (d, 1H), 6.03 (br s, 1H), 5.44 (br s, 2H), 4.12 (d, 1H), 3.49–3.45 (m, 1H), 3.29 3.26 (m, 2H), 3.01–2.96 (m, 3H), 2.88 (s, 6H), 2.69 (br m, 2H), 2.44 (br m, 1H). ¹³C¹H NMR (100 MHz, DMF-d₇): δ 168.4, 152.2, 136.6, 130.01, 129.99, 129.8, 128.9, 128.2, 123.9, 119.7, 115.6, 57.7, 55.1, 47.3, 45.2, 42.5, 39.6. ¹⁹⁵Pt¹H NMR (86 MHz, DMF-d₇): δ –2345. IR (KBr, cm⁻¹): 3351 m, 3270 m, 3192 m, 3141 m, 2946 w, 1659 vs, 1571 w, 1547 w, 1318 s, 1095 w, 788 s, 629 w, 576 w. ESI-MS (negative-ion mode): m/z 657.8 ([M–H]⁻, calcd. 658.1). Anal. Calcd. for 2, C₁₈H₂₇Cl₂N₅O₃PtS: C, 32.78; H, 4.13; N, 10.62. Found: C, 32.92; H, 4.12; N, 10.77.

Synthesis of [Pt(edBz₂)Cl₂] (3). A solution of CDI (0.465 g, 2.87 mmol) in 50 mL of DMF was added to a suspension of [Pt(chedra)Cl₂] (0.619 g, 1.40 mmol) in 16 mL of DMF. The resulting mixture was heated at 60 °C for 10 min, at which point a yellow solution resulted, and then sparged with N₂ for 5 min. Benzylamine (0.307 g, 2.87 mmol) in 40 mL of DMF was added in a
dropwise manner to this solution containing the activated platinum complex. After stirring for 12 h, the solution was concentrated to 15 mL under reduced pressure and elevated temperature (60 °C). The addition of 20 mL of water afforded the desired compound as an off-white solid, which was isolated by filtration and washed sequentially with 5 mL of water, 2–5 mL of ethanol, and 2 × 5 mL of diethyl ether (Et₂O) before being dried in vacuo. Yield: 0.594 g (68%). M.p. > 280 °C (gradual browning), 302–307 °C (dec into black liquid). 

\(^1\)H NMR (400 MHz, DMF-\(d_7\)): \(R,R/S,S + R,S\) diastereomers (1:1) \(\delta 8.64 (2H, two\ overlapping\ triplets, amide NH), 7.36–7.24 (multiplet, 10H, aromatic protons), 6.22 + 6.15 (2H, broad singlets, coordinating NH), 4.48–4.38 (m, 4H, benzyl CH₂), 4.31–4.16 (2H, two doublets, CH adjacent to amide), 3.80–3.62 (two doublet of doublets, 2H, CH adjacent to amide), 3.21–3.11 (broad multiplet, 2H, CH₂ ethylenediamine backbone), 2.72–2.66 (broad multiplet, 2H, CH₂ ethylenediamine backbone). 

\(^13\)C{\(^1\)H} NMR (100 MHz, DMF-\(d_7\)): \(R,R/ S,S + R,S\) diastereomers (1:1) \(\delta 168.1 + 168.0, 139.50 + 139.48, 128.7, 127.82, 127.79, 127.3, 55.6 + 54.7, 55.1, 42.91. \(^{195}\)Pt\(^{1}\)H} NMR (86 MHz, DMF-\(d_7\)): \(R,R/S,S + R,S\) diastereomers (1:1) \(\delta -2347, -2362.\) IR (KBr, cm\(^{-1}\)): 3340 m, 3165 m, 3111 m, 2949 w, 1685 m, 1662 s, 1555 m, 1496 w, 1452 w, 1419 m, 1358 w, 1261 m, 1078 w, 1025 w, 986 w, 860 w, 748 w, 695 w, 581 w, 453 w. ESI-MS (negative-ion mode): \(m/z\) 582.9 ([M–2H–Cl]⁻, calcd. 583.1), 619.0 ([M–H]⁻, calcd. 619.1), 1239.1 ([2M–H]⁻, calcd. 1239.2). Anal. Calcd. for 3, C\(_{20}\)H\(_{25}\)Cl\(_2\)N\(_4\)O\(_2\)Pt: C, 38.72; H, 4.22; N, 9.03. Found: C, 38.71; H, 4.13; N, 8.96.

**Synthesis of [Pt(edBz)Cl\(_4\)] (4).** [Pt(edBz)Cl\(_2\)] (0.120 g, 0.250 mmol) was dissolved in 10 mL of DMF with gentle heating at 60 °C. To this solution, iodobenzene dichloride (0.072 g, 0.26 mmol) in 1 mL of DMF was added dropwise. The solution changed color immediately from pale yellow to bright yellow. After stirring 3 h at rt, a 25-mL portion of diethyl ether was added, and the resulting bright yellow solid was collected by filtration. It was further washed with diethyl
ether and then dried in vacuo. Yield: 0.086 g (63%). M.p. 188–198 °C (dec). $^1$H NMR (400 MHz, DMF-$d_7$): $\delta$ 8.90 (t, 1H), 7.92 (br s, unresolved $^{195}$Pt satellites, 1H), 7.68 (br s, unresolved $^{195}$Pt satellites, 1H), 7.39–7.27 (m, 5H), 7.10 (br s, $^2J_{PH} = 56$ Hz, 1H), 4.51–4.43 (m, 3H), 3.93–3.83 (m, 1H), 3.36–3.20 (br m, 3H), 2.88 (br m, 1H). $^{13}$C{$^1$H} NMR (100 MHz, DMF-$d_7$): $\delta$ 166.7, 139.2, 128.7, 127.8, 127.4, 59.2, 54.5, 47.8, 43.2. $^{195}$Pt{$^1$H} NMR (86 MHz, DMF-$d_7$): $\delta$ –382. IR (KBr, cm$^{-1}$): 3224 m, 3075 m, 3027 m, 2961 w, 1656 vs, 1571 m, 1496 vw, 1453 w, 1409 w, 1329 w, 1298 w, 1266 w, 1203 vw, 1131 vw, 1098 w, 1048 w, 750 m, 701 m, 662 w, 613 w, 595 w, 575 w. ESI-MS (negative-ion mode): $m/z$ 542.5 ([M–H]$^-$, calcd. 542.0), 1086.6 ([2M–H]$^-$, calcd. 1086.9). Anal. Calcd. for 4, C$_{11}$H$_{17}$Cl$_4$N$_3$OPt: C, 24.28; H, 3.15; N, 7.72. Found: C, 24.66; H, 3.36; N, 7.43.

**Synthesis of [Pt(edDs)Cl$_4$] (5).** A solution of iodobenzene dichloride (0.107 g, 0.390 mmol) in 2 mL of DMF was added dropwise to a solution of [Pt(edDs)Cl$_2$] (0.250 g, 0.379 mmol) in 16 mL of DMF. The color of the solution changed immediately from pale yellow to pale orange. After stirring 12 h at rt, 80 mL of diethyl ether was added to form a sticky residue on the flask bottom. The diethyl ether and DMF were decanted, and 30 mL of water was added to the residue. The resulting solid was collected by filtration. It was suspended sequentially in water, methanol, and diethyl ether before being dried in vacuo. Yield: 0.103 g (33%). M.p. 245–254 °C (dec). $^1$H NMR (400 MHz, DMF-$d_7$): $\delta$ 8.57–8.53 (m, 2H), 8.41 (d, 1H), 8.23 (d, 1H), 8.01 (t, 1H), 7.90 (br s, 1H), 7.71–7.61 (m, 3H), 7.32 (d, 1H), 6.92 (br s, $^2J_{PH} = 58$ Hz, 1H), 4.35–4.30 (m, 1H), 3.74–3.68 (m, 1H), 3.37–3.29 (m, 3H), 3.16–3.05 (m, 5H), 2.89 (s, 6H). $^{13}$C{$^1$H} NMR (100 MHz, DMF-$d_7$): $\delta$ 166.7, 152.0, 136.6, 130.0, 129.9, 129.8, 128.9, 128.2, 123.9, 119.8, 115.6, 58.9, 54.3, 47.7, 45.2, 42.3, 40.0. $^{195}$Pt{$^1$H} NMR (86 MHz, DMF-$d_7$): $\delta$ –386. IR (KBr, cm$^{-1}$): 3346 s, 3307 m, 3185 m, 3075 m, 2956 w, 2872 w, 2790 w, 1688 vs, 1613 w, 1573 m, 1543 m,
1480 w, 1450 m, 1431 m, 1412 m, 1316 s, 1254 w, 1234 w, 1158 s, 1141 vs, 1085 m, 1063 m, 1041 m, 960 m, 942 m, 910 w, 839 w, 814 m, 788 s, 681 w, 627 s, 574 s, 554 w, 499 w. ESI-MS (negative-ion mode): m/z 728.5 ([M–H]−, calcd. 728.0), 1458.6 ([2M–H]−, calcd. 1459.0). Anal. Calcd. for 5, C18H27Cl4N3O3PtS: C, 29.60; H, 3.73; N, 9.59. Found: C, 29.55; H, 3.71; N, 9.36.

Synthesis of [Pt(edBz2)Cl4] (6). To a suspension of [Pt(edBz2)Cl2] (200 mg, 0.322 mmol) in 5 mL of DMF, a solution of iodobenzene dichloride (91 mg, 0.33 mmol) in 1 mL of DMF was added in a dropwise manner. The suspension became a bright yellow solution, which was allowed to stir at rt for 1 h. The solution was filtered and 200 mL of Et2O was added. After 10 min, a fine yellow solid deposited. This solid was isolated by vacuum filtration, washed twice with 10 mL of Et2O, and then dried under vacuum. Yield: 0.108 g (49%). M.p. > 200 °C (gradual browning), 255–265 °C (dec into black char). 1H NMR (400 MHz, DMF- d7): R,R/S,S + R,S diastereomers (3:1) δ 8.95 + 8.92 (triplets, 2H, NH amide), 7.39–7.28 (overlapping multiplets, 12H, 5H aromatic + NH), 4.50 + 4.45 (doublets, 4H, benzyl CH2), 4.23–3.80 (multiplets, 4H, CH2 adjacent to amide), 3.60–3.20 (broad multiplets, 4H, CH2 ethylenediamine backbone). 13C{1H} NMR (100 MHz, DMF-d7): R, R/S,S + R,S diastereomers (3:1) δ 166.6 + 166.5, 139.2 + 139.1, 128.7, 127.8, 127.4, 57.4 + 57.2, 55.0 + 54.3, 43.3 + 43.2. 195Pt{1H} NMR (86 MHz, DMF-d7): R,R/S,S + R,S diastereomers (3:1) δ –370 (minor), –378 (major). IR (KBr, cm−1): 3440 m, 3294 m, 3153 w, 3105 w, 2924 w, 2876 w, 1657 s, 1584 w, 1571 m, 1495 vw, 1450 w, 1384 w, 1410 w, 1324 w, 1277 w, 1216 vw, 1068 w, 758 m, 704 m, 508 w. ESI-MS (negative-ion mode): m/z 580.9 ([M–4H–3Cl]−, calcd. 581.1), 616.9 ([M–3H–2Cl]−, calcd. 617.1), 652.9 ([M–2H–Cl]−, calcd. 653.1), 689.0 ([M–H]−, calcd. 689.0), 1381.1 ([2M–H]−, calcd. 1380.9). Anal. Calcd. for 6, C20H26Cl4N4O2Pt: C, 34.75; H, 3.79; N, 8.10. Found: C, 34.70; H, 3.66; N, 8.20.
**X-Ray Crystallography.** Single crystals of 1·DMF and 4·DMF were grown by vapor diffusion of diethyl ether into DMF solutions. Single crystals of 3 and 6 were grown by slow evaporation of a DMF solution and vapor diffusion of water into a DMF solution, respectively. Single crystals were mounted in Paratone oil on cryoloops and frozen under a 100 K KRYO-FLEX nitrogen cold stream. In general, data were collected on a Bruker APEX CCD X-ray diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) controlled by the APEX2 software package. For crystals other than those of 3, absorption corrections were applied using SADABS. The structures were solved using direct methods and refined on $F^2$ with the SHELXTL-97 software package. Structures were checked for higher symmetry using PLATON. All non-hydrogen atoms were located and refined anisotropically. Hydrogen atoms were placed in idealized locations and given isotropic thermal parameters equivalent to either 1.5 (terminal CH$_3$ hydrogen atoms) or 1.2 times the thermal parameter of the atom to which they were attached. X-ray crystallographic data collection and refinement parameters are presented in Table 3.1.
### Table 3.1. X-Ray Crystallographic Data Collection and Refinement Parameters for 1-DMF, 3, 4-DMF, and 6.

<table>
<thead>
<tr>
<th></th>
<th>1-DMF</th>
<th>3</th>
<th>4-DMF</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>formula</td>
<td>C\textsubscript{14}H\textsubscript{24}Cl\textsubscript{2}N\textsubscript{4}O\textsubscript{2}Pt</td>
<td>C\textsubscript{20}H\textsubscript{26}Cl\textsubscript{2}N\textsubscript{4}O\textsubscript{2}Pt</td>
<td>C\textsubscript{14}H\textsubscript{24}Cl\textsubscript{4}N\textsubscript{4}O\textsubscript{2}Pt</td>
<td>C\textsubscript{20}H\textsubscript{26}Cl\textsubscript{4}N\textsubscript{4}O\textsubscript{2}Pt</td>
</tr>
<tr>
<td>fw</td>
<td>546.36</td>
<td>620.44</td>
<td>617.26</td>
<td>691.34</td>
</tr>
<tr>
<td>space group</td>
<td>\textit{P2}\textsubscript{1}/c</td>
<td>\textit{P}1</td>
<td>\textit{P2}\textsubscript{1}/c</td>
<td>\textit{P}4\textsubscript{2}2\textsubscript{1}</td>
</tr>
<tr>
<td>(a), Å</td>
<td>13.7730(9)</td>
<td>9.2382(10)</td>
<td>14.4637(8)</td>
<td>11.3811(3)</td>
</tr>
<tr>
<td>(b), Å</td>
<td>12.6440(8)</td>
<td>12.9438(14)</td>
<td>11.2251(6)</td>
<td>18.4022(11)</td>
</tr>
<tr>
<td>(c), Å</td>
<td>11.2843(7)</td>
<td>18.045(2)</td>
<td>13.3621(8)</td>
<td>18.045(2)</td>
</tr>
<tr>
<td>(\alpha), deg</td>
<td>80.2930(17)</td>
<td>102.6200(10)</td>
<td>88.9920(18)</td>
<td>109.8980(10)</td>
</tr>
<tr>
<td>(\beta), deg</td>
<td>1917.6(2)</td>
<td>2126.5(4)</td>
<td>2039.9(2)</td>
<td>2383.63(17)</td>
</tr>
<tr>
<td>(\gamma), deg</td>
<td>90.0000(16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V), Å(^3)</td>
<td>4</td>
<td>4 1.123, -0.447</td>
<td>4.783, -2.435</td>
<td>2.011, -0.894</td>
</tr>
<tr>
<td>(\rho_{\text{calcld.}}, \text{g/cm}^3)</td>
<td>1.892</td>
<td>1.938</td>
<td>2.010</td>
<td>1.926</td>
</tr>
<tr>
<td>(T), °C</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
</tr>
<tr>
<td>(\mu) (Mo (K\alpha)), mm(^{-1})</td>
<td>7.608</td>
<td>6.874</td>
<td>7.418</td>
<td>6.360</td>
</tr>
<tr>
<td>(\theta) range, deg</td>
<td>1.52-29.61</td>
<td>1.60 to 25.06</td>
<td>1.50-25.05</td>
<td>2.10 to 28.71</td>
</tr>
<tr>
<td>total no. of data</td>
<td>41701</td>
<td>58892</td>
<td>31699</td>
<td>50603</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>5382</td>
<td>7479</td>
<td>3604</td>
<td>3092</td>
</tr>
<tr>
<td>no. of parameters</td>
<td>210</td>
<td>518</td>
<td>228</td>
<td>142</td>
</tr>
<tr>
<td>completeness to (\theta), (%)</td>
<td>99.8</td>
<td>99.4</td>
<td>99.8</td>
<td>99.9</td>
</tr>
<tr>
<td>(R1^a) (%)</td>
<td>2.49</td>
<td>8.00</td>
<td>3.42</td>
<td>2.42</td>
</tr>
<tr>
<td>(wR2^b) (%)</td>
<td>3.95</td>
<td>12.58</td>
<td>6.98</td>
<td>4.11</td>
</tr>
<tr>
<td>(R1^a) (%) for (I &gt; 2\sigma)</td>
<td>1.77</td>
<td>4.93</td>
<td>2.81</td>
<td>1.92</td>
</tr>
<tr>
<td>(wR2^b) (%) for (I &gt; 2\sigma)</td>
<td>3.72</td>
<td>11.07</td>
<td>6.66</td>
<td>4.11</td>
</tr>
<tr>
<td>GOFC</td>
<td>1.074</td>
<td>1.024</td>
<td>1.086</td>
<td>1.058</td>
</tr>
<tr>
<td>max, min peaks, eÅ(^{-3})</td>
<td>1.123, -0.447</td>
<td>4.783, -2.435</td>
<td>2.011, -0.894</td>
<td>0.817, -0.558</td>
</tr>
<tr>
<td>Flack parameter</td>
<td></td>
<td></td>
<td></td>
<td>-0.012(7)</td>
</tr>
</tbody>
</table>

\(^a\) \(R1 = \Sigma |F_o| - |F_i|/\Sigma |F_o|\). \(^b\) \(wR2 = \{\Sigma [w(F_o^2 - F_c^2)]^2/\Sigma [w(F_o^2)]\}^{1/2}\). \(^c\) GOFC = \{\Sigma [w(F_o^2 - F_c^2)]/(n - p)\}^{1/2}\) where \(n\) is the number of data and \(p\) is the number of refined parameters.

Crystals of 3 all displayed signs of non-merohedral twinning in the form of split reflections. After full data collection, the program CELL\_NOW\(^{37}\) was used to look for additional domains. Two domains were found that accounted for 97% of the harvested reflections. The second domain was rotated by 6.8° about the \(c^*\) axis. The data were integrated over both domains using SAINT.\(^{38}\) An absorption correction was applied with the program TWINABS.\(^{39}\)

The corrected data were then analyzed for systematic absences and higher metric symmetry with
XPREP\textsuperscript{40} to determine the space group. The structure was solved with the SHELXTL-97 software package and refined using data from both domains. The second domain refined to a scale factor of 8.33\%. Two molecules of 3 are present in the asymmetric unit in the space group P\bar{1}. Restraints on the directionality and size of the thermal displacement parameters of the nitrogen and carbon atoms of the ethylenediamine backbone of one of the molecules in the asymmetric unit were applied. The largest electron density peak and hole are 4.78 and \(-2.44\) e-A\(^3\), located 1.19 Å from Pt2 and 0.78 Å from Pt1, respectively. This large residual density might in part be due the presence of additional twin domains. It should also be noted that the space group utilized was P\bar{1}. Both b and c are close to 90°, suggesting that a higher metric symmetry monoclinic space group might be more appropriate to describe the structure. Furthermore, the presence of two molecules of 3 in the asymmetric unit also raises concerns as to whether these two species are symmetry-related in a monoclinic space group. Structure solution in \(P2_1/c\) gave basic atomic connectivity. During refinement, however, thermal ellipsoids attained unreasonable sizes and many became non-positive definite upon anisotropic refinement, a problem not encountered in P\bar{1}. Furthermore, the merging and refinement statistics and the standard deviations of the bond distances and angles for the \(P2_1/c\) solution and refinement were substantially worse than those in the triclinic space group. The two molecules in the asymmetric unit in the P\bar{1} solution exhibit only one obvious conformational difference. In particular, the tilting of the five-membered chelate rings is different, as discussed in more detail below. Searches for higher symmetry space groups with PLATON on the P\bar{1} solution were unsuccessful.

**Theoretical Calculations.** Density functional theory (DFT) calculations were performed with the GAUSSIAN 03 (Rev.D01) software package.\textsuperscript{41} All calculations were carried out using the
B3LYP functional. The LANL2DZ basis set and effective core potential was used for the Pt atom and the 6-31++G(d,p) basis set was used for all other atoms. Geometries of 2 and 5 were optimized in the gas phase and established as local minima by frequency calculations, which revealed no imaginary values. The conductor-like polarizable continuum model (CPCM) was applied for subsequent molecular orbital and time-dependent DFT (TDDFT) calculations to simulate solvation of the compounds in water. The TDDFT calculations were used to determine the natures, energies, and oscillator strengths of the 50 lowest energy singlet excited states.

3.3. Results and Discussion

Synthesis and Characterization. The platinum(II) complexes, 1 and 2, were prepared by an amide coupling reaction between [Pt(edma)Cl₂] and benzylamine or Ds-en, respectively. Similarly, using two equiv of the necessary coupling reagents, benzylamine was coupled to the dicarboxylic acid complex [Pt(edda)Cl₂] (Scheme 3.1). This amide coupling reaction can potentially be used to tether other desired amines to the core platinum(II) moieties. It is analogous to the amide coupling chemistry that has been employed to conjugate chemical moieties to the platinum(IV) complex, c,c,t-[Pt(NH₃)₂Cl₂(O₂CC₂H₄CO₂H)₂], a derivative of cisplatin. Similar platinum(II) and platinum(IV) complexes bearing ethylenediamine-N,N'-diester ligands have been reported previously. In these cases, however, the esterified ligands were prepared prior to metallation. For a series of platinum(II) peptide conjugates that were synthesized on a solid-phase support, it was necessary to protect the platinum-chelating unit during the synthesis of the peptide fragment. The advantage of performing the coupling reaction directly on the coordinated ligand, as demonstrated here, is that it eliminates the need for protecting groups during organic or peptide synthesis. We envision that this synthetic
approach will be general and provide access to a range of interesting functionalized platinum(II) complexes.

Scheme 3.1. Syntheses of 1–3.

The oxidation of the platinum(II) complexes with iodobenzene dichloride in DMF cleanly afforded the expected diaminotetrachloroplatinum(IV) analogs (Scheme 3.2). Alternatively, the use of Br₂ as an oxidant for 3 gave rise to a mixture of diaminotetrahalidoplatinum(IV) complexes varying in respective ratios of chloride to bromide.⁵³ Previously described methods to generate diaminotetrachloroplatinum(IV) complexes utilize a two-step reaction sequence.⁵⁴ The first step is formation of a trans-hydroxo complex by oxidation with aqueous H₂O₂. The following step is the ligand substitution of the hydroxide ligand carried out by hydrochloric acid. The use of iodobenzene dichloride to access diaminotetrachloroplatinum(IV) complexes in a single step, described here, is advantageous.
Verification of successful amide bond formation for 1–3 is provided in part by IR spectroscopy. The strong stretching frequency of the free carboxylic acid near 1720 cm\(^{-1}\) present in the starting materials is absent in the amide-containing products. Instead lower energy C=O stretching frequencies are present at 1654, 1659, and 1662 cm\(^{-1}\) for 1–3, respectively, which correspond to the newly formed amides. The platinum(IV) complexes, 4–6, display similar IR spectroscopic features. New Pt–Cl stretching modes, which typically range between 300 and 400 cm\(^{-1}\), expected for these complexes could not be observed within the window of the spectrometer used (4000–400 cm\(^{-1}\)). Elemental analysis and ESI-MS further validate the proposed compositions and structures of 1–6.

The \(^1\)H and \(^{13}\)C NMR spectra obtained in DMF-\(d_7\) of 1, 2, 4, and 5 are consistent with the proposed structures. The aliphatic regions of the \(^1\)H NMR spectra are characterized by complex splitting patterns due diastereotopic protons on and near the ethylenediamine backbone. The proton of the amide group resonated between 8.2 and 9.0 ppm in the four complexes as a triplet. Upon formation of the platinum(IV) complexes, resonances due to the coordinated NH and NH\(_2\) groups shift downfield, consistent with oxidation of the platinum center (Figure 3.1). Additionally, the NH resonances of the coordinated secondary amine in the platinum(IV)
complexes display satellites arising from coupling to the $^{195}$Pt nucleus ($I = 1/2, 33\%$), with $^2J_{PH} = 56$ and $58$ Hz for 4 and 5, respectively. Platinum(IV) complexes give rise to well-resolved Pt satellites, unlike those of platinum(II).\textsuperscript{55} The $^{13}$C NMR spectra display all anticipated resonances.

![Figure 3.1. $^1$H NMR spectra in the aromatic region of 1 (top in red) and 4 (bottom in blue) recorded in DMF-$d_7$ at 20 °C with a field strength of 400 MHz. The asterisk marks the signal due to a protic impurity in the solvent.](image)

For 3 and 6, NMR spectroscopy in DMF-$d_7$ revealed the presence of two species in solution. Because both coordinating nitrogen atoms of 3 and 6 are chiral, both the enantiomeric $R,R/S,S$ and meso $R,S$ diastereomers exist. The two distinct, yet chemically similar, species observed in solution are assigned as these two diastereomers. The ratio of these diastereomers for 3 observed by NMR spectroscopy is 1:1, whereas for 6 it is 3:1. As for 1, 2, 4, and 5 amide protons resonate between 8.5 and 9.0 ppm. The NH resonances of the coordinated amine ligand in the $^1$H NMR spectrum are shifted downfield relative to those of 3 and are largely obscured by
overlapping resonances of the aromatic protons. This overlap prevents observation of $^{195}$Pt coupling to the NH protons, as was detected for 4 and 5.

The $^{195}$Pt NMR spectra of 1 and 2 reveal resonances at $-2339$ and $-2345$ ppm, respectively. For 3, two peaks, corresponding to the two diastereomers, are present in a 1:1 ratio at $-2347$ and $-2362$ ppm. These values are typical for platinum(II) in an N$_2$Cl$_2$ coordination environment, and the similarity between them indicates that the peripheral substituents on the amide do not significantly affect shielding at the platinum nucleus. Upon oxidation, a large downfield shift occurs as expected. Compounds 4 and 5 resonate at $-382$ and $-386$ ppm, respectively. The signal of the major diastereomer of 6 is at $-378$ ppm in the $^{195}$Pt NMR spectrum, whereas that of the minor diastereomer resonates if found at $-370$ ppm. As with the platinum(II) complexes, the close proximity of all the resonances indicates that the coordination environments of the platinum centers are substantially the same with almost no effect due to the peripheral amide substituents.

X-Ray Crystal Structures. Single crystals of 1-DMF, 3, 4-DMF, and 6 were analyzed by X-ray diffraction and structurally characterized. Selected interatomic distances and angles for 1-DMF and 3 are collected in Table 3.2. These structures are shown in Figure 3.2. Both 1 and 3 attain a square planar coordination geometry as expected for platinum(II). The platinum(II)-ligand distances are typical as well. A molecule of DMF is in the crystal lattice of 1 and forms a hydrogen bonding interaction with the NH group of the amide linkage, with $O_{DMF} - N_{amide} = 2.83$ Å. Also present in the lattice of both 1 and 3 are short intermolecular Pt--Pt interactions, marked by Pt--Pt distances of 3.30 and 3.38 Å, respectively. The X-ray crystal structure of the analogous compound, [Pt(en)Cl$_2$] (en = ethylenediamine), has been determined for two distinct polymorphs. A careful crystal packing analysis of these polymorphs revealed close Pt--Pt
intermolecular interactions, similar to those observed for 1 and 3. In the orthorhombic form of [Pt(en)Cl₂],⁵⁷ the Pt...Pt separation is 3.38 Å, whereas the Pt...Pt separation in the triclinic polymorph⁵⁸ is 3.42 Å. These Pt...Pt interactions are partially supported by hydrogen bonds between the chloride ligands and coordinated amine protons of the neighboring molecules. As mentioned above, 3 exists as two possible diastereomers in solution. Both molecules of 3 in the asymmetric unit are meso-R,S diastereomers as conveyed by their nitrogen centers. A significant difference between them is the canting of the five-membered chelate ring. The conformation of non-planar five-membered chelate rings produces two different chiral orientations of the ligand, referred to as λ and δ (Figure 3.3).⁵⁹ One of the molecules in the asymmetric unit has the λ chelate ring conformation, whereas the other displays a δ chelate ring conformation. No disorder occurs in the ethylenediamine backbone, confirming that the two molecules are crystallographically distinct and not symmetry related.

Figure 3.2. Solid-state molecular structures of 1 and 3. Ellipsoids are drawn at 50% probability levels. The DMF molecule in the lattice with 1 is omitted. The other molecules of 3 in the asymmetric unit is not shown.
Table 3.2. Selected Interatomic Distances (Å) and Angles (°) for 1 and 3.

<table>
<thead>
<tr>
<th></th>
<th>1·DMF</th>
<th>3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt1—Cl1</td>
<td>2.3142(5)</td>
<td>2.310(2)</td>
</tr>
<tr>
<td>Pt1—Cl2</td>
<td>2.3246(6)</td>
<td>2.303(2)</td>
</tr>
<tr>
<td>Pt1—N1</td>
<td>2.024(2)</td>
<td>2.060(8)</td>
</tr>
<tr>
<td>Pt1—N2</td>
<td>2.0425(18)</td>
<td>2.061(8)</td>
</tr>
<tr>
<td>N1—Pt1—N2</td>
<td>84.14(7)</td>
<td>84.3(3)</td>
</tr>
<tr>
<td>N1—Pt1—Cl1</td>
<td>90.87(5)</td>
<td>94.1(2)</td>
</tr>
<tr>
<td>N1—Pt1—Cl2</td>
<td>174.38(5)</td>
<td>175.2(2)</td>
</tr>
<tr>
<td>N2—Pt1—Cl1</td>
<td>174.97(6)</td>
<td>178.3(2)</td>
</tr>
<tr>
<td>N2—Pt1—Cl2</td>
<td>90.28(6)</td>
<td>90.9(2)</td>
</tr>
<tr>
<td>Cl1—Pt1—Cl2</td>
<td>94.72(2)</td>
<td>90.74(9)</td>
</tr>
</tbody>
</table>

Atoms are labeled as indicated in Figure 3.2. Numbers in parentheses are estimated standard deviations of the last significant figures. Two structurally similar molecules of 3 are present in the asymmetric unit. The distances and angles of only one of those are presented in this table.

Figure 3.3. Depiction of the λ and δ isomers of 3 found in the asymmetric unit. Hydrogen atoms and phenyl rings are omitted for clarity.
The platinum(IV) complexes 4 and 6 attain expected octahedral coordination geometries, as shown in Figure 3.4. Selected interatomic distances and angles for these complexes are collected in Table 3.3. The coordination spheres are comprised of four chloride ligands and the chelating diamine. Compound 4, like 1, crystallizes with a molecule of DMF. This DMF molecules also hydrogen bonds to the amide proton with $O_{\text{DMF}}$-$\cdot$-$N_{\text{amide}} = 2.81 \text{ Å}$. The platinum atom of 6 resides on a crystallographic 2-fold symmetry axis in the chiral space group $P4_32_12$. The 2-fold axis requires that both coordinating nitrogen atoms attain the same stereochemistry, and for the crystal studied the complex is the $S,S$ enantiomer. The fact that the Flack parameter refined to a value near zero confirms this choice. The conformation of the chelate ring is $\delta$.

Figure 3.4. Solid-state molecular structures of 4 and 6. Ellipsoids are drawn at 50% probability levels. The DMF molecule in the lattice with 1 is omitted.
Table 3.3. Selected Interatomic Distances (Å) and Angles (°) for 4 and 6.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distances</td>
<td>Angles</td>
</tr>
<tr>
<td>Pt1—N1</td>
<td>2.017(4)</td>
<td>173.63(15)</td>
</tr>
<tr>
<td>Pt1—N2</td>
<td>2.109(6)</td>
<td>94.22(14)</td>
</tr>
<tr>
<td>Pt1—Cl1</td>
<td>2.3041(15)</td>
<td>91.87(14)</td>
</tr>
<tr>
<td>Pt1—Cl2</td>
<td>2.3216(14)</td>
<td>85.70(14)</td>
</tr>
<tr>
<td>N1—Pt1—N2</td>
<td>91.43(5)</td>
<td>91.06(6)</td>
</tr>
<tr>
<td></td>
<td>83.30(18)</td>
<td>90.09(5)</td>
</tr>
<tr>
<td>N1—Pt1—Cl1</td>
<td>91.12(12)</td>
<td>91.05(5)</td>
</tr>
<tr>
<td></td>
<td>88.69(13)</td>
<td>91.12(12)</td>
</tr>
<tr>
<td>N1—Pt1—Cl3</td>
<td>87.86(13)</td>
<td>91.05(5)</td>
</tr>
</tbody>
</table>

\(^a\) Atoms are labeled as indicated in Figure 3.4. Numbers in parentheses are estimated standard deviations of the last significant figures.

**Photophysical Properties of 2 and 5.** The absorption and emission properties of the fluorescent compounds 2, 5, and Ds-en were measured in both DMF and phosphate-buffered saline (PBS) at pH 7.4. The results are summarized in Table 3.4. In DMF, the absorption and emission maxima of the three compounds are nearly identical. This result is expected because the optical properties of both compounds are dominated by relatively intense \(\pi-\pi^*\) transitions of the dansyl fluorophore. In moving from DMF to water, the absorption maxima of the three compounds are blue-shifted by 10 nm, and the emission maxima are red-shifted by approximately 40 nm. As seen in Table 3.4, the emission quantum yields depend on both the presence of the platinum center and its oxidation state, as well as the solvent. In DMF, the platinum(II) center of 2 slightly quenches the emission, with the quantum yield dropping to 27% from the 40% value measured for free Ds-en. The relatively high quantum yield of 27% is in contrast to the results described in Chapter 2 in which the presence of a platinum(II) center lowered the quantum yield of the dansylated di-2-pyridyl(methane) ligand by a factor of 15. In the previous system, however, the dansyl fluorophore was positioned much more closely to the platinum(II) center. Quenching of
Dansyl fluorescence by platinum(II) through the heavy-atom effect has been shown to depend on the spatial separation between the two moieties. The higher quantum yield of 2 most likely derives from the long distance of the dansyl fluorophore from the platinum(II) center. The emission quantum yield of the platinum(IV) complex, 5, in DMF is only 1.6%, significantly less than that of 2. This result indicates 5 to be a good candidate for displaying the desired fluorescence turn-on response upon reduction to Pt(II).

Table 3.4. Photophysical Properties of 2, 5, and Ds-en in DMF and Buffered Aqueous Solution.

<table>
<thead>
<tr>
<th>Compound</th>
<th>DMF $\lambda_{\text{max}}$ (nm), $\varepsilon \times 10^3$ (M$^{-1}$-cm$^{-1}$)</th>
<th>PBS $\lambda_{\text{max}}$ (nm), $\varepsilon \times 10^3$ (M$^{-1}$-cm$^{-1}$)</th>
<th>DMF $\lambda_{\text{em}}$ (nm), $\Phi$</th>
<th>PBS $\lambda_{\text{em}}$ (nm), $\Phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>339, 4.8</td>
<td>328, 4.6</td>
<td>520, 0.27</td>
<td>560, 0.033</td>
</tr>
<tr>
<td>5</td>
<td>333, 5.19</td>
<td>325, 5.1</td>
<td>520, 0.16</td>
<td>560, 0.0027</td>
</tr>
<tr>
<td>Ds-en</td>
<td>333, 4.7</td>
<td>328, 4.6</td>
<td>520, 0.40</td>
<td>555, 0.032</td>
</tr>
</tbody>
</table>

In moving from DMF to PBS, significant fluorescence quenching occurs for all three compounds. The quantum yields of Ds-en and 2 drop to values of 3.2% and 3.3%, respectively. This result is expected because the emission of the dansyl fluorophore depends strongly on solvent polarity. Importantly, the increased quenching effect of the platinum(IV) compound, 5, carries over to the aqueous environment where the 0.27% quantum yield for emission is still much lower than that of 2. In water, 5 will therefore elicit a fluorescence turn-on response when reduced to platinum(II), as described below.

**DFT Calculations.** Time-dependent DFT (TDDFT) calculations were performed to gain an understanding of the origin of the decreased emission of 5 relative to that of 2. The frontier molecular orbital diagrams of 2 and 5 are shown in Figure 3.5. The HOMO for both complexes is a dansyl-based $\pi$ orbital. The LUMOs of 2 and 5 are different, however. In the case of 2, the
LUMO, like the HOMO, is a dansyl-based π orbital. In 5, the octahedral coordination geometry shifts the energies of the $d_{xy}$ and $d_{z^2}$ metal orbitals to an energy that lies between those of the dansyl-based occupied and unoccupied orbitals, resulting in a LUMO that is metal-based. To understand how these molecular orbitals affect the photophysical properties of the complexes, the 50 lowest energy singlet excited states were computed for both 2 and 5. Both compounds are computed to have an identical, dansyl-based excited state in the visible region (3.32 eV, 373 nm, $S_3$ for 2, $S_{16}$ for 5) with a large oscillator strength ($f = 0.1741$), corresponding to the HOMO-LUMO transition for 2 and the HOMO-LUMO+2 transition for 5 (Figure 3.5). Because this excited state transition is the only one that is strongly allowed following irradiation with visible light ($\lambda > 310$ nm, $f > 0.01$) and is localized entirely on the dansyl moiety, it is therefore responsible for emission as it relaxes to the ground state. If lower-energy excited states are available, however, relaxation can occur non-radiatively through them. In 2, only two excited states, both ligand field (d–d) in character, are lower in energy than the allowed HOMO–LUMO transition. Compound 5, on the other hand, has 15 excited states that are lower in energy than its allowed HOMO–LUMO+2 transition. Of these 15, the first and second lowest-energy excited states can be described as $\pi$ (dansyl) $\rightarrow$ d $\sigma^*$ (Pt) charge-transfer states ($S_1$, HOMO–LUMO and $S_2$, HOMO–LUMO+1). As shown on the right side of Figure 3.5 by the black downward arrows, these excited states provide plausible non-radiative decay pathways that can be rationalized based on molecular orbital energies alone. These competing non-radiative pathways lower the overall quantum yield for emission of 5, relative to that of 2.
Figure 3.5. Frontier Kohn-Sham molecular orbital diagram for 2 and 5. The high oscillator transition of the dansyl group, calculated by TDDFT, is indicated.

Response to Reducing Agents. Figure 3.6 displays the emission turn-on response induced by treatment of 5 with a 10-fold excess of glutathione in PBS at pH 7.4. Substitution of other biological reducing agents, cysteine and ascorbic acid, for glutathione afforded nearly identical results. The magnitude and speed of the turn-on response is the same for the three reducing agents. Within a minute after addition of the reducing agents, the turn-on response is complete, with no further increase in emission for up to 15 min. The kinetic behavior of this turn-on response is consistent with the ability of ascorbic acid to rapidly reduce a similar compound, [Pt(en)Cl₂]. The turn-on response upon reduction affords a 6.3-fold increase in integrated...
emission intensity. Control experiments were carried out in which 5 was treated with the non-reducing amino acid glycine, and the platinum(II) complex, 2, was similarly treated with glycine as well as the three biological reducing agents. The emission of 2 did not change upon introduction of any of the four compounds, as anticipated. When 5 was treated with glycine, a 1.2-fold increase in emission was observed after several scans and minutes. In an additional control experiment, 5 alone in buffer showed a similar emission increase after several scans in the fluorimeter. This small but noticeable emission increase most likely arises from photoreduction of 5 in the fluorimeter. Such photoreduction of the metal center is consistent with the proposed fluorescence quenching mechanism by which non-radiative relaxation occurs through the population of metal-based orbitals.

![Emission spectra](image)

**Figure 3.6.** Emission spectra of 5 before (solid black) and after (dashed red) the addition of 10 equiv of glutathione in pH 7.4 phosphate-buffered saline. The small peak at 680 nm arises from scattered excitation light ($\lambda_{ex} = 340$ nm).
3.4. Summary and Conclusions

The platinum(II) complexes [Pt(edma)Cl₂] and [Pt(edda)Cl₂] can be readily coupled to amine functional groups through amide bond formation at the non-coordinating carboxylic acid moieties of the ligands, as demonstrated here in the syntheses of 1 and 3 from benzylamine. The convenient amide coupling chemistry employed for their syntheses can be used to access many different derivatives with the goal of either making effective anticancer agents or complexes that act as mechanistic probes. Fluorophore-labeled construct 2 was prepared as just such a probe by using this synthetic strategy. The clean oxidation of these three complexes was demonstrated with the hypervalent iodine reagent iodosobenzene dichloride. The fluorescent platinum(II)/platinum(IV) pair, 2 and 5, are useful for investigating the reduction of platinum(IV) complexes. Compound 5 elicits an emission turn-on response upon reduction to 2. Compound 5 therefore has the potential to serve as a mechanistic probe to monitor the reduction platinum(IV) complexes in biology. In theory, such a turn-on response should enable both spatial and temporal resolution of the platinum(IV) reduction event by fluorescence microscopy. Unfortunately, the practical applicability of this complex in living cells is limited by its low quantum yield in water and the rapid rate of reduction. As a first generation platinum(IV) redox sensor, however, 5 provides a proof of concept and starting point for the design of more effective sensors of the properties of platinum(IV) complexes in biological milieu.

3.5. References


Chapter 4

In Vitro Anticancer Activity of Platinum(II) Complexes with β-
Diketonate Leaving Group Ligands

Reproduced in part with permission from Wilson, J. J.; Lippard, S. J. J. Med. Chem. 2012, 55,
5326-5336. Copyright 2012 American Chemical Society.
4.1. Introduction

As described in Chapter 1, the administration of cisplatin produces toxic side effects,\(^1\) the amelioration of which inspired the discovery of the second-generation chemotherapeutic agent, carboplatin (Chart 4.1). Cisplatin and carboplatin operate by the same mechanism. Labile ligands in the coordination sphere, chloride for cisplatin and 1,1-cyclobutanedicarboxylate (CBDCA) for carboplatin, are displaced by water or other nucleophiles, and the activated \textit{cis-}\textit{diammineplatinum(II)} moiety binds to purine bases in nuclear DNA.\(^2\) The resulting platinum-DNA adducts, chiefly 1,2-intrastrand cross-links, ultimately lead to the death of cancer cells through transcription inhibition and downstream effects.\(^3\) Because cisplatin and carboplatin bear the same NH\(_3\) nonleaving group ligands, the resulting DNA adducts are identical,\(^4\) and the drugs therefore exhibit the same spectrum of activity.\(^5\) Carboplatin, however, is significantly less toxic than cisplatin. The typical patient dose for carboplatin is approximately ten times greater than that of cisplatin (400 mg/m\(^2\) versus 40 mg/m\(^2\)), and the dose-limiting toxic side effect of carboplatin is myelosuppression in contrast to nephrotoxicity for cisplatin.\(^6\) These important clinical differences reveal that the leaving group ligands can play an important role in modulating toxic side effects. Further modification of these units may therefore be of value in the search for new platinum anticancer drug candidates.

As anticipated, the properties of carboplatin spurred the design of cisplatin analogues having other leaving groups.\(^7\)\(^{-20}\) The departing ligands of platinum anticancer agents influence not only the aquation rate but also the lipophilicity of the resulting complex. Both parameters, as exemplified by carboplatin, influence overall pharmacokinetic properties. Leaving group modifications have been applied to a class of cytotoxic platinum(II) complexes in which the remaining ligands have trans stereochemistry.\(^21\) Exchange of the original halide leaving groups...
of these trans platinum(II) compounds with carboxylates led to new complexes with improved aqueous solubility, hydrolytic stability, and cellular uptake.\textsuperscript{22-26}

Acetylacetonate (acac) and related $\beta$-diketonates form a diverse class of ligands with many applications in inorganic chemistry. The primary $O,O'$ bidentate chelating mode of these ligands is analogous to that of the CBDCA ligand of carboplatin, thus warranting consideration of their use as leaving group ligands for novel platinum anticancer drug candidates. Although $\beta$-diketonates have been used as supporting ligands for titanium(IV)\textsuperscript{27,28} and organometallic ruthenium(II) anticancer agents,\textsuperscript{29-32} few studies describe their potential utility in platinum-based therapeutic agents. In an early investigation, the complex \[ \text{[Pt(1,2-diaminocyclohexane)(acac)](NO₃)} \] was synthesized, but it displayed poor antitumor activity in vivo.\textsuperscript{31} On the basis of the structure-activity relationships at the time,\textsuperscript{34} the authors attributed this poor activity to the positive charge of the complex. More recently, the complex, \[ \text{[Pt(O,O'-acac)(\gamma-acac)(dimethylsulfide)]} \], was investigated for its anticancer properties.\textsuperscript{35-39} This nonconventional anticancer platinum complex apparently exerts its biological properties through a mechanism other than DNA binding. Only the $\gamma$-coordinated acac ligand behaves as a leaving group.

Chapters 2 and 3 have focused on strategies to modify the non-leaving group ligands of platinum(II) complexes. In this Chapter, this trend is shifted to exploring the modifications of leaving group ligands. As alluded to above, $\beta$-diketonates are explored as leaving group ligands for platinum(II) anticancer complexes. Five such cis-diammineplatinum(II) complexes bearing different $\beta$-diketonates were prepared and characterized (Chart 4.1). The particular variations on the $\beta$-diketonate ligands were selected to systematically modify the electronic properties and hydrophobicity of the resulting platinum complexes through the use of trifluoromethyl and
phenyl groups, respectively. We discovered that both of these properties have a direct effect on the overall cytotoxicity of the resulting complexes in cancer cells.

![Chemical structures](chart)

**Chart 4.1.** Structures of cisplatin, carboplatin, and β-diketonate compounds 1–5 described in this Chapter.

### 4.2. Experimental Methods

**General Considerations.** All synthetic procedures were performed under normal atmospheric conditions without exclusion of oxygen or moisture. Cisplatin and Ba(acac)$_2$·H$_2$O were purchased from Strem Chemicals and used as received. The β-diketonate ligands were obtained from Alfa Aesar and used as received. Distilled water and analytical grade DMF were employed as solvents.
Physical Measurements. NMR measurements were recorded on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility at 20 °C. ^1^H and ^1^3^C{^1^H} NMR spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane, SiMe₄ (δ = 0 ppm). ^1^9^5^Pt{^1^H} and ^1^9^F{^1^H} NMR spectra were referenced externally using standards of K₂PtCl₄ in D₂O (δ = −1628 ppm relative to Na₂PtCl₆) and trifluorotoluene (δ = −63.72 ppm relative to CFCl₃), respectively. Fourier transform infrared (FTIR) spectra were recorded with a ThermoNicolet Avatar 360 spectrophotometer running the OMNIC software. Samples were prepared as KBr disks. Electrospray ionization mass spectrometry (ESI-MS) measurements were acquired on an Agilent Technologies 1100 series LC-MSD trap. Graphite furnace atomic absorption spectrometry (GFAAS) was carried out using a Perkin-Elmer A Analyst600 GFAAS. Elemental analyses were performed by a commercial analytical laboratory.

Synthesis of [Pt(acac)(NH₃)₂](SO₄)₀.₅ (I). A suspension of cisplatin (0.500 g, 1.67 mmol) and Ag₂SO₄ (0.499 g, 1.60 mmol) in 15 mL of water was stirred at room temperature in the dark for 12 h. The resulting solids were filtered to remove white AgCl, and to the filtrate, a 10 mL aqueous solution of Ba(acac)₂·H₂O (0.268 g, 0.800 mmol) was added dropwise, inducing the precipitation of insoluble BaSO₄. This suspension was stirred at room temperature for 3 h and then filtered. The orange–brown filtrate was concentrated to dryness under reduced pressure at 60 °C to afford a brown residue. The residue was dissolved in 5 mL of MeOH at 65 °C and filtered through Celite to remove an insoluble impurity. Treatment of this MeOH solution with 15 mL of Et₂O yielded the desired compound as a white solid, which was isolated by centrifugation and washed an additional two times with 10 mL of Et₂O before drying in vacuo. Yield: 0.438 g (70%). Mp >140 °C (gradual darkening and decomposition). ^1^H NMR (400 MHz,
MeOD-d₄): δ 5.58 (s, 1H), 1.85 (s, 6H). ¹³C(¹H) NMR (100 MHz, MeOD-d₄): δ 185.6, 103.3, 26.1. ¹⁹⁵Pt(¹H) NMR (86 MHz, MeOD-d₄): δ −1593. IR (KBr, cm⁻¹): 3446 m, 3179 s, 3134 s, 3075 m, 1564 s, 1525 vs, 1427 w, 1382 m, 1331 w, 1304 m, 1284 w, 1194 w, 1112 vs, 1024 w, 951 w, 938 w, 869 w, 831 w, 772 vw, 659 m, 619 m, 541 w, 480 w. ESI-MS (pos. ion mode, MeOH): m/z 328.1 ([M]+, calcd. 328.1). Anal. Calcd. for 1, C₅H₁₁N₂O₄Pt⁵S₀.₅: C, 15.96; H, 3.48; N, 7.44. Found: C, 15.82; H, 3.51; N, 6.94.

**Synthesis of [Pt(tfac)(NH₃)₂](SO₄)₀.₅ (2).** A suspension of cisplatin (0.500 g, 1.67 mmol) and Ag₂SO₄ (0.499 g, 1.60 mmol) in 15 mL of water was stirred at room temperature in the dark for 12 h. The resulting suspension was filtered to remove white AgCl, and to the filtrate, a 10 mL hot (70 °C) aqueous solution of Ba(OH)₂·8H₂O (0.252 g, 0.800 mmol) and Htfac (0.246 g, 1.60 mmol) was added dropwise, inducing the precipitation of insoluble BaSO₄. The yellow suspension was stirred at room temperature for 3 h and then filtered. The bright yellow filtrate was concentrated to dryness under reduced pressure at 60 °C to afford the desired compound as a yellow solid. The yellow solid was resuspended in 5 mL of cold H₂O (≈ 4°C), filtered, and washed sequentially with 5 mL of cold H₂O and twice with 5 mL of Et₂O before being dried in vacuo. Yield: 0.314 g (44%). Mp >190 °C (gradual darkening and decomposition). ¹H NMR (400 MHz, MeOD-d₄): δ 6.10 (s, 1H), 2.00 (s, 3H). ¹⁹F(¹H) NMR (376 MHz, MeOD-d₄): δ −76.43. ¹⁹⁵Pt(¹H) NMR (86 MHz, MeOD-d₄): δ −1497. IR (KBr, cm⁻¹): 3341 m, 3101 s, 2917 w, 1592 s, 1526 m, 1458 w, 1367 w, 1304 vs, 1240 m, 1194 m, 1138 vs, 876 w, 805 w, 743 w, 668 w, 618 m, 448 w. ESI-MS (pos. ion mode, MeOH): m/z 382.0 ([M]+, calcd. 382.0). Anal. Calcd. for 2, C₅H₁₀F₃N₂O₄PtS₀.₅: C, 13.96; H, 2.34; N, 6.51. Found: C, 13.81; H, 2.48; N, 6.41.

**Synthesis of [Pt(bzac)(NH₃)₂](NO₃) (3).** A mixture of cisplatin (0.500 g, 1.67 mmol) and AgNO₃ (0.553 g, 3.25 mmol) in 10 mL of water was stirred in the absence of light for 12 h at
room temperature. The resulting suspension was filtered to remove white AgCl, and a hot aqueous solution (70 °C, 10 mL) of Hbzac (0.264 g, 1.63 mmol) and NaOH (0.065 g, 1.6 mmol) was added dropwise to the filtrate, forming a yellow suspension, which was allowed to stir at room temperature for 4 h. The mixture was concentrated in vacuo at 60 °C to a volume of 8 mL and then filtered to collect the desired compound as a pale yellow solid. This solid was suspended in 10 mL of Et₂O and isolated by centrifugation twice to remove an ether-soluble impurity, before being dried in vacuo. Yield: 0.400 g (53%). Mp 160 °C (gradual darkening), 210–215 °C (dec). ¹H NMR (400 MHz, MeOD-d₄): δ 7.95 (d, 2H), 7.55 (t, 1H), 7.41 (app t, 2H), 6.31 (s, 1H), 2.00 (s, 3H). ¹³C{¹H} NMR (100 MHz, MeOD-d₄): δ 187.5, 178.2, 138.0, 132.7, 129.9, 128.4, 100.3, 26.7. ¹⁹⁵Pt{¹H} NMR (86 MHz, MeOD-d₄): δ -1572. IR (KBr, cm⁻¹): 3435 w, 3279 s, 3200 m, 3116 m, 1599 w, 1584 m, 1548 s, 1514 vs, 1490 m, 1451 m, 1397 s, 1383 s, 1341 vs, 1312 vs, 1221 w, 957 w, 878 w, 779 m, 713 m, 689 w, 671 w, 580 v, 483 v, 1341 vs, 1312 vs, 1221 w, 957 w, 878 w, 779 m, 713 m, 689 w, 671 w, 580 v, 483 v. ESI-MS (pos. ion mode, MeOH): m/z 390.1 ([M]+, calcd. 390.1), 842.0 ([2M+NO₃]+, calcd. 842.1).


**Synthesis of [Pt(dbm)(NH₃)₂(NO₃)] (5).** Cisplatin (750 mg, 2.50 mmol) and AgNO₃ (828 mg, 4.87 mmol) were stirred in 6 mL of DMF at room temperature for 12 h in the absence of light. The resulting suspension was filtered to remove AgCl. The pale yellow filtrate was added dropwise to Na₂CO₃ (321 mg, 3.00 mmol) and Hdbm (560 mg, 2.50 mmol) in 5 mL of DMF. This mixture was stirred at room temperature for 3.5 h and then filtered. The filtrate was concentrated to dryness under vacuum at 60 °C to obtain an orange oily residue. This residue was dissolved in 10 mL of MeOH and filtered. The addition of Et₂O (40 mL) afforded the desired product as a yellow solid, which was isolated by filtration, washed with cold H₂O (4 °C, 20 mL) and Et₂O (20 mL), and dried under vacuum. Yield: 0.306 g (24%). Mp 221–229 °C
(dec). $^1$H NMR (400 MHz, MeOD-$d_4$): $\delta$ 8.07 (d, 4H), 7.60 (t, 2H), 7.47 (t, 4H), 6.96 (s, 1H).
$^{13}$C{$^1$H} NMR (100 MHz, MeOD-$d_4$): $\delta$ 179.9, 138.5, 132.9, 130.0, 128.4, 97.5. $^{195}$Pt{$^1$H} NMR (86 MHz, MeOD-$d_4$): $\delta$ -1528. IR (KBr, cm$^{-1}$): 3438 w, 3278 m, 3198 m, 3058 w, 1589 m, 1530 vs, 1487 s, 1454 m, 1385 s, 1318 s, 1237 m, 1075 w, 1024 w, 999 w, 945 w, 827 w, 757 m, 706 m, 670 m, 596 vw, 559 vw. ESI-MS (pos. ion mode, MeOH): $m/z$ 452.0 ([M]+, calcd. 452.1), 965.8 ([2M+NO$_3$]+, calcd. 966.2). Anal. Calcd. for 5·H$_2$O, C$_{15}$H$_{19}$N$_3$O$_6$Pt: C, 33.84; H, 3.60; N, 7.89. Found: C, 34.17; H, 3.24; N, 7.99.

**X-ray Crystallography.** Vapor diffusion of diethyl ether into methanol solutions afforded single crystal of 3 and 4. The single crystals were mounted in Paratone oil on a cryoloop and frozen under a 110 or 100 K KRYO-FLEX nitrogen cold stream. Data were collected on a Bruker APEX CCD X-ray diffractometer with graphite-monochromated Mo-$K\alpha$ radiation ($\lambda = 0.71073$ Å) controlled by the APEX2 software package.$^{40}$ Absorption corrections were applied using SADABS.$^{41}$ The structures were solved using direct methods and refined on $F^2$ with the SHELXTL-97 software package.$^{42,43}$ Structures were checked for higher symmetry using PLATON.$^{44}$ All non-hydrogen atoms were located and refined anisotropically. Hydrogen atoms were placed in idealized locations and given isotropic thermal parameters equivalent to either 1.5 (terminal CH$_3$ or NH$_3$ hydrogen atoms) or 1.2 times the thermal parameter of the atom to which they were attached. Crystallographic data collection and refinement parameters are provided in Table 4.1.
Table 4.1. X-Ray Crystallographic Data Collection and Refinement Parameters for 3 and 4.

<table>
<thead>
<tr>
<th></th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>formula</td>
<td>$\text{C}<em>{10}\text{H}</em>{12}\text{N}<em>{3}\text{O}</em>{5}\text{Pt}$</td>
<td>$\text{C}<em>{10}\text{H}</em>{12}\text{F}<em>{3}\text{N}</em>{3}\text{O}_{5}\text{Pt}$</td>
</tr>
<tr>
<td>fw</td>
<td>452.34</td>
<td>506.32</td>
</tr>
<tr>
<td>space group</td>
<td>$P\text{ca}2_1$</td>
<td>$P\text{2}_1/c$</td>
</tr>
<tr>
<td>$a$, Å</td>
<td>9.4898(12)</td>
<td>18.336(2)</td>
</tr>
<tr>
<td>$b$, Å</td>
<td>15.218(2)</td>
<td>7.1871(9)</td>
</tr>
<tr>
<td>$c$, Å</td>
<td>8.9436(12)</td>
<td>7.1871(9)</td>
</tr>
<tr>
<td>$\beta$, deg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V$, Å$^3$</td>
<td>1291.6(3)</td>
<td>1400.5(3)</td>
</tr>
<tr>
<td>$Z$</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$\rho_{\text{calc}}, \text{g cm}^{-3}$</td>
<td>2.326</td>
<td>2.401</td>
</tr>
<tr>
<td>$T$, °C</td>
<td>-173(2)</td>
<td>-173(2)</td>
</tr>
<tr>
<td>$\mu(\text{Mo Kα}), \text{mm}^{-1}$</td>
<td>10.884</td>
<td>10.081</td>
</tr>
<tr>
<td>$\theta$ range, deg</td>
<td>2.53–29.06</td>
<td>2.26–28.48</td>
</tr>
<tr>
<td>total no. of data</td>
<td>25499</td>
<td>26078</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>3436</td>
<td>3530</td>
</tr>
<tr>
<td>no. of parameters</td>
<td>176</td>
<td>201</td>
</tr>
<tr>
<td>completeness to θ (%)</td>
<td>99.8</td>
<td>99.4</td>
</tr>
<tr>
<td>$R1^a$ (%)</td>
<td>1.44</td>
<td>2.78</td>
</tr>
<tr>
<td>$wR2^b$ (%)</td>
<td>2.89</td>
<td>3.91</td>
</tr>
<tr>
<td>$R1^a$ (%) for $I &gt; 2\sigma$</td>
<td>1.31</td>
<td>1.94</td>
</tr>
<tr>
<td>$wR2^b$ (%) for $I &gt; 2\sigma$</td>
<td>2.85</td>
<td>3.67</td>
</tr>
<tr>
<td>GOF$^c$</td>
<td>1.062</td>
<td>1.045</td>
</tr>
<tr>
<td>max, min peaks, eÅ$^{-3}$</td>
<td>0.879, -0.532</td>
<td>0.959, -0.765</td>
</tr>
<tr>
<td>Flack parameter</td>
<td>0.011(7)</td>
<td></td>
</tr>
</tbody>
</table>

Solution Stability Measurements. The platinum complexes were dissolved in water or pH 7.4 PBS containing 10% D$_2$O to a concentration of ~1 mM. The solutions were transferred to NMR tubes and incubated in a 37 °C water bath. $^1$H and $^{19}$F NMR spectra were obtained at various time points during the incubation. A small amount of 1,4-dioxane ($\delta = 3.75$ ppm) was included in the samples as an internal standard for referencing $^1$H NMR spectra and a sealed capillary containing aqueous KF ($\delta = -120.46$ ppm) was used for referencing $^{19}$F NMR spectra. The
methyl group of 2 in the $^1$H NMR spectra was integrated relative to the 1,4-dioxane reference at different time points and used for kinetic analyses. The trifluoromethyl group of 4 in the $^{19}$F NMR spectra was integrated relative to the KF standard at different time points and used for kinetic analyses. Under the assumption of first-order kinetics, approximate half-lives were determined by fitting a line to a plot of ln[Pt] versus time and using the integrated first-order rate law, $\ln[Pt] = -kt + [Pt]_0$ and the relationship, $t_{1/2} = \ln 2/k$, where $[Pt]_0$ is the starting concentration of complex, $k$ is the pseudo-first-order rate constant, $t$ is time, and $t_{1/2}$ is the half-life. Water suppression for $^1$H NMR spectra was accomplished with presaturation. The pH of the solutions was measured at the beginning and end of the desired incubation time using a DG-111-SG glass electrode calibrated with standard buffers.

**Partition Coefficient Measurements.** Water or PBS were stirred vigorously with $n$-octanol for 24 h and then centrifuged for 5 min to obtain octanol-saturated water and water-saturated octanol. The platinum complexes were dissolved in the octanol-saturated water, or PBS for cisplatin, to a typical concentration of 0.03 to 3 mM and then mixed with water-saturated octanol in volumetric ratios of 1:1, 1:2, and 2:1 in duplicate. The mixtures were vortexed for 0.5 h and then centrifuged for 5 min. The layers were separated carefully using a fine-gauge needle and then analyzed for Pt content by GFAAS. The partition coefficient was taken as a ratio of the concentration of platinum in the octanol layer to that in the aqueous layer ($P = c_{cel}/c_{water}$). The reported error is the standard deviation of the six measurements obtained from this protocol.

Calculated ligand log $P$ values were obtained from the online program ALOGPS 2.1 available at the Virtual Computational Chemistry Laboratory. Only the keto tautomeric form of the protonated ligand was used for these calculations. In addition to using the ALOGPS algorithm, this program also calculates log $P$ values using several other algorithms for
comparison. The values presented here are the average log $P$ values computed for all the algorithms, and the errors reported are the standard deviation of these values.

**Cell Lines and Culture Conditions.** HeLa (human cervical cancer), A549 (human lung cancer), U2OS (osteosarcoma), and MCF-7 (human breast cancer) cells were grown as adherent monolayers in growth medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cultures were grown in 25-cm$^2$ flasks in an in an incubator at 37 °C with a humidified atmosphere composed of 5% CO$_2$.

**Cytotoxicity Assays.** The colorimetric MTT assay was used to determine the cytotoxicity of cisplatin and the $\beta$-diketonate platinum complexes. Trypsinized cells were seeded into a 96-well plate at a cell density of 2000 cells/well in 200 μL of growth medium and incubated for 24 h. The medium was then removed, and 200 μL of new growth medium containing various concentrations of the platinum complexes was added. After 72 h, the medium was removed, 200 μL of a 0.8 mg/mL solution of MTT in DMEM was added, and the plate was incubated further for 4 h. The DMEM/MTT mixture was aspirated, and 200 μL of DMSO with 10% pH 10.5 glycine buffer was added to dissolve the resulting purple formazan crystals. The absorbance of the plates was read at 555 nm. Absorbance values were normalized to the platinum-free control wells and plotted as [Pt] versus % viability. IC$_{50}$ values were interpolated from the resulting curves. The reported IC$_{50}$ values are the averages from at least three independent experiments, each of which consisted of six replicates per concentration level. Dilutions of the platinum complexes in growth medium were made from concentrated (1-3 mM) solutions in distilled water for $\beta$-diketonate platinum complexes or pH 7.4 PBS for cisplatin.
Compound 4 was submitted to the National Cancer Institute (NCI) in July 2011 for single-dose testing in the NCI-60 tumor cell screen. These tests were carried out by the NCI using their established protocols.48

**Cellular Uptake Studies.** The total platinum uptake per cell was determined using slight modifications of a previously described protocol.49 In two 6-well plates, $3 \times 10^5$ HeLa cells per well were seeded in 3 wells with 2.5 mL of growth medium. In one of the plates, the additional 3 wells were filled with 2.5 mL of growth medium to act as blanks for nonspecific platinum adsorption on the surface of the well. After incubating for 48 h, each well in the plate containing both cells and cell-free medium was treated with 0.278 mL of a 100-μM solution of the desired compound to give an exposure concentration of 10 μM. The cells and cell-free blanks were incubated with the Pt complex for 4 h. Meanwhile, the cells in the other plate were counted with trypan blue after detachment with trypsin in order to obtain the number of cells per well. After the 4-h incubation period, the growth medium was aspirated, and all six wells were washed twice with 3 mL of pH 7.4 PBS and then treated with exactly 0.5 mL of hot (~90 °C) concentrated nitric acid for 2 h. This solution was analyzed by GFAAS to determine the total Pt content per well. The amount of Pt per cell was calculated by subtracting the average amount of Pt found in the blank wells from the average amount of Pt found in the cell-containing wells and normalizing to the average number of cells per well.

**Intracellular DNA Platination Measurements.** Five million HeLa cells were seeded in a 100 mm Petri dish containing 9 mL of growth medium. On the following day, the cells were treated with the platinum complex at 100-μM concentration for 4 h. The growth medium was then replaced with platinum-free medium, and the cells were incubated for an additional 16 h. Cells, both floating and attached, were collected and combined. Trypsin (2 mL) was used to detach the
cells. The combined cells were centrifuged for 5 min at 4 °C, and the resulting cell pellet was washed twice with 3 mL of ice-cold PBS. DNAzol (1 mL, genomic DNA isolation reagent, MRC), containing 100 μg/mL RNase A, was used to lyse the cell pellet. DNA was precipitated with 0.5 mL of absolute ethanol, washed with 75% ethanol (2 × 0.75 mL), and redissolved in 200 μL of 8 mM NaOH. The DNA concentration was determined by UV-visible spectroscopy, and platinum was quantified by GFAAS. The reported values are the average of at least three independent experiments with the error reported as the standard deviation.

4.3. Results

Synthesis and Characterization. The platinum β-diketonate complexes were synthesized by three different routes using cisplatin as a common starting material (Scheme 4.1). In all three approaches, cisplatin was first activated by treatment with the appropriate Ag(I) salt to remove the chloride ligands as insoluble AgCl and generate reactive solvated cis-diammineplatinum(II) cations. Subsequent treatment with the appropriate salt of the β-diketonate ligand afforded 1–5 (Scheme 4.1). The disparate solubilities of the β-diketonate ligands and the final platinum(II) complexes necessitated the use of the three slightly different routes shown in Scheme 4.1, as will be described in more detail in Section 4.4 below.

Characterization of the complexes was achieved by NMR and IR spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and elemental analysis. The presence of nitrate as the counterion for 3–5 was verified both by the characteristic N–O stretching band in the IR spectrum near 1383 cm⁻¹ and by an ESI-MS m/z peak corresponding to the nitrate adduct, [2M + NO₃]⁺. In the mass spectra of 1 and 2, only the molecular ion arising from the cationic platinum complex was observed. The IR spectra of 1–5 are consistent with O,O'-coordination of
the \( \beta \)-diketonate ligands. Vibrational frequencies between 1560 and 1590 cm\(^{-1} \) correspond to C=O stretches\(^{50} \) and indicate a decrease in C=O bond order, consistent with \( \pi \) electron delocalization through the six-membered chelate ring. Elemental analyses are in agreement with the anticipated molecular formulas.

\begin{align*}
H_3N\text{Pt}^+\text{Cl}^- + Ag_2SO_4 & \rightarrow H_2O - 2AgCl(s) \rightarrow \left[H_3N\text{Pt}^+\text{OH}_2\right](\text{SO}_4)_{0.5} \\
H_3N\text{Pt}^+\text{Cl}^- + 2AgNO_3 & \rightarrow H_2O - 2AgCl(s) \rightarrow \left[H_3N\text{Pt}^+\text{OH}_2\right](\text{NO}_3)_2 \\
H_3N\text{Pt}^+\text{Cl}^- + 2AgNO_3 & \rightarrow \text{DMF} - 2AgCl(s) \rightarrow \left[H_3N\text{Pt}^+\text{DMF}\right](\text{NO}_3)_2
\end{align*}


The complexes were also studied by multinuclear NMR spectroscopy. The low solubility of 2 in aqueous and organic solvents precluded acquisition of its \( ^{13} \)C NMR spectrum. For the other complexes, all expected resonances were observed in the \( ^{13} \)C NMR spectra. The trifluoromethyl groups of 2 and 4 appear at \(-76.43 \) and \(-76.17 \) ppm, respectively, in the \( ^{19} \)F NMR spectra. The \( ^1 \)H NMR spectra display the expected resonances except for the protons of the coordinated \( \text{NH}_3 \) ligands, which are not observed, presumably due to rapid exchange with deuterons of the methanol-\( d_4 \) NMR solvent. Protons at the gamma position of the \( \beta \)-diketonate ligands resonate between 5.58–6.94 ppm in the five complexes, as summarized in Table 4.2. The
$^{195}\text{Pt}$ NMR spectra of the complexes are marked by a single peak that ranges from $-1593$ to $-1454$ ppm depending on the β-diketonate ligand (Table 4.2).

Table 4.2. Selected $^1\text{H}$, $^{13}\text{C}$, $^{19}\text{F}$, and $^{195}\text{Pt}$ NMR Chemical Shifts (ppm) for 1–5.

<table>
<thead>
<tr>
<th>compound</th>
<th>$\delta^1\text{H}, \gamma$ position$^a$</th>
<th>$\delta^{13}\text{C}, \gamma$ position$^a$</th>
<th>$\delta^{195}\text{Pt}^b$</th>
<th>$\delta^{19}\text{F}^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.58</td>
<td>103.3</td>
<td>$-1593$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.10</td>
<td>n.d.$^d$</td>
<td>$-1497$</td>
<td>$-76.43$</td>
</tr>
<tr>
<td>3</td>
<td>6.32</td>
<td>100.3</td>
<td>$-1572$</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.72</td>
<td>96.5</td>
<td>$-1454$</td>
<td>$-76.17$</td>
</tr>
<tr>
<td>5</td>
<td>6.96</td>
<td>97.5</td>
<td>$-1528$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Referenced to SiMe$_4$ at $\delta = 0$ ppm. $^b$ Referenced to Na$_2$PtCl$_6$ at 0 ppm. $^c$ Referenced to CFCl$_3$ at 0 ppm. $^d$ Not determined.

Single crystals of 3 and 4 were obtained by vapor diffusion of diethyl ether into methanol solutions of the compounds, enabling their structures to be determined by X-ray crystallography. The results are presented in Figure 4.1, with relevant bond lengths and angles reported in Table 4.3. The structures confirm the expected square planar coordination geometries of the platinum(II) center and the $O,O'$-coordination mode of the β-diketonate ligands. The nitrate counterions are engaged in hydrogen bonding interactions with the protons of the coordinated NH$_3$ ligands (not shown).
Figure 4.1. Solid-state molecular structures of the complex cations of 3 and 4. Ellipsoids are drawn at the 50% probability level. Gray shaded ellipsoids represent carbon atoms, orange shaded ellipsoids fluorine atoms, and open green circles hydrogen atoms. The identities of the other atoms are denoted.

Table 4.3. Selected Interatomic Distances (Å) and Angles (°) for 3 and 4.

<table>
<thead>
<tr>
<th></th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptl–N1</td>
<td>2.034(2)</td>
<td>2.017(3)</td>
</tr>
<tr>
<td>Ptl–N2</td>
<td>2.030(2)</td>
<td>2.015(2)</td>
</tr>
<tr>
<td>Ptl–O1</td>
<td>1.9956(17)</td>
<td>2.005(2)</td>
</tr>
<tr>
<td>Ptl–O2</td>
<td>1.9907(18)</td>
<td>1.988(2)</td>
</tr>
<tr>
<td>O1–Ptl–O2</td>
<td>95.20(7)</td>
<td>94.69(9)</td>
</tr>
<tr>
<td>O1–Ptl–N1</td>
<td>86.81(8)</td>
<td>88.69(10)</td>
</tr>
<tr>
<td>O1–Ptl–N2</td>
<td>177.62(9)</td>
<td>176.19(10)</td>
</tr>
<tr>
<td>O2–Ptl–N1</td>
<td>177.79(8)</td>
<td>176.54(10)</td>
</tr>
<tr>
<td>O2–Ptl–N2</td>
<td>85.37(8)</td>
<td>86.12(10)</td>
</tr>
<tr>
<td>N1–Ptl–N2</td>
<td>92.65(9)</td>
<td>90.55(11)</td>
</tr>
</tbody>
</table>

Atoms are labeled as shown in Figure 4.1. Numbers in parentheses are estimated standard deviations of the last significant figures.

Lipophilicity. To quantify the lipophilicity of the new platinum complexes, water-octanol partition coefficients ($P$) were measured using the shake-flask method. The resulting log $P$ values are reported in Table 4.4 and graphically compared in Figure 4.2. The measured log $P$ of cisplatin is -2.21, consistent with literature values. The most lipophilic complex is 5, with a log
$P$ value of $0.0 \pm 0.1$. The overall order of lipophilicity follows the sequence $5 > 4 > 3 > 2 > 1$. The phenyl groups in 3, 4, and 5 have a larger effect on the lipophilicity than the trifluoromethyl groups of 2 and 4. Substitution of a phenyl for a methyl group on the $\beta$-diketonate ligand leads to an increase of lipophilicity by approximately 1 log $P$ unit, whereas the analogous substitution for a trifluoromethyl group leads to an increase of approximately 0.3 log $P$ units.

The log $P$ values of the free $\beta$-diketonate ligands in the keto form were calculated using the online program ALOGPS 2.1\textsuperscript{45} available at the Virtual Computational Chemistry Laboratory.\textsuperscript{46,47} The values are displayed in Table 4.4. The calculated ligand log $P$ values are linearly proportional to the experimentally measured complex log $P$ values (Figure 4.2). As expected, more lipophilic ligands give rise to more lipophilic complexes.

Table 4.4. Experimentally Measured Complex and Computed Ligand Log $P$ Values.

<table>
<thead>
<tr>
<th>compound</th>
<th>measured complex log $P^{a}$</th>
<th>calculated ligand log $P^{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$-2.67 \pm 0.08$</td>
<td>$0.09 \pm 0.35$</td>
</tr>
<tr>
<td>2</td>
<td>$-2.28 \pm 0.07$</td>
<td>$0.72 \pm 0.24$</td>
</tr>
<tr>
<td>3</td>
<td>$-1.30 \pm 0.09$</td>
<td>$1.47 \pm 0.43$</td>
</tr>
<tr>
<td>4</td>
<td>$-0.98 \pm 0.03$</td>
<td>$2.15 \pm 0.54$</td>
</tr>
<tr>
<td>5</td>
<td>$0.0 \pm 0.1$</td>
<td>$3.08 \pm 0.33$</td>
</tr>
<tr>
<td>cisplatin</td>
<td>$-2.21 \pm 0.06$</td>
<td>n.d.\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Errors are estimated from the standard deviations of repeated experiments. \textsuperscript{b} Errors are the standard deviations obtained from the use of seven different computational algorithms as employed by ALOGPS 2.1. \textsuperscript{c} Not determined.
Aquation and Anation Rates. The stabilities of 1–5 in water and phosphate-buffered saline (PBS), pH 7.4, at 37 °C were determined by NMR spectroscopy. The compounds bearing nonfluorinated β-diketonate ligands, 1, 3, and 5, exhibited no changes in their $^1$H NMR spectra in either medium for up to 25 d. Compounds 2 and 4, which both have a trifluoromethyl group in the ligand backbone, slowly decomposed in water and PBS. In water, the $^1$H NMR chemical shift at 2.00 ppm of the methyl group of 2 decayed with a half-life of 58 d and concomitant appearance of a new resonance at 2.22 ppm. Over the course of this reaction, the pH changed from 7.5 to 6.3. For 4, the rate of decomposition was faster. The $^{19}$F NMR chemical shift at $-74.4$ ppm of 4 disappeared with a half-life of 29 d, accompanied by the appearance of a new resonance at $-76.6$ ppm. The aliphatic region of the $^1$H NMR spectrum at this final time point is
marked by a sharp singlet at 2.67 ppm, which is not observed upon the initial dissolution of the complex. The pH of this solution remained at 7.5 for the duration of the experiment.

In pH 7.4 PBS, the rates of decomposition increased. The half-lives of 2 and 4 in this medium are 3.4 and 1.8 d, respectively. For 4, the final product is the same as that observed following aquation in non-buffered water as characterized by a $^{19}$F NMR chemical shift at $-76.6$ ppm and a sharp singlet in the $^1$H NMR spectrum at 2.67 ppm. Complex 2 displays more complex reactivity in PBS. Although the major final product is the same as that observed for aquation in pure water ($^1$H $\delta = 2.22$ ppm), two other minor products were observed by $^1$H NMR spectroscopy having CH$_3$ proton resonances at 1.91 and 1.56 ppm. In addition, an intermediate with a CH$_3$ proton resonance at 2.25 ppm was apparent.

With the goal of characterizing the aquation and anation products of 2 and 4, the NMR spectra of the free ligands of these complexes in PBS were investigated. Initially, the $^1$H NMR spectrum of sodium trifluoracetylacetonate, Na(tfac), in PBS displayed a CH$_3$ resonance at 2.25 ppm. Upon further incubation at 37 $^\circ$C for 6 d, this resonance at 2.25 ppm decayed and was replaced by a major resonance at 2.22 ppm and two minor resonances at 1.91 and 1.56 ppm. Because these signals are the same as those observed during the aquation and anation of 2, we propose that the free tfac ligand is displaced from the platinum center as an intermediate with a chemical shift of 2.25 ppm and then undergoes ligand decomposition reactions. For the ligand of 4, 4,4,4-trifluorobenzoylacetonate (Htfbz), two peaks in its $^{19}$F NMR spectrum at $-76.37$ and $-86.7$ ppm were initially observed. These two signals possibly correspond to the keto and enol tautomers of the compound. After 6 d at 37 $^\circ$C, the major species in solution is a compound characterized by a singlet in the $^1$H NMR spectrum at 2.67 ppm and a signal in the $^{19}$F NMR spectrum at $-76.41$ ppm. These spectroscopic signals are consistent with the major species
observed after the aquation and anation of 4. Thus, as for 2, dissociation from the platinum center precedes ensuing hydrolytic decomposition of the fluorinated β-diketonate ligand.

**Cancer Cell Cytotoxicity.** The antiproliferative activities of 1–5 and cisplatin were determined in HeLa (human cervical cancer), A549 (human lung cancer), U2OS (human osteosarcoma), and MCF-7 (human breast cancer) cell lines by the MTT assay. The cells were treated continuously for 72 h. The resulting 50% growth inhibitory concentration (IC_{50}) values are summarized in Table 4.5 and graphically depicted in Figure 4.3. For the four cell lines tested, 1 is the least cytotoxic of the five complexes, with IC_{50} values ranging from 24 to 76 μM. The IC_{50} values of 3 vary between 7 and 33 μM, indicating slightly greater cytotoxicity than 1. Complexes 2, 4, and 5 exhibit cytotoxicities comparable to that of cisplatin. The IC_{50} values for these compounds are generally <10 μM, except for 2 in MCF-7 cells where the IC_{50} is 15 μM.

**Table 4.5.** IC_{50} Values of 1–5 and Cisplatin in HeLa, A549, U2OS, and MCF-7 Cell Lines and Cellular Uptake in HeLa Cells.

<table>
<thead>
<tr>
<th>compound</th>
<th>HeLa</th>
<th>A549</th>
<th>U2OS</th>
<th>MCF-7</th>
<th>HeLa cellular uptake (ng Pt/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32 ± 5</td>
<td>28 ± 9</td>
<td>24 ± 2</td>
<td>76 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>2.9 ± 0.7</td>
<td>2.2 ± 0.6</td>
<td>4.1 ± 0.8</td>
<td>15 ± 2</td>
<td>29 ± 9</td>
</tr>
<tr>
<td>3</td>
<td>24 ± 4</td>
<td>7 ± 2</td>
<td>8 ± 2</td>
<td>33 ± 6</td>
<td>83 ± 8</td>
</tr>
<tr>
<td>4</td>
<td>3 ± 1</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.5</td>
<td>4.3 ± 0.3</td>
<td>160 ± 20</td>
</tr>
<tr>
<td>5</td>
<td>6.7 ± 0.7</td>
<td>1.3 ± 0.3</td>
<td>2.7 ± 0.7</td>
<td>3.1 ± 0.5</td>
<td>520 ± 80</td>
</tr>
<tr>
<td>cisplatin</td>
<td>2.1 ± 0.1</td>
<td>3.2 ± 0.6</td>
<td>5 ± 2</td>
<td>14 ± 3</td>
<td>14 ± 2</td>
</tr>
</tbody>
</table>

^aErrors are standard deviations determined from at least three independent experiments. ^bThe errors are determined by error propagation equations, as described in ref 49.
Because 4 is on average the most cytotoxic of the five complexes, it was submitted to the NCI for evaluation in the NCI-60 tumor cell panel screen. In this screen, a single-dose cytotoxicity measurement is performed in 60 cell lines with distinctive drug sensitivity profiles. This process can identify drug candidates with unique anticancer activity spectra based on which cell lines are sensitive or resistant to a compound of interest. Using the COMPARE algorithm, activity spectra can be quantitatively correlated with those of other compounds in the NCI database. Pearson correlation coefficients are used to evaluate similarities in activity spectra. Correlations coefficients close to one indicate compounds with similar mechanisms of action and resistance profiles. The average cell growth percentage of the 60 cell lines after 48 h of treatment with 10 μM 4 was 89.65%, indicating slight growth inhibitory action. Compound 4 showed the greatest efficacy against central nervous system (CNS) cancer, where the average cell growth was 64.52%. Results of the COMPARE algorithm are summarized in Table 4.6.

Figure 4.3. Graphical representation of IC₅₀ values for 1–5 and cisplatin in HeLa, A549, U2OS, and MCF-7 cell lines.

![Graphical representation of IC₅₀ values for 1–5 and cisplatin in HeLa, A549, U2OS, and MCF-7 cell lines.](image-url)
Table 4.6. Results of COMPARE Analysis of 4.

<table>
<thead>
<tr>
<th>rank</th>
<th>PCC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NSC no.</th>
<th>name</th>
<th>biological mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.589</td>
<td>95466</td>
<td>PCNU</td>
<td>alkylation agent</td>
</tr>
<tr>
<td>2</td>
<td>0.569</td>
<td>750</td>
<td>busulfan</td>
<td>type II topoisomerase inhibitor</td>
</tr>
<tr>
<td>3</td>
<td>0.559</td>
<td>301739</td>
<td>mitoxantrone</td>
<td>type II topoisomerase inhibitor</td>
</tr>
<tr>
<td>4</td>
<td>0.534</td>
<td>355644</td>
<td>anthrapyrazole</td>
<td>alkylation agent</td>
</tr>
<tr>
<td>5</td>
<td>0.506</td>
<td>178248</td>
<td>chlorozotocin</td>
<td>alkylation agent</td>
</tr>
<tr>
<td>6</td>
<td>0.501</td>
<td>357704</td>
<td>cyanomorpholino-ADR</td>
<td>alkylation agent</td>
</tr>
<tr>
<td>7</td>
<td>0.495</td>
<td>3088</td>
<td>chlorambucil</td>
<td>alkylation agent</td>
</tr>
<tr>
<td>8</td>
<td>0.491</td>
<td>132313</td>
<td>dianhydrogalactitol</td>
<td>alkylation agent</td>
</tr>
<tr>
<td>9</td>
<td>0.489</td>
<td>119875</td>
<td>cisplatin</td>
<td>alkylation agent</td>
</tr>
<tr>
<td>10</td>
<td>0.485</td>
<td>353451</td>
<td>mitozolamide</td>
<td>alkylation agent</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pearson correlation coefficient. <sup>b</sup>Compound identification number utilized by the NCI.

**Cellular Uptake.** The uptake of cisplatin and 1–5 by HeLa cells after a 4 h exposure time at 10 μM concentration was measured by GFAAS. The results are tabulated in Table 4.5. The cellular uptake correlates with the lipophilicity of the compound in an exponential fashion (Figure 4.4). The most lipophilic complex, 5, is taken up to the greatest extent (520 ± 80 ng Pt/10<sup>6</sup> cells), whereas the least lipophilic complex is taken up the least (6 ± 2 ng Pt/10<sup>6</sup> cells). All complexes except 1 have larger accumulation in HeLa cells than cisplatin under similar conditions.
**Figure 4.4.** Plot of complex log $P$ versus cellular uptake in HeLa cells. The blue line is an exponential fit of the data.

**Intracellular DNA Platination.** Because platinum-based drugs exert their cytotoxic effects by the formation of DNA crosslinks, the extent to which 1–5 bind to intracellular DNA was measured. HeLa cells were treated with 100 µM of the platinum complexes for 4 h and then incubated for an additional 16 h in platinum-free growth medium. The nuclear DNA was isolated, and the quantity of bound platinum was measured by GFAAS. The results of this study are shown in Table 4.7. The extent of DNA platination follows the order $5 \approx 4 >$ cisplatin $> 3 \approx 2 > 1$. 

---

189
Table 4.7. Intracellular DNA Platination in HeLa Cells Induced by Cisplatin and 1–5.\textsuperscript{a}

<table>
<thead>
<tr>
<th>compound</th>
<th>Pt/DNA (pmol/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cisplatin</td>
<td>1.58 ± 0.97</td>
</tr>
<tr>
<td>1</td>
<td>0.063 ± 0.012</td>
</tr>
<tr>
<td>2</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td>3</td>
<td>0.35 ± 0.24</td>
</tr>
<tr>
<td>4</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>5</td>
<td>23 ± 10</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Reported errors are the standard deviations from at least three experiments.

4.4. Discussion

The use of \( \beta \)-diketonate ligands as leaving groups for traditional \textit{cis-}
diammineplatinum(II) anticancer agents is relatively unexplored. Here, we investigated the
physical properties and anticancer efficacy for a small set of compounds in this class. The five \( \beta \)-
diketonate ligands chosen for this study were systematically varied, by addition of either a
phenyl ring or a trifluoromethyl group (Chart 4.1). Such variations allowed us to monitor how
alterations of the hydrophobicity and electron-withdrawing properties of the leaving group ligand
translate into differences in anticancer efficacy in vitro.

The three synthetic methodologies employed to prepare 1–5 are outlined in Scheme 4.1. For 1 and 2, the sulfate salt of the \textit{cis-}
diamminediaquaplatinum(II) cation was utilized. The
treatment of this cation with Ba(acac)\(_2\) or Ba(tfac)\(_2\) produced 1 or 2, each of which was
subsequently isolated from the aqueous solution, and BaSO\(_4\) as an insoluble white solid. For the
syntheses of 3 and 4, the nitrate salt of the \textit{cis-}
diamminediaquaplatinum(II) cation was treated
with Na(bzac) or Na(tfbz). The NaNO\(_3\) byproduct of the reaction was washed away using cold
water with little product loss because 3 and 4 are only sparingly soluble in water. The low
aqueous solubility of the ligand Hdbm necessitated the use of \( N,N \)-dimethylformamide (DMF) as
the solvent. Activation of cisplatin in DMF with AgNO\(_3\) followed by treatment of Na(dbm)
afforded 5 after the appropriate reaction workup, albeit in lower yields. The preparation of the analogous hexafluoroacetylacetonate (hfac) complex was also attempted. None of the three synthetic methodologies described here provided access to the desired platinum-hfac complex. After reaction workup, unidentified green-blue residues were obtained. We propose that the difficulties associated with obtaining this complex may arise from the lability of the strongly electron-withdrawing hfac ligand.

Characterization data including ESI-MS, elemental analysis, IR spectroscopy, and multinuclear NMR spectroscopy are consistent with the proposed structures and elemental compositions of 1–5. Of particular interest are the $^{195}\text{Pt}$ NMR chemical shifts of the new complexes. This parameter is very sensitive to the platinum coordination environment and can span a region of $>13,000$ ppm. In the present case, the $^{195}\text{Pt}$ NMR chemical shifts of 1–5 range over $139$ ppm (Table 4.2), indicating that peripheral substituents on the β-diketonate ligands influence the electron density at the platinum center. Complex 1 has the most shielded Pt nucleus, which resonates at $-1593$ ppm, whereas 4 is most deshielded with the $^{195}\text{Pt}$ chemical shift appearing at $-1454$ ppm. The chemical shift for this class of compounds appears to depend on the electron-withdrawing strength of the ligand. The trifluoromethyl substituents of 2 and 4 deshield the Pt nucleus by approximately 100 ppm relative to their analogues, 1 and 3, which lack trifluoromethyl groups. The phenyl groups of 3, 4, and 5 also deshield the Pt nucleus, but to a lesser degree than the trifluoromethyl groups. Compared to their analogues having no phenyl groups, 3, 4, and 5 are deshielded by 20–44 ppm. The solid-state molecular structures of 3 and 4 display the expected coordination geometries (Figure 4.1). The complexes are structurally similar, with comparable interatomic distances and angles (Table 4.3). A related compound,
[Pt(acac)(trans-1R,2R-diaminocyclohexane]) (acac), has also been structurally characterized, and its bond metrics resemble those of 3 and 4.

An important physical property that affects the biodistribution of a drug candidate is its lipophilicity. The lipophilicity of a compound can be quantitatively evaluated by its log $P$ values, where $P$ is the water–octanol partition coefficient. More positive log $P$ values correspond to more lipophilic complexes, whereas more negative log $P$ values correspond to more hydrophilic ones. The log $P$ values for cisplatin and 1–5 are given in Table 4.4 and Figure 4.2. The addition of lipophilic groups to the β-diketonate ligands increases the log $P$ values of the resulting complex. Thus, the lipophilicity can be tuned with the appropriate choice of functional groups. For comparison, the log $P$ values of the free ligands in their diketo forms were computed with an online program. A linear correlation exists between the calculated log $P$ values of the ligands and the experimentally measured log $P$ values of the complexes, as shown in Figure 4.2. The slope of the best-fit line is 0.90. This value, which is close to 1, indicates that coordination of the β-diketonate ligand to the cis-diammineplatinum(II) moiety affects its lipophilicity in an additive manner, consistent with the additive properties of substituent hydrophobicity constants. The intercept of the best fit line is −2.8, which can be interpreted as the substituent hydrophobicity constant for the [Pt(NH$_3$)$_2$]$^+$ moiety in this class of compounds.

After establishing that different substituents on the β-diketonate ligands have a predictable and significant effect on the lipophilicities of 1–5, we investigated how these substituents can modulate the reactivities of the complexes. The stabilities of 1–5 in water and in pH 7.4 phosphate-buffered saline at 37 °C were assessed by NMR spectroscopy. In either water or PBS, there was no sign of decomposition for 1, 3, and 5 for up to 25 d. Compounds 2 and 4 decomposed in water with half-lives of 58 and 29 d, respectively, and in PBS with half-lives of
3.4 and 1.8 d, respectively. The observation that the non-fluorinated complexes, 1, 3, and 5, appear to have an indefinite lifetime in water and aqueous buffer indicates that the strongly electron-withdrawing properties of the trifluoromethyl groups in 2 and 4 are responsible for the increased reactivities of these complexes. In this context, however, it should be noted that 2 and 4 are still significantly more inert than cisplatin, which has an aqueous solution half-life of 2 h.\(^5\) In PBS, which contains 137 mM NaCl and 10 mM Pi, the half-lives of 2 and 4 decrease to 3.4 and 1.8 d indicating that the high ion concentration plays a role in their reactivities. Carboplatin, which is stable for up 60 d in water,\(^6\) has increased reactivity in the presence of high phosphate and chloride ion concentrations, the chemistry proceeding by direct anion attack on the complex and not requiring an aqua intermediate.\(^6\) A similar process most likely explains the increased rates of decomposition of 2 and 4 in PBS relative to pure water as the solvent.

The antiproliferative activities of the complexes were assessed in four human cancer cell lines. IC\(_{50}\) values are displayed in Table 4.5 and graphically compared in Figure 4.3. The general trend of cytotoxicity among the four cell lines follows the order 1 < 3 < 2 ~ 4 ~ 5. Compounds 2, 4, and 5 have IC\(_{50}\) values comparable to or lower than those of cisplatin. The differences in observed cytotoxicities among the five complexes can be attributed to variations in lipophilicity and reaction kinetics conferred by the p-diketonate leaving group ligands because the DNA-binding cis-diammineplatinum(II) fragment is the same in all five complexes as well as cisplatin.

The cellular uptake properties of the five complexes and cisplatin were measured in HeLa to see whether this parameter might be the dominant factor in determining cytotoxicity. The results are summarized in Table 4.5. As shown in Figure 4.4, the cellular uptake is a function of lipophilicity of the platinum complex. This correlation suggests that these complexes are dependent on a passive diffusion uptake mechanism. Given that these complexes are cations,
they may also take advantage of organic cation transporters for selective transport into cancer cells, like oxaliplatin.\textsuperscript{61} No direct correlation, however, was observed between cellular uptake and IC\textsubscript{50} values for the five complexes. This result indicates that the reaction kinetics of the complexes must also play a role in their biological activities. Consistent with this hypothesis is the observation that the fluorinated complexes, 2 and 4, exhibit comparable or better cytotoxicities than 5 despite the fact that over twice as much 5 is taken up by cells. The increased reactivities of 2 and 4 in water and buffer are probably why they display comparable cytotoxicity to 5, even though they are present at significantly lower concentrations within the cell. A similar correlation between aquation rates and activity is observed in a series of carboplatin derivatives bearing fluorinated CBDCA leaving group ligands.\textsuperscript{13} There is rapid aquation of the fluorinated CBDCA relative to that of the nonfluorinated ligands, which is most likely responsible for the increased cytotoxic activity of the fluorinated derivatives relative to underivatized carboplatin.\textsuperscript{13}

The amount of platinum found on the DNA of HeLa cells treated with cisplatin and 1–5 (Table 4.7) is not directly correlated with the IC\textsubscript{50} values of the complexes, although the least active complex, 1, does induce the least amount of DNA platination. However, for determination of the IC\textsubscript{50} values the cells were treated for a continuous 72 h period, whereas for the DNA platination measurements, cells were treated at a high concentration for 4 h and then allowed to incubate for an additional 16 h in the absence of platinum. During this 16 h post-treatment period, efflux of platinum complexes out of the cell might become an important factor. Additionally, cellular targets other than DNA could modulate in part the cytotoxic activities of 1–5. The balance between lipophilicity and reactivity in the formation of Pt-DNA adducts is still apparent, however. Complexes 2 and 3 platinate DNA to a similar extent (\(\approx 0.3\) pmol Pt/\(\mu\)g DNA), as do complexes 4 and 5 (14–23 pmol Pt/\(\mu\)g DNA). Cellular accumulation studies
indicate that 3 and 5 are taken up to a much greater extent than 2 and 4, owing to their greater lipophilicities. The similar DNA platination levels of these complexes probably reflects the higher reactivities of the fluorinated complexes 2 and 4, which compensates for their lower intracellular abundance.

Among the compounds studied, 4 has the optimal balance of lipophilicity and reactivity. The average IC$_{50}$ value of this complex in the four cells lines tested is 2.6 μM, the lowest among the five complexes tested. For this reason, 4 was submitted to the National Cancer Institute for testing in the NCI-60 tumor cell panel screen. As described above, the NCI-60 tumor cell panel screen utilizes 60 different cell lines with distinct sensitivity profiles and measures the cell growth inhibitory action of a compound of interest, initially at a single dose. Differential cell growth inhibition among the 60 cell lines indicates the compound's spectrum of activity, which can be quantitatively compared to that of other compounds in the database via the COMPARE algorithm. For 4, the average cell growth percent among the 60 cell lines after a single dose (10 μM) treatment was 89.65% relative to the complex-free control. The apparent lower activity of 4 in the NCI-60 tumor cell screen compared to that observed in our laboratory is most likely the result of different experimental conditions. The shorter incubation time (48 h), the use of RPMI as culture medium, different initial cell densities, and use of the sulforhodamine B (SRB) assay by the NCI screen are plausible reasons for the difference. In fact, higher IC$_{50}$ values are typically measured with the SRB compared to the MTT assay because the former measures total protein content instead of mitochondrial activity. The COMPARE algorithm was utilized to investigate how the spectrum of activity of 4 matches that of other anticancer agents in the NCI database. Table 4.6 lists the ten anticancer compounds with the highest correlation coefficients with the spectrum of activity of 4. Eight of these ten compounds are known DNA alkylating
agents. Cisplatin is one of the top ten with a Pearson correlation coefficient of 0.489. These correlations indicate that 4 and the related β-diketonato complexes act, as anticipated, by binding DNA, with the β-diketonate ligands serving as the leaving groups. The relatively high correlation with the activity of cisplatin is consistent with the formation of similar DNA cross-links. The Pearson correlation coefficient comparing the spectra of activity of cisplatin and carboplatin is 0.798 and is therefore much greater than that for 4 and cisplatin. The β-diketonate ligand of 4 apparently has a larger influence on the cell line selectivity than the CBDCA ligand of carboplatin. This observation may have important implications in the design of new cisplatin analogues, which differ only by the nature of their leaving group ligands.

4.5. Summary and Conclusions

This Chapter describes the first systematic study of the physical properties and in vitro anticancer efficacy of a series of cis-diammineplatinum(II) complexes with β-diketonate leaving group ligands. Our results indicate that modifications of the β-diketonate ligands predictably affect both the lipophilicity and reactivity of the resulting platinum complexes. The lipophilicity of these compounds is important because it dictates the degree of cellular uptake, whereas optimal reactivity kinetics ensure that a significant amount of platinum can bind to DNA or other cellular targets within the biologically relevant time frame. Of the compounds presented here, 4 exhibited the greatest cytotoxicity on average. The trifluorobenzoylacetonate (tfbz) ligand of 4 carries both a phenyl and trifluoromethyl group. These two groups appear to provide an optimal combination of lipophilicity and reactivity for biological activity. The NCI-60 tumor cell screen revealed 4 to have a spectrum of activity similar to that of other alkylating agents, a category that often includes cisplatin. Like carboplatin, which has a similar spectrum of activity as that of
cisplatin and is used as a less toxic alternative, the present β-diketonate complexes may find similar roles in future pharmaceutical applications.

4.6 References

(40) APEX2, Version 2008-4.0; Bruker AXS, Inc.: Madison, WI, 2008.
Chapter 5

Acetate-Bridged Dinuclear Platinum(III) Complexes Derived from Cisplatin

5.1. Introduction

Chapters 2-4 focused on the chemistry of platinum in the +2 oxidation state, presenting modifications of both leaving and non-leaving group ligands. Beginning in this Chapter and continuing in Chapter 6, higher valent platinum complexes are explored. Multinuclear platinum complexes in the rare +3 oxidation state are described. It is well established that platinum typically attains oxidation states of 0, +2, or +4. The +3 oxidation state, however, is much less common for mononuclear complexes of this element, and only few such species are known.1-4 In contrast, platinum(III) compounds with metal–metal bonds are more common.5-7 The unpaired electrons of the platinum(III) units couple to form a metal–metal σ bond, thus eliminating any radical character and increasing the stability of the platinum(III) centers.

Early interest in dimeric and oligomeric platinum(III) complexes was motivated by the discovery8 that such species are a component of the deeply colored class of compounds known as the platinum blues,9,10 some members of which display anticancer properties.11,12 Since then, a large array of ligand-bridged dinuclear platinum(III) complexes have been synthesized and characterized,5-7 as well as several unbridged platinum(III) dimers with unsupported metal–metal bonds.13-18 In addition to their potential utility as anticancer agents,19-22 dinuclear platinum(III) complexes have found use as photoluminescent materials23-25 and catalysts.26

In this chapter, the previously reported dinuclear platinum(II) complex cis-[Pt(II)(NH₃)₂(μ-OAc)₂Pt(II)(NH₃)₂](NO₃)₂, [1](NO₃)₂,27 is utilized as a synthon for new platinum(III) species. Full structural and multinuclear NMR spectroscopic characterization of [1](NO₃)₂ and its two-electron oxidation products, cis-[ClPt(III)(NH₃)₂(μ-OAc)₂Pt(III)(NH₃)₂Cl](NO₃)₂, [2](NO₃)₂, and cis-[BrPt(III)(NH₃)₂(μ-OAc)₂Pt(III)(NH₃)₂Br](NO₃)₂, [3](NO₃)₂, are presented. Electrochemical studies
of these complexes are also reported. Lastly, the oxidation of \([1](\text{NO}_3)_2\) with PhI(\text{O}_2\text{CCF}_3)_2 and XeF_2 was explored.

5.2. Experimental Methods

**General Methods and Materials.** Cisplatin was purchased from Strem Chemicals and used as received. Silver(I) nitrate was obtained from Alfa Aesar, and bromine and bis(trifluoroacetoxy)iodobenzene were acquired from Sigma Aldrich. Iodobenzene dichloride was prepared by a previously reported method. Reactions were carried out under normal atmospheric conditions with no precautions taken to exclude moisture or oxygen unless otherwise stated.

**Physical Measurements.** NMR measurements were made on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility. \(^1\text{H}\) and \(^{13}\text{C}\{^1\text{H}\}\) NMR spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane, SiMe_4 (\(\delta = 0\) ppm). \(^{195}\text{Pt}\{^1\text{H}\}, \(^{19}\text{F}\{^1\text{H}\},\) and \(^{14}\text{N}\{^1\text{H}\}\) NMR spectra were referenced externally using standards of K_2PtCl_4 in D_2O (\(\delta = -1628\) ppm relative to Na_2PtCl_6), trifluorotoluene (\(\delta = -63.72\) ppm relative to CFCl_3), and NH_4Cl in 0.1 M HCl (\(\delta = 0\) ppm), respectively. Fourier transform infrared (FTIR) spectra were recorded with a ThermoNicolet Avatar 360 spectrophotometer running the OMNIC software. Samples were prepared as KBr disks. Melting points were measured on a Meltemp apparatus and are reported uncorrected. Electrochemical measurements were carried out utilizing a VersaSTAT3 potentiostat from Princeton Applied Research accompanied by the V3 Studio software. A three-electrode cell comprising a glassy carbon (GC) working electrode, a Pt wire auxiliary electrode, and a Ag/AgCl reference electrode was used. The electrolyte was 0.1 M (Bu_4N)(PF_6) (TBAP) in \(N,N\)-
dimethylformamide (DMF). Under the experimental conditions described, the reversible ferrocene/ferricenium redox couple occurred at 0.55–0.56 V versus the reference. Elemental analyses were performed by a commercial analytical laboratory.

Synthesis of cis-[Pt\(^{II}\)(NH\(_3\))\(_2\)(\(\mu\)-OAc)\(_2\)Pt\(^{II}\)(NH\(_3\))\(_2\)](NO\(_3\))\(_2\), [1](NO\(_3\))\(_2\). This compound was synthesized as previously reported\(^{27}\) with slight modifications. A suspension of cisplatin (1.00 g, 3.33 mmol) and AgNO\(_3\) (1.10 g, 6.48 mmol) in 15 mL of water was stirred for 16 h in the absence of light at room temperature. The resulting mixture was filtered to remove AgCl. To the filtrate was added 80% acetic acid (0.250 g, 3.33 mmol). Storage of this solution at 4 °C for 3 days afforded the desired compound as thin brown needles, which were isolated by filtration and washed with 5 mL of diethyl ether. Yield: 151 mg, 13%. Four additional crops could be isolated from the filtrate by continued storage at 4 °C over the course of several weeks. Yield of additional crops: 270 mg, 24%. Mp 194–200 °C (dec). \(^1\)H NMR (DMF-\(d_7\), 400 MHz): \(\delta\) 5.07 (br s, 12H), 1.97 (s, 6H). \(^{13}\)C\({^{\{1\}}H}\) NMR (DMF-\(d_7\), 100 MHz): \(\delta\) 185.9, 22.3. \(^{14}\)N\({^{\{1\}}H}\) NMR (DMF-\(d_7\), 29 MHz): \(\delta\) 356, −86 (\(W_{1/2} \approx 300 \text{ Hz}\)). \(^{195}\)Pt\({^{\{1\}}H}\) NMR (DMF-\(d_7\), 86 MHz): \(\delta\) −1401. IR (KBr, cm\(^{-1}\)): 3432 m, 3284 m, 1541 s, 1442 s, 1384 vs, 1303 s, 1042 w, 839 w, 704 w, 536 w.

Synthesis of cis-[ClPt\(^{III}\)(NH\(_3\))\(_2\)(\(\mu\)-OAc)\(_2\)Pt\(^{III}\)(NH\(_3\))\(_2\)Cl](NO\(_3\))\(_2\cdot3\)DMF, [2](NO\(_3\))\(_2\cdot3\)DMF. To a brown suspension of [1](NO\(_3\))\(_2\) (100 mg, 0.143 mmol) in 3 mL of DMF was added a solution of PhICl\(_2\) (40 mg, 0.15 mmol) in 1 mL of DMF. The brown suspension became a bright-yellow solution immediately upon the addition of PhICl\(_2\). Vapor diffusion of Et\(_2\)O into this yellow solution at 4 °C over the course of 16 h afforded bright-yellow crystals. The supernatant was decanted, and the crystals were rinsed three times with 2 mL of Et\(_2\)O before being dried in
vacuo. Yield: 130 mg, 92%. Mp 101–106 °C (dec). $^1$H NMR (DMF-$d_7$, 400 MHz): δ 6.44 (t, 12H, $^1$J$_{NH} = 49$ Hz), 2.33 (s, 6H). $^{13}$C{$^1$H} NMR (DMF-$d_7$, 100 MHz): δ 195.2, 22.4. $^{14}$N{$^1$H} NMR (DMF-$d_7$, 29 MHz): δ 355, −65 ($W_{1/2} \approx 50$ Hz). $^{195}$Pt{$^1$H} NMR (DMF-$d_7$, 86 MHz): δ −76 (quintet, $^1$J$_{PN} = 226$ Hz). IR (KBr, cm$^{-1}$): 3443 m, 3097 s, 1648 s, 1585 m 1540 m, 1496 w, 1384 vs, 1289 m, 1106 w, 1044 w, 911 w, 722 w, 665 w. Anal. Calcd. for [2](NO$_3$)$_2$·3DMF, C$_{13}$H$_{39}$Cl$_2$N$_9$O$_3$Pt$_2$: C, 15.76; H, 3.97; N, 12.73. Found: C, 15.65; H, 3.93; N, 12.56.

**Synthesis of cis-[BrPt$^{III}$(NH$_3$)$_2$(μ-OAc)$_2$Pt$^{III}$(NH$_3$)$_2$Br](NO$_3$)$_2$·3DMF, [3](NO$_3$)$_2$·3DMF. To a brown suspension of [1](NO$_3$)$_2$ (58 mg, 0.083 mmol) in 2 mL of DMF was added a freshly prepared 1.23 M solution of Br$_2$ in DMF (75 µL, 0.092 mmol of Br$_2$). The mixture became a dark-orange-red solution immediately. Vapor diffusion of Et$_2$O into the orange-red solution at 4 °C over the course of 16 h afforded bright-orange crystals. The supernatant was decanted, and the crystals were washed three times with 5 mL of Et$_2$O before being dried in vacuo. Yield: 80 mg, 89%. Mp 78–85 °C (dec). $^1$H NMR (DMF-$d_7$, 400 MHz): δ 6.43 (t, 12H, $^1$J$_{NH} = 44$ Hz), 2.31 (s, 6H). $^{13}$C{$^1$H} NMR (DMF-$d_7$, 100 MHz): δ 196.2, 22.6. $^{14}$N{$^1$H} NMR (DMF-$d_7$, 29 MHz): δ 355, −67 ($W_{1/2} \approx 45$ Hz). $^{195}$Pt{$^1$H} NMR (DMF-$d_7$, 86 MHz): δ −216 (quintet, $^1$J$_{PN} = 232$ Hz). IR (KBr, cm$^{-1}$): 3437 w, 3150 m, 1648 s, 1611 m, 1540 w, 1496 w, 1384 vs, 1352 m, 1286 w, 1107 w, 1043 w, 903 w, 720 w, 662 w. By combustion analysis, [3](NO$_3$)$_2$ analyzed as a solvate containing 2.75, rather than three, DMF molecules per formula unit as observed by X-ray crystallography and $^1$H NMR spectroscopy. Presumably, a small fraction of DMF was lost from the lattice under vacuum or during transport of the sample. Anal. Calcd. for [3](NO$_3$)$_2$·2.75$DMF, C$_{12.25}$H$_{37.25}$Br$_2$N$_{8.75}$O$_{12.75}$Pt$_2$: C, 13.86; H, 3.54; N, 11.55. Found: C, 13.85; H, 3.52; N, 11.34.

**Synthesis of cis-[(O$_2$CCF$_3$)Pt$^{III}$(NH$_3$)$_2$(μ-OAc)$_2$Pt$^{III}$(NH$_3$)(μ-NH$_2$)]$_2$(NO$_3$)$_4$·4DMF, [4](NO$_3$)$_4$·4DMF. To a suspension of [1](NO$_3$)$_2$ (62 mg, 0.089 mmol) in 1 mL of DMF was
added a solution of PhI(O₂CCF₃)₂ (39 mg, 0.091 mmol) in 1 mL of DMF. The mixture, initially a
dark-red suspension, became a yellow solution after 10 min at room temperature. Vapor
diffusion of Et₂O into this yellow solution afforded yellow-orange crystals after 16 h at room
temperature. The supernatant was decanted, and the crystals were washed three times with 2 mL
of Et₂O before being dried in vacuo. Yield: 68 mg, 40%. Mp: >180 °C (gradual darkening),
195–202 °C (dec). IR (KBr, cm⁻¹): 3432 br m, 3122 br m, 1651 vs, 1542 m, 1454 s, 1421 s, 1384
vs, 1301 m, 1267 m, 1190 m, 1138 w, 1083 w, 837 vw, 800 vw, 722 w, 666 vw, 521 vw,
Found: C, 14.97; H, 3.10; N, 11.94.

**Reaction of [1](NO₃)₂ with XeF₂.** In a nitrogen-filled glovebox, [1](NO₃)₂ (10 mg, 0.014 mmol)
was suspended in 1 mL of dry DMF. A solution of XeF₂ (3 mg, 0.02 mmol) in 1 mL of dry DMF
was added. A yellow solution formed immediately with a small amount of dark brown
precipitate. The solution was separated from the precipitate by decantation. Vapor diffusion of
Et₂O into this solution over 16 h at room temperature under N₂ afforded thin yellow needles
suitable for analysis by single crystal X-ray diffraction. Upon drying under vacuum and exposure
to air, the needles decomposed to a brown oily residue.

**X-ray Crystallography.** Single crystals, mounted in Paratone oil on a cryoloop, were cooled to
100 K under a nitrogen cold stream. A Bruker APEX CCD X-ray diffractometer controlled by
the APEX2 software package²⁹ with a graphite-monochromated Mo Kα radiation source (λ =
0.71073 Å) was used for data collection. Data were integrated with SAINT³⁰ and then corrected
for absorption with SADABS.³¹ Space-group determination was carried out by analysis of the
metric symmetry of the unit cell and systematic absences in the diffraction pattern using the
program XPREP.³² Structures were solved and refined against F² with the SHELXTL-97
software package. Non-hydrogen atoms were located on difference Fourier maps. Hydrogen atoms were placed at idealized locations with displacement parameters constrained to be either 1.2 or 1.5 times (for terminal CH$_3$ or NH$_3$ groups) the thermal parameter of the atoms to which they were attached. Specific refinement details are provided below, and relevant X-ray crystallographic data collection and refinement parameters are reported in Table 5.1.

Vapor diffusion of Et$_2$O into a DMF solution of [1](NO$_3$)$_2$ afforded a mixture of thin yellow needle-like crystals and orange shards. An orange shard was selected for X-ray diffraction analysis because the yellow needles were too small to afford appreciable diffraction. The asymmetric unit consists of the [1]$^{2+}$ cation, a nitrate anion situated on a general position at full occupancy, a nitrate anion residing on a crystallographic 2-fold axis, and a nitrate anion disordered with a water molecule about a crystallographic inversion center. For the nitrate anion disordered about the inversion center, the bond distances and angles were restrained to be the same as those of the non-disordered nitrate anion. The occupancy factors of the disordered nitrate and water atoms were 50:50, as necessitated by the crystallographic inversion center about which they are disordered. The hydrogen atoms of this half-occupancy water molecule could not be located on the difference Fourier map and were therefore not included in the final model. The remaining largest electron density peak (1.32 e·Å$^{-3}$) and hole (−1.53 e·Å$^{-3}$) are 0.77 and 0.66 Å from Pt1, respectively.

Yellow crystals of [2](NO$_3$)$_2$·3DMF were grown by vapor diffusion of Et$_2$O directly into the DMF solution of the crude reaction mixture. The asymmetric unit comprises one molecule of [2](NO$_3$)$_2$ and three molecules of DMF. One molecule of DMF is disordered over two orientations. The bond lengths and angles of the two disordered components were restrained to be the same as one another, as were the thermal displacement parameters. The site occupancy
factors of the disordered components refined to a ratio of 71:29. The remaining largest electron density peak (3.64 e·Å⁻³) and hole (−1.83 e·Å⁻³) are 0.77 and 0.64 Å from Pt2, respectively.

Orange crystals of [3](NO₃)₂·3DMF were grown by vapor diffusion of Et₂O directly into a DMF solution of the crude reaction mixture. The asymmetric unit comprises two molecules of [3](NO₃)₂ and six molecules of DMF. One molecule of DMF is disordered over two positions. The bond lengths and angles of the two disordered components were restrained to be the same as one another, as were the thermal displacement parameters. The site occupancy factors of the disordered components were allowed to refine freely and converged at a ratio of 52:48. The central nitrogen atom of one of the nitrate counterions (N9) gave non-positive definite ellipsoids upon anisotropic refinement. Attempts to constrain the ellipsoid parameters to match those of other well-behaved nitrate nitrogen atoms in the structure gave rise to spurious large electron density peaks and holes in the difference Fourier map near these atoms. In the final model, N9 was therefore refined isotropically without restraints or constraints. We hypothesize that the difficulty in refining N9 may arise from a small amount of bromide ion at the site of the nitrate counterion, which would lead to the observed contraction of the N9 thermal displacement parameter. The remaining largest electron density peak (1.67 e·Å⁻³) and hole (−1.60 e·Å⁻³) appear at 0.66 Å from Pt4 and 0.63 Å from Pt2, respectively.

Pale-yellow crystals of [4](NO₃)₄·4DMF were obtained by vapor diffusion of Et₂O directly into a DMF solution of the crude reaction mixture. Half of the tetranuclear complex cation is related to the other half by a crystallographic inversion center. Also in the asymmetric unit are the two nitrate counterions and two molecules of DMF. One of the DMF molecules is disordered over two orientations. Similarity restraints were used to model this disorder and the site occupancy factors refined to a ratio of 56:44. The axial trifluoroacetate ligands of [4]⁺ were
also disordered. One carbon (C1) atom and one oxygen (O2) atom were modeled in different orientations using the appropriate similarity restraints. The disordered components refined to site occupancy factors of 52:48. After full anisotropic refinement and placement of the hydrogen atoms, residual electron density was still present about a crystallographic inversion center. This electron density was modeled with the SQUEEZE algorithm as part of the crystallographic program PLATON.35 Two solvent-accessible voids of 300 Å³ with 91 e⁻ were excluded from this model with SQUEEZE. We hypothesize that this electron density corresponds to disordered diethyl ether molecules that are readily lost under reduced pressure. The largest remaining electron density peak (4.85 e Å⁻³) and hole (−1.76 e Å⁻³) were located 1.07 and 0.85 Å from Pt2 and Pt1, respectively.

Yellow needles, 5, of the reaction products of [1](NO₃)₂ and XeF₂ were obtained by the vapor diffusion of Et₂O into a DMF solution. The X-ray crystal structure revealed an infinite platinum chain, which propagates down the crystallographic c-axis. Only one nitrate counterion could be definitely located on the difference map. A large amount of unresolvable electron density remained in the crystal lattice. This density was modeled with SQUEEZE. The volume of the void was 1209 Å³ and the electron density corresponded to 1062 e⁻.
Table 5.1. X-Ray Crystallographic Data Collection and Refinement Parameters.

<table>
<thead>
<tr>
<th></th>
<th><a href="NO%E2%82%83">1</a>₂·0.5H₂O</th>
<th><a href="NO%E2%82%83">2</a>₂·3DMF</th>
<th><a href="NO%E2%82%83">3</a>₂·3DMF</th>
<th><a href="NO%E2%82%83">4</a>₂·4DMF</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C₂₉H₃₈N₆O₁₀·5Pt₂</td>
<td>C₁₃H₅₂Cl₂N₂O₁₃Pt₂</td>
<td>C₁₃H₅₂Br₂N₂O₁₃Pt₂</td>
<td>C₂₄H₆₂F₆N₁₆O₂₈Pt₄</td>
<td>C₄₈H₁₈N₂O₇Pt₂</td>
</tr>
<tr>
<td>Mw</td>
<td>708.42</td>
<td>990.61</td>
<td>1079.53</td>
<td>1917.26</td>
<td>638.41</td>
</tr>
<tr>
<td>Space group</td>
<td>C2/c</td>
<td>P2₁/c</td>
<td>P2₁/c</td>
<td>C2/c</td>
<td>P6₃22</td>
</tr>
<tr>
<td>a, Å</td>
<td>19.1794(9)</td>
<td>13.3959(5)</td>
<td>24.2462(12)</td>
<td>35.401(4)</td>
<td>17.7995(9)</td>
</tr>
<tr>
<td>b, Å</td>
<td>15.7804(7)</td>
<td>10.3272(4)</td>
<td>10.5186(5)</td>
<td>13.8473(14)</td>
<td>11.5444(11)</td>
</tr>
<tr>
<td>c, Å</td>
<td>10.8811(5)</td>
<td>25.3699(10)</td>
<td>26.2471(13)</td>
<td>12.8506(13)</td>
<td>10.7367(2)</td>
</tr>
<tr>
<td>β, deg</td>
<td>100.4520(10)</td>
<td>121.7120(10)</td>
<td>113.6230(10)</td>
<td>107.367(2)</td>
<td>113.6230(10)</td>
</tr>
<tr>
<td>V, Å³</td>
<td>3238.6(3)</td>
<td>2985.7(2)</td>
<td>6133.0(5)</td>
<td>6012.3(11)</td>
<td>3167.5(4)</td>
</tr>
<tr>
<td>Z</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Density, g·cm⁻³</td>
<td>2.906</td>
<td>2.204</td>
<td>2.338</td>
<td>2.118</td>
<td>2.008</td>
</tr>
<tr>
<td>T, °C</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
</tr>
<tr>
<td>μ(Mo Kα), mm⁻¹</td>
<td>17.320</td>
<td>9.610</td>
<td>11.788</td>
<td>9.384</td>
<td>13.256</td>
</tr>
<tr>
<td>θ range, deg</td>
<td>1.68–30.10</td>
<td>1.79–29.33</td>
<td>1.83–28.92</td>
<td>1.59–25.13</td>
<td>1.32–27.17</td>
</tr>
<tr>
<td>Total no. of data</td>
<td>35449</td>
<td>61247</td>
<td>124152</td>
<td>45478</td>
<td>56908</td>
</tr>
<tr>
<td>No. of unique data</td>
<td>4748</td>
<td>8166</td>
<td>16123</td>
<td>5366</td>
<td>2354</td>
</tr>
<tr>
<td>No. of parameters</td>
<td>233</td>
<td>387</td>
<td>755</td>
<td>424</td>
<td>86</td>
</tr>
<tr>
<td>Completeness to θ (%)</td>
<td>99.4</td>
<td>99.7</td>
<td>99.6</td>
<td>99.7</td>
<td>99.7</td>
</tr>
<tr>
<td>R1 (%)</td>
<td>2.27</td>
<td>3.72</td>
<td>5.55</td>
<td>7.78</td>
<td>5.21</td>
</tr>
<tr>
<td>wR2 (%)</td>
<td>3.83</td>
<td>6.59</td>
<td>7.21</td>
<td>14.86</td>
<td>10.40</td>
</tr>
<tr>
<td>R1(%) for I &gt; 2σ</td>
<td>1.82</td>
<td>2.86</td>
<td>3.74</td>
<td>5.36</td>
<td>4.27</td>
</tr>
<tr>
<td>wR2(%) for I &gt; 2σ</td>
<td>3.70</td>
<td>6.22</td>
<td>6.66</td>
<td>13.61</td>
<td>10.06</td>
</tr>
<tr>
<td>GOFc</td>
<td>1.036</td>
<td>1.119</td>
<td>1.105</td>
<td>1.071</td>
<td>1.139</td>
</tr>
<tr>
<td>Max, min peaks, eÅ⁻³</td>
<td>1.320, -1.529</td>
<td>3.643, -1.828</td>
<td>1.673, -1.599</td>
<td>4.849, -1.761</td>
<td>1.183, -0.868</td>
</tr>
<tr>
<td>Flack parameter</td>
<td>0.03(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a R1 = Σ|F₁| - |F₂|/Σ|F₁|.  
*b wR2 = Σ[w(Fo² - Fc²)²]/Σ[w(Fo²)²]^(1/2).  
*c GOF = {Σ[w(Fo² - Fc²)²]}/(n - p)^(1/2) where n is the number of data and p is the number of refined parameters.

**Computational Studies.** Geometries were optimized in the gas-phase starting from coordinates obtained by X-ray crystallography. Frequency calculations based on optimized geometries were employed to ensure convergence to local minima on the potential energy surface. For [1]²⁺ and [1](NO₃)⁺, geometry optimization required the use of an ultrafine integration grid in order to achieve convergence at a local minimum. All geometry optimization and frequency calculations were carried out using the Gaussian03 software package with the hybrid functional PBE0. ³⁷
The LANL2DZ basis set and effective core potential\textsuperscript{38} were utilized for platinum atoms, and the 6-31+G(d,p) basis set\textsuperscript{39} was used for the other elements.

Electric field gradient (EFG) parameters and \(^{14}\text{N}\) NMR chemical shifts were computed with the program ORCA\textsuperscript{40} using optimized geometries obtained from the Gaussian03 calculations. The PBE0 functional was applied for these calculations as well. The all-electron basis set, def2-TZVP(-f), and its corresponding decontracted auxiliary basis set, def2-TZVP/J,\textsuperscript{41} were used for all atoms. The zeroth-order regular approximation (ZORA)\textsuperscript{42} was applied to correct for relativistic effects. Solvation effects were modeled with the conductor-like screening model (COSMO)\textsuperscript{43} for DMF. Isotropic shielding parameters for \(^{14}\text{N}\) nuclei were computed with the IGLO approach,\textsuperscript{44,45} as implemented in ORCA. The isotropic shielding of \(\text{NH}_4^+\) was calculated at the same level of theory with simulated solvation in water and was used as a reference to convert other values to ppm.

5.3. Results and Discussion

Synthesis. Treatment of the cis-diamminediaquaplatinum(II) cation with 1 equiv of acetic acid in water afforded the acetate-bridged dinuclear platinum(II) complex [I]\textsuperscript{2+} (Scheme 5.1), as previously described.\textsuperscript{27} At 4 °C, the nitrate salt of this compound precipitates from aqueous solution as brown needles. The initial yield of the compound was only 13%. More crops, however, could be isolated from the filtrate, increasing the total yield to 37%. In aqueous solution, a mixture of acetic acid and the cis-diamminediaquaplatinum(II) cation gives rise to multiple species that differ in the binding mode and stoichiometry of the acetate ligand.\textsuperscript{46}
Scheme 5.1. Synthesis of [1](NO₃)₂.

When a solution of [1](NO₃)₂ in DMF was allowed to react with slightly greater than 1 equiv of the two-electron-oxidizing agents PhICl₂ or Br₂, nitrate salts of the halide-capped platinum(III) dimers, [2]²⁺ and [3]²⁺, respectively, were obtained (Scheme 5.2). Although partially oxidized chains of [1]²⁺ have previously been isolated following bulk electrolysis,⁴⁷ compounds [2]²⁺ and [3]²⁺ are the first examples of discrete cis-diammineplatinum(III) dinuclear complexes bridged by acetate ligands. Previously employed bridging ligands for dinuclear cis-diammineplatinum(III) complexes are primarily monoanionic nitrogen and oxygen donors, including α-pyridonate,⁴⁸-⁵² α-pyrrolidonate,⁵³-⁵⁵ pyrimidines,⁵⁶-⁶⁰ and amidates.⁶¹-⁶⁴ Somewhat analogous carboxylate-bridged dinuclear cis-dimethylplatinum(III) complexes have been reported,⁶⁵-⁶⁸ and carboxylate-bridged cyclometalated diplatinum(III) complexes have also recently been described.⁶⁹-⁷¹
Scheme 5.2. Syntheses of [2](NO₃)₂ and [3](NO₃)₂.

Description of Crystal Structures. Dinuclear platinum(II) cation [1]²⁺ has previously been structurally characterized as the SiF₆²⁻ salt.²⁷ Here, crystals of [1]²⁺ as the nitrate salt were obtained by vapor diffusion of Et₂O into a DMF solution. Two different crystal forms deposited under these conditions: very fine yellow needles, which were too small for X-ray diffraction studies, and larger orange shards, which were selected for analysis by X-ray diffraction. The structure of the orange cation [1]²⁺ is shown in Figure 5.1, and selected structural features are summarized in Table 5.2. As expected, the structure is an acetate-bridged dimer. There are two nitrate counterions per platinum(II) dimer, consistent with a +2 oxidation state of the platinum ions and indicating the stability of the complex in the presence of oxygen. The distance between Pt1 and Pt2 (atoms labeled in Figure 5.1) is 2.92149(18) Å. This value is significantly longer than those of dinuclear platinum(III) complexes, which generally range from 2.5 to 2.7 Å,⁵ indicating the absence of a formal metal–metal bond between the two d⁸ metal ions.⁷² The tilt angle (τ) between adjacent platinum coordination planes is 31.9°, and the average torsion angle (ω) about the Pt–Pt vector is 5.5°. These values for [1]²⁺ compare favorably to those in the related α-pyridonate head-to-tail-bridged dinuclear platinum(II) complex [Pt₂(NH₃)₄(C₂H₄NO)₂](NO₃)₂, which are 2.8981(5) Å and 28.8°, respectively.⁷³ The torsion angle in the α-pyridonate complex is 13.0°.⁷³
As indicated in Figure 5.1, dimers of $[1]^{2+}$ stack in the crystal lattice to form infinite chains. The Pt–Pt separation between Pt1 and its symmetry equivalent, Pt1A, is 3.1523(2) Å, and the corresponding value for the Pt2/Pt2A pair is 3.1324(3) Å. Hydrogen-bonding interactions between oxygen atoms of the bridging acetates and protons of the ammine ligands stabilize the interaction between Pt2 and Pt2A, for which the torsion angle is 180° and the N–O distances are 2.97 and 3.00 Å. At the Pt1–Pt1A interface, the torsion angle is 41.5° and the nearest N–O distance is 3.19 Å, which rules out hydrogen bonding as a contributor to this interaction. Closed shell d$^8$–d$^8$ interactions most likely promote this close contact. The structure of the...
previously reported SiF$_6^{2-}$ salt of [1]$^{2+}$ also includes infinite stacking of platinum(II) dimer
cations,\textsuperscript{27} which excludes crystal packing as an explanation for the phenomenon

The solid-state molecular structures of [2]$^{2+}$ and [3]$^{2+}$ reveal discrete halide-capped cis-
diammineplatinum(III) dimers, with the platinum atoms bridged by two acetate ligands (Figure
5.2). Selected structural features of these and related dinuclear platinum complexes are given in
Table 5.2. The two-electron oxidation of [1]$^{2+}$ to generate [2]$^{2+}$ or [3]$^{2+}$ is accompanied by an
approximate 0.3 Å shortening of the Pt–Pt distance and a 10° decrease in the tilt angle,
signifying the removal of two electrons from a strongly metal–metal antibonding orbital (vide
infra). The Pt–Pt distance in [2]$^{2+}$ is 2.5997(2) Å. There are two molecules of [3]$^{2+}$ in the
asymmetric unit, with Pt–Pt distances of 2.6004(3) and 2.6052(3) Å. The small difference in the
Pt–Pt bond lengths between [2]$^{2+}$ and [3]$^{2+}$ indicates that the axial ligands have a negligible trans
influence. This result is similar to that for the head-to-tail $\alpha$-pyridonate-bridged platinum(III)
dimers,\textsuperscript{49} where the Pt–Pt bond lengthens by only 0.014(1) Å upon changing axial ligands from
chloride to bromide.\textsuperscript{51} For similar head-to-head $\alpha$-pyrrolidonate-bridged dimers, the Pt–Pt bond
elongates by 0.024(1) Å when chloride is replaced by bromide.\textsuperscript{77,78} For [2]$^{2+}$ and [3]$^{2+}$, the trans
influence of the Pt–Pt bond on the axial Pt–ligand bond is clearly noticeable, however. The
Pt–Cl bond lengths in [2]$^{2+}$ are 2.4321(12) and 2.4017(11) Å, >0.1 Å larger than typical Pt–Cl
bond distances in most platinum(II) and platinum(IV) structures. A similar effect occurs in [3]$^{2+}$,
where the Pt–Br distances (2.5399–2.5597 Å) are greater than typical 2.45 Å Pt–Br distances in
other compounds.
Figure 5.2. Solid-state molecular structures of the cations [2]$^{2+}$ (left) and [3]$^{2+}$ (right). Only one of the cations in the asymmetric unit of [3]$^{2+}$ is shown. Thermal ellipsoids are drawn at the 50% probability level.

The torsion angles in [1]$^{2+}$-[3]$^{2+}$ are all less than 5.5°, indicating nearly eclipsed geometries. The α-pyridonate-bridged platinum(II) complexes exhibit moderate torsion angles (13.0°), which increase upon oxidation to ~28° (Table 5.2). The α-pyrrolidonate-bridged platinum complexes like [1]$^{2+}$-[3]$^{2+}$ remain in eclipsed conformations (ω < 5°) irrespective of the oxidation state (Table 5.2). Structurally analogous carboxylate-bridged platinum(III) dimers with cis-methyl groups instead of ammines exhibit relatively large torsion angles (22–25°). The small torsion angles of [1]$^{2+}$-[3]$^{2+}$ are therefore most likely not a consequence solely of the bridging acetate ligands. Ammine ligands are much stronger hydrogen-bond donors than unlike methyl groups. Indeed, significant hydrogen-bonding interactions are present in the crystal lattices of [1]$^{2+}$-[3]$^{2+}$ with DMF and nitrate ions. These hydrogen-bonding interactions may also stabilize the eclipsed conformations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pt oxidation state</th>
<th>Pt–Pt dist, Å</th>
<th>Pt–L$_{axial}$ dist, Å</th>
<th>$\tau$ ° deg</th>
<th>$\omega$ ° deg</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Pt(OC\text{C}CH}_3\text{)(NH}_3\text{)(NO}_3\text{)]}_2$</td>
<td>2</td>
<td>2.92149(18)</td>
<td>31.9</td>
<td>5.5</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>$[\text{Pt(OC\text{C}CH}_3\text{)(NH}_3\text{)(SiF}_4\text{-4H}_2\text{O}$</td>
<td>2</td>
<td>2.9713(8)</td>
<td>36.3</td>
<td>7.2</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>HT-$[\text{Pt(C}_5\text{H}_4\text{NO})(\text{NH}_3\text{)(NO}_3\text{)]}_2$-$2\text{H}_2\text{O}$</td>
<td>2</td>
<td>2.8981(5)</td>
<td>28.8</td>
<td>13.0</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>HH-$[\text{Pt(C}_5\text{H}_4\text{NO})(\text{NH}_3\text{)(SO}_4\text{-H}_2\text{O}$</td>
<td>2</td>
<td>2.9749 (11)</td>
<td>29.9</td>
<td>4.3</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>$[\text{PtCl}_2(\text{OC\text{C}CH}_3\text{)(NH}_3\text{)(NO}_3\text{)]}_2$-3DMF, $[2]$(NO$_3$)$_2$-3DMF</td>
<td>3</td>
<td>2.5997(2)</td>
<td>2.4321(12)</td>
<td>2.4017(11)</td>
<td>19.3</td>
<td>1.4</td>
</tr>
<tr>
<td>$[\text{PtBr}_2(\text{OC\text{C}CH}_3\text{)(NH}_3\text{)(NO}_3\text{)]}_2$-3DMF, $[3]$(NO$_3$)$_2$-3DMF</td>
<td>3</td>
<td>2.6004(3)</td>
<td>2.5590(7)</td>
<td>2.5465(7)</td>
<td>18.4</td>
<td>3.5</td>
</tr>
<tr>
<td>HT-$[\text{PtCl}_2(\text{C}_5\text{H}_4\text{NO})(\text{NH}_3\text{)(NO}_3\text{)]}_2$</td>
<td>3</td>
<td>2.568(1)</td>
<td>2.444(2)</td>
<td>2.429(4)</td>
<td>19.5</td>
<td>27.4</td>
</tr>
<tr>
<td>HT-$[\text{PtBr}_2(\text{C}_5\text{H}_4\text{NO})(\text{NH}_3\text{)(NO}_3\text{)]}_2$-0.5H$_2$O</td>
<td>3</td>
<td>2.582(1)</td>
<td>2.573(1)</td>
<td>2.562(1)</td>
<td>20.5</td>
<td>29.4</td>
</tr>
<tr>
<td>HH-$[\text{PtCl}_2(\text{C}_5\text{H}_4\text{NO})(\text{NH}_3\text{)(SO}_4\text{-2H}_2\text{O}$</td>
<td>3</td>
<td>2.6235 (13)</td>
<td>2.410 (5)</td>
<td>2.446 (5)</td>
<td>16.3</td>
<td>0.4</td>
</tr>
<tr>
<td>HH-$[\text{PtBr}_2(\text{C}_5\text{H}_4\text{NO})(\text{NH}_3\text{)(NO}_3\text{)]}_2$</td>
<td>3</td>
<td>2.6476 (4)</td>
<td>2.5647 (9)</td>
<td>2.5889 (8)</td>
<td>18.1</td>
<td>1.0</td>
</tr>
<tr>
<td>$[\text{Pt(C}_5\text{H}_4\text{N}_2\text{)(OC\text{C}CF}_3\text{)(CH}_3\text{)]}_2$</td>
<td>3</td>
<td>2.557(1)</td>
<td>2.09(2)</td>
<td>2.17(2)</td>
<td>17.6</td>
<td>22</td>
</tr>
<tr>
<td>$[\text{Pt(C}_5\text{H}_4\text{N}_2\text{)(OC\text{C}CH}_3\text{)(CH}_3\text{]}_2$</td>
<td>3</td>
<td>2.529(1)</td>
<td>2.200(11)</td>
<td>15.6</td>
<td>24.8</td>
<td>68</td>
</tr>
</tbody>
</table>

$^a$ $\tau$ is the tilt angle between adjacent platinum coordination planes. $^b$ $\omega$ is the average torsion angle about the Pt–Pt vector. $^c$ This work. $^d$ The features of both molecules in the asymmetric unit are reported.

**Multinuclear NMR Spectroscopy.** Further characterization of $[1]^{2+}$–$[3]^{2+}$ was provided by $^1$H, $^{13}$C, $^{14}$N, and $^{195}$Pt NMR spectroscopy (Table 5.3). The $^{13}$C NMR spectra each display two signals for the inequivalent carbon atoms of the bridging acetate ligands. Although the methyl group resonance, which occurs near 22 ppm for $[1]^{2+}$–$[3]^{2+}$, is not affected by the platinum oxidation state, the central carboxylate carbon shifts downfield by approximately 10 ppm, from 185.9 to 195.2 and 196.2 ppm, upon oxidation to $[2]^{2+}$ and $[3]^{2+}$, respectively.
Table 5.3. Multinuclear NMR Spectroscopic Data for $[1]^{2+}$–$[3]^{2+}$.a

<table>
<thead>
<tr>
<th>compound</th>
<th>$^1\text{H}$ $\delta$ NH$_3$, ppm</th>
<th>$^1J_{\text{NH}}$, Hz</th>
<th>$^{13}\text{C}$ $\delta$ CH$_3$CO$_2^-$</th>
<th>$^{14}\text{N}$ $\delta$ NH$_3$, ppm</th>
<th>$^{195}\text{Pt}$ $\delta$, ppm</th>
<th>$^{1J}_{\text{N-PT}}$, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[1]^{2+}$</td>
<td>5.07</td>
<td>n.o. b</td>
<td>185.9</td>
<td>-86</td>
<td>-1401</td>
<td>n.o. b</td>
</tr>
</tbody>
</table>

a All spectra were acquired at room temperature in DMF-$d_7$. b Not observed under these experimental conditions.

The $^1\text{H}$ NMR spectra of $[1]^{2+}$–$[3]^{2+}$ display sharp singlets for the CH$_3$ group of the bridging acetate near 2 ppm and broad resonances between 5 and 7 ppm for the coordinated NH$_3$ groups. Upon oxidation of $[1]^{2+}$, the methyl group resonance shifts from 1.97 to 2.33 and 2.31 ppm for $[2]^{2+}$ and $[3]^{2+}$, respectively, indicating that the electron-deficient platinum(III) center has an appreciable effect on the electron density at the protons of the methyl group. A similar effect is observed for the protons of the NH$_3$ ligands. The greater deshielding effect of the platinum(III) centers shifts the resonances of these protons from 5.07 ppm in $[1]^{2+}$ to 6.44 ppm in $[2]^{2+}$ and $[3]^{2+}$. Whereas the resonance appears as a broad singlet for $[1]^{2+}$, it is a broadened triplet in $[2]^{2+}$ and $[3]^{2+}$ (Figure 5.3). The triplet splitting pattern is due to coupling with the quadrupolar $^{14}\text{N}$ nucleus ($I = 1$, 99.63% natural abundance). Quadrupolar relaxation by the $^{14}\text{N}$ nucleus broadens the lines and leads to poorly resolved multinuclear coupling. At constant temperature and solvent viscosity, the quadrupolar relaxation rate depends on the asymmetry and magnitude of the electric field gradient (EFG) at the nucleus.81 Because the solvent and temperature used for all NMR measurements were the same, the ability to resolve $^1\text{H}$–$^{14}\text{N}$ coupling in $[2]^{2+}$ and $[3]^{2+}$ denotes a significant change in the EFG at the coordinated amines in the platinum(III) complexes. Values for $^{1J}_{\text{NH}}$ of $[2]^{2+}$ and $[3]^{2+}$ are 49 and 44 Hz, respectively, slightly smaller than those observed for cis-diamminedichloroplatinum(IV) complexes bearing axial aromatic carboxylate ligands, which range from 53 to 54 Hz.82
Figure 5.3. NH₃ region of the ¹H NMR spectra of [1]²⁺-[3]²⁺ in DMF-d₇ recorded at room temperature and at a frequency of 400 MHz.

Proton-decoupled ¹⁴N NMR spectra were also recorded. ¹⁴N NMR spectral analyses of platinum complexes have been used to study reactions of cisplatin with components of human blood plasma. More recently, this methodology was applied to investigate photoreactive anticancer platinum(IV) azido complexes. Figure 5.4 shows a comparison of the ¹⁴N NMR spectra of [1]²⁺-[3]²⁺ in the NH₃ region. Not shown are sharp signals that appear at 355 ppm, due to the nitrate counterions for all three complexes. The NH₃ signal of [1]²⁺ shifts downfield upon oxidation to form [2]²⁺ and [3]²⁺, from −86 to −65 and −67 ppm, respectively, consistent with the higher oxidation state in the latter complexes. The coordinated ammine ligands in platinum(IV) azido complexes shift even farther downfield, to ~−40 ppm. The ¹⁴N NMR resonance of the coordinated NH₃ group in [1]²⁺ is significantly broader ($W_{1/2} \approx 300$ Hz) than those of the
platinum(III) complexes $[2]^{2+}$ and $[3]^{2+}$ ($W_{1/2} \approx 50$ and 45 Hz). Because quadrupolar relaxation is the dominant relaxation mechanism for $^{14}$N nuclei, the sharpening of the signal in $[2]^{2+}$ and $[3]^{2+}$ most likely reflects a decrease in the quadrupolar relaxation rate induced by changing the EFG upon oxidation, as discussed above. Shoulders are also present in the NH$_3$ signal of $[2]^{2+}$ and $[3]^{2+}$. These shoulders are due to coupling to the $^{195}$Pt nucleus ($I = 1/2$), which is 33% abundant.

![NMR spectra](image)

**Figure 5.4.** $^{14}$N{H} NMR spectra of $[1]^{2+}$–$[3]^{2+}$ in DMF-$d_7$ recorded at room temperature and at a frequency of 29 MHz.

The proton-decoupled $^{195}$Pt NMR spectra of $[1]^{2+}$–$[3]^{2+}$ are shown in Figure 5.5. The broad peak for $[1]^{2+}$ occurs at $-1401$ ppm, a value similar to those of other platinum(II) complexes having $O_2N_2$ coordination environments. The $^{195}$Pt NMR chemical shifts for $[2]^{2+}$ and $[3]^{2+}$ are $-76$ and $-216$ ppm, respectively, within the region expected for a dinuclear
platinum(III) complex. The 140 ppm upfield shift of [3]$^{2+}$ relative to [2]$^{2+}$ is consistent with the empirical observation that softer ligands shift $^{195}$Pt NMR resonances to more negative values. The signals of [2]$^{2+}$ and [3]$^{2+}$ appear as quintets, which arise from coupling to two equivalent $^{14}$N nuclei. The presence of such $^{14}$N coupling in the spectra of [2]$^{2+}$ and [3]$^{2+}$, but not [1]$^{2+}$, is most likely a consequence of decreased quadrupolar relaxation at the nitrogen nuclei in the latter. Chemical shift anisotropy (CSA) of $^{195}$Pt plays a large role in its relaxation and attendant NMR line width. The octahedral coordination geometries of [2]$^{2+}$ and [3]$^{2+}$ produce less CSA than the square-planar geometry of [1]$^{2+}$. Smaller CSAs for [2]$^{2+}$ and [3]$^{2+}$ decrease the line width and are another factor that enables observation of $^{14}$N–$^{195}$Pt coupling. The values for $^1J_{NPt}$ for [2]$^{2+}$ and [3]$^{2+}$ are 226 and 232 Hz, respectively, larger than that of the platinum(IV) complex cis,cis,trans-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$], 194 Hz, and those of several cis-diamminedichloroplatinum(IV) complexes having axial aromatic carboxylate ligands, which range from 172 to 179 Hz.
Electrochemistry. The cyclic voltammogram of $[1]^{2+}$ in DMF with 0.1 M TBAP is shown in Figure 5.6. An irreversible oxidation is observed at 1.20 V vs Ag/AgCl. The return scan shows a peak at 0.53 V, presumably corresponding to the reduction of the oxidized species obtained on the forward scan. In contrast to the irreversible oxidation observed for $[1]^{2+}$, a number of ligand-bridged dinuclear platinum(II) complexes exhibit reversible oxidations on the cyclic voltammetry (CV) time scale.\textsuperscript{48,50,94-96} Even at scan rates as low as 10 mV·s\textsuperscript{-1} and as high as 1000 mV·s\textsuperscript{-1} and at −20 °C, the oxidation of $[1]^{2+}$ remained irreversible.
Figure 5.6. Cyclic voltammogram of [1](NO₃)₂ in DMF with 0.1 M TBAP obtained at a scan rate of 100 mV·s⁻¹. The arrows mark the initial potential and scan direction. Potentials are referenced to Ag/AgCl.

The cyclic voltammograms of [2]²⁺ and [3]²⁺, obtained under the same conditions, are shown in Figure 5.7. The initial scan toward negative potential for [2]²⁺ reveals a reduction with a peak potential of 0.12 V. Return scans to positive potentials are marked by weak oxidation features at 0.90 and 1.40 V. On the second and subsequent cycles, the irreversible reduction peak broadens and shifts slightly negative, to −0.05 V. At −20 °C and at both fast (1000 mV·s⁻¹) and slow (10 mV·s⁻¹) scan rates, no reversibility is observed. The initial reduction peak potential of [3]²⁺ is 0.18 V. This peak does not shift after the first cycle or subsequent cycles. The return scan shows an irreversible peak at 0.90 V that is much more pronounced than those observed for [2]²⁺. As with [1]²⁺ and [2]²⁺, no reversibility was observed at −20 °C at different scan rates. It is somewhat surprising that the reduction peak potentials of [2]²⁺ and [3]²⁺ are so similar. The axial halide ligands appear to have little influence on this peak potential. This observation is in
contrast to a previous electrochemical study on lantern-type pyrimidinethiolate-bridged platinum(III) dimers, in which complexes bearing axial bromide ligands were reduced at potentials that were 100 mV more positive than analogous complexes with axial chloride ligands. Also strange is the fact that the return oxidation features are different. Intuitively, it is expected that the reduction products of $[2]^{2+}$ and $[3]^{2+}$ might be $[1]^{2+}$. Because the return oxidation does not occur at 1.20 V, as observed in the CV of $[1]^{2+}$, it appears that different reduction products are being formed for $[2]^{2+}$ and $[3]^{2+}$.

To test this hypothesis, $[2]^{2+}$ and $[3]^{2+}$ were reduced chemically. These reactions were followed by $^1$H NMR spectroscopy. Reduction with zinc powder produced the platinum(II) dimer $[1]^{2+}$ as the major product. In contrast, the use of SnCl$_2$·2H$_2$O as the reducing
agent resulted in the release of the \( \text{NH}_3 \) ligands as ammonium ions, as evidenced by the presence of a sharp 1:1:1 triplet centered at 7.68 ppm. These results demonstrate that the reduction products of \([2]^{2+}\) and \([3]^{2+}\) depend on the nature of the reducing agent and suggest that the electrochemical reduction within the diffusion layer of the electrode could reduce \([2]^{2+}\) and \([3]^{2+}\) to species other than \([1]^{2+}\).

**DFT Calculations.** The geometries of \([1]^{2+}\)–\([3]^{2+}\) were optimized in the gas phase using the hybrid functional PBE0 and the 6-311+G(d,p) basis set for atoms other than platinum. The LANL2DZ basis set and effective core potential were used for the platinum atoms. Table 5.4 compares structural features of the calculated and experimentally determined geometries. At this level of theory, the Pt–Pt interactions are poorly modeled. This shortcoming is manifest in computed Pt–Pt distances and tilt angles that are significantly greater than the experimental ones. For the platinum(III) species, the Pt–Pt separation is overestimated by 0.10–0.14 Å, whereas for \([1]^{2+}\), this distance exceeds experimental values by 0.64 Å. The larger discrepancy between the computed and experimental Pt–Pt distances for platinum(II) is consistent with a recent observation that standard hybrid DFT functionals have difficulty in modeling weak \(d^8-d^8\) interactions between platinum(II) centers.\(^{98}\) Otherwise, the computed platinum–ligand distances agree well with experimental values. Notably, the trans influence of the metal–metal bond in \([2]^{2+}\) and \([3]^{2+}\) is recapitulated, as revealed by the long computed platinum–halide distances.

Because the crystal structures of \([1]^{2+}\)–\([3]^{2+}\) indicate significant hydrogen bonding between the coordinated ammine ligands and the solvent or counterions, additional geometry optimizations were carried out, at the same level of theory, in which a nitrate counterion was explicitly added as a hydrogen-bonding partner for the ammines. The addition of the nitrate counterion leads to optimized geometries in which the Pt–Pt distances are significantly shorter.
For $[2]^{2+}$ and $[3]^{2+}$, the Pt–Pt distances in these optimized ion pairs are only overestimated by 0.06 and 0.08 Å, respectively. The Pt–Pt separation in the nitrate adduct of $[1]^{2+}$ exceeds experimental values by 0.22 Å. Although still quite large, this result marks a significant improvement over that computed for the free ion $[1]^{2+}$ (0.64 Å). The presence of the nitrate ion had little effect on the other platinum–ligand bond distances.

### Table 5.4. Comparison of Selected Structural Features of $[1]^{2+}$–$[3]^{2+}$ Determined Experimentally by X-ray Diffraction and Calculated by DFT.$^a$

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt–Pt</td>
<td>2.921</td>
<td>3.561</td>
<td>3.138</td>
<td>2.600</td>
<td>2.705</td>
<td>2.660</td>
<td>2.603</td>
<td>2.743</td>
<td>2.686</td>
</tr>
<tr>
<td>Pt–X</td>
<td>2.046</td>
<td>2.035</td>
<td>2.052</td>
<td>2.031</td>
<td>2.032</td>
<td>2.048</td>
<td>2.028</td>
<td>2.033</td>
<td>2.050</td>
</tr>
<tr>
<td>Pt–Oacetate</td>
<td>2.024</td>
<td>2.056</td>
<td>2.045</td>
<td>2.024</td>
<td>2.070</td>
<td>2.042</td>
<td>2.022</td>
<td>2.070</td>
<td>2.044</td>
</tr>
<tr>
<td>Pt–Namine</td>
<td>31.9</td>
<td>70.11</td>
<td>42.5</td>
<td>19.3</td>
<td>32.8</td>
<td>23.7</td>
<td>19.0</td>
<td>33.2</td>
<td>24.0</td>
</tr>
</tbody>
</table>

$^a$ Experimental values reported are the averages of chemically equivalent distances or angles found in the asymmetric unit of the X-ray structure. Distances are in Å, angles in degrees. $^b$ Tilt angle between adjacent platinum coordination planes in the binuclear unit. $^c$ Average twist or torsion angle about the Pt–Pt vector.

Molecular orbitals relevant to the Pt–Pt bonding interactions of the dimers are shown in Figure 5.8. The molecular orbitals of the free ions and the nitrate ion pairs of $[1]^{2+}$–$[3]^{2+}$ were qualitatively similar. Because the optimized geometries of the nitrate ion pairs are closer to the experimental ones than to those of the isolated cations, the molecular orbitals of the ion pairs are shown. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of $[2]^{2+}$ and $[3]^{2+}$ are $\sigma$ and $\sigma^*$ orbitals derived from the symmetric and antisymmetric combination of the $d_{z^2}$ orbitals of the platinum centers. The HOMOs of $[2]^{2+}$ and $[3]^{2+}$ signify the formal Pt–Pt single bond. Furthermore, the HOMOs are antibonding with respect to the axial ligands, thus giving rise to the long Pt–X axial bond lengths. For $[1]^{2+}$, which is reduced by two electrons relative to $[2]^{2+}$ and $[3]^{2+}$, the HOMO is the $\sigma^*$ orbital. Population of
this strongly Pt–Pt antibonding molecular orbital in [1]\(^{2+}\) is consistent with the large Pt–Pt atomic separation and absence of a formal metal–metal bond. The symmetric combination of the d\(_{z^2}\) orbitals is found to be lower in energy as the HOMO–5.

![Frontier Kohn-Sham molecular orbitals](image)

**Figure 5.8.** Relevant frontier Kohn-Sham molecular orbitals of [1]\(^{2+}\)–[3]\(^{2+}\), optimized as the ion pair with a single nitrate counterion. The nitrate ions included in the calculation are omitted for clarity. Isovalues are drawn at 0.04 au.

DFT calculations were further employed to understand the NMR spectral properties of [1]\(^{2+}\)–[3]\(^{2+}\). \(^{14}\)N NMR chemical shifts and EFG parameters of the \(^{14}\)N nuclei were computed for the optimized geometries of the nitrate ion pair and are collected in Table 5.5. The computed \(^{14}\)N NMR chemical shifts ppm, in excellent agreement with the experimentally measured chemical shift of 355 ppm. Calculated values for the coordinated ammine ligands are approximately 50 ppm farther downfield from experimentally measured values. Given that the known window for
$^{14}\text{N}$ NMR chemical shifts in diamagnetic compounds spans a region of 1100 ppm,\textsuperscript{88} this seemingly large 50 ppm deviation represents only a 4.5% absolute error. Importantly, the calculation successfully predicts the downfield shift of the $^{14}\text{N}$ resonance observed upon the oxidation of [1]$^{2+}$ to [2]$^{2+}$ or [3]$^{2+}$.

The quadrupolar relaxation rate of a $^{14}\text{N}$ nucleus determines its observed spectral line width ($W_{1/2}$). Therefore, the line width is proportional to $\eta$, the asymmetry of the EFG at the nucleus, the nuclear quadrupole coupling constant (NQCC), a measure of the magnitude of the EFG at the nucleus, and $\tau_q$, the correlation of the molecule in solution, according to Equation 5.1. The EFG parameters $\eta$ and NQCC can be readily calculated with DFT methods. The values computed for the $^{14}\text{N}$ atoms of the coordinated ammine ligands are listed in Table 5.5. The asymmetries at the EFG ($\eta$) of ammine ligands are similar for all three complexes. The NQCC of [1](NO$_3$)$_2^+$, however, is more than twice as large as the corresponding values for [2](NO$_3$)$_2^+$ and [3](NO$_3$)$_2^+$ and therefore the more important contributor to the large observed $W_{1/2}$ of [1]$^{2+}$. If the correlation times are assumed to be equal for the three complexes, the ratio of $^{14}\text{N}$ NMR line widths can be calculated from Equation 5.1, using the computed values for $\eta$ and NQCC. This calculation predicts that, for the platinum(III) complexes, the line widths are equal, whereas the same signal for the platinum(II) complex is broader by a factor of 6. This value is in agreement with experimental observations; the $W_{1/2}$ value for [1]$^{2+}$ ($\approx$ 300 Hz) is close to 6 times greater than those of [2]$^{2+}$ and [3]$^{2+}$, which are 50 and 45 Hz, respectively.

**Equation 5.1.**

$$W_{1/2} \approx \left(1 + \frac{\eta^2}{3}\right) \cdot (\text{NQCC})^2 \cdot \tau_q$$
Table 5.5. DFT-Computed Isotropic $^{14}$N NMR Chemical Shifts and EFG Parameters for the Coordinated Ammine Ligands.$^a$

<table>
<thead>
<tr>
<th></th>
<th>$^{14}$N $\delta_{\text{calc}}$, ppm</th>
<th>NQCC, MHz</th>
<th>$\eta$</th>
<th>(\left(1 + \frac{\eta}{3}\right)^2 \cdot (\text{NQCC})^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="NO$_3$">1</a>$_2^+$</td>
<td>-29.8</td>
<td>-0.858</td>
<td>0.450</td>
<td>1.05</td>
</tr>
<tr>
<td><a href="NO$_3$">2</a>$_2^+$</td>
<td>-17.2</td>
<td>-0.400</td>
<td>0.493</td>
<td>0.173</td>
</tr>
<tr>
<td><a href="NO$_3$">3</a>$_2^+$</td>
<td>-19.0</td>
<td>-0.394</td>
<td>0.585</td>
<td>0.173</td>
</tr>
</tbody>
</table>

$^a$ The reported values are averages of those calculated for the four distinct ammine ligands.

Synthesis and Characterization of the Tetranuclear Complex [4](NO$_3$)$_4$. Treatment of [1](NO$_3$)$_2$ in DMF with a slight excess of the hypervalent iodine reagent PhI(O$_2$CCF$_3$)$_2$, followed by crystallization via vapor diffusion of Et$_2$O, afforded an apparently homogeneous crop of yellow-orange crystalline material. Analysis by X-ray diffraction revealed the compound to be the amido-bridged tetranuclear platinum compound [4](NO$_3$)$_4$, illustrated in Figure 5.9 and Scheme 5.3, the interatomic distances and angles of which are summarized in Table 5.6.

Scheme 5.3. Synthesis of [4](NO$_3$)$_4$. 

228
Two acetate-bridged diplatinum(III) units are connected by two bridging amido ligands to form the observed tetranuclear complex. A crystallographic inversion center relates the amido-bridged dimers. The amido groups could alternatively be assigned as hydroxo ions because the X-ray scattering power of nitrogen and oxygen are similar. Reasonable and similar thermal displacement parameters were obtained from isotropic refinements using either assignment, with insignificant changes in refinement statistics or geometry. The crystal packing was therefore studied to make the assignment. Both hydrogen atoms of the proposed bridging amido ligands act as hydrogen-bond donors to a nitrate counterion and a DMF molecule in the crystal lattice. A hydroxide bridge would hydrogen bond as a donor on one side and an acceptor on the other. Therefore, our assignment of the bridging atoms as amido groups is most likely correct. The coordination sphere of the inner two platinum centers (Pt2 and Pt2A) is derived from two oxygen atoms of bridging acetate ligands in equatorial positions, a nitrogen atom from an ammine ligand in an equatorial position, and two amido ligands, one in an equatorial position and one in the axial position. The bridging amido ligand that is coordinated in the axial position is significantly elongated relative to that in the equatorial position [2.136(10) vs 2.002(9) Å]. This elongation of the axial ligand is due to the strong trans influence of the Pt-Pt bond [Pt1–Pt2, 2.5561(7) Å]. The outer two platinum atoms are coordinated to trifluoroacetate ligands in the axial position. The Pt–O bond is 2.210(9) Å. This value is significantly longer than those typically observed for Pt–TFA bonds in platinum(II) and (IV) compounds, ~2.0 Å. The significant elongation of this bond may be due to the additive trans influence of the metal–metal bond and the amido ligand. Longer Pt–TFA bonds of around 2.3 Å have been observed in multinuclear complexes with strong Tl–Pt bonds. The [4]4+ structure is analogous to those of the α-pyrrolidonate/amido-bridged tetranuclear platinum(III) complex.
[(NO$_3$)(NH$_3$)$_2$Pt$_{III}$($C_4H_6$NO)$_2$Pt$_{III}$($NH_3$)(μ-NH$_2$)$_2$($NO_3$)$_2$].$^{105}$ The difference between this complex and [4]$^{4+}$ is the presence of acetate rather than α-pyrrolidonate as bridging ligands and trifluoroacetate instead of nitrate as axial ligands for the outer platinum atoms. In a subsequently described analogue, the α-pyrrolidonate ligands are oriented in a head-to-tail fashion.$^{54}$ The central bridging ligands were assigned as hydroxo rather than amido.$^{54}$ Analysis of the CIF file deposited in the CSD of the head-to-tail structure indicates that the bridging ligand donates two hydrogen bonds with nitrate counterions, suggesting that these ligands are incorrectly assigned as hydroxo rather than amido.

**Figure 5.9.** Solid-state molecular structure of [4]$^{4+}$. Ellipsoids are drawn at the 50% probability level. Unlabeled gray and green ellipsoids correspond to carbon and fluorine atoms, respectively. Hydrogen atoms and the minor component of disorder in the trifluoroacetate group have been omitted for clarity.

**Table 5.6.** Selected Structural Features of [4]$^{4+}$.$^a$  

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt1–Pt2</td>
<td>2.5561(7)</td>
<td>Pt1–O5</td>
<td>2.021(8)</td>
<td>Pt2–O4</td>
<td>2.057(7)</td>
<td>Pt2–N4</td>
<td>2.002(9)</td>
<td>Pt1–O3</td>
<td>2.043(8)</td>
<td>Pt1–N2</td>
<td>2.006(9)</td>
</tr>
</tbody>
</table>

$^a$ Atoms are labeled as indicated in Figure 5.9. Numbers in parentheses are estimated standard deviations in the last significant figures. Interatomic distances are reported in Å. $^b$ τ, the tilt angle in degrees between the coordination planes of Pt1 and Pt2. $^c$ ω, the average torsion angle in degrees about the Pt1–Pt2 vector.
The $^1$H and $^{19}$F NMR spectra of \([4]^{4+}\) indicate complex solution behavior. Broad, ill-defined peaks occur between 5.5 and 7.5 ppm in the $^1$H NMR spectrum of \([4]^{4+}\) in DMF-$d_7$ at 25 $^\circ$C. These features most likely correspond to protons from the ammine and bridging amido ligands. The proton chemical shifts of $\mu$-$\text{NH}_2$ ligands in platinum(II) complexes range from $-1$ to 3 ppm depending on the solvent and nature of the peripheral ligands.\(^{106-109}\) In platinum(IV) complexes, the protons of these groups resonate farther downfield, between 4.5 and 5.5 ppm.\(^{110,111}\) On the basis of the chemical shifts observed for the $\mu$-$\text{NH}_2$ ligands in \([4]^{4+}\), it appears that the metal–metal-bonded diplatinum(III) centers produce deshielding effects similar to those of the more electron-deficient platinum(IV) centers. The CH$_3$ resonances of the two inequivalent bridging acetate ligands appear at 2.58 and 2.30 ppm. Notably, these peaks display shoulders at 2.54 and 2.27 ppm. Also present in the CH$_3$ region are several small peaks between 2.45 and 2.30 ppm that integrate to one-tenth the area of those of the major CH$_3$ resonances. The minor peaks are present consistently in solutions of crystalline material obtained from different syntheses. The $^{19}$F NMR spectrum of \([4]^{4+}\) at 25 $^\circ$C similarly displays two major peaks, at $-76.09$ and $-76.62$ ppm, and two minor ones, at $-75.72$ and $-75.80$ ppm. The two major peaks integrate to a ratio of 1:1.4, whereas the minor peaks integrate to a ratio of 0.04:0.06. At 45 $^\circ$C, the two major CH$_3$ resonances coalesce into sharp singlets, losing the shoulders observed at room temperature (Figure 5.10). The NH region of the $^1$H NMR spectrum also displays subtle changes at higher temperatures. The major peaks in the $^{19}$F NMR spectrum at 45 $^\circ$C are significantly broadened (Figure 5.10), suggesting interconversion between two species. Upon return of the temperature to 25 $^\circ$C, the peaks in the $^1$H NMR spectrum display shoulders again and the peaks in the $^{19}$F NMR spectrum return as sharp singlets, indicating the process to be reversible. At temperatures greater than 65 $^\circ$C, the two $^{19}$F peaks coalesce completely. At these
higher temperatures, there is significant decomposition of the starting material, however, as evidenced by a number of new species in the $^1$H NMR spectrum upon returning to 25 °C.

The $^{19}$F NMR signal of NaTFA appears at $-76.00$ ppm in DMF-$d_7$. Therefore, the peak observed at $-76.09$ ppm might arise from the TFA ion in solution. The addition of 10 equiv of NaTFA to a solution of [4]$^{4+}$ results in coalescence and a slight upfield shift of the CH$_3$ signals in the $^1$H spectra, and the corresponding $^{19}$F NMR spectra display a change in the intensities of the peaks (Figure 5.11). The peak near $-76$ ppm increases dramatically due to the presence of excess TFA ion, and the minor peak at $-75.80$ ppm increases as well. These data suggest that the axial TFA ligands are labile and that in solution the complex exists as a mixture of different species bearing different axial ligands. As demonstrated in another control experiment, the addition of 40 equiv of NaNO$_3$ produces no significant changes in either the $^1$H or $^{19}$F NMR spectra, thereby suggesting that it is most likely DMF that competes with TFA for axial coordination to the tetranuclear platinum ion.
Figure 5.11. $^1$H (left) and $^{19}$F (right) NMR spectra of $[4]^2{^{+}}$ before (bottom) and after (top) the addition of 10 equiv of NaTFA in DMF-$d_7$.

The electrochemistry of $[4](NO_3)_4$ in DMF was investigated by CV at a glassy carbon (GC) working electrode with 0.1 M TBAP as the supporting electrolyte. Cyclic voltammograms of $[4](NO_3)_4$ are highly dependent on the polishing state of the electrode. With thorough polishing between scans, fairly reproducible voltammograms were obtained, as shown in Figure 5.12; however, the voltammogram evolved over the course of multiple scan cycles, as indicated by the arrows of Figure 5.12. The initial scan shows an irreversible reduction comprising several broad peaks that occur at an onset potential of near 0.4 V and a peak potential of approximately 30 mV. The presence of what appear to be multiple peaks may reflect the presence of multiple species of $[4]^4{^{+}}$ in solution or sequential reduction of individual platinum centers. After the initial reduction of $[4]^4{^{+}}$, an irreversible oxidation feature appears near 1.2 V. Subsequent cycles lead to the growth of a reduction feature near 0.5 V and the decay of the initial reduction feature at 30 mV. The oxidation feature at 1.2 V and the reduction at 0.5 V are consistent with those of the platinum(II) dimer $[1]^2{^{+}}$ and suggest that $[1]^2{^{+}}$ is an electrochemical reduction product of $[4]^4{^{+}}$. 233
The consistent decrease in the peak current upon multiple cycles and the need for stringent electrode polishing between experiments indicate that other reduction products may be adsorbed to or deposited on the electrode surface.

![Cyclic voltammogram](image)

**Figure 5.12.** Cyclic voltammogram of $[4](\text{NO}_3)_4$ in DMF with 0.1 M TBAP as the supporting electrolyte. The potential is reported relative to Ag/AgCl, and the voltammogram was obtained at a scan rate of 100 mV·s$^{-1}$. The red arrow indicates the starting potential and initial scan direction. The bold red trace is the first scan cycle. The following scans are in black. Changes in the current as the potential is cycled are indicated by black arrows.

**Reaction of $[1](\text{NO}_3)_2$ with XeF$_2$.** In an attempt to access a platinum(III) dimer with axial fluoride ligands, $[1](\text{NO}_3)_2$ was treated with a source of solid fluorine, XeF$_2$. Following introduction of XeF$_2$, the brown suspension of $[1](\text{NO}_3)_2$ in DMF became a bright yellow solution. Crystallization by vapor diffusion of diethyl ether gave a homogeneous batch of thin yellow needles. One of these needles was analyzed by X-ray diffraction. The structure reveals infinite chains of acetate-bridged platinum dimers that propagate parallel to the crystallographic
c-axis (Figure 5.13). A column of nitrate counterions also propagate in the same direction. A large solvent accessible void with diffuse electron density is present in the lattice. This void of disordered electron density prevented the definitive location of all counterions in the crystal lattice and, therefore, the oxidation states of the platinum ions. The intradimer Pt–Pt separation is 2.9354(8) Å and the interdimer Pt–Pt separation is 3.1435(8) Å. These values compare favorably to those in the confirmed platinum(II) dimer 1 where they are 2.92149(18) and 3.1523(2) Å, respectively. Oxidation of these platinum dimers should decrease the bond lengths as electrons are removed from a Pt–Pt antibonding orbital. Thus, it appears that treatment of [I](NO₃)₂ with XeF₂ afforded no net oxidation or reaction. Given that an immediate solution color change occurs after [I](NO₃)₂ is treated with XeF₂ and that XeF₂ is an extremely strong oxidizing agent, it is plausible that the initially oxidized platinum(III) product is unstable and reductively decomposes back to [I](NO₃)₂. The reductive decomposition could occur through disproportionation, photoreduction, or by oxidation of the solvent. Recently, it was reported that treatment of an acetate-bridged palladium(II) dimer with XeF₂ affords a compound with infinite palladium chains.¹¹² The short 2.72 Å Pd–Pd separation confirms the +3 oxidation state of the palladium ions in the chain. Notably, these chains are thermally unstable. Such thermal instability may also be a feature of the species formed initially upon oxidation of [I](NO₃)₂ with XeF₂.
Figure 5.13. Crystal packing of the infinite Pt chain obtained from the reaction of XeF₂ with [1](NO₃)₂ viewed down the crystallographic c-axis with residual electron density in green (top). Visualization of the Pt chain with relevant Pt–Pt distances labeled (bottom).

5.4. Summary and Conclusions

The oxidative reactivity of the acetate-bridged cis-diammineplatinum(II) complex [1]²⁺ was explored. Treatment of [1](NO₃)₂ with halogen-based oxidants affords the first discrete diacetate-bridged cis-diammineplatinum(III) complexes, [2](NO₃)₂ and [3](NO₃)₂. Complexes [1]²⁺–[3]²⁺ were fully characterized by multinuclear NMR spectroscopy, X-ray crystallography, electrochemical methods, and DFT calculations. A novel tetranuclear amido-bridged platinum(III) species, [4]⁴⁺, was also characterized structurally by X-ray crystallography and in
solution by NMR spectroscopy. The complexes are new additions to the growing family of dinuclear platinum(III) complexes derived from cisplatin.

5.5. References

Chapter 6

Synthesis, Characterization, and Cytotoxicity of Platinum(IV)

Dicarbamate Complexes

6.1. Introduction

Having covered chemistry of platinum in the +2 and +3 formal oxidation states in previous Chapters, in the final chapter of this thesis, we describe new synthetic developments in the realm of platinum(IV) anticancer prodrugs. Even though all of the clinically approved platinum-based drugs employ this element in the +2 oxidation state, compounds with platinum in the +4 oxidation state have become of interest for their potential therapeutic properties. In particular, they have shown considerable promise both for oral administration and for reduction of systemic toxicity. The orally administered platinum(IV) complex satraplatin progressed as far as phase III in clinical trials. It is hypothesized that the increased stability of these complexes, due to their low-spin d⁶ electronic configuration, aids in their survival of the acidic environment of the gastrointestinal tract before being absorbed into the bloodstream. They operate by a mechanism similar to that of the first- and second-generation platinum(II) analogues. An activation step, reduction from platinum(IV) to platinum(II), must occur before aquation and DNA binding, however (Scheme 6.1).

Scheme 6.1. Reductive activation of platinum(IV) prodrugs.

In addition to their kinetic stability, another favorable property of platinum(IV) complexes relative to their platinum(II) counterparts is the presence of two additional coordination sites that can be modified to alter their pharmacokinetic properties. By varying the
two axial ligands, one can predictably alter the redox potential\textsuperscript{15-17} and lipophilicity\textsuperscript{8,9} of the platinum(IV) complex while leaving the DNA-binding \textit{cis}-diammineplatinum moiety unaltered. Furthermore, the axial coordination positions serve as binding sites for other biologically active ligands, which may have synergistic effects with platinum therapy, as demonstrated by us\textsuperscript{10-13} and by others.\textsuperscript{14-16} The ability to tether platinum(IV) complexes via the axial ligands to various nanodelivery devices for increased cellular uptake and selectivity\textsuperscript{17-25} is another advantage. The design of new platinum(IV) anticancer complexes, however, is limited by the current synthetic methodology.\textsuperscript{26-32} Most of the newly tested platinum(IV) complexes bear either chloro, hydroxo, or carboxylato axial ligands. The development of new synthetic methodologies for accessing the platinum(IV) manifold can expand the range of complexes having novel properties.

The synthesis of platinum(IV) complexes with axial methyl, ethyl, and isopropyl carbamate ligands was described over ten years ago.\textsuperscript{26} Since then, their biological properties have only rarely been explored,\textsuperscript{33} and further investigations of the scope of this synthetic methodology have not been pursued. In this chapter, a modification and an expansion of this approach through the synthesis of eight new platinum(IV) complexes of both alkyl and aryl carbamates as well as a brief investigation of their biological activity is reported. Computational density functional theory (DFT) studies were undertaken to gain a deeper understanding of the electronic structure of these new complexes. The results presented here indicate that platinum(IV) carbamates are a promising new class of anticancer drug candidates.

6.2. Experimental Methods

\textbf{General Materials and Methods.} All reactions were carried out under normal atmospheric conditions. Solvents were used as received without additional drying or purification. All
isocyanates were used as received from commercial vendors. The compounds cis,cis,trans-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$], cis,cis,trans-[Pt(NH$_3$)$_2$Cl$_2$(O$_2$CCH$_3$)$_2$] (I), and cis-[Pt(NH$_3$)$_2$Cl$_2$] (3) were synthesized as previously described$^{34,35}$ using cisplatin purchased from Strem Chemicals, Inc., as the starting material.

**Physical Measurements.** NMR measurements were recorded on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility at 20 °C with deuterated dimethyl sulfoxide (DMSO-$d_6$) as the solvent. All NMR chemical shifts ($\delta$) are reported in parts per million (ppm) and referenced as described below. $^1$H and $^{13}$C$^{'1}$H NMR spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane, SiMe$_4$ ($\delta = 0$ ppm). $^{195}$Pt$^{'1}$H and $^{19}$F$^{'1}$H NMR spectra were referenced externally using standards of K$_2$PtCl$_4$ in D$_2$O ($\delta = -1628$ ppm) and trifluorotoluene ($\delta = -63.72$ ppm), respectively. Fourier transform infrared spectra were recorded with a ThermoNicolet Avatar 360 spectrophotometer running the OMNIC software. Samples were prepared as KBr disks. Cyclic voltammograms were obtained at room temperature using a VersaSTAT3 potentiostat from Princeton Applied Research accompanied by the V3 Studio software. A three-electrode system was used comprising glassy carbon as the working electrode, a Pt wire as the auxiliary electrode, and a Ag/AgCl reference electrode. Samples were prepared as 2 mM solutions in N,N-dimethylformamide (DMF) with 0.1 M (n-Bu$_4$N)PF$_6$ as the supporting electrolyte. Reported values are peak potentials of the irreversible reduction event at a scan rate of 100 mV·s$^{-1}$. Under the conditions described here, the reversible ferrocene/ferricenium redox couple was consistently found between 0.54 and 0.55 V vs Ag/AgCl. Electrospray ionization mass spectrometry (ESI-MS) measurements were acquired on an Agilent Technologies 1100 series LC-MSD trap. Elemental analyses were carried out by a commercial analytical laboratory.
Synthesis of cis,cis,trans-[Pt(NH₃)₂Cl₂(O₂CCF₃)₂] (2). The compound cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂] (0.144 g, 0.429 mmol) was suspended in 2 mL of trifluoroacetic anhydride. The mixture was stirred for 1 h at room temperature, open to air, at which point the volatile anhydride had evaporated, leaving a white residue. A 2-mL volume of tetrahydrofuran (THF) was added to the residue, and the resulting yellow solution was filtered through Celite. Pentane (~10 mL) was layered on top of the THF solution, and the mixture was kept at -40 °C for 1 h to afford pale-yellow microcrystals of 2. These microcrystals were collected by vacuum filtration and washed with pentane before being dried in vacuo. Yield: 0.127 g (56%). Mp: 194–196 °C.

1H NMR (400 MHz): δ 6.63 (br s, 6H). 13C{¹H} NMR (100 MHz): δ 161.8 (q, JCF = 37 Hz), 111.7 (q, JCF = 288 Hz). 19F{¹H} NMR (377 MHz): δ -73.6. 195Pt{¹H} NMR (86 MHz): δ 1182. IR (KBr, cm⁻¹): 3426 m br, 3280 s, 3232 s, 3197 m, 1722 vs, 1559 w, 1382 m, 1331 m, 1212 s, 1162 vs, 1034 w, 859 w, 781 m, 739 m, 524 w. Anal. Calcd. for 2, C₄H₆Cl₂F₆N₂O₄Pt: C, 9.13; H, 1.15; N, 5.32. Found: C, 9.38; H, 1.21; N, 5.31.

General Synthesis of cis,cis,trans-[Pt(NH₃)₂Cl₂(O₂CNHR)₂]. To a suspension of cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂] (0.20 g, 0.60 mmol) in 1 mL of DMF was added a 1-mL DMF solution containing 4 mol equiv of the isocyanate. The resulting mixture was stirred for 12 h at room temperature, resulting in the formation of a homogeneous solution. The solution was filtered, and the desired product was precipitated by the addition of diethyl ether. The solid was collected by either filtration or centrifugation. To remove residual DMF, the solid was suspended in water for 30 min, isolated by centrifugation, resuspended in ethanol, isolated by centrifugation, resuspended in diethyl ether, isolated by centrifugation, and finally dried under vacuum.

Compound 4. R = tert-butyl. White solid. Yield: 0.153 g (48%). Mp: 238–245 °C (dec). 1H NMR (400 MHz): δ 6.65 (br, 6H), 6.05 (br, 2H), 1.17 (s, 18H). 13C{¹H} NMR (100 MHz): δ
162.8, 49.4, 29.3. ¹⁹⁵Pt{¹H} NMR (86 MHz): δ 1276. IR (KBr, cm⁻¹): 3387 vs, 3301 m, 3220 s, 2975 m, 2931 w, 1640 vs, 1629 vs, 1505 vs, 1462 m, 1393 w, 1366 m, 1281 s, 1211 s, 1079 m, 943 m, 788 w, 727 w, 645 w, 434 w. 


**Compound 5.** R = cyclopentyl. White solid. Yield: 0.240 g (72%). Mp: 208–214 °C (dec). ¹H NMR (400 MHz): δ 6.67 (br, 6H), 6.55 (br, 2H), 3.78-3.71 (m, 2H), 1.68-1.34 (m, 16H). ¹³C{¹H} NMR (400 MHz): δ 163.4, 52.7, 32.5, 23.3. ¹⁹⁵Pt{¹H} NMR (86 MHz): δ 1274 (major), 1262 (minor). IR (KBr, cm⁻¹): 3402 s, 3354 vs, 3243 vs, 2959 s, 2869 m, 1629 vs, 1509 vs, 1358 m, 1297 s, 1252 s, 1099 w, 1037 w, 1008 w, 953 w, 782 w, 581 w. ESI-MS (negative-ion mode): m/z 555.0 ([M–H]⁻, calcd. 555.1). Anal. Calcd. for 5, C₁₂H₂₆Cl₂N₄O₄Pt: C, 25.91; H, 4.71; N, 10.07. Found: C, 25.76; H, 4.66; N, 10.29.

**Compound 6.** R = cyclohexyl. White solid. Yield: 0.287 g (81%). Mp: 228–233 °C (dec). ¹H NMR (400 MHz): δ 6.67 (br, 6H), 6.47 (br, 2H), 3.19 (br, 2H), 1.71-1.50 (m, 10H), 1.22-1.02 (m, 10H). ¹³C{¹H} NMR (400 MHz): δ 163.0, 50.0, 33.1, 25.3, 24.9. ¹⁹⁵Pt{¹H} NMR (86 MHz): δ 1274 (major), 1263 (minor). IR (KBr, cm⁻¹): 3375 vs, 3304 vs, 3243 vs, 2959 s, 2869 m, 1629 vs, 1509 vs, 1358 m, 1297 s, 1252 s, 1099 w, 1037 w, 1008 w, 953 w, 782 w, 581 w. ESI-MS (negative-ion mode): m/z 583.0 ([M–H]⁻, calcd. 583.1). Anal. Calcd. for 6, C₁₄H₃₆Cl₂N₄O₄Pt: C, 28.77; H, 5.17; N, 9.59. Found: C, 28.89; H, 5.20; N, 9.44.

**Compound 7.** R = phenyl. Yellow solid. Yield: 0.214 g (60%). Mp: 171–176 °C (dec). ¹H NMR (400 MHz): δ 9.12 (br, 2H), 7.47 (d, 4H), 7.18 (t, 4H), 6.86 (t, 2H), 6.79 (br, 6H). ¹³C{¹H} NMR (100 MHz): δ 160.7, 140.7, 128.3, 121.0, 118.0. ¹⁹⁵Pt{¹H} NMR (86 MHz): δ 1265. IR (KBr, cm⁻¹): 3243 s br, 1654 vs, 1595 s, 1514 s, 1438 s, 1393 s, 1315 s, 1227 s, 1045 m, 1025 m,
754 m, 691 m. ESI-MS (negative-ion mode): \(m/z\) 570.9 ([M–H]−, calcd. 571.0). Anal. Calcd. for 7, C\(_{14}\)H\(_{18}\)Cl\(_2\)N\(_4\)O\(_4\)Pt: C, 29.38; H, 3.17; N, 9.79. Found: C, 29.56; H, 3.07; N, 9.67.

**Compound 8.** R = p-tolyl. Pale-orange solid. Yield: 0.229 g (64%). Mp: 149–151 °C (dec). \(^1\)H NMR (400 MHz): \(\delta\) 9.02 (br, 2H), 7.36 (d, 4H), 6.98 (d, 4H), 6.79 (br, 6H), 2.20 (s, 6H). \(^{13}\)C\(^{\{1\}H}\) NMR (100 MHz): \(\delta\) 160.8, 138.2, 129.7, 128.7, 118.1, 20.4. \(^{195}\)Pt\(^{\{1\}H}\) NMR (86 MHz): \(\delta\) 1264. IR (KBr, cm\(^{-1}\)): 3404 s, 3353 s, 3217 br vs, 2924 w, 1663 vs, 1635 s, 1592 m, 1522 vs, 1502 m, 1404 m, 1314 s, 1289 s, 1250 m, 1223 vs, 1041 s, 817 m, 776 m, 740 w, 582 w. ESI-MS (negative-ion mode): \(m/z\) 599.0 ([M–H]−, calcd. 599.1), 1199.0 ([2M–H]−, calcd. 1199.1). Anal. Calcd. for 8, C\(_{16}\)H\(_{22}\)Cl\(_2\)N\(_4\)O\(_4\)Pt: C, 32.01; H, 3.69; N, 9.31. Found: C, 32.14; H, 3.75; N, 9.51.

**Compound 9.** R = p-anisole. Pale-orange solid. Yield: 0.190 g (50%). Mp: 115–117 °C (dec). \(^1\)H NMR (400 MHz): \(\delta\) 8.98 (br, 2H), 7.37 (d, 4H), 6.77 (br d, 1OH), 3.68 (s, 6H). \(^{13}\)C\(^{\{1\}H}\) NMR (100 MHz): \(\delta\) 160.9, 153.8, 134.0, 119.5, 113.5, 55.1. \(^{195}\)Pt\(^{\{1\}H}\) NMR (86 MHz): \(\delta\) 1265. IR (KBr, cm\(^{-1}\)): 3223 s br, 2835 w, 1647 vs, 1515 vs, 1410 m, 1298 s, 1223 vs, 1178 m, 1029 s, 827 m, 777 w, 739 w, 652 w, 583 w, 527 w. ESI-MS (negative-ion mode): \(m/z\) 630.9 ([M–H]−, calcd. 631.0). Anal. Calcd. for 9, C\(_{16}\)H\(_{22}\)Cl\(_2\)N\(_4\)O\(_4\)Pt: C, 30.39; H, 3.51; N, 8.86. Found: C, 30.45; H, 3.48; N, 8.73.

**Compound 10.** R = 4-fluorophenyl. Pale-yellow solid. Yield: 0.237 g (65%). Mp: 208–210 °C (dec). \(^1\)H NMR (400 MHz): \(\delta\) 9.21 (br, 2H), 7.48-7.45 (m, 4H), 7.02 (app t, 4H), 6.78 (br, 6H). \(^{13}\)C\(^{\{1\}H}\) NMR (100 MHz): \(\delta\) 160.7, 156.9 (d, \(^1\)J\(_{CF}\) = 236 Hz), 137.1, 119.5, 114.7 (d, \(^2\)J\(_{CF}\) = 22.0 Hz). \(^{19}\)F\(^{\{1\}H}\) NMR (377 MHz): \(\delta\) –125.2 (s, 2F). \(^{195}\)Pt\(^{\{1\}H}\) NMR (86 MHz): \(\delta\) 1265. IR (KBr, cm\(^{-1}\)): 3367 s, 3238 s br, 1657 vs, 1512 vs, 1407 s, 1389 m, 1305 m, 1259 s, 1213 vs, 1157 w, 1101 w, 1037 s, 832 s, 653 m, 584 w, 513 w. ESI-MS (negative-ion mode): \(m/z\) 606.9 ([M–H]−,
calcd. 607.0), 1216.0 ([2M–H]–, calcd. 1215.0). Anal. Calcd for 10, C14H16Cl2F2N4O4Pt: C, 27.64; H, 2.65; N, 9.21. Found: C, 27.42; H, 2.50; N, 9.02.

**Compound 11.** R = 1-naphthyl. Pale-orange solid. Yield: 0.093 g (46%, using 0.30 mmol of the starting material). Mp: 152–157 °C (dec). 1H NMR (400 MHz): δ 9.01 (s, 2H), 8.19-8.17 (m, 2H), 7.68-7.65 (m, 2H), 7.67 (d, 2H), 7.62 (d, 2H), 7.50-7.40 (m, 6H), 6.82 (br, 6H). 13C{1H} NMR (100 MHz): δ 161.9, 135.5, 133.7, 127.9, 127.5, 125.7, 125.6, 125.2, 123.5, 123.4, 120.6. 195Pt{1H} NMR (86 MHz): δ 1269. IR (KBr, cm⁻¹): 3407 s, 3355 s, 3211 s br, 3063 m, 1654 vs, 1566 m, 1490 s, 1410 s, 1341 s, 1247 s, 1101 w, 999 w, 791 m, 772 s, 544 w. ESI-MS (negative-ion mode): m/z 669.8 ([M–H]–, calcd. 671.1). Anal. Calcd. for 11, C22H22Cl2N4O4Pt: C, 39.30; H, 3.30; N, 8.33. Found: C, 39.44; H, 3.29; N, 8.39.

**X-ray Crystallographic Studies.** Single crystals were mounted in Paratone oil on a cryoloop and frozen under a 110 or 100 K KRYOFLEX nitrogen cold stream. Data were collected on a Bruker APEX CCD X-ray diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) controlled by the APEX2 software package. Absorption corrections were applied using SADABS. The structures were solved using direct methods and refined on F² with the SHELXTL-97 software package. Structures were checked for higher symmetry using PLATON. All non-hydrogen atoms were located and refined anisotropically. Unless otherwise stated, hydrogen atoms were placed in idealized locations and given isotropic thermal parameters equivalent to either 1.5 (terminal CH₃ or NH₃ hydrogen atoms) or 1.2 times the thermal parameter of the atom to which they were attached. Structure refinement was carried out using established strategies. Crystallographic data collection and refinement parameters are shown in Tables 6.1 and 6.2.
Colorless plates of 4 suitable for diffraction were grown by slow evaporation of a concentrated solution of DMSO. Three molecules of DMSO and 4 comprise the asymmetric unit. One of the three DMSO molecules is disordered over two orientations. This disorder was refined with the use of similarity restraints on both the geometry and thermal ellipsoids. The ratio of disordered components refined to 0.3:0.7. The highest residual electron density peak is located 0.09 Å from C16A, which is part of the disordered DMSO molecule. The largest residual electron density hole is located 0.78 Å from the platinum atom.

Large, colorless prisms of 5 were obtained by vapor diffusion of water into a DMF solution. Along with 5, two molecules of DMF are in the asymmetric unit. Validation of this structure using checkCIF gave rise to an A-level alert regarding the presence of larger than normal carbon atom thermal ellipsoids. The large ellipsoids arise from unresolved conformational disorder of the cyclopentyl ring. No attempt was made to model the disorder as two distinct conformations. The highest residual electron density peak and hole are located 0.80 and 0.78 Å from the platinum atom, respectively.

Slow evaporation of a DMSO solution of 6 afforded colorless blocks. The asymmetric unit includes two molecules of DMSO and a molecule of 6. One of the DMSO molecules was disordered over two orientations. The disorder was refined with similarity restraints on the geometry and ellipsoids. The ratio of disordered components refined to 0.17:0.83. A B-level alert was generated by checkCIF pointing to the presence of large carbon thermal ellipsoids. As with 5, these large ellipsoids can be attributed to a small amount of conformational disorder in the cyclohexyl ring. The highest residual electron density peak is located 0.80 Å from the platinum atom, and the largest residual electron density hole is located 0.46 Å from C10, which is part of the cyclohexyl ring.
Vapor diffusion of water into a DMSO solution of 7 afforded yellow plates suitable for diffraction. Compound 7 crystallized with two molecules of DMSO, one molecule of water, and two molecules of the Pt(IV) complex per asymmetric unit. The hydrogen atoms of the water molecule could be located on a difference Fourier map and were included, constraining the O–H bond distances to 0.84 Å and restraining the thermal ellipsoids to 1.5 times those of the oxygen atom. As with 5 and 6, an A-level alert was generated by checkCIF regarding larger than normal carbon ellipsoids. The large carbon ellipsoids involve only one of the four phenyl rings in the asymmetric unit. Instead of attempting to model the disorder over two sites, the ellipsoids were allowed to represent the disorder, which involves a slight rotation of the phenyl ring. The highest residual electron density peak and hole are located 0.94 and 0.75 Å from the platinum atom, respectively.

Pale yellow plates of 8 were grown by vapor diffusion of water into a DMSO solution. The asymmetric unit comprises two molecules of 8, four molecules of DMSO, and 1.48 molecules of water. Three molecules of DMSO and one water molecules are well ordered. The hydrogen atoms of the well ordered water molecule could be located on a difference Fourier map and were included, constraining the O–H bond distances to 0.84 Å and restraining the thermal ellipsoids to 1.5 times those of the oxygen atom. A DMSO molecule is disordered over two positions. In one of those positions, an additional partially occupied water molecule is also incorporated into the lattice. The two DMSO molecules were refined with similarity restraints on their geometries, and the occupancy factor of the water molecule was assigned the same free variable as the non-clashing disordered DMSO. The occupancy factor of the DMSO/water pair refined to 0.48, with the single DMSO molecules occupying the lattice the rest of the time. The hydrogen atoms of the partially occupied water could not be located in the difference Fourier
map and are not included in the model. Level A alerts were generated by checkCIF. These alerts signal the presence of impossibly close contacts between the disordered DMSO molecules in different unit cells. However, the alerts can be disregarded because the roughly 50:50 components of the disorder must alternate from cell to cell in the lattice in order to prevent physically impossible close contacts. The highest residual electron density peak and hole are located 0.89 and 0.59 Å from S300, which is part of the disordered DMSO molecules.

Pale yellow needles of 9 were obtained from the vapor diffusion of diethyl ether into an acetone solution. A molecule of acetone and 9 are present in the asymmetric unit. Structure refinement proceeded smoothly with no disorder or other issues. The highest residual electron density peak and hole are located 0.80 and 1.45 Å from the platinum atom, respectively.

Vapor diffusion of diethyl ether into a DMF solution of 10 afforded pale yellow needles. Within the asymmetric unit, two molecules of 10 and seven molecules of DMF are present. Two of the DMF molecules are disordered over two orientations. Using similarity restraints, both cases of disorder were refined successfully. The ratio of disordered components refined to 0.42:0.58 and 0.21:0.79. The highest residual electron density peak and hole are located 0.84 and 0.72 Å from the Pt1 and Pt2, respectively.

Large yellow blocks of 11 were grown by vapor diffusion of water into a DMF solution. Aside from one molecule of 11, three molecules of DMF also reside in the asymmetric unit. One of these DMF molecules is disordered over two orientations. The disorder was refined with similarity restraints, and the ratio of disordered components refined to 0.35:0.65. The highest residual electron density peak and hole are located 0.85 and 0.74 Å from the platinum atom, respectively.

<table>
<thead>
<tr>
<th></th>
<th>4-3DMSO</th>
<th>5-2DMF</th>
<th>6-2DMSO</th>
<th>7:DMSO-0.5H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>formula</td>
<td>C_{10}H_{22}Cl_{2}N_{4}O_{7}PtS</td>
<td>C_{10}H_{22}Cl_{2}N_{4}O_{7}Pt</td>
<td>C_{10}H_{22}Cl_{2}N_{4}O_{7}PtS</td>
<td>C_{10}H_{22}Cl_{2}N_{4}O_{7}PtS</td>
</tr>
<tr>
<td>fw</td>
<td>766.72</td>
<td>702.55</td>
<td>740.67</td>
<td>659.45</td>
</tr>
<tr>
<td>space group</td>
<td>P1</td>
<td>P1</td>
<td>Pbcn</td>
<td>P2_1/c</td>
</tr>
<tr>
<td>a, Å</td>
<td>8.9361(10)</td>
<td>9.0048(9)</td>
<td>25.5442(17)</td>
<td>11.5530(5)</td>
</tr>
<tr>
<td>b, Å</td>
<td>10.5917(12)</td>
<td>12.6877(13)</td>
<td>10.7982(7)</td>
<td>31.3292(13)</td>
</tr>
<tr>
<td>c, Å</td>
<td>16.762(2)</td>
<td>13.2385(14)</td>
<td>20.7211(13)</td>
<td>13.5070(6)</td>
</tr>
<tr>
<td>a, deg</td>
<td>89.100(2)</td>
<td>116.484(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β, deg</td>
<td>89.454(2)</td>
<td>93.069(2)</td>
<td></td>
<td>109.7380(10)</td>
</tr>
<tr>
<td>γ, deg</td>
<td>72.258(2)</td>
<td>92.288(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, Å³</td>
<td>1510.8(3)</td>
<td>1348.4(2)</td>
<td>5715.5(6)</td>
<td>4601.6(3)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>ρ.calcd, g·cm⁻³</td>
<td>1.685</td>
<td>1.730</td>
<td>1.721</td>
<td>1.904</td>
</tr>
<tr>
<td>T, °C</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
</tr>
<tr>
<td>μ(Mo Kα), mm⁻¹</td>
<td>5.066</td>
<td>5.444</td>
<td>5.281</td>
<td>6.458</td>
</tr>
<tr>
<td>θ range, deg</td>
<td>2.02-28.25</td>
<td>1.72-29.73</td>
<td>1.59-29.18</td>
<td>1.73-25.11</td>
</tr>
<tr>
<td>total no. of data</td>
<td>30171</td>
<td>29870</td>
<td>118407</td>
<td>75374</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>7386</td>
<td>7585</td>
<td>7718</td>
<td>8189</td>
</tr>
<tr>
<td>no. of parameters</td>
<td>337</td>
<td>304</td>
<td>323</td>
<td>546</td>
</tr>
<tr>
<td>completeness to θ (%)</td>
<td>98.9</td>
<td>98.7</td>
<td>100.0</td>
<td>99.8</td>
</tr>
<tr>
<td>R1 (%)</td>
<td>2.91</td>
<td>2.38</td>
<td>4.27</td>
<td>3.44</td>
</tr>
<tr>
<td>wR2 (%)</td>
<td>5.50</td>
<td>5.00</td>
<td>5.66</td>
<td>4.96</td>
</tr>
<tr>
<td>R1 (%) for I &gt; 2σ</td>
<td>2.32</td>
<td>2.17</td>
<td>2.48</td>
<td>2.45</td>
</tr>
<tr>
<td>wR2 (%) for I &gt; 2σ</td>
<td>5.42</td>
<td>4.92</td>
<td>4.99</td>
<td>4.67</td>
</tr>
<tr>
<td>GOF</td>
<td>1.040</td>
<td>1.039</td>
<td>1.021</td>
<td>1.044</td>
</tr>
<tr>
<td>max, min peaks, e·Å⁻³</td>
<td>1.749, -1.229</td>
<td>1.879, -2.044</td>
<td>1.672, -0.787</td>
<td>1.264, -0.733</td>
</tr>
</tbody>
</table>

a R1 = Σ||F_o| - |F_c||/Σ|F_o|.

b wR2 = \{Σ[w(F_o^2 - F_c^2)^2]/Σ[w(F_o^2)]\}^{1/2}.

c GOF = \{Σ[w(F_o^2 - F_c^2)^2]/(n-p)\}^{1/2} where n is the number of data and p is the number of refined parameters.
Table 6.2. Summary of X-Ray Crystallographic Data Collection and Refinement Parameter for 8-11.

<table>
<thead>
<tr>
<th></th>
<th>8-2DMSO-0.74H2O</th>
<th>9-acetone</th>
<th>10 3.5DMF</th>
<th>11 3DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>formula</td>
<td>C26H35Cl2N4O5.74PtS2</td>
<td>C19H25Cl2N4O7Pt</td>
<td>C24H30.5Cl2F2N7.5O7.5Pt</td>
<td>C31H43Cl3N7O3Pt</td>
</tr>
<tr>
<td>fw</td>
<td>769.55</td>
<td>690.44</td>
<td>864.13</td>
<td>891.71</td>
</tr>
<tr>
<td>space group</td>
<td>P1</td>
<td>P2_1/c</td>
<td>P2_1</td>
<td>P1</td>
</tr>
<tr>
<td>a, Å</td>
<td>11.6514(7)</td>
<td>18.9166(10)</td>
<td>18.0923(7)</td>
<td>11.7178(14)</td>
</tr>
<tr>
<td>b, Å</td>
<td>14.7016(8)</td>
<td>9.5089(5)</td>
<td>7.0254(3)</td>
<td>12.0190(15)</td>
</tr>
<tr>
<td>c, Å</td>
<td>18.4270(11)</td>
<td>13.7632(7)</td>
<td>26.4980(11)</td>
<td>13.2152(16)</td>
</tr>
<tr>
<td>α, deg</td>
<td>68.7800(10)</td>
<td>87.170(2)</td>
<td>87.170(2)</td>
<td>87.170(2)</td>
</tr>
<tr>
<td>β, deg</td>
<td>78.9840(10)</td>
<td>92.5790(10)</td>
<td>104.0310(10)</td>
<td>77.548(2)</td>
</tr>
<tr>
<td>γ, deg</td>
<td>84.3020(10)</td>
<td>24.732.2(2)</td>
<td>3267.6(2)</td>
<td>1757.6(4)</td>
</tr>
<tr>
<td>V, Å³</td>
<td>2586.6(3)</td>
<td>2473.2(2)</td>
<td>3267.6(2)</td>
<td>1757.6(4)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>ρ calc., g·cm⁻³</td>
<td>1.771</td>
<td>1.854</td>
<td>1.757</td>
<td>1.685</td>
</tr>
<tr>
<td>T, °C</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
<td>-173(2)</td>
</tr>
<tr>
<td>μ(Mo Kα), mm⁻¹</td>
<td>5.234</td>
<td>5.935</td>
<td>4.524</td>
<td>4.199</td>
</tr>
<tr>
<td>total no. of data</td>
<td>60845</td>
<td>51159</td>
<td>68723</td>
<td>35819</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>15609</td>
<td>6409</td>
<td>16640</td>
<td>8716</td>
</tr>
<tr>
<td>no. of parameters</td>
<td>706</td>
<td>304</td>
<td>903</td>
<td>457</td>
</tr>
<tr>
<td>completeness to Θ (%)</td>
<td>98.7</td>
<td>99.7</td>
<td>99.8</td>
<td>98.8</td>
</tr>
<tr>
<td>R1 (%)</td>
<td>4.36</td>
<td>2.82</td>
<td>2.83</td>
<td>2.27</td>
</tr>
<tr>
<td>wR2 (%)</td>
<td>8.05</td>
<td>4.83</td>
<td>5.70</td>
<td>4.21</td>
</tr>
<tr>
<td>R1 (%) for I &gt; 2σ</td>
<td>3.16</td>
<td>2.10</td>
<td>2.27</td>
<td>1.91</td>
</tr>
<tr>
<td>wR2 (%) for I &gt; 2σ</td>
<td>7.18</td>
<td>4.57</td>
<td>5.08</td>
<td>4.09</td>
</tr>
<tr>
<td>GOF</td>
<td>1.029</td>
<td>1.030</td>
<td>1.039</td>
<td>1.037</td>
</tr>
<tr>
<td>max, min peaks, e·Å⁻³</td>
<td>3.119, –2.625</td>
<td>2.131, –0.468</td>
<td>1.221, –1.191</td>
<td>0.942, –0.712</td>
</tr>
</tbody>
</table>

\[ R1 = \sum |F_o| - |F_c|/\sum |F_o| \]
\[ wR2 = \{\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2\}^{1/2} \]
\[ GOF = \{\sum w(F_o^2 - F_c^2)^2/(n-p)\}^{1/2} \]

where n is the number of data and p is the number of refined parameters.
Theoretical Calculations. DFT calculations were performed using the Gaussian 03 (revision D01) software package. Geometry optimizations, frequency calculations, and molecular orbital generations were all carried out using the B3LYP functional. For the light atoms (carbon, hydrogen, chlorine, nitrogen, oxygen, and fluorine), the 6-31++G(d,p) basis set was used, and for platinum, the LANL2DZ basis set and effective core potential were used. No solvation models were employed; the results described for all complexes are in the gas phase. Frequency calculations were carried out on all optimized geometries to verify the absence of imaginary values. To determine adiabatic electron affinities, an additional set of geometry optimizations and energy calculations were performed for the analogous monoanionic platinum(III) complexes with a doublet spin state. The difference in total electronic energy between the platinum(III) anion and neutral platinum(IV) complexes is the adiabatic electron affinity of the latter. The geometry optimization of the one-electron-reduced analogue of 3 gave rise to a structure with an imaginary frequency. Hence, its adiabatic electron affinity was not computed.

Cell Lines and Culture Conditions. Human A549 (lung carcinoma) and human MRC-5 (normal lung fibroblasts) were grown as adherent monolayers in a growth medium consisting of Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cultures were grown in 25-cm² flasks in an incubator at 37 °C with a humidified atmosphere composed of 5% CO₂.

Cytotoxicity Assays. The colorimetric MTT assay was used to determine the cytotoxicity of cisplatin and compounds 4–11. Trypsinized A549 and MRC-5 cells were seeded into a 96-well plate at cell densities of 1500 and 2500 cells/well, respectively, in 200 μL of growth medium and were incubated for 24 h. The medium was then removed, and 200 μL of new growth medium containing various concentrations of the platinum complexes was added. After 72 h, the medium
was removed, 200 μL of a 0.8 mg/mL solution of MTT in DMEM was added, and the plate was incubated for an additional 4 h. The DMEM/MTT mixture was aspirated, and 200 μL of a mixture of 90% DMSO and 10% glycine buffer, pH 10.5, was added to dissolve the purple formazan crystals. The absorbance of the plates was read at 570 nm. Absorbance values were normalized to the platinum-free control wells and plotted as [Pt] versus % viability. IC₅₀ values were interpolated from the resulting curves. The reported IC₅₀ values are the averages from at least three independent experiments, each of which consisted of three replicates per concentration level. Dilutions of the platinum(IV) compounds in growth medium were prepared from concentrated solutions (10–20 mM) in DMSO. Cisplatin was diluted from a phosphate-buffered saline solution (≈ 2 mM). Final DMSO concentrations in all cases was ≤ 1%, a content not substantially toxic to cells.

6.3. Results and Discussion

Synthesis and Characterization. The synthesis of platinum(IV) carbamate complexes was accomplished by treating cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂] with the desired isocyanate in a DMF solution (Scheme 6.2). Because the starting complex, cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂], is largely insoluble in DMF, the progress of the reaction was monitored visually by observing conversion of the reaction mixture from a suspension to a homogeneous solution. The synthesis of the analogous methyl ethyl, and isopropyl carbamate complexes has been reported previously. In this prior study, the authors prepared these complexes by suspending cis,cis,trans-[Pt(NH₃)₂Cl₂(OH)₂] in neat isocyanate with no additional solvent. In cases where isocyanate is expensive or toxic, the use of only a slight excess, as demonstrated in the present work, is a clear advantage.
Platinum(IV) dicarbamates are either white (4–6) or pale-yellow to pale-orange (7–11) solids. Compounds 7–11 are the first reported platinum(IV) complexes bearing aryl carbamate ligands and thus extend the scope of the chemistry beyond simple alkyl isocyanates. Compounds 4–11 exhibit good solubility in DMF and DMSO, moderate solubility in THF, acetonitrile, and acetone, and poor solubility in water and halogenated organic solvents. In solution, the aryl carbamate complexes, 7–11, decompose to dark-brown solutions when exposed to ambient light over the course of several hours. The alkyl carbamate complexes, 4–6, remain stable in solution even in the presence of light. The aromatic substituents of 7–11 most likely play a role in the
photodecomposition of the complexes. In the absence of light, all of the compounds are stable in solution.

Characterization of compounds 4–11 was accomplished by NMR spectroscopy, IR spectroscopy, mass spectrometry, elemental analysis, and X-ray crystallography (vide infra). Elemental analyses of the complexes are in good agreement with expected values, and electrospray ionization mass spectrometry gave rise to the expected [M–H]$^-$ signals, further validating the molecular formulas of these compounds. The IR spectra displayed characteristic C=O stretching frequencies ranging from 1647 to 1663 cm$^{-1}$ for the aryl carbamate complexes and from 1628 to 1629 cm$^{-1}$ for the alkyl carbamate complexes. All complexes also display N-H stretching frequencies derived from the ammine ligands, which appear as a broad series of bands near 3200 cm$^{-1}$.

The $^1$H, $^{13}$C, and $^{19}$F (for 10) NMR spectra of the complexes display all expected resonances. $^{195}$Pt and selected $^1$H NMR chemical shifts are summarized in Table 6.3. The signal corresponding to the NH proton of the carbamate ligands is observed between 8.97 and 9.21 ppm for the aryl carbamate complexes, 7–11, and between 6.05 and 6.55 ppm for the alkyl carbamate complexes, 4–6. This 3 ppm shift reflects significant deshielding of the NH carbamate resonance relative to the alkyl substituents by the aryl substituents. The proton resonances of the coordinated ammine ligands appear in all complexes as broad peaks ranging from 6.67 to 6.82 ppm. These values are consistent with ammine coordination to a platinum(IV) center; protons of ammines coordinated to platinum(II) centers typically lie farther upfield, between 3 and 5 ppm.$^{47}$
Table 6.3. $^{195}$Pt and Selected $^1$H NMR Shifts for 4–11 in DMSO-$d_6$ at 20 °C.

<table>
<thead>
<tr>
<th>compound</th>
<th>$\delta$ $^{195}$Pt, ppm</th>
<th>$\delta$ $^1$H, carbamate NH, ppm</th>
<th>$\delta$ $^1$H, NH$_3$, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1276</td>
<td>6.05</td>
<td>6.65</td>
</tr>
<tr>
<td>5</td>
<td>1275 (major), 1262(minor)</td>
<td>6.55</td>
<td>6.67</td>
</tr>
<tr>
<td>6</td>
<td>1276 (major), 1263 (minor)</td>
<td>6.47</td>
<td>6.67</td>
</tr>
<tr>
<td>7</td>
<td>1265</td>
<td>9.12</td>
<td>6.79</td>
</tr>
<tr>
<td>8</td>
<td>1264</td>
<td>9.02</td>
<td>6.79</td>
</tr>
<tr>
<td>9</td>
<td>1265</td>
<td>8.98</td>
<td>6.77</td>
</tr>
<tr>
<td>10</td>
<td>1265</td>
<td>9.21</td>
<td>6.78</td>
</tr>
<tr>
<td>11</td>
<td>1269</td>
<td>9.01</td>
<td>6.82</td>
</tr>
</tbody>
</table>

The $^{195}$Pt NMR spectra of 7–11 display a single resonance in the range 1264–1269 ppm. Given that the known window for $^{195}$Pt NMR shifts is $>$15,000 ppm, the small variance in chemical shifts among these complexes indicates that the peripheral substituents of the aryl rings have little effect on the magnetic environment of the platinum nucleus. The alkyl carbamate complex, 4, exhibits a single $^{195}$Pt NMR resonance in DMSO-$d_6$ at 1276 ppm. These chemical shifts are in the range expected for platinum(IV) complexes$^{48-50}$ and are close to related platinum(IV) alkyl and aryl carboxylate complexes, which fall between 1000 and 1300 ppm. Although both the $^1$H and $^{13}$C NMR spectra of 5 and 6 are consistent with the presence of a single species in solution, the $^{195}$Pt NMR spectra at 20 °C in DMSO-$d_6$ display two resonances at approximately 1276 and 1262 ppm in relative intensities of approximately 2:1. As the temperature of the NMR sample is increased, the two resonances eventually coalesce between 50 and 65 °C, as shown in Figure 6.1 for 5. Consistent with the known temperature dependence of $^{195}$Pt NMR chemical shifts,$^{51}$ the peaks are shifted downfield at higher temperatures as well.
This fluxional process could also be monitored by $^1$H NMR spectroscopy, as shown in Figure 6.1 for 5. The NH resonance of the carbamate ligand exhibits significant temperature dependence, shifting upfield by 0.5 ppm at 80 °C. A small peak in the spectrum near 5.8 ppm broadens into the baseline at 35 °C. This peak most likely corresponds to the NH resonance of a minor conformational isomer. The aliphatic region of the $^1$H NMR spectrum is unaffected by changes in temperature. The broad peak of the coordinated NH$_3$ protons is only slightly affected by an increase in temperature; a small upfield shift occurs and shoulders due to coupling to $^{14}$N (I = 1) become visible. The changes in the $^{195}$Pt and $^1$H NMR spectra as a function of the temperature are fully reversible; after increasing the temperature to 80 °C, the original spectra can be obtained at 20 °C.
As shown in Chart 6.1, three possible conformational isomers exist for the complexes depending on the orientation of substituents about the C–N bond of the carbamate ligand. It is not clear why only two of these isomers are observed by $^{195}$Pt NMR spectroscopy, but it is possible that two of the isomers have very similar chemical shifts and are therefore not resolved as distinct peaks. Another possibility is that one of the conformational isomers is significantly less stable than the other two and never accumulates in a high enough concentration to be observed under equilibrium conditions. The use of variable-temperature $^{195}$Pt NMR spectroscopy to distinguish between chemically similar stereoisomers has been reported before.\textsuperscript{52-55} Our findings here similarly validate this method as a valuable tool for distinguishing isomers that could not otherwise be discerned by the more commonly used $^1$H and $^{13}$C NMR spectroscopy.

![Chart 6.1. Structures of possible isomers of platinum(IV) dicarbamate complexes.](image)

**X-ray Crystal Structures.** Complexes 4–11 were all characterized by X-ray crystallography and are the first such structurally characterized platinum carbamate complexes. Relevant bond distances and angles for the platinum carbamate complexes are listed in Table 6.4, and the
structures are shown in Figures 6.2 and 6.3. The bond distances are typical; the Pt–Cl bond lengths are close to 2.3 Å and the Pt–O/N distances are ~2.0 Å. All complexes display the expected octahedral coordination geometry for platinum(IV). In addition, the structures all have the same stereochemistry as that of the starting platinum(IV) hydroxo compound (cis,cis,trans), which is retained upon formation of the carbamate ligands.

Table 6.4. Selected Interatomic Distances (Å) and Angles (deg) for 4–11.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7(^b)</th>
<th>8(^b)</th>
<th>9</th>
<th>10(^b)</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt1-Cl1</td>
<td>2.3145(7)</td>
<td>2.3324(6)</td>
<td>2.3075(7)</td>
<td>2.3272(10)</td>
<td>2.3367(9)</td>
<td>2.3187(6)</td>
<td>2.3201(11)</td>
<td>2.3176(6)</td>
</tr>
<tr>
<td>Pt1-Cl2</td>
<td>2.3326(8)</td>
<td>2.3170(6)</td>
<td>2.3151(7)</td>
<td>2.3202(10)</td>
<td>2.3324(9)</td>
<td>2.3271(6)</td>
<td>2.3127(11)</td>
<td>2.3126(6)</td>
</tr>
<tr>
<td>Pt1-N1</td>
<td>2.037(2)</td>
<td>2.0423(19)</td>
<td>2.042(2)</td>
<td>1.993(3)</td>
<td>2.034(3)</td>
<td>2.029(2)</td>
<td>2.054(4)</td>
<td>2.0299(17)</td>
</tr>
<tr>
<td>Pt1-N2</td>
<td>2.037(2)</td>
<td>2.038(2)</td>
<td>2.041(2)</td>
<td>1.995(3)</td>
<td>2.033(3)</td>
<td>2.038(2)</td>
<td>2.048(3)</td>
<td>2.0349(18)</td>
</tr>
<tr>
<td>Pt1-O1</td>
<td>2.0117(19)</td>
<td>2.0088(16)</td>
<td>2.026(2)</td>
<td>1.993(3)</td>
<td>2.000(3)</td>
<td>2.0230(18)</td>
<td>2.0209(18)</td>
<td>2.0065(13)</td>
</tr>
<tr>
<td>Pt1-O3</td>
<td>2.0062(19)</td>
<td>1.9970(16)</td>
<td>2.001(2)</td>
<td>1.995(3)</td>
<td>2.002(3)</td>
<td>2.0113(18)</td>
<td>1.9936(19)</td>
<td>2.0161(13)</td>
</tr>
<tr>
<td>N1-Pt1-N2</td>
<td>90.00(10)</td>
<td>90.02(8)</td>
<td>93.17(10)</td>
<td>92.21(13)</td>
<td>89.81(14)</td>
<td>91.70(9)</td>
<td>93.49(10)</td>
<td>92.42(7)</td>
</tr>
<tr>
<td>Cl1-Pt1-Cl2</td>
<td>92.57(3)</td>
<td>91.99(2)</td>
<td>91.48(3)</td>
<td>92.68(4)</td>
<td>94.64(3)</td>
<td>90.90(2)</td>
<td>89.24(3)</td>
<td>91.33(2)</td>
</tr>
<tr>
<td>O1-Pt1-N1</td>
<td>96.54(9)</td>
<td>86.31(7)</td>
<td>97.67(10)</td>
<td>96.66(12)</td>
<td>91.99(13)</td>
<td>91.27(8)</td>
<td>91.90(14)</td>
<td>91.81(6)</td>
</tr>
<tr>
<td>O1-Pt1-Cl2</td>
<td>90.12(6)</td>
<td>87.53(5)</td>
<td>90.47(6)</td>
<td>86.18(8)</td>
<td>88.04(9)</td>
<td>88.69(6)</td>
<td>89.58(10)</td>
<td>88.74(4)</td>
</tr>
<tr>
<td>O3-Pt1-N2</td>
<td>94.89(9)</td>
<td>89.31(8)</td>
<td>93.21(9)</td>
<td>93.15(12)</td>
<td>92.70(13)</td>
<td>91.94(8)</td>
<td>91.40(14)</td>
<td>96.96(6)</td>
</tr>
<tr>
<td>O3-Pt1-Cl1</td>
<td>87.04(6)</td>
<td>88.30(5)</td>
<td>89.64(6)</td>
<td>88.14(8)</td>
<td>87.07(9)</td>
<td>87.92(5)</td>
<td>87.67(9)</td>
<td>84.12(4)</td>
</tr>
<tr>
<td>O1-Pt1-O3</td>
<td>174.06(8)</td>
<td>174.22(7)</td>
<td>174.17(9)</td>
<td>174.94(11)</td>
<td>168.70(11)</td>
<td>174.20(7)</td>
<td>174.72(8)</td>
<td>173.33(6)</td>
</tr>
</tbody>
</table>

\(^a\)The numbers in parentheses are the estimated standard deviations of the last significant figures. Atoms are labeled as indicated in Figures 6.2 and 6.3. \(^b\)Two molecules per asymmetric unit are present in the crystal lattice. The parameters shown here are only for one of those molecules.
Figure 6.2. Solid-state molecular structures of 4–7. Ellipsoids are drawn at the 50% probability level.

Figure 6.3. Solid-state molecular structures of 8–11. Ellipsoids are drawn at the 50% probability level.
A common feature among the eight complexes is the presence of intramolecular hydrogen bonding between the oxygen atom of the axial carbamate ligands and the equatorial ammine ligands. Three different geometries are observed for this interaction, as shown in Figure 6.4. In the first geometry, the oxygen atoms lie in a plane bisecting the N–Pt–N angle and interact with both coordinated ammines equally. This situation occurs for 6, both molecules in the asymmetric unit of 7, one of the molecules in the asymmetric unit of 8, and 11. The geometry in which both of the oxygen atoms are twisted to opposite sides and hydrogen bond with the different ammines is observed only in the case of 5. In the final case, one of the oxygen atoms is twisted to the side and interacts with only one of the coordinated ammines. This geometry is the most common hydrogen-bonding motif for this class of compounds and occurs in the remaining structures.

Figure 6.4. The three intramolecular hydrogen bonding motifs observed in the crystal structures of 4–11. These three examples are compounds 8 (left), 7 (middle), and 5 (right).
As discussed above, different conformational isomers, reflecting alternative orientations of the substituents about the carbamate C–N bond, exist (Chart 6.1). The most commonly observed isomer is that with a syn/syn ligand orientation, observed for 5, 6, both molecules in the asymmetric unit of 7, both molecules in the asymmetric unit of 8, and one of the molecules in the asymmetric unit of 10. The anti/syn isomer occurs in 4, 9, and the other molecule in the asymmetric unit of 10. The naphthyl carbamate complex 11 crystallized exclusively as the anti/anti isomer. The occurrence of all three possible isomers throughout the crystal structures of 4–11 reflects a small energy difference between isomers in solution, as observed by NMR spectroscopy.

**Cyclic Voltammetry.** The biological activity of platinum(IV) complexes is mediated by their redox chemistry. In most cases, platinum(IV) complexes, unlike their platinum(II) progeny, do not bind directly to DNA or other biological nucleophiles. The redox potential of platinum(IV) complexes is therefore believed to be an important factor in their efficacy as antitumor agents. With this possibility in mind, we studied redox potentials of 1–11 by cyclic voltammetry.

Because of the limited aqueous solubility of 4–11, the cyclic voltammograms were recorded in DMF using 0.1 M \((n-\text{Bu}_4\text{N})\text{PF}_6\) as the supporting electrolyte. For comparison, the electrochemical properties of compounds 1–3 were also investigated by cyclic voltammetry in the same solvent and electrolyte system. As expected for the platinum(IV)/platinum(II) redox couple, all of the compounds exhibit a single irreversible reduction event in the potential window of +0.4 to −1.2 V vs Ag/AgCl. The peak potentials \((E_p)\) for these processes obtained at a scan rate of 100 mV·s\(^{-1}\) are reported in Table 6.5, and the corresponding cyclic voltammograms are shown in Figure 6.5. With careful polishing of the glassy carbon working electrode, reproducible voltammograms were obtained with only minor deviations in the peak potentials. No systematic
shift in the peak potentials upon multiple cycles was observed, as was observed for 1 when measured in aqueous solution (see Appendix A).

Because these processes are irreversible, thermodynamic values for the redox potentials cannot be readily extracted from a single voltammogram. The observed peak potentials reflect a combination of both the kinetic overpotential and the thermodynamic reduction potential, as discussed in more detail in Appendix A. Correlations of peak potentials with structure-activity relationships\textsuperscript{8} and reduction kinetics\textsuperscript{6} of platinum(IV) complexes have been observed. Hence, although the peak potential is not clearly defined as a thermodynamic or kinetic parameter, it is still of use for characterizing potential platinum(IV) anticancer agents. For a given set of equatorial ligands on a platinum(IV) center, the peak potential in water predictably changes as the axial ligands are varied.\textsuperscript{5,7,57} Trifluoroacetate ligands produce the most easily reduced (most positive peak potential) platinum(IV) complexes, followed by chloride ligands, and then acetate ligands in that order. Axial hydroxo ligands, although not investigated here, give rise to platinum(IV) complexes that exhibit peak potentials more negative than those with acetate ligands. For example, the precursor complex \textit{cis,cis,trans-}[Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(OH)\textsubscript{2}] is reported to have a very negative peak potential at \(-880\) mV in aqueous media.\textsuperscript{57} In moving from water to DMF, we find here that this general trend still exists for the chloride and carboxylate ligands, as \(E_p(2) > E_p(3) > E_p(1)\). The peak potentials themselves, however, are shifted significantly from those measured in water. In water, the measured peak potentials of 1 and 3 have been determined by others to be \(-640\) and \(-260\) mV, respectively,\textsuperscript{57} and the peak potential of 2 was measured to be \(10\) mV. In DMF, these potentials are \(-720\) (1), \(-350\) (2), and \(-450\) (3) mV, indicating that this solvent change shifts the peak potential by up to \(300\) mV in the negative direction. Even though the potentials measured in DMF are shifted significantly from those measured in aqueous

265
solution, a comparison of peak potentials for the carbamate complexes 4–11 to those for 1–3 is valuable for understanding the relative stability of the complexes in the biological milieu.

![Figure 6.5. Cyclic voltammograms of 1–11. Data obtained for 2 mM solutions of the complexes in DMF with 0.1 M (Bu₄N)(PF₆) as the supporting electrolyte. The scan rate was 100 mV/s.](image-url)
Table 6.5. Peak Reduction Potentials for 1–11 Measured by Cyclic Voltammetry in DMF.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_p$, V vs. Ag/AgCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$-0.72 (-0.64)^a b$</td>
</tr>
<tr>
<td>2</td>
<td>$-0.35 (0.01)^a c$</td>
</tr>
<tr>
<td>3</td>
<td>$-0.45 (-0.26)^a b$</td>
</tr>
<tr>
<td>4</td>
<td>$-0.85$</td>
</tr>
<tr>
<td>5</td>
<td>$-0.85$</td>
</tr>
<tr>
<td>6</td>
<td>$-0.82$</td>
</tr>
<tr>
<td>7</td>
<td>$-0.73$</td>
</tr>
<tr>
<td>8</td>
<td>$-0.71$</td>
</tr>
<tr>
<td>9</td>
<td>$-0.72$</td>
</tr>
<tr>
<td>10</td>
<td>$-0.66$</td>
</tr>
<tr>
<td>11</td>
<td>$-0.63$</td>
</tr>
</tbody>
</table>

$^a$ Values in parentheses are for those measured in aqueous solution. $^b$ Ref. 57. $^c$ This work

Compounds 7–9 display nearly identical peak potentials near $-720$ mV. This similarity indicates that electron-donating groups in the para position of the aryl carbamate ligands have little effect on the redox potentials of these compounds. Notably, the peak potentials of these compounds are indistinguishable from that of 1. Thus, the aryl carbamate ligands confer the same degree of stabilization to the $4^+$ oxidation state as the acetate ligand. Compound 10 exhibits a higher peak potential at $-660$ mV. This more positive potential is attributed to the electron-withdrawing fluorine atom on the aromatic ring of the carbamate ligand, which favors reduction. The least negative peak potential of the series, at $-630$ mV, is displayed by the naphthyl carbamate complex 11. The reason for 11 having a peak potential approximately 100 mV more positive than those of 7–9 is not entirely clear. A possible explanation is that the increased steric bulk from the large naphthyl group favors ligand dissociation and consequently reduction. Alternatively, the kinetic component of the peak potential may be increased by a favorable interaction of the $\pi$-aromatic ligands with the glassy carbon electrode during the electron transfer process. The alkyl carbamates 4–6 have peak potentials between $-820$ and $-850$ mV. The alkyl substituents on the carbamate ligand stabilize the $4^+$ oxidation state by about 100
mV relative to the aryl substituents. This observation indicates in part that alkyl carbamates are stronger electron donors and are more capable of stabilizing the electron-poor 4+ oxidation state than aryl carbamates at least from the heterogeneous electron transfer conditions probed in this experiment.

Theoretical Calculations. Geometry optimizations were carried out for 1–11 at the DFT B3LYP theoretical level. For the carbamate complexes, 4–11, geometry optimizations were computed only for the anti/anti isomers (Chart 6.1). Although no symmetry restraints were placed on the geometry optimizations, all optimized structures attained nearly perfect $C_2v$ symmetry, with the principal 2-fold axis bisecting the Cl–Pt–Cl and NH$_3$–Pt–NH$_3$ angles in the equatorial plane. In this configuration, intramolecular hydrogen bonding occurs between the oxygen atom of the carbamate ligands and both of the coordinated ammine ligands, as observed experimentally in several of the crystal structures.

The frontier Kohn-Sham molecular orbitals (FMOs) of 1–3 are shown in a qualitative molecular orbital diagram in Figure 6.6. The expected two-over-three d-orbital splitting for a nearly octahedral transition-metal complex is predicted by these computations. The LUMO and LUMO+1 are nearly degenerate and are $d_{z^2}$ and $d_{xy}$ $\sigma^*$ in character. The HOMO to HOMO–3 are also close in energy. These orbitals are $d_{x^2-y^2}$, $d_{xz}$, and $d_{yz}$ $\pi^*$, and Cl 3p nonbonding in character. The HOMO-LUMO gaps of 1–3 are 4.16, 3.97, and 3.70 eV, respectively. These values correspond to the magnitude of the d-orbital splitting and reflect the ordering of the acetate, trifluoroacetate, and chloride ligands in the spectrochemical series.
The FMOs of 6, 7, and 11 are shown in the qualitative molecular orbital diagram in Figure 6.7. The FMOs of the other alkyl carbamate complexes, 4 and 5, are qualitatively similar to those of 6, as are the FMOs of the other aryl carbamate complexes, 8–10, to complex 7. Like
1–3, the LUMO and LUMO+1 of the carbamate complexes are d_{z^2} and d_{xy} σ* in character. The HOMOs, however, are ligand-localized π orbitals. The presence of these orbitals leads to smaller HOMO-LUMO gaps for the aryl carbamate complexes which range from 2.52 to 2.99 eV, compared to those of 1–3. This result is consistent with the observation that 7–11 are sensitive to light. The smaller HOMO-LUMO gap may render dissociative excited states accessible by the energy provided by visible light.

![Image of molecular orbital diagram](image)

**Figure 6.7.** Qualitative (energies not drawn to scale) Kohn-Sham molecular orbital diagram for 6 (left), 7 (middle), 11 (right). The x-, y-, and z-axes are defined as the bisector of the Cl_{eq}–Pt–Cl_{eq} and NH_{3}–Pt–NH_{3} angles, the bisector of the Cl_{eq}–Pt–NH_{3} angles, and the line comprising the Pt atom and its two axial ligands, respectively.
The gas-phase adiabatic electron affinities of 1, 2, and 4–11 were computed by optimizing the geometry of the one-electron reduced platinum(III) species as a doublet anion and then subtracting its total electronic energy from the neutral platinum(IV) species. The results are summarized in Table 6.6. The optimized geometries of the platinum(III) species give rise to stationary points on the potential energy surface, as evidenced by the lack of imaginary frequencies, except in the case of 3. A comparison of the geometrical parameters of the calculated platinum(IV) and platinum(III) structures is presented in Table 6.6. Upon reduction to platinum(III), two mutually trans bonds are elongated significantly, whereas the other bonds are only altered slightly. This result is expected, for the addition of another electron to the closed-shell platinum(IV) species would require population of an antibonding orbital. For most cases, the axial platinum-carbamate bond is lengthened by approximately 0.4 Å in the platinum(III) complex, which is consistent with the conventional view of platinum(IV) reduction depicted in Scheme 6.1. For 6–8, however, significant bond elongation occurs for the equatorial, mutually trans platinum-chloride and platinum-ammine bonds. These bonds are elongated by approximately 0.34 Å. This result suggests that in some cases elimination of the equatorial ligands may be a viable reductive pathway of platinum(IV) and is consistent with experimental observation of this pathway by others. The computed gas-phase adiabatic electron affinities correlate well with the observed peak potentials for reduction. In Figure 6.8, the peak potentials are plotted as a function of the electron affinity. The relationship is roughly linear (R² = 0.887), and as expected, larger electron affinities correlate with more positive peak potentials.
Table 6.6. The Computed Adiabatic Electron Affinities and the Difference in Computed Interatomic Distances between the Neutral Pt(IV) Complex and the Anionic Pt(III) Complex.\(^a\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Electron Affinity, eV</th>
<th>Pt–Cl1</th>
<th>Pt–Cl2</th>
<th>Pt–N1</th>
<th>Pt–N2</th>
<th>Pt–O1</th>
<th>Pt–O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.754</td>
<td>0.03021</td>
<td>0.03005</td>
<td>-0.01439</td>
<td>-0.01425</td>
<td>0.39707</td>
<td>0.39701</td>
</tr>
<tr>
<td>2</td>
<td>3.791</td>
<td>0.02479</td>
<td>0.02553</td>
<td>-0.02139</td>
<td>-0.02078</td>
<td>0.40872</td>
<td>0.41175</td>
</tr>
<tr>
<td>4</td>
<td>2.576</td>
<td>0.02695</td>
<td>0.02698</td>
<td>-0.00836</td>
<td>-0.00832</td>
<td>0.39533</td>
<td>0.39631</td>
</tr>
<tr>
<td>5</td>
<td>2.570</td>
<td>0.02719</td>
<td>0.02718</td>
<td>-0.00791</td>
<td>-0.00790</td>
<td>0.39420</td>
<td>0.39422</td>
</tr>
<tr>
<td>6</td>
<td>2.321</td>
<td>0.36070</td>
<td>0.03682</td>
<td>-0.01119</td>
<td>0.33653</td>
<td>0.02840</td>
<td>0.02918</td>
</tr>
<tr>
<td>7</td>
<td>2.683</td>
<td>0.33739</td>
<td>0.03196</td>
<td>-0.01319</td>
<td>0.33328</td>
<td>0.03518</td>
<td>0.03507</td>
</tr>
<tr>
<td>8</td>
<td>2.631</td>
<td>0.33878</td>
<td>0.03234</td>
<td>-0.01287</td>
<td>0.33366</td>
<td>0.03467</td>
<td>0.03458</td>
</tr>
<tr>
<td>9</td>
<td>2.901</td>
<td>0.02454</td>
<td>0.02454</td>
<td>-0.00784</td>
<td>-0.00787</td>
<td>0.39023</td>
<td>0.39024</td>
</tr>
<tr>
<td>10</td>
<td>3.129</td>
<td>0.02412</td>
<td>0.02419</td>
<td>-0.00897</td>
<td>-0.00884</td>
<td>0.39158</td>
<td>0.39165</td>
</tr>
<tr>
<td>11</td>
<td>3.060</td>
<td>0.02305</td>
<td>0.02306</td>
<td>-0.00790</td>
<td>-0.00792</td>
<td>0.39017</td>
<td>0.39028</td>
</tr>
</tbody>
</table>

\(^a\) Atoms are labeled as shown in Figures 6.2 and 6.3.

Figure 6.8. Plot of the computed adiabatic electron affinity versus the experimentally measured reduction peak potentials. The black line is the linear regression of the data.
**Biological Properties.** The cytotoxicities of compounds 4–11 and cisplatin against human lung carcinoma (A549) and human normal lung (MRC-5) cells were measured by the MTT assay. The results in the form of 50% growth inhibitory concentrations (IC$_{50}$ values) are shown in Table 6.7. In A549 cells, most of the platinum(IV) carbamates exhibit a level of cytotoxicity that is similar to that of cisplatin. The IC$_{50}$ values range from 3 to 6.7 µM, compared to an IC$_{50}$ of 7.0 µM for cisplatin. Compounds 4 and 5, bearing tert-butyl and cyclopentyl carbamate ligands, showed greater cytotoxicity than the other compounds, with the IC$_{50}$ values being 1.0 and 0.6 µM, respectively. In the noncancerous lung fibroblasts (MRC-5), all of the complexes, except for 4 and 5, were slightly less cytotoxic than cisplatin. The IC$_{50}$ of cisplatin in this cell line was 4.3 µM, and those of 6–11 ranged from 4.8 to 16.0 µM. The cytotoxicities of 4 and 5 in the lung fibroblasts were marked by IC$_{50}$ values of 2.8 and 2.3 µM, indicating that they are approximately a factor of two less cytotoxic in the healthy cells compared to the cancerous cells, as observed for the other carbamate complexes. The results here demonstrate that, like the platinum(IV) complexes bearing axial chloro, hydroxo, and acetato ligands, this newly synthesized class of platinum(IV) complexes bearing axial carbamate ligands also contains viable anticancer drug candidates.
Table 6.7. Cytotoxieties of Cisplatin and 4–11 in A549 and MRC-5 Cells.

<table>
<thead>
<tr>
<th>compound</th>
<th>IC\textsubscript{50} (\mu M)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A549</td>
</tr>
<tr>
<td>4</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>6.7 ± 2.9</td>
</tr>
<tr>
<td>7</td>
<td>6.7 ± 2.1</td>
</tr>
<tr>
<td>8</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>9</td>
<td>5.3 ± 2.5</td>
</tr>
<tr>
<td>10</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>11</td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td>cisplatin</td>
<td>7.0 ± 2.6</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 50% cell growth inhibitory concentrations as measured by the MTT assay after 72 h exposure. Values are the average of at least three independent experiments, and the reported errors are the corresponding standard deviations.

6.4. Summary and Conclusions

The syntheses of eight new platinum(IV) carbamate complexes are described. The general reactivity of both aryl and alkyl isocyanates with the important platinum(IV) synthon \textit{cis,cis,trans-[Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(OH)\textsubscript{2}]} provides a valuable synthetic methodology for the design of new platinum(IV) complexes with novel properties. It also offers a route for the functionalization of isocyanate-bearing nanomaterials through the formation of a platinum-carbamate bond. In contrast to the more common platinum(IV) carboxylate complexes, platinum(IV) carbamates adopt different isomeric forms depending on the rotational orientation of the ligand, as revealed by NMR spectroscopy and X-ray crystallography, and their ligand-based orbitals are intermediate in energy between those of the empty and filled metal d orbitals, as determined by
DFT calculations. Electrochemical studies of platinum(IV) carbamates display redox potentials similar to those of platinum(IV) acetates, such as satraplatin. This result suggests that platinum(IV) carbamate complexes should exhibit stability properties in biological milieu similar to those of the acetate complexes. The fact that platinum(IV) carbamates display cytotoxicities similar to or better than that of cisplatin reveals their therapeutic potential. Finally, DFT computational analyses indicate that the redox potential of platinum(IV) complexes can be correlated with their computed electron affinities.

6.5. References

Appendix A

Aqueous Electrochemistry of a Platinum(IV) Prodrug
A.1. Introduction

As discussed in Chapter 6, platinum(IV) anticancer agents have recently gained interest as alternatives for clinically used first and second generation platinum(II) analogs.1-3 This interest stems from their octahedral coordination geometry, which allows for two additional ligand-binding sites for derivation, and their kinetic inertness, which imparts a greater in vivo lifetime and the possibility for oral administration. Platinum(IV) anticancer agents are pro-drugs of platinum(II) complexes. Reduction of platinum(IV) results in loss of two ligands and formation of a DNA-binding platinum(II) species. The kinetics of this reductive activation step in vitro and in vivo are of great interest because they dictate whether there is any benefit in utilizing a platinum(IV) complex over its platinum(II) analog. Premature reduction in the bloodstream or gastrointestinal tract will lead to a platinum(II) species with its attendant biodistribution properties. If reduction occurs too slowly, the platinum(IV) complex may be cleared before it can bind to DNA in tumor tissues.

Various researchers, including us, have utilized cyclic voltammetry as a way to measure the propensity of a given platinum(IV) complex to be reduced.4-13 Correlations between reduction rates and biological activities with cyclic voltammetric data have been found in some cases,4,7,14-16 thus validating the methodology as an important tool for characterizing anticancer platinum(IV) complexes. The change in coordination geometry of a platinum(IV) complex from octahedral to square-planar upon reduction is reflected by an irreversible reduction peak in the cyclic voltammogram. Only in a few cases with specially designed ligands have reversible Pt(IV)/Pt(II) or Pt(IV)/Pt(III) couples been observed.17,18

Irreversible electrochemical processes, as probed by cyclic voltammetry, are inherently more complex than reversible ones. For reversible processes, the thermodynamic reduction
potential of a compound can be determined based on the midpoint of the anodic and cathodic peak potentials. Since no return oxidation wave is observed in the CVs of platinum(IV) complexes, the midpoint, and hence the thermodynamic, potential cannot be determined upon visual inspection of the CV. In the literature, the commonly cited value for Pt(IV) reduction potentials are the irreversible peak potentials, which depend on scan rate along with other factors (Equation A.1). The peak potential is not purely a thermodynamic value. It is, however, mathematically related to it. The equation describing the peak potential for an irreversible process is described by Equation A.1, where $E_p$ is the peak potential, $E^\circ$ is thermodynamic standard reduction potential, $R$ is the gas constant, $T$ is the temperature, $F$ is Faraday’s constant, $\alpha$ is the transfer coefficient, $n_a$ is the number of electrons transferred, $D_\text{ox}$ is the diffusion coefficient of the compound being studied, $k^\circ$ is the standard rate constant, and $\nu$ is the scan rate. It is clear from this equation that the rate of electron transfer from the electrode surface to the platinum(IV) complex ($k^\circ$) affects the $E_p$ observed by cyclic voltammetry.

**Equation A.1.**

$$E_p = E^\circ - \frac{RT}{\alpha n_a F} \left[ 0.780 + \ln \left( \frac{D_\text{ox}^{1/2}}{k^\circ} \right) + \ln \left( \frac{\alpha n_a F n}{RT} \right) ^{1/2} \right]$$

The loss of two ligands accompanies the reduction of platinum(IV) to platinum(II). In the design of new platinum(IV) anticancer constructs, it has been assumed that only the axial ligands are lost. Axial ligands have been used to attach platinum(IV) pro-drugs to various nanodelivery devices under the assumption that, upon reduction, cisplatin will be released, leaving the axial ligands behind (Scheme A.1). Recent studies, however, have shown that elimination of both equatorial and axial ligands can occur upon reduction by components of cellular extracts. Furthermore, the products obtained when glutathione was used as a reducing agent were
glutathione-bound platinum(II) complexes. These observations have implications for the design of future platinum(IV) constructs.

Scheme A.1. Possible reduction pathways for a platinum(IV)-nanodelivery conjugate.

Here, we describe our investigation of the electrochemical reduction in aqueous solutions of cis,cis,trans-[Pt(NH₃)₂Cl₂(OAc)₂] (1). Although 1 has previously been studied by cyclic voltammetry in aqueous solutions, we report further studies here utilizing both a glassy carbon and platinum working electrode. In contrast to the results obtained in organic solvent, it is shown that significantly different peak potentials are obtained depending on the nature of the working electrode in water. Furthermore, it also appears that 1 is reduced by four electrons at the glassy carbon electrode, thereby forming a layer of platinum metal on the electrode surface. Additionally, the electrochemical reduction products of 1 were investigated by ¹H NMR spectroscopy after their formation by bulk electrolysis. In accord with previous work, the NMR spectroscopic studies suggest that both equatorial and axial ligands are lost upon reduction.
A.2. Experimental Methods

Compound 1 was synthesized and characterized as previously described.\(^5\) Electrochemical measurements were carried out utilizing a VersaSTAT3 potentiostat from Princeton Applied Research accompanied by the V3 Studio software. Three electrode cells were used for cyclic voltammetry (CV) and chronoamperometry experiments. The working electrode was either a glassy carbon (GC) or platinum disk (Pt) electrode purchased from Princeton Applied Research. A platinum wire and Ag/AgCl in saturated NaCl were used as counter and references electrodes, respectively. Phosphate-buffered saline (PBS) at pH 7.4, which contains 12 mM HPO\(_4^{2-}\) and 137 mM NaCl, was used as the electrolytic solution. Before acquiring scans, solutions were purged with N\(_2\) for ~5 min, and then kept under a blanket of N\(_2\) during measurements. The working electrodes were routinely cleaned between experiments by polishing with alumina paste, rinsing with water, and then sonicating for ~2 min in clean water. Additionally, the glassy carbon electrode was electrochemically activated by holding the potential at -1 V in a clean PBS solution for 10 min to possibly reduce off C=O functionalities on the surface. This electrochemical activation step gave similar results to experiments carried out without it. Hence, this activation was not used routinely for electrode cleaning. Under the experimental conditions described here, the \([\text{Fe}^{II}(\text{CN})_6]^{4-}/[\text{Fe}^{III}(\text{CN})_6]^{3-}\) couple was consistently observed as at +0.22 V. All CVs were obtained at a scan rate of 100 mV/s at ambient temperature (22–25°C).

Bulk electrolysis experiments were carried out in a three-electrode, two-compartment H-cell, where the compartments were separated by a fine glass frit. One compartment contained the working electrode and reference electrode, and the other compartment contained the counter
electrode. The working electrode was carbon felt, the reference electrode was Ag/AgCl in saturated NaCl, and the counter electrode was a Pt mesh. The electrolytic solution used was unbuffered 0.2 M NaNO₃ in water. No buffer was used for these initial experiments in order to avoid the binding of buffer components to the reduced platinum(II) species. Electrolysis was carried out with magnetic stirring. A concentration of ~10 mM of 1 was utilized. The current was monitored during the electrolysis and used to determine the endpoint as indicated by its return to background levels upon the consumption of all electroactive species. Aliquots were removed from the electrolysis cell during different time points for analysis by ¹H NMR spectroscopic analysis. For ¹H NMR spectral analysis, 10% D₂O was added for locking and shimming and 1,4-dioxane was added as an internal reference (δ = 3.75 ppm). Water suppression was accomplished by pre-saturation of the HOD signal at a power level of 55 dB.

A.3. Results

Cyclic Voltammetry in Aqueous Solution. The cyclic voltammogram of 1 in pH 7.4 PBS using a GC working electrode is shown in Figure A.1. As expected, a single irreversible peak was observed near −630 mV, consistent with the observations of others. Multiple CV scans on the same sample between +1 and −1 V lead to a gradual shift in the peak potential to more positive values. Eventually, after more than 10 cycles, the peak potential stabilized to a value of approximately −180 mV. Polishing the electrode with alumina paste and rinsing it with water returned the peak potential to within 80 mV of the original −630 mV value. Multiple cycles, as before, shifted the peak potential back to a stable value around −180 mV. This general behavior is reproducible. Polishing the electrode appears to return it to its original state. The values of the peak potential themselves, however, varied by up to 80 mV during six different trials.
Figure A.1. Cyclic voltammograms of 1 in pH 7.4 PBS at a scan rate of 100 mV/s using a GC working electrode. The first cycle is bold and red. Subsequent cycles are black. Black arrows indicate the features that grew in after multiple consecutive cycles. The red arrow indicates the initial scan direction.

The cyclic voltammogram of 1 in pH 7.4 PBS obtained using a Pt disk working electrode is shown in Figure A.2. The CV displays an irreversible peak near -200 mV. Multiple cycles on the same solution gave rise to poorly overlaid surfaces, but no systematic shift in potential was observed as occurred for the glassy carbon electrode. The apparent poor signal-to-noise ratio of this peak may be attributed to the higher background current of the Pt disk electrode, which begins to catalyze proton reduction at approximately -0.5 V.
Figure A.2. Cyclic voltammogram of 1 in pH 7.4 PBS at a scan rate of 100 mV/s using a Pt disk working electrode. The black arrow indicates the initial scan direction.

Blank runs using freshly polished GC and Pt disk working electrodes in pH 7.4 PBS are displayed in Figure A.3. Both electrodes displayed a negative potential limit where the current increases dramatically. This current increase is most likely due to proton reduction. The onset for this catalytic feature occurs at $-0.5$ V for the Pt disk electrode and $-1.25$ V for the GC electrode. The lower potential for proton reduction at the Pt electrode is consistent with the well known utility of platinum as an effective catalyst for this reaction.
Figure A.3. Cyclic voltammograms of a blank solution of pH 7.4 PBS obtained at a scan rate of 100 mV/s using either a glassy carbon (red) or platinum (black) working electrode.

A GC electrode was modified by running multiple CV cycles on a PBS solution of 1. The resulting modified electrode was rinsed with water and transferred to a clean solution of pH 7.4 PBS. The blank acquired on the 1-modified glassy carbon electrode is shown in Figure A.4 together with a blank run on a polished glassy carbon electrode for comparison. The blank shows the onset of a catalytic current near -0.5 V, similar to that observed for the Pt disk electrode. No irreversible peak near -180 mV due to 1 in solution was observed. These results suggest that the reduction of 1 at the glassy carbon electrode results in the deposition of a new material, which fundamentally alters the electrode surface and gives rise to electrocatalytic activity. A likely candidate for the nature of the deposited material is metallic platinum.
Figure A.4. Cyclic voltammograms of a blank pH 7.4 PBS solution obtained at a scan rate of 100 mV/s using either clean glassy carbon electrode (black) or a glassy carbon electrode that has been modified by repeated CV cycles in a solution containing 1 (red).

**Chronoamperometry.** Proton reduction was monitored by chronoamperometry (Figure A.5). The potential was held at -1 V, where proton reduction should occur readily for Pt electrodes, but to a lesser degree for GC. The current response for a clean GC electrode in pH 7.4 PBS was approximately 6 µA. The current for a Pt disk electrode stabilized at 60 µA, indicating faster proton reduction relative to the GC electrode. The GC electrode modified by 1 displayed efficient proton reduction marked by a current of approximately 70 µA, comparable to that of the Pt disk electrode. Slow bubble formation was observed at the electrode surface for the Pt disk and 1-modified GC electrode consistent with the formation of hydrogen gas upon proton reduction. Polishing the 1-modified GC electrode reduced the observed current to 27 µA. This result indicates that the adsorbed species can be partially removed by polishing. Additional polishing and rinses with nitric acid were necessary to restore the current closer to that of the
clean glassy carbon. The apparent difficulty in removing the adsorbed species by conventional polishing might also explain variances observed in the peak potentials of cyclic voltammograms of 1 and related platinum(IV) complexes.

Figure A.5. Current versus time traces obtained when holding the potential at -1 V vs Ag/AgCl in a clean pH 7.4 PBS solution using different electrodes.

**Bulk Electrolysis and Characterization of Reduction Products.** Since the initial peak potential for the reduction of 1 observed by cyclic voltammetry occurs near -630 mV on a GC electrode, a bulk electrolysis experiment was carried out by holding the potential constant at a value of -600 mV. Upon complete consumption of 1, as indicated by the return of the current to background levels, a grey precipitate had formed. Analysis of the solution by $^1$H NMR spectroscopy revealed only the presence of free acetate at $\delta = 1.90$ ppm. The pH of the resulting solution was between 11 and 12.

Bulk electrolysis experiments at a more positive potential, -0.35 V, were undertaken. Upon completion of the electrolysis, no visible precipitate had formed. During the course of the
electrolysis the pH of the solution increased from 4.1 to 10.5. Figure A.6 shows the $^1$H NMR spectra of the solution during different time points in the electrolysis. The CH$_3$ resonance of 1 at 2.10 ppm decayed and three new peaks appeared at 2.00, 1.97, and 1.90 ppm. All three of these peaks were present at the end of the electrolysis as well. The peak at 1.90 ppm is assigned to free acetate, whereas the peaks at 2.00 and 1.97 ppm can be assigned to the platinum(II) complexes cis-[Pt(NH$_3$)$_2$(OAc)$_2$] and cis-[Pt(NH$_3$)$_2$(OAc)(OH$_2$)]$^+$, respectively (Scheme A.2). These assignments were verified by independent preparation of cis-[Pt(NH$_3$)$_2$(OAc)$_2$], which exists in equilibrium with its aquated form, cis-[Pt(NH$_3$)$_2$(OAc)(OH$_2$)]$^+$.32

Figure A.6. $^1$H NMR spectra of the bulk electrolysis products of 1 at -0.35 V vs Ag/AgCl at different time points.
Scheme A.2. Electrochemical reduction pathways of 1.

A.4. Discussion

The peak potential of 1 during the first CV cycle using a GC working electrode observed in these studies was consistent with previously reported values. Literature values span -570 mV to -690 mV.\textsuperscript{5,7,16} In our studies, we observed values ranging from -560 to -640 mV. The peak potential measured using a Pt working electrode was found near -200 mV. There is no literature precedent for cyclic voltammetry of 1 or related platinum(IV) anticancer complexes with a Pt working electrode. The apparent avoidance of a platinum working electrode by the platinum anticancer drug development community may stem from the limited potential region in which the electrode can be used in aqueous solutions. At -0.5 V, the Pt working electrode begins to catalyze reduction of protons, thus preventing the observation of reduction events at more negative potentials. The large positive shift in potential in moving from a GC to a Pt working electrode is noteworthy. Peak potential shifts of up to 600 mV have been observed for compounds of the formula, \textit{trans}-[Pt(NH\textsubscript{3})\textsubscript{4}X\textsubscript{2}]\textsuperscript{2+} where X is a halide, upon switching from a GC
to a Pt electrode. This dependence on the composition of the working electrode implies that electron transfer from the electrode surface to 1 occurs via an inner-sphere mechanism in which 1 is directly in contact with the surface. The different potentials observed may result from different adsorption energies and kinetics of 1 on the different surfaces. Other researchers have previously attributed this large shift in potential due to a change in electrochemical mechanism from an $E2C$ (initial 2-e\textsuperscript{-} transfer followed by a chemical reaction) to an $ECE$ mechanism (1-e\textsuperscript{-} transfer, chemical reaction, then 1-e\textsuperscript{-} transfer) when switching from Pt to GC. They propose that the $E2C$ mechanism operates by a two-electron inner-sphere electron transfer at the Pt electrode, whereas the $ECE$ mechanism operates by an initial one-electron outer-sphere electron transfer at the Pt electrode.

Multiple CV cycles appear to alter the composition of the GC electrode and the resulting peak potential readout for 1 (Figure A.1). The observation of the onset of a catalytic current at approximately $-0.5$ V and the shift of the peak potential to $-0.2$ V suggest that the GC electrode is being converted to a Pt electrode, presumably through electrodeposition of platinum metal from 1. That the modified electrode itself, rather than a species in solution, was inducing the catalytic proton reduction current was confirmed by transferring the 1-modified electrode to a clean PBS solution. Running CV cycles on the clean solution still gave rise to the catalytic current, which was comparable to that observed for a Pt disk electrode (Figure A.4). Furthermore, the modified GC electrode showed comparable efficiency to the Pt electrode in proton reduction when the potential was held at $-1$ V (Figure A.5). The direct electrodeposition of platinum from $[PtCl_6]^{2-}$ on carbon-based electrodes is known and has been used for the formation of adsorbed platinum nanoparticles for catalytic purposes. Three redox couples are invoked to explain the deposition of Pt\textsuperscript{0} from $[PtCl_6]^{2-}$: 291
\[
\text{Pt}^{IV}\text{Cl}_6^{2-} + 4e^- \rightarrow \text{Pt}^0 + 6\text{Cl}^-
\]
(1)
\[
\text{Pt}^{IV}\text{Cl}_6^{2-} + 2e^- \rightarrow \text{Pt}^{II}\text{Cl}_4^{2-} + 2\text{Cl}^-
\]
(2)
\[
\text{Pt}^{II}\text{Cl}_4^{2-} + 2e^- \rightarrow \text{Pt}^0 + 4\text{Cl}^-
\]
(3)

The standard reduction potentials at 25 °C for the three couples are 0.74, 0.72, and 0.76 V versus NHE for (1), (2), and (3), respectively.\(^4\) The similarities of all three couples indicate that all three processes occur within a narrow potential window. Hence, they would most likely not be resolved as individual peaks in a cyclic voltammetry experiment. Although the corresponding couples for 1 are not known, a similar phenomenon may explain the apparent observation of platinum deposition and only the presence of a single peak in the cyclic voltammogram.

Bulk electrolysis studies were carried out to investigate the reduction products of 1. Reduction at −0.6 V led to the formation of a black precipitate, and only free acetate ion was observed by \(^1\)H NMR spectroscopy. The black precipitate may be platinum black formed upon reduction from Pt(IV) to Pt(0). The observation of only free acetate by \(^1\)H NMR spectroscopy is consistent with such an hypothesis. Additionally, the relatively basic pH measured at the end of electrolysis might be due to the release of ammonia from the platinum coordination sphere upon full reduction. The electrolysis at −0.35 V appears to reduce 1 by only two electrons, because the platinum(II) compounds \(cis-[\text{Pt(NH}_3)_2(\text{OAc})_2]\) (2) and \(cis-[\text{Pt(NH}_3)_2(\text{OAc})(\text{OH}_2)]^+\) (3) remained in solution after the current returned to background levels. Cisplatin most likely is also formed. However, owing to rapid proton exchange of the coordinated ammine ligands, cisplatin cannot be detected by \(^1\)H NMR spectroscopy. The use of \(^195\)Pt NMR spectroscopy to detect cisplatin was precluded by the low concentrations employed for these studies. The increase in pH of the solution is not fully understood. Free acetate ion is expected to increase the...
pH only slightly. The compounds cis-[Pt(NH$_3$)$_2$(OAc)$_2$] and cis-[Pt(NH$_3$)$_2$(OAc)(OH$_2$)]$^+$ observed after electrolysis probably arise from the cis elimination of the chloride ligands upon the reduction of 1 (Scheme A.2). Alternatively, both axial acetate ligands could be lost and then exchange with the chloride ligands of cisplatin. Given the lability of the acetate ligand on platinum(II) complexes, this pathway is disfavored. The observation of cis elimination here is consistent with literature.$^{24,25,43}$

A.5. Summary and Conclusions

Significantly different peak potentials for the reduction of 1 are observed depending on the nature of the working electrode. This difference is assumed to be a result of different heterogeneous rate constants, $k^o$ (Equation A.1), for electron transfer from the electrode surface to 1. Different $k^o$ values could result from different mechanisms of electron transfer at the electrode surface. Inner-sphere electron transfer mediated by bridging halide ligands has been documented for platinum(IV) complexes at the surface of electrodes.$^{33,35,44-46}$ The relative energies of such surface-bound halide-bridged intermediates and the rates at which they form would certainly be different depending on the composition of the surface. The large errors (up to 100 mV)$^{14}$ in observed peak potentials measured in aqueous solution might be a reflection of the sensitivity of the inner-sphere platinum reduction event to the nature of the electrode surface.

Cyclic voltammetry at a GC electrode in aqueous solution leads to a four-electron reduction of 1 and deposition of metallic platinum. The electrodeposition of Pt(IV) on carbon-based electrodes has been observed previously.$^{34-41}$ One explanation for this phenomenon is that the 2-e$^-$ and 4-e$^-$ reduction events occur at similar potentials such that CV cannot resolve them. Another possibility is that the overpotential required for the 2-e$^-$ reduction of platinum(IV) is
greater than the potential required for the 4-e\textsuperscript{−} reduction. Thus, by the time a current response is observed in the CV, the potential is sufficiently reducing to form platinum metal.

The bulk electrolysis of 1 occurs with both cis and trans elimination of two ligands. This observation may have implications for the design of future platinum(IV) anticancer agents. In the case of 1, the axial ligands are acetates. Since acetate is a fairly labile ligand for platinum(II), the resulting cis-diaminediacetoplatinum(II) complex should still readily bind to DNA. On the other hand, if cis halide elimination were to occur for cct-[Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(OH)\textsubscript{2}], the inert complex, cis-[Pt(NH\textsubscript{3})\textsubscript{2}(OH)\textsubscript{2}] would result. The products observed in this study, however, were the result of reduction at an electrode surface. The product distribution should depend on the mechanism of reduction and therefore the chemical nature of the reducing agent. As has been shown recently, reduction of platinum(IV) complexes with ascorbic acid yields different products than those obtained by the reduction with glutathione.\textsuperscript{27,29}

The reduction of platinum(IV) complexes to platinum(II) and platinum(0) should be thermodynamically favored for most reducing agents, because the standard reduction potentials for these processes are quite positive (+0.68 to 1.18 V versus NHE).\textsuperscript{47} As with the aquation of cisplatin, the reduction of platinum(IV) appears to be a kinetically controlled process. In most cases, the reduction of platinum(IV) proceeds through inner-sphere electron transfer mechanisms via a ligand-bridged activated complex.\textsuperscript{48-53} The rate-determining step in these cases is formation of this ligand-bridged activated complex. For outer-sphere electron transfer reactions of platinum(IV), the rate-determining step is a one-electron transfer and would therefore be dictated by the Pt(IV)/Pt(III) redox couple. Outer-sphere electron-transfer reactions of platinum(IV) are much slower than inner-sphere reactions,\textsuperscript{54} and therefore likely play only a minor role in the biological setting. The heterogeneous electron transfer kinetics at an electrode surface are

294
fundamentally different from the homogeneous reduction reactions that occurs in the biological setting. However, useful correlations between the electrochemical peak potential and homogeneous reduction rates and anticancer activity have been noted.\textsuperscript{4,7,14-16} The observed correlation between electrochemical peak potential and reduction rate by ascorbic acid\textsuperscript{14} may arise due to similar halide-bridged intermediates either at the electrode surface or at an oxygen atom of ascorbic acid. There are several cases, however, in which this correlation is not present,\textsuperscript{53,55} confirming that heterogeneous electron-transfer kinetics at the electrode surface do not always predict the homogeneous electron transfer kinetics in solution. Correlations of the biological activity with peak potential may relate to the electronic properties of the platinum(IV) centers. Electronic properties of medicinal compounds, typically in the form of a Hammett parameter, can often be related to biological activity in structure-activity relationships.\textsuperscript{56} Because the peak potential in organic solvent correlates with computed electron affinity,\textsuperscript{13} and in part with LUMO energy,\textsuperscript{57} this property may also be useful for modeling and predicting anticancer activity of platinum(IV) complexes. The measured peak potential also correlates in some cases with the lipophilicity of the platinum(IV) complexes.\textsuperscript{58} Hence, relationships between peak potential and biological activity may be due to differences in lipophilicity between the compounds.

A.6. References


295
Appendix B

Targeting the Mitochondria with Platinum Anticancer Agents using Mitochondria-Penetrating Peptides
B.1. Introduction

The biological target of the widely used anticancer drug cisplatin is nuclear DNA.¹ There is ample evidence to support this hypothesis, the most convincing of which is the observation that cells with decreased nuclear DNA repair capacities are hypersensitive to cisplatin.²⁻⁴ Nevertheless, the electrophilic properties of cisplatin and its aquated analogues enable this and related platinum-based drugs to bind to a wide range of intracellular nucleophiles, such as proteins,⁵ RNA,⁶ and phospholipids.⁷ The importance, if any, of these off-target binding interactions on the mechanism of action of platinum-based drugs remains poorly understood.

Because of their central role in mediating apoptosis, mitochondria are actively being explored as a potential anticancer drug targets.⁸⁻¹¹ The mitochondria contain their own circular DNA (mtDNA).¹² It is therefore of interest to consider the degree to which platinum-based drugs bind to mtDNA and how these mtDNA lesions affect their overall mechanism of action. Previous studies revealed that greater quantities of cisplatin bind to mtDNA than to genomic DNA extracted from mammalian cells.¹³,¹⁴ The role of the mtDNA adducts is debated in the literature. On one hand, mitochondrial DNA damage by cisplatin in various tissues has been implicated as a mediator for the toxic side effects of the drug, including neuropathy,¹⁵ ototoxicity,¹⁶ and developmental toxicity.¹⁷,¹⁸ In contrast, other studies have proposed that the mitochondrial and not nuclear DNA is the critical target of cisplatin in potentiating its anticancer activity.¹⁹,²⁰ The mitochondria also appear to play in role in mediating cellular resistance to cisplatin. Cisplatin-resistant cell lines have elevated mitochondrial membrane potentials,²¹,²² sustain less damage to mtDNA when treated with cisplatin,²³ and exhibit substantially less mitochondrial uptake of cisplatin²⁴ compared to the non-resistant parent lines. Taken together, the abovementioned
results indicate a complicated role of the mitochondria in mediating the cellular response to cisplatin.

To investigate more precisely the importance of the mitochondria as a target for platinum-based chemotherapy, it would be advantageous to have a platinum complex that selectively localizes to this organelle. The use of cell-penetrating peptides (CPPs)\textsuperscript{25,26} to direct the platinum complex through the cell membrane into the desired organelle provides a viable strategy for preparing such a construct. In particular, mitochondria-penetrating peptides (MPPs),\textsuperscript{27} a subclass of CPPs, can be utilized. MPPs are typically short peptide sequences comprising alternating lipophilic and cationic residues. Here, we describe our preliminary investigations of a platinum(II) complex conjugated to the N-terminus of an MPP with the sequence $rF_xrF_xrF_xr$, where $r$ is $d$-arginine and $F_x$ is $l$-cyclohexylalanine. This work is a collaborative effort with the lab of Professor Shana O. Kelley at the University of Toronto. The chemistry described provides a general route to Pt-peptide conjugates that are attached to leaving group ligands. The biological activity of this construct in wild-type and cisplatin-resistant A2780 ovarian cancer cells is presented.

\textbf{B.2. Experimental Methods}

\textbf{General Materials and Methods.} Small-molecule synthesis was carried out under normal atmospheric conditions with no precautions to exclude moisture or air. Cisplatin was purchased from Strem Chemicals and used as received. HATU ($O$-(7-azabenzotriazol-1-yl)-$N,N,N',N''$-tetramethyluronium hexafluorophosphate) was purchased from Oakwood Chemicals and used as received. Succinylacetone (Hsuccac) was synthesized from ethyl acetate and levulinic acid as previously described.\textsuperscript{28} Peptides were synthesized and provided in the solid-state, attached to Rink Amide resin, by the Kelley lab.
Physical Measurements. NMR spectra were acquired on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility (DCIF). $^1$H and $^{13}$C NMR spectra were referenced to residual protons or the carbon nucleus of the deuterated methanol ($^1$H $\delta = 3.31$ ppm, $^{13}$C $\delta = 49.1$ ppm), and signals are reported versus TMS. For $^1$H NMR spectra acquired in D$_2$O, signals were referenced to an internal standard of 1,4-dioxane ($\delta = 3.75$ ppm). $^{195}$Pt NMR spectra were referenced externally to a standard of K$_2$PtCl$_4$ in D$_2$O ($\delta = -1628$ ppm). For FTIR spectra, samples were prepared as KBr disks and data was recorded with a ThermoNicolet Avatar 360 spectrophotometer running the OMNIC software. Electrospray ionization mass spectrometry (ESI-MS) measurements were acquired on an Agilent Technologies 1100 series LC-MSD trap. Solutions used for biological studies were dissolved in MilliQ water (or PBS for cisplatin) and sterile filtered. The platinum concentrations of the solutions were determined by graphite-furnace atomic absorption spectroscopy (GFAAS) using a Perkin-Elmer AAAnalyst600 spectrometer. Elemental analyses were performed by a commercial analytical laboratory.

Synthesis of [Pt(succac)(NH$_3$)$_2$](NO$_3$). Cisplatin (500 mg, 1.67 mmol) and AgNO$_3$ (552 mg, 3.25 mmol) were stirred together in 10 mL of H$_2$O in the absence of light at room temperature for 16 h. The resulting mixture was filtered to remove AgCl. To the filtrate, a solution of NaOH (67 mg, 1.7 mmol) and succinylacetone (269 mg, 1.70 mmol) in 5 mL of H$_2$O was added dropwise. After stirring at rt for 5 h, the resulting solution was concentrated to dryness at 60 ºC under reduced pressure to afford an orange oil. This oil was dissolved in 3 mL of H$_2$O, acidified with three drops of 25% HNO$_3$. Acetone (50 mL) was added, and the resulting turbid white suspension was stirred for approximately 3 min, resulting in the deposition of an oily orange-brown residue. The turbid supernatant was decanted and mixed with 50 mL of a 1:1 (v/v) mixture of acetone and diethyl ether. Upon stirring at rt for approximately 5 min, an orange-
brown residue deposited again. The cloudy supernatant was decanted and poured directly into 150 mL of diethyl ether. The mixture was stored at -40 °C for 1.5 h and filtered to collect a white solid. The white solid was washed with 2 × 10 mL diethyl ether and then dried in vacuo. Yield: 203 mg (28%). Mp 159-164 °C (dec). $^1$H NMR (400 MHz, MeOD-d$_4$): δ 5.65 (s, 1H), 4.44 (br s, 6H), 2.58 (t, 2H), 2.44 (t, 2H), 1.88 (s, 3H). $^{13}$C NMR (100 MHz, MeOD-d$_4$): δ 186.5, 186.3, 176.7, 102.7, 35.1, 31.0, 26.1. $^{195}$Pt NMR (86 MHz, MeOD-d$_4$): δ -1570. IR (KBr, cm$^{-1}$): 3432 m, 3285 s, 1708 m, 1563 s, 1524 s, 1384 vs, 1356 s, 1309 s, 1202 w, 1175 m, 1039 w, 807 w, 645 w. ESI-MS (+ mode, MeOH): m/z 386.1 (calcd. for [Pt(succac)(NH$_3$)$_2$]$: 386.1). Anal. Calcd. for C$_7$H$_{15}$N$_3$O$_7$Pt: C, 18.75; H, 3.37; N, 9.37. Found: C, 19.04; H, 3.38; N, 9.27.

**Synthesis of Pt-MPP, [Pt(succac)(NH$_3$)$_2$]-r(Fir)$_3$-CONH$_2$.** A 50-µmol portion of solid FMOC-r(Fir)$_3$ on Rink Amide resin was placed in a fritted 2.5-mL Torviq disposable syringe and swelled with 2 mL of anhydrous DMF for 1 h. The N-terminal FMOC group was removed by treating the resin with a solution of 25% 4-methylpiperidine in DMF (v/v) for a period of 30 min, and the deprotected resin was washed with 5 × 1.5 mL of DMF. A mixture of 90 mg (200 µmol) [Pt(NH$_3$)$_2$(succac)]NO$_3$ and 76 mg (200 µmol) HATU was dissolved in 1.5 mL of 10% N,N-diisopropylethylamine (DIPEA) in DMF (v/v) and immediately added to the deprotected resin. The reaction vessel was shaken at rt for 60 min, and the solution was then expunged from the syringe. The resin was washed sequentially with 5 × 1.5 mL of DMF and 5 × 1.5 mL CH$_2$Cl$_2$ and then dried in vacuo. The dry resin was treated with a TFA/water/triisopropylsilane 95/2.5/2.5% (v/v) solution for 90 min to cleave Pt-MPP from the Rink Amide resin. Pt-MPP was precipitated from the cleavage solution with diethyl ether. The resulting solid Pt-MPP was separated by centrifugation and purified by semi-preparative HPLC with a C$_{18}$ reverse-phase column (9.4 mm × 250 mm). A two-solvent system (A = 0.1% TFA (v/v) in H$_2$O; B = 0.1% TFA (v/v) in
acetonitrile) was employed for purification according to the following protocol: isocratic flow, 10 % B, 0–5 min; gradient, 10-50% B, 5–35 min. The flow-rate was kept constant at 3 mL min⁻¹ throughout the purification. Fractions from sequential runs containing Pt-MPP were pooled and lyophilized. The purity of the final product was assessed via analytical HPLC (C₁₈, 4.6 mm x 250 mm), according to the following protocol: after a 5 min isocratic wash (10 % B), a linear gradient of 10–50% B was run over 30 min (35 min total) at a flow rate of 1 mL min⁻¹. HPLC, tᵣ = 23.8 min, >95% pure. ESI-MS (pos. ion mode): m/z 1583.8 ([M+HTFA]⁺, calcd. 1583.8), 1469.8 ([M]⁺, calcd. 1469.8), 735.3 ([M+H]²⁺, calcd. 735.4), 490.5 ([M+2H]³⁺, calcd. 490.3), 368.1 ([M+3H]⁴⁺, calcd. 368.2). ¹⁹⁵Pt NMR (86 MHz, D₂O): δ = 1591 ppm.

Synthesis of Pt-MPP(TAMRA), [Pt(succac)(NH₃)₂]⁺-r(Fₓr)₃K(5/6-carboxytetramethylrhodamine)-CONH₂. A 50-µmol portion of solid FMOC-r(Fₓr)₃K(5-6)-TAMRA) on Rink Amide resin was labeled with 200 µmol (90 mg) of [Pt(NH₃)₂(succac)]NO₃ using the procedure described above for the preparation of Pt-MPP. The purity of the two isomers (5 and 6) of Pt-MPP(TAMRA) were assessed via analytical HPLC (C₁₈, 4.6 mm x 250 mm), according to the same protocol as for Pt-MPP. The 5-isomer (tᵣ = 26.5 min) was found to be >95% pure based on the integrated chromatogram. The 6-isomer (tᵣ = 25.7 min) was judged to be 94% pure based on the integrated chromatogram and not deemed sufficiently pure for further biological studies. Characterization for 5-isomer: ESI-MS (pos. ion mode): m/z 1005.7 ([M+H]⁺, calcd. 1005.5), 670.8 ([M+2H]²⁺, calcd. 670.9), 503.3 ([M+3H]³⁺, calcd. 503.4). ¹⁹⁵Pt NMR (86 MHz, D₂O): δ = 1589 ppm.

X-ray Crystallography. Single crystals of succinylacetone and [Pt(succac)(NH₃)₂](NO₃) were grown from hexanes/Et₂O and MeOH/Et₂O, respectively. Crystals were selected and mounted in n-paratone oil on a cryoloop, frozen under a 100 K KRYO-FLEX nitrogen cold stream. A Bruker
APEX CCD X-ray diffractometer with graphite-monochromated Mo–Kα radiation ($\lambda = 0.71073$ Å) controlled by the APEX2 software package was utilized for data collection. Data were corrected for absorption with SADABS. The SHELXTL-97 software package was used to solve the structures with direct methods and further refine them against $R^2$. All non-hydrogen atoms were located on the difference map and refined anisotropically. The enol and carboxylic acid hydrogen atoms of succinylacetone were located on the difference map and semi-freely refined with constraints on the O–H distances (0.84 Å) and isotropic thermal displacement parameters of the hydrogen atoms (1.5 times that of the oxygen atoms to which they are attached). The carboxylic acid hydrogen atom of [Pt(succac)(NH$_3$)$_2$](NO$_3$) was also located and refined similarly. Other hydrogen atoms were placed at calculated positions and isotropic displacement parameters were constrained to be either 1.2 or 1.5 (terminal hydrogens) times those of the atoms to which they are bound. Crystallographic data collection and refinement parameters are collected in Table B.1.

Cell Culture. A2780 and A2780CP70 (ovarian wild-type and cisplatin-resistant) cell lines were obtained from Fox Chase Cancer Center and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were incubated in a humidified atmosphere containing 5% CO$_2$ at 37 °C.

Cytotoxicity Assays. The colorimetric MTT assay was used to assess cytotoxicity. In a 96-well plate, 2000 cells per well were seeded in 100 µL of growth medium and allowed to attach overnight. The growth media was then aspirated and replaced with 100 µL of growth medium containing varying concentrations of the platinum complexes. Six wells were used per concentration level. After 72 h, the platinum-containing media was aspirated and replaced with 200 µL of RPMI-1640 containing 0.67 mg/mL MTT. The cells were further incubated for 4 h.
The MTT solution was removed, and the resulting purple formazan crystals were dissolved in 100 µL of 25:1 mixture of DMSO and aqueous ammonia. The absorbance was measured at 550 nm. The absorbance values were normalized to the Pt-free wells (100% cell viability) and are plotted versus platinum concentration. From the resulting curves, 50% growth inhibitory concentration (IC$_{50}$) values were determined by interpolation. These experiments were repeated at least three times, and the reported IC$_{50}$ values are the averages of these trials with the error estimated from the resulting standard deviations.

Table B.1. X-ray Crystallographic Data Collection and Refinement Parameters for Succinylacetone and [Pt(succac)(NH$_3$)$_2$](NO$_3$).

<table>
<thead>
<tr>
<th></th>
<th>succinylacetone</th>
<th><a href="NO$_3$">Pt(succac)(NH$_3$)$_2$</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>formula</td>
<td>C$<em>7$H$</em>{10}$O$_4$</td>
<td>C$<em>7$H$</em>{15}$N$_3$O$_7$Pt</td>
</tr>
<tr>
<td>fw</td>
<td>158.15</td>
<td>448.31</td>
</tr>
<tr>
<td>space group</td>
<td>$P2_1/c$</td>
<td>$P2_1/c$</td>
</tr>
<tr>
<td>$a$, Å</td>
<td>21.050(4)</td>
<td>5.1808(3)</td>
</tr>
<tr>
<td>$b$, Å</td>
<td>6.5228(14)</td>
<td>9.5216(6)</td>
</tr>
<tr>
<td>$c$, Å</td>
<td>5.2953(11)</td>
<td>25.5478(16)</td>
</tr>
<tr>
<td>$\beta$, deg</td>
<td>90.273(3)</td>
<td>91.4100(10)</td>
</tr>
<tr>
<td>$V$, Å$^3$</td>
<td>727.1(3)</td>
<td>1259.88(13)</td>
</tr>
<tr>
<td>$Z$</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$\rho$ calcd, g·cm$^{-3}$</td>
<td>1.445</td>
<td>2.364</td>
</tr>
<tr>
<td>$T$, °C</td>
<td>-173(2)</td>
<td>-173(2)</td>
</tr>
<tr>
<td>$\mu$(Mo Kα), mm$^{-1}$</td>
<td>0.119</td>
<td>11.168</td>
</tr>
<tr>
<td>$\Theta$ range, deg</td>
<td>1.93–29.40</td>
<td>1.59–28.77</td>
</tr>
<tr>
<td>completeness to $\Theta$ (%)</td>
<td>99.8</td>
<td>100</td>
</tr>
<tr>
<td>total no. of data</td>
<td>14465</td>
<td>24630</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>2002</td>
<td>3257</td>
</tr>
<tr>
<td>no. of parameters</td>
<td>107</td>
<td>169</td>
</tr>
<tr>
<td>no. of restraints</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>R1$^a$ (%) (all data)</td>
<td>3.73</td>
<td>3.23</td>
</tr>
<tr>
<td>wR2$^b$ (%) (all data)</td>
<td>10.70</td>
<td>4.52</td>
</tr>
<tr>
<td>R1$^a$ (%) (I &gt; 2σ)</td>
<td>3.53</td>
<td>2.30</td>
</tr>
<tr>
<td>wR2$^b$ (%) (I &gt; 2σ)</td>
<td>10.49</td>
<td>4.25</td>
</tr>
<tr>
<td>GOF$^c$</td>
<td>1.070</td>
<td>1.029</td>
</tr>
<tr>
<td>max, min peaks, e·Å$^3$</td>
<td>0.510, -0.195</td>
<td>1.850, -0.905</td>
</tr>
</tbody>
</table>

$^a$ R1 = $\Sigma|F_o| - |F_c|/\Sigma|F_o|$. $^b$ wR2 = $\{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}$\(^{1/2}\). $^c$ GOF = $\{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}$\(^{1/2}\).
**Cell Imaging Studies.** A2780 and A2780CP70 cells were seeded in an imaging dish in 2 mL of growth medium. At \( \approx 60\% \) confluency, the cells were imaged. The growth media was swapped with premixed media containing 1 or 10 \( \mu \)M of the Pt-MPP(TAMRA) construct, and the cells were allowed to incubate with the dye for 1 h. The organelle dyes, Mito-tracker Green and Hoechst 33258, were added to the cells at 1.25–2.5 \( \mu \)M concentrations and allowed to incubate for 30 min. At the end of the incubation period, the media was aspirated, and the cells were washed with 3 \( \times \) 1 mL PBS and submerged in 2 mL of dye-free DMEM for imaging. The imaging experiments were performed using a Zeiss Axiovert 200M inverted epifluorescence microscope equipped with an EM-CCD digital camera (Hamamatsu) and a MS200 XY Piezo Z stage (Applied Scientific Instruments). The light source was an X-Cite 120 metal-halide lamp (EXFO) and the fluorescence images were obtained with an oil-immersion objective at 63\( \times \) magnification. The microscope was operated by the Volocity software program of Perkin-Elmer. Colocalization of the dyes was investigated with using the program ImageJ\textsuperscript{34}, using a previously described protocol.\textsuperscript{35}

**B.3. Results and Discussion**

**Synthetic Strategy.** Conjugates of platinum-based anticancer agents and peptides have previously been prepared and characterized, the first of which was reported in 2000.\textsuperscript{36} These conjugates can be categorized based on the attachment point of the peptide to the platinum agent, which occurs either at the non-leaving group ligand (amine) of a platinum(II) complex,\textsuperscript{36-45} leaving group ligand (carboxylate) of a platinum(II) complex,\textsuperscript{46} or through the axial ligands of platinum(IV) complex.\textsuperscript{47-51} By attaching a peptide to the non-leaving group ligands of a platinum anticancer agent, the reactivity of the platinum complex with DNA is altered,\textsuperscript{52} and the nature of
the resulting DNA adducts and cellular responses are also presumably different from the parent drug. Different amine ligands, as in the case of oxaliplatin, give rise to platinum anticancer complexes with different spectra of activity and resistance profiles. Although desirable for the preparation of new platinum chemotherapeutics, these features are not preferred if one is seeking to investigate only how the change in cellular localization, directed by a conjugated peptide, affects biological activity of an anticancer agent. For our purposes then, the MPP could either be attached to the leaving group ligand of a platinum(II) complex or to an axial ligand of a platinum(IV) complex. Platinum(IV) complexes need to be reduced to platinum(II) before binding to DNA or other cellular targets can occur.\(^\text{53,54}\) Two ligands are lost upon reduction. The commonly held notion that two axial ligands are exclusively eliminated upon reduction has been challenged by recent studies, which show that equatorial chloride ligands can also be lost.\(^\text{55,56}\) Hence, the reduced product is not necessary reflective of the parent platinum(II) agent. Furthermore, the reduction kinetics of platinum(IV) complexes are also dependent on the nature of the cell line used,\(^\text{57}\) and some platinum(IV) compounds are reduced immediately by components of the cellular growth medium.\(^\text{58}\)

Because of the abovementioned complications for the other two approaches, we sought to use a leaving group ligand as an attachment point for the MPP. The work discussed in Chapter 4 demonstrated the utility of β-diketonate ligands as leaving groups in platinum anticancer agents.\(^\text{59}\) Hence, we sought a ligand that contained both a β-diketonate unit for coordination to platinum(II) and a carboxylic acid or amine functionality for conjugation to a peptide via standard coupling chemistry. The compound succinylacetone, which can be prepared in one step from commercial reagents without the need for column chromatography,\(^\text{28}\) met both of those criteria and was selected for further studies.
Synthesis and Characterization of \([\text{Pt}(\text{succac})(\text{NH}_3)_2](\text{NO}_3)\). The synthetic route utilized for the preparation of \([\text{Pt}(\text{succac})(\text{NH}_3)_2](\text{NO}_3)\) is one that was described in Chapter 4 for other cis-diammine(\(\beta\)-diketonate)platinum(II) complexes.\(^{59}\) As shown in Scheme B.1, the diaqua analog of cisplatin was treated with the deprotonated succinylacetone to obtain the expected product. The previously reported workup protocol for this class of compounds, which calls for the evaporation of the aqueous solution, dissolution of the resulting residue in methanol, and precipitation of the product with diethyl ether, failed to give pure material. Instead, brown solids were typically obtained that did contain the desired product, as evidenced by ESI-MS, but of insufficient purity for further use and studies. We hypothesize that the difficulty in isolating pure material arises from the free carboxylic acid on the succinylacetone ligand, which can also coordinate to platinum(II), forming a number of undesired byproducts. To access pure compound, an alternative workup protocol was devised. A concentrated aqueous solution of the reaction mixture was acidified with \(\text{HNO}_3\) to ensure full protonation of the carboxylic acid group. A series of precipitations was carried out using acetone, an acetone:ether mixture, and diethyl ether. The first two precipitations afforded oily orange-brown residues and white slightly turbid supernatant. Pure compound could be isolated from the supernatant upon the addition of further volumes of diethyl ether as a white solid. Presumably, the major impurities have lower solubility in these acetone:water mixtures than the desired compound.

\[
\begin{align*}
\text{H}_3\text{N}^+\text{Pt}^2\text{Cl}_2 \quad &\quad \text{H}_2\text{O} \quad 2 \text{AgNO}_3 \quad \text{H}_2\text{O} \quad -2 \text{AgCl} \quad \left[\text{H}_3\text{N}^+\text{Pt}^2\text{OH}_2\right]^{\text{(NO}_3)\text{)}_2} \quad + \quad \text{H}_3\text{N}^+\text{Pt}^2\text{OH}_2 \\
\text{H}_3\text{N}^+\text{Pt}^2\text{Cl}_2 \quad &\quad \text{H}_2\text{O} \quad \text{H}_2\text{O} \quad \text{H}_3\text{N}^+\text{Pt}^2\text{OH}_2 \\
\end{align*}
\]

\(\text{Scheme B.1. Synthesis of } [\text{Pt}(\text{succac})(\text{NH}_3)_2](\text{NO}_3).\)

308
Elemental analysis and ESI-MS are in accord with the proposed formula. Multinuclear NMR spectroscopy further established the purity and identity of the compound. The \( ^1H \) and \( ^{13}C \) NMR spectra display all expected resonances for the proposed structure of this compound. Notably, the NH\(_3\) protons of the coordinated ammine ligands, which readily exchange with protic or deuteric solvents, could be observed in MeOD-\( d_4 \) as a broad feature at 4.44 ppm if spectra were acquired within half an hour of preparation of the NMR sample. The observed chemical shift of 4.44 ppm is in the expected region for NH\(_3\) protons coordinated to platinum(II)\(^{60}\). The \( ^{195}Pt \) NMR spectrum of [Pt(succac)(NH\(_3\)_2)](NO\(_3\)) shows a single broad peak centered at -1570 ppm. This resonance is shifted downfield by approximately 20 ppm from the related complex, [Pt(acac)(NH\(_3\)_2)](SO\(_4\))\(_{0.5}\)\(^{69}\). Although for \( ^{195}Pt \) NMR spectroscopy, which spans a chemical shift region of 13,000 ppm\(^{61,62}\), this 20 ppm shift is relatively minor, it is still outside the bounds of the expected error for this measurement (1–5 ppm). Hence, the free carboxylic acid of [Pt(succac)(NH\(_3\)_2)](NO\(_3\)) has a subtle effect on the coordination sphere of the platinum center, possibly by an intra- or intermolecular hydrogen-bonds with the coordinated ammine ligands, as observed in the solid-state crystal structure (vide infra).

Succinylacetone and [Pt(succac)(NH\(_3\)_2)](NO\(_3\)) were both characterized crystallographically. These solid-state molecular structures are shown in Figure B.1. Succinylacetone crystallizes as its enol tautomer, as verified the disparate C–O bond distances, which are indicative of one single C–O bond and one double C=O bond. Furthermore, the hydrogen atom of the enol could be definitively located on the difference map. The crystal structure of [Pt(succac)(NH\(_3\)_2)](NO\(_3\)) confirms the expected coordination of the succac ligand through the \( \beta \)-diketonate unit rather than the carboxylic acid. The hydrogen atom of the
carboxylic acid was located on the difference map as well. The delocalized nature of the β-diketonate ligand is ascertained from the similar C–O and C–C bond distances that comprise the six-member chelate ring. A network of hydrogen bonds span the crystal lattice of [Pt(succac)(NH₃)₂](NO₃). The terminal carboxylic acid does not engage in carboxylic acid dimer interactions, but instead forms hydrogen bonds with an ammine ligand of a neighboring complex and a nitrate counterion.

![Diagram of molecular structures](image)

**Figure B.1.** Solid-state molecular structures of succinylacetone (top) and [Pt(succac)(NH₃)₂](NO₃) (bottom). Bond distances displayed in the figure are in Angstroms. For [Pt(succac)(NH₃)₂](NO₃), the nitrate counterion has been omitted for clarity. Ellipsoids are drawn at the 50% probability level. Selected interatomic distances (Å) and angles (°) for [Pt(succac)(NH₃)₂](NO₃): Pt1–O1, 1.994(3); Pt1–O2, 1.992(3); Pt1–N1, 2.029(3); Pt1–N2, 2.021(3); O1–Pt1–O2, 95.46(10); N1–Pt1–N2, 91.43(13); O1–Pt1–N1, 85.74(12); O2–Pt1–N2, 87.37(12).
Preparation of Pt-MPP and Pt-MPP(TAMRA). The Pt-MPP conjugates were prepared directly on the solid-phase resin (Rink Amide). The resins, which contained the MPP and the TAMRA-labeled MPP attached via the C-terminus, were soaked in a DMF solution containing an excess of [Pt(succac)(NH₃)$_2$](NO₃) preactivated with the coupling reagent HATU. The resin was then washed, and the peptides were cleaved from the solid-phase support with 95% TFA. Preparative HPLC allowed isolation of the expected Pt-conjugates in greater than 95% purity. Conjugation to an analogous peptide labeled with the carboxytetramethylrhodamine (TAMRA) fluorophore was also carried out for later use in fluorescence cell imaging studies. The TAMRA dye was attached by an amide bond via the amine side-chain of an additional lysine residue. The TAMRA-labeled peptide was provided as a mixture of isomers, which differed in the attachment point of the peptide to the bottom ring of the TAMRA dye (5 or 6 position). Only one of the isomers was successfully isolated with >95% purity. The final constructs, Pt-MPP and Pt-MPP(TAMRA), are shown in Chart B.1.

Pt-MPP

Pt-MPP(TAMRA)

Chart B.1. Schematic drawing of the two Pt-peptide constructs reported herein.
The conjugates were characterized by ESI-MS and NMR spectroscopy. The mass spectra displayed m/z peaks of the +1, +2, and +3 ions, which were all marked by isotopic fine structure characteristic of the six naturally abundant isotopes of platinum. $^{195}\text{Pt}$ NMR spectra in D$_2$O could be obtained for both constructs as well. As shown in Figure B.2, both species display a single resonance near $-1590$ ppm. These values are identical to that of $[\text{Pt(acac})(\text{NH}_3)_2](\text{SO}_4)_{0.5}$, which is found at $-1593$ ppm.$^{59}$ Hence, the diammine(β-diketonate) coordination sphere of the platinum atoms is retained in the peptide-conjugates, even after cleavage from the resin with 95% TFA.$^1$ H NMR spectra were also obtained. Diagnostic peaks for the proton at the γ-position of the β-diketonate ligand were found at 5.68 and 5.61 ppm as sharp singlets for Pt-MPP and Pt-MPP(TAMRA), respectively. Furthermore, the CH$_3$ resonances of the succinylacetone ligand were located at 1.88 and 1.83 ppm for the labeled and unlabeled constructs. The aromatic region of the $^1$H NMR spectrum of Pt-MPP(TAMRA) displayed all the expected signals for the TAMRA dye. Based on previous NMR studies of isomerically pure TAMRA dyes,$^{63}$ the aromatic signals are assigned as shown in Figure B.3. The singlet of H$_f$ in CD$_3$OD resonates at 7.97 ppm for the 6 isomer of carboxytetramethylrhodamine and at 8.73 ppm for the 5 isomer.$^{63}$ Hence, our construct contains isomerically pure 5 isomer as drawn in Chart B.1. because this resonance is observed at 8.63 ppm.
Cytotoxicity Studies. For cytotoxicity studies, the wild-type and cisplatin-resistant ovarian cancer cell lines A2780 and A2780CP70 were used. In addition to testing cisplatin and Pt-MPP, [Pt(acac)(NH₃)₂](SO₄)₀.₅ and [Pt(succac)(NH₃)₂](NO₃) were also investigated. These two
complexes were used as peptide-free controls. Their ligand substitution kinetics are expected to be more comparable to that of Pt-MPP because the three constructs all employ similar β-diketonate leaving group ligands. The results of these assays using a 72 h incubation period are summarized in Table B.2, where IC₅₀ values are collected, and displayed in Figure B.4, which shows several representative dose-response curves. In Table B.2, resistance factors (R.F.’s), which are defined as the ratios of IC₅₀ values of the resistant to sensitive cell lines, are given as well.

**Table B.2. IC₅₀ Values and Resistance Factors (R.F.’s) Measured After a 72 h Incubation Period.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (μM)ᵃ</th>
<th>R.F.ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2780</td>
<td>A2780CP70</td>
<td></td>
</tr>
<tr>
<td>cisplatin</td>
<td>0.60 ± 0.08</td>
<td>5.2 ± 1.4</td>
</tr>
<tr>
<td>Pt-MPP</td>
<td>7.5 ± 0.3</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td><a href="SO%E2%82%84">Pt(acac)(NH₃)₂</a>₀.₅</td>
<td>13.9 ± 1.9</td>
<td>122 ± 10</td>
</tr>
<tr>
<td><a href="NO%E2%82%83">Pt(succac)(NH₃)₂</a>₂</td>
<td>220 ± 40</td>
<td>855 ± 35</td>
</tr>
</tbody>
</table>

ᵃ Values are the average from at least independent experiments and reported errors are the standard deviations. ᵇ R.F. = resistance factor, defined as IC₅₀(resistant)/IC₅₀(sensitive)

Cisplatin effectively kills A2780 cells at submicromolar concentration levels. In the resistant line, however, the IC₅₀ value increases by almost one order of magnitude. [Pt(acac)(NH₃)₂](SO₄)₀.₅ is less effective than cisplatin with IC₅₀ values in both cell lines that are approximately 20 times greater. The least cytotoxic compound tested is [Pt(succac)(NH₃)₂](NO₃) as indicated by its IC₅₀ values, which are greater than 200 μM in both cell lines. In the wild-type cell line, the Pt-MPP is less cytotoxic than cisplatin, but more so than [Pt(acac)(NH₃)₂](SO₄)₀.₅. In the resistant cell line, however, Pt-MPP is equitoxic with cisplatin. Additionally, the resistance factor of this construct is 0.7 ± 0.2, thus indicating that it avoids traditional cisplatin resistance.
mechanisms in this cell line. The conjugation of the MPP, therefore, increases the efficacy of the 
$\text{[Pt(\beta\text{-diketonate})(\text{NH}_3)_2]^+}$ structure and circumvents cellular resistance.

![Graphs](image)

**Figure B.4.** Representative dose-response curves for the treatment of A2780 (black squares) and A2780CP70 (red circles) cells lines with (A) cisplatin, (B) $\text{[Pt(acac)(NH}_3)_2(SO}_4)_0.5$. (C) $\text{[Pt(succac)(NH}_3)_2(NO}_3$), and (D) Pt-MPP.

**Cell Imaging Studies.** Imaging studies of the TAMRA-labeled Pt-MMP were carried out in both resistant and sensitive A2780 cell lines. Both 1 and 10 $\mu$M incubation concentrations were used for these studies. Representative images for both cell lines and concentrations are shown in Figures B.5 and B.6, and Pearson’s correlation coefficients, describing the degree of colocalization of Pt-MPP(TAMRA) with the organelle dyes, are collected in Table B.3.
Figure B.5. Images of A2780 cells treated with (A) 1 or (B) 10 μM Pt-MPP(TAMRA) for 1 h visualized by (i) DIC, (ii) blue channel (Hoechst 33258), (iii) green channel (Mito-tracker green), and (iv) the red channel (TAMRA). Scale bar = 32 μM.

Figure B.6. Images of A2780CP70 cells treated with (A) 1 or (B) 10 μM Pt-MPP(TAMRA) for 1 h visualized by (i) DIC, (ii) blue channel (Hoechst 33258), (iii) green channel (Mito-tracker green), and (iv) the red channel (TAMRA). Scale bar = 32 μM.
Table B.3. Pearson’s Correlation Coefficients (PCC) for Colocalization of Pt-MPP(TAMRA) and the Organelle Dyes.

<table>
<thead>
<tr>
<th>Cell Line, Concentration</th>
<th>Mitochondrial PCC</th>
<th>Nuclear PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2780, 1 μM</td>
<td>0.36 ± 0.08</td>
<td>-0.03 ± 0.04</td>
</tr>
<tr>
<td>A2780, 10 μM</td>
<td>0.45 ± 0.08</td>
<td>0.0 ± 0.03</td>
</tr>
<tr>
<td>A2780CP70, 1 μM</td>
<td>0.40 ± 0.18</td>
<td>0.03 ± 0.06</td>
</tr>
<tr>
<td>A2780CP70, 10 μM</td>
<td>0.53 ± 0.13</td>
<td>0.0 ± 0.01</td>
</tr>
</tbody>
</table>

For both cell lines, Pt-MPP(TAMRA) shows moderately good localization to the mitochondria as judged by Pearson’s correlation coefficients for overlap with the mitochondrial dye. Pearson’s correlation coefficients range from −1 to 1, where 1 indicates a perfect colocalization and −1 signifies opposite localization. The extent of the localization appears to be dependent on the concentration of construct with better mitochondrial localization observed at the 10 μM concentration level. Additionally, these results clearly show that Pt-MPP(TAMRA) does not go the nucleus; Pearson’s correlation coefficients for overlap with the nuclear stain are all effectively 0. Some punctate staining was observed in the red channel. Several of these intense, sharp signals occur in extracellular locations. The precise origin of these punctate features remains uncertain, but they could arise from precipitation of the peptide, possibly due to aggregation. In the absence of these signals, the Pearson’s correlation coefficients for mitochondrial localization should increase.

B.4. Conclusions and Ongoing Work

Cellular resistance to cisplatin can arise at one or many different points during the operative lifetime of the drug. Pre-target resistance occurs as a result of either decreased cellular uptake or increased intracellular sequestration by off-target nucleophiles such as glutathione. When cisplatin locates and binds to DNA, on-target resistance pathways, which includes increased repair of platinum-DNA adducts, take effect. Post-target pathways occur if the cell fails to trigger apoptosis or other cell death pathways in response to DNA damage induced by
cisplatin. The mechanisms of cisplatin resistance in the A2780CP70 cell line have been the subject of many investigations. From these previous reports, it appears that many of the abovementioned factors contribute to cisplatin resistance in this cell line. Post-target resistance, however, is not important because A2780CP70 cells can activate the apoptotic cell death pathway. Additionally, cellular uptake does not appear to be responsible for this resistance either because at short exposure times (1–4 h) cisplatin is taken up to almost equal extents in the wild-type and resistant cell lines. On the other hand, the resistant cell line does exhibit increased glutathione levels by comparison to the sensitive line, which suggests that sequestration of the active drug by these thiols could be a contributing factor to the resistance.

Further studies found that the resistant cell line can more efficiently remove Pt-DNA cross-links, and that resistance can be overcome by inhibiting the nucleotide excision repair pathway. Given these known resistance mechanisms in A2780CP70 cells, the question naturally arises as to how Pt-MPP can circumvent them whereas the other platinum agents tested in our study cannot. Because both cisplatin and our Pt-MPP constructs contain the same non-leaving group unit, the nature of the resulting DNA adducts and the cellular response to them should be identical. Hence, an on-target circumvention of resistance can be ruled out. Additionally, the similar structure of our construct to that of [Pt(acac)(NH$_3$)$_2$](SO$_4$)$_{0.5}$ suggests that both species should be equally susceptible to deactivation by glutathione. Therefore, a difference in this off-target mechanism of resistance is most likely not operable. Two other possibilities exist; either the Pt-MMP construct exhibits enhanced cellular uptake in the resistant cell line and/or it is hitting a completely different target that is not susceptible to the same resistance mechanisms as the conventional platinum target, nuclear DNA. The cell localization
studies using the TAMRA-labeled conjugate indicate that the construct localizes to the mitochondria to a much higher extent than the nucleus. This result suggests that the platinum may be avoiding traditional resistance pathways by targeting the mitochondria. This conclusion, however, assumes that the TAMRA dye and additional lysine residue do not significantly alter the cellular distribution properties of the original construct. Caution must be used in invoking such an assumption because large dyes can significantly alter the localization properties of metal-peptide conjugates. Preliminary organelle fractionation studies using cells treated with the unlabeled Pt-MPP, however, do indicate a subtle increase in mitochondrial uptake of Pt as measured by GFAAS, corroborating these fluorescence imaging studies.

The preliminary results of this investigation can be compared to those of another system, where the anticancer, DNA-alkylating agent chlorambucil was attached to an MPP. Like our platinum conjugate, the chlorambucil-MPP construct exhibited good cytotoxicity in chlorambucil-resistant cell lines. Additionally, the chlorambucil-MPP conjugate, in contrast to Pt-MMP, was significantly more cytotoxic than the parent drug, chlorambucil, in all cell lines tested. The lower toxicity of Pt-MPP compared to cisplatin most likely arises from slow ligand substitution kinetics imparted by the β-diketonate ligand. It may be more appropriate to compare the anticancer activity to [Pt(acac)(NH₃)₂]⁺, which should have similar aquation and DNA-binding kinetics. For this comparison, it is clear that attachment of the MPP leads to an increase in activity, consistent with the chlorambucil-MPP studies.

Work carried out by our collaborators in the University of Toronto has revealed some additional interesting aspects of this construct. The polymerase chain reaction (PCR) was carried out using mitochondrial and genomic DNA extracted from cells treated with Pt-MPP. Results of this study indicate that Pt-MPP impedes PCR of mitochondrial DNA to a greater extent than
genomic DNA in comparison to those from cisplatin-treated cells. Therefore, it appears that Pt-MPP damages mitochondrial DNA more effectively than cisplatin. Additionally, cell cycle analysis revealed that cisplatin arrests cells in the G2 phase, consistent with literature reports, whereas Pt-MPP leads to no alteration or arrest of the cell cycle. These results support the hypothesis that Pt-MPP attacks a different target than cisplatin.

In summary, the conjugation of platinum anticancer compound to an MPP creates an agent that is able to circumvent traditional cisplatin-resistance mechanisms, most likely by interacting with a target different from genomic DNA. We demonstrated the utility of the complex, [Pt(succac)(NH₃)₂](NO₃), for attachment to an MPP via standard solid-phase peptide synthetic methods. A number of other CPPs can also conceivably used as well thus indicating that [Pt(succac)(NH₃)₂](NO₃) will have a greater utility for making novel anticancer platinum compounds.

B.5. References

Isonishi, P.; Andrews, P.; Cullen, K.; Yang, Z.; Schumaker, L.; Guo, Z.; Schneider, A.; Borrelli, F.; Barragan, F.; Carrion-Salip, D.; Dodd, J.; van Zutphen, J.; Barragan, F.; Moreno, V.; Marchan, V.


Groessl, M.; Zava, O.; Dyson, P. J. Metallomics 2011, 3, 591-599.


321
(74) Sorenson, C. M.; Eastman, A. Cancer Res. 1988, 48, 4484-4488.
Appendix C

Synthesis and Characterization of Several Novel Platinum Complexes
C.1. Introduction

In this final appendix, several novel platinum compounds that were synthesized and characterized during the course of this thesis work are described. The structures of these compounds, along with their abbreviated names, are shown in Chart C.1.

**Complexes with DCA Ligands.** The first set of compounds presented here was inspired by work in our lab on mitaplatin, \(\text{cis,cis,trans-[Pt(NH}_3)_2\text{Cl}_2(DCA)_2}\) where DCA = dichloroacetate\(^1\). Like mitaplatin, the compounds presented here, \(\text{cis-[Pt(NH}_3)_2(DCA)_2}\) and \(\text{cis-[Pt(NH}_3)_2(DCA)_4}\), are coordinated to DCA. DCA is an inhibitor of pyruvate dehydrogenase kinase (PDK)\(^2\). A result of inhibition of PDK is the switching of the cancer cell metabolic pathway from aerobic glycolysis, which occurs in the cytosol, (Warburg Effect) to oxidative phosphorylation, which takes place in the mitochondria. This shift restores normal mitochondrial function and decreases the mitochondrial membrane potential, allowing apoptosis-inducing factors (AIFs) to escape this organelle and trigger apoptosis\(^3,4\). Mitaplatin, a combination of cisplatin and 2 equiv of DCA, exhibits selectivity for killing cancerous over non-cancerous cells lines, presumably because of the ability of DCA to target the unique cancer metabolic pathway.

We were interested to determine whether \(\text{cis-[Pt(NH}_3)_2(DCA)_2}\) and \(\text{cis-[Pt(NH}_3)_2(DCA)_4}\) would display similar anticancer properties and set out to prepare them.

**Using BIPhMe for Platinum Chemistry.** The bidentate ligand \(^{2,2'}\)-bis(1-methylimidazolyl)phenylmethoxymethane, or BIPhMe, was reported by our lab over 20 years ago\(^5\). Since then, this ligand has been used for the preparation of multinuclear Fe, Mn, and Zn complexes\(^5,6\). The compound \(\text{[Pt(BIPhMe)Cl}_2]\) was pursued because related platinum(II) bis(imidazole) complexes bind to nucleobases and exhibit in vitro anticancer activity\(^7,10\). It was hypothesized that \(\text{[Pt(BIPhMe)Cl}_2]\) might also have anticancer properties.

324
**Amide-Coupling to Functionalize Platinum(II) Complexes.** The amide-coupling chemistry of [Pt(edma)Cl₂] discussed in Chapter 3 was used to tether several biologically active molecules to the platinum(II) core.

Nitroimidazoles are well known hypoxia-targeting and radio-sensitizing agents.¹¹ These agents undergo a 4-electron reduction selectively in hypoxic tissue to form electrophilic hydroxylamines, which can alkylate DNA or other targets.¹² Several platinum-nitroimidazole complexes were previously reported.¹³⁻¹⁶ These constructs, however, contain a direct bond between the nitrogen atom of the nitroimidazole and the platinum(II) center. This bond can potentially disrupt functions of both the platinum(II) center and the nitroimidazole group. Here, a nitroimidazole was attached remotely to the [Pt(edma)Cl₂] core. The utility of this complex, nitro-Pt, may lie in its ability, by virtue of the nitroimidazole group, to selectively target hypoxic tumor tissue.

Nitrogen mustards comprise a well studied class of anticancer agents¹⁷ that operate by forming interstrand DNA cross-links.¹⁸ Modified versions of the clinically used nitrogen mustards, mustine and chlorambucil, were covalently attached to [Pt(edma)Cl₂] via amide bond coupling. The presence of both the platinum(II) unit, which forms primarily 1,2-intrastrand DNA-cross-links, and the nitrogen mustards, which prefer to form interstrand cross-links, was proposed to lead to a potent DNA-damaging cytotoxic agent.
C.2. Experimental Methods

Materials and Methods. The compounds, cis-[Pt(DMSO)$_2$Cl$_2$], cis-[Pt(NH$_3$)$_2$(OH)$_4$], 2-(2-methyl-5-nitro-1H-imidazolyl)ethylamine dihydrochloride, $N^1,N^1$-bis(2-chloroethyl)propane-1,3-diamine dihydrochloride, and 4-(3-aminopropyl)-$N,N$-bis(2-chloroethyl)aniline dihydrochloride were prepared as previously reported. BIPhMe, synthesized by known methods, was kindly provided by Mr. Eric Victor. Other materials and reagents were purchased from commercial vendors and used as received. Solvents were analytical grade, but otherwise not rigorously dry. Reactions were carried out under normal atmospheric conditions.

Physical Measurements. NMR spectra were acquired on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility (DCIF). $^1$H and $^{13}$C NMR spectra were referenced to residual protons or the carbon nucleus of the solvent, and signals are reported.
versus tetramethylsilane, Me₄Si (δ = 0). ¹⁹⁵Pt NMR spectra were referenced externally to a standard of K₂PtCl₄ in D₂O (δ = −1628 ppm). For FTIR spectra, samples were prepared as KBr disks and data were recorded with a ThermoNicolet Avatar 360 spectrophotometer running the OMNIC software. Electrospray ionization mass spectrometry (ESI-MS) measurements were acquired on an Agilent Technologies 1100 series LC-MSD trap. Elemental analyses were carried out by a commercial analytical laboratory.

**Synthesis of cis-[Pt(DCA)₂(NH₃)₂].** A mixture of cisplatin (0.10 g, 0.33 mmol) and Ag₂SO₄ (0.10 g, 0.32 mmol) was suspended in 13 mL of distilled H₂O and stirred for 12 h in the absence of light. The resulting mixture was filtered through a pad of Celite on a glass frit to remove solid AgCl and yield a colorless filtrate containing cis-[Pt(NH₃)₂(H₂O)₂](SO₄). An aqueous solution of Ba(DCA)₂, prepared by mixing Ba(OH)₂·8H₂O (0.10 g, 0.73 mmol) and dichloroacetic acid (0.10 g, 0.78 mmol) in 2 mL of H₂O, was added dropwise with vigorous stirring. A white precipitate, presumably insoluble BaSO₄, formed immediately, and the mixture was left to stir at room temperature. After 3 h, the mixture was filtered, and the colorless filtrate was lyophilized to yield an off-white powder (0.16 g, 99%). IR (KBr, cm⁻¹): 3425 m sh, 3276 s br, 1660 vs, 1356 s, 1217 m, 819 m, 787 m, 738 m, 669 m. ¹H NMR (400 MHz, DMSO-d₆ with NaDCA added): δ 6.46 (2H, s), 4.79 (6H, br s). ESI-MS (neg. and pos. ion mode): m/z 356.9 ([M−DCA]+, calcd. 356.0), 612.8 ([M+DCA]⁻, calcd. 612.8).

**Synthesis of cis-[Pt(DCA)₄(NH₃)₂].** cis-[Pt(OH)₄(NH₃)₂] (0.24 g, 0.80 mmol) was suspended in 10 mL of CH₂Cl₂. Dichloroacetic anhydride (0.96 g, 4.0 mmol) in 10 mL of CH₂Cl₂ was added, and the mixture was stirred at room temperature for 1.5 h, resulting in the formation of a homogeneous pale yellow solution. The CH₂Cl₂ was removed under reduced pressure to give a thick yellow oil. Upon the addition of 25 mL of H₂O followed by vigorous stirring and
sonication, a white precipitate formed. The precipitate was collected by filtration and washed with 5 mL of Et<sub>2</sub>O. Yield: 0.38 g, 63%. IR (KBr, cm<sup>-1</sup>): 3568 m, 3497 w, 3231 m br, 1679 vs, 1559 w, 1324 s, 1196 s, 953 m, 818 s, 783 m, 748 m, 673 w. <sup>1</sup>H NMR (400 MHz, DMSO-<d>: δ 6.94 (6H, br s), 6.62 (2H, s), 6.53 (2H, s). <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, DMSO-<d>): δ 169.8, 169.7, 66.6, 64.8. <sup>195</sup>Pt{<sup>1</sup>H} NMR (86 MHz, DMSO-<d>): δ 2076. ESI-MS (neg. ion mode): m/z 739.0 ([M-H]<sup>-</sup>, calcd. 738.7).

Synthesis of [Pt(BIPhMe)C<sub>2</sub>]. To a suspension of cis-[Pt(DMSO)C<sub>2</sub>]<sup>-</sup> (0.118 g, 0.279 mmol) in 2 mL MeOH, a solution of BIPhMe (80 mg, 0.28 mmol) in 1 mL of MeOH was added dropwise. After stirring for 16 h at rt, the resulting suspension was filtered to collect the product as a pale yellow solid. The material was washed with 2 × 3 mL MeOH and 2 × 3 mL diethyl ether, prior to drying in vacuo. Yield: 134 mg, 88%. <sup>1</sup>H NMR (400 MHz, DMF-<d>): δ 8.10 (d, 2H), 8.00 (d, 2H), 7.58 (t, 2H), 7.51 (d, 2H), 7.47 (t, 1H), 3.71 (s, 6H), 3.19 (s, 3H). <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, DMF-<d>): δ 140.1, 136.7, 129.5, 129.4, 126.8, 125.3, 124.7, 81.0, 53.1, 36.1. <sup>195</sup>Pt{<sup>1</sup>H} NMR (86 MHz, DMF-<d>): δ -2170. ESI-MS (pos. ion mode): m/z 570.9 ([M+Na]<sup>+</sup>, calcd. 571.0), 1118.9 ([2M+Na]<sup>+</sup>, calcd. 1119.1).

Synthesis of Must1-Pt. To a solution of [Pt(edma)C<sub>1</sub>]<sup>-</sup> (300 mg, 0.781 mmol) in 6 mL of DMF was added a solution of 1,1'-carbonyldiimidazole (131 mg, 0.809 mmol) in 6 mL of DMF. The resulting mixture was stirred at 60 °C for 10 min and then sparged with N<sub>2</sub>. At room temperature, a solution of <i>N</i><sup>2</sup>,<i>N</i><sup>2</sup>-bis(2-chloroethyl)propane-1,3-diamine dihydrochloride (220 mg, 0.809 mmol) in 9 mL of DMF was added in a dropwise manner. The resulting mixture was stirred at room temperature for 16 h, and then concentrated to a volume of ~3 mL under vacuum at 60 °C. The concentrated solution was filtered through Celite, and the desired compound was precipitated by the addition of 10 mL of H<sub>2</sub>O. The pale yellow solid was collected by filtration.
and washed sequentially with 2 × 5 mL of water, 2 × 5 mL of ethanol, and 2 × 5 mL of diethyl ether before being dried under vacuum. Yield: 148 mg (33%). \(^1\)H NMR (DMF-\(d_7\), 400 MHz): \(\delta\) 8.13 (t, 1H), 6.07 (br s, 1H), 5.47 (br s, 2H), 4.17 (d, 1H), 3.66 (t, 4H), 3.60 (dd, 1H), 3.27 (q, 2H), 3.30 (br m, 1H), 2.89 (t, 4H), 2.77 (br m, 2H), 2.66 (t, 2H), 2.50 (br m, 1H), 1.65 (m, 2H).

\(^{13}\)C\(^{\text{\{\text{H}\}}\) NMR (DMF-\(d_7\), 100 MHz): \(\delta\) 168.0, 57.8, 56.4, 55.2, 52.0, 47.3, 42.8, 37.3, 27.7.

\(^{195}\)Pt\(^{\text{\{\text{H}\}}\) NMR: \(\delta = -2338\). ESI-MS (MeOH, pos. ion mode): \(m/z\) 586.9 ([M+Na]\(^+\), calcd. 587.0), 1151.9 ([2M+Na]\(^+\), calcd. 1153.1 (100%), 1152.1 (99.7%)). Anal. Calcd. for C\(_{11}\)H\(_{22}\)Cl\(_4\)N\(_4\)OPt: C, 23.37; H, 4.28; N, 9.91. Found: C, 23.22; H, 4.20; N, 9.72.

**Synthesis of Must2-Pt.** To a solution of [Pt(edma)Cl\(_2\)] (227 mg, 0.591 mmol) in 5 mL of DMF was added a solution of 1,1’-carbonyldiimidazole (101 mg, 0.62 mmol) in 5 mL of DMF. The resulting mixture was stirred at 60 °C for 10 min and then sparged with N\(_2\). At room temperature, a solution of 4-(3-aminopropyl)-N,N-bis(2-chloroethyl)aniline dihydrochloride (216 mg, 0.62 mmol) in 8 mL of DMF was added in a dropwise manner. The resulting mixture was stirred at room temperature for 16 h, and then concentrated to a volume of ~3 mL under vacuum at 60 °C. The concentrated solution was filtered through Celite, and the desired compound was precipitated by the addition of 10 mL of H\(_2\)O. The pale yellow solid was collected by filtration and washed sequentially with 3 mL of water, 2 × 3 mL of ethanol, and 2 × 3 mL of diethyl ether before being dried under vacuum. Yield: 237 mg (63%). \(^1\)H NMR (400 MHz, DMF-\(d_7\)): \(\delta\) 8.19 (t, 1H), 7.11 (d, 2H), 6.75 (d, 2H), 6.09 (br s, 1H), 5.49 (br s, 2H), 4.19 (d, 1H), 3.78 (s, 8H), 3.64 (m, 1H), 3.22 (m, 2H), 3.05 (br m, 1H), 2.78–2.69 (br m, 2H), 2.57–2.53 (br m, 3H), 1.79–1.72 (m, 2H). \(^{13}\)C\(^{\text{\{\text{H}\}}\) NMR (100 MHz, DMF-\(d_7\)): \(\delta\) 168.0, 145.0, 130.1, 129.7, 112.5, 55.8, 55.25, 53.1, 47.3, 41.5, 38.9, 32.0, 31.7. \(^{195}\)Pt\(^{\text{\{\text{H}\}}\) NMR: \(\delta = -2338\). ESI-MS (pos. ion mode): \(m/z\)

**Synthesis of Nitro-Pt.** To a solution of [Pt(edma)Cl₂] (300 mg, 0.781 mmol) in 6 mL of DMF was added a solution of 1,1'-carbonyldiimidazole (128 mg, 0.790 mmol) in 6 mL of DMF. The resulting mixture was stirred at 60 °C for 10 min and then sparged with N₂. At room temperature, a solution of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyamine dihydrochloride (197 mg, 0.810 mmol) in 8 mL of DMF was added in a dropwise manner. The solution was concentrated to 6 mL under vacuum at 60 °C and then filtered. The addition of 14 mL of water precipitated the desired compound as a pale yellow solid, which was collected by centrifugation and washed sequentially with 5 mL of H₂O, 2 × 5 mL of ethanol, and 2 × 5 mL of diethyl ether, before being dried under vacuum. Yield: 220 mg (53%). ¹H NMR (DMF-d₇, 400 MHz): δ 8.45 (t, 1H), 8.00 (s, 1H), 6.06 (br s, 1H), 5.45 (br s, 2H), 4.56–4.42 (m, 2H), 4.18 (d, 1H), 3.77 (m, 1H), 3.60–3.50 (m, 1H), 2.90 (br m, 1H), 2.69 (br m, 2H), 2.51 (s, 3H), 2.37 (br m, 1H). ¹³C{¹H} NMR (DMF-d₇, 100 MHz): δ 168.9, 151.9, 139.3, 133.3, 57.8, 54.9, 47.3, 45.9, 38.6, 13.9. ¹⁹⁵Pt{¹H} NMR: δ –2343. ESI-MS (MeOH, pos. ion mode): m/z 558.9 ([M+Na]⁺, calcd. 559.0), 1094.8 ([2M+Na]⁺, calcd. 1095.1). Anal. Calcd. for C₁₀H₁₈Cl₂N₆O₃Pt: C, 22.40; H, 3.38; N, 15.67. Found: C, 22.58; H, 3.42; N, 15.46.

**X-Ray Crystallography.** Single crystals were mounted in Paratone oil on a cryoloop and frozen under a 110 K KRYO-FLEX nitrogen cold stream. Data were collected on a Bruker APEX CCD X-ray diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) controlled by APEX2 software package. Empirical absorption corrections were applied using SADABS. The structures were solved using either direct methods or Patterson methods and refined on R² using the SHELXTL-97 software package. Structures were checked for higher symmetry
using PLATON. All non-hydrogen atoms were located and refined anisotropically. In general, hydrogen atoms were placed in idealized locations and given isotropic thermal parameters equivalent to either 1.5 (NH₃ protons) or 1.2 times the thermal parameter of the atom to which they are attached. X-ray crystallographic data collection and refinement parameters are collected in Table C.1.

A water molecule crystallized in the lattice with cis-[Pt(NH₃)₂(DCA)₂]. The hydrogen atoms were located on the map and refined semi-freely with constraints on the O–H distances and hydrogen atom thermal displacement parameters. The hydrogen atoms of the ammonia ligands were also found and refined in a similar manner. A water molecule was also present in the lattice with [Pt(BIPhMe)Cl₂]. The hydrogen atoms, however, could not be discerned from the difference Fourier map and were therefore omitted from the final model. The inability to find these hydrogen atoms is somewhat consistent with the apparent lack of hydrogen bonds, which might give long-range crystallographic order and directionality of the water molecule, within the crystal lattice.

Two molecules of cis-[Pt(NH₃)₂(DCA)₄] crystallized in the asymmetric unit along with one molecule of water. The hydrogen atoms of the water molecules were located and refined as described above. Two DCA ligands of one complex and three of the other one in the asymmetric unit exhibited significant orientational disorder, primarily involving the chlorine atoms. The refinement of this disorder was aided by similarity restraints of the interatomic distances and angles of the disordered components. The directionality and magnitude of the thermal displacement parameters of these disordered ligands were also restrained. Despite these efforts to satisfactorily refine the disorder, the final model contained several chlorine and carbon atoms with ellipsoids that were abnormally large or oblate, resulting in the generation of several Level
A, B, and C CheckCIF alerts. Additionally, four solvent-accessible voids with unidentifiable residual electron density remained in the unit cell on the 4 site symmetry special positions. The SQUEEZE algorithm of PLATON\textsuperscript{28} was employed to account for this diffuse electron density. Each of these voids was 174 Å\textsuperscript{3} in volume and contained density equivalent to 55 electrons. This density is proposed to arise from 5.5 disordered water molecules.

Table C.1. X-Ray Crystallographic Data Collection and Refinement Parameters for cis-[Pt(NH\textsubscript{3})\textsubscript{2}(DCA)\textsubscript{2}]\textsubscript{2}H\textsubscript{2}O, cis-[Pt(NH\textsubscript{3})\textsubscript{2}(DCA)\textsubscript{4}]6H\textsubscript{2}O, and [Pt(BIPhMe)Cl\textsubscript{2}]\textsubscript{1}H\textsubscript{2}O.

<table>
<thead>
<tr>
<th>formula</th>
<th>cis-[Pt(NH\textsubscript{3})\textsubscript{2}(DCA)\textsubscript{2}]\textsubscript{2}H\textsubscript{2}O</th>
<th>cis-[Pt(NH\textsubscript{3})\textsubscript{2}(DCA)\textsubscript{4}]6H\textsubscript{2}O</th>
<th>Pt(BIPhMe)Cl\textsubscript{2}]\textsubscript{1}H\textsubscript{2}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>fw</td>
<td>503.03</td>
<td>749.88</td>
<td>564.33</td>
</tr>
<tr>
<td>space group</td>
<td>P\textsubscript{t}</td>
<td>14/1</td>
<td>P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}</td>
</tr>
<tr>
<td>a, Å</td>
<td>6.7906(11)</td>
<td>25.2649(5)</td>
<td>10.8521(5)</td>
</tr>
<tr>
<td>b, Å</td>
<td>9.4294(15)</td>
<td>12.4965(6)</td>
<td>13.3279(6)</td>
</tr>
<tr>
<td>c, Å</td>
<td>11.1410(18)</td>
<td>27.8478(11)</td>
<td>13.3279(6)</td>
</tr>
<tr>
<td>a, deg</td>
<td>68.628(2)</td>
<td>75.903(2)</td>
<td>72.458(2)</td>
</tr>
<tr>
<td>β, deg</td>
<td>72.458(2)</td>
<td>72.458(2)</td>
<td>72.458(2)</td>
</tr>
<tr>
<td>γ, deg</td>
<td>626.20(17)</td>
<td>17775.7(9)</td>
<td>1807.44(15)</td>
</tr>
<tr>
<td>V, Å\textsuperscript{3}</td>
<td>8.626(2)</td>
<td>7.458(2)</td>
<td>7.458(2)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>ρ\textsubscript{calc} g cm\textsuperscript{-3}</td>
<td>2.668</td>
<td>2.242</td>
<td>2.074</td>
</tr>
<tr>
<td>T, °C</td>
<td>-163(2)</td>
<td>-163(2)</td>
<td>-173(2)</td>
</tr>
<tr>
<td>μ(Mo Kα), mm\textsuperscript{-1}</td>
<td>12.602</td>
<td>7.316</td>
<td>8.077</td>
</tr>
<tr>
<td>Θ range, deg</td>
<td>1.99-26.37</td>
<td>1.61-28.70</td>
<td>2.23-29.66</td>
</tr>
<tr>
<td>total no. of data</td>
<td>11439</td>
<td>187020</td>
<td>37973</td>
</tr>
<tr>
<td>no. of unique data</td>
<td>2567</td>
<td>11496</td>
<td>5073</td>
</tr>
<tr>
<td>no. of parameters</td>
<td>169</td>
<td>655</td>
<td>230</td>
</tr>
<tr>
<td>completeness to Θ (%)</td>
<td>99.9</td>
<td>100.0</td>
<td>99.8</td>
</tr>
<tr>
<td>R1\textsuperscript{a} (%)</td>
<td>1.42</td>
<td>3.19</td>
<td>1.67</td>
</tr>
<tr>
<td>wR2\textsuperscript{b} (%)</td>
<td>3.51</td>
<td>7.09</td>
<td>3.24</td>
</tr>
<tr>
<td>R1\textsuperscript{a} (%) for I &gt; 2σ</td>
<td>1.39</td>
<td>2.80</td>
<td>1.56</td>
</tr>
<tr>
<td>wR2\textsuperscript{a} (%) for I &gt; 2σ</td>
<td>3.50</td>
<td>6.88</td>
<td>3.20</td>
</tr>
<tr>
<td>GOF\textsuperscript{c}</td>
<td>1.173</td>
<td>1.031</td>
<td>1.039</td>
</tr>
<tr>
<td>max, min peaks, e Å\textsuperscript{-3}</td>
<td>1.126, -0.756</td>
<td>2.884, -2.100</td>
<td>0.458, -0.609</td>
</tr>
<tr>
<td>Flack parameter &amp; -0.014(4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} R1 = \Sigma ||F_o|| - |F_c|/Σ|F_o|. \textsuperscript{b} wR2 = \{Σ[w(F_o^2 - F_c^2)^2]/Σ[w(F_o^2)]\}^{1/2}. \textsuperscript{c} GOF = \{Σ[w(F_o^2 - F_c^2)])/n(n - p)\}^{1/2} where n is the number of data and p is the number of refined parameters.
C.3. Results and Discussion

New Analogues of Mitaplatin. The platinum(II) version of mitaplatin, \( \text{cis-}[\text{Pt(NH}_3\text{)}_2\text{(DCA)}_2] \), was prepared using methods that are well established for the synthesis of \( \text{cis-diam(m)inedicarboxylatoplatinum(II)} \) complexes.\(^{29}\) Briefly, \( \text{cis-}[\text{Pt(NH}_3\text{)}_2\text{(OH)}_2](\text{SO}_4) \), obtained in situ from cisplatin and \( \text{Ag}_2\text{SO}_4 \) in water, was treated with \( \text{Ba(DCA)}_2 \), formed from \( \text{Ba(OH)}_2 \cdot 8\text{H}_2\text{O} \) and dichloroacetic acid. Insoluble \( \text{BaSO}_4 \) results and the water-soluble product can be recovered by removal of the solvent after filtration.

Lyophilization was necessary because rotary evaporation at 60 °C decomposed the material, giving insoluble platinum black. Additionally, there were occasional instances in which lyophilization of the filtrate gave a pale-green powder with the strong smell of dichloroacetic acid. Because of this olfactory clue, it is believed that a certain amount of decomposition occurred due to the presence of excess acid. The green color could also possibly arise from photoreduced \( \text{Ag} \), carried over from the first step of the reaction. Samples of \( \text{cis-}[\text{Pt(NH}_3\text{)}_2\text{(DCA)}_2] \) prepared in this manner are consistently contaminated with free DCA, possibly as the counterion of residual \( \text{Ba}^{2+} \) ions. Because of the high solubility of \( \text{cis-}[\text{Pt(NH}_3\text{)}_3\text{(DCA)}_2] \) in most polar solvents, attempts to remove excess DCA by washing with water or alcohols resulted in dissolution of most of the product. Hence, the preparation of this compound still needs optimization. The \(^1\text{H NMR spectrum in DMSO-}\text{d}_6 \) indicates the presence of two species in solution in addition to residual DCA; a major peak at 6.48 ppm and a minor one at 6.28, both sharp singlets. The addition of excess NaDCA, however, leads to the disappearance of the minor peak and an increase in the major one. Therefore, it is proposed that \( \text{cis-}[\text{Pt(NH}_3\text{)}_2\text{(DCA)}_2] \) is in equilibrium with its monosolvated form, \( \text{cis-}[\text{Pt(NH}_3\text{)}_2\text{(DMSO)(DCA)}]^+ \).
The addition of excess DCA can shift this equilibrium to \(\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{DCA})_2]\), as observed by \(^1\text{H}\) NMR spectroscopy.

The solid-state molecular structure of \(\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{DCA})_2]\), shown in Figure C.1, reveals the expected square-planar coordination geometry that is characteristic of platinum(II) complexes. A molecule of water is also present in the crystal lattice. The orientations of the carbonyl groups of the DCA ligands are nearly perpendicular to the coordination plane, obviating any intramolecular hydrogen bonding with the coordinated ammine ligands. Instead, they form hydrogen bonds with the water molecule and the ammine ligands of neighboring complexes in the lattice.

![Solid-state molecular structure of cis-[Pt(NH₃)₂(DCA)₂]](image)

**Figure C.1.** Solid-state molecular structure of \(\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{DCA})_2]\). Ellipsoids are drawn at the 50\% probability level. The water molecule in the asymmetric unit is omitted for clarity. Selected distances (Å) and angles (°): Pt1–O1, 2.036(2); Pt1–O3, 2.0309(19); Pt1–N1, 2.013(2); Pt1–N2, 2.040(3); O1–Pt1–O3, 87.04(8); N1–Pt1–N2, 90.46(11); O1–Pt1–N2, 93.56(10); O3–Pt1–N1, 88.73(9).

The preparation of the tetrakis(DCA) complex, \(\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{DCA})_4]\), was accomplished by treating \(\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{OH})_4]\) with an excess of dichloroacetic anhydride in \(\text{CH}_2\text{Cl}_2\). After an
hour, the suspension of cis-[Pt(NH₃)₂(OH)₄] converts to a homogeneous solution, signaling completion of the reaction. This general protocol for the preparation of cis-diamminetetracarboxylatoplutonium(IV) complexes is previously reported.³⁰,³¹ ¹H NMR spectroscopy of the complex is consistent with the presence of two inequivalent (axial and equatorial) DCA ligands that resonate at 6.62 and 6.53 ppm. The ¹⁹⁵Pt NMR spectrum reveals a single resonance at 2076 ppm, consistent with the formulation of the complex as a Pt(IV) species with an N₂O₄ coordination sphere.³²

The solid-state molecular structure of cis-[Pt(NH₃)₂(DCA)₄] is shown in Figure C.2. Only one of the molecules that is present in the asymmetric unit is shown. Both molecules in the asymmetric unit exhibited conformational disorder of the DCA ligands, giving rise to some poorly behaved thermal ellipsoids. The immediate coordination sphere of the platinum(IV) center, however, is not directly involved in this disorder. The structure reveals the expected octahedral geometry that is characteristic for complexes of platinum(IV). The ammine ligands are arranged in a cis orientation, indicating that the stereochemistry of the starting material, cis-[Pt(NH₃)₂(OH)₄], is retained.
**Figure C.2.** Solid-state molecular structure of cis-[Pt(NH$_3$)$_2$(DCA)$_4$]. Only one molecule in the asymmetric unit is shown. Only major components of the disordered DCA ligands are shown. Green, red, and grey ellipsoids correspond to chlorine, oxygen, and carbon atoms respectively. Ellipsoids are drawn at the 50% probability level. Selected distances (Å) and angles (°): Pt1–O1, 2.018(3); Pt1–O3, 2.023(3); Pt1–O5, 2.003(3); Pt1–O7, 1.998(3); Pt1–N1, 2.034(3); Pt1–N2, 2.025(3); N1–Pt1–N2, 88.46(13); O1–Pt1–O3, 83.05(10); O5–Pt1–O7, 169.68(11).

**Preparation of [Pt(BIPhMe)Cl$_2$].** The synthesis of [Pt(BIPhMe)Cl$_2$] was accomplished by the treatment of cis-[Pt(DMSO)$_2$Cl$_2$] with BIPhMe in MeOH. This protocol is analogous to that described in Chapter 2 for the preparation of dichloroplatinum(II) complexes of modified di-2-pyridylmethane ligands. The $^1$H NMR spectrum of [Pt(BIPhMe)Cl$_2$] indicates that the complex has $C_s$ symmetry in solution. Even though the BIPhMe ligand forms a six-membered chelate ring and the substituents on the bridgehead carbon atom are inequivalent, only one conformational isomer is observed in DMF-$d_7$ at room temperature. This result is in contrast to that for the di-2-pyridylmethane complexes described in Chapter 2, for which both exo and endo conformers
were detected under similar conditions. The $^{195}$Pt NMR shift of [Pt(BIPhMe)Cl$_2$] is $-2170$ ppm, similar to those observed for the complexes of Chapter 2, which range from $-2057$ to $-2199$ ppm.

The structure of [Pt(BIPhMe)Cl$_2$] is shown in Figure C.3. A notable difference between this structure and those of the di-2-pyridylmethane complexes is the greater planarity of the chelate ring. The average root-mean-square deviation (RMSD) of the atoms comprising this ring and from the best-fit plane is $0.153 \text{ Å}$ for [Pt(BIPhMe)Cl$_2$]. For [Pt(Ts-dpm)Cl$_2$] and [Pt(Ds-dpm)Cl$_2$], these values are $0.341$ and $0.358 \text{ Å}$, respectively. The larger values for the dpm compounds arise from the distorted boat-like conformation of the chelate ring. The greater planarity observed for [Pt(BIPhMe)Cl$_2$] may explain the inability to detect the conformational exo and endo isomers.

![Figure C.3](image-url)

**Figure C.3.** Solid-state molecular structure of [Pt(BIPhMe)Cl$_2$]. Ellipsoids are drawn at the 50% probability level. A water molecule in the asymmetric unit is omitted for clarity. Selected distances (Å) and angles (°): Pt1–Cl1, 2.3078(7); Pt1–Cl2, 2.2977(7); Pt1–N1, 2.010(2); Pt1–N2, 2.010(2); Cl1–Pt1–Cl2, 88.70(2); N1–Pt1–N2, 88.29(8); Cl1–Pt1–N1, 92.25(6); Cl2–Pt1–N2, 90.92(6).
Nitroimidazole- and Nitrogen Mustard-Platinum Conjugates. The amide-coupling chemistry described in Chapter 3 was used to attach a nitroimidazole group and two different nitrogen mustards to a diaminedichloroplatinum(II) core. The synthetic protocol was essentially unchanged from that described in Chapter 3, demonstrating the scope of this reaction. Furthermore, all compounds were obtained as analytically pure material, suitable for biological studies. Preliminary cytotoxicity assays with \textbf{must1-Pt} and \textbf{must2-Pt} revealed approximate IC$_{50}$ values of 80 and 2 µM, respectively, in HeLa cells. Because of solubility issues, however, DMF was used as a cosolvent, and dose-response curves never reached concentrations that completely killed all cells. Hence, this assay needs to be repeated. The nitroimidazole complex was not tested because our lab does not currently have an established protocol for culturing cells under hypoxic conditions. The hypoxia-targeting properties of the nitroimidazole, therefore, could not be assessed. A potential pitfall of these and related compounds is the modification of the non-leaving group ligands, which may negatively affect the DNA-binding properties of the complexes. The use of succinylacetone as a leaving group, as described in Appendix B, may provide a more favorable method to make functionalized platinum complexes.

C.4. Summary

The synthesis and characterization of several new platinum complexes are reported. The Pt-DCA complexes represent new additions to a growing class of dual-threat platinum anticancer agents. [Pt(BIPhMe)Cl$_2$] is the first platinum complex bearing the BIPhMe ligand. \textbf{Nitro-Pt}, \textbf{must1-Pt}, and \textbf{must2-Pt} illustrate the utility of the amide-coupling chemistry described in Chapter 3 for the preparation of novel bifunctional platinum anticancer agents.
C.5. References

Biographical Note

The author was born on April 10, 1986 in Santa Clara, California to Richard and Anne Wilson. After spending his first years of life in San Jose, CA, he moved with his grandmother, Magdalena “Chacho” Lavayen, to Dana Point, CA where he remained until graduating from high school. After high school, Justin enrolled in U.C. Berkeley as an undeclared major in the College of Letters and Science. Inspired by his first semester of freshman chemistry, Justin switched to the College of Chemistry to pursue chemistry. In the College of Chemistry, he carried out research in the lab of Professor Jeffrey R. Long on the topic of single-molecule magnets. After graduating from U.C. Berkeley with highest honors and receiving the College of Chemistry Departmental Citation, Justin moved to the east coast to pursue his Ph.D at the Massachusetts Institute of Technology, where he worked in the lab of Professor Stephen J. Lippard. His thesis topic focused on the synthesis and characterization of novel platinum complexes as potential anticancer drug candidates. After receiving his Ph.D, Justin will carry out postdoctoral research at Los Alamos National Laboratory.
Justin J. Wilson  
Curriculum Vitae

Education

2008–2013 Massachusetts Institute of Technology  
Ph.D Inorganic Chemistry  
Research Advisor: Professor Stephen J. Lippard  
GPA: 5.00 (out of 5.00)

2004 – 2008 University of California, Berkeley  
B.S. Chemistry, Awarded with Highest Honors  
Undergraduate Research Advisor: Professor Jeffrey R. Long  
GPA: 3.97 (out of 4.00)

Research Experience

2009–2013 Graduate Research Assistant - Massachusetts Institute of Technology  
- Synthesis and characterization of platinum coordination compounds as potential anticancer drug candidates. Routinely utilized single crystal X-ray diffraction, multi-dimensional and -nuclear NMR spectroscopy, atomic absorption spectroscopy, and electrospray-ionization mass spectrometry. Used mammalian tissue culture to determine cytotoxicity and cellular uptake of new platinum compounds.

2006–2008 Undergraduate Research Assistant – U.C. Berkeley  
- Synthesized multinuclear cyanide-bridged transition metal clusters using inert atmosphere Schlenk and glove box techniques. The new clusters were characterized by IR spectroscopy and single crystal X-ray diffraction using synchrotron radiation when necessary. The single-molecule magnetic properties of the clusters were investigated by SQUID magnetometry.

Teaching and Mentoring Experience

2012–2013 Undergraduate Research Mentor for Maria Chan – MIT  
- Trained Maria, a third year undergraduate chemical engineering major at MIT, in synthetic inorganic chemistry and mammalian cell culture techniques.

- Instructed Jennifer, a third year undergraduate chemistry major at MIT, in a variety of techniques crucial for independent research, leading to a first-author publication.

Spring 2010 Teaching Assistant, “Physical Methods in Inorganic Chemistry” – MIT
- Graded and helped prepare problem sets a graduate-level class on physical methods in inorganic chemistry. Helped students understand methods such as vibrational, electron paramagnetic resonance, X-ray, and Mössbauer spectroscopies.

Fall 2009  **Teaching Assistant, “Principles of Bioinorganic Chemistry” – MIT**
- Graded and helped prepare problem sets for a graduate-level class on bioinorganic chemistry. Helped organize the end-of-term student-run seminar symposium.

Spring 2009  **Teaching Assistant, “Laboratory Chemistry” – MIT**
- Supervised a laboratory section of ~20 undergraduate students and provided instructional sessions on laboratory experiments and techniques. Graded lab reports.

Fall 2008  **HHMI Teaching Assistant Fellow, “Principles of Chemical Science” – MIT**
- Provided three 50-minute discussion sections per week on lecture material to a group of ~25 undergraduate students on the subject off first-semester freshman chemistry. Graded exams and weekly problem sets.

Spring 2008  **Teaching Assistant, “General Chemistry” – UC Berkeley**
- Supervised a laboratory section of ~30 undergraduate students and provided a weekly 50-minute discussion section on lecture material and a 15-minute instructional session on the laboratory experiments. Graded exams and weekly lab reports. Subject matter was that of a first semester freshman chemistry course.

**Honors and Awards**

2012  David H. Koch Graduate Fellowship
2012  Morse Travel Grant
2011  Society for Biological Inorganic Chemistry Student Travel Grant
2011  International Precious Metals Institute Metalor Technologies Graduate Student Award
2009  Honorable Mention NSF Graduate Fellowship
2008  Howard Hughes Medical Institute Teaching Assistant Fellow
2008  UC Berkeley College of Chemistry, Chemistry Departmental Citation

**Professional Societies**

2011 – Present  Society for Biological Inorganic Chemistry
2009 – Present  American Chemical Society, Inorganic Division
Publications


Ulf-Peter Apfel, Daniela Buccella, Justin J. Wilson, Stephen J. Lippard. “Detection of Nitric Oxide and Nitroxy1 with Benzoresorufin-Based Fluorescent Sensors” Inorg. Chem. 2013, 52(6), 3285-3294.


- Referee-Recommended “Hot Article”


- Featured in MIT News, July 11, 2012


Patents


Presentations


